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14. ABSTRACT Prostate cancer (PCa) is the second highest cancer mortality among male cancer in USA. This disease is still incurable because PCa once becomes metastatic and develops drug resistance when cancer cells undergo epithelial-to-mesenchymal transition (EMT) and acquire cancer stem cell (CSC) phenotypes. Emerging evidence has shown that the presence of metastatic PCa is associated with CSC phenotype that is likely associated with its resistance to radiation or chemotherapy. The preliminary data from this study clearly demonstrate that several tumor suppressor microRNAs (miRNAs) involved in regulating these processes are often degraded by IFIT5. Also, elevated IFIT5 is associated with PCa malignancy. Thus, this study will delineate the mechanism of IFIT5 in tumor suppressor miRNA degradation, and examine IFIT5 gene regulation. By determining its clinical correlation, this study will provide valuable biomarker(s) for lethality of PCa, which will have an immediate impact on patient prognosis and selection for more suitable agent. The outcome of this study will provide a better understanding of miRNAs biogenesis associated with aggressive PCa exhibiting CSC phenotypes. Most importantly, the long-term the impact of this study will generate more effective therapeutic strategy of CRPC.					
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1. INTRODUCTION

MicroRNAs (miRNAs) are small non-coding RNA molecules that regulate gene expression by post-transcriptional degradation or translational repressions by recognizing the 3'-UTR sequence of target mRNA from the specific seed sequence (ca. 2-7 nucleotides) of miRNAs (1). miRNAs have been shown to regulate approximate 60% protein-coding genes via post-transcriptional suppression by facilitating mRNA degradation, or translational inhibition. In general, miRNAs are initially transcribed into a long primary transcript by RNA polymerase II similar to cellular mRNA, and sequentially processed by Drosha and Dicer-mediated endonuclease cleavage to become mature miRNA (2-4). Nevertheless, miRNA biogenesis becomes more complicated when miRNAs are derived from the same gene cluster controlled by the same promoter and yet some is processed with different efficiency at the precursor or mature level (5, 6), which adds more complexities into the scheme of gene regulation.

Epithelial-to-mesenchymal transition (EMT) is considered an initial step for cancer cells to acquire the metastatic potentials. In prostate cancer, many studies have demonstrated the relationship of the onset of EMT phenotypes with cancer metastasis. Knowing EMT as a normal physiologic process takes place during embryonic development, therefore, the cancer cells undergoing EMT appear to have higher potential to acquire cancer stem cell (CSC) phenotypes (7). However, the molecular mechanism(s) associated with EMT or CSC in prostate cancer is not fully understood.

Our preliminary data clearly indicated that elevated IFIT5 is associated with malignant prostate cancer and IFIT5 can target many miRNAs with tumor suppressive function in preventing EMT and CSC, in which IFIT5 can be as a potential therapeutic target. Therefore, it is critical to dissect the mechanism of IFIT5 in degrading miRNA or the regulation of IFIT5. Also, significant clinical correlation of elevated IFIT5 expression in prostate cancer specimens prompt us to explore IFIT5 as prognostic marker for prostate cancer.

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2. KEYWORDS

Epithelial-to-mesenchymal transition, cancer stem cell, IFIT5, XRN1 microRNA, microRNA turn over.

3. ACCOMPLISHMENTS

Major goals and accomplishments

Aim 1 Dissect the mechanism of IFIT5-mediated miRNA turnover.

Major Task: Unveil new machinery of miRNA turnover.

Milestone: Manuscript on mechanism of miRNA turnover.

A manuscript is under 1st revision in Cancer Research.

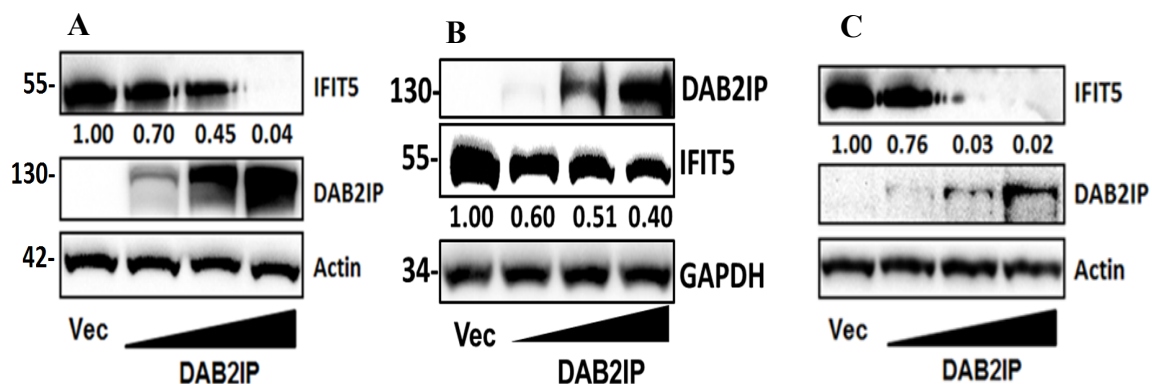
Aim 2 Determine the regulation of IFIT5 gene in prostate cancer progression.

Major Task: Identify key regulator(s) and inducer(s) of IFIT5 gene expression.

Subtask 1: Determine the inhibitory mechanism of DAB2IP in regulating IFIT5 expression induced by IFN.

From screening of many prostate cancer cell lines, it appeared that the steady-state levels of IFIT5 mRNA and protein were inversely correlated with DAB2IP. Since DAB2IP is involved in regulating the transduction of many signaling pathway. Therefore, we hypothesized that DAB2IP can suppress IFIT5 protein expression. As shown in Figure 1, the inhibitory effect of DAB2IP on IFIT5 expression is demonstrated by the ectopic expression of DAB2IP in LAPC4-KD (Figure 1A), C4-2Neo (Figure 1B), and LNCaP cells (Figure 1C).

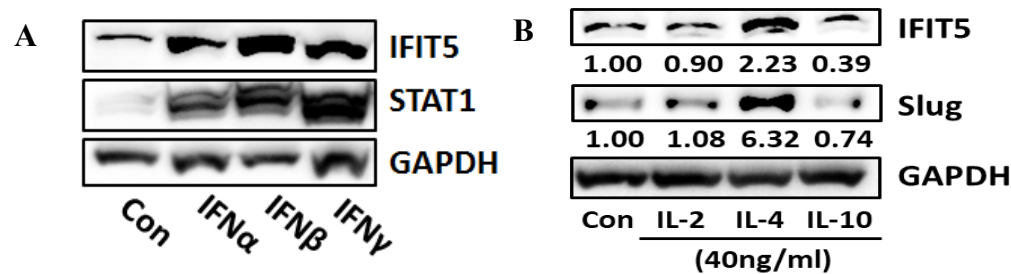
Figure 1 Suppression of IFIT5 protein expression by ectopic transfecting DAB2IP into prostate cancer cell lines. LAPC4-KD (A), C4-2Neo (B), and LNCaP (C) cells after normalizing with the control vector (Vec).



Subtask 2: Examine the mechanism of IFIT5 gene regulation in response to paracrine/juxtacrine stimulation.

IFIT5 is known as a bona fide interferon γ (IFN γ)-induced gene; IFN γ is commonly produced by immune cells that are often infiltrated and surrounding tumor. Also, IFN gene family has several subtypes. Therefore, we decided to examine other paracrine factors such as different cytokines (IL). As shown in Figure 2, IFN- α and β have the same ability of inducing IFIT5 expression compared with IFN- γ . In addition, IL-4 is also able to induce IFIT5 expression, indicating that the regulation of IFIT5 expression can be regulated by cytokine critical for prostate cancer progression.

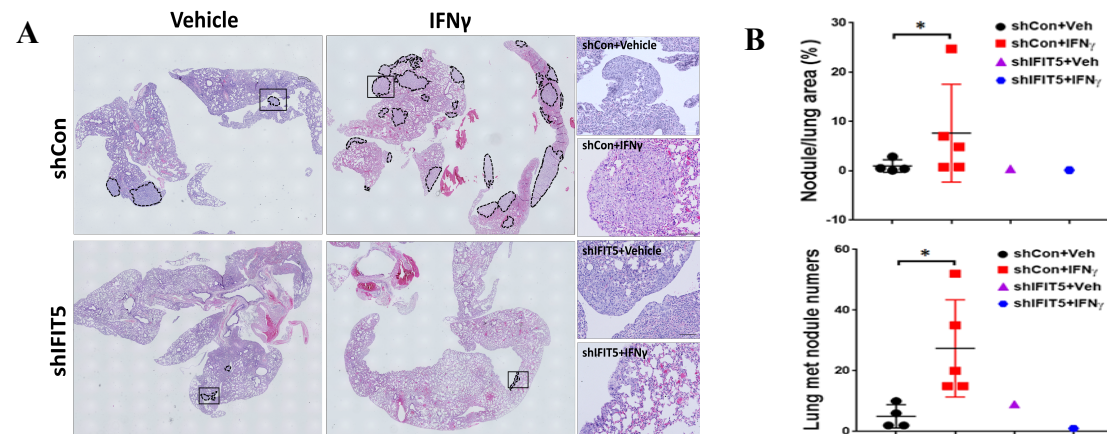
Figure 2 The effect of IFN and cytokine on IFIT5 expression. (A) Induction of IFIT5 and STAT1 in LNCaP cells treated with IFN- α and IFN- β (20 ng/ml) for 24hrs. (B) Induction of IFIT5 and Slug in PC3 cells treated with IL-2, IL-4 and IL-10 (40ng/ml) for 24hrs.



Subtask 3: Determine the role of IFIT5 in prostate cancer progression

To examine the *in vivo* effect of IFN γ on prostate cancer metastasis and the role of IFIT5 in this event, we treated control and IFIT5-knockdown PC3 cells with IFN γ for 48hrs then cells were injected intravenously into SCID animal via tail vein. IFN γ treatment significantly increases the number and size of metastatic nodules at lung parenchyma, in contrast, loss of IFIT5 dramatically reduces metastasis of prostate cancer with or without IFN γ .

Figure 3 The stimulatory effect of IFN γ on prostate cancer metastasis. (A) H&E staining of lung tissue derived from mice receiving tail vein intravenous injection of IFIT5-knockdown PC3 cells (PC3-shIFIT5) pretreated with vehicle (Veh, PBS) or IFN γ (20 ng/ml), compared to PC3 cells transfected with control vector (shCon). The black dotted line-circles region indicate the presence metastatic nodules observed at lung parenchyma. Representative tumor nodules from each group are shown at right side panels (scale bar =100 μ m). (B) Comparison of tumor nodule numbers and comparative area ratio in the lung parenchyma among each group.



