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REVIEW ARTICLE

Epigenetic regulation of prostate cancer

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Abstract Prostate cancer is (PCa) the second leading cause of cancer death in males in the United State, with 174,650 new cases and 31,620 deaths estimated in 2019. It has been documented that epigenetic deregulation such as histone modification and DNA methylation contributes to PCa initiation and progression. EZH2 (enhancer of zeste homolog 2), the catalytic subunit of the Polycomb Repressive Complex (PRC2) responsible for H3K27me3 and gene repression, has been identified as a promising target in PCa. In addition, overexpression of other epigenetic regulators such as DNA methyltransferases (DNMT) is also observed in PCa. These epigenetic regulators undergo extensive post-translational modifications, in particular, phosphorylation. AKT, CDKs, PLK1, PKA, ATR and DNA-PK are the established kinases responsible for phosphorylation of various epigenetic regulators.

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Introduction

While a majority of studies have demonstrated that accumulation of genetic mutations will result in cancer initiation and progression, epigenetic changes without altering DNA sequences, which have been under intensive research in recent years, also contribute to activation of oncogenes and inactivation of tumor suppressors, and subsequently

leading to the development of cancer.¹ Epigenetics is usually defined as a heritable change in gene expression without alteration in DNA sequence, including three primary epigenetic mechanisms - DNA methylation, covalent modification of histones and non-coding RNAs. It has been noted that heritable epigenetic marks can be dynamically regulated in response to any change in physiological conditions. Therefore, failure of the appropriate maintenance of these marks will highly possibly result in disease states such as cancer.² Epigenetic modifiers refer to adding or removing DNA methylation or histone modifications, which are defined as writers or erasers respectively. Besides writers and erasers, some epigenetic effectors can also be recruited, and affect the final epigenetic programs which we call them “readers”. Increasing data has shown that the

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activity of these epigenetic modifiers are also under regulation of some posttranslational modifications, such as phosphorylation, which might determine the final biological outcome.³ In this review, we will take a comprehensive look of current findings of the regulation of the epigenetic modifiers by phosphorylation during carcinogenesis. We will also further discuss phosphorylation of one critical "writer"- enhancer of zeste homolog 2 (EZH2), which is a histone methyltransferase mainly mediating trimethylation of histone H3 at Lys 27 (H3K27me3), in prostate cancer, and the idea of therapeutic strategies based on EZH2 phosphorylation in prostate cancer treatment.

Epigenetic modification in cancer

DNA methylation aberrations have been firstly and mostly linked to cancer initiation and progression among the epigenetic alterations, which is featured with genome-wide hypomethylation and hypermethylation of clusters of CpGs, known as CpG islands.⁴ The role of global DNA hypomethylation in tumorigenesis has been well established. It occurs in various cancers and becomes an important reason resulting in increased genomic instability and inappropriate activation of oncogenes.⁵ Hypermethylation of CpG islands in the promoters of tumor suppressor genes to silence their expressions has been well noted to contribute to tumorigenesis. Many tumor suppressor genes, which are usually involved in various tumor-associated cellular processes including DNA repair, cell cycle, apoptosis, etc., have been observed to undergo CpG island hypermethylation. These genes, such as cell cycle related gene RB, the DNA repair protein BRCA1, and the tumor suppressor p53, are observed in different types of cancer like esophageal cancer, colorectal and gastric cancers, in which they are commonly mutated.^{6–9}

DNA methylation is maintained by a family of enzymes which are called DNA methyltransferases (DNMTs). There are four members of the DNMT family, including DNMT1, DNMT3A, DNMT3B and DNMT3L. Among them, DNMT1 plays the main role in maintaining the methylation status of DNA; at the same time, DNMT3A and DNMT3B are known to encode the *de novo* methyltransferases which will methylate the unmethylated DNA, while DNMT3L, unlike the other DNMTs, has no enzymatic activity.¹⁰ A number of studies have demonstrated a relationship between alterations of DNMTs and tumorigenesis. Overexpression of DNMTs has been well reported in a variety of human cancers via correlating with aberrant DNA methylation. Consequently, overexpression of DNMTs tends to result in increased metastasis and poor prognosis. Highly expressed DNMT1 has been found in numerous patient specimens such as esophageal squamous cell carcinoma and pancreatic cancer. Similarly, increased DNMT3A or DNMT3B is involved in liver cancer, BRCA1-mutated breast tumor, intestinal neoplasia and prostate tumors.^{11–17} In addition to overexpression of DNMTs, somatic mutations in DNMTs are also reported as an important contributor to malignant transformation. These mutations have been observed in colon cancers or acute myeloid leukemia, thus leading to disruption of normal DNA methylation and subsequently tumor promotion.^{18–20} While deletion of DNMTs in mouse

models has shown a lethal phenotype, several recent studies based on the conditional knockout approach demonstrated that loss of DNMTs also participates in development of peripheral T cell lymphoma (PTCL) or AML.^{21–23}

