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14. ABSTRACT

Ovarian cancer's high mortality rate is largely due to the absence of effective targeted therapies. In addressing this, our project centered on the therapeutic potential of targeting UCA1, a long non-coding RNA. Our comprehensive research included gene amplification analysis, qRT-PCR, RNA-pulldown, mass spectrometric analysis, and in vivo xenograft models. We identified a two-fold mechanism of UCA1 upregulation: gene amplification and activation by growth factors like Lysophosphatidic Acid (LPA). Notably, we discovered that multiple LPA-receptor sub-types stimulate UCA1 expression, with their collective silencing markedly reducing UCA1 levels. Additionally, UCA1 functions as a competing endogenous RNA (ceRNA), inhibiting let-7 miRNAs, which in turn upregulates oncogenes such as c-Myc and Ras. In vivo studies confirmed that UCA1 silencing increases let-7 miRNAs, underscoring its significant role in ovarian cancer progression. Importantly, UCA1 interacts with spliceosome proteins, influencing the alternative splicing of key genes involved in cancer cell invasion, including MENA in addition to interacting with PRC family of protein to suppress the expression of tumor suppressors. Our findings not only deepen the understanding of UCA1's role in ovarian cancer but also spotlight it as a promising target for innovative therapeutic approaches, contributing significantly to the field of precision medicine in oncology.

15. SUBJECT TERMS None listed.

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1. INTRODUCTION

Ovarian cancer stands as the eighth most prevalent cancer among women and is the fifth leading cause of cancer-related deaths [1, 2]. This alarming mortality rate is primarily attributed to the disease's heterogeneous nature and the absence of effective targeted therapies [3, 4]. Our research has been focused on unveiling the oncogenic pathways influenced by the gep oncogene, specifically the α-subunit of Gα12, encoded by GNA12. This exploration led to the discovery of a previously unrecognized role of UCA1, a long noncoding RNA (lncRNA) with oncogenic properties, in the initiation and progression of ovarian cancer. Since the identification of lncRNAs in 2005 [5], their involvement in cancer biology has only recently begun to be fully appreciated. A number of these lncRNAs have been found to actively participate in gene regulation [5, 6]. While Recent studies highlight the oncogenic role of various lncRNAs in several cancers[7-12], their role in ovarian cancer is poorly understood. Our findings, indicating the upregulation of UCA1 by LPA through the gep oncogenes, take on added significance considering the substantial elevation of LPA levels in ovarian cancer patients [13]. Given that lncRNAs can control diverse gene sets involved in specific cellular responses through both cytosolic and nuclear regulatory mechanisms, the UCA1 signaling pathway, which is the focus of our study, is poised to be a crucial factor in the development, progression, and treatment resistance of ovarian cancer. We pursued three specific aims in this project: 1) Elucidating the mechanism through which gep-oncogenes activate UCA1 expression; 2) Deciphering the process by which UCA1 modulates oncogenic signaling; and 3) Investigating UCA1's role in the growth of ovarian cancer in vivo, as well as evaluating its potential as a therapeutic target using preclinical mouse models. The successful completion of this project is expected to significantly contribute to identifying new therapeutic targets and developing novel treatment strategies for ovarian cancer. Moreover, assessing the therapeutic potential of UCA1 inhibition in combination with cisplatin or epigenetic inhibitors promises to enhance treatment options, potentially overcoming therapy resistance in ovarian cancer patients.

2. KEYWORDS:

Ovarian Cancer; Gep oncogene, GNA12 gene; Long Non-coding RNA; Urothelial Cancer associated-1 (UCA); Spliceosome; microRNA; Mammalian Enabled (MENA); Therapy-resistance; Cancer epigenome; Lysophosphatidic Acid (LPA); Polycomb Group Proteins; Tumor-suppressors.

3. ACCOMPLISHMENTS:

What were the major goals of the project?

The major goals of this project were centered on elucidating the mechanisms and therapeutic potentials associated with UCA1 in ovarian cancer. Firstly, we aimed to define the process by which Lysophosphatidic Acid (LPA) stimulates UCA1 expression. This involved understanding the interaction between LPA and the gep oncogenes, and how this interaction leads to the activation of UCA1. Our second goal was to unravel the mechanisms by which UCA1 regulates oncogenic signaling. This step was crucial in comprehending the broader impact of UCA1 on cellular processes and its potential role in cancer progression. Moreover, the project sought to thoroughly analyze the role of UCA1 in the growth of ovarian cancer in vivo. This included assessing the therapeutic potential of UCA1, both as a standalone target and in conjunction with its downstream effectors. This approach was anticipated to expand the range of therapeutic options, potentially overcoming therapy resistance in ovarian cancer. The successful completion of these objectives was expected to significantly impact the development of novel therapeutic targets and treatment regimens for ovarian cancer.

What was accomplished under these goals?

The primary objective of our research was to elucidate the mechanisms by which UCA1 regulates oncogenic signaling in ovarian cancer and to identify potential therapeutic targets. This project was structured around

specific aims, focusing on understanding UCA1's role in cancer progression, therapy resistance, and exploring avenues for targeted treatments.

Aim 1. Define the Mechanism by Which LPA Stimulates UCA1 Expression

Gene Amplification Analysis: Our initial focus was on the genetic basis for UCA1 overexpression. We identified a key mechanism: We discovered a dual mechanism for UCA1 expression upregulation in ovarian cancer: genomic amplification (Figure 1) and stimulation by growth factors, including LPA (Figure 2).

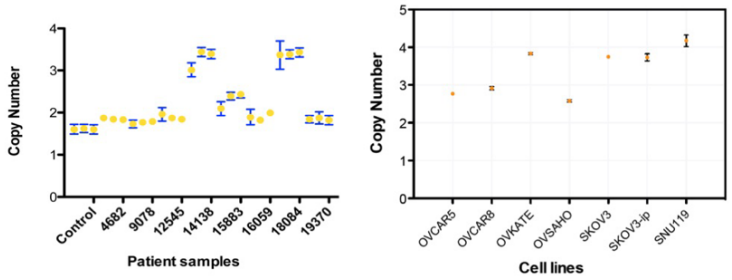
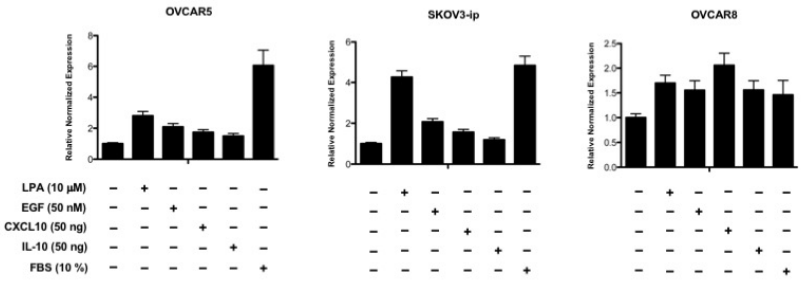


Figure 1. Copy Number Variation Analysis for UCA1*. CNV analysis was carried out using the genomic DNA from ovarian cancer cells derived from patient samples (Left Panel) or from the HGSCO cell lines (right Panel). Droplet PCR methods was used to determine the copy number variation.*

Figure 2. Stimulation of UCA1 expression be Growth Factors and Cytokines. *A panel of HGSOC cells were stimulated with LPA, EGF, CXCL10, IL-10, or FBS for 6-hrs as indicated. Expression of UCA1 was monitored by qRT-PCR.*



Our further analyses revealed that LPA-receptor sub-types (LPAR1, LPAR2, and LPAR3) are capable of stimulating UCA1 expression, with a significant attenuation upon their simultaneous silencing.

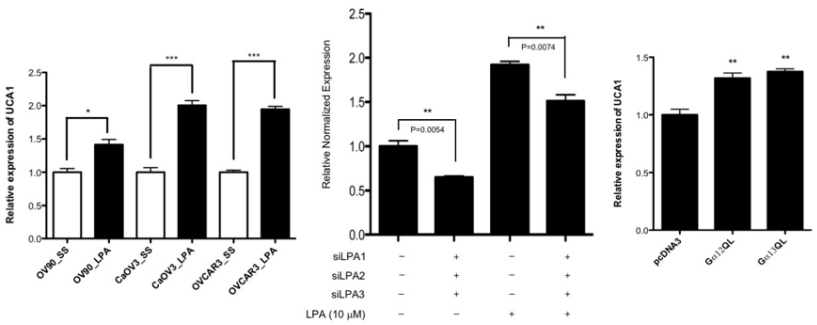


Figure 3. Expression of UCA1 by LPA, LPAR, and gep oncogene. *OV90, CaOV3, and OVCAR3 cells, after 24 hours of serum starvation, were treated with 10 μM LPA for 16 hours and analyzed for UCA1 expression via qRT-PCR (Left Panel). In parallel, SKOV3-ip cells were transfected with siControl or siLPA1/2/3, stimulated with LPA (10 μM) post-transfection, and UCA1 expression was quantified by RT-qPCR, normalized against GAPDH (Middle Panel). Additionally, SKOV3-ip cells with overexpressed constitutively active Ga12 and Ga13 were analyzed for UCA1 expression, compared to vector control cells (Right Panel).*

Interpretation and Significance: Our finding that the increased expression of UCA1 in ovarian cancer involves gene amplification as well as gene expression was crucial as it provided a genetic explanation for UCA1 overexpression, laying the groundwork for understanding its regulatory functions in ovarian cancer. The stimulation of UCA1 by growth factors like LPA suggests potential therapeutic targets. Inhibiting these pathways could be a strategy to control UCA1 expression and its oncogenic effects. This suggests that targeting the LPA pathway could be a viable strategy for modulating UCA1 expression, offering new insights into

therapeutic interventions. This suggests that targeting the LPA pathway could be a viable strategy for modulating UCA1 expression, offering new insights into therapeutic interventions.

Aim 2. Define the mechanism by which UCA1 regulates oncogenic signaling:

Functional Interaction with miRNAs: We discovered that UCA1 functions as a competing endogenous RNA (ceRNA), interacting with let-7 miRNAs (Figures 4A-E). This interaction quenches the activity of let-7 miRNAs, leading to increased expression of their target oncogenes, such as c-Myc and Ras.

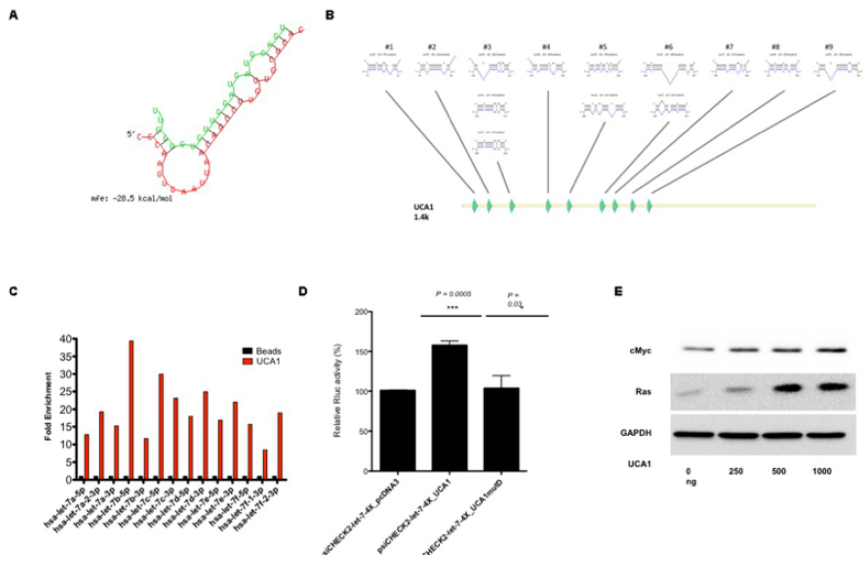


Figure 4. Functional quenching of Let-7 miRNA by UCA1. *A. In silico analysis indicating the interaction between UCA1 and Let-7 miRNA. B. Sequence analysis of UCA1 indicating the Let-7 interaction sites. In silico analysis derived interaction of the different members of Let-7 family of miRNAs are presented. C. Co-precipitation of Let-7family of miRNAs was monitored by qRT-PCR of the streptavidin bead precipitated biotinylated UCA1 in OVCAR8 lysates. D. Let-7 sensor (psiCHECK2-let-7 4x) was transfected into HEK 293 cells, together with putative sponge plasmid encoding UCA1, followed by luciferase activity assays. Numbers are mean ± SD (n = 3). E. OVCAR8 cells were transfected with 0, 250, 500, or 1000 ng of UCA1-expression construct for 48 hrs. Expression of Ras and cMyc, representative targets Let-7 miRNA target proteins were monitored by immunoblot analysis. Results are from a typical experiment.*

Identification of UCA1-Targeted Tumor Suppressors: We have also identified and validated the interaction of UCA1 with polycomb repressive complex (PRC) proteins, including EZH2, Suz12, Ring1A, and Ring1B, using crosslinking-immunoprecipitation (CLIP) assays. Further investigations expanded this interaction to other PRC2 complex proteins like AEBP2, EED, JARID2, and RbAp46/48 (Figure 5). These results reinforce UCA1's role in repressing tumor suppressor genes via interactions with multiple PRC family members.

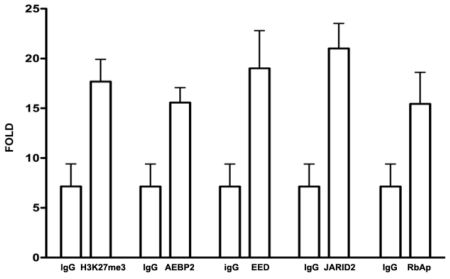


Figure 5. UCA1 interactions with PRC proteins. *Crosslinked complexes were immunoprecipitated using IgG, or antibodies to H3K27me3, AEBP2, EED, JARID2, or RbAp46/48 from OVCR8 lysates. After the reversal of the crosslinking, RNA was reverse transcribed and RT-qPCR was performed for the expression of UCA1. Fold enrichment (Fold) is presented for the respective polycomb group of proteins with UCA1, in comparison to the IgG control*

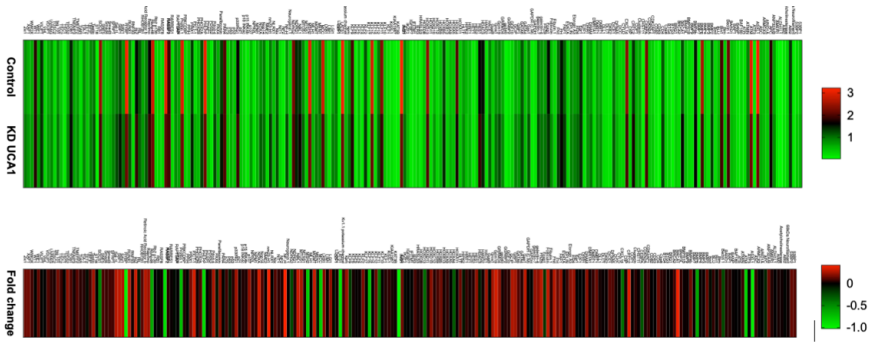


Figure 6. Changes of PRC-target protein detected by RPPA. Top Panel. Heat map of protein levels detected by RPPA. The levels of each protein are presented with colors, with red for highest and green for lowest. Bottom Pane. Fold change in the protein levels are calculated and presented with red for highest and green

for lowest. RPPA analysis was carried out three times and the representative heat map is presented.

Employing an antibody array (RPPA) approach, we identified 18 tumor suppressor genes significantly repressed by UCA1 (Figures 6). This repression highlights UCA1's role in promoting tumorigenesis in ovarian cancer. The RPPA analysis provided a visual representation of the protein expression changes, underscoring UCA1's extensive regulatory impact.

Pathway and Network Analysis: Pathway analyses using Cytoscape software revealed that UCA1-targeted tumor suppressors are predominantly involved in crucial biological processes and regulatory pathways, including Notch and Hippo signaling. This novel finding positions UCA1 as a key regulator of Notch signaling in ovarian cancer, a pathway previously known for its role in the disease but lacking a defined genetic activation mechanism. Further network analysis our results indicated that for significant proteins (BRCA2, RARB, HES1, and RUNX1) constituted major signaling hubs underlying UCA1 effect on ovarian cancer cell (Fig. 7).

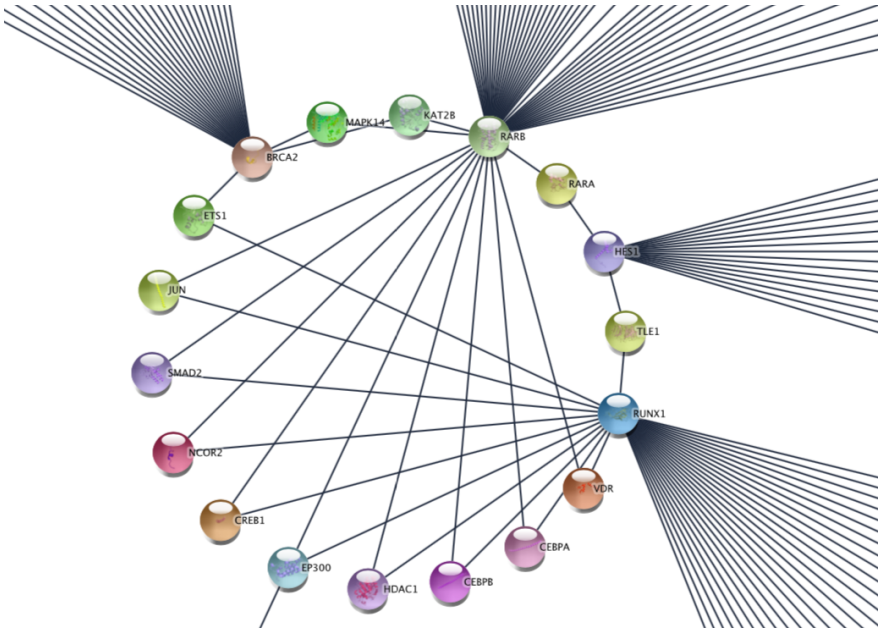


Figure 7. UCA1 targeted hub genes in ovarian cancer cells.

To validate that these tumor suppressor genes are indeed repressed by UCA1, we analyzed the expression levels of these proteins upon silencing. As shown in Fig. 8, the silencing of UCA1 led to an increase in the levels of RARB, HES1, BRCA2, and RUNX1. These tumor suppressors, identified through RPPA analysis, can serve as biomarkers for disease progression or therapeutic response. Targeting the pathways leading to their repression could provide new therapeutic avenues.

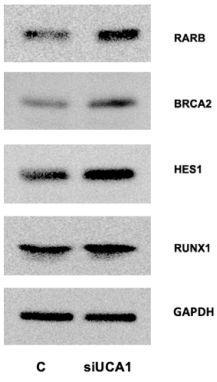


Figure 8. Silencing of the UCA1 increases the expression of RARB, BRCA2, HES1, and RUNX1. *OVCAR8 cells in which UCA1 was silenced (siUCA1) along with non-targeting sh-control cells (C) were serum lysed at 48 hrs following transfection of the siRNAs. Expressions of RARB, BRCA2, HES1, and RUNX1 were monitored by the sequential immunoblots using the respective antibodies. Stripped blot was also probed with GAPDH to monitor equal levels of protein loading.*

Of the candidate genes suppressed by UCA1, deletions or decreased expressions o TP53BP1, BRCA2, and NEFH are known to be associated with poor prognosis an therapy resistance in ovarian cancer [14-18]. Therefore, we tested the expression of thes genes in OVCAR8 and OVCAR3 cells independent of array results. To test the expressio.. of UCA1 was knocked down using two different siRNAs. The lysates were processed for the analyses of the expression of TP53BP1, NEFH, or BRCA2. As shown in Figure 9 panel A and B, the silencing of UCA1 led to an increase in the expression of TP53BP1, BRCA2, and NEFH, thus suggesting the role of UCA1 in suppressing the expression of these PRC1/2 targeted tumor-suppressor genes presumably via PRC1/2.

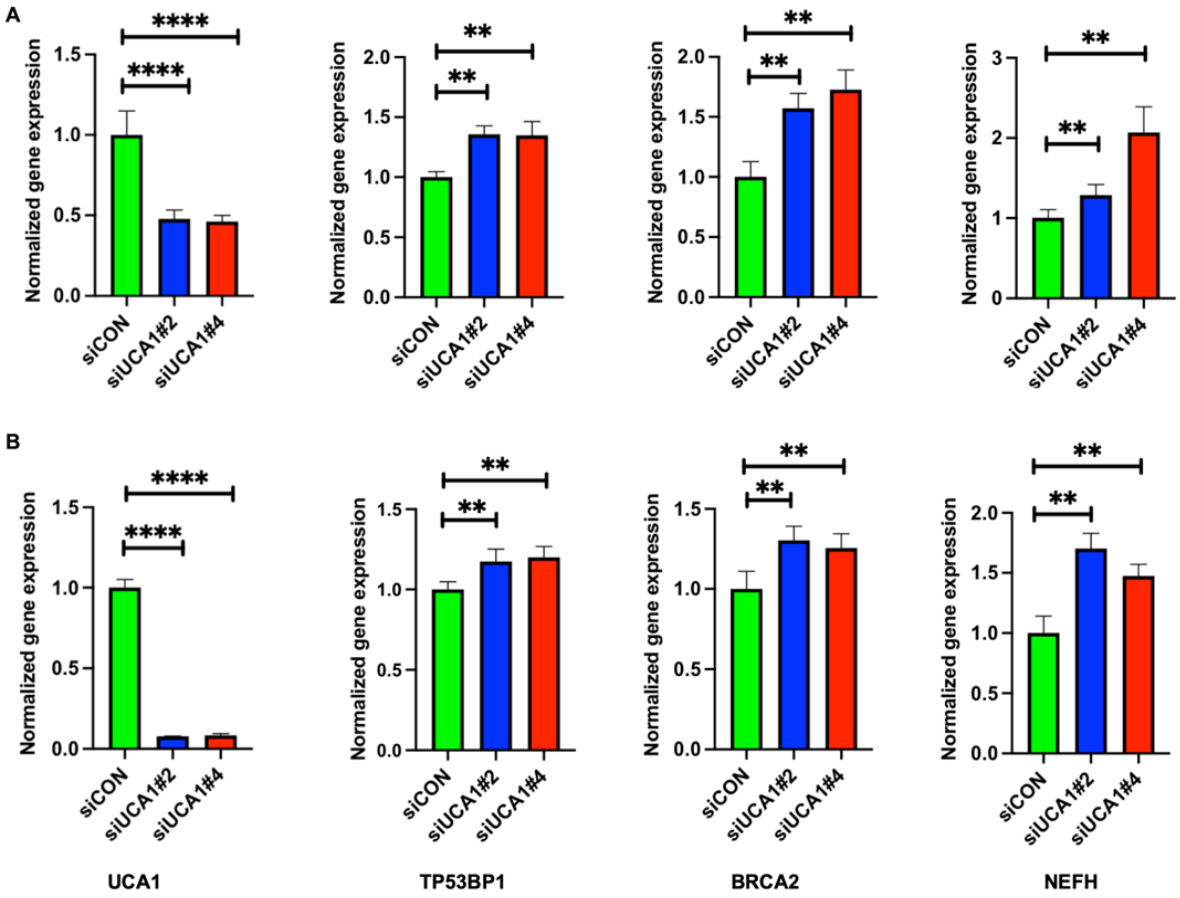


Figure 9. Knockdown of UCA1 Relieves the Repression of Tumor Suppressor Genes. *Upregulated expression of representative tumor-suppressor genes upon silencing of UCA1 was validated using OVCAR3 (A) and OVCAR3 (B) cells in which the expression of UCA1 was silenced using two different siRNAs targeting UCA1. Cells transfected with non-targeting scrambled siRNA pool were used as the control (SiCON). After validating the silencing of UCA1 with the respective siRNAs, upregulated genes were validated by monitoring the expression of TP53BP1, BRCA2, and NEFH by qRT-PCR. Statistical significance was determined by Student's t-test (**P < 0.005; ***P < 0.0005; ****P < 0.0001).*

ChIRP-Seq Analysis for UCA1-Targeted Gene Identification: Through ChIRP-Seq analysis, we identified a broad array of genes as potential UCA1 targets in the chromatin of the cancer genome, suggesting UCA1's involvement in diverse pathways and processes linked to cancer. The high-density data derived from the ChIRP sequencing is subjected to bioinformatic data analysis. Initial bioinformatic analysis has identified that UCA1 occupancy can be correlated with 2000 genes in the genome. Reasoning that the functional networking of these genes could provide insight into the mechanism by which UCA1 promotes oncogenic growth of ovarian cancer cells, we carried out pathway and network analyses. Since pathway enrichment analysis places UCA1 to genes involved in many critical pathways involved in cancer. They include genes involved in diverse metabolic pathways, protein synthesis, and pathways associated with spliceosome regulation (Figure 9)

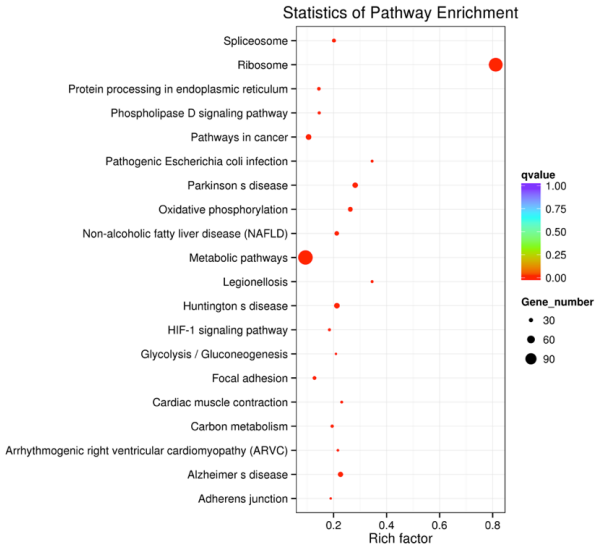
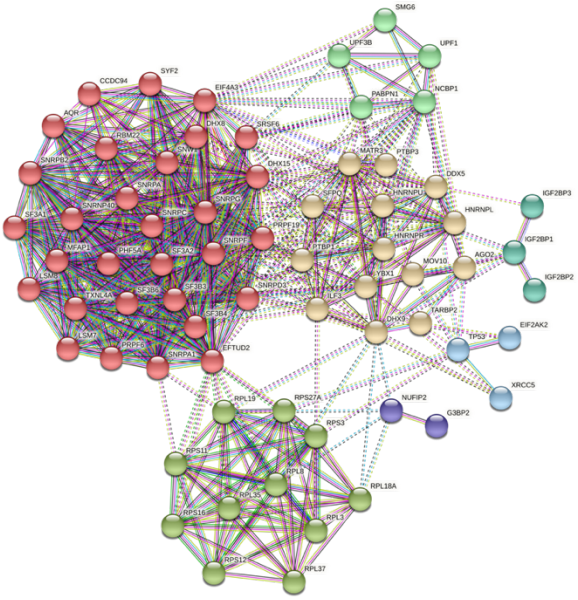


Figure 9. Pathway Enrichment Analysis of UCA1-occupancy sites. Kegg pathway analyses of gene enriched by UCA1-ChiRP cluster them into different groups.

Investigation of UCA1 Interactomes and Spliceosome Regulation: Our research focused on identifying therapeutic targets within the UCA1-regulated transcriptomic network in ovarian cancer, leading us to explore UCA1's interactome in OVCAR8 cells, a high-grade serous ovarian cancer (HGSOC) cell line. We conducted RNA-pulldown assays followed by liquid chromatography-mass spectrometry using biotinylated UCA1. Our findings revealed that UCA1 associates with proteins critical for mRNA splicing. Using STRING and Cluster algorithms for interactome analysis, we uncovered UCA1's potential role in activating oncogenic alternative splicing (AS), which appears to promote ovarian cancer growth and progression (Figure 11). This was particularly evident from the observed correlation between UCA1 expression and the altered ratio of MENA (ENAH) splice variants: the anti-invasive MENA11a and the pro-invasive MENAΔ11a/Δ6 (Figure 12). Remarkably, UCA1 knockdown in OVCAR8 cells resulted in the increased expression of the anti-invasive MENA11a variant. In line with this, treatments using spliceosome inhibitors or MENA silencing reduced the invasive potential of ovarian cancer cells. These results collectively highlight the UCA1-regulated AS switch as a key driver in ovarian cancer progression, presenting it as a promising target for precision cancer therapies.



Cluster 1	30	AQR,CCDC94,DHX15,DHX8,EFTUD2,EIF4A3,LSM7,LSM8,MFAP1,PHF5A,PRPF19,PR...
Cluster 2	14	AGO2,DDX5,DHX9,HNRNPL,HNRNPR,HNRNPU,ILF3,MATR3,MOV10,PTBP1,PTBP3,S...
Cluster 3	11	RPL18A,RPL19,RPL3,RPL35,RPL37,RPL8,RPS11,RPS12,RPS16,RPS27A,RPS3
Cluster 4	5	NCBP1,PABPN1,SMG6,UPF1,UPF3B
Cluster 5	3	IGF2BP1,IGF2BP2,IGF2BP3
Cluster 6	3	EIF2AK2,TP53,XRCC5
Cluster 7	2	G3BP2,NUFIP2

Figure 11. Protein-Protein Interaction Network of UCA1-interacting Proteins. *A. Using web-based STRING application, PPI network of the UCA1-interactome was constructed. Clustering of the interactome was carried out using MCL clustering algorithm. Clusters are color-coded. B. the constituent proteins in the respective clusters are listed.*

Alternative Splicing and Cancer Progression: A hallmark discovery of our project has been the identification of UCA1's involvement in alternative splicing, particularly impacting the MENA gene. This finding underscores the complexity of cancer progression and highlights UCA1's role in the post-transcriptional modification of key proteins.

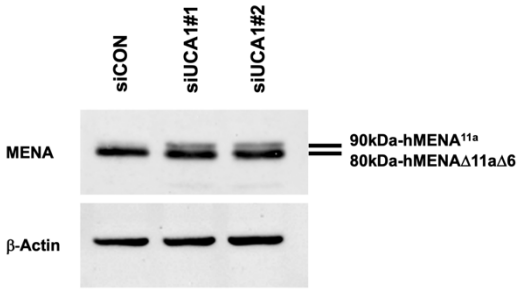


Figure 12. Expression MENA11a upon silencing of UCA1. *This figure displays the impact of UCA1 silencing on the expression of MENA splice variants in OVCAR8 cells, as analyzed by Western blotting. β-actin is used as the loading control. The experimental setup includes a scrambled siRNA control (siCon) and two distinct UCA1-targeting siRNAs (siUCA1#1 and siUCA1#2), illustrating the changes in MENA11a expression post-UCA1 silencing.*

Therapeutic Targeting of Spliceosomes in ovarian cancer. Since our results indicated a role for UCA1 in regulating the splicing switch in ovarian cancer cells, we sought to test whether small molecular inhibitors of spliceosomes can be used to inhibit ovarian cancer growth. Previous studies have shown that small molecular inhibitors of spliceosomes such as isoginkgetkin, pladienolide B, and indacaterol inhibits the growth of many cancer cells [19, 20]. Therefore, we tested whether the inhibition of spliceosome activity can attenuate the invasive migration of OVCAR8. Our results indicated that all of the tested inhibitors attenuated the invasive migration of OVCAR8 cells. However, pladienolide B that inhibits SF3B complex, more potently inhibited the invasive migration of OVCAR8 cells (Figure 13).

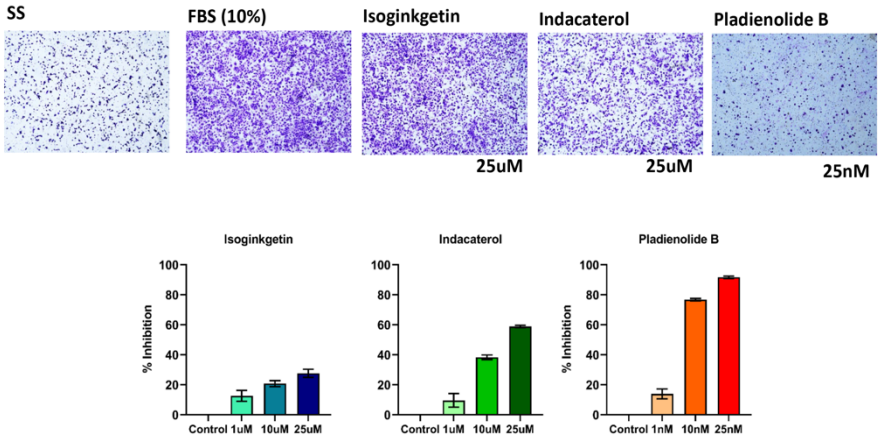


Figure 13. Effects of spliceosomal inhibitors on migration of ovarian cancer cells. *OVCAR8 cells, which were serum starved for 24h, was suspended in 200µl (5x10^4 cells) serum-free media and placed in the upper well of the transwell insert. Each well of the companion plate contained 500 µL media containing serum-free media (control) or serum-free media with 10% FBS (control) or serum-free media with 10% FBS containing 1µM, 10µM or 25 µM each of Isoginkgetin or Indacaterol or 1nM, 10nM and 25nM of Pladienolide B. The cells were incubated for 24 h. Non-migrating cells on the proximal side of the inserts were removed with a cotton swab and the migrated cells on the distal side of the insert were fixed stained with Hemacolor. Images were obtained of random fields of view at 4X magnification. The images shown are representative of the three replicate fields. Migrated cells were quantified from six different fields at 20x magnification from each well and averaged to three independent wells (mean ± SEM; n = 3) and plotted as percent inhibition over control with 10%FBS in serum free media.*

Similarly, shRNA to MENA drastically inhibited the invasive migration of OVCAR8 cells (Figure 14)

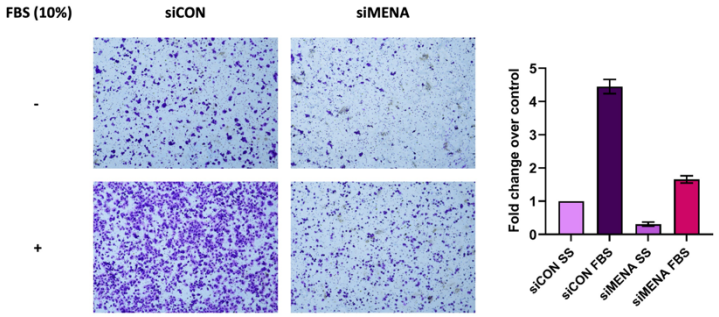


Figure 5. Effects of MENA on migration of ovarian cancer cells. *OVCAR8 cells were transfected with siRNA for MENA or scrambled siRNA control. After 24h of transfection, the cells were serum starved for 24h. Cells were then suspended in 200µl (5x10^4 cells) serum-free media and placed in the upper well of the transwell insert. Each well of the companion plate contained 500 µL media containing serum-free media or serum-free media with 10% FBS. The cells were incubated for 24 h. Non-migrating cells on the proximal side of the inserts were removed with a cotton swab and the migrated cells on the distal side of the insert were fixed stained with Hemacolor. Images were obtained of random fields of view at 4X magnification. The images shown are representative of the three replicate fields. Migrated cells were quantified from six different fields at 20x magnification from each well and averaged to three independent wells (mean ± SEM; n = 3) and plotted as fold change of migrated cells over control (siCON cells in serum free media). SS denotes serum free media and FBS denotes serum free media with 10% FBS.*

The successful inhibition of ovarian cancer cell invasiveness using spliceosome inhibitors highlights the therapeutic potential of targeting the UCA1-spliceosome-MENA axis. This approach is a testament to the effectiveness of precision medicine strategies in oncology. This finding paves the way for clinical trials testing spliceosome inhibitors in ovarian cancer, offering a promising new treatment modality for patients with UCA1-driven tumors.

Conclusion and Project Fulfillment:

Comprehensive Understanding of UCA1's Role in Ovarian Cancer: Our project's journey to decode the role of UCA1 in ovarian cancer has led to several pivotal discoveries, each contributing to a holistic understanding of this complex disease. One of the key aspects of this research was the implementation and analysis of Reverse Phase Protein Array (RPPA), which significantly enriched our findings.

1. **Elucidation of UCA1 Expression and Oncogenic Signaling:** The dual mechanism driving UCA1 expression—genomic amplification and stimulation by growth factors, especially LPA—was a critical discovery. Additionally, our insight into UCA1's modulation of let-7 miRNAs and its involvement in alternative splicing, particularly affecting the MENA gene, has provided a nuanced understanding of cancer progression.
2. **Pioneering RPPA Analysis:** The use of RPPA allowed for a high-throughput, quantitative analysis of protein interactions and expression levels affected by UCA1. Our findings from the RPPA analysis revealed that UCA1 silencing relieved the repression of 18 key tumor suppressor genes. This information is pivotal in understanding the epigenetic influence of UCA1 in ovarian cancer and identifies potential markers for prognosis and therapy resistance.The RPPA analysis not only confirmed our hypotheses about UCA1's role in gene expression regulation but also provided tangible targets for future therapeutic interventions, underscoring the utility of this technology in cancer research.
3. **Innovative Therapeutic Strategies and Epigenomic Mapping:** The project has opened new avenues in cancer treatment, particularly through the exploration of spliceosome inhibitors. Additionally, our extensive epigenomic mapping via ChIRP-Seq analysis offers a comprehensive view of UCA1's impact on the cancer genome.
4. **Targeting the UCA1-Spliceosome-MENA Axis:** Our studies with spliceosome inhibitors, informed by the understanding of UCA1's role in mRNA splicing, have shown promising results in attenuating ovarian cancer cell invasiveness. This approach exemplifies the potential of targeting specific molecular pathways for cancer therapy.
5. **Rich Resource for Future Research:** The ChIRP-Seq analysis has provided an extensive dataset, mapping UCA1's influence across the cancer epigenome. This resource is invaluable for identifying novel targets and understanding the broader implications of UCA1 in cancer biology.

Implications for Precision Medicine and Future Resarch: The cumulative findings of our project have profound implications for precision medicine. By delineating UCA1's diverse roles, we have identified multiple potential targets for personalized treatment strategies. Our research not only meets the project's objectives but also serves as a catalyst for future explorations into targeted therapies and personalized medicine in ovarian cancer.

Project Legacy and Future Directions: As we conclude, we reflect on a legacy marked by scientific discovery and innovation. The methodologies and insights gained, especially through RPPA analysis, have not only enhanced our understanding of ovarian cancer but also set a new standard for cancer research. The project lays a foundation for future studies to build upon, with the ultimate goal of improving patient outcomes and advancing the field of oncology. In summary, this project has achieved its objectives and more, significantly advancing our understanding of ovarian cancer and opening new paths for treatment. The findings, particularly from the RPPA analysis, represent a substantial contribution to cancer research, with lasting implications for future studies and clinical practice.

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4. IMPACT

This project has significantly advanced our understanding of UCA1 in ovarian cancer, from its regulation and function to its potential as a therapeutic target. By bridging gaps in knowledge and introducing innovative therapeutic strategies, the project has made substantial contributions to the fields of oncology and precision medicine. The findings not only enhance the scientific community's understanding of lncRNA roles in cancer but also offer hope for improved patient outcomes through **more targeted and effective treatments.**

What was the impact on the development of the principal discipline(s) of the project?

1. **Dual-Regulation of UCA1:** Our research uncovered a dual-regulation mechanism of UCA1 involving gene amplification and growth factor stimulation. This key discovery enhances the understanding of lncRNA regulation in ovarian cancer, offering new perspectives on its role in cancer biology.

2. **UCA1-let7 Interaction:** We demonstrated that UCA1 acts as a competing endogenous RNA, modulating the activity of let-7 miRNAs. This interaction leads to the upregulation of oncogenes such as c-Myc and Ras, providing insight into the molecular mechanisms of oncogenesis driven by lncRNAs.
3. **RPPA Analysis - Pathways and Tumor Suppressors:** Through Reverse Phase Protein Array (RPPA) analysis, we identified critical pathways and tumor suppressors regulated by UCA1. This has significantly contributed to the understanding of how lncRNAs like UCA1 can influence key cancer-related pathways and tumor suppressor genes.
4. **ChiRP Sequencing Insights:** Utilizing ChiRP sequencing, we explored the broader genomic impact of UCA1, revealing its extensive role in the ovarian cancer epigenome. This comprehensive approach has identified numerous potential targets for therapy and furthered our understanding of the genomic landscape influenced by UCA1.
5. **Alternative Splicing of MENA:** Our findings on the alternative splicing of MENA, regulated by UCA1, highlight a novel aspect of lncRNA function in cancer progression. This has implications for developing targeted therapies aimed at splice variants influencing cancer cell invasiveness.

What was the impact on other disciplines?

1. **Advancement in Therapeutic Strategies in Oncology:** The project's findings have significantly contributed to the advancement of therapeutic strategies in the field of oncology. The demonstration of siRNA and spliceosome inhibitors' effectiveness in targeting UCA1-regulated pathways supports the development of novel, precise treatment modalities for ovarian cancer. The integration of our RPPA analysis data has been instrumental in this context, revealing key proteins and pathways modulated by UCA1, which can be targeted therapeutically.
2. **Development of lncRNA-Targeted Therapies:** We are currently developing innovative therapeutic strategies targeting UCA1, including siRNA-UCA1-encapsulated targeted nanoparticles. This approach is based on our findings from RPPA analysis and epigenomic studies, which have identified specific pathways and tumor suppressors regulated by UCA1. The outcomes of these studies could establish a groundbreaking lncRNA-targeted therapeutic paradigm for ovarian cancer.
3. **Alignment with Precision Medicine:** Our research aligns with the evolving field of precision medicine, aiming to offer patient-specific treatments informed by genetic, environmental, and lifestyle factors. The discoveries concerning UCA1's dual-regulation, interaction with let-7 miRNAs, and the impact on MENA's alternative splicing are pivotal in developing these targeted therapies. Furthermore, our ChiRP sequencing data, revealing UCA1's extensive role in the cancer epigenome, enriches our understanding of its function and potential as a therapeutic target.
4. **Broader Applications Beyond Ovarian Cancer:** The potential of these therapeutic strategies extends beyond ovarian cancer, offering valuable insights and frameworks applicable to other types of cancers where lncRNAs play critical roles. Our comprehensive approach, combining RPPA analysis and cancer epigenome studies, provides a template for exploring lncRNA functions and therapeutic targeting in various cancer forms.

What was the impact on technology transfer?

- Our research on UCA1's regulatory mechanisms holds significant promise for technology transfer, particularly in the realm of RNA-based therapeutics. The knowledge gained about UCA1's function and its network in ovarian cancer cells provides a valuable foundation for the development of new RNA-based drugs.
- Since Splicing Switch Oligonucleotides (SSO)-based therapeutics are being developed for the treatment of many different diseases including cancer [21, 22], SSO based on UCA1-target spicing sequence can be developed for ovarian cancer therapy. Therefore, our studies will have a significant impact on

technology transfer focused on the the development of new class of SSO-based therapeutics for ovarian cancer.

- The translational potential of these findings is vast, with the possibility of revolutionizing ovarian cancer treatment protocols. These innovative therapeutic approaches, stemming from our understanding of UCA1's interactions and impact on gene expression, could be pivotal in treating other lncRNA-influenced malignancies as well.
- The integration of our findings into pharmaceutical development pipelines could lead to the creation of a new class of therapeutics, bolstering the arsenal against cancer with more targeted and effective treatments.

What was the impact on society beyond science and technology?

- Our research has the potential to significantly enhance cancer treatment and patient care. By developing therapies that are more targeted and specific to the molecular profile of a patient's cancer, we can reduce the side effects often associated with conventional treatments.
- The paradigm shift towards personalized and targeted cancer treatments, as evidenced by our project, has immense implications for patient quality of life. By improving the efficacy of treatments and minimizing collateral damage to healthy cells, these therapies could lead to better patient outcomes and quicker recoveries.
- The societal impact of these advancements is substantial. By reducing the physical, emotional, and financial burden of cancer treatments on patients and their families, our research contributes to a more sustainable and efficient healthcare system. The identification of UCA1 as a key target in ovarian cancer treatment is a step forward in precision oncology, with the potential to transform the landscape of cancer care and benefit society at large.

5. CHANGES/PROBLEMS:

Actual or anticipated problems or delays and actions or plans to resolve them

During the period of 2019 t0 2021, we were hampered drastically by the COVID-19 lock-down and the departure of postdoctoral associates to their respective countries due to COVID-19 outbreak during this period as well. However, we could resolve these issue through the recruitment of new research personnel during the follwing.

Changes that had a significant impact on expenditures

Nothing to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to Report.

Significant changes in use or care of human subjects

Nothing to Report.

Significant changes in use of biohazards and/or select agents

Nothing to Report.

Publications, conference papers, and presentations

Journal publications

Published:

1. Ha JH, Jayaraman M, Yan M, Dhanasekaran P, Isidoro C, Song YS, Dhanasekaran DN. Identification of GNA12-driven gene signatures and key signaling networks in ovarian cancer. Oncol Lett. 2021 Oct;22(4):719. doi: 10.3892/ol.2021.12980. Epub 2021 Aug 10. PMID: 34429759 (Ref. [23]).
   Federal support including this grant is acknowledged.

2. Ha JH, Jayaraman M, Yan M, Dhanasekaran P, Isidoro C, Song YS, Dhanasekaran DN. GNAi2/gip2-Regulated Transcriptome and Its Therapeutic Significance in Ovarian Cancer. Biomolecules. 2021 Aug 14;11(8):1211. doi: 10.3390/biom11081211. PMID: 34439877 (Ref. [24]). Federal support including this grant is acknowledged

3. Nadhan R, Dhanasekaran DN. Decoding the Oncogenic Signals from the Long Non-Coding RNAs. Onco 2021, 1, 176-206. (Ref. [25]). Federal support including this grant is acknowledged.

4. Nadhan R, Isidoro C, Song YS, Dhanasekaran DN. Signaling by LncRNAs: Structure, Cellular Homeostasis, and Disease Pathology. Cells. 2022 11:2517. doi: 10.3390/cells11162517. PMID: 36010595 (Ref. [26]). Federal support including this grant is acknowledged.

5. Nadhan R, Dhanasekaran DN. Regulation of Tumor Metabolome by Long Non-Coding RNAs. Journal of Molecular Signaling. 2022; 16: 1, pp. 1–19. DOI: https://doi.org/10.55233/1750-2187-16-1 (Ref. [27]). Federal support including this grant is acknowledged.

These publications are included under "Appendices".

Under Revision for Resubmission:

1. Ha JH, Radhakrishnan R, Jayaraman M, Yan M, Fung KM, Moxley KM, Sood AK, Isidoro C, Bhattacharya R, Mukherjee P, Song YS, Dhanasekaran DN. Unraveling a GPCR-lncRNA-miRNA Nexus: Identification of an Aberrant Therapeutic Target in Ovarian Cancer (Under revision for resubmission to *Science Advances*).

   This manuscript , which is under revision, is included under "Appendices.

2. Jayaraman M, Ha JH, Radhakrishnan R, Song YS, Bhattacharya R, Mukherjee P, Dhanasekaran DN. Transcriptomic analysis of lncRNAs and mRNAs in ovarian cancer (Under revision for resubmission to Cells).

Manuscript Under Preparation:

1. Jayaraman M, Ha JH, Radhakrishnan R, Song YS, Dhanasekaran DN. Regulation of ovarian cancer epigenome by UCA1. Jayaraman M, Ha JH, Radhakrishnan R, Song YS, Dhanasekaran DN. Transcriptomic analysis of lncRNAs and mRNAs in ovarian cancer (Under revision for resubmission to *Biomolecules*).

2. Nadhan R, Ha JH, Radhakrishnan R, Jayaraman M, Isidoro C, Bhattacharya R, Mukherjee P, Song YS, Dhanasekaran DN. Oncogenic Splice-switching by the Long Non-coding RNA UCA1 In Ovarian Cancer (In preparation for submission to Cancer Communications).

Books or other non-periodical, one-time publications.

1. **Book Chapter:** Nadhan R, Isidoro C, Song YS, and Dhanasekaran DN. Long non-coding RNAs in Cancer, Handbook of Oncobiology: From Basic to Clinical Sciences. R. C. Sobti et al. (eds.), Springer Nature Singapore Pte Ltd. 2023. https://doi.org/10.1007/978-981-99-2196-6_37-1. (Ref. [28]). Federal support including this grant is acknowledged.

2. **Published AACR Meeting Abstract:** Nadhan R, Ha JH, Jayaraman M, Kashyap S, Dhanasekaran, DN. Ovarian cancer cell-derived exosomal UCA1 reprograms glucose metabolism in stromal fibroblasts [abstract]. In: Proceedings of the American Association for Cancer Research Annual Meeting 2023; Part 2 (Clinical Trials and Late-Breaking Research); 2023 Apr 14-19; Orlando, FL. Philadelphia (PA): AACR; Cancer Res 2023;83(8_Suppl):Abstract nr LB039.

These publications are included under "Appendices".

Other publications, conference papers, and presentations.

1. "LncRNA UCA1 Regulation of Ovarian Cancer Genesis and Progression", December 28, 2020; Fifth Meeting of the International Society of Precision Cancer Medicine, Virtual Conference, Seoul, S. Korea.
2. "GPCR, lncRNA, miRNA: An Emerging Pathological Alliance in Ovarian Cancer", November 01, 2021; Winthrop P. Rockefeller Cancer institute, University of Arkansas Medical Center, Little Rock, AR.
3. "LncRNAs and Spliceosomes", November 12, 2021; Webinar on Non-Coding RNAs, Virtual Conference, University of Novara, Italy.
4. "Oncogenic Splice-switching by LncRNAS in Ovarian Cancer", September 28-30, 2022; 6th Cancer World Congress, Lisbon, Portugal
5. "Ovarian Cancer Cell-derived Exosomal UCAl Reprograms Glucose Metabolism in Stromal Fibroblasts", April 15, 2023; AACR Meeting, Orlando, Florida.
6. "ExoLnc Signaling: Reprogramming Cancer Metabolism in the TME by UCA1", October 5-7, 2023; First International Cancer Science Congress, Palermo, Italy.
7. "LncRNAs and Oncometabolism", November 1-3, 2023; International Conference hosted by the Journal of Traditional and Complimentary Medicine, Taipei, Taiwan.

Website(s) or other Internet site(s)

Nothing to Report.

Technologies or techniques

Nothing to Report.

Inventions, patent applications, and/or licenses

Nothing to Report.

Other Products

- **Data or databases**

7. PARTICIPANTS

What individuals have worked on the project?

No Change from previous annual submission:

Name: Dr. Danny Dhanasekaran
Project Role: Principal Investigator
Nearest person month worked: 1
Contribution to Project: Supervised the study.

Name: Dr. Kar-Ming Fung
Project Role: Co-Investigator
Nearest person month worked: 1

Name: Dr. Katherine Moxley
Project Role: Co-Investigator
Nearest person month worked: 1
Contribution to Project: Collaborator

Name: Dr. Jihee Ha
Project Role: Postdoctoral Fellow
Nearest person month worked: 2
Contribution to Project: Carried out the studies under the PI's supervision.

Name: Dr. Revathy Nadhan
Project Role: Postdoctoral Fellow
Nearest person month worked: 12
Contribution: Carried out the studies under the PI's supervision.

Name: Dr. Ramesh Rajagopal
Project Role: Consultant
Nearest person month worked: 2
Contribution to Project: Need-based consultancy

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

No Change.

What other organizations were involved as partners?

Not applicable.

8. SPECIAL REPORTING REQUIREMENTS

Not Applicable

9. APPENDICES:

PDF files of the following published papers and a manuscript under revisionare attached following the references section:

Published Papers

1. Ha JH, Jayaraman M, Yan M, Dhanasekaran P, Isidoro C, Song YS, Dhanasekaran DN. Identification of GNA12-driven gene signatures and key signaling networks in ovarian cancer. Oncol Lett. 2021 Oct;22(4):719. doi: 10.3892/ol.2021.12980. Epub 2021 Aug 10. PMID: 34429759.
2. Ha JH, Jayaraman M, Yan M, Dhanasekaran P, Isidoro C, Song YS, Dhanasekaran DN. GNAi2/gip2-Regulated Transcriptome and Its Therapeutic Significance in Ovarian Cancer. Biomolecules. 2021 Aug 14;11(8):1211. doi: 10.3390/biom11081211. PMID: 34439877.
3. Nadhan R, Dhanasekaran DN. Decoding the Oncogenic Signals from the Long Non-Coding RNAs. Onco 2021, 1, 176-206.
4. Nadhan R, Isidoro C, Song YS, Dhanasekaran DN. Signaling by LncRNAs: Structure, Cellular Homeostasis, and Disease Pathology. Cells. 2022 11:2517. doi: 10.3390/cells11162517. PMID: 36010595.
5. Nadhan R, Dhanasekaran DN. Regulation of Tumor Metabolome by Long Non-Coding RNAs. Journal of Molecular Signaling. 2022; 16: 1, pp. 1–19. DOI: https://doi.org/10.55233/1750-2187-16-1.
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Manuscript under revision:

1. Ha JH, Radhakrishnan R, Jayaraman M, Yan M, Fung KM, Moxley KM, Sood AK, Isidoro C, Bhattacharya R, Mukherjee P, Song YS, Dhanasekaran DN. Unraveling a GPCR-lncRNA-miRNA Nexus: Identification of an Aberrant Therapeutic Target in Ovarian Cancer (Under revision for resubmission to *Science Advances*).

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26. Nadhan R, Isidoro C, Song YS, Dhanasekaran DN: Signaling by LncRNAs: Structure, Cellular Homeostasis, and Disease Pathology. *Cells* 2022, 11(16).
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Identification of *GNA12*-driven gene signatures and key signaling networks in ovarian cancer

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Abstract. With the focus on defining the oncogenic network stimulated by lysophosphatidic acid (LPA) in ovarian cancer, the present study sought to interrogate the oncotranscriptome regulated by the LPA-mediated signaling pathway. LPA, LPA-receptor (LPAR) and LPAR-activated G protein 12 α-subunit, encoded by G protein subunit α 12 (*GNA12*), all serve an important role in ovarian cancer progression. While the general signaling mechanism regulated by LPA/LPAR/*GNA12* has previously been characterized, the global transcriptomic network regulated by *GNA12* in ovarian cancer pathophysiology remains largely unknown. To define the LPA/LPAR/*GNA12*-orchestrated oncogenic networks in ovarian cancer, transcriptomic and bioinformatical analyses were conducted using SKOV3 cells, in which the expression of *GNA12* was silenced. Array analysis was performed in Agilent SurePrint G3 Human Comparative Genomic Hybridization 8x60 microarray platform. The array results were validated using Kuramochi cells. Gene and functional enrichment analyses were performed using Database for Annotation, Visualization and Integrated Discovery, Search Tool for Retrieval of Interacting Genes and Cytoscape algorithms. The results indicated a paradigm in which *GNA12* drove ovarian cancer progression by upregulating a pro-tumorigenic network with *AKT1*, *VEGFA*, *TGFB 1*, *BCL2L1*, *STAT3*, insulin-like growth factor 1 and growth hormone releasing hormone as critical hub and/or bottleneck nodes. Moreover, *GNA12* downregulated a growth-suppressive network involving proteasome 20S subunit (PSM) β6, *PSM* α6, *PSM* ATPase 5, ubiquitin conjugating enzyme E2 E1, *PSM* non-ATPase 10, NDUFA4 mitochondrial complex-associated, NADH:ubiquinone oxidoreductase subunit B8 and anaphase promoting complex subunit 1 as hub or bottleneck nodes. In addition to providing novel insights into the LPA/LPAR/*GNA12*-regulated oncogenic networks in ovarian cancer, the present study identified several potential nodes in this network that could be assessed for targeted therapy.

Introduction

Ovarian cancer is the seventh most common cancer type in women worldwide, with 250,000 new cases diagnosed worldwide annually, and it lead to 185,000 deaths in 2020 (1). Recent analysis has estimated that the deaths associated with ovarian cancer would be as high as 13,770 by 2021 in the USA alone (2). The high mortality rate in ovarian cancer is associated with a late diagnosis, as well as a lack of an effective targeted therapy (3,4).

The observation that lysophosphatidic acid (LPA) synthesized by cancer cells acts as an endogenous growth factor, and that LPA-mediated signaling pathways serve a tumor-promoting role across numerous cancer types, including ovarian cancer, is clinically significant (5,6). LPA was initially identified as a platelet derived bioactive phospholipid that stimulated the proliferation of fibroblasts involved in wound healing (7). Subsequent studies have shown that LPA stimulates multiple signaling pathways underlying cell proliferation, migration, and survival via specific G-protein coupled receptors and the associated heterotrimeric G proteins (8,9). Of the different G proteins that could be activated by LPA-receptors (LPARs), G protein 12 (G12) has been identified as the major conduit involved in LPA-mediated mitogenic signaling (10-12). In

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Abbreviations: LPA, lysophospatidic acid; LPAR, lysophosphatidic acid receptor

Key words: ovarian cancer, lysophosphatidic acid, G protein subunit α 12, gene expression, genomics, signaling-network

ovarian cancer, cancer cells synthesize and release LPA into the tumor microenvironment (TME). LPA present in the TME promotes cancer progression and metastasis via the activation of specific LPA-receptors (LPARs) that are present in multicellular components of the TME (13,14). In cancer cells, LPA stimulates an autocrine signaling loop via the activation of cancer cell-bound G-protein coupled LPARs. Although G protein coupled receptors, such as LPARs, have proven to be highly amenable for drug development, targeting LPARs in ovarian cancer has been challenging. High concentrations of LPA in the intraperitoneal ascites surrounding the ovarian cancer tissue and the close proximity of the LPA-synthetic machinery to LPARs on the surface of ovarian cancer cells have impeded LPAR-targeted therapeutic strategies in ovarian cancer. Recent studies from several laboratories, including ours, have reported that the α-subunit of the oncogene G-protein G12, encoded by the gene G protein subunit α 12 (*GNA12*), is the major conduit involved in transmitting oncogenic signals in numerous cancer types, including ovarian cancer (15-20). It has been revealed that either LPAR-stimulated activation or mutational activation of *GNA12*, referred to as the *gep* oncogene, induces the oncogenic proliferation of ovarian cancer cells (15,16).

Tumorigenesis and tumor progression often involve the deregulation of multiple pathways, impacting a cell-wide signaling network rather than an alteration in a single gene or pathway (21). Therefore, we hypothesized that the transcriptomic analysis based on the aggregated expression of genes associated with multiple pathways co-regulated by *GNA12* could provide additional insights into the LPA/LPAR/*GNA12*-induced oncogenic signaling network in ovarian cancer. Based on this rationale, the present study aimed to investigated *GNA12*-orchestrated effects in ovarian cancer pathobiology using micro-array based transcriptomic analysis. Herein, the results from pathway-based bioinformatics analyses are shown in order to define the co-regulatory signaling circuits regulated by *GNA12* in ovarian cancer. Using SKOV3 cells in which *GNA12* had been silenced, transcriptomic profiling was conducted to identify the differentially expressed genes (DEGs). Moreover, array results were validated by monitoring the expression levels of representative DEGs via reverse transcription-quantitative (RT-q) PCR analysis in *GNA12*-silenced-Kuramochi cells. Further Gene Ontology (GO) enrichment and protein-protein interaction (PPI) network analyses were performed using web-based Database for Annotation, Visualization and Integrated Discovery (DAVID) and Search Tool for Retrieval of Interacting Genes (STRING), as well as Cytoscape software applications. In addition to providing a novel insight into the organizational structure of LPA/LPAR/*GNA12*-driven transcriptomic network in ovarian cancer, the present study has identified specific hub and bottleneck nodes that can be targeted individually or collectively for effective targeted adjuvant therapy for ovarian cancer.

Materials and methods

Cell lines and culture. High grade serous carcinoma cell line Kuramochi and non-serous ovarian carcinoma cell line SKOV3 were obtained from American Type Culture Collection (Manassas, VA) and the cells were authenticated by short tandem repeat analysis as described (13). Kuramochi cells were maintained in Roswell Park Memorial Institute (RPMI)-1640 medium (Cellgro) and SKOV3 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Cellgro), both at 37˚C in a 5% CO_2 incubator. In both cases, the media were supplemented with 10% FBS (Gemini Bio-Products), 50 U/ml penicillin, 50 μg/ml streptomycin (Cellgro). For LPA-stimulation studies, 18.1 LPA (1-oleoyl-2-h ydroxy-sn-glycero-3-phosphate; cat. no. 85730), was obtained from Avanti Polar Lipids (Alabaster, AL). LPA was dissolved in 10 mM stock solutions in phosphate buffered saline containing 1% BSA and stored at -80˚C until use.

Human cell lines and methods used in this study were approved by the Institutional Review Board for the protection of the Human Subjects of the University of Oklahoma (approval no. 9599).

Transfection methods. Silencing of *GNA12* in SKOV3 cells was carried out as described in our previously publication (16). Briefly, non-target scrambled control shRNA pLKO.1 vector construct (Sigma-Aldrich; Merck KGaA (SHC002) and pLKO.1 vector construct targeting *GNA12*/Gα12 (RHS3979-98491914; Open Biosystems) were stably transfected into SKOV3 cells using Amaxa Biosystems Nucleofector II, according to the instructions of the manufacturer. The stably transfected NS control and Gα-silenced clones were selected with puromycin (2 μg/ml; MP Biomedicals) and single clones were picked, expanded to obtain stable cell lines. Prior to the array analysis, the silencing of *GNA12*-expression was ascertained by immunoblot analysis. Silencing of GNA12 in Kuramochi cells were carried out using siRNAs targeting *GNA12* (siGENOME Human *GNA12* siRNA SMARTpool; cat. no. M-008435-00-0005) and non-targeting scrambled control siRNAs control (siGENOME Non-Targeting siRNA Pool; cat. no. D-001206-13-05) were obtained from Dharmacon/Horizon Discovery. Kuramochi cells were transfected with siRNA using Lipofectamine RNAiMAX reagent (Invitrogen, Life Technologies) as recommended by the manufacturer. Kuramochi cells were seeded in 6-well plates at a density of $1x10^5$ cells per well and incubated for 24 h. Lipofectamine RNAiMAX reagent (9 μl) in 300 μl of Opti-MEM (Invitrogen, Life Technologies) was incubated for 5 min at room temperature. siRNA was added to the Opti-MEM-lipofectamine RNAiMAX solution to a final concentration of 100 nM. The mixture was added to the cell culture, and after 48 h incubation for gene silencing, the cells were collected for RT-qPCR studies. Expression of *GNA12* in the transfectants was monitored by RT-qPCR analysis.

Transcriptomic analysis. Transcriptome profiles were obtained using Agilent SurePrint G3 Human Comparative Genomic Hybridization 8x60 microarray platform. SKOV3-shScr (non-specific scrambled shRNA control) and SKOV3-sh*GNA12* cells were cultured for 24 h, followed by 16 h of serum starvation. These cells were stimulated with LPA (10 μM) for 16 h and total RNA was extracted using Qiagen RNeasy mini kit (Qiagen) following the manufacturer's protocol. Agilent QuickAmp labeling kit was used to label RNA samples with Cy3-CTP and hybridized to the array slides following the manufacturer's protocol. The hybridized

array slides were scanned using Agilent SureScan scanner at 2 microns resolution. The spot intensity was extracted using Agilent Feature Extraction version 11.0 software. Further, gene expression analysis was carried out using Agilent GeneSpring GX version 13.0. Differentially expressed genes (DEGs), with a cut-off value of ≥5-fold change compared to control cells, were used for further bioinformatic analyses.

Bioinformatics analysis. Gene ontology Enrichment analysis of the DEGs was carried out using the web-based annotation tool DAVID (https://david.ncifcrf.gov/home.jsp) (22). Protein-Protein Interaction Networks Functional Enrichment Analysis was carried out using web-based (https://string-db.org/) STRING database (23). The upregulated genes and downregulated genes were analyzed separately with the highest confidence interaction score (0.9) and <10 degree of interaction. Significant modules in the PPI network was analyzed further using Cytoscape software application (24). The hub and bottle neck nodes of the PPI network were identified using the cytoHubba plugin in Cytoscape (25). Multiple algorithms of cytoHubba including Degree, Maximal Clique Centrality and (MCC), maximum neighbourhood component (MNC), Edge Percolated Component (EPC), EcCentricity, Closeness, Betweenness, and Clustering Coefficient were used to identify the hub nodes of the PPI networks (26). BottleNeck algorithm of cytoHubba was used to identify the bottleneck nodes of the network.

RT-qPCR analysis. Total RNA was extracted using Qiagen RNeasy kit (Qiagen) following the manufacturer's instructions. cDNA synthesis was carried out using an iScript™ cDNA Synthesis Kit (Bio-Rad). Real-time quantitative PCR (RT-qPCR) was carried out using the cDNA from the above step using appropriate primers (Table SI) and SoAdvanced Universal SYBR Green Supermix (Bio-Rad) in a BioRad CFX96 Real time PCR detection system. The raw Cq values were normalized against GAPDH, housekeeping gene.

Immunoblot analysis. Antibodies to GNA12 (sc-409), GAPDH (CB1001), peroxidase-conjugated anti-rabbit IgG (W401B) were obtained from Santa Cruz Biotechnology Inc., Abcam and Promega Corporation, respectively. Immunoblot analysis was carried out according to our previously published methods (12) and developed with a Kodak Image Station 4000 MM.

Statistics. All required statistical analyses were performed using GraphPad Prism by two-tailed unpaired Student's t-test with Welch's correction. Statistics used in bioinformatics such as P-values and False Discovery Rates were calculated using the built-in statistical programs of the respective analytical tools.

Results

Identification of DEGs. Our previous studies have shown that LPA/LPAR stimulates ovarian cancer growth and cell proliferation via the activation of GNA12, encoded by the gene *GNA12* or its mutationally activated configuration known as the *gep* oncogene (15,16). To obtain an understanding of the transcriptomic network regulated by *GNA12*, the expression of *GNA12* was silenced in SKOV3 cells using shRNAs targeting *GNA12*. These cells were stimulated with LPA and the DEGs in *GNA12*-silenced cells compared with those in the scrambled shRNA control group were identified using an Agilent array. With a cut-off value of ≥5-fold change, compared with control cells, *GNA12*-silenced cells had 313 downregulated genes and 293 upregulated genes (Fig. 1A and B). Of the 313 downregulated genes, 145 genes were found to be protein-encoding genes (Table SII). Similarly, among the 293 upregulated genes, 186 genes were found to be protein-encoding genes (Table SIII). Other genes were represented by either long non-coding RNAs or pseudogene transcripts (Tables SIV).

Next, the current study aimed to validate the array results in a cell lines that represent high grade ovarian serous ovarian carcinoma (HGSOC). The expression of *GNA12* was silenced in Kuramochi cells, a HGSOC cell line, using specific siRNAs targeting *GNA12*. After determining the efficacy of *GNA12* silencing in these cells (Fig. 1C), RT-qPCR analysis was conducted to validate the expression levels of the DEGs. Downregulated genes were validated by monitoring the expression levels of the representative growth-promoting genes ankyrin repeat domain 1 (*ANKRD1*), bone marrow stromal cell antigen 2 (*BST2*) and cancer antigen 1 (*CAGE1*), whereas the upregulated genes were validated by monitoring the expression levels of growth-repressive representative genes, namely autophagy-related 16-like 1 (*ATG16L1*), spindlin family member 3 (*SPIN3*), thrombopoietin (*THPO*) and tetraspanin 16 (*TSPAN16*). It was found that silencing of *GNA12* led to the decreased expression of *ANKRD1*, *BST2* and *CAGE1* (Fig. 1D), along with the increased expression of *ATG16L1*, *SPIN3*, *THPO* and *TSPAN16*, thereby validating the array results (Fig. 1E).

GO enrichment analysis of DEGs. It should be noted that the genes downregulated after silencing of *GNA12* represent the genes whose expression was induced by *GNA12*, whereas the upregulated genes represent the genes whose expression was repressed by *GNA12 in situ*. Therefore, defining the functional relationships among the downregulated as well as the upregulated DEGs could provide insights into the mechanism via which *GNA12* promotes ovarian cancer progression. Since GO enrichment analysis can provide information on the functional relationship among a large set of genes, GO analysis under the three sub-ontologies, namely biological processes (GO:BP), molecular functions (GO:MF) and cellular components (GO:CC), was conducted. GO enrichment analyses of the DEGs were performed using the web-based DAVID analytical tool (22). In GO:BP, the upregulated genes were significantly enriched in BP involving 'cell adhesion', 'proliferation' and 'cell motility' (Table I). These BP were associated with the known oncogenic functions of *GNA12* in oncogenic cell proliferation and migration. In GO:CC, CC including 'plasma membrane' and 'actin-based cellular projections' formed the major categories, which was consistent with the role of *GNA12* in actin cytoskeletal reorganization underlying cell invasion (20). In GO:MF, the topmost enriched categories were 'macromolecular interaction', 'chromatin and nucleic acid interaction' and 'transcriptional activation' (Table I), thus

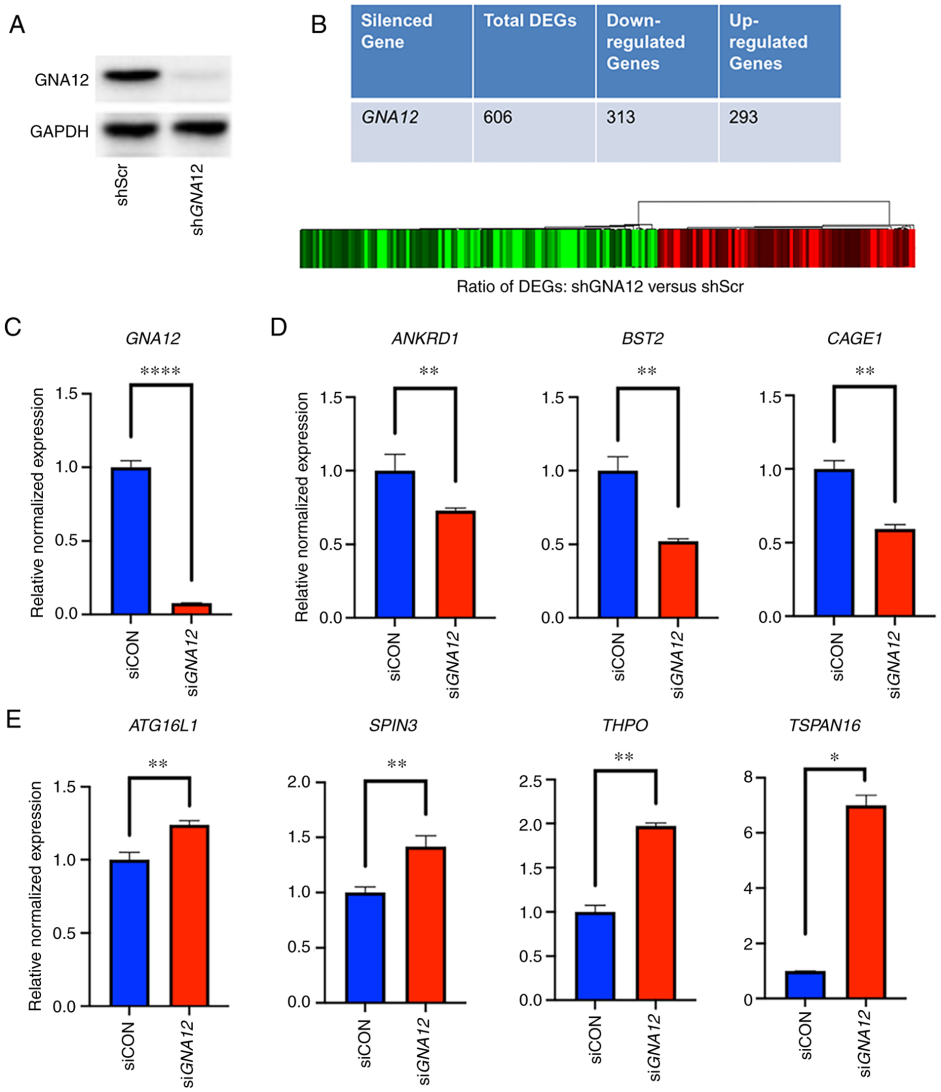

Figure 1. Heatmap of the DEGs and validation. (A) Validation of shRNA-mediated *GNA12*-silencing in SKOV3 cells. Expression of *GNA12* was stably silenced in SKOV3 cells using sh*GNA12* compared with control cells stably expressing non-targeting scrambled shRNAs. (B) Heatmap of the DEGs; the ratio comparing control cells (scrambled shRNA) and GNA12-silenced cells is presented as a heat map. Red, black and green colors represent upregulated, unchanged and downregulated expression, respectively. Total number of DEGs with >5-fold-change compared with control values and the number of down-regulated and upregulated DEGs are presented as a table. (C) Array results were validated by RT-qPCR using siRNA-mediated *GNA12*-silenced Kuramochi cells compared with non-targeting scrambled siRNA controls. (D) Downregulated DEGs were validated by monitoring the expression of the representative genes *ANKRD1*, *BST2* and *CAGE1* by RT-qPCR. (E) Expression of the upregulated DEEGs was validated by monitoring the expression of the representative upregulated genes *ATG16L1, SPIN3, THPO,* and *TSPAN16* by RT-qPCR. *P<0.05, **P<0.005, ****P<0.0005. DEGs, differentially expressed genes; sh, short hairpin; GNA12, G protein subunit α 12; Scr, scrambled; RT-qPCR, CON, control; ANKRD1, ankyrin repeat domain 1; BST2, bone marrow stromal cell antigen 2; CAGE1, cancer antigen 1; RT-qPCR, reverse transcription-quantitative PCR; ATG16L1, autophagy-related 16-like 1; SPIN3, spindlin family member 3; THPO, thrombopoietin; TSPAN16, tetraspanin 16.

validating the role of GNA12-mediated network in molecular interactions leading to oncogenic transcriptional events.

With regards to genes that were downregulated in *GNA12*-silenced cells, GO:BP showed enrichment of categories associated with the overall negative regulation of cellular and BP including different aspects of 'cell death' and 'proteolytic processes' that can be linked with growth-inhibition (Table II). In GO:CC, the enriched categories included 'membrane components of the cells' and components associated with 'autophagosome membrane' and 'extracellular matrix'. This finding was in agreement with the notion that the primary site of action of GNA12 is closer to cell surface membrane (27). In GO:MF, the enriched categories were associated with 'protein binding' and peptidase functions including 'exopeptidase', 'metallopeptidase' and 'metalloexopeptidase' (Table II), which are often associated with programmed cell death (28).

Analysis of PPI networks and pathways. To further investigate the functional interactions among the proteins encoded by the DEGs, PPI network functional enrichment analysis was conducted using the STRING database (23). The upregulated and downregulated genes were analyzed separately, with the highest confidence interaction score (0.9) and <10 degrees of interaction. The PPI network downregulated in *GNA12*-silenced cells was constructed by screening 186 nodes and 306 edges (Fig. 2). The most significant module in the PPI network was determined using Cytoscape software (24). Kyoto Encyclopedia of Genes and Genomes

Table I. GO enrichment analysis of downregulated genes in *GNA12*-silenced cells.

A, GO: Biological process

Term	Description	Gene count	P-value
GO:0048518	Positive regulation of Biological Process	45	$6.3x10^2$
GO:0048583	Regulation of response to Stimulus	24	$8.7x10^2$
GO:0007155	Cell adhesion	21	$2.1x10^2$
GO:0008283	Cell proliferation	19	$4.9x10^1$
GO:0048870	Cell motility	15	$4.9x10^1$

B, GO: Cellular component

Term	Description	Gene count	P-value
GO:0005886	Plasma membrane	45	$6.7x10^2$
GO:0071944	Cell periphery	45	$9.0x10^2$
GO:0005576	Extracellular region	42	$4.8x10^2$
GO:0042995	Cell projection	18	$9.3x10^2$
GO:0098862	Cluster of actin-based cell projections	4	$7.8x10^2$

C, GO: Molecular function

Term	Description	Gene count	P-value
GO:0044877	Macromolecular complex binding	15	$6.0x10^2$
GO:0001067	Regulatory region nucleic acid binding	10	$9.4x10^2$
GO:0000981	Transcription factor activity	9	$8.5x10^2$
GO:0003982	Chromatin binding	8	$5.7x10^2$
GO:0001228	Transcriptional activator activity	6	$7.2x10^2$

GO, Gene Ontology; GNA12, G protein subunit α 12.

(KEGG) analyses indicated that the major pathways defined by *GNA12*-dependent genes were pathways involved in 'cancer', 'PI3K/AKT signaling', 'chemotherapy resistance' and 'FoxO signaling' (Table III). Reactome analysis expanded this further into pathways associated with signaling involving 'tyrosine kinases', 'VEGF signaling', 'PI3K/AKT signaling', 'cell surface interactions at the vascular wall', 'cancer associated aberrant signaling by PI3K' and 'signaling by receptor tyrosine kinases' (Table III).

A similar PPI network construction was performed with the genes upregulated after silencing of *GNA12* by screening a total of 202 nodes and 964 edges from the STRING portal (Fig. 3). KEGG pathway analyses indicated that the network, which was repressed by *GNA12*, was primarily involved in 'metabolism', 'oxidative phosphorylation', 'proteasomal proteolysis', 'cell cycle arrest' and 'transcriptional misregulation in cancer' (Table IV). Reactome pathways analysis revealed that pathways regulated in this network included 'metabolism of proteins', 'cell cycle checkpoints', 'cellular stress response', 'anaphase-prophase complex (APC/C) mediated degradation of mitotic proteins' and 'ubiquitin-dependent degradation of cyclin D', all of which could be associated with growth-inhibition (Table IV).

Identification of the hub and bottleneck nodes. The network was further analyzed to identify the critical genes that define the hub nodes of the PPI network using the cytoHubba plugin in Cytoscape (25). The multiple algorithms of the cytoHubba, including Degree, MCC, MNC, EPC, EcCentricity, Closeness, Betweenness and Clustering Coefficient, were used to identify the hub nodes of the PPI networks (26). The intersecting genes identified by the different algorithms were tabulated (Table V). Results from this analysis identified *AKT1*, *VEGFA*, *BCL2L1*, *TGFB1* and *STAT3* as the top five hub nodes (Fig. 2, Insert; Table V). In addition to hub nodes, the identification of bottleneck nodes has equal or more importance in PPI networks due to their role as the key 'connector proteins' (29,30). Therefore, the bottleneck nodes of the network were extracted using the BottleNeck algorithm in cytoHubba application of Cytoscape. The results demonstrated that *VEGFA*, AKT1 and *STAT3* were defined the bottleneck nodes, in addition to *IGF1* and *GHRH* (Fig. 2, Insert).

A similar analysis was conducted to extract the hub nodes and the bottleneck nodes of the upregulated genes. The results identified proteasome 20S subunit (PSM) β 6 (*PSMB6*), *PSM* α 6 (*PSMA6*), *PSM* ATPase *5* (*PSMC5*), ubiquitin conjugating enzyme E2 E1 (*UBE2E1*) and *PSM* non-ATPase 10 (*PSMD10*),

Table II. GO enrichment analysis of upregulated genes in *GNA12*-silenced cells.

A, GO: Biological process

Term	Description	Gene count	P-value
GO:0048519	Negative regulation of biological process	46	$6.4x10^{2}$
GO:0048523	Negative regulation of cellular process	45	$3.2x10^{2}$
GO:0006508	Proteolysis	20	$7.5x10^{2}$
GO:0010941	Regulation of cell death	18	$8.8x10^{1}$
GO:0042981	Regulation of apoptotic process	17	$8.7x10^{1}$

B, GO: Cellular component

Term	Description	Gene count	P-value
GO:0016020	Membrane	85	$4.9x10^{2}$
GO:0071944	Cell periphery	51	$5.8x10^{2}$
GO:0005886	Plasma membrane	50	$5.9x10^{2}$
GO:0031012	Extracellular matrix	10	$2.3x10^{2}$
GO:0000421	Autophagosome membrane	3	$2.0x10^{2}$

C, GO: Molecular function

Term	Description	Gene count	P-value
GO:0005488	Binding	122	$9.6x10^{2}$
GO:0008233	Peptidase	10	$9.8x10^{2}$
GO:0008237	Metallopeptidase	5	$6.1x10^{2}$
GO:0008238	Exopeptidase	4	$5.5x10^{2}$
GO:0008235	Metalloexopeptidase	3	$8.9x10^{2}$

GO, Gene Ontology; GNA12, G protein subunit α 12.

the genes involved in proteasomal proteolysis, as the hub nodes (Fig. 3, Insert; Table VI). *PSMA6* and *PSMB6* were also identified as bottleneck nodes, along with NDUFA4 mitochondrial complex associated *(NDUFA4)*, NADH:ubiquinone oxidoreductase subunit B8 (*NDUFB8*) and anaphase promoting complex subunit 1 (*ANAPC1*) genes (Fig. 3, Insert; Table VI).

Biological significance of the hub and bottleneck nodes. The biological significance of the hub and bottleneck node genes in relation to ovarian cancer was determined via cBioPortal analysis (31,32). First, the hub and bottleneck genes downregulated in *GNA12*-silenced cells were examined (Table V). The oncoprint profile of these genes indicated that they were either amplified or upregulated in at least 4-10% of the patients with ovarian cancer (Fig. 4). Functional annotation of these genes, as shown in Table V, indicated that the aberrant increased expression of these genes was associated with cancer growth, progression, metastasis and therapy resistance in ovarian cancer (33-45).

Next, the hub and bottleneck genes upregulated in *GNA12*-silenced cells were examined (Table VI). These nodes, representing the genes that would have been suppressed by *GNA12,* were found to be associated with proteasome-mediated context-specific apoptotic pathways and therapy resistance in numerous cancer types (46-57). Taken together, the functional analyses of the hub and bottleneck genes indicated that the genes downregulated in *GNA12*-silenced cells coded for pro-tumorigenic proteins, while the genes upregulated upon silencing of *GNA12* encoded anti-tumorigenic proteins (Fig. 5).

