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INVITED REVIEW

Prostate Cancer

B lymphoma Moloney murine leukemia virus insertion region 1: an oncogenic mediator in prostate cancer

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B lymphoma Moloney murine leukemia virus insertion region 1 (BMI1), a core member of polycomb repressive complex 1 (PRC1), has been intensely investigated in the field of cancer epigenetics for decades. Widely known as a critical regulator in cellular physiology, BMI1 is essential in self-renewal and differentiation in different lineages of stem cells. BMI1 also plays a significant role in cancer etiology for its involvement in pathological progress such as epithelial–mesenchymal transition (EMT) and cancer stem cell maintenance, propagation, and differentiation. Importantly, overexpression of BMI1 is predictive for drug resistance, tumor recurrence, and eventual therapy failure of various cancer subtypes, which renders the pharmacological targeting at BMI1 as a novel and promising therapeutic approach. The study on prostate cancer, a prevalent hormone-related cancer among men, has promoted enormous research advancements in cancer genetics and epigenetics. This review summarizes the role of BMI1 as an oncogenic and epigenetic regulator in tumor initiation, progression, and relapse of prostate cancer.

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INTRODUCTION

Prostate cancer (PCa) is the second most commonly diagnosed cancer affecting men worldwide.¹ The incidence of PCa in the developed world is strikingly higher than that in developing countries. In the United States, PCa is the most prevalent malignancy and the third fatal carcinoma to cause cancerous mortality among males.² Despite the conspicuous medical advances including surgery and radiotherapy amongst the current treatment options, PCa still poses a great medical challenge to clinicians and researchers. Patients diagnosed with early-stage PCa are treated with radical or partial prostatectomy and combined local radiation therapy.³ Whereas androgen deprivation therapy (ADT) remains the mainstream treatment for advanced and metastatic PCa and demonstrates benefits and better survival for patients, a considerable portion of tumors will inevitably evolve into castration-resistant prostate cancer (CRPC).⁴ Androgen and androgen receptor (AR)-directed therapies such as abiraterone acetate and enzalutamide (MDV3100) are initially effective in treating CRPC.⁵ Typical chemotherapy drug docetaxel, combined with prednisone, also demonstrated positive therapeutic effects on clinical treatments on CRPC and advanced refractory PCa.⁶ However, PCa tumors will eventually develop drug resistance in which AR signaling plays a critical role. The possible mechanisms include AR amplification, mutations, and activation.^{7–9} Prostate cancer stem cells, which are resistant to most standard therapies, are also considered to be a major mechanism of cancer progression and recurrence.¹⁰ Other mechanisms such as epithelial–mesenchymal transition (EMT) and alteration of

glucocorticoid receptor (GR) are also reported to be key players of the advanced PCa.^{11,12} Metastasis and relapse will cause high mortality among those patients.¹³

PCa is defined as a multifactorial disease since the comprehensive understanding of its etiology remains elusive. Age and genetic predisposition rank as the top risk factors. Latest studies have discovered that epigenetic aberrations also play an indispensable role in the tumorigenesis of PCa.¹⁴ The epigenetic changes are the result of postreplication modification in DNA and/or posttranslational modification of DNA-associated proteins. B lymphoma Moloney murine leukemia virus insertion region 1 (BMI1), a widely known epigenetic regulator in a series of physiological stemness-associated functions, is also found to exert its oncogenic effects during the onset of malignant changes. *BMI1* was isolated as an oncogene that cooperates with *c-MYC*, a proto-oncogene, in the oncogenesis of murine lymphomas.¹⁵ Furthermore, BMI1 transcriptionally represses p16^{Ink4A} and p19/p14^{Arf}, two tumor suppressors coded by *Ink4a/Arf* (*CDKN2A*) with regard to their effects to curb cell proliferation in response to aberrant mitogenic signaling.^{16–18}

BMI1 plays a critical role in the regulation of gene expression and cellular processes, including cell cycle progression, DNA damage repair, EMT, as well as self-renewal, differentiation, and malignant transformation of stem cells.¹⁹ Following the intimate correlation between BMI1 overexpression and malignancy, the fact that BMI1 serves as an oncogenic regulator inspires researchers to develop new promising therapeutic approaches. In the context of PCa, tumors with high malignant grades are highly correlated with corresponding

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upregulation of BMI1 detected in tumor specimens.²⁰ As an indicator of poor clinical outcome, BMI1 is more of a multifaceted regulator in the network of genetic and epigenetic modifications. This review accordingly aims to provide a comprehensive overview of the relevance and involvement of BMI1 in PCa initiation, progression, recurrence, and potential therapeutic approaches.

