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14. ABSTRACT

Ovarian cancer is a major cause of cancer-related mortality in women, with high recurrence rates and resistance to chemotherapy. Discovering new therapeutic targets is crucial, especially for recurrent, chemotherapy-resistant cases. Cancer cells interact with neighboring normal fibroblasts (NOFs), inducing their conversion into cancer-associated fibroblasts (CAFs) through paracrine signaling, but the underlying mechanism remains unclear. This study hypothesizes that lysophosphatidic acid (LPA) triggers metabolic reprogramming, essential for NOF to CAF transdifferentiation. The research aims to unveil the LPA-LPAR-HIF1α signaling nexus and identify potential therapeutic targets. The study's three specific aims encompass understanding LPA-induced metabolic programming and myofibroblast differentiation, delineating the role of LPA-induced HIF1α in fibroblast transcriptional reprogramming, and assessing the therapeutic potential of candidate genes and pseudohypoxia signaling nodes in ovarian cancer. In pursuit of these objectives, we conducted a comprehensive investigation of LPARs, Gα-subunits, and the long non-coding RNA XIST, leading to the identification of XIST's novel role in CAF differentiation and potential signaling targets that can disrupt CAF differentiation. Our results also shed light on the intricate interplay between LPA signaling and XIST-mediated gene expression, providing insights into the mechanism underlying HIF1α expression and CAF differentiation. Collectively, our progress during this period has uncovered new avenues for therapeutic interventions targeting the CAF differentiation pathway. This research holds promise for advancing ovarian cancer treatment by better understanding and potentially disrupting the mechanisms that contribute to chemotherapy resistance and recurrence.

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TABLE OF CONTENTS

1. INTRODUCTION

Ovarian cancer presents a formidable public health challenge as the leading cause of mortality among gynecological cancers, with a five-year survival rate of only 46% for localized disease and a dismal 29% for the distant-stage disease [1]. The lack of early symptoms contributes to delayed diagnoses, limiting treatment options and reducing survival rates. To address this critical challenge, our research endeavors have been intently focused on unraveling the role of Lysophosphatidic acid (LPA), a lipid growth factor, in ovarian cancer. Our investigations have led us to a pivotal discovery, identifying a connection between LPA-induced pseudohypoxic responses and the pathobiology of both cancer cells and cancer-associated fibroblasts (CAFs) [2,3]. CAFs, a major component of the tumor stroma, wield significant influence over tumor progression, metastasis, and resistance [4-9]. Originating from diverse cell types within the tumor microenvironment, normal peritumoral fibroblasts undergo transformation into CAFs through paracrine signaling initiated by cancer cells [4,7-11]. Bidirectional communications between cancer cells and CAFs play a major role in therapy resistance and disease prognosis in many cancers [12]. Thus, it is of critical interest to define the mechanism through which cancer cells activate the quiescent fibroblasts in the tumor microenvironment (TME) to CAFs, enabling the development of a therapeutic strategy for co-targeting cancer cells and CAFs [13-15]. Although several distinctive functional and phenotypic changes set CAFs apart from their normal counterparts, the key differentiating feature of CAFs is their metabolic shift towards aerobic glycolysis, leading to the production and secretion of lactate and pyruvate, which support cancer cell growth [16-18].

Recent findings from our laboratory have unraveled the role of ovarian cancer cell-derived LPA in inducing a pseudohypoxic response in both ovarian cancer cells and CAFs, resulting in the upregulation of HIF1a expression [2,3]. LPA-induced pseudohypoxia triggers metabolic reprogramming and the transformation of peritumoral fibroblasts into CAFs via HIF1α [2,3]. These findings define LPA-LPAR-HIF1α signaling nexus as a promising therapeutic target for the development of targeted therapies in ovarian cancer. As LPA induces pseudohypoxia and metabolic changes in both cancer cells and peritumoral fibroblasts, the LPA-LPAR-HIF1α axis emerges as a unique and promising signaling node for the development of a multi-target drug development strategy targeting both cancer cells and CAFs in ovarian cancer. Thus, it can be surmised that mechanistic understanding of the signaling network underlying CAF differentiation and ovarian cancer progression holds immense promise for developing more effective and targeted treatments for ovarian cancer, ultimately improving the quality of life for ovarian cancer patients.