Discussion

Autocrine and paracrine signaling by LPA serve a determinant role in cancer development and progression, and this is evident in the context of ovarian cancer (15,16). Our previous studies have reported that activation of GNA12 by LPA/LPAR signaling or mutational activation of *GNA12* into the *gep* oncogene serve a critical role in the oncogenic proliferation of ovarian cancer cells (15,16). In the present study, the key signaling pathways and critical genetic nodes that are aberrantly regulated by LPA/LPAR/*GNA12* signaling in ovarian cancer were identified. In the cellular model used, the genes that were downregulated upon silencing of *GNA12* represent the genes that would be upregulated by an intact LPA/LPAR/*GNA12* signaling pathway, whereas the genes that were upregulated upon silencing

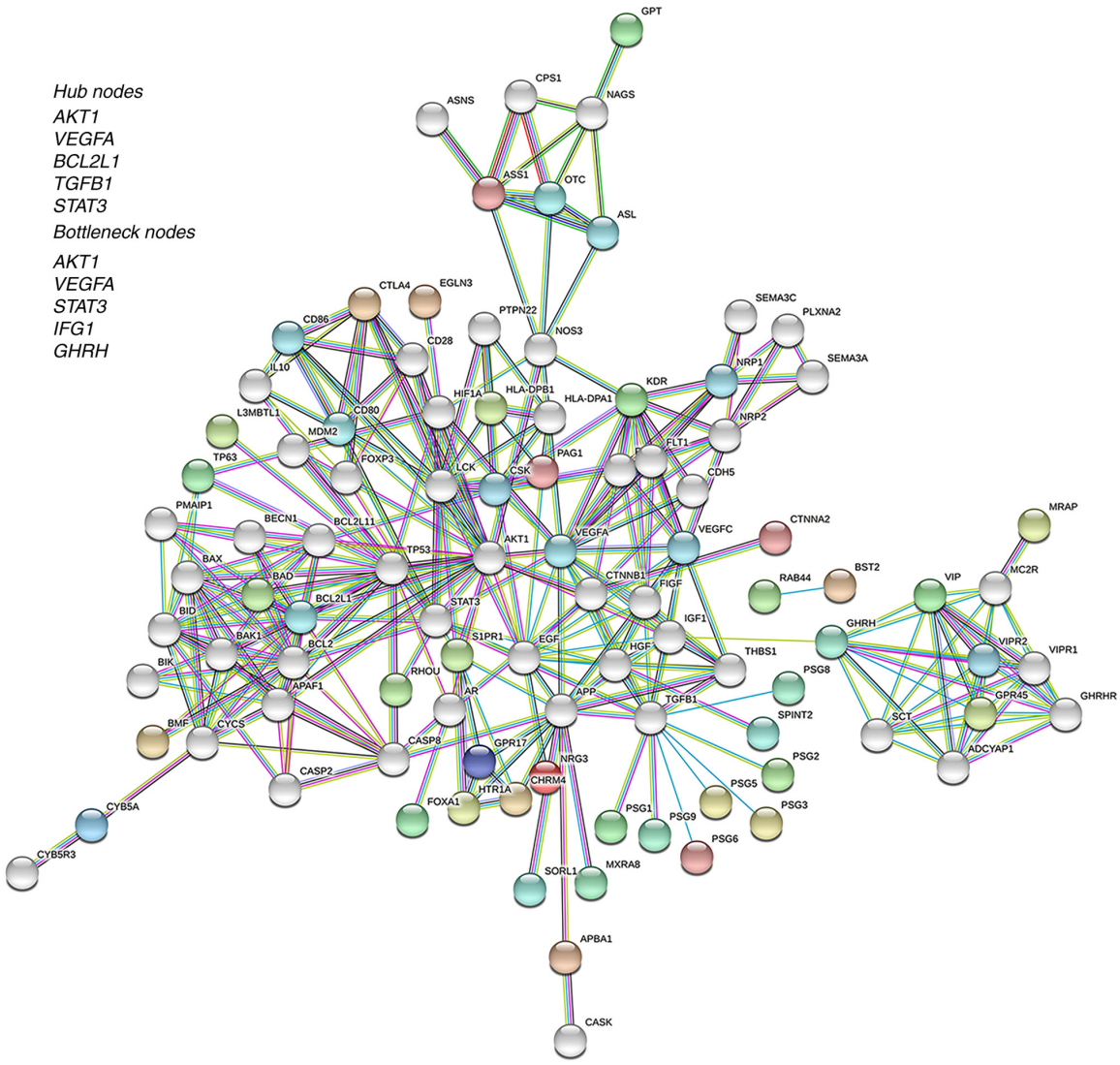

Figure 2. PPI network of downregulated genes. Using the web-based Search Tool for Retrieval of Interacting Genes tool, a PPI network of the genes downregulated in GNA12-silenced cells was constructed. Query proteins and their first shell interactions are denoted by colored nodes. Second shell interactions are in grey. Nodes of similar color identifies the specific cluster of interacting nodes. Predicted functional interactions are indicated by the connecting lines. The colors of the lines represent the types of evidence that were used to predict the PPI associations as follows: Red, known gene fusions; green, gene neighborhood; blue, gene co-occurrence; purple, experimental data; yellow, text-mining; light blue, protein homology; aqua marine, curated database; and black, co-expression. Hub and bottleneck nodes identified by the cytoHubba plugin in Cytoscape application are presented as the inset. PPI, protein-protein interaction; GNA12, G protein subunit α 12; IGF1, insulin-like growth factor 1; GHRH, growth hormone-releasing hormone.

GNA12 represent the genes that would be suppressed by this signaling pathway. The array analyses results indicated that genes that were upregulated by GNA12 (downregulated in *GNA12*-silenced cells) were mostly pro-tumorigenic, while genes that were downregulated by GNA12 (upregulated in *GNA12*-silenced cells) were growth-inhibitory. These array results were corroborated by the findings demonstrating that *GNA12* silencing decreased the expression of pro-tumorigenic genes, along with a coincident increase in the expression of growth-suppressive genes. *ANKRD1*, *BST1* and *CAGE1* have been previously shown to have oncogenic role in different cancer types, including ovarian cancer. *ANKRD1* has been revealed to promote drug-resistance and epithelial-mesenchymal transition (EMT) in multiple cancer cells, including ovarian cancer cells (58,59). Moreover, *BST1* has been observed to induce EMT in ovarian cancer cells (59,60), while *CAGE1* is known to promote both proliferation and migration in different cancer cells (61). By contrast, the genes upregulated upon silencing of *GNA12*, have been shown to exert a tumor suppressive role in different cancer types. For example, *SPIN3* has been identified as a tumor suppressor gene that induces apoptosis in human seminoma cancer cells (62). Furthermore, *ATG16L1* has been reported to be involved in promoting autophagic cell death in ovarian cancer cells (63). THPO, encoded by *THPO*, has also been observed to induce apoptosis in a context specific manner (64). While the cellular function of *TSPAN16* remains to be fully defined, its weak expression profile in cancer cells has been considered to be indicative of its tumor suppressing potential (65). Collectively, *GNA12* appears to stimulate a pro-tumorigenic network, along with the simultaneous suppression of a growth-inhibitory network.

The synergistic network organization was further clarified by the current results from the GO enrichment analyses. While GO enrichment in CC was in accordance with the known

Table III. Pathway analysis of downregulated genes in *GNA12*-silenced cells.

A, Kyoto Encyclopedia of Genes and Genomes pathway

Term	Description	Gene count	False discovery rate
hsa05200	Pathways in cancer	28	$2.43x10^{11}$
hsa04151	PI3K-AKT signaling pathway	18	$2.34x10^{7}$
hsa01524	Platinum drug resistance	13	$5.69x10^{11}$
hsa01521	EGFR tyrosine kinase inhibitor resistance	13	$2.35x10^{9}$
hsa04068	FoxO signaling pathway	9	$3.60x10^{5}$

B, Reactome pathway

Term	Description	Gene count	False discovery rate
HSA-9006934	Signaling by receptor tyrosine kinases	24	$1.29x10^{9}$
HSA-194138	Signaling by VEGF	12	$7.95x10^{8}$
HSA-2219528	PI3K/AKT signaling in cancer	10	$9.32x10^{7}$
HSA-202733	Cell surface interactions at the vascular wall	9	$1.70x10^{4}$
HSA-2219530	Constitutive signaling by aberrant PI3K in cancer	7	$7.17x10^{5}$

GNA12, G protein subunit α 12.

Table IV. Pathway analysis of upregulated genes in GNA12-silenced cells.

A, Kyoto Encyclopedia of Genes and Genomes pathway

Term	Description	Gene count	False discovery rate
hsa-0110	Metabolic pathways	34	$5.57x10^{6}$
hsa00190	Oxidative phosphorylation	26	$1.79x10^{22}$
hsa03050	Proteasome	24	$5.40x10^{29}$
hsa04218	Cell cycle	12	$2.25x10^{5}$
hsa05202	Transcriptional misregulation in cancer	7	$2.32x10^{4}$

B, Reactome pathway

Term	Description	Gene count	False discovery rate
HSA-392499	Metabolism of proteins	40	$7.46x10^{5}$
HSA-69620	Cell cycle checkpoints	33	$6.55x10^{24}$
HSA-5668541	Cellular responses to stress	35	$2.69x10^{21}$
HSA-174178	APC/C:Cdh1 mediated degradation of Cdc20 and other APC/C:Cdh1 targeted proteins in late/early mitosis	30	$8.83x10^{35}$
HSA-75815	Ubiquitin mediated degradation of cyclin D	27	$2.49x10^{33}$

APC/C, anaphase-promoting complex; Cdh, CDC20 homolog; GNA12, G protein subunit α 12.

role of GNA12 in transmitting the plasma membrane-located LPA/LPAR signaling from the cell periphery, novel insights could be gained via the analyses of GO BP and MF. The GO:BP enrichment indicated that the pathways upregulated by GNA12, thus downregulated in *GNA12*-silenced cells, were associated with critical BP and MF associated with cancer progression, metastasis and therapy resistance. In fact, GO:BP, such as 'positive regulation of biological process' (GO:0048518), has already been shown to be associated with chemoresistance in HGSOC (55). Other GO:BPs, such as 'cellular response to stimulus' (GO:0048583), 'cell adhesion' (GO:0007155), 'cell proliferation' (GO:0008283) and 'cell

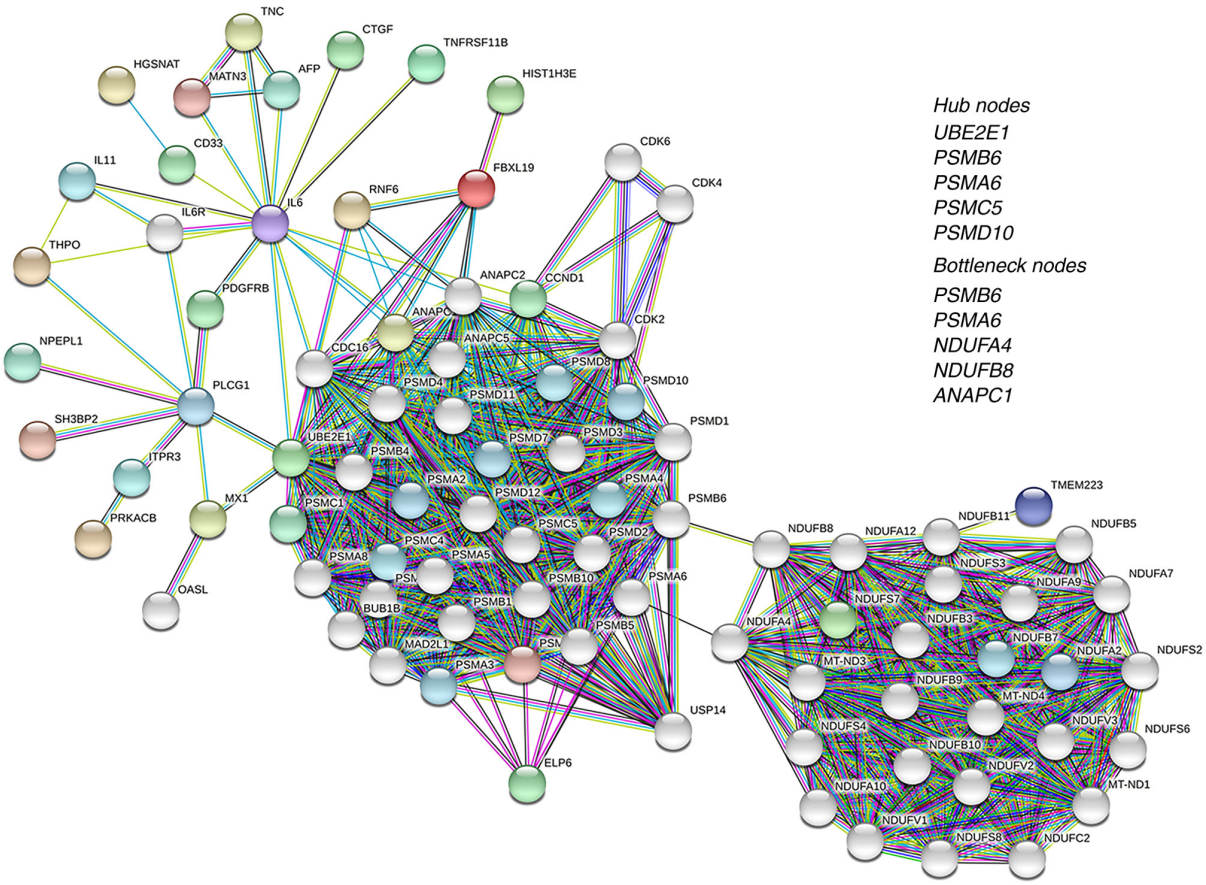

Figure 3. PPI network of upregulated genes. A PPI network of the genes upregulated in GNA12-silenced cells is presented. Colored nodes represent the query proteins and first shell interaction and white nodes denote second shell interactions. Nodes of similar color identifies the specific cluster of interacting nodes. Predicted functional interactions are indicated by the connecting lines. The colors of the lines represent the types of evidence that were used to predict the PPI associations as follows: Red, known gene fusions; green, gene neighborhood; blue, gene co-occurrence; purple, experimental data; yellow, text-mining; light blue, protein homology; aqua marine, curated database; and black, co-expression. Hub and bottleneck nodes of the PPI network derived from the use of cytoHubba plugin in Cytoscape application are presented as the inset. PPI, protein-protein interaction; GNA12, G protein subunit α 12; UBE2E1, ubiquitin conjugating enzyme E2 E1; PSM, proteasome 20S subunit; NDUFA4, NDUFA4 mitochondrial complex-associated; NDUFB8, NADH:ubiquinone oxidoreductase subunit B8; ANAPC1, anaphase promoting complex subunit 1.

motility' (GO:0048870), have been associated with cancer growth, recurrence and therapy resistance in numerous cancer types (56,57,66,67). Together with the indicated functions in GO:MF enrichments, the current novel findings emphasize the oncogenic role of GNA12 in transmitting signals from the LPA/LPAR signaling pathway located in the cellular periphery to a set of highly consequential nuclear events. Another significant analytical suggestion from the results was that genes suppressed by *GNA12*, as evidenced by their upregulation in *GNA12*-silenced cells, show GO enrichments in BP and MF associated with 'negative regulation of cell growth processes'. The GO:MF enrichment also suggested the potential role of *GNA12* in suppressing proteolysis associated with negative cell proliferative.

Ovarian cancer is characterized by a heterogeneous histopathology with the manifestation of dissimilar genetic and pathway alterations. Major pathways that are aberrantly altered in a range of subtypes include TP53 (68,69), PI3K/AKT (70-72), VEGF (73,74), EGFR (75,76) and FoxO signaling (77). It was significant that KEGG and Reactome pathway analyses of the DEGs upregulated in *GNA12*-silenced cells directly linked *GNA12* to these multiple tumor-promoting pathways. KEGG and Reactome pathways upregulated in *GNA12*-silenced cells involved pathways associated with cell cycle check points, including 'APC/C mediated proteasomal degradation of mitotic proteins'. Thus, the pathway analyses present an oncogenic paradigm orchestrated by GNA12 in which mutational or LPA/LPAR activation of *GNA12* leads to the stimulation of a pro-tumorigenic network, while concurrently suppressing an anti-tumorigenic network involving anti-mitotic, anti-proliferative and cellular stress pathways. This was further substantiated by the analysis of the hub and bottleneck signaling nodes derived from the PPI networks of the DEGs, especially the ones that were downregulated in *GNA12*-silenced cells. In line with the oncogenic role of *GNA12*, these genes were upregulated in distinct subsets of patients with ovarian cancer and were critically involved in ovarian cancer pathobiology. *AKT1* gene is frequently upregulated in ovarian cancer and is associated with paclitaxel resistance in patients with ovarian cancer (33). Furthermore, the upregulation of *VEGFA* has been shown to be associated with distant metastasis and resistance to chemotherapy in patients with ovarian cancer (34-36). Increased expression of *TGB1* has been correlated with EMT, tumor growth and metastasis in ovarian cancer (37,38), while overexpression of *BCL2L1* is associated with anti-apoptosis effects and

Table V. Hub and bottleneck genes downregulated in *GNA12*-silenced cells.

Genes	Nodes	Function	(Refs.)
AKT	Hub and bottleneck	Amplified in ovarian cancer patients; confers resistance to paclitaxel.	(33)
VEGFA	Hub and bottleneck	Overexpression in ovarian cancer patients; tumor angiogenesis, associated with distant metastasis and resistance to chemotherapy.	(34-36)
STAT3	Hub and bottleneck	Tumor cell growth; survival, growth, stemness and tumor angiogenesis.	(41)
BCL2L1	Hub	Anti-apoptosis; confers platinum resistance.	(39,40)
TGFB1	Hub	Tumor growth, epithelial-mesenchymal transition and metastasis.	(37,38)
IGF1	Bottleneck	Overexpressed in ovarian cancer; tumor cell proliferation; immunosuppressive role.	(42,43)
GHRH	Bottleneck	Endogenous synthesis in ovarian cancer cells; ovarian cancer growth; tumor vascularization.	(44,45)

GNA12, G protein subunit α 12; IGF1, insulin-like growth factor 1; GHRH, growth hormone-releasing hormone.

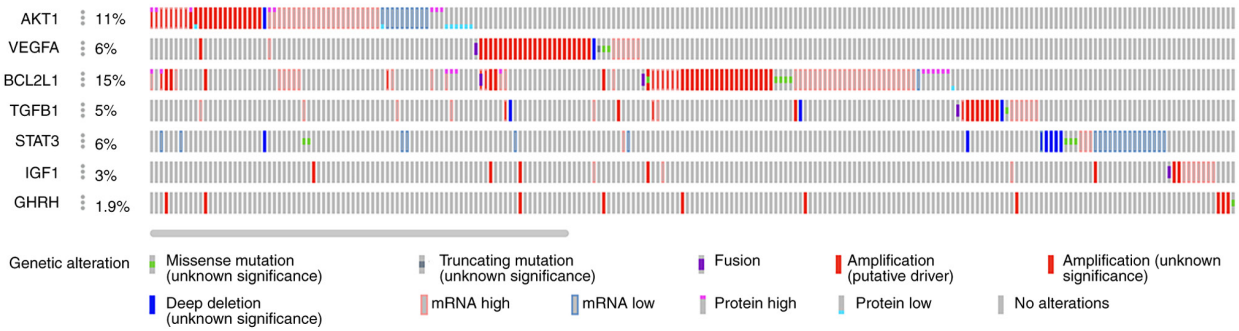

Figure 4. Genomic and expression profile of hub and bottleneck genes in patients with ovarian cancer. Genomic and expression profile of the hub and bottleneck genes in ovarian cancer patients were visualized in OncoPrint at cBioPortal web-portal. IGF1, insulin-like growth factor 1; GHRH, growth hormone-releasing hormone.

platinum resistance (39,40). In addition, *STAT3* signaling in ovarian cancer has been reported to be associated with tumor cell proliferation, survival, stemness and angiogenesis (41), while the overexpression of *IGF1* has been revealed to stimulate the proliferation of ovarian cancer cells, along with its immunosuppressive role in ovarian cancer (42,43). In a similar manner, *GHRH*, which is endogenously produced in ovarian cancer cells, is involved in ovarian cancer growth and tumor vascularization (44,45). The oncogenic role for these genes has also been shown by the oncoprint profiles of these genes generated using the CBioPortal. In fact, *in silico* data-mining indicated that all of the *GNA12*-regulated hub and bottleneck nodes identified her such as *AKT*, *VEGFA*, *BCL2L1*, *TGFb1*, *IGF1*, and *GHRH* are associated with poor prognosis in ovarian cancer (78-83).

A new paradigm emerging from the current analysis showed the potential role of *GNA12* in suppressing the proteasome pathway. It is of interest to note that the proteasomal proteolytic machinery has been reported to be required for the rapid onset of death receptor-induced apoptosis in a context-specific manner (47,48). In fact, it has been documented that the overexpression of PSMA6 and PSMC5 was associated with chemoresistance in prostate cancer and radiation-therapy resistance in lung cancer, respectively (49,50). Moreover, *ANAPC1*, a hub/pathway gene in this network, is part of the APC/C, which is involved in cell cycle arrest at G_1 phase. APC/C is an E3-ubiquitin ligase that regulates cell cycle arrest by marking cell cycle proteins, such as cyclins, for degradation by proteasomes during cell cycle exit (53,54). *UBE2E1*, which was identified here as the hub and pathway gene, encodes an E2-ubiquitin conjugating enzyme. It has been revealed that UBE2E1 can complex with polycomb repressive complex 1 (PRC1), the E3 ligase complex responsible for histone H2A ubiquitination and gene silencing (46). With the established role of PRC1 complex in the silencing of tumor suppressor genes (84), it can be considered that *UBE2E1* serves an active role in PRC1-mediated silencing of tumor suppressor genes.

One of the hallmarks of cancer involves metabolic reprogramming in cancer cells, with a shift towards aerobic glycolysis. It is known that along with the glycolytic shift, cancer cells concomitantly suppress mitochondrial oxidative phosphorylation (85). While the mechanism via which cancer cells proactively resort to glycolytic shift is beginning to be understood, the role of an active signaling mechanism involved in suppressing oxidative phosphorylation has thus far remained uncharacterized. In this regard, *NDUFA4* and *NDUFB8*, which have been identified as bottleneck genes in the *GNA12*-suppressed network, are highly relevant. NDUFA4 and NDUFB8 are subunits of complex IV and complex I of the mitochondrial electron transport chain, and are essential components involved in mitochondrial oxidative phosphorylation (86-88). The decreased expression of NDUFA4 has been correlated with the suppression of oxidative phosphorylation and stimulation of glycolysis in renal

Table VI. Hub and bottleneck genes upregulated in *GNA12*-silenced cells.

Genes	Nodes	Function	(Refs.)
PSMB6	Hub and bottleneck	20S proteasome subunit; context specific apoptosis.	(47,48)
PSMA6	Hub and bottleneck	20S proteasome subunit; context specific apoptosis; confers chemosensitivity.	(49)
PSMC5	Hub	26S proteasome regulatory subunit; confers radiosensitivity.	(50)
PSMD10	Hub	26S proteasome regulatory subunit; oncogene, but involved in context specific apoptosis.	(47,48)
UBE2E1	Hub	PRC1 mediated gene silencing.	(46)
NDUFA4	Bottleneck	Subunit of complex IV of the mitochondrial electron transport chain; reduced expression associated with metabolic reprograming in renal cell carcinoma.	(84)
NDUFB8	Bottleneck	Accessory subunit of NADH dehydrogenase complex I of the mitochondrial electron transport chain; reduced expression associated with metabolic reprograming in breast cancer cells.	(91)
ANAPC1	Bottleneck	Cell cycle arrest; mitotic checkpoint regulator.	(53,54)

PRC1, polycomb repressive complex 1; GNA12, G protein subunit α 12; PSM, proteasome 20S subunit; UBE2E1, ubiquitin conjugating enzyme E2 E1; NDUFA4, NDUFA4 mitochondrial complex-associated; NDUFB8, NADH:ubiquinone oxidoreductase subunit B8; ANAPC1, anaphase promoting complex subunit 1.

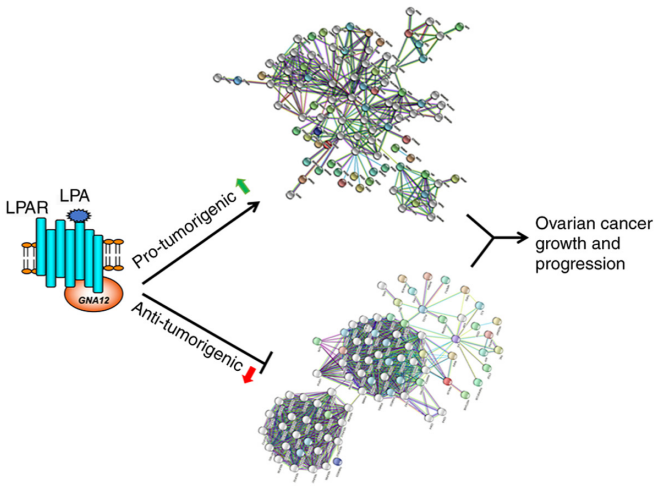

Figure 5. Schematic representation of *GNA12*-driven genetic networks in ovarian cancer. LPA-LPAR-stimulation or mutational activation of *GNA12* drove the coordinated regulation of two genetic networks. *GNA12* activated a pro-growth genetic network with the genetic nodes involved in cell proliferation and simultaneously downregulated a growth-suppressive network defined by pro-apoptotic and growth-suppressive genes. Sinergy between these networks promoted aggressive growth and progression of ovarian cancer. LPA, lysophosphatidic acid; LPAR, lysophosphatidic acid receptor; GNA12, G protein subunit α 12.

cell carcinoma (89,90). Similarly, low expression of NDUFB8 has been associated with impaired oxidative phosphorylation, along with a shift towards aerobic glycolysis in breast cancer cells (91). These findings, along with the current results that NDUFA4 and NDUFB8 were identified as bottleneck nodes, revealed the previously uncharacterized signaling node via which the LPA/LPAR/*GNA12* signaling network could suppress oxidative phosphorylation to promote glycolytic shift in ovarian cancer cells. Thus, taken together, *GNA12* appears to promote ovarian cell proliferation by suppressing multi-faceted anti-tumorigenic signaling nodes.

In summary, the present results provided novel insights into the mechanism via which GNA12, stimulated by LPA/LPAR or mutational activation, could coordinate the upregulation of a growth promoting signaling network, while simultaneously regulating the downregulation of a growth-suppressive signaling network, to promote ovarian cancer growth. While the dysregulation of numerous different pathways is known in ovarian cancer, the core signal processing unit that connects the signaling nodes into a coordinated oncogenic network remains unknown. The current study demonstrated such a role for the LPA/LPAR/*GNA12* signaling unit in ovarian cancer. The present study identified the duplex signaling mode of GNA12 via which the pro-tumorigenic signaling network was upregulated, along with the simultaneous downregulation of growth-suppressive signaling network in ovarian cancer. It has been realized that the therapeutic targeting of a single pathway may not be an effective treatment strategy for multiple type of cancer. This is especially true in the case of ovarian cancer, due to its subtype and pathway heterogeneity. In this context, the current finding that the LPA/LPAR/*GNA12* signaling nexus regulated multiple hub and bottleneck nodes of an extensive oncogenic network suggested that this axis may be a potential target for the development of network-targeted combination therapeutic strategies for treating ovarian cancer.

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Availability of data and materials

The microarray data presented in the present study are deposited at the Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/; accession no. GSE173214. Oncoprint data supporting the reported results used the Ovarian Serous Cystadenocarcinoma (TCGA, Firehose Legacy) dataset available https://www.cbioportal.org.

Authors' contributions

CI, YSS, JHH, MJ and DND conceptualized the study. MY, JHH and MJ developed the methodology. JHH and PD validated the experiments. MY, JHH and PD performed the formal analysis. DND procured resources. MY, MJ, PD and JHH curated and analyzed the data. DND wrote and prepared the original draft. DND, YSS and CI were responsible for writing, revising and editing the manuscript. DND supervised, acquired funds and was project administrator. All authors have read and approved the final version of the manuscript. DND, YSS, MJ, JHH and CI confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Approved by the Institutional Review Board of the University of Oklahoma (approval no. #9599).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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GNAi2/gip2-Regulated Transcriptome and Its Therapeutic Significance in Ovarian Cancer

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Abstract: Increased expression of *GNAi2*, which encodes the α-subunit of G-protein i2, has been correlated with the late-stage progression of ovarian cancer. *GNAi2*, also referred to as the proto-oncogene *gip2*, transduces signals from lysophosphatidic acid (LPA)-activated LPA-receptors to oncogenic cellular responses in ovarian cancer cells. To identify the oncogenic program activated by *gip2*, we carried out micro-array-based transcriptomic and bioinformatic analyses using the ovarian cancer cell-line SKOV3, in which the expression of *GNAi2/gip2* was silenced by specific shRNA. A cut-off value of 5-fold change in gene expression ($p < 0.05$) indicated that a total of 264 genes were dependent upon *gip2*-expression with 136 genes coding for functional proteins. Functional annotation of the transcriptome indicated the hitherto unknown role of *gip2* in stimulating the expression of oncogenic/growth-promoting genes such as KDR/VEGFR2, CCL20, and VIP. The array results were further validated in a panel of High-Grade Serous Ovarian Carcinoma (HGSOC) cell lines that included Kuramochi, OVCAR3, and OVCAR8 cells. Gene set enrichment analyses using DAVID, STRING, and Cytoscape applications indicated the potential role of the *gip2*-stimulated transcriptomic network involved in the upregulation of cell proliferation, adhesion, migration, cellular metabolism, and therapy resistance. The results unravel a multi-modular network in which the hub and bottleneck nodes are defined by ACKR3/CXCR7, IL6, VEGFA, CYCS, COX5B, UQCRC1, UQCRFS1, and FYN. The identification of these genes as the critical nodes in *GNAi2/gip2* orchestrated onco-transcriptome establishes their role in ovarian cancer pathophysiology. In addition, these results also point to these nodes as potential targets for novel therapeutic strategies.

Keywords: ovarian cancer; *GNAi2*; *gip2*; transcriptome; gene expression; bio-informatics

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1. Introduction

Despite major advances in anticancer drug development research and newer treatment modalities, ovarian cancer lags behind other cancers by failing to show a significantly improved survival rate over the years [1,2]. This is primarily due to the late diagnosis of the disease, which is further compounded by the therapy resistance of the recurrent disease [3,4]. While targeted therapy is emerging as an important deterrent to overcome drug resistance in many cancers including ovarian cancer, it requires a better understanding of the disease mechanism and causative factors involved in disease progression [4,5]. Plasticity of tumor cells, as well as pathway-bypass mechanisms involving the expression

and/or activation of surrogate signaling molecules, were observed to blunt the effectiveness of an otherwise proven targeted therapy [6,7]. In this context, it is of interest to note that the α-subunit of G protein i2, which is encoded by the gene *GNAi2* and often referred to as *gip2* proto-oncogene, shows a biased increased expression in the late stages of ovarian cancer [8]. The role of *gip2* in ovarian cancer pathobiology becomes all the more important considering the fact that GNAi2 is activated by lysophosphatidic acid (LPA), an endogenous growth factor in ovarian cancer. Such a context-specific expression of *gip2* along with its potent role in activating diverse oncogenic signaling responses such as cell proliferation, EMT, stemness, and invasive metastatic migration necessitates a need to define the global transcriptomic changes associated with the expression of *gip2* so that potential therapeutic nodes can be identified. With this rationale, we sought to gain insight into the onco-transcriptome stimulated by *gip2* in ovarian cancer cells and its functional role in ovarian cancer progression and/or therapy resistance. We report the results from micro-array-based transcriptomic analysis and gene set enrichment analysis on defining *gip2*-dependent transcriptomic network along with the associated hub and bottleneck genes in ovarian cancer cells.

Transcriptome profiling of SKOV3 cells in which the expression of *gip2* was silenced indicated the *gip2*-dependent expression of 264 genes, of which 136 were found to be coding for functional proteins. Many of these genes, totaling 78, are known to be associated with the hallmarks of cancer. The array results were validated by monitoring the expression of *KDR/VEGFR2*, *CCL20*, and *VIP*, as a representative set of pro-tumorigenic genes in the high-grade serous ovarian carcinoma cell lines Kuramochi and OVCAR8. Gene Ontology (GO) enrichment and protein–protein interaction (PPI) network analyses of the dataset from the transcriptome were carried out using the web-based Database for Annotation, Visualization and Integrated Discovery (DAVID), Search Tool for Retrieval of Interacting Genes (STRING), and Cytoscape applications. Gene enrichment analysis indicated the oncogenic role of *gip2* in inducing the expression of genes involved in cell proliferation, adhesion, and migration. PPI network analysis identified the critical pathways regulated by the transcriptome, which include cellular energetics, oncogenic signaling, and therapy resistance. Further network analysis using Cytoscape application identified the potential pro-tumorigenic role of hub and bottleneck genes, namely *CYCS*, *VEGFA*, *IL6*, *UQCRFS1*, *UQCRC1*, *COX5B*, *ACKR3*/*CXCR7*, and *FYN*, in *gip2*-orchestrated onco-transcriptome. Functional annotation of the hub and bottleneck genes indicated a triplex signaling mode driving ovarian cancer progression. This involves the activation of a network cluster that plays a stimulatory role in cancer cell metabolism, cell proliferation, and invasive migration. In addition to providing new insights into the network organization of *gip2*-stimulated onco-transcriptome in ovarian cancer, the results provide a molecular basis for investigating the therapeutic potential of the hub and bottleneck nodes such as those of *UQCRFS1*, *ACKR3*/*CXCR7*, and *FYN* for the development of second-line targeted therapy in ovarian cancer.

2. Materials and Methods

2.1. Cell Lines and Culture

High-grade serous carcinoma cell lines OVCAR3 and non-serous ovarian carcinoma cell line SKOV3 were acquired from American Type Culture Collection (ATCC, Manassas, VA, USA), OVCAR8 and Kuramochi cells were procured from the National Cancer Institute (NCI, Bethesda, MD, USA) and Japanese Collection of Research Biosources Cell Bank (JCRB, Osaka, Japan) respectively. Routine authentication of the cell lines was carried out by short tandem repeat analysis as described [9]. Cell-culture conditions and the use of SKOV3 cell lines expressing shRNAs targeting *GNAi2/gip2* and non-targeting scrambled shRNA were previously described [10]. OVCAR3, OVCAR8, and Kuramochi cells were maintained in Roswell Park Memorial Institute (RPMI) 1640 medium (Cellgro, Manassas, VA, USA) whereas SKOV3 cells were cultured in Dulbecco's modified Eagle's (DMEM) Medium (Cellgro, Manassas, VA, USA) supplemented with 10% FBS (Gemini Bio-Products,

West Sacramento, CA, USA), 50 U/mL penicillin, 50 µg/mL streptomycin (Cellgro, Manassas, VA, USA) at 37 °C and 5% CO_2. siRNAs targeting *GNAi2/gip2* (siGENOME Human *GNAi2* siRNA SMARTpool; Cat # M-008435-00-0005) and non-targeting scrambled control siRNAs control (siGENOME Non-Targeting siRNA Pool; Cat # D-001206-13-05) were purchased from Dharmacon/Horizon Discovery, Lafayette, CO. *GNAi2/gip2*-specific siRNA and siCon were transfected into Kuramochi, OVCAR3 and OVCAR8 cells using Lipofectamine RNAiMAX (Invitrogen, Life Technologies, Carlsbad, CA, USA) as recommended by the manufacturer. The knockdown of *gip2* in the transfectants was confirmed using RT-PCR. LPA (1-oleoyl-2-hydroxy-sn-glycero-3-phosphate) used in the study was prepared as 10 mM stock solution in PBS containing 1% BSA and stored at −80 °C until use.

2.2. Transcriptomic Analysis

Serum-starved stable SKOV3-shScr (nonspecific scrambled shRNA control) and SKOV3-sh*gip2* cells were stimulated with LPA (10 µM) for 16 h. Qiagen RNeasy mini kit (Qiagen, Carlsbad, CA) was used to extract total RNA following the manufacturer's protocol. Agilent SurePrint G3 Human Comparative Genomic Hybridization 8 × 60 microarray platform was employed to generate the transcriptomic profile of these stable cell lines. Complementary RNAs were labeled with Cy3-CTP using the Agilent Quick Amp labeling kit (Agilent, CA) and hybridized to the array slides following the manufacturer's protocol. A total of six samples (3 control and 3 experimental) were used in the microarray platform. Agilent SureScan scanner was used to scan the array slides at 2 microns resolution and the spot intensity extracted using Agilent Feature Extraction version 11.0 software. Delineation of gene expression between the cell lines was established using Agilent GeneSpring GX version 13.0. *gip2*-dependent genes, with ≥ a 5-fold decrease over the control cells, were used for further bioinformatic analyses.

2.3. Bioinformatic Analysis

Multiple web-based enrichment and network analysis were employed to define the pathways and network their interactions. Database for Annotation, Visualization and Integrated Discovery (DAVID) annotation tool (https://david.ncifcrf.gov/home.jsp) (accessed on 21 July 2021) was used for the Gene Ontology Enrichment analysis [11]. Protein–protein interaction (PPI) network analysis was carried out employing the web-based Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (https://string-db.org/) (accessed on 21 July 2021) with the high confidence interaction score (0.7) and <10 degrees of interaction limit [12]. Cytoscape software application was used to identify the significant modules in the PPI network [13]. The hub and bottleneck nodes of the PPI network were identified using the cytoHubba plugin in Cytoscape [14]. While the hub nodes of the PPI network were identified using Degree, MCC, MNC, EPC, EcCentricity, Closeness, Betweenness, and Clustering Coefficient algorithms, the bottleneck nodes were identified using the BottleNeck algorithm of the cytoHubba plugin [15].

2.4. RT-qPCR Analysis

Total RNA was extracted using Qiagen RNeasy kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. cDNA synthesis was carried out using iScript™ cDNA Synthesis Kit (BioRad, Hercules, CA, UAS). Real-time quantitative PCR (RT-qPCR) was carried out using the cDNA from the above step using the SsoAdvanced Universal SYBR Green Supermix (BioRad, Hercules, CA, USA) in a BioRad CFX96 Real-time PCR detection system. The raw Cq values were normalized against GAPDH, a housekeeping gene. The primers used in this study are shown in Table S1.

2.5. Immunoblot Analysis

Immunoblot Analysis. Antibodies to GNAi2 (sc-409), GAPDH (CB1001), peroxidase-conjugated anti-rabbit IgG (W401B) were from Santa Cruz Biotechnology Inc (Dallas, TX, USA), Abcam (Cambridge, MA, USA) and Promega Corporation (Madison, WI, USA)

respectively. Immunoblot analysis was carried out using our previously published methods [10].

2.6. Statistics

All gene expression studies were tested by a two-tailed Student's t-test with Welch's correction using GraphPad Prism software (La Jolla, CA, USA). *p* values and False Discovery Rates in the bioinformatic analysis were from built-in statistical analytical tools within respective programs.

3. Results

3.1. Characterization of GNAi2/gip2-Dependent Transcriptome

Our previous studies have shown that LPA-LPAR activation or mutational activation of GNAi2/gip2 stimulates proliferation, EMT, invasive migration, and metabolic reprogramming of ovarian cancer cells [9,16–19]. To gain better insight into the global oncogenic network regulated by gip2, we carried out transcriptome profiling of SKOV3 ovarian cancer cells in which the expression of gip2 was silenced using shRNAs [10]. The cells were stimulated with LPA and the genes that showed a decrease in the gip2-silenced cells compared to the scrambled shRNA control group were identified using Agilent array. With the fold change cut-off value of $\geq$5 compared to control cells, gip2-silenced cells showed a downregulation of 264 genes (Figure 1A; GEO Accession No: GSE173214). Of the downregulated genes, only 135 genes were found to be protein-encoding genes (Table S2). Others were represented by genes encoding uncharacterized transcripts or the ones encoding pseudogenes, anti-sense RNAs, or non-coding RNAs (GEO Accession No: GSE173214). Analysis of the genes through data mining from published literature indicated that 78 of these genes are known to play an oncogenic role in different cancers (Table S3). More interestingly, querying these genes in TCGA ovarian cancer dataset (TCGA, Firehose Legacy) via CBioPortal, indicated that 61 of these genes showed increased expression in ovarian cancer (Table S4) and 40 of these genes showed co-occurrence in their expression profiles (Table S5), thus further validating our results in ovarian cancer patient subgroup. These array results were experimentally validated by monitoring the expression of a representative set of pro-tumorigenic genes in the SKOV3 cell line and high grade serous ovarian carcinoma (HGSOC) cell lines Kuramochi and OVCAR8. Expression of gip2 was silenced using shRNA (SKOV3 cells) or siRNAs specifically targeting gip2 (Kuramochi and OVCAR8 cells). Expression of KDR/VEGFR2, VIP, and CCL20-a representative set of genes from the array results-were monitored by RT-PCR. As shown in Figure 1B–D, silencing of gip2 decreased the expression of all these genes in SKOV3 as well as Kuramochi and OVCAR8 cells, thus validating the array results.

3.2. Gene Ontology Enrichment Analysis of gip2-Dependent Genes

In the gip2-silenced cellular model system used here, the genes that show decreased expression upon the silencing of gip2-the gip2-dependent genes-are in fact defined as the genes that would be stimulated by gip2 in situ in ovarian cancer cells. Consistent with this premise, functional annotation of these genes indicated that at least 50% of the genes (a total of 78 genes out of 136 protein-coding genes) were found to play an oncogenic role in different cancers (Table S2). Reasoning that the functional networking of these genes could provide insight into the mechanism by which gip2 promotes neoplastic growth of ovarian cancer cells, we carried out pathway and network analyses. Since Gene Ontology (GO) enrichment analysis could provide information on the functional relationship among a large set of genes, GO analysis of biological processes (GO:BP), molecular functions (GO:MF) and cellular components (GO:CC) were carried out using DAVID database [11]. In GO:BP ontology, the gip2-dependent genes were significantly enriched in biological processes involving cell adhesion, proliferation, and cell motility (Table 1). In GO:CC ontology, cellular periphery including plasma membrane and membrane region formed the major

categories. In GO:MF ontology, the topmost enriched categories were molecular transducer activity, receptor signaling activity, and lipid and tyrosine kinase binding (Table 1).

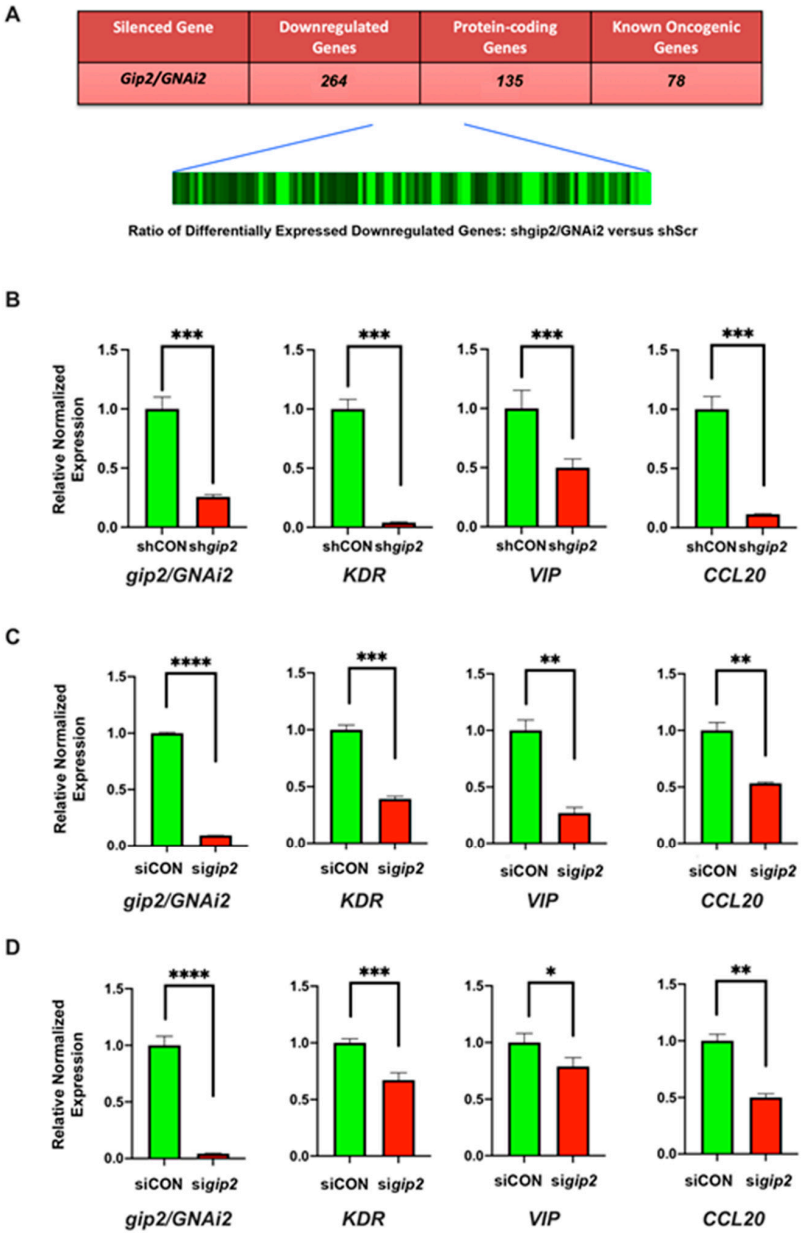

Figure 1. Heatmap of genes downregulated upon silencing of GNAi2/gip2 and validation. (**A**) Heatmap and the downregulated genes. The ratio comparing control cells (scrambled shRNA) and gip2-silenced cells is presented as a heat map. Black and green bands represent unchanged and downregulated expression, respectively. Total number of genes that show > 5-fold reduction in the expression compared to control values is presented as a table insert. *Array results were* validated by RT-PCR methods using SKOV3 (**B**) Kuramochi (**C**) and OVCAR8 (**D**) cells in which the expression of gip2 was silenced (sigip2) using specific shRNA or siRNA. In SKOV3 cells, cells stably expressing scrambled shRNA (shCON) were compared with cells in which gip2/GNAi2 was silenced with the stable expression of specific shRNA targeting gip2/GNAi2 (shgip2). In Kuramochi and OVCAR8 cells, cells transfected with non-targeting scrambled siRNA pool were used as the control group (siCON). Downregulated genes were validated by monitoring the expression of the representative genes KDR, VIP, and CCL20 by RT-PCR. Statistical significance between gip2-knockdown and scrambled siRNA cells was determined by Student's *t*-test (* $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$, **** $p < 0.0001$ACKR3).

Table 1. Gene ontogeny enrichment analysis of differentially expressed genes.

Category	Term	Description	Gene Count	*p*-Value
GO: BP	GO:0050896	Response to stimulus	67	2.9E−2
	GO:0009605	Intracellular signal transduction	25	7.1E−2
	GO:0042127	Regulation of cell proliferation	19	1.3E−1
	GO:0007155	Cell adhesion	17	9.5E−2
	GO:0016477	Cell migration	14	5.2E−2
GO:CC	GO:0005886	Plasma membrane	46	1.7E−2
	GO:0071944	Cell periphery	46	2.5E−2
	GO:0005576	Extracellular region	41	3.1E−2
	GO:0042995	Extracellular space	20	2.8E−3
	GO:0098862	Membrane region	6	8.5E−2
GO:MF	GO:0060089	Molecular transducer activity	18	3.8E−2
	GO:0038023	Signaling receptor activity	18	3.8E−2
	GO:0004888	Transmembrane signaling receptor activity	15	5.2E−2
	GO:0003982	Lipid binding	10	2.5E−2
	GO:0001228	Protein tyrosine kinase binding	3	5.5E−2

Gene ontology (GO) enrichment analysis was carried out with the genes downregulated in SKOV3 cells upon silencing of gip2 using the DAVID gene annotation tool. GO term enrichment analyses in terms of the three sub-ontologies: Biological Process (BP), Cellular Component (CC), and Molecular Function (MF) are presented.

3.3. Analysis of PPI Networks and Pathways

To further investigate the functional interactions among the proteins encoded by the gip2-dependent genes, downregulated in gip2-silenced cells, we carried out Protein–Protein Interaction Networks Functional Enrichment Analysis using STRING database [12]. Gip2-dependent genes were analyzed with a high confidence interaction score (0.7) and < 10 degrees of interaction. The PPI network was constructed by screening 185 nodes and 473 edges (Figure 2). The most significant modules in the PPI network were determined using the Cytoscape software application [13].

Using the web-based STRING tool, the PPI network of the genes downregulated in gip2-silenced cells was constructed. Query proteins and their first shell interactions are denoted by colored nodes. Second shell interactions are in while. Key pathway clusters defined by specific nodes are denoted by different colors as follows: Red, metabolic pathway; Blue, Pathways in Cancer; Green, Cytokine–Cytokine Receptor Interactions; Yellow, Oxidative Phosphorylation; Violet, apoptosis; and Aqua Marine, Platinum Resistance. Predicted functional interactions are indicated by the connecting lines. The colors of the lines represent the types of evidence that were used to predict the PPI associations. They are as follows: Red-known gene fusions; Green-gene neighborhood; Blue-gene co-occurrence; Purple-experimental data; Yellow-text-mining; Light Blue-protein homology; Aqua Marine-curated database; and Black-co-expression.

KEGG analyses indicated that the top pathways defined by the gip2-dependent genes were: 1. Metabolic pathways; 2. Oxidative phosphorylation; 3. Pathways in cancer; 4. Platinum resistance; and 5. EGFR-inhibitor resistance (Table 2). Reactome analysis expanded this further into pathways associated with 1. Respiratory electron transport; 2. Apoptosis; 3. Hemostasis; 4. Mitochondrial protein support; and 5. Interleukin-6 signaling (Table 2).

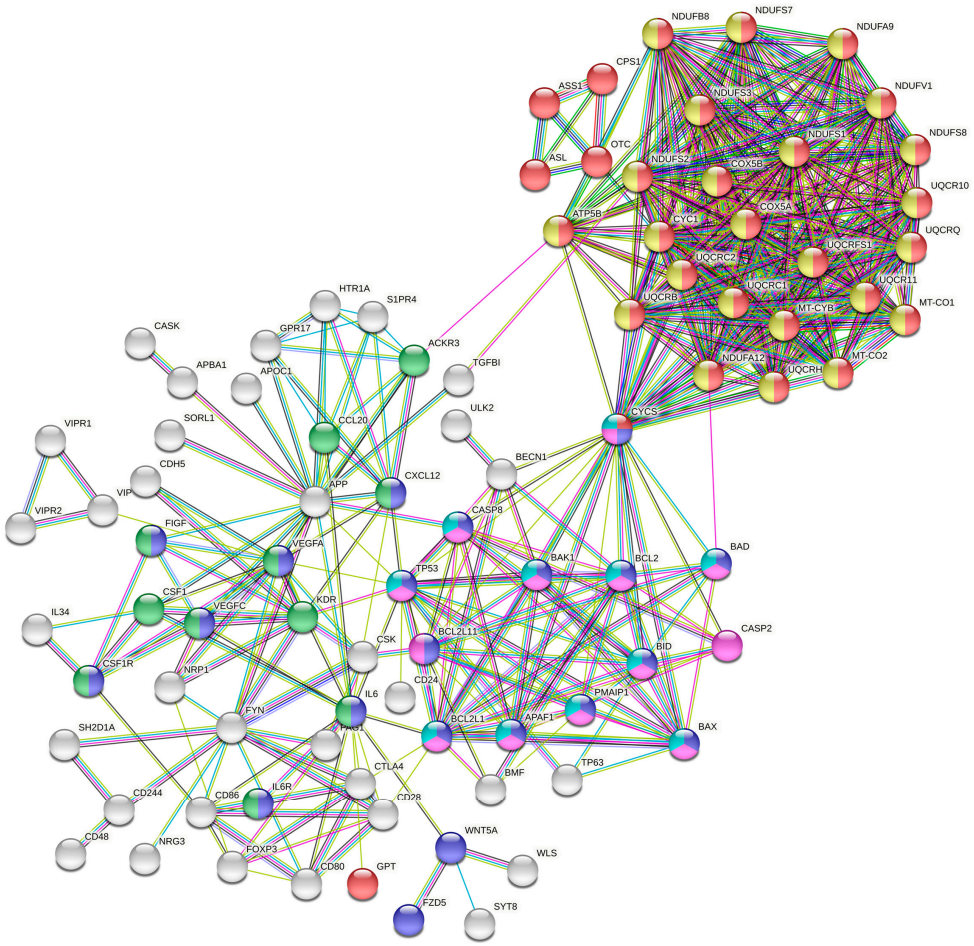

Figure 2. Protein–Protein Interaction Network of gip2-dependent genes.

Table 2. KEGG and Reactome pathway analysis of differentially expressed genes. KEGG and Reactome pathway enrichment analyses were carried out with the genes downregulated in gip2-silenced SKOV3 cells using STRING and Cytoscape applications. Major pathways of the PPI identified in downregulated and upregulated genes along with the gene count and false discovery rate are presented.

Pathway	Term	Description	Gene Count	False Discovery Rate
KEGG Pathway	hsa01100	Metabolic pathways	36	5.40e−08
	hsa00190	Oxidative phosphorylation	24	6.13e−21
	hsa05200	Pathways in cancer	21	5.83e−07
	hsa01524	Platinum drug resistance	11	5.45e−09
	hsa01521	EGFR tyrosine kinase inhibitor resistance	9	1.75e−06
Reactome Pathway	HSA-1428517	TCA Cycle and Respiratory electron transport	21	7.61e−16
	HSA-109581	Apoptosis	14	1.62e−08
	HSA-109582	Hemostasis	14	1.67e−02
	HSA-1268020	Mitochondrial protein import	4	2.76e−02
	HSA-1059683	Interleukin-6 signaling	2	3.69e−02

3.4. Identification of the Hub and Bottleneck Nodes

We probed the network further to identify the critical genes that define the hub nodes of the network using the cytoHubba plugin in Cytoscape [13]. We applied the multiple algorithms of the cytoHubba including Degree, MCC, MNC, EPC, EcCentricity, Closeness,

Betweenness, and Clustering Coefficient to identify the hub nodes of the PPI networks [14]. The intersecting genes identified by the different algorithms were tabulated. Results from this analysis identified CYC5, VEGFA, COX5B, UQCRFS1, and IL6 as the top five hub nodes of the network (Table 3). In addition, we identified the bottleneck nodes of the network since they are considered as the "connector" or "choke points" in PPI networks [19,20] using the BottleNeck algorithm in cytoHubba application of Cytoscape. The results identified CYCS, VEGFA, and IL6 along with ACKR3/CXCR7 and FYN as the top five bottleneck node genes of the network (Table 3). Functional annotation of these genes points to the diverse oncogenic roles of these genes in many different cancers including ovarian cancer (Table 3).

Table 3. Hub and bottleneck genes of the PPI network. The top five genes derived from the PPI network using MCC, MNC, Degree, EPC, and EcCentricity algorithms of the CytoHubba plugins in Cytoscape and top five bottleneck genes determined from the BottleNeck algorithm are presented.

Genes.	Nodes	Function	References
CYC5	Hub and Bottleneck	Increased oxidative phosphorylation and pro-survival cellular events	Huttemann et al., 2011
VEGFA	Hub and Bottleneck	Overexpression in ovarian cancer patients; tumor angiogenesis, associated with distant metastasis and resistance to chemotherapy	Guan et al., 2019; Sopo et al., 2019; Li et al., 2020
IL6	Hub and Bottleneck	Ovarian Cancer Growth, stemness, and therapy resistance	Wang et al., 2018; Azar et al., 2020
UQCRFS1	Hub	Oncogenic reprogramming of metabolism role in Pancreatic cancer	Kaneko et al., 2003; Ohashi et al., 2004; Owens et al., 2011; Jun et al., 2012
UQCRC1	Hub	Oncogenic reprogramming of metabolism role in Pancreatic cancer	Wang et al., 2020
COX5B	Hub	COX5B-mediated metabolic reprogramming is Associated with poor prognosis in many cancers	Gao et al., 2017; Chu et al., 2020
ACKR3/CXCR7	Bottleneck	Chemokine receptor activated in many cancers to promote invasive metastasis.	Neves et al., 2019; Smit et al., 2020
FYN	Bottleneck	Mediates oncogenic cell proliferation, migration, EMT, and therapy resistance in many cancers.	Saito et al., 2010; Lee et al., 2018; Yu et al., 2020

Gip2-dependent expression of the hub and bottleneck genes were validated by monitoring the expression of IL6 and UQCRC1 in gip2-silenced SKOV3, OVCAR8 and OVCAR3 cells by RT-PCR analysis. As shown in Figure 3, gip2-silencing led to the decreased expressions of both IL6 and UQCRC1 in all the tested cell lines (Figure 3).

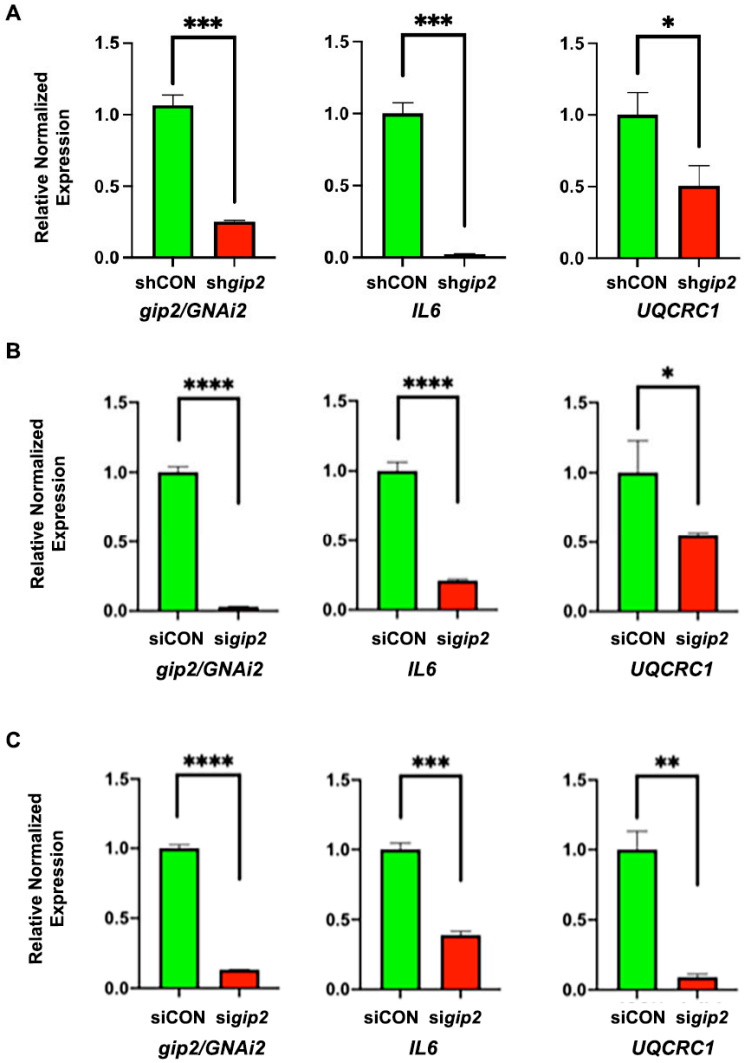

Figure 3. Validation of gip2-dependent hub and bottleneck genes. Downregulation of Hub and Bottleneck node genes upon silencing of gip2 was validated using SKOV3 (**A**), OVCAR8 (**B**) and OVCAR3 (**C**) cells in which the expression of gip2 was silenced using specific shRNA (SKOV3) or siRNAs (OVCAR8 and OVCAR3). In the case of SKOV3 cells, cells expressing non-targeting scrambled RNA were used as control group (shCON). In OVACR8 and OVCAR3 cell, cells transfected with non-targeting scrambled siRNA pool were used as the control group (SiCON). Downregulated genes were validated by monitoring the expression of the representative bottleneck gene IL6 and hub gene UQCRC1. Statistical significance was determined by Student's *t*-test (* $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$, **** $p < 0.0001$).

3.5. Significance of the Hub and Bottleneck Nodes

Next, we investigated the biological significance of the hub and bottleneck node genes in relation to ovarian cancer through cBioPortal analysis [20,21]. Oncoprint profile of CYCS, VEGFA, IL6, UQCRC1, UQCRFS1, COX5B, ACKR3/CXCR7, and FYN indicated that these genes were either amplified or overexpressed in 4–25% of the ovarian cancer patients (Figure 4A). Increased amplification or expression seen with UQCRFS1 in ovarian cancer patients, prompted us to carry out in silico analysis of its expression profile and overall survival rate of the ovarian cancer patients who show the altered expression of UQCRFS1. RNASeq data and overall survival plot were obtained through CBioPortal analyses. As shown in Figure 4B, increased expression of UQCRFS1 (Figure 4B) correlated with the reduced overall survival of ovarian cancer patients (Figure 4C). More intriguingly, increased expression of gip2 was not observed in UQCRFS1-patients. It is possible that

the initiating event involved in the expression of UQCRFS1 is the activation of gip2 rather than an increased expression of gip2 as in the case of other oncogenes [22]. Together with the functional annotation of the other hub and bottleneck genes, these results substantiate the potential role of the gip2-dependent hub/bottleneck nodal network in ovarian cancer pathophysiology.

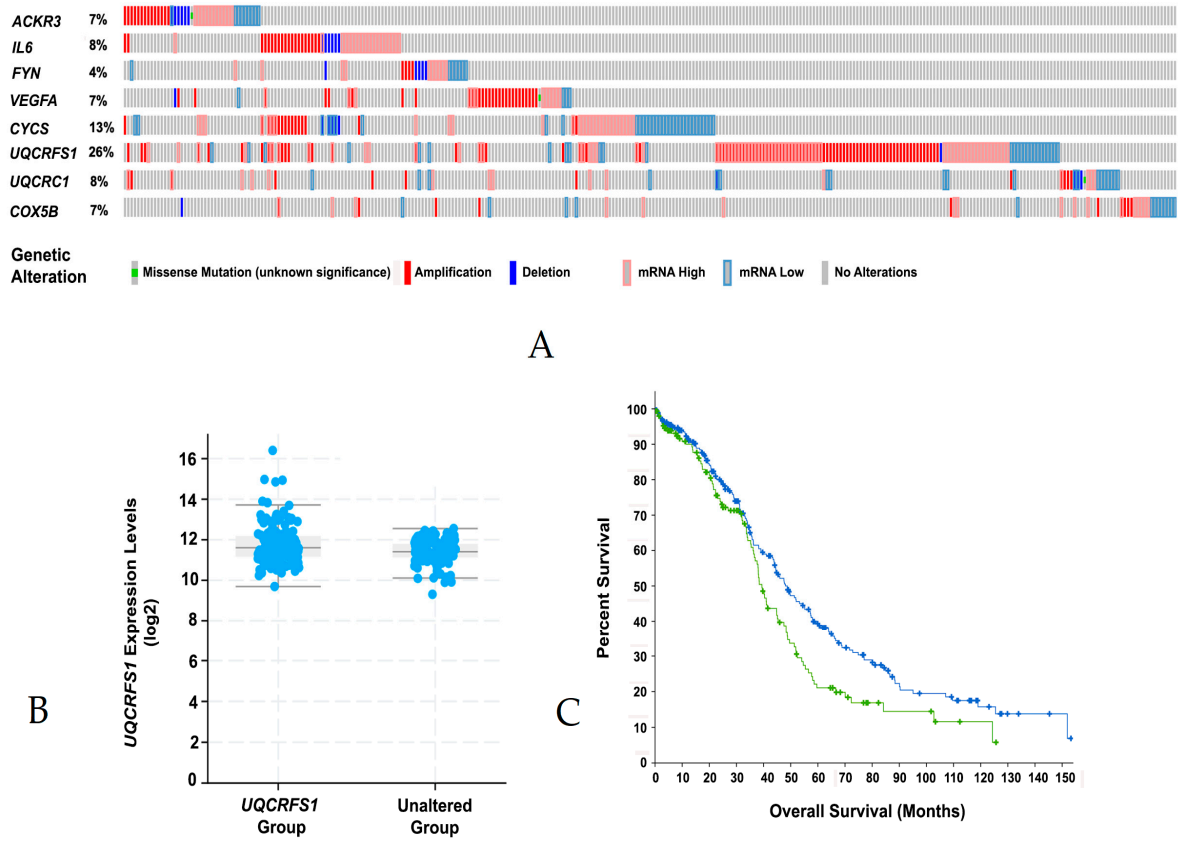

Figure 4. Genomic and expression profile of hub and bottleneck genes in Ovarian cancer patients. Genomic and expression profiles of the hub and bottleneck genes in ovarian cancer patients were visualized in OncoPrint at cBioPortal web-portal (**A**). Plots depicting the expression of the hub node gene UQCRFS1 with the *p*-value of 2.54e−8 were obtained from the CBioPortal (**B**). Overall survival of patients with UQCRFS1 amplification or overexpression (Log Rank Test *p*-value of 3.9e−2) was extracted from the CBioPortal (**C**).

4. Discussion

Effective targeted therapy in ovarian cancer has remained elusive primarily due to the heterogenous subtypes and aberrant signaling pathways associated with the disease [23,24]. While PARP inhibitors and anti-angiogenic agents have provided relief to many patients, the emergence of therapy resistance, especially in late-stage disease, has remained an unsurmountable clinical problem [25–28]. This has necessitated the critical need to have a clear understanding of the molecular events associated with different subtypes and stages of the disease so that context-specific therapeutic strategies can be developed. Although recent studies have identified major pathways and genetic risk factors associated with ovarian cancer [29], causative factors involved in sustaining tumor growth in the advanced stages of ovarian cancer are largely unknown. In this regard, the observation that *GNAi2/gip2* shows increased expression in advanced ovarian cancers is quite significant as it suggests the possibility that *gip2* could play a critical, if not unique, role in advanced ovarian cancers. Therefore, we investigated whether *gip2*-dependent transcriptome in ovarian cancer can be probed to identify any novel targets for ovarian cancer therapy. Results from such analysis, presented here, establish the critical role of the *gip2*-dependent transcriptomic network

in pathways related to cell survival, proliferation, metastasis, adhesion, and cancer cell metabolism (Tables S2 and S3).

While the individual pathways activated by *gip2* have been cataloged in the past, the extent to which *gip2* is involved in regulating a comprehensive transcriptomic network to facilitate ovarian cancer progression has not been fully understood until now. Our results presented here provide the first evidence to show the synergistic signaling nodes regulated by *gip2* in promoting ovarian cancer growth (Table S2). Functionally, the transcriptomic nodes regulated by *gip2* range from pathways involved in cellular energetics to evading cell death. The genes validated in the HGSOC cells lines OVCAR8 and Kuramochi substantiate this point (Figure 1). *KDR* gene, which encodes vascular endothelial growth factor receptor 2, was shown to promote oncogenic signaling pathways in many different cancers including ovarian cancer [30,31]. Vasoactive Intestinal Peptide, encoded by *VIP*, is known to be involved in autocrine as well as paracrine signaling loop that promotes cancer growth in multiple cancer types [32]. *CCL20* gene encodes the chemokine C-C Motif Chemokine Ligand 20 and it was identified to play a role in metastasis and therapy resistance of ovarian cancer cells [33,34].

In addition to these genes, GO enrichment analysis of the data has identified several novel correlates associated with ovarian cancer growth and progression. Results from GO:CC enrichment analysis are in conformity with the known cellular and signaling locale of GNAi2 in transducing the signals from the membrane-bound LPAR when activated by LPA (Table 1). Similarly, the predicted molecular functions such as molecular transducer activity, signaling receptor activity, transmembrane signaling receptor activity, lipid binding, and protein tyrosine kinase binding, can all be related to the signal-transducing activity of *gip2* (Table 1). More interestingly, GO:BP analysis unravels certain novel aspects of *gip2*-regulated biological processes in ovarian cancer transcriptome. While the role of *gip2* in biological processes such as response to stimulus and intracellular signal transduction has been well characterized, gene enrichment in GO:BPs such as cell proliferation, adhesion, and migration provide a wider glimpse into the transcriptomic landscape activated by *gip2* in ovarian cancer cells. One of the characteristic features of late-stage ovarian cancer is the peritoneal dissemination of ovarian cancer cells, which precedes peritoneal and hematogenous metastasis of ovarian cancer [35]. Biological processes underlying peritoneal dissemination and subsequent metastasis involve cell migration, adhesion, and subsequent tumor angiogenesis [36]. While the molecular components of these pathways have been characterized to a certain extent, the integration of these pathways into a transcriptomic network and the master regulator that modulates the expression of the downstream signaling nodes have remained unknown. Results from the gene enrichment analyses point to such a regulatory role for *gip2* in the transcriptomic reprogramming in ovarian cancer cells.

Organizational features of the *gip2*-orchestrated transcriptomic network are more clearly discernible with the results from the PPI network analysis. Two major clusters can be identified in the PPI network: one that promotes cancer growth through multiple cancer-specific signaling pathways and the other that promotes cancer cell metabolism (Figure 2). The tumorigenic pathways cluster encompasses genes involved in cell proliferation, suppression of apoptosis, and platinum resistance. This cluster is further augmented by signaling circuits involving cytokines, chemokines, and their cognate receptors that are known to play a critical role in cancer cell proliferation, adhesion, invasive migration, and metastasis. The metabolism cluster, on the other hand, primarily includes the genes that encode proteins/enzymes involved in oxidative phosphorylation (Figure 2). Analyses of the hub and bottleneck signaling nodes of the PPI network provide further insight into the *gip2*-regulated transcriptome. The finding that the silencing of *gip2* leads to the reduced expressions of all of these hub and bottleneck genes underscores the critical roles of these nodes in the onco-transcriptome (Figure 3). Functional annotation of these hub and bottleneck genes as well as the Oncoprint analyses of these genes add further support to their tumorigenic roles in ovarian cancer (Table 3; Figure 4A).

The known roles of *VEGFA*, *ACKR3*, *IL6* and *FYN* establish them as the major regulators of cancer pathways cluster in the network. Vascular Endothelial Growth Factor A, encoded by *VEGFA*, was found to be overexpressed in many cancers including ovarian cancer. *VEGFA* expression and its activation of VEGFRs were correlated to metastasis, tumor angiogenesis, and chemotherapy resistance in ovarian cancer [37–39]. Similarly, autocrine and paracrine signaling pathways stimulated by Interleukin-6, encoded by *IL6*, were shown to be associated with cancer cell proliferation, migration, stemness, and therapy resistance in ovarian cancer [40,41]. Likewise, the signaling pathways activated by CXCR7, a chemokine receptor encoded by *ACKR3*, were shown to be critically involved in the migration and invasive metastasis of multiple cancers [42,43]. Fyn kinase, encoded by *FYN*, stimulates a wide array of oncogenic pathways including cell proliferation, migration, EMT, and therapy resistance in many cancers [44–46]. Similarly, *CYCS*, *UQCRC1*, *UQCRFS1*, and *COX5B* genes present themselves as the regulatory nodes in the metabolism cluster of the network. Cytochrome C, encoded by *CYCS*, is a major component of the electron transport chain of mitochondria and it is tightly associated with the pro-survival pathways in normal as well as cancer cells [47]. *UQCRC1* encodes the mitochondrial ubiquinol-cytochrome c reductase core protein I, which is part of Complex I of the mitochondrial respiratory chain and was shown to be critically involved in the oncogenic reprogramming of metabolic pathways in pancreatic cancer [48]. *UQCRFS1*, another hub gene identified here, encodes the mitochondrial Ubiquinol-Cytochrome C Reductase/Rieske Iron-Sulfur polypeptide 1 and it is a key subunit of Complex III of the mitochondrial respiratory chain. Its overexpression and amplification were observed in different cancers including ovarian cancer [49–52]. Its overexpression was implicated in the aggressive phenotype of breast cancer [50,51]. *COX5B* gene encodes cytochrome c oxidase, and it is another critical component of the mitochondrial respiratory chain. Overexpression of *COX5B* is associated with a poor prognosis in many cancers [53,54]. The observation that these critical nodes are part of the gene cluster involved in mitochondrial oxidative phosphorylation gains further significance in light of the recent findings that mitochondrial oxidative phosphorylation is upregulated in many cancers including ovarian cancer [55–57]. Thus, the comprehensive analysis of the oncogenic network regulated by *gip2*indicates the activation of a transcriptomic network that involves cell metabolism, suppression of cell death, invasive metastasis, and tumor angiogenesis that cumulatively leads to aggressive ovarian cancer growth and progression (Figure 5).

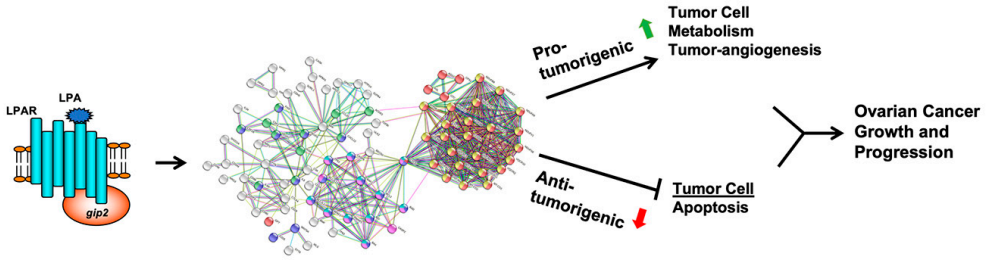

Figure 5. Gip2-regulated onco-transcriptome in ovarian cancer. LPA-LPAR-stimulation or mutational activation of *gip2* drove the coordinated regulation of two major network clusters. Gip2-stimulated pro-tumorigenic network comprised of nodes involved in tumor cell metabolism and angiogenesis synergizes with the suppression of anti-tumorigenic network consisting of pro-apoptotic and growth-suppressive nodes. LPA; lysophosphatidic acid; LPAR, lysophosphatidic acid receptor; *gip2*, G protein subunit α i2.

It should be noted that the limitation of the results is that the transcriptome network depicted here is derived from the data using a single ovarian cancer cell line. Nevertheless, the findings that (1) Representative genes are validated experimentally in HGSOC cell lines (Figures 1 and 3); (2) More than 50% of the genes in the network (78 genes) were shown to be dysregulated in many cancers including ovarian cancer (Table S3); (3) Analysis of these

genes in TCGA ovarian cancer dataset indicated that 61 of these genes showed increased expression in ovarian cancer (Table S4); and (4). Forty of these genes showed co-occurrence in their expression profiles (Table S5) provide external validation of the potential role of these network genes in HGSOC cells and the ovarian cancer patient subgroup. The identification of CYCS, VEGFA, IL6, UQCRC1, UQCRFS1, COX5B, ACKR3/CXCR7, and FYN as pro-tumorigenic nodes designates them as the novel and potentially druggable targets for effective targeted adjuvant therapy for ovarian cancer. In this context, the role of *UQCRFS1* as a hub node warrants special mention. The query of this gene in cBioPortal indicated that *UQCRFS1* is either amplified or overexpressed in 26% of ovarian cancer patients (Figure 4A). More strikingly, the cBioPortal analysis indicates further that the aberrant expression of *UQCRFS1* could be correlated with the reduced overall survival of ovarian cancer patients (Figure 4B). While the therapeutic potentials of *VEGFA*, *ACKR3*, and *IL6* have been already investigated or exploited [43,58,59], our analysis presented here points to *UQCRSF1* as a, thus far unidentified, potential node for the development of novel therapeutics.

5. Conclusions

In summary, the results presented here provide a paradigm in which *GNAi2/gip2*-dependent transcriptome promotes aggressive cancer growth during advanced stages of ovarian cancer through the gene network that stimulates cell metabolism, invasive metastasis, tumor angiogenesis along with the suppression of cell death. Considering the late-stage expression profile of *GNAi2/gip2* in ovarian cancer, the hub and bottleneck nodes identified here, especially the metabolic signaling nodes such as *UQCRFS1*, should provide newer targets for the development of second-line targeted therapy for advanced ovarian cancers.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/biom11081211/s1, Table S1: RT-qPCR Primers; Table S2: *Gip2*-dependent Genes Downregulated upon Silencing of *gip2*; Table S3: Oncogenic Profile of gip2-dependent Genes; Table S4: Network Gene Alteration Frequency in Ovarian Cancer Patients; Table S5: Co-occurrence of Network Genes in Ovarian Cancer Patients.

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Data Availability Statement: The microarray data presented in the present study are deposited at the Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/ accessed on 21 July 2021); accession no. GSE173214. Oncoprint data supporting the reported results used the Ovarian Serous Cystadenocarcinoma (TCGA, Firehose Legacy) dataset available at https://www.cbioportal.org (accessed on 21 July 2021).

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Review

Decoding the Oncogenic Signals from the Long Non-Coding RNAs

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Simple Summary: Long non-coding RNAs (lncRNA), which are often referred to as "Genomic Dark Matter", are emerging as critical molecules involved in the regulation of multiple cellular events. Their aberrant expressions and activities are correlated with tumorigenesis and tumor growth in many cancers. The documented potential of lncRNAs to regulate diverse oncogenic events identifies them as promising candidates for targeted therapy in cancer. In addition, the expression profiles of lncRNAs have the potential to serve as diagnostic or prognostic markers in many cancers. Therefore, an insight into the functional role of lncRNAs in cancer could aid in the development of novel diagnostic, prognostic, and therapeutic strategies to combat cancer. This review is focused on providing a succinct treatise on the functional role of lncRNAs in the genesis, growth, and progression of different cancers.

Abstract: Cancer is one of the leading causes of death worldwide. Multifactorial etiology of cancer and tumor heterogeneity are the two most acute challenges in existing diagnostic and therapeutic strategies for cancer. An effective precision cancer medicine strategy to overcome these challenges requires a clear understanding of the transcriptomic landscape of cancer cells. Recent innovative breakthroughs in high-throughput sequencing technologies have identified the oncogenic or tumor-suppressor role of several long non-coding RNAs (lncRNAs). LncRNAs have been characterized as regulating various signaling cascades which are involved in the pathobiology of cancer. They modulate cancer cell survival, proliferation, metabolism, invasive metastasis, stemness, and therapy-resistance through their interactions with specific sets of proteins, miRNAs and other non-coding RNAs, mRNAs, or DNAs in cells. By virtue of their ability to regulate multiple sets of genes and their cognate signaling pathways, lncRNAs are emerging as potential candidates for diagnostic, prognostic, and therapeutic targets. This review is focused on providing insight into the mechanisms by which different lncRNAs play a critical role in cancer growth, and their potential role in cancer diagnosis, prognosis, and therapy.

Keywords: lncRNA; cancer; oncogene; tumor-suppressor; miRNA; tumorigenesis

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1. Introduction

Long non-coding RNAs (lncRNAs) form a heterogenous family of non-coding RNAs (ncRNAs) that comprises almost 98% of the human transcriptome [1,2]. Linear non-coding RNAs that are greater than 200 nt in length are classified as lncRNAs. The family of lncRNAs includes long intergenic non-coding RNAs (lincRNAs), the anti-sense lncRNAs, the sense lncRNAs, the intronic lncRNAs, and the bi-directional lncRNAs [3]. Each of these subtypes carries out diverse functions, adopting different modes of action, either within the nucleus or in the cytoplasm [3]. Despite being not translated into proteins, lncRNAs actively contribute to all the major cellular processes, which span from cellular homeostasis to the cellular pathology underlying many diseases, including cancer [1,2,4–6].

Through their various modes of action, lncRNAs contribute significantly to the different, but synergistic, oncogenic pathways underlying cancer genesis, progression, and therapy resistance [7]. Recent observations that lncRNAs traverse through circulation, either in the form of circulating tumor cells or exosomal lncRNAs, also point to their role in long-distance signaling, including the processes involved in distant metastasis [8–12]. In addition, the findings that lncRNAs can be detected in blood, plasma, and urine also identify them as potential diagnostic, prognostic, or therapeutic biomarkers [13,14].

Despite the significant progress in diagnostic and therapeutic strategies over the years, cancer still ranks as the leading cause of death worldwide [15]. While increased understanding of the disease has led to the development of novel diagnostic methodologies and targeted therapies for some cancers, disease recurrence and therapy resistance contribute significantly to cancer death [16,17]. Disease recurrence and therapy resistance often arise from the evolution of rescue pathways that facilitate cancer cells to bypass treatment strategies [17]. Effective therapeutic strategies are further compounded by inter- and intra-tumoral heterogeneity in cancer [18,19]. Personalized or precision cancer medicine is emerging as an effective therapeutic strategy that can overcome the changes associated with inter- and intra-tumoral heterogeneity in cancer [20,21]. However, this requires a clear understanding of the multi-omics landscape of the cancer cells from a given patient, so that effective therapies can be launched to combat the specific aberrant or asynchronous pathway(s) presented by the patient. Thus, defining the mechanistic role of lncRNAs in the cancer transcriptome is crucial for the development of an effective targeted therapy. In this review, we present a comprehensive overview on the mechanistic role of lncRNAs in different oncogenic responses, so that the diagnostic, prognostic, and/or therapeutic potential of lncRNAs can be incorporated into precision cancer medicine strategies.

2. The Superfamily of lncRNAs and Cancer

The superfamily of lncRNAs consists of more than 70,000 annotated lncRNAs in the human genome [22]. Functionally, lncRNAs can be defined as the multifunctional regulators of gene expression. Aberrant expression of lncRNAs and the resultant dysregulated expression of oncogenes and tumor-suppressor genes are associated with tumorigenesis and tumor progression. The mechanisms by which lncRNAs execute their oncogenic function involve the modulation of gene expressions through cis- and trans-modes of epigenetic regulation, the regulation of chromatin topology, serving as RNA decoys, acting as scaffolds for proteins and RNAs, and sequestering microRNAs (miRNAs) by acting as competing endogenous RNAs (ceRNAs) [21–25]. They also play significant roles in enhancing the expression of neighboring genes [18]. Through their various modes of action, lncRNAs contribute to several oncogenic processes. These include cell proliferation, genomic instability, invasive migration, epithelial-to-mesenchymal transition (EMT), stem cell differentiation, maintenance of pluripotency, tumor progression and suppression, DNA damage response and repair, and metabolic reprogramming [19,26–44].

Specifically, lncRNAs play a functional role in several key spatiotemporal events involved in gene expression. The functional roles of lncRNAs include the maintenance of spliceosome machinery, the inhibition of gene transcription by direct binding to the gene, regulating gene expression through the modulation of transcription factor recruitment, inducing epigenetic modifications to regulate gene expression, regulating the functions of its interacting partners such as RNA, DNA, and proteins, regulating messenger RNA (mRNA) translation, regulating mRNA decay, and preventing the miRNA-mediated mRNA inhibition as a ceRNA. However, these functional roles need not be mutually exclusive. To a certain extent, the mechanisms by which lncRNA alters gene expression are dependent on the cellular location of the lncRNAs (Figure 1).

