Chapter 4

The Landscape of Histone Modification in Cancer Metastasis

Zhaoping Qiu, Jianlin Wang and Yadi Wu

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.78383

Abstract

Metastasis represents one of the most devastating aspects of cancer. Epithelial to mesenchymal transition (EMT) has been shown to play a critical role in tumorigenic metastasis. During metastatic progression, both genetic and epigenetic modifications endow cancer cells with properties that modulate the capacity for metastatic success. Histone modification is profoundly altered in cancer cells and contributes to cancer metastasis by controlling different metastatic phenotypes. Here, we first review histone modifications and discuss their roles in EMT and metastasis, with a particular focus on histone methylation and acetylation. Second, we review the major histone modification enzymes that control chromatin in cancer metastasis. Third, we discuss the transcriptional regulation concerted by these enzymes with EMT transcription factors at different molecular layers. Finally, we discuss pharmacologic manipulation of histone modification enzymes for metastasis treatment. A comprehensive understanding of histone modification in metastasis will not only provide new insights into our knowledge of cancer progression and metastasis, but also offer a novel approach for the development of innovative therapeutic strategies.

Keywords: EMT, epigenetic, histone modification, metastasis, inhibitor

1. Introduction

Approximately 90% of cancer deaths are caused by metastasis [1]. Cancer metastasis is an exceedingly complex process involving tumor cell motility, intravasation, and circulation in the blood or lymph system, extravasation, and growth in new tissues and organs [2, 3]. During invasion, tumor cells lose cell–cell adhesion, gain mobility and leave the site of the primary tumor to invade adjacent tissues. In intravasation, tumor cells penetrate through the endothelial barrier and enter the systemic circulation through blood and lymphatic vessels. In

extravasation, cells that survive the anchorage-independent growth conditions in the bloodstream attach to vessels at distant sites and leave the bloodstream. Finally, in metastatic colonization, tumor cells form macrometastases in the new host environment [2, 3]. All of these steps, from initial breakdown of tissue structure, through increased invasiveness, and ultimately distribution and colonization throughout the body, are developmental characteristics of the processes, epithelial to mesenchymal transition (EMT) and mesenchymal to epithelial transition (MET). EMT is a distinctive morphogenic process that occurs during embryonic development, chronic degeneration and fibrosis of organs, and tumor invasion and metastasis [4–6]. The similarity of genetic controls and biochemical mechanisms that underlie the acquisition of an invasive phenotype and the subsequent systemic spread of cancer cells highlights the concept that tumor cells usurp this developmental pathway for metastatic dissemination. In total, EMT provides tumor cells with the proclivity for early metastasis, renders them resistance to therapeutics and endows cells with cancer stem cell (CSC)-like traits [6].

The hallmark of EMT is the loss of E-cadherin expression, an important caretaker of the epithelial phenotype. Loss of E-cadherin expression is often correlated with the tumor grade and stage because it results in the disruption of the cell–cell adhesion and an increase in the nuclear β-catenin. Several transcription factors have been implicated in the regulation of EMT, including the zinc finger proteins of the SNAIL family (SNAIL1/2/3), the basic helix–loop–helix (HLH) factor TWIST (TWIST1/2, E12/E47), and two double zinc finger and homeodomain ZEB family (ZEB1/ZEB2). These factors act as a molecular switch for the EMT program by repressing a subset of common genes that encode cadherins, claudins, integrins, mucins, plakophilin, occludin and ZO1, and thereby induce EMT.

EMT is a dynamic process that preserves plasticity [6]. In this instance, the reprogramming of gene expression provides a rapid and dynamic regulatory mechanism to switch between the epithelial and mesenchymal conditions during cancer progression. Consistent with this, these EMT-activating transcriptional factors (EMT-TFs) are liable proteins that turn over rapidly and do not have long residence times at their binding sites. Interestingly, disseminating cells orchestrate a metastatic cascade without a concomitant need for genomic mutations, which indicates that this dissemination is epigenetically templated. Both EMT and epigenetic modification (DNA methylation and histone modifications) are dynamic and efficient processes during development, differentiation and carcinogenesis. These studies indicate that the epigenetic mechanism plays an important role in modulating the induction of EMT and tumor metastasis.