2. KEYWORDS:

Ovarian Cancer; Lysophosphatidic acid (LPA); Cancer-associated fibroblast (CAF), Tumor microenvironment (TME); Hypoxia-inducible factor 1-alpha (HIF1α); Pseudohypoxia; Metabolic reprogramming, Glycolysis; Long non-coding RNA (lncRNA); XIST

3. ACCOMPLISHMENTS:

What were the major goals of the project?

The overarching goals of the proposed studies are to define the mechanism by which LPA-HIF1a signaling axis stimulates CAF-differentiation and assess the therapeutic potential of targeting CAF-differentiation pathway using FDA-approved drugs. The major goals of the project are 1) Define the mechanism by which LP induces metabolic reprogramming and CAF differentiation in ovarian cancer; 2) Define the mechanism by which HIF1a induces transcriptional reprogramming in fibroblasts; and 3) Evaluate the therapeutic potential of targeting CAF-transdifferentiation pathway using FDA approved drugs.

What was accomplished under these goals?

The results of this study have led to significant accomplishments in unraveling the intricate signaling pathways and regulatory elements involved in LPA-induced metabolic responses and cancer-associated fibroblast (CAF) differentiation. Through a comprehensive interrogation of LPA receptors (LPARs), Gα-subunits, and the long non-coding RNA XIST, potential signaling targets to disrupt CAF-differentiation - and thus tumor progression - have been identified. Our findings also shed light on the complex interplay between LPA signaling and XIST-mediated gene expression, providing insights into the mechanism underlying HIF1α expression and CAF differentiation. Additionally, the discovery of potential molecular signatures and biomarkers of CAF differentiation such as XIST opens avenues for personalized treatment strategies in cancer management. These accomplishments offer promising opportunities for technology transfer and may lead to advancements in cancer therapeutics, patient care, and our understanding of the tumor microenvironment, ultimately benefiting both cancer patients and the broader society.

Major Activities:

The major activities carried out under this period focused mainly on aim 1 sub-tasks 1 and 43. During this funding period, we completed the subtasks 1A – IC prior to the start date of the project.

Specific Aim 1: Define the mechanism by which LPA-induces metabolic programming and CAF-differentiation.	**Timeline** **Months**
Major Task 1: Characterize the receptor and the mechanism underlying HIF1α expression involved in CAF Differentiation	
Subtask 1A: Submission of institution's IRB approval and related material for DoD's HRPO approval. Subtask 1B: Submission of institution's IRB approval and related material for DoD's HRPO approval. Subtask 1C: Receive ACURO approval before initiating animal experiments.	1-6
Subtask 2: Identify LPA-receptor that stimulates the transdifferentiation of fibroblasts Cell lines used: NOF151, CAF148, CAF147 [OUHSC]	1-9
Subtask 3: Define the mechanisms underlying LPA-induced upregulation of HIF1α Cell lines used: Multiple cell lines [ATCC] and human ovarian fibroblast cell lines (ATCC)	6-18

Our primary focus was to identify the LPA-receptor involved in fibroblast transdifferentiation. Our previous studies established that the metabolic reprogramming of fibroblasts towards aerobic glycolysis is an early event in the transdifferentiation to cancer-associated fibroblasts (CAFs). Therefore, we monitored LPA-stimulated glycolysis as a functional marker for CAF differentiation,

Identify LPA-receptor (LPAR) that stimulates the transdifferentiation of fibroblasts. As proposed under the specific aim 1.1, we investigated whether the silencing of a specific LPAR attenuated the expression of HIF1α-mediated aerobic glycolysis. LPAR1, LPAR2, and LPAR3 are the major LPAR-subtypes expressed by normal

ovarian fibroblasts (NOFs) and CAFs. Therefore, we tested whether knockdown of LPAR1, LPAR2, or LPAR3 using the respective siRNAs resulted in the reduction in LPA-stimulated glycolytic response. Briefly, expression of individual LPARs in NOF151 or CAF148 cells were silenced using transiently transfected siRNAs targeting the respective LPARs or scrambled siRNAs (control) for 48 hrs. These cells were stimulated with 5 µM LPA for 6 hrs and LPA-induced glycolytic shift was measured using Seahorse XFe96 analyzer. Results from this analysis indicated that that LPA induced an increase in glycolysis and glycolytic capacity as anticipated. However, knocking down any individual receptor did not inhibit the glycolytic shift induced by LPA (Figure 1).