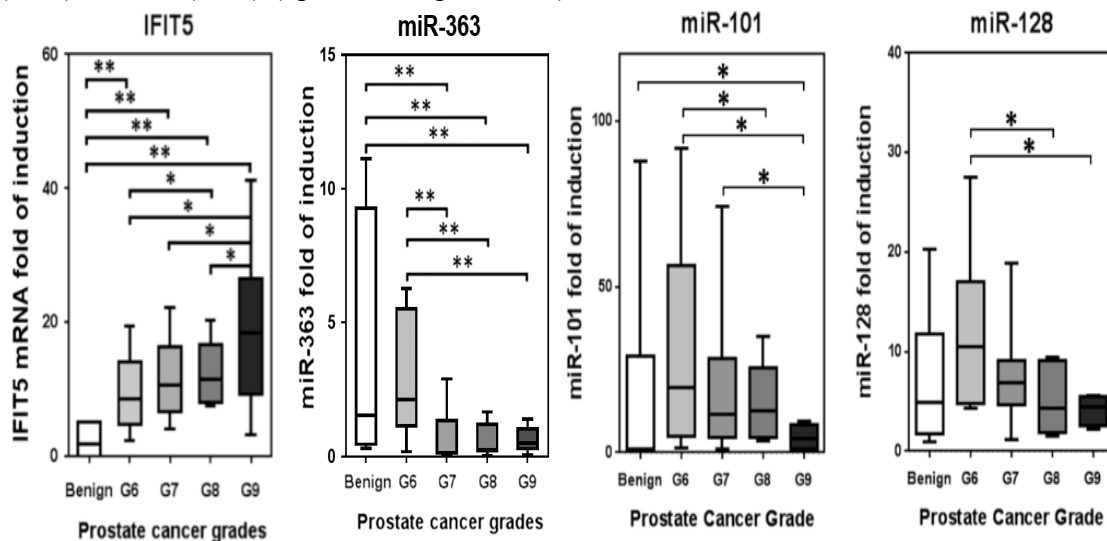
Aim 3 Evaluate IFIT5 as a prognostic marker in prostate cancer patients.

Major Task: Determine IFIT5 as a potential prognostic marker for prostate cancer.

Subtask 1: Collect clinical specimens and Study the clinical correlation of IFIT5 and miRNAs in prostate cancer progression.

In this aim, we proposed to collect clinical specimens for 30 months and determine IFIT5 mRNA and miRNA (-363, -101, and -128) levels in these specimens for clinical correlation. While we are still collecting patient specimens, we have decided to perform preliminary study to determine sample size. As shown in Figure 4, from 41 clinical specimens, we were able to demonstrate a positive correlation of IFIT5 mRNA with different tumor grades. In contrast, there is an inverse correlation of miRNA (-363, -101, and -128) levels with higher tumor grade. Overall data support our hypothesis.

Figure 4 The expression of IFIT5 mRNA and mature miR-363, miR-101 and miR-128 in clinical prostate cancer specimens. Relative expression of IFIT5 mRNA and mature miR-363, miR-101 and miR-128 level in human prostate cancer specimens derived from different grades including benign (N=10), G6 (N=9), G7 (N=9), G8 (N=6) and G9 (N=7) (* $p < 0.05$, ** $p < 0.0001$).



What opportunities for training and professional development have the project provided?

This project provides excellent training opportunities for molecular cell biology, tumor biology and pathohistologic techniques.

How were the results disseminated to communities of interest?

Currently, we have published two manuscripts. Also, one manuscript is under revision and one abstract is submitted to annual SBUR meeting in 2018.

What do you plan to do during the next reporting period to accomplish the goals?

Currently, our progress is right on the target based on original SOW; we have completed Specific Aim 1 and the majority of Aim 2 and submitted one manuscript. For the next reporting period, we expect to delineate (1) the role of IFIT5 in cancer stem cell development (2) the correlation of IFIT5 with clinical manifestation of human prostate cancer.

4. IMPACT

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

Our study unveils the tumorigenic role of IFN in prostate cancer progression, which raises concerns of using IFN as a therapeutic agent. This study also demonstrates that several tumor suppressor microRNAs (miRNAs) involved in regulating these processes are often degraded by IFIT5. Also, elevated IFIT5 is associated with prostate cancer malignancy. Thus, this study will delineate the mechanism of IFIT5 in tumor suppressor miRNA degradation, and examine IFIT5 gene regulation. By determining its clinical correlation, this study will provide valuable biomarker(s) for lethality of prostate cancer, which will have an immediate impact on patient prognosis and selection for more suitable agent.

In addition, we have discovered a new function of IFIT5 complex as many tumor suppressor miRNA degradation machinery in prostate cancer. This discovery can certainly be applied onto other cancer types such as renal and liver cancers. In addition, the knowledge derived from this study can be used for designing better miRNA for therapeutic purpose.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS

Nothing to report.

6. PRODUCTS

Lo, U., Lee, C.F., Lee, M.S., Hsieh, J.T. (2017) The role and mechanism of epithelial-to-mesenchymal transition in prostate cancer progression. *Int. J. Mol. Sci.*, 18: pii: E2079. PMID28973968

Lin CJ, Lo UG, Hsieh JT. (2018) The regulatory pathways leading to stem-like cells underlie prostate cancer progression. *Asian J Androl.* doi: 10.4103/aja.aja_72_18. PMID: 30178777

Lo, U., Pong, R.C., Yang, D., Gandee, L., Hernandez, E., Dang, A., Lin, C-J., Santoyo, J., Ma, S-H., Sonavane, R., Huang, J., Tseng, S-F., Moro, L., Arbin A.A., Kaour, P., Raj, G., He, D., Lai, C.H., Lin, H., Hsieh, J.T. (2018) Interferon- γ induces

epithelial-to-mesenchymal transition in prostate cancer via a new mechanism of microRNA processing. Cancer Research. (revised).

7. PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

Name: U-Ging Lo

Project Role: Research Scientist

No Change

Name: Payal Kapur

Project Role: collaborator

No Change

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

Nothing to Report.

What other organizations were involved as partners?

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

None.

9. APPENDIX

- (1) Lo, U., Lee, C.F., Lee, M.S., Hsieh, J.T. (2017) The role and mechanism of epithelial-to-mesenchymal transition in prostate cancer progression. Int. J. Mol. Sci., 18: pii: E2079. PMID28973968
- (2) Lin CJ, Lo UG, Hsieh JT. (2018) The regulatory pathways leading to stem-like cells underlie prostate cancer progression. Asian J Androl. doi: 10.4103/aja.aja_72_18. PMID: 30178777



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INVITED REVIEW

Prostate Cancer

The regulatory pathways leading to stem-like cells underlie prostate cancer progression

Chun-Jung Lin, U-Ging Lo, Jer-Tsong Hsieh

Prostate cancer (PCa) is the most common cause of malignancy in males and the third leading cause of cancer mortality in the United States. The standard care for primary PCa with local invasive disease mainly is surgery and radiation. For patients with distant metastases, androgen deprivation therapy (ADT) is a gold standard. Regardless of a favorable outcome of ADT, patients inevitably relapse to an end-stage castration-resistant prostate cancer (CRPC) leading to mortality. Therefore, revealing the mechanism and identifying cellular components driving aggressive PCa is critical for prognosis and therapeutic intervention. Cancer stem cell (CSC) phenotypes characterized as poor differentiation, cancer initiation with self-renewal capabilities, and therapeutic resistance are proposed to contribute to the onset of CRPC. In this review, we discuss the role of CSC in CRPC with the evidence of CSC phenotypes and the possible underlying mechanisms.

Asian Journal of Andrology (2018) 20, 1–8; doi: 10.4103/aja.aja_72_18; published online: ???

Keywords: cancer stem cell; castration-resistant prostate cancer; neuroendocrine differentiation; transdifferentiation

INTRODUCTION

Prostate cancer (PCa) remains the most commonly diagnosed cancer, from the 2017 cancer statistic report, there were 180 890 estimated new cases in 2017, and PCa is also the third leading cause of death, with 26 120 estimated deaths in the United States.

Clinical treatment for primary PCa includes radical prostatectomy and radiotherapy. Androgen deprivation therapy (ADT) is commonly used to decrease the androgen-dependent tumor burden of metastatic PCa (mPCa). Castration-resistant PCa (CRPC) is defined as the reappearance of cancer lesion from metastatic site(s) often with rising prostate-specific antigen (PSA) in patient's serum. CRPC is recognized as the end-stage disease since patients do not respond to chemotherapeutics very well with average 6-month to 1-year survival. Although recent introduction of second-line anti-androgen agents has prolonged patients' survival, PCa eventually develops therapy-resistant phenotypes. Clinically, therapy-resistant tumors can be divided into several different phenotypes such as neuroendocrine, androgen receptor (AR) variants, and AR hyperactivation due to gene amplification and/or mutation, which raise a critical question for the cell origin of these subtypes. Many studies have demonstrated the presence of stem-like population in normal prostate and abnormal prostate,^{1–3} which raises the potential role of cancer stem cell (CSC) in cancer progression. In this article, we have summarized the potential pathways associated with CSC leading to CRPC and the possible therapeutic strategies to improve the clinical outcomes of PCa patients.

CANCER STEM CELL IN CRPC

Cell markers for CSC

The CSC theory, as a potential mechanism for CRPC, has raised significant attention in recent years. In general, embryonic stem

cell is pluripotent with the ability of developing into different tissue types. Somatic stem cell in each organ is considered to be quiescent most of time with limited number and capable of self-renewing and differentiation maintained in a homeostatic balance. Isaacs and Coffey⁴ first proposed that prostate stem cell resides in basal cell population; its expansion underlies the development of benign prostatic hyperplasia (BPH). Further study suggested that enrich stem cell is in the proximal duct of prostate.⁵ During normal prostate development, androgen binding to the AR in surrounding stromal cells plays a key role of basal stem cell different into luminal cell population.⁶ Recent study using gene-tracing technology indicated that luminal cell population has stem cells as well with less potency than basal stem cell.⁷ For CSC, its self-renewal ability allows a single cell remained after therapies to repopulate entire tumor population as therapeutic resistance. Further, the pluripotency of CSC is capable of differentiating into different cell types such as neuroendocrine. It has been reported that prostate CSC is likely derived from basal cell population.⁸ Normal prostate gland can be divided into epithelial, stromal, and neuroendocrine cells; the epithelial cells include luminal and basal cells. Among all these heterogeneous cell types in the normal prostate, the gene expression profile of basal cell is highly correlated with that of stem cell. Noticeably, the basal cell gene profiles are enriched in advanced, anaplastic, castration-resistant, and metastatic PCa in the human PCa sample set.⁹ However, it is still unclear whether clonal expansion and/or adaptation through epithelial-to-mesenchymal transition (EMT)^{10–12} or transdifferentiation¹³ during therapies results in the expansion of CSC.