BMI1 AS A CORE COMPONENT OF POLYCOMB REPRESSIVE COMPLEX 1 (PRC1)

Two common assets of epigenetic modifiers known as trithorax group (TrxG) and polycomb group (PcG) are respectively responsible for transcriptional activation and repression.^{21,22} PcG proteins form mainly two large multimeric polycomb repressive complexes which are, namely, PRC1 and PRC2. In the company of other epigenetic regulators, these two complexes silence downstream genes through histone tail posttranslational modifications.^{23,24} BMI1 is one of the core components of PRC1 together with RING1A (RNF1 or RING1), RING1B (RNF2 or RING2), chromobox proteins (CBXs), and other variable subunits. RNF2 functions as an E3 ubiquitin ligase and monoubiquitylates histone 2A at lysine 119 residue (H2AK119ub) and thus subsequently represses the expression of downstream targets.²⁵ BMI1 was a cofactor to stimulate the ubiquitylating activities of RNF2.²⁶ Collaborating with other polycomb group allies, BMI1 functions as an epigenetic repressor which remodels chromatin by the monoubiquitination of histone 2A at lysine 119 and subsequent methylations at histone.²⁷ BMI1 was first identified in 1991 as a frequent target of Moloney virus insertion in virally accelerated B-lymphoid tumors of E mu-myc transgenic mice.¹⁵ BMI1 represses the *INK4a/ARF* tumor suppressor which encodes the p16^{Ink4a} and p19/p14^{Arf}.^{28,29}

Gene and protein structure of BMI1

BMI1 was initially isolated as an oncogene which collaborates with *c-Myc* in the tumorigenesis of murine retrovirus-induced leukemia.^{15,30} BMI1, which is composed of 9 introns and 10 exons, is localized on the short arm of chromosome 10 (10p11.23) and extends over 10 kb (Figure 1a).³¹ Human BMI1 protein is highly conserved and composed of 326 amino acids. BMI1 has a species-conserved N-terminal RING finger, a central helix-turn-helix (HTH) domain, a C-terminal proline-glutamic acid-serine-threonine (PEST) domain, and two nuclear localization signal (NLS) motifs KRRR and KRMK (Figure 1b).³² The N-terminal RING motif of BMI1 does not endow BMI1 with E3 ligase abilities, but its association with RING1B is required for enhancing the E3 ligase activities of RING1B.^{33,34} The HTH domain is significant for BMI1 localization at DNA strand break and crucial for the recruitment of DNA damage repair and inhibition of cellular senescence.^{15,35-37} The PEST domain, which is namely rich in proline (P), glutamic acid (E),

serine (S), and threonine (T), is necessary for BMI1 protein turnover.¹⁹ PEST domain also inhibits BMI1 degradation and thus promotes its oncogenic capacities including EMT.³²

BMI1 EXPRESSION AND REGULATION

BMI1 is ubiquitously expressed in nearly all organs and tissues and highly expressed in the brain, spinal cord, thymus, lungs, kidneys, blood, bone marrow, gonads, placenta, and stem cells of diverse lineages.³⁸ Its expression is observed to be elevated in many cancer subtypes. BMI1 is involved in many physiological and pathological processes, and thus its transcription and expression levels are tightly regulated by diverse factors (Table 1).

BMI1 is regulated by many transcription factors and epigenetic regulators. Transcription factor twist family bHLH transcription factor 1 (TWIST1), zinc finger E-box binding homeobox 1 (ZEB1), E2F transcription factor 1 (E2F1), c-Myc, N-Myc, Sp1 transcription factor (Sp1), spalt-like transcription factor 4 (SALL4), and NANOG positively regulate BMI1 expression. TWIST1 directly regulates BMI1 transcription,³⁹ whereas ZEB1 indirectly upregulates BMI1 by repressing miR-200.⁴⁰ TWIST1 and ZEB1 also are EMT promoting transcription factors. C-Myc and N-Myc were shown to upregulate BMI-1 mRNA by binding to the E-box within the BMI1 promoter in a dose-dependent manner.⁴¹ E2F1 was reported to regulate BMI1 and PRC2 components, but not other PRC1 subunits.⁴² Sp1 and SALL4 bind to the BMI1 promoter and thus upregulate its expression.^{43,44} NANOG, a transcription factor critically involved with self-renewal of embryonic stem cells, was reported to positively regulate BMI1 by binding to its promoter.⁴⁵ BMI1 expression was shown to be suppressed by Kruppel-like factor 4 (KLF4), Mel-18 (polycomb group ring finger 2 [PCGF2]), and histone deacetylase inhibitors (HDACi).⁴⁶⁻⁴⁸

More importantly, posttranscriptional regulations of BMI1 mRNA enable its maturation, transport to the correct loci, and final translation of its encrypted information to protein. Such regulations require the interaction of RNA binding protein with mRNA elements localized within 5'- and 3'- untranslated regions (UTRs) of precursor mRNA (pre-mRNA). The 5'- and 3'-UTRs are also subject to pharmacological inhibition. Moreover, microRNAs (miRNAs) also participate in the repression of mRNA translation by either silencing or directly degrading the target mRNA. Dysregulation of BMI1 associated miRNAs is often related to disordered cancer cell proliferation. During prostate carcinoma progression, miR-128, -200b, -200c, -221, -30d, -15a, and -16 have been reported to suppress the overall pathogenesis of PCa through targeting BMI1.⁴⁹⁻⁵³ These miRNAs are tumor suppressors which may be potential clinical classification biomarkers and therapeutic targets in PCa. BMI1 plays a critical role in epigenetic modification by posttranscriptional regulation of miRNAs.