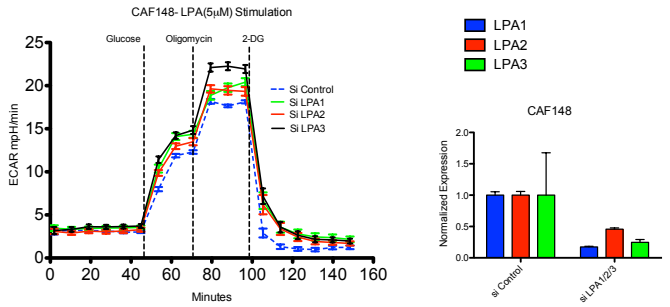


Fig. 1. Role of LPAR1, LPAR2, and LPAR3 in LPA-induced CAF Differentiation. *CAF148 cells were transfected with siRNA targeting LPAR1, LPAR2, LPAR3 or control nontargeting siRNA for 48 h, following which they were stimulated with 5 µM of LPA for 6 hours. ECAR flux over time, glycolysis and glycolytic capacity were plotted. Results (Mean and SEM; n=6 to 22 parallel determinations) from a representative analysis (n=3 independent experiments. The knockdown efficiency was monitored by RT-qPCR with the primers for the respective LPARs.*

Considering the possibility that silencing one subtype of LPARs may activate another as a compensatory adaptive mechanism, we co-silenced LPAR1, LPAR2, and LPAR3. Surprisingly, even the co-silencing of all three LPAR subtypes failed to reduce LPA-stimulated aerobic glycolysis in both the normal ovarian fibroblast cell line (NOF151) and the CAF cell line (CAF148) (Figure 2).

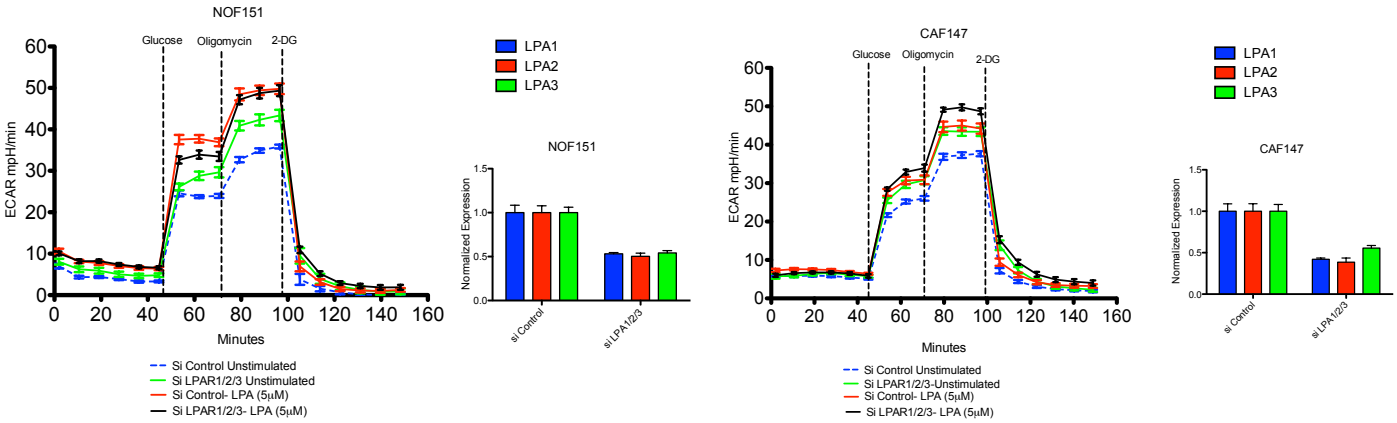


Fig. 2. Effect of simultaneous knock down of LPAR1/2/3 receptors in ovarian fibroblasts. *NOF151 or CAF148 cells were transfected with the pooled siRNAs targeting LPAR1, LPAR2, and LPAR3 along with the control nontargeting siRNA for 48 h, following which they were stimulated with 5 µM of LPA for 6 hours. ECAR flux over time, glycolysis and glycolytic capacity were plotted. Results (Mean and SEM; n=6 to 22 parallel determinations) from a representative analysis (n=3 independent experiments). The knockdown efficiency was monitored by RT-qPCR with the primers for the respective LPARs.*

These results suggested to us that LPA-stimulation of aerobic glycolysis may involve other subtypes of LPA receptors. Ovarian NOFs and CAFs also express LPAR4, LPAR5, and LPAR6 (Figure 3).

