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Histone modifications: implications in renal cell carcinoma

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Abstract

In 2012, an estimated 64,770 men and women were diagnosed with malignancy of the kidney and renal pelvis, of which 13,570 succumbed to their disease. Common genetic aberrations in renal cell carcinomas (RCCs) include loss of function of the *VHL* gene in clear-cell RCC, overexpression of the *c-MET* gene in papillary RCC type I, deficiency in the *FH* gene in papillary RCC type II and loss of heterozygosity of the *BHD* gene in chromophobe RCC. Recent studies illustrate epigenetic silencing of *VHL*, as well as alterations in histone modifications and their governing enzymes. The possibility of reversing these epigenetic marks has resulted in efforts to target these changes by utilizing inhibitors of HDACs, DNA methyltransferases and, recently, histone methyltransferases in preclinical and clinical studies. This article focuses on potential therapeutic interventions, and the implications of histone modifications and related enzyme alterations in RCC.

Keywords

DNA methyltransferase; epigenetics; HDAC; histone acetylation; histone methylation; renal cell carcinoma

Epigenetic mechanisms mediated by HDACs, histone acetyltransferases (HATs) and histone methyltransferases (HMTs) play critical roles in various biological and cellular processes, including cell proliferation, angiogenesis, hypoxia-related effects and cell cycle regulation. Furthermore, numerous studies have emphasized the deregulation of these enzymes in multiple cancer types, highlighting them as potential novel therapeutic targets for the treatment of cancer. Targeting these enzymes and, thus, reversing their epigenetic modifications has the potential of reactivating tumor suppressor genes or suppressing

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oncogenes, thus, affecting tumor growth or progression. In renal cell carcinomas (RCCs), evaluation of patient tumors has demonstrated epigenetic modifications of tumor suppressor genes (including *VHL* and p53), as well as alterations in histone methylating and demethylating enzymes (i.e., *EZH2*, *PBRM1* and *UTX*). The discovery of downstream targets and the pathways affected is ongoing and providing valuable insight in comprehending the biology of RCC. This article discusses histone-modifying enzymes, the resulting epigenome and how we can exploit these aberrations to create novel therapeutic avenues for patients with RCC.

Histone modifications

DNA, histones and nonhistone proteins are condensed into a highly complex nucleoprotein structure known as chromatin, which is either in a compact heterochromatin form or a more open euchromatin form, which is associated with transcriptionally active genes [1].

Histones on the N-terminus are altered by acetylation, methylation, phosphorylation, ubiquitination, sumoylation, deamination or ADP ribosylation [2,3]. The different histone residues and their modifications result in either transcriptionally active or repressive marks. For example, methylation of H3K4, H3K26 and H3K79 is associated with active marks, whereas methylation of H3K9, H3K27 and H4K20 is associated with repressive marks [4].

Histone modifications & their enzymes

Acetylation—Acetylation, the most frequent histone modification [5], often occurs on lysine residues. The neutralization of the basic charge of the histone tails by acetylation reduces their affinity for DNA, thus altering histone–histone interactions between adjacent nucleosomes, as well as the interactions of histones with other regulatory proteins by creating new binding surfaces [6,7]. Acetylated histones usually result in transcriptionally active chromatin, whereas deacetylated histones result in transcriptionally inactive chromatin [7]. Acetylation status (on histone H2, H2 variants, H3 and H4) is affected by two classes of enzymes: HATs and HDACs. HDAC enzymes are classified into: class I (HDAC1, 2, 3 and 8; found in the nucleus), class II (HDAC4, 5, 6, 7 and 9; translocating between the nucleus and the cytoplasm), class III (Sirtuins) and class IV (HDAC11) [1,8]. Class I, II and IV are homologous in their structure and sequence, and their catalytic activity depends on the presence of zinc ions [1]. Sirtuins, however, have no structural or sequence homology to the other HDAC classes, and require NAD⁺ for their catalytic activity [9]. HATs are subdivided into: the GNAT family; the MYST family; and the CBP/p300 family [1,10]. Studies have shown that a small percentage of these enzymes not only act on histones, but also affect nonhistone proteins, such as p53 and pRB [11,12]. Inhibitors developed against epigenetic-modifying enzymes include inhibitors of HATs and HDACs, which are either class-specific or pan-HDACis (discussed later in the article).

Methylation—In contrast to acetylation, methyl groups can be added in a mono, di or tri manner. Lysine residues can be mono-, di- or tri-methylated [13], whereas arginine residues can only be mono- or di-methylated [14]. Increasing the amount of methylation at any particular site intensifies the activation or repressive mark at that site [4], and these levels are maintained by HMTs and recently discovered histone demethylases (HDMTs). HMTs, for lysine residues, are enzymes from the SET domain-containing family of proteins, including SUV39H1, SUV39H2, SET7 and SET9; certain enzymes of this family can methylate histones as well as nonhistone proteins (including p53 and ER- α) [15,16]. Methylation can either lead to transcriptional repression (i.e., H3K9 methylation by SUV39H1 and SUV39H2) or activation (i.e., H3K4 methylation by SET7/9) [15]. One of the first HDMTs to be discovered was LSD1, followed by the discovery of the Jumonji AT-rich interactive domain (JARID1)- and Jumonji C domain (JMJC)-containing family of

HDMTs [17,18]. The JARID1 and JMJC family of proteins (~30 members), along with their mechanism of actions, have been reviewed in papers by Di Stefano and Dyson, and Berry and Janknecht [18,19]. The JMJC domain demethylases act on H3K4, H3K9, H3K27, H3K36 or H3K20, and are dependent on Fe²⁺ and α -ketoglutarate for their activity [19,20]. The JMJC family of demethylases can demethylate mono-, di or tri-methylated residues; however, LSD1 mainly acts on mono- or di-methylated residues [19]. Methylation on histone H3 has two distinct effects: on mDNA it serves as an activation mark, whereas at the rDNA locus it acts as a repression mark [21]. The most widely studied phenomenon of repression is the silencing of the X chromosome in females due to increased levels of the histone methylation marks H3K27Me3 and H3K9Me2 [22,23]. Arginine residues on histones can be methylated by the PRMT1 class of proteins, such as the CARM1 complex [15] on histone H3 residues 2, 17 and 26 [24]. Recent studies have also identified arginine demethylases, such as PADI4, that convert methylarginine to citrulline by the removal of an imine group associated with the methyl residue [25,26].