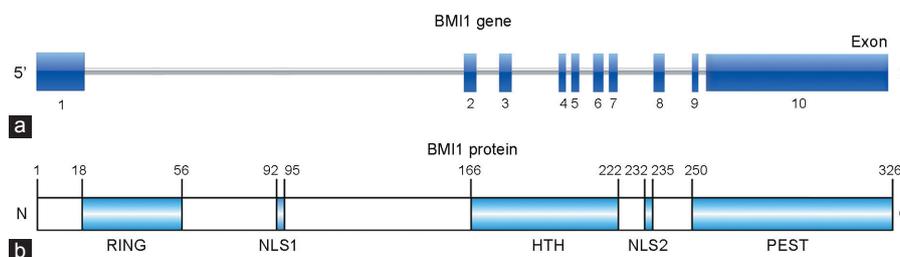


Figure 1: Gene and protein structure of human BMI1. (a) BMI1 gene consists of 10 exons and 9 introns. (b) BMI1 protein starts from N-terminal RING domain required for interaction with RING1A/B, two NLS domains, HTH domain required for BMI1 localization at DNA damage site, to C-terminal PEST which is critical for BMI1 turnover. BMI1: B lymphoma Moloney murine leukemia virus insertion region 1; NLS: nuclear localization signal; HTH: helix-turn-helix; PEST: proline-glutamic acid-serine-threonine.

Table 1: Regulators of B lymphoma Moloney murine leukemia virus insertion region 1 and their functions

Regulator	Feature	Function	Reference
Transcriptional regulation			
TWIST1	Transcription factor, EMT marker	Direct upregulation of BMI1 transcription levels	Yang <i>et al.</i> ³⁹
ZEB1	Transcription factor, EMT marker	Indirect upregulation of BMI1 by repressing miR-200	Liu <i>et al.</i> ⁴⁰
E2F1	Transcription factor	Direct upregulation of BMI1 transcription levels	Nowak <i>et al.</i> ⁴²
c-Myc	Transcription factor	Upregulation of BMI1 mRNA by binding to the E-box within its promoter	Huang <i>et al.</i> ⁴¹
N-Myc	Transcription factor	Upregulation of BMI1 mRNA by binding to the E-box within its promoter	Huang <i>et al.</i> ⁴¹
Sp1	Transcription factor	Upregulation of BMI1 mRNA by binding to its promoter	Wang <i>et al.</i> ⁴³
SALL4	Transcription factor	Upregulation of BMI1 mRNA by binding to its promoter	Yang <i>et al.</i> ⁴⁴
NANOG	Transcription factor	Upregulation of BMI1 mRNA by binding to its promoter	Xie <i>et al.</i> ⁴⁵
KLF4	Transcription factor	Repression of BMI1 expression by binding to its promoter	Yu <i>et al.</i> ⁴⁶
Mel-18	Polycomb group protein	Indirect downregulation of BMI1 by repressing c-Myc expression	Guo <i>et al.</i> ⁴⁷
Posttranscriptional regulation			
miR-128, -128a, -130b	MicroRNA	Downregulation of BMI1 in brain cancer	Palumbo <i>et al.</i> , ¹²⁰ Venkataraman <i>et al.</i> , ¹²¹ Zhu <i>et al.</i> ¹²²
miR-320a	MicroRNA	Downregulation of BMI1 in nasopharyngeal cancer	Qi <i>et al.</i> ¹²³
miR-203, -452, -487b	MicroRNA	Downregulation of BMI1 in nonsmall cell lung cancer	Chen <i>et al.</i> , ¹²⁴ He <i>et al.</i> , ¹²⁵ Xi <i>et al.</i> ¹²⁶
miR-15a	MicroRNA	Downregulation of BMI1 in gastric cancer	Wu <i>et al.</i> ¹²⁷
miR-31, -200, -495, -630	MicroRNA	Downregulation of BMI1 in breast cancer	Cho <i>et al.</i> , ¹²⁸ Kopp <i>et al.</i> , ¹²⁹ Wang <i>et al.</i> , ¹³⁰ Gong <i>et al.</i> ¹³¹
miR-128, -200b, -200c, -221, -30d, -15a and -16	MicroRNA	Downregulation of BMI1 in prostate cancer	Jin <i>et al.</i> , ⁴⁹ Yu <i>et al.</i> , ⁵⁰ Cao <i>et al.</i> , ⁵¹ Xuan <i>et al.</i> , ⁵² Musumeci <i>et al.</i> ⁵³
miR-215, -218	MicroRNA	Downregulation of BMI1 in colorectal cancer	Jones <i>et al.</i> , ¹³² He <i>et al.</i> ¹³³
miR-16	MicroRNA	Downregulation of BMI1 in lymphoma	Teshima <i>et al.</i> ¹³⁴
miR-218, -203	MicroRNA	Downregulation of BMI1 in melanoma	Wei <i>et al.</i> , ¹³⁵ Chang <i>et al.</i> ¹³⁶
Posttranslational regulation			
MAPKAP kinase3	BMI1 phosphorylation	The dissociation of BMI1 with chromatin and repression of downstream targets	Voncken <i>et al.</i> ¹³⁷
AKT	BMI1 phosphorylation	Enhancement of the oncogenic capacities of BMI1 in prostate cancer	Nacerddine <i>et al.</i> ⁵⁴
βTrCP	BMI1 ubiquitination	BMI1 degradation	Sahasrabudhe <i>et al.</i> ¹³⁸
CBX4	BMI1 sumoylation	Recruitment of BMI1 to DNA damage site	Ismail <i>et al.</i> ⁵⁵
OGT	BMI1 O-GlcNAcylation	Stabilization of BMI1	Li <i>et al.</i> ⁵⁶