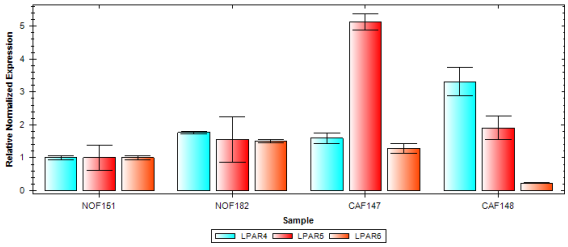


Fig. 3. Expression of LPAR4, LPAR5, and LPAR6 in Ovarian Fibroblasts. *Expression pf LPAR4, LPAR5, and LPAR6 was monitored by RT-qPCR using the RNAs extracted from NOF151, NOF182, CAf147, and CAF148 cells using previously published methods from our lab* [2,3].

Therefore, we investigated whether silencing these receptors could abrogate LPA-induced glycolysis in NOF151. However, knocking down these LPARs also failed to reduce LPA-induced glycolytic response (Figure 4).

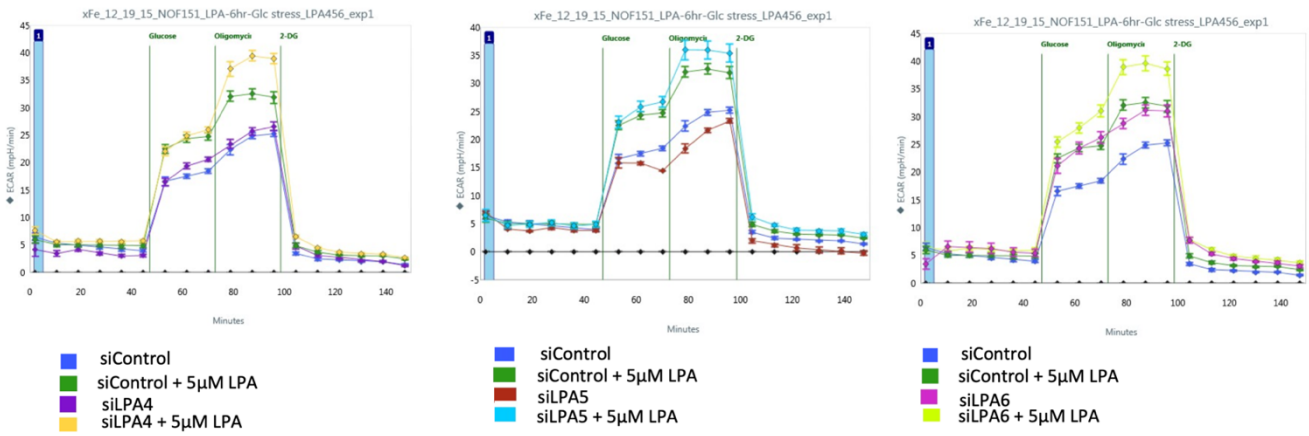


Fig. 4. Role of LPAR1, LPAR2, and LPAR3 in LPA-induced CAF Differentiation. *CAF148 cells were transfected with siRNA targeting LPAR1, LPAR2, LPAR3 or control nontargeting siRNA for 48 h, following which they were stimulated with 5 µM of LPA for 6 hours. ECAR flux over time, glycolysis and glycolytic capacity were plotted. Results (Mean and SEM; n=6 to 22 parallel determinations) from a representative analysis (n=3 independent experiments). The knockdown efficiency was monitored by RT-qPCR with the primers for the respective LPARs.*

Anticipate Results and Alternative Strategies:

Contrary to our anticipated results, none of the LPAR knockdowns attenuated LPA-induced aerobic glycolysis in cancer-associated fibroblasts. Despite targeted reductions in LPAR expression, the metabolic reprogramming driven by LPA remained largely unaffected. These results suggest the existence of intricate and redundant signaling mechanisms, leading to metabolic adaptations in cancer-associated fibroblasts, which are not solely dependent on individual LPARs. While our results do not present a direct answer to the role of individual LPARs in LPA-induced aerobic glycolysis, they hold significant clinical implications. The complexity and redundancy observed in the LPA-induced metabolic response emphasize the need to explore alternative signaling pathways and novel regulatory elements governing cancer-associated fibroblast metabolism.

We'll carry out the following experiments to define the complexity underlying LPA-induced metabolic responses.

- Redundancy of LPARs: It is possible that the redundancy among LPA receptors involves receptors other than LPAR1–LPAR6. To further explore this, we will perform combination knockdown experiments targeting LPAR1–LPAR6. Notably, the combination knockdown of LPAR4–LPAR6 has not been conducted yet, and its examination may yield valuable insights into the functional redundancy among these receptors.

