Review Article

Epigenetic regulation in bladder cancer: development of new prognostic targets and therapeutic implications

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Abstract: Epigenetic and genetic alterations contribute to cancer initiation and progression. Epigenetics refers to heritable alterations in gene expression without DNA sequence changes. Epigenetic changes include reversible alterations in DNA methylation, chromatin modification, nucleosome positioning, and non-coding RNA profiles. In bladder cancer, many epigenetic changes have been reported to exhibit correlation with cancer progression and malignances. In particular, both DNA hypermethylation and hypomethylation have been reported to be associated with higher bladder cancer rates or advanced tumor stages. The association of changes in histone modifying enzyme expression with bladder cancer stages has been extensively studied. MicroRNA (miRNA) expression also has been studied in bladder cancer diagnosis and disease progression. The use of epigenetics for the diagnosis of and therapeutic target screening in bladder cancer is an emerging and rapidly evolving field. The reversible nature of epigenetic changes may facilitate additional therapeutic options in the future.

Keywords: Bladder cancer; epigenetics; DNA methylation; histone modification; microRNA (miRNA)

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Introduction

Bladder cancer is one of the leading cancers of the urinary tract. It is often diagnosed at advanced stages of the disease and high rates of recurrence are the most difficult obstacle for patient treatment (1). To explore early diagnostic and possible therapeutic targets for bladder cancer, understanding the regulatory system of gene expression is one of the most significant steps. Among many transcriptional regulatory mechanisms, modulation of epigenetic modifications has been studied most extensively over the last decade. Epigenetic modifications to DNA or its associated proteins result in changes in gene expression without altering DNA sequences (2). These characteristics make regulation of epigenetic mechanisms more flexible and important therapeutic targets for cancer treatments. Epigenetic regulatory modification includes DNA methylation alterations to gene promoter regions, histone modifications, and microRNA (miRNA) expression changes. DNA methylation is an enzymatic process involving covalent modifications of DNA with methyl groups using S-adenosyl methionine (SAM) as the methyl group donor and addition of this moiety to the 5-carbon atom of the cytosine base. DNA methylation typically acts to repress gene transcription. In plants and other organisms, DNA methylation is generally found in three different sequence contexts: CG (or CpG), CHG, or

CHH (where H corresponds to A, T, or C). In mammals, however, DNA methylation almost exclusively occurs at CpG dinucleotide sites, with the cytosine on both strands usually being methylated. The DNA methylation process is known to be associated with development, aging, and carcinogenesis (3). In tumorigenesis, hypermethylation of tumor suppressor genes and hypomethylation of oncogenes have been characterized. Histone modification includes acetylation, methylation, carbonylation, ubiquitination, SUMOylation, and phosphorylation of histone side chains (4). The functional unit of chromatin, the nucleosome, consists of four histone proteins identified as H2A, H2B, H3, and H4. Each of these proteins exhibit 'tail' extensions and these can be modified by a number of histone modifying enzymes. The addition of specific chemical groups to histone tails usually alters the binding affinity of the tails to DNA and results in changes in the conformation of the chromatin structure to regulate RNA transcription of genes. Histone acetylation usually indicates active gene transcription, whereas histone methylation is associated with both gene repression and activation, depending on the specific target histone residues. In this review, genome-wide histone modification and expression of key histone modifying enzymes associated with bladder cancer will be discussed. A miRNA is a small non-coding RNA molecule found in various species including plants, animals, and some viruses. They are approximately 18 to 25-nucleotide-long and are synthesized and processed in the nucleus. The function of miRNAs involves RNA silencing and post-transcriptional regulation of gene expression (5). It has been estimated that at least 30% of human genes may be regulated by interactions with miRNAs. It is established that each mRNA can interact with multiple miRNAs and that each miRNA may regulate multiple mRNAs. miRNAs play an important role in tumorigenesis by activating oncogenes or restraining tumor suppressor genes (6). We will explore and discuss epigenetic regulation of tumor-related genes in bladder cancer, and possible therapeutic and diagnostic targets for bladder cancer therapy. The diagnosis approach for bladder cancer is typically invasive causing discomfort to patients and only provides a generalized outcome for patients. The need for non-invasive markers and demand for improved screening techniques are urgent. We will also discuss a number of epigenetic biomarkers that have emerged recently as candidates for more effective diagnosis for bladder cancer.