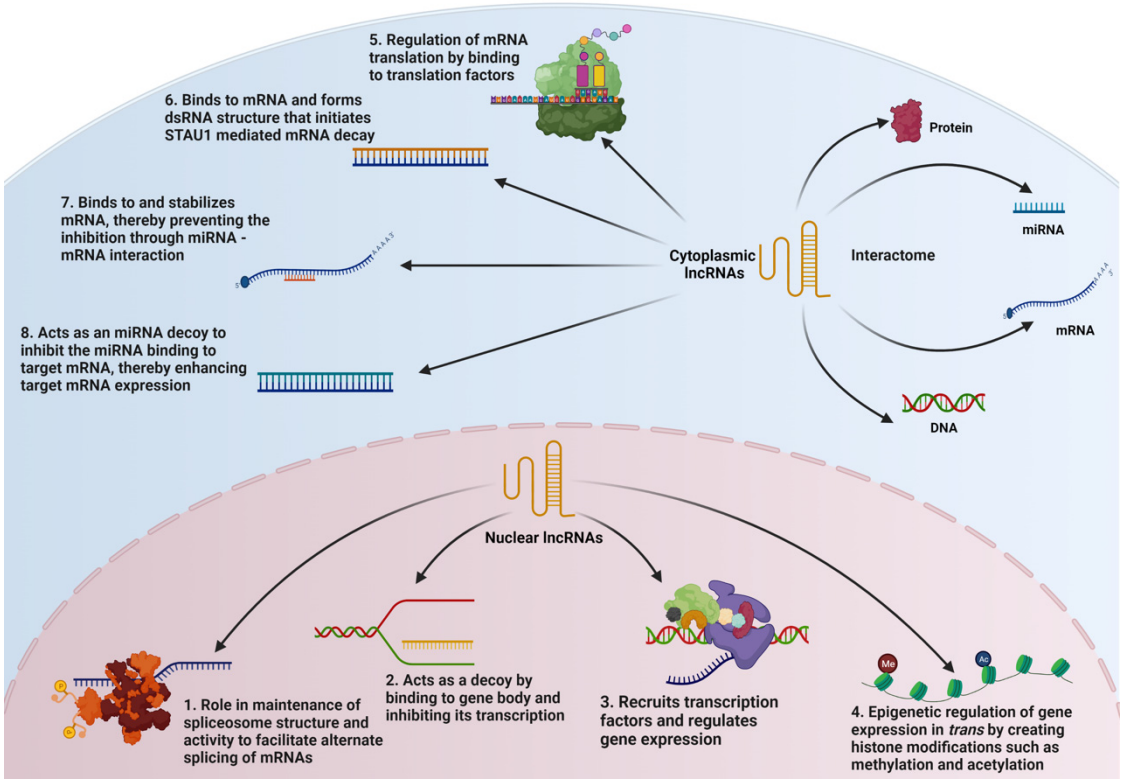

Figure 1. Nuclear and Cytosolic Functions of lncRNAs. The nuclear functions of lncRNAs include (1) role in mRNA splicing; (2) inhibition of gene transcription by direct binding to the gene; (3) regulating gene expression through modulation of transcription factor recruitment; and (4) inducing epigenetic modifications to regulate gene expression. The cytoplasmic functions of lncRNAs include (5) regulating mRNA translation; (6) mediating mRNA decay; (7) preventing the miRNA-mediated mRNA inhibition through mRNA stabilization involving lncRNA-mRNA duplex formation; and (8) preventing miRNA-mediated mRNA inhibition by binding to the miRNA as a competing endogenous RNA though the respective interactomes.

Based on their role in oncogenic pathways, lncRNAs can be grouped into oncogenic lncRNAs and tumor-suppressor lncRNAs (TS-lncRNAs). In addition, some lncRNAs are grouped as dual-function lncRNAs due to their functional ability to be either oncogenic or tumor-suppressive in a context-specific manner.

Oncogenic, Tumor-Suppressor, and Dual-Function lncRNAs

A large number of lncRNAs have been reported to function as oncogenes in many cancers [23]. Increased expression of lncRNAs is seen in many cancers, either through gene amplification or dysregulated constitutive expression [24]. Oncogenic lncRNAs promote tumorigenic cell growth through the activation of multiple pathways involved in cell proliferation, cell survival, genomic instability, EMT, invasive metastasis, tumor angiogenesis, evading immune surveillance, cancer cell stemness, and/or radio/chemotherapy resistance (Table S1). Similarly to the multi-targeted effects of oncogenic lncRNAs, TS-lncRNAs inhibit the oncogenic growth of cells by suppressing the many different facets of oncogenic pathways (Table S2). Presumably, this involves the lncRNA-mediated suppression of expression or the activities of many of the oncogenic proteins, lncRNAs, and miRNAs.

There are also lncRNA variants that exhibit the dual functions of being either an oncogene or a tumor suppressor in a cellular or spatiotemporal context-dependent manner [25]. The plasmacytoma variant translocation 1 (PVT1), as well as the neuroblastoma-associated transcript 1 (NBAT1) locus, produce different splice variants of lncRNAs, which act as either oncogenes or as tumor suppressors, affecting the MYC levels [26–29]. Furthermore, the metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) locus produces multiple splice variants of MALAT1, in addition to the full length MALAT1. These splice

variants play both oncogenic and tumor-suppressor roles in a cell-type/cancer-specific manner [30,31]. In addition, H19, the NEAT1 (Nuclear Enriched Abundant Transcript 1), and BANCR (BRAF-activated non-protein coding RNA) are a few other lncRNAs that exhibit both oncogenic and tumor-suppressive functions in various tumor and cellular conditions [32–37]. Furthermore, the cancer cell type and its metabolic state serve as decisive factors to determine the exact role of the lncRNAs, be it either an oncogenic or a tumor-suppressive mode of action [23].

3. Role of lncRNAs and Cancer

Cancer can be defined as a pathophysiological state in which the cancer cells manifest an uncontrolled proliferation potential, breaking free from the cellular homeostatic regulatory mechanisms. The slow evolution of normal cells into cancer cells involves the accumulation of genetic and epigenetic changes, and various oncogenic selection processes [38,39]. The differences between normal cells and cancer cells have been defined as the hallmarks of cancer [40,41]. Major traits defined by the hallmarks of cancer include sustained cell proliferation, resistance to cell death, genomic instability, invasive metastasis, tumor angiogenesis, and metabolic reprograming. LncRNAs affect most, if not all, of the hallmarks of cancer in promoting cancer genesis and growth. A broad spectrum of oncogenic activities, regulated by different families of lncRNAs, act in concert with each other, thus cumulatively contributing to cancer genesis and progression (Figure 2).

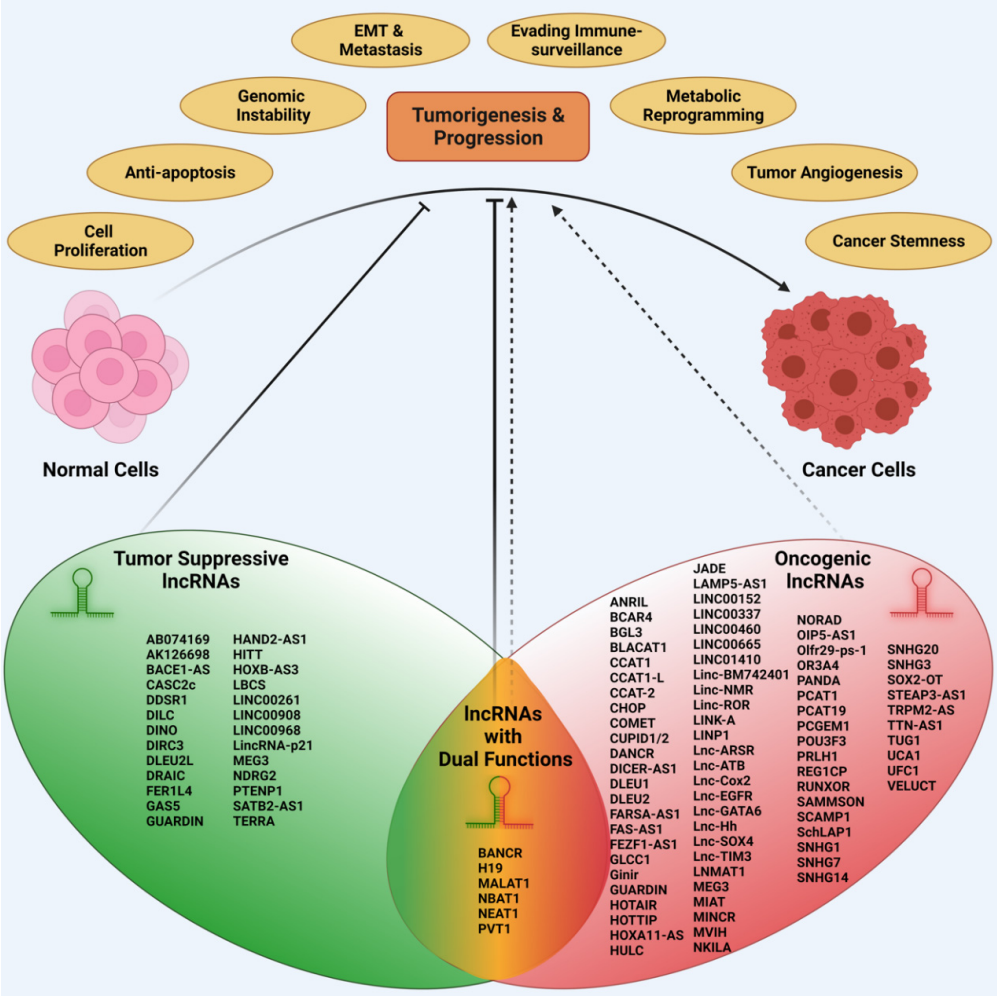

Figure 2. Oncogenic versus Tumor Suppressive Roles of lncRNAs in cancer genesis and progression. Based on their contributory roles in cancer pathobiology, lncRNAs can be classified into three superfamilies, namely oncogenic lncRNAs, tumor-suppressor lncRNAs, and dual-function lncRNAs. Representative examples of the lncRNAs with their respective roles are categorized under the corresponding subfamilies in the figure.

3.1. lncRNAs in Cancer Cell Proliferation and Apoptosis

At normal physiological conditions, cells have stringent control over cell proliferation and apoptosis to maintain cellular, as well as organismal, homeostasis. Oncogenes have been known to disrupt cellular homeostasis and confer survival advantages to malignant cells by stimulating pro-mitotic signaling pathways and/or inhibiting apoptotic signaling pathways. Likewise, aberrant expressions of lncRNAs have been shown to provide survival advantages to cancer cells by stimulating the activation of specific pro-mitotic signaling pathways and/or inhibiting apoptotic signaling pathways. The pro-mitotic function of lncRNAs mostly involves their ability to act as a scaffold for chromatin-modifying protein complexes such as polycomb-repressive complex 2 (PRC2) and mixed-lineage leukemia 1 (MLL1), and the associated histone marks involved in gene silencing or activation. An example of this mode of action can be seen with HOTTIP (HOXA Transcript at the Distal Tip) lncRNA, which shows increased expression in many cancers (Table S1). In pancreatic cancer, the oncogenic role of HOTTIP is correlated with its ability to interact with MLL1, a H3K4 methyl transferase, and WD-repeat-containing protein 5 (WDR5), a methyl transferase adaptor [42]. Using a panel of pancreatic cancer cells, it has been shown that HOTTIP provides a scaffold for MLL1 and WDR5 that leads to the increased expression of the AURKA gene, which encodes Aurora-A kinase. Specifically, HOTTIP-MLL1 interaction leads to the increased expression of the potent oncogene AURKA [43], presumably through H3K4-trimethylation [44]. UCA1 (Urothelial Cancer Associated 1), which is overexpressed in many cancers, utilizes a different epigenetic mechanism to promote oncogenic proliferation, as evidenced in gastric cancer cells. UCA1 interacts with the enhancer of zest homologue 2 (EZH2), a histone methyl transferase, to induce repressive H3K27-trimethylation in the promoter regions of tumor-suppressor genes such as p27 and protein sprout homologue 1 (SPRY1), thereby promoting cell proliferation. Thus, the silencing of UCA1 has been shown to suppress cell proliferation, as well as the xenograft tumor growth of gastric cancer cells [45]. Similarly, EZH2-mediated suppression of the phosphatase and tensin homologue (PTEN), has been attributed to the oncogenic role of the lncRNA UFC1 (Ubiquitin Fold Modifier Conjugating Enzyme 1) in the proliferation of non-small cell lung carcinoma (NSCLC) cells, as well as xenograft tumor growth [46]. More interestingly, exosomally transmitted UFC1 could also promote the epigenetic silencing of PTEN via EZH2 in this cancer model [47]. In some instances, oncogenic lncRNAs regulate the transcription of genes by recruiting specific transcription factors and associated proteins to the promoters of the target genes. The lncRNA REG1CP (Regenerating Family Member 1 Gamma Pseudogene), which is overexpressed in colorectal cancer (CRC), forms an RNA–DNA triplex at the regenerating family member 3 alpha (REG3A) promoter region and recruits fanconi anemia J helicase to unwind the DNA. This enables the glucocorticid receptor-α mediated transactivation of REG3A expression and the subsequent REG3A-mediated proliferation of CRC cells and CRC xenograft tumor growth [48].

A primary mechanism through which lncRNAs promote cell proliferation is through the sequestration of specific miRNAs. The lncRNA PVT1 sequesters the tumor-suppressor miRNA, miR-543, to block its inhibitory effect on the expression of the oncogenic trichorhinophalangeal syndrome-1 gene. This leads to the increased proliferation and migration of breast cancer cells [49]. Similarly, UCA1 stimulates the proliferation of prostate cancer cells by sequestering miR-143. Using DU145, a prostate cancer cell line, it has been shown that the silencing of UCA1 results in the suppression of xenograft tumor growth and it is associated with the ability of UCA1 to sequester the tumor-suppressive miRNA miR-143 that targets pro-mitogenic MYO6 [50]. The mechanistic role for such a sequestration of tumor-suppressive miRNAs by lncRNAs has been shown in many other cancers by different lncRNA-miRNA combinations. The lncRNA CCAT1 sequesters miR-155, and thus upregulates c-Myc expression and the proliferation of acute myeloid leukemia (AML) cells [51]. In breast cancer cells, NEAT1 sequesters miR-141-3p to upregulate the expression of the mitogenic transcription factor, Kruppel-like factor 12 (KLF12) [52]. The lncRNAs, STEAP3-AS1 (Antisense RNA 1 to Six-Transmembrane Epithelial Antigen of

Prostate-3), UFC1, and ANRIL (Antisense Non-coding RNA in the INK-4 Locus), also exhibit miRNA sequestration properties through which an array of oncogenes are upregulated, triggering cell proliferation in the cellular models of colon, pancreatic and gastric cancers, respectively [53–55].

In addition to the oncogenic lncRNAs, TS-lncRNAs play a critical role in cancer cell proliferation. In many cancers, the genes encoding TS-lncRNAs are deleted or their expressions are drastically reduced. SATB2-AS1 (Special AT-rich Sequence Binding protein-2 antisense RNA 1) and DIRC3 (Disrupted in Renal Carcinoma 3) are examples of lncRNAs which take part in the epigenetic regulation of genes involved in the inhibition of cell proliferation. Using a panel of CRC cell lines, it has been shown that the lncRNA, SATB2-AS1, serves as a scaffold for the recruitment of the transcriptional co-activator p300. SATB2-AS1-recruited p300 activates the expression of the tumor-suppressor gene SATB2 through the acetylation of H3K27 and H3K9 at the promoter site. However, downregulated expression of SATB2-AS1, often seen in CRC, leads to decreased levels of SATB2 with a resultant increase in cell proliferation [56]. In melanocytes, DIRC3, which is localized in the nucleus, enhances the transcription of the tumor-suppressor protein, insulin-like growth factor binding protein-5 (IGFBP5). DIRC3 acts as a decoy chromatin locus of IGFBP5 to prevent SRY box transcription factor-10 (SOX10)-mediated repression of IGFBP5. However, in melanoma cells, the melanocyte-inducing transcription factor and SOX10 complex represses the expression of DIRC3 through the repressive histone acetylation and methylation of the DIRC3 promoter. With the downregulation of DIRC3, SOX10 is able to repress the expression of IGFBP5, thus promoting the survival and growth of melanoma cells [57]. The sequestration of miRNAs by TS-lncRNAs also plays a role in tumor growth. The lncRNA MEG3 (Maternally Expressed-3) has been shown to exert its tumor-suppressive effect through the sequestration of miR-95-5p, which targets the tumor suppressor SOX11. In hepatocellular carcinoma (HCC), the downregulation of MEG3 leads to the increase in the levels of miR-9-5p. Subsequently, the expression of tumor suppressor SOX-11, which is a target of miR-9-5p, is thus suppressed, which augments cell proliferation and anti-apoptotic signals in HCC [58]. A similar role has been demonstrated with the TS-lncRNA, HAND2-AS1 (Heart and Neural Crest Derivatives Expressed Transcript 2 Antisense RNA 1), whose expression is downregulated in many cancers [59]. In vitro studies using a panel of HCC cell lines have shown that the lncRNA HAND2-AS1 sequesters miR-3118, which targets the suppressor of cytokine signaling 5 (SOCS5). The downregulation of HAND2-AS1 in HCC cells enhances the activity of miR-3118, and the subsequent suppression of its target SOCS5. SOCS5 is an inhibitor of JAK/STAT signaling. Hence, the downregulated HAND2-AS1 results in augmented JAK/STAT signaling, which contributes to enhanced proliferation, invasion, and migration in HCC cells [59]. The TS-lncRNA, GAS5 (Growth Arrest Specific-5), acts as a ceRNA to sequester miR-196a-3p in breast cancer cells. The lower levels of GAS5 in triple-negative breast cancers (TNBCs) increase the miR-196a-3p levels to inhibit the expression of the forkhead box O1 (FOXO1) gene. This activates the phosphatidyl inositol-3kinase (PI3K)-AKT kinase signaling pathway, which triggers numerous oncogenic pathways [60]. In addition to miRNA sequestration, TS-lncRNAs regulate mRNA translation by acting as scaffold in the recruitment of various translation factors. This function also plays a role in cancer cell proliferation and survival. The TS-lncRNA GAS5 interacts with the eukaryotic translation initiation factor, eIF4E and directly inhibits its recruitment to the translation site, thereby attenuating c-Myc translation and the downstream activation of c-Myc-regulated oncogenic events. This inhibition is relieved, and cell growth is promoted with the reduced expression of GAS5 in lymphoma cells [61]. In these cells, GAS5 is also found to interact directly with c-Myc mRNA to inhibit its translation. Such direct TS-lncRNA-mRNA interaction is also observed in the case of lincRNA-p21 [62]. In HeLa cells, it has been shown that lincRNA-p21 directly interacts with the mRNAs of the oncogenes JUNB and CTNNB1/β-catenin to inhibit their translation [63]. In addition, it binds to the polysomal translational machinery and the translational repressors RCK and FMRP to further suppress the translation of JUNB and CTNNB1 [62,63]. Reduced

expression of lincRNA-p21 eases these constraints on JUNB and CTNNB1 expressions, which leads to oncogenic cell growth. A few other TS-lncRNAs also affect the stability of mRNAs that encode specific oncogenes. The tumor-suppressor role of NBAT1 in HCC cells has been attributed to its ability to physically associate with insulin growth factor-2 binding protein-1 to prevent its stabilizing interaction with c-Myc mRNA. Reduced expression of NBAT1, in turn, enhances the stability of c-Myc mRNA, triggering the activation of c-Myc regulated oncogenic signaling cascades [27].

The lncRNA interactome is so diverse that these interactions could determine their role in tumorigenesis and tumor progression. DRAIC (Downregulated RNA in Cancer) has been identified as a TS-lncRNA in castration-resistant prostate cancers [64]. It interacts with the IκB kinase (IKK) subunits, thereby preventing the interaction between these subunits, their phosphorylation, and the subsequent activation of nuclear factor kappa B (NF-κB). When DRAIC levels are lower, it leads to NF-κB activation and the NF-κB-pathway-mediated proliferation of prostate cancer cells [64]. A variation of this theme is seen with the NBAT1 and CASC15-003 (Cancer Susceptibility 15) lncRNAs in neuroblastoma [65]. In low-risk neuroblastoma cells, both NBAT1 and CASC15-003 show higher expressions, predicting better prognosis. In contrast, decreased expression of NBAT1 and CASC15-003 with poor prognosis was observed in high-risk neuroblastoma. A decrease in the expression levels of NBAT1 and CASC15-003 were accompanied with the increased expression levels of N-Myc (MYCN). It has been demonstrated that NBAT1 and CASC15-003 interact with ubiquitin-specific peptidase-36 (USP36), a de-ubiquitinating protein, to prevent its interaction with N-Myc. Since USP36-N-Myc interaction prevents the ubiquitin-mediated proteolytic degradation of N-Myc, increased expression of NBAT1 and CASC15-003 inhibits MYC-mediated oncogenic pathways. However, reduced levels of NBAT1 and CASC15-003 result in the enhanced expression of N-Myc and N-Myc-regulated oncogenic activities in neuroblastoma [65]. In summary, lncRNAs regulating cell proliferation and survival signals can serve as significant therapeutic targets, as well as precise biomarkers for cancers.

3.2. lncRNAs and Genomic Instability

Genome instability is a hallmark of cancer which is characterized by defects in either DNA replication fidelity, chromosome segregation, and/or DNA damage repair (DDR) mechanisms. In cancer cells, genomic instability involves the complex DNA damage response (DDR) pathways, in which lncRNAs also play a definitive role. DDR involves DNA repair enzymes, tumor suppressors, apoptotic proteins, kinases, and cell cycle checkpoint factors. Defective DDR, such as in cancers, enhance the mutational load as well as genomic instability, and adversely affects therapy with DNA-damaging agents. The prominent mammalian DDR pathways include homologous recombination repair (HRR), non-homologous end joining (NHEJ), base excision repair (BER), nucleotide excision repair (NER), and mismatch repair (MMR). The major sensors of DNA damage include ATM (ataxia telangiectasia mutated), ATR (ataxia telangiectasia and Rad3 related) and DNA-PKs (DNA-dependent protein kinase), while p53 serves as the most significant transcriptional regulator to modulate cell cycle arrest and apoptosis in case of DNA damages. LncRNAs are either induced directly owing to DNA damage, or indirectly via intermediates such as p53, which is triggered upon DNA damage sensing, thereby regulating the cell cycle and DNA repair, as well as DDR events [66,67].

DDR genes and associated DNA repair mechanisms play a major role in the maintenance of genomic stability [68]. Several lncRNAs have been documented to play a role in the genomic instability of cancer cells through diverse mechanisms. The p53-responsive lncRNA, GUARDIN, acts as a scaffold for the breast cancer type 1 susceptibility protein/BRCA1-associated ring domain 1 (BRCA1/BARD1) complex, promoting DDR in many different cancer cells, including those of CRC, osteosarcoma, and lung cancer [69]. GUARDIN also sequesters miR-23a to upregulate telomere repeat-binding factor-2 (TRF2) levels which prevent chromosomal end-to-end joining in these cells [69]. In addition, GUARDIN acts as a scaffold for the breast cancer type 1 susceptibility protein/BRCA1

associated ring domain 1 (BRCA1/BARD1) complex, promoting DDR. The reduction of GUARDIN sensitizes cancer cells to chemotherapy [70]. JADE (Gene for Apoptosis and Differentiation in Epithelia) is an ATM-induced lncRNA, which is expressed upon DNA damage in cancers. It transcriptionally activates the expression of Jade-1 protein, which forms a complex with the histone acetyltransferase enzyme, human acetylase binding to ORC1 (HBO1), to stimulate histone 4-specific acetyltanferase activity. This leads to the expression of several other DDR genes promoting genomic instability and tumorigenesis in breast cancer cells [71]. The lncRNA SNHG1 (Small Nucleolar RNA Host Gene 1) promotes genomic instability by targeting p53 stability. In doxorubicin-treated colorectal cancer cells, SNHG1 sequesters heterogenous nuclear ribonucleoprotein C (hnRNPC) from interacting with p53, which leads to the inactivation of p53 with the resultant activation of DNA repair pathways [72]. The lncRNA, ANRIL, binds to and stabilizes ATR proteins, preventing it from ubiquitination. Thus, the complex promotes DNA double-strand repair through HRR in cancer cells [73]. The lncRNA, PRLH1, (p53-regulated lncRNA for HRR 1), which is upregulated in p53-mutated HCC, modulates the HRR pathway by binding to and stabilizing the ring finger protein, RNF169, to recruit HRR factors aiding DDR [74].

Several p53-independent lncRNAs regulate DDR in cancers by acting as scaffold for DNA-repair-associated proteins. LINP1 (LncRNA in NHEJ 1) regulates NHEJ by serving as scaffold for Ku70/80 and major DNA-PKs in TNBCs. Upon DNA damage, LINP1 translocates from the cytoplasm to the nucleus, and its depletion sensitizes these cancers to radiotherapy [75]. In multiple myeloma, MALAT1 binds to poly-ADP-ribose polymerase 1 (PARP1) and DNA ligase III in regulating the alternate-NHEJ repair mechanism [76]. Single-nucleotide polymorphisms at cyclin D1 (CCND1)-promoter regions enhance the levels of lncRNAs CUPID1 and CUPID2 (CCND1 upstream intergenic DNA repair 1 and 2), which play a significant role in promoting HRR over NHEJ through interaction with p53-binding protein 1 (53BP1) and BRCA1 [77]. The lncRNA, BGL3 (beta globin locus transcript 3), promotes DDR through its interaction with PARP1 and BARD1 proteins. It acts as scaffold for the BRCA1/BARD1 complex and other DDR, as well as HRR proteins, to stimulate DNA damage repair pathways in breast, osteosarcoma, and colon cancer cells [78]. BGL3 depletion has been shown to sensitize breast cancer cells to DNA damage-mediated therapy [78]. Another oncogenic lncRNA, Ginir (Genomic Instability Inducing RNA), interferes with karyogenesis, leading to the formation of multinucleated cells, during cell division in multiple myeloma cells. This is primarily due to its regulatory role over centrosomal functions through its interaction with the centrosomal protein, Cep112. Ginir-Cep112 association impairs BRCA1-Cep112 interaction, leading to dysregulated DDR and genomic instability in multiple myeloma, both in vitro and in vivo [79]. NORAD (Non-coding RNA Activated by DNA damage) is yet another lncRNA that actively regulates DDR in cancer cells. It acts as a decoy for various RNA-binding proteins, such as PUMILIO and RBMX, to prevent them from inhibiting the expression of DDR proteins [80]. In colon cancer cells, as well as colon organoids from mouse models, it has been reported that the lncRNA, CCAT2 (colon cancer associated transcript 2), enhances the expression of genes involved in ribosomal biogenesis, including the myc-induced expression of block of proliferation 1 (BOP1). In addition, CCAT2 directly interacts with BOP1 to increase its stability. BOP1 enhances the expression and activation of Aurora Kinase B, which leads to chromosome instability and consequent tumorigenesis [81]. Overexpressed NEAT1 has been observed to promote nuclear paraspeckle formation, enhancing the ATR signaling in osteosarcoma and TNBC cell lines. This facilitates the rapid replication of cancer cells, while its silencing sensitizes TNBC cells to chemotherapy [82].

Several of the TS-lncRNAs also play a role in regulating genomic stability. LincRNA-p21 has been reported to activate p53-induced p21 expression through the interaction with hnRNP-K, which further induces the expression of various DDR and G1/S cell cycle checkpoint genes mediated via PRC2. The deficiency of lincRNA-p21 disrupts this DDR system in many cancers [83]. A greater number of lncRNAs exert their functional roles, acting as scaffold for various proteins to facilitate DDR. The lncRNA, DINO (Damage induced

Non-coding), a transcriptional target of p53, binds and stabilizes p53, thus preventing the latter from ubiquitin-mediated proteolytic degradation, thereby facilitating error-proof DDR in CRC cell lines. The in vivo knockout mouse models affirmed that DINO-mediated stabilization of p53 further promotes the expressions of DDR genes such as DDB2, RRM2b, and GADD45a [84]. It has also been shown that DINO can reactivate dysfunctional p53 in human papilloma virus-positive cervical cancer cells via ATM/checkpoint kinase-2 (CHK2)-mediated DDR [85]. The lncRNA, DDSR1 (DNA damage sensitive RNA 1), an ATM-induced lncRNA upon DNA damage, acts as scaffold that binds to BRCA1 and hnRNP U-like 1 (hnRNPUL1) to facilitate the resection of DNA ends for the double-strand-break repair. The downregulation of DDSR1 results in BRCA1 accumulation at the damage site, resulting in deregulated HRR and DDR [86]. However, the seemingly tumor-suppressor function of DDSR1 is contrasted by the observation that the reduced expression of DDSR1 is correlated with the increased proliferation rate in multiple cancer cells [86]. The lncRNA, HITT (HIF-1α Inhibitor at Translational level), appears to be the first reported lncRNA that inhibits ATM activity in human cancers. HITT interacts with ATM, and prevents its association with the meiotic recombination 11 (MRE11)-RAD50-Nibrin (NBS1) complex, thereby inhibiting ATM recruitment and its activity at the damage sites, both in vitro and in vivo. Thus, HITT can sensitize colon cancer cells to chemotherapy through the inhibition of HRR [87]. The lncRNA, TERRA (Telomere Repeat Containing RNA), which is induced by tumor suppressors, such as p53, promotes genomic stability in normal cells. [88]. TERRA recruits TRF2 and associated factors at telomere ends to induce telomere stability [88]. Reduced expression of TERRA, as well as increased activity of TERRA, has been associated with the genomic instability in many cancers, including breast cancer [88,89]. From these results, it can be surmised that the modulation in TERRA levels in cancer cells leads to telomerase instability. Thus, lncRNAs play a major role in regulating various DDR components involved in genomic instability through multiple mechanisms. Further studies can reveal the precise roles in critical steps to maintain genome integrity, which can aid cancer therapy.

3.3. lncRNAs and Metabolic Reprogramming

Metabolic reprogramming is one of the major hallmarks of cancer, which involves the regulation of diverse cellular metabolic pathways, such as that of carbohydrates, lipids, amino acids, and nucleotides, so as to support cancer cell proliferation and survival. Since the cancer cells survive within a stressful tumor microenvironment, this condition can trigger various metabolic signals which can modulate the lncRNA levels. Such lncRNAs, in turn, regulate the expression and activity of various metabolic signaling pathway components to alter the cellular metabolism, termed as metabolic reprogramming, aiding tumorigenesis.

The major energy metabolism in cancer cells is through glucose utilization, whose rate is enhanced either by increasing the glucose uptake or by increasing the dependency on aerobic glycolysis rather than depending on mitochondrial oxidative phosphorylation, termed as Warburg effect [90]. To stimulate aerobic glycolysis, cancer cells increase the uptake of glucose through several lncRNAs. The lncRNAs, PCGEM1 (Prostate Cancer Gene Expression Marker 1) in prostate cancers, CRNDE (Colorectal Neoplasia Differentially Expressed Non-protein Coding Gene) in CRC, LINK-A (Long Intergenic RNA for Kinase Activation) in triple-negative breast cancers, and NRCP (lncRNA Ceruloplasmin) in ovarian as well as breast cancers, enhance the glucose uptake in respective cancer cells [91–94]. Specifically, CRNDE has been shown to stimulate glucose uptake by increasing the expression of glucose transporter-4 [92]. LncRNAs also regulate the key enzymes involved in glucose metabolism such as hexokinase 2 (HK), aldolase, pyruvate kinase, and lactate dehydrogenase (LDH). Hexokinase, which is encoded by HK1 or HK2 genes, is the first enzyme in the glycolytic pathway. In HCC cells, TUG1 (Taurine Upregulated 1) sequesters miR-455-3p, which represses the expression of the AMP-activated protein kinase β-2 (AMPKβ2). AMPKβ2, thus relieved from the inhibitory effects of miR-455-3p, stimulates HK2 expression and

glycolysis [95]. In gallbladder cancer cells, PVT1 overexpression enhances glycolysis by quenching miR-143, which targets HK2, thereby increasing the expression levels of HK2 with the resultant increase in glycolysis [96]. In esophageal carcinoma, HOTAIR (HOX Transcript Antisense Intergenic RNA) has been shown to upregulate the expression of HK2 by sequestering both miR-125 and miR-143, which target HK2 [97]. Increased expression of HK2 is correlated with the overexpression of HOTAIR in pancreatic adenocarcinoma [98]. LncRNA-mediated stimulation of glycolysis also involves the increased expression of the PKM gene which encodes pyruvate kinase. In cellular as well as murine models of CRC, antisense lncRNA to FEZ family zinc finger-1 (FEZF1-AS1) increases the stability of both cytoplasmic and nuclear PKM2 by physically associating with PKM2 [99]. While the cytoplasmic PKM2 interaction increases the aerobic glycolysis of CRC cells, nuclear interaction leads to the hyperactivation of the signal transducer and the activator of transcription 3 (STAT3) signaling. Another lncRNA, PCGEM1, has been shown to increase the levels of PKM2 and lactate dehydrogenase A (LDHA) to promote prostate cancer cell survival [100]. In prostate cancer cells, it has been reported that PCGEM1 directly binds to the promoters of c-Myc and its target genes to stimulate the transactivation of PKM2 and LDHA genes. In CRC, glucose starvation induces the lncRNA, GLCC1 (glycolysis associated lncRNA of colorectal cancer 1), which directly interacts with HSP90 to stabilize c-Myc and transcriptionally activate the expression of LDHA [101]. The lncRNA, lnc-IGFBP4-1, has also been shown to promote glucose metabolism and cancer cell proliferation in lung cancers by stimulating the expression of LDHA and HK2, as well as the PDK1 gene which encodes pyruvate dehydrogenase kinase 1 [102].

In addition to glucose metabolism, lncRNAs are also involved in rewiring lipid metabolism in cancer cells. Lipid metabolism is essential for cancer cells not only due to its role in aiding proliferation and by being a cell membrane component, but by also being a source for cellular energy and a precursor to many important signaling molecules. The lncRNAs, HULC (Highly Upregulated in Liver Cancer) and TINCR (Terminal Differentiation Induced lncRNA), stimulate acyl-CoA metabolism to promote tumor growth [103,104]. Using cellular as well as murine models of HCC, it has been demonstrated that HULC increases the activity of acetyl CoA synthase (ACS), which leads to increased triacylglycerol synthesis. Specifically, HULC increases the expression of the ACS-long chain family member 1 (ACSL1) subunit of ACS through the upregulation of the transcription factor peroxisome proliferator-activated receptor alpha (PPARA), which transactivates the ACSL1 promoter. The mechanism by which HULC upregulates the expression of PPARA is through the epigenetic silencing of miR-9, which targets PPARA [103]. Using a panel of nasopharyngeal carcinoma (NPC) cell lines and a xenograft mouse model, it has been shown that TINCR interacts with adenosine triphosphate citrate lyase to protect it from ubiquitin degradation, which leads to the upregulation of acetyl CoA and an overall increase in lipid biosynthesis [104].

Several lncRNAs have been shown to converge on the regulation of fatty acid synthase (FASN), a key enzyme involved in lipid biogenesis. In the prostate cancer cell line LNCaP, the lncRNA, PCGEM1, which regulates glucose metabolism, acts in co-ordination with the androgen receptor and c-Myc to regulate fatty acid biosynthesis by increasing the expression of FASN [100]. HOXD-AS1 or HAGLR (HOXD antisense growth-associated long non-coding RNA), which is elevated in NSCLC, enhances the expression of FASN, thereby increasing the levels of free fatty acids [105]. In NPC cells, HOTAIR has been shown to induce the expression of FASN [106]. In the U2OS osteosarcoma cell line, PVT1 upregulates the expression of FASN by quenching miR-195, which targets FASN [107]. Cellular lipid metabolism is strictly regulated by the transcription factors, sterol regulatory element-binding proteins (SREBPs). In the HCC cell line HepG2, MALAT1 has been shown to associate with SREBP-1c to enhance its stability. MALAT1-stabilized SREBP-1c promotes the transcription of genes involved in lipid metabolism, such as FASN, ATP citrate synthase, stearoyl CoA desaturase 1 and acetyl-CoA carboxylase 1, leading to cellular lipid accumulation [108].

In addition to their role in reprogramming glucose and lipid metabolism in cancer cells, a host of lncRNAs target amino acid metabolism. Of the amino acids, glutamine is the most abundant and significant one which actively takes part in the bioenergetic and biosynthetic pathways in cancer cells [109]. Glutamine, taken up by the cancer cells, undergoes glutaminolysis, in which it is converted into glutamate and α-ketoglutarate. These metabolites from glutaminolysis are used to generate ATP via the tricarboxylic acid cycle, and to provide an anabolic carbon backbone for the synthesis of lipids, amino acids, and nucleotides. The key enzyme involved in glutaminolysis is glutaminase. In CRC cells, the lncRNA CCAT2 enhances the expression of GAC, a gene that encodes glutaminase-c isoform, to augment the glutamine metabolism [110]. In bladder cancer cells, UCA1 has been shown to sequester miR-16 and upregulates the expression of glutaminase 2 (GLS2). This has been correlated with a reactive oxygen species (ROS) synthesis that protects cells from oxidative toxicity, in addition to the promotion of glutaminolysis in bladder cancers [111]. HOTTIP has been shown to enhance glutamine metabolism in a panel of cancer cells by upregulating GLS1 expression to promote tumorigenesis [112]. In glioma cells, upregulated HOTAIR promotes glutamine metabolism by sequestering miR-126 and relieving its inhibition on the expression of GLS [113]. In the prostate cancer cell line, LnCAP, PCGEM1 promotes chromatin recruitment of c-Myc, for the transactivation of its target genes that regulate the glutamine metabolism [100]. LncRNAs such as TUG1 also regulate other enzymes involved in glutamine metabolism. In intrahepatic cholangiocarcinoma cells, TUG1 induces sirtuin-3 (Sirt3)-mediated glutamate dehydrogenase (GDH) levels, thereby enhancing glutaminolysis and synthesis of metabolic intermediates to promote tumor progression [114]. It is significant to note here that the lncRNA, PVT1-5, in lung cancer, has been demonstrated to promote increased amino acid uptake by promoting the expression of the neutral amino acid exchanger, solute carrier family 7 member 5 (SLC7A5). SLC7A5 mediates the transport of neutral amino acids in exchange for the intracellular glutamine. PVT1-5 increases the expression levels of SLC7A5 by sequestering miR-126 that targets SLC7A5 [115]. However, a similar lncRNA-mediated mechanism for the enhanced uptake of glutamine by cancer cells remains to be established.

The role of lncRNAs in directly targeting molecules involved in nucleotide synthesis remains poorly understood. Increased expression of the linc-NMR (long intergenic non-coding RNA–nucleotide metabolism regulator) is observed in many cancers. Its overexpression has been correlated with cell proliferation, invasion, and metastasis of the respective cancer cells. Using the HCC cell line, HLE, it has been observed that the silencing of linc-NMR expression induces G0/G1 phase cell cycle arrest, which is accompanied by a drastic reduction in the levels of all the deoxy nucleotide triphosphates. Further analyses have indicated that linc-NMR binds to and regulates Y-box-binding protein-1 (YBX1), and YBX1 in turn regulates the key enzymes in pyrimidine metabolism, such as the ribonucleotide reductase 2, thymidylate synthetase, and thymidine kinase 1 [116]. Detailed analysis of the lncRNA-regulated metabolic reprogramming would help in unraveling them as potential biomarkers and therapeutic targets in cancer.

3.4. lncRNAs and Evasion of Immune Surveillance

The human immune system includes both adaptive and innate immune responses. Adaptive immune responses in the host involve the production of antigen-responsive lymphocytes, mainly T-lymphocytes or T-cells and B-lymphocytes or B-cells, which protect the host from similar kinds of antigenic exposure or re-infection with the same pathogen. In adaptive immune responses, T-cell activation further triggers numerous T-cell subsets, such as the CD8+ cytotoxic T-lymphocytes (CTLs), which eliminate the cancer cells via the release of various cytokines such as tumor necrosis factors (TNFs) and interleukins (ILs) [117]. Several lncRNAs target CTLs to promote immune evasion by cancer cells. The lncRNA, NEAT1, which is upregulated in the PBMCs of HCC patients, enhances the apoptotic death of CTLs so that the cytolysis of cancer cells is inhibited. The paradigm that emerges from both in vitro and in vivo studies with the use of the HCC model system

indicates that NEAT1 binds to miR-155, quenching its repression on the expression of T-cell immunoglobulin and mucin-domain containing-3 (Tim3), which promotes apoptosis in CTLs, thus attenuating the CTL-mediated cytolysis of cancer cells [118]. A different mechanism appears to be used by the lncRNA, lnc-TIM-3, to inhibit the cytolytic function of CTLs in HCC [119]. Using the ectopically expressed lnc-TIM3, it has been shown that it binds to and inhibits the interaction of TIM3 with the HLA-B-associated transcript 3 (BAT3) and BAT3-mediated downstream signaling. This leads to CD8+T cell exhaustion that contributes to compromised anti-tumor immunity and tumor progression [119]. Thus, the increased expression of both NEAT1 and lnc-TIM3 in the CTLs in HCC could contribute to the inactivation of functional CTLs through different mechanisms. In breast cancer, the lncRNA NKILA (NF-κB-interacting lncRNA) is involved in promoting the activation-induced cell death of CTLs and type 1 helper T-cells to facilitate immune evasion by cancer cells. NKILA has been shown to induce the tumor-antigen-activated death of T-cells by physically interacting and inhibiting the activities of IκBα and NF-κB [120]. Although the role of the lncRNA, lincMAF4 (Long-intergenic Non-coding Macrophage-activating Factor Transcriptional Regulator RNA 4) in cancers remains to be established, lincMAF4 could play a role in cancer-immune evasion through its potent inhibitory effect on T-cell differentiation [121]. It has been shown that lincMAF4 can epigenetically silence the transcriptional activation of the transcription factor MAF [121]. Since MAF is involved in the functional differentiation of regulatory T-cells (Tregs) and T-helper cells through the transcriptional activation of interleukin-4 and -10 [122], the lincMAF4-MAF link can be anticipated to have a potential role in cancer cells' immunoevasion.

Amongst the T-cell-mediated adaptive immune response, one of the prominent tumor-infiltrating lymphocytes (TILs) that aid tumor invasion by creating immune suppressive milieu in the tumor microenvironment are the Tregs, whose maintenance and differentiation are critically regulated by lncRNAs. Studies with HCC patient-derived CD4+ T cells and xenograft mouse models indicate that lnc-EGFR (long non-coding epidermal growth factor receptor) plays a role in Treg differentiation and suppresses CTLs by binding to EGFR, so as to inhibit the signaling axis involving the transcription factors' early growth response gene 1, and transcription factor forkhead box p3 (Foxp3) [123]. Since there are two splice variants of lnc-EGFR, namely lnc-EGFR-1 and lnc-EGFR-3, it is not clear whether both lnc-EGFRs can bind to EGFR to inhibit tumor immunoescape. A mechanism based on the ability of the lncRNA, FLICR, to epigenetically suppress the expression of FOXP3 to alleviate its tumor-suppressive functions in Tregs, has also been proposed as a potential mechanism through which cancer cells can escape immune surveillance [124]. Recent studies have shown that indolamine-2, 3-dioxygenase (IDO) is an immunoregulatory enzyme involved in tryptophan metabolism. By catabolizing tryptophan into kynurenine, IDO suppresses T-cell activities and promotes immune tolerance for cancer cells. The lncRNA, SNHG1, increases the levels of IDO in HCC cells by sequestering miR-448, which targets IDO, thus suppressing the T-cell-mediated immune response [125]. In addition, lnc-SOX5 has been reported to upregulate IDO through an unknown mechanism in CRC cells, and this has been shown to play an immuno-suppressive role promoting CRC xenograft tumor growth [126]. Immune checkpoint proteins such as programmed death ligand-1 (PD-L1), as well as cytotoxic T-lymphocyte associated protein-4 (CTLA4), are also targeted by lncRNAs to evade immune surveillance. In silico analyses have indicated a role for lncRNA RP11-571M6.8 in reducing the expression levels of PD-1, PD-L1 and CTLA4 [127]. Direct evidence for the ability to modulate the expression levels of PD-1 is shown by LINC00473, SNHG20, and MALAT1 in different cancers [128–130]. LINC00473 upregulates the expression of PD-L1 in pancreatic cancer cells by sequestering PD-L1-targeting miR-195-5p [128]. In esophageal squamous-cell carcinoma (ESCC), the lncRNA, SNHG20, enhances the expression as well as the activation of ATM, Janus kinase (JAK), and PD-L1, thus aiding compromised anti-tumor immunity [129]. The lncRNA, MALAT1, regulates the NF-κB-dependent innate immune response, in addition to sequestering miR-195 and upregulating PD-L1, resulting in CTL apoptosis as well as an immune escape

in large B-cell lymphoma [130]. Similar to the effects of the T-cell-mediated immune response, B-cell humoral responses are also regulated by lncRNAs. The aberrant levels of soluble FAS receptors in B-cell lymphoma have been associated with the PRC2-EZH2-mediated repression of FAS gene expression by lncRNA FAS-AS1 in B-cell lymphoma cells [131]. The lncRNA, DLEU2 (deleted in lymphocytic leukemia-2), controls B-cell proliferation by sequestering the miRNA15a/16-1 axis, which targets several cyclins and cyclin-dependent kinases [132]. The lncRNA, MIAT (Myocardial Infarction-Associated Transcript), is essential for B-cell survival and proliferation through its positive regulation on the transcription factor Oct-4 (octamer-binding transcription factor-4) [133]. Thus, MIAT can play a determinant role in B-cell immunity against cancer.

The major immune cells involved in innate immune responses are natural killer cells (NKs), macrophages, and myeloid-derived suppressor cells (MDSCs). The sequestration of miRNA is one of the mechanisms through which lncRNAs modulate the differentiation and/or activities of these cells. In the case of NK cells, TS-lncRNA GAS5 has been shown to exert its inhibitory effect on NK cells through the sequestration of miR-554. GAS5 binds to miR-554, which targets the runt-related transcription factor-3 (RUNX3) and interferon-γ (IFN-γ) in liver cells. Thus, the decreased levels of GAS5 in HepG2 and Huh7 liver cancer cells result in the reduced expression of the transcription factor RUNX3 and interferon-γ levels due to increased miR-544 activity. This results in the overall reduction in the number of CD107a+ NK cells. Both of these events suppress NK cell-mediated cytotoxicity against HCC cells [134]. In the case of macrophages, lncRNAs have been shown to be involved in the pathobiology of both M1 and M2 macrophages. Activated M1 macrophages are involved in anti-tumor immune responses, whereas M2 polarization/differentiation results in the formation of tumor-associated macrophages (TAMs) that facilitate tumor progression. Using RAW264.7 macrophages, it has been shown that lincRNA-COX2 (Long Intergenic Non-coding RNA to Cyclooxygenase 2) promotes the anti-tumor functionality of M1 macrophages. In these cells, the lipopolysaccharide-induced M1 polarization and maintenance of M1-phenotype of the macrophages are very much dependent on lincRNA-COX2, and the silencing of lincRNA-COX2 results in the attenuation of M1 macrophage-mediated inhibition of HCC cell proliferation. The resultant strengthening of the functionality of M2 macrophages promotes HCC growth, both in vitro and in vivo [135]. A few of the lncRNAs are directly involved in promoting M2-polarization and the immunoescape of cancer cells. The lncRNA, LNMAT1 (Lymph Node Metastasis Associated Transcript 1), acts as scaffold and recruits hnRNPL to the promoter of C-C-motif chemokine ligand-2 (CCL2), which results in the H3K4-trimethylation-mediated transcriptional activation of CCL2, as well as CCL2-responsive genes. This promotes the M2-polarization of macrophages and upregulated expression of vascular endothelial growth factor-C (VEGF-C), which triggers lymphatic metastasis in bladder cancer [136]. The CCL2 conduit is also utilized by HOTAIR to promote M2-polarization in many different cancers [137]. The lncRNA MM2P (modulator of macrophage M2 polarization) is overexpressed in M2 macrophages. MM2P has been shown to promote the M2 polarization of macrophages by reducing the levels of phosphorylated STAT6 through a (yet to be defined) phosphatase-dependent mechanism [138]. Validating this paradigm, the silencing of MM2 negatively affects the M2-driven tumorigenesis and tumorangiogenesis of osteosarcoma xenograft tumor growth in mice [138]. In contrast to the lncRNAs that promote M2 polarization, the TS-lncrRNA, CASC2c, has been shown to inhibit the M2 polarization of macrophages through a different mechanism in glioblastoma multiforme context. CASC2c inhibits M2 polarization in glioblastoma multiforme cells by acting in concert with miR-338-3p to inhibit the expression and release of the coagulation factor X (FX), a pro-M2-polarization factor [139]. The subsequent disruption of the paracrine loop involving FX leads to a reduction in M2 polarization of macrophages.

MDSCs are another heterogeneous class of immune cells which accumulate in cancers and enhance tumor progression [140]. Upon stimulation by pro-inflammatory cytokines, such as IL-6 and granulocyte-macrophage colony-stimulating factor (GM-CSF), lncRNA

olfactory receptor 29 pseudogene 1 (Olfr29-ps-1) is upregulated via JAK3/STAT3 signaling. Olfr29-ps-1 sequesters miR-214-3p and facilitates the differentiation of monocyte-MDSCs [141]. The lncRNA lnc-CHOP (Long Non-coding C/EBP Homologous Protein) interacts with the CHOP-CCAAT-enhancer-binding protein β (C/EBPβ) complex and induces the H3K4me3-mediated transcriptional activation of the genes encoding arginase 1, cyclooxygenase 2, NADPH oxygenase 2, and nitric oxide synthase 2, favoring the immunosuppression and differentiation of monocyte-MDSCs [142]. In lung cancers, the lncRNA RUNXOR (RUNX1 overlapping RNA) reduces RUNX1 expression, favoring MDSC differentiation and tumor progression [143]. The hypoxia-induced lncRNA, PVT1, was upregulated in monocyte-MDSCs as well as granulocyte-MDSCs, which increase ROS and Arg1 expression, thereby enhancing immunosuppressive functions in lung cancer cell lines as well as the murine model of lung cancer [47]. Thus, lncRNAs that regulate tumor-immune responses are emerging as precise immunotherapeutic targets for cancers.

3.5. lncRNAs in EMT and Metastasis

The advancing stages of cancers are marked by their prominent characteristics involving EMT and the invasive migration of cancer cells, as well as metastasis to distant sites, creating secondary tumors. Many different lncRNAs are critically involved in regulating the expression of genes involved in these pathological events. A host of oncogenic lncRNAs induce the EMT of cancer cells through epigenetic mechanisms. The lncRNA, HOTAIR, induces epigenetic histone marks through its interaction with either PRC2 or LSD1. Using both in vitro cellular and in vivo mouse models, it has been established that the HOTAIR-PRC2 complex induces a repressive H3K27 methylation on tumor-suppressive genes such as junctional adhesion molecule-2, protocadherin-10, PTEN, or protocadherin beta-5 [30]. On the other hand, in cellular as well as murine models of breast cancer, the HOTAIR-LSD1 complex induces H3K4 demethylation histone marks at the promoters or enhancers of oncogenic genes such as LDHA, Cyclin A, or eIF4E, to promote EMT [56]. Likewise, the MALAT1-PRC2 complex represses the expression of tumor-suppressor genes such as p21 and p27 through H3K27 trimethylation, to promote EMT and metastasis in mantle cell lymphoma cell lines [30]. Both in vitro and in vivo studies in bladder cancer have shown that H19 interacts with EZH2 to repress nucleoside diphosphate kinase 1, a Wnt-signaling antagonist, via H3K27 trimethylation, to enhance EMT through Wnt signaling [144]. The lncRNA, SChLAP1 (SWI/SNF complex antagonist associated with prostate cancer 1), binds to EZH2 and induces repressive H3K27 trimethylation on the promoters of miR-340-5p and miR-143-3p, which target the DNA methyltransferase, DNMT3a. This leads to the upregulation of DNMT3a, which methylates the promoters of tumor-suppressor genes that suppress metastasis, thus promoting cancer metastasis in prostate cancer cells [145]. The lncRNA, UFC1, interacts with EZH2 to induce H3K27me3-repressive methylation on the PTEN promoter to inhibit its expression and thereby promote the invasive metastasis of NSCLC cells, both in vitro and in vivo [46].

Many of the lncRNAs also utilize their roles as ceRNAs to modulate EMT and metastasis. HOTAIR acts through the sequestration of miR-23b-3p in HCC and the sequestration of miR-1227-5p in gastrointestinal (GI) cancer cell lines and tumor xenografts to upregulate the expressions of zinc finger E-box-binding homeobox-1 (ZEB1) and collagen type V alpha 1 chain (COL5A1), respectively, to induce EMT and metastasis [146]. MALAT1 acts as a ceRNA to sequester miR-126-5p, through which it promotes the expressions of snail family transcriptional repressor 2 (SLUG) and the transcription factor, TWIST, to induce EMT and metastasis in CRC [147]. Using HCC cell lines, it has been shown that both MALAT1 and lncRNA-ATB stimulate the expression of the ZEB family of transcription factors through the sequestration of two different miRNAs to promote EMT. While MALAT1 sequesters miR-143-3p to upregulate the expression of ZEB1, lncRNA-ATB competitively binds to miR-200 to upregulate ZEB1/2 [148]. In both the in vitro as well as the in vivo models of GI cancer, PVT1 has been shown to sequester miR-30a-5p, through which it relieves the inhibitory effect of miR-30a-5p on the expression of snail family transcriptional repressor 1

(SNAIL), ZEB1/2 and neural-cadherin (N-cadherin), to facilitate EMT and metastasis [29]. In osteosarcoma, both in vitro and in vivo studies have shown that TGF-β secreted by cancer-associated fibroblasts induce the lncRNA TUG1, which sequesters miR-143-5p to upregulate hypoxia-inducible factor-1α (HIF-1α), and promotes metastasis [149]. In cellular as well as murine models of CRC, H19 is shown to sequester miR-138 and miR-200a to upregulate ZEB1/2 to promote EMT [144]. Similar lncRNA, miRNA, and target gene nexuses are seen to promote EMT and metastasis in many other cancers. The lncRNA HULC has been shown to act through the miR-200a-3p/ZEB1 axis in HCC cell lines and mouse models [150]. LINC00460 has been observed to quench miR-433-3p to increase the expression of ANXA2, Vimentin, and N-Cadherin to induce EMT in both cellular and xenograft mouse models of colon cancers [151]. NEAT1 has been shown, both in vitro and in vivo, to restrain miR-141-3p from inhibiting the expression of KLF12 in breast cancers [152]. UFC1 has also been shown to sequester miR-34a to inhibit its repressive effect on the expression of CXCL-10 involved in invasive metastasis [153].

In addition to epigenetic and ceRNA roles, lncRNAs also act as scaffold to regulate EMT and metastasis. Increased levels of HOXA11-AS serve as scaffold for LSD1 or DNMT1, as well as ceRNA for miR-1297, which targets EZH2 to promote tumor invasion and EMT in GI cancers [154]. Using a panel of GI cancer cell lines and xenograft mouse models, the HOXA11-AS/DNMT1/EZH2 complex has been shown to repress KLF2 expression, while the HOXA11-AS/LSD1/EZH2 complex represses PRSS8 expression. The decreased expression of the tumor suppressors KLF2 and PRSS8 leads to an overall increase in the proliferation and migration of the GI cancer cells [154]. In breast cancer, the lncRNA, BCAR4 (Breast Cancer Anti-estrogen Resistance-4), is overexpressed in breast cancer cells and mediates C-C motif ligand-21 (CCL21) and C-C motif chemokine receptor-7 (CCR7)-induced EMT [28]. BCAR4 acts as scaffold for phospho-Gli-2 and protein phosphatase 1 nuclear targeting subunit (PNUTS), and Smad nuclear-interacting protein-1 (SNIP1), to regulate p300-mediated histone acetylation as well as RNA Pol II activities. Activated phospho-Gli2 stimulates the expression of the transforming growth factor beta 1 (TGFβ1), IL-6, protein patched homologue-1 (PTCH1), and mucin 5AC (MUC5AC) to promote EMT and metastasis [155]. The lncRNA CCAT2 interacts with the transcription factor TCF7L2 (Transcription Factor 7 like 2) to transactivate the expression of WNT target genes, including MYC, to promote invasive cell migration in colon cancer cells [156].

TS-lncRNAs are also involved in the regulation of invasion, migration, EMT and metastasis in cancers. In HCC cells, lincRNA-p21 has been shown to inhibit the repressive effect of miR-9 on the expression of E-cadherin to suppress the invasive migration of HCC cells. Thus, the reduced expression of lincRNA-p21 is correlated with the invasive migration of HCC cells [157]. In the context of glioma and TNBC, the expression levels of the lncRNA, GAS5, are inversely correlated with the size, staging, and metastasis of the respective cancers. GAS5 acts as a ceRNA to quench miR-196a-5p that targets FOXO1 expression, thereby increasing the expression of FOXO1 and FOXO1-mediated inhibition of PI3K/AKT activation, involved in the growth and invasive migration of glioma stem cells and TNBC cells [60,61,158].

3.6. lncRNAs and Tumor Angiogenesis

Angiogenesis is defined as the process associated with the formation of new blood vessels. Cancer growth and progression, including metastatic tumor growth, require angiogenesis to provide nutrients and oxygen for the growing tumors, in addition to the disposal of metabolic wastes. Numerous growth factors, such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), transforming growth factor (TGF), and angiopoietins, as well as signaling cascades such as STAT-3, Wnt/β-Catenin, mTOR (mechanistic target of rapamycin), and NF-κB also engage in activating the pro-angiogenic mechanisms. In addition to cancer cells, numerous other cell types within the tumor microenvironment, such as TAMs, are also involved in initiating angiogenesis, thereby recruiting several cell types such as endothelial progenitor cells,

haematopoietic stem cells, and mesenchymal stem cells to initiate blood vessel formation and thence, angiogenesis [159]. The hypoxic conditions in tumors activate the hypoxia-inducible factors (HIFs), which are translocated to the nucleus and escape its degradation in the cytoplasm, which further transactivates the expression of multiple genes to activate angiogenesis. A broad spectrum of lncRNAs regulate these pro-angiogenic factors, so as to regulate tumor angiogenesis.

The lncRNA, PVT1, binds to phospho-STAT3 and prevents it from ubiquitin-mediated proteolytic degradation, which then upregulates VEGFA expression, enhancing tumor angiogenesis in gastric cancer cells [160]. In cellular and murine models of lung carcinoma, LINC00665 has been shown to interact with the Y-box-binding transcription factor YB-1, to protect it from ubiquitin-mediated proteolytic degradation. YB-1, thus stabilized, binds to the promoters of angiopoietin-4, angiopoietin-like 3, and VEGFA to enhance their expressions and promote tumor angiogenesis [161]. Similarly, lncRNA olfactory receptor family 3 subfamily A member 4 pseudogene (OR3A4) promotes tumor angiogenesis by associating with the transcription factor AGGF1 (angiogenic factor with G-patch and FHA domains 1), which transactivates the expression of VEGF, FGF and Ang1 in HCC cells [162]. Tumor angiogenesis in HCC appears to also involve the lncRNA, MVIH (microvascular invasion in hepatocellular carcinoma), through a different mechanism. Using HCC tissues and cell lines, MVIH has been shown to physically interact with phosphoglycerate kinase (PGK) to block its secretion. Since the secreted PGK1 is known to inhibit angiogenesis, the MVIH-mediated block in PGK1 secretion relieves this inhibition and promotes tumor angiogenesis [163]. LncRNA-CamK-A (calcium-dependent kinase activation) in breast cancer cells activates PNCK through physical association to stimulate the expression of cytokines such as IL-6, IL-8, and VEGF through the NFκB signaling pathway. These cytokines promote tumor angiogenesis, as demonstrated by a patient-derived xenograft model using TNBC cells overexpressing lncRNA-CamK-A [164].

The lncRNA-mediated sequestration of specific miRNAs also plays a role in tumor angiogenesis. Tumor angiogenesis in gastric cancer has been shown to involve the ability of LINC01410 to sequester miR-532-5p, which represses the expression NCF-2 and NF-κB. The consequent upregulation of neutrophil cytosolic factor-2 (NCF-2)- and NF-κB-mediated signaling pathways promote angiogenesis in gastric cancers [165]. The lncRNA, TUG1, acts through the miR-299/VEGFA axis in glioblastoma, while it upregulates HIF-1α through miR-143-5p in osteosarcoma to promote angiogenesis [149]. The lncRNA, MALAT1, also promotes angiogenesis, through the miR-140/VEGFA axis in HCC, or through the miR-145/VEGFA axis in breast cancer cells [148]. Other examples of lncRNAs which act through miRNA sequestration to promote angiogenesis include DANCR (Differentiation Antagonizing Non-protein Coding RNA) through the miR-145/VEGF axis, SCAMP1 (Secretory Carrier Membrane Protein 1) through the miR-137/CXCL-12 axis, and MEG3 through the miR-421/PDGFR-A (platelet-derived growth factor receptor-A) axis, all of which are regulated in ovarian cancers [166–168]. In HCC, the lncRNA HULC stimulates an increase in the activation of sphingosine kinase 1 (SPHK1), a kinase that promotes tumor angiogenesis [169]. In cytosol, HULC sequesters miR-107 to upregulate the levels of the transcription factor E2F1, which targets SPHK1. In the nucleus, HULC promotes the expression of SPHK1 by recruiting E2F1 onto the SPHK1 promoter, [169].

In addition to the cancer cells, lncRNAs also influence the cells in the tumor microenvironment, as well as the cancer stem cells, to regulate angiogenesis. The lncRNA-MM2P promotes the macrophage M2-mediated angiogenesis through the direct binding and stabilization of phospho-STAT-6. The depletion of lncRNA-MM2P reduces osteosarcoma xenograft tumor growth and tumor angiogenesis [138,170]. Furthermore, the lncRNA, POU3F3 (POU class 3 homeobox 3), released through exosomes from tumor cells, is reported to be internalized by endothelial cells to enhance endothelial cell-based angiogenic signaling through the upregulation of bFGF (basic fibroblast growth factor), VEGFA, bFGFR (basic FGF receptor), and Angio in glioma cells [171]. However, the detailed mechanism behind the upregulation of these pro-angiogenic factors is yet to be elucidated. In

HCC, CD90+ cancer stem cells release exosomal H19, which modulates endothelial cells by upregulating the expression of VEGF and VEGFR (VEGF receptor), thereby promoting angiogenesis [172]. Certain angiogenic lncRNAs also epigenetically regulate critical angiogenic factors. For instance, the lncRNA, SNHG14, induces cytoplasmic polyadenylate-binding protein 1 (PABPC1) expression via H3K27 acetylation. Increased levels of PABPC1 inhibit PTEN signaling, presumably through its inhibitory effect on PTEN mRNA stability, which further induces angiogenesis in both the cellular and xenograft models of HCC [127]. In CRC cells, LINC00337 recruits DNMT1 to suppress CNN1 (calponin 1) expression, thus relieving its repressive effect on VEGF-mediated angiogenesis [173,174]. In TAMs, the lncRNA, MALAT1, stimulates FGF-2 expression and associated paracrine signaling pathways to promote angiogenesis in thyroid cancers [175]. Although the mechanism by which MALAT1 upregulates the expression of FGF2 is not defined, MALAT1 has been shown to promote tumor angiogenesis in neuroblastoma via FGF2 [176].

TS-lncRNAs also contribute to the regulation of tumor-angiogenic pathways. In vitro and in vivo studies have shown that the lncRNA, GAS5, inhibits the Wnt/β-catenin pathway through the downregulation of β-catenin, Cyclin D1, and c-Myc in CRC, thereby attenuating angiogenesis [177]. The lincRNA-p21 has also been reported to regulate angiogenesis under hypoxic conditions in NSCLC through the modulation of MMP-2 (matrix metalloproteinase 2), FGF-2, VEGFA, and PDGFB (platelet-derived growth factor β) expressions [178]. MEG3 has tumor-suppressive functions and inhibits cancer cell proliferation, as well as induces apoptosis, while its knockdown promotes angiogenesis. In breast cancer cells, MDA-MB-231 and MCF-7, as well as nude mouse xenograft models, it has been shown that MEG3 suppresses AKT pathways and downstream effectors such as MMP-9 and VEGFA, to inhibit angiogenesis [179]. Furthermore, HIF-1A-AS2, an antisense lncRNA to HIF-1α, binds to and inhibits HIF-1α, to inhibit tumor angiogenesis in the OVCAR-8 ovarian cancer cell line, as well as the LNCaP prostate cancer cell line and tumor xenografts [180].

3.7. lncRNAs and Cancer Stemness

Cancer stemness plays a critical role in disease recurrence and therapy resistance [181]. Many different lncRNAs significantly contribute to cancer stemness. Cancer stemness is marked by the expression of several unique transcription factors [182]. A large number of lncRNAs have been shown to modulate the expression of one or more of these transcription factors to confer or maintain cancer stemness [183,184]. The antisense lncRNA, LAMP5-AS1 (antisense-to-lysosomal-associated membrane protein family member 5), interacts with and activates methyl transferase activity of the enzyme disruptor of telomerase-silencing-1 (DOT1L), promoting H3K79 di- and tri-methylations on the promoters of DOT1L-regulated genes to augment cancer stemness in mixed-lineage leukaemia [185]. The lncRNA-mediated sequestration of miRNA also plays a role in the regulation of cancer stemness. UCA1, transmitted exosomally from the cervical cancer cell line CaSki, has been shown to sequester miR122-5p and upregulate SOX2 expression, which promotes cancer stemness in these cells [186]. UCA1 has also been shown to promote stemness in glioma cells by inducing the expression of SLUG through the sequestering of both miR-1 and miR-203a [187]. Using CRC cells, it has been reported that H19 upregulates the expression of c-Myc, SOX-2, and Oct-4 to induce stemness. In this model, it has also been shown that the exosomal-derived H19 from the cancer-associated fibroblasts upregulates the expression of stemness-associated factors through the β-catenin signaling cascade by sequestering miR-141 [188]. Stemness in CRCs is also mediated by the lncRNA FARSA-AS1 (Phenylalanyl-tRNA Synthetase Subunit Alpha Antisense RNA1) [189]. Here, FARSA-AS1 increases the expression levels of SOX-9 by sequestering miR-18b-5p. More interestingly, SOX-9 also increases the expression levels of FARSA-AS1 by activating its transcription by binding to its promoter, thus establishing a sustained positive feedback loop to maintain cancer stemness. The lncRNA, MALAT1, has been shown to modulate cancer stemness through the quenching of miR-375 to upregulate the expression of YAP1 (Yes-associated

transcriptional regulator) in liver cancer cells [190]. In gastric cancer cells, linc-ROR (long intergenic non-coding RNA—regulator of reprogramming) has been reported to enhance the expression of stemness-associated transcription factors such as Oct4, SOX-2, and Nanog through the sequestration of miRNAs [191].

In the tumor-initiating cells of renal cell carcinoma, lncARSR (lncRNA activated in renal cell carcinoma with sunitinib resistance) interacts with YAP1 and inhibits YAP1 phosphorylation, facilitating the nuclear translocation of YAP1. YAP1 upregulates the expression of Oct-4, Nanog, and SOX-2 to promote the self-renewal of CSCs and metastasis [192]. Several lncRNAs promote cancer stemness in breast cancer cells through multiple mechanisms. HOTAIR represses the expression of HoxD10 through PRC2-mediated H3K27 trimethylation, which leads to a decrease in the levels of miR-7. The resultant increased expression and activation of STAT3 promotes stemness in breast cancer cells via c-Myc and TWIST [146,193]. The lncRNA-Hh, which is regulated by TWIST, promotes stemness in MCF7 breast cancer cells by stimulating hedgehog signaling-mediated expression of Oct-4 and Sox-2 [194]. Examples of the lncRNAs acting through hedgehog signaling also include lnc-HDAC2 and lnc-HOXA10. In the liver-cancer-tumor-initiating cells, lnc-HDAC2 activates the hedgehog signaling pathway, and lnc-HOXA10 initiates the transcriptional activation of HOXA10. Together, these lncRNAs stimulate the self-renewal and tumor progression of their CSCs [195]. In the context of liver cancer, lncRNA-SOX4 has been shown to recruit STAT3 to the SOX4 promoter to enhance its expression, thereby promoting stemness [196]. In CRC cells, lncGATA6 (lncRNA GATA binding protein 6) recruits a nucleosome remodeling factor (NURF) complex to the promoter of Ehf (ETS homologous transcription factor) to induce its expression, which leads to Lgr4/5 expression, LGR4/5-mediated activation of Wnt signaling, and CSC renewal and maintenance [197].

TS-lncRNAs are also associated with cancer stemness. Reduced expression of DILC (lncRNA Downregulated in Liver Cancer Cells), a TS-lncRNA, has been correlated with cancer stemness in HCC [198]. DILC inhibits IL-6 expression by binding to its promoter and thereby attenuating the STAT3 signaling and cancer stemness. Similarly, lncRNA-LBCS (lncRNA Bladder and Prostate Cancer Suppressor) is downregulated in bladder cancer stem cells. This lncRNA, otherwise, would bind to hnRNPK and EZH2, acting as scaffold, to induce the H3K27-repressive tri-methylation of SOX-2 and repress its expression [199].

3.8. lncRNAs and Therapy Resistance

Therapy resistance contributes greatly to cancer mortality. A number of lncRNAs have been reported to play a role in either promoting or inhibiting therapy resistance in cancers. Generally, lncRNAs that promote radiosensitivity include those with tumor-suppressive activity, and the primary mechanism appears to be through the sequestration of miRNAs that target tumor suppressors. NEAT1 enhances chemosensitivity in nasopharyngeal carcinoma through the sequestering of miR-101, which targets EMP2 (epithelial membrane protein 2 [200]. Similarly, OIP5-AS1 has been shown to play a role in the chemoresistance of CRCs via its interaction with miR-369. Using the CRC cell lines LoVo and SW280, it has been shown that the sequestering of miR-369 by OPI5-AS1 leads to the increased expression of dual-specificity tyrosine phosphorylation-regulated kinase-1A (DYRK1A), which is involved in suppressing cell proliferation and therapy resistance [201]. LincRNA-p21 has been shown to promote sensitivity to radiation therapy by inhibiting the expression of β-catenin at both the mRNA and protein levels in gastric as well as CRC cells [202,203]. As can be predicted, oncogenic lncRNAs enhance the radiotherapy resistance in numerous cancers. UCA1 regulates the cell-cycle signaling molecules such as focal adhesion kinase, AKT, FGR-tyrosine kinase, AMP-activated protein kinase, and AMPKα1 to contribute to radiotherapy resistance in prostate cancer [204]. MALAT1 confers radiotherapy resistance in NPC by sequestering miR-1 to enhance the expression of SLUG, whereas LINC00963 promotes radiotherapy resistance in breast cancer by sequestering miR-324-3p to increase the expression of activated CDC42 kinase 1 [205]. In the case of resistance to chemotherapy, it could be against a single drug or to a broader spectrum of drugs. Many lncRNAs

confer drug resistance, either by stimulating signaling pathways that can override drug sensitivity or by increasing the expression of proteins involved in drug efflux, such as multiple-drug resistance (MDR) or multi-drug-resistance-associated proteins (MRP) [206]. Several lncRNAs, such as MALAT1 and HOTAIR, induce drug resistance by modulating the expressions of proteins involved in the influx and efflux of therapeutic agents, or by altering specific signaling pathways. MALAT1 was first identified as overexpressed in lung cancer metastasis, and induces MDR by upregulating MRP1, as well as MDR1 proteins and STAT3 activation, to induce drug efflux [207]. MALAT1 has been associated with gefitinib resistance in lung cancer by sequestering miR-200a, thereby upregulating ZEB-1 expression, which is involved in the regulation of MDR proteins [208]. However, in gastric cancers, MALAT1 enhances ZFP91 (zinc finger protein 91 homologue) expression through the sequestration of miR-22-3p, thereby inducing oxaliplatin resistance [209]. HOTAIR has been reported to induce doxorubicin resistance in breast cancers via the activation of PI3K/AKT/mTOR pathways and upregulating the MDR1, MRP1 and ABCB1 (ATP-binding cassette subfamily B member 1) expressions [210]. In addition, HOTAIR also induces cisplatin resistance in NSCLC via the upregulation of MDR1, MRP1, and Wnt/β-catenin signaling [211]. HOTAIR induces paclitaxel and doxorubicin resistance in gastric cancers by sequestering miR-217, targeting glypican 5 (GPC5) and protein tyrosine phosphatase non-receptor type 14 (PTPN14) [212]. It also induces cisplatin resistance in gastric cancers by targeting miR-126, which targets and activates PI3K/AKT/MRP1 proteins [213]. It has been reported that the natural product, curcumin, inhibited the HOTAIR/miR-20a-5p/WT-1 transcription factor signaling axis to reduce the adriamycin resistance in acute myeloid leukemia (AML), thus suggesting the potential role of the HOTAIR/miR-20a-5p/WT-1 axis in conferring adriamycin resistance in AML [214].

In gastric cancers, UCA1 sequesters miR-27b, which upregulates cyclin G1 (CCNG1). CCNG1 enhances p53 expression, as well as downregulates miR-508-5p, thereby inducing MDR [215,216]. Several other lncRNAs, such as TRPM2-AS (antisense lncRNA to transient receptor potential cation channel subfamily M member 2), which acts through the miR-138-5p/EGFR/PI3K/AKT axis in NSCLC; lncRNA OIP5-AS1, which acts through miR-340-5p/LPAATβ (lysophosphatidic acid β)/PI3K/AKT/mTOR axis in osteosarcoma; lncRNA H19, which acts through the miR-107/HMGB1 (high mobility group box 1) axis in laryngeal squamous cell carcinoma; lncRNA SNHG7, which acts through miR-34a in breast cancers; lncRNA BLACAT1 (bladder cancer-associated transcript 1), which acts through the miR-519d-3p/CREB1 (cAMP-responsive element-binding protein 1) axis in CRCs; lncRNA TTN-AS1 (antisense lncRNA to titin), which acts through miR-16-5p/Cyclin E1 in HCC, lncRNA DICER-AS1 (antisense lncRNA to DICER ribonuclease), which acts through miR-34a-5p in osteosarcoma; and lncRNA PCGEM1, which acts through the miR-129-5p/EVT1 (ETS variant gene 1) axis, alter the MDR proteins to confer drug resistance [206].

In addition, some of these lncRNAs overcome drug resistance by stimulating the expression of signaling proteins that can overcome drug sensitivity. The exosomal lncRNA, SOX2-OT (SOX-2 overlapping transcript), sequesters miR-627-3p, which targets Smad2/3/4, and activates Smad signaling. This promotes macrophage M2 polarization, resulting in the production of TAMs to enhance resistance to EGFR-TKIs (EGFR-tyrosine kinase inhibitors) in NSCLC [217]. The extra-vesicular NEAT1 sequesters miR-141-3p and upregulates KLF12 to induce resistance to paclitaxel, cisplatin, and 5-fluorouracil in breast cancer model [152]. The exosomal HOTTIP inhibits miR-214, thereby upregulating karyopherin subunit alpha 3 (KPNA3) expression to promote mitomycin resistance in CRC cancers [218]. The lncRNA, UCA1, induces tamoxifen resistance in breast cancers by associating with EZH2 to generate repressive H3K27me3 methylation on the p21 promoter, thereby downregulating p21 expression and activating PI3K/AKT signaling [219]. UCA1 induces cisplatin resistance in gastric cancers through its association with EZH2 and the activation of PI3K/AKT signaling through upregulation of p-PI3K and p-Akt [220]. UCA1 has also been reported to activate Wnt/β-catenin signaling, resulting in both tamoxifen and cisplatin resistance in breast and bladder cancers, respectively [221,222]. HOTAIR upregulates DNMT3b lev-

els and induces the promoter hypermethylation of PTEN, which downregulates PTEN expression, leading to drug resistance in AML [223]. LINC00665 interacts with EZH2 and induces Akt expression to activate the PI3K/AKT pathway, thereby leading to the gefitinib resistance in NSCLC [224]. LINC00152 interacts with EZH2 to induce ZEB1 expression, thereby leading to EMT and oxaliplatin resistance in esophageal cancers [225]. In gliomas, the lncRNA DLEU1 (Deleted in Lymphocytic Leukemia 1) generates temozolomide resistance by regulating autophagy [226]. Similarly, HOTAIR has been reported to induce autophagy and imatinib resistance in GI cancers through the inhibition of miR-130a and the subsequent upregulation of ATG2B (autophagy-related 2B) levels, which promotes autophagy-mediated cell survival [227]. The lncRNA, PCAT-1 (Prostate Cancer Associated Transcript-1), activates AKT and GSK-3 (glycogen synthase kinase-3) phosphorylation, resulting in signaling which augments gefitinib resistance [228]. PVT1 inhibits kelch-like ECH-associated protein 1 (Keap1)–nuclear factor erythroid 2-related factor 2 (Nrf-2) association and stabilizes the latter, resulting in doxorubicin resistance in TNBCs [229]. Though these studies have shown an increased drug sensitivity through the knockdown of the lncRNAs, the exact mechanism has yet to be defined.

In addition to the oncogenic lncRNAs described above, TS-lncRNAs play a role in regulating drug resistance in cancers. The downregulated expression of GAS5 is inversely correlated with ABCB1 expression, as shown through silencing studies. GAS5, otherwise, targets miR-221-3p and upregulates DKK2 (Dickkopf Wnt Signaling Pathway Inhibitor 2) expression to inhibit the Wnt/β-catenin pathway, to promote adriamycin resistance in breast cancers [230]. In HCC, the downregulated GAS5 levels contribute to doxorubicin resistance via the activation of miR-222-3p and downregulation of PTEN [231]. In NSCLC, it has been demonstrated that the lncRNA AC078883.3 levels are downregulated, which increases miR-19a activity that targets PTEN. Similarly, downregulation of lncRNA TP53TG1 (TP53 Target 1), which otherwise suppresses miR-18a, reduces PTEN levels. Both these lncRNAs have been reported to contribute to cisplatin resistance in NSCLC through the regulation of PTEN [232,233]. The lncRNA FER1L4 (Fer-1-like Family Member 4 Pseudogene) levels are downregulated in ovarian cancers, which induces paclitaxel resistance through the activation of mitogen-activated protein kinase (MAPK) signaling [234]. The lncRNA, LINC00968 is downregulated in breast cancers and contributes to drug resistance through its inhibitory effects on Wnt signaling. LINC00968 interacts with the transcription factor HEY1 and inhibits Wnt2, thereby leading to Wnt/β-catenin signaling [235]. In pancreatic cancers, the lncRNA DLEU2L targets miR-210-3p and upregulates BRCA2 to reduce the gemcitabine resistance [236]. Targeting these oncogenic—as well as tumor-suppressive—lncRNAs, and their associated axes, will be an ideal therapeutic strategy to combat the therapy resistance in cancers.

4. Perspective and Conclusions

LncRNAs form a diverse class of non-coding RNAs which regulate the cellular processes, both at the physiological as well as pathophysiological states in the human body. They regulate cellular homeostasis through the modulation of gene expressions and protein levels, as well as their stability and interactions. As discussed here, lncRNAs critically participate in diverse cell signaling cascades, bringing out their multitudinous roles, ranging from embryonic development to disease biogenesis, and most importantly, cancer genesis and progression. From the initial oncogenic signals triggering the transformation of normal cells to the tumorous state, through the regulation of diverse functional attributes that promote tumor progression and metastasis, to the response to anti-cancer therapies, lncRNAs perform a cardinal role (Figure 3).

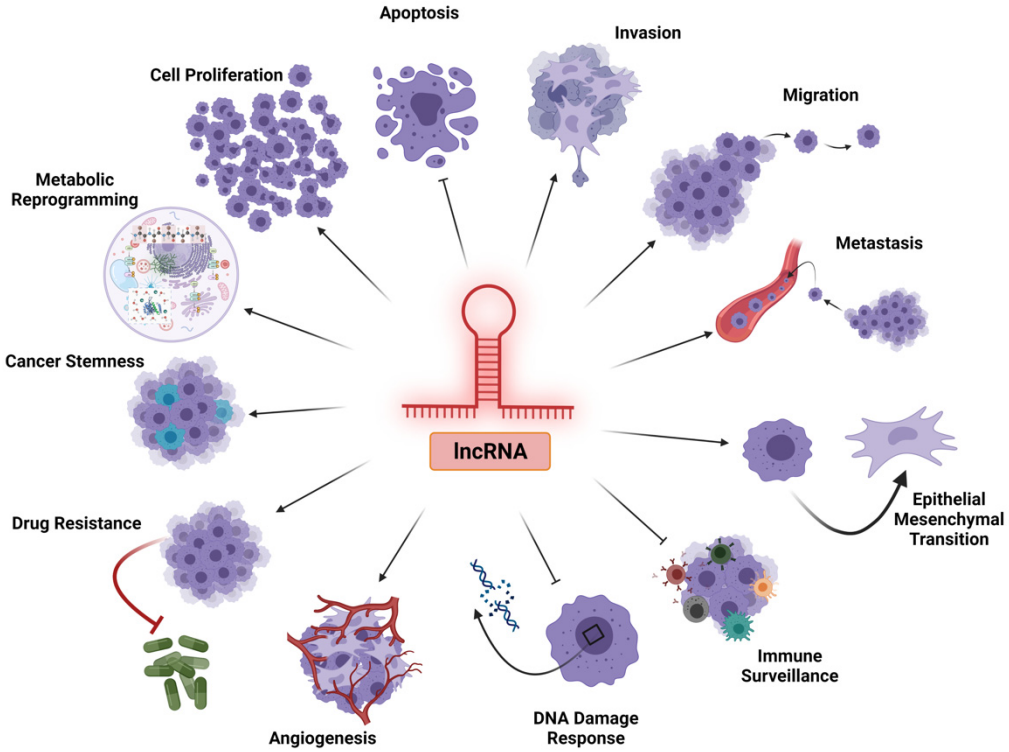

Figure 3. Multifacted role of lncRNAs in tumorigenesis and tumor progression. The diverse classes of lncRNAs play a role in tumor progression by variably regulating the major arms of tumorigenesis, either by promoting cell proliferation, invasion, migration, metastasis, EMT, angiogenesis, drug resistance, cancer stemness, and metabolic reprogramming, or by inhibiting apoptosis, immune surveillance, and efficient DNA damage response within the cancer cells.

Non-coding RNAs were considered to be the dark matter of the genome, as they could not be translated into proteins and carry out cellular functions. The advent of the existence of functional non-coding RNAs such as that of ribosomal RNAs (rRNAs), transfer RNAs (tRNAs), lncRNAs, small interfering RNAs (siRNAs), miRNAs, guide RNAs (gRNAs), piwi-interacting RNAs (piRNAs), and small nucleolar RNAs (snRNAs) has revolutionized the significance of transcriptomes. Deeper understanding of transcriptomes has transfigured the whole paradigm of cancer pathobiology. lncRNAs are now recognized as an active component of complex cellular regulatory networks, playing significant roles in cancer genesis, progression, and therapy resistance. Since lncRNAs lack sequence conservation, multiple factors such as secondary, tertiary, and quaternary structure–function relationships in their respective interactomes must be taken into account, in addition to their linear primary sequence. Thus, targeting lncRNAs in precision cancer medicine requires a clear understanding of their functional roles and underlying mechanisms in relation to their interactomes in specific cancers, as well as in normal cells. The present review has discussed the salient functional features of lncRNAs in cancer biology. However, it should be noted that further analyses of the expression profiles and functional mechanisms of lncRNAs could unravel them as effective diagnostic, prognostic, and therapeutic targets for cancers. This calls for conjoint research demanding various omics technologies such as genomics, transcriptomics, proteomics, and metabolomics, that would help towards deciphering the precise upstream and downstream events underlying the aberrant expression of lncRNAs in different cancers. With the advent of newer high-throughput technologies, the wait is not long for when tailored therapy involving lncRNAs will translate into clinics.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/onco1020014/s1, Table S1: Functional role of oncogenic lncRNAs in cancer; Table S2: Functional role of tumor-suppressor lncRNAs in cancer.