Several markers have been commonly used to identify CSC population including CD44, stem cell antigen (Sca-1 or Ly6A), prominin-1 (CD133), and ATP-binding cassette subfamily G member 2 (Junior blood group) (ABCG2). CD44 is often associated

with CSC, which is a cell membrane receptor that is involved in cell-cell interactions, adhesion, and migration. Especially, cells expressing CD44, but lacking CD24 (CD44⁺CD24⁻) PCa cells, were identified as the CSC population with *in vivo* and *in vitro* models.¹⁴ These CD44⁺CD24⁻ PCa cells have the ability of forming spheres and producing tumor from a single cell, which is known as stem cell self-renewal ability. Sca-1 is a mouse glycosyl phosphatidylinositol-anchored surface protein that expressed by stem cells or progenitor cells. However, the human homolog of Sca-1 has not been identified yet. Studies in mouse model demonstrated that cells with Sca-1 expression have tumor-initiating ability, and tumor cells expressing higher Sca-1 were correlated with their aggressive phenotype.^{15,16} CD133 is a transmembrane glycoprotein, and is known as a marker for basal stem cell as well as PCa-initiating cell. Richardson *et al.*¹⁷ reported that a subset of CD133⁺ population exhibited higher clonogenic potential than CD133⁻ population. Furthermore, these CD133⁺ populating can fully differentiate to prostatic acini from *in vivo* animal model. In addition, studies demonstrated that CD133 involved in cell growth, cell development, and tumor progression, in which the expression of CD133 was significantly increased in cancer-initiating cells using patient-derived primary cell model.¹⁸ ABCG2 is the ATP-binding cassette membrane transporter. Patient-derived cells with high ABCG2 expression correlated with cell that expresses stem cell markers, and these subsets of cells have shown to gain multidrug resistance and be responsible for the recurrence of PCa.¹⁹ Although these CSC markers²⁰ listed in this review are indeed correlated with CSC population associated with cancer progression, recurrence, and therapy resistance, there is still lacking a specific PCa CSC marker.

Molecular signaling pathways lead to CSC in CPRC

Three signaling pathways have been suggested to be critical for CSC development including Wnt, Sonic Hedgehog, and Notch signaling pathways. Several reports have demonstrated that targeting these signaling pathways along with conventional treatment can prevent the emergence of CRPC.^{21,22}

Wnt

In the canonical of Wnt pathway, Wnt ligands bind to Frizzled and low-density lipoprotein receptor-related protein (LRP) 5/6, which activate downstream molecular targets, leading to the accumulation and nuclear translocation of β -catenin, subsequently affecting cell survival; while, in the noncanonical pathways, Wnt activates downstream effectors and activates targeted gene expression and cytoskeleton rearrangement, resulting in altered cell survival. Abnormal Wnt signaling has been found in several cancer types, including brain, breast, and colorectal cancer.²³ In PCa, elevated β -catenin expression was often found in the nucleus of cancer cells.²⁴ Importantly, Wnt signal regulates self-renewal ability of several cell models including LNCaP, C42B, and PC3 cell in an AR-independent manner,^{25,26} while downregulated Wnt/ β -catenin pathway significantly suppresses stem cell-like properties.²⁷ Furthermore, Wnt3 has been shown to increase the expression of its downstream effectors, as well as CSC markers including CD133 and CD44, which subsequently lead to sphere formation.²⁵ In addition, Zhang *et al.*²³ demonstrated that human telomerase reverse transcriptase (hTERT)-expressing PCa cells have higher Wnt/ β -catenin activity and can thereby regulate the self-renewal and differentiation activity of PCa cells. Collectively, Wnt plays a key role in CSC development in CRPC (Figure 1a).

Sonic Hedgehog

Sonic Hedgehog signaling pathway is a conserved process that controls cell renewal and cell survival. Hedgehog signaling is initiated by hedgehog family ligands (Sonic, Desert, and Indian). These ligands bind to membrane receptors Patched (Ptch1 and 2) and Smoothened on the primary cilium, leading to the activation and nuclear translocation of glioma-associated oncogene homolog (Gli) (Gli 1, 2, and 3), which trigger the expression of targeted genes that regulate cell survival. Abnormal Hedgehog signaling pathway has been found in several cancer types, including brain, gastrointestinal, lung, breast, and prostate cancers.^{28,29} Some studies also demonstrated that Hedgehog involved in tumor progression and CSC proliferation.³⁰⁻³² Importantly, using several PCa cell line models,²⁹ *i.e.*, LNCaP, Du145, PC3, 22Rv1, and

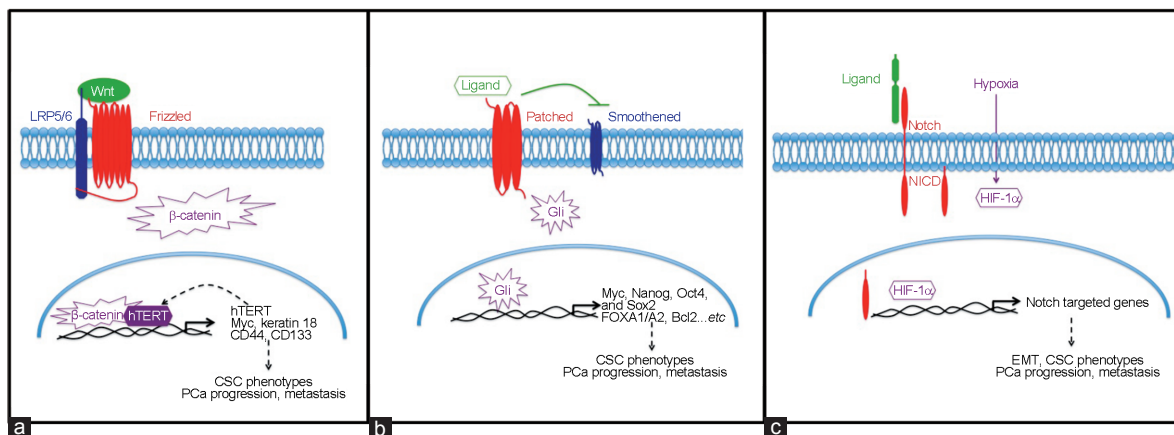


Figure 1: Schematic representation of signal contributes to CSC phenotype. (a) Wnt proteins bind to both frizzled receptor proteins and the co-receptor LRP5/6. This binding further facilitates the activation of β -catenin. Activated β -catenin translocates into nucleus and promotes Wnt downstream gene transcription. Furthermore, Wnt/ β -catenin induced hTERT also acts as a transcriptional factor, resulting a positive feedback in the enhanced expression of Wnt target genes that leads to cancer-promoting functions and CSC phenotypes. (b) Sonic Hedgehog pathway is initiated by binding one of the three secreted Hedgehog ligands to its receptor. This binding releases Smoothened that modulates the expression of three Gli zinc-finger transcription factors. (c) The Notch receptor is activated by ligand binding, which is presented by a neighboring cell. Notch activation releases an active fragment, NICD. NICD then translocates into the nucleus and promotes the expression of targeted genes. Notch-dependent signaling induces several genes associated with differentiation, survival, stemness, and EMT, which all relate to PCa progression and metastasis. Further, Notch signaling is often activated by hypoxia through HIF-1 α . CSC: cancer stem cell; PCa: prostate cancer; hTERT: human telomerase reverse transcriptase; LRP: low-density lipoprotein receptor-related protein; Gli: glioma-associated oncogene homolog; Sox 2: sex determining region Y-box 2; FOXA1/A2: forkhead box A1/A2; NICD: notch intracellular domain; HIF-1 α : hypoxia-inducible factor 1 subunit alpha; EMT: epithelial-to-mesenchymal transition; Bcl2: B-cell lymphoma 2; Oct4: octamer-binding transcription factor 4.

LAPC4, as well as xenograft model (CWR22),³⁰ several Gli-related genes have been shown to correlate with the progression of CRPC. These Gli-related genes, including *CDKN2A/p16/INK4A*, *Myc*, and *CDK2*, promote AR-independent tumor cell growth^{33,34} and lead to tumor recurrence.³⁵ Data from PCa patients-derived tissue microarray and PCa cell line models (PC3, LNCaP, and Du145) indicated that forkhead box A1 (FOXA1) was found to promote the progression of CRPC.^{36,37} Cell model (LNCaP, PC3, Du145, and LAPC4) also showed that the expression of B-cell lymphoma 2 (Bcl2) correlates with therapy resistance ability in CRPC.³⁸ Importantly, increased Hedgehog signaling was found in PCa CSC population (CD44⁺CD24⁻) *in vivo* and *in vitro*.¹⁴ Taken together, Hedgehog downstream signaling pathway contributes to CRPC progression and therapy-resistant phenotypes of CSC (Figure 1b).

Notch

Notch signaling pathway has been extensively studied and has been correlated to promote CSC phenotype in several cancers. Notch signaling is mediated by four Notch receptors, including Notch1–4, and five ligands including delta-like (DLL) 1, DLL 3, DLL4, Jagged 1, and Jagged 2, were involved. Various types of cancers exhibit dysfunction of the Notch pathway in their cells.³⁹ Importantly, Notch interacts with AR pathway and the phosphoinositide 3-kinase (PI3k)/Akt pathway, which are the two main signaling pathways in controlling prostate development and carcinogenesis.^{40–42} Studies from cell culture models and human PCa specimens have demonstrated that higher Notch ligand, Jagged 1-Notch1 signaling contributes to PCa progression and metastasis, and promotes EMT and CSC phenotype.^{43–46} Higher Notch3 expression was found in CRPC, which was induced by hypoxia condition.⁴⁷ Recently, Notch4-targeted silencing, leading to the inhibition of nuclear factor kappa B (NF- κ B) activity, also showed a promising anti-PCa growth and anti-EMT effect.^{44,48,49} These reports all point out the importance of Notch signal in PCa progression and the initiation of CSC phenotype (Figure 1c).

Caveolin-1 (Cav-1)

The Caveolin protein family including caveolin-1, -2, and -3 is the major component of caveolae; Cav-1 is the first member identified and has been extensively characterized. It is known to regulate multiple cellular functions, including cell cycle, signal transduction, endocytosis, and cholesterol trafficking and efflux. Cav-1 levels are correlated with PCa progression and metastasis.^{50–52} Extensive studies using PCa cell line (Du145, LNCaP, and 22Rv1), and human BPH sample model, also indicated that Cav-1 signaling contributes to therapy resistance and the reoccurrence of PCa.^{52–54} Using xenograft model, Cav-1 can be secreted into circulation to enhance tumor growth in a paracrine manner, implying that Cav-1 can be a secretory protein.^{55,56} Interestingly, other study has demonstrated that Cav-1 can elevate the Notch-1-Akt-NF-kappaB pathway, leading to chemoresistance in ovarian cancer.⁵⁷ Studies from PCa cell models (LNCaP, PC3, and Du145) and immunostaining of PCa specimens have indicated that Cav-1 can interact with LRP6 to regulate Wnt- β -catenin signaling.^{58,59} Moreover, Cav-1 contributes to the expression of CSC-related genes including *Oct4*, *Nanog*, *CD44*, *CD133*, and *ABCG2* in lung cancer cell and breast cancer models,^{59,60} which all strongly suggest that Cav-1 signaling involved in the induction of CSC phenotypes. However, additional data are required to link Cav-1 directly to CSC generation in CRPC.