βTrCP: beta-transducin repeat containing protein; AKT: also known as PKB, protein kinase B; BMI1: B lymphoma Moloney murine leukemia virus insertion region 1; CBX4: chromo box protein 4; E2F1: E2F transcription factor 1; KLF4: Kruppel-like factor 4; OGT: O-linked N-acetylglucosamine (GlcNAc) transferase; MAPKAP kinase3: mitogen-activated protein kinase-activated protein kinase 3; Mel-18: also known as PCGF2, polycomb group ring finger 2; SALL4: spalt-like transcription factor 4; Sp1: Sp1 transcription factor; TWIST1: twist family BHLH transcription factor 1; ZEB1: zinc finger E-box binding homeobox 1; EMT: epithelial-mesenchymal transition; NANOG: Nanog homeobox protein

Posttranslational regulation is also crucial for the physiologic functions of BMI1. AKT kinase phosphorylates BMI1 and enhances its oncogenic potential in an Ink4a/Arf-independent manner in PCa.⁵⁴ CBX4 mediates BMI1 sumoylation at lysine 88 to enhance its recruitment at damaged DNA sites.⁵⁵ Furthermore, BMI1 directly interacted with O-linked N-acetylglucosamine (GlcNAc) transferase (OGT), an enzyme which catalyzes the O-linked beta-N-acetylglucosamine (O-GlcNAcylation), an uncommon type of posttranslational modification.⁵⁶ O-GlcNAcylation inhibits BMI1 from degrading via ubiquitin proteasomal degradation pathway and therefore stabilizes BMI1 protein and enhances its oncogenic potential.

THE CLINICAL FEATURES OF BMI1 IN PROSTATE CANCER

Increasing evidence suggests that BMI1 is highly correlated with unfavorable prognosis, low survival, and poor outcome of cancer. BMI1 overexpression was first discovered to be associated with poor prognosis in hematopoietic malignancies,^{57,58} since then its elevation has been reported in a plethora of cancer types including nonsmall cell lung cancer,⁵⁹ gastric cancer,⁶⁰ nasopharyngeal carcinoma,⁶¹ breast cancer,⁶² as well as PCa.^{63,64} According to the broad microarray analyses, BMI1 serves as a diagnostic and prognostic biomarker predicting

metastasis, chemotherapy resistance, and tumor recurrence in a plethora of cancer types.^{65,66}

The oncogenic capacities of BMI1 have been unveiled in PCa. Elevation of BMI1 mRNA was detected in human PCa cell lines, xenografts derived from PCa cells and human PCa specimens.⁶⁶ PCa with adverse pathologic and clinical features is highly correlated with BMI1 overexpression. Tumors graded with Gleason scores of 8 or higher are associated with significant upregulation of BMI1, and the presence of BMI1 in lower grade PCa specimen is highly predictive for prostate-specific antigen (PSA) recurrence.²⁰ Microarray meta-analyses have discovered that the presence of BMI1 in PCa specimens often indicates tumor metastasis and poor prognosis. The study also identified a BMI1 pathway with concordant profiles in PCa metastasis.⁶⁶ BMI1 was observed to be enriched in a population of PCa cells with higher tumor initiating capacities. Also, BMI1 expression was highly correlated with the therapy failure and poor survival in five types of epithelial tumors including PCa.⁶⁷

Lack of predictive biomarkers for diagnosing and monitoring PCa has been a medical challenge. Although PSA has been widely recognized as the biomarker for the diagnosis, surveillance, and prognosis of PCa, it has several disadvantages such as inadequate

sensitivity and bias of racial and ethnic differences. Secretory BMI1 protein has been discovered to be a conceivably reliable serum biomarker for the diagnosis, progression monitoring, and prognosis of human PCa.⁶⁸

BMI1 IN TUMOR INITIATION WITH REGARD TO NORMAL STEM CELLS

Stem cells are generally divided into two types: embryonic stem cells (ESCs) and adult stem cells (ASCs). ESCs, derived from the blastocyst in early mammalian embryos, are pluripotent stem cells that can differentiate into any cell types. BMI1 is highly expressed in ESCs and associated with morphogenesis during embryonic development. *Bmi1*-null mutant mice exhibited neurological abnormalities, hematopoietic defects, and lymphatic dysplasia.⁶⁹