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Review

Signaling by LncRNAs: Structure, Cellular Homeostasis, and Disease Pathology

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Abstract: The cellular signaling network involves co-ordinated regulation of numerous signaling molecules that aid the maintenance of cellular as well as organismal homeostasis. Aberrant signaling plays a major role in the pathophysiology of many diseases. Recent studies have unraveled the superfamily of long non-coding RNAs (lncRNAs) as critical signaling nodes in diverse signaling networks. Defective signaling by lncRNAs is emerging as a causative factor underlying the pathophysiology of many diseases. LncRNAs have been shown to be involved in the multiplexed regulation of diverse pathways through both genetic and epigenetic mechanisms. They can serve as decoys, guides, scaffolds, and effector molecules to regulate cell signaling. In comparison with the other classes of RNAs, lncRNAs possess unique structural modifications that contribute to their diversity in modes of action within the nucleus and cytoplasm. In this review, we summarize the structure and function of lncRNAs as well as their vivid mechanisms of action. Further, we provide insights into the role of lncRNAs in the pathogenesis of four major disease paradigms, namely cardiovascular diseases, neurological disorders, cancers, and the metabolic disease, diabetes mellitus. This review serves as a succinct treatise that could open windows to investigate the role of lncRNAs as novel therapeutic targets.

Keywords: lncRNA; miRNA; epigenetics; cardiovascular disease; diabetes; cancer; Alzheimer's; Parkinson's; neurology; tumorigenesis

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1. Introduction

The genetic flow of information from DNA to protein via RNA has been held as the central dogma of molecular biology [1,2]. While this fundamental tenet of molecular biology is shaken to a certain extent by the discovery of reverse transcriptases that reverse the flow of information from RNA to DNA [3,4], proteins have remained as the critical downstream molecules that execute and regulate diverse cellular functions. The primary function of RNA has long been considered as the transcriber of genetic information from DNA and the template upon which the proteins can be built in the ribosomes [1]. However, the advent of transcriptomics and next generation sequencing technologies have unraveled the functional significance of several newer species of non-coding RNAs, that were once considered transcriptional noises or the dark matter of the genome. They include a family of microRNAs (miRNAs), circular RNAs (circRNAs), piwi-interacting-RNAs (piRNAs), small nucleolar RNAs (snoRNAs), and long non-coding RNAs (lncRNAs). LncRNAs are the non-coding RNAs that are greater than 200 nucleotides in length. In general, lncRNAs lack open reading frames and are not generally translated into proteins with a

few exceptions of micropeptide-encoding lncRNAs [5]. To date, he GENCODE v35 has annotated 48,684 lncRNA transcripts and 17,957 lncRNA genes, while the NONCODE v5 has annotated the existence of 172,216 lncRNA transcripts and 96,308 lncRNA genes [6,7]. LncRNAs have been classified as sense-, antisense-, bidirectional-, intronic-, and intergenic-lncRNAs based on their genomic locations [8–10]. Sense lncRNAs are the ones whose sequence overlaps with the sense-strand of the protein coding sequence of a gene whereas antisense-lncRNAs are the ones whose sequence overlaps with the opposite antisense strand of a protein coding gene. Bidirectional lncRNAs are defined by the lncRNAs whose transcription is initiated by the same promoter region as that of the protein coding gene, but in the opposite direction. Intronic lncRNAs are the ones that are derived from the intron of another transcript. Long intergenic non-coding RNAs or the lincRNAs are those lncRNAs whose genomic loci do not overlap with any of the coding regions of the genome. However, in this review we have grouped all the sub-classes of lncRNAs as 'lncRNAs'. Recent studies have documented a wide variety of mechanisms by which the lncRNAs regulate cellular homeostasis through their active as well as modulatory roles in diverse cell signaling pathways. In line with their critical roles, aberrant expression of lncRNAs have been shown to be associated with the pathogenesis and progression of several diseases.

Signaling by lncRNAs has the potential to interface with multitudinous factors of the cell signaling network due to their diverse functional roles such as serving as decoys, guides, scaffolds, and effector molecules. Recent progress in lncRNA research has unraveled the dynamics of lncRNA-mediated mechanisms in cellular homeostasis as well as pathogenesis. Analysis of lncRNA- interactomes that encompass proteins, RNAs, and lipids have pointed to the potential of lncRNAs as therapeutic targets. In this review, we discuss the structural and functional aspects of lncRNAs and emphasize the role of lncRNA signaling in human disease pathogenesis, focusing on four major disease paradigms, namely the cardiovascular diseases, neurological disorders, cancers, and diabetes.

2. Structural Features of lncRNAs

To explore the multifarious role of lncRNAs as signaling molecules that can regulate diverse cardinal signaling cascades, a deeper understanding of the structural hierarchies of lncRNAs is very much required. Results from several studies have indicated that the structure of lncRNAs define their functional interactions with other macromolecules including DNA, RNA, and proteins. It appears that the primary and secondary as well as tertiary structures of lncRNAs play context-specific roles in the structural and functional organization of lncRNAs. Possible diversities in their primary, secondary, and tertiary structural conformations of lncRNAs that relate to their functional roles are discussed here (Figure 1).

2.1. Primary Structure

The primary structures of the lncRNAs are defined by their linear nucleotide sequences. Across the species, lncRNAs do not exhibit sequence conservation in their primary sequences other than with their promoter regions [11]. In general, lncRNAs are transcribed by RNA polymerase II from either the intergenic or the exonic regions or even from the distal protein coding regions of the genome. Certain lncRNAs are also transcribed by RNA polymerase III [12]. Once they are transcribed, they undergo post-transcriptional modifications, similar to the messenger RNAs (mRNAs), such as 5′ capping, 3′ poly-A tailing, and alternate splicing [13–15]. However, a few of the lncRNAs show specific primary sequence modifications that can be associated with their potential secondary structural motifs. One such specialized sequence is the presence of poly-adenylated nuclear (PAN) regions at the 3′ end of the lncRNAs MALAT1 and NEAT1 [16,17]. Unlike the classic poly-A tails, the poly-A rich region of MALAT1 and NEAT1 is preceded by two Uracil-rich motifs separated by a set of nucleotides, thus capable of forming a stem loop structure. These stem loop structures protect the poly-A coded region at 3′ end from deadenylation and associated degradation, conferring an enhanced stability to these lncRNAs [16,17]. Another specialized primary

sequence that has been observed in a few of the lncRNAs (such as lincRNA-p21) is the presence of the transposable elements [18]. The most commonly observed transposable element in the lncRNAs is Alu retrotransposon, belonging to the family of short interspersed nuclear elements [15,18]. The lincRNA-p21 has been reported to possess two inverted repeats of Alu elements contributing to their helical structures. Mutation in these repeat elements has been shown to prevent the co-localization of lincRNA-p21 with NEAT1 in the nuclear paraspeckle structures [18].

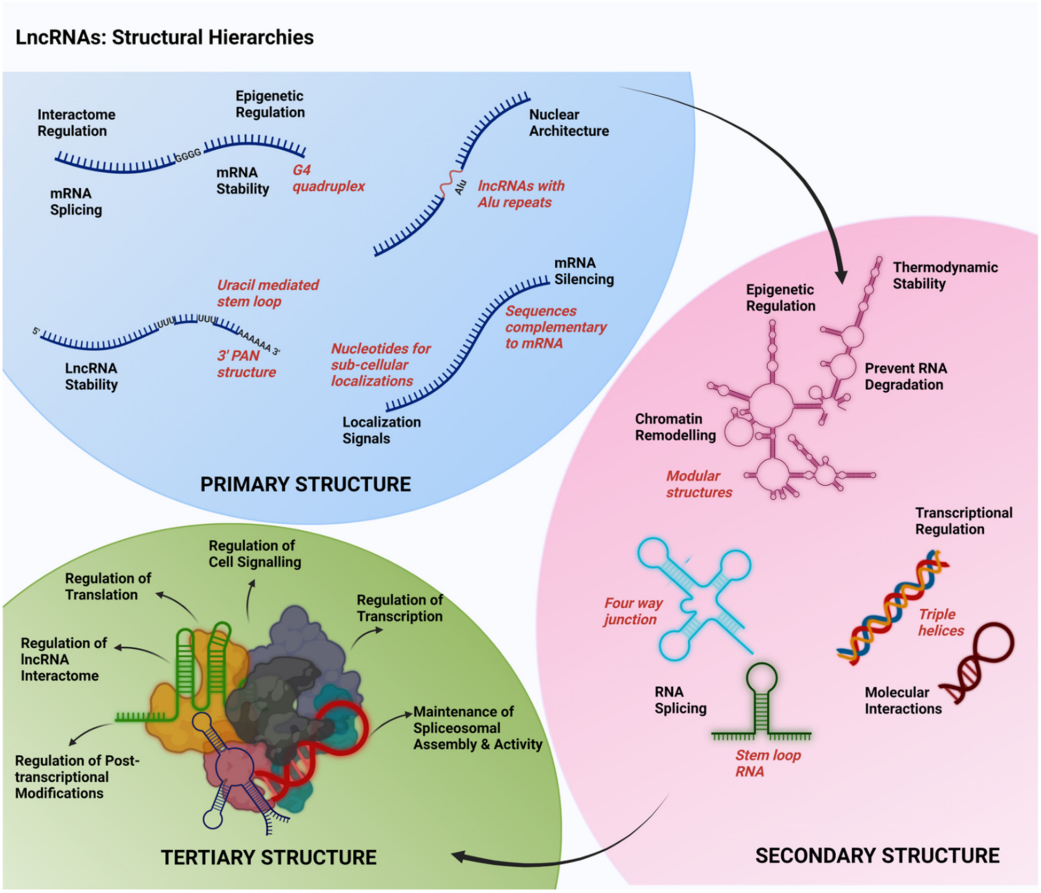

Figure 1. Structural hierarchies of lncRNA. The figure depicts the various levels of structural organizations of lncRNAs inclusive of their specialized modifications that provide them with their functional advantages. The primary structure of lncRNAs involve numerous specialized sequence modifications such as the presence of G4 quadruplexes, 3′ poly-A motifs, specific localization signals as well as presence of Alu retrotransposons that aid them in carrying out focussed functions. The secondary structure involves the modified primary sequences that attain improved structural hierarchies such as modules/domains, stem loops, triple helices, or even circular structures. Furthermore, the wide interactome of lncRNAs help them in bringing out broader levels of functions such as regulation of cell signaling, transcription, post-transcriptional modifications, and translation as well as maintenance of spliceosome assembly and activity.

Specific sequences such as the presence of Guanine-quadruplexes (G4) has also been reported to be associated with certain lncRNAs such as NEAT1, GSEC, and REG1CP [19]. The presence of G4 has been reported to affect the translation of mRNAs such as that of VEGF (vascular endothelial growth factor) and of TGFβ2 (transforming growth factor β2). This could be either through the modulation of the splicing or stability of mRNAs [19]. G4 also contributes to the association with polycomb repressive complexes (PRCs) and subsequent chromatin modifications (as shown by the lncRNA HOTAIR) through in vitro transcription and oligomer based experiments [20]. The lncRNA TERRA, which possess G-rich telomeric repeat sequences, has been identified with the G4 [21]. The presence of G4

enables TERRA to pair with Cytosine-residues in the DNA and form RNA-DNA duplexes resulting in R-loop structures. These R-loop structures help in the regulation of the telomere lengths in the differentiated cells [22].

Several other primary structure specific sequences can be attributed to the RNA- and DNA- interacting functions of lncRNAs. Primary sequences of the lncRNAs complementary to the miRNAs are attributed to the competitive endogenous RNA (ceRNA) function of lncRNAs that help in sequestration and inhibition of miRNAs [23]. There are nuclear localization sequences in lncRNAs such as PVT1, MALAT1, and BORG that facilitate their nuclear localization and associated functions [24]. Certain lncRNAs (e.g., MALAT1) possess sequences complementarity to the recognition motifs in U1 snRNPs (small nuclear ribonucleoproteins), which aid their binding and recruitment to the chromatin regions to facilitate pre-mRNA splicing [25]. Thus, the primary sequence of the lncRNAs provides clues to their predicted secondary structural modifications as well as sequence-dependent interactions with other RNAs and proteins.

2.2. Secondary Structure

Since lncRNAs exhibit a low sequence conservation across various species, the specificity of their interactome and functional diversity are rather dependent on their secondary and tertiary structures [26]. LncRNAs possess numerous secondary structural modifications that enhance their thermodynamic stability. These include bulges, junctions, hairpin loops, stem loops, internal loops, helices, subdomains, and pseudoknots, most of which involves the non-Watson Crick base pairing [27]. In some cases, the formation of secondary structure involves ribose backbone interactions [27–29]. These secondary structures determine their functional interactions with proteins, DNAs, and other RNAs [30]. Numerous studies have shown that several lncRNAs have modular structures that are comprised of ten to a hundred nucleotides in length. LncRNAs such as SRA, MEG3, and HOTAIR possess such modules in their secondary structure. These modules are not just independent folding units, but serve as independent functional units as well. For instance, HOTAIR possess four modules, namely D1-D4, wherein the 5′ D1 module interacts with PRC2, while the 3′ D4 module interacts with lysine-specific demethylase 1 (LSD1) to carry out their chromatin associated epigenetic regulatory functions [30,31]. HOTAIR, which has four independent folding modules/domains, possesses 56 helical regions, 19 junctions, 34 internal loops, and 38 terminal loops [30]. The multi-way junctions that are formed at the junctures of the stem loop structures are also significant as they define numerous tertiary structures as well as interactions of the lncRNAs. For instance, in human MEG3, the highly conserved H10 and H11 stems are oriented by the three-way junction J3 in their D2 module [31]. These junctions confer rigidity to the lncRNA secondary structures. The internal loops modulate the flexibility of the helical stems, which would in turn enhance numerous intramolecular as well as intermolecular interactions of the lncRNAs. The asymmetric right hand turns (RHTs) are examples of recurrent internal loops which possess specific 3′ and 5′ single stranded nucleotide stretches that interacts non-canonically to form the functional units. In U6 snRNP, such RHTs serve as the receptor for pre-messenger RNA splicing protein [32].

One of the prominent secondary structures of lncRNAs is the triple helix structure. It is a clover-leaf or four-way junction structure located at the 3′end, which protects the lncRNAs from degradation. During normal instances, post transcriptional processing of lncRNA involves the cleavage and polyadenylation specificity factor 73 (CPSF73)-mediated endonucleolytic cleavage and subsequent polyadenylation, which is essential for the RNA stability. However, a triple helix is formed when the cleavage is induced by Ribonuclease P, instead of CPSF73, thereby removing a conserved tRNA structure from the 3′ end. Further processing is carried out by Ribonuclease Z that generates a short adenine rich strand, resulting in a UAA triple helix at the 3′ end. LncRNAs such as MALAT1 and NEAT1 exhibit a similar triple helical structure at their 3′ end, which enhances their thermodynamic stability [16,17,33–35]. Another secondary structure is the double stem loop structure, which is mostly associated with the lncRNAs involved in chromatin remodeling. Such

lncRNAs act in *trans* by interacting with the chromatin modifying enzymes. GAS5 and HOTAIR are examples of lncRNAs that possess a double stem loop structure to carry out chromatin remodeling [36–38]. For instance, GAS5 possesses A-form double helical structure that interacts with DNA binding domain of steroid receptors in a sequence specific manner to repress the steroid-mediated transcriptional mechanisms [39].

It should be noted here that the predicted secondary structures of many of the lncRNAs are based on in vito studies. They are derived from structural modeling using computational methods or biophysical, chemical, and enzymatic probing using purified lncRNAs [15,40]. The limitations of these analyses are that they do not take into account the endogenous interacting partners of lncRNAs that nucleate and/or stabilize the secondary structures of the lncRNAs in vivo. However, the complementary approaches using structural modeling and enzymatinc foot printing have to a great extent provided clues to the functional role of secondary structures [40].

2.3. Tertiary Structure

In addition to the secondary structure, the tertiary structure also attributes to their diverse interactome, which enables them to carry out their varied and dynamic functions. Major structural features that contribute to the tertiary structures of lncRNAs include T-loops, kink and hook turns, tetraloop receptor interactions, ribose zippers, minor groove triples and A-minor interactions, pseudoknots and kissing loops, G-quadruplexes, and/or triple helical modifications [41]. Though recent findings from several laboratories are unfolding the diverse tertiary structures in lncRNAs and their association with various functions, the current knowledge on these is limited. One of the lncRNAs whose 3-dimensional structure has been well explored is MEG3. A conserved hairpin H11 in D2 module/domain and the region H25-H27 within the D3 module/domain are the prominent RNA motifs necessary for p53 stimulation. Further, six of the repeated sequences in H27 are complementary to the sequences in the terminal loop H11 and they form pseudoknot structures by base pairing, which is indispensable for its compact conformation that facilitates its functional significance in p53 signaling pathway [31,41]. Thus, the tertiary structures mainly attribute to the interactome of the lncRNAs, which further determines their cellular functional roles.

3. LncRNAs: Functional Mechanisms

With these abundant structural modifications, lncRNAs exhibit diverse mechanisms of action in regulating diverse cellular responses. Known mechanisms of action for lncRNAs at the molecular levels, broadly based on their sub-cellular localization, are discussed below.

3.1. Nuclear Functions

Within the nucleus, lncRNAs carry out functional roles such as modulation of nuclear architecture, epigenetic regulation of gene expression, regulation of transcription as well as mRNA splicing, dosage compensation, and genomic imprinting (Figure 2).

3.1.1. Nuclear Architecture

LncRNAs located in the nucleus contribute to the maintenance of nuclear architecture [42]. They have been shown to be involved in the regulation of nucleosome positioning, chromosome positioning, and chromatin looping [42]. Such spatial regulation by lncRNAs contributes to the modulation of gene expression through transcriptional and post-transcriptional modifications of mRNAs in the nucleus that can impact their translation in the cytoplasm [42]. LncRNAs also play a major role in nuclear speckle formation [43]. Nuclear speckles are dynamic granular structures that are present in the inter-chromatin region and play a dominant role in the transcription of mRNA, especially in their splicing [43]. Functionally, nuclear speckles consist of splicing machinery inclusive of the spliceosomes, the serine/arginine rich (SR) factors, and snRNPs that translocate to the active regions of transcription to facilitate the mRNA splicing [43]. LncRNA MALAT1 is reported to associate with the nuclear speckle structures, acting as a scaffold to recruit the nuclear speckles to

the active transcription sites for facilitating the mRNA splicing [44]. Nuclear paraspeckles are another sub-nuclear structure composed of RNA-protein structures present in the inter-chromatin region. They facilitate the nuclear retention of those specific mRNAs that have undergone either adenosine to inosine (A to I) editing or have several inverted repeats to form double-stranded RNA structures [45]. LncRNA NEAT1 is one of the key components of the nuclear paraspeckles. It interacts with proteins such as proline and glutamine rich splicing factor (PSF/SFPQ) and non-POU domain containing octamer binding protein ($p54^{nrb}$/NONO) as well as paraspeckle component 1 (PSPC1/PSP1) protein to orchestrate the formation of nuclear paraspeckles. NEAT1 also helps in the positioning of the nuclear paraspeckles to the active transcription sites. This aids in the nuclear retention of double-stranded mRNAs that are formed out of the inverted repeats. Such retentions are reported to be associated with regulations in circadian rhythm, stress responses, viral infections, and pluripotency [46].

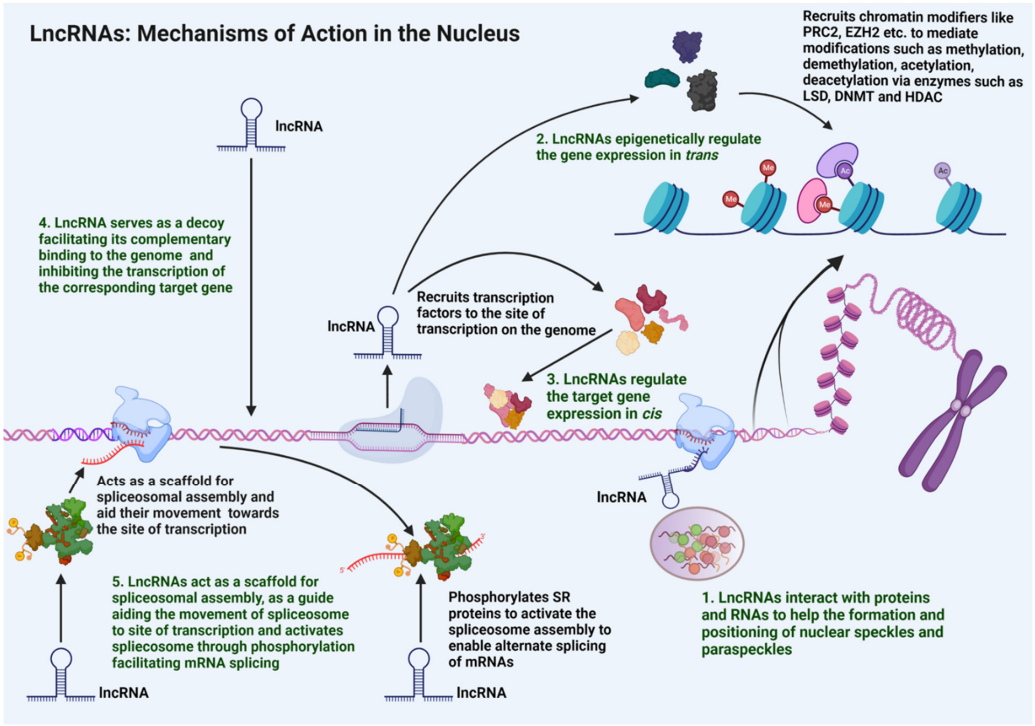

Figure 2. Mechanisms of nuclear lncRNA activity. The figure describes modes of action through which nuclear lncRNAs carry out their functions. (1) LncRNAs interact with several proteins and RNAs to aid the maintenance of nuclear architecture; (2) LncRNAs epigenetically regulate expressions of several genes by acting in *trans*; (3) LncRNAs regulate the gene expression by recruiting various transcription factors to the genetic locus; (4) LncRNAs also inhibit the gene expression by serving as decoy, through binding to the respective genetic loci directly; (5) LncRNAs serve as a scaffold for spliceosomal assembly as well as aid its movement across genetic loci to facilitate the mRNA splicing.

LncRNAs also take part in chromosome positioning. LncRNA FIRRE is located on both the X-chromosome and is transcribed even from the inactivated X-chromosomes, escaping the X-chromosome inactivation. FIRRE localizes the inactivated X-chromosome near the nucleolus, thereby helping the chromosome positioning [47]. LncRNAs aid chromatin looping to modify the gene expression. The lncRNA LUNAR1 is associated with the Notch occupied insulin-like growth factor 1 receptor (IGF1R)-enhancer regions as well as its own promoter through chromatin looping. This recruits the mediator molecules to the IGF1R target promoter to activate its expression [48,49].

3.1.2. Dosage Compensation and Genomic Imprinting

LncRNAs also contribute to the dosage compensation as well as genomic imprinting in the cells. Dosage compensation is the process of inactivating one of the X chromosomes by silencing most of its gene expression in the female germline (XX) to compensate with the male germline (XY). One of the few genes that is transcribed and which is involved in dosage compensation is the lncRNA XIST. XIST is transcribed from the X-chromosome that undergoes inactivation. With the adenine-rich repeat domains, XIST transcript interacts with other proteins to promote the silencing of other genes on the X-chromosome, to inactivate them and result in dosage compensation. XIST can bind to PRC2/PRC1 complex to recruit DNA methyl transferases (DNMTs) and methylate the gene promoters to inactivate the expression of those genes. XIST can also bind to SMRT/HDAC1 associated repressor protein (SHARP), split end protein (SPEN), and silencing mediator for retinoid or thyroid hormone receptor (SMRT) protein complexes to recruit histone deacetylases (HDACs) to the genes, so as to suppress their expression [50–52].

Genomic imprinting labels gene expression to parental origin with modifications. LncRNA H19 is paternally imprinted and expressed from the maternal allele [53]. In addition, H19 takes part in imprinting of other genes by recruiting and interacting with methyl-CpG-binding domain protein 1 (MBD1). The H19-MBD1 complex recruits histone lysine methyl transferases to generate histone H3 lysine 27 trimethylation (H3K9me3) chromatin repression marks in their target gene loci such as those of insulin-like growth factor-2 (IGF2), solute carrier family 38 member 4 (SLC38A4), and paternally expressed gene 1 protein (PEG1), thereby suppressing the expression of either their paternal or maternal allelic genomic regions [53,54].

3.1.3. Epigenetic Regulation

Nuclear lncRNAs play a critical role in chromatin remodeling through which the accessibility of chromatin regions to various components of transcriptional machinery and the resultant expression of specific gene loci are stringently regulated. LncRNAs can act as histone modifiers by modulating the activity of methylating-, demethylating-, or even acetylating-enzymes [55]. LncRNA HOTAIR inhibits the gene expression at homeobox (HOX) D cluster loci via PRC2 interaction, which is an example of chromatin repression [36]. HOTAIR interacts with PRC2, which facilitates H3K27me3-mediated repressive histone methylation through DNMTs. In breast cancer, HOTAIR is overexpressed and interacts with PRC2 to induce the repressive histone methylation, which suppresses the expressions of several tumor-suppressor genes, thereby resulting in tumor invasion and metastasis [37,56,57]. HOTAIR can mediate chromatin remodeling in PRC2 independent manner as well [58]. In human embryonic kidney cells as well as the kidney epithelial and mesenchymal cells, HOTAIR interacts with LSD1 to displace it from the promoters and enhancers of its target genes, thereby switching the normal epithelial phenotype to the mesenchymal one [59]. Several other lncRNAs also take part in regulating the methylome of the cancer cells, such as DACOR. In normal cells, the tumor suppressor lncRNA DACOR interacts with and activates DNMTs, resulting in repressive methylation of numerous oncogenes. This hypermethylation of the oncogene promoters reduces their expression, thus preventing any tumor initiation. Downregulation of DACOR, as seen in colon cancer, leads to the reduction of DNMT activity and subsequent hypomethylation of oncogene promoters that leads to the overexpression of oncogenes and tumor progression [56,57].

3.1.4. Regulation of Transcription

LncRNAs regulate the transcription of various genes, by acting either as *cis*- or *trans*-elements. In the *cis*-mode, lncRNAs regulate the expression of genes in their respective chromosomal neighborhood. At least three different cis-regulatory mechanisms through which lncRNAs could modulate gene expression have been identified [60]. The first mechanism is where the lncRNA transcript directly mediates the recruitment of specific transcription factors so as to modulate gene regulation. For instance, during X-chromosome

inactivation, XIST recruits specific gene silencing factors through its adenine repeat-rich domain to silence the genes present on the X chromosome [51]. The second mechanism is where the lncRNA directly modulates the expression of the neighboring gene. For example, the *IGF2R* (insulin like growth factor 2 receptor) gene, which is paternally imprinted, is associated with its anti-sense lncRNA, AIRN [61]. AIRN sequence spans part of the *IGF2R* gene as well as its promoter region. Thus, the binding of Airn transcript to *IGF2R* genomic locus silences the *IGF2R* expression [61]. LncRNA PANDA is antisense to the cyclin-dependent kinase inhibitor 1, p21 (*CDK1N1A*) gene that encoded cyclin-dependent kinase inhibitor, p21. Binding of PANDA to *CDKN1A* suppresses the p21 expression to promote cell proliferation [62]. The third mechanism is where the DNA sequence within the lncRNA loci can activate or repress the expression of genes in their vicinity. The lincRNA-p21, a TP53 induced lncRNA, is expressed in response to DNA damage. The lincRNA-p21 locus, whether it is being transcribed or not, activates the expression of the *CDKN1A* gene since the promoter regions of *CDK1N1A* lie within the lincRNA-p21 locus [63]. The enhancer lncRNAs (or e-lncRNAs), which arise from sequences within the enhancer regions of the neighboring genes, also take part in modulating the expression of genes in their vicinity. LncRNA PAUPAR represses the expression of the Paired Box gene 6 (*PAX6*) gene by acting in *cis*, since the PAUPAR locus contains certain enhancer elements for *PAX6*. Further, the PAUPAR-PAX6 level modulates several other enhancers, repressors, and promoter elements in executing transcriptional regulation of genes, so as to balance the cell cycle and modulate neural differentiation [64].

Several lncRNAs exhibit trans-regulatory mechanisms in controlling the expression of genes present in distal locations on the chromosomes. A classic example is provided by HOTAIR, which is transcribed from the *HOXC* locus. HOTAIR interacts with PRC2 and the lysine demethylase LSD1 to repress the gene expression at the distant HOXD locus, contributing to an epithelial–mesenchymal transition (EMT) in cancer cells [65,66]. A similar trans-regulatory mechanism of gene expression has been seen with lncRNAs such as MALAT1 and TUG1 [67].

3.2. Cytosolic Functions

The cytoplasmic lncRNAs play a major role in the regulation of both the transcriptional and translational machineries. Functions regulated by lncRNAs include the regulation of mRNA stability, translational regulations, sequestration of miRNAs, and functional modulation of proteins through specific interactomes (Figure 3).

3.2.1. Regulation of mRNA Stability and Translation

For the maintenance of upregulated levels of cellular proteins, mRNA stability is a significant factor. LncRNAs play a major role in regulating the mRNA stability. It has been reported that the interactions of lncRNAs with numerous mRNAs either enhances their stability or promote their degradation [68]. In staufen, double-stranded RNA binding protein 1 (STAU1)-mediated mRNA decay, the STAU1 binds to its target mRNAs in its double-stranded form to initiate the degradation signals. The TINCR lncRNA binds to the STAU1 target mRNAs through the base pairing of the Alu repeats in both these RNAs, thereby forming a double-stranded RNA structure that in turn is degraded by the STAU1-mediated decay mechanism [69,70]. In detail, the TINCR binds with kruppel-like factor 2 (KLF2) mRNA, which stimulates the STAU1-mediated decay of KLF2 mRNA. This would adversely affect the expression of KLF2-regulated genes such as *CDKN1A* and *CDKN2B* that encode cyclin-dependent kinase inhibitors p21 and p15, respectively, leading to augmented tumorigenesis in gastric cancers [71,72]. LncRNAs also modulate the non-sense-mediated degradation (NMD) of mRNAs. Up-frameshift suppressor 1 homolog (UPF1) protein is a critical component of NMD pathway. LncRNAs SNHG6 and SNAI3-AS1 bind to UPF1 and recruit it to the mRNA that codes for the protein known as small mothers against decapentaplegic homolog 7 (SMAD7) and enables the degradation of SMAD7 mRNA in

hepatocellular carcinoma via the NMD process. Since SMAD7 is a negative regulator of the TGF/SMAD pathway, this degradation promotes TGF-mediated EMT in these cancers [73].

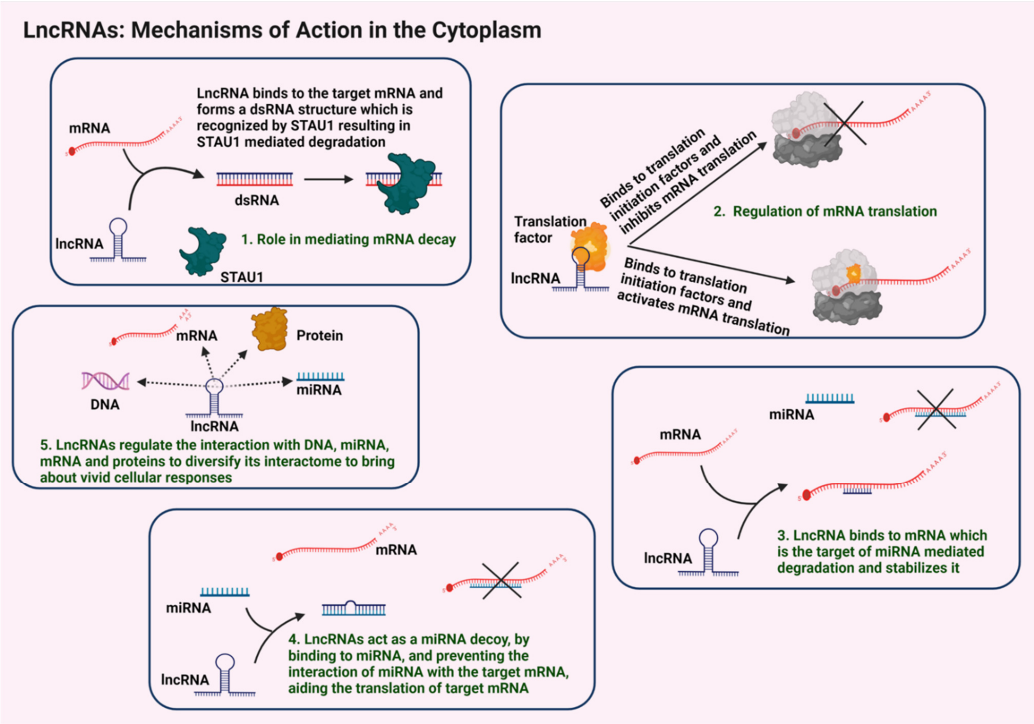

Figure 3. Mechanisms of cytoplasmic lncRNA activity. The figure describes the modes of action through which cytoplasmic lncRNAs carry out their functions. (1) LncRNAs contribute to different modes of mRNA degradation, thereby regulating the mRNA stability; (2) LncRNAs regulate mRNA translation by binding to the translation factors; (3) LncRNAs bind to mRNA and enhance their stability by preventing their miRNA-mediated inhibition; (4) LncRNAs bind to miRNAs and prevent their binding to the mRNAs; (5) LncRNAs also regulate the activity of their diverse interactomes that facilitate to carry out their functions within the cytoplasm.

LncRNAs also take part in regulation of translation within the cytoplasm by binding to several translation factors. LncRNA BC1 binds to the eukaryotic initiation factor 4A (eIF4A) and inhibits its helicase activity, thereby negatively regulating the mRNA translation [74]. LncRNA GAS5 interacts with translation initiation factor eIF4E and inhibits the translation of MYC gene that encodes c-Myc in lymphomas [75].

3.2.2. Sequestration of miRNAs

Many of the cytosolic lncRNAs act as ceRNAs to sequester miRNAs that bind to and inhibit the target mRNAs, which possess corresponding miRNA response elements (MRE). Such lncRNA-miRNA binding suppresses the inhibitory action of miRNA on their target mRNAs, subsequently resulting in the expression of those genes targeted by the respective miRNAs. The lncRNAs that exhibit such sponging or inhibitory effect on specific miRNAs are termed as ceRNAs [76]. For example, under conditions of oxidative stress, the lncRNAs H19 and HULC act as ceRNAs by binding to miRNA let7a/b and miR-372/373 respectively, thereby inhibiting their activities. These interactions indirectly activate the expression of inflammatory cytokine interleukin-6 (IL6) as well as chemokine receptor type 4 (CXCR-4) that are targeted by these miRNAs. These inflammatory signals augment tumorigenesis in cholangiocarcinoma [77,78].

3.2.3. Regulation of lncRNA-Specific Interactomes

Cytoplasmic lncRNA interactome includes numerous proteins. LncRNAs often play a role in regulating the activities of their interacting partners. For instance, pumilio RNA binding family member 1/2 (PUM1/2) proteins bind to specific mRNAs to trigger their degradation. However, the binding of the lncRNA NORAD to PUM1/2, reduces its availability to facilitate mRNA degradation [79]. Consistent with this functional role, PUM1/2 activity is increased upon the knockdown of NORAD, leading to the modulation of numerous mitotic regulators with the resultant chromosomal instability and aneuploidy [79]. With these aforesaid mechanistic roles, aberrant signaling by lncRNAs is critically involved in the genesis and progression of various human diseases, as discussed below.

A cautionary note here is that the functional roles of the lncRNAs have been deduced from the ectopic overexpression of the lncRNA or deletion of their expression. Differential localization of the overexpressed lncRNAs and compensatory activities of other lncRNAs in deletion experiments could contribute to erroneous conclusions. Therefore, complementary approaches are often required to assign a function to a specific lncRNA.

4. LncRNAs in Human Diseases

Recent findings on the structural and functional diversities of lncRNAs and their cognate interactomes have unraveled their potential role in the pathogenesis of many different human diseases. In addition to the structural and functional diversities that have been discussed in this review, we explore the divergent roles of lncRNAs in the induction and progression of major human diseases such as cardiovascular diseases, neurological disorders, cancer, and metabolic diseases such as diabetes. Major lncRNAs identified to play a role in these diseases are listed in Table 1.

4.1. LncRNAs in Cardiovascular Diseases

Cardiovascular diseases (CVD) are the leading cause of mortality worldwide. The major stress responses result in either the autophagy, apoptosis, necrosis, or the hypertrophy of cardiomyocytes, leading to CVD. Several lncRNAs are expressed during diverse stages of development, differentiation, and maturation as well as pathogenesis of the cardiomyocytes (Figure 4).

Table 1. Major LncRNAs in disease pathology.

Disease	Upregulated LncRNAs	Downregulated LncRNAs
Cardiovascular Diseases	APF [80] BANCR [81] CHAER [82] CHAST [83] CHRF [84] CHROME [85] Giver [86] HOTTIP [87] LINC00968 [88] lnc-Ang362 [89] lnc-AK098656 [90] MIAT [91] MIRT1 [92] KCNQ1OT1 [93] UCA1 [94]	FTX [95] GAS5 [96] HOTAIR [97] lincRNA-p21 [98,99] MHRT [100] NEXN-AS1 [101] SENCR [102]

Table 1. *Cont.*

Disease	Upregulated LncRNAs	Downregulated LncRNAs
Neurological Diseases	BACE1-AS [103,104] C9ORF72-AS [105] EBF3-AS [106] GAS5 [107] LINC00507 [108] lincRNA-p21 [109] MALAT1 [110] MEG3 [104] MSNP1-AS [111] NEAT1 [104,112] SHANK2-AS [113] SNHG1 [114–116] SNHG14 [117] SOX21-AS1 [118] SYNGAP1-AS [119] TUG1 [104] XIST [104,120]	DISC1-AS [121] H19 [122] HAR1 [104] MALAT1 [123] MEG3 [124] MIAT/GOMAFU [125]
Cancer	ANRIL [126] BGL3 [127] CCAT1 [128] CCAT2 [129] CRNDE [130] DLEU2 [131] HULC [132] HOTAIR [133,134] linc-NMR [135] LINC00337 [136] LINC00963 [137] lnc-Hh [138] LNMAT1 [139] MALAT1 [140–143] MVIH [144] NEAT1 [145] POU3F3 [146] PVT1 [147,148] REG1CP [149] UCA1 [150–154]	GAS5 [155] LBCS [156] lincRNA-p21 [157,158]
Diabetes	ANRIL [159] ARAP1-AS2 [159] Bhmt-AS [160] CDKN2B-AS1 [159] HOTAIR [159,161] IGF2-AS [162] KCNQ1OT1 [163] LEGLTBC [164] LINC00968 [165] lnc-p3134 [166] lnc-Rpph1 [167] MALAT1 [168–172] MEG3 [173,174] MIAT [175] NONRATT021972 [176] NR-033515 [159] PVT1 [177] RNCR3 [178] uc.48+ [179,180]	ARAP1-AS1 [159] BANCR [159] CASC2 [159] GAS5 [181,182] H19 [183–188] HOTAIR [189] LUCAT1/SCAL1 [190] MEG3 [191,192] MIRT2 [193] SHGL [194] SNHG7 [195] SRA [159] TUG1 [196,197] ZEB1-AS1 [198]

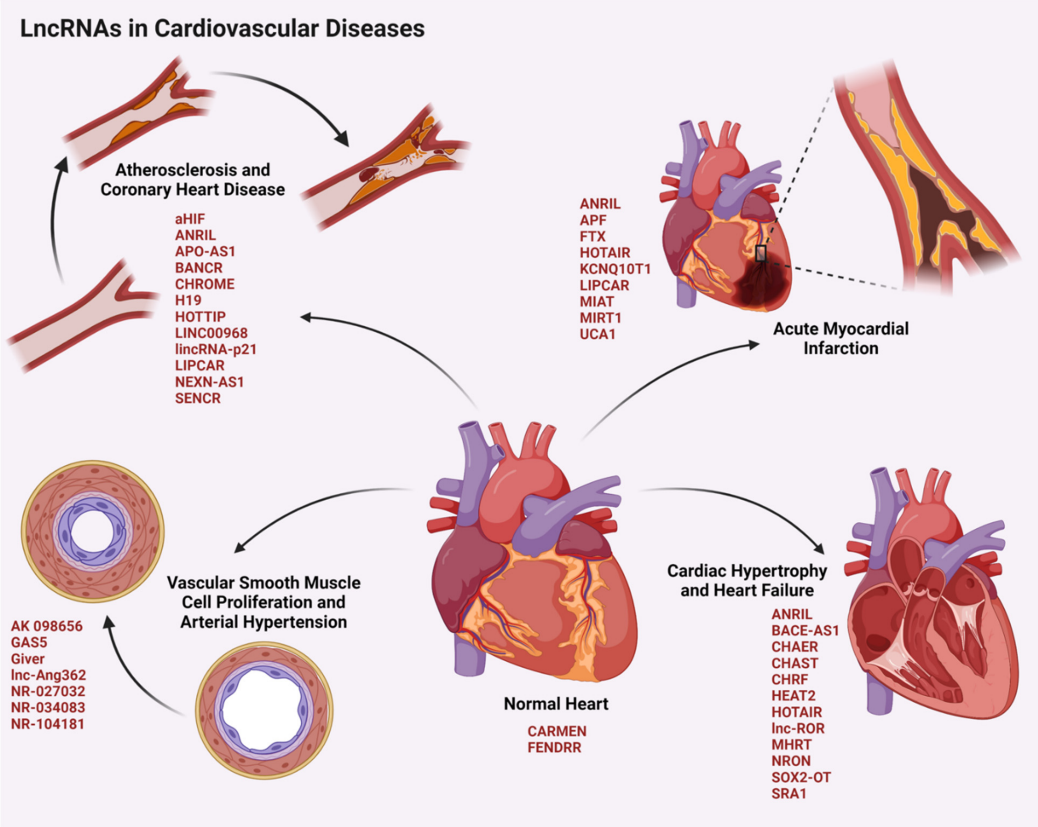

Figure 4. LncRNAs in cardiac physiology and pathology. The figure depicts the representative lncRNAs that aid in normal cardiac functioning as well as during cardiovascular complications such as arterial hypertension, atherosclerosis, coronary heart disease, acute myocardial infarction, cardiac hypertrophy, and heart failure.

LncRNAs expressed in the cardiac tissues mostly exhibit a cell/tissue-specific expression profile, which affects the cardiovascular development in the normal physiological conditions. For instance, lncRNA CARMEN regulates the cell fate, cellular differentiation, and homeostasis in human cardiac precursor cells [199]. Whereas the lncRNA FENDRR, which is expressed in the lateral mesoderm of the heart, is required for the heart wall development through its interactions with PRC2, trithorax group of proteins (TrxG), and mixed lineage leukemia protein (MLL) in modifying the chromatin state and gene expressions [200]. In addition to the normal developmental functions, lncRNAs also serve as a critical regulator of cardiovascular pathology, which includes conditions such as arterial hypertension, coronary heart disease, acute myocardial infarction, and heart failure [201].

4.1.1. LncRNAs in Arterial Hypertension

Arterial hypertension (AHT) remains one of the most common forms of CVDs in addition to forming the basal triggering signal for other classes of CVDs. It arises mainly due to defects in proliferation, differentiation, and migration of vascular smooth muscle cells (VSMCs), which are the critical contractile elements of the blood vessel wall. In vivo experiments in rats with angiotensin-II (Ang-II) treatment revealed a role for the lncRNA lnc-Ang362. Overexpression of lnc-Ang362 elevates the expression of miR-221/222 leading to the activation of Nuclear factor kappa B (NF-κB) signaling. Enhanced NF-κB signaling promotes proliferation and migration of VSMCs to aggravate AHT [89]. Ang-II induces senescence of endothelial progenitor cells (EPCs), which play an important role in the repair of vascular endothelial damage, in hypertensive patients. The lincRNA-p21 was shown to protect EPCs from Ang-II damage by activating the SESN2/AMPK/TSC2 pathway and transcriptional activity of p53 that eventually enhanced autophagy. Another significant

lncRNA that promotes AHT is Giver. In response to elevated Ang-II, the transcription factor known as nuclear receptor subfamily 4 group A member (NR4A3) is recruited to the promoter of Giver, which enhances the expression of Giver during AHT. Giver interacts with RNA Pol II and reduces the repressive histone H3K27me3 methylation of genes involved in oxidative stress such as *NOX1* that encodes NADPH Oxidase 1 as well as those involved in inflammation such as *IL6*, *CCL2*, and *TNF* (tumor necrosis factor), to promote their expression and thereby AHT [86]. LncRNAs that act as a scaffold to affect the protein stability and degradation also regulate AHT. For instance, lncRNA AK098656 interacts with 26S proteasomal components and facilitates the interaction of cytoskeletal VSMC specific contractile protein myosin heavy chain-11 with the proteasomal system to promote its degradation. This reduces the contractility of the vessels and enhancement of AHT [90]. Certain lncRNAs such as GAS5 are downregulated during AHT. Under normal conditions, GAS5 is expressed in endothelial cells (EC) and VSMCs. It regulates the vascular functions through β-catenin pathway. It acts as a ceRNA for miR-21 and elevates the expression of programmed cell death 4 (PDCD4) protein, a miR-21 target. PDCD4, in turn, attenuates AHT stimulation through the inhibition of platelet derived growth factor-bb (PDGF-bb)-induced VSMC proliferation and migration [96].

4.1.2. LncRNAs in Coronary Heart Diseases

Several lncRNAs have been identified to be associated with coronary heart diseases (CHDs). The TNFα-induced lncRNA BANCR is overexpressed in VSMCs during CHD. It activates the c-Jun N-terminal kinase (JNK) pathway and promotes the proliferation and migration of VSMCs, which lead to atherosclerosis and CHD [81]. A similar effect has been exhibited by the TNFα/PDGF-bb-induced lncRNA HOTTIP, which is upregulated in CHD. However, this lncRNA enhances the proliferation and migration of the ECs, which is mainly through the Wnt-β-catenin signaling cascade [87]. The upregulation of lncRNA LINC00968 in response to enhanced levels of oxidized LDL (low density lipoprotein) enhances the proliferation and migration of the ECs to aggravate CHD by sequestering miR-9-3p [88]. In addition to the proliferation and migratory signals, metabolic reprogramming is also regulated by lncRNAs that promotes CHD. For instance, lncRNA CHROME, which is upregulated in CHD, is involved in the regulation of cellular and systemic cholesterol homeostasis. It is overexpressed in response to the higher levels of dietary cholesterol uptake through the activity of LXRs (Liver-X receptors). CHROME has also been identified to interact with argonaute-2 (Ago-2) protein that is involved in the sequestration or decay of its miRNA targets [85]. CHROME sequesters miR-27b/33a/33b/128, thereby upregulating the expression of miR-27b/33a/33b/128-target genes that encode cholesterol efflux transporter proteins such as ATP-binding cassette subfamily A member 1, a member of ATP-binding cassette transporters. These transporters aid cholesterol efflux from macrophages. The extruded cholesterol accumulates either in the tissues or deposit within the arterial walls causing atherosclerosis and CHD. In addition, CHROME-mediated sequestration of miRNAs leads to the expression of genes involved in HDL (high density lipoproteins) synthesis, which plays a pathogenic role in promoting atherosclerosis and CHD.

In contrast to the upregulation of pathogenic lncRNAs, lncRNAs with anti-atherosclerotic effects are downregulated in CHD. The antisense lncRNA, NEXN-AS1 is downregulated in atherosclerosis and CHD. NEXN-AS1 directly interacts with chromatin remodeling protein known as bromodomain adjacent to zinc finger domain 1A (BAZ1A) and induces the expression of *NEXN* gene that encodes nexilin F-actin binding protein. NEXN inhibits the oligomerization of Toll-like receptor-4 and the down-stream NF-κB signaling, which further inhibits the expression of inflammatory cytokines as well as cell adhesion molecules by ECs. This suppresses the adhesion of monocytes to the ECs and prevents atherosclerosis and CHD [101]. Likewise, the lncRNA SENCR, which is involved in the maintenance of the vascular endothelial cell integrity, is downregulated in CHD. SENCR maintains vascular EC adheren junctions through its interaction with cytoskeleton-associated protein 4. Although the mechanism underlying the downregulation of SENCR is not known, decrease in the

expression of SENCR leads to defective EC differentiation and vascular permeability functions [102]. The downregulation of lincRNA-p21 has also been shown to be associated with atherosclerosis and CHD. LincRNA-p21, induced by p53, regulates the expression of several p53-downstream target genes, especially those involved in cell cycle arrest and apoptosis through its direct interaction with heterogeneous nuclear ribonucleoprotein K. LincRNA-p21 also interacts with mouse double minute 2 homolog protein, MDM2, to release its inhibitory effects on p53. Relieved p53 interacts with p300 and facilitates its recruitment to the promoter/enhancer sites of target genes in vascular smooth muscle cells [99]. Thus, the decreased expression of lincRNA-p21 in CHD results in enhanced cell proliferation and anti-apoptotic signaling along with the accelerated neointima formation in carotid arteries, all of which promotes atherosclerosis [99]. Some of the lncRNAs such as H19, ANRIL, and lincRNA-p21 exhibit genetic polymorphisms that enhance the risk of CHD in the patients [201].

4.1.3. LncRNAs in Acute Myocardial Infarction

Acute myocardial infarction (AMI) results from the acute obstruction in the coronary artery due to atherosclerosis. The decreased blood flow due to atherosclerotic plaques in the coronary artery leads to reduced blood supply and oxygen to the myocardial tissues. This leads to myocardial ischemia as well as myocardial necrosis, and subsequently, an AMI or a heart attack. The pathological complications in patients are not limited to AMI incidence, but continues with the post-AMI difficulties such as cardiac fibrosis, ventricular remodeling, inflammatory responses, and ischemic–reperfusion injuries (IR injury) as well as apoptosis and autophagy of cardiomyocytes. Several lncRNAs are upregulated during the conditions of AMI that governs the complications post-AMI in patients. LncRNA MIAT is upregulated under conditions of AMI. It sequesters miR-24 and induces the expression of pro-fibrotic genes such as Furin and TGF-β1, which promotes proliferation of cardiac fibroblasts, accumulation of collagen and cardiac interstitial fibrosis. Thus, MIAT acts as a pro-fibrotic lncRNA, worsening the cardiac functions by promoting cardiac fibrosis during the post-AMI conditions [91]. Inflammation and apoptosis of the cardiomyocytes are the two worst cellular events that worsen the cardiac functions post-AMI and determine the fate of the heart. LncRNA MIRT1, which is upregulated in cardiac fibroblasts during AMI, has been reported to regulate the apoptosis of cardiomyocytes and myocardial inflammation. Silencing of MIRT1 in cardiac fibroblasts has been shown to inhibit the pro-apoptotic factors such as Bax, Bcl2, and caspases in cardiomyocytes. In vitro and in vivo studies have shown that knocking down MIRT1 can inhibit the macrophage infiltration into the myocardium thus attenuating the myocardial inflammation post-AMI. In addition, MIRT1 also promotes the NF-κB pathway that promotes the infiltration of inflammatory cells into the infarcted myocardium as well as peritoneal macrophage migration that results in worsening the post-AMI effects [92]. One of the post-AMI complications is the risk for ischemia–reperfusion (IR) injuries, in which the reperfusion of blood to the ischemic regions could cause tissue damages that can even lead to cardiac as well as multi-organ failures. IR injury is associated with enhanced oxidative and mitochondrial as well as endoplasmic stress that result in apoptosis of cardiomyocytes. LncRNA UCA1 overexpression has been reported to protect these cells from IR injury related endoplasmic reticulum stress and inhibit apoptosis in cardiomyocytes [94]. Furthermore, lncRNA APF (autophagy promoting factor), which is upregulated post-AMI, sequesters miR-188-3p and upregulates the expression of autophagy related 7 (ATG7) protein. ATG7 is critical for the autophagy of cardiomyocytes, thereby aggravating the injury after AMI [80]. In the same line, the lncRNA KCNQ1OT1 (KCNQ1 overlapping transcript 1), which is upregulated in MI patients and IR mouse models, was shown to promote autophagy-associated apoptosis of cardiomyocytes by sponging miR-26a-5p, which targets ATG12 [93].

Some of the lncRNAs such as HOTAIR and FTX are downregulated in post-AMI patients. HOTAIR sequesters miR-1 and regulates the expression of pro-apoptotic genes encoding B-cell lymphoma 2 apoptosis regulator, BCl2-associated X apoptosis regulator,

and caspases to prevent apoptosis of cardiomyocytes [97]. FTX is also involved in cardioprotection through the inhibition of apoptosis in cardiomyocytes through the sequestration of miR-29b-1-5p to upregulate the expression of Bcl2-like 2 protein [95]. Thus, the downregulation of HOTAIR and FTX following AMI appears to accentuate myocardial injury.

4.1.4. LncRNAs in Heart Failure

The terminal form of CVD is heart failure (HF), where the heart loses its ability to carry out its function of pumping blood across the human body. It arises as a result of the cumulative effects of other CVD attributes including the loss of ability of the heart to either contract or relax. In cardiomyocytes, elevated levels of lncRNA CHRF in response to Ang-II sequesters miR-489 and upregulates the expression of myeloid differentiation primary response 88 protein, encoded by *MYD88* gene. The latter induces cardiac hypertrophy and apoptosis in cardiomyocytes, leading to HF [84]. The lncRNA CHAER is also upregulated during HF. It interacts directly with PRC2, and prevents H3K27me3-repressive tri-methylation on target genes that promote cardiac hypertrophy such as those encoding MYH7, atrial natriuretic factor, and skeletal alpha actin, thereby augmenting mTOR signaling for HF [82]. Another significant lncRNA affecting cardiac hypertrophy and HF is CHAST, which is upregulated in cardiomyocytes upon receiving aortic constriction and HF signals. This negatively regulates the expression of the *PLEKHM1* gene that encodes pleckstrin homology domain-containing family M member 1 protein, which modulates autophagy and endocytic trafficking. Though the exact detailed mechanism is yet to be elucidated, the in vivo studies have shown that CHAST could promote cardiac remodeling and hypertrophy leading to HF [83]. Along with the increased expression of HF-promoting lncRNAs, a drastic downregulation was seen with MHRT, a cardioprotective lncRNA. MHRT is critically involved in the structural and functional homeostasis of the heart. Under normal physiological conditions, MHRT binds to brahma related gene 1 (BRG1) and inhibits its helicase activity. MHRT-BRG1 interaction prevents the action of BRG1/HDAC/poly ADP ribose polymerase (PARP) complex on chromatin modification of its target genes that alter the cardiac contractility, thus keeping the cardiac function intact. However, under conditions of extreme CHD and AMI leading to HF, MHRT is downregulated through the epigenetic repression by BRG1/HDAC/PARP complex on *MHRT* locus. Resultant dysregulation of the genes that were stringently regulated by MHRT along with BRG1-mediated cardiac remodeling leads to the loss of cardiac contractility and HF [100].

In addition to these causative lncRNAs that are being interrogated as therapeutic targets in CVDs, certain lncRNAs are being investigated as circulatory biomarkers for the different CVDs. First of its kind was the analysis of lncRNA LIPCAR levels in plasma, which was correlated with aberrant left ventricular remodeling post-AMI and enhanced risk of HF post-AMI [202,203]. In addition, several lncRNAs such as NRON, MHRT, ANRIL, BACE-AS1, HEAT-2, HOTAIR, linc-ROR, SOX-2-OT, and SRA1 have been investigated for their role in being biomarkers for HF [201]. The major circulatory lncRNA biomarker candidates for AHT includes GAS5, NR-027032, NR-034083, and NR-104181, while for CHD, it includes aHIF, APO-AS1, and LIPCAR [201]. Furthermore, ANRIL, KCNQ1OT1, ANRIL, and MIAT have been studied as lncRNA biomarkers in circulation for AMI [201]. Though the precise functional roles of these lncRNAs in cardiac functions are yet to be determined, further explorations on their mechanistic role in disease progression as well as their roles as biomarkers would undoubtedly bring them into clinical settings in the near future.

4.2. LncRNAs in Neurological Disorders

The central nervous system possesses the largest number of lncRNAs, which constitutes almost 40% of the total lncRNAs present in the human body [26]. They show greater conservation in the brain, along with the highest degree of spatial and temporal specificities than any other locations in the human body [104]. Under normal physiological conditions, lncRNAs regulate the development and differentiation of the neuronal cells and

the nervous system. For instance, lncRNA DALI augments the expression of the *POU3F3* gene encoding the transcription factor POU class 3 homeobox 3 and acts as a cis-acting lncRNA. In addition, it forms a complex with POU3F3 to promote the activation of multiple genes to aid in neural differentiation as a trans-acting lncRNA [204]. On the contrary, BDNF-AS, an anti-sense lncRNA, binds to PRC2 and recruits the complex to the *BDNF* (brain-derived neurotrophic factor) gene locus to inhibit its expression. This adversely affects the BDNF-mediated axonal growth and differentiation [205]. Not just with the normal physiological conditions, lncRNAs execute various pathological roles as well, resulting in several neurodegenerative disorders [104,121]. These include diseases such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and Huntington's disease, as well as neurodevelopmental/neuropsychiatric disorders such as autism spectrum disorder, and schizophrenia (Figure 5).

4.2.1. LncRNAs in Alzheimer's Disease

Alzheimer's disease (AD) contributes to almost 50% of the dementia cases worldwide [206,207]. It is characterized by the deposition of β-amyloid plaques (Aβ-plaques) and the hyper-phosphorylation of Tau proteins that leads to neurofibrillary tangles, leading to neuronal inflammation as well as apoptosis and abrupt neuronal loss in the brain [104]. Aβ-plaques are formed from the amyloid precursor protein (APP), primarily by the action of beta site amyloid precursor protein cleaving enzyme 1 (BACE1). The antisense lncRNA BACE1-AS, which is highly upregulated in AD, binds to BACE1 and stabilizes it, to enhance the Aβ-plaque production [103]. LncRNA XIST, which is also highly expressed in AD, promotes Aβ-plaque formation. XIST sequesters miR-124 that targets BACE1, thus enhancing the expression levels of BACE1 and resultant Aβ-plaque formation [120]. Hyper-phosphorylation of Tau proteins accelerates the pathogenesis of AD as the Tau aggregation results in neurotoxicity and resultant loss of neuronal function, in addition to its role in promotion of Aβ plaque formation. LINC00507, which is upregulated in AD, promotes hyper-phosphorylation of Tau proteins by activating glycogen synthase kinase 3 beta (GSK3β) signaling cascade. It sequesters miR-181c-5p to enhance the expression of microtubule associated protein tau (MAPT), the gene encoding Tau protein, as well as tau tubulin kinase 1 (TTBK1) that phosphorylates Tau [108]. In addition, lncRNA SOX21-AS1 has also been reported to be upregulated in conditions of Aβ 1–42 induced AD pathogenesis. Though the exact downstream targets are not yet elucidated, it sequesters miR-107 to enhance Tau phosphorylation as well as promotes the neuronal apoptosis [118]. Interestingly, lncRNA SOX21-AS1 is currently investigated as a biomarker for AD [118].

Neuronal apoptosis is one of the prominent downstream effects of Tau phosphorylation and Aβ plaque deposition that worsens the AD pathogenesis. LncRNA SNHG1 is upregulated in AD and is induced by Aβ 25–35 deposition. It sequesters miR-137 and upregulates the expression of kringle containing transmembrane protein 1, which induces neuronal apoptosis that aggravates the AD pathogenesis [114]. Further, neuronal apoptosis is also enhanced by upregulated levels of lnc-EBF3-AS, which enhances the expression of its downstream target early B-cell transcription factor 3 (EBF3). EBF3 induces apoptosis of neurons in AD through upregulation of caspase activity and Bax proteins, while downregulating the BCL2 level [106]. Certain lncRNAs such as MALAT1 are downregulated in AD as well [123]. MALAT1 sequesters inflammatory miRNAs such as miR-125b and miR-155. Further, it enhances the expression of interleukin (IL)10 while downregulating the expressions of IL1β and TNFα. Furthermore, it alters the Janus kinase-signal transducer and activator of transcription (STAT), NF-κB, JNK, and p38 mitogen activated protein kinase pathways to modulate neuro-inflammation associated with AD [123]. In addition to MALAT1, lncRNA MEG3 is also downregulated in AD. Overexpression of MEG3 has been shown to reduce Aβ 25–35 deposition and the oxidative stress along with reduction of inflammatory signals through downregulation of IL1β, IL6, and TNFα as well as inhibition of Phosphoinositide 3-kinases (PI3K)/protein kinase B (Akt) signaling cascades in the

hippocampus tissues of AD rat models [124]. Thus, the downregulation of MEG3 can be correlated with neuro-inflammation associated AD.

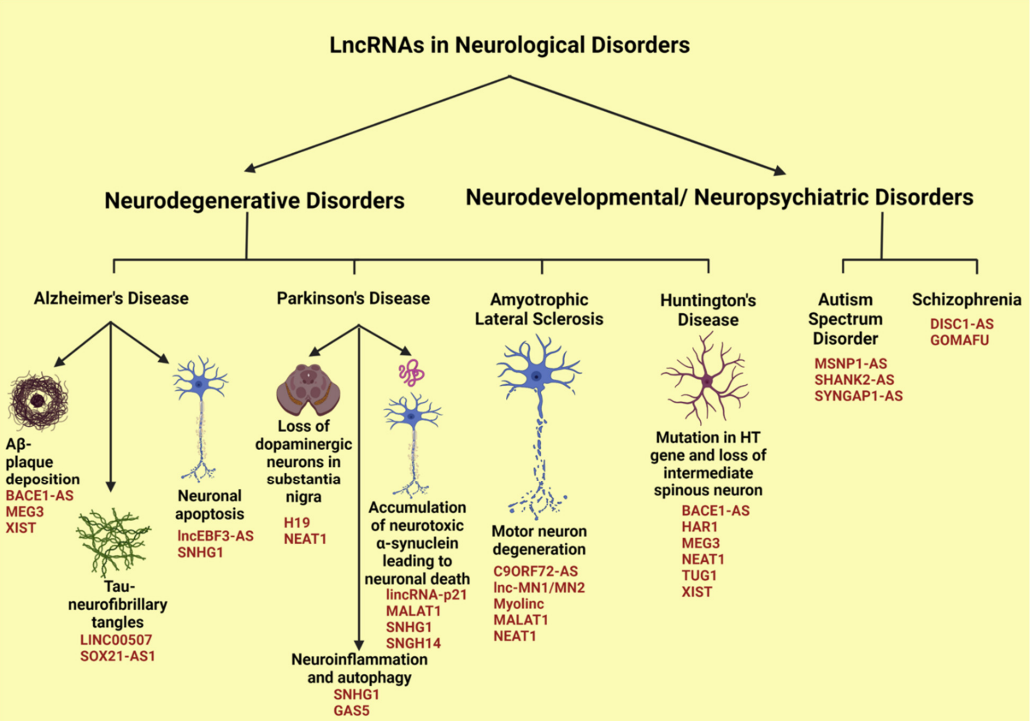

Figure 5. LncRNAs in neurological disorders: The figure represents lncRNAs involved in neurodegenerative disorders such as Alzheimer's disease, Parkinson disease, amyotrophic lateral sclerosis, and Huntington's disease, as well as the neurodevelopmental/neuropsychiatric disorders such as autism spectrum disorder and schizophrenia.

4.2.2. LncRNAs in Parkinson's Disease

Parkinson's disease (PD) is another progressive neurodegenerative disease affecting elderly people characterized by loss of dopaminergic neurons in the substantia nigra of the brain. This disease condition is also characterized by the accumulation of α-synuclein, a pre-synaptic neuronal protein which in its abnormal insoluble form is neurotoxic, resulting in neuronal death [104]. Two major lncRNAs, namely H19 and NEAT1, have been demonstrated to be associated with loss of dopaminergic neurons in PD. The expression levels of H19 are reduced in PD. LncRNA H19 sequesters miR-301b-3p and upregulates the expression of hypoxanthine phosphoribosyl transferase 1 (HPRT1) as well as tyrosine hydroxylase. These enzymes activate Wnt/β-catenin signaling pathway through upregulation of genes such as *NGN2*, *NURR1* (nuclear receptor related protein 1), *PITX3* (pituitary homeobox 3), and *NEUROD1* (neuronal differentiation1), that prevent the loss of dopaminergic neurons. Therefore, the downregulation of H19 in PD results in the attenuation of this pathway and loss of dopaminergic neurons in PD [122]. Increased levels of α-synuclein are associated with the etiology as well as the progression of PD including the loss of dopaminergic neurons associated with PD. The elevated levels of SNHG14 sequester miR-133b to enhance the expression of α-synuclein, a miR-133b target [117]. In addition to promoting the expression levels of α-synuclein, SNHG1 can also promote its oligomerization. SNHG1 sequesters miR-15b-5p and enhances the expression levels of SIAH-family E3 ubiquitin protein ligase 1 (SIAH1), which further interacts with ubiquitin-conjugating enzyme H8 (UbcH8), an ubiquitin binding enzyme. SIAH1-UbcH8 ubiquitinates α-synuclein and promotes its aggregation, thereby worsening PD [115]. By increasing the expression and oligomerization of α-synuclein, SNHG1 plays a pivotal role in PD pathology. Several other lncRNAs also promote PD progression by modulating the expression and/or oligomerization of α-synuclein. LncRNA MALAT1, which is observed to be elevated in PD, binds

to and enhances the α-synuclein stability, aggravating PD [110]. Further, the upregulated levels of lincRNA-p21 in PD sequesters miR-1277-5p and augments α-synuclein expression and aggregation, which contribute to the pathogenesis of PD [109].

Similar to AD, neuro-inflammation also worsens PD as it results in release of pro-inflammatory cytokines leading to neuronal apoptosis. The major lncRNAs affecting neuro-inflammation in PD are SNHG1 and GAS5, which act through miRNA sequestration. SNHG1 acts through miR-7 while GAS5 acts through miR-223-3p to upregulate NLRP3 (NOD-like receptor protein 3), which is involved in promoting inflammatory response in microglial cells [107,116]. In addition to neuro-inflammation, autophagy also contributes to aggravating the PD [208]. LncRNA SNHG1, which is upregulated in PD, sequesters miR-221/222 and indirectly regulates p27/mammalian target of rapamycin (mTOR) as well as microtubule associated proteins 1A/1B light chain 3B (LC-III) autophagic regulator expressions to inhibit autophagy, while promoting neuronal cytotoxicity in PD [209].

In comparison to other upregulated lncRNAs whose overexpression worsens the PD, elevated levels of NEAT1 exhibits neuroprotective roles during PD. NEAT1 is associated with nuclear paraspeckle formation and its upregulation is correlated with larger number of nuclear paraspeckles in dopaminergic neurons during PD. Under conditions of PD, these dopaminergic neurons result in loss of mitochondria and elevated oxidative stress that can damage the neuronal cells. However, NEAT1-mediated nuclear paraspeckle formation entraps numerous RNA and protein molecules within these structures, serving as a protective mechanism to the oxidative stress-mediated neuronal cell death. It has also been noted that NEAT1-assembled nuclear paraspeckles can entrap LRKK2, which mediates oxidative stress-mediated neuronal cell death. Interestingly, expression levels of NEAT1 are correlated further with gender-based incidence of PD. It has been observed that PD is less frequent in women. Since higher levels of estrogen induce NEAT1 that prevent the loss of dopaminergic neurons through its neuroprotective effects, the low incidence of PD in women has been correlated with estrogen-stimulated increase in the expression of NEAT1 [112]. However, this needs to be validated further with more experimental and epidemiological studies.

4.2.3. LncRNAs in Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder which affects the motor neurons in the brain stem, spinal cord, and motor cortex. The major gene mutations associated with ALS involve the genes *TDP43* and *C9ORF72* that encode transactive response DNA binding protein 43 and chromosome 9 open reading frame 72 protein, respectively. LncRNA NEAT1 serves as a scaffold for the interaction between RNA binding protein, TDP43 and fused in sarcoma/translocated in liposarcoma (FUS/TLS) protein, which forms the augmented number of accessory spots seen within the motor neuron nuclei, a property exhibited by the ALS patients [210]. Other lncRNAs that interact with TDP43 resulting in the pathogenesis of PD include MALAT1, Myolinc, and lnc-MN1/MN2, whose detailed mechanism of action are yet to be analyzed [104]. With regard to *C9ORF72* gene, GGGGCC (G4C2) repeat expansions at 5′ UTR of the gene results in not only the loss of function of the native protein, but also the generation of toxic proteins which aggravates ALS [105]. The antisense lncRNA, C9ORF72-AS has been identified to interact with the C9ORF72-mRNA. The abnormal GC repeats forms G-quadruplex structures that act as toxic molecules during ALS [105]. However the precise role of the interaction between C9ORF72-AS and C9ORF72-mRNA in the pathogenesis of ALS has not yet been elucidated [105].

4.2.4. LncRNAs in Huntington's Disease

Huntington's disease (HD) is an autosomal dominant neurodegenerative disease characterized by abnormal CAG repeats in the *HTT* gene that encodes the Huntington protein, as well as selective loss of intermediate spinous neurons in the striatum. The mutant HTT proteins promote neurodegeneration mainly through dysregulated transcription,

misfolded proteins, and oxidative stress as well as dysfunctional mitochondria [104]. The major lncRNAs upregulated in HD are MEG3, XIST, BACE1-AS, TUG1, and NEAT1, whereas the downregulated lncRNAs include HAR1. These lncRNAs have been reported to enhance the levels of mutant HTT protein, thereby aggravating the HD. However, the functional mechanism has not yet been identified [104].

4.2.5. LncRNAs in Autism Spectrum Disorder

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that is heterogeneous in genetic defects, ranging from single nucleotide variants to chromosomal abnormalities. The disease exhibits varied behavioral patterns, defective communicative skills as well as reciprocal social disconnections [121]. Major lncRNAs associated with ASD are the antisense lncRNAs such as SYNGAP1-AS, MSNP1-AS and SHANK2-AS, all of which are upregulated in ASD. The SYNGAP1-AS locus falls within the parent gene *SYNGAP1*, which codes for synaptic Ras GTPase activating protein-1, a critical protein involved in synaptic function and cognition. SYNGAP-AS1 reduces the expression of SYNGAP1 affecting the impairment of cortical function in the ASD patients [119]. Similarly, the lncRNA MSNP1-AS inhibits the expression of moesin, a neuronal factor involved in nuclear architecture and immune response. Upregulated MSNP1-AS reduces moesin levels and inhibits moesin-regulated RhoA, Rac, and PI3K/Akt pathways. These effects adversely affect the morphology and function of neurites, thus contributing significantly to ASD pathogenesis [111]. The antisense lncRNA SHANK2-AS inhibits the expression of SH3 and multiple ankyrin repeat domains 2 protein (SHANK2) in ASD. SHANK2 is a postsynaptic scaffolding protein involved in the structural and functional organization of diverse signaling pathways involved in post-synapse formation and development. The attenuation of SHANK2-promoted proliferative signals along with the propagation of apoptotic signals in neuronal cells drastically reduces the number as well as the length of neurites often observed in ASD [113].

4.2.6. LncRNAs in Schizophrenia

Schizophrenia (SZ) is a neuropsychiatric disorder characterized by delusions, psychosis, depression, and dysphoria. Though the causative factors are not well described, numerous genetic, epigenetic as well as environmental factors have been shown to influence the SZ pathogenesis [121]. The most widely studied lncRNA associated with SZ is MIAT (also known as GOMAFU), which is downregulated in SZ. It interacts with splicing factors encoded by the genes QK1 (quaking homolog 1) and SRSF1 (serine/arginine rich splicing factor 1) to regulate their splicing activities. Hence, the downregulation of MIAT critically affects the global splicing, leading to alternative splicing of different pre-mRNAs. GOMAFU also regulates the expressions of *DISC1* (disrupted in schizophrenia 1) and *ERBB4* (Erb-B2 receptor tyrosine kinase 4) genes. Dysregulated alternative splicing of *DISC1* and *ERBB4* mRNAs mediated by MIAT has been associated with SZ. In vivo studies have also linked the reduction of MIAT expression with behavioral changes observed in SZ [125]. LncRNA DISC1-AS is also downregulated in SZ, which results in augmented DISC1 expression. DISC1 is involved in neurotransmitter signaling, especially the dopamine trajectories, by serving as a scaffold protein with a diverse interactome. Consequently, downregulation of DISC1 contributes SZ pathogenesis [121]. Thus, a greater in-depth analysis of the role of lncRNAs in regulating the gene expressions as well as cellular architecture and signaling can shed light on the link between the developmental and degenerative neurological disorders, which can be employed for tailored RNA-based therapeutic approaches.

4.3. LncRNAs in Cancer

Cancer has been defined as a pathological condition characterized by an uncontrolled proliferation of the cells, disrupting the tissue homeostasis. Major hallmarks of cancer include the self-sufficiency of growth signals, insensitivity of anti-growth signals, evading apoptosis, sustained angiogenesis, limitless replicative potential, tumor invasion and metastasis, repro-

gramming of energy metabolism, and the evasion of immune responses [211,212]. Recent studies have unequivocally demonstrated that noncoding RNAs play a cardinal role in modulating these cancer phenotypes through both genetic and epigenetic mechanisms [213–215]. Specifically, several lncRNAs have been identified as oncogenes while many others have been identified as tumor suppressors [213,216]. An in depth analysis of lncRNAs and their critical role in tumorigenesis has been recently reviewed [213]. Therefore, only a brief outline on the mechanism by which lncRNAs regulate diverse oncogenic phenotypes is presented here (Figure 6).

4.3.1. LncRNAs and Cell Proliferation

During normal physiological conditions, there exists an equilibrium between cell proliferation and apoptosis that maintains the cellular homeostasis. However, the oncogenic signals enhance the pro-mitotic as well as anti-apoptotic signaling, that favor the aberrant cell proliferation in cancers. Tilting the cellular homeostatic balance towards cell survival and proliferation is carried out by lncRNAs through multiple mechanisms. The LncRNA UCA1, which is upregulated in gastric cancers, recruits EZH2 (enhancer of zeste homolog 2) to the promoters of the genes that encode p27 (*CDKN1B*) and the sprouty RTK signaling antagonist 1 (*SPRY1*) to mediate the repressive H3K27me3-trimethylation. This suppresses the expression of *CDKN1B* and *SPRY1* along with their tumor-suppressive activities, thus promoting gastric cancer cell proliferation [150]. In contrast to the epigenetic regulation of gene expression, lncRNA REG1CP, which is overexpressed in colorectal cancers, recruits FANCJ (Fanconi anemia of complementation group J) helicase at the *REG3A* (regenerating family member 3 alpha) promoter region. This association promotes the unwinding of DNA at the genetic locus of *REG3A* to promote its transcription, thus facilitating colon cancer cell proliferation [149]. In acute myeloid leukemia, lncRNA CCAT1 sequesters miR-155 to upregulate the downstream target c-Myc and subsequent signaling to augment cell proliferation [128]. The tumor suppressor lncRNA GAS5 is downregulated in triple negative breast cancers. GAS5 sequesters miR-196a-3p to elevate the expression of the transcription factor forkhead box O1 (FOXO1) expression, which inhibits the PI3K/Akt associated oncogenic signaling [155]. The downregulation of GAS5 in TNBC cell has an opposite effect with the resultant increase potentiation of PI3K/Akt-mediated oncogenic signaling.

4.3.2. LncRNAs and Genomic Instability

Genomic instability and defective DNA damage response pathways are hallmarks of cancers. The scaffolding function of lncRNAs plays a primary role in the modulation of genomic instability in cancers. LncRNA ANRIL interacts with the DNA damage sensing protein ATR (ataxia telangiectasia) and prevents its ubiquitination. Thus, the homologous recombination-aided DNA double strand break repair remains intact in cancer cells facilitating uninterrupted cancer cell proliferation [126]. LncRNA BGL3 acts as a scaffold for BRCA1 (breast cancer type 1 susceptibility protein)/BARD1 (BRCA1 associated ring domain 1) and interacts with poly (ADP-ribose) polymerase 1 or PARP1 in facilitating a homologous recombination repair. This lncRNA has been investigated as a therapeutic target due to its depletion has been reported to sensitize cancer cells to therapeutic approaches that induce DNA damage [127]. One of the tumor-suppressor lncRNAs affecting genomic instability is the lincRNA-p21, which is downregulated in cancers. Under normal conditions, lincRNA-p21 interacts with heterogeneous nuclear ribonucleoprotein (hnRNP)-K and activates p53-mediated p21 expression, which further regulates the expression of various genes involved in cell cycle checkpoint regulation. This regulation is disrupted in cancers due to downregulated levels of lincRNA-p21 [157].

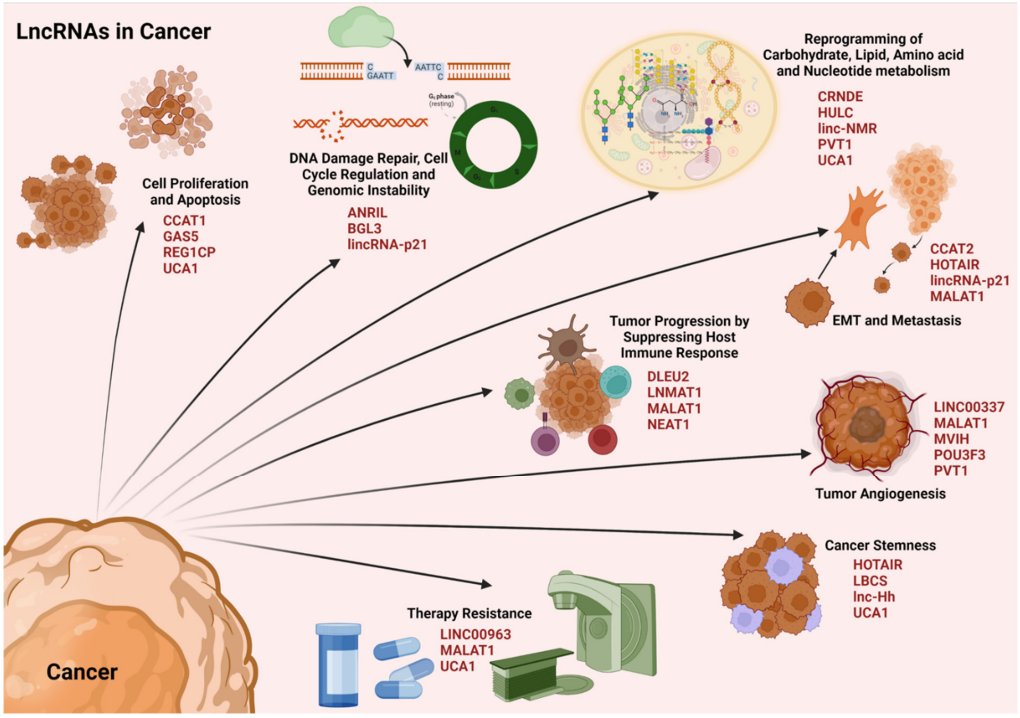

Figure 6. LncRNAs regulating tumorigenesis. The figure depicts the mechanisms by which representative lncRNAs regulate diverse cancer phenotypes such as cell proliferation and apoptosis, genomic instability, DNA damage repair, metabolic reprogramming, epithelial–mesenchymal transition and metastasis, host immune responses, tumor angiogenesis, cancer stemness, and therapy resistance.

4.3.3. LncRNAs and Metabolic Reprogramming

Metabolic reprogramming of the cells is a cardinal facet during oncogenic transformation as well as cancer progression. The changes occur in carbohydrate, lipid, amino acid, and nucleotide metabolisms in the cancer cells. The major source of energy for the cancer cells is glucose, and the proliferating cancer cells utilize glucose mainly through the time-efficient aerobic glycolysis instead of the energy-efficient mitochondrial oxidative phosphorylation, well explained as the Warburg effect. Numerous lncRNAs promote glucose metabolism either by enhancing levels of glucose transporters, as for instance, lncRNA CRNDE that upregulates GLUT1 (glucose transporter 1) expression in colorectal cancers, or through upregulating enzymes in the aerobic glycolytic pathway, as for instance, lncRNA PVT1 that enhances hexokinase levels by sequestering miR-143 [130,147]. Lipid metabolism is also significant in tumorigenesis as lipids act as membrane components, signaling molecules as well as energy source. For instance, lncRNA HULC is upregulated in hepatocellular carcinoma where it sequesters miR-9 and elevates peroxisome proliferator-activated receptor alpha (PPARA) levels. The transcription factor PPARA further binds to the promoter of *ACSL1* (acyl-CoA synthetase long chain family member 1) gene and transactivates its expression, which is a major subunit of acetyl CoA synthase involved in triacylglycerol synthesis [132]. The major amino acid whose metabolism is altered in cancer cells is glutamine, as it serves as the source for glutamate and α-ketoglutarate by the action of glutaminase (GLS) enzyme. LncRNA UCA1, which is upregulated in bladder cancers, promotes glutaminolysis through enhancing the expression of GLS2, which codes for glutaminase enzyme via miR-16 sequestration [151]. The studies on lncRNAs affecting nucleotide metabolism are very scarce. In hepatocellular carcinoma, the elevated levels of linc-NMR has been reported to interact with Y-box binding protein 1 (YBX1) and regulate the enzymes in pyrimidine metabolism such as ribonucleotide reductase regulatory subunit M2 (RRM2), thymidylate synthetase (TYMS), and thymidine kinase 1 (TK1) to enhance the deoxynucleotide triphosphate availability, favoring cancer cell proliferation [135].

4.3.4. LncRNAs and Immune Evasion

Immune suppression is one of the major pre-requisites for cancer cell proliferation and progression. The immune response in the human body occurs either through adaptive or innate mechanisms. The adaptive mechanism employs either activated T-cells or B-cells to evoke immunity of a previously recognized pathogen or pathogenic state. Activated CD8+ cytotoxic T lymphocytes kill the cancer cells through the release of TNFs and ILs, besides other mechanisms.

The upregulation of lncRNA NEAT1 promoted $CD8^+$ T cell apoptosis via the sequestration of miR-155 and consequent increase in Tim-3 (T cell immunoglobulin and mucin domain containing protein 3). This results in immune evasion and the growth of hepatocellular carcinoma [145]. LncRNA MALAT1 sequesters miR-195 to enhance the expression of programmed death ligand 1 (PDL-1) expression, which facilitates the apoptosis of cytotoxic T-lymphocyte apoptosis and immune escape, favoring tumorigenesis [140]. LncRNAs play a major role in the regulation of B-cell-mediated humoral immune responses as well. For instance, lncRNA DLEU2 sequesters miR-15a/16 to regulate B-cell proliferation through the upregulation of its downstream target genes involved in proliferation such as *CCND2/3* and *CCNE* in chronic lymphocytic leukemia [131]. Major immune cells associated with the innate immune responses are the macrophages, natural killer (NK) cells, and myeloid derived suppressor cells (MDSCs). The M2 polarization of macrophages promotes the tumor progression by recruiting MDSCs in the tumor microenvironment, thus suppressing the immune responses. LncRNA LNMAT1 recruits hnRNPL to the promoter of *CCL2* gene to activate its expression through H3K4-me3 trimethylation. Subsequent CLL2-promoted infiltration of M2-type tumor associated macrophages and the upregulation of CCL2-responsive genes enhance cancer progression through immune evasion in bladder cancers [139].