MicroRNAs contribute to CSC properties of PCa cell

Emerging evidence has implied that microRNA (miRNA) regulation

is crucial in promoting or repressing cancer metastasis via regulating the characteristics of CSCs. In particular, dysregulation of miRNAs is associated with tumor initiation and progression of PCa. A coordinated downregulation of miR-34a, let-7b, miR-106a, and miR-200 family has been observed in the progenitor stem cell population of PCa (Table 1).⁶¹

miR-34, a p53 downstream target gene, is known as a tumor-suppressor miRNA. Frequent hypermethylation of miR-34 has been observed in many malignancies with p53 mutation. Cheng *et al.*⁶² using conditional knockout/transgenic mouse model demonstrated that inactivation of both p53 and miR-34a in mouse prostate epithelium leads to the expansion of the prostate stem cell compartment, as well as development of early invasive adenocarcinoma and high-grade prostatic intraepithelial neoplasia. Consistent with their *in vivo* observations, combined deficiency of both miR-34 and p53 leads to accelerated EMT-dependent growth, enhanced self-renewal capacity, and increased cell motility in prostate stem/progenitor cells derived from the proximal region of prostatic ducts.⁶² In addition, miR-34a is known to be a key negative regulator of CD44, an adhesion molecule that is a key player in metastasis. CSCs derived from multiple malignant tumors have shown high expression of CD44. These CD44-positive CSC populations have colonogenic, tumor-initiating, and metastatic capacities. Liu *et al.*⁶³ demonstrated that systemic delivery of miR-34a can inhibit PCa metastasis and regeneration by targeting CD44. A recent study done by Bucay *et al.*⁶⁴ also revealed that another CD44-targeting miRNA, miR-383, is frequently downregulated due to loss of the chromosome 8p22 locus in the progression of PCa. Functionally, miR-383 is shown to inhibit tumor-initiating potential and metastasis of CD44-positive PCa cells by direct targeting of CD44.⁶⁴

miR-320 is found significantly downregulated in the progression of PCa; reduction of miR-320 associated with increased β -catenin expression has been observed in a CD44-high subpopulation of PCa cells and clinical prostatic tumor specimens. By global gene expression profiling, we reported that ectopic expression of miR-320 in PCa cells leads to suppression of CSC markers such as CD133, CD117, CXCR4, and ABCG2, as well as downstream target genes of Wnt/ β -catenin pathway.²⁷ Functionally, miR-320 deficiency facilitates the CSC properties including tumor-sphere formation, chemo-resistance, and tumorigenic abilities. Overall, this study strongly suggested that miR-320 is a potent regulator of tumor-initiating cells in prostate.

Similar to miR-320, expression level of miR-7 is also significantly reduced in a subpopulation of CD133-positive/CD44-positive PCa cells, which possess CSC-like features and are sufficient for tumorigenesis based on a limited dilution analysis. On the other hand, restoration of miR-7 in PCa cell lines results in sustained inhibition of CSC characteristics and impaired tumorigenesis via targeting Kruppel-like factor 4 (*Klf4*). Overall, this study implies the critical role of miR-7 in regulating the properties of PCa stem cell.⁶⁵

Meanwhile, loss of the let-7 family has been observed in PCa tissue specimens, particularly in high-grade tumor. Kong *et al.*⁶⁶ demonstrated an inverse correlation between let-7 and enhancer of Zeste homolog 2 (EZH2), a putative let-7 family target that is highly expressed in CSCs of many malignancies and is known to regulate expansion and maintenance of CSC.⁶⁷ Functionally, let-7 is shown to diminish both colonogenic ability and sphere-forming capacity via targeting *EZH2* in PCa cells.⁶⁶

Similar to let-7, expression level of miR-100 is also significantly decreased particularly in bone metastatic PCa specimens. Wang *et al.*⁶⁸ suggested that miR-100 regulates spheroid and colony formation of PCa cells by targeting argonaute 2, RISC catalytic component (Ago2), leading to suppression of stemness markers such as c-Myc, CD44, *Klf4*, and *Oct4*. This indicates that loss of miR-100 may promote the stemness

Table 1: MicroRNAs involved in prostate cancer progression to castration-resistant prostate cancer

MicroRNAs	Target gene	Impacts on PCa progression	Reference
miR-34	<i>CD44</i>	Inhibits PCa metastasis, regeneration, and carcinogenesis	62,63
miR-383	<i>CD44</i>	Inhibits tumor-initiating potential and metastasis of CD44-positive PCa cells	64
miR-320	<i>CD133, CD117, CXCR4, ABCG2</i>	Suppress tumor-sphere formation, chemoresistance, and tumorigenic abilities of prostatic CSCs	27
miR-7	<i>Klf4</i>	Inhibits stemness properties and impairs tumorigenesis of PCa stem-like cells	65
let-7	<i>EZH2</i>	Diminishes colonogenic ability and sphere-forming capacity of PCa cells	61,66
miR-100	<i>AGO2</i>	Regulates spheroid and colony formation of PCa cells	68,69
miR-200b	<i>Bmi-1</i>	Suppresses proliferation and migration, as well as enhances chemosensitivity of PCa cells to docetaxel	70
miR-141	<i>Klf9</i>	Facilitates spheroid formation and proliferation of PCa cell	73
miR-143	<i>Oct4, Sox2, Klf4, FNDC3B</i>	Inhibits cell viability and colony formation of bone metastatic PC3 cells. Suppresses tumor sphere formation and CSC marker expression in PC-3 cells	74,76-78
miR-145			
miR-128	<i>BMI-1, NANOG, TGFBRI</i>	Reduces sphere formation and colonogenic potential of PCa cells	79
miR-663		Enhances cell proliferation, invasion and neuroendocrine differentiation characteristics in PCa cells	108

PCa: prostate cancer; CRPC: castration-resistant PCa; CSC: cancer stem cell; Sox2: sex determining region Y-box 2; Bmi-1: B-cell-specific Moloney murine leukemia virus insertion site 1; TGFBRI: transforming growth factor beta receptor 1; Klf4: Kruppel-like factor 4; FNDC3B: fibronectin type III domain containing 3B; EZH2: enhancer of Zeste homolog 2; Klf9: Kruppel like factor 9; CXCR4: C-X-C chemokine receptor type 4; ABCG2: ATP-binding cassette subfamily G member 2 (Junior blood group); AGO2: argonaute 2, RISC catalytic component

properties of PCa. On the contrary, by screening miRNA expression in PCa patient-derived xenograft tumor lineages, a recent study done by Nabavi *et al.*⁶⁹ demonstrated that several miRNAs (miR-100-5p, miR-411-5p, and miR-185-5p) are associated with the regression to dormancy status after ADT. Particularly, miR-100 has been recognized as a key component contributing to initiation and evolution of CRPC; it is believed that miR-100 is critical for the cell survival upon AR deprivation in AR-positive PCa cell lines.⁶⁹

The miR-200 family (miR-200a/b/c and miR-141) is known for targeting mesenchymal transcription factors leading to inhibition of EMT. In particular, Yu *et al.*⁷⁰ reported that miR-200b is significantly downregulated in PCa *in vivo* and in advanced PCa cell lines (LNCaP, PC3, and DU145), as well as patient samples (BPH) *in vitro*. Ectopic expression of miR-200b sensitizes PCa cells to chemotherapeutic reagent, docetaxel, by targeting the gene, B-cell-specific Moloney murine leukemia virus insertion site 1 (*Bmi-1*),⁷⁰ which is a critical regulator of CSC properties in several malignancies such as breast and gastric cancers.^{71,72} In contrast, miR-141-3p has an opposite regulatory role. Li *et al.*⁷³ utilized miR-141-3p mimics to demonstrate its effect on facilitating spheroid formation and proliferation of PCa cell line (PC3). The impact of miR-141 on promoting PC3 stemness is associated with the upregulation of Oct4, Bmi-1, sex-determining region Y-box 9 (Sox9), and CD44, suggesting that miR-141 can target a common repressor of these genes.⁷³ Certainly, more detailed studies are needed to unveil the mechanism of action of miR-141.

Both miR-143 and miR-145 are known to regulate bone metastasis of PCa. Huang *et al.*⁷⁴ reported that both miR-143 and miR-145 can inhibit colony formation, suppress sphere-forming capacity, and reduce expression of CSC markers (CD133, CD44, Oct4, c-Myc, and Klf4) in bone-metastasis-derived PCa cell line. The finding of this study strongly suggested that miR-143 and miR-145 may play a crucial role in regulating CSCs in bone metastatic PCa. Accumulating studies have indicated that miR-145 negatively regulates pluripotency of embryonic stem cells via targeting several stemness markers such as Oct4, Sox2, and Klf4.^{75,76} Ozen *et al.*⁷⁷ demonstrated that ectopic expression of miR-145 leads to reduced cell renewal of PCa cell lines by targeting Sox2 gene expression. By screening the miRNA expression between 3D-sphere and 2D-adherent PCa cells, Fan *et al.*⁷⁸ unveiled that progressively elevated miR-143 is found in the sphere-re-adherent culture of PCa cells, suggesting that miR-143 is involved in stem cell formation.

In contrast to those miRNAs with CSC-promoting activities, it is significantly reduced miR-128 in PCa compared to benign prostate tissue that may have an opposite function. Indeed, Jin *et al.*⁷⁹ demonstrated that overexpression of miR-128 leads to diminished CSC properties by reducing sphere formation and clonogenic potential in PCa cells. Mechanistically, miR-128 is shown to target on several self-renewal genes such as *BMI-1*, *NANOG*, and transforming growth factor beta receptor 1 (*TGFBRI*).⁷⁹ Overall, this study highly suggested that miR-128 regulates tumor initiation in PCa by limiting the CSC properties mediated by BMI-1 and NANOG.

NEUROENDOCRINE DIFFERENTIATION (NED) IN CRPC AND CSC

Prostate NED carcinoma is considered as a type of prostatic epithelial neoplasms that have NED feature,⁸⁰ which usually identified by histopathological examination with NED markers. Therefore, NED can be found in small cell carcinoma, carcinoid, and carcinoid-like tumors, as well as prostatic adenocarcinoma (**Figure 2**). Importantly, NED has been found in recurrent CRPC after second line of ADT.^{81,82} Although small cell carcinoma in prostate only counts for less than 2% of total PCa population,⁸⁰ neuroendocrine prostate cancer (NEPC) is detected in 10%–20% of CRPC population.⁸³ Interestingly, a study with PCa cells (Du145 and PC3) and xenograft models demonstrates that CD44⁺ is selectively expressed in neuroendocrine cells and these cells are responsible for PCa recurrence.⁸⁴ NEPC is an end stage of CRPC and most patients survive less than a year after recurrence.⁸⁵ Clinically, NEPC is identified based on histology features. In general, the tumor cell morphology was similar to high-grade neuroendocrine cancers, which have high numbers of mitotic cells with nuclear molding and chromatin-like “salt and pepper” similar to small cell.^{81,86} In addition, there are neuroendocrine markers that can be used for validation.