2. Epigenetics and histone modification

2.1. Epigenetic and chromatin structure

The term “epigenetics” was first coined by Conrad H. Waddington in his Principles of Embryology textbook in 1942 to designate a process in which gene regulation modulated development. The final definition of epigenetics was confirmed in the Epigenetic Meeting held by the Banbury Conference Center and Cold Spring Harbor Laboratory in 2008 as “a stably heritable phenotype resulting from changes in a chromosome without alterations in

http://dx.doi.org/10.5772/intechopen.78383

the DNA sequence." In general, epigenetic regulation includes changes that impact histone modification, DNA methylation, histone variants, chromatin looping, noncoding RNAs and nucleosomal occupancy and remodeling.

Genomic DNA is tightly packaged in chromatin by both histone and nonhistone proteins in the nucleus of eukaryotic cells. The basic chromatin subunits, nucleosomes, are formed by wrapping 146 base pairs (bp) of DNA around an octamer of four core histones: H2A, H2B, H3, and H4. Whereas the nucleosomal core is compact, eight flexible lysine-rich histone tails protrude from the nucleosome that modulate internucleosomal contacts and provide binding sites for nonhistone proteins. From the perspective of gene transcription, chromatin structure can be divided into two distinct categories: euchromatin and heterochromatin. "Euchromatin" is an open chromatin structure that affords accessibility of transcription factors to DNA, resulting in gene activation. In contrast, "heterochromatin" is a closed chromatin structure with a low interaction between transcription factors and the genome, leading to gene repression.

2.2. Histone modifications and histone code hypothesis

The histone code hypothesis was first proposed by Strahl and Allis in 2000. They suggested that "multiple histone modifications, acting in a combinatorial or sequential fashion on one or multiple histone tails, specify unique downstream functions" [7]. The histone "language," based on this "histone code," is encoded in these modifications and read by chromatin-associated proteins. So far, several histone post-translational modifications (PTMs) have been identified, including acetylation, methylation, phosphorylation, ubiquitination, sumoylation, ADP ribosylation, proline isomerization, biotinylation, citrullination and their various combinations [8]. These modifications constitute a unique "code" to regulate histone interactions with other proteins and thereby allow modification (either overcoming or solidifying) of the intrinsic histone barrier to transcription. Accordingly, with these modifications, the various proteins that add, recognize and remove these PTMs, termed writers, readers and erasers, respectively, have been identified and structurally characterized. While "writer" and "eraser" enzymes modify histones by catalyzing the addition and removal of histone PTMs, respectively; "reader" proteins recognize these modified histones and 'translate' the PTMs by executing distinct cellular programs. In addition, numerous core histone chaperones also facilitate core histone deposition or removal from chromatin. Histone modifications control dynamic transitions between transcriptionally active or silent chromatin states, and regulate the transcription of genetic information encoded in DNA (the "genetic code") [9]. Analyses of genome-wide profiles of histone modifications and gene expression identified three distinct types of configurations: repressed, active and bivalent. First, the closed chromatin configuration is linked with suppression of gene transcription, the repressed state. Second, an open chromatin configuration is associated with active gene transcription, the active state. Third, bivalent chromatin consists of domains that have both repressive and active histone markers, predominately on developmental genes, which allows phenotypic plasticity before committing to a specific cell fate.

During EMT, histone modifications provide a regulatory platform to orchestrate the repression or activation between epithelial and mesenchymal genes. Here, we only focus on the well-studied histone acetylation and methylation, and discuss their diverse regulation and role in transcriptional reprogramming of tumor metastasis (**Table 1**).

Modification				Writer	Eraser
Acetylation	H3K9, H3K27			P300, PCAF, MOF, Tip60	HDAC
Methylation	Lysine	Activating	H3K4	MLL1–4, Set 1a, Set 1b, Ash1L, Set7/9, and SMYD	KDM1, KDM2, KDM5, JARID2 and NO66
			H3K36	KMT3, NSD2, NSD3 and SMYD	KDM2, KDM4/JMJD and NO66
			H3K79	KMT4	PHF8
		Repressing	H3K9	KMT1	KDM1, KDM3, KDM4, PHF8, and JHDM1D
			H3K20	KMT5, KMT7 and SET8	Unknown
			H3K27	PRC2	KDM6, UTY and JHDM1D
	Arginine	H4R3me2a, H3R8, H3R2me2a,H4R3me2s		PRMT1, PRMT2, PRMT3, CARM1/PRMT4, PRMT5, PRMT6, PRMT7, PRMT8, and PRMT9	Unknown

Table 1. Histone modifying enzymes involved in metastasis.