Most adult tissues harbor stem cells responsible for the self-regeneration and differentiation to maintain homeostasis and repair after tissue injury.⁷⁰ BMI1 has been implicated in the modulation of self-renewal in ASCs of several lineages. A potential association between BMI1 and stem cell properties was suggested after increased BMI1 mRNA levels were observed in adult human hematopoietic stem cells (HSCs). Besides, defective self-renewal capability of HSCs was detected in *Bmi1*^{-/-} mice.⁵⁸ BMI1 was found highly expressed in human HSCs and then decreased when HSCs differentiate into certain lineages.⁵⁷ Similarly, it has been reported that BMI1 plays an essential role in the self-renewal and pluripotency of neural stem cells (NSCs). Downregulation of BMI1 could cause compromised self-renewal and propagating capacities in NSCs both *in vivo* and *in vitro*.⁷¹

The prostate is a glandular and solid organ whose development highly relies on the serum androgen level. Normally, mature prostatic epithelium consists of three basic cell types: basal, luminal (secretory),

and neuroendocrine which are distinguished from their specific marker expression and corresponding features. Basal cells, which are located on the basement membrane in prostatic epithelium, are characterized by the expression of cytokeratin 5 and 14 (CK5 and CK14), CD44, and p63.^{64,72-75} A small-scale population of basal cells has been recognized as the prostate stem cells (PrSCs). PrSCs have the self-renewal abilities, and they can differentiate into luminal cells, induce tissue regeneration, and also play an important role in tumor initiation.⁷⁶ PrSCs are more likely to survive ADT and propagate in castrated mice. BMI1 mRNA levels were observed to be 7-fold higher in the basal/stem cell population than in the luminal cells. Immunohistochemical analyses of adult prostate sections showed that BMI1 overexpression was in line with the coexpression of the basal marker CK5, which demonstrates that BMI1 enriches in the PrSCs and executes their maintenance.⁷⁷ It has been reported that BMI1 marked a population of luminal epithelial cells that exhibited prostate regenerative and cancer initiating potential as well as the castration-resistance.⁷⁸

BMI1 IN PROSTATE CANCER PROGRESSION AND RECURRENCE

The mechanism of progression and recurrence of PCa are complicated. Cancer stem cells (CSCs) transformation and EMT, in which BMI1 plays a pivotal role, facilitate diverse phenotypes of tumor cells and enhance the capacities of metastasis and drug resistance.⁷⁹ Besides, BMI1 is also involved in cellular processes such as cell cycle progression and DNA damage repair.^{80,81} Whereas numerous current chemotherapy drugs are targeting cell cycle and DNA repair, BMI1 overexpression has been proven to be involved in corresponding chemoresistance.^{82,83} Overall, BMI1 is an oncogenic regulator in the complicated mechanism network of PCa progression and recurrence (Figure 2).

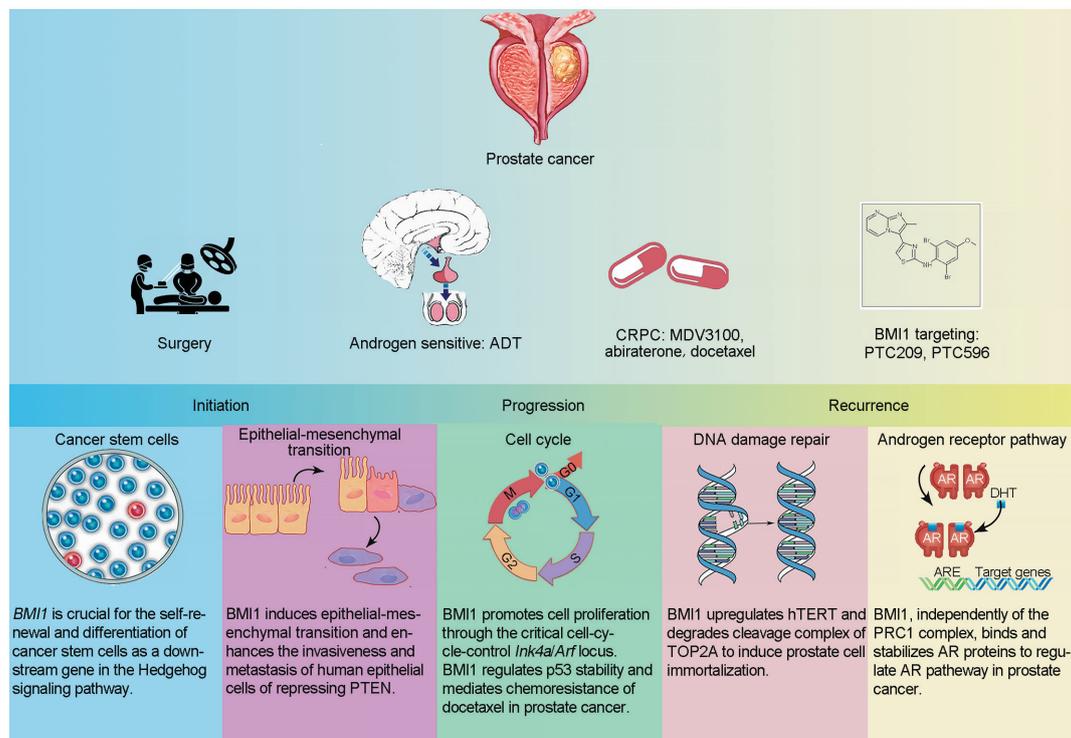


Figure 2: Schematic representation of BMI1 participation in the initiation, progression, and relapse of prostate cancer. ADT: androgen deprivation therapy; AR: androgen receptor; BMI1: B-cell-specific MMLV insertion region 1; CRPC: castration-resistant prostate cancer; PRC1: polycomb repression complex 1; PTEN: phosphatase and tensin homolog; hTERT: human telomerase reverse transcriptase; TOP2A: topoisomerase 2-alpha.