DNA methylation in bladder cancer

Cancer initiation and progression are often accompanied by profound changes in DNA methylation which are marked by genome-wide hypomethylation and site-specific CpG island promoter hypermethylation (7). Hypomethylation occurs mainly in repetitive DNA sequences such as tandem and interspersed repeats and in segmental duplications. This leads to activation of cancer-causing genes, with global DNA hypomethylation often being associated with metastatic disease. Hypermethylation is associated with CpG islands and mediates silencing of tumor suppressor genes commonly associated with cancer development. Activity of DNA methyltransferases (DNMTs) generates a pattern of methylated CpG di-nucleotides at the 5' region of the promoter of the gene involved.

In bladder cancer, a large number of genes have been shown to undergo promoter hypermethylation. These loci include *GSTP1*, *TIG1*, *TIMP-2*, *p14*^{ARF}, *RUNX3*, *APC*, *SFRP2*, *RASSF1A*, *MGMT*, *FHIT*, *CDH13*, and *RARB* (8-17) (*Table 1*). In particular, the *RUNX3* gene was implicated as a tumor suppressor and exhibited significant elevations of methylation levels in bladder cancer upon analysis of 124 tumor tissue samples (14). A prognostic indicator in patients with non-muscle invasive bladder cancer (NMIBC) was found by Yoon *et al.* (20). Their analysis of 136 human bladder specimens (8 normal controls and 128 NMIBCs) showed clinical relevance of the radial spoke head 9 homolog (*RSPH9*) gene methylation status as an independent predictor of disease recurrence and progression. Another report suggested that hypermethylation of *PCDH10* and *PCDH17* was related to bladder cancer development and cancer-specific survival (21,22). A recent paper reported that the combination of *PCDH17* and *POU4F2* methylation in urine sediment DNA yielded high sensitivity and specificity (90% and 93.96%, respectively) in detecting bladder cancer (19). Renard *et al.* showed that identification of *TWIST1* and *NID2* methylation can be employed for detecting bladder cancer with high sensitivity and specificity (93% and 90%, respectively) in urine samples (18). Targeted epigenetic analysis using tumor biopsies (n=105) and urine samples (n=113) revealed 95 oncogenic mutations and 189 hypermethylation events. They revealed that three methylation markers, including *APC*, *RASSF1A*, and *SFRP2*, together with genetic mutation of *FGFR3* provided sensitivity of 90% for tumor specimens and 62% for urine samples, with 100% specificity respectively (23).

Table 1 List of hypermethylated gene in bladder cancer

Cellular function	Gene name	Full name	References
Cell cycle control genes	*STAT1*	Signal transducer and activator of transcription 1	(16)
	p14^{ARF}	Cyclin-dependent kinase inhibitor 2A, isoform 4 (CDKN2A)	(7,10,11,15,16)
	p15	Cyclin-dependent kinase inhibitor 2B (CDKN2B)	(9)
Apoptosis related genes	*DAPK1*	Death associated protein kinase 1	(8,9,16,17)
	EDNRB	Endothelin receptor type B	(12)
	TNFRSF25	TNF receptor superfamily member 25	(12)
	TERT	Telomerase reverse transcriptase	(12)
Functional tumor suppressor genes	*RASSF1A*	Ras association (RalGDS/AF-6) domain family member 1	(8,10,12)
	RUNX3	Runt related transcription factor 3	(14)
	MLH1	MutL homolog 1	(7)
	FHIT	Fragile histidine triad	(7,8)
DNA damage repair genes	*COX2*	Cyclooxigenase-2	(16)
	GSTP1	Glutathione S-transferase pi1	(7,11,17)
	MGMT	O-6-methylguanine-DNA methyltransferase	(7,8,11)
Cell differentiation-related genes	*RARB*	Retinoic acid receptor-b	(8,9)
	TIG1	Tazarotene induced 1'RARRES1 (retinoic acid receptor responder 1)	(17)
	TWIST1	Twist family bHLH transcription factor 1	(18)
Cell invasion genes	*CDH1*	Cadherin 1	(7,8)
	CDH4	Cadherin 4	(12)
	CDH13	Cadherin 13	(8)
Wnt antagonist genes	*APC*	Adenomatosis polyposis coli	(7,8,10,12,17)
	WIF1	WNT inhibitory factor 1	(12)
	SFRP2	Secreted frizzled related protein 2	(13)
Other functions	*NID2*	Nidogen 2	(18)
	POU4F2	POU domain, class 4, transcription factor 2	(19)
	RSPH9	Radial spoke head 9 homolog	(20)