- Non-LPAR-mediated Mechanism: LPA-induced aerobic glycolysis might not be solely mediated by canonical LPA receptors. There could be alternative receptors that mediate this response. Recent studies have shown that LPA stimulates the nuclear receptor, peroxisome proliferator-activated receptor-gamma (PPARγ), in ovarian cancer cells to upregulate the expression of the zinc transporter ZIP4. To investigate this possibility, we will employ the PPARγ-selective inhibitor GW9662 or knockdown PPARγ in the fibroblasts to determine whether these interventions abrogate LPA-induced aerobic glycolysis.

- Temporal Dynamics of LPA-LPAR Signaling Cascade: It is conceivable that the signaling pathways activated by LPARs involve a series of events that occur over different time scales. Consequently, downstream events, such as the aerobic glycolytic shift triggered by LPAR activation, may become less sensitive to LPAR knockdown at certain time points. To address this temporal aspect, we will pursue two independent analyses. Firstly, we will conduct a comprehensive time-course analysis to assess how LPA-induced aerobic glycolysis and downstream signaling change over time. By identifying the minimal time point required for LPA-stimulated aerobic glycolysis, we can discern the critical time frame for LPAR involvement in this pathway. Subsequently, we will employ this identified time point in the knockdown assays to unveil the specific LPA receptor(s) involved in the observed metabolic response.

Define the mechanisms underlying LPA-induced upregulation of HIF1α. In this subtask, we investigated the molecular mechanisms by which LPA induces the upregulation of HIF1α in NOFs. LPA is known to play a crucial role in cancer progression and metabolic reprogramming, including the induction of aerobic glycolysis. To elucidate the specific G-protein alpha (Gα) subunits downstream of LPARs responsible for mediating HIF1α expression, we carried out gene knockdown experiments targeting individual α-subunits. NOFs were cultured under standard conditions to investigate the effects of LPA on HIF1α expression. Specific siRNAs targeting G-protein alpha subunits (Gαi2, Gα13, Gαq, and Gα12) were transfected into NOFs to selectively downregulate their expression. After siRNA transfection, cells were treated with LPA for 6 hrs to evaluate the impact of G-protein alpha subunit knockdown on HIF1α induction. Protein levels of HIF1α were monitored by Western blotting to quantify the extent of HIF1α upregulation following LPA treatment.

Our study demonstrated that LPA induces HIF1α expression in cancer-associated fibroblasts and this effect is contingent upon specific G-protein alpha subunits downstream of LPARs. Among the tested G-protein alpha subunits (Gαi2, Gα13, Gαq, and Gα12), Gαi2, Gα13, and Gαq were found to play critical roles in mediating LPA-induced HIF1α expression (Figure 5). Knockdown of Gαi2, Gα13, or Gαq significantly attenuated the upregulation of HIF1α in response to LPA treatment. However, intriguingly, knockdown of Gα12 had no significant effect on LPA-induced HIF1α expression. (Figure 5).

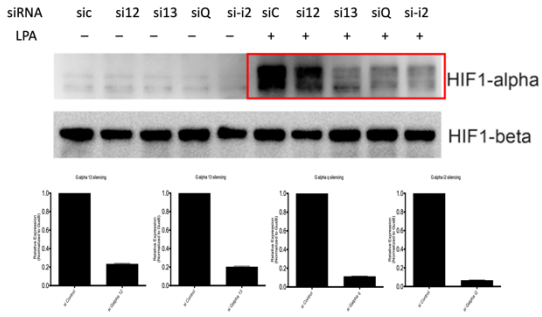


Figure 5. Role of G proteins in the expression of HIF1α. *NOF151 cells were transfected with siRNA targeting Ga12, Ga13, GaQ, Gai2 or control nontargeting siRNA for 48 h, following which they were stimulated with 5 μM of LPA for 6 hours. HIF1a and HiF1b levels were monitored by western blot using the lysates derived from the transfectants. Knockdown efficiency was monitored by RT-qPCR with the primers for the respective Ga-subunits.*

Our findings provide novel insights into the complex signaling network activated by LPA in cancer-associated fibroblasts. The involvement of Gαi2, Gα13, and Gαq in LPA-induced HIF1α upregulation suggests a convergence of distinct G-protein-coupled receptor signaling pathways, each contributing to the regulation of HIF1α expression. These results indicate the existence of multiple G-protein-dependent pathways governing LPA-mediated HIF1α induction, underscoring the intricacy of LPA signaling in the context of cancer metabolism. The identification of Gαi2, Gα13, and Gαs as crucial mediators of LPA-induced HIF1α expression highlights potential targets for therapeutic interventions aimed at disrupting LPA-driven metabolic reprogramming in cancer-associated fibroblasts. By delineating specific G-protein alpha subunits involved in HIF1α regulation, this study paves the way for future investigations to decipher the precise downstream signaling cascades and potential crosstalk with other pathways implicated in cancer metabolism.