4.3.5. LncRNAs and Epithelial–Mesenchymal Transition

EMT and metastasis form the critical phases in tumor progression. LncRNA HOTAIR interacts with PRC2 and induces H3K27me3-mediated repressive methylation on the promoter of tumor suppressive genes such as *PTEN* (phosphatase and tensin homolog) and *JAM2* (junctional adhesion molecule B). It can also interact with LSD1 to induce H3K4-demethylation to activate the gene expression on the promoter of *LDHA* (lactate dehydrogenase A) and CCNA1 (cyclin A) genes, thereby promoting EMT and metastasis [133]. LncRNA MALAT1 sequesters miR-126-5p and enhances the expression of Slug and Twist to facilitate EMT and metastasis in colorectal cancers [141]. By acting as a scaffold, lncRNA CCAT2 interacts with the transcription factor termed transcription factor 7-like 2 (TCF7L2). TCF7L2 transactivates the expressions of c-Myc and oncogenic miRNAs such as miR-17-5p and miR-20a, thus aiding metastasis in colorectal cancers [129]. Several tumor suppressor lncRNAs that affect EMT and metastasis are downregulated in cancers, one of these being lincRNA-p21. Normally, lincRNA-p21 sequesters miR-9 to upregulate the expression of E-cadherin, a suppressor of EMT. However, the downregulation of lincRNA-p21 relieves this inhibition on miR-9 and the ensuing downregulation of E-cadherin contributes to tumor invasion in hepatocellular carcinoma [158].

4.3.6. LncRNAs and Tumor Angiogenesis

Angiogenesis is significant for cancer genesis and progression as it aids nutrient and oxygen supply to the proliferating cancer cells. Increase in VEGFA levels mediated by STAT3-signaling plays a critical role in tumor angiogenesis. LncRNA PVT1 enhances the stability of STAT3 by preventing it from ubiquitin-mediated proteolysis, thus promoting tumor angiogenesis [148]. In hepatocellular carcinoma, lncRNA MVIH inhibits the secretion of phosphoglycerate kinase 1 (PGK1) through a direct interaction, which prevents the inhibitory effects of PGK1 on tumor angiogenesis. PGK1 acts as a disulphide reductase that reduces the disulfides in protease plasmin, which in turn promotes the release of the angiogenic inhibitor angiostatin [144]. In breast cancer, lncRNA MALAT1 sequesters miR-140 in order to upregulate VEGFA expression to promote tumor angiogenesis [142]. Regulation

of tumor angiogenesis by lncRNAs also involves epigenetic mechanisms. LINC00337 recruits DNMT1 to the promoter of the tumor-suppressive gene, *CNN1* (calponin 1), to repress its expression in colorectal cancers, which further promotes VEGF-mediated tumor angiogenesis [136]. LncRNAs also regulate the tumor microenvironment to promote tumor angiogenesis. The exosomal lncRNA POU3F3 released by glioma cells upregulates VEGFA and bFGF (basic fibroblast growth factor) in endothelial cells, thus promoting angiogenesis [146].

4.3.7. LncRNAs and Cancer Stemness

Cancer stemness is the key feature that contributes greatly to cancer relapse and therapeutic resistance. LncRNA-Hh promotes sonic hedgehog–glioma-associated oncogene homolog 1 pathway to upregulate the expression of transcription factors, SRY-related HMG box transcription factor 2 (Sox-2) and octamer-binding transcription factor 4 (Oct-4), to enhance the self-renewal of cancer stem cells and mammosphere formation in breast cancers [138]. In cervical cancers, the elevated levels of UCA1 sequester miR-122-5p to upregulate Sox-2 expression, thereby facilitating cancer stemness [152]. LncRNA HOTAIR recruits PRC2 to the promoter of gene encoding miR-7 to induce repressive H3K27me3 methylation. Further, the upregulation of its downstream targets such as c-Myc and Twist through miR-7/SETDB1 (SET domain bifurcated histone lysine methyl transferase 1)/STAT3 axis enhances the stemness in breast cancers [133,134]. The tumor suppressor lncRNA LBCS is downregulated in bladder cancers, which otherwise recruits hnRNPK and EZH2 to the promoter of *SOX2* gene to induce H3K27me3 repressive tri-methylation, thus suppressing *SOX2* expression [156].

4.3.8. LncRNAs and Therapy Resistance

LncRNAs regulate therapy resistance in cancers through different mechanisms. LncRNAs MALAT1 and LINC00963 contribute to radioresistance in nasopharyngeal cancers and breast cancers, respectively. While the former acts via miR-1/Slug axis, the latter acts through miR-324-3p/activated Cdc42 associated kinase1 (ACK1) axis to enhance radioresistance [137,143]. Slug, which is an oncogenic transcription factor, and ACK1, which is an oncogenic receptor tyrosine kinase, promote downstream signaling facilitating radioresistance in these cancers. LncRNA UCA1 sequesters miR-27b and upregulates *CCNG1* (cyclin G1) expression, which subsequently elevates p53 level, and downregulates miR-508-5p to induce multidrug resistance in gastric cancers [153,217]. UCA1 also recruits EZH2 to the promoter of *CDKN1A/p21* gene to suppress its expression and activate the PI3K/Akt pathway, thereby contributing to tamoxifen resistance in breast cancers [218]. Certain lncRNAs also upregulate the drug efflux transporters to induce drug resistance in cancers. LncRNA MALAT1 upregulates multidrug resistance protein 1 (MRP1) and multidrug resistance 1 (MDR1) proteins to enhance drug efflux and, thereby, inducing multidrug resistance in lung cancers [219].

An in depth analysis of the functional associations of different lncRNAs with the different cancers and cancer sub-types could identify them as druggable targets for cancer drug discovery and tailored cancer therapy.

4.4. LncRNAs in Diabetes Mellitus

Diabetes is one of the most common metabolic disorders with chronic impacts. Diabetes is a broader term which encompasses the group of diseases that manifest impairment in glucose utilization [220]. In general, chronic diabetes is categorized as type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) whereas T1DM is characterized by defective insulin synthesis primarily due to pancreatic β-cell destruction whereas T2DM is characterized by progressive loss in insulin secretion along with insulin insensitivity [221,222]. Since T2DM comprises more than 90% of all diabetic cases, the role of lncRNAs in T2DM is discussed here. However, the correlates discussed here are likely to have an impact on prediabetes and gestational diabetes since both prediabetes and

gestational diabetes often progress towards T2DM. LncRNAs are involved in regulating multiple pathological complications associated with DM (Figure 7).

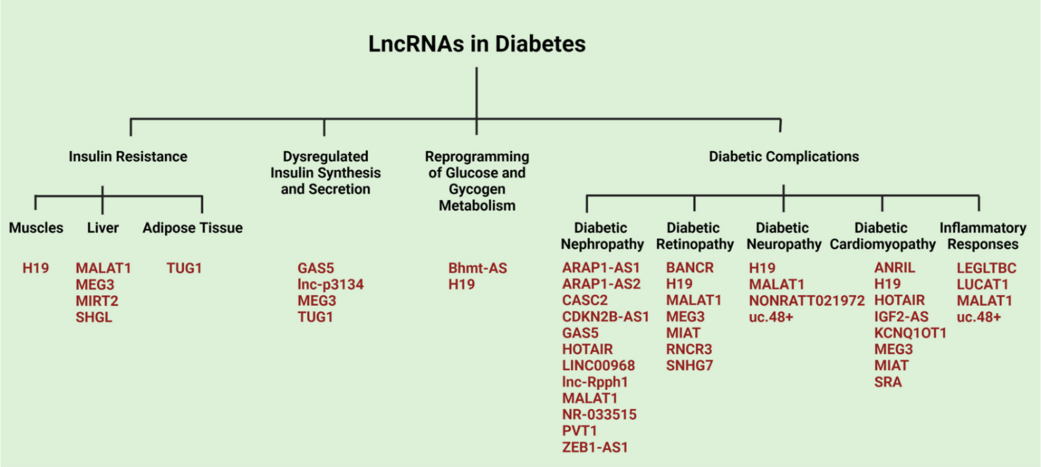

Figure 7. LncRNAs in diabetes mellitus. Major lncRNAs affecting the diverse phenotypes of diabetes mellitus such as insulin resistance, deregulated insulin synthesis and secretion, reprogramming of glucose, and glycogen metabolisms are depicted. In addition, lncRNAs involved in diabetic complications such as diabetic nephropathy, diabetic retinopathy, diabetic neuropathy, diabetic cardiomyopathy, and inflammatory responses are presented.

Aberrant expression of lncRNAs can be attributed to insulin resistance, insulin synthesis, and reprogramming of glucose metabolism. In addition, dysregulated lncRNAs contribute significantly to the ailments associated with DM such as diabetic nephropathy, diabetic retinopathy, diabetic neuropathic pain, diabetic cardiomyopathy cardiovascular, and inflammatory complications [159].

4.4.1. LncRNAs in Insulin Resistance and Synthesis

Several lncRNAs are associated with insulin resistance in DM. In the muscles, the major pathway involved in glucose uptake, fatty acid oxidation, and insulin sensitivity is the AMPK (5′ AMP-activated protein kinase) signaling cascade. LncRNA H19, which promotes AMPK signaling, is downregulated in DM. H19 inhibits let-7 expression, thereby promoting the expression of genes involved in insulin sensitivity such as *DUSP27* (dual specificity phosphatase 27), *IDE* (insulin degrading enzyme), *INSR* (insulin receptor), and *IRS2* (insulin receptor substrate 2) [184]. In the muscles, downregulation of H19 contributes to insulin resistance by dysregulating the β-oxidation of fatty acids in mitochondria and accumulating in the fatty acids. Under normal physiological conditions, H19 recruits hnRNPA1 to the mitochondria, which promotes the translation of carnitine palmitoyl transferase 1 beta (CPT1B) and peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC1A) mRNAs that are required for the β-oxidation of fatty acids [183]. LncRNAs have also been shown to regulate the hepatic glucose and lipid metabolisms, thereby contributing to hepatic insulin resistance and DM. The major transcription factor required for lipid biosynthesis in liver is sterol regulatory element-binding transcription factor 1c (SREBP-1c). LncRNA MALAT1, which is overexpressed in DM, binds to and stabilizes SREBP-1c protein to promote lipogenesis and hepatic insulin resistance in vivo [168]. LncRNA MEG3 contributes to hepatic insulin resistance in DM by sequestering miR-214 and upregulating the transcriptional factor ATF4 (activating transcription factor 4), which promotes gluconeogenesis through upregulation of glucose-6-phosphatase and phosphoenolpyruvate carboxy kinase enzymes [173]. In regulating gluconeogenesis, lncRNA SHGL recruits hnRNPL to promote calmodulin expression, that inhibits gluconeogenesis. However, SHGL is downregulated in DM, thus impairing gluconeogenesis in DM [194]. LncRNA MIRT2 sequesters miR-34-5p and upregulates its target ubiquitin specific peptidase 10 (USP-10).

USP-10 interacts with sirtuin-6 to counteract hepatic insulin resistance. With the downregulation of MIRT2 in DM, this process is reversed and insulin resistance is promoted in DM [193]. In addition to muscles and liver, adipose tissue also exhibits insulin resistance in DM. LncRNA TUG1 sequesters miR-204 and augments SIRT1 expression, which enhances insulin sensitivity in adipose tissue through activated GLUT4/PPARγ/Akt signaling. The downregulation of TUG1 in DM leads to decreased insulin sensitivity [196].

LncRNAs also affect the pancreatic β-cell function as well as the insulin synthesis. The downregulated MEG3 levels in DM reduce the expression of transcription factors musculoaponeurotic fibrosarcoma oncogene family protein A (MAFA) and pancreatic and duodenal homeobox 1 (PDX1), which in turn inhibit insulin synthesis and secretion from the pancreatic β-cells [191]. Further, the reduced levels of lncRNA GAS5 in DM also adversely affect the MAFA and PDX1 expressions as well as cell cycle arrest to inhibit the pancreatic insulin synthesis [181]. LncRNA-p3134 promotes the expression of MAFA, PDX1, GLUT2, and TCF7L2 in pancreatic β-cells, which enhances insulin secretion in response to higher glucose levels. This points out to the protective role of lncRNA-p3134 from toxic effects of enhancing the rate of glucose-induced insulin secretion from pancreatic cells, while remaining elevated in DM [166]. Inhibition of lncRNA TUG1 in the pancreatic β-cells contributed to defective insulin synthesis through either promoting the β-cell apoptosis or through reduction in transcription factors such as MAFA and PDX1, which plays a role in insulin synthesis [197].

4.4.2. LncRNAs in Glucose Metabolism

The role of lncRNAs in reprogramming glucose metabolism also contributes to DM. In DM, glucose levels are elevated in the liver due to defective glycolysis, and increased gluconeogenesis or glycogenolysis. This can be correlated with the downregulation of lncRNA H19 in DM. Downregulation of H19 enhances the expression level as well as nuclear localization of FOXO1 transcriptional factor. FOXO1 enhances the transcription of gluconeogenic genes thereby promoting hepatic glucose accumulation and DM pathogenesis [185]. Hepatic gluconeogenesis is also promoted by the lncRNA Bhmt-AS, which is upregulated in DM. Bhmt-AS, an antisense lncRNA, stabilizes the expression of betaine homocysteine S-methyl transferase enzyme, which is involved in hepatic gluconeogenesis [160]. In addition to modulating predisposing factors and signaling pathways promoting DM, numerous lncRNAs are also associated with microvascular and macrovascular complications associated with the disease, which includes diabetic nephropathy, diabetic retinopathy, diabetic neuropathic pain, diabetic cardiomyopathy, and inflammatory complications [159].

4.4.3. LncRNAs in Diabetic Nephropathy

Diabetic nephropathy (DN) is one of the microvascular complications of DM damaging the renal tissue and functions. Primarily, the proliferation and fibrosis of the mesangial cells contribute to diabetic nephropathy. LncRNAs LINC00968 and GAS5 recruit EZH2 to the promoter of *CDKN1A* and *MMP9* genes (matrix metalloproteinase 9), respectively, to repress their expression. LINC00968 is upregulated while GAS5 is downregulated under conditions of DN. However while LINC00968-mediated downregulation of p21 promotes proliferation and fibrosis of mesangial cells through enhancing the expression of ECM proteins, thus aggravating DN, GAS5-mediated inhibition of MMP9 expression reduces the inflammatory signals to attenuate renal fibrosis and prevent the burden of DN [165,182]. LncRNA PVT1, which recruits EZH2 to the promoter of *FOXA1* to induce H3K27me3-mediated repressive tri-methylation, is overexpressed during conditions of DN. The lower FOXA1 levels, in turn, regulates apoptotic genes and facilitates podocyte apoptosis and nephropathy in DM [177]. LncRNA Rpph1 overexpression enhances its interaction with galectin 3 and activates galectin-3/mitogen-activated protein kinase kinase/extracellular signal-regulated kinase pathway aiding proliferation and inflammation of the mesangial cells [167]. LncRNA MALAT1 sequesters miR-145 and induces the expression of the transcription factor zinc finger E-box binding homeobox 2 (ZEB2) expression, which promotes the transcription of

ECM genes aiding renal fibrosis [169]. Synergizing with this mechanism, lncRNA ZEB1-AS1 that suppresses renal fibrosis, is downregulated in DN. Normally, ZEB1-AS1 sequesters miR-217 and upregulates the transcription factor MAFB (musculoaponeurotic fibrosarcoma oncogene homolog B) along with reduction in ECM proteins such as fibronectin and collagen, to inhibit renal fibrosis in DM [198]. The downregulation of ZEB1-AS1 favors renal fibrosis in DM.

4.4.4. LncRNAs in Diabetic Retinopathy

Diabetic retinopathy (DR) is another major complication associated with DM that damages the retinal cells and vision. LncRNA RNCR3, which is upregulated in DR, sequesters miR-185-5p and augments the expression of the transcription factor KLF2 to promote retinal endothelial cell proliferation, migration, and tube formation. This further leads to augmented acellular capillaries, increased vascular leakage and inflammatory responses that result in retinal reactive gliosis and DM induced retinal neurodegeneration [178]. LncRNA MALAT1 sequesters miR-125b to upregulate downstream target gene expressions, such as that of vascular endothelial cadherin, aiding proliferation and vascular permeability of retinal microvascular endothelial cells, to adversely affect the vision by promoting neovascularisation [170]. In contrast to these upregulated lncRNAs, levels of lncRNAs H19, SNHG7, MEG3, and BANCR are downregulated in DR. LncRNA H19 sequesters miR-93 to upregulate the expression of X-box binding protein 1, resulting in reduction of inflammatory cytokines and inflammation in retinal epithelial cells. Downregulation of H19 leads to the reversal of the inhibitory control in DR [186]. Similarly, the downregulation of SNHG7 relieves its inhibition of retinal endothelial cell angiogenesis via the miR-543-SIRT1-VEGF signaling axis [186,195]. Likewise, the downregulation of MEG3 releases its inhibitory control to prevent DR through the reduction of VEGF and TGF-β1 expressions in retinal cells [192].

4.4.5. LncRNAs in Diabetic Neuropathic Pain

Diabetic neuropathic pain (DNP) is a prominent chronic complication of DM. LncRNA NONRATT021972 is upregulated in patients with DNP. It increases the expression of TNFα as well as the expression of purinergic receptors (P2X) 3 and 7 to aggravate the DNP conditions [176]. Elevated levels of lncRNA uc.48+ enhances P2X3 receptor expression to aggravate DNP. P2X3 signaling activates downstream ERK1/2 and TNFα to bring out excitatory transmission signals that contribute to DNP [179]. Another major symptom or disease complication associated with DM is the chronic wounds in the patients. LncRNA H19 has been reported to augment the wound healing process in DM. It activates fibroblasts through recruitment of EZH2 to induce H3K4me3 methylation on the promoter of *HIF1A* gene that encodes hypoxia inducible factor 1α promoter to augment its expression and fasten the wound healing process [187]. Thus, the reduced levels of H19 in DM contribute significantly to the impaired wound-healing process. Though lncRNA MALAT1 is overexpressed in DM, it has been shown that transplantation of MALAT1-overexpressing human mesenchymal stem cells improves the wound healing in DM patients. In these stem cells, MALAT1 sequesters miR-205-5p to augment VEGF levels to promote wound healing in DM [223].

4.4.6. LncRNAs in Diabetic Cardiac Myopathy

Diabetic cardiac myopathy (DCM) is characterized by the presence of myocardial abnormalities leading to cardiac complications, such as heart failure, in patients with DM. Similar to the other allied complications of DM, the incidence and progression of DCM is also regulated by several lncRNAs. The lncRNA KCNQ1OT1, which is upregulated under conditions of DCM, has been shown to sequester miR-214-3p and alter caspase-1 expression in cardiomyocytes. This resulted in the pyroptosis-mediated death of cardiomyocytes, deregulated cytoskeletal structures, and enhanced calcium overload, all of which contribute to the worsening of cardiac functions [163]. LncRNA MIAT sequesters miR-22-3p to

upregulate DAPK2 (death associated protein kinase 2) expression, resulting in the apoptosis of cardiomyocytes along with the worsening of the left ventricular function in diabetic animal models [175]. Furthermore, MEG3, which is upregulated in DCM, sequesters miR-145 to induce the expression of the apoptotic protein known as programmed cell death 4 (PDCD4). PDCD4 is a cardinal regulator of cellular apoptosis that alters BAX/BCL2 levels to induce cardiomyocyte apoptosis in DM [174]. LncRNA IGF2-AS inhibits IGF2 as well as VEGF expression in myocardial microvascular endothelial cells, thereby inhibiting angiogenesis, and affecting the cardiovascular system during DM [162]. In addition to these upregulated lncRNA, several lncRNAs that have cardioprotective roles are downregulated in DCM. In normal cellular homeostasis of cardiomyocytes, H19 downregulates DIRAS3 (distinct subgroup of the Ras family member 3) expression through the recruitment of EZH2 to induce H3K27me3 methylation on the *DIRAS3* promoter [188]. DIRAS3 inhibits cell proliferation and promotes autophagy-mediated cell dormancy [224]. Downregulation of H19 observed in DCM leads to the depression of DIRAS3 and the resultant inhibition of Akt-signaling pathway and autophagy of cardiomyocytes, promoting DCM [188]. Likewise, the downregulation of HOTAIR results in the reduced viability of cardiomyocytes through inactivation of PI3K/Akt signaling cascades [189].

4.4.7. LncRNAs in Diabetes-Associated Inflammation

Several lncRNAs also modulate inflammatory signals that can directly impinge on the pathological complications associated with DM. LncRNA MALAT1 mediates the activation of NLRP3-mediated inflammation resulting in cardiovascular complications in DM [171]. The macrophage polarization also contributes to inflammatory responses associated with DM. The levels of circulating lncRNA uc.48+ are elevated in DM patients. It enhances the P2X7 receptor expression and elevates the levels of the pro-inflammatory cytokines, IL-1β and TNFα, promoting apoptosis and inhibition of the proliferation of macrophages during DM [179,180]. In the cases of cerebral IR injury associated with DM, MALAT1 worsens the condition through the activation of MyD88/IRAK1 (interleukin 1 receptor associated kinase 1)/TRAF6 (TNF receptor associated factor 6) signaling cascade resulting in an inflammatory response in microglia [172]. LncRNA LEGLTBC sequesters miR-34a and upregulates SIRT1 expression to promote apoptosis in INS-1 beta cells, thereby adversely affecting the insulin synthesis and release, aggravating DM [164]. In instances of diabetic lung disorders, lncRNA LUCAT1/SCAL1 is reduced in the serum considerably, which otherwise inhibits iNOS (inducible nitric oxide synthase) expression and nitric oxide production in the lung cells during DM [190]. Higher levels of nitric oxide in the lungs result in the activation of platelets to stimulate chronic inflammation. These conditions lead to severe damages in the endothelium of lung capillaries as well as causing microangiopathy, worsening the diabetic lung disease.

In addition to their causative and/or synergistic role in diabetes progression and complications, some of the lncRNAs have been identified as potential biomarkers for complications associated with DM. Upregulated levels of lncRNA-ARAP1-AS2, NR-033515, CDKN2B-AS1, and HOTAIR as well as downregulated levels of lncRNA-ARAP1-AS1, CASC2, GAS5, and ZEB1-AS1 have been proposed as biomarkers for DN [159]. The observations that lncRNA BANCR is downregulated while lncRNA MIAT is elevated in DM patients with DR identify them as predictive biomarkers for DR [159]. Similarly, lncRNA SRA, whose level is lower, and lncRNA ANRIL, whose level is higher in DM patients with cardiovascular complications, are being investigated as biomarkers for DM with cardiovascular ailments [159]. Thus, the diverse mechanisms of action exhibited by lncRNAs in regulating metabolic disorders, such as diabetes, attributes to its several allied disease complications as well as opens the path in exploring them as biomarkers, thus providing theoretical insights to manage DM.

5. Conclusions

Cellular homeostasis is maintained through a vast array of cellular signaling networks that act in concert to elicit coordinated responses in response to internal/external cues. Dysregulation of the network forms the etiological basis for most, if not all, of the diseases. Therefore, identifying the aberrant signaling nodes and the causative factors could serve as potential therapeutic targets. A greater part of our current therapeutic strategies rely on the data arising from mere 2% of the genome, which codes for the proteins. Recent studies, as discussed here, have shown that the non-coding RNAs, especially the lncRNAs, play cardinal roles in the genesis and progression of multifactorial human diseases. Each of the human diseases are complex with respect to the interconnected network of signaling pathways and their crosstalks. LncRNAs mediate the crosstalk between these diverse signaling cascades with their diverse interactome including proteins, RNAs and lipids. Aberrant expression or asynchronous signaling by lncRNAs and their interactome contribute not only to the pathogenesis and progression of diseases, but also to therapy resistance. Furthermore, similar to the SNPs (single nuclear polymorphisms) in coding regions of the genome, mutations in lncRNAs are also being unraveled to contribute to disease risks in humans. The systematic investigation of lncRNA profiles in diverse human disease types would pioneer the efforts in developing lncRNA-based disease biomarkers as well as therapeutic targets. However, extensive in vitro and in vivo experimental validations are required for definitive conclusions on the regulatory as well as biological roles of lncRNAs and the effects of targeting the critical lncRNAs for therapy. Unraveling the diverse lncRNA-based therapeutic strategies would undoubtedly shed light on the existing complexities of several targeted therapeutics that so far have been unsuccessful in suppressing the disease or even in combating the multifaceted therapeutic resistance. Considering the multitudes of genetic and epigenetic events regulated by lncRNAs, development of a personalized therapeutic approach can be refined by investigating the therapeutic targeting lncRNAs alone or in combination with other targeted therapeutic agents.

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REVIEW

Regulation of Tumor Metabolome by Long Non-Coding RNAs

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Tumorigenesis necessitates enhanced ability for macromolecular biosynthesis, higher energy/ATP levels and co-ordinated redox balance systems within the cancer cells as well as the cellular components of the tumor microenvironment. Cancer cells have adapted several mechanisms to accommodate their growing need for energy and macromolecular building blocks. A critical mechanism that sustains cancer growth and progression is metabolic reprogramming. Metabolic reprogramming refers to the process that changes the pattern of metabolism in these cells that regulates cancer energetics and metabolic biosynthesis. Recent studies have shown a role for long non-coding RNAs (lncRNAs) in the metabolic reprogramming of multiple cell types in cancer tissue. LncRNAs play a decisive role in the reprogramming of glucose, lipid, amino acid, and nucleotide metabolisms to promote tumor growth. Emerging tumorigenic role of lncRNAs and the diverse mechanisms through which they reprogram tumor metabolome is comprehensively discussed here.

Keywords: Cancer; lncRNA; tumor metabolome; metabolomics; targeted therapy

Introduction

Tumorigenesis requires metabolic reprogramming of the cells for meeting their higher energy demands as well as for the production of anabolic intermediates, together which signifies metabolic reprogramming as one of the major cancer hallmarks. The augmented catabolism of glucose, lipids and amino acids facilitate the higher proliferative rates of cancer cells by enhancing the energy supply as well as overpowering the apoptotic signals [1]. Under conditions of nutritional stress or hypoxic conditions, the cells of the tumor microenvironment (TME) also undergo metabolic reprogramming, so as to enable their own survival as well as inducing metabolic changes within other cells favouring tumorigenesis [2]. Recent researches have shown that metabolic reprogramming of the cancer cells and the cells in the tumor stroma remodel the TME into an acidic immunosuppressive environment as well [3]. Metabolomics, which sheds light to metabolic reprogramming, have evolved as a promising arm to decipher novel biomarkers and therapeutic targets empowering the concept of precision medicine for cancers.

Similar to the diverse components such as proteins and mRNAs involved in regulating the cancer cell metabolism, long non-coding RNAs (lncRNAs) are emerging as a major regulator of cancer metabolome [4]. LncRNAs are non-coding RNAs that are greater than 200 nucleotides in length, but cannot be translated into proteins [4]. For a longer time period in the scientific research, lncRNAs were considered to be dark matters of the genome, since it composed larger fractions of the genome than the coding ones and was thought to be not involved in any cellular processes [4]. However, recent studies have unravelled their tremendous potential as a regulatory node which actively takes part in physiological and pathological signaling in the humans [5]. LncRNAs carry out indispensable role in regulating almost all the cancer hallmarks, including metabolic reprogramming [6]. They can act as miRNA sponges, guides, scaffolds and even mediate interactions with several other nucleic acids or proteins to bring about their functions. In this review, we try to portray the major lncRNAs involved in reprogramming of glucose, lipid, amino acid as well as nucleotide metabolisms in numerous cancers, which would strengthen the notions on considering lncRNAs as precise therapeutic targets favouring advancements in cancer therapy.

LncRNAs and Reprogramming of Glucose Metabolism in Cancers

LncRNAs are involved in the regulation of multiple pathways involved in the reprogramming of glucose metabolism in tumor cells (**Figure 1**). The primary energy source for highly proliferating cancer cells is glucose. Under normal physiological conditions, cells utilize glucose through glycolysis and then proceed to the mitochondrial oxidative phosphorylation for production of ATP, in the presence of oxygen. In the absence of oxygen, cells mostly rely on glycolysis for energy production [7]. However, even in

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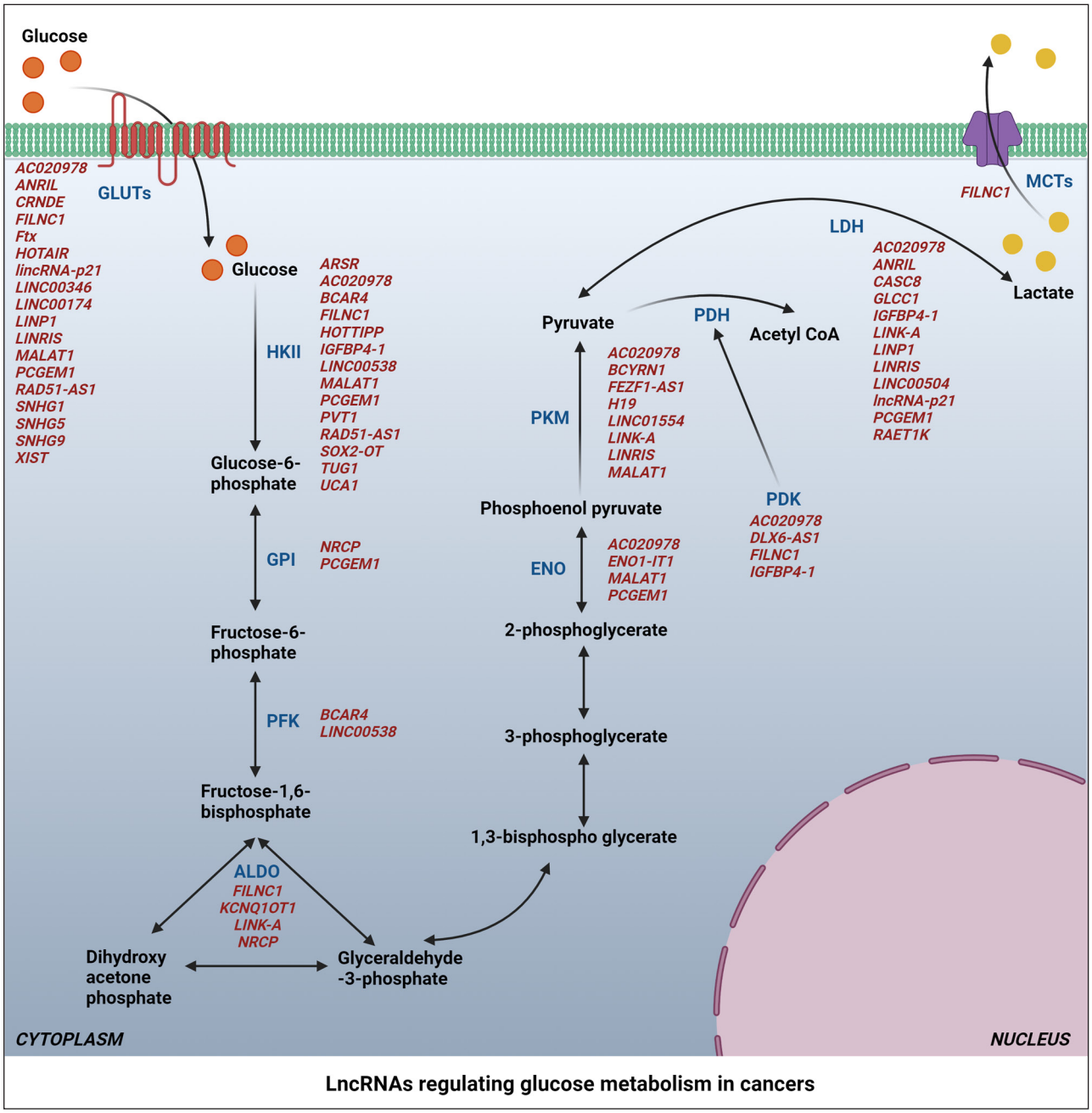

Figure 1: LncRNAs regulating glucose metabolism in cancers. The figure illustrates the representative lncRNAs that regulate various enzymes at different stages of glucose metabolism in cancers. (ALDO-aldolase; ENO-enolase; GLUTs-glucose transporters; GPI-phosphoglucoisomerase; HKII-hexokinase II; LDH-lactate dehydrogenase; MCTs-monocarboxylate transporters; PDK-pyruvate dehydrogenase kinase; PDH-pyruvate dehydrogenase; PFK-phosphofructokinase; PKM-pyruvate kinase).

the presence of oxygen, cancer cells rely on rapid glycolysis for ATP synthesis that fuels the uncontrolled proliferation of the cancer cells. This phenomena is termed as Warburg effect or aerobic glycolysis [8]. Cancer cells employ this mechanism by enhancing their glucose uptake and lactate release. The acidic TME facilitates the cancer progression, as well. Glycolytic intermediates are utilized as substrates for various cellular anabolic processes [9]. Furthermore, utilization of aerobic glycolysis provides additional survival advantage to the cancer cells for the fact that they produce lesser amounts of reactive oxygen species (ROS) that can induce apoptotic signal in cancer cells [10]. LncRNAs regulate glucose metabolism in cancer cells as well as the TME by regulating the pathways at different stages such as enhancing glucose transporters as well as modulating the enzymes involved in diverse glucose metabolic pathways.

The initial step involved in regulation of glucose metabolism in cancer cells is through the expression of glucose transporters, GLUTs, which are transmembrane glycoproteins that promotes the glucose entry into the cancer cells. LINC00346 sequesters miR-148a/b to upregulate GLUT1 expression to enhance glucose uptake for aerobic glycolysis in breast cancers [11]. The elevated levels of lncRNA CRNDE has been reported to upregulate the GLUT4 levels that enhances the glucose import into the

cancer cells favouring aerobic glycolysis. In addition, it also enhances the levels of transcription factor MLXIPL (MLX interacting protein like) that subsequently elevates the transcription of several downstream genes involved in glucose metabolism [12]. LncRNA Ftx transcriptionally and post-transcriptionally promotes PPARγ (peroxisome proliferator-activated receptor gamma) expression, which further enhances transcription of GLUT1 and GLUT4 facilitating enhanced glucose import into cancer cells for aerobic glycolysis [13]. In cancer cells, hypoxia induces HIF-1α (hypoxia inducible factor 1 alpha) mediated upregulation of lincRNA-p21, which further interacts with HIF-1α to upregulate transcription of GLUT1 and LDHA (lactate dehydrogenase A) to promote glycolysis [14]. In prostate cancers, lncRNA PCGEM1 activates c-Myc mediated upregulation of GLUT1 to enhance the glucose uptake, along with augmenting the expression of glycolytic genes such as HKII (hexokinase II), GPI (glucose-6-phosphate isomerase), ENO1 (enolase1), and LDHA to favour aerobic glycolysis for tumor progression [15]. Furthermore, lncRNA HOTAIR upregulates GLUT1 expression through activation of mTOR pathway in hepatocellular carcinoma and promotes glucose uptake and glycolysis [16]. LncRNA lncp23154 inhibits miR-378a-3p expression to upregulate GLUT1 levels in oral squamous cell carcinoma [17]. In glioblastoma, lncRNA XIST sequesters miR-126 to upregulate IRS1 (insulin receptor substrate 1) and activate PI3K/AKT signaling, which favours GLUT1 and GLUT3 expression [18]. Similarly, LINC00174, SNHG9, SNHG5 and SNHG1 also facilitates enhanced aerobic glycolysis in glioma through enhancing expression of several glycolytic genes by sequestration of their miRNA targets [19–22].

After the entry of glucose into the cancer cells, the next step is the glycolysis. Hexokinase II (HKII) is the first major enzyme in the glycolytic pathway that catalyzes the conversion of glucose to glucose-6-phosphate. In hepatocellular carcinoma, lncRNA TUG1 enhances miR-455-3p expression by downregulating p21 levels, which acts as transcriptional repressor of miR-455-3p. The elevated levels of miR-455-3p inhibits its target AMPKβ2 (5' AMP activated protein kinase beta 2) mRNA that activates p-mTOR (mammalian target of rapamycin) and subsequent downstream target HKII. This promotes aerobic glycolysis in hepatocellular carcinoma [23]. Furthermore, in gall bladder cancer, lncRNA PVT1 sequesters miR-143 to augment the expression of the miR target HKII, facilitating enhanced glycolysis and tumorigenesis [24]. Hypoxia induced lncRNA HOTTIPP expression in non-small cell lung cancers sequesters miR-615-3p to upregulate HMGB3 (high mobility group box 3) expression that regulates HKII expression as well as glucose uptake, lactate secretion and aerobic glycolysis in these cancer cells [25]. LncRNA TUG1 promotes HKII expression and aerobic glycolysis in osteosarcoma [26]. In bladder cancer cells, lncRNA UCA1 regulates the expression of HKII to facilitate aerobic glycolysis through two independent mechanisms. One is through promotion of HKII transcription via mTOR-STAT3 (signal transducer and activator of transcription 3) signaling and the other is via sequestration of miR-143 and resultant upregulation of miR target HKII [27]. UCA1 has been reported to upregulate HKII expression by sequestering miR-203 in oesophageal cancers, as well [28]. Furthermore, UCA1 enhances radioresistance in cervical cancer cells through promotion of HKII/glycolysis signaling pathways [29]. In colorectal adenocarcinoma, lncRNA RAD51-AS1 is downregulated, which otherwise sequesters miR-29b-3p and miR-29c-3p and upregulates NDRG2 (N-Myc downstream regulated gene 2) expression. During cancerous conditions NDRG2 is downregulated and it enhances expression of GLUT1 and HKII favouring aerobic glycolysis [30]. Furthermore, lncARSR sequesters miR-34a-5p to upregulate HKII expression and subsequent aerobic glycolysis to facilitate tumor progression in colorectal cancers, both *in vitro* and *in vivo* [31]. In hepatocellular carcinoma, lncRNA HOTAIR sequesters miR-130a-3p to upregulate HIF-1α expression, which subsequently promotes transcription of HKII to favour aerobic glycolysis [32].

The next steps in glycolysis involves isomerization of glucose-6-phosphate to fructose-6-phosphate by GPI (glucose-6-phosphate isomerase). Fructose-6-phosphate is phosphorylated to fructose-1,6-bisphosphate by the enzyme phosphofructokinase (PFK). Aldolases splits fructose-1,6-bisphosphate to glyceraldehyde-3-phosphate and dihydroxyacetone phosphate in glycolysis. In ovarian cancers, lncRNA NRCP is overexpressed that binds to STAT1 and RNA Pol II to enhance the expression of glycolysis genes such as GPI as well as aldolases, ALDOA and ALDOC [33]. LncRNA KCNQ1OT1 sequesters miR-34c-5p to promote ALDOA expression and aerobic glycolysis in osteosarcoma [34]. LncRNA LINC00538/YIYA interacts with CDK6 and phosphorylates PFKFB3; active PFKFB3 and HKII promotes aerobic glycolytic pathway in breast cancers [35]. Another lncRNA, BCAR4, transcriptionally induced by yes-associated protein (YAP), also upregulates expression of HKII and PFKFB3 through interaction with GLI2 mediating their transcription to promote glucose uptake and lactate production in triple negative breast cancers (TNBCs) [36].

Enolase (ENO) catalyzes the conversion of 2-phsophoglycerate to phosphoenolpyruvate. In colorectal cancers, the microbiota containing *Fusobacterium nucleatum* activates transcription of lncRNA ENO1-IT1 by enhancing the binding of SP1 transcription factor to its promoter. ENO1-IT1 mediates KAT7, histone acetyltransferase, mediated histone modification to promote the expression of ENO1 that augments the aerobic glycolysis in these cancers [37].

Pyruvate kinase is the rate limiting as well as the final step of glycolysis catalysing the conversion of phosphoenolpyruvate to pyruvate. It is coded by PKM gene which has two isoforms, namely PKM1 and PKM2. PKM2 favours aerobic glycolysis while PKM1 upregulation favours oxidative phosphorylation. In colorectal cancers, lncRNA FEZF1-AS1 binds to and stabilizes the PKM2 expression thereby promoting aerobic glycolysis [38]. In ovarian cancers, lncRNA H19 sequesters miR-324-5p to upregulate PKM2 expression favouring aerobic glycolysis [39]. In non-small cell lung cancers, hypoxic conditions induces lncRNA AC020978 expression that enhances PKM2 expression and activity. Furthermore, PKM2 mediated

HIF-1α transcriptional activity promotes the expression of HIF-1α target genes such as GLUT1, pyruvate dehydrogenase kinase 1 (PDK1), HKII, ENO1 and LDHA that promotes glucose uptake and aerobic glycolysis [40]. In hepatocellular carcinoma, lncRNA LINC01554 is downregulated owing to miR-365a-3p mediated inhibition, which otherwise enhances the proteasomal degradation of PKM2 and lowering its expression to reduce the glycolysis [41]. LncRNA BCYRN1 sequesters miR-149 to upregulate PKM2 expression that favours aerobic glycolysis in non-small cell lung cancers [42]. MALAT1 is overexpressed in hepatocellular carcinoma. MALAT1 activates mTORC1 signaling to activate TCF7L2 (transcription factor 7 like 2) transcription factor that upregulates the transcription of genes involved in aerobic glycolysis such as GLUT1, HKII, ENO1 and PKM2, while reducing the expression of genes coding for gluconeogenesis enzymes such as G6PC (glucose-6-phosphatase) and PCK1 (phosphoenolpyruvate carboxykinase 1) [43]. LncRNA SOX2-OT sequesters miR-195-5p to enhance the expression of HKII, thereby promoting aerobic glycolysis in hepatocellular carcinoma [44].

Pyruvate dehydrogenase kinases (PDK) phosphorylates pyruvate dehydrogenase (PDH) and inhibits its activity, thereby preventing entry of pyruvate to TCA cycle and favouring aerobic glycolysis mediated lactate production for ATP synthesis. In gastric cancers, lncRNA DLX6-AS1 sequesters miR-4290 that targets PDK1. Subsequently, PDK1 expression is upregulated and contributes to enhanced glucose uptake and lactate production [45]. Pyruvate is converted to lactate favouring energy production for rapidly proliferating cells and is catalysed by lactate dehydrogenase (LDH), encoded by LDHA and LDHB genes. LncRNA GLCC1 is upregulated in colorectal cancers. It acts as scaffold for the interaction of HSP90 to c-Myc proteins to stabilize the latter and facilitating c-Myc mediated transcription of LDHA favouring higher glycolysis for meeting energy needs [46]. Similar mechanism have been reported for LINC00504 in promoting glycolysis in colon cancer through c-Myc stabilization [47]. In bladder cancer, lncRNA CASC8 is downregulated, which otherwise binds to FGFR1 and prevents the FGFR1 mediated LDHA phosphorylation that activates LDHA activity [48]. While lncRNA ANRIL enhances GLUT1 and LDHA expression via activation of AdipoR1 pathway in acute myeloid leukemia, it activates the same in nasopharyngeal carcinoma via activation of mTOR signaling pathway [49, 50]. However, lncRNA LINP1 enhances GLUT1 and LDHA expression in acute myeloid leukemia through upregulation of HNF4α (hepatocyte nuclear factor 4 alpha) and AMPK (AMP activated protein kinase)/WNT5A signaling cascades [51]. In hepatocellular carcinoma, under hypoxic conditions, HIF-1α promotes lncRNA RAET1K expression which sequesters miR100-5p to upregulate LDHA expression [52]. In TNBCs lncRNA LINK-A interacts with BRK and LRRK2 kinases to phosphorylate and stabilize HIF-1α that further activates the transcription of glycolytic genes such as ALDOA, PKM2 and LDHA to promote aerobic glycolysis [53]. In lung cancer cells, lnc-IGFBP4-1 enhances the expression of glycolytic enzymes HKII, PDK1 and LDHA to promote aerobic glycolysis for tumor progression [54]. In colorectal cancer lncRNA LINRIS interacts with IGF2BP2 (insulin growth factor 2 binding protein 2) and prevents its ubiquitin mediated degradation. IGF2BP2 then binds to c-Myc and stabilizes it further to enhance the transcription of c-Myc regulated glycolytic genes such as GLUT1, PKM2 and LDHA, thereby promoting aerobic glycolysis for tumorigenesis [55].

Cancer cells maintains optimum lactate fluxes mainly through the monocarboxylate transporters (MCTs). In renal cell carcinoma, lncRNA FILNC1 is downregulated, which otherwise binds to AUF1 transcription factor and downregulate c-Myc expression. Lower FILNC1 levels is correlated with higher c-Myc and upregulation of c-Myc regulated glycolysis target gene expressions such as GLUT1, GLUT3, HKII, ALDOC, MCT4 lactate transporter, PDK1, PDK4. Thus, FILNC1 deficiency promotes glucose uptake and lactate production for energy needs in renal cell carcinoma [56].

The circNRIP1, a circular lncRNA, sequesters miR-149-5p to activate AKT1/mTOR signaling, which enhances glucose uptake, lactate and ATP production, indicative of enhanced glycolysis in gastric cancers [57]. In ovarian cancers, the elevated levels of lncRNA GHET1 induces vHL mediated HIF-1α expression under hypoxic conditions that enhances the glucose uptake and lactate production in these cancer cells [58]. In cetuximab resistant colorectal cancer cells, it has been reported that LINC00973 enhanced the aerobic glycolysis and thereby contributing to chemoresistance in these cells as evidenced by augmented glucose uptake and lactate production [59]. In hepatocellular carcinoma DDX11-AS1-miR-195-5p-MACC1 axis promotes glucose uptake and lactate production [60]. In oesophageal cancers, LINC00184 recruits DNMT (DNA methyl transferase) to PTEN promoter and inhibits PTEN expression. This promotes AKT activity which enhances glucose uptake, lactate production and ATP synthesis [61]. LncRNA PDIA3P interacts with c-Myc and promotes transcription of G6PD (glucose-6-phosphate dehydrogenase), thereby promoting pentose phosphate pathway in multiple myeloma [62].

LncRNAs and Reprogramming of Lipid Metabolism in Cancers

Lipids are one of the major cellular macromolecules as it forms the structural part of plasma membrane, plays role in energy production, affects membrane fluidity, mediates post-translational modifications, acts as signaling mediators and even play cardinal role in pathogenesis of cancers. Though there are diverse classes of lipids, studies on lncRNAs associated with fatty acids, triglycerides, cholesterol and sphingolipids are the major ones linking lncRNAs to reprogrammed lipid metabolism in cancers (**Figure 2**).

Though normal cells prefer exogenous fatty acids, cancer cells prefer *de novo* synthesis of fatty acids as it would act as an energy source for proliferating cancer cells. In addition, they could serve as major source for NADPH in volved in the anti-oxidant response that favor tumorigenesis. Major enzymes involved in fatty acid biosynthesis, utilizing citrate from TCA (tricarboxylic acid) cycle, that are

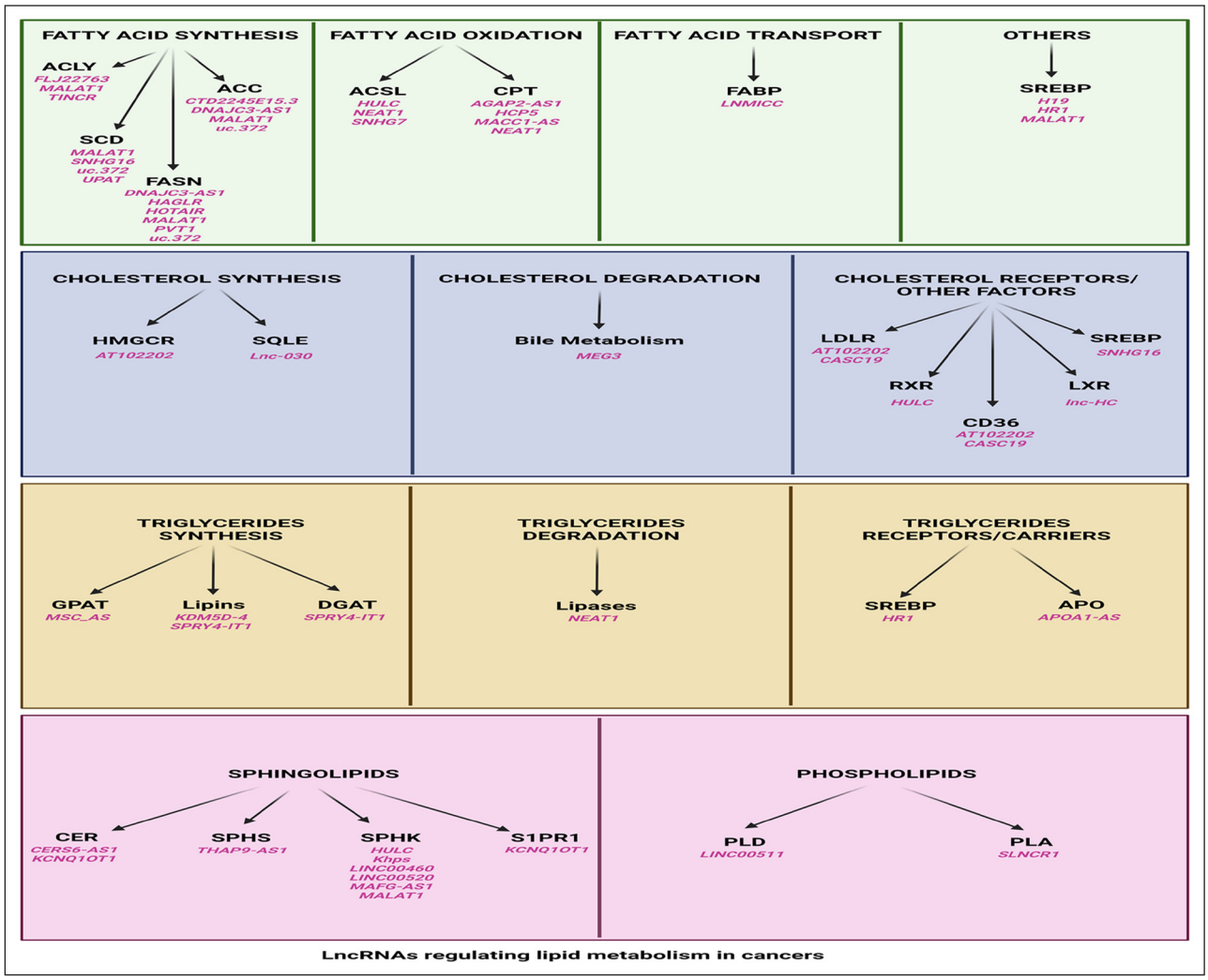

Figure 2: LncRNAs regulating lipid metabolism in cancers. The figure depicts various lncRNAs affecting lipid metabolism in numerous cancers. Lipids undergoing metabolic reprogramming are grouped into four classes namely the fatty acids, the cholesterol, the triglycerides as well as the sphingolipids and the phospholipids. LncRNAs regulate each of these lipid classes in their synthesis, degradation as well as transport and other regulatory stages. (ACLY-ATP citrate lyase; ACC-acetyl CoA carboxylase; FASN-fatty acid synthase; ACSL-acyl CoA synthetase long chain; SCD-stearoyl CoA desaturase; CPT1-carnitine palmitoyltransferase 1; SREBPs-sterol regulatory element binding proteins; HMGCR-3-hydroxy-3-methyl-glutaryl-CoA reductase; FABP-fatty acid binding proteins; SQLE-squalene epoxidase; LDLR-low density lipoprotein receptor; LXR-liver X receptor; RXR-retinoid X receptor; APO-apolipoproteins; GPAT- glycerol-3-phosphate acyltransferase; DGAT-diacylglycerol acyl transferase; CER-ceramidases; SPHK-sphingosine synthase; SIPR1-sphingosine-1-phosphate receptor 1; SPHS-sphingosine synthase; PLD-phospholipase D; PLA-phospholipase A).

upregulated in cancers includes ATP citrate lyase (ACLY), acetyl coA carboxylase (ACC), fatty acid synthase (FASN) and acyl CoA synthetase-long chain (ACSL). Initial step of fatty acid biosynthesis is the conversion to citrate to acetyl CoA in the mitochondria by ACLY enzyme. LncRNA TINCR is upregulated in nasopharyngeal carcinoma, which binds to ACLY to prevent its ubiquitin mediated proteasomal degradation, thereby enhancing fatty acid synthesis and proliferation, chemoresistance and metastasis in these cancers [63]. In human gastric cancer cell lines, it has been reported that lncRNA FLJ22763 is downregulated, which otherwise reduces the ACLY expression and thereby fatty acid biosynthesis. However the exact mechanism is yet to be elucidated [64]. Acetyl CoA is carboxylated to malonyl-CoA in the next step for fatty acid biosynthesis that is catalysed by ACC, which has two isoforms namely ACC1 and ACC2. While ACC1 promotes fatty acid synthesis, ACC2 inhibits fatty acid oxidation. While lncRNA DNAJC3-AS1 activates PI3K/AKT pathway to enhance expression of ACC and FASN in colorectal cancers, lncRNA CTD2245E15.3 activates ACC1 activity by preventing inhibitory phosphorylation at Ser-117 site of ACC1 in non-small cell lung cancers to promote lipogenesis [65, 66]. FASN is the major enzyme involved in synthesis of palmitate in a NADPH dependent mechanism. FASN catalyzes the conversion of acetyl CoA and malonyl coA to palmitate. In human osteosarcoma U2OS cells, the upregulated PVT1 levels sequester miR-195 to enhance FASN expression and favour de novo fatty acid biosynthesis enabling cancer cell proliferation, attenuation of apoptosis

and tumor progression [67]. Over-expression of lncRNAs HAGLR and HOTAIR in non-small cell lung cancers and nasopharyngeal carcinoma respectively has been reported to upregulate FASN expression and fatty acid biosynthesis, which was correlated with higher free fatty acid levels in these cancers promoting tumor progression through an unknown mechanism [68, 69]. Yet another enzyme involved in synthesis of monounsaturated fatty acids is SCD (stearoyl-CoA desaturase) that has two isoforms, namely SCD1 and SCD5. It has been reported that lncRNA SNHG16 promotes c-Myc mediated SCD expression by acting as a competing endogenous RNA to sequester miRNA that targets SCD by forming complex with Ago-HuR proteins in colorectal cancers [70]. In hepatocellular carcinoma, overexpression of lncRNA uc.372 prevents maturation of miR-195 and miR-4668 by binding to their pri-miRNAs. This further relieves the inhibition of these miRNAs on their targets leading to upregulated target expressions such as ACC and FASN for miR-195 and SCD1 and CD36 for miR-4668 contributing to fatty acid synthesis and tumorigenesis [71]. In colon cancers, the lncRNA UPAT is upregulated which binds to UHRF1 (ubiquitin like with PHD and ring finger domains 1) and prevents the ubiquitin mediated proteasomal degradation to enhance UHRF1 stability. SCD1 is a downstream target of PAT and UHRF1, the regulation of which is yet to be elucidated [72]. Fatty acid transporters such as FABPs (fatty acid binding proteins) are also deregulated by lncRNAs to promote fatty acid metabolism in cancers. The lncRNA LNMICC, which is upregulated in cervical cancers, recruits NPM1 (nucleophosmin 1) transcription factor to FABP5 promoter to enhance its expression. Higher levels of FABP5 have shown to promote epithelial to mesenchymal transition (EMT) and metastasis in these cancers through reprogramming of fatty acid metabolism [73]. Not just the fatty acid biosynthesis, lncRNAs also regulate fatty acid oxidation. Fatty acid oxidation produces acetyl CoA, which in turn, produces NADPH through TCA cycle and coupled oxidative phosphorylation. NADPH acts as antioxidant as well as source of energy for the cancer cells. For these, fatty acids are initially converted to fatty acyl CoA by ACSL isoforms that facilitate their entry into mitochondria for oxidation. The upregulated HULC levels in hepatocellular carcinoma induces methylation on the promoters of miR-9, thereby attenuating its expression. Subsequently miR-9 target PPAR-α (peroxisome proliferator-activated receptor alpha) is upregulated, which acts as a transcription factor to enhance the expression of ACSL1 that favours conversion of fatty acids to fatty acyl CoA and fatty acid oxidation that promotes cancer progression [74]. In thyroid cancers, lncRNA SNHG7 is upregulated, which sequesters miR-449a and enhances the expression of miR target ACSL1, thereby promoting fatty acid oxidation in thyroid cancer cells [75]. In docetaxel resistant prostate cancer cells, NEAT1 levels are upregulated. NEAT1 sponges miR-34a-5p and miR-204-5p, which otherwise targets ACSL4 expression. Upregulated NEAT1 and ACSL4 levels contribute to proliferation, migration and docetaxel resistance in these cancers through promoting fatty acid oxidation [76]. CPT1 (carnitine palmitoyltransferase 1) is another major enzyme that regulates fatty acid oxidation and subsequent ATP-NADPH generation process. The mesenchymal stem cell induced lncRNAs HCP5 and MACC1-AS functions through miRNA sequestration to upregulate CPT1 expression in gastric cancer cells. HCP5 sequesters miR-3619-5p and upregulates its target, the transcription factor, PGC-1α (PPARγ coactivator 1 alpha), which further enhances CPT1 expression by activating PGC-1α-CEBPB (CCAAT enhancer binding protein beta) complex at the CPT1 promoter [77]. However, it has also been reported that mesenchymal stem cell secreted TGF-β1 (transforming growth factor-β1) acts through TGF-β receptor and SMAD2/3 pathway to upregulate the lncRNA MACC1-AS that sequesters miR-145-5p to upregulate the expression of miR target, CPT1 [78]. In both these events, CPT1 upregulation, enhances fatty acid oxidation and contributes to stemness as well as chemoresistance in gastric cancers. Furthermore the mesenchymal stem cells induce lncRNA AGAP2-AS1 in breast cancers which enhances CPT1 expression in two ways. One is by stabilizing the CPT1 mRNA through AGAP2-AS1-HuR complex, while the other is by sequestering miR-15a-5p to upregulate CPT1 expression, thereby promoting stemness and trastuzumab resistance in breast cancers [79]. Furthermore, in breast cancer cells, lncRNA NEAT1 sequesters miR-107 to upregulate CPT1 expression to promote fatty acid oxidation and synthesize ATP for energy needs favouring growth and metastasis in breast cancer cells [80]. LncRNAs also effect the expressions of fatty acid synthesis regulatory molecules such as SREBPs (sterol regulatory element binding proteins). SREBP-1a and -1c enhances fatty acid biosynthesis. LncRNAs H19 and MALAT1 are reported to enhance the stability of SREBP-1c and thereby lipid accumulation. H19 acts as a scaffold for the binding of SREBP-1c mRNA to PTBP1, which enhances the stability of SREBP-1c as well as its transcriptional activity in hepatocytes [81]. However, MALAT1 enhances nuclear SREBP-1c protein stability by direct interaction through the ubiquitin mediated proteasome signaling in hepatoma. Subsequently SREBP-1c targets such as SCD1, FASN, ACC1 and ACLY are upregulated that promotes fatty acid biosynthesis and insulin resistance [82]. In addition, lncRNAs HR1 and Gm16551 have been identified as repressors of SREBP-1c that adversely affects fatty acid biosynthesis in hepatic cells; however their roles in human cancers are yet to be elucidated [83, 84].

Cholesterol and triglycerides are the next class of lipids, which mainly acts as storage lipids and are cardinal for energy production as well as signaling processes. HMGCR (3-hydroxy-3-methyl-glutaryl-CoA reductase) catalyzes the first rate limiting step in cholesterol biosynthesis which utilizes acetyl CoA to synthesize mevalonate. Studies on hepatocyte cell line, HepG2, shows that knocking down of lncRNA AT102202 enhances HMGCR expression and cholesterol synthesis [85]. SREBP2 is the transcription factor that facilitates HMGCR and LDL (low density lipoprotein) receptor expression. LncRNA SNHG16, which is upregulated in pancreatic cancers, sequesters miR-195 and enhances the expression of SREBP2, thus promoting cholesterol metabolism for energy needs [86]. Mevalonate

is converted sequentially to isoprenoids and then to squalene, which cyclizes to lanosterol by action of SQLE (squalene epoxidase). Lanosterol acts as the immediate precursor for cholesterol biosynthesis. In breast cancer stem cells, lnc-030 binds to PCBP2 (poly rC-binding protein 2) to stabilize the SQLE mRNA, which enhances cholesterol biosynthesis as well as downstream PI3K/AKT signaling pathways to facilitate stemness [87]. The upregulated levels of lncRNA HULC acts through miR-9-PPARA-ACSL1 axis to enhance cholesterol biosynthesis, which acts as a feedback loop for enhancing HULC expression through activation of RXRA (retinoid X receptor alpha) receptors in hepatocellular carcinoma [74]. LDL receptors are involved in the exogenous cholesterol uptake into the cells during cholesterol insufficiency. In non-small cell lung cancers, the elevated levels of lncRNA CASC19 sequesters miR-301b-3p to upregulate LDL receptor expression that promotes exogenous cholesterol uptake by the cancer cells favouring cancer cell proliferation and metastasis [88]. In addition, cholesterol efflux transporters mediate cholesterol transport from the cells, which are anti-tumorigenic signals. Under conditions of surplus cholesterol, LXRs (liver X receptors) are activated that promote the expression of ABC transporters to facilitate cholesterol export. High cholesterol levels in hepatocytes induces transcription factor CEBPB mediated upregulation of lncRNA-HC, which in turn binds to ribonucleoprotein, hnRNPA2B1 to form a complex. This complex binds to mRNA of CYP7A1 or ABCA1 (ATP binding cassette transporter A1) transporters that facilitate cholesterol efflux from the cells [89]. In addition to the cholesterol export, excess cholesterol accumulation in cells are resolved by conversion to bile acids mediated by bile acid sensor, FX receptors and its target genes involved in synthesis of bile acids, such as CYP8B1 and CYP7A1. Cholestasis is a pathological condition seen during liver diseases, including liver cancers, where in defective bile acid secretion results in its accumulation in liver leading to liver injury. In hepatocellular carcinoma, lncRNA MEG3 is downregulated, which otherwise would acts as a scaffold for the binding of PTBP1 (polypyrimidine tract binding protein 1) with SHP (small heterodimer partner) mRNA, leading to the SHP mRNA decay. SHP attenuates bile acid synthesis and prevents cholestasis. However, since MEG3 levels are low, it leads to enhanced SHP levels which enhances bile acid synthesis and cholestasis, as shown in liver cancers, both *in vitro* and i*n vivo*. Furthermore, higher SHP levels prevents the binding of CREBP (cAMP response element binding protein) to the MEG3 promoter to inhibit the MEG3 expression, reciprocally [90]. Though direct studies reporting reprogramming of cholesterol transport in cancers are still under research, several studies have correlated the roles of prominent oncogenic lncRNAs upregulated in cancers and their roles in regulating this factor in significant cellular components of cancer milieu such as monocytes, macrophages or even human hepatocytes, which is correlated with enhanced cholesterol metabolism and associated inflammations. One of the oncogenic lncRNAs mostly upregulated in cancers is NEAT1. In a study on human macrophage cell line THP-1, NEAT1 has been reported to play role in cholesterol metabolism. Oxidized LDL induces NEAT1 expression and paraspeckle formation which is mediated through p38 and NF-κB signaling pathways. Furthermore, NEAT1 also mediates the oxidized LDL induced TNFα (tumor necrosis factor alpha) secretion through regulation of MAPK (mitogen activated protein kinases) and NF-κB (nuclear factor-κB) pathways as well as adversely affecting the CD36 expression by affecting mRNA stability. These events inhibit the lipid uptake by macrophages [91]. In addition, in human monocyte derived macrophages, the upregulated levels of lncRNA RP11-728F11.4 promotes FXYD6 (FXYD domain containing ion transport regulator 6) expression, which enhances the CD36 levels and thereby the lipid uptake and accumulation in these cells [92].

Triglycerides are prominent lipid molecules serving as storage lipids as well as signaling intermediates. Fatty acids are converted sequentially to fatty acyl CoA, LPA (lysophosphatidic acid), phosphatidate, diacylglycerol and then finally to triglycerides. Fatty acyl CoA is converted to LPA by GPAT (glycerol-3-phosphate acyltransferase) enzyme. In lung adenocarcinoma, lncRNA MSC-AS sequesters miR-33b-5p to upregulate the miR target GPAT and facilitate triglyceride synthesis [93]. Conversion of phosphatidate to diacylglycerol is catalysed by phosphatidic acid phosphohydrolases (PAPs) or lipins. Diacylglycerol and fatty acyl CoA are then esterified to triglycerides by diacylglycerol acyl transferase (DGAT). LncRNA SPRY4-IT1 is upregulated in melanomas, which inhibits the activity of lipin2 as well as DGAT2, thereby lowering the triglyceride synthesis in these cancer cells. The study also shows that silencing of SPRY4-IT1 enhances lipid accumulation and lipotoxicity mediated apoptosis in these cancer cells [94]. In hepatocellular carcinoma, silencing of lnc-KDM5D-4 enhances lipin2 activity favouring lipid biosynthesis, though the mechanism is yet unknown [95]. The regulatory elements SREBP-1c also effects triglyceride levels in cancer cells. Exogenous supplementation of lncRNA HR1 in liver cancer cells inhibited AKT phosphorylation and resultant translocation of FOXO1 (forkhead box O1) from nucleus. Nuclear accumulation of FOXO1 antagonizes LXR elements in SREBP-1c promoter to downregulate its expression and prevent triglyceride synthesis as well as accumulation for energy supply to inhibit tumor progression [96]. Triglyceride degradation to free fatty acids are carried out by lipases namely ATGL (adipose triglyceride lipase), HSL (hormone sensitive lipase) and MAGL (monoacylglycerol lipase). In hepatocellular carcinoma, lncRNA NEAT1 is upregulated that sequesters miR-124-3p to enhance expression of ATGL and promote triglyceride catabolism in cancers [97]. Apolipoproteins (APO) are class of lipid binding proteins in circulation, and APOA1 is one of the prominent high density lipoproteins. In hepatocellular carcinoma cells, lncRNA APOA1-AS mediates interaction between SUZ12 and APO gene favouring H3K27 trimethylation and upregulation of APOA1 expression [98].

Sphingolipids are important structural components of plasma membrane and their metabolites such as sphingosine-1-phosphate and ceramides serve as signaling intermediates. Conversion of dihydrosphingosine to

dihydroceramide is catalysed by CERSs (ceramide synthases). In breast cancers, the anti-sense lncRNA CERS6-AS1 binds to IGF2BP3 (insulin like growth factor 2 binding protein 3) to maintain the stability of CERS6 mRNA [99] and enhances CERS6 activity promoting tumor progression [99]. Ceramides can be converted to sphingomyelin by sphingomyelin synthase. In oesophageal squamous carcinoma, elevated levels of lncRNA THAP9-AS1 sequesters miR-335-5p to upregulate sphingosine synthase 2 expression favouring tumor progression [100]. Ceramide can be converted to sphingosine-1-phosphate by ceramidases. In hepatocellular carcinoma, lncRNA KCNQ1OT1 sequesters miR-146a-5p to upregulate ACER3, (alkaline ceramidase 3) expression to attenuate apoptosis and radiosensitivity in these cancer cells [101]. Furthermore, in hepatocellular carcinoma, lncRNA KCNQ1OT1 sequesters miR-149 to upregulate S1PR1 (sphingosine-1-phosphate receptor1) expression that facilitates migration and invasion of cancer cells, *in vitro* and *in vivo* [102]. In addition to ceramidases, sphingosine kinases (SPHK) also convert ceramide to sphingosine-1-phosphate. Several lncRNAs regulate SPHK expressions through sequestration of miRNAs in diverse cancers. While lncRNA MALAT1 sequesters miR-124-3p in osteosarcomas, lncRNA MAFG-AS1 sequesters miR-125b-5p in bladder cancer to upregulate SPHK1 expression [103, 104]. LncRNA Khps, which is antisense to SPHK1 is upregulated in osteosarcoma. Khps binding upstream to SPHK1 promoter that facilitates the binding of p300/CBP (CREB binding protein) histone acetyl transferase complex to the promoter site and open the chromatin. This leads to transcription of both Khps and SPHK1 favouring tumor progression [105]. Furthermore, in hepatocellular carcinoma, lncRNA HULC sequesters miR-107 to upregulate transcription factor E2F1 expression and facilitate its recruitment to SPHK1 promoter to augment its expression that favours angiogenesis [106]. LncRNA LINC00460 sequesters miR-613 to upregulate SPHK1 expression in colorectal cancers [107]. However, in papillary thyroid carcinoma, LINC00460 and LINC00520 sequesters miR-613 and miR-577 respectively to upregulate SPHK2 expression [108, 109].

Phospholipid metabolism are also regulated by lncRNAs in cancers. Phospholipases are enzymes that catalyse the hydrolysis of acyl and phosphate esters of numerous phospholipids. Phospholipase D (PLD) hydrolyses phosphatidyl choline to phosphatidic acid. In cervical cancers, LINC00511 facilitates binding of RXRA transcription factor to PLD1 promoter, to upregulate PLD1 expression, thereby favouring cancer cell proliferation [110]. Phosphatidic acid is further hydrolysed to free fatty acids and lysophospholipids by phospholipase A1 (PLA2). In non-small cell lung cancers, lncRNA SLNCR1 interacts with secretory PLA2 to regulate migration and invasion of cancer cell lines [111].

LncRNAs and Reprogramming of Amino Acid Metabolism in Cancers

Several lncRNAs coordinate the reprogramming of amino acid metabolism in tumor cells (**Figure 3**). Amino acids serve as the building blocks of proteins. They form the essential components of diverse signaling cascades that facilitate the maintenance of cellular homeostasis as well as disease pathogenesis. Amino acids are transported into the cells through membrane bound transporters. In cancers, amino acid metabolism as well as the levels of amino acid transporters are altered to favour tumorigenesis. Amino acids also serve as an alternate source of energy for the rapidly proliferating cancer cells. Glutamine is one of the most abundant amino acids in the human body. Cancer cells utilize higher amounts of glutamine as its serves as an energy source, helps nucleotide synthesis as it serves as source of nitrogen, facilitates synthesis of non-essential amino acid biosynthesis and also serves as source of carbon source for entering into TCA cycle. Exogenous glutamine enters into the cells through ASCT2 transporters, which are then deaminated to glutamate by glutaminase (GLS) enzyme. There are two isoforms namely GLS1 or KGA (glutaminase kidney isoform) and GLS2 or GAC (glutaminase isoform C). Glutamate catabolism is further carried out by glutamate dehydrogenase (GDH) or glutamate transaminases, namely GPT (glutamate pyruvate transaminase) and GOT (glutamate oxaloacetate transaminase). These reactions are accompanied with production of NADH, NADPH, ammonium and biosynthesis of non-essential amino acids. Glutamine also serves as substrate for glutathione synthesis that mediates ROS homeostasis. Hence the major reprogramming within the amino acid metabolism occurs for glutamine in cancer cells.

GLS is one of the most deregulated enzymes in cancers that affects glutamine metabolism. In prostate cancers, lncRNA PCGEM1 acts as a co-activator of c-Myc to transcriptionally induce the GLS expression, which favours the deamination of glutamine to glutamate for tumorigenesis [15]. Furthermore, lncRNA CCAT2 has been identified of exhibiting allele specific activity in regulating the alternate splicing of GLS in colorectal cancers. CCAT2 acts as a scaffold to facilitate the interaction between GLS pre-mRNA with cleavage factor CFIm. This enhances the alternate splicing favouring the production of GAC isoform over KGA to promote cancer cell proliferation and metastasis in colorectal cancers, both *in vitro* and *in vivo* [112]. The role of lncRNA HOTTIPP in regulating glutamine metabolism has been studied in hepatocellular carcinoma. The study reports that miR-192 and miR-204 antagonizes HOTTIP and attenuates its functions. However, HOTTIPP, which is upregulated in hepatocellular carcinoma, also regulates GLS1 expression to enhance glutaminolysis in this cancer favouring tumorigenesis [113]. The circular lncRNA circ0000517 is upregulated in non-small cell lung cancer which sequesters miR-330-5p to upregulate YY1 (ying yang 1) protein and thereby upregulate the GLS expression to promote glutaminolysis in non-small cell lung cancer [114]. Similarly circ-PITX1 also induced enhanced glutaminolysis in non-small cell lung cancers as evidenced by the extracellular acidification rates by seahorse metabolic assays [115]. LncRNA HOTAIR is upregulated in glioma and it sequesters miR-126-5p to enhance the GLS expression that favour glutaminolysis and tumor progression. In addition, since glutamate acts as precursor for glutathione, it enhanced glutathione production,

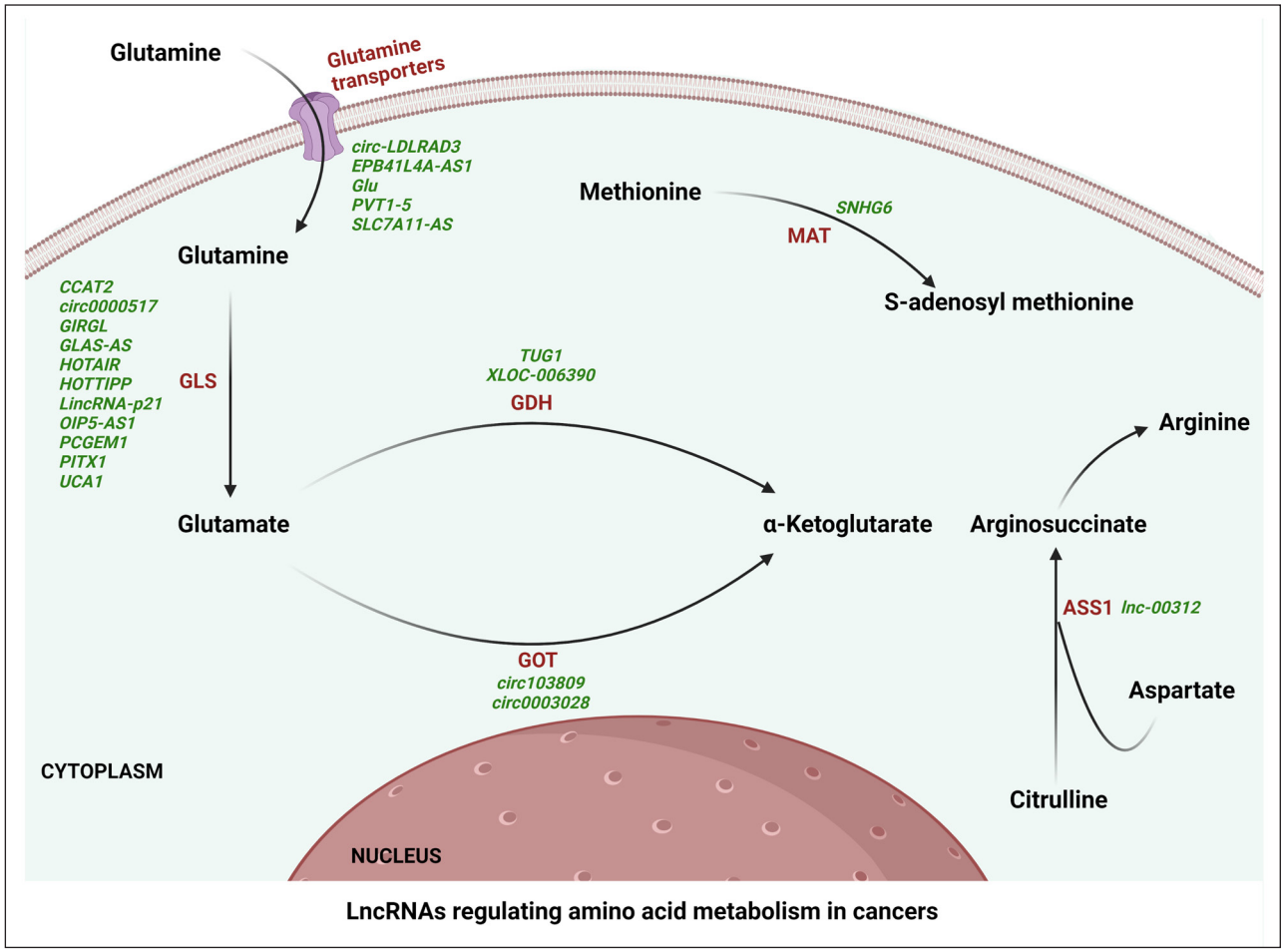

Figure 3: LncRNAs regulating amino acid metabolism in cancers. The figure presewnts an overviee of various lncRNAs that affect the metabolic reprogramming of amino acids namely glutamine, arginine and methionine in cancers. (ASS1-arginine succinate synthase 1) GDH-glutamate dehydrogenase; GLS-glutaminase; GOT-glutamine oxaloacetate transferase; MAT-methionine adenosyltransferases).

as well [116]. Furthermore, lncRNA UCA1 plays cardinal role in reprogramming the glutamine metabolism in bladder cancers. The upregulated UCA1 sequesters miR-16 and enhances the expression of miR-16 target GLS2, which in turn favours ROS homeostasis, redox balance and promotion of mitochondrial glutaminolysis [117]. LincRNA-p21 is a tumor suppressor which is downregulated in bladder cancers. Though the exact mechanism is not known yet, exogenous supplementation of lincRNA-p21 lowers GLS levels and glutaminolysis, thus regulating the glutamine metabolism [118]. The antisense lncRNA, OIP5-AS1 promotes glutaminolysis in melanoma by sequestering miR-217, thereby upregulating the miR target GLS. Thus, OIP5-AS1 enhances glutamate, α-ketoglutarate and ATP levels, in addition to glutamine consumption in melanoma [119].

Certain lncRNAs express during glutamine stress conditions to help the cancer cells to adapt during the stress by downregulating GLS expression. Two such lncRNAs are GLAS-AS and GIRGL. During glutamine deficiency or stress in pancreatic cancers, the lncRNA GLS-AS, which is antisense to GLS is downregulated. GLS-AS, otherwise, inhibits GLS by binding to the mRNA post-transcriptionally. It also attenuates GLS-c-Myc binding which affects c-Myc stability adversely. Under conditions of glutamine stress, c-Myc cannot bind to GLS-AS promoter to attenuate GLS-AS expression. Thus, GLS-AS-GLS-c-Myc axis acts reciprocally to favour the tumor progression under conditions of glutamine stress in pancreatic cancers [120]. In colorectal cancers, under conditions of glutamine deficiency, c-Jun mediated upregulation of lncRNA GIRGL occurs. GIRGL binds to stress protein CAPRIN1 (cell cycle associated protein 1) thereby affecting the stability of GLS1 mRNA by inducing liquid-liquid phase separation of this complex into stress granules. These processes favours cancer cells to adapt to glutamine deficiency [121].

Glutamine metabolism also involves activity of GOT1 and GOT2 enzymes as these transaminases help in the production of α-ketoglutarate which supplements resources to TCA cycle. The circ-103809 and circ-0003028 sequesters miR-377-3p and miR-1298-5p to upregulate the expressions of GOT1 and GOT2 respectively in non-small cell lung cancers to promote glutamine metabolism favouring tumor progression [122, 123]. GDH is also regulated by lncRNAs. In intrahepatic cholangiocarcinoma, the lncRNA TUG1 is upregulated, which sequesters miR-145. This in turn enhances the expression of miR-145 target SIRT3 (sirtuin 3) which augments the GDH activity and thereby glutamine consumption as well as ATP production to promote cancer [124]. LncRNA XLOC-006390-c-Myc axis regulates

GDH expression in pancreatic cancers. This lncRNA interacts with c-Myc to enhance its stability by preventing ubiquitin mediated degradation, thus making c-Myc available at the promoter of GDH to enhance the GDH expression and glutaminolysis in pancreatic cancers [125].

Glutamine or glutamate transporters are also regulated by lncRNAs to favour deregulated glutamine metabolism in cancers. LncRNA EPB41L4A-AS1 is downregulated in liver cancers and is regulated by p53 and PGC-1α (peroxisome proliferator activated receptor gamma coactivator 1-alpha). Lower expression of this lncRNA or its deletion in cancers renders HDAC2 (histone deacetylase 2) free of its interaction with EPB41L4-AS1in nucleolus and facilitates its entry to nucleoplasm. HDAC2 then epigenetically repress vHL and VDAC1 (voltage dependent anion channel 1) expressions through histone modifications. ROS was increased, which leads to transcriptional activation of ATF4 (activating transcription factor 4) /P-eIF2α and subsequent enhancement of SNAT5 (system N amino acid transporter 5) glutamine transporters. These events enhances glutamine consumption by the cancer cells, increases glutaminolysis and glutamine dependency. Other than SNAT5, upregulation of transporter ASCT2 (alanine serine cysteine transporter 2), GLS as well as ME1/2 were also observed that contributed to altered glutamine metabolism. Furthermore, the study has also shown that depletion of this lncRNA enhances sensitivity of these cancers to GLS inhibitors [126]. The circ-LDLRAD3 lncRNA enhances glutamine metabolism by sequestering miR-137 to upregulate SLC1A5, mitochondrial glutamine transporter expression. This favours glutamine dependent ATP synthesis and glutathione production favouring tumor progression in non-small cell lung cancers [127]. In addition, lncRNAs PVT1-5 and MINCR also exhibit similar mechanism of action by sequestering miR-126 to upregulate glutamine transporter SLC7A5 to promote glutaminolysis in lung cancers and non-small cell lung cancers respectively [128, 129]. In triple negative breast cancers, 17-β estradiol activates the G-coupled estrogen receptor to downregulate lncRNA Glu expression, which otherwise would bind to VGLUT2 (vesicular glutamate transporter 2). Lnc-Glu binds to VGLUT2 to inhibit the latter and thereby attenuating glutamate secretion from the TNBC cells. However, in TNBCs, active VGLUT2 promotes glutamate secretion to promote cancer cell invasion and metastasis [130].

Yet another amino acid whose metabolism is deregulated in cancers is arginine. Arginine succinate synthase 1 (ASS1) is the central enzyme indispensable for arginine synthesis, regulation of the urea cycle and in addition, regulation of aspartate to urea conversion. In renal cell cancers, lncRNA00312 is downregulated, which otherwise sequesters miR-34a-5p to upregulate ASS1 expression [131]. The amino acid cysteine acts as rate-limiting factor for glutathione synthesis and hence is cardinal for maintaining redox balance in cancer cells. One of the major transporters for extracellular cysteine is SLC7A11, which also transports the intracellular glutamate, thus favouring glutathione synthesis. The antisense lncRNA SLC7A11-AS is downregulated in epithelial ovarian cancers, which otherwise would inhibit the SLC7A11 expression. The higher levels of SLC7A11 contributes to enhanced redox homeostasis through glutathione production and also regulates glutamine metabolism in cancer cells to favour tumor progression [132]. LncRNAs also regulate the methionine cycle in cancers. LncRNA SNHG6 sequesters miR-1297, thereby upregulating the expression of miR target MAT2A, which codes for methionine adenosyltransferases in hepatocellular carcinoma. These enzymes catalyse the synthesis of S-adenosylmethionine through methionine cycle affecting global genome methylation that favours hepatocarcinogenesis [133].