Cell markers for NED in CRPC

There are several general NED markers that are currently used to diagnose NEPC, and the presence of at least one of these is diagnostic of the condition. These markers include (1) neuron-specific enolase (NSE), a cell-specific isoenzyme of the glycolytic enzyme enolase, and it is one of the most reliable markers for the diagnosis of small cell in lung cancer;⁸⁷ (2) synaptophysin (SYP), a major synaptic vesicle protein, is usually combined with the neuroendocrine secretory protein, chormogranin A (ChgA), for diagnosis;⁸⁸ (3) ChgA, a secretory

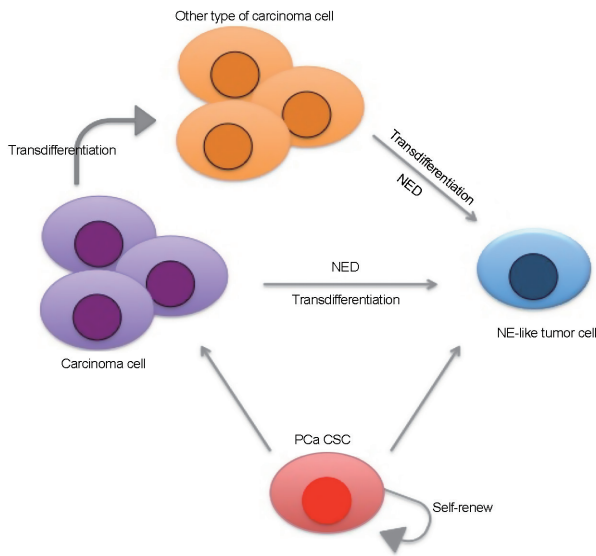


Figure 2: Schematic representation of the relationship of CSC, PCa cells, and NE-like cell. CSC maintains its multipotency to form different types of tumor cell and is capable of self-renewing to expand its progeny. NE-like tumor cell can originate from the same type of tumor cells that undergo NED or transdifferentiation from other type of tumor cell (*i.e.*, adenocarcinoma to small cell adenocarcinoma with NE phenotype). CSC: cancer stem cell; PCa: prostate cancer; NE: neuroendocrine; NED: neuroendocrine differentiation.

protein found in the neuroendocrine cells, has commonly used in detecting neuroendocrine tumors;⁸⁹ (4) CD56, also known as neural cell adhesive molecule (NCAM), which belongs to a group of cell surface glycoproteins involved in cell-cell adhesion.⁹⁰ On the other hand, NEPC cells often lack the expression of luminal markers such as PSA and prostatic acid phosphatase.⁹¹ Recently, FOXA2 has also been suggested to be a reliable marker in diagnosis of NEPC.⁹²

Molecular mechanisms leading to NED in CRPC

Several studies have indicated that Myc (N-Myc or c-Myc) plays a key role in NEPC and also Myc gene amplification or protein overexpression is often detected in clinical specimens.⁹³ Recently, Lee *et al.*⁹⁴ have indicated that ectopic expression of N-Myc in basal cell population of prostate potentiates NEPC through the activation of Akt signaling pathway with human patient sample model *in vivo* and *in vitro*. Other embryonic transcription factors such as FOXA1 and FOXA2 are associated with NEPC. FOXA1 is known as a pioneering factor in modulating AR activity, and is also involved in prostate epithelial differentiation by altering chromatin tertiary structure. However, the loss of FOXA1 is found to facilitate NEPC through the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) signaling pathways in both cell line model (TRAMP-C and 22Rv1) and human patient sample.⁹⁵ In contrast, FOXA2 expression was detected in NEPC, but not in primary and metastatic PCa samples (NCI-H660, PC3),⁹² and it cooperates with hypoxia-inducible factor 1 subunit alpha (HIF-1 α) activity to facilitate NED in PCa cell model (PC3, NCIH660, LAPC4, and LNCaP).⁹⁶ In addition, other key regulators in neuronal differentiation such as BRN2, POU-domain transcription factor, have recently shown to regulate Sox2, which contributes to NED in PCa cell model (PC3, NCI-H660, LNCaP, and LAPC4).⁹⁷

Under the influence of tumor microenvironment, studies with mouse PCa *in vitro* and *in vivo* model, as well as LNCaP cell model, have found that interleukin 6/signal transducer and activator of

transcription 3 (IL-6/STAT3) pathway associated with aurora kinase A (AURKA), N-Myc, and EZH2 have been shown to induce NEPC.^{98,99} Mechanistically, in LNCaP cell model, autophagy process is involved in IL-6-induced NED and therapy-resistant phenotype in PCa model;¹⁰⁰ thus, targeting autophagy synthesis or related signaling pathways can block neuroendocrine cell differentiation.^{101,102} In addition, secretory protein secretogranin II (SgII), a neuroendocrine secretory protein-like ChgA that is widely distributed throughout the neuroendocrine system, has been shown to induce NEPC phenotype.¹⁰³ G-protein coupled receptor kinase 3 (GRK3) expression correlated with PCa progression has been shown to increase neuroendocrine phenotypes associated with ADT resistance in PCa cells (NCI-H660, PC3, LNCaP, and VCaP).¹⁰⁴ In contrast to oncogenic factors, loss of retinoblastoma 1 (RB1) and tumor protein p53 (TP53) further facilitates ADT resistance of PCa cells that exhibit several NED markers in both clinical patient sample and mouse model.^{105,106}

MicroRNAs correlated with NED in PCa

Dang *et al.*¹⁰⁷ demonstrated that the treatment of enzalutamide or casodex increases the infiltration of mast cells secreting C-C motif chemokine ligand 8 (CCL8) and IL-8 that can promote NED phenotypes by upregulating miR-32 in PCa cells. Indeed, overexpression of miR-32 leads to enhanced NED characteristics in PCa cells associated with elevated NSE. Overall, this study demonstrates that the potential impact of immune cells on the emergence of NED in PCa is mediated by microRNA.¹⁰⁷

Meanwhile, by examining PCa tissue specimens, Jiao *et al.*¹⁰⁸ revealed a significant upregulation of miR-663 in CRPC. Functionally, overexpression of miR-663 in LNCaP cell results in enhanced cell proliferation, invasion, and NED. Clinically, expression level of miR-663 is correlated with PCa grade, node, and metastasis (TNM) staging of PCa,¹⁰⁸ suggesting miR-663 as a potent regulator in NEPC.

In addition, Liang *et al.*¹⁰⁹ reported that hypoxia can promote NED in PCa cell lines such as LNCaP and PC3 by inducing the expression of a polycistronic miRNA cluster containing miR-106b, miR-93, and miR-25, which can suppress the expression of neuron-restrictive silencer factor, also known as RE1-Silencing Transcription factor (*REST*) gene, but increase of pro-neuronal genes such as paired mesoderm homeobox protein 2A (*PHOX2A*), absent small and homeotic disks protein 1 (*ASH-1*), and *ChgA*. Clinically, an inverse correlation between miR-106b-25 and *REST* is observed in PCa with high Gleason grade.¹⁰⁹ Altogether, this study suggested that loss of *REST* due to elevation of miR-106b-25 under hypoxia condition might promote NED in PCa.

TRANSDIFFERENTIATION IN CRPC

Transdifferentiation is the process of cell conversion from one type to another type (Figure 2). For example, pancreatic progenitor cells transdifferentiate into hepatocyte-like cells.¹¹⁰ Recent data clearly show that somatic cells can undergo reprogramming process by exogenously introducing key transcription factors such as Sox2, Klf4, c-Myc, Oct4, Nanog, and lin-28 homolog A (LIN28).^{111,112} For cancer cells, particularly, high-grade poorly differentiated cancer cells exhibiting CSC phenotypes can transdifferentiate into different cell types by turning on similar genes endogenously.¹¹³ For example, loss of Rb1 and TP53 underlying cancer lineage plasticity¹⁰⁶ is mediated by increased Sox2 expression in these cells.¹¹⁴ Similarly, loss of PTEN and TP53 facilitates ADT resistance and initiates transdifferentiation event in adenocarcinoma, which is evidenced by elevated NED markers.¹¹⁵ All these data conclude that CSC is the result of cancer cell de-differentiation through genetic alteration and/or epigenetic

alteration from tumor microenvironment. In CRPC, accumulating evidence supports that CSC plays a central role of therapeutic resistance, NED. Thus, developing anti-CSC strategy is expected to improve the survival of CRPC patients.

CONCLUSION

The presence of CSC has been identified in hematopoietic and testicular cancers but less known in solid tumors, particularly PCa. Accumulating data support the critical role of prostate CSC in disease progression. Despite these progresses, there is still lacking human PCa-specific CSC marker(s). Further, the underlying mechanisms of transdifferentiation as well as NED in CSC are not fully understood, which will be critical for further developing better therapeutic strategies.

AUTHOR CONTRIBUTIONS

CJL and UGL prepared manuscript. JTH outlined framework and finalized manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

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Review

The Role and Mechanism of Epithelial-to-Mesenchymal Transition in Prostate Cancer Progression

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Abstract: In prostate cancer (PCa), similar to many other cancers, distant organ metastasis symbolizes the beginning of the end disease, which eventually leads to cancer death. Many mechanisms have been identified in this process that can be rationalized into targeted therapy. Among them, epithelial-to-mesenchymal transition (EMT) is originally characterized as a critical step for cell trans-differentiation during embryo development and now recognized in promoting cancer cells invasiveness because of high mobility and migratory abilities of mesenchymal cells once converted from carcinoma cells. Nevertheless, the underlying pathways leading to EMT appear to be very diverse in different cancer types, which certainly represent a challenge for developing effective intervention. In this article, we have carefully reviewed the key factors involved in EMT of PCa with clinical correlation in hope to facilitate the development of new therapeutic strategy that is expected to reduce the disease mortality.

Keywords: epithelial-to-mesenchymal transition; metastasis; prostate cancer progression

1. Introduction

The plasticity of cellular phenotypic transformation is fundamental to embryonic development. During gastrulation stage, the reprogramming process of epithelial-to-mesenchymal transition (EMT) mainly governs the phenotypic change of polarized ectodermal epithelial cells into migratory mesenchymal cells that ultimately constitute the mesodermal layer of the embryo [1]. EMT occurs by breakdown of cell-to-cell or cell-to-extracellular matrix (ECM) adherence at the polarized epithelium lining. E-cadherin is a major component of epithelial adherence junction and acts as the master gatekeeper of EMT. Loss of E-cadherin, considered to be the key step to initiate EMT, leads to collapse of intercellular mechanical communication. In contrast, critical mesenchymal markers such as vimentin and N-cadherin, as well as several E-cadherin transcriptional repressors including zinc finger proteins Snail/SNAI1 and Slug/SNAI2, twist-related protein 1 (Twist 1) and zinc finger E-box-binding homeobox 1 and 2 (ZEB1 and ZEB2) are highly elevated during EMT (Figure 1), leading to acquisition of mesenchymal phenotype of enhanced cell mobility [2]. EMT is critical for tissue remodeling during embryonic morphogenesis [3–6]; however, this reprogramming process is also observed in different pathological process such as organ fibrosis, wound healing and carcinoma progression. In particular, primary carcinoma cells switch from epithelial characteristics to mesenchymal-like phenotype while responding to either intrinsic genetic and molecular alteration or extrinsic microenvironmental stimuli, which leads to the invasion into surrounding stroma and subsequent vasculature, ultimately colonization at a distant pre-metastatic niche [6,7]. Particularly, the role of EMT in metastasis has

been demonstrated in many cancer types including prostate cancer (PCa) to elicit their metastatic potentials [2,8,9], which is supported by significant correlation between TGF- β and EMT-related genes detected from circulating prostate cancer cells of PCa patients [10]. PCa is the most common male malignancy and the second leading cause of cancer mortality in the men of US. Current treatments for primary prostatic tumor involve radical prostatectomy, external radiotherapy, brachytherapy, and androgen deprivation therapy (ADT). The major cause of PCa mortality is the onset of metastatic castration-resistant PCa (mCRPC). Although none of these therapeutic strategies are curative for PCa, surgery and radiation remain the most effective regimen for patients with organ-confined disease. It is known that PCa is a multifocal disease with heterogeneous cell population. Thus, understanding cellular and molecular mechanisms underlying metastatic dissemination of PCa, such as EMT could generate potential therapeutic strategies to prevent PCa related mortality. In this review, we will discuss several key players in driving EMT in PCa and the different mechanisms that produce distinct signaling cascades to modulate gene transcription or epigenetic regulation, and post-transcriptional regulation by microRNA (miRNA) or long non-coding RNAs (lncRNAs).