Specific Aim 2: Delineate the mechanism by which HIF1α induces transcriptional reprogramming in fibroblasts.	
Major Task 2: Identification of the LPA-HIF1α-target genes involved in CAF differentiation.	
Subtask 1: Role of LPA-induced pseudohypoxia mediated transcriptional programming	12-24

Identification of target genes involved in CAF differentiation. While our investigation into the role of LPARs and Gα-subunits in LPA-induced HIF1α expression provides valuable insights into the complexity of LPA signaling in CAFs, we sought to investigate additional downstream regulatory elements contributing to HIF1α-expression and CAF differentiation. Through transcriptomic analyses comparing NOFs and CAFs, we identified XIST, a long non-coding RNA as the major transcript significantly upregulated in CAFs (Figure 6, Top Panel). XIST has been reported to play a role in the expression of hypoxia-inducible HIF1α [19] and Yes-associated protein, YAP [20]. Given that YAP is involved in CAF differentiation [21] and LPA has been shown to activate YAP [22], we hypothesized that XIST might play a pivotal role in the metabolic reprogramming and differentiation of CAFs. Although XIST has been shown to upregulate HIF1α to promote tumorigenesis and tumor progression in colorectal carcinoma [19], its role in CAF differentiation and ovarian cancer pathogenesis remains largely unexplored. To investigate this, we first validated the expression of XIST in CAFs compared to NOFs, confirming a robust increase in XIST expression in CAFs (Figure 6, Bottom Panel).

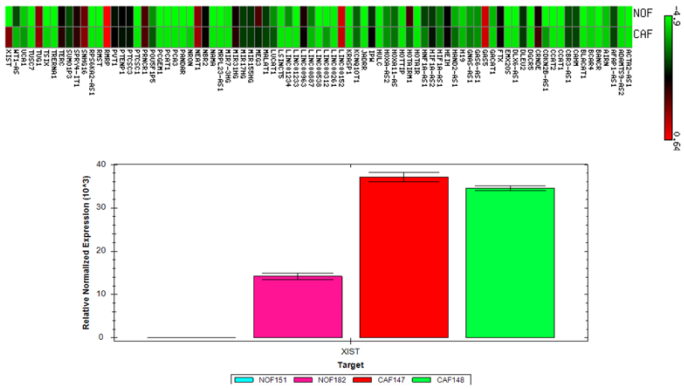
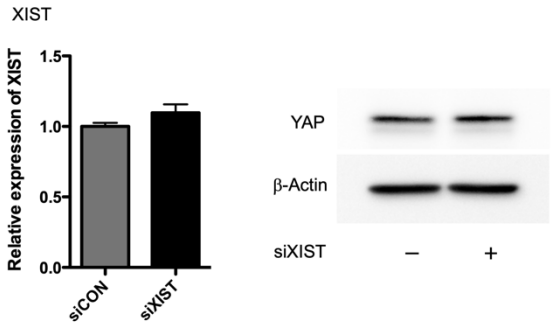


Fig. 6. Identification XIST as CAF differentiation gene. *Total RNA isolated from NOF151 and CAF 147 c3ells were reverse transcribed to cDNA and differentially expressed lncRNA genes were identified using RT² lncRNA PCR Array (Qiagen. Valencia, CA). The lncRNA expression data from CAF147 was compared with that of NOF151 at QIAGEN web portal at GeneGlobe. The heatmap obtained from the analysis is presented (Top Panel). Expression of XIST in NOF151, NOF182, CAF147, and CAF148 was monitored by RT-qPCR using the RNAs extracted from NOF151, NOF182, CAf147, and CAF148 cells using previously published methods.*

It is significant to note that NOF182, a partially transdifferentiated fibroblast cells, showed partial increase in XIST levels compared to the completed differentiated CAF147 and CAF148.



Next, we examined the impact of XIST knockdown on YAP expression levels, as YAP is known to play a crucial role in CAF differentiation. Remarkably, knocking down XIST in NOFs led to a clear decrease in YAP expression levels (Figure 7).