The N-terminal tails of histones, in which lysine and arginine residues are distributed, are subject to a variety of covalent posttranslational modifications (PTMs), such as acetylation, phosphorylation and methylation.²⁴ PTMs of proteins are highly dynamic in response to the altered contexts to ensure the histone modification in balance which is critical for maintaining genome integrity.²⁵ Many different combinations of PTMs on multiple residues, which defined as "histone code" and regulated by enzymes as "writers" and "erasers", can precisely govern specific cellular responses, such as cell cycle or signal transductions.^{26,27}

Misregulation of histone PTMs, including acetylation, methylation and phosphorylation, have been extensively linked to a variety of cancer types.²⁸ In tumors, such a misregulation results in the abnormal activation of oncogenes or the repression of tumor suppressors. And the PTMs-induced inappropriate activation or inactivation depends on which residues are modified and which type of modifications occur.²⁹ Generally speaking, lysine acetylation can open up chromatin structure and subsequently tend to activate the transcription of its target genes.²⁴ Therefore, histone acetyltransferase (HATs) should promote transcription whereas histone deacetylases (HDACs) should be anti-transcriptional. Alterations and mutations occur on HATs (e.g. MOZ or CBP/EP300) or HDACs have been reported to correlate with a poor clinical outcome in cancer patients.^{30–32} Besides the histone acetylation "writers" HATs or "erasers" HDACs, the histone acetylation "readers" the BET (bromodomain and extraterminal domain) proteins, such as BRD4, show increased expression in a variety of cancers. Consequently, the BET inhibitors, like JQ1, have demonstrated great therapeutic efficacy for the treatment of cancers, such as leukemia.^{33,34} Unlike histone acetylation, histone methylation will not exclusively act as a transcriptional activator or repressor; the activation or inactivation depends upon the open or close of the chromatin structure arising from which residue is modified and the degree of its methylation.³⁵ Similar with histone acetylation, tumor cells are also found common alterations of histone methylation and its histone methyltransferases (HMTs). For example, H3K27 trimethylation contributes to the aberrant silencing of multiple tumor suppressor genes and is associated with poor diagnosis of patients, and correspondingly, its main HMT, EZH2, is overexpressed in these cancers such as prostate cancer and breast cancer.³⁶ Similarly, the dysregulation of other HMTs and the methylation patterns (e.g. G9a and H3K9me3), have been found in cancers as well.³⁷ Histone demethylases have been recently identified to have a linkage to cancer. For example, LSD1, the demethylase for H3K4 and H3K9 residues, has been found overexpressed in many types of cancer.³⁸ Histone phosphorylation is a dynamic process catalyzed by several distinct kinases that are depending on different amino acid residues in histone.³⁹ Histone phosphorylation occurs with the change of many cellular processes, such as cell cycle, DNA damage

repair, and apoptosis, therefore its misregulation often leads to tumorigenesis. Accordingly, the kinases regulating the histone phosphorylation are always found overexpressed in cancers. For example, high PRK1 level, which mediates H3T11 phosphorylation, correlates with high stages of prostate cancer.⁴⁰

Noncoding RNAs (ncRNAs) has also been regarded as a crucial epigenetic modification gaining intensive attention. With technical development, many types of ncRNAs have been identified, including microRNAs (miRNAs), PIWI-interacting RNAs (piRNAs), small nucleolar RNAs (snoRNAs), the complicated long ncRNAs (lncRNAs), and the newly validated circular RNAs (circRNAs), etc.⁴¹ Increasing evidence has shown that ncRNAs are of essential importance for the development of human diseases notably cancers via their crucial regulatory potency in gene expression at the transcriptional, post-transcriptional, or translational processes. Among them, the most deeply studied miRNAs and lncRNAs, aberrantly expressed in a variety of cancers, play oncogenic or tumor suppressive functions in these processes.^{42,43}