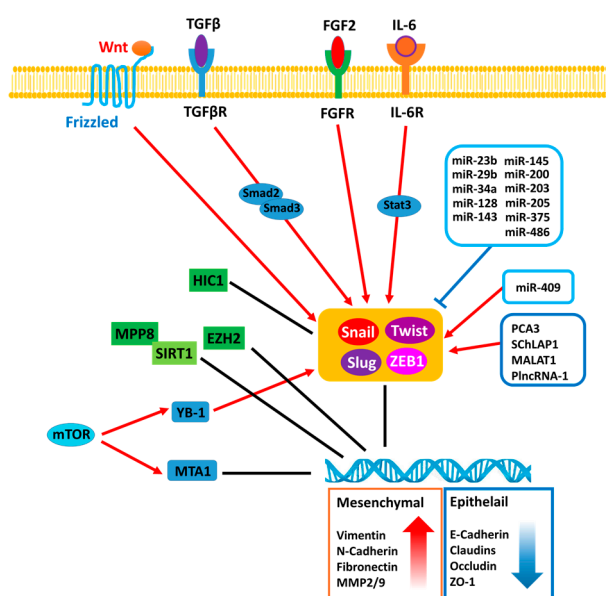


Figure 1. The regulatory mechanisms associated with epithelial-to-mesenchymal transition (EMT) in prostate cancer (PCa). MPP8: M-phase phosphoprotein 8; SIRT1: NAD-dependent deacetylase sirtuin-1; HIC1: Hypermethylated in cancer 1; EZH2: Enhancer of zeste homolog 2; YB-1: Y-box binding protein 1; MTA1: Metastasis Associated 1 protein; mTOR: Mammalian target of rapamycin; TGF β R: Transforming growth factor beta receptor; FGFR: Fibroblast growth factor receptor; IL-6R: Interleukin 6 receptor; Snail: Zinc finger protein SNAI1; Slug: Zinc finger protein SNAI2; ZEB1: Zinc finger E-box-binding homeobox 1; Twist: Twist-related protein 1.

Accumulating studies have demonstrated that activation of EMT transcription factors induces acquisition of stem cell properties in epithelial cells and contributes to the emergence of tumor-initiating cell population in several cancer types such as breast and pancreatic cancer [11–13]. In PCa, one study also indicates that *N*-cadherin can increase prostate tumor spheroid formation by elevating expression of stemness markers such as *c-Myc*, *Klf4*, *Sox2* and *Oct4* via *ErbB* signaling pathway [14]. In addition, the ectopic expression of *Semaphorin 3 C* can concurrently enhance the invasiveness and stemness in normal prostate epithelial cells, and that mesenchymal markers such as *N*-cadherin and *Vimentin* are highly upregulated in CD44-positive populations, compared to CD44-negative ones [15]. Using transgenic mouse model, prostate tumor cells with mesenchymal characteristics displayed enhanced invasiveness and stemness [16]. However, using primary prostate cancer-derived cells, there is no significant correlation between stemness and the expression level of EMT markers such as

vimentin and *N*-cadherin [17], suggesting that more studies are needed to delineate the regulation of EMT leading to PCa stemness.

EMT-related transcription factors such as Snail, Slug and Twist are shown to confer chemo-resistance in ovarian, breast and nasopharyngeal carcinoma [18–20]. In PCa, a study demonstrated that reintroduction of E-cadherin significantly sensitizes chemo-resistant PCa cell lines to paclitaxel [21]. A recent study showed that Skp2-mediated Twist stabilization can facilitate the acquisition of chemo-resistant to paclitaxel or doxorubicin during PCa progression toward CRPC [22]. In addition, ZEB1 has been shown to promote the chemo-resistance in Paclitaxel-resistant PCa [23]. Taken together, the onset of EMT can lead to PCa cells acquiring drug resistance during progression.

2. The Signal Pathways Leading to Epithelial-To-Mesenchymal Transition (EMT) in Prostate Cancer (PCa)

The tumor surrounding microenvironment has been shown to play an important role in eliciting EMT of carcinoma cell through paracrine/endocrine fashion. Many extracellular signals are responsible for cell–cell communication that alter PCa cell behavior through a receptor-dependent manner. Several peptide hormones such as TGF- β , IL-6, FGF and Wnt are detected in prostatic stromal cells, which are associated with cancer progression [24–27] (Table 1). Among these factors existing in the tumor microenvironment, TGF- β is one of the most well characterized EMT inducer in PCa. It is known that TGF- β can promote EMT via induction of vimentin, fibronectin and suppression of E-cadherin level in vitro [28,29]. It appears that the canonical pathway of TGF- β plays a key role in increasing the expression of EMT transcriptional factor such as (Snail1/2 or ZEB1), which is supported by the evidence that E74-like factor (Elf5), a member of the large E-twenty-six (ETS) transcription factor family, can directly bind to Smad3 and block EMT [30]. Noticeably, the expression of this protein is associated with and E-cadherin expression in PCa specimens [30]. In addition to the canonical pathway, TGF- β -induced Twist expression appears to be mediated by non-canonical pathway of Stat3 in PCa cell lines such as PC3 and DU145 [31,32].

IL-6 is also demonstrated to be an EMT inducer leading to PCa invasiveness [33] and its elevated level is found in metastatic specimens of PCa patients [34]. A study investigating TRAMP (transgenic adenocarcinoma of the mouse prostate) mouse-derived PCa cell lines demonstrated elevated IL-6 levels in hormone resistant cells. Knocking down IL-6 can increase E-cadherin expression and decrease vimentin expression via Stat3 pathway, which also reduces tumor invasion in vivo [35]. Consistently, in LNCaP cells, IL-6 can induce cell migration as well as altered mesenchymal morphology. Mechanistically, IL-6 can induce Twist expression via State3 pathway and lead to the increased fibronectin expression and the inhibited E-cadherin expression [36]. In addition, a similar effect of IL-6 on EMT has been reported using BPH cells [37].

Accumulating evidence has demonstrated that fibroblast growth factor (FGF) family is associated with EMT in PCa. An in vitro study using PCa cell lines demonstrated that FGF2 increases mesenchymal markers, *N*-cadherin, vimentin and decreases epithelial marker, E-cadherin, leading to cell invasion [38]. Moreover, by using a transgenic mouse-expressing FGF9 in PrECs crossed with the TRAMP mouse model, the authors found that forced expression of FGF9 can accelerate the PCa progression in TRAMP mice. Mechanistically, FGF9 derived from LNCaP cells is shown to activate c-Jun dependent TGF- β secretion from prostatic stromal cells, which in turns triggers EMT of LNCaP cells in a paracrine manner [39].

PCa is a typical androgen-dependent disease and ADT is a standard treatment for patients with metastatic disease. It is known that androgen receptor (AR) can induce the expression of several proteases such as MMP2/9 and TMPRSS2 underlying cell invasion [40–42]. In contrast, AR ablation has been shown to induce EMT genes [43]. Mechanistically, AR directly represses Snail gene expression by directly binding to its responsive elements of the promoter. Thus, enzalutamide can promote PCa EMT by de-repressing Snail [44]. Similarly, AR-negative PCa cell lines, PC3 and DU145, show higher mesenchymal gene expression and lower epithelial characteristics than androgen-dependent PCa

cell lines, LNCaP [45]. By using patient-derived xenograft model, a study demonstrated that both *N*-cadherin and vimentin become elevated after ADT [46]. Furthermore, ADT can affect EMT gene expression partly due to the emergence of AR variants [47,48]. For example, one of AR variant such as AR-V7 has been shown to induce mesenchymal genes such as ZEB1 and vimentin, and stem cell marker, Nanog, leading to metastasis [49–51].

Clinically, there is a positive correlation between AR and β -catenin in high-grade PCa [52]. It certainly implies Wnt/ β -catenin signaling is associated to PCa [53]. Indeed, DAB2IP, a potent tumor suppressor, is able to block Wnt-induced EMT by facilitating β -catenin degradation resulted in increasing E-cadherin expression through the canonical pathway [54]. It has been reported that up-regulation of Frizzled 8 (FZD8), which is a subtype of Wnt family receptor, induces PCa to metastasize to bone [55]. Moreover, osteoblast derived Wnt-induced secreted protein-1 (WISP1) facilitated PC3 and DU145 invasion through up-regulation of VCAM-1 [56]. In addition, non-canonical Wnt signaling could also contribute EMT, for example, in high-grade PCa specimens exhibiting elevated Wnt5A, *N*-cadherin and vimentin expression but no change in E-cadherin expression [57].

Table 1. Microenvironment soluble factors involved in EMT progression of PCa.

Soluble Factors	Role in EMT	Impacts on PCa Progression	Reference
TGF- β 1	Inducer	Invasion, Migration, Metastasis, Sphere formation	[28–32]
BMP	Inducer	Sphere formation	[58]
IL-6	Inducer	Invasion, Metastasis, Sphere formation, Tumor incidence	[33–35,37]
FGF	Inducer	Invasion, Metastasis	[38,39]
AR	Suppressor	EMT Suppression	[24,43,44,52]
AR variants	Inducer	Metastasis	[47–50]
Wnt/ β -catenin	Inducer	Invasion, Metastasis, Stemness	[42,52,53,55–57]

EMT: epithelial-to-mesenchymal transition; PCa: prostate cancer.