Phosphorylation—The linker histone H1 mostly undergoes phosphorylation [21]. Phosphorylation of either H1 or H3 is thought to be key in both transcriptional regulation and mitosis [6,27].

Ubiquitination—Histone H2B undergoes ubiquitination [21], with monoubiquitination of H2BK123 during transcription driving methylation of H3K4 and H3K79 [25].

Certain histone marks are found in particular phases of the cell cycle. For instance, diacetylation of histone H4 at K4 and K12 in S phase correlates with histone depositions, whereas phosphorylation of histone H2A (at S1 and T119) and H3 (at T3, S10 and S28) occurs during mitosis [28]. Histone modifications can also interact with DNA modifications to change transcriptional activation. For example, repressive DNA methylation marks are found in the same region as loss of histone acetylation [20]. Studies have also investigated the effect of DNA methylation in the recruitment of several corepressor complexes containing HDAC enzymes and histone modifications, such as H3K9 methylation on the p16 promoter region occurring before CpG methylation [29].

Agents targeting histone-modifying enzymes

HDACis

The most widely studied inhibitors of histone modifiers are HDACis. HDACis developed for class I, II and IV of the HDACs are divided into four classes based on their structure: hydroxamic acids (e.g., panobinostat), cyclic peptides (e.g., romidepsin), short chain fatty acids (e.g., valproic acid) and benzamides (e.g., entinostat) [10]. These inhibitors can be further subdivided according to their specificity for certain classes of HDAC enzymes, thus adding to the complexity of class-specific versus pan-HDACis in the context of clinical use [10]. Agents developed for class III HDACs/Sirtuins include both activators and inhibitors. Activators of Sirtuins, such as resveratrol and synthetically derived SRT1720, have been thought to provide benefit for Type 2 diabetes, cardiovascular disease, neurodegeneration, inflammation and cancer [8]. Inhibitors of Sirtuins, such as salermide and FK866, have efficacy in leukemic cell lines and act by p53-dependent or -independent mechanisms [30,31].

A list of HDACis studied in RCC are listed in Table 1.

HAT inhibitors

Natural compounds such as anacardic acid, garcinol, curcumin and epigallocatechin-3-gallate have been shown to inhibit p300 and PCAF *in vitro* [32,33]. Other HAT inhibitors and their catalytic mechanisms have been described in detail in a review by Furdas *et al.* [34].

HMT inhibitors

One of the first HMT inhibitors (HMTis) developed, chaetocin, showed some specificity for the SUV39 class of HMTs [11]. Recent HMTis include inhibitors of EZH2, SMYD2 and SET7/9 [35]. HMTis that have been identified are classified into those targeting: the SET domain-containing family of proteins (e.g., SAH, sinefugin and chaetocin), non-SET domain HMT DOT1L (e.g., EPZ004777) and arginine methyltransferase inhibitors, which are derivatives of pyrazole amide or analogs of 1,5-diphenyl-1,4-pentadien-3-one that have differential activities when tested *in vitro* [36].

HDMT inhibitors

Although selectivity has been a critical issue, monoamine oxidases and bisguanidine polyamine analogs have been developed as LSD1 inhibitors; and α -ketoglutarate analogs and HIF inhibitors have been shown to have inhibitory effects on the JMJC family of HDMTs [37,38].

Histone modifications & cancer

In various cancer types, including breast, lung and colon, loss of monoacetylation at K16 occurs along with predominant trimethylation of K20 of histone 4 at DNA repetitive sequences [39]. There has been no evidence of these histone alterations and their association with disease progression or prognosis in these cancers [39]. However, the authors speculate that these modifications, similar to DNA hypomethylation and CpG island hypermethylation, may represent additional markers of transformation from normal cells to a malignant phenotype [39]. It has recently been established that certain cancers are characterized by different histone marks and deregulation of histone-modifying enzymes. More specifically, the loss of acetylation and methylation of histones H3 and H4 occur in several cancers, whereas HDAC1, 2 and 6 are overexpressed in prostate, cervical and breast carcinomas, respectively [1,39]. In esophageal and lung cancer, the overexpression of JMJD2C is thought to reduce H3K9Me2 and H3K9Me3 levels, which contributes to tumorigenesis [3]. In another study with prostate cancer samples, a correlation between the overexpression of LSD1 and increasing tumor grade was established [40,41]. SIRT1 deficiency in mice leads to hyperacetylation of p53 and several developmental defects [42]. Acetylation of p53 disrupts its association with Mdm2, thereby allowing p53 to function in response to DNA damage [43]. *In vitro* studies using HDACis on prostate cancer cell lines have shown that p53 acetylation leads to cell cycle arrest through p21 activation [44,45]. Similarly, HDAC1 has been shown to interact with the Rb-E2F complex that is responsible for the regulation of cell cycle genes, including cyclin E [2]. Studies showing differential expression of histone-modifying enzymes, such as HDAC1, 5 and 7A, SIRT1 and SUV39H1 in tumor colorectal samples, and EZH2 and HDAC4 overexpression in breast cancer, have also been established [15]. The aforementioned examples provide the rationale for developing inhibitors targeting histone-modifying enzymes.