3. Histone acetylation

Evidence has established that histone acetylation is associated with gene activation. A genome-wide study demonstrated that all forms of histone acetylation are positively correlated with gene expression [10]. Histones contain amino acids with basic side chains that are positively charged and attracted to the negatively charged genomic DNA. Ultimately, histone acetylation reduces the positive charge on histones and decreases the interaction between nucleosomes and DNA. Generally, histone acetylation is greater in the promoters of active genes and influences both the initiation and elongation of gene transcription. Histone acetylation also stabilizes the binding of chromatin remodeling factors at promoter regions and induces nucleosomes unfolding as well as reduces nucleosome occupancy. The acetylation state of a chromatin leads to the structural modification of the nucleosome. Acetylated (or hyperacetylated) chromatin is in a relaxed confirmation and associated with active transcription. In contrast, deacetylated (or hypoacetylated) chromatin is condensed and supercoiled, and is associated with transcriptional silencing (and, in the context of cancer, the inhibition of tumor suppressor genes).

Histone acetylation is a rapid and reversible process controlled by histone acetyltransferases (HATs) and histone deacetylases (HDAC)s. The HATs transfer acetyl groups from acetyl-coenzyme A (CoA) to the ε-amino groups of lysine residues in histone tails, which results in gene activation. HATs contain a bromodomain that recognizes and binds to acetylated histones, categorized into three major families, GNAT (GCN5 and PCAF), MYST (Tip60 and MOF), and CBP/p300. The HDACs remove acetyl groups from lysine residues, leading to gene silencing. Sequence homology, subcellular location, and the features of the catalytic site have been used to classify the 18 members of the human HDAC family into 4 groups: class

I (HDACs 1, 2, 3, and 8), class II (HDACs 4, 5, 6, 7, 9, and 10), class III (SIRT1, SIRT2, SIRT3, SIRT4, SIRT5, SIRT6, and SIRT7), and class IV (HDAC11) [11]. Class I HDACs have sequence homology to class II HDACs and class IV HDACs but not class III HDACs. Class I, II, and IV HDACs are zinc-dependent, whereas class III HDACs are nicotinamide adenine dinucleotide $(NAD)^+$-dependent. Genome-wide mapping of the binding of HATs and HDACs to the human genome demonstrate that these enzymes regulate the activation and repression of transcription, respectively. The dysfunctional balance between acetylation and deacetylation is clearly associated with human disease and tumorigenesis.

p300 cooperates in an epigenetic manner with a DOT1L-c-Myc complex to induce EMT in breast metastasis [12]. The elevated level of p300-DOT1L-c-Myc is associated with the acquisition of CSC-like properties during breast carcinogenesis, which implies that p300 functions as a potential oncogene to influence the clinical outcome of breast cancer. In addition, transforming growth factor-beta (TGF-β) and WNT co-operated to mediate EMT. TGF-β induces the translocation of β-catenin to the nucleus where it binds to T- cell Factor (TCF); this complex recruits p300/CBP to assemble a transcriptional complex on target gene promoters that promotes EMT signaling. Intriguingly, over-expression of SNAIL/SLUG up-regulates TGF-β-receptor 2 (TGFBR2) expression with an increase of H3K9 acetylation on TGFBR2 promoter to increase TGF-β signaling [13]. In contrast, however, p300 was reportedly recruited by the hepatocyte nuclear factor (HNF) 3 to the E-cadherin promoter, increasing expression, and thus reducing the metastatic potential of breast cancer cells [14]. Similarly, the p300-CBP-associated factor (PCAF) has functions that can differ among cancer types. PCAF is an anti-oncogene and its expression is down-regulated and negatively correlated with tumor metastasis in hepatocellular carcinoma (HCC) [15]. This complex plays an important role in suppressing EMT and HCC metastasis and by targeting Gli1 [16]. However, it was also reported that PCAF acetylates the enhancer of zeste homolog 2 (EZH2) at K348 to augment EZH2 stability, and thus promotes lung cancer cell migration and invasion [17]. These reports indicate that the role of PCAF is context-dependent. In several breast cancer cell lines, hMOF catalyzes promoter H4K16 acetylation, which is critical to maintain expression of EMT-related tumor suppressor genes [18]. Consistent with this, MOF also acetylates the histone demethylase lysine-specific histone demethylase 1 (LSD1), to suppress EMT, indicating that MOF is a critical suppressor of EMT and tumor progression [19]. Recently, we found that Tip60 appears to be an important regulator of TWIST activity by acetylating at H3K73 and H3K76 of the GK-X-GK motif, resulting in an interaction between BRD4 and TWIST, hence promoting the aggressiveness of basal-like breast cancer (BLBC) [20].