Accumulating evidence has demonstrated that interplay between signal transduction pathways in response to external stimuli is a critical mechanism to drive the development of metastatic CRPC. In particular, signaling pathways involved in the initiation of EMT often lead to suppression of E-cadherin, resulting in enhanced cell proliferation and metastasis. Particularly, the phosphoinositide 3-kinase (PI3K)-Akt signaling pathway integrates external growth factor stimulations with internal cellular processes. TGF- β can initiate EMT by dissociation of E-cadherin/catenin complexes from the actin cytoskeleton via PI3K/Akt signaling [58]. In this study, the authors demonstrated that TGF- β treatment induces PI3K activation, phosphorylation on either α - or β -catenin associated with E-cadherin localized at the actin cytoskeleton. Dissociation of phosphorylated α - or β -catenin molecules from the E-cadherin results in diminished cell–cell adhesion, as well as enhanced cell migration and invasion. Moreover, TGF- β treatment also leads to significant down-regulation of E-cadherin protein level, accompanied by dramatic change of cancer cell shape from epithelial-like to spindle-like morphology. In contrast, PTEN is a PI3K-Akt signaling regulator involved in stabilization of adherent junctions via de-phosphorylation of β -catenin. TGF- β treatment causes PTEN dissociation from β -catenin, and thus reduced β -catenin dephosphorylation further facilitates PI3K-induced β - and α -catenin phosphorylation, leading to reduction of E-cadherin/catenin complex at the adherent junction and transformation into a more mesenchymal-like phenotype. Meanwhile, bone morphogenetic protein-7 (BMP-7) is shown to induce activation of PI3K and ERK signaling and contributes to the morphological conversion of bone metastatic PCa cell line in both 2D monolayer and 3D spheroid culture system [59]. In particular, the authors observed a significant down-regulation of E-cadherin, accompanied by up-regulation of both Twist and Slug in 3D-cultured PC3 spheroids after exposure to BMP7 treatment. In contrast, inhibition of both Akt and ERK signaling cascades abolishes BMP7-mediated EMT in PC3 cells by diminishing cell migration motility. Overall, this study suggests that initiation of EMT by BMP7 can be regulated through PI3K and ERK signaling in PCa.

Moreover, another critical downstream effector of Akt during prostate tumorigenesis is the mammalian target of rapamycin (mTOR) kinase. Hyper-activation of mTOR is observed in nearly 100%

of advanced PCa [60,61]. In a study using ribosomal profiling approach, mTOR signaling mediates the translation of a specific repertoire of PCa genes involved in cell proliferation, metabolism and invasion. Based on the profiling outcome, mTOR translationally regulates genes including YB-1 (Y-box binding protein 1), vimentin, and MTA1 (metastasis associated 1) that are mainly involved in PCa invasion and metastasis [62]. Notably, ectopic expression of YB-1 can enhance translational activation of Snail and Twist, leading to down-regulation of E-cadherin and enhanced cell migration motility. In contrast, loss of YB-1 results in significant reduction of several mesenchymal factors such as Twist, N-cadherin and Snail [63]. Overall, this study demonstrated a critical impact of YB-1 on EMT, MTA1 is a chromatin remodeler playing an important role in PCa invasiveness. A study using MTA1 transgenic knock-in mouse model displayed an inverse correlation between MTA1 and E-cadherin level in the murine prostate tissue, and that MTA1 is shown to suppress E-cadherin expression at post-transcriptional level [64]. Meanwhile, another study also showed that MTA1 impacts on the invasiveness of PCa cells through regulating E-cadherin expression [65]. Functionally, ectopic expression of MTA1 leads to increased invasive capacity of untransformed prostate epithelial cell line [64,66,67]. In addition, studies using clinical specimen and tumor model also demonstrated a significant elevation of MTA1 in highly aggressive PCa, and that loss of MTA1 diminishes PCa invasion, bone metastasis and angiogenesis [68–70]. Overall, these studies demonstrated a significant role of MTA1 and YB-1 in the EMT of PCa leading to metastatic progression of the disease. In addition, different protein components of the mTOR complex such as mTORC1 and mTORC2 exhibit its significant impact on PCa metastasis. Loss of the mTORC1 or mTORC2 complex components, Raptor or Rictor, leads to attenuation of PCa migration and invasion due to elevated E-cadherin and β -catenin expression and reduced mesenchymal markers such as N-cadherin and vimentin [71]. Moreover, it appears that paclitaxel-resistant PCa cells are invasive [21]; this is initiated by EMT via Notch-1 signaling and suppression of E-cadherin expression. Overall, this study implies that Notch-1 signaling facilitates the mesenchymal phenotype associated with the acquisition of chemo-resistance in PCa cells.

3. Transcription Factors Associated with EMT

E-cadherin is an essential cell–cell adhesion molecule involved in maintaining the epithelial integrity of the carcinoma cell. Hence, loss of E-cadherin becomes a critical step for EMT initiation and is mainly regulated by several transcriptional repressors such as Snail [72], Slug [73], Twist [74,75] and ZEB1 [76].

Snail is a zinc-finger protein binding to E-box sequences of the E-cadherin promoter [72]. Aberrant up-regulation of Snail has been observed in many malignancies such as breast cancer, ovarian cancer [77,78], colorectal cancer [79,80] and PCa [44,81]. In particular, elevation of Snail protein expression has been seen in both enzalutamide-resistant PCa cell line as well as highly metastatic PCa patient specimens [81]. Particularly, ectopic expression of Snail results in enhanced elevation of both AR and AR variants, which might be an initial cause of enzalutamide resistance in PCa lines, suggesting the impact of Snail on the recurrence of metastatic CRPC. In addition to its suppressor function, Snail may facilitate cancer metastasis via enhancing the protein expression and enzymatic activity of urokinase-type plasminogen activator (uPA) leading to enhanced motility in PCa cell lines [82]. In addition, Snail impacts on the expression level of several tight junction protein components by repressing the promoter activity of claudins and occludin genes, and inhibiting Zona occludin 1 (ZO-1) expression at post-transcriptional level [83,84]. Overall, the impact of Snail on EMT process and cell adhesion molecules demonstrates its crucial regulatory role in the disease progression of PCa.

Slug is a dominant regulator of EMT in many cancers including PCa. In addition to acting as an E-cadherin transcriptional repressor, Slug also regulates other factors leading to EMT in PCa. An in vitro study showed that Slug up-regulates both CXCL12 and CXCR4 and impacts on CXCL12/CXCR4 signaling downstream target gene, MMP9, leading to highly invasiveness of PCa [85], implying that up-regulation of autocrine CXCL12 is a critical mechanism underlying Slug-mediated migration and invasion of PCa. In primary PCa, Slug/SNAI2 gene expression are often down

regulated due to the promoter methylation; the expression of Slug is restored or elevated in the invasion front of high grade PCa and lymph node metastases [86]. In addition, Slug can suppress several metastasis-suppressor genes such as KISS1. Particularly, KISS1 is able to inhibit EMT by via suppressing *N*-cadherin and vimentin, and increasing E-cadherin expression then diminish tumor cell migration and invasion motility [87]. Clinically, loss of KISS1 is widely observed in primary and metastatic PCa compared with benign tissue. Restoring KISS1 expression in highly metastatic PCa cell lines results in diminishing cell invasion motility [88]. Taken together, Slug is a highly potent promoter for PCa metastasis via EMT induction, cytokine production and metalloprotease secretion.

Twist is a basic helix-loop-helix protein that plays critical roles during development and tumorigenesis. Many studies have demonstrated that Twist can activate EMT, and that it enhances cell migration via binding to the promoter of the E-cadherin gene. In the past, the mechanistic association between Twist and transcriptional repression of E-cadherin has been shown in many malignancies including esophageal squamous cell carcinoma [89], bladder cancer [90], breast cancer [91] and PCa [92]. Clinically, Twist is found highly expressed in malignant prostatic tissue when compared to BPH tissue, and its protein level is significantly correlated with Gleason grades and metastasis [75]. Meanwhile, overexpression of Twist at the marginal area of prostatic tumor has been correlated with capsule invasion and biochemical recurrence (BCR) in PCa patients receiving radical prostatectomy [93]. In addition to acting as E-cadherin repressor, Twist also facilitates EMT by regulating *N*-cadherin expression [94]. This study demonstrated that β 1 integrin-mediated nuclear translocation of Twist is capable of inducing *N*-cadherin transcriptional activation via binding of Twist to the E-box regulatory element within the *N*-cadherin gene, suggesting that Twist acts as pivotal transcription factor in the metastatic progression of PCa.

ZEB1 is a zinc finger homeodomain transcriptional repressor that regulates skeletal patterning during development and suppresses E-cadherin transcriptional activity in multiple malignancies.

Clinically, ZEB1 is elevated in high-grade prostatic tumors, compared to benign or lower grade PCa specimens [95]. A recent study demonstrated that ZEB1 is physically associated with the histone H4K20-specific methyltransferase, SET8. Mechanistically, SET8-induced H4K20 methylation is implied to exert a dual function in ZEB1-regulated gene expression. Functionally, ZEB1 and SET8 cooperatively trigger EMT by suppression of E-cadherin and induction of vimentin in PCa cells, leading to the invasive potential of PCa [96]. Moreover, an in vitro study has demonstrated that elevation of ZEB1 and loss of E-cadherin is concurrently observed in a subpopulation of PC3 cells that acquired trans-endothelial migration characteristics in vitro, compared to the parental cell line. In contrast, loss of ZEB1 partially restores the epithelial phenotype and reduces trans-endothelial extravasation of PC3 cells [76]. Overall, this study suggests that ZEB1 is a critical regulator of EMT and mediates vascular extravasation of PCa cells during the disease progression.

Forkhead box (FOX) proteins constitute a large family of 19 subgroups of transcriptional regulators that contain an evolutionary conserved DNA binding domain (Forkhead or winged-helix). Among them, FOXA1 is known as a pioneering transcription factor for AR [97–99]. However, FOXA1 loss is often detected in metastatic PCa specimen [100] because FOXA1 has an AR-independent function on suppressing EMT via regulating Slug in PCa cells. In contrast, FoxO family is able to block EMT in malignant cells of multiple cancers [101,102]. Clinically, emerging evidence has demonstrated an inverse correlation between FoxOs level and PCa grade as well as tumor dissemination, indicating its suppressor role in PCa metastasis [103]. Mechanistically, the transcriptional activity of FoxO3a is negatively regulated by PI3K/Akt signaling through post-translational phosphorylate modification. During PCa progression, progressive activation of Akt leads to increased phosphorylation of FoxO3a, which impacts on its nuclear localization and hence FoxO3a-dependent transcriptional activity is further inhibited [104]. Functionally, FoxO3a can directly compete with T-cell factor (TCF) for the interaction with β -catenin, leading to inhibition of β -catenin/TCF transcriptional activity and thus reduction in expression of β -catenin-target genes, such as ZEB1 and Snail. Moreover, knockdown of FoxO3a leads to elevation of *N*-cadherin, fibronectin, ZEB1 and vimentin in highly metastatic

PC3 cells [105]. Overall, these data demonstrated a crucial role of FoxO family in PCa metastasis via targeting EMT factors.