Histone modifications, histone-modifying enzymes & RCC

A frequently occurring feature of tumor cells growing as a mass is the ability to grow in low oxygen levels or hypoxia. In recent years, it has been established that correlations exist

between HIF isoforms and histone-modifying enzymes. Qian *et al.* have demonstrated that the interaction between HDAC4 and HIF-1 α protects HIF-1 α from proteosomal degradation. This is antagonized by LAQ284 (no longer in clinical development), which increases acetylation of HIF-1 α , thereby reducing HIF-1 α stability in RCC cell lines [46]. While HIF-1 α has been shown to interact with HDACs, other HMTs, such as JMJD1A, are transcriptionally induced by HIF-2 α under hypoxic conditions in various RCC cell lines [47–49]. JARID1C is an additional HIF-targeted HDMT and is believed to be a tumor suppressor gene in *VHL* null tumors [50]. This, along with the consistent overexpression of JMJD1A in RCC, as compared with normal tissue [48,49], indicates that histone-modifying enzymes are especially significant in RCCs with *VHL* mutations or deletions.

Epithelial-to-mesenchymal transition (EMT) is believed to play a major role in renal fibrosis [51]. In this particular context, upon treatment of human renal proximal tubular epithelial cells with TGF- β (the primary inducer of EMT in the kidney and other organs [12]) and an HDACi, such as trichostatin A, increased global acetylation, thus, preventing EMT in these cells [51]. These results suggest that HDAC inhibition suppresses TGF- β -induced EMT, thereby indicating a potential role for HDACis in reversing EMT.

HDACs are also essential in the regulation of the cell cycle and apoptosis through their effects on nonhistone proteins, such as pRB and p53 [11,12]. Treatment of 786-O cell lines (*in vitro* and *in vivo*) with class I and II HDACi panobinostat resulted in G₂/M arrest and apoptosis indirectly through depletion of Aurora A and B kinases [26]. In addition, HDACi treatment enhanced the apoptotic activity of Ad5-TRAIL in renal proximal tubular cells [52]. HDACis in combination with other agents, including rapamycin (an inhibitor of mTORC1), have also been shown to have enhanced tumor inhibitory growth in xenografts of RCC [53]. However, while HDACis display strong anti-tumor activity toward certain renal cell lines *in vitro* and xenograft tumor models *in vivo*, they do not exhibit similar toxicity or activity levels across various renal cell lines [54]. For instance, romidepsin treatment induced differential levels of acetylation in different renal cell lines suggesting that HDACi activity varies with cellular context [54].

Several individual studies have recently reported *SETD2*, a HMT, as a tumor suppressor gene in RCC [55–57]. An initial study consisting of a large-scale next-generation sequencing of primary RCC genomes identified somatic truncating mutations in *SETD2* (~3%) [56]. *SETD2* mutations were associated with a decrease in H3K36 trimethylation [55], *VHL* mutations and/or hypoxic phenotype [56], as well as a decrease in chromatin remodeling complex protein PBRM1 in clinical trials [57]. It is also important to note that *SETD2* is located on the short arm of the human chromosome 3 (3p21.31), in close proximity to the *VHL* gene, and the 3p region is frequently altered in clear-cell RCCs (ccRCCs) [19]. Mutations in enzymes governing chromatin remodeling are often found in advanced-stage disease; however, these mutations do not correlate with survival [58]. The H3K27 methyltransferase *EZH2* (which is associated with aggressiveness in breast cancer and overexpressed in metastatic prostate cancer) [13] is overexpressed in renal tumor patient samples, compared with their adjacent nontumor tissues [15]. Silencing *EZH2* affects renal cell viability *in vitro* and overall/recurrence-free survival [59]. Conversely, HDMTs governing H3K27 methylation status, *UTX* and *JMJD3*, are overexpressed in ccRCC compared with the adjacent nontumor tissue, which corresponds to the decreased H3K27 methylation in ccRCC [60].

Histone methylation/acetylation levels and the enzymes that modify these play several important roles in different aspects of transcription and cell cycle regulation; however, phosphorylation of H3 is associated with chromosome condensation and segregation during mitosis [61]. In addition, premature chromatin condensation is prevented by a decrease in

H3 phosphorylation and not H1 phosphorylation [62]. In renal proximal tubular epithelial cells, 2,3,5-tris(glutathion-S-yl)hydro-quinone induces phosphorylation of H3 through signaling via reactive oxygen species, which causes premature chromatin condensation and, ultimately, cell death [63]. Table 2 summarizes the various histone marks and histone-modifying enzymes that are commonly found in RCC.

Histone modifications & DNA modifications in RCC

DNA methylation can result in the recruitment of protein complexes containing HDACs, which can further result in the alteration of chromatin structure [64]. This is illustrated in a study using the DNA demethylating agent 5-aza-2'-deoxycytidine and/or genistein, which reactivates the tumor suppressor gene *BTG3* by increasing acetylation of H3 and H4, transcription-activating methylation marks H3K4Me2 and H3K4Me3 at the *BTG* transcription start site [65]. The combination also reduced the repressive methylation marks H3K9Me2 and H3K9Me3 [65]. Enhanced anti-tumor activity has been found in RCC models treated with trichostatin A and entinostat in combination with all-*trans* retinoic acid and *cis*-retinoic acid, respectively, by reactivation of RAR- β_2 [66,67]. Minardi *et al.* demonstrated tumor tissues to have increased global DNA methylation (observed by immunohistochemistry staining), as compared with their nontumor counterparts. Grade 3 and 4 tumors also presented with a higher percentage of DNA methylation [68]. However, this mark was not found to be a prognostic indicator in the study [68]. Different pathways in the cellular context are affected by the above-mentioned modifications and these are summarized in Figure 1.

Histone modifications as prognostic indicators in RCC

Histone modifications have been demonstrated to play an important role in cancer progression. In addition, several histone modifications (mentioned below) are indicative of tumor grade, progression or clinical outcomes. Ellinger *et al.*, reported correlation of lower levels of global H3K4 methylation in 193 RCC tumor samples with poor prognosis and a shorter period of progression-free survival; and the clinically aggressive papillary type II RCC had a lower H3K4 methylation score compared with papillary type I [4]. In the same data set, it was found that low global H3K9 and H4K20 methylation status correlated with higher tumor stage and grade; in addition, lower H3K9Me1 levels were associated with worse progression-free as well as cancer-specific survival [69]. In a different sample set, H3K18Ac and H3K4Me were found to be important markers of recurrence in patients with localized disease and independent predictors for the progression in RCC for all patients, respectively [70]. Global levels of acetylated histone H3 are decreased in RCC compared with nontumor counterparts, with decreased acetylation correlating with increasing nuclear grade and pathological stage of tumors [54,68]. Although there was no correlation with survival, Seligson *et al.* found that patients with localized RCC tumors (grade 1 and 2) who had lower staining of H3K4Me2 and H3K18Ac marks had poorer clinical outcomes [71].