Fig. 7. XIST regulates the expression of YAP1. *NOF182 ce3lls were transfected with siRNA targeting XIST or control nontargeting siRNA for 48 h, following which the cell lysates were analyzed for XIST and YAP1 expression. YAP1 levels were monitored by western blot assay using the lysates derived from the transfectants. Knockdown efficiency of XIST was monitored by RT-qPCR with the primers for XIST.*

Our results provide a novel mechanistic link between XIST and the regulation of HIF1α, a critical transcription factor orchestrating LPA-induced pseudohypoxia. The combined findings from our study highlight a synergistic interplay between LPA signaling and XIST-mediated metabolic reprogramming in CAFs. While LPA signaling via specific Ga-subunits drives aerobic glycolysis and HIF1α induction, XIST amplifies the expression of HIF1α and YAP, reinforcing CAF differentiation. It is significant to note that the Knockdown of XIST resulted in a downregulation of YAP, which is known to be a downstream effector of LPA signaling. This raises the possibility that XIST may act as a molecular link bridging the effects of LPA signaling to the upregulation of YAP and the subsequent promotion of CAF differentiation. The identified role of XIST in upregulating HIF1α and promoting CAF differentiation highlights its potential significance in shaping the tumor microenvironment. These findings point to potential of targeting XIST and LPA signaling as potential therapeutic strategies to disrupt the pro-tumorigenic functions of cancer-associated fibroblasts and improve cancer treatment outcomes.

4. IMPACT

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

- **Cancer Biology and Metabolism:** The study's insights into complex signaling pathways in LPA-induced responses and CAF differentiation significantly advance our understanding of cancer cell-stromal interactions. This knowledge can potentially pave the way for novel therapeutic strategies targeting the tumor microenvironment to inhibit tumor growth and metastasis.
- **Translational Oncology:** By identifying potential drug targets within LPARs and Gα-subunits, the research provides a crucial foundation for the development of targeted therapies to disrupt CAF-driven metabolic support. This has implications for translational oncology, with the potential to improve treatment outcomes and patient survival rates.
- **RNA Biology and Non-Coding RNAs:** The discovery of XIST's role in regulating HIF1α and YAP in CAFs is a paradigm-shifting observation and it offers valuable insights into the impact of non-coding RNAs on cancer-associated pathways. This expanding knowledge of RNA biology could potentially lead to the development of novel RNA-based therapeutics for cancer treatment.
- **Molecular Cell Signaling:** Through the detailed exploration of LPARs and Gα-subunits in LPA-induced responses, the findings significantly contribute to the field of molecular cell signaling. Understanding the redundancy and potential involvement of other receptors in LPA-stimulated glycolysis highlights the complexity of these signaling networks and their role in cellular responses.
- **Personalized Medicine in Cancer:** The identification of molecular signatures and biomarkers reflecting CAF behavior opens up new possibilities for personalized medicine in cancer treatment. Tailoring therapies based on individual tumor characteristics may enhance treatment efficacy, minimize side effects, and ultimately improve patient outcomes.
- **Interdisciplinary Research Collaboration:** The multidisciplinary approach in our study, involving cancer biology, molecular biology, and bioinformatics, fosters collaboration among researchers, clinicians, and industry experts. This cross-disciplinary collaboration accelerates the translation of research findings into potential clinical applications, promoting technology transfer and advancements in cancer treatment and patient care.

What was the impact on other disciplines?

The impact of the study has the potential to extend beyond the principal disciplines and has implications for other fields as well:

- **Pharmacology and Drug Development**: The identification of potential drug targets in LPARs and Gα-subunits for disrupting CAF-driven metabolic support opens new avenues in pharmacology and drug development.
- **Bioinformatics and Computational Biology:** The integration of bioinformatics and computational biology in our research can contribute to data analysis, network modeling, and the identification of

potential biomarkers. This interdisciplinary approach can enhance the understanding of complex biological systems and the development of computational tools for cancer research.

- **Systems Biology:** Our investigation into the intricate signaling networks and regulatory elements involved in LPA-induced responses and CAF behavior aligns with systems biology principles. It contributes to the understanding of system-level behavior in cancer biology and provides insights into potential therapeutic intervention points.
- **Cancer Immunology:** Insights gained from studying the tumor microenvironment, particularly the interactions between cancer cells, stromal cells, and immune cells, have implications for immunology. Understanding how LPA signaling influences these interactions could contribute to the development of immunotherapeutic approaches in cancer treatment.
- **Clinical Oncology:** The potential development of targeted therapies and personalized treatment strategies resulting from our research can have great impact on clinical oncology. These advancements may translate into more effective and tailored treatment options for cancer patients, potentially improving patient outcomes and overall survival rates.