Genomic DNA hypomethylation is considered to be a biomarker for bladder cancer with elevated long interspersed element-1 (*LINE-1*) hypomethylation (24). *LINE-1* hypomethylation was found to be associated with oxidative stress which may contribute to the promotion of urothelial cell carcinogenesis (25).

Although some obstacles arising from the employment of bisulfite treatment need to be surmounted, including DNA isolation efficiency, leukocyte DNA contamination, and loss of DNA templates, these DNA hypermethylation markers may improve the understanding of tumorigenesis and the prognostic value of DNA-based diagnosis in noninvasive detection of bladder cancer. Several chemical agents used for bladder cancer treatment exhibit DNA methyltransferase inhibitor activity. 5-Fluoro-2-deoxycytidine is such an inhibitor and was used together with tetrahydrouridine, a cytosine deamination inhibitor, and they synergistically inhibited bladder cancer growth (26). The combination of DNA methyltransferase inhibitor and tetrahydrouridine for bladder cancer treatment is now in phase II clinical trials.

When the anti-metabolite gemcitabine was tested against bladder cancer cell lines, the DNA methylation status of several epigenetically silenced genes, including *GSTP1*, *IGFBP3*, and *RASSF1A*, was changed (27)

MicroRNA regulation in bladder cancer growth

Since their discovery in the early 21st century, miRNAs have been intensively studied in various fields, including development, differentiation, metabolism, and cancer progression. miRNAs, a class of noncoding RNAs, is approximately 18 to 25-nucleotide-long and can be synthesized and processed in the nucleus. They are encoded within intergenic regions or within introns or exons of protein-coding genes of the genome. The miRNA sequence is initially transcribed to a several-kb-long primary transcript, which is subsequently processed by diverse enzymes, including exportin-5 and its catalytic partner. After translocation to the cytosol, miRNAs are enzymatically processed by Dicer to approximately 22-nucleotide-long mature miRNAs. These can bind matching mRNAs and regulate their stability (28). In tumor tissues, the expression of some miRNAs has been reported to be related to cell proliferation, differentiation, and apoptotic processes. The overexpression of these miRNAs can diminish expression of tumor suppressor genes and is referred to as oncogenic miRNAs.

Ten human micro-RNAs, including miR-223, miR-26b, miR-221, miR-103-1, miR-185, miR-23b, miR-203, miR-17-5p, miR-23a, and miR-205, were reported to be significantly up-regulated in bladder cancer specimens. In the same study, only miR-26b was shown to exhibit decreased abundance (29). A recent study suggested that miR-143 expression levels were suppressed in human bladder cancer tissues and cells, and such diminished expression levels were negatively correlated with bladder cancer clinical staging. The authors proposed that the functional target of miR-143 in bladder cancer was insulin-like growth factor-1 receptor (*IGF-1R*), and this was correlated with patient survival (30). Another study reported that miR-24-3p was highly expressed in bladder cancer tissues and functioned to regulate *DEDD*, a member of the death effector domain-containing protein family. *DEDD* was seen to be expressed at low levels in bladder cancer. Western blotting and RT-PCR experiments revealed that miR-24-3p inhibited apoptosis and promoted the growth and proliferation of bladder cancer cells (31). The expression level of miR-5195-3p was also down-regulated in bladder cancer samples, and the target of miR-5195-3p was identified as the Krüppel-like transcription factor-5 (*KLF5*), a proven proto-oncogene (32). After bioinformatics screening for epigenetic regulators of Survivin, an important enzyme in bladder cancer development, miR-138-5p was confirmed to act as a 3'-UTR-targeting modulator of Survivin (33). The functions of miR-138-5p in bladder cancer growth and invasive characteristics were confirmed in a xenograft mouse model. miR-106a also regulated cell cycle and proliferation processes in bladder cancer (34).