Conclusion

Evaluation of the recent literature indicates that genetic changes occurring in RCC are often accompanied by epigenetic modifications as indicated by loss of the tumor suppressor gene *VHL*, which targets HIF- α for degradation thereby regulating the JMJD1 family of HMTs by HIF-dependent mechanisms [47–50]. Mutations in histone-modifying enzymes, such as *PBRM1*, *SETD2* and *UTX*, and overexpression of others, such as *EZH2* and *JMJD1A*, are now being reported in the literature [55–60]. In RCC, as described above, certain histone modifications correspond to progression-free survival and also correlate with the pathological characteristics of the tumor. However, these are observed as global changes and are being investigated for alterations at the individual gene level, and without functional

validation of histone modifiers it is difficult to predict whether they drive tumorigenesis. *PBRM1* has been reported to reduce proliferation and induce cell cycle arrest following the induction of p21 in breast cancer [72]. Truncating mutations of *PBRM1* in RCC [57], loss of *PBRM1* in renal tumor cell lines along with its association in aggressive tumors [73] have been reported. This suggests that loss of *PBRM1* in RCC can similarly lead to increased cell proliferation and chromosomal instability, which can contribute to RCC progression. Tumor intraheterogeneity studies in RCC show that *SETD2* mutations (although diverse in nature) are common, albeit in a small patient cohort [74]. Mutations in *PBRM1* and *SETD2*, located on the short arm of the 3p region (loss of which is most commonly associated with RCC development [75]), correlate with worse cancer-specific survival [76] and are thought to have tumor suppressive properties [76]. Competing histone modifiers may have an additional effect on the overall levels of methylation or acetylation and should be studied in tandem. The correlation between these changes, their downstream histone alterations and the resulting epigenome, off-target effects and, thus, their importance in RCC development or progression have not been thoroughly established. Dot1l in MLL-driven leukemia [77], LSD1 in medulloblastoma [78], and EZH2 in prostate and lymphomas [79,80] have been shown to be drivers of tumorigenesis. A recent study also showed that certain mutations in H3K27 leads to alteration of methylation status and drives tumorigenesis of pediatric gliomas [81]. However, studies establishing the functional relevance in RCC (using knock-in or knockout cell lines or transgenic mice models) need to be performed to establish the correlation between histone modifiers and tumor formation/progression. These studies will provide a valuable insight into the epigenetic changes occurring in RCC and, with the functional validation of histone modifiers, will lead to the identification of novel therapeutic targets.

Future perspective

Epigenetic changes and enzymes governing these changes in RCC are still in the nascent stage of discovery. Histone acetylation and methylation are dynamic changes and the interplay between these changes needs to be further delineated. Hence, variations in different histone marks observed together may possibly need correlation studies for biomarker discovery or the development of prognostic markers. The dynamic nature of histone modifications also presents a considerable challenge in determining the order of changes that finally lead to the transformation of normal cells into a malignant phenotype. This, along with the intratumor heterogeneity that exists in RCC [74], requires studies of not only multiple tumor sites within the same disease, but also indepth analysis of cell lines *in vitro* for understanding the genes and/or pathways involved in RCC development and progression. Histone-modifying enzymes may have redundant functions and enzyme alterations may not always correspond to their target histone modifications. Hence, it is not only important to develop agents targeting these enzymes, but to also understand how the epigenome is affected by these agents. Furthermore, the off-target effects of these agents have not been elaborated upon. In particular, inhibitors of enzymes that target histone alterations may have differential effects not only on different tumor types, but are also dependent largely upon cellular context (primary vs metastatic tumors). Histone modifications are often studied as a global effect, however, they will need to be examined at the individual gene level to understand the actual effects of these variations. Even if there are no changes in the overall epigenome, alterations at specific gene locations may lead to activation or suppression of an oncogene versus a tumor suppressor gene, respectively. Therefore, advanced proteomics analysis, including studying the chromatin structure at specific gene locations in tumors, will provide further understanding of changes in specific genes and pathways involved in RCC progression.

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References

Papers of special note have been highlighted as:

■ of interest

1. Ellis L, Atadja PW, Johnstone RW. Epigenetics in cancer: targeting chromatin modifications. *Mol Cancer Ther.* 2009; 8(6):1409–1420. [PubMed: 19509247]
2. Hake SB, Xiao A, Allis CD. Linking the epigenetic 'language' of covalent histone modifications to cancer. *Br J Cancer.* 2004; 90(4):761–769. [PubMed: 14970850]
3. Wang GG, Allis CD, Chi P. Chromatin remodeling and cancer, part I: covalent histone modifications. *Trends Mol Med.* 2007; 13(9):363–372. [PubMed: 17822958]
4. Ellinger J, Kahl P, Mertens C, et al. Prognostic relevance of global histone H3 lysine 4 (H3K4) methylation in renal cell carcinoma. *Int J Cancer.* 2010; 127(10):2360–2366. [PubMed: 20162570]
5. Turner BM. Histone acetylation and an epigenetic code. *BioEssays.* 2000; 22(9):836–845. [PubMed: 10944586]
6. Iizuka M, Smith MM. Functional consequences of histone modifications. *Curr Opin Genet Dev.* 2003; 13(2):154–160. [PubMed: 12672492]
7. Grant P. A tale of histone modifications. *Genome Biol.* 2001; 2(4):3.1–3.6.
8. Milne JC, Denu JM. The Sirtuin family: therapeutic targets to treat diseases of aging. *Curr Opin Chem Biol.* 2008; 12(1):11–17. [PubMed: 18282481]
9. Bolden JE, Peart MJ, Johnstone RW. Anticancer activities of histone deacetylase inhibitors. *Nat Rev Drug Discov.* 2006; 5(9):769–784. [PubMed: 16955068]
10. Cortez CC, Jones PA. Chromatin, cancer and drug therapies. *Mutat Res.* 2008; 647(1–2):44–51. [PubMed: 18691602]
11. Greiner D, Bonaldi T, Eskeland R, Roemer E, Imhof A. Identification of a specific inhibitor of the histone methyltransferase SU(VAR)3–9. *Nat Chem Biol.* 2005; 1(3):143–145. [PubMed: 16408017]
12. Zeisberg M, Kalluri R. The role of epithelial-to-mesenchymal transition in renal fibrosis. *J Mol Med (Berl).* 2004; 82(3):175–181. [PubMed: 14752606]
13. Kleer CG, Cao Q, Varambally S, et al. EZH2 is a marker of aggressive breast cancer and promotes neoplastic transformation of breast epithelial cells. *Proc Natl Acad Sci USA.* 2003; 100(20):11606–11611. [PubMed: 14500907]
14. Sato A, Asano T, Ito K, Sumitomo M, Asano T. Suberoylanilide hydroxamic acid (SAHA) combined with bortezomib inhibits renal cancer growth by enhancing histone acetylation and protein ubiquitination synergistically. *BJU Int.* 2011; 109(8):1258–1268. [PubMed: 21895936]
15. Ozdag H, Teschendorff A, Ahmed A, et al. Differential expression of selected histone modifier genes in human solid cancers. *BMC Genomics.* 2006; 7(1):90. [PubMed: 16638127]
16. Pradhan S, Chin HG, Estève P-O, Jacobsen SE. SET7/9 mediated methylation of non-histone proteins in mammalian cells. *Epigenetics.* 2009; 4(6):383–387. [PubMed: 19684477]
17. Chi P, Allis CD, Wang GG. Covalent histone modifications – miswritten, misinterpreted and mis-erased in human cancers. *Nat Rev Cancer.* 2010; 10(7):457–469. [PubMed: 20574448]
18. Di Stefano L, Dyson NJ. The emerging roles for histone demethylases in the modulation of signaling pathways. *Biomol Concepts.* 2013; 4(1):13–27.
19. Berry WL, Janknecht R. KDM4/JMJD2 histone demethylases: epigenetic regulators in cancer cells. *Cancer Res.* 2013; 73(10):2936–2942. [PubMed: 23644528]
20. Nightingale KP, O'Neill LP, Turner BM. Histone modifications: signalling receptors and potential elements of a heritable epigenetic code. *Curr Opin Genet Dev.* 2006; 16(2):125–136. [PubMed: 16503131]

21. He H, Lehming N. Global effects of histone modifications. *Brief Funct Genomic Proteomic*. 2003; 2(3):234–243. [PubMed: 15239926]
22. Bártová E, Krejčí J, Harnířová A, Galiová G, Kozubek S. Histone modifications and nuclear architecture: a review. *J Histochem Cytochem*. 2008; 56(8):711–721. [PubMed: 18474937]
23. Li E. Chromatin modification and epigenetic reprogramming in mammalian development. *Nat Rev Genet*. 2002; 3(9):662–673. [PubMed: 12209141]
24. Mahalingam D, Medina EC, Esquivel JA, et al. Vorinostat enhances the activity of temsirolimus in renal cell carcinoma through suppression of survivin levels. *Clin Cancer Res*. 2010; 16(1):141–153. [PubMed: 20028765]
25. Latham JA, Dent SYR. Cross-regulation of histone modifications. *Nat Struct Mol Biol*. 2007; 14(11):1017–1024. [PubMed: 17984964]
26. Cha TL, Chuang MJ, Wu ST, et al. Dual degradation of Aurora A and B kinases by the histone deacetylase inhibitor LBH589 induces G₂-M arrest and apoptosis of renal cancer cells. *Clin Cancer Res*. 2009; 15(3):840–850. [PubMed: 19188154]
27. Cheung P, Allis CD, Sassone-Corsi P. Signaling to chromatin through histone modifications. *Cell*. 2000; 103(2):263–271. [PubMed: 11057899]
28. Peterson CL, Laniel MA. Histones and histone modifications. *Curr Biol*. 2004; 14(14):R546–R551. [PubMed: 15268870]
29. Francois F. DNA methylation and histone modifications: teaming up to silence genes. *Curr Opin Genet Dev*. 2005; 15(5):490–495. [PubMed: 16098738]
30. Cea M, Soncini D, Fruscione F, et al. Synergistic interactions between HDAC and Sirtuin inhibitors in human leukemia cells. *PLoS One*. 2011; 6(7):e22739. [PubMed: 21818379]
31. Lara E, Mai A, Calvanese V, et al. Salermide, a Sirtuin inhibitor with a strong cancer-specific proapoptotic effect. *Oncogene*. 2009; 28(8):1168–1168.
32. Choi K-C, Jung MG, Lee YH, et al. Epigallocatechin-3-gallate, a histone acetyltransferase inhibitor, inhibits EBV-induced B lymphocyte transformation via suppression of RelA acetylation. *Cancer Res*. 2009; 69(2):583–592. [PubMed: 19147572]
33. Cui L, Miao J, Furuya T, et al. Histone acetyltransferase inhibitor anacardic acid causes changes in global gene expression during *in vitro* plasmodium falciparum development. *Eukaryot Cell*. 2008; 7(7):1200–1210. [PubMed: 18487348]
34. Furdas SD, Kannan S, Sippl W, Jung M. Small molecule inhibitors of histone acetyltransferases as epigenetic tools and drug candidates. *Arch Pharm (Weinheim)*. 2012; 345(1):7–21. [PubMed: 22234972]
35. Wagner T, Jung M. New lysine methyltransferase drug targets in cancer. *Nat Biotechnol*. 2012; 30(7):622–623. [PubMed: 22781684]
36. Zagni C, Chiacchio U, Rescifina A. Histone methyltransferase inhibitors: novel epigenetic agents for cancer treatment. *Curr Med Chem*. 2013; 20(2):167–185. [PubMed: 23210854]
37. Lohse B, Kristensen JL, Kristensen LH, et al. Inhibitors of histone demethylases. *Bioorg Med Chem*. 2011; 19(12):3625–3636. [PubMed: 21596573]
38. Sayegh J, Cao J, Zou MR, et al. Identification of small molecule inhibitors of jumonji AT-rich interactive domain 1B (JARID1B) histone demethylase by a sensitive high-throughput screen. *J Biol Chem*. 2013; 288(13):9408–9417. [PubMed: 23408432]
39. Fraga MF, Ballestar E, Villar-Garea A, et al. Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. *Nat Genet*. 2005; 37(4):391–400. [PubMed: 15765097]
40. Metzger E, Wissmann M, Yin N, et al. LSD1 demethylates repressive histone marks to promote androgen-receptor-dependent transcription. *Nature*. 2005; 437(7057):436–439. [PubMed: 16079795]
41. Kurdistani SK. Histone modifications as markers of cancer prognosis: a cellular view. *Br J Cancer*. 2007; 97(1):1–5. [PubMed: 17592497]
42. Cheng H-L, Mostoslavsky R, Saito SI, et al. Developmental defects and p53 hyperacetylation in Sir2 homolog (SIRT1)-deficient mice. *Proc Natl Acad Sci USA*. 2003; 100(19):10794–10799. [PubMed: 12960381]

43. Li M, Luo J, Brooks CL, Gu W. Acetylation of p53 inhibits its ubiquitination by Mdm2. *J Biol Chem.* 2002; 277(52):50607–50611. [PubMed: 12421820]
44. Roy S, Packman K, Jeffrey R, Tenniswood M. Histone deacetylase inhibitors differentially stabilize acetylated p53 and induce cell cycle arrest or apoptosis in prostate cancer cells. *Cell Death Differ.* 2005; 12(5):482–491. [PubMed: 15746940]
45. Kai L, Samuel SK, Levenson AS. Resveratrol enhances p53 acetylation and apoptosis in prostate cancer by inhibiting MTA1/NuRD complex. *Int J Cancer.* 2010; 126(7):1538–1548. [PubMed: 19810103]
46. Qian DZ, Kachhap SK, Collis SJ, et al. Class II histone deacetylases are associated with VHL-independent regulation of hypoxia-inducible factor 1 α . *Cancer Res.* 2006; 66(17):8814–8821. Demonstrates that class II HDACs not only associate with HIF, but also regulate HIF-1 α activity. [PubMed: 16951198]
47. Beyer S, Kristensen MM, Jensen KS, Johansen JV, Staller P. The histone demethylases JMJD1A and JMJD2B are transcriptional targets of hypoxia-inducible factor HIF. *J Biol Chem.* 2008; 283(52):36542–36552. Demonstrates that histone demethylases are targets of HIF, an important pathway most frequently upregulated in renal cell carcinomas. [PubMed: 18984585]
48. Krieg AJ, Rankin EB, Chan D, Razorenova O, Fernandez S, Giaccia AJ. Regulation of the histone demethylase JMJD1A by hypoxia-inducible factor 1{alpha} enhances hypoxic gene expression and tumor growth. *Mol Cell Biol.* 2010; 30(1):344–353. [PubMed: 19858293]
49. Guo X, Shi M, Sun L, et al. The expression of histone demethylase JMJD1A in renal cell carcinoma. *Neoplasma.* 2011; 58(2):153–157. [PubMed: 21275466]
50. Niu X, Zhang T, Liao L, et al. The von Hippel-Lindau tumor suppressor protein regulates gene expression and tumor growth through histone demethylase JARID1C. *Oncogene.* 2012; 31(6):776–786. [PubMed: 21725364]
51. Yoshikawa M, Hishikawa K, Marumo T, Fujita T. Inhibition of histone deacetylase activity suppresses epithelial-to-mesenchymal transition induced by TGF- β 1 in human renal epithelial cells. *J Am Soc Nephrol.* 2007; 18(1):58–65. [PubMed: 17135397]
52. Vanoosten RL, Earel JK Jr, Griffith TS. Enhancement of Ad5-TRAIL cytotoxicity against renal cell carcinoma with histone deacetylase inhibitors. *Cancer Gene Ther.* 2006; 13(6):628–632. [PubMed: 16456549]
53. Verheul HMW, Salumbides B, Van Erp K, et al. Combination strategy targeting the hypoxia inducible factor-1 α with mammalian target of rapamycin and histone deacetylase inhibitors. *Clin Cancer Res.* 2008; 14(11):3589–3597. [PubMed: 18519793]
54. Kanao K, Mikami S, Mizuno R, Shinojima T, Murai M, Oya M. Decreased acetylation of histone H3 in renal cell carcinoma: a potential target of histone deacetylase inhibitors. *J Urol.* 2008; 180(3):1131–1136. [PubMed: 18639283]
55. Duns G, Van Den Berg E, Van Duivenbode I, et al. Histone methyltransferase gene *SETD2* is a novel tumor suppressor gene in clear cell renal cell carcinoma. *Cancer Res.* 2010; 70(11):4287–4291. [PubMed: 20501857]
56. Dalglish GL, Furge K, Greenman C, et al. Systematic sequencing of renal carcinoma reveals inactivation of histone modifying genes. *Nature.* 2010; 463(7279):360–363. Demonstrates that histone demethylases are targets of HIF, an important pathway most frequently upregulated in renal cell carcinomas. [PubMed: 20054297]
57. Varela I, Tarpey P, Raine K, et al. Exome sequencing identifies frequent mutation of the SWI/SNF complex gene *PBRM1* in renal carcinoma. *Nature.* 2011; 469(7331):539–542. *PBRM1* mutations were found in 41% of clinical samples, suggesting that it plays an important role in renal cell carcinomas. [PubMed: 21248752]
58. Hakimi AA, Chen Y-B, Wren J, et al. Clinical and pathologic impact of select chromatin-modulating tumor suppressors in clear cell renal cell carcinoma. *Eur Urol.* 2012; 63(5):848–854. [PubMed: 23036577]
59. Sakurai T, Bilim VN, Ugolkov AV, et al. The enhancer of zeste homolog 2 (EZH2), a potential therapeutic target, is regulated by miR-101 in renal cancer cells. *Biochem Biophys Res Commun.* 2012; 422(4):607–614. [PubMed: 22609199]