LncRNAs and Reprogramming of Nucleotide Metabolism in Cancers

Nucleotides that includes both purines and pyrimidines constitute the genomic material are cardinal for the uncontrolled cell proliferation seen in cancers. Though there are significant advances in research on reprogramming on glucose or lipid or amino acid metabolisms in cancer, the data on nucleotide metabolic reprogramming is scarce. Similar to the interconnected glucose/lipid/amino acid metabolisms in cancer, certain reports have also shown how amino acid and nucleotide metabolisms are in par with each other. It has been shown that under conditions of glutamine starvation, in glioblastoma, the upregulation of glutamine synthetase provides glutamine prototrophy to the cells and enhances the purine biosynthesis, favouring de novo nucleotide synthesis for cancer cell proliferation. This has been proved by the *in vitro*, orthotopic glioblastoma model as well as patient data [134]. It has been reported that over 30% of cytoplasmic glutamine undergoes catabolism to glutamate that aids nucleotide biosynthesis. Another study explains an elaborated genome scale metabolic model created by analysis of uptake and release fluxes in the cellular level proves that enhanced release of cytoplasmic glutamate to the ECM enhances the nucleotide metabolism, especially the pyridine biosynthesis in liver cancers to sustain their cellular proliferation and tumor growth [135]. These data serves as a treatise on predicting the possible roles of those lncRNAs affecting GS expression to regulate nucleotide metabolism as well (**Figure 4**).

Thymidylate synthase (TS) acts as a major enzyme in DNA synthesis by catalysing the rate limiting step involving reductive methylation of dUMP to dTMP. In colorectal cancers, lncRNA XIST is upregulated and induces the expression of TS enzyme through an unknown mechanism, so as to enhance pyrimidine biosynthesis facilitating DNA replication in the proliferating cancer cells. The higher levels of XIST induced TS also confers resistance to 5-FU therapy in these cancers [136]. In addition, the lncRNA TUG1 also enhances TS expression by sequestering miR-197-3p and thereby conferring 5-FU resistance in colorectal cancer cells [137]. In colorectal cancers, yet another mechanism was shown wherein lncRNA HOTAIR epigenetically silenced miR-218 expression to activate VOPP1 (vesicular over-expressed in cancer pro-survival protein 1) expression and NF-κB signaling. These subsequently enhanced TS expression to confer 5-FU resistance in these cancers

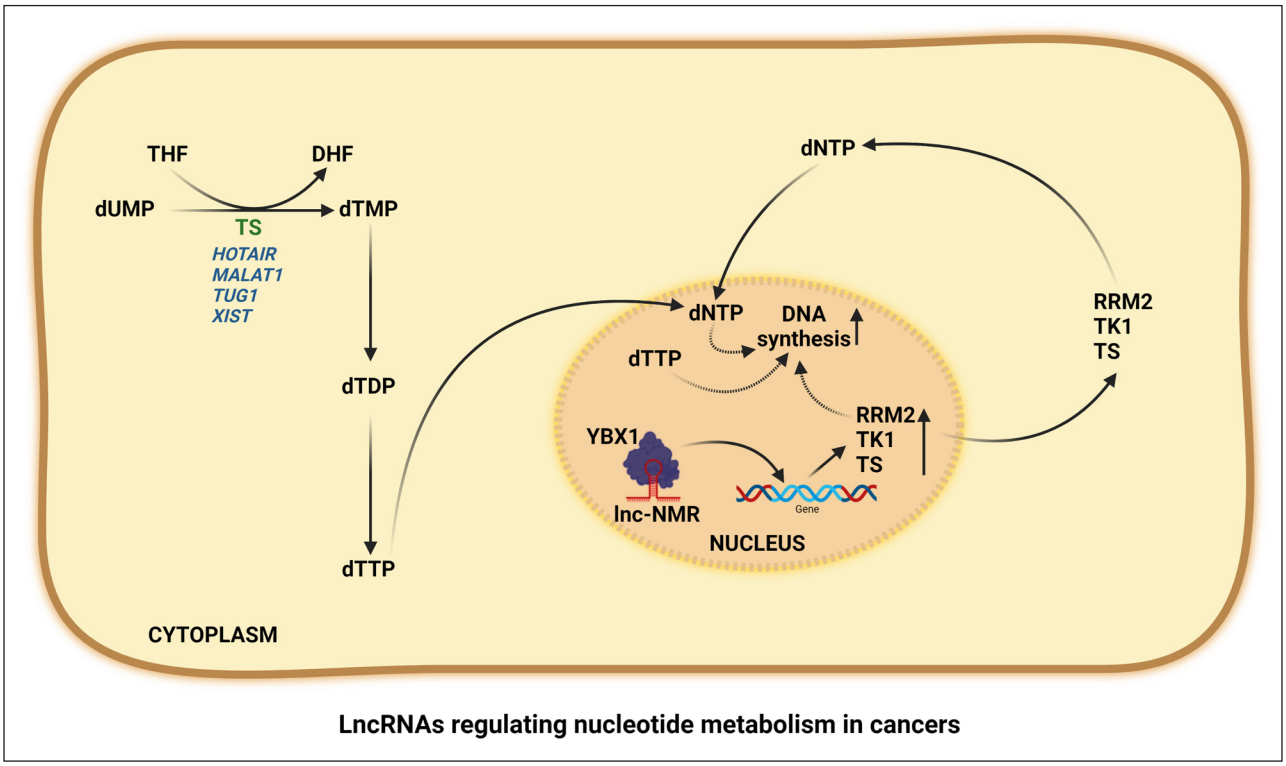

Figure 4: LncRNAs regulating nucleotide metabolism in cancers. The figure presents a summary of mechanisms by which different lncRNAs modulate the reprogramming of nucleotide metabolism in cancers. The major enzymes involved in nucleotide metabolism that are regulated by lncRNAs includes TS (thymidylate synthase), TK1 (thymidylate kinase 1) and RRM2 (ribonucleotide reductase subunit 2).

[138]. In glioblastoma multiforme, lncRNA MALAT1 is over-expressed. MALAT1 sequesters miR-203a-3p, thereby upregulating its target TS. This signaling axis confers resistance to temozolomide in glioblastoma [139]. These reports explore the roles of lncRNAs such as XIST, TUG1, HOTAIR and MALAT1 in regulating the TS expression and thereby the nucleotide metabolism in cancers.

An elaborated report on the effect of lncRNAs in regulating the nucleotide metabolism in cancers have been reported through unveiling the role of lncRNA, linc-NMR, in hepatocellular carcinoma [140]. Linc-NMR is upregulated in hepatocellular carcinoma. It interacts with the transcriptional factor, YBX1 (Y-box binding protein 1), and regulates YBX1 activity. Linc-NMR-YBX1 complex is recruited to the promoters of the genes coding for three major enzymes indispensable for nucleotide biosynthesis, namely, RRM2 (ribonucleotide reductase subunit 2), TK1 (thymidylate kinase 1) and TS. Elevated levels of these enzymes increases the dNTP levels in the cancer cells leading to enhanced cell proliferation and tumorigenesis. Thus, linc-NMR-YBX1-RRM2-TK1-TS axis regulates proliferation-senescence axis in hepatocellular carcinoma, which can serve as an ideal therapeutic target for numerous cancers [140].

Conclusion

Cancer cells reprogram their macromolecular metabolism to sustain their uncontrolled proliferation and tumorigenic attributes. The reprogrammed metabolisms serves as a better energy source for the rapidly proliferating cells in cancers. They also enable the cells with surplus metabolic intermediates that can facilitate cellular biosynthesis. LncRNAs form a critical group of regulatory molecules that reprogram various cellular metabolisms for cancer progression. In this review, we have detailed on the role of various lncRNAs in reprogramming of glucose, lipid, amino acid and nucleotide metabolisms in diverse cancers. Ranging from basic research to clinical trials, lncRNAs serve as potent therapeutic targets for cancers. The review provides us a concise knowledge of employing several of these lncRNAs as diagnostic, prognostic or therapeutic cancer biomarkers. Further advancements in employing lncRNAs are therapeutic targets would bring about betterments in personalized cancer therapy.

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Competing Interests

The authors have no competing interests to declare.

Author Contributions

Conceptualization: D.N.D.; Writing—original draft preparation: R.N.; Writing, revising, and editing: D.N.D. and R.N. All authors have read and agreed to the published version of the manuscript.

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Long Non-coding RNAs in Cancer

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Abstract

Long non-coding RNAs (lncRNAs) are emerging as cardinal biomarkers for diagnosis, prognosis, and therapy of cancers as well as precise therapeutic targets against cancers. Through their interactions with multiple macromolecules in the cell, they regulate diverse array of cellular responses. Dysregulated expression and functional regulation of the lncRNAs have been associated with the genesis and progression of many cancers. Their potential to form vast network of interactomes involving DNA, RNA, and proteins enables them to have a complex functional repertoire. LncRNAs localize within the nucleus, cytosol, and other subcellular compartments to execute their functions in regulating tumor genesis and progression. LncRNAs act as molecular signals, guides, scaffolds, and decoys in executing either the tumor-promoting or -suppressing roles within the cancer cells. However, further advancements are required for pre-clinical validations that can warrant lncRNA-based therapeutics for the clinical trials. This book chapter reviews the diverse mechanisms of action, both within the nucleus and the cytoplasm, that enables them to regulate tumorigenesis.

Keywords

Cancer · Oncogene · Tumor suppressor gene · LncRNA · miRNA · Epigenetics · Histone-modification · Chromatin modeling · DNA-methylation · mRNA-splicing · Spliceosomes · Transcription · Translation

Introduction

Cancer genesis, progression, and metastasis involves the synergistic coordinated signaling by different cell types in the tumor microenvironment (TME). Signals that promote tumor growth are propagated by numerous oncogenes as well as tumor suppressor genes. In addition to these classical protein-coding oncogenes and tumor suppressor genes, non-coding RNAs are emerging as major players that regulate multiple facets of cancer pathobiology ranging from tumorigenesis, tumor growth,

metastasis, and leading all the way to therapy resistance. The superfamily of non-coding RNAs include ribosomal RNAs (rRNAs), transfer RNAs (tRNAs), micro RNAs (miRNAs), circular RNAs (circRNAs), small interfering RNAs (siRNAs), small nucleolar RNAs (snoRNAs), piwi interacting RNAs (piRNAs), and long non-coding RNAs (lncRNAs) (Ma et al. 2013). While the mechanisms by which non-coding RNAs such as miRNAs regulate tumor growth are well characterized, the mechanisms by which lncRNAs might regulate tumorigenesis and tumor growth are being realized only now (Ramírez-Moya et al. 2022).

In general, non-coding RNAs that are longer than 200 nucleotides are classified as lncRNAs. Based on their genomic loci, lncRNAs are further classified into different subtypes. They can be either a) intronic lncRNAs that are transcribed from introns of any protein coding genes; b) intergenic lncRNAs that are transcribed from sites in between two protein coding regions; c) overlapping/sense lncRNAs that are transcribed from regions overlapping the protein coding regions; or d) antisense lncRNAs that are transcribed in opposite direction with regard to protein coding sequence (Ma et al. 2013). A recent estimate indicates that in the human genome, 19,951 genes code for proteins and as many as 17,948 genes code for lncRNAs (Camilleri-Robles et al. 2022). Since, a single lncRNA gene locus codes for multiple lncRNA variants, NONCODE database estimates that human genome codes for 102,000 lncRNAs (Zhao et al. 2016). In spite of the fact that they do not get translated into functional proteins, lncRNAs have been shown to be involved in the regulation of multitudinous cellular functions (Nadhan et al. 2022). Aberrant expression of lncRNAs is correlated with the pathobiology of various diseases including cardiovascular diseases, diabetes, neurological disorders, and cancers (Nadhan et al. 2022). In cancer, lncRNAs have been identified as master regulators of the diverse hallmarks of cancer (Nadhan and Dhanasekaran 2021). They serve as cardinal regulators of oncogenesis by modulating the cancer cell proliferation, anti-apoptotic signals, migration, invasion, metastasis, epithelial to mesenchymal transition (EMT), metabolic reprogramming, immune evasion, drug resistance, and maintenance of cancer stemness (Nadhan and Dhanasekaran 2021; Nadhan and Dhanasekaran 2022). Similar to functional protein coding genes, lncRNAs can be classified as either oncogenic lncRNAs or tumor suppressor lncRNAs. In some cases, an individual lncRNA can behave as oncogene or tumor suppressor depending on the cellular context. This chapter provides an insight into the different mechanisms through which lncRNAs can regulate tumorigenesis and tumor progression in different cancers.

Cellular Functions of LncRNAs

LncRNAs have a high structural complexity owing to their unconserved primary sequences between species as well as varied secondary and tertiary structures (Graf and Kretz 2020). The structural complexity endows them with the ability to carry out their diverse functions (Zampetaki et al. 2018). These functions include DNA-transcription, mRNA-splicing, post-transcriptional modification of mRNAs,

mRNA-translation, post-translational modifications of proteins, scaffolding function, organization of interactomes, maintenance of redox balance, metabolic regulation, stress response regulation, localization of proteins and RNAs, cell cycle regulation, stimulation of stemness and genomic imprinting, and pluripotency (Ma et al. 2013). They act as guide molecules for numerous RNA binding proteins to precisely localize them for carrying out their cellular functions such as transcriptional regulation and chromatin modification (Wang and Chang 2011). LncRNAs also act as scaffolds in recruiting major cellular proteins to specific locations to bring about their functions (Wang and Chang 2011). They serve as decoys by interacting with specific effector molecules such as chromatin modifiers, transcription factors, and miRNAs to attenuate their function (Wang and Chang 2011). Functional role of lncRNAs are spatiotemporal and cellular context-specific (Wang and Chang 2011). LncRNAs are transcribed by RNA polymerase II (RNA Pol II) and undergo post-transcriptional modifications such as 5′ capping and 3′ polyadenylation, similar to those of the mRNAs (Ma et al. 2013). Following their synthesis, many of the lncRNAs are retained within the nucleus, while others are translocated to the cytoplasm. Even within the nucleus and cytoplasm, lncRNAs localize to specific nuclear structures and cellular organelles, respectively (Bridges et al. 2021). In some instances, they are also packaged into extracellular vesicles (Pathania and Challagundla 2021). Based on their context-specific cellular localizations, which are often dynamic and non-mutually exclusive, lncRNAs execute either cytoplasmic or nuclear functions (Fig. 1).

Nuclear Functions of LncRNAs

In the nucleus, lncRNAs play role in bringing about epigenetic regulations, such as histone modifications, DNA methylations, and chromatin remodeling, and regulate transcription through enhancer looping as well as associating with the transcription factors, in addition to regulating the alternate splicing of mRNA. It should be noted here that lncRNAs can regulate gene expression either in *cis*- or *trans* regulatory modes. In *cis* mode of action, lncRNAs act as regulators of genes which are in close proximity on the genome, while in the *trans* mode they regulate effector gene expressions at a distant locus from that of the lncRNA locus.

LncRNAs and Histone Methylation

Histone modifications are associated with the regulation of gene expressions by either activating or by repressing the expression of corresponding gene. LncRNAs interact with several histone modifiers to regulate target gene expressions (Fig. 2). The major regulatory proteins that interact with lncRNAs for modifying the histones include polycomb-repressive complex (PRC) proteins, lysine-specific demethylases (LSDs), mixed lineage leukemia (MLL) complex proteins, WD-repeat protein-5 (WDR5), histone deacetylases (HDACs), and p300 histone acetyltransferases.

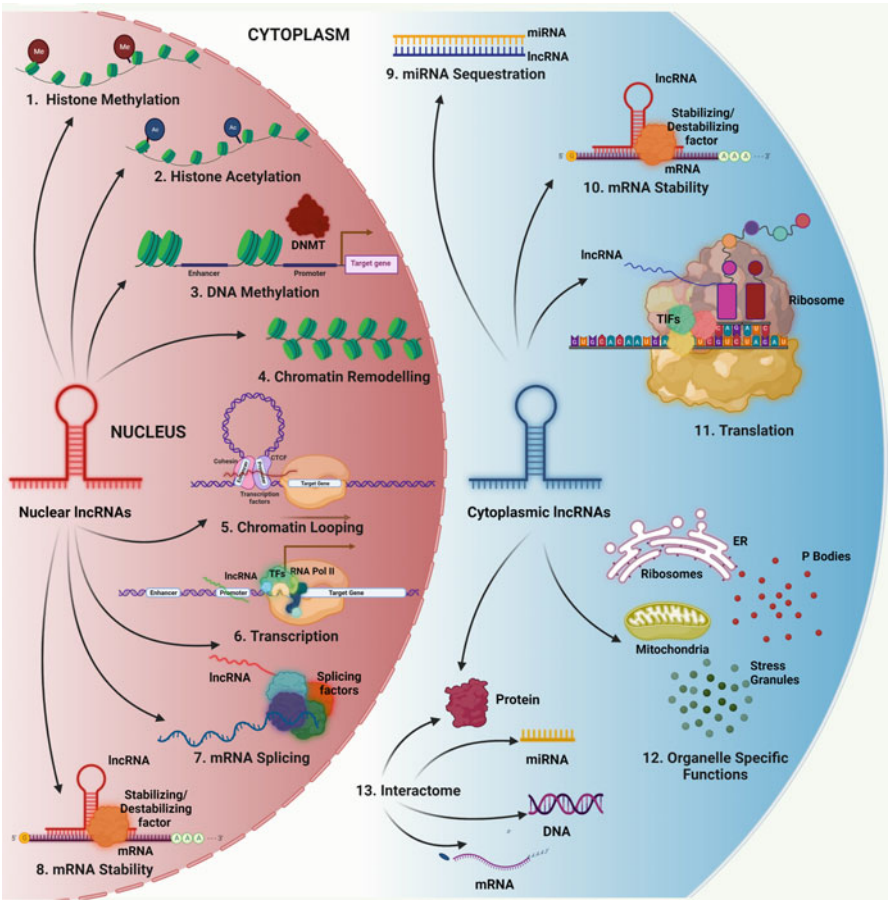

Fig. 1 LncRNAs: nuclear and cytoplasmic functions. LncRNAs modulate oncogenic responses through distinct nucleus-specific and cytoplasm-specific mechanisms. The nuclear mechanisms include regulation of (1) histone methylation, (2) histone acetylation, (3) DNA methylation, (4) chromatin remodeling, (5) chromatin looping, (6) transcription, (7) mRNA alternative splicing, and (8) mRNA stability. The cytoplasmic mechanisms include regulation of (9) miRNA sequestration, (10) mRNA stability, (11) translation, (12) organelle-specific functions, and (13) maintaining the diverse interactome to regulate protein functions (CTCF-CCCTC binding factor; DNMT-DNA methyl transferase; ER-Endoplasmic reticulum; LncRNAs-long non-coding RNAs; mRNA-messenger RNA; miRNA-micro RNA; P bodies-Processing bodies; RNA Pol II-RNA polymerase II; TFs-Transcription factors; and TLFs-Translation factors)

LncRNAs mediate the histone modifications through their interactions with these protein partners primarily through the formation of DNA-RNA triple helix (Schmitz et al. 2010; Mondal et al. 2015) as depicted in Fig. 2.

The PRCs are the major histone-modifying complexes, of which PRC1 modifies the histone structure by monoubiquitination of histone 2A lysine 119 (H2AK119ub1) and PRC2 modifies the histone structure by di- (H3K27me2)

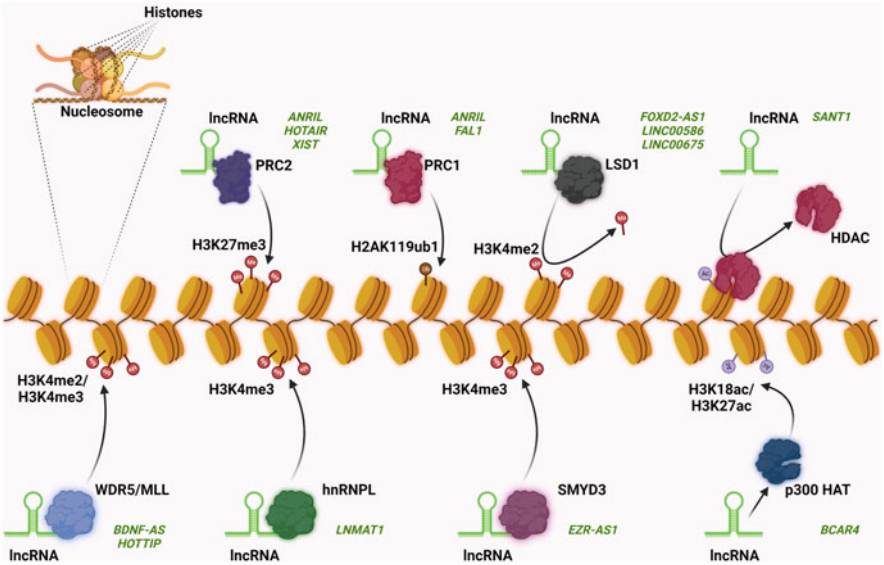

Fig. 2 Regulation of histone modifications by LncRNAs. LncRNAs regulate histone methylation through their interactions with diverse histone modifiers. LncRNA-associated histone methylations include repressive H3K27me3 mediated by PRC2 complex, and active H3K4me2/H3K4me3 mediated by WDR5/MLL, hnRNPL, or SMYD3. LncRNA-associated histone ubiquitinations such as H2AK119ub1 are mediated by PRC1 complex while histone demethylations are mediated by LSD1. LncRNA-facilitated histone acetylation is mediated by p300 HAT, and histone deacetylation is mediated by HDAC. Examples of lncRNAs that regulate each mechanism of histone modifications are enlisted adjacent to each of the mechanisms (green font). The abbreviations used are the following: H3K27me3 – Histone 3 Lysine 27 trimethylation; H3K4me2 – Histone 3 Lysine 4 di-methylation; H3K4me3 – Histone 3 Lysine 4 tri-methylation; H2AK119ub1 – Histone 2A Lysine 119 monoubiquitination; HAT – Histone acetyltransferases; HDAC – Histone deacetylases; hnRNPL – Heterogenous nuclear ribonucleoprotein L; LncRNA – Long non-coding RNA; LSD1 – Lysine Demethylase 1; MLL – Mixed Lineage Leukemia proteins; PRC-Polycomb Repressive Complexes; SMYD3-SET and MYND domain containing protein 3; and WDR5-WD repeat domain 5

or tri-methylation of histone 3 lysine 27 (H3K27me3) (Cohen et al. 2020; Guo et al. 2021). Majority of the histone modifications mediated by lncRNAs are through their interactions with PRC2, serving either as a guide RNA or as a scaffold, to induce repressive methylations at H3K27. LncRNA XIST, which is upregulated in neuroblastoma, interacts with Enhancer of Zeste Homolog 2 (EZH2), the catalytic component of the PRC2 complex, to enhance H3K27me3 over gene locus of tumor suppressor gene, *DKK1*, encoding Dickkopf Wnt signaling pathway inhibitor 1 (Zhang et al. 2019). The repressive histone methylation at the *DKK1* promoter locus inhibits its expression leading to the elevated mitotic, and invasive oncogenic signaling in neuroblastoma (Zhang et al. 2019). Such EZH2-mediated H3K27-trimethylation is used by several lncRNAs to repress the expressions of various tumor suppressor genes. Notable examples are the repression of the tumor suppressor gene *CDH1* (gene coding for E-cadherin) by HOTAIR in nasopharyngeal

carcinoma, *IκBα* (nuclear factor kappa B inhibitor-alpha), by HOTAIR in breast cancer stem cells and *p14/p15/p16* by ANRIL in prostate cancer (Yang et al. 2021; Wang et al. 2022a; Aguilo et al. 2011; Yap et al. 2010).

LncRNAs interact with PRC1 proteins to bring about histone modifications by acting in *cis* and *trans*. LncRNA ANRIL also interacts with PRC1 and guides it to target genes by recognizing the H3K27me3-repressive methylation marks. PRC1 is involved in the ubiquitination of histone H2A, which in addition to H3K27-repressive methylations act together for further chromatin compaction that prevents binding of any transcriptional machinery to repress the target gene expression. In prostate cancer cells, ANRIL promotes the translocation of PRC1 complex to the genetic loci of *p14, p15*, and *p16*, acting in *cis*, through its interaction with chromobox protein homolog 7 (CBX7) component of PRC1. Resultant H2AK119ub1 ubiquitination at the respective genetic loci leads to the complete repression of *p14, p15,* and *p16* gene expressions promoting tumorigenic cell cycle progression (Yap et al. 2010). In a minor variation of the theme, lncRNA FAL1 act in *trans* to repress the expression of *CDKN1A* in ovarian cancer through its interaction with the B lymphoma Mo-MLV insertion region 1 (BMI1) component of PRC1 complex. This interaction recruits PRC1 to the genetic locus of *CDKN1A* that encodes cyclin-dependent kinase inhibitor 1A or p21 to suppress its expression (Hu et al. 2014a). Since PRC1-mediated histone ubiquitination is one of the mechanisms to augment the histone methylation effects in repressing the expression of targets, it is possible that several other lncRNAs utilize such mechanism to repress the expression of specific target genes.

Another major protein involved in lncRNA-mediated histone methylation is the LSD1, the lysine demethylase enzyme. LSD1 acts along with HDACs in specifically demethylating either one or two methyl groups of the transcriptionally active H3K4me2-marks on gene promoters (Hayward and Cole 2016). In cervical cancer cells, lncRNA FOXD2-AS1 is upregulated and is stabilized by the interaction with METTL3 (m^6A methyltransferase like 3). FOXD2-AS1 interacts with LSD1 and recruits it to the genetic locus of *p21* that removes the active transcription marks on H3K4 (H3Kme2) to suppress its expression, thereby favoring proliferation and migration of cervical cancer cells (Ji et al. 2021). A similar role for the lncRNA LINC00586 has been proposed in LSD1-mediated derepression of tumor suppressor gene *ASXL1* (additional sex combs like 1 transcriptional regulator) in colorectal cancer cells. Using both the in vitro and in vivo models of CRC, it has been reported that the lncRNA LINC00586 promotes the demethylation of H3K4me2-marks on *ASXL1*, thereby suppressing its expression to promote EMT in colorectal cancer (Liu et al. 2022a). Likewise, the mechanism underlying the tumor suppressor role of lncRNA LINC00675 in gastric cancer has been attributed to its ability to mediate LSD1-mediated H3K4-demethylation of *SPRY4* (sprout homolog 4) oncogenic locus (Pan et al. 2020).

Yet another interacting partner of lncRNAs involved in histone modifications is the WDR5, a component of MLL 1–4 complex that facilitates the complex assembly and activity (Trievel and Shilatifard 2009). The MLL complex is involved in H3K4 di- or tri- methylation that acts as an active transcription mark for the gene loci

facilitating their enhanced expression. LncRNA HOTTIP, which is upregulated in pancreatic adenocarcinoma, binds to WDR5-MLL1 complex and recruits it for H3K4me3-methylation that leads to the upregulated expression of oncogenic targets such as *CYP26B1* (cytochrome P450 family 26 subfamily B member 1), *CHI3L1* (chitinase 3 like protein 1), *CLIC5* (chloride intracellular channel 5), and *UCP2* (uncoupling protein 2) favoring tumor growth and invasion (Wong et al. 2020). In peritoneal metastasis of gastric cancers, aberrantly overexpressed lncRNA BDNF-AS interacts with WDR5 and facilitates MLL-mediated H3K4 methylation to promote *FBXW7* (F-box and WD repeat domain containing 7) expression. FBXW7, further, ubiquitinates VDAC3 (voltage-dependent anion-selective channel protein 3) to regulate ferroptosis in these cancer cells (Huang et al. 2022). Ferroptosis is a type of programmed cell death that depends on high amounts of iron accumulation as well as lipid peroxidation, which is being recently explored as mechanism of targeted therapy in cancers (Li et al. 2020a).

Several other lncRNAs initiate H3K4 methylations in an MLL-independent manner as well. In lymph node metastasis of bladder cancer, lncRNA LNMAT1 recruits hnRNPL to *CCL2* genetic locus and induces active H3K4me3 marks. This enhances *CCL2* (CC-motif chemokine ligand 2) expression and promotes macrophage infiltration and VEGFC (vascular endothelial growth factor C)-mediated lymph node metastasis (Chen et al. 2018). In esophageal squamous cell carcinoma, lncRNA EZR-AS1 recruits SMYD3 (SET and MYND domain containing protein 3) histone methyl transferase to the genetic locus of *EZR* (ezrin) to facilitate H3K4me3 active transcription marks, favoring *EZR* expression and tumor progression (Zhang et al. 2018a).

LncRNAs and Histone Acetylation

In addition to histone methylations and PRC1-mediated ubiquitin addition, histone acetylation is yet another significant lncRNA-mediated histone modification that regulates gene expression (Fig. 2). LncRNAs are involved in regulating histone acetylation which are active transcription marks. In breast cancer cells, the lncRNA BCAR4 interacts with the transcription factor, SNIP1 (SMAD nuclear interacting protein 1), to relieve its suppressive effects on p300 histone acetyltransferase and induce H3K18 acetylation at the loci of Gli2-target genes such as *IL6* (interleukin 6) and *PTCH1* (protein-patched homolog 1) to enhance their expression as well as invasion and metastasis in these cancer cells (Xing et al. 2014). Similarly, BCAR4-mediated histone H3K18 acetylation also promotes Gli2-mediated upregulation of glycolytic enzymes *HKII* (hexokinase II) and *PFKFB3* (6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3), to promote tumor progression through Hippo-YAP (Yes associated protein) signaling (Zheng et al. 2017). In contrast to histone acetyltransferases, HDACs repress the target gene expression by mediating the histone deacetylation. LncRNA SANT1 acts in *cis* to upregulate *SLC47A2* expression in renal cell carcinoma. SANT1 acts as a decoy for HDACs by acting through removal of the SFPQ (splicing factor proline and glutamine rich)/E2F1 (E2F

transcription factor 1)/HDAC1 complex from promoter loci of *SLC47A2* (solute carrier family 47 member 2), which would otherwise deacetylate the histones and suppress the expression of the latter. Thus, SANT1 enhances *SLC47A2* expression that promotes tumorigenesis in renal cell carcinoma cells (Gao et al. 2019).

LncRNAs and DNA-Methylation

In addition to histone methylation as discussed above, lncRNAs regulate DNA methylation at the promoters of numerous genes to modulate their expressions (Fig. 3). The major enzymes with which lncRNAs interact to regulate DNA methylation include DNA methyltransferases (DNMTs) and members of ten eleven translocation enzyme family (TETs). In addition, lncRNAs can contribute to

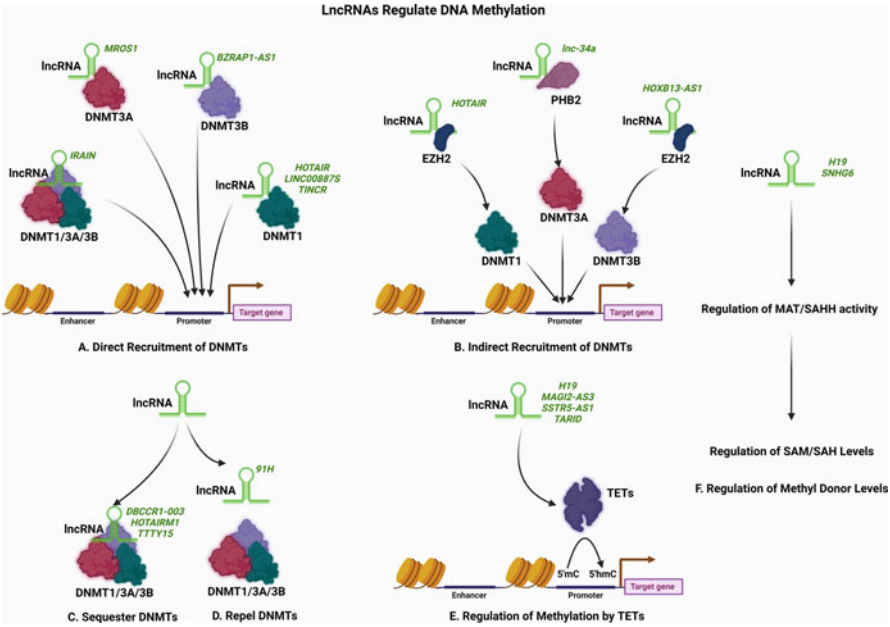

Fig. 3 Regulation of DNA methylation by lncRNAs. LncRNAs regulate DNA methylations through DNMTs as well as TETs to control target gene expressions in cancer cells. They can recruit DNMTs either (**a**) directly or (**b**) indirectly through interactions with EZH2 as well as PHB2 proteins. (**c**) LncRNAs can either sequester DNMTs to prevent their association with target gene promoters (**d**) or repel them from the target gene promoters. (**e**) They can also recruit TETs to target specific gene promoters. (**f**) LncRNAs can also regulate the activity of SAHH and MAT enzymes to regulate the levels of methyl donors such as SAM and SAH, which indirectly affects the target gene promoter methylations. Examples of lncRNAs that regulate each mechanism of DNA methylations are presented (green font). The abbreviation are the following: DNMT – DNA Methyltransferases; EZH2 – Enhancer of Zeste Homolog 2; LncRNA – Long non-coding RNA; MAT – Methionine Adenosyl Transferase; PHB2 – Prohibitin 2; SAM – S-Adenosyl Methionine; SAH – S-Adenosyl Homocysteine; SAHH – S-Adenosyl Homocysteine Hydrolase; and TET – Ten Eleven Translocation enzymes

DNA-methylation process indirectly by regulating the levels of methyl donors, SAM (S-adenosyl methionine) and SAH (S-adenosyl homocysteine).

There are five different classes of DNMTs, namely DNMT1, DNMT2, DNMT3A, DNMT3B, and DNMT3L, of which DNMT1, DNMT3A, and DNMT3B are the major ones involved in mammalian genome methylation (Zhang and Xu 2017). LncRNAs play a major role in regulating the methylome of cancer genome through its role as guiding molecules for DNMTs to the target tumor suppressor genes. Thus, lncRNAs are actively involved in DNMT-mediated methylation at the promoters of tumor suppressor genes and subsequent attenuation of their expression that leads to tumor genesis and/or progression. LncRNA HOTAIR, which is upregulated in chronic myelocytic leukemia, recruits DNMT1 to the promoter of *PTEN* that encodes phosphatase and tensin homolog. The *PTEN* promoter hypermethylation reduces the expression of this tumor suppressor, which in turn contributes to enhanced proliferation, migration, and invasion of these cancer cells (Song et al. 2021). Another interesting function has been reported to be associated with lncRNA LINC00887 in tongue squamous cell carcinoma. LINC00887 has two variants, namely, 887S short variant and 887 L long variant, both of which are involved in regulation of their downstream target carbonic anhydrase IX (CA9) that is upregulated during hypoxic conditions and plays a major role in pH regulation. Both these variants have contradictory functions within the cancer cells. Under conditions of hypoxia, while 889 L induces hypoxia-inducible factor 1 alpha (HIF1α)-mediated *CA9* expression, 887S mediates the recruitment of DNMT1 to *CA9* promoter to methylate its promoter and suppress its expression. The balance between these variants regulate tumorigenesis during hypoxia in tongue squamous cell carcinoma (Shen et al. 2021). Higher MROS1 in oral cancer cells recruit DNMT3A to the promoter of tumor suppressor, *PRUNE2* (prune homolog 2), and suppress its expression. This favors nodal metastases of these cancers (Su et al. 2021). In hepatocellular carcinoma, lncRNA BZRAP1-AS1 recruited DNMT3B to the promoter of anti-angiogenic molecule *THBS1* (thrombospondin 1) to suppress its expression, thereby promoting tumor-angiogenic signaling in these cancer cells (Wang et al. 2019a). LncRNAs recruit DNMTs to regulate the expression of tumor suppressor miRNAs, as well. LncRNA TINCR has been shown to recruit DNMT1 to the promoter of miR-503-5p to downregulate the miRNA expression, which in turn enhances EGFR levels and promotes JAK-STAT-EGFR signaling and tumorigenesis (Wang et al. 2021a).

Similar to these oncogenic lncRNAs, tumor suppressor lncRNAs also act as a guide to regulate target gene expression. LncRNA IRAIN is downregulated in renal carcinoma, which otherwise recruits DNMT1, DNMT3A, and DNMT3B to the promoter of *VEGFA* (vascular endothelial growth factor A) to suppress its expression via promoter hypermethylation. Thus, lower IRAIN levels upregulate *VEGFA* levels that promote tumorigenesis (Li et al. 2020b).

In addition to the direct recruitment of DNMTs, lncRNAs act as a scaffold for interaction with other epigenetic modifiers such as EZH2 and PHB2 to recruit DNMTs to target gene promoters. In chondrosarcoma, HOTAIR-EZH2 complex facilitates the recruitment of DNMT1 to the promoters of miR-454-3p to

downregulate its expression. Subsequently, miRNA targets, *STAT3* and *ATG12* (autophagy-related 12), are upregulated and contribute to oncogenic signaling (Bao et al. 2017). In colon cancer stem cells, lnc-34a associates with PHB2 (prohibitin 2) to recruit DNMT3A to methylate miR-34a promoter. In addition to the promoter methylation, lnc-34a also mediates histone deacetylation by recruiting HDACs to the miRNA locus. Together, promoter hypermethylation and histone deacetylation attenuate miR-34a expression. These events promote colon cancer stem cell renewal and tumor growth, both in vitro and in vivo (Wang et al. 2016). In glioma, lncRNA HOXB13-AS1 recruits DNMT3B via EZH2 to *HOXB13* (homeobox B 13) promoter, which is a tumor suppressor, to attenuate its expression (Xiong et al. 2018).

Acting as decoy molecules, lncRNAs regulate DNA methylation by the sequestration of DNMTs to prevent promoter hypermethylation-induced oncogene repressions. This role is exemplified in glioblastoma multiforme, with the increased levels of HOTAIRM1 that sequesters DNMTs to prevent promoter-methylation of the oncogene *HOXA1,* in order to promote its expression (Li et al. 2018a). In breast cancer cells, an antisense lncRNA transcript to *H19/IGF2* (insulin-like growth factor 2) gene, named as 91H, is highly expressed along with *H19* and *IGF2*. LncRNA 91H repels the association of DNMTs at their promoter, acting as a DNMT decoy, thereby enhancing *H19/IGF2* expressions (Vennin et al. 2017).

Tumor suppressor lncRNAs also play role in regulating tumor progression by acting as DNMT decoy. In non-small cell lung cancer cells, lncRNA TTTY15 sequesters DNMT3B to regulate the tumor suppressor gene *TBX4* (T-box transcription factor 4) expression (Lai et al. 2019). Similarly, in bladder cancer cells, lncRNA DBCCR1-003 is downregulated, which otherwise would sequester DNMT1 and prevent promoter methylation of tumor suppressor, *DBCCR1* (deleted in bladder cancer chromosome region 1) to induce G0/G1 cell cycle arrest and apoptosis in these cancer cells (Qi et al. 2016).

Other than DNMTs, yet another prominent enzyme involved in regulation of DNA methylation is the TETs, which catalyze the hydroxylation of 5′ methyl cytosine to 5′ hydroxy methyl cytosine at the gene promoter sites that serves as a mechanism for demethylation. Two studies have reported the role of lncRNA TARID in regulating transcription factor 21 (*TCF21*) expression (Arab et al. 2019; Arab et al. 2014). TARID recruits the demethylation factor, Growth Arrest and DNA Damage inducible 45 Alpha (GADD45A), thymine DNA glycosylase, and TET1 at the promoter of *TCF21* to induce demethylation through the hydrolysis of 5'-mC to 5'-hmC at the promoter, so as to activate the expression of *TCF21*. In a similar fashion, lncRNA H19 has been reported to facilitate the targeting of TET3 to the promoter sites of its target genes such as *HMGA2* (high mobility group A2), *MED12* (mediator complex subunit 12), and others involved in extracellular matrix remodeling for the progression of uterine leiomyoma (Cao et al. 2019a). Tumor-suppressive lncRNAs such as SSTR5-AS1 and MAGI2-AS3 also act as a guide for TETs to regulate target gene expressions. In laryngeal squamous cell carcinoma, lncRNA SSTR5-AS1 is downregulated, which otherwise interacts with TET1 and augments its translocation to the promoter of *CDH1* encoding E-cadherin to

facilitate the demethylation of 5'-mC thereby attenuating EMT (Wang et al. 2019b). In acute myeloid leukemia stem cells, lncRNA MAGI2-AS3 as well as LRIG1 (leucine-rich repeats and immunoglobulin-like domains protein 1) is downregulated. MAGI2-AS3 would otherwise recruit TET2 to the promoter of *LRIG1* (leucine-rich repeats and immunoglobulin-like domains 1), a tumor suppressor gene (Chen et al. 2020).

In addition to regulation of target gene promoter methylations, lncRNAs regulate the levels of methyl donors, as well, to modulate methylations. LncRNAs such as H19 and SNHG6 regulate the expression levels of *SAHH* that encodes the enzyme S-adenosyl homocysteine hydrolase and *MAT* that codes for methionine adenosyl transferase to regulate methyl donor levels (SAM and SAH), respectively, in cancer cells to modulate target gene promoter methylations and their subsequent expressions. SAHH enzyme is involved in the hydrolysis of SAH to yield homocysteine and adenosine. SAHH also acts as an inhibitor of DNMTs, thereby attenuating DNMT-mediated promoter methylations across the genome. LncRNA H19 inhibits the *SAHH* expression and enzyme activity and promotes the SAH levels, which in turn inhibits the activity of SAM-dependent DNMTs. This reduces the promoter methylation on gene loci of several oncogenes that promote tumor progression (Zhou et al. 2015; Wang et al. 2019c; Fu et al. 2018). In contrast, MAT enzymes convert methionine to SAM that acts as a methyl donor for SAM-dependent DNMTs, which enhances promoter hypermethylation. In hepatocellular carcinoma, lncRNA SNHG6 inhibits *MAT1A* expression, which reduces the SAM levels and attenuates SAM-dependent DNMTs. This leads to promoter hypomethylation of oncogenes and their over expression (Guo et al. 2018).

LncRNAs and Chromatin Remodeling

Chromatin remodeling plays a major role in the regulation of gene transcription as well as DNA replication by dynamically altering the chromatin architecture to provide access to densely packed DNA in the chromosome for transcription or replication (Magaña-Acosta and Valadez-Graham 2020). The major chromatin remodeling multiprotein complexes are SWItch/Sucrose Non Fermentable complex (SWI/SNF), INO80-SWI2/SNF2-Related 1 (INO80/SWR1) complex, Imitation SWI/SNF (ISWI) complex, and Chromodomain Helicase DNA binding protein (CHD) complex (Neve et al. 2021). LncRNAs interact with members of these chromatin remodeling complexes to bring about changes in gene expression (Fig. 4).

In renal cancer cells, the lncRNA IL7-AS acts as a scaffold for p300 histone acetyl transferase and SWI/SNF complex through its interaction with Brahma-related gene 1 (BRG1), the catalytic helicase subunit of SWI/SNF family, and guides it to the promoter regions of downstream target genes such as *CCL2, CCL5, CCL7,* and *IL6* to upregulate their expressions for tumor progression (Liu et al. 2019a). In liver cancer stem cells, the lncRNA, lnc-BRM, interacts with SMARCA2 (BRM/Brahma ATPase) and initiates BRG1-BRM switch at the promoter of *YAP1* to promote YAP signaling. Transcription factor KLF4 (kruppel like factor 4)-mediated

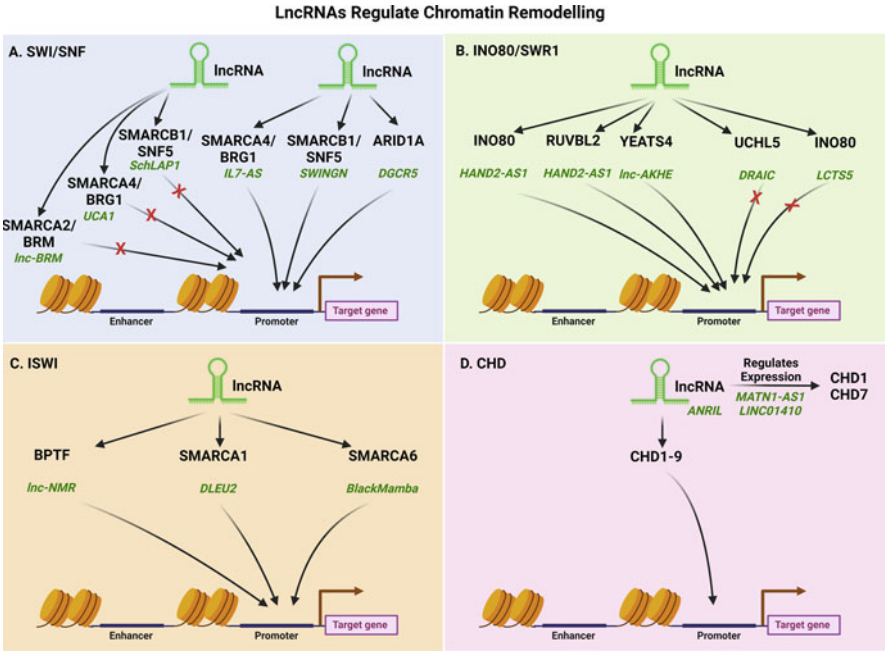

Fig. 4 Chromatin remodeling by lncRNAs. LncRNAs interact with SWI/SNF, INO80/SWR1, ISWI, and CHD family of chromatin remodelers to regulate chromatin remodeling that affect the target gene expressions in cancer cells: (**a**) LncRNAs interact with SWI/SNF family members such as SMARCA4/BRG1, SMARCB1/SNF5, and/or ARID1A to recruit them to specific genetic loci. A few lncRNAs interact with SMARCA2/BRM, SMARCA4/BRG1, and SMARCB1/SNF5 to sequester them and prevent their interaction at the target genetic loci; (**b**) LncRNAs interact with INO80SWR1 family members such as INO80, RUVBL2, and YEATS4l to recruit them to the target genetic loci while few others interact with UCHL5 and INO80 to sequester them in order to prevent their interaction at the target genetic loci; (**c**) LncRNAs can also interact with ISWI family members such as BPTF, SMARCA1, or SMARCA6 to recruit them to the target genetic loci; (**d**) LncRNAs also interact with CHD family members to recruit them to target genetic loci facilitating chromatin remodeling. Examples of lncRNAs that regulate each mechanism of chromatin remodeling are enlisted in green font. The abbreviations are the following: BPTF – Bromodomain PHD finger Transcription Factor; BRG1 – Brahma-Related Gene 1; BRM – Brahma ATPase; CHD – Chromodomain Helicase DNA binding protein complex; INO80/SWR1-INO80-SWI2/SNF2-Related 1 complex; ISWI – Imitation SWI/SNF complex; LncRNA – Long non-coding RNA; RUVBL2 – RUVB Like 2; SMARC – SWI/SNF-related Matrix-Associated actin-dependent Regulator of Chromatin; SWI/SNF – SWItch/Sucrose Non Fermentable complex; UCHL5 – Ubiquitin C-terminal Hydrolase L5; and YEATS4 – YEATS domain containing 4

BRG1-SWI/SNF complex activates *YAP1* expression which further triggers activation of downstream targets including *ANKRD1* (ankyrin repeat domain 1), *BIRC5* (baculoviral IAP repeat containing 5), *FOXM1* (forkhead box protein M1), and *SOX9* (SRY box transcription factor 9) that helps to maintain stemness in these cancer cells (Zhu et al. 2016). In breast cancer cells, the tumor suppressor lncRNA DGCR5 is downregulated and its tumor suppressor role is attributed to its interaction with ARID1A protein of the SWI/SNF complex. In non-cancerous cells, DGCR5

promotes the localization of SWI/SNF complex to the promoter of *p21*, thereby upregulating its expression and subsequent inhibition of tumor progression (Fang et al. 2019). In contrast, lncRNA UCA1 has been reported to sequester BRG1 to prevent its chromatin remodeling activity at the promoter site of *CDKN1A* gene that encodes cyclin-dependent kinase inhibitor 1A, p21. Resultant inhibition of *p21* expression promotes tumor progression in bladder cancer cells (Wang et al. 2014). In a somewhat similar fashion, the lncRNA SchLAP1 interacts with SNF5 member of SWI/SNF family to disrupt the recruitment of SWI/SNF complex across the genome to favor tumor progression in prostate cancer cells (Prensner et al. 2013).

In liver cancer stem cells, lncRNA HAND2-AS1 guides INO80 and ATP-dependent helicase, RUVBL2 (RuvB-like 2), to the promoter of *BMPR1A* (bone morphogenetic protein receptor-type 1A), thereby enhancing its expression and subsequent activation of SMAD (suppressor of mothers against decapentaplegic)-mediated BMP (bone morphogenetic protein) signaling for maintaining stemness (Wang et al. 2019d). Similarly, lncRNA, lnc-AKHE, which is upregulated in hepatocellular carcinoma, acts as a guide RNA. It interacts with YEATS4 (YEATS domain containing 4) component of INO80 complex to aid in its recruitment to *NOTCH2* (neurogenic locus notch homolog protein 1) promoter, thereby enhancing the expression of *NOTCH2* as well as members of NOTCH2 signaling such as *HES1* (hairy and enhancer of split 1) and *HEY1* (hairy/enhancer-of-split related with YRPW motif protein 1) promoting tumorigenesis (Huang et al. 2018a). Tumor suppressor lncRNAs also interact with INO80 complex members to inhibit their activity. In gastric cancer cells, the tumor suppressor lncRNA DRAIC is downregulated and its loss leads to the upregulation of NFRKB (nuclear factor related to kappa B binding protein). This is attributed to the ability of DRAIC to sequester UCHL5 component of INO80 complex, thereby preventing UCHL5-mediated ubiquitination and degradation of oncogenic NFRKB in gastric cancer cells (Zhang et al. 2020). In non-small cell lung cancer cells, the increased expressions of *CXCL5* (CXC motif chemokine ligand 5), *MAP3K1* (mitogen-activated protein kinase kinase kinase 1), and *ZNF703* (zinc finger protein 703) are correlated with the downregulation of LCTS5, a tumor suppressor lncRNA. In non-small cell lung cancer cells, INO80/SWR1 complex promotes the active transcription of several oncogenes including *CXCL5*, *MAP3K1*, and *ZNF703*. While LCTS5 sequesters INO80 and disrupts the formation of INO80/SWR1 complex and subsequent transcriptional activation of the oncogenes in non-malignant cells, its loss in non-small cell lung cancer cells reverse this process (Wang et al. 2020).

In esophageal squamous cell carcinoma, lnc-NMR guides BPTF (bromodomain PHD finger transcription factor)-NURF (nucleosome remodeling factor) complex to chromatin, thereby promoting *MMP3* and *MMP10* expressions involved in cancer cell migration, invasion, and chemoresistance (Li et al. 2018b). In colorectal cancer cells, lncRNA DLEU2 interacts with SMARCA1 (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 1) and guides the NURF complex to promoter sites of *KPNA3* (karyopherin subunit alpha 3) oncogene to facilitate its expression as well as tumor progression (Liu et al. 2018). The lncRNA BlackMamba interacts with SMARCA6-HELLS (helicase lymphoid

specific) complex to guide it to the promoters of genes regulating cell architecture and morphology to upregulate their expressions and thereby promote tumorigenesis (Fragliasso et al. 2020).

The fourth family involved in chromatin remodeling is the CHD complex which is involved in recruiting several histone deacetylase or demethylase enzymes on the genetic loci to regulate multiple functions including transcription (Neve et al. 2021; Murawska and Brehm 2011). A direct role for lncRNAs in CHD-mediated chromatin remodeling and subsequent effect on the expression of specific oncogene or tumor suppressor gene remains poorly understood. However, it has been shown that the lncRNA ANRIL-guided CHD targeting to H3K27me3 sites of *p16-INK4a* is involved in inhibiting its expression through CHD-mediated chromatin compaction (Ren et al. 2016). In addition, several lncRNAs have been shown to modulate the expression levels of CHD family members, thus impacting indirectly on the expression of the target genes of CHD-complex (Zhu et al. 2020; Lu et al. 2020). While lncRNA MATN1-AS1 sequesters miR-200b/c to upregulate *CHD1* expression in glioma, lncRNA LINC01410 sequesters miR-23c to upregulate *CHD7* expression in endometrial cancer cells for tumor progression (Zhu et al. 2020; Lu et al. 2020).

LncRNAs and Chromatin Looping

LncRNAs play a major role in chromatin looping, which is critically involved in bringing distal transcriptional regulatory elements in close proximity to influence gene expression (Fig. 5). Specifically, the lncRNAs that arise from the enhancer regions of several genetic loci actively play a role in chromatin looping. This class of lncRNAs, defined as enhancer RNAs (eRNAs), can arise from unidirectional or bidirectional transcription from enhancers. In addition, lncRNAs that possess enhancer-like functions are also included in this class (Schmitz et al. 2016; Kim et al. 2015). The eRNAs along with the cohesion complex proteins promote chromatin looping by topologically connecting the enhancer region and promoter sites in *cis* configuration. Such enhancer-promoter loops form an active transcription site to recruit RNA polymerase, transcription factors, and other regulatory factors to modulate gene transcription (Schmitz et al. 2016). The lncRNA HOTTIP is upregulated in several cancers and is transcribed as an eRNA from the enhancer region of *HOXA* locus. Acting in *cis*, HOTTIP, interacts with the adaptor protein WDR5 to facilitate the recruitment of MLL complex to the promoter region of *HOXA* locus by chromatin looping. These events enhance expression of *HOXA* genes such as *HOXA13*, *HOXA11*, and *HOXA10*, which are potent oncogenes (Wang et al. 2011; Luo et al. 2017). Yet another example is KLK3e, an eRNA which is transcribed from the enhancer region of KLK3. KLK3e possesses androgen response element III which is required for androgen receptor (AR) and mediator 1 for AR-dependent gene expression. Facilitating chromatin looping to promote the topological interaction of *KLK3* enhancer and *KLK2* promoter and bringing together AR, mediator 1, and RNA Pol II to the promoter site, KLK3e stimulates the transcriptional activation of *KLK2*. Thus, KLK3e acts as a scaffold for AR-dependent complex for mediating AR-mediated

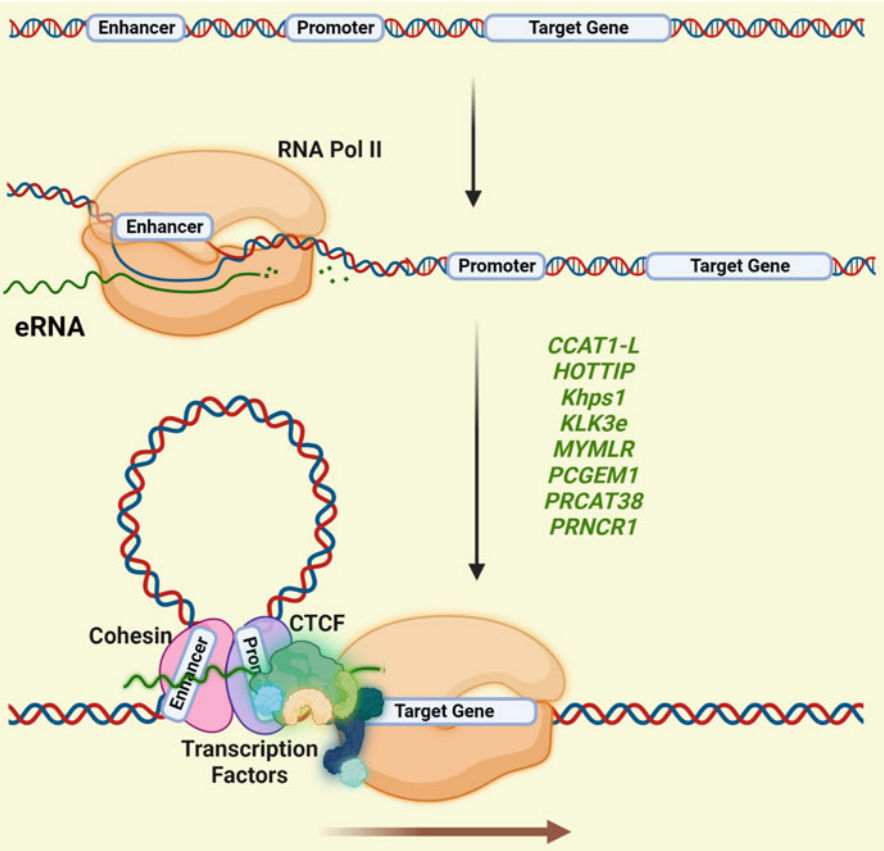

Fig. 5 Chromatin looping by lncRNAs. LncRNA-mediated chromatin looping plays a role in regulations involving distally located enhancers and genetic loc. Representative examples of lncRNAs involved in chromatin looping to modulate target gene expressions in cancer cells are presented (green font). Abbreviations: CTCF-CCCTC binding factor; LncRNA-Long non-coding RNA

gene expression through chromatin looping and modification of chromatin architecture (Hsieh et al. 2014). Studies have shown that lncRNA Khps1, which is an antisense transcript from the genetic locus of the oncogene *SPHK1* that encodes sphingosine kinase 1, is upregulated in numerous cancer cells. SPHK1 expression prevents apoptosis and promotes tumor growth in these cancer cells. Khps1 promotes the expression of SPHK1 by guiding p300/CBP (CREB binding protein) complex to *SPHK1* promoter that activates histone acetylation and opens up chromatin structure for E2F1 binding. The recruitment of p300/CBP as well as E2F1 transcription complex to the *SPHK1* transcription start site is facilitated by Khps1-mediated chromatin looping, specifically with the upstream homopurine sites of SPHK1, thereby forming a DNA-RNA triple helix (Postepska-Igielska

et al. 2015). LncRNA PRCAT38 and oncogene *TMPRSS2* (transmembrane serine protease 2) are located on chromosome 21 and are overexpressed in prostate cancer cells.

The eRNAs can also act in *trans* to regulate chromatin looping. Studies on castrate-resistant prostate cancer cells show that lncRNAs PRNCR1 and PCGEM1, which are upregulated in these cancer cells, play role in enhancer looping to activate transcription of distant genetic loci in both AR-dependent and independent manner (Yang et al. 2013). PRNCR1 binds to the C-terminus of the acetylated AR and promotes DOT1 like histone lysine methyl transferase (DOT1L)-mediated methylation of AR. This leads to the recruitment of PCGEM1 to the N-terminus of the methylated AR. PCGEM1, in turn, recruits Pygopus 2 adaptor which together promotes enhancer looping and enhanced expression of AR-responsive genes including *KLK2*, *PSA*, *TMPRSS2*, *FKBP5* (FK506 binding protein 5), and *NKX3-1* (Nk3 homeobox 1). These events occur even in a ligand-independent manner in the presence of truncated AR (Yang et al. 2013). In colorectal cancer cells, CCAT1-L lncRNA promotes chromatin looping favoring *MYC* expression. CCAT1-L locus has been identified to be a super enhancer region. It interacts with CTCF (CCCTC binding factor) to promote long-range looping for interactions between *MYC* locus and their enhancer sites (Xiang et al. 2014).

LncRNAs and DNA-Transcription

LncRNAs regulate gene transcription by diverse mechanisms, which include direct binding to the promoters of target genes, facilitating the binding of RNA Pol II to the promoter of target genes, and facilitating the localization of transcription factors to the transcriptional start sites (Fig. 6). An example for the direct binding of lncRNAs to the promoters of target genes is seen with the lncRNA ARHGAP5-AS1. In chemoresistant gastric cancer cells, ARHGAP5-AS1 directly binds to the promoter of Rho GTPase activating protein 5 gene, *ARHGAP5*, and enhances its transcription (Zhu et al. 2019a).

An example of direct regulation of RNA Pol II is seen in the case of lncRNA SLERT. The DEAD Box RNA helicase 21 (DDX21) encircles the RNA Pol II to inhibit its activity and suppress the transcription of rRNA locus. However, in ovarian carcinoma cells, lncRNA SLERT interacts with DDX21 to relieve its blocking of RNA Pol II so as to enable RNA Pol II-mediated transcription of rRNA genes and tumor progression (Xing et al. 2017). LncRNAs also regulate RNA Pol II indirectly by recruiting chromatin-modifying enzymes to alter chromatin structure, which in turn can facilitate the binding of RNA Pol II to the promoters of target genes. In hepatocellular carcinoma, lncRNA AY interacts with H1FX (histone 1 FX) and reduces its occupancy at the *ITGAV* (integrin alpha V) promoter. In addition, AY increases the transcriptional activating H3K4me3 and H3K9/14 acetylation, while reducing the repressive H3K27me3. Together, these events lead to chromatin remodeling that permits RNA Pol II binding and enhanced transcription of *ITGAV* underlying tumor growth and metastasis (Kang et al. 2019). Under certain

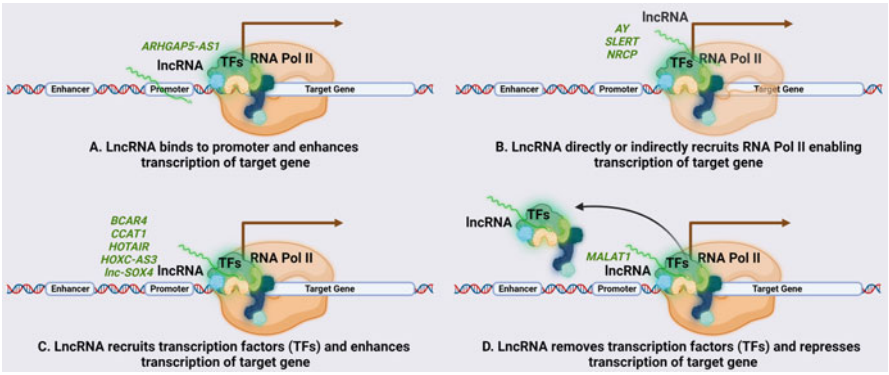

Fig. 6 **Transcriptional regulation by lncRNAs**. LncRNAs modulate the expression of oncogenes and tumor suppressor genes through multiple mechanisms: (**a**) LncRNAs directly bind to target gene promoter to enhance the transcription (ARHGAP5-AS1); (**b**) LncRNAs interact with and recruit RNA Pol II to promoter loci to promote target gene transcription (AY, SLERT, and NRCP); (**c**) LncRNAs interact with and recruit transcription factors to the transcription start site to promote target gene transcription (BCAR4, CCAT1, HOTAIR, HOXC-AS3, and lnc-SOX4); and (**d**) LncRNAs interact with and sequester transcription factors, thereby preventing their recruitment to target gene loci and repress their transcription (MALAT1). Examples of lncRNAs that regulate gene transcription are listed (green font). Abbreviations: LncRNAs – Long non-coding RNAs; RNA Pol II – RNA Polymerase II; and TFs-Transcription Factors

instances, lncRNAs can interact with both RNA Pol II and transcription factor to enhance target gene expressions. In ovarian cancer cells, the lncRNA NRCP interacts with and recruits transcription factor STAT1 and RNA Pol II, to the promoter of *GPI* gene that encodes glucose-6-phosphate isomerase. In this case, NRCP acts as a scaffold for STAT1 and RNA Pol II to transactivate the expression of *GPI*, which is critically involved in elevated glycolysis and metabolic reprogramming of cancer cells for higher energy needs (Rupaimoole et al. 2015).

LncRNAs can interact with transcription factors and act as guide molecules to target transcription factors to the target genetic loci. In the tumor-initiating cells of hepatocellular carcinoma, lnc-SOX4 acts as a guide for STAT3 to target it to the promoter of *SOX4* oncogene in order to elevate its expression and facilitate migration and invasion (Chen et al. 2016a). In gastric cancer cells, lncRNA HOXC-AS3 acts as a guide for YBX1 (Y-box binding protein 1) to the CCAAT box in the promoters of the target oncogenes such as *CDK2, IGFBP4* (insulin-like growth factor 2 binding protein 4), *MAPK4, MMP7, HOXB13, ATF5* (activating transcription factor 5), *MMP24, HDAC5, BIRC2*, and *WNT10B* (Wnt family member 10B) (Zhang et al. 2018b). LncRNAs also act as a scaffold to mediate the interaction of multiple transcription factors and proteins at the target gene loci. LncRNA HOTAIR acts a scaffold for LSD1 and transcription factors, HBXIP (hepatitis B X interacting protein), as well as c-Myc and recruits them to the promoter sites of c-Myc-regulated target genes such as *LDHA* (lactate dehydrogenase A), *CCNA2* (CyclinA2), and *eIF4E* (eukaryotic translation initiation factor 4E) to promote proliferation of breast

cancer cells (Li et al. 2016). In squamous cell carcinoma, CCAT1 acts as a scaffold for transcription factors, TP63 and SOX2, and recruits them to the super enhancer region of *EGFR* to activate *EGFR* expression (Jiang et al. 2018). In breast cancer cells, BCAR4 interacts with the transcription factors, SNIP1 and PNUTS (protein phosphatase 1 nuclear targeting subunit), to promote Gli-responsive gene expression, thus activating the Gli-mediated signaling involved in metastasis. Here, BCAR4 interacts with SNIP1 to relieve the inhibition of p300 histone acetyltransferase that stimulates H3K18 acetylation and expression of Gli target genes such as *IL6, PTCH1, TGFβI* (transforming growth factor beta I), and *MUC5AC*. In addition, BCAR4 also interacts with PNUTS and activates PP1 (protein phosphatase 1) phosphatase that further relieves the inhibition on RNA Pol II at the promoters of the target genes, which enhances their transcription and favors tumor progression (Xing et al. 2014). LncRNAs also act as decoys to sequester transcription factors so as repress the transcriptional activation of specific target genes. In vitro and in vivo studies in breast cancers have shown that the tumor suppressor lncRNA MALAT1 interacts with pro-metastatic transcription factor TEAD (TEA domain family member 1) preventing its interaction with YAP by acting as an RNA decoy. This prevents the transcription of YAP-responsive genes such as *VEGFA* and *ITGB4* (integrin subunit beta 4) that are involved in tumor progression and metastasis (Kim et al. 2018).

LncRNAs and mRNA Splicing

Precursor mRNA splicing is a critical step in the maturation of messenger RNAs in which the introns are removed and specific exons are spliced to form mature mRNA. LncRNAs play a cardinal role in both normal splicing as well as aberrant alternative splicing involved in tumorigenesis and tumor progression (Fig. 7). Splicing factors are present in the nucleoplasm and are localized to structures called as "nuclear speckles." They act as splicing factor storage sites as well as site for their recycle. In addition, nuclear speckles contain several transcription factors, RNA binding proteins, and chromatin remodelers as well as RNAs and are associated with active chromatin sites (Saitoh et al. 2004). LncRNA MALAT1 is present in nuclear speckles and facilitates the recruitment of splicing SR (serine arginine rich) factors to the chromatin region at the 5′ end of pre-mRNA to enable alternate splicing (Engreitz et al. 2014). Furthermore, the m^6A-methylated MALAT1 lncRNA interacts with the nuclear m^6A reader YTHDC1 (YTH domain containing 1) within the nuclear speckles, which is indispensable for maintaining the homeostasis of these nuclear speckle structures, and also in facilitating the alternative splicing of numerous genes involved in metastasis of esophageal squamous cell carcinoma (Wang et al. 2021b). Yet, another structure within the nucleus, which harbors numerous RNA binding proteins, nuclear retained RNAs, and proteins for enabling pri-miRNA processing, is the nuclear paraspeckle. Major proteins associated with NEAT1 in the nuclear paraspeckles are NONO (non-POU domain containing octamer binding), SFPQ, and TDP43 (transactive response DNA binding protein 43) that are involved

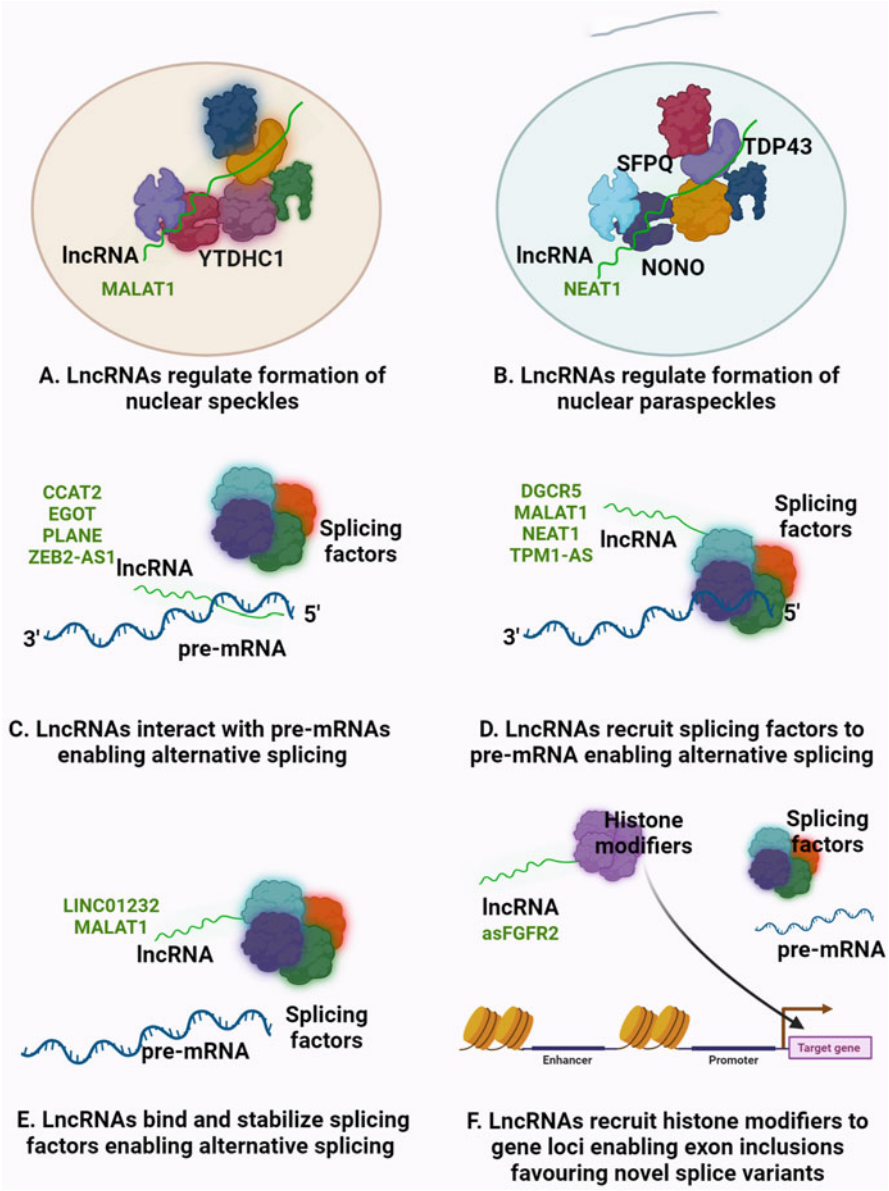

Fig. 7 Regulation of mRNA splicing by lncRNAs. LncRNAs interact with spliceosomal machinery of cancer cells to promote alternative splicing of mRNAs through multiple but non-mutually exclusive mechanisms. LncRNAs regulate the formation of nuclear microbodies such as nuclear speckles (**a**) and paraspeckles (**b**), where lncRNAs promote alternative splicing of pre-mRNAs through their interactions with specific spliceosomal proteins. LncRNAs can directly interact with specific pre-miRNAs (**c**) or with specific splicing factors to promote oncogenic alternative splicing, exon-skipping, and/or slice-switching (**d**). LncRNAs could also stabilize splicing factors to promote

in the formation of nuclear paraspeckle structures as well as enabling alternative splicing (Smith et al. 2020).

LncRNAs can switch the splicing sites of pre-mRNAs, in addition to recruiting specific splicing factors, to promote such alternative splicing of mRNAs that give rise to different splice variants from the same gene loci either by intron/exon inclusion or intron/exon skipping. Splice-switching by lncRNAs can occur both in *cis* and *trans* configurations. An instance of lncRNA acting in *cis* to promote alternative splicing is seen with lncRNA EGOT in breast cancer cells. EGOT is an intronic antisense transcript of ITPR1. EGOT, through its fragment 2 (324–645 nt) within exon 1, binds to both the pre-mRNA of ITPR1 (inositol 1,4,5-trisphosphate receptor type 1) and hnRNPH1 (heterogeneous nuclear ribonucleoprotein H1) protein to promote alternative splicing of pre-ITPR1 mRNA and higher expression of ITPR1. ITPR1 is involved in promoting autophagy, and these events promote autophagosome accumulation in the cancer cells, thereby sensitizing these cancer cells to paclitaxel therapy (Xu et al. 2019). In colorectal cancer cells, ZEB2-AS1, a natural antisense transcript to ZEB2 (zinc finger E box binding homeobox 2), acts in *cis*, to promote ZEB2 expression. However, this mediated through regulating the alternative splicing of ZEB2 mRNA by enabling the intron inclusion at 5'UTR in ZEB2. Snail1 promotes lncRNA ZEB2-AS1 expression that binds with ZEB2 pre-mRNA at the 5'UTR region to block the internal ribosomal entry sites (IRES) within the 5'UTR. This blocks the spliceosome recognition site within the 5'UTR, thus retains the IRES site within the 5' UTR of ZEB2 mRNA. These events promote ZEB2 translation, thereby inducing EMT and metastasis in these cancer cells (Beltran et al. 2008). LncRNAs also act in *trans* to interact with pre-mRNAs and regulate alternative splicing. In colorectal cancer cells, lncRNA CCAT2 acts as a scaffold that promotes the binding of glutaminase (GLS) pre-mRNA and CFIm (cleavage factor I complex) to enable alternative splicing of GLS pre-mRNA. GLS enzyme, which is involved in metabolizing glutamine for energy needs within the cancer cells, has two isoforms, namely, the GAC (glutaminase isoform C) and KGA (glutaminase kidney isoform) variants. Furthermore, allelic selection that favors CCAT2-G allele-variant expression promotes alternative splicing of GLS pre-mRNA to produce GAC variant. Thus, the binding of CCAT2-G allele lncRNA and CFIm complex to GLS pre-mRNA facilitates the poly(A) site selection within the intron 14, thereby driving the production of GAC splice variant of GLS, which promotes metastasis in these cancer cells by reprogramming glutamine metabolism for higher energy needs (Redis et al. 2016). In multiple cancers, lncRNA PLANE

Fig. 7 (continued) specific alternative splicing of pre-mRNA (**e**). Few of the lncRNAs recruit histone modifiers to specific genetic loci to open up the chromosome in order to enable alternative splicing pre-mRNAs through exon inclusions (**f**). Examples of lncRNAs that regulate mRNA splicing are in green font. The abbreviations are the following: lncRNAs – long non-coding RNAs; mRNA – messenger RNA; NONO – Non-POU domain containing octamer binding; SFPQ – Splicing Factor Proline and glutamine rich; TDP43 – Transactive response DNA binding Protein 43; and YTDHC1 – YTH N6-Methyladenosine RNA Binding Protein C1

binds to tumor suppressor NCOR2-202 (nuclear receptor co-repressor 2) pre-mRNA, in *trans*, to recruit hnRNPM (heterogeneous nuclear ribonucleoprotein M) to modulate alternative splicing and attenuate its expression (Teng et al. 2021).

LncRNAs can interact with splicing factors to modulate splicing variants. They either recruit them to promote alternative splicing of specific exons or sequester them to block splicing of specific exons. In esophageal squamous cell carcinoma, lncRNA DGCR5 interacts with spliceosomal factor, SRSF1 (serine arginine rich splicing factor 1), and stabilizes it to promote alternate splicing of Mcl1 (myeloid cell leukemia 1) pre-mRNA. There are two splice variants for Mcl1, namely, the Mcl1S, which is pro-apoptotic and Mcl1L, which is anti-apoptotic in function. DGCR5 promotes SRSF1-mediated alternative splicing of Mcl1 pre-mRNA to produce Mcl1L variant, through exon 2 inclusion, which enhances proliferation, migration, and invasion in these cancer cells (Duan et al. 2021). LncRNA TPM1-AS is downregulated in esophageal cancer cells, which would otherwise bind to RBM4 (RNA binding motif protein 4) splicing factor and prevent its association with TPM1 (tropomyosin 1) pre-mRNA. These events adversely affect the exon 2a inclusion in TPM1 to augment V1, V3, V4, and V5 variants, while downregulating V2 and V7 variants, thereby suppressing the tumor progression (Huang et al. 2017).

LncRNAs stabilize the splicing factors or activate them to regulate alternative splicing of mRNA. LncRNA MALAT1, which is located in the nuclear speckles and highly overexpressed in numerous cancers, has been extensively reported of playing role in alternate splicing. They interact with serine-/arginine-rich proteins such as SRSF1/2/3/5 and regulate their degree of phosphorylation to modulate their transport and distribution between nuclear speckles and site of transcription at target genes. MALAT1-SRSF1 complex has been reported to interact with mutant p53 and ID4 (inhibitor of DNA binding protein 4) proteins to regulate alternate splicing of VEGFA pre-mRNA. SRSF1 stabilizes the interaction of mutant p53-ID4 to MALAT1 and facilitates MALAT1 distribution from nuclear speckles to chromatin at VEGFA pre-MRNA. These events attenuate anti-angiogenic VEGFA165b isoform expression and promote angiogenesis in breast cancer cells (Pruszko et al. 2017). In colorectal cancer cells, MALAT1 acts as SFPQ decoy and binds to SFPQ-PTBP2 (polypyrimidine tract binding protein 2) complex to release the proto-oncogene PTBP2, which further initiates downstream oncogenic events to promote tumorigenesis (Ji et al. 2014). LINC01232 stabilizes hnRNPA2/B1 (heterogeneous nuclear ribonucleoprotein A2/B1) splicing factor by preventing ubiquitin-mediated degradation, thereby facilitating alternate splicing of A-Raf (A-rapidly accelerated fibrosarcoma) to promote MAPK/ERK signaling and metastasis in pancreatic cancer cells (Meng et al. 2020).

LncRNAs also mediate histone modifications to regulate alternative splicing. In lung cancer cells, the lncRNA, asFGFR2, recruits EZH2 and SUZ12 of PRC2 complex and histone demethylase KDM2a (lysine demethylase 2a) to the *FGFR2* (fibroblast growth factor receptor 2) gene locus to inhibit the binding of repressive adaptor complex MRG15-PTB. This results in the inclusion of exon IIIb to form FGFR2-IIIb splice-variant, which promotes epithelial phenotype to attenuate tumor progression (Gonzalez et al. 2015).

LncRNAs and Nuclear mRNA Stability

LncRNAs affect the mRNA stability both within the cytoplasm and the nucleus. Though majority of these events happen within the cytoplasm, studies have also shown that lncRNAs regulate mRNA stability within the nucleus (Fig. 8). LncRNA PDCD4-AS1 affects the stability of its sense mRNA, PDCD4 (programmed cell death 4), a tumor suppressor in breast cancer cells. PDCD4-AS1 binds to PDCD4 mRNA to form a duplex and prevents the interaction of HuR-KSRP (KH-type splicing regulatory protein) complex that would destabilize the PDCD4 mRNA. These events promote PDCD4 expression and associated tumor-suppressive effects (Jadaliha et al. 2018). In colorectal cancer cells, lncRNA FOXC2-AS1 binds to FOXC2 mRNA to enhance the mRNA stability in the nucleus that promotes cancer cell proliferation, invasion, and migration by enhancing the intracellular Ca2+ levels (Pan and Xie 2020). Similar antisense- and sense-RNA duplex-mediated mRNA stability involving other lncRNA-mRNA pairs have been observed to play a role in many other cancer cells. TALAM1 is the natural antisense transcript for MALAT1 lncRNA, and both are upregulated in breast cancer cells. MALAT1 and TALAM1 interact with each other to synergistically enhance their expression levels. Experimental studies using breast cancer cells have shown that the duplex formation

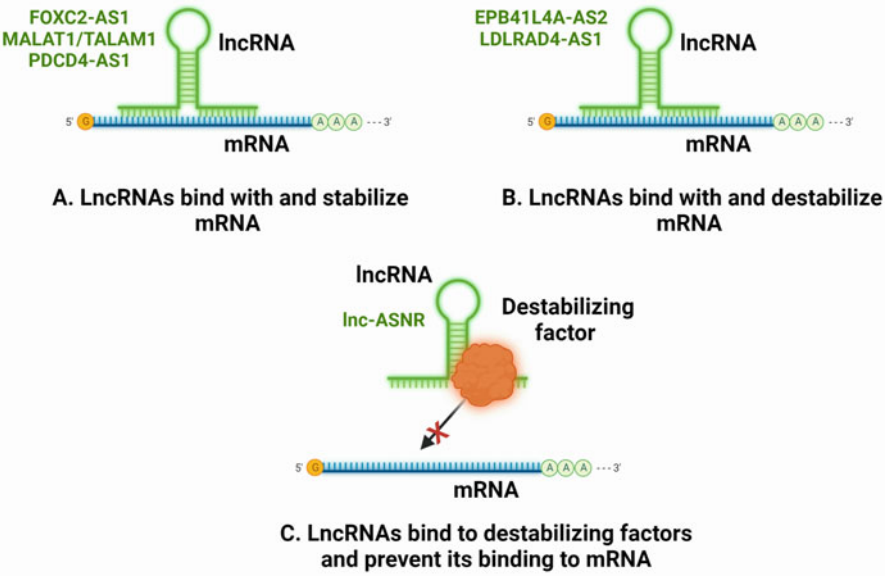

Fig. 8 Regulation of nuclear mRNA stability by lncRNAs. LncRNAs regulate the mRNA stability within the nucleus by interacting with either the mRNA or mRNA stabilizing/destabilizing factors: LncRNAs can interact directly with mRNA either to stabilize (**a**) or destabilize them (**b**). LncRNAs could also interact with mRNA destabilizing factors and prevent them from interacting with mRNA, thereby enhancing their stability (**c**). (Examples of the lncRNAs that regulate mRNA stability are in green font)

between MALAT1 and TALAM1 at their transcription sites greatly contribute to their stability. In addition to enhancing the stability of MALAT1 through RNA-duplex formation, TALAM1 stabilizes MALAT1 by promoting RNAse P-mediated 3′-end processing that facilitates the formation of the stabilizing triple helix structure in the 3′-end of MALAT1 (Gomes et al. 2019). In contrast, lncRNA LDLRAD4-AS1 interacts with LDLRAD4 mRNA in the nucleus to adversely affect the mRNA stability. Subsequent reduction in the expression levels of tumor suppressor LDLRAD4 results in the upregulation of Snail and subsequently EMT in colorectal cancer cells (Mo et al. 2020). LncRNA lnc-ASNR acts as a decoy for AU-rich element RNA binding protein 1 (AUF1) and sequesters AUF1 in colon adenocarcinoma cells. This reduces the availability of AUF1 within the cytoplasm that would otherwise bind to anti-apoptotic Bcl2 mRNA to adversely affect its stability (Chen et al. 2016b).