Despite their clinical significance in tumorigenesis, little is known about the function of miRNAs in cancer metastasis. Noteworthy, various studies regarding miRNA functions and involvement in bladder cancer metastasis were published over several years. Immunohistological screening of miRNAs by tissue microarray revealed that expression of miR-3658 was upregulated in bladder cancer tissues, and is associated with lymph node invasion, distant metastasis, and histological grading (35). Chemokine receptor type 7 (*CCR7*) is known to be correlated with lymphatic metastasis and poor clinical prognosis in bladder cancer. MiR-199a-5p was found to target the 3'-UTR of *CCR7* and this correlation was further strongly linked to the miRNA/chemokine axis that regulates bladder cancer cell metastasis (36). miR-3713 also regulates *MMP9* in bladder cancer and significantly lower levels have been found in bladder cancer tissues (37). Data mining reports using public databases also suggested a correlation between miRNAs and several EMT genes. Two miRNAs, miR-214-3p and miR-145-5p were reported to target and modulate *MMP-9* and *NGAL* genes (38). miR-451 was found to regulate bladder cancer cell migration and invasion by directly targeting c-Myc, and apoptosis promoted by miR-451 may participate in this process (39). When miR-106a was expressed in bladder cancer cell lines, migration and invasive capacity of these cell lines decreased. miR-101 was found to be less expressed in bladder cancer tissues and bladder epithelial cell lines (40,41). TargetScan analysis and a luciferase assay confirmed that miR-101 negatively regulates c-FOS levels. Bladder cancer cell proliferation and invasion were inhibited by miR-101. The above mentioned miRNAs dysregulated in bladder cancer were listed in *Table 2*.

There are some therapeutic candidate inhibitor miRNAs potentially useful for bladder cancer treatment. Gambogic acid (GA), an inhibitor of zeste homolog 2 (EZH2), was shown to induce bladder cancer cell apoptosis, mediated through miR-101 (42). This result established GA as a

Table 2 Dysregulated miRNAs reported in bladder cancer

miRNA name	Status in bladder cancer	Target gene	References
miR-143	Suppressed	Insulin-like growth factor-1 receptor (*IGF-1 R*)	(30)
miR-24-3p	Up regulated	Death effector domain-containing protein (*DEDD*)	(31)
miR-5195-3p	Suppressed	Krüppel-like transcription factor-5 (*KLF5*)	(32)
miR-138-5p	Suppressed	Survivin	(33,34)
miR-3658	Up regulated	metastatic genes (not known)	(35)
miR-199a-5p	Suppressed	Chemokine receptor type 7 (*CCR7*)	(36)
miR-3713	Suppressed	Metrics metalloprotein 9 (*MMP9*)	(37)
miR-214-3p	Suppressed	*MMP-9* and Neutrophil gelatinase-associated lipocalin (*NGAL*)	(38)
miR-145-5p	Suppressed	*MMP-9* and *NGAL*	(38)
miR-451	Suppressed	*MYC*	(39)
miR-101	Suppressed	*FOS*, *MET*	(40,41)

possible combination therapeutic drug for bladder cancer treatment. Another natural product, honokiol, isolated from the Magnolia plant, inhibited bladder cancer cell proliferation, survival, cancer stemness, migration, and invasion through down-regulation of *EZH2* expression. This effect was associated with miR-143, a tumor suppressor micro RNA (43).

Histone modification in bladder cancer

Histones are small, highly-conserved proteins and comprise the key protein components of chromatin condensation, which packs DNA into chromosome domains. Histone modification includes methylation, acetylation, phosphorylation, ubiquitination, and SUMOylation of particular amino acids, usually at N-terminal tails. They are known to play crucial roles in regulating many fundamental biological processes, including gene expression, DNA replication, and DNA damage repair (44). Acetylation of histone residues usually represents active chromatin regions, whereas methylation may be associated with both gene activation and repression, depending on the specific lysine residue position (45). In bladder cancer, a growing number of papers are being published regarding the relationship between histone modifications and cancer progression (*Table 3*). Genetic profiling recently revealed that 89% of bladder cancers exhibit altered histone modification pathways, and 64% of cancers harbor alterations in the *SWI/SNF* complex, which is responsible for chromatin remodeling so as to activate or inactivate gene transcription (62).