60. Shen Y, Guo X, Wang Y, et al. Expression and significance of histone H3K27 demethylases in renal cell carcinoma. *BMC Cancer*. 2012; 12(1):470. [PubMed: 23057811]
61. Richon V, Zhou X, Rifkind R, Marks P. Histone deacetylase inhibitors: development of suberoylanilide hydroxamic acid (SAHA) for the treatment of cancers. *Blood Cells Mol Dis*. 2001; 27(1):260–264. [PubMed: 11358386]
62. Ajiro K, Nishimoto T. Specific site of histone H3 phosphorylation related to the maintenance of premature chromosome condensation. Evidence for catalytically induced interchange of the subunits. *J Biol Chem*. 1985; 260(29):15379–15381. [PubMed: 4066674]
63. Tikoo K, Lau SS, Monks TJ. Histone H3 phosphorylation is coupled to poly-(ADP-Ribosylation) during reactive oxygen species-induced cell death in renal proximal tubular epithelial cells. *Mol Pharmacol*. 2001; 60(2):394–402. [PubMed: 11455028]
64. Nan X, Ng HH, Johnson CA, et al. Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. *Nature*. 1998; 393(6683):386–389. [PubMed: 9620804]
65. Majid S, Dar AA, Ahmad AE, et al. BTG3 tumor suppressor gene promoter demethylation, histone modification and cell cycle arrest by genistein in renal cancer. *Carcinogenesis*. 2009; 30(4):662–670. [PubMed: 19221000]
66. Touma SE, Goldberg JS, Moench P, et al. Retinoic acid and the histone deacetylase inhibitor trichostatin inhibit the proliferation of human renal cell carcinoma in a xenograft tumor model. *Clin Cancer Res*. 2005; 11(9):3558–3566. [PubMed: 15867260]
67. Wang X-F, Qian DZ, Ren M, et al. Epigenetic modulation of retinoic acid receptor β 2 by the histone deacetylase inhibitor MS-275 in human renal cell carcinoma. *Clin Cancer Res*. 2005; 11(9):3535–3542. [PubMed: 15867257]
68. Minardi D, Lucarini G, Filosa A, et al. Do DNA-methylation and histone acetylation play a role in clear cell renal carcinoma? Analysis of radical nephrectomy specimens in a long-term follow-up. *Int J Immunopathol Pharmacol*. 2011; 24(1):149–158. [PubMed: 21496397]
69. Rogenhofer S, Kahl P, Holzapfel S, Von Ruecker A, Mueller SC, Ellinger J. Decreased levels of histone H3K9me1 indicate poor prognosis in patients with renal cell carcinoma. *Anticancer Res*. 2012; 32(3):879–886. [PubMed: 22399607]
70. Mosashvili D, Kahl P, Mertens C, et al. Global histone acetylation levels: prognostic relevance in patients with renal cell carcinoma. *Cancer Sci*. 2010; 101(12):2664–2669. [PubMed: 20825416]
71. Seligson DB, Horvath S, McBrien MA, et al. Global levels of histone modifications predict prognosis in different cancers. *Am J Pathol*. 2009; 174(5):1619–1628. [PubMed: 19349354]
72. Xia W, Nagase S, Montia AG, et al. BAF180 is a critical regulator of p21 induction and a tumor suppressor mutated in breast cancer. *Cancer Res*. 2008; 68(6):1667–1674. [PubMed: 18339845]
73. Pawłowski R, Mühl SM, Sulser T, Krek W, Moch H, Schraml P. Loss of PBRM1 expression is associated with renal cell carcinoma progression. *Int J Cancer*. 2013; 132(2):E11–E17. [PubMed: 22949125]
74. Gerlinger M, Rowan AJ, Horswell S, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N Engl J Med*. 2012; 366(10):883–892. Compared different tumor sites within the primary tumor with its metastatic tumors and showed diverse genetic alterations, indicating intratumor heterogeneity in clinical tumors. [PubMed: 22397650]
75. Van Den Berg A, Buys CHCM. Involvement of multiple loci on chromosome 3 in renal cell cancer development. *Genes Chromosomes Cancer*. 1997; 19(2):59–76. [PubMed: 9171996]
76. Hakimi AA, Ostrovskaya I, Reva B, et al. Adverse outcomes in clear cell renal cell carcinoma with mutations of 3p21 epigenetic regulators BAP1 and SETD2: a report by MSKCC and the KIRC TCGA research network. *Clin Cancer Res*. 2013; 19(12):3259–3267. [PubMed: 23620406]
77. Bernt Kathrin M, Zhu N, Sinha Amit U, et al. MLL-rearranged leukemia is dependent on aberrant H3K79 methylation by DOT1L. *Cancer Cell*. 2011; 20(1):66–78. [PubMed: 21741597]
78. Pajtler K, Weingarten C, Thor T, et al. The KDM1A histone demethylase is a promising new target for the epigenetic therapy of medulloblastoma. *Acta Neuropathol Comm*. 2013; 1(1):19.
79. Koh CM, Iwata T, Zheng Q, Bethel C, Yegnasubramanian S, De Marzo AM. Myc enforces overexpression of EZH2 in early prostatic neoplasia via transcriptional and post-transcriptional mechanisms. *Oncotarget*. 2011; 2(9):669–683. [PubMed: 21941025]