Tumor suppressor lncRNAs have also been shown to regulate mRNA stability in the nucleus. In nasopharyngeal carcinoma, lncRNA EPB41L4A-AS2 is downregulated, which otherwise would bind to YBX1 and guide it to Snail mRNA. This adversely affects the Snail mRNA stability that enhances the E-cadherin expression to reverse EMT (Du et al. 2021).

Cytoplasmic Functions of LncRNAs

LncRNAs that are translocated from the nucleus to the cytoplasm localize diffusively either in the cytosol or specifically in different subcellular organelles (Bridges et al. 2021; Krause 2018). Dependent on their cellular locales, they carry out different functions. In general, cytoplasmic lncRNAs modulate the expression levels of oncogenes, other growth promoting genes, and tumor suppressors through a variety of mechanisms such as miRNA sequestration, regulating the mRNA stability, and controlling the mRNA translation (Graf and Kretz 2020; Wang and Chang 2011; Schmitz et al. 2016; Chandra Gupta and Nandan Tripathi 2017). In addition, a few of the lncRNAs have been characterized to modulate the activities of different proteins by nucleating the organization of pathway-specific interactome and/or providing scaffolding function for protein-protein interactions (Graf and Kretz 2020).

LncRNAs and miRNA Sequestration

One of the major mechanisms through which lncRNAs regulate cellular processes during physiological as well pathological conditions is through modulating gene expressions via miRNA sequestration that occurs within the cytoplasm (Fig. 9). Such lncRNAs are termed as competing endogenous RNAs (ceRNAs), as they serve as miRNA decoys. The miRNAs that possess mRNA binding sites specifically bind to target mRNAs either to degrade the mRNA via AGO2-RISC (argonaute 2-RNA-induced silencing complex) complex or leading to attenuation of mRNA translation. The ceRNAs possess miRNA binding elements and bind to specific

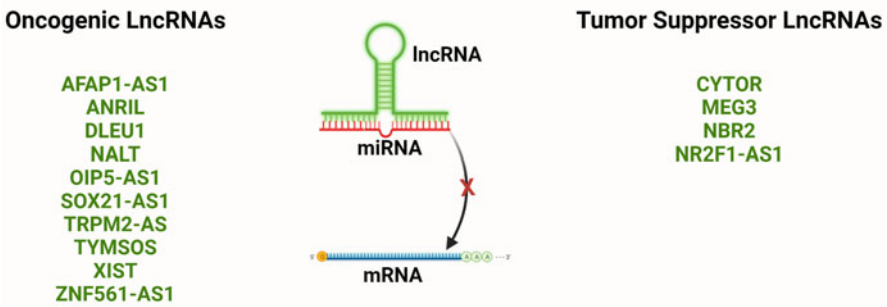

Fig. 9 Functional modulation of miRNAs by lncRNAs. In the cytosol, lncRNAs act as competing endogenous RNAs (ceRNAs) to sequester miRNAs, thus inhibiting their destabilizing effects to regulate target gene expression by miRNA sequestration. Oncogenic lncRNAs sequester tumor-suppressive miRNAs whereas tumor suppressor lncRNAs sequester oncogenic miRNAs. Examples of lncRNAs are in green fonts. (LncRNAs – Long non-coding RNAs; mRNA – messenger RNA; and miRNA – microRNA)

miRNAs, thereby inhibiting the miRNA-mRNA binding, which favors greater mRNA expression and translation. The resultant upregulation of target mRNA and their protein levels either promote or suppress tumor progression.

Most, if not all, of the cytoplasmic lncRNAs promote the expression of target oncogenes or inhibit the repression of tumor suppressor genes through miRNA sequestration. Since varied number of lncRNAs and their target miRNAs in different cancer contexts have already been catalogued extensively (Ratti et al. 2020), only a few representative examples are presented here. In pancreatic cancer cells, STAT6-mediated upregulation of lncRNA SOX21-AS1 sequesters miR-576-5p and enhances *SOX21* expression. SOX21-AS1 also interacts with USP10 and deubiquitinates SOX21 protein and stabilizes it to promote tumorigenesis (Yu et al. 2022). In colorectal cancer cells, lncRNA NALT sequesters miR-574-5p to upregulate miR target *PEG10* (paternally expressed 10) to promote proliferation and migration in these cancer cells, both in vitro and in vivo (Ye et al. 2022). Studies have shown that lncRNA CASC9 sequesters miR-874-3p to enhance *SOX12* expression that promotes cancer cell invasion in osteosarcoma (Qiu et al. 2022). LncRNA TYMSOS sequesters miR-130a-5p and upregulates *MARCKSL1* (myristoylated alanine-rich C-kinase substrate like protein 1) expression, which promotes EMT in thyroid cancer cells via PI3K/Akt signaling (Jia et al. 2022). LncRNA XIST sequesters miR-506-3p to upregulate *FOXP1* expression, thereby promoting autophagy, carboplatin resistance, and tumor growth in ovarian cancer cells (Xia et al. 2022). In prostate cancer cells, lncRNA TRPM2-AS, the natural antisense transcript to TRPM2 (transient receptor potential melastatin 2), sequesters miR-497-5p to enhance the expression of *FOXK1* (forkhead box protein K1) that promotes paclitaxel resistance (Shi et al. 2022a). In hepatocellular carcinoma, lncRNA ZNF561-AS1 sequesters miR-302a-3p to upregulate *PDGFD* (platelet-derived growth factor

D) expression, thereby favoring angiogenesis in these cancer cells (Zheng et al. 2022). Transcription factor YY1-mediated upregulation of lncRNA DLEU1 in cholangiocarcinoma results in sequestration of miR-149-5p resulting in enhancing *YAP1* expression. YAP1 serves as transcriptional coactivator to enable TEAD2-mediated *SOX2* expression that maintains stemness in these cancer cells (Li et al. 2022a). In laryngeal carcinoma, AFAP1-AS1 natural antisense transcript lncRNA sequesters miR-320a to enhance the expression of *RBPJ* (recombination signal binding protein for immunoglobulin kappa J) to promote stemness and chemoresistance (Yuan et al. 2018). In triple negative breast cancer cells, lncRNA ANRIL sequesters miR-125a to enhance the expression of glycolytic enzyme enolase, *ENO1*. This promotes aerobic glycolysis and contributes to chemoresistance (Ma et al. 2022). Under conditions of hypoxia, lncRNA OIP5-AS1 sequesters miR-124-5p to upregulate *IDH2* (isocitrate dehydrogenase 2) levels that upregulates HIF-1α expression and associated aerobic glycolysis in cervical cancer cells (Li et al. 2021).

Tumor suppressor lncRNAs also employ miRNA sequestration mechanism in regulating tumorigenesis. In cervical cancer cells, lncRNA NR2F1-AS1 is downregulated, which otherwise would sequester miR-642a-3p and upregulate *NR2F1* (nuclear receptor subfamily 2 group F member 1) expression that promotes cancer cell proliferation, migration, and invasion (Zhang et al. 2022a). In colon cancer cells, lncRNA CYTOR sequesters miR-105 to upregulate PTEN that promotes metastasis (Zhang et al. 2021). In cervical cancer cells, lncRNA MEG3 is downregulated, which otherwise sequesters miR-21 and upregulates miR-target *PTEN*. In these cancer cells, since MEG3 is downregulated, PTEN levels are low and contribute to cisplatin resistance (Du et al. 2022). In multiple myeloma, lncRNA NBR2 levels are lower. However, NBR2 overexpression upregulates *DLC1* (deleted in liver cancer 1) expression through miR-561-5p sequestration that represses aerobic glycolysis through AMPK/mTOR signaling pathway, thereby inhibiting tumor growth in these cancer cells (Wang et al. 2022b).

LncRNAs and Cytoplasmic mRNA Stability

Cytoplasmic lncRNAs are also involved in enhancing the stability of mRNAs in the cytoplasm through several mechanisms (Fig. 10). LncRNA-mediated sequestration of miRNAs, as discussed above, can also enhance mRNA stability. In these contexts, the lncRNAs act as miRNA decoys and enhance mRNA stability for their enhanced expression. In addition, lncRNAs have been shown to increase the stability of mRNAs through their direct interactions with specific mRNAs. This mechanism is utilized primarily by the antisense lncRNAs to protect their sense counterparts. In glioma, the antisense lncRNA PTB-AS, in association with SND1 (staphylococcal nuclease domain containing protein 1), binds to 3' UTR of its sense mRNA, PTBP1, which codes for polypyrimidine tract binding protein 1, an RNA binding protein that promotes tumor progression in glioma. This interaction prevents miR-9 binding to

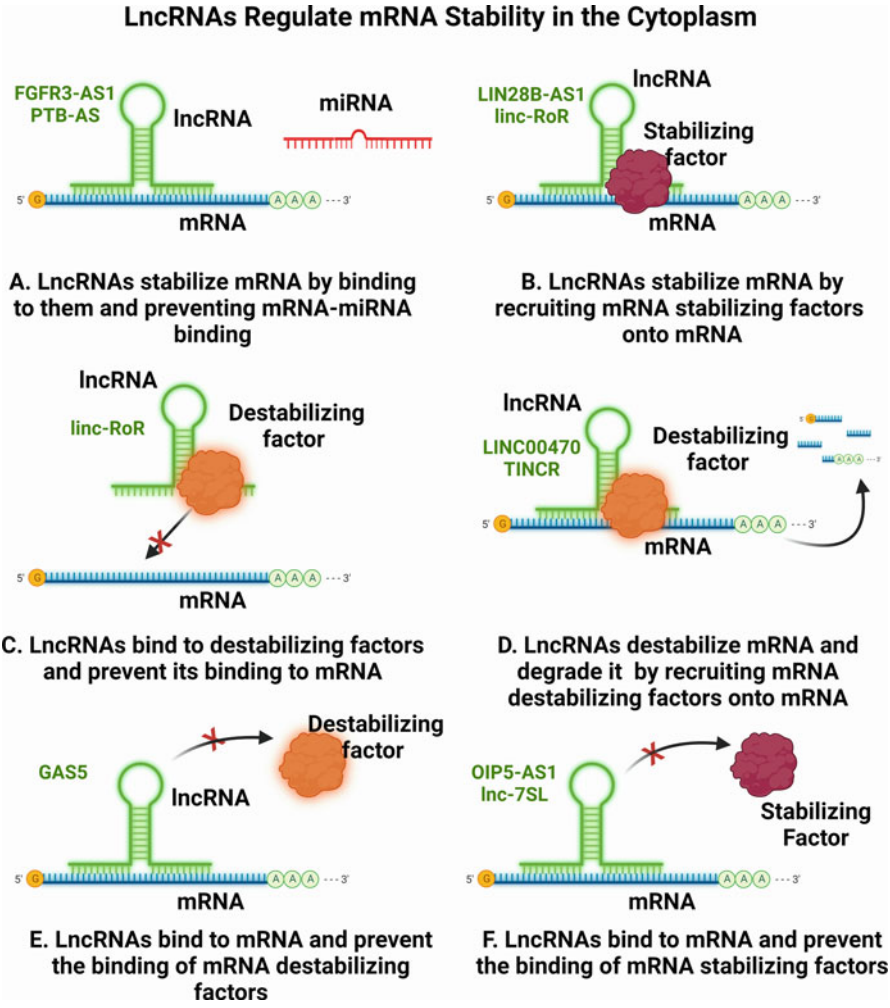

Fig. 10 Regulation of cytoplasmic mRNA stability by lncRNAs. LncRNAs regulate the mRNA stability within the cytoplasm through multiple mechanisms. Antisense lncRNAs can stabilize their counterpart sense-mRNAs through their direct association that prevents targeting by destabilizing miRNAs (**a**). Alternatively, lncRNAs can recruit mRNA-stabilizing factors to the mRNAs and enhance their stability (**b**). LncRNAs can also bind to mRNA-destabilizing factors and prevent their binding with mRNAs to enhance their stability (**c**). Some lncRNAs reduce the stability of target mRNAs by recruiting specific mRNA-destabilizing factors onto mRNA (**d**). Few of the lncRNAs could bind to the cognate sense-mRNAs and prevent the binding of mRNA-destabilizing factors so as to enhance the stability of the constituent mRNA (**e**). Other lncRNAs bind to mRNA and inhibit the binding of mRNA-stabilizing factors to destabilize the mRNA (**f**). Examples of lncRNAs that regulate mRNA stability within the cytoplasm are presented in green font

PTBP1 mRNA, thereby promoting PTBP1 expression (Zhu et al. 2019b). A similar mechanism has been reported in osteosarcoma cells with lncRNA FGFR3-AS1 and FGFR3 mRNA. FGFR3AS1-FGFR3 interaction blocks the miRNA binding sites in FGFR3 mRNA and enhances its stability (Sun et al. 2016).

Another mechanism through which lncRNAs promote mRNA stability is through their interaction with specific cytoplasmic RNA-binding proteins. This involves the binding of lncRNAs to proteins such as IGF2BPs and hnRNPs that promote RNA stability or proteins that promote RNA decay and degradation such as YTHDF2 (YTH N6 methyl adenosine RNA binding protein 2) and STAU1. In lung adenocarcinoma cells, lncRNA LIN28B-AS1 binds to IGF2BP1, a chaperone that promotes stability for specific mRNAs, and guides it to LIN28B mRNA to enhance the mRNA stability (Wang et al. 2019e). In colon cancer context, linc-RoR facilitates the association of hnRNP1 to c-Myc mRNA to stabilize it and promote its translation (Huang et al. 2016). LncRNAs interact with destabilizing RBPs and prevent them from binding to oncogenic mRNAs. Linc-RoR interacts with AUF1 (AU-rich element RNA binding factor 1) to prevent its interaction with c-Myc that would destabilize the c-Myc mRNA (Huang et al. 2016).

LncRNAs can also interact with specific stabilizing RBPs to inhibit their stabilizing effects on tumor suppressor mRNAs. Since m^6A methylations in mRNAs and lncRNAs affect their stability, lncRNAs modulate the stability of mRNAs through their interactions with m^6A readers such as YTHDF2. In gastric cancer cells, LINC00470 interacts with PTEN mRNA and promotes association with m^6A writer METTL3 by acting as a scaffold. This elevates m^6A methylation on PTEN mRNA, which promotes interaction with YTHDF2 that facilitates the mRNA degradation, thus adversely affecting the mRNA stability within the cytoplasm (Yan et al. 2020). An opposing "methylation eraser" strategy is utilized by the GAS5-AS1 and GAS5 in cervical cancer context (Wang et al. 2019f). In cervical cancer cells, GAS5-AS1 as well as GAS5 expressions are downregulated, which would otherwise interact with each other and promote association with ALKBH5 (AlkB homolg 5 RNA demethylase) demethylase, by acting as a scaffold, to reduce the methylation on GAS5 mRNA. This reduces the YTHDF2-mediated GAS5 RNA degradation within the cytoplasm, so as to attenuate tumor growth in cervical cancer cells (Wang et al. 2019f). In gastric cancer cells, lncRNA TINCR binds to STAU1 as well as KLF2 (kruppel like factor 2) mRNA within the cytoplasm, which promotes STAU1-mediated decay of pro-apoptotic KLF2 that facilitates tumorigenesis (Xu et al. 2015).

Several other lncRNAs interact with HuR to modulate the stability of mRNAs. In a panel of cancer cells, it has been reported that the upregulated levels of lncRNA 7SL interact with TP53 mRNA at the 3' UTR to prevent HuR binding to the mRNA. This destabilizes the TP53 mRNA, leading to degradation and downregulated TP53 expression in these cancer cells (Abdelmohsen et al. 2014). In colorectal cancer cells, lncRNA OCC1 and OIP5-AS1 are downregulated, which would otherwise interact with HuR. While OCC1 promotes HuR degradation through mediating the binding of β-TrCP1 (β transducin repeat containing protein) E3 ubiquitin ligase, OIP5-AS1 sequesters HuR to prevent its binding to mRNA targets (Lan et al. 2018; Kim et al. 2016).

LncRNAs and mRNA Translation

LncRNAs regulate ribosomal complex biogenesis to regulate translation (Fig. 11). LncRNA SLERT which is upregulated in numerous cancers interacts with helicase DDX21 to relax the helicase and reduces their multimerization. Thus, SLERT acts as a decoy for DDX21 and prevents the DDX21 helicase from hijacking the transcribed ribosomal DNAs. These events promote ribosomal assembly and enhanced translation of several oncogenes to promote tumor progression (Wu et al. 2021). LncRNA, lnc-NB1, binds to ribosomal protein, RPL35 (ribosomal protein L35), to promote E2F1 expression. This augments N-Myc translation as well as stability to promote tumorigenesis (Liu et al. 2019b).

LncRNAs can directly interact with mRNAs to regulate translation. In colorectal cancer cells, Snail-induced ZEB2, transcriptionally represses CDH1 expression to promote EMT. In these cells, increased expression of *ZEB2* is mediated by the

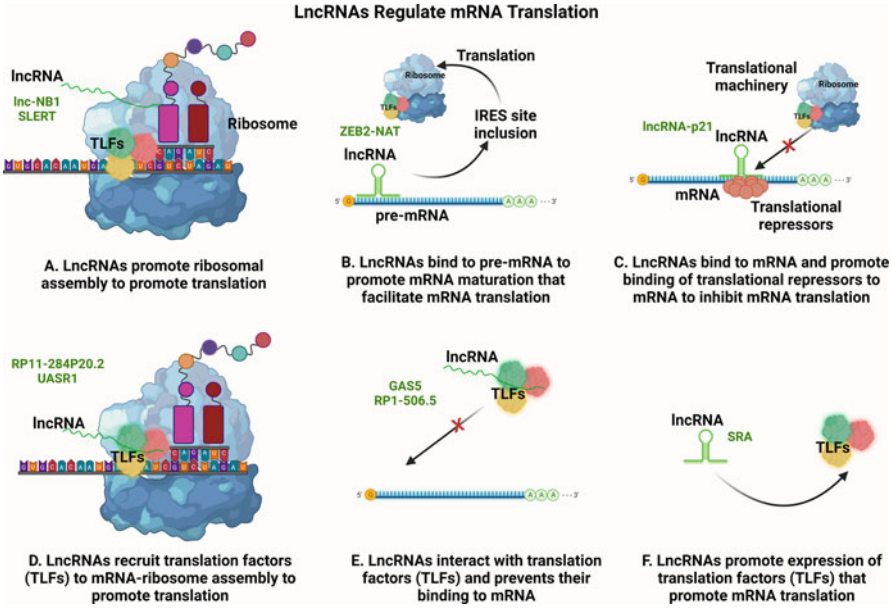

Fig. 11 Regulation of mRNA translation by lncRNAs. Different mechanisms are utilized by lncRNAs to modulate the translation of mRNAs in the cytosol. LncRNAs regulate ribosomal assembly by interacting with ribosomal components to promote mRNA translation (**a**). LncRNAs can bind to pre-mRNAs and regulate its maturation, thus promoting their translation (**b**). Some lncRNAs bind with mRNAs and promote the binding of translational repressors to inhibit mRNA translation (**c**). LncRNAs could also bind to translation factors and recruit them to mRNA to promote translation (**d**). On the contrary, some lncRNAs bind to translation factors and prevent their binding to mRNA thereby inhibiting their translation (**e**). LncRNAs could also regulate the expression of translation factors to promote mRNA translation (**f**). Examples of lncRNAs that regulate mRNA translation are in green. The abbreviations are the following: lncRNAs – long non-coding RNAs; mRNA – messengerRNA; TLFs – Translation Factors; IRES – Internal Ribosome Entry Site

lncRNA ZEB2-NAT through an IRES-dependent translation mechanism. ZEB2-NAT binds with ZEB2 mRNA and prevents the splicing out of an intronic IRES site in the 5' UTR of ZEB2, thereby enhancing IRES-dependent translation of ZEB2 mRNA and its expression (Beltran et al. 2008). It has been observed that one of the tumor-suppressive mechanism by lncRNA-p21 involves its ability to inhibit the translation of the mRNAs of oncogenic *CTNNB1* (catenin beta 1) and *JUNB* by facilitating the association of translational repressors RCK and FMRP to the respective mRNAs to repress their translation. However, the destabilization of lncRNA-p21 by HuR in cervical cancers relieves this derepression, promoting the translation of CTNNB1 and JUNB with the resultant activation of downstream oncogenic signaling (Yoon et al. 2012). PYCARD-AS1 lncRNA interacts with PYCARD mRNA at the 5'UTR to inhibit ribosomal assembly for attenuating PYCARD translation in breast cancer cells (Miao et al. 2019).

LncRNAs also directly interact with translation factors to regulate mRNA translation. In hepatocellular carcinoma, lncRNA RP11-284P20.2 acts as a guide and recruits eIF3B (eukaryotic translation initiation factor 3B) to c-Met mRNA to promote its translation, thereby enhancing c-Met-mediated proliferative and invasive signals in these cancer cells (Fang et al. 2020). In breast cancer cells, lncRNA RP1-506.5 interacts with translation initiation complex factor eIF4E, acts as its decoy, and inhibits its binding to eIF4G (eukaryotic translation initiation factor 4G). This prevents the translation of p27kip1, which is a transcriptional repressor of Snail1. Thus, Snail-mediated EMT and metastasis are stimulated in these cancer cells (Jia et al. 2019). LncRNA GAS5 is downregulated in cancer cells, which otherwise binds to eIF4E and prevents the translation of oncogenic c-Myc and adversely affects the transcription of subsequent downstream oncogenic c-Myc targets to inhibit tumor progression (Hu et al. 2014b).

In addition, lncRNAs regulate translation by affecting the expression of translation factors, as well. In endometrial cancer cells, lncRNA SRA enhances the expression of *4E-BP1* (eIF4E binding protein 1) through an unknown mechanism, which promotes translation of β-catenin. This enhances Wnt/β-catenin signaling enabling EMT and metastasis in endometrial cancer cells (Park et al. 2020). LncRNAs also regulate signaling pathways that promote translation events. PI3K/AKT/mTOR pathway, specifically mTORC1, can promote translation events by phosphorylating eIF4E inhibitor, 4E-BP1, and ribosomal protein S6 kinase (S6K). Phospho-4E-BP1 releases eIF4E to interact with other components for translation initiation, while phospho-S6K phosphorylates eIF4B (eukaryotic translation initiation factor 4B) and recruits it eEF4A (eukaryotic translation elongation factor 4A) to promote translation. LncRNA UASR1 promotes translation of oncogenes in breast cancer cells by activating PI3K/AKT/mTOR pathway to enhance the levels of p-4E-BP1 and p-p70S6K (Cao et al. 2019b).

LncRNAs and Signaling Pathway Modulation

LncRNAs possess diverse secondary structures that enable them to bind with DNA, RNA, and proteins to orchestrate and/or to be an integral component of diverse

context-specific interactomes. As discussed above, the net outcome of lncRNA-mediated interactions is coordinated network of gene expression that promotes tumorigenesis, tumor growth, and tumor progression. LncRNA-mediated cytoplasmic interactome and scaffolds play a crucial role in the modulation of signaling pathways in cancer cells. In addition to these regulations on gene expression, lncRNAs serve as a scaffold for diverse protein interactomes, modulating the functional activation of the interacting partners. In p53 mutant pancreatic cancer cells, LINC00857 serves as a scaffold for facilitating the interaction between FOXM1 and OTUB1 proteins. This interaction promotes the activity of deubiquitinase, OUTB1, and prevents FOXM1 from ubiquitin-mediated proteasomal degradation, thereby promoting the stability as well as activity of oncogene FOXM1 and associated metastasis in pancreatic cancers (Zhang et al. 2023). LncRNA IFITM4P, which is upregulated in oral squamous cell carcinoma, serves as a scaffold for SASH1 adaptor protein and TAK1 kinase, promoting the phosphorylation and activation of TAK1. The phosphorylated TAK1, further, interacts with NF-κB to induce the expression PDL1. In addition to NF-κB, nuclear IFITM4P-KDM5A histone demethylase-mediated PTEN expression, together, contributes to enhanced PDL1 expression in the nucleus. This promotes immunosuppressive signals that facilitates the cancer cells to overcome anticancer immune signals (Shi et al. 2022b). Elevated levels of SLCO4A1-AS1 act as scaffold for interaction between Cdk2 and Hsp90, which promotes Cdk2 protein stability. Active Cdk2 promotes c-Myc signaling cascades by phosphorylating c-Myc and promotes tumor progression in colorectal cancer cells (Zhang et al. 2022b). LncRNA HULC interacts with YBX-1 and ERK to promote ERK-mediated YBX-1 phosphorylation that facilitates its release from YBX-1-bound oncogenic mRNAs. The binding of YBX-1 to mRNAs prevent them from undergoing translation. YBX-1-free mRNAs such as that of CyclinD1, CyclinE1, and MMP3 undergo translation and promote tumor progression and chemoresistance in hepatocellular carcinoma (Li et al. 2017). Another interesting observation is the organization of a functional interactome by the lncRNA glycoLINC to orchestrate metabolic reprogramming in hepatocellular carcinoma. Elevated levels of lncRNA glycoLINC serve as a scaffold for the glycolytic pathway enzymes PGAM1, ENO1, PKM2, and LDHA. This interactome, involving the enzymes in the later stages of glycolysis, promotes glycolytic flux, reduces OXPHOS, and enhances ATP synthesis, thereby contributing to cancer cell survival during the conditions of serine deprivation (Zhu et al. 2022).

Tumor suppressor lncRNAs also serve as scaffold to regulate the functions of their interacting proteins. In gastric cancer cells, lncRNA SDCBP2-AS1 is downregulated, which would otherwise serve as a scaffold for hnRNPK and mediate hnRNPK-β-catenin protein interactions. SDCBP2-AS1 inhibits the SUMOylation of the hnRNPK and promotes ubiquitination of both hnRNPK and β-catenin, leading to their degradation. In these cancer cells, while this lncRNA is downregulated, hnRNPK will be actively SUMOylated that prevents β-catenin ubiquitination and degradation. Active β-catenin will be then translocated to nucleus that promotes transcription of downstream target genes. Thus, this lncRNA inhibits tumor progression and metastasis in these cancer cells (Han et al. 2022).

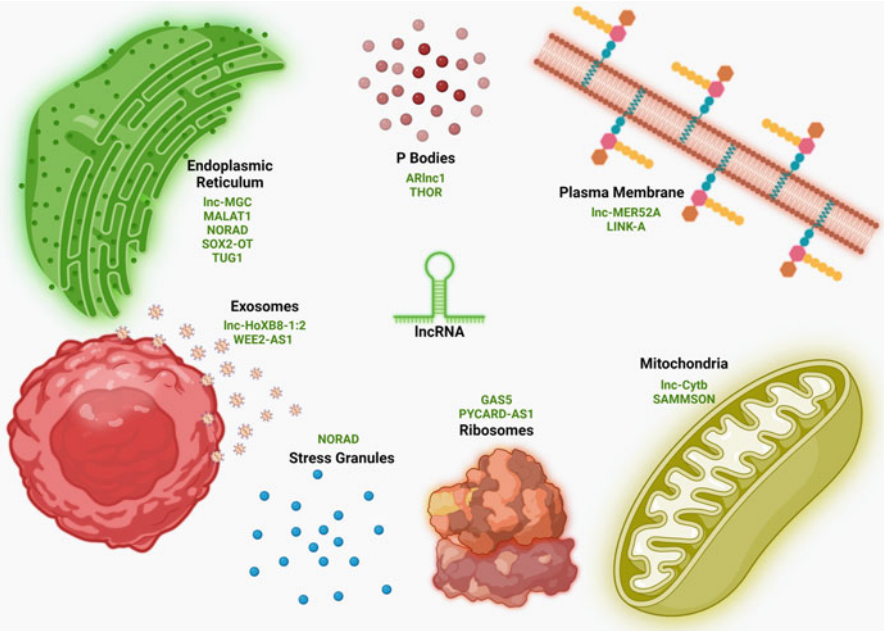

Fig. 12 Organelle-specific LncRNAs. Several lncRNAs have been identified to be associated with subcellular components and cellular organelles such as endoplasmic reticulum, ribosomes, mitochondria, P-bodies and stress granules, plasma membrane, and extracellular vesicles. Their functional roles in cancer context are beginning to be understood only recently (detailed in the text)

Organelle-Specific Functions of lncRNAs

In addition to the critical roles of lncRNAs in the nucleus, lncRNAs have been shown to be associated with other organelles. Within the cytoplasm of the cancer cells, lncRNAs can localize to numerous subcellular compartments other than the cytosol (Fig. 12). These include endoplasmic reticulum, ribosomes, mitochondria, and cell membrane as well as cytoplasmic microbodies such as P bodies and stress granules. The release of lncRNAs through exosomes that serve as a cardinal means of intercellular contact between tumor cells and the components of the TME makes it significant to be addressed as another subcellular compartment to which lncRNAs localize.

LncRNAs of Endoplasmic Reticulum

Endoplasmic reticulum (ER) serves as the primary site for protein synthesis and folding. ER is the site where most of the post-translational modifications that regulate the protein functions occur. Recent study has shown that TUG1 and NORAD, two lncRNAs dysregulated in cancer cells, were localized to ER (Fazal et al. 2019).

Though TUG1 acts both as oncogene and tumor suppressor in a context-specific manner in cancers, it has previously been reported to be localized to nucleus and have functions in regulating gene expression (Wang et al. 2017). LncRNA NORAD has been known to be upregulated in numerous cancers and is extensively studied for its association with Pumilio proteins in regulating genomic stability (Soghli et al. 2021). However, the roles of both these lncRNAs within ER have not been elucidated yet. Furthermore, ER stress that occurs during abnormal cancer cell proliferation results in unfolded protein response (UPR) through three sensors which can be either inositol requiring enzyme 1 (IRE1), protein kinase RNA like ER kinase (PERK), or activating transcription factor 6 (ATF6). Numerous lncRNAs such as MALAT1, lnc-MGC, and SOX2-OT have been reported to be upregulated during these responses. However, their functional localization to ER in regulating the ER stress-mediated oncogenic signals are yet to be characterized (Ebrahimi et al. 2022).

LncRNAs of Ribosome

The association of several lncRNAs with ribosomal components as well as ribosomal assembly has been described above. Majority of the lncRNAs that are transported to cytoplasm were associated with polysome complexes involved in mRNA/lncRNA decay (Carlevaro-Fita et al. 2016). It has been shown that lncRNAs are transported to ribosomes for their decay similar to nonsense-mediated decay of mRNAs. This mechanism is attributed to the downregulation of tumor suppressor lncRNA GAS5 lncRNA in numerous cancer cells (Tani et al. 2013; Ji et al. 2019). However, the earlier description of the role of lncRNAs in regulating translation have been detailed.

LncRNAs of Mitochondria

Mitochondria is the site for OXPHOS energy production, ROS generation, and apoptosis, as well as calcium storage. Hence, the mitochondrial function plays a central role in tumorigenesis and progression. Several lncRNAs have been shown to regulate mitochondrial processes to regulate tumor growth. LncRNAs seen within mitochondria can arise from either mitochondrial genome or nuclear genome. Lnc-Cytb arises from the mitochondrial genome and has been reported to shuttle between mitochondria and nucleus in hepatocellular carcinoma, though the exact role of this lncRNA has not been identified yet (Rackham et al. 2011; Zhao et al. 2019). Nuclear genome-encoded lncRNA SAMMSON is upregulated in melanoma in response to transcription factor SOX10. SAMMSON interacts with mitochondrial membrane and facilitates the mitochondrial import of p32. SAMMSON-p32 interaction enhances pro-oncogenic signals by regulating mitochondrial 16S rRNA maturation, maintaining mitochondrial membrane potential, regulating mitochondrial respiratory chain, regulating mitochondrial OXPHOS, and controlling expression of mitochondrial DNA-encoded proteins such as COX2 and ATP6, as well as maintaining the structure and integrity of mitochondria (Leucci et al. 2016).

LncRNAs of Cytoplasmic Microbodies

Similar to the nuclear microbodies such as nuclear speckles and paraspeckles, which has been described earlier, cytoplasmic microbodies also exist such as P-bodies and stress granules. They act as sites for storage, trafficking, processing, and metabolism of numerous RNAs. P-bodies are the sites where untranslated mRNAs associate with RNAi, miRNA, or RNA decay complexes (Hubstenberger et al. 2017). One of the major proteins abundant in the P bodies is IGF2BP1, which is known to regulate mRNA stability. LncRNA THOR has been reported to be associated with IGF2BP1, while ARlnc1 has been reported to be interacting with HuR at the P-bodies (Pitchiaya et al. 2019). These lncRNAs have been reported to interact with these protein partners in cancer cells to regulate mRNA stability (Pitchiaya et al. 2019; Huang et al. 2018b; Zhang et al. 2018c). However, detailed studies have to be carried out in unraveling the roles of P-bodies in mediating lncRNA-protein-mRNA interactions.

Stress stimuli within the cancer cells trigger translational arrests that accumulate large number of untranslated mRNAs within the stress granules. One of the prominent lncRNAs found abundantly in stress granules is the NORAD. Recently, reports have shown that AU-rich elements in the NORAD transcript facilitate binding of AU-rich element binding proteins such as TIA1 or TIAR that are required for stress granule formation (Namkoong et al. 2018). LncRNA has also been shown to interact with ATP helicase, eIF4A, to prevent its recruitment to stress granules (Tauber et al. 2020). However, the functional role of stress granule-localized oncogenic lncRNAs, such as NORAD, in regulating tumor progression, remains to be defined.

LncRNAs of Plasma Membrane

Plasma membrane that limits and protects the cells from extracellular milieu is an integral part of cell signaling mechanisms due to the embedded receptors, transporters, and other myriads of signaling proteins. Numerous lncRNAs have also been observed to be localized to the plasma membrane lipid bilayers. These lncRNAs are actively involved in promoting oncogenic signaling in many cancer cells. The critical role of plasma membrane-localized lncRNAs in several other oncogenic contexts are beginning to emerge only now. Representative examples include those of the lncRNAs LINK-A and lncMER52A. In triple negative breast cancer cells, LINK-A was found to be associated with PIP3 (phosphatidyl inositol-3,4,5-triphosphate) on the lipid membrane, upper conditions of hypoxia, to activate AKT-mediated signaling cascade that promotes tumorigenesis (Lin et al. 2017; Lin et al. 2016). This endows resistance to AKT inhibitors in these cancer cells. Furthermore, in hepatocellular carcinoma cells, lncMER52A was found to interact with p120 catenin and promote its stability as well as the downstream Rho-GTPase oncogenic signals. The p120 catenin is a key member of adherens junctions involving the outer plasma membrane that promotes cell-cell contact (Wu et al. 2020).

LncRNAs of Extracellular Vesicles

Extracellular vesicles are heterogenous collection of membrane-bound vesicles that originate from endoplasmic reticulum or plasma membrane. They include apoptotic bodies (50–5 μm), microvesicles (200 nm–1 μm), macrovesicles (500 nm–2 μm), and exososmes (50–100 nm). Of these different EVs, the oncogenic role of exosomes are well characterized. In cancer context, exosomes act as transporters for diverse cargo including nucleic acids and proteins to mediate intercellular communication during tumorigenesis. Recent studies have shown that exosomal cargo also includes lncRNAs. The exosomal cargo release can be either from the cancer cells to other cell types within the TME or vice versa (Jahan et al. 2022). The exosomes released from neuroendocrine-differentiated colorectal cancer cells expressed higher levels of lnc-HoXB8-1:2, which is transferred to macrophages. The lncRNA sequesters miR-6825-5p to enhance *CXCR3* expression, thereby augmenting macrophage M2 polarization and TAM (tumor-associated macrophage) infiltration into tumors aiding tumor progression (Li et al. 2022b). LncRNA WEE2-AS1 is highly expressed in the exosomes derived from the cancer-associated fibroblasts in colorectal cancer cells. When WEE2-AS1-rich exosomes are transferred to the cancer cells, they promote tumor progression by serving as a scaffold for MOB1A and Praja 2, E3 ubiquitin protein ligase complex within the cancer cells. This interaction would result in ubiquitin-mediated degradation of MOB1A. MOB1A is a prominent component of the Hippo signaling pathway that attenuates tumor progression by promoting apoptosis. MOB1A degradation, thus, inhibits the Hippo signaling, while activating the nuclear translocation of YAP to activate YAP signaling pathways that promotes tumor growth (Yang et al. 2022). Exosomes are currently explored as cancer biomarkers as well as studied as drug delivery vehicles for anticancer therapy (Liu et al. 2022b).

Conclusion and Perspective

LncRNAs represent a coordinated signaling network that regulates tumor cells as well as tumor microenvironment to modulate the process of tumorigenesis. They act as guides, scaffolds, and decoys to carry out their functional diversity. Advancements in lncRNA research have led to their identification as critical biomarkers for cancer diagnosis, prognosis, and therapy as well as employing them as precise therapeutic targets. Though several other non-coding RNAs such as miRNAs are being explored as therapeutic targets in clinical trials, lncRNAs are yet to reach these stages. However, lncRNAs possess diverse and complex functional repertoire that provides immense strategies to employ them as therapeutic targets. They can be targeted at transcriptional, post-transcriptional and even functional levels to bring about the therapeutic effects. Strategies such as employing antisense oligos against NATs (Natural Antisense Transcripts) have shown promising effects in reactivation of NAT-targeted tumor suppressor genes. Though RNA-based therapeutics has been reported with adverse effects such as specificity, tolerance, and delivery, an interdisciplinary research

involvement which explores their immunogenicity and biodistribution can overpower these drawbacks. Research advancements with lncRNAs in tumor progression serve as a promising preclinical evidence that warrants lncRNA-based therapeutics into clinical trials.

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FRONT MATTER

Title

- **Unraveling a GPCR-lncRNA-miRNA Nexus: Identification of an Aberrant Therapeutic Target in Ovarian Cancer**

- Cancer Nexus: GPCR-lncRNA-miRNA Hub

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Abstract

Ovarian cancer, a leading cause of gynecological malignancy mortality, lacks early diagnostic methods and a comprehensive understanding of molecular mechanisms. Lysophosphatidic acid (LPA)-mediated signaling is pivotal in ovarian cancer progression. Previous transcriptomic analysis identified Urothelial Carcinoma Associated-1 (UCA1) as an LPA-stimulated long non-coding RNA (lncRNA). Here, we elucidated the tripartite synergy among LPA-receptors (LPARs), UCA1, and let-7 miRNAs in ovarian cancer growth regulation. Elevated UCA1 expression promoted proliferation, invasive migration, and therapy-resistance in high grade serous ovarian carcinoma cells, while UCA1 knockdown attenuated these responses. UCA1 showed an inverse correlation with patient survival and therapy resistance in ovarian cancer samples. Mechanistically, UCA1 acted as a molecular sponge for let-7 miRNAs, impairing their tumor-suppressive activity. Importantly, intratumoral injection of UCA1-specific siRNAs suppressed cisplatin-refractory ovarian cancer xenografts. Our findings emphasize the potential of the LPAR-UCA1-let-7 axis as a promising therapeutic target for ovarian cancer.

Teaser

Unveiling the Oncogenic Nexus: LPAR, UCA1, and let-7 miRNAs — A Crucial Link in Cancer Progression and Targeted Therapy.

MAIN TEXT

Introduction

Ovarian cancer, a heterogeneous group of malignancies, stands as the primary cause of death among gynecological cancers (*1, 2*). Despite advances in chemotherapy and surgical procedures, the overall five-year survival rate for ovarian cancer patients remains low (*3*), highlighting the pressing need for the identification of an early-stage biomarker and a deeper understanding of the disease's molecular mechanisms. Recent studies, including our own, have revealed the crucial role of lysophosphatidic acid (LPA)-mediated signaling pathways in promoting ovarian cancer cell proliferation and migration (*4-9*). In our recent transcriptome analysis focusing on genes regulated by LPA, we identified Urothelial Carcinoma Associated-1 (UCA1) as the most highly expressed LPA-responsive long noncoding RNA (lncRNA) in ovarian cancer cells (*4*). LncRNAs, as a family of non-protein coding RNA molecules, have demonstrated critical involvement in gene regulation and cellular processes (*10-12*). More specifically, UCA1 has been implicated in various biological processes, including cell proliferation, migration, apoptosis, and tumorigenesis in cancers (*13-15*). The observation that LPA stimulates the expression of UCA1 in ovarian cancer cells led us to hypothesize that UCA1 plays a pivotal role in the pathogenesis of ovarian cancer and that targeting its expression could hold diagnostic as well as therapeutic potential. Therefore, in the present study, we focused on elucidating the regulatory role of UCA1 in the pathobiology of ovarian cancer. The primary objective of this study is to investigate the functional role of the aberrantly expressed UCA1 in ovarian cancer, providing novel insights into the pathobiology and paving the way for the development of potential diagnostic and therapeutic strategies.

Results from our investigations using high-grade serous ovarian carcinoma (HGSOC) cell lines and patient-derived ovarian cancer cells indicate that LPA stimulates UCA1 expression and that UCA1 is involved in LPA-mediated proliferation and migration of ovarian cancer cells. Furthermore, we discovered that LPA stimulates UCA1 expression through a pathway involving the putative gep proto-oncogene, Gα12. Our analysis of an ovarian cancer tissue microarray (TMA), constructed from Oklahoma ovarian cancer patients revealed a role for UCA1 in therapy resistance, which correlated with its ability to confer cisplatin resistance to ovarian cancer cells. Additionally, we found that UCA1 acts as a competing endogenous RNA (ceRNA) for the let-7 family of microRNAs (miRNAs), attenuating their tumor suppressive effects and increasing the expression of let-7-targeted oncogenes, such as c-Myc, Ras, and IL-6. Furthermore, intratumoral injection of synthetic small interfering RNA (siRNA) to UCA1, silencing the expression of UCA1, alleviated the suppression on let-7 miRNA levels and attenuated ovarian cancer xenograft tumor growth along with survival advantage in an *in vivo* ovarian cancer xenograft mouse model.

Understanding the molecular mechanisms underlying ovarian cancer progression and therapy resistance, specifically focusing on the LPAR-UCA1-let-7 axis, has the potential to yield groundbreaking insights for the development of targeted therapeutic approaches in ovarian cancer treatment. Our results shed light on the intricate interactions within the

GPCR-lncRNA-miRNA axis and its contribution to ovarian cancer progression. Moreover, the results from *in vivo* ovarian cancer xenograft mouse models using synthetic siRNA against UCA1 indicate its potential as a therapeutic target for ovarian cancer treatment. In addition, understanding the molecular mechanisms underlying UCA1's role in ovarian cancer progression may lead to the development of innovative biomarker-based approaches for early detection and improved patient stratification.

Results

LPA stimulates the expression of UCA1 in ovarian cancer cells

Previous studies, including our own, have demonstrated that LPA plays a pivotal role in promoting proliferation and migration in various cell types (*4-9, 16-19*). Our microarray-based transcriptomic analysis previously identified UCA1 as one of the major transcripts upregulated by LPA through the Gα12 signaling pathway in the ovarian cancer cell line SKOV3 (*7*). To validate these findings in the context of HGSOC, we treated a panel of HGSOC cell lines with LPA (10 μM) to examine its effect on UCA1 expression. Consistent with our previous observations, LPA stimulation resulted in a significant upregulation of UCA1 expression in all tested ovarian cancer cell lines (Figure 1A). As LPA exerts its specific responses through the activation of G-protein coupled LPA-receptors (LPARs), we investigated the involvement of LPARs in UCA1 stimulation using OVCAR8 cells. Treatment with LPA antagonists effectively inhibited the upregulation of UCA1, indicating LPAR-mediated expression of UCA1 (Figure 1B). The observation that neither of the LPAR1/3 antagonists, Ki16425, and LPAR2 antagonist, H2L5186303, completely inhibited UCA1 expression suggested the potential involvement of receptor sub-type permissiveness in inducing UCA1 expression. While Ki16425 and H2L5186303 showed partial inhibition of UCA1 expression, the LPAR1-specific antagonist, ONO-7300243, exhibited the least efficacy in inhibiting UCA1 expression (Figure 1B), implying a dominant role of LPAR2 and LPAR3 in inducing UCA1 expression by LPA.

Our initial array analysis indicated that the upregulation of UCA1 is dependent on the α-subunit of the G protein G12 (Gα12), as evidenced by the reduced expression of UCA1 in Gα12-silenced cells in response to LPA. To further confirm the role of Gα12 in LPA-mediated UCA1 stimulation, we tested whether the overexpression of a GTPase activity-deficient, constitutively activated mutant, Gα12QL, could induce UCA1 expression independent of LPA-LPAR signaling. Our results from a panel of ovarian cancer cells indicated that the expression of Gα12QL increased UCA1 expression, providing evidence for the downstream role of Gα12 in transducing the LPA-induced upregulation of UCA1 (Figure 1C).

These findings provide mechanistic insights into the LPA-mediated stimulation of UCA1 expression and highlight the contribution of LPARs and G□12 signaling in this process. Overexpression of UCA1 has been implicated in many cancers and has attracted significant attention (*20-23*). However, its expression profile and role in ovarian cancer development remain largely unknown. To address this, we first examined the expression of UCA1 in a panel of ovarian cancer cell lines. As shown in Figure 1D, increased expression of UCA1, albeit at varying levels, was observed in all the tested cell lines. Additionally, we analyzed ovarian cancer patient-derived cells from two cohorts, which also exhibited elevated expression of UCA1 (Figure 1E).

UCA1 promotes the proliferation of ovarian cancer cells.

We further investigated whether the aberrant overexpression of UCA1 or LPA-induced expression of UCA1 directly influences aggressive growth in terms of proliferation and invasive migration of ovarian cancer cells. To assess this, we performed *in vitro* phenotypic assays using ovarian cancer cell lines with UCA1- overexpression or UCA1-knockdown.

Using Click-iT-EdU cell proliferation assay, which monitors the incorporation of the thymidine analogue 5-ethynyl-2'-deoxyuridine into DNA, we demonstrated that UCA1 knockdown with siRNAs inhibited LPA-stimulated proliferation of both SKOV3.ip (Figure 2A) and OVCAR8 cells (Figure 2B). Using IncuCyte cell proliferation assay, we could also demonstrate that siRNAs to UCA1 inhibited both the FBS- and LPA-stimulated growth rate of OVCAR8 cells (Figure 2C).

Next, we examined the cellular responses regulated by UCA1, drawing analogy from previous findings in bladder cancer, where ectopic overexpression of UCA1 correlated with the regulation of proliferation-specific genes (*24, 25*). Therefore, we tested whether UCA1 promotes the proliferation-specific genes in ovarian cancer. As shown in Figure 2D, the transient expression of a vector encoding UCA1 led to increased expression of proliferation-specific cyclin D1 (*CCND1*) and cyclin D3 (*CCND3*) genes, along with a concomitant decrease in the CDK-inhibitor p27. Conversely, treating the cells with siRNAs to UCA1 inhibited the expression of cyclin D1 and the proliferation marker Ki67 (Figure 2E).

UCA1 promotes the migration of ovarian cancer cells.

Considering our previous studies demonstrating that LPA stimulates epithelial-mesenchymal transition (EMT) to promote cell migration (*7*), we assessed the expression of EMT-specific genes such as Slug, Twist, Vimentin, and Fibronectin in response to UCA1-expression in SKOV3.ip cells. As shown in Figure 3A, the transient expression of UCA1 led to increased expressions of all the tested EMT-genes. These genes, though carrying out different functions involved in the EMT of cancer cells, collectively promote the invasive migration of tumor cells. Consequently, we conducted a series of comprehensive assays using different ovarian cancer cell lines to investigate the role of UCA1 in ovarian cancer cell migration.

First, we carried out IncuCyte Scratch Wound assay using UCA1-silenced OVCAR8 cells. The results demonstrated that the silencing of UCA1 effectively inhibited LPA-induced migration of OVCAR8 cells (Figure 3B). Subsequently, we interrogated the role of UCA1 in LPA-stimulated invasive migration using the Transwell Invasive Migration assay with UCA1-silenced COV318 cells. Consistent with our previous findings, the knockdown of UCA1 led to a significant reduction in the invasive migration of COV318 cells in response to LPA (Figure 3C). Similar results were obtained with SKOV3.ip cells (Supplementary Figure 1).

To further elucidate the role of UCA1 on cell migration, we employed Automated Live Cell Tracking assay using UCA1-silenced OVCAR8 cells in Operetta High Content Imaging system. Strikingly, our results revealed a drastic inhibition of cell migration upon UCA1 knockdown (Figure 3D; Supplemental Video 1 & 2). These findings underscore the crucial involvement of UCA1 in promoting both the innate and LPA-induced motogenic potential of ovarian cancer cells, emphasizing its significance in facilitating tumor metastasis.

Expression of UCA1 in ovarian cancer patients and therapy resistance

Transitioning from the cellular context to the clinical relevance of UCA1, our investigation focused on defining the expression profiles of UCA1 in ovarian cancer cells and patients. Utilizing cBioPortal (*26, 27*), *in silico* analysis revealed amplified expression in 15% of ovarian cancer patients, correlating with poor patient survival (Supplementary Figure 2).

To further investigate the clinical significance of UCA1 expression, we analyzed its expression in a custom-built ovarian cancer TMA (*28*). This TMA was designed to examine UCA1 expression in a cohort of 125 patients diagnosed with HGSOC. The original tissue samples were collected between 2000 and 2011 from patients with advanced-stage disease and subsequent recurrence during follow-up. Cases were individually reviewed by a gynecologic pathologist, and 1 mm cores in triplicate were taken from tumor blocks for the TMA using a standardized protocol.

The median age of the population was 65 years, 73% had an optimal cytoreductive surgery (<1 cm gross residual), and 98% were treated with chemotherapy. The median progression-free survival (PFS) for the entire cohort was 14 months, with a median overall survival (OS) of 42 months. We divided the entire population into four cohorts based on the duration of PFS to allow comparisons between various definitions of platinum resistance and sensitivity. UCA1 expression in the TMA was monitored using the RNAScope method of *in situ* hybridization with a UCA1-specific probe, and the average UCA1 expression value was recorded. RNAScope-based *in situ* hybridization analysis revealed significant overexpression of UCA1 in ovarian cancer tissue compared to adjacent normal ovarian epithelial tissue (Figure 4A). Analysis of UCA1 expression based on median values or high and low quartile scores was correlated with clinical and pathological variables, PFS, and OS using appropriate statistical tests, including chi-square, Wilcoxon rank sums, and log-rank tests as indicated. Furthermore, we conducted a multiple variable analysis using the Cox proportional hazards method to assess the independent significance of variables in the model. Statistical evaluations were performed using SAS 9.2 programming. Scoring the expression of UCA1 in correlation with sample annotations in the TMA indicated a median OS of 51 months for UCA1 low-expressing patients, contrasting with 40 months for UCA1-high expressors (Figure 4B). These observations suggest that UCA1 overexpression can be correlated with therapy resistance and accelerated disease recurrence in ovarian cancer patients.

To investigate whether UCA1 contributes to therapy resistance in the ovarian cancer context, we interrogated its role using the syngeneic, cisplatin-sensitive, and cisplatin-resistant TYK-nu cell lines. Figure 4C demonstrated relatively higher expression of UCA1 in cisplatin-resistant TYK-nu.CP-r cells compared to the cisplatin-sensitive TYK-nu cell line, suggesting a potential role of UCA1 in conferring resistance to chemotherapy.

To further validate the role of UCA1 in therapy resistance, we examined whether UCA1 silencing could enhance cisplatin sensitivity in ovarian cancer cells. Silencing UCA1 led to increased cisplatin-mediated DNA damage response in a dose-dependent manner, indicating a causative role for UCA1 in the cisplatin-resistant phenotype of ovarian cancer cells (Figure 4D). DNA-damage response was monitored by histone H2AX phosphorylation, a marker for DNA-damage repair (*29*). We also tested whether the knockdown of UCA1 could enhance sensitivity in another HGSOC cell line, OVCAR8. To test, we exposed UCA1-silenced and control OVCAR8 cells to varying concentrations of cisplatin and monitored their viability. Notably, the UCA-knockdown cells displayed heightened sensitivity to cisplatin, as evidenced by decreased cell viability compared to control cells with a decrease in IC50 values for cisplatin (Figure 4E).

Spheroid formation is an important characteristic of cancer cells, resembling the three-dimensional architecture observed in tumors. *In vitro* spheroid formation assay has emerged as a valuable tool to assess aggressive cancer behavior associated with tumor progression, metastasis, and therapy resistance (*30-32*). Therefore, we investigated whether UCA1 modulates this critical process in ovarian cancer cells. We found that UCA1 knockdown disrupted the formation and growth of OVCAR4 spheroids, as shown in Figure 4F, highlighting the pivotal role of UCA1 in facilitating spheroid formation and maintenance. Together, these findings implicate the role of UCA1 in promoting aggressive behavior and therapy resistance in ovarian cancer.

UCA1 Modulates Downstream Targets by Interacting with let-7 microRNAs

To gain insight into the underlying molecular mechanisms driving the diverse pathological responses associated with UCA1 in ovarian cancer, we explored its potential interactions with miRNAs, which add a critical regulatory layer in cellular processes. Leveraging previous studies on the lncRNA H19 and *in silico* analysis using the BiBiServ portal (*33, 34*), we carried out *in silico* analysis to identify putative binding sites for specific miRNAs within the sequence of UCA1. Our analysis revealed the presence of nine distinct binding sites for members of the let-7 family of miRNAs (Supplementary Figure 3). To validate the *in silico* finding, we synthesized biotinylated UCA1 RNA and incubated it with the OVCAR8 cell lysate. Following overnight incubation, UCA1 was precipitated using streptavidin. To assess the ability of UCA1 to quench let-7 miRNAs, we performed let-7-specific qRT-PCR on the UCA1 precipitates. Our initial analysis demonstrated the detection of various let-7 family members in the UCA1 precipitates, albeit at varying levels (Figure 5A).

It has been established that miRNAs exist as miRNA ribonucleoprotein complexes in which Argonaute-2 (Ago-2), protein is a major constituent (*35*). Therefore, we employed an Ago-2-based crosslinking and immunoprecipitation (CLIP) assay to monitor the interaction between the lncRNA and bound specific miRNAs following previously published methods (*34, 36, 37*). The assay involved formaldehyde crosslinking of cell lysates, immunoprecipitation of Ago-2, and subsequent quantification of UCA1 and let-7 miRNAs within the immunoprecipitated Ago-2 complex. Such an analysis indicated the presence of UCA1 in Ago-2 precipitates (Figure 5B). Remarkably, the CLIP assay results also provided evidence for the co-precipitation of UCA1 and let-7 miRNAs coexisting in the Ago2 complex, lending support to the potential role of UCA1 in "sponging" and potentially quenching the activity of let-7 miRNAs (Figure 5B).

These findings suggest the paradigm in which UCA1 inhibit the tumor suppressor activity of let-7 miRNAs by sequestering them through the specific let-7 interacting sites. This would also suggest that deleting these sites could rescue the function of let-7 miRNAs. To validate such a paradigm, we constructed a mutant construct of UCA1 in which all the nine potential let-7 binding sites were mutated (Supplementary Figure 3) and assessed its ability to inhibit the activity of let-7 miRNAs. This analysis was carried out using a let-7 activity reporter plasmid, psiCHECK2-let-7-4X (*34*). This reporter construct contains DNA encoding four repeats of the let-7 target sequence at the 3'UTR of the Renilla luciferase mRNA transcript. Increased levels of let-7 miRNAs would lead to their binding to the 3'UTR of Renilla luciferase mRNAs, resulting in the degradation of mRNA encoding Renilla luciferase and hence its activity. To demonstrate, HEK293 cells were transfected with the let-7 activity reporter along with either UCA1 or the UCA1 mutant construct (UCA1mutD). Luciferase activities were measured 36 hours post-transfection using the Promega Dual-Luciferase Reporter Assay System. As shown in Figure 5C, cells expressing UCA1 (Lane 2) exhibited increased luciferase activity, presumably resulting from the quenching of let-7 miRNAs by UCA1 leading to the derepression of the luciferase reporter. Contrarily, cells expressing the mutant UCA1 construct showed decreased luciferase activity (Lane 3), indicating the inability of the mutant UCA1 to quench let-7 miRNAs. These findings were further validated by demonstrating that the exogenous expression of UCA1 led to increased expression levels of canonical let-7 targets, such as Ras and c-Myc (Figure 5D). Consistent with these findings, knockdown of UCA1 resulted in the decreased expression of IL-6, yet another target gene of let-7 miRNAs (Figure 5E). These experimental results provide compelling evidence supporting the hypothesis that UCA1 modulates let-7 activity by potentially binding and quenching let-7 miRNAs. This interaction, as indicated by the alteration in luciferase activity and the regulation of let-7 target genes, highlights the intricate regulatory role of UCA1 in modulating downstream pathways involved in ovarian cancer progression.

Therapeutic Potential of UCA1 siRNA in Suppressing Ovarian Cancer Xenograft Tumor Growth

To assess the therapeutic potential of targeting UCA1 in ovarian cancer, we investigated the effect of UCA1 knockdown on xenograft tumor growth using specific UCA1 siRNA. Our aim was to elucidate the *in vivo* therapeutic impact of UCA1 inhibition and its effect on ovarian cancer xenograft tumors derived from cisplatin-refractory OVCAR8 cells (*38*). OVCAR8 cells were subcutaneously injected, and tumor size was monitored until the average volume reached approximately 50 mm^3. Subsequently, the tumors were intratumorally injected with either scrambled siRNA or UCA1-specific siRNA (10 µg/tumor suspended in 50 µl MaxSuppressor In-Vivo RNA-LANCEr II carrier) at 4-day intervals for a total of 4 injections. Tumor volume was measured every 4 days starting from the 19th day after OVCAR8 cell injection until the 38th day when the mice were euthanized.

The measurements presented in Figure 6A and 6B provide a quantitative analysis of tumor volumes over time. The mean tumor volume change is plotted, allowing for a comprehensive assessment of the overall trend in tumor growth or regression following treatment with scrambled siRNA or UCA1-specific siRNA. Quantitative analysis of tumor volumes was performed, presenting the mean tumor volume change over time (Figure 6A). Additionally, the individual tumor volumes, along with interquartile ranges, were depicted for each treatment group (Figure 6B), providing insight into the distribution and variability of tumor responses to the respective treatments. Subcutaneous tumors were harvested from euthanized animals, photographed, and overall tumor growth was recorded. Our results clearly showed that the intratumoral administration of siUCA1 reduced the xenograft tumor growth (Figure 6C).

To gain a deeper understanding of the mechanistic impact of UCA1 silencing, we analyzed key factors including UCA1 levels, let-7 miRNAs, proliferation markers, and apoptotic markers in xenograft tissues. First, we confirmed the effective knockdown of UCA1 in xenograft tumor tissues using RNAScope analysis (Figure 6D). Next, we evaluated the levels of let-7 miRNAs in response to UCA1 knockdown. Quantitative RT-PCR analysis revealed an overall increase in let-7 miRNA levels in siUCA1-treated xenografts, particularly let-7b-3p, let-7d-3p, and let-7f-1-3p (Figure 6E).

To explore the impact on cellular proliferation, we quantified mitotic figures in xenograft tissues using immunostaining for phosphorylated histone H3 (pHH3), a marker for mitotic cell proliferation (*39*). Xenografts from mice treated with siUCA1 exhibited reduced mitotic figures, indicating a decrease in cellular proliferation (Figure 6F). Additionally, we analyzed cellular apoptosis in the xenograft tumor tissues by performing a TUNEL assay. The results revealed increased staining for DNA breaks, indicating elevated levels of apoptotic events upon UCA1 silencing (Figure 6G). Collectively, these results provide compelling evidence for the therapeutic potential of targeting UCA1 in suppressing ovarian cancer growth. The significant reduction in xenograft tumor growth following UCA1 knockdown, along with the alterations in let-7 miRNA levels, cellular proliferation, and apoptosis, highlights the intricate regulatory role of UCA1 in modulating downstream pathways involved in ovarian cancer progression.

Discussion

Our study unravels a complex interplay between GPCR signaling, the oncogenic lncRNA UCA1, and the let-7 family of tumor suppressor miRNAs in the pathobiology of ovarian cancer. Our results have identified a novel role for LPA in inducing the expression of the oncogenic lncRNA UCA1 (Figure 1A). LPA has been shown to promote ovarian cancer aggressiveness and chemoresistance (*4-9, 31*). The observation that LPA induces significant upregulation of UCA1 indicates the importance of LPA-mediated signaling pathways in driving UCA1 expression and potentially contributing to the aggressive behavior of ovarian cancer. The findings that the LPAR antagonists inhibit LPA-induced upregulation of UCA1 points to LPAR-mediated stimulation of UCA1-expression by LPA (Figure 1B). Interestingly, the partial inhibition of UCA1 expression by LPAR1/3 antagonists Ki16425, and LPAR2 antagonist H2L5186303, suggests potential receptor sub-type permissiveness. Notably, the LPAR1-specific antagonist, ONO-7300243, exhibited the least efficacy in inhibiting UCA1 expression (Figure 1B), indicating a dominant role of LPAR2 and LPAR3 in inducing UCA1 expression by LPA. These findings underscore the importance of specific LPARs in driving the upregulation of UCA1 and provide valuable insights into the molecular mechanisms underlying UCA1's activation in ovarian cancer. The crucial mediator of LPAR-induced UCA1 upregulation was identified as the G protein α-subunit Gα12. The present observation that the overexpression of the constitutively active mutant Gα12QL significantly increased UCA1 expression in ovarian cancer cells (Figure 1C) is in line with our previous findings that the knockdown of Gα12 reduces the expression of UCA1, firmly establishing the pivotal role of Gα12 in transducing LPAR-induced upregulation of UCA1 in ovarian cancer cells.

While our present study does not focus into the specific mechanisms by which Gα12 upregulates UCA1 expression, our previous findings provide insights into a plausible signaling pathway that could connect Gα12 activity to UCA1 regulation. Specifically, our prior work indicates that Gα12 is involved in the modulation of the transcription factor cyclic AMP-response element binding protein (CREB) through Ras-dependent signaling in ovarian cancer cells (*17*). Moreover, our computational analysis using Tfsitescan (*40*) revealed the presence of a CREB-binding site located at position -41 upstream of UCA1's transcription start site (Supplementary Table 5). This raises the intriguing possibility that Gα12 could influence CREB activity, which in turn might impact the expression of UCA1 through CREB. While further experimental validation is required to confirm the direct involvement of CREB in UCA1 upregulation, these combined insights suggest this as a potential signaling conduit downstream of Gα12.

Our study also provides valuable insights into the functional significance of UCA1 in ovarian cancer. Elevated expression of UCA1, observed in both ovarian cancer cell lines, patient derived ovarian cancer cells, and patient samples (Figure 1D & E; Figure 4A), underscores its potential as a critical regulatory molecule in ovarian cancer progression. This finding is particularly significant as UCA1 promotes cell proliferation (Figure 2), migration (Figure 3), and therapy resistance in ovarian cancer cells (Figure 4), emphasizing

its multifaceted impact on the disease. Further clinical support is garnered through TMA analysis, revealing that increased UCA1 expression in a subset of ovarian cancer patients correlates with poor survival outcomes (Figure 4A & B). Moreover, the TCGA dataset indicates UCA1 gene amplification in at least 15% of HGSOC patients (Supplementary Figure 2), and the 19p13.12 region, the genetic locus of UCA1, is amplified in 19.5% of HGSOC patients (*41*). Additionally, our research has identified increased copy numbers of the UCA1 gene in several HGSOC cell lines and ovarian cancer patient tumor samples (Supplementary Figure 4). These findings point towards dual mechanisms underlying the aberrant expression of UCA1 involving gene amplification and signaling-induced. The observation that LPA stimulates the expression of UCA1 in the cell lines with varying UCA1 copy numbers indicate that these mechanisms are non-mutually exclusive. The multifunctional nature of UCA1 in promoting cell proliferation, migration, and therapy resistance, along with its potential correlation with patient survival outcomes highlights the significant role of aberrantly expressed UCA1 in shaping the disease course of ovarian cancer, thus underscoring its clinical relevance.

Mechanistically, our study uncovered a compelling interaction between the oncogenic lncRNA UCA1 and the tumor suppressive miRNAs of let-7-family. UCA1 was found to bind and potentially sequester let-7 miRNAs, exerting a regulatory influence on downstream pathways involved in ovarian cancer progression. This interaction was validated through *in vitro* RNA pull down and CLIP assays, providing evidence for UCA1's role in acting as a "sponge" for let-7 miRNAs (Figure 5 A & B), thereby derepressing downstream targets. Our study also poses an intriguing question regarding the requirement for UCA1 in attenuating the activity of tumor suppressor miRNAs, considering that the expression of such miRNAs is typically reduced in cancer. The observation of substantial let-7 miRNA levels in ovarian cancer cells suggests that the sequestration of let-7 miRNAs by UCA1 plays a critical role in the pathobiology of ovarian cancer. To preemptively address this question, we measured the levels of Let-7 miRNAs in both ovarian cancer cell lines and patient-derived ovarian cancer cells. Surprisingly, our analysis revealed substantial levels of let-7 miRNAs in both the groups of cells (Supplementary Figure 5), suggesting that the quenching of let-7 miRNA is, perhaps, critically required for tumorigenesis and tumor progression in ovarian cancer. Generally, tumor suppressor genes are known to be downregulated or functionally impaired in cancer, leading to uncontrolled cell growth and tumor progression. However, emerging evidence suggests that the regulation of tumor suppressor pathways is more complex than initially thought, involving not only genetic alterations but also epigenetic and post-transcriptional mechanisms. Our study sheds light on one such mechanism, where UCA1, an oncogenic lncRNA, interacts with and modulates the activity of let-7 miRNAs (Figure 5C & D). This novel finding adds to the growing understanding of the intricate mechanisms underlying the deregulation tumor suppressor pathways, encompassing not only genetic alterations but also epigenetic and post-transcriptional mechanisms.

More importantly, our findings hold profound implications for therapeutic strategies. Targeting UCA1 offers a promising approach to disrupt the GPCR-lncRNA-miRNA

network and counteract the aggressive behavior of ovarian cancer cells (Figure 6). *In vivo* experiments using UCA1-specific siRNA demonstrated its efficacy in suppressing ovarian cancer xenograft tumor growth (Figure 6 A-C) through the modulation of UCA1 levels (Figure 6D) that leads to increased let-7 miRNA expression (Figure 6E), reduced cellular proliferation (Figure 6F), and increased apoptosis (Figure 6G). These observations highlight the therapeutic potential of targeting UCA1 and its associated regulatory network in ovarian cancer treatment. In this context, it is of intertest to note that the increased UCA1 expression correlates with reduced overall survival in various cancers, including prostate adenocarcinoma, melanoma, and lung adenocarcinoma (Supplementary Figure 6). Considering the documented elevation of LPAR signaling across multiple cancers, including prostate adenocarcinoma, melanoma, and lung adenocarcinoma, it is plausible that the paradigm outlined here (Figure 7) plays a pivotal role in the pathogenesis of a broader spectrum of cancers. In the current clinical landscape, a multitude of long non-coding RNAs (lncRNAs) are being investigated as therapeutic targets or diagnostic biomarkers for various cancers (*10*). In fact, UCA1 and the lncRNA WRAP53 are in clinical trials for their utility as diagnostic biomarkers for hepatocellular carcinoma (*42*).

In conclusion, our findings illuminate the intricate roles of LPA-signaling, UCA1, and let-7 miRNAs in ovarian cancer, shedding light on the complex regulatory network underpinning ovarian cancer aggressiveness. The multifaceted role played by UCA1 in promoting tumor progression, metastasis, therapy resistance, and modulation of downstream oncogenic pathways highlights its potential as a promising therapeutic target. The demonstrated *in vivo* efficacy of inhibiting UCA1 to suppress tumor growth in *in vivo* models further emphasizes its significance as a candidate for clinical interventions. By providing comprehensive evidence supporting the critical role of UCA1 in promoting aggressive behavior and therapy resistance in ovarian cancer, our findings extend beyond the confines of this study, resonating with broader implications for the field of cancer biology and precision medicine. These results open new avenues for the development of targeted therapeutic strategies that could ultimately translate into improved outcomes for ovarian cancer patients.

Materials and Methods

Reagents, Cells, and Cell Culture

All the ovarian cancer cell lines used in this study, namely, SNU119, OVCAR3, OVCA429, OV90, CaOV3, OVCAR5, OVCAR8, OVCAR4, OVKATE, OVSAHO, SKOV3, SKOV3-ip, HeyA8, COV318, COV362, TYK-nu, and control Fallopian tube derived epithelial cell line FTE188 have been previously described (*5-7, 9, 30*). TYK-nu.CP-r cell line was obtained from the JCRB Cell Bank, Tokyo, Japan. All cell lines were authenticated by short tandem repeat analysis (*43*) at the Stephenson Cancer Center's Center of Biomedical Research Excellence (SCC-COBRE) Cell line Authentication Research Core Facility. Cells were monitored for mycoplasma contamination using previously published PCR-based protocol (*44*). HEK293 cells were maintained in Dulbecco's modified Eagle's (DMEM) Medium (Cellgro, VA, USA); FTE188 cells were maintained in MCDB105:M199 (1:1) Medium (Thermo Fisher Scientific, MA, USA); TYK-nu.CP-r cells were maintained in Minimum Essential Medium (MEM) (Cellgro, VA, USA). All cells were maintained at 37 °C in a 5% CO_2 incubator. All media were supplemented with 10% FBS (Gemini Bio-Products, CA, USA), 50 U/mL penicillin, 50 µg/mL streptomycin (Cellgro, VA, USA). Patient-derived cell lines ASC110515, ASC102315, ASC092214, ASC080414, ASC011215, ASC092514, ASC082516, and ASC050316 were isolated from the ascites samples of patients at the Stephenson Cancer Center, University of Oklahoma Health Science Center (OUHSC), Oklahoma City, Oklahoma, USA using previously published protocols (*5*). The study was approved by the OUHSC Office of Human Research Participant Protection (HRPP) Institutional Review Board and samples were collected with the informed consent from the patients. SNU3236, SNU3237 and SNU3298 cells were normal fallopian tube derived epithelial cells, while SNUA5, SNUA8 and SNUA39 cells were ovarian cancer cells isolated from ascites samples of patients at the Seoul National University (SNU), Seoul, South Korea. The study was approved by the Office of Human Research Participant Protection Institutional Review Board of the Seoul National University, Seoul, S. Korea and samples were collected with the informed consent from the patients. The ascites-derived ovarian cancer cells from OUHSC were maintained in MCDB:DMEM (1:1) and those from SNU were maintained in DMEM:F12 (1:1) supplemented with 10% FBS, 50 U/mL penicillin and 50 µg/mL streptomycin (Cellgro, VA, USA). All cultures were maintained at 37 °C in a 5% CO_2 incubator. Construction and use of pcDNA3 vectors encoding constitutively activated mutant of G□12 (Ga12Q229L or Ga12QL) has been previously described (*45*). pcDNA3 vector encoding UCA1 was amplified from the total RNA from OVCAR8 cells and the 1.4 kilobase fragment was ligated into BamH1 and EcoR1 sites of the pcDNA3 vector. Lysophosphatidic acid (LPA, 1-oleoyl-2-hydroxy-sn-glycero-3-phosphate), obtained from Avanti Polar Lipids (Birmingham, UK) was dissolved into 5 mmol/L stock solutions in PBS with 0.1% BSA and stored at −80°C until use. LPAR antagonists, Ki16425 (3-[[[4-[4-[[[1-(2-chlorophenyl) ethoxy]carbonyl]amino]-3-methyl-5-isoazoly]phenyl] methyl]thio]-propanoic acid) was purchased from Cayman Chemicals (MI, USA); ONO-7300243 was purchased from

Selleckchem (TX, USA), and H2L5186303 was purchased from TOCRIS (MN, USA). Antibodies, the vendors, and the dilution used for immunoblots, CLIP-assays, and IHC staining are as indicated (Supplementary Table 1). PCR primers used in the study were obtained from IDT Inc., IA, USA (Supplementary Table 2). The siRNAs used for the *in vitro* and *in vivo* experiments were procured from Dharmacon, Horizon Discovery, CO, USA (Supplementary Tables 3 & 4).

Tumor Spheroid Formation Assay

The tumor spheroid formation assay was performed following the previously published protocols (*30*). OVCAR4 cells were transfected with UCA1-siRNAs and scrambled siRNA control and seeded into 6-well plates. Post-24 h transfection, cells were plated at the density of 1×10^4 cells/200 µl per well in 96-well Corning Round bottom ULA plates (Corning, NY, USA). Cells were incubated for 6 days at 37°C, 5% CO_2, and 95% humidity. The spheroid images were acquired at 4x magnification in Olympus CK50 microscope.

Cell Proliferation Assays

Click-iT™ EdU Cell Proliferation Assay: Cell proliferation was monitored by determining the incorporation of the thymidine analog 5-ethynyl- 2′-deoxyuridine (EdU) incorporation into DNA using Click-iT™ EdU Plus Alexa Fluor 488 Cell Proliferation Assay kit (Cat. No. C10637; Invitrogen, MA). Cells were transfected with UCA1-siRNA or and scrambled siControl (Supplementary Table 3) for 24 h. Cells were then serum starved for 6 h, followed by LPA (5 µM) treatment for 18 h. EdU was added to the wells 2 h prior to the end of the experiment, which was followed by Click-iT Plus EdU Alexa Flour 488 Imaging Kit protocol (Cat. No. C10637). The cells were fixed using 3% formaldehyde, permeabilized with 0.1% Triton-X buffer and stained with Alexa Flour 488 dye with the excitation wavelength of 488 nm and emission wavelength of 525 nm. Nuclei were stained with 4'6-diamino-2-phenylindole (DAPI), which has the excitation and emission wavelengths of 350 nm and 470 nm respectively. The stained cells were imaged at 10x magnification in Operetta High-Content Imaging System (Perkin Elmer, MA, USA). The percentage of S-phase cells were calculated using Harmony High-Content Imaging and Analysis Software by counting the EdU-positive ells over the total number of nuclei in the given fields.

Incucyte® Live Cell Proliferation Analysis: Cell proliferation analysis was performed using IncuCyte® S3 Live-Cell Analysis system (Sartorius, Germany). OVCAR8 cells were transfected with UCA1-siRNAs or scrambled siRNA control using Lipofectamine RNAiMax reagent (Invitrogen, MA, USA) and seeded into 96-well plates (1×10^4 cells/well) in appropriate growth media incubated in IncuCyte® S3 Live-Cell Analysis System (37 °C; 5% CO_2) for 24 h. The cells were serum starved for 16 h and treated with either LPA (5 µM) or 10% FBS for 72 h. Effects on cell proliferation were monitored by imaging the cells at 24 h intervals. At each time point, images were taken from eight random wells (4 images/well/group) in the phase bright-field channel at 10x magnification. Cell proliferation was monitored by analyzing the occupied area (Number of cells per field) of cell images over time, using Cell-by-Cell Analysis Software Module of the IncuCyte® software using Adherent Cell-by-Cell application.

Cell Viability Assay

OVCAR8 cells were transfected with UCA1-siRNAs or non-targeting siRNA control and seeded into 6-well plates. After 24 h of transfection, cells were plated in a 96-well plate at a density of 0.8 x 10^4 cells/well. Following overnight incubation, cells were treated with cisplatin at concentrations ranging from 0.001-100 μM, along with the respective vehicle control (0.01% DMSO), and incubated for 48 hours. The Cell Counting Kit 8 (CCK-8) reagent (Sigma Aldrich, MO, USA) containing a water-soluble tetrazolium salt was added to the wells (1:10) and incubated for 2 h. The absorbance of the reduced formazan product was measured at 450 nm using an EnVision Multilabel Plate Reader (Perkin Elmer, MA, USA). Cell survival percentage was calculated, and the IC50 was determined by nonlinear regression analysis using the three-parameter logic sigmoidal dose curve equation in GraphPad Prism software.

Cell Migration Assays

Incucyte® Live Cell Scratch Wound Migration Assay: OVCAR8 cells were transfected with UCA1-siRNAs or scrambled siRNA control using Lipofectamine RNAiMax reagent and seeded into an Incucyte® Imagelock 96-well plate (1×10^4 cells/well) in appropriate growth media. The cells were incubated in the IncuCyte® S3 Live-Cell Analysis System (37 °C; 5% CO2) for 24 h. Subsequently, a wound was created in each well using the 96-pin Woundmaker Tool, followed by incubation in serum-free medium with or without LPA (5 μM). Cell migration was monitored by imaging the wound healing at the 48th hour, using the phase bright-field channel at 10 × magnification. Quantification of cell migration was performed by analyzing the wound width over time using the IncuCyte® Scratch Wound Analysis Software Module.

Invasive Migration Assay: Invasive migration of ovarian cancer cells were monitored using collagen-coated transwell assay as previously described (*9*). In brief, transwells with 8-□m pore were coated with Collagen type 1 (B D Biosciences, NJ, USA) (0.5 g/L) and incubated overnight at 4°C. After transfection with UCA1-specific siRNAs or non-targeting control siRNAs for 24 h, the cells were incubated in serum-free medium for an additional 24 hrs. 5×10^4 transfectants were suspended in 200 μL serum-free media and added to the collagen-coated inserts. The inserts were then placed in a 24-well companion plate containing 500 μL of serum-free media with either vehicle control (0.1% BSA) or LPA (5 μM). After incubation for 20 h, non-migrating cells on the proximal side of the inserts were removed with a cotton swab, and the migrated cells on the distal side were fixed with methanol for 10 min and stained with Hemacolor® Rapid Staining Kit (EMD Millipore, MA, USA). Images of migrated cells were captured at 10× magnification in 5 random fields for each group using an Olympus CK50 microscope.

Automated Single Cell Tracking Analysis: OVCAR8 cells were transfected with the UCA1-siRNAs or scrambled siRNA control and plated into 6-well plates. Post-24 h transfection, the cells were seeded in the serum free medium at a density of $1x10^4$ cells/well into a 96 well collagen coated cell carrier plate (Perkin Elmer, MA, USA) and incubated overnight. Cell migration was stimulated by 10% FBS. Live single cell imaging was performed using an Operetta High-Content Imaging System equipped with temperature (37ºC) and CO_2 (5%) regulation. The morphology of migrating cells was analyzed by acquiring digital phase contrast images using a 10x objective, at 15 minutes interval, for 24

h. Analysis algorithm in the Harmony High-Content Imaging and Analysis Software was used to track and estimate the mean square displacement (MSD) of cells over time in each of the wells to assess the cell migration potential.

Let-7-Luciferase Reporter Assay

Luciferase reporter assay was performed in a 48-well plate scale as previously described with minor modifications (*34*). Let-7 sensor (psiCHECK2-let-7 4x) was purchased from Addgene (Plasmid 20930). Briefly, 20 ng of let-7 sensor (psiCHECK2-let-7 4x) was cotransfected with vector control (pcDNA3), full length UCA1 (pcDNA3-UCA1), or UCA1mutD (pcDNA3-UCA1mutD) into HEK293 cells ($2x10^4$ cell/well) according to previously published protocols (*34, 46*). Luciferase activities were measured 36 h post-transfection using Promega Dual-Luciferase Reporter Assay System (E1910) according to the manufacturer's protocol. Renilla luciferase activity was normalized against Firefly luciferase activities and presented as percentage of inhibition.

Immunoblotting

Cells were lysed using RIPA buffer (PBS pH 7.4, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 50 mM sodium fluoride, 1 mM phenylmethylsulfonyl fluoride, 0.2 mM sodium vanadate, 2 mg/mL leupeptin, 2 mg/mL pepstatin A, and 4 mg/mL aprotinin). Protein quantification, SDS-PAGE separation, and immunoblotting with specific antibodies were performed as described previously [8, 46]. Images were developed using either Kodak Image Station 4000MM (Kodak, NY, USA) or Bio-Rad ChemiDoc MP Imaging System (Bio-Rad, CA, USA).

Quantitative RT-PCR

Total RNA was isolated from the cells using RNAeasy Mini kit (Qiagen, MD, USA). The cDNA was synthesized using iScript cDNA synthesis kit (Bio-Rad, CA, USA) following the manufacturer's protocol in a 20 µl reaction mix with 1 µg RNA. Real-time quantitative PCR (qRT-PCR) was performed using the cDNA from the above step using SsoAdvanced SYBR green PCR kit (Bio-Rad, CA, USA) in a Bio-Rad CFX96 Real time PCR Detection System using specific primers (Supplementary Table 2). The data were normalized to the values obtained with the house-keeping genes. Results were expressed as fold change in gene expression over the control, using the $2^{-\Delta\Delta Ct}$ method.

CNV Analysis

To analyze the copy number variations (CNVs) of UCA1 in HGSOC cell lines and patient tumor tissue samples, we employed droplet digital PCR (ddPCR) following previously published methods (*47*). DNA from HGSOC cell lines was extracted using the Quick DNA Mini-Prep Plus Kit (Zymo Research, CA, USA), adhering to the manufacturer's guidelines and utilizing $5x10^6$ cells for each sample. For FFPE tumor tissues, the HighPrep™ FFPE Tissue DNA Kit (Mag-Bio, MD, USA) was used as per its protocol, incorporating ten 4 µm thick sections from FFPE blocks of each patient's ovarian cancer tissue. The quality and quantity of the procured DNA were subsequently assessed using a micro-volume spectrophotometer (DeNovix Inc., DE, USA). For ddPCR analysis, the genomic DNA was first digested with the HinDIII restriction enzyme. The CNVs of UCA1 were assessed using the TaqMan Copy Number assay probe (Hs01909129_s1, ThermoFisher Scientific, MA, USA), with RPP30 (dHsaCP2500350, Bio-Rad, CA, USA) Hsa probe functioning as a

reference. A 20 µL PCR reaction mixture was prepared, combining ddPCR™ Supermix (Bio-Rad, CA, USA), 5 ng of the template, and the designated probes. This mixture was then dispensed into a DG8 cartridge (Bio-Rad, CA, USA) alongside 70 µL of droplet generation oil. Post droplet creation, the resultant droplets were transferred to a 96-well PCR plate and subjected to PCR, initiating with a denaturation at 95 °C for 10 minutes, followed by 40 cycles at 94 °C for 30 seconds and 60 °C for 1 minute, and concluding with an elongation at 98 °C for 10 minutes. The produced droplets from each well were read on a QX100 droplet reader (Bio-Rad, CA, USA), and the data was processed with QuantaSoft™ version 1.7.4.0917 (Bio-Rad, CA, USA) to represent CNVs of UCA1 in each sample using absolute quantification.

RNA Pull-down Analysis

RNA pull-down analysis was performed according to previously published methods (*46*). Biotinylated UCA1 was prepared as follows. UCA1 sequence from pcDNA3 expression vector was amplified using PCR and subsequently cloned into the pcDNA3 vector (Invitrogen, Waltham, MA). After linearization, the construct was subjected to *in vitro* transcription using biotin RNA labelling mix (Roche, Basel, Switzerland) and T7 RNA polymerase (New England Biolabs) for UCA1 sense or SP6 RNA polymerase (New England Biolabs, MA, USA) for anti-sense strand of UCA1. The resulting RNA transcripts were then treated with RNase-free DNase I (Roche) following manufacturer's protocol and purified using the RNeasy Mini Kit (Qiagen, MD, USA). Biotinylated RNAs, both sense and antisense UCA1 RNAs, were incubated with cytosolic extracts for 2 hours at 4°C. RNA was extracted using a Direct-Zol RNA mini Prep kit (Zymo Research, CA, USA), according to the manufacturer's protocol. Reverse transcription was performed using a miScript II RT kit (Qiagen, MD, USA) and qRT-PCR was performed with the human Let-7 miScript primer assay (Qiagen, MD, USA) in a Bio-Rad CFX96 Real time PCR Detection System.