Histone acetylation in bladder cancer

A recent study on histone acetylation reported that global H3 acetylation (H3Ac) levels decreased in bladder cancer patients, whereas H3K18Ac and H4Ac levels were similar between normal and cancer group (63). Another report examining tissue microarray involved 174 bladder cancer patients and investigated expression levels of histone deacetylases (*HDACs*) 1, 2 and 3. High-grade noninvasive papillary bladder tumors were associated with elevated expression levels of *HDAC-1* and *HDAC-2* (46). In particular, high grade tumors, in combination with high expression of *HDAC-1*, exhibited poorer prognoses than other tumors did. These results indicate that inhibition of *HDAC* has potential in bladder cancer therapy. Romidepsin, a histone deacetylase inhibitor effective in T-cell lymphoma therapy, was reported to have suppressive effects on bladder cancer growth in combination with tetrahydrouridine, and currently, is in phase I trials for bladder cancer treatment (64). Combined treatment with the histone deacetylase inhibitor OBP-801 and celecoxib demonstrated bladder cancer cell growth inhibition and promoted apoptosis (65). The authors showed that this combined treatment was dependent on the expression of death receptor 5 (*DR5*), and knockdown of *DR5* significantly suppressed cell apoptosis with this combined treatment regime. Another histone deacetylase inhibitor, AR-42, has

Table 3 Histone modifying enzymes involved in bladder cancer growth

Name	Enzyme activity	Regulating genes, enzymes or pathways	References
HDAC-1, 2, 3	Histone deacetylase	Promoter histones	(46)
KDM5a	H3K4 demethylase	E2R/RB1 pathway	(47,48)
WDR5	H3K4 methyltransferase	cyclins, *UHMK1, MCL1, BIRC3*	(49)
MLL	H3K4 methyltransferase	Hox	(50)
KDM6a	H3K27 demethylase	*IGFBP3*	(51,52)
NSD1	H3K36 methyltransferase	*MEIS1*	(53)
SETD2	H3K36 methyltransferase	*PTEN, TP53, ATR, RAD50, POLN, XRCC1*	(53)
LSD1	H3K4 demethylase,	AR, ER pathway	(54,55)
	Non-histone enzyme demethylation	p53*, DNMT1*	
SMYD3	H3K4 methyltransferase	*BCLAF1*	(56)
EZH2	H3K27 methyltransferase	*DKK-1, RAD51, BRCA1, RUNX3, DAB2IP*	(57,58)
G9a	H3K9 methyltransferase	AMPK/mTOR pathway	(59)
PRMT6	H3R2 methyltransferase	Not specified	(60)
PRMT1	Arginine methyltransferase	INCENP*	(61)

*, non-histone enzyme modification.

shown promise in combination with cisplatin in treating bladder cancer in a mouse model and in *in vitro* stem cell culture (66). The histone deacetylase inhibitor trichostatin A re-sensitized the otherwise gemcitabine-resistant urothelial carcinoma cells via suppression of triglyceride (TG)-interacting factor and down-regulating Akt signaling (67). Transient receptor potential cation channel, subfamily M, member 2 (TRPM2) protein is reportedly controlled by histone deacetylase inhibition, with its promoter acetylation status (68). *TRPM2* up-regulation by an HDAC inhibitor promoted apoptosis in bladder cancer cell lines, and *TRPM2* promoter acetylation changes were monitored by chromatin immunoprecipitation (ChIP) experiments. A proteomic approach revealed that histone deacetylase inhibitors (including romidepsin, trichostatin A, vorinostat) induced bladder cancer cell death, which is associated with chromatin modification and modulation of protein expression of some 6,003 proteins (69).