80. McCabe MT, Ott HM, Ganji G, et al. EZH2 inhibition as a therapeutic strategy for lymphoma with EZH2-activating mutations. *Nature*. 2012; 492(7427):108–112. [PubMed: 23051747]
81. Chan K-M, Fang D, Gan H, et al. The histone H3.3K27M mutation in pediatric glioma reprograms H3K27 methylation and gene expression. *Genes Dev*. 2013; 27(9):985–990. [PubMed: 23603901]
82. Jones J, Juengel E, Mickuckyte A, et al. Valproic acid blocks adhesion of renal cell carcinoma cells to endothelium and extracellular matrix. *J Cell Mol Med*. 2009; 13(8b):2342–2352. [PubMed: 19067765]
83. Kato Y, Yoshimura K, Shin T, et al. Synergistic *in vivo* antitumor effect of the histone deacetylase inhibitor MS-275 in combination with interleukin 2 in a murine model of renal cell carcinoma. *Clin Cancer Res*. 2007; 13(15):4538–4546. [PubMed: 17671140]
84. Rogenhofer S, Kahl P, Mertens C, et al. Global histone H3 lysine 27 (H3K27) methylation levels and their prognostic relevance in renal cell carcinoma. *BJU Int*. 2011; 109(3):459–465. [PubMed: 21810159]
85. Fritzsche F, Weichert W, Roske A, et al. Class I histone deacetylases 1, 2 and 3 are highly expressed in renal cell cancer. *BMC Cancer*. 2008; 8(1):381. [PubMed: 19099586]

Executive summary

Histone modifications & their enzymes

- Histones can be acetylated, methylated, phosphorylated or ubiquitinated. Enzymes governing acetylation and methylation are extensively studied and are categorized as histone acetyltransferases/HDACs and histone methyltransferases/demethylases, respectively.

Agents targeting histone-modifying enzymes

- Inhibitors have been developed to target the above-mentioned enzymes and are currently being investigated for their specificity.

Histone modifications & cancer

- Different cancers present with modifications at specific histone residues and have differential expression of histone-modifying enzymes.

Histone modifications, histone-modifying enzymes & renal cell carcinoma

- Specifically, renal cell carcinomas are characterized by overexpression of class I HDACs, JMJD1A and EZH2, and mutations in *SETD2* and *PBRM1*.

Histone modifications as prognostic indicators in renal cell carcinoma

- Renal cell carcinomas also have alterations in histone methylation at specific residues; however, only a small percentage of these alterations can be used as prognostic or diagnostic marks.

Figure 1. Pathways in renal cell carcinoma affected by changes in histone marks, histone-modifying enzymes or their inhibitors, including the mTOR pathway, retinoic acid pathway, epithelial-to-mesenchymal transition, cell cycle and hypoxia

These effects and their significance in RCC are discussed in detail in the text.

CRA: *Cis*-retinoic acid; EMT: Epithelial-to-mesenchymal transition; PFS: Progression-free survival; RCC: Renal cell carcinoma; TSA: Trichostatin A.

Table 1

Agents targeting HDAC enzymes studied in renal cell carcinoma.

Agent	Target	Treatment condition	Model	Mode of action	Ref.
Vorinostat [†]	Class I, II and IV HDACs	In combination with proteasome inhibitor bortezomib	<i>In vitro/in vivo</i>	Histone acetylation and protein ubiquitination	[14]
		In combination with temsirolimus	<i>In vitro/in vivo</i>	Reduced survivin	[24]
Panobinostat [†]	Class I, II and IV HDACs	Single agent	<i>In vitro</i>	Aurora kinase inhibition	[26]
Dacinostat	Class I, II and IV HDACs	Single agent	<i>In vitro</i>	HIF-1 α acetylation	[46]
Valproic acid	Class I, II and IV HDACs	In combination with IFN- α	<i>In vivo</i>	Reduced adhesion to endothelium and ECM	[82]
TSA	Class I, II and IV HDACs	Single agent	<i>In vitro</i>	Markers of EMT reduced	[51]
		In combination with Ad5-TRAIL	<i>In vitro</i>	Apoptosis induction	[52]
		In combination with ATRA	<i>In vitro/in vivo</i>	RAR- β re-expression	[66]
Entinostat [†]	Class I HDACs	In combination with IL-2	<i>In vivo</i>	Reduced Tregs	[83]
		In combination with CRA	<i>In vitro/in vivo</i>	RAR- β re-expression	[67]
Romidepsin	Class I, II and IV HDACs	Single agent	<i>In vitro</i>	H3 acetylation and apoptosis	[54]

[†] Agents that have been tested, as a single agent or in combination, in cell lines or xenograft models of renal cell carcinoma.

ATRA: All-trans retinoic acid; CRA: Cis-retinoic acid; ECM: Extracellular matrix; EMT: Epithelial-to-mesenchymal transition; TSA: Trichostatin A.

Table 2
Changes in different histone marks and histone modifiers occurring in renal cell carcinoma.

Histone modifiers/histone marks	Sample type	Results	Samples (n)	p-value	Ref.
H3K27 methylation	Patient RCC tumors	Lower levels of methylation correlated with higher grade and relapse	142	<0.005	[84]
H3K4 methylation	Patient RCC tumors	Independent predictor of RCC progression-free survival	142	0.039	[4]
H3K18 acetylation	Patient RCC tumors	Independent predictor of cancer progression after surgery for localized disease	142	0.003	[70]
Histone H3 acetylation	Patient RCC tumors	Decreased acetylated histone H3 associated with higher-grade/advanced stage	20	<0.05	[54]
PRBM1	Patient RCC tumors	Truncating mutations in 41% of clinical samples	257	–	[57]
JARID1C	Patient RCC tumors	Truncating tumors in 3% of clinical samples	407	–	[56]
SETD2	Patient RCC tumors and cell lines	Premature terminating codon of <i>SETD2</i> consistent with decreased H3K36Me3	7 out of 10 cell lines, 3% of 407 cases	–	[55–57]
HDAC1 and HDAC2	Patient RCC tumors	Overexpression of HDAC1 and HDAC2 correlated with higher proliferation (Ki-67 staining)	60% of 106 cases	<0.005 for Ki-67	[85]

RCC: Renal cell carcinoma.