What was the impact on technology transfer?

Our novel findings on LPARs, Gα-subunits, and XIST in CAF-differentiation are poised to have a transformative impact on technology transfer in the field of cancer research and therapeutics. Specific drug targets to disrupt CAF-driven metabolic support and key insights into the intricate tumor microenvironment have been elucidated, offering promising opportunities for technology transfer to the industry. The potential development of innovative therapeutic interventions, personalized medicine approaches, and the discovery of novel biomarkers make this research a catalyst for collaboration among academia, clinicians, and industry experts, accelerating the translation of research findings into practical applications for improved cancer treatments. In summary, the work represents a significant step forward in enhancing cancer care and has far-reaching implications for advancing cancer research and technology transfer.

What was the impact on society beyond science and technology?

The impact of the present study extends beyond science and technology, potentially benefiting society in various ways:

1. **Improved Cancer Treatment Outcomes:** The study's findings on LPARs, Gα-subunits, XIST, and their roles in cancer-associated fibroblasts (CAFs) provide insights into the tumor microenvironment and potential therapeutic targets. This may lead to the development of more effective and targeted cancer treatments, ultimately improving patient outcomes and quality of life for those affected by cancer.

2. **Advancements in Personalized Medicine:** The identification of molecular signatures and biomarkers reflecting CAF behavior may contribute to the advancement of personalized medicine approaches in cancer treatment. Tailoring therapies based on individual tumor characteristics can lead to more precise and less invasive treatments, reducing side effects and enhancing patients' well-being.

3. **Enhanced Understanding of Tumor Microenvironment:** The study's comprehensive investigation into LPARs, Gα-subunits, and XIST sheds light on the complex interactions within the tumor microenvironment can pave the way for new research and therapeutic strategies that address the dynamic relationship between cancer cells, stromal cells, and the immune system.

4. **Empowering Cancer Research and Advocacy:** The research contributes valuable knowledge to the scientific community and cancer advocacy groups. It can provide a foundation for further research in related fields and empowers advocates to raise awareness about the importance of understanding the tumor microenvironment in cancer progression and treatment.

5. **Potential Economic and Societal Benefits:** Advancements in cancer research and treatment can have substantial economic and societal impacts. Improved cancer treatments may reduce healthcare costs and alleviate the burden on patients, families, and healthcare systems. Additionally, effective cancer treatments may enable patients to maintain productivity and contribute to society positively.

6. **Addressing Global Health Challenges:** Cancer is a significant global health challenge, affecting millions of people worldwide. By providing insights into the molecular mechanisms driving CAF differentiation, the study contributes to the collective efforts to address and combat cancer as a global health concern.

In summary, the impact of the present study extends to society beyond science and technology by potentially improving cancer treatment outcomes, advancing personalized medicine, enhancing the understanding of the tumor microenvironment, and contributing to global health efforts. It also has the potential to empower cancer research and advocacy, leading to positive societal and economic benefits, and promoting patient-centric and ethical cancer care.

5. CHANGES/PROBLEMS:

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

To acerating extent, we were hampered drastically by the COVID-19 lock-down and the recent departure of a postdoctoral associate to his native country due to COVID-19 outbreak during this period as well. However, we have resolved these issue through the recruitment of new research personnel.

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.

6. PRODUCTS:

Publications, conference papers, and presentations

Journal publications

Nothing to report

Other publications, conference papers, and presentations.

"Interrogating Tumor Metabolome: Identification of Novel Therapeutic Targets in Ovarian Cancer", July 15, 2022; 2022 Cancer and Metabolism Symposium, University of Kentucky, Lexington, KY

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?

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

Name: Dr. **Yan Daniel Zhao, Ph.D**
Project Role: Co-Investigator
Nearest person month worked: 1
Contribution to Project: Statistical Analysis

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. Kathleen Moore
Project Role: Co-Investigator
Nearest person month worked: 1
Contribution to Project: Analysis of translational data

Name: Dr. Kenneth Buetow
Project Role: Co-Investigator
Nearest person month worked: 1
Contribution to Project: Bioinformatic Data Analysis

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?

Arizona State University, Tempe, AZ 85287

8. SPECIAL REPORTING REQUIREMENTS

Not Applicable

9. APPENDICES:

None

10. References

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