Cancer stem cells

Tumor tissues are composed of heterogeneous groups of cells. CSCs are cancer cells that possess characteristics as normal stem cells, specifically the abilities to differentiate into all cell types in a particular tumor sample. The pluripotency of CSCs remarkably enhances the capacities to give rise to heterogeneous phenotypes;⁸⁴ therefore, the tumor recurrence subsequent to tumor shrinkage is inevitable in the conventional treatments as chemotherapies and radiotherapies. Metastasis and tumor relapse are highly unmanageable due to the substantial resilience of CSCs. Evidences have indicated that BMI1 is crucial for the self-renewal and differentiation of CSCs. BMI1 is also reported to be crucial for the self-renewal, proliferation, and differentiation of leukemia stem cells (LSCs).⁸⁵ Intriguingly, BMI1-expressing LSCs were prone to inducing leukemia when transplanted into irradiated mice. On the contrary, BMI1-null LSCs presented limited proliferative and tumorigenic potential.⁸⁷ Similarly, BMI1 has been proposed to maintain the self-renewal and pluripotency of bronchioalveolar CSCs⁸⁶ and breast cancer stem cells.⁸⁷ Besides, *BMI1* has been demonstrated as a downstream gene in the Hedgehog signaling pathway, which is known to be associated with the self-renewal, differentiation, and canceration of breast and prostate stem cells.⁸⁷⁻⁸⁹ Patients diagnosed with PCa are often treated with ADT. Although this treatment is mostly followed by the androgen independence and recurrence of PCa, the resolution to CRPC may lie in the PCa stem cells, which have the ability to survive ADT and replenish the tumor with cells which have more aggressive phenotypes.

Epithelial-mesenchymal transition

The EMT is the primary mechanism in which cancer cells acquire malignant phenotypes that induce metastasis and recurrence. EMT in cancer cells causes many adverse alterations including loss of epithelial cell marker, activation of specific growth factors, and gain of mesenchymal phenotypes.⁹⁰ Cancer cells which have experienced EMT exhibit similar characteristics as CSCs.^{91,92} BMI1 plays a crucial part in the process of EMT. Upregulation of BMI1 represses the tumor suppressor phosphatase and tensin homolog (PTEN) by which BMI1 induced EMT and enhanced the invasiveness and metastasis of human nasopharyngeal epithelial cells, whereas silencing BMI1 expression reversed EMT.⁹³ TWIST1 is a significant EMT marker which positively regulates BMI1 expression. The knockdown of either TWIST1 or BMI1 blocked both EMT and stem-like properties,⁹⁴ indicating that BMI1 is essential as a downstream target of TWIST1 during EMT.

Cell cycle

The normal cell cycle progression consists of four phases and its regulation demands cell cycle checkpoints to ensure the accurate division. BMI1, being a PcG protein and transcriptional repressor, also plays an important role in cell cycle regulation. BMI1 promotes cell proliferation through the critical cell-cycle control locus *Ink4a/Arf*. It negatively regulates tumor suppressors p16 and p19^{Arf} which induce G1 and G2 phase arrest.^{17,95} CDK4/6 activity and pRb-p53 pathway are promoted by BMI1 repressing p16 and p19^{Arf}. Primary embryonic fibroblasts deficient of *Bmi1* were impaired in cell cycle progression and underwent premature senescence.¹⁷ BMI1 also directly regulates p53 stability, which is independent of *Ink4a/Arf* locus, further enhancing its duties in cell cycle progression and cellular proliferation.^{96,97} Docetaxel causes cell cycle arrest and blocks mitosis by inhibiting mitotic spindle assembly. BMI1 was reported to mediate docetaxel chemoresistance in PCa, and silencing BMI1 enhances the sensitivity of PCa cells to docetaxel.⁹⁸

DNA damage repair

Genome integrity is vulnerable to intra- and extracellular sources such as oxidative particles generated by metabolic activities and environmental radiation. DNA damage repair is a series of processes by which cells identify and rectify the damage to the genome structure. The crucial role of BMI1 has been demonstrated in the cellular response to DNA damage. BMI1 is recruited to DNA breaks and enriched at the chromatin after irradiation. BMI1 tethers RING1B to DNA lesions and further stimulates H2A ubiquitination and subsequent DNA repair.³⁶ While some polycomb proteins, including MEL18, CBX6, CBX7, and CBX8, have been reported to be recruited to the DNA damage sites in a poly(ADP ribose) polymerase (PARP)-dependent manner,⁹⁹ the localization of BMI1 has been proven to be independent upon PARP1.⁸¹ DNA topoisomerase 2-alpha (TOP2A) covalently binds to double strand DNA breaks, and then TOP2A-DNA cleavage complex forms DNA lesions to trigger cell cycle arrest and cell death.⁸² BMI1/RING1A complex degraded TOP2A cleavage complex; therefore, inhibiting the E3 ligase activities of BMI1/RING1A significantly increased antitumor potency of TOP2 drugs.⁸³ BMI1 enhanced telomerase activity by upregulating human telomerase reverse transcriptase (hTERT) to induce PCa immortalization, which is another mechanism to exhibit BMI1's oncogenic capacities.¹⁰⁰