Cross-linking and Immunoprecipitation (CLIP) Assay

CLIP assay was performed according to previously published protocols (*34, 36, 37, 48*). OVCAR8 cell pellets were resuspended in 10 ml of PBS and cross-linked using formaldehyde at a final concentration of 1 % for 10 min. Cross-linking was quenched using glycine (pH 7.0) at a final concentration of 0.25 M. Cell pellet was resuspended in RIPA buffer with protease inhibitor cocktails and incubated on ice for 15 min. The cells were then subjected to sonication on ice for 12 seconds each for three times at a setting of 3 (output 15 W) using Branson Digital Sonifier 250. Cells were incubated on ice for 2 min in between each sonication cycle. Cell lysate was centrifuged at 14,000 rpm in a micro-centrifuge for 20 min at 4ºC to remove insoluble materials. The lysate was then pre-cleared by incubation with 25 µl each of protein A/G beads (Sigma Aldrich, MO, USA) at 4°C for 2 h. The cleared lysate was mixed with Ago-2 antibody and incubated at 4°C for 2 h. The beads were collected and washed with RIPA buffer 5 times at 10000 rpm for 30 sec each at 4°C. To reverse the crosslinks, 100 µl of elution buffer (50 mM Hepes, pH 7.4, 5 mM EDTA, 100 mM NaCl, 1% SDS, 10 mM DTT) was added to the washed beads and the sample was heated at 70°C for 45 min. At the end of heating, the tube was kept on ice until RNA extraction. RNA was isolated from the immunoprecipitated beads. The cross linked, immunoprecipitated RNA was reverse transcribed and an RT-PCR was carried out for

UCA1 (1.4 kb). The products were run in 1 % agarose gels and visualized using a BioRAD gel doc Ez imager. The cDNA was used for qRT-PCR for UCA1 and let-7 family of miRNAs. Primers for UCA1 used for the qRT-PCR analysis are provided in the supplementary data (Supplementary Table 2). The qRT-PCR for let-7 family of miRNAs was performed with the human Let-7 miScript primer assay (Qiagen, MD, USA).

Tissue Microarray Analysis

The Biospecimen Core of the SCC-COBRE constructed the Tissue Microarray (TMA) for this study. The TMA comprised formalin-fixed paraffin-embedded (FFPE) blocks derived from tumor tissues of 125 patients with high-grade serous ovarian carcinoma (HGSOC), as described previously (*28*). In brief, the TMA included samples from four different groups of patients: (1) 25 platinum-refractory patients who did not achieve a complete clinical response (CR) after primary therapy; (2) 50 platinum-resistant patients, including 25 who achieved a CR but experienced recurrence within 6 months from the last use of platinum (platinum-resistant), and 25 who achieved a CR but experienced recurrence between >6 months and <12 months from the last use of platinum (platinum intermediate group); and (3) 50 patients with platinum-sensitive disease, defined as those who achieved a CR and experienced recurrence >12 months from the last use of platinum in their primary therapy. Prior to participation, written informed consent was obtained from all enrolled patients, and the study protocol received approval from the Institutional Review Board at OUHSC. Tissue cores of 1 mm diameter were extracted, in triplicates, from these blocks and were precisely placed in a new paraffin block using Veridiam Tissue Arrayer (Verdiam Inc, CA, USA). Each TMA had 70-80 spots with triplicates built within them. Paraffin sections of the TMA block were Hematoxylin and Eosin stained. The stained sections were read by a Pathologist and the individual cores were verified for tumor cells. A total of 6 TMAs covered all the patient samples with repetitions.

RNAScope Analysis

RNA *in situ* hybridization technology, RNAScope™ was performed on the FFPE sections of each TMA as well as *in vivo* tumor tissues, with target-specific UCA1 probe (ACD Biotechne, CA), following the manufacturer's protocol on an Automated Leica BOND-RX IHC Stainer kit (Leica Biosystems, IL, USA). Briefly, FFPE tissue sections are pretreated with RNAScope™ pretreatment reagent to unmask the target RNA and permeabilize the cells. RNAScope™ target probe pool with 20 gene-specific double-Z probe pairs were hybridized to target RNA molecules. RNAScope™ detection reagents amplified the hybridization signals via sequential hybridization of amplifiers. Post-amplification, probes were detected with chromogen, 3, 3′-diaminobenzidine tetrahydrochloride (DAB) staining. Each UCA1 molecule appears as a puncta within the cell region. The TMA slides were scanned using an Aperio ScanScope® Scanner (Leica Biosystems, CA, USA) at 20x magnification. Using ImageScope™ and TMA lab™ module (Leica Biosystems, CA, USA), individual tissue core spots were assigned to their respective patient identity. Using Spectra™ Software (Leica Biosystems, CA, USA), these puncta were enumerated and average UCA1 expression value was recorded. Analysis of UCA1 expression based on median values or high and low quartile scores was correlated with clinical and pathological variables, PFS, and OS using appropriate statistical tests, including chi-square, Wilcoxon

rank sums, and log-rank tests. A multiple variable analysis using the Cox proportional hazards method to assess the independent significance of variables in the model was also performed. Statistical evaluations were performed using SAS 9.2 programming.

***In vivo* Xenograft Analysis**

Nu/Nu nude female mice (5–6 weeks old) were purchased from Taconic Labs Inc. and were housed in a barrier facility under 12-hour light/dark cycle under pathogen-free conditions, with food and water ad libitum. All experiments were performed with the approval of the University of Oklahoma Health Science Center Institutional Animal Care and Use Committee. OVCAR8 cells were subcutaneously injected at a density of 2 x 10^6 cells/injection into the on the right flank of nude female mice, and tumor size was monitored until the average volume reached approximately 50mm^3. Subsequently, the tumors were intra-tumorally injected with customized siRNAs purchased from Dharmacon™ (Supplementary Table 4), which were specially processed post-synthesis through counter-ion (Na^+) exchange, sterile filtration, desalting and endotoxin testing, facilitating *in vivo* applications. UCA1-specific siRNA or non-targeting control scrambled siRNAs (10 µg/tumor; suspended in 50 µl MaxSuppressor In-Vivo RNA-LANCEr II carrier) were injected at 4-day intervals, for a total of 4 injections. Tumor volume was measured every 4 days, starting from the 19th day after OVCAR8 cell injection, until the 38th day when the mice were euthanized. Subcutaneous tumors were harvested, photographed, and processed by formalin fixation and paraffin embedding for further analysis.

Phosphohistone H3 (pHH3) Immunohistochemical Staining

FFPE tissues were sectioned at 4 µm thickness and mounted onto positively charged slides. The slides were dried overnight at room temperature and incubated at 60°C for 45 minutes, followed by deparaffinization and rehydration in an automated Leica ST5020 Multistainer (Leica Biosystems, CA, USA). Subsequently, these slides were transferred to the Leica Bond-III stainer (Leica Biosystems, CA, USA) and treated for target retrieval at 100°C for 20 minutes with a retrieval buffer, pH 6.0. Endogenous peroxidase was blocked using peroxidase-blocking reagent, followed by the incubation with the primary antibody, pHH3 (Supplementary Table 1). As the secondary antibody, polymeric horseradish peroxidase-IgG was added. The substrate chromogen, 3,3'-Diaminobenzidine (DAB) detects the complex as brown precipitate, while hematoxylin counterstain the cell nuclei (blue). The slides were then dehydrated on Leica ST5020 Slide Stainer (Leica Biosystems, CA, USA) and mounted with Leica MM24 mounting media. Antibody-specific positive and negative (omission of primary antibody) controls were parallel stained. IHC was carried out with the control siRNA and UCA1-siRNA injected (*in vivo*) xenograft tumor sections. Number of cells in mitotic phase represented as mitotic figures were counted using ImageJ software in 5 fields of each mouse tumor sample and 5 mice tumor mean ± SEM is presented.

***In situ* Apoptosis Assay**

The detection of apoptotic cells in UCA1 knocked down xenograft mice model was performed by TUNEL (Terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling) Assay using *In situ* cell death detection kit (Sigma Aldrich, MO, USA) according to the manufacturer's protocol. In brief, FFPE tissues were sectioned at 4 µm thickness and mounted onto positively charged slides. The slides were dried overnight at room

temperature and incubated at 60°C for 45 minutes followed by deparaffinization and rehydration in an Automated Leica ST5020 Multistainer. Further, the sections were protease treated, permeabilized and labeled with fluorescein-dUTP on the DNA strand breaks by Terminal deoxynucleotidyl Transferase (TdT) using the manufacturer's protocol. The nucleus was labelled with DAPI (5 ug/ml) in PBS. The stained sections were mounted with ProLong™ Gold Antifade Mountant (ThermoFisher, MA, USA). The labelled tissue sections were imaged for DAPI (Excitation wavelength: 350 nm; Emission wavelength: 470 nm) and Fluorescein (Excitation wavelength: 488 nm; Emission wavelength: 525 nm) on Operetta® High Content Imaging System (Perkin Elmer, MA, USA) at 20x magnification. The micrographs show the nucleus stained with DAPI in blue and the apoptotic cells were stained green.

Statistical Analysis

All the experiments were repeated at least three times and the results shown are from a representative analysis. Error bars indicate mean ± SEM. All statistical analysis was performed using GraphPad Prism (La Jolla, CA, USA) by two-tailed student's *t*-test with Welch's correction. Statistical significance between treatment and control groups was determined by Student's t test (**$p < 0.005$; *** $p < 0.0005$; **** $p < 0.0001$).

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Author contributions: Each author's contribution(s) to the paper should be listed (we suggest following the CRediT model with each CRediT role given its own line. No punctuation in the initials.

Conceptualization: DND, CI, YSS, RB, PM
Methodology: RR, JHH, RN, MY, MJ, RG
Investigation: JHH, RR, RN, MY, MJ, RG
Bioinformatic analysis: CX, MJ, MY, DND
Supervision: DND
Writing—original draft: DND
Writing—review & editing: RN, CI, RB, PM, RR, YSS, DND

Competing interests:

All other authors declare they have no competing interests.

Data and materials availability:

All data are available in the main text or the supplementary materials.

Figures and Tables

FIGURE 1

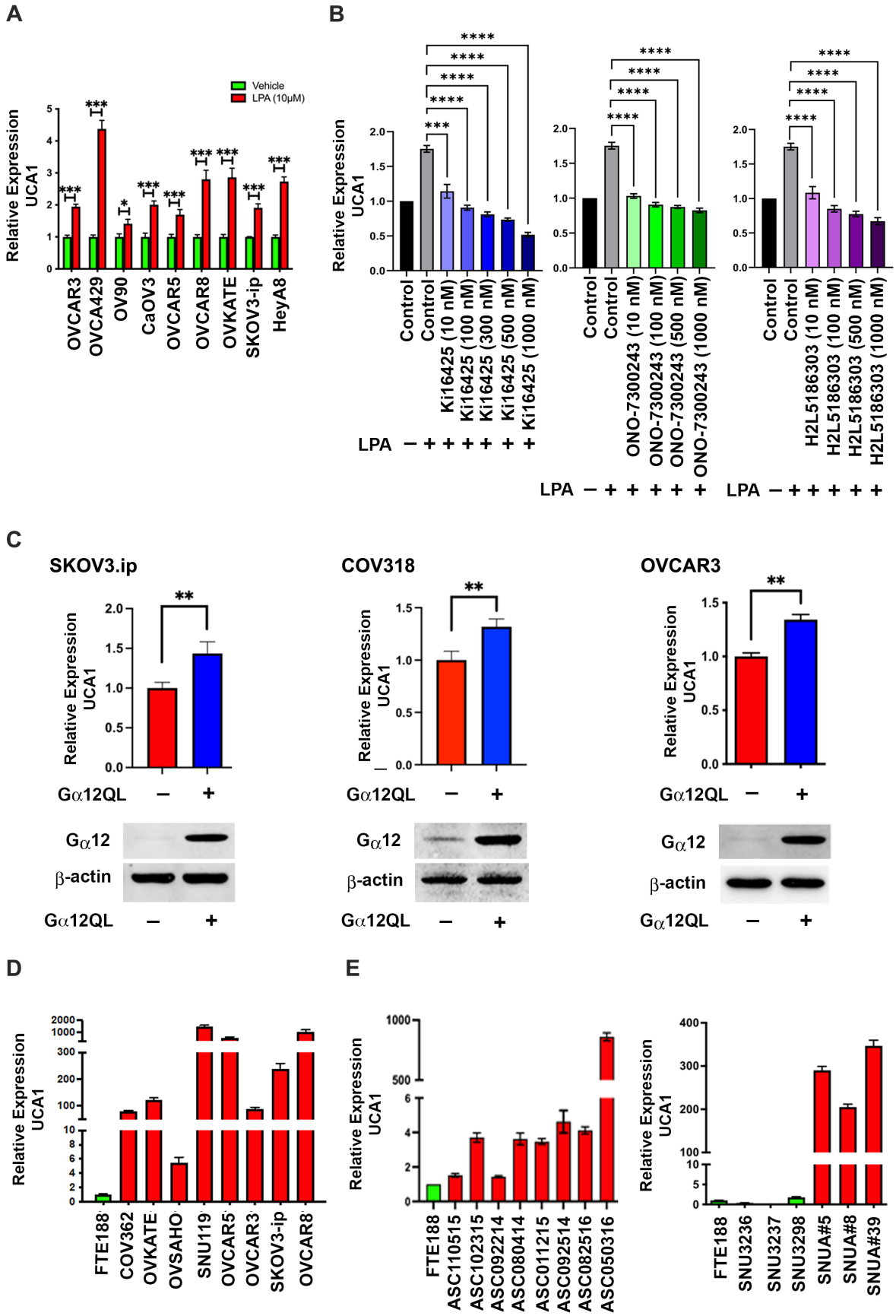
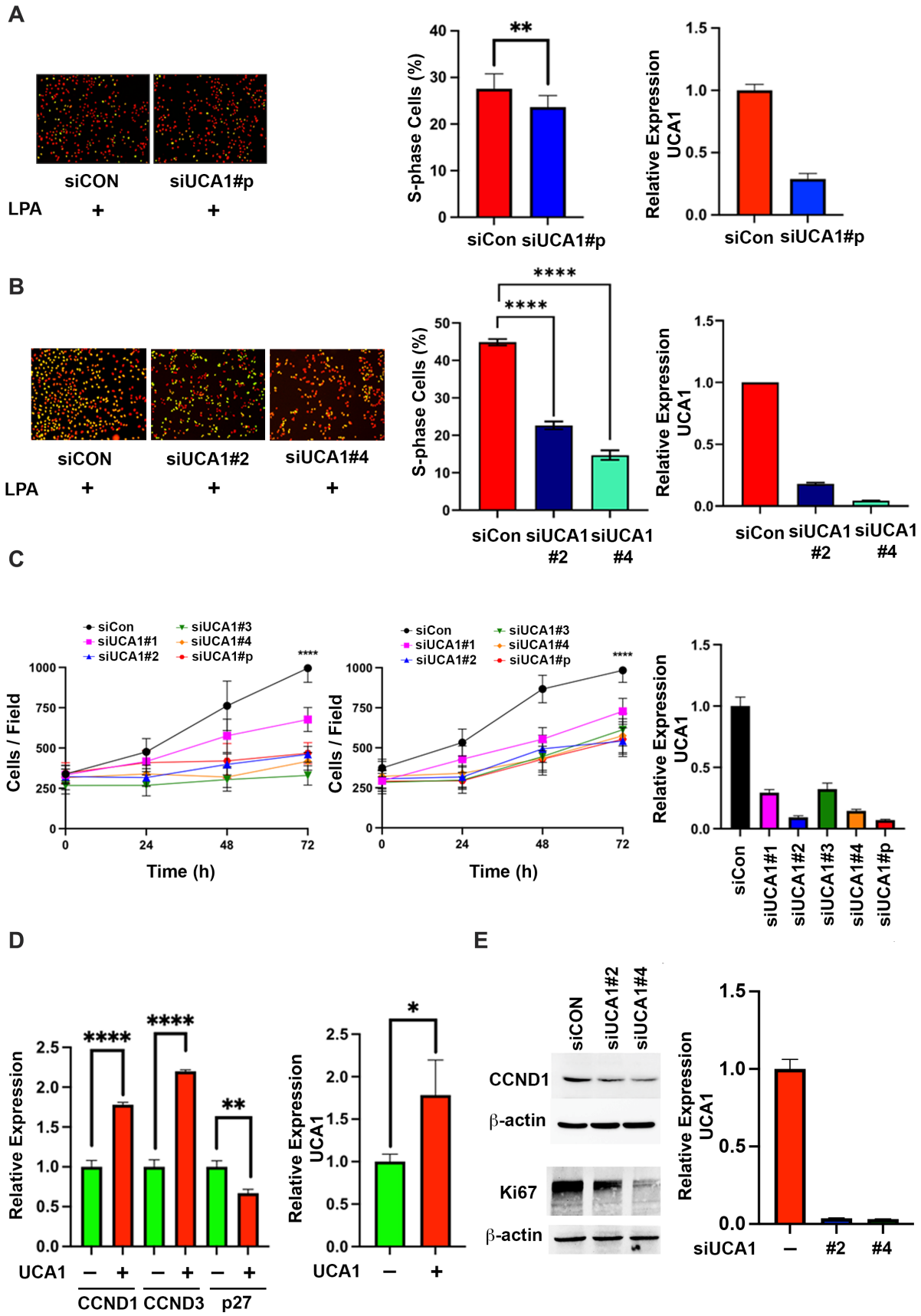


Fig. 1. LPA stimulates UCA1 expression in ovarian cancer cells

A) UCA1 expression was measured by qRT-PCR in a panel of HGSOC cell lines stimulated with LPA (10 μM). **B)** UCA1 expression was measured by qRT-PCR in LPA (5 μM) treated OVCAR8 cells, pretreated with or without LPAR antagonists, Ki16425 (*Left Panel*), ONO-7300243 (*Middle Panel*), or H2L518303 (*Right Panel*). **C)** UCA1 expression was monitored by qRT-PCR in Gα12QL over-expressed SKOV3.ip (*Left Panel*), COV318 (Middle Panel), and OVCAR3 (Right Panel) cells. Gα12QL over-expression in these cell lines were validated by western blotting. **D)** Expression of UCA1 was measured by qRT-PCR in a panel of HGSOC cell lines along with the control, fallopian tube epithelial cell line, FTE188. UCA1 expression was normalized to UCA1 expression in FTE188. **E)** Expression of UCA1 in patient-derived ovarian cancer cells from cohort-1 consisting of ASC110515, ASC102315, ASC092214, ASC011215, ASC092514, ASC082516 and ASC050316 (*Left Panel*) and cohort-2 consisting of SNU#A5, SNUA#8 and SNU#39. (*Right Panel*). SNU3236, SNU3237, and SNU3298 cells formed addition group of control FTE cell lines. UCA1 expression was normalized to UCA1 expression in fallopian tube epithelial cell line, FTE188. Each experiment was repeated three times. Error-bars indicate mean ± SEM. Statistical significance between treatment and control groups was determined by Student's t test (** $p< 0.005$; *** $p< 0.0005$; **** $p< 0.0001$).

FIGURE 2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Relative Expression UCA1
UCA1 – +
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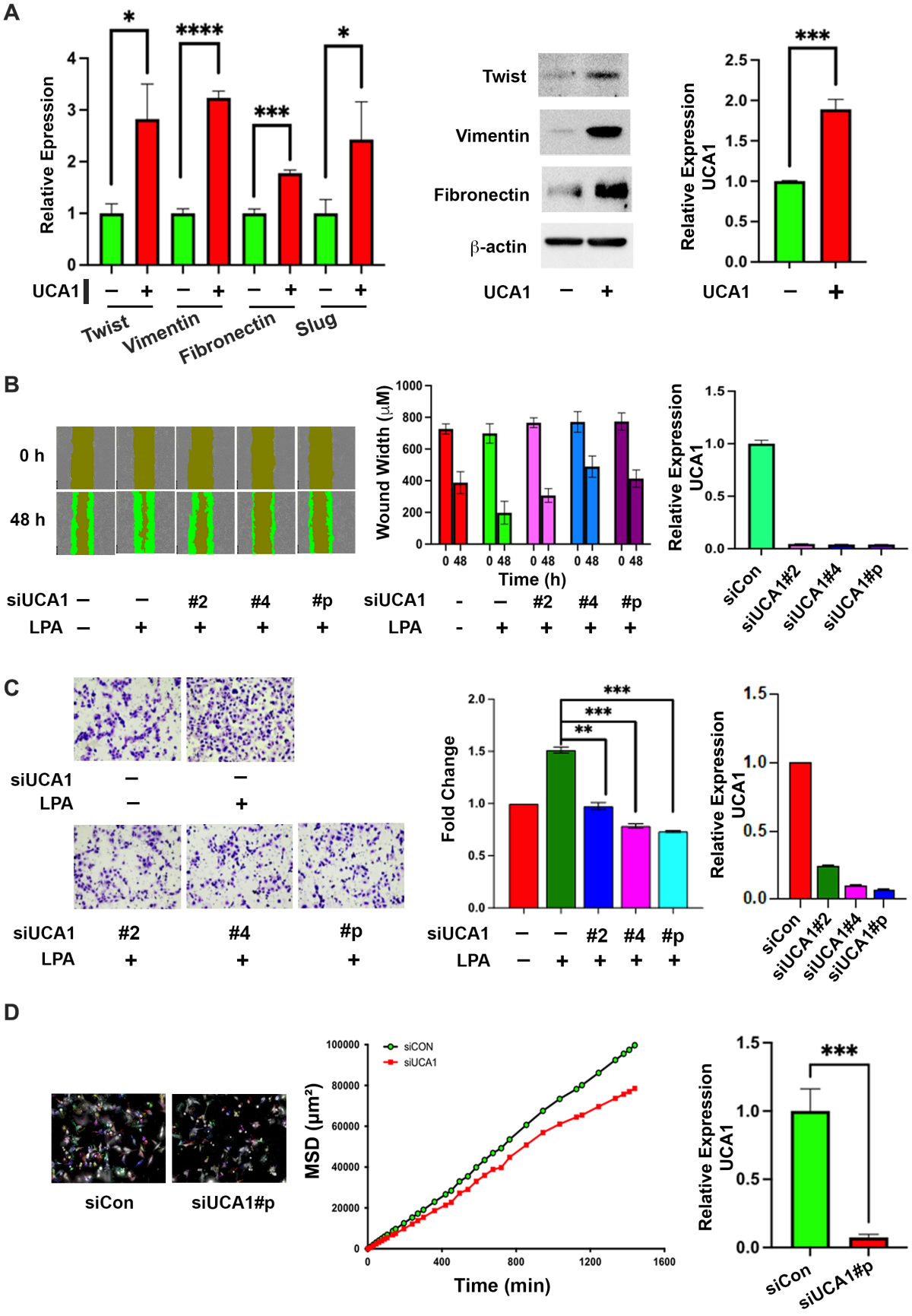
CCND1
β-actin
Ki67
β-actin
Relative Expression UCA1
siUCA1 – #2 #4

84

Fig. 2. UCA1 promotes ovarian cancer cell proliferation

A) Cell proliferation in UCA1-silenced SKOV3.ip and **B**) OVCAR8 cells, stimulated with LPA (5 μM), was assessed with Click-iT EdU proliferation assay using Operetta High Content Imaging system. EdU-positive (green) S-phase cells and DAPI stained nuclei (red) were imaged (*Left Panel*). Percentage of S-phase cells are quantified and represented as graph (*Middle Panel*). UCA1 silencing in these cells were confirmed using qRT-PCR (*Right Panel*). **C)** Cell proliferation in UCA1 silenced OVCAR8 cells treated with either 10%FBS (*Left Panel*) or LPA (5μM) (*Middle Panel*) was monitored using IncuCyte S3 live-Cell Analysis system. UCA1 silencing in OVCAR8 cells was confirmed using qRT-PCR (*Right Panel*). **D)** mRNA expressions of CCND1, CCND3 and p27 in UCA1 over-expressing SKOV3.ip cells were evaluated by qRT-PCR (*Left Panel*). UCA1 over-expression in SKOV3.ip cells was validated by qRT-PCR (*Right Panel*). **E)** Protein levels of CCND1 and Ki67 in UCA1 silenced OVCAR8 cells were obtained by immunoblot analysis (*Left Panel*). UCA1 silencing in OVCAR8 cells was validated by qRT-PCR (*Right Panel*). Each experiment was repeated three times. Error bars indicate mean ± SEM. Statistical significance between treatment and control groups was determined by Student's t test (**$p< 0.005$; *** $p< 0.0005$; **** $p< 0.0001$).

FIGURE 3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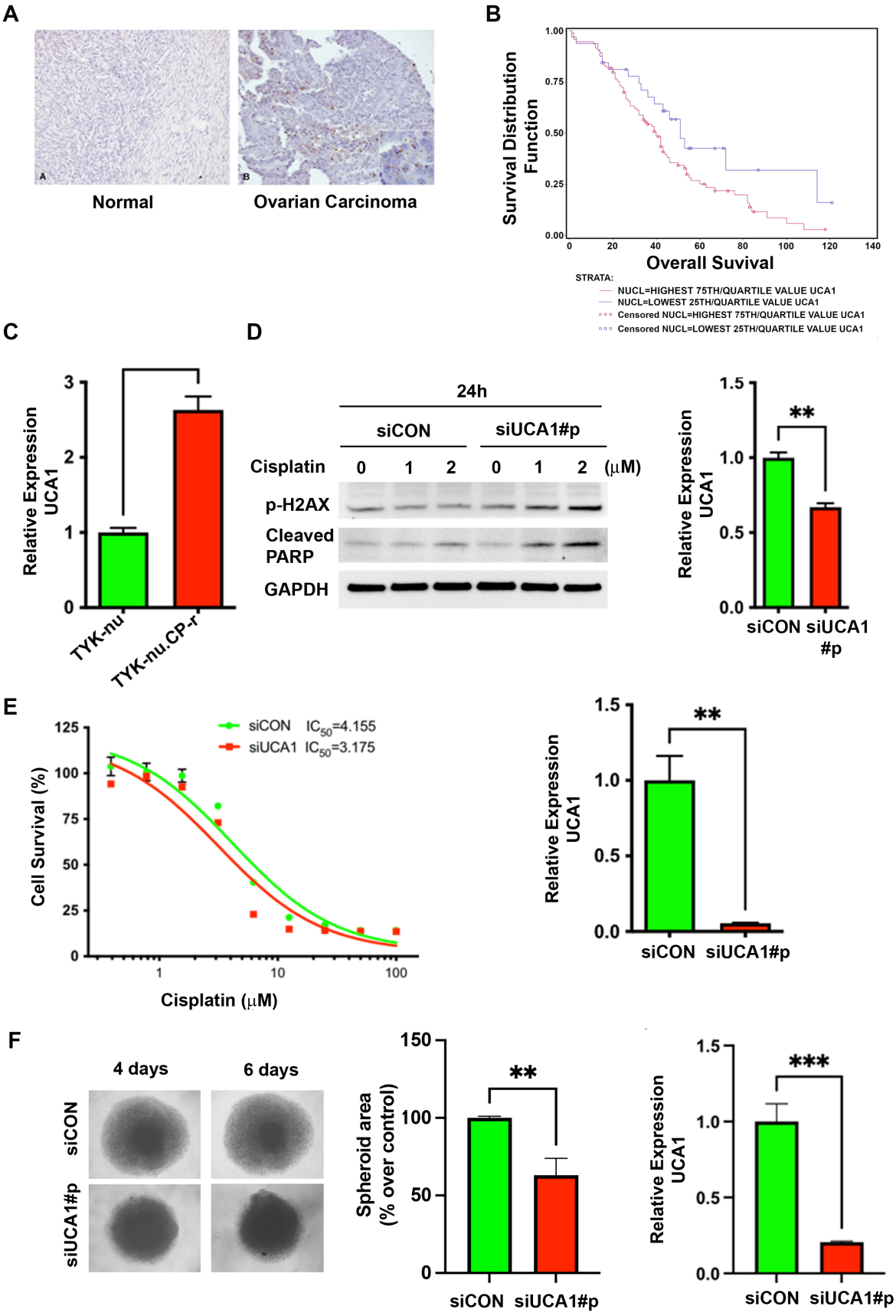
Twist
Vimentin
Fibronectin
Slug
Twist
Vimentin
Fibronectin
β-actin
UCA1
Relative Expression UCA1
B
0 h
48 h
Wound Width (μM)
Time (h)
siUCA1
LPA
#2
#4
#p
Relative Expression UCA1
siCon
siUCA1#2
siUCA1#4
siUCA1#p
C
siUCA1
LPA
Fold Change
Relative Expression UCA1
D
siCon
siUCA1#p
siCON
siUCA1
MSD (μm²)
Time (min)
Relative Expression UCA1

Fig. 3. UCA1 promotes ovarian cancer cell migration

A) Expression levels of Twist, Vimentin, Fibronectin and Slug in UCA1 over-expressing SKOV3.ip cells were monitored by qRT-PCR (*Left Panel*). Protein levels of Twist, Vimentin and Fibronectin, in UCA1 over-expressing SKOV3.ip cells were obtained by immunoblot analysis (*Middle Panel*). UCA1 over-expression in SKOV3.ip cells was validated by qRT-PCR (*Right Panel*). **B)** Incucyte-based Scratch Wound assay was performed to determine cell migration of UCA1 silenced OVCAR8 cells treated with or without LPA (5 μM) using the IncuCyte S3 Live-Cell Analysis system (*Left Panel*). The rate of wound healing over time was quantified using the IncuCyte Scratch Wound Analysis Software Module (*Middle Panel*). UCA1 silencing in OVCAR8 cells was validated using qRT-PCR (*Right Panel*). **C)** Invasive migration of LPA (5 μM) treated UCA1-silenced COV318 cells was monitored by transwell migration assay. Migrated cells were stained and imaged at 10x magnification (*Left Panel*). The fold change of invaded cells over control is plotted (*Middle Panel*). UCA1 silencing in the COV318 cells was validated by qRT-PCR (*Right Panel*). **D)** Automated single cell tracking was used to monitor the migration of UCA1 silenced OVCAR8 cells, stimulated with 10% FBS, using live cell imaging on Operetta High Content Imaging system (*Left Panel*). Migrating cancer cells were tracked continuously for 24 h, and the mean square displacement (MSD) was calculated using Harmony® High Content Imaging and Analysis Software (*Middle Panel*). UCA1 silencing in the OVCAR8 cells was confirmed by qRT-PCR (*Right Panel*). Each experiment was repeated three times. Error bars indicate mean ± SEM. Statistical significance between treatment and control groups was determined by Student's t test (**$p < 0.005$; *** $p < 0.0005$; **** $p < 0.0001$).

FIGURE 4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Ovarian Carcinoma
B
Survival Distribution Function
Overall Suvival
STRATA:
NUCL=HIGHEST 75TH/QUARTILE VALUE UCA1
NUCL=LOWEST 25TH/QUARTILE VALUE UCA1
Censored NUCL=HIGHEST 75TH/QUARTILE VALUE UCA1
Censored NUCL=LOWEST 25TH/QUARTILE VALUE UCA1
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Cleaved PARP
GAPDH
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siCON IC50=4.155
siUCA1 IC50=3.175
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Cisplatin (μM)
Relative Expression UCA1
siCON siUCA1#p
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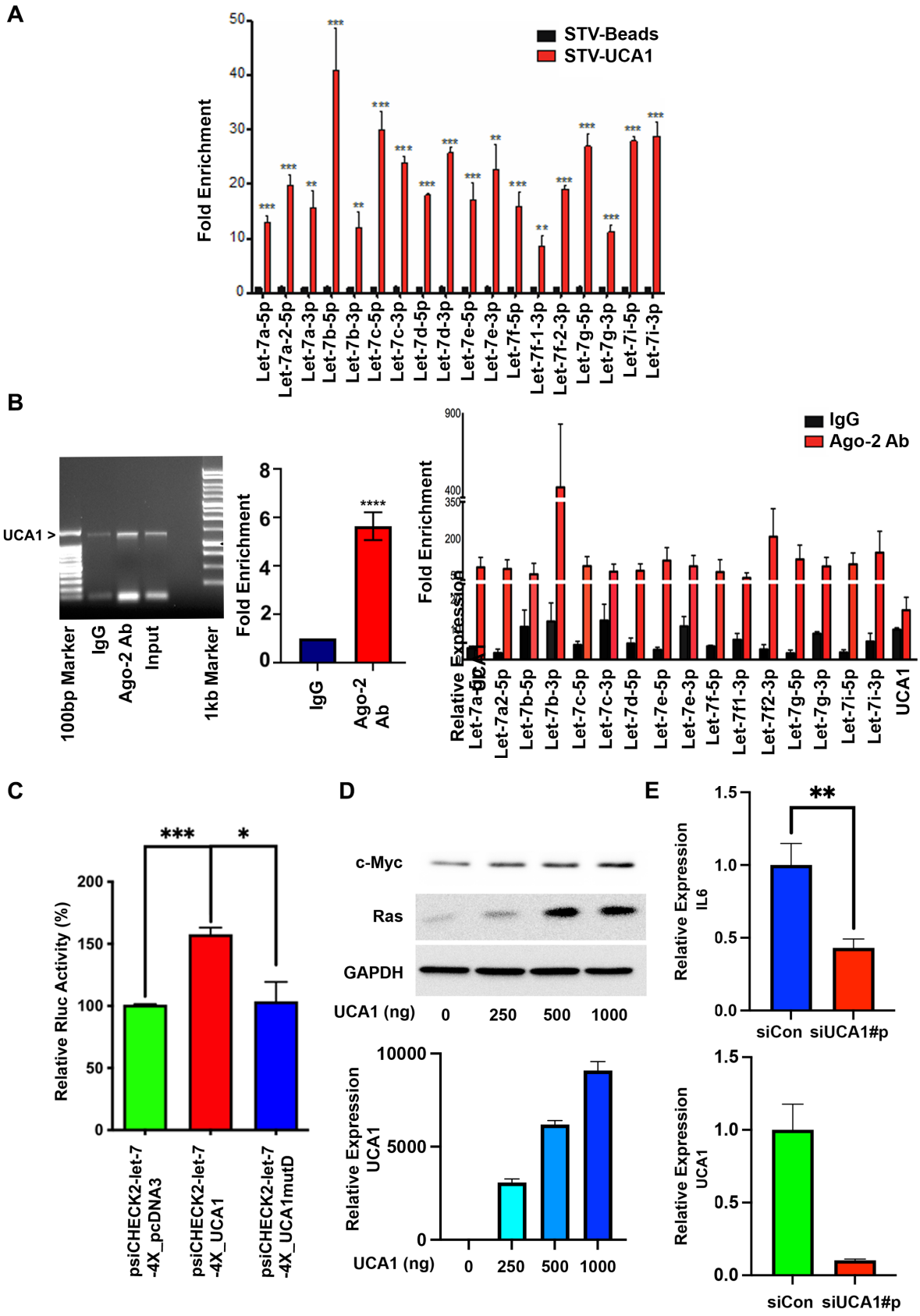
4 days
6 days
siCON
siUCA1#p
Spheroid area (% over control)
siCON siUCA1#p
Relative Expression UCA1
siCON siUCA1#p

126

Fig. 4. UCA1 promotes therapy resistance in ovarian cancer cells

A) Expression of UCA1 in the formalin-fixed paraffin embedded tissue samples from ovarian cancer patients and their adjacent normal ovarian epithelial tissues was monitored using RNAScope™ based *in situ* hybridization analysis. **B)** Kaplan-Meier plot for survival of OUHSC ovarian cancer patients, correlated with the UCA1 expression. RNAScope on FFPE sections of each TMA with target-specific UCA1 probe was carried out. After amplification steps, probes were detected with DAB staining. The TMA slides were scanned into an Aperio ScanScope Scanner (Leica Biosystems, CA, USA). Using TMA lab software, individual tissue core spots were extracted and designated their respective patient identity. Using Image J (NIH, MD, USA), these punctate dots were enumerated and UCA1 expression levels classified. Red denotes low UCA1 expression whereas Blue denotes high UCA1 expression. **C)** UCA1 expression was monitored by qRT-PCR in TYK-nu and cisplatin resistant TYK-nu.CP-r cells. **D)** Protein levels of p-H2AX and cleaved-PARP in cisplatin (0-2 μM) treated UCA1-silenced TYK-nu.CP-r cells were obtained by immunoblot analysis (*Left Panel*). UCA1 silencing in TYK-nu.CP-r cells was confirmed by qRT-PCR (*Right Panel*). **E)** Cell viability in UCA1-silenced OVCAR8 cells, treated with cisplatin (0.001-100 μM), was measured using tetrazolium salt-based CCK-8 assay (*Left Panel*). IC_{50} titres were calculated by nonlinear regression analysis using the three parameter logic sigmoidal dose curve equation on GraphPad Prism software. IC_{50} was determined to be 4.155μM and for 3.175μM respectively for siRNA- and siUCA1 transfected cells. UCA1 silencing in OVCAR8 cells was validated by qRT-PCR (*Right Panel*). F) OVCAR4 cells were grown as 3-D spheroids with or without UCA1 silencing. Spheroids were imaged at 4x magnification in an Olympus CK50 microscope (*Left Panel*). Spheroid size on day 6 was quantified by determining the spheroid spread area using ImageJ software. The data is presented as % reduction of spheroid size from siUCA1-treated cells compared to the spheroids from control siRNA-treated cells (*Middle Panel*). UCA1 silencing in OVCAR4 cells was confirmed by qRT-PCR (*Right Panel*). Each experiment was repeated three times. Error bars indicate mean ± SEM. Statistical significance between treatment and control groups was determined by Student's t test (**$p< 0.005$; *** $p< 0.0005$; **** $p< 0.0001$).

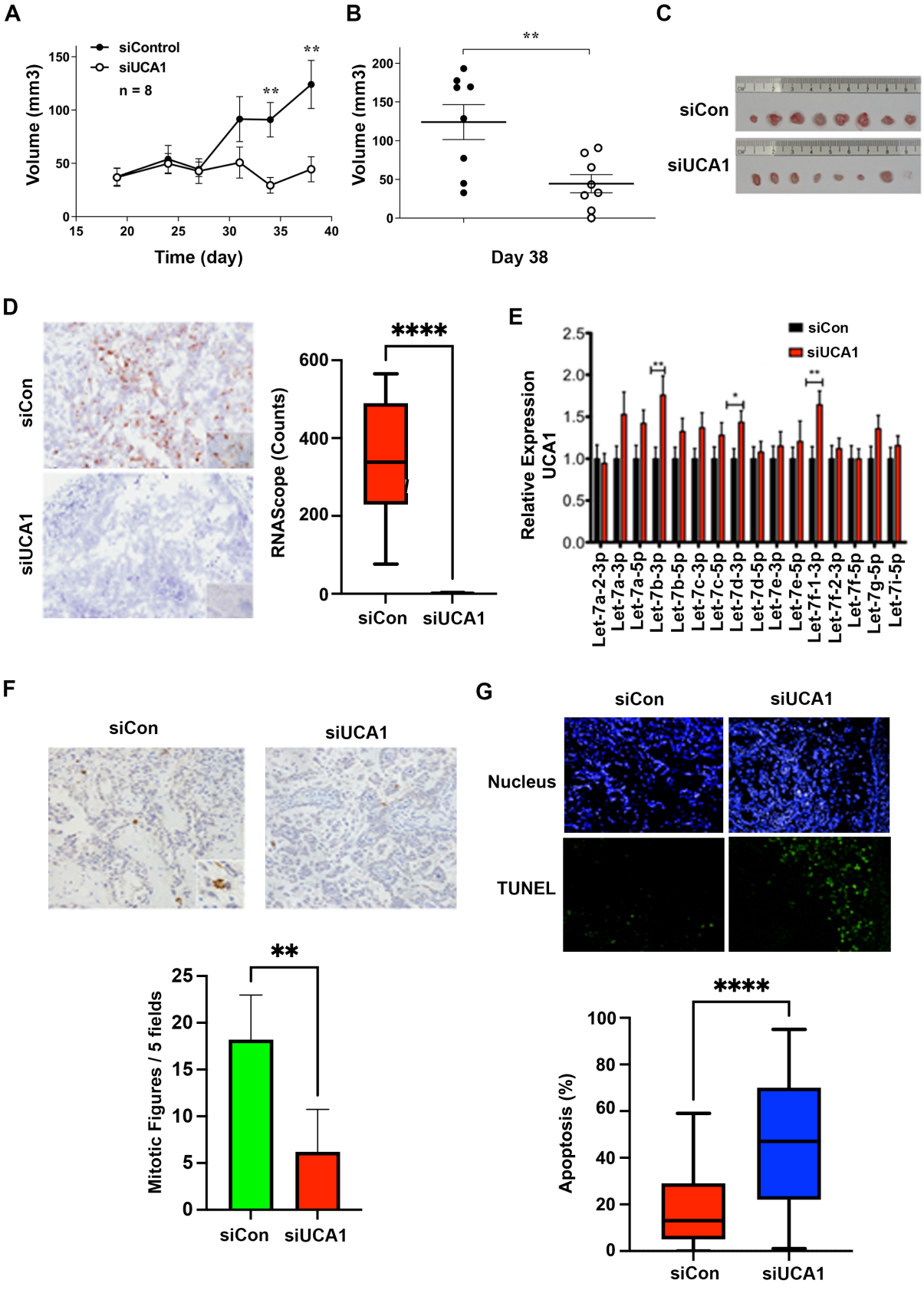

A
STV-Beads
STV-UCA1
Fold Enrichment
Let-7a-5p
Let-7a-2-5p
Let-7a-3p
Let-7b-5p
Let-7b-3p
Let-7c-5p
Let-7c-3p
Let-7d-5p
Let-7d-3p
Let-7e-5p
Let-7e-3p
Let-7f-5p
Let-7f-1-3p
Let-7f-2-3p
Let-7g-5p
Let-7g-3p
Let-7i-5p
Let-7i-3p
B
UCA1 >
100bp Marker
IgG
Ago-2 Ab
Input
1kb Marker
Fold Enrichment
IgG
Ago-2 Ab
IgG
Ago-2 Ab
Relative Expression
UCA1
C
Relative Rluc Activity (%)
psiCHECK2-let-7-4X_pcDNA3
psiCHECK2-let-7-4X_UCA1
psiCHECK2-let-7-4X_UCA1mutD
D
c-Myc
Ras
GAPDH
UCA1 (ng) 0 250 500 1000
Relative Expression UCA1
UCA1 (ng) 0 250 500 1000
E
Relative Expression IL6
siCon siUCA1#p
Relative Expression UCA1
siCon siUCA1#p

56

Fig. 5. UCA1 modulates the function of let-7 family of miRNAs in ovarian cancer

A) OVCAR8 cell lysates were incubated with biotinylated UCA1 and biotinylated UCA1 in the lysates was pulled down using streptavidin magnetic beads. The presence of UCA1 and let-7 miRNAs were monitored by qRT-PCR. **B)** CLIP assay was carried out to detect Ago-2 bound UCA1 and let-7 miRNAs. Following *in vivo* cross-linking by formaldehyde, immunoprecipitation conducted OVCAR8 cell lysates using Ago-2 antibodies or pre-immune IgG. Crosslinking was reversed, RNAs in the immunoprecipitates were extracted, and RT-PCR was carried out for Ago-2-bound UCA1 (1.4 kb) and UCA1 present in the lysate (input). Non-specific binding of UCA1 to antibodies were monitored using IgG control. The products were analyzed on 1% agarose gels and visualized on a BioRAD Gel-Doc-Ez system (*Right Panel*). Fold enrichments of Ago-2-bound UCA1, against IgG controls, are presented (*Middle Panel*). Let-7 miRNAs bound to Ago-2 complex was monitored by qRT-PCR with the human let-7 miScript primer assay (Qiagen, MD, USA). Fold enrichment for let-7 miRNAs against IgG control, is presented (*Right Panel*). **C)** Let-7 reporter plasmid (psiCHECK2-let-7 4x_pcDNA3) was transfected into HEK293 cells along with the pcDNA3 vector encoding UCA1 or let-7 binding-site mutated UCA1 (UCA1mutD). Renilla luciferase activity, determined at 36 h post-transfection was normalized against firefly luciferase activity and presented as percentage over the control vector values. **D)** HEK293 cells were transfected with varying amount of UCA1-expression vectors for 36 hrs. Expression of let-7 target genes c-Myc and Ras in response to ectopic expression of UCA1 was monitored by immunoblot analysis of the lysate from the HEK293 cells. Stripped blot was probed with GAPDH to monitor equal protein-loading. **E)** Expression of let-7 target gene IL-6 was monitored following the knockdown of UCA1. IL-6 expression (*Top Panel*) and the silencing of UCA1 by the respective siRNAs (*Bottom Panel*) was determined by qRT-PCR. Error bars indicate mean ± SEM. Each of the presented experiments is repeated thrice and the statistical significance between treatment and control groups was determined by Student's t test (**$p< 0.005$; *** $p< 0.0005$; **** $p< 0.0001$).

FIGURE 6


A
siControl
siUCA1
n = 8
Volume (mm3)
Time (day)
B
Volume (mm3)
Day 38
C
siCon
siUCA1
D
siCon
siUCA1
RNAScope (Counts)
E
siCon
siUCA1
Relative Expression UCA1
Let-7a-2-3p
Let-7a-3p
Let-7a-5p
Let-7b-3p
Let-7b-5p
Let-7c-3p
Let-7c-5p
Let-7d-3p
Let-7d-5p
Let-7e-3p
Let-7e-5p
Let-7f-1-3p
Let-7f-2-3p
Let-7f-5p
Let-7g-5p
Let-7i-5p
F
siCon
siUCA1
Mitotic Figures / 5 fields
G
siCon
siUCA1
Nucleus
TUNEL
Apoptosis (%)

183

Fig.6. Therapeutic potential of targeting UCA1 in ovarian cancers

A) Nu/Nu nude female mice were injected with OVCAR8 cells subcutaneously ($5x10^6$ cells) to generate tumors. At day 19, when the tumors reached approximately 50 mm^3, the mice were randomized into 2 groups and intratumorally administered with scrambled siRNA or UCA1-specific siRNA (10 µg/tumor suspended in 50 µl MaxSuppressor In-Vivo RNA-LANCEr II carrier) at 4-day intervals for a total of 4 injections. Tumor volume was measured every 4 days until the 38th day when the mice were euthanized. The mean tumor volume was plotted. **B)** Individual tumor volumes, along with interquartile ranges, were plotted for each treatment group at day 38. **C)** At day 38, subcutaneous tumors were harvested from euthanized animals, photographed, and tumor growth was recorded. **D)** FFPE blocks derived from the tumor tissue were analyzed for UCA1 expression using RNAScope method and a representative image is presented (*Left Panel).* The data was quantified by enumerating the spots obtained by the RNAScope analysis (*Right Panel*). **E)** From frozen xenograft tissues, RNA was isolated and let-7 miRNA levels were monitored by qRT-PCR with the human let-7 miScript primer assay system (Qiagen, MD, USA). **F).** Mitotic figures as indices for cell proliferation were monitored by pHH3 IHC staining of the FFPE sections of the xenograft tumors from control and siUCA1 treatment groups. Magnification of original images is 20x and the inset in left image is 60x. Representative image is presented (*Top Panel*). Results are quantified using image tumors derived from three animals each from the control and siUCA1 injected groups (*Bottom Panel*). **G)** Apoptosis was monitored by TUNEL assay using the FFPE sections of xenograft tumors from control and siUCA1 treatment groups. Micrographs indicating the nuclei (blue) and apoptotic cells (green) were obtained using Operetta High Content Imaging system at 20x magnification (*Top Panel*). Apoptotic cells were enumerated and calculated as % over the total nuclei/field (*Bottom Panel*). All the experiments presented here were repeated at least thrice. Error bars represent mean ± SEM. Statistical significance between treatment and control groups was determined by Student's t test (**$p< 0.005$; *** $p< 0.0005$; **** $p< 0.0001$).

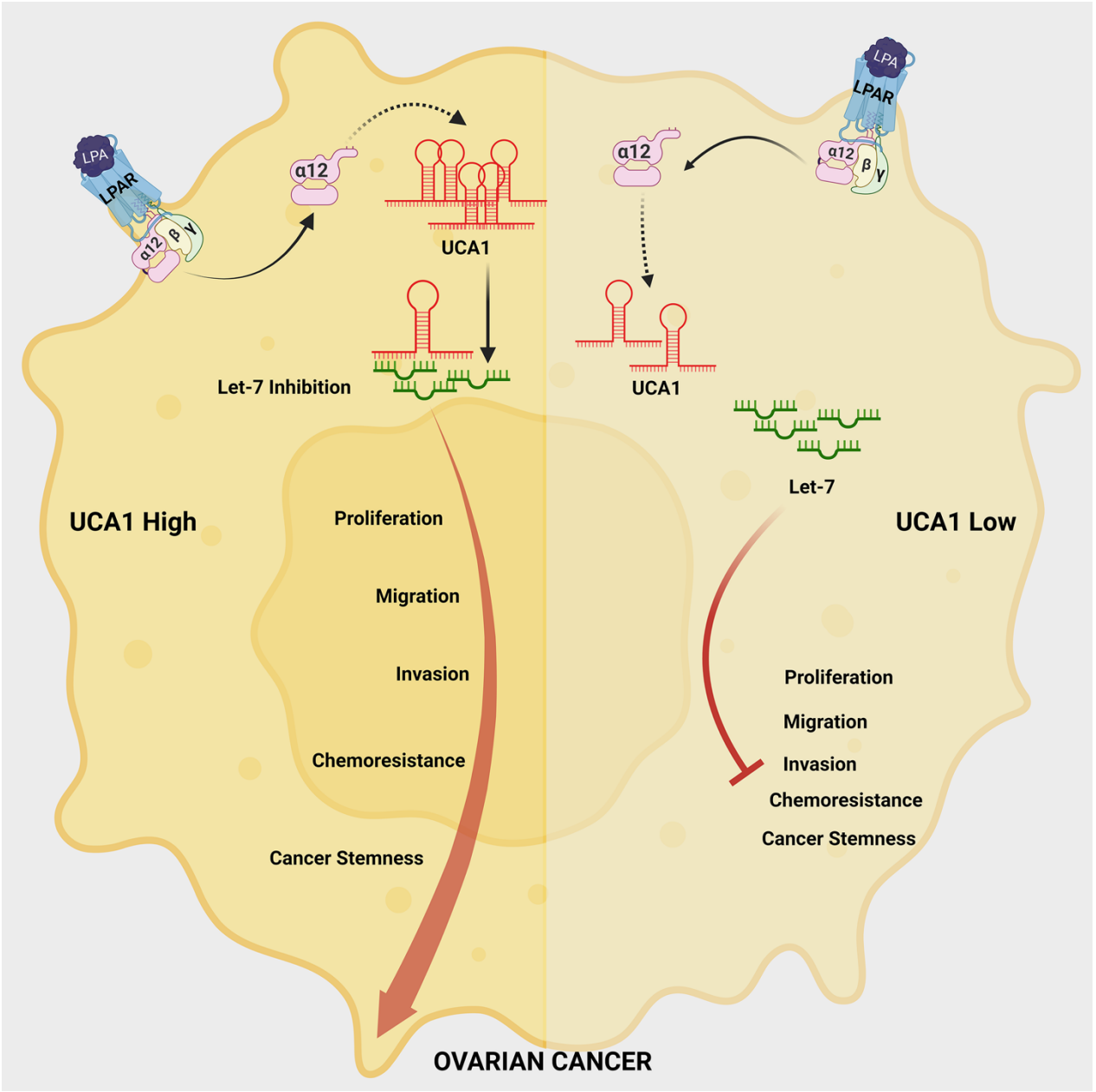


Fig. 7. LPA-LPAR-UCA1-Let-7 axis regulates ovarian cancer progression

LPA binds to LPARs to stimulate UCA1 expression through Gα12 protein in ovarian cancer cells. UCA1, thus upregulated, sequesters let-7 family of miRNAs, relieving the tumor suppressor activity of let-7 miRNAs. Resultant increase in the expression of let-7 target oncogenes facilitate ovarian cancer progression by stimulating oncogenic cell proliferation, invasion, migration, chemoresistance and cancer stemness. During cellular homeostasis, when UCA1 levels are low, the let-7 miRNA activity is higher, leading to attenuation of the expression of its oncogenic targets, to inhibit the cancer mediating signals in the ovary. This figure was created using BioRender.com under academic license.

Supplementary Materials for

Unraveling a GPCR-lncRNA-miRNA Nexus: Identification of an Aberrant Therapeutic Target in Ovarian Cancer

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This PDF file includes:

Figs. S1 to S6
Tables S1 to S5

Other Supplementary Materials for this manuscript include the following:

Movies S1 to S2

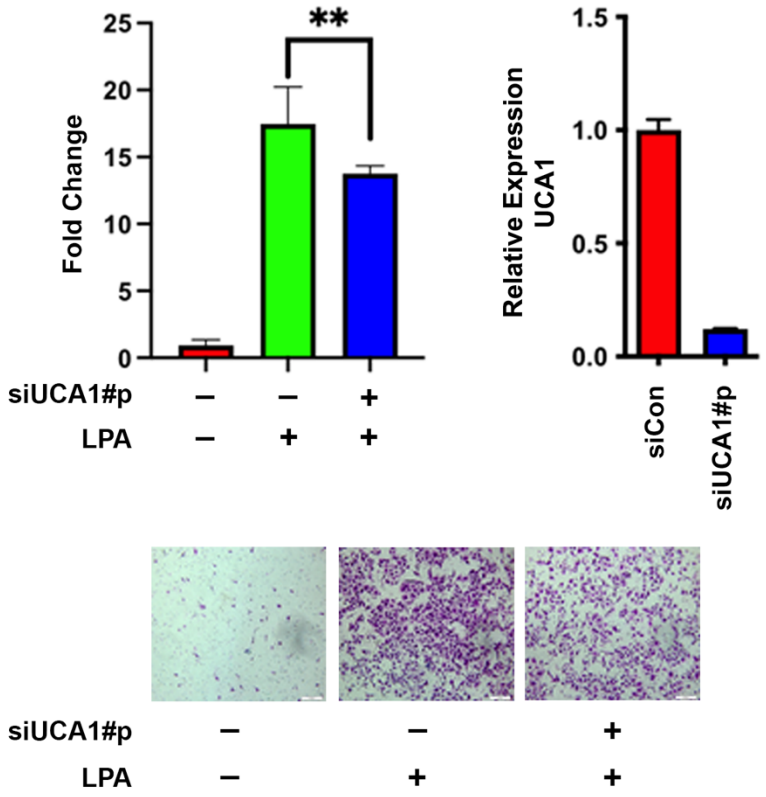

Fig. S1. UCA1 promotes invasive migration in SKOV3.ip cells. Invasive migration of LPA (5 μM) treated UCA1 silenced SKOV3.ip cells was monitored by transwell migration assay. Migrated cells were stained and imaged at 100× magnification (Top Panel). The fold change of invaded cells over control is plotted (Bottom Left Panel). UCA1 silencing in the SKOV3.ip cells were validated by qRT-PCR (Bottom Right Panel). Every experiment was repeated three times. Error bars indicate mean ± SEM. Statistical significance between treatment and control groups was determined by Student's t test (**p< 0.005; *** p< 0.0005; **** p< 0.0001).

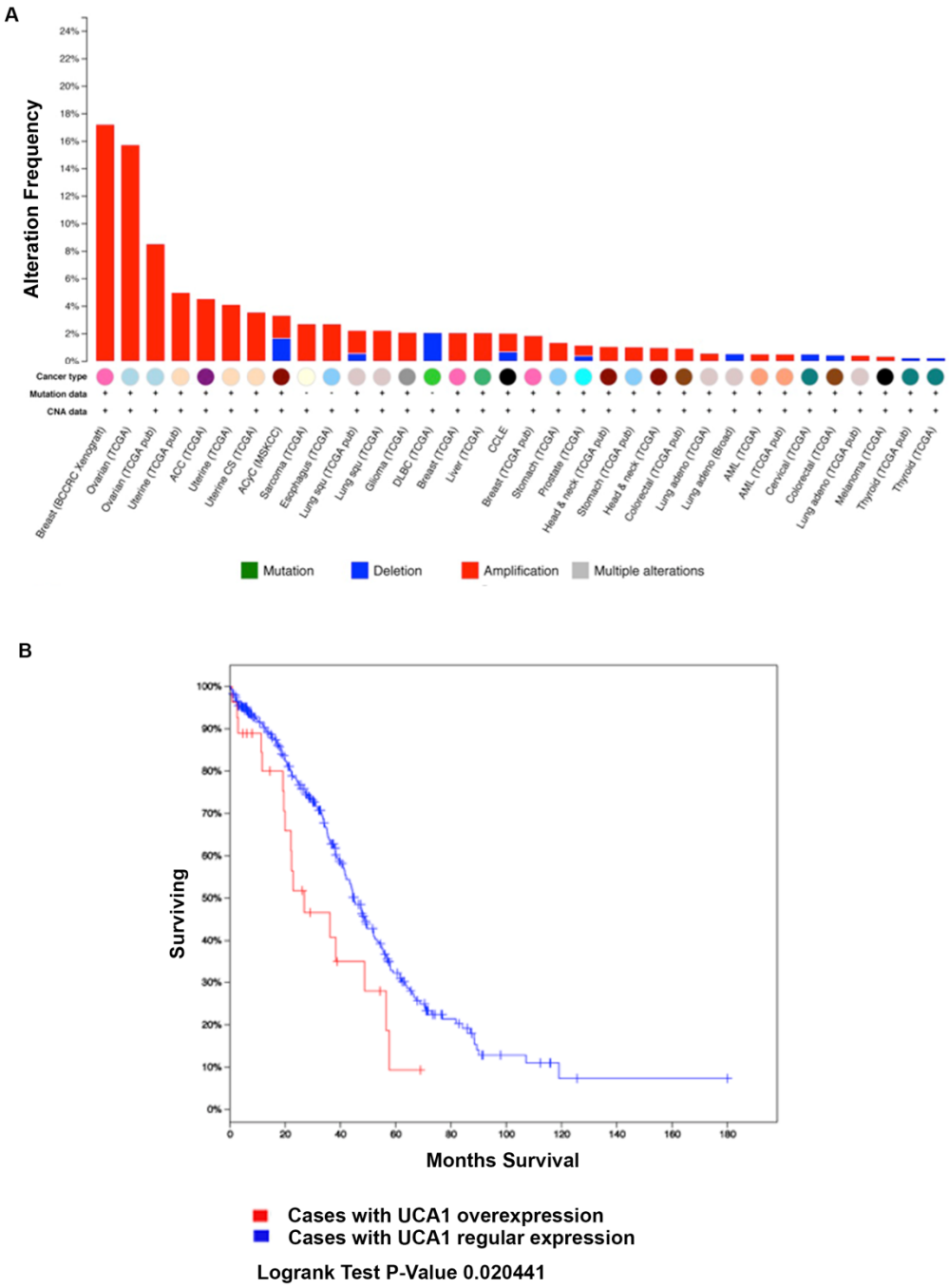

Fig. S2. Expression of UCA1 in Cancer patients: A) UCA1 expressions in pan cancer types obtained from cBioportal. B) Kaplan Meier plot for overall survival of patients with ovarian cancer, correlated with their UCA1 expressions, obtained from cBioportal (31, 32).

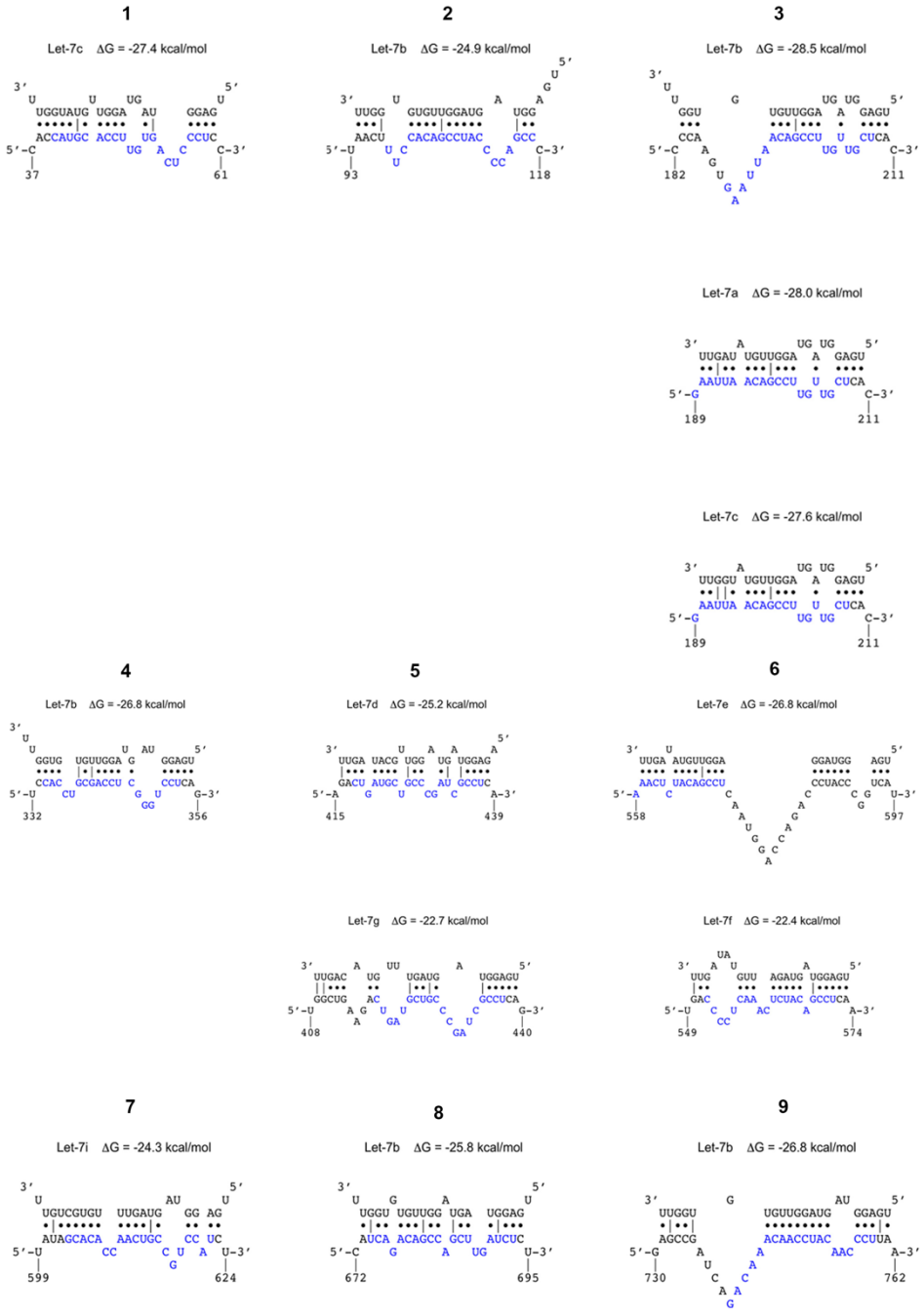

Fig. S3. *In Silico* Analysis of let-7 binding sites in UCA1. Sequences of nine putative binding sites for let-7 miRNAs and UCA1 as obtained from RNA Hybrid program available in BiBiServ portal (34).

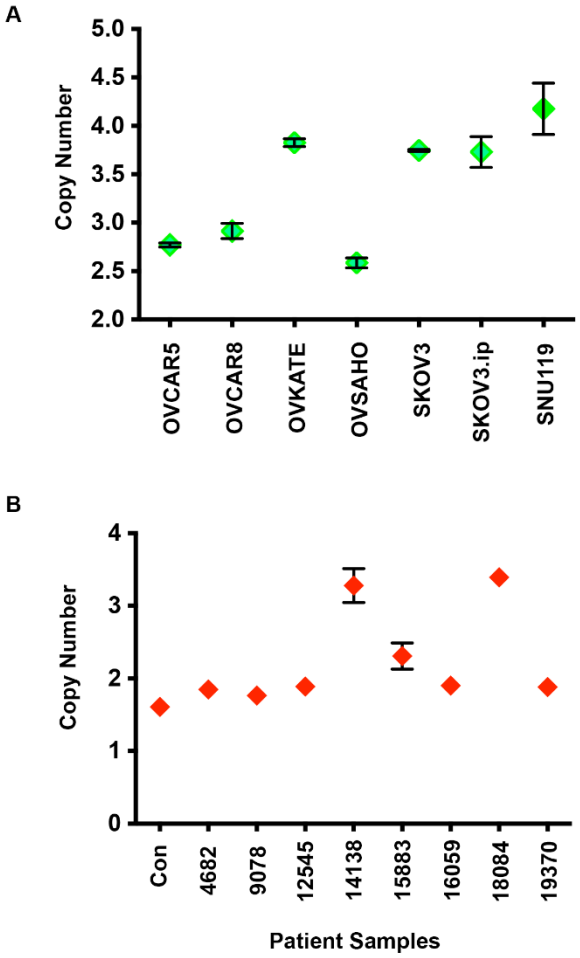

Fig. S4. Copy number variations of UCA1 in HGSOC cell lines and ovarian cancer patient tumor samples. Copy number variations evaluated utilizing the genomic DNA isolated from the different A) HGSOC cell lines and B) ovarian cancer patient tumor samples from OUHSC, using droplet digital PCR system.

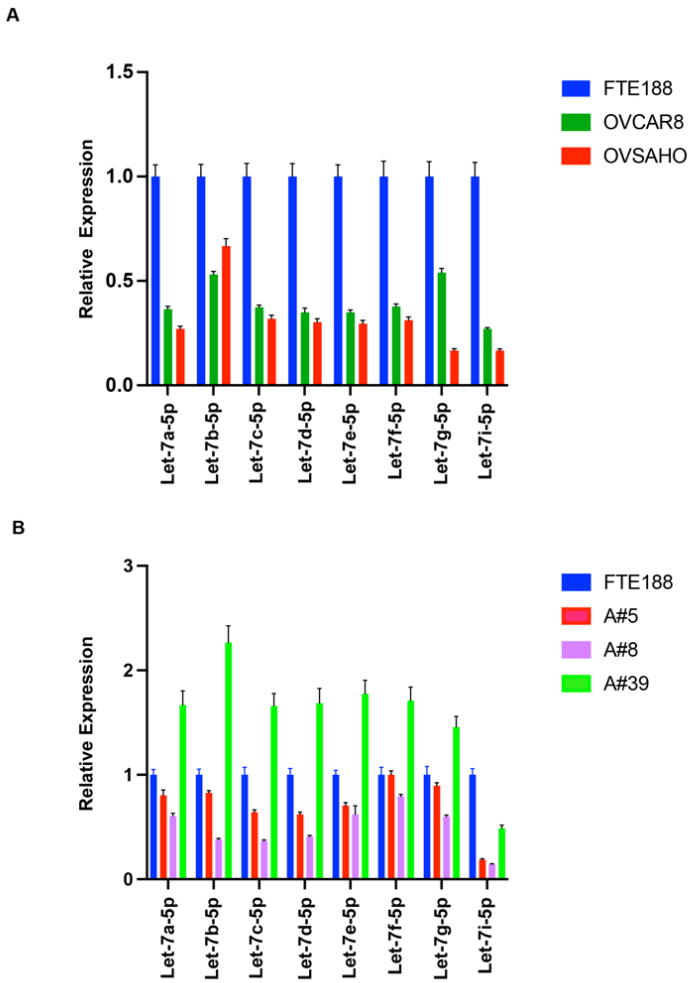

Fig. S5. Let-7 expression in HGSOC cell lines and patient-derived ovarian cancer cells. A) miRNA expressions for let-7 family in a panel of HGSOC cell lines were monitored by qRT-PCR (Top Panel). B) miRNA expressions for let-7 family in a panel of ascites derived cancer cells from SNU ovarian cancer patients were monitored by qRT-PCR (Bottom Panel). Statistical significance, determined by two-way ANOVA and Dunnett's multiple comparison post-hoc test, are denoted by ****, $p < 0.0001$, applicable to all the let-7 miRNA family expressions in the ovarian cancer cell samples, when compared over the control FTE188 cells.

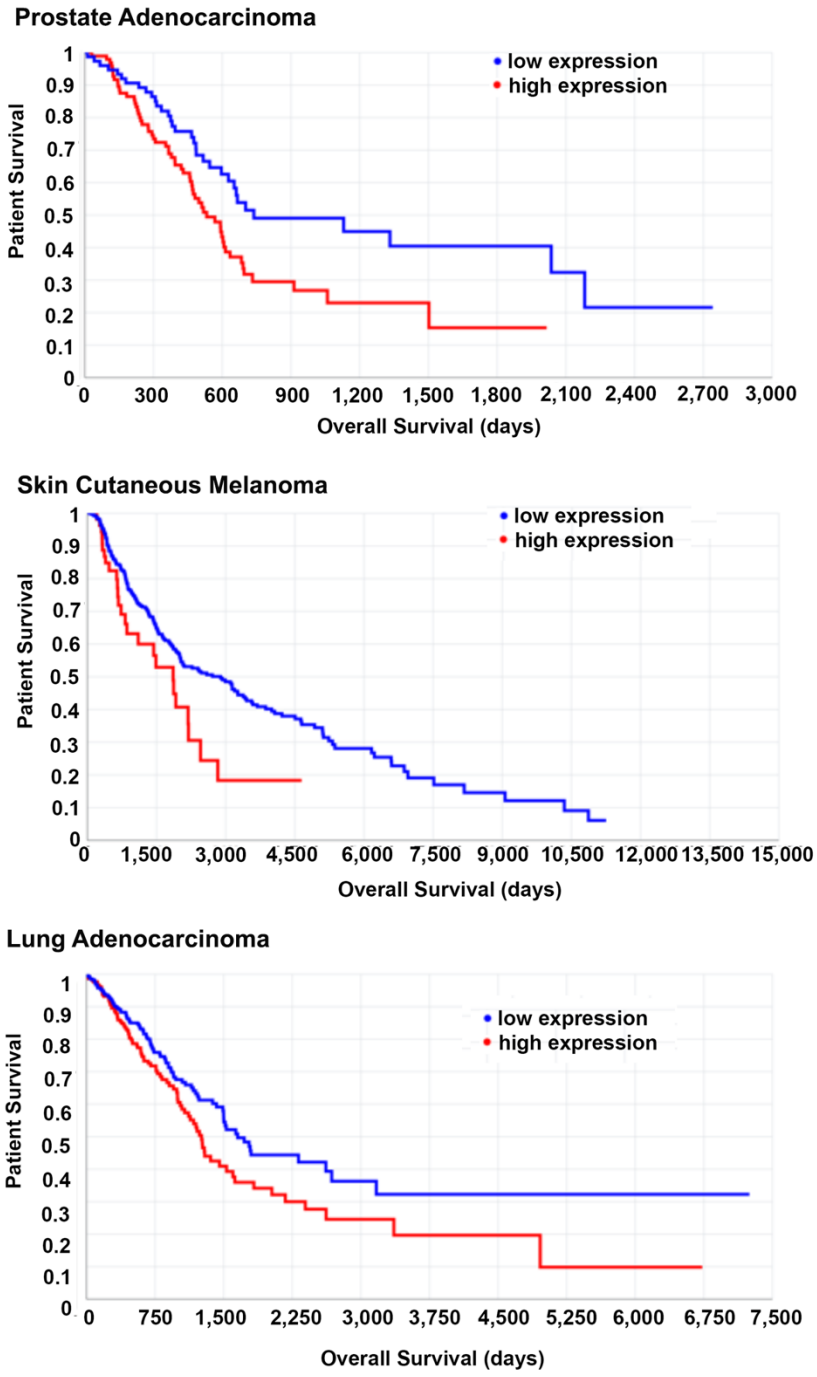

Fig. S6. UCA1 expression and overall survival of patients. Overall survival of patients in relation to UCA1 expression was analyzed using the publicly available TCGA dataset. Kaplan-Meier survival analysis dat was generated using the online tool at the web-analysis portal: https://www.tcga-survival.com/

Table S1. List of antibodies

a. Immunoblot Analysis:

Antibodies	Catalog Number and Vendor	Dilution
β-actin	#8457 (Cell Signaling Technologies, MA, USA)	1:5000
Cleaved PARP	#9532 (Cell Signaling Technologies, MA, USA)	1:1000
c-Myc	#5605 (Cell Signaling Technologies, MA, USA)	1:1000
Gα12	#sc-409 (Santa Cruz Biotechnology, CA, USA)	1:1000
GAPDH	#51332 (Cell Signaling Technologies, MA, USA)	1:5000
p-H2AX	#9718 (Cell Signaling Technologies, MA, USA)	1:1000
Ras	#sc-35 (Santa Cruz Biotechnology, CA, USA)	1:1000
Anti-mouse HRP IgG	#NA931V (GE Health Care, IL, USA)	1:20000
Anti-rabbit HRP IgG	#NA9340V (GE Health Care, IL, USA)	1:20000

b. Immunoprecipitation

Antibodies	Catalog Number and Vendor	Dilution
Ago-2	#39853 (Active Motif, CA, USA)	1:50
IgG Mouse	#sc-69786 (Santa Cruz Biotechnology, CA, USA)	1:50

c. Immunohistochemistry

Antibodies	Catalog Number and Vendor	Dilution
pHH3	#369A-17 (Sigma Millipore, MO, USA)	1:300

Table S2. List of qRT-PCR Primers

Vendor: IDT Inc., IA, USA

Target Gene	Sequence
CCND1	Forward: 5'-TCCTCTCCAAAATGCCAGAG-3' Reverse: 5'-GGCGGATTGGAAATGAACTT-3'
CCND3	Forward: 5'-CTCTGTGCTACAGATTATACC-3' Reverse: 5'-GCCAGCAGCTCTGTGAGC-3'
Fibronectin	Forward: 5'-AAACCAATTCTTGGAGCAGG-3' Reverse: 5'-CCATAAAGGGCAACCAAGAG-3'
IL-6	Forward: 5'-CAATCTGGATTCAATGAGGAGAC-3' Reverse: 5'-CTCTGGCTTGTTCCTCACTACTC-3'
p27	Forward: 5'-AGGACACGCATTTGGTGGA-3' Reverse: 5'-TAGAAGAATCGTCGGTTGCAGGT-3'
Slug	Forward: 5'-GGTCAAGAAGCATTTCAAC-3' Reverse: 5'-GGTAATGTGTGGGTCCGA-3'
Twist	Forward: 5'-GGAGTCCGCAGTCTTACGAG-3' Reverse: 5'-TCTGGAGGACCTGGTAGAGG-3'
UCA1	Forward: 5'-TAAAGCCATGCCCATCAGACAGC-3' Reverse: 5'-GGGATGGCCATTTGGAAGGAGTG-3'
Vimentin	Forward: 5'-CGTGAATACCAAGACCTGCTC-3' Reverse: 5'-GGAAATGTTTGGAAGAGGCAG-3'

Table S2. List of qRT-PCR Primers

Vendor: IDT Inc., IA, USA

Target Gene	Sequence
CCND1	Forward: 5'-TCCTCTCCAAAATGCCAGAG-3' Reverse: 5'-GGCGGATTGGAAATGAACTT-3'
CCND3	Forward: 5'-CTCTGTGCTACAGATTATACC-3' Reverse: 5'-GCCAGCAGCTCTGTGAGC-3'
Fibronectin	Forward: 5'-AAACCAATTCTTGGAGCAGG-3' Reverse: 5'-CCATAAAGGGCAACCAAGAG-3'
IL-6	Forward: 5'-CAATCTGGATTCAATGAGGAGAC-3' Reverse: 5'-CTCTGGCTTGTTCCTCACTACTC-3'
p27	Forward: 5'-AGGACACGCATTTGGTGGA-3' Reverse: 5'-TAGAAGAATCGTCGGTTGCAGGT-3'
Slug	Forward: 5'-GGTCAAGAAGCATTTCAAC-3' Reverse: 5'-GGTAATGTGTGGGTCCGA-3'
Twist	Forward: 5'-GGAGTCCGCAGTCTTACGAG-3' Reverse: 5'-TCTGGAGGACCTGGTAGAGG-3'
UCA1	Forward: 5'-TAAAGCCATGCCCATCAGACAGC-3' Reverse: 5'-GGGATGGCCATTTGGAAGGAGTG-3'
Vimentin	Forward: 5'-CGTGAATACCAAGACCTGCTC-3' Reverse: 5'-GGAAATGTTTGGAAGAGGCAG-3'

Table S3. List of siRNAs used in the *in vitro* studies

Vendor: Dharmacon™, Horizon Discovery, CO, USA

siRNA	**Sequence**
siControl	ON-TARGETplus Non-targeting Control Pool #1
siUCA1#1	Target Sequence: 5'-UGUUAGAGGGCUUGGGACA-3' Antisense: 5'-UGUCCCAAGCCCUCUAACA-3'
siUCA1#2	Target Sequence: 5'-CACCCUAGCUGGACGAUCA-3' Antisense: 5'-UGAUCGUCCAGCUAGGGUG-3'
siUCA1#3	Target Sequence: 5'-ACCCUAGACCCGAAACUUA-3' Antisense: UAAGUUUCGGGUCUAGGGU-3'
siUCA1#4	Target Sequence: 5'-GAUUAGGCCGAGAGCCGAU-3' Antisense: 5'-AUCGGCUCUCGGCCUAAUC-3'
siUCA1#p (pool)	Lincode SMARTpool Human UCA1 (Cat# R-188002-00-0020)

Table S4. Custom siRNAs used in the *in vivo* studies

Vendor: Dharmacon™, Horizon Discovery, CO, USA

siRNA	Sequence (21mer)
UCA1-1	Sense: 5' U.G.U.U.A.G.A.G.G.G.C.U.U.G.G.G.A.C.A.U.U 3' Antisense: 5' U.G.U.C.C.C.A.A.G.C.C.C.U.C.U.A.A.C.A.U.U 3'
UCA1-2	Sense: 5' C.A.C.C.C.U.A.G.C.U.G.G.A.C.G.A.U.C.A.U.U 3' Antisense: 5' U.G.A.U.C.G.U.C.C.A.G.C.U.A.G.G.G.U.G.U.U 3'
UCA1-3	Sense: 5' A.C.C.C.U.A.G.A.C.C.C.G.A.A.A.C.U.U.A.U.U 3' Antisense: 5' U.A.A.G.U.U.U.C.G.G.G.U.C.U.A.G.G.G.U.U.U 3'
UCA1-4	Sense: 5' G.A.U.U.A.G.G.C.C.G.A.G.A.G.C.C.G.A.U.U.U 3' Antisense: 5' A.U.C.G.G.C.U.C.U.C.G.G.C.C.U.A.A.U.C.U.U 3'
Scrambled Control LNC-C1	Sense: 5' U.A.G.C.G.A.C.U.A.A.A.C.A.C.A.U.C.A.A.U.U 3' Antisense: 5' U.U.G.A.U.G.U.G.U.U.U.A.G.U.C.G.C.U.A.U.U 3'
Scrambled Control LNC-C1	Sense: 5' U.A.A.G.G.C.U.A.U.G.A.A.G.A.G.A.U.A.C.U.U 3' Antisense: 5' G.U.A.U.C.U.C.U.U.C.A.U.A.G.C.C.U.U.A.U.U 3'
Scrambled Control LNC-C1	Sense: 5' A.U.G.U.A.U.U.G.G.C.C.U.G.U.A.U.U.A.G.U.U 3' Antisense: 5' C.U.A.A.U.A.C.A.G.G.C.C.A.A.U.A.C.A.U.U.U 3'
Scrambled Control LNC-C1	Sense: 5' A.U.G.A.A.C.G.U.G.A.A.U.U.G.C.U.C.A.A.U.U 3' Antisense: 5' U.U.G.A.G.C.A.A.U.U.C.A.C.G.U.U.C.A.U.U.U 3'

Table S5. Transcription factor sites in UCA1 promoter

Site (Length)	Position	Score	Gaps	Occurrence	Exp Value
c-Myb (8)	-20	8	0	1	4.19E-01
v-MCS (6)	-20	6	0	1	6.21E-01
CAC-binding-PBG (7)	-26	7	0	1	5.86E-02
ASE (7)	-28	7	0	1	5.86E-02
Sp1-AS(2) (10)	-29	10	0	1	9.38E-04
Sp1(11)	-29	8	0	1	5.07E-01
MDR1-HT-p53-sit (11)	-36	11	0	1	2.34E-04
CREB (8)	-41	8	0	1	1.50E-02
Ad-conserved-se (6)	-41	6	0	1	2.15E-01
ATF (6)	-41	6	0	1	2.15E-01
CREB (6)	-41	6	0	1	2.15E-01
E4F1 (7)	-42	7	0	1	5.86E-02
E4TF1 (7)	-42	7	0	1	1.14E-01
YY1 (9)	-45	8	0	1	8.04E-01
HC3 (6)	-50	6	0	1	2.15E-01
enhancer_core (8)	-54	8	0	1	1.14E-01
TEF (6)	-54	6	0	1	2.15E-01
TEF-1 (8)	-55	7	0	1	3.83E-01
C/EBP-IE1/2 (10)	-60	10	0	1	9.38E-04
C/EBP (9)	-61	7	0	1	3.82E-01
Sp1-YBX1 (6)	-66	6	0	1	2.15E-01
Isl-1_site (7)	-73	7	0	1	6.20E-01
CHX10 (8)	-74	8	0	1	1.14E-01
S8_site (11)	-76	10	0	1	7.46E-03
gamma-IRE (8)	-88	6	0	1	9.79E-01
Pet-1 (11)	-89	8	0	1	2.54E-01
Ets-Rh50-distal (7)	-89	7	0	1	5.86E-02
Msx1 (7)	-89	6	0	1	2.15E-01
CTF/NF-1a (7)	-92	7	0	1	5.86E-02
CTF/NF-1b (7)	-92	7	0	1	5.86E-02
CTF/NF-I-t (7)	-92	7	0	1	5.86E-02
NFI-NFI (7)	-92	7	0	1	5.86E-02

Transcription factor sites in UCA1 promoter was analyzed using Tfsitescan server (http://www.ifti.org/cgi-bin/ifti/Tfsitescan.pl) following previously published method (45). Transcriptioin factor binding sites with 100 bp upstream of transcription start sites are presented. Exp Value indicates the expected number of occurrences of a match between a transcription factor binding motif and a match by random chance.

Movie S1. OVCAR8 cells transfected with scrambled control siRNA. Automated time-lapse video captures cell migration over 24 hours post-FBS stimulation (10%) using Operetta High-Content Imaging. Trailing 'tail' indicates motility tracks.

Movie S2. OVCAR8 cells transfected with UCA1-specific siRNAs. Cell migration tracked over 24 hours post-FBS stimulation (10%) using Operetta High-Content Imaging. Trailing 'tail' shows motility tracks.