Histone lysine methylation in bladder cancer

In 2011, a report showed that global histone H4K20 tri-methylation is related to cancer-specific survival in muscle-invasive bladder cancer patients (70). Furthermore, numerous specific histone modification enzymes are reported to be involved in bladder cancer growth and proliferation. Depletion of the H3K4 demethylase KDM5a resulted in cell growth retardation through co-regulation of the E2F/RB1 pathway in bladder cancer (47,48). One of the core components of the MLL/SET1 complex is WDR5, which exerts methyltransferase activity. WDR5 was found to be highly expressed in bladder cancer tissues, and overexpression of WDR5 promoted bladder cancer cell growth *in vitro* and *in vivo* (49). Whole-exome sequencing of 37 bladder cancer patient tissue samples revealed that *MLL*, *EP400*, *PRDM2*, *ANK3*, and *CHD5* were differentially altered in recurrent bladder tumors (50). The authors also showed that MLL-mutated bladder cancer cells using CRISPR/Cas9 genetic modification demonstrated increased drug-resistance to epirubicin (a chemotherapeutic drug employed for patients with bladder cancer). Additionally, alterations in the *MLL* gene were involved in bladder cancer relapse. Another massive exome sequencing using 54 bladder cancer samples revealed that *KDM6A* and BRCA1-associated protein-1 (*BAP-1*) mutations frequently co-occurred in tumors. Depletion of KDM6a in bladder cancer cell lines enhanced anchorage independent growth and cell migration, but not monolayer growth (51,52). These data showed that *KMD6A* plays a role in

tumor growth and suppression of cell migration. When a NF-kB inhibitor, dimethylaminoparthenolide (DMAPT) was tested for potential effects on bladder cancer cell proliferation, H3K36 trimethylase NSD1 (KMT3B) and SETD2 (KMT3A) levels increased and cell death was induced (53). Lysine-specific demethylase 1 (*LSD1*) was found to be highly expressed in bladder cancer stem cells, and knock down of *LSD1* in bladder cancer cell resulted in significant suppression of cancer proliferation (54). A study involving Drosophila unveiled the function of LSD1 in Pumilio (PUM1), a post-transcriptional repressor that plays important roles in bladder cancer development (55). Up-regulation of the histone methyl transferase SMYD3 promoted bladder cancer progression by targeting BCL2-associated transcription factor 1 (*BCLAF1*) promoter (56). The authors demonstrated that SMYD3 physically interacted with the *BCLAF1* promoter and upregulated its expression by accumulating di- and tri-methylation of H3K4. Upregulated BCLAF1 increased autophagy and bladder cancer cell growth. Enhancer of EZH2 belongs to the Polycomb repressive complex 2, acting as its catalytic subunit, and which exerts gene silencing effects through the trimethylation of H3K27. EZH2 was reported to be overexpressed in bladder cancer and involved in development and progression of bladder cancer (57,58). G9a H3K9 methyltransferase is known as an oncogene in bladder cancer, and inhibition of G9a induced autophagic cell death via the AMPK/mTOR pathway in bladder cancer cell lines (59).

A small-molecule G9a inhibitor, BIX-01294, was reported to inhibit tumor cell growth and also induce apoptosis of bladder cancer cells through the ER stress pathway, resulting in *PMAIP1* up-regulation and *MCL1* down-regulation (71). Emodin, a natural product, which has the ability to inhibit bladder cancer cell line growth, was found to enhance H3K27 trimethylation. Today, various inhibitors targeting HKMTs and HKDMs are being increasingly developed for cancer treatment (72).

Histone arginine methylation in bladder cancer

Histone arginine methylation in bladder cancer has, to date, been studied rather less than has histone lysine methylation. PRMT6 is a type I protein arginine methyltransferase, and is reported to act on H3R2, which has been identified as a gene repressive epigenetic mark. PRMT6 was reported to be overexpressed in bladder cancer (60). Protein arginine methyltransferase PRMT1 was observed to be overexpressed in bladder cancer (61) and promotes mitosis of cancer cells through inner centromere protein (INCENP) arginine methylation (61). Although less well-studied than lysine methylation, arginine methylation may also contribute to the initiation, progression and development of bladder cancer and likely represents a promising target for therapeutic drug screening.