These observations collectively suggest that BMI1 emerges as an inherent regulator for the maintenance of both normal stem cells and cancer stem cells. BMI1 is at the crossroads of physiological processes and malignant alterations. This suggests that BMI1 may play a pivotal role in the progression of PCa.

BMI1 AND AR SIGNALING PATHWAY IN PROSTATE CANCER

PCa therapy highly depends on androgen signaling pathway blockage considering that AR signaling pathway is profoundly involved in initiation, progression, and relapse of PCa. AR, also known as nuclear receptor subfamily 3, group C, member 4 (NR3C4), belongs to nuclear receptors that are activated by steroid hormones as testosterone and 5 α -dihydrotestosterone (DHT).¹⁰¹ AR consists of three major functional domains: the N-terminal domain (NTD), the DNA binding domain (DBD), and the C-terminal ligand binding domain (LBD). Intracellularly, testosterone and DHT exert their biological effects through binding to AR, which results in translocation of AR into the nucleus. In the nucleus, receptor dimers bind to androgen response elements (AREs) in the promoter of target genes (*e.g.*, PSA and transmembrane serine protease 2 [*TMPRSS2*]) and modulate their expression, leading to responses such as PCa maintenance and differentiation.¹⁰²

The AR signaling pathway is intimately linked to normal development and functioning of the prostate, moreover, cancerous progression. Recent evidence suggests that 159 mutations found in AR gene are correlated with PCa. Research also indicates that shorter AR CAG repeats in NTD may lead to greater risk of PCa in males.¹⁰³ Most prostate tumors are dependent on androgen at initial diagnosis, making ADT the favored treatment to block the AR signaling and cause cancer cell death.¹⁰⁴ However, patients may gradually develop hormone-refractory disease (CRPC). Four possible mechanisms of CRPC development are proposed as increased sensitivity of the AR to its agonists, AR mutations that render the receptor responsive to alternate nonandrogen ligands, ligand-independent AR activation, and other AR-independent mechanisms.¹⁰¹ AR splice variants (AR-Vs) are identified in cell lines and tumor tissues derived from patients with CRPC.¹⁰⁵ The presence of AR-V7 is strongly associated with drug resistance in a cohort study. By detecting AR-V7 in circulating tumor

cells with reverse transcription-quantitative real-time polymerase chain reaction (RT-qPCR) assay and examining PSA level in patients, AR-V7-positive patients were found to express higher AR mRNA and PSA and were more likely to have lower Eastern Cooperative Oncology Group (ECOG) performance score and unfavorable clinical outcome from enzalutamide and abiraterone therapy.¹⁰⁶ While AR variants, including but not limited to AR-V7 and AR-V^{6567s}, play a crucial role in CRPC indisputably, the molecular mechanisms downstream of AR that mediate CRPC have not been comprehensively revealed.

A few studies have focused on the relation between PcG proteins and AR signaling pathway. EZH2 expression was proven to be repressed by androgens through retinoblastoma (RB) and p130-dependent pathways.¹⁰⁷ Higher protein levels of BMI1 were detected in androgen-independent PC3 and DU145 cells compared to androgen-dependent LNCaP cells, which implies that BMI1 might exert its oncogenic effects by participating in AR signaling pathway.¹⁰⁰ Despite the fact that BMI1 and AR are both abundantly expressed in PCa cells, the association between BMI1 and AR has remained unclear until very recently a study reported that BMI1, independently of the PRC1 complex, binds and stabilizes AR proteins to regulate the AR pathway in PCa.¹⁰⁸ According to this study, AR protein levels together with the protein and transcript levels of AR-activated targets, such as PSA and TMPRSS2, were downregulated after depletion of BMI1 in AR-positive PCa cells; meanwhile, AR-repressed targets were increased, suggesting that BMI1 acts as a transcriptional activator through its binding with AR. IP-IB analysis revealed that AR-NTD, but not other two domains, is essential for AR-BMI1 interaction. Besides, BMI1 compromised the interaction between AR-NTD and MDM2 dose dependently, confirming that BMI1 competitively inhibits the interaction between MDM2 and the AR-NTD. In addition, BMI1 interacts with AR-V7, AR-V^{6567s}, and other AR variants since all AR variants contain the AR-NTD domain. Moreover, targeting BMI1 could significantly inhibit tumor growth of xenografts which were resistant to castration and enzalutamide treatment.¹⁰⁸ In conclusion, BMI1 is a potential and novel therapeutic target for CRPC.