As a post-transcriptional regulator of gene expression, miRNAs have been extensively believed abnormally expressed in tumors. The dysregulation of miRNAs would affect several cancer hallmarks for tumor initiation and progression which include sustaining cell cycle and proliferative signaling, avoiding from cell death, and intriguing invasion and metastasis.⁴⁴ Cell-cycle regulation is highly rely on cyclins, cyclin-dependent kinases (CDKs) and their inhibitors. In a variety of human tumors, the CDK inhibitor p27^{Kip1}, p21^{CIP1} and p16^{INK4a} have been found to be directly regulated by miRNAs such as miR-221/222 miR-663, miR-302 and miR-24 which are highly upregulated in tumors.^{45–47} In addition, miRNAs can also regulate expression of CDKs and cyclin not just inhibitors. Cyclin D1 and CDK4 are repressed by miRNA-545 leading to cell-cycle arrest in lung cancer.⁴⁸ MiRNAs have been known to take part in resisting apoptosis. Dysregulation of several miRNAs involved in p53 functions, such as miR-17–92, has been identified and confers tumor cells resistant to chemotherapy-induced cell death.⁴⁹ It has also been shown that miRNAs are fully involved in cancer metastasis. For example, a number of TGF- β -associated miRNAs, such as miR-155, engage in the process of TGF- β -induced epithelial–mesenchymal transition (EMT) which have been widely accepted as a key step in the cancer metastasis.^{50,51}

Similar with miRNAs, increasing research has revealed lncRNAs playing an emerging role in tumor suppression or oncogenesis in recent years. It has been well demonstrated that aberrant lncRNAs affect cancer cell proliferation and metastasis by remodeling chromatin structure and regulating oncogenes or tumor suppressors transcriptionally and post-transcriptionally.⁵² A good example of lncRNAs mediating epigenetic modifications is the HOX antisense intergenic RNA (HOTAIR) which has been applied as a predictive marker for metastasis in a variety of cancer, such as breast cancer.⁵³ HOTAIR working with the chromatin-modifying complexes PRC2 which catalyzes H3K27 trimethylation, affects the transcription of target genes via a PCR2-lncRNA binding.⁵⁴ Besides influencing PRC2 enzymatic function, HOTAIR can also enhance PLK1-mediated proteasomal

degradation of SUZ12 which is a core component of PRC2 complex during hepatitis B virus-induced liver carcinogenesis.⁵⁵

Phosphorylation of epigenetic modifiers in cancer

Epigenetic modifiers, including “writers”, “erasers” and “readers”, play a crucial role in maintaining the dynamic balance of epigenetic modification patterns, usually depending on their activities. It has been well established that these modifiers are also under regulation of post-translational modifications (PTMs), and their activity are affected by these PTMs consequently.³ Among the PTMs, the importance of phosphorylation contributing to epigenetic events in response to environmental changes has been widespread accepted. These phosphorylation is catalyzed and mediated by many kinases including protein kinase B (PKB/Akt), cyclin-dependent kinases (CDKs), polo-like kinase 1(PLK1), protein kinase A (PKA), AMP-activated protein kinase (AMPK), casein kinase 2 (Ck2) and ataxia telangiectasia and Rad3 related kinase (ATR), etc. Phosphorylation-mediated regulation of modifiers may directly activate or suppress their enzymatic activity, or indirectly regulate the interaction between modifiers with other proteins or RNAs, or make chromatin structure tight or loose.⁵⁶ And several epigenetic modifiers have been found either aberrantly hyperphosphorylated or hypophosphorylated in cancer cells, including DNA methyltransferases, histone methyltransferases, histone demethylases, histone acetyltransferases and deacetylases.

DNMTs especially DNMT1 have been known to be phosphorylated to regulate the protein stability and enzymatic activity. AKT and PKC kinases were reported to phosphorylate DNMT1 at Ser127/143 and Ser127, respectively, which will disrupt the interactions of DNMT1 with PCNA and UHRF1 in human cells to promote tumorigenesis.^{57,58} In addition, it has been reported that GSK3 β can interact with DNMT1 and then phosphorylate DNMT1 at Ser410 and Ser414, and finally promote β TrCP-induced proteasomal degradation of DNMT1.⁵⁹ There are not many reports showing the phosphorylation of DNMT3s, however, the kinase CK2 tends to phosphorylate DNMT3A, and decrease the global genomic methylation levels.⁶⁰

The histone methyltransferase EZH2 has been shown to be phosphorylated by several kinases, such as AKT, AMPK, CDKs, or Janus kinase 3 (JAK3), in various types of cancer. AKT-mediated phosphorylation of EZH2 at Ser21 results in loss of methylation of H3K27 and increase of expressions of genes used to be silent by H3K27me3. The AKT-mediated phosphorylation of EZH2 promotes expression of several critical oncogenes, and is involved in the development of prostate cancer, uterine cancer and glioblastoma tumorigenesis.^{61–63} AMPK can also phosphorylate EZH2 at Thr311 to disrupt the polycomb repressive complex 2 (PRC2), in which EZH2 is the core component, and thus suppresses methyltransferase activity in both ovarian and breast cancers.⁶⁴ In addition, CDK1/2 have also been reported to phosphorylate EZH2 at Thr350 and Thr487, which will not only inhibit the enzymatic activity, but also block EZH2

binding to its target region, and will highly increase the risk of tumorigenesis.^{65,66} Phosphorylation of EZH2 by JAK3 induces a noncanonical function of EZH2 to promote transcriptional activation in natural killer/T-cell lymphoma.⁶⁷ Collectively, regulation of EZH2 by phosphorylation is highly correlated with tumorigenesis with regard to its activity. We will further discuss about the role of EZH2 and its phosphorylation in prostate cancer and castration-resistant prostate cancer (CRPC) in the subsequent session.

Although contribution to cancer by the phosphorylation of H3K27 histone methyltransferase has been extensively studied, phosphorylation of histone methyltransferases that add methyl groups to other residues of histone tail, such as H3K4, was also reported. For instance, ATR phosphorylates MLL (H3K4 methyltransferase) on Ser516 in response to environmental stress in the S phase, resulting in its degradation and finally contributing to human MLL leukaemia.⁶⁸ Several studies also demonstrated the unusual phosphorylation events occurring on arginine methyltransferases. For example, in myeloproliferative neoplasms, Janus kinase 2 (JAK2) oncogenic mutant V617F can phosphorylate protein arginine methyltransferase 5 (PRMT5), consequently decreasing its activity and increasing expression of genes that are inhibited by PRMT5.⁶⁹

Histone demethylases also appear to be regulated by phosphorylation, although how phosphorylation affects the activities still remains to be elucidated. Protein kinase A (PKA)-induced phosphorylation of H3K9me2 demethylase PHD finger protein 2 (PHF2) at Ser1056 results in its increased binding to a DNA-binding protein ARID5B, reduction of methylation on ARID5B, and decrease of gene transcription.⁷⁰ CDK1 can catalyze phosphorylation of another demethylase, PHD finger protein 8 (PHF8) at Ser33 and Ser84, leading to disruption of PHF8 with chromatin in acute promyelocytic leukemia.⁷¹

Phosphorylation can also regulate the activities of HATs and HDACs, and therefore affect the gene transcription via histone acetylation patterns. The histone acetyltransferase CBP is phosphorylated by CDK2 in a cell cycle-dependent manner. One study demonstrated that the DNA-dependent protein kinase (DNA-PK) phosphorylates hGCN5, which possesses HAT activity, and the phosphorylation suppresses its HAT activity.⁷² In addition, in response to DNA damage, the HAT activity of activating transcription factor 2 (ATF2) is phosphorylated.⁷³ The PI3K/AKT pathway also stimulates p300 phosphorylation at Ser1834 and its transcriptional activator potential.⁷⁴

Like HMTs, HDACs have been commonly believed that their activities are tightly regulated by phosphorylation. The HDAC family members, including HDAC1, HDAC2, HDAC3, HDAC4, HDAC5, HDAC6 and HDAC8, are phosphorylated by many kinases, such as CK2, PKA, extracellular signal regulated kinase (ERK1/2), etc. within different residues, to influence the structure, stability, acetyltransferase activity, binding with partners, or cellular localization, and ultimately leading to either pro- or anti-tumorigenesis.^{75,76} I will take two recent findings as examples. Activated PI3K/AKT pathway in breast cancer cells can lead to the phosphorylation of the p70 S6 kinase (S6K1) and transcriptionally regulate estrogen receptor α (ER α) expression.⁷⁷ Moreover, c-Jun N-terminal kinase (JNK)-

mediated phosphorylation of HDAC3 in triple-negative breast cancer (TNBC) might affect the sensitivity of HDAC inhibitors in treatment.⁷⁸

EZH2 methyltransferase and its phosphorylation in prostate cancer

In 2019, prostate cancer (PCa) has become the most common cancer with 174,650 newly diagnosed cases and the second leading cause of death associated with cancer or cancer-related factors in males in the United States with estimated 31,620 deaths.⁷⁹ Androgen deprivation therapy (ADT) is the routinely used approach to treat PCa patients. Although patients initially respond to ADT well, castration-resistant prostate cancer (CRPC) eventually occurs in most of these patients after several years and then develops into even worse metastasis.⁸⁰ It has been well established that androgen receptor (AR) signaling is enhanced and plays an important role in CRPC.⁸¹ Subsequently, the AR inhibitors, such as enzalutamide and abiraterone, have been approved by the Food and Drug Administration (FDA) for the treatment of late stage PCa.⁸² However, enzalutamide resistance eventually develops for almost all cases, making the disease almost incurable. Therefore, how to overcome enzalutamide resistance of CRPC has been under intensive research, and new targets and mechanism-based strategies are urgently needed to treat these patients.^{83–85}

As we described above, EZH2, the catalytic subunit of PRC2 complex, plays a critical role in repressing gene expression by mediating H3K27me3. Many studies have demonstrated that there is a tight linkage between EZH2 and oncogenesis, and that EZH2-mediated trimethylation triggers silencing of tumor suppressor genes in cancer.⁸⁶ Besides acting as a transcriptional suppressor, emerging evidence has shown the uncanonical role of EZH2 towards transcription activation of some genes, whose expression seems to be PRC2-independent. EZH2 has been identified as either a direct transcription activator or coactivator binding with other transcription factors to promote expression of several oncogenes. For example, the transcription levels of genes in NOTCH pathway, NF- κ B pathway or Wnt pathway are directly or indirectly regulated by EZH2 in breast cancer and colon cancer, respectively, which are independent of EZH2 methyltransferase activity^{87–90} (see Fig. 1).

In PCa especially in CRPC, EZH2 is overexpressed and promotes cancer cell proliferation and invasion, making EZH2 an attractive anti-cancer drug target. Similar with other types of cancer, aberrant PTMs, such as phosphorylation, of EZH2 are also found in PCa (Fig. 2). For example, AKT-mediated phosphorylation of EZH2 at Ser21 induces a functional switch from a PRC2-dependent transcription repressor to a PRC2-independent transcription coactivator working with AR to promote the development of CRPC.^{61,63} In addition to AKT kinase, CDK1/2 can also phosphorylate EZH2 at Thr350 during S and G2/M phases. Phosphorylation of Thr350 promotes PCa cell proliferation and migration. Consequently, blocking Thr350 phosphorylation is important for abrogation of the oncogenic activity of EZH2.^{65,66}

Polo-like kinase 1 (PLK1), a regulator of various stages of mitosis, has been shown to be overexpressed in various

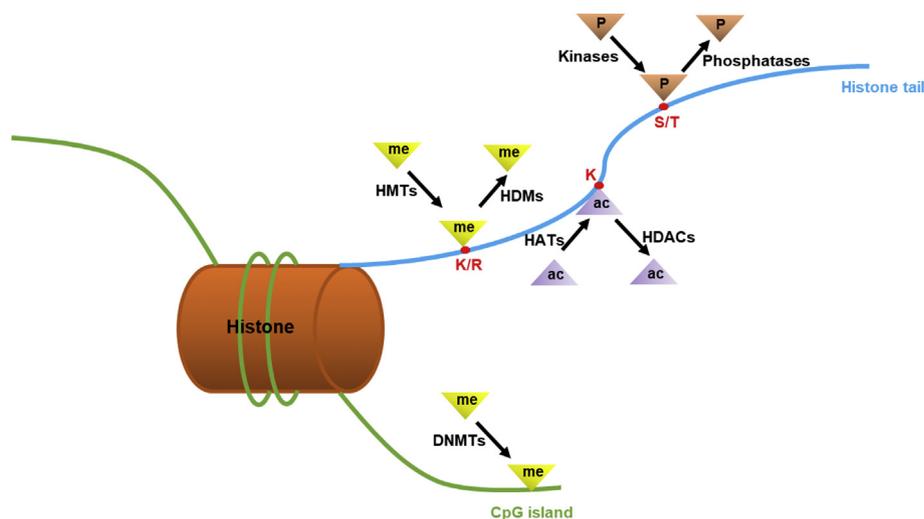


Figure 1 The dynamic epigenetic modifications on DNA and histone tail. Enzymes coordinately regulate the epigenetic modifications by adding or removing epigenetic hallmarks. Deregulation of the enzymes can lead to oncogenesis.

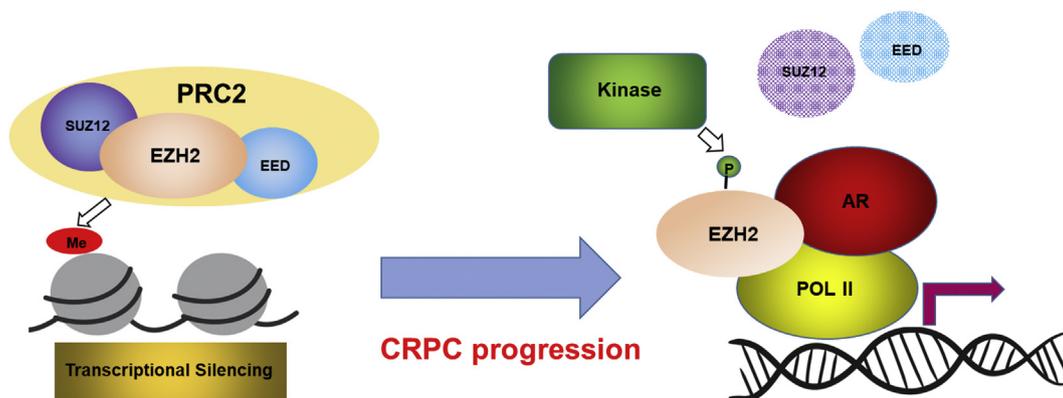


Figure 2 A model of the EZH2 functional switch by its hyper phosphorylation in CRPC. Deregulation of EZH2 phosphorylation can change EZH2 from a transcriptional repressor depending upon PRC2 to a transcriptional co-activator cooperating with AR which is independent of PRC2, finally contributing to CRPC progression.

types of cancers. Our lab has made a series of discoveries to show that Plk1 plays a critical role in different aspects of PCa, including its initiation, progression and therapy resistance.⁹¹ It was shown that PLK1 directly phosphorylates SUZ12, another component of PRC2 complex, and that PLK1 phosphorylation of SUZ12 abolishes its interaction with EZH2 and the PRC2 function.⁵⁵ Whether PLK1 also directly phosphorylates EZH2 is unknown.

It is worth of mentioning that EZH2 has an influence on DNA methylation by direct association with DNMTs.⁹² DNA methylation abnormal changes can be highly present in advanced stages of prostate cancer, and changes are tied together with alterations of EZH2 activity in silencing tumor suppressors. Thus, if not all, modifications, like PTMs, that affect EZH2 activity will also affect DNA methylation. For example, one study has shown that EZH2 directly interacts with DNMTs and contributes to DNA hypermethylation of selected target genes (particularly GSTP1 and RARB2) by qRT-PCR in 47 primary prostate cancers.⁹³ Additionally, EZH2 and DNMT3B have been shown to mediate

hypermethylation of HOXB13 in prostate cancer cells, and both EZH2 and DNMT3B could be repressed by an anticancer agent, all-trans retinoic acid (ATRA) flowing a reduced methylation of HOXB13.⁹⁴

Conclusions

PCa is the most commonly diagnosed malignant neoplasm of males in the United States, and ADT is an effective treatment for patients with PCa. However, most patients ultimately develop resistance and cancer relapse. Treatment for CRPC is very limited. Therefore, exploring novel cellular mechanisms controlling progression of PCa is very critical for identifying new targets and eventually developing efficient strategies to treat CRPC. Expression of EZH2 is often upregulated in CRPC, thus EZH2 has been proposed as a target for CRPC. Importantly, it has been demonstrated that EZH2 becomes hyperphosphorylated in CRPC cells.⁶³ However, very few studies reported the effect of PLK1-dependent phosphorylation on epigenetic modifications.

Our ongoing study is expected to fill in this knowledge gap by determining whether and how PLK1-dependent phosphorylation of various epigenetic regulators contributes to PCa progression and drug resistance.

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Conflict of Interest

The authors declare no conflict of interests.

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