Clinical approaches targeting epigenetic regulators

Several molecules targeting epigenetic alterations have been developed for anticancer therapy (*Table 4*). Among them, some small molecule inhibitors have been approved by the U.S. Food and Drug Administration, and were proven therapeutically effective in various cancers (73). DNMT inhibitor 5-aza-2'-deoxycytidine (5-aza-CR; Vidaza®; decitabine) was approved for myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML); in addition, it completed phase 2 trials for treatment of bladder cancer (74), and it is in phase 1 trials in combination with tetrahydrouridine for bladder cancer treatment (64). Inhibition of DNA methylation with cytosine deamination (5-fluoro-2-deoxycytidine with tetrahydrouridine) have finished phase 2 trials in bladder cancer (26). Romidepsin (depsipeptide or Istodax), a HDAC inhibitor approved for cutaneous T-cell lymphoma (CTCL) in 2009, has been in phase 2 trials for treatment of many cancer types, including bladder cancer (75). Another HDAC inhibitor, vorinostat (SAHA, Zolinza®, MK-0683) was approved for CTCL in 2006, passed phase 2 trials as a monotherapy for bladder cancer and completed phase 1 trials as a combination therapy with docetaxel (76). Valproic acid, a short-chain fatty acid, which exhibits histone deacetylase inhibitory activity, is currently in phase 1–2 clinical trials either alone or in combination with other drugs, such as azacitidine, retinoic acid, temozolomide, and karenitecin, for treatment of some solid tumors (77). Tazemetostat (an EZH2 histone *N*-methyl transferase inhibitor) is being studied in ongoing clinical trials. For the diagnostic use of epigenetic regulators for treating bladder cancer patients, the following article may be a good reference. Targeted exome sequencing of 50 bladder cancer patients revealed that many epigenetic regulator genes were mutated in cancer specimens, and this results can be used for targeted therapy for bladder cancer patients at very high risk of metastasis and death (78). Such high-throughput technologies and clear understanding of epigenetic regulators could open the way for the use

Table 4 Epigenetic inhibitors clinically studied in bladder cancer

Inhibitor	Other names	Comments
DNA methyltransferase inhibitors (DNMTs)		
5-aza-2'-deoxycytidine	Decitabine, 5-aza-CR	Approved for MDS and AML
	Vidaza	Phase 2 trials for bladder cancer
		Phase 1 trials in combination with tetrahydrouridine
5-fluoro-2-deoxycytidine	FdCyd	Phase 2 trials in combination with tetrahydrouridine
HDAC inhibitor		
Romidepsin	depsipeptide, Istodax	Approved for CTCL
		Phase 2 trials for bladder cancer
Vorinostat	SAHA, Zolinza,	Approved for CTCL
	MK-0683	Phase 2 trials for bladder cancer
		Phase 1 trials in combination with docetaxel
Valproic acid		
		Phase 2 trials for bladder cancer
		Phase 1 trials in combination with azacitidine, retinoic acid, temozolomide, karenitecin
Histone methyl transferase inhibitor		
Tazemetostat		EZH2 inhibitor
		Phase 1 trials in genetically defined solid tumors

MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; CTCL, cutaneous T-cell lymphoma; EZH2, enhancer of zeste homolog 2.

of personalized targeted therapies for bladder cancer treatment.

Conclusions

Invasive bladder cancer is characterized by high rates of mutation in many cancer related genes. Identifying epigenetic changes and their multiple and significant roles in bladder cancer development represent highly useful targets for patient diagnosis and drug screening. Epigenetic profiling also can allow identification of patients' risk of tumor invasiveness using less intrusive urine tests. Epigenetic modifiers such as histone deacetylase and methyl transferases have come into focus as novel drug targets for bladder cancer therapy. The importance of epigenetics in bladder cancer has now been fully recognized and the research in this arena has matured evolved over the years. The use of high throughput methods has made it possible to scrutinize epigenetic processes (and their effects) at the global level than those involving individual genes. Next-generation sequencing techniques combined with chromatin immune-precipitation (Chip-seq) have enabled studies of the histone modification status of bladder cancer patients at a global level. In addition, next-generation sequencing also allowed studies of DNA methylation status at the single nucleotide level. Employing these epigenetic data will help profoundly in designing more potent treatment strategies in bladder cancer. Moreover, the intrinsic reversibility nature of epigenetic modifications will afford exciting new opportunities for the development and establishment of improved, novel strategies for bladder cancer prevention, detection and therapeutic intervention. An important note is that, due to the crucial functions of epigenetic control and regulation in normal (as well as abnormal) physiology, inhibition could potentially exert toxic effects on non-diseased organs. Hence, toxicological and combination therapy studies should be performed carefully in the development of new drugs targeting epigenetic pathways.

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