Dysfunctional class I HDAC expression and activity is associated with cancer metastasis. HDAC1 regulates invasiveness by increasing matrix metalloproteinase (MMP) expression. Furthermore, HIF-2α is a transcriptional regulator of the *HDAC1* gene, and hypoxia increase HIF-2α and HDAC1 expression [21]. TGF-β-driven E-cadherin silencing and EMT in human pancreatic cancer cells also depend on HDAC activity [22]. HDAC1 and HDAC2 form a transcriptional repressor complex with SNAIL to downregulate the E-cadherin expression of pancreatic cancer cells during metastasis. Intriguingly, SNAIL also recruits the HDAC1/2-containing SIN3A complex to deacetylate histones on the E-cadherin promoter for gene

silencing [23]. In a similar manner, SLUG recruits the HDAC1-containing CtBP complex to silence genes by binding to the E-box on the BRCA2 promoter [24]. ZEB1/2 also recruits the CtBP/HDAC1 complex to the E-cadherin promoter, while ZEB1 recruits SIRT1, a class III HDAC, to silence E-cadherin and promote EMT and metastasis in prostate cancer cells [25, 26]. Moreover, ZEB1-induced EMT is accomplished by repression of other epithelial genes, including EPCAM, ESRP1 and RAB25, and is accomplished by a reduced acetylation of H3K9 and H3K27 at their promoters. In fact, evidence suggests that H3K27 deacetylation is a key epigenetic event in ZEB1-induced transcriptional reprogramming [27]. In addition to the EMT transcriptional factors, HDAC1 co-purifies with TCF12 and promotes migration and invasion; elevated expression of TCF12 and HDAC1 correlate with a poor prognosis in gallbladder cancer. These findings suggest that this HLH transcription factor TCF12 could also target HDACs for epithelial gene silencing during EMT [28]. The expression of the HDAC1 and HDAC3 correlates with nuclear receptor (NR) (i.e., ER and PR) status. HDAC assembles a complex with ERα that binds to the SLUG promoter to repress expression [29]. Interestingly, HDAC6 and SIRT1 counteract the p300-catalyzed acetylation on Cortactin, which enhances its F-actin binding ability to facilitate EMT and tumor progression [30]. Conversely, HDAC6 and ERα are co-localized in the cytoplasm of renal cell carcinoma (RCC) cells and HDAC6 enhances cell motility by decreasing acetylated α-tubulin expression [21]. Loss of α-Tubulin acetylation by HDAC6 is also associated with TGF-β-induced EMT [31].

Recently, the clinical relevance of HDACs and the therapeutic potential of HDAC inhibitors (HDACi) have been reported. HDACi can generally be classified into hydroximates, cyclic peptides, aliphatic acids, and benzamides [32], and grouped according to their specificity. Thus far three HDACi: vorinostat (SAHA), romidepsin (Istodax) and PTCL (Belinostat or Beleodaq) are approved by the FDA for some T-cell lymphomas [33]. However, these molecules have not produced favorable and expected outcomes in solid tumors. Currently, a number of small molecules HDACi were investigated in clinical trials with variety of solid neoplasms, including breast cancer, either alone or in combination with hormonal treatments. Entinostat (MS-275), a benzamide with high specificity for the class I HDACs, is currently in a phase II/III trial for advanced ER^+ breast cancer [34, 35]. Vorinostat exerts EMT reversal effects by restoring the expression of E-cadherin. An expanded screen on 41 HDACi further identified 28 HDACi compounds, such as the class I-specific inhibitors Mocetinosat, Entinostat and CI994, that restore E-cadherin and ErbB3 expressions in ovarian, pancreatic and bladder carcinoma cells [36]. Mocetinostat, but not other HDACi, specifically interferes with ZEB1 function, restores miR-203 expression, represses stemness properties, and induces sensitivity against chemotherapy by restoring histone acetylation on the E-cadherin promoter [37]. Given that persistent genes activation may require targeting of multiple epigenetic silencing machineries, a combination of HDACi with anticancer drugs and/or radiotherapy demonstrate synergistic or additive effects in clinical trials. For example, HDACi have been utilized in combination with 5 Aza-dC as a synergistic strategy [38]. However, recent reports also found that HDACi could promote EMT in prostate and nasopharyngeal cancer cells [39, 40], indicating the application of HDACi in anti-cancer therapy is cancer-context dependent and may limit application.

http://dx.doi.org/10.5772/intechopen.78383

4. Histone methylation

Histone methylation occurs at specific lysine or arginine residues on the histone tails. This modification is associated with either transcriptional activation or repression. Histone methylation does not change the electrostatic charge of histones or affect the chromatin structure. The functