

Review

Role of Chromatin Modifying Complexes and Therapeutic Opportunities in Bladder Cancer

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Received 11 September 2021

Accepted 14 February 2022

Pre-press 28 February 2022

Published 3 June 2022

Abstract.

BACKGROUND: Chromatin modifying enzymes, mainly through post translational modifications, regulate chromatin architecture and by extension the underlying transcriptional kinetics in normal and malignant cells. Muscle invasive bladder cancer (MIBC) has a high frequency of alterations in chromatin modifiers, with 76% of tumors exhibiting mutation in at least one chromatin modifying enzyme [1]. Additionally, clonal expansion of cells with inactivating mutations in chromatin modifiers has been identified in the normal urothelium, pointing to a currently unknown role of these proteins in normal bladder homeostasis.

OBJECTIVE: To review current knowledge of chromatin modifications and enzymes regulating these processes in Bladder cancer (BCa).

METHODS: By reviewing current literature, we summarize our present knowledge of external stimuli that trigger loss of equilibrium in the chromatin accessibility landscape and emerging therapeutic interventions for targeting these processes.

RESULTS: Genetic lesions in BCa lead to altered function of chromatin modifying enzymes, resulting in coordinated dysregulation of epigenetic processes with disease progression.

CONCLUSION: Mutations in chromatin modifying enzymes are wide-spread in BCa and several promising therapeutic targets for modulating activity of these genes are currently in clinical trials. Further research into understanding how the epigenetic landscape evolves as the disease progresses, could help identify patients who might benefit the most from these targeted therapies.

Keywords: Bladder cancer, chromatin modifications, epigenetics

INTRODUCTION

Bladder cancer (BCa) is the fourth most common cancer in men and the sixth most common cancer

overall, with an average age of diagnosis around 73 years [2, 3]. Most BCa (~90%) develops from the uninterrupted proliferation of cells in the stratified urothelium, a specialized epithelial layer that lines the urinary tract [4–7]. Next generation sequencing of BCAs has identified a heterogeneous somatic mutation landscape, with one of the highest mutation

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burdens, estimated at 8.2 mutations per MB (median) [8–12].

In the 2017 Cancer Genome Atlas (TCGA) MIBC cohort, after the *TP53/RB* tumor suppressor pathway, chromatin modifier genes are the second most frequently mutated class of genes [11]. Recent studies investigating early events in bladder tumorigenesis have identified the clonal expansion of cells with widespread somatic mutations in chromatin regulatory genes within normal urothelium [13, 14]. Identification of a high frequency of mutations in chromatin modifying enzymes in independent cohorts has heightened the need to understand the function of these gene alterations in influencing disease aggressiveness and predicting treatment outcomes in BCa patients [11, 15–22].

REGULATION OF CHROMATIN STRUCTURE

The core nucleosome structure consists of 146 bp of DNA wrapped around a histone octamer containing two molecules of H2A, H2B, H3 and H4 (Fig. 1) [23–25]. Tail-like structures protruding from the core-histones are prime sites for post-translational modifications (PTMs) that regulate chromatin structure. Chromatin packaging and accessibility is a determining factor for all cellular events from cell division during organism development to uncontrolled cell division during neoplastic transformation [26–28].

Enzymatic processes that regulate chromatin organization are targets of dysregulation in cancer. Unlike tumor suppressors or oncogenes, loss of function mutations in chromatin regulatory genes may have unique effects on the transcriptional landscape

depending on the cell type. More importantly, in a manner unique to cell-of-origin, epigenetic status impacts chromatin packaging which regulates the genomic mutational landscape [29, 30].