4. Epigenetic Regulation of EMT

Epigenetic regulation is considered as a key initial step in mammalian development. Since EMT occurs during embryogenesis, it is conceivable that epigenetics also plays a critical role in pathologic EMT. Accumulating evidence has demonstrated that both hyper- and hypomethylation of DNA are involved in the deregulation of several genes contributing to PCa progression [106–108]. In particular, aberrant DNA hypermethylation in cancer may lead to inactivation of tumor suppressor genes, leading to increased invasiveness of PCa. HIC1 is a tumor suppressor gene located at 17p13.3, a chromosomal region that is frequently hyper-methylated or deleted in human tumors. HIC1 acts as a transcriptional repressor involved in the suppression of SIRT1 and the regulation of TP53-dependent apoptotic DNA-damage responses [109]. A study using PCa specimens showed that high frequency of HIC1 gene hypermethylation is observed in metastatic PCa, compared to primary and benign tissue. Moreover, hypermethylation of HIC1 gene in PCa cells leads to induction of cell migration and metastasis by promoting EMT via enhancing both Slug and CXCR4 expression that are crucial to PCa metastasis [110]. Meanwhile, restoring HIC1 expression in several PCa cell lines markedly inhibits cell proliferation, migration and invasion in vitro, as well as reduces tumor growth, tissue metastasis and bone destruction in vivo [111,112]. Clearly, epigenetic modification of HIC1 promoter can impact EMT induction in PCa.

Moreover, histone modification of critical genes has similar effect on EMT induction during PCa metastasis. The histone methyltransferase, MMSET/WHSC1 (Multiple Myeloma SET domain), is capable of facilitating EMT in PCa cells via induction of Twist1, which in turn suppresses E-cadherin expression [113]. In addition, Zeste homolog 2 (EZH2) is a critical component of Polycomb repressive complex 2 (PRC2) and causes gene silencing by increasing histone methylation. Increased level of EZH2 has been observed in PCa and many other cancer types. Particularly, transcriptional repression of E-cadherin by EZH2 is often observed in highly aggressive PCa [114–116]. In addition, EMT-related transcription factor can be an epigenetic regulator to orchestrate EMT process. SIRT1 is, known as class III Histone deacetylase, also characterized as an EMT-related transcription factor. By silencing of SIRT1 can cause down-regulation of ZEB1. In addition, recruitment of SIRT1 at the promoter region of E-cadherin can be facilitated by the presence of ZEB1 in PCa cells, leading to transcriptional suppression of E-cadherin [117]. A recent study demonstrated that silencing of SIRT1 can suppress PCa cell migration and invasion via down-regulation of Vimentin and N-cadherin, leading to subsequent up-regulation of E-cadherin [118]. Overall, SIRT1 is a unique epigenetic regulator as well as EMT-related transcription factor in PCa.

5. MicroRNA Associated with EMT during PCa Progression

MicroRNAs (miRNAs) are small non-coding RNA molecules regulating gene expression via post-transcriptional silencing of target genes. miRNA regulation is highly associated with multiple biological processes such as differentiation, proliferation, migration, survival and invasion. Several miRNAs are known to target transcription factors contributing to the mesenchymal phenotype in PCa (Table 2). For incidence, members of the miR-200 family (miR-200a, miR-200b, miR-200c, miR-141 and miR-449) are markedly down-regulated during PCa progression and are shown to suppress EMT mainly by inhibiting E-cadherin repressors such as ZEB1 and ZEB2 at the post-transcriptional level [119–121]. Both miR-203 and miR-205 are known to restore epithelial phenotype in PCa cells by targeting Slug/SNAI2 and ZEB2. Clinically, expression level of miR-203 is significantly attenuated in bone metastatic PCa specimens compared with benign tissue, while miR-205 is found to be decreased dramatically in lymph node metastasis when compared to primary prostatic tumor [122,123]. Meanwhile, by miRNA microarray analysis, miR-508-5p, miR-145, miR-143, miR-33a and miR-100 were found to be significantly down-regulated in metastatic PCa compared to

the primary tumor. In particular, miR-143 and miR-145 derived from the same cluster are shown to reverse EMT and reduce PCa cell migration and invasion by targeting fibronectin and ZEB2 [124,125]. Moreover, several mesenchymal factors such as *N*-cadherin, Twist and Snail are regulated by miR-29b, which is also down-regulated significantly in PCa cell lines and PCa patient specimens when compared to normal prostate epithelial cells and adjacent benign tissue, respectively. Ectopic expression of miR-29b in PCa cells is capable of suppressing PCa invasiveness in vitro, and diminishing secondary colonization at the lungs and liver following intravenous injection in vivo, suggesting miR-29b acts as an anti-metastatic miRNA that is down-regulated during PCa progression [126]. Meanwhile, miR-23b is found to be a methylation-silenced tumor suppressor that inhibits EMT via directly targeting Src kinase and Akt. Moreover, this study also demonstrated that ectopic expression of miR-23b in PC3 cells causes decline in mesenchymal markers vimentin and Snail, and increase of epithelial marker, E-cadherin [127]. Similarly, miR-34a is a tumor suppressive miRNA implicated in EMT and cancer stemness in multiple tumors. A study showed that miR-34a is negatively correlated with PCa migration and invasion by targeting lymphoid enhancer-binding factor-1 (LEF1), a key transcription factor involved in regulation of cell proliferation and invasion. This study also demonstrated that ectopic expression of miR-34a causes the down regulation of *N*-cadherin and Snail, and induction of E-cadherin in LNCaP and C4-2B cell lines, overall suggesting that miR-34a-LEF1 regulation plays an important role in the metastatic progression of PCa [128]. In addition, miR-486 is significantly down-regulated in metastatic C4-2 cells as well as disseminated tumors in PCa patients, compared to parental LNCaP cell and localized PCa tissues, respectively. Functionally, miR-486 is demonstrated to target Snail by post-transcriptional suppression and functionally inhibit PCa cell migration and invasion [129]. Findings from this study suggest that miR-486 negatively mediates the migration and invasion potential of PCa via targeting Snail.

In contrast to tumor suppressor miRNAs, aberrant expression of oncogenic miRNAs is observed in highly aggressive PCa associated with EMT. A study using intra-cardiac inoculation of PCa cells in mice demonstrated the oncogenic role of miR-409 in PCa bone metastasis [130]. Inhibition of miR-409 in highly metastatic PCa cells reverses EMT process by increasing E-Cadherin expression, reducing *N*-cadherin level, and causing morphological change to the epithelial phenotype.

In addition, several studies demonstrated a negative feedback loop between miRNA and EMT transcription factors. For example, ZEB2 known as a direct target of miR-145 can also suppress miR-145 at transcription level. This double negative feedback loop between ZEB2 and miR-145 determines the invasiveness and stemness properties of PCa and contributes to the bone metastasis [131]. Moreover, ZEB1 can suppress the transcription of miR-375 that can inhibit EMT-elicited cell migration and invasion via targeting YAP1 [132].

Table 2. MicroRNAs involved in the EMT and metastatic progression of PCa.

MicroRNAs	Role in EMT	Target	Impacts on PCa Progression	Reference
miR-200b	Suppressor	ZEB1, ZEB2	Suppress cell proliferation, EMT, invasion, and inhibit prostate tumor growth and metastasis.	[120,133,134]
miR-141	Suppressor	ZEB1, CD44, EZH2, Rac1	Inhibits cell sphere formation, invasion, and suppresses tumor regeneration and metastasis.	[119]
miR-203	Suppressor	ZEB2, Bmi, Survivin, RunX2	Suppress prostate tumor metastasis, inhibit cell proliferation, EMT, and invasion motility	[122]
miR-205	Suppressor	c-SRC, ZEB1, ZEB2	Attenuate cell proliferation, invasion and tumor growth	[123,135,136]
miR-143	Suppressor	Fibronectin, ZEB2, MMP13	Suppress cell invasion and migration	[125,137]
miR-145	Suppressor	Fibronectin, ZEB2	Repress cell bone metastasis, invasion and migration	[125]
miR-29b	Suppressor	N-cadherin, Twist1, Snail	Suppress cell invasion, migration and attenuate prostate tumor lung metastasis	[126]
miR-23b	Suppressor	Slug, Vimentin, Src	Suppress cell migration, invasion and attenuate prostate tumorigenicity	[127]
miR-34a	Suppressor	LEF1, N-cadherin, Snail	Attenuate cell invasion and migration	[128,138]
miR-486	Suppressor	Snail	Suppresses migration and invasion of cells.	[129]
miR-409	Inducer	STAG2, RBL2, RSU1, NPRL2	Increase invasiveness and aggressiveness, and promotes tumorigenicity, EMT and stemness of prostate tumor	[130]

6. Long Non-Coding RNA Regulation of EMT in PCa

Long non-coding RNAs (lncRNAs), such as the prostate specific prostate cancer antigen 3 (PCA3/DD3), also plays a critical role in PCa EMT. Silencing of PCA3 in LNCaP cells modulates the expression pattern of several cancer-related genes coding EMT markers such as MTA2 and PLAUR. Meanwhile, PCA3 is shown to facilitate PRKD3-mediated invasion and migration via competitive sponging of miR-1261 [139]. In addition, SChLAP1 (Second Chromosome Locus Associated with Prostate-1) is prevalently expressed in a subset of metastatic PCa, compared to localized primary PCa. Mechanistically, SChLAP1 is able to enhance PCa metastasis by altering the cellular localization and gene regulation of tumor-suppressive SWI/SNF (Switch/Sucrose Nonfermenting) chromatin-modifying complex through interaction with SNF5 [140]. In addition, a recent study also demonstrates that SChLAP1 can modulate the MAPK1 signaling pathway, leading to accelerating cell proliferation and enhancing metastatic potential of PCa in vitro and in vivo [141]. Another highly up-regulated lncRNA in PCa is Metastasis-associated Lung Adenocarcinoma Transcript 1 (MALAT1) that is shown to enhance EZH2-mediated repression of Polycomb-dependent target gene, E-Cadherin. Mechanistically, by interacting with the Polycomb protein enhancer of EZH2, MALAT1 is capable of facilitating EZH2 recruitment to target genes, such as E-cadherin and DAB2IP, resulting in enhanced EZH2-mediated migration and invasion in aggressive CRPC cell lines [142–144]. PlncRNA-1 has been shown to induce N-cadherin expression through modulating TGF- β 1 signaling, and hence increase PCa cell migration and invasion motility [145].

7. Conclusions

The initiation of EMT is considered the initial step leading to cancer metastasis that is expected to contribute to the poor prognosis of cancer patient. Thus, targeting EMT is likely to improve the overall survival of a patient. EMT is a highly regulated process that can be engaged by the reciprocal interaction between tumor surrounding microenvironment and cancer cells. Through extensive survey in PCa, several key inducers associated with the specific signaling pathways and their regulations have been reported. With respect to the role of EMT in cancer metastasis, stemness, and chemo-resistance, apparently, these key regulators can be druggable targets to be a new generation of cancer medicine as

a targeted therapeutic strategy. In this case, small molecule inhibitor such as EZH2 inhibitor or certain unique miRNA such as miR-200 [146] and miR-145 [147] can be further tested in vivo to evaluate their efficacy and validate their mechanism of action.

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