PHARMACOLOGICAL TARGETING OF BMI1

As discussed above, BMI1 exerts oncogenic functions during cancer progression, and it is presumably concluded that BMI1 targeting should have quintessential antitumor effects. 2-pyridine-3-yl-methylene-indan-1,3-dione (PRT4165), a PRC1 inhibitor, was discovered as a molecule inhibitor of BMI1/RING1B-mediated polyubiquitylation as it inhibited E3 ligase activities of PRC1.¹⁰⁹ PRT4165 has synergistic effects with anthracyclines to increase the persistence of TOP2A-DNA cleavage complex.⁸³ However, PRT4165 only targets the PRC1-dependent enzymatic activities of BMI1 and does not affect the expression levels of BMI1.¹¹⁰

Knocking down BMI1 on tumor cells with small hairpin RNA (shRNA) inhibited cell growth and efficiently reversed the status of chemoresistance.⁹⁸ PTC-209, a novel small molecule inhibitor of BMI1, has been reported to reduce BMI1 expression through selectively targeting the BMI1 transcript via its 3'-UTR. The anticancer effects of PTC-209 were mediated by cell cycle exit and subsequent apoptosis in colorectal cancer stem cells.¹¹¹ Most cancer chemotherapies are aimed at the cell cycle. The cell cycle arrest has further been investigated in biliary tract cancer cells. Under PTC-209 treatment, most cells were arrested in the G0/G1 phase of the cell cycle and cells in the S-phase were significantly reduced.¹¹² Breast cancer 1, early onset (BRCA1), meiotic recombination 11 homolog A (MRE11A), and RAD51 recombinase (RAD51), which are responsible for the DNA repair machinery, were observed to be inhibited

by PTC-209 treatment.^{112,113} Both the transcript levels and protein levels of BMI1 in lung cancer cell lines were downregulated upon PTC-209 treatment.¹¹⁴ Targeting of BMI1 with PTC-209 also showed potent therapeutic effects in multiple myeloma.¹¹⁵

Treatment of human PCa cells, stem cells, and patient-derived stem cells with PTC-209 induced BMI1 downregulation and subsequent inhibitory effects on cell proliferative abilities. BMI1 expression is tightly controlled by posttranscriptional processes mapping to the 5'- and 3'-UTRs.¹¹⁶ PTC-209 exerts its antiproliferative effects by directly modulating BMI1's posttranscriptional mechanisms. PTC-209 could directly bind to the pockets formed in the BMI1 RNA fold structure, and BMI1 UTR luciferase reporter assay was utilized to indicate that PTC-209 selectively inhibits the BMI1 UTR-regulatory reporters. PTC-209 treatment critically reduces BMI1 protein levels, but not a panel of 245 kinases and 21 phosphatases that may exhibit epigenetic capacities. PTC-209 impaired both the cell survival and clonogenic capacities in therapy-resistant PCa and pharmacologically inhibited the tumor growth in mouse xenografts of PCa tumor cells.¹¹⁷

PTC596, a well-tolerated drug currently in phase I clinical trial, has shown higher specificity of BMI1 inhibition and less side effects in animal cancer models.¹¹⁸ PTC596 induced degradation of BMI1 and eventually caused CSC depletion. PTC596 has been proven to downregulate MCL-1 and induces p53-independent apoptosis in acute myeloid leukemia progenitor cells.¹¹⁹

We have implied that BMI1 level is predictive for the malignancy and poor prognosis of PCa.⁶⁶⁻⁶⁸ Thus, pharmacological inhibition of BMI1 impedes cancer progression and therefore is a promising strategy for targeting PCa, especially after hormone therapy resistance.

CONCLUSIONS

Mounting evidence has indicated that BMI1, the key component of PRC1, plays a pivotal oncogenic role in the tumor initiation, progression, and relapse of prostate cancer, as well as many other cancer categories. BMI1 dysregulation has been associated with evolution and progression of a cohort of carcinoma subtypes mostly by endowing tumor cells with proliferative capacities. This review elucidates the oncogenic functions of BMI1 identified in prostate cancer. BMI1 is a critical oncogenic modulator involved in the initiation, progression, and relapse of prostate cancer. BMI1 is also a biomarker for the clinical diagnosis, management, and prognosis as well as a therapeutic target in therapy-resistant prostate cancer. BMI1 inhibitors such as PTC209 and PTC596 have demonstrated potent therapeutic effects on several types of solid tumors including prostate cancer. In spite of noteworthy progress made on pharmacological targeting of BMI1, the treatment of advanced-stage prostate cancer necessitates further development on drugs with higher specificity and less adverse effects.

BMI1 is a transcriptional silencer as a collaborator of RING1B to enhance its E3 ligase activities. The interaction between BMI1 and AR signaling pathway has been demonstrated to occur in absence of other PRC1 members, implying that BMI1 may have multiple functions in PRC1-independent manner. The capacities of BMI1 which are independent of PRC1 have yet to be revealed.

AUTHOR CONTRIBUTIONS

Qipeng Liu reviewed literature and drafted the manuscript. Qiaqia Li created the figures. SZ, YY, and QC edited the manuscript. QC supervised the whole work. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declared no competing interest.



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