The distorted genetic and epigenetic landscape of cancer cells supports tumor evolution by amplifying cellular signals that allow for uninhibited tumor growth [31–33]. During carcinogenesis, tumor cells create their own micro-environmental niche, that is distinctive in its metabolic requirements and signaling pathways [31]. Each of these adaptations helps the tumor gain a survival advantage while evading the immune system. The fast pace of modifying chromatin compaction is frequently exploited by cancer cells to achieve an altered transcriptional landscape [34]. Over the decades, pathologists have observed hyper-chromatic nuclei in malignant cells, likely a reflection of the massive number of organizational changes that take place during neoplastic transformation [35, 36]. With development of high sensitivity assays, the effect of chromatin packing on transcriptional plasticity and its influence on tumor heterogeneity and therapeutic outcome is better appreciated [37, 38].

Chromatin modifying proteins include chromatin remodeling complexes and DNA and histone modifying proteins. Chromatin remodeling complexes, using the energy from ATP hydrolysis, reposition nucleosomes to increase accessibility of DNA binding proteins to DNA whereas histone modifying proteins edit post-translational modifications (PTMs) on histone tails [39, 40]. Histone PTMs are a minimalistic way to control transcription at specific loci. DNA can also be directly epigenetically modified with repercussions on the transcriptional output at any specific locus (Fig. 2). PTMs and DNA modifications

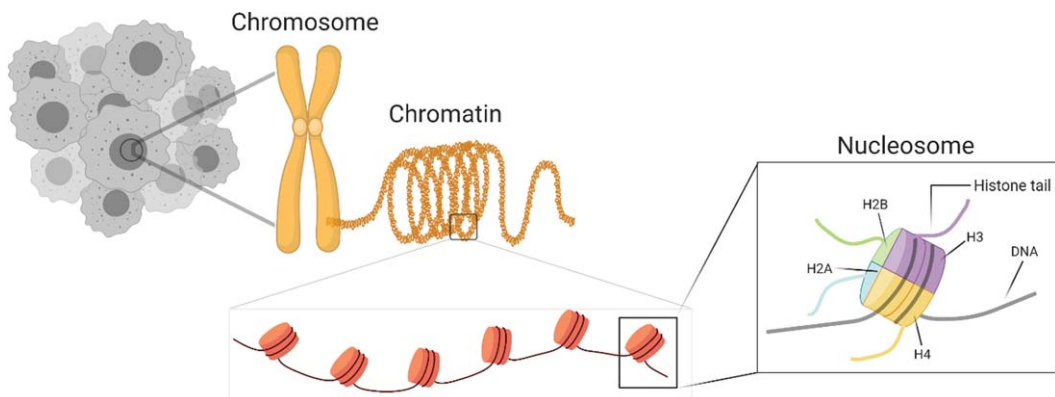


Fig. 1. Overview of chromatin organization in a nucleus.

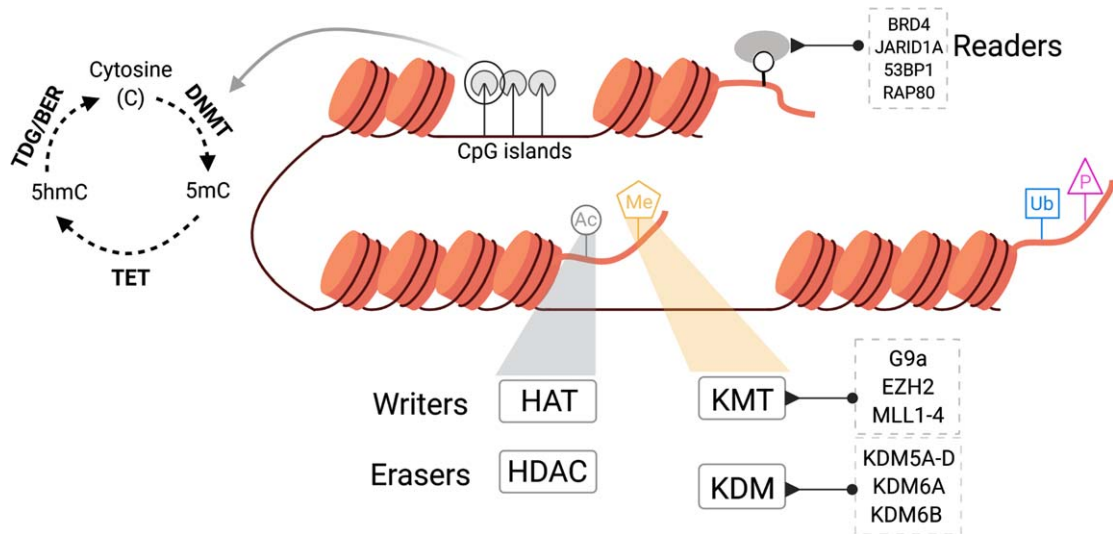


Fig. 2. Overview of chromatin regulatory processes and the enzymes that regulate these in mammalian cells.

can weaken or strengthen non-covalent interactions between histones and DNA and local chromatin composition, thereby allowing or restricting access to selected sections of DNA. Densely packed heterochromatin is largely inaccessible to transcription machinery, while euchromatin, which is more loosely packed, contains accessible sections of the genome with a high transcriptional output. Analogous to other signaling pathways, chromatin organization is coordinated by chromatin modifying enzymes in response to external cues such as developmental stimuli [28, 41, 42].

Chromatin modifying enzymes can be readers, writers, or erasers, aptly classified by their unique actions. Readers interpret the covalent modifications present on chromatin, writers add chromatin marks and erasers remove post translational modifications from the chromatin. Each of these enzymes lay down unique post-translational modifications such as methylation (Me), acetylation (Ac), phosphorylation (P), and ubiquitination (Ub) that inimitably modulate chromatin structure (Fig. 2). In this review, we highlight the state of epigenetic dysregulation in BCa focusing on current knowledge and therapeutic opportunities available to target chromatin modifiers.

DNA METHYLATION

Overview

Methylation is a dynamic, reversible, and non-sequence altering means of DNA modification that

determines accessibility and transcription of DNA [43, 44]. Commonly found near gene promoters, DNA methylation is associated with transcriptional silencing. Cytosines found in palindromic CpG dinucleotide repeats are prime sites for DNA methylation. Promoters containing CpG islands, generally unmethylated, can be targeted by aberrant DNA methylation during cellular transformation, which is associated with transcriptional silencing of tumor suppressor genes. In a metastable phenomenon, DNA methylation patterns established at the time of tissue patterning and differentiation are passed on as epigenetic traits across cell generations [45]. Studies have identified silencing of genes by DNA methylation to be as common as loss-of-function mutations, if not a more prevalent mechanism of transcriptional repression in cancer [46, 47].

Regulators of DNA methylation

DNA methylation landscape is the output of DNA methyltransferases (*DNMT1*, *DNMT3A*, *DNMT3B*) and demethylase (*TET1*, *TET2*, *TET3*) activity [48, 49]. DNA methyltransferases add methyl groups at carbon-5 position of cytosine (5mC) while TET enzymes oxidize 5mC to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fc) and 5-carboxylcytosine (5caC) [50]. Mutations in DNA methyltransferases are found in 6% of MIBC tumors and 10% of MIBC cases harbor mutations in DNA demethylases. While several studies have explored DNA methylation status as a prognostic

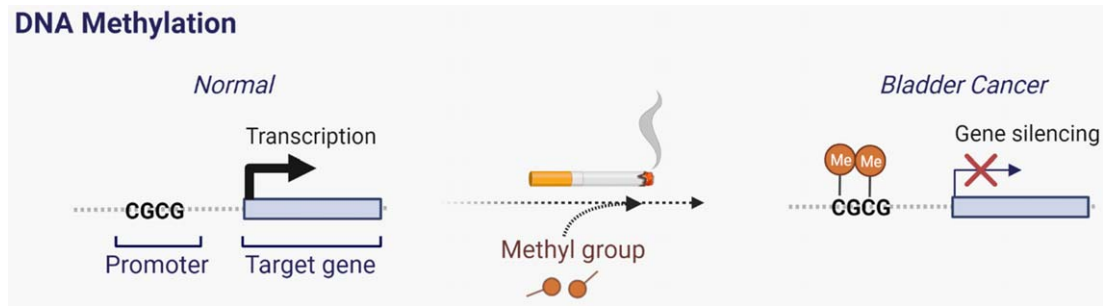


Fig. 3. Carcinogen exposure leads to promoter hypermethylation in BCa.

marker for BCa staging, subtyping, diagnosis or therapy outcome, the role of genes most affected by aberrant DNA methylation in triggering carcinogenesis remains unclear.

Causes of derailed DNA methylation in BCa

Epidemiological studies have identified a correlation between smoking and the risk of developing Bca [51–53]. Studies analyzing DNA methylation patterns in urothelial carcinomas have identified a CpG island hyper methylator phenotype to be associated with the intensity of cigarette smoking (Fig. 3).

Furthermore, this increase in methylation was associated with silencing of tumor suppressor genes in high grade invasive bladder tumors [1, 54–57].

Additionally, an increase in methylated metabolites is observed in high grade tumors from smokers. Overall, there is strong evidence to suggest that external stimuli (such as smoking) can alter DNA methylation landscape in BCa [49, 55, 58]. In-depth analysis of variation in local DNA methylation levels between normal and disease state in larger cohorts can help establish prognostic biomarkers for early diagnosis of BCa.

Insights from clinical trials with DNA methylation inhibitors

Given the multidimensional effects of DNA methylation, inhibition of this process has wide ranging outcomes from inhibition of proliferation to induction of an anti-viral state manifested by de-repression of genes involved in antigen presentation and inflammatory responses [59]. Inhibition of DNA methylation has been explored as a therapeutic strategy in blood cancers and solid tumors. Cytidine analogues, azacytidine and decitabine have been approved for patient

use in acute myeloid leukemia and myelodysplastic syndrome [60].

With the goal of targeting the altered DNA methylation landscape, S110, a derivative of decitabine showed tumor regression in mouse xenograft models [61]. DNMT1 inhibitors are currently in clinical trials for advanced BCa (NCT00030615, NCT02030067, NCT01478685) [62–64]. Results from a phase I trial designed to test the efficacy of 5-azacytidine combination with sodium phenylbutyrate failed to identify any clinical benefit associated with the combination in a cohort of patients with refractory solid tumors [65]. *In-vitro* studies in BCa cells exposed to decitabine point to enhanced expression of tumor antigens such as cancer testis antigens (CTA) leading to activation of immune-regulatory pathways. This opens up the avenue for utilizing immunotherapy to enhance the efficacy of DNMT inhibitors [66, 67].

REGULATION OF TRANSCRIPTIONAL STATE BY HISTONE MODIFICATIONS

Overview

Charged histone tails extending from nucleosome cores are prime sites for post-translational modifications. Several histone modifications such as deamination, citrullination, ADP-ribosylation, and O-GlcNAcylation play critical roles in regulation of chromatin structure during diverse cellular processes. Among these the most widely studied histone modifications are acetylation and methylation which are reviewed here [68].

Histone acetylation and deacetylation

Altered state of histone acetylation has been identified as a distinguishing feature of human tumors

[69]. Relaxation of chromatin allows transcription machinery to access DNA thereby increasing transcriptional output from the locus. Acetyl marks added to positively charged lysine residues on histone tails neutralize electrostatic interactions, thereby allowing for localized unwrapping of chromatin. Acetylation is often associated with open chromatin, occurring mostly at promoter and enhancer elements and is regulated by activity of writers: histone acetyltransferases (also referred to as lysine acetyltransferases, HAT) and erasers: histone deacetylases (HDAC).

HATs belong to two major subgroups classified based on their functions: nuclear type-A HATs and cytoplasmic type-B HATs. Nuclear type-A HATs are comprised of three protein families: GNAT (*KAT2A*, *KAT2B*), MYST (*KAT5*, *KAT6A*, *KAT6B*, *KAT7*, *KAT8*) and p300/CBP (*KAT3A*, *KAT3B*) each with unique substrate specificity that regulates acetylation events leading to gene expression [70]. Cytoplasmic type-B HATs coordinate acetylation activities on newly formed histones prior to nucleosome assembly. Approximately 36% of MIBC tumors in TCGA cohort have a mutation in one or more HAT enzymes [11].

To date, 18 enzymes with histone deacetylase activity have been identified. Depending on structural homology and function, these enzymes are broadly classified into four classes: Class I (*HDAC 1-3* and *HDAC8*), Class II (*HDAC 4-7*, *HDAC9* and *HDAC10*), Class III (*SIRT 1-7*) and Class IV (*HDAC11*) [71].

Elevated expression levels of HDAC-1, HDAC-2 and HDAC-3 are noted in high grade urothelial tumors, however a definitive function of these proteins in bladder carcinogenesis remains unclear [72]. Silencing of histone deacetylases has been shown to inhibit cell growth *in-vitro* [73]. Additionally, studies investigating the usefulness of HDAC inhibitors in *in-vitro* models and animal studies have observed a decrease in cellular proliferation and a reduction in tumor volumes [74]. However, despite the encouraging pre-clinical results, HDAC inhibitors have failed to show efficacy in BCa patients (NCT02236195, NCT00363883) [75–78].

Histone methylation and demethylation

Histone methylation mainly occurs on the positively charged tails of histones H3 and H4 at sites of lysine and arginine residues [79]. The location of the methylated residue in the sidechain and the number of methyl-groups present on the amino-acid

residue determine the effect of the modification on chromatin conformation and hence transcriptional state of the locus. Methylation at H3K4, H3K36 and H3K79 is associated with transcriptional activation whereas H3K9 and H3K27 are transcriptional repressor marks. H3K4me1 is highly enriched at enhancers while H3K4me3 is predominantly present at active promoters [80]. Histone methylation is coordinated by activity of histone methyltransferases and demethylases. More than 30 genes function as writers of histone methylation and approximately 18 genes belong to the KDM family of lysine demethylases [81].

Key players in histone methylation

The most well-studied proteins for their role in regulating transcription through histone methylation are the Polycomb (PcG) and Trithorax (TrxG) group of proteins [82, 83]. PcG and TrxG group of proteins play contrasting roles in regulating transcription. PcG proteins which encompass two major complexes, PRC1 and PRC2, repress transcription through histone methylation at H3K27 (PRC2) and ubiquitination (PRC1). TrxG proteins made up of COMPASS and COMPASS-like protein complexes behave as histone methyltransferases. *SET1A* (*KMT2F*), *SET1B* (*KMT2G*) and *MLL1* (*KMT2A*), *MLL2* (*KMT2D*), *MLL3* (*KMT2C*), *MLL4* (*KMT2B*) make up the catalytic subunits of distinct multi-subunit COMPASS complexes, each with discrete functions. Furthermore, *KDM6A*, a histone H3K27 demethylase, has also been identified as a member of the COMPASS complex [84]. Proteins within these structures regulate assembly, stability, and activity of the enzymatic complex [85]. Each of these enzymatic complexes regulates H3K4 methyltransferase in a unique manner impacting different functions. For example, SET1 is responsible for H3K4me3 at promoters of housekeeping genes, MLL1/2 complexes deposit H3K4me2/me3 at promoters of Hox genes and are hence crucial for the gene expression pattern of developmental genes and MLL3/4 protein complexes facilitate H3K4me1 marks at transcriptional enhancers.

Consequence of dysregulated histone methylation in BCa

Analysis of 11,000 tumors from 33 different types of cancer identified alterations in methyltransferases in 49% and alterations in demethylases in 30% of

MIBC tumors [86]. In BCa, alterations in one or more histone methyltransferases and demethylases were identified in 48% and 36% of MIBC cases, respectively [11].

Role of H3K4 methyltransferases in BCa

Somatic mutations in the *MLL* genes occur in 46% of MIBC tumors [87]. The enzymatic SET domain within the MLL (KMT) family members confers histone methyltransferase activity to this group of proteins. While a relatively small fraction of MIBC tumors have mutations in the SET domain of these genes, a significantly large fraction of tumors harbor nonsense mutations or truncating deletions resulting in a non-functional protein.

Given the crucial role of *MLL* gene family during development and hematopoiesis, studying the role of *MLL* genes in cancer using mouse models has been a difficult task [88, 89]. While deletion of *Mll1* is embryonic lethal, targeted deletion of *Mll1* SET domain is sufficient to rescue viability, but mice exhibit visible developmental defects [89, 90]. Dependent on the developmental stage at which *Mll2* is deleted, embryos are either non-viable or develop into mice with developmental defects [91].

Mll3 deletion is embryonically lethal and like *Mll1*, deletion of *Mll3* SET domain has been shown to rescue viability in these mice, however, ureteral epithelial tumors were observed in nearly 50% of mice carrying a homozygous deletion of *Mll3* SET domain. In a p53 +/- background, this phenotype was exacerbated with 100% of mice exhibiting accelerated carcinogenesis with formation of aggressive tumors [92–94]. Downregulation of *MLL3* has been demonstrated to influence expression of DNA damage response and repair genes, with higher genome instability observed in cells with low levels of *MLL3* [95]. Levels of *MLL2* are lower in carcinomas compared to adjacent normal tissues, in addition, loss of *MLL2* has been associated with a decrease in expression of tumor suppressors *PTEN*, *P53* [96].

It is important to note that mutations in *MLL3* are found in patients with Kleefstra syndrome, an intellectual disability disorder, de novo mutations in *MLL2* are found in patients with Kabuki syndrome and heterozygous loss of function mutations in *MLL1* are detected in patients with Wiedemann-Steiner syndrome [97–101]. While a small frequency of patients with Kabuki syndrome have developed cancer, further research is needed to determine whether mutations in *MLL2* predispose to cancer

[102]. Future mechanistic studies will be crucial to determine whether loss-of-function of *MLL* methyltransferases is sufficient to drive bladder carcinogenesis or if epigenetic reorganization caused due to loss of *MLL* activity creates an accommodating environment to accelerate oncogenic transformation in cases with synchronized loss of tumor suppressor genes.

Role of H3K9 and H3K27 methyltransferases in BCa

A unique multi-regional epigenetic silencing phenotype driven by variable H3K9 and H3K27 methylation levels was found to be associated with the carcinoma *in-situ* expression signature in MIBC [103]. Studies have identified a correlation between global H4K20 trimethylation levels and stage of BCa, with lower levels of H4K20me1/2/3 observed with progression to higher stage cancer [104]. In an independent cohort, a decrease in H3K9 and H3K27 methylation levels was observed in cancer tissue compared to normal controls and a further decrease in these levels was observed with disease stage [105]. Studies have identified a basal and luminal sub-type specific H3K4me1 peak pattern in MIBC tumors [106, 107]. Overall, evidence from multiple studies points to a decrease in transcriptional repressive marks with an increase in stage and invasiveness of BCa.

E2F1 has been identified as a regulator of EZH2 and SUZ12 expression levels and a high expression of the E2F1-EZH2-SUZ12 pathway is associated with aggressive Bca [108]. BRD4 has also been identified as an indirect transcriptional regulator of EZH2 [109]. Several studies have shown an increase in expression of EZH2 to be associated with more aggressive disease [110–113]. Ramakrishnan et al. have noted an *EZH2* dependency in SWI/SNF mutated BCa cell-lines. Furthermore, using cell-line derived xenograft models, they demonstrated combination treatment with EZH2i and cisplatin to be an effective therapy for tumors with SWI/SNF mutations, and hypothesized EZH2i cell death in animal models to be dependent on immune responses via natural killer (NK) cell signaling [114]. A recent study identified a role for the *PRC2* complex in evading immune surveillance by suppression of MHC-I antigen processing genes and a consequent increase in anti-tumor immunity upon pharmacological inhibition of this complex [115].

Methylation at H3K9 is associated with transcriptional repression. G9a, a H3K9 methyltransferase

is over-expressed in BCa and its inhibition has been shown to impact cell survival by inducing autophagy [116–119]. Crosstalk between G9a and DNA methyltransferase DNMT1 has been shown to induce transcriptional silencing of target genes [120]. High levels of G9a and EZH2 have been detected in tumors from BCa patients who do not respond to anti-PD-1 immunotherapy [121].

Targeting histone methylation

Inhibition of hyperactive EZH2 has been explored as a therapeutic avenue in several different cancers [122]. EZH2 has two pockets for binding to S-adenosyl methionine (SAM), the methyl group donor for methylation reactions. Most EZH2 inhibitors currently in development target this SAM binding site within the SET domain [122]. Tazemostat, a SAM competitive inhibitor of EZH2 has recently been approved for treatment of advanced epithelioid sarcoma and refractory follicular lymphoma [123]. Several other inhibitors that function like tazemostat through competitive inhibition with SAM are currently in clinical trials [122]. Few studies have identified a role for EZH2 in epigenetic silencing of tumor immune signaling pathways [124, 125]. Pharmacological inhibition of EZH2 could enhance immunogenicity and sensitize tumors to immune checkpoint blockade approaches. Clinical trials investigating EZH2 inhibition along with anti-PD-1 immunotherapy (pembrolizumab) are currently ongoing (NCT03854474) [126]. Combinatorial inhibition of G9a and DNMT using the recently described dual G9a/DNMT inhibitor CM-272 has also been shown to effectively kill BCa cells *in-vitro* and *in vivo* in an immunocompetent transgenic mouse model of metastatic MIBC [121]. High levels of G9a and EZH2 have been detected in tumors from BCa patients who do not respond to anti-PD-1 immunotherapy. Combinatorial treatment with CM-272 and anti-PD-L1 was shown to decrease incidence of tumor as well as metastasis in a genetically engineered mouse model of MIBC.

Key players in histone demethylation

The process of demethylation in eukaryotes is carried out by two families of histone demethylases: Lysine specific demethylases (LSD) and Jumonji C domain (JMJC) containing proteins [127]. Each of these proteins differs in their substrate specificity [128]. KDM6 removes methyl groups at H3K27 site

and KDM5 family of proteins remove methyl groups at H3K4 site.

Role of demethylases in BCa

The *KDM5* family is altered in 9% of MIBC cases and *KDM6A* mutations are found in 26% of patients in TCGA MIBC cohort. A large fraction of these mutations have been identified to be loss-of-function mutations.

KDM6A (*UTX*) is a lysine demethylase located on the X-chromosome. Mutations in *KDM6A* are found at a higher frequency in low-grade NMIBC tumors (52%) in comparison to high grade tumors (24%) [129]. Additionally, a higher frequency of *KDM6A* mutations are detected in non-invasive bladder tumors from females compared to males [130]. In a mouse model, loss of *KDM6A* has been shown to increase risk of BCa incidence in female mice [131]. Ahn et al have proposed a compensatory role for *KDM6A* homolog *UTY* in *KDM6A* deficient cells [132]. It remains to be determined if combinatorial loss of *KDM6A* and *UTY* is a requirement for initiation of bladder carcinogenesis in males. Kobatake et al described activation of inflammatory pathways in *KDM6A* deficient mice and a cooperative effect between *KDM6A* and *p53* on bladder tumorigenesis [133]. EZH2 hyper-dependence has been noted in tumors with loss-of-function alterations in *KDM6A* [134]. Given the high frequency of *KDM6A* mutations in BCa, this opens up the avenue for EZH2 inhibitors in treatment of *KDM6A* mutated BCa [135]. However, further research will be needed to determine if all *KDM6A* mutations will create epigenetic susceptibility to EZH2i.

In addition to *KDM6A*, several other histone demethylases have been studied in BCa. For example, JMJD1A (*KDM3A*) expression is elevated in BCa and has been associated with metabolic reprogramming that contributes to BCa progression [136–138]. High levels of *KDM5B* are detected in BCa tissues and have been shown to regulate expression of *E2F1*, thereby impacting cell proliferation [139]. High levels of *KDM7A* have been associated with increased proliferative capacity of BCa cells and cisplatin resistance *in vitro* [140].

Given the high frequency of loss-of-function mutations in chromatin modifying genes, the function of these proteins has been classified as oncogenic driver or tumor suppressive in BCa. However, high frequency of mutations in this group (in particular *MLL2* and *KDM6A*) have also been identified in nonmalignant

nant bladder urothelium. These results suggest that while clonal expansion of cells with mutations in chromatin modifiers is high in the normal urothelium, these cells are non-malignant and require additional events to trigger the process of carcinogenesis [13, 14]. For example, *FGFR3* activation and *KDM6A* loss-of-function mutations commonly co-occur in MIBC. Recently, *KDM6A* loss of function mutations have been demonstrated to create a more permissive environment for *FGFR3* activating mutations to drive bladder tumorigenesis [141]. While further work is needed to understand the mechanisms that drive this cooperativity, these results are encouraging and point to new roles for chromatin modifying enzymes in BCa.

Conclusions and future outlook

Dysregulation of the epigenomic landscape results in aberrant gene expression and is associated with a wide spectrum of diseases from developmental disorders to cancer [142–144]. Endeavors dedicated to sequencing tumors at different stages have identified genetic and epigenetic changes that define the evolving landscape of BCa [1, 11, 15]. While our review is focused primarily on DNA and histone methylation and histone acetylation, chromatin remodeling proteins are also frequently mutated in BCa and provide additional opportunities for therapeutic intervention [145, 146]. More mechanistic questions of epigenetic regulators require further investigation, it remains clear that chromatin modifying enzymes play a crucial role in maintaining bladder homeostasis. Even prior to malignant transformation, mutations in chromatin modifying enzymes are found at a high frequency in the urothelium, pointing to a currently unidentified role that these enzymes play in non-malignant urothelium [13, 14]. Further research dedicated to identifying dependencies specific to chromatin modifying enzymes in a BCa model may improve the range of therapeutic strategies that can be used to target this subset of tumors.

ACKNOWLEDGMENTS

The authors have no acknowledgments

FUNDING

JJM is supported by a grant from the VHA BX003692, AP is supported by a grant from NCI: K99CA234434, LC is an American Urologic Asso-

ciation Research Scholar and a grant from the Society of Women in Urology.

AUTHOR CONTRIBUTIONS

KM: Conception, performance of work, interpretation of results, writing and review of article. LC: performance of work, interpretation of results, writing and review of article, AP: Conception, performance of work, interpretation of results, writing and review of article, JM: Conception, performance of work, interpretation of results, writing and review of article. All authors had access to data and manuscript.

CONFLICTS

LC, KM: No conflicts of interest to report, AP: owns stock with Epizyme, JM: JJM participated in advisory boards for Ferring, AstraZeneca, BMS, Janssen, Foundation Medicine, and Merck and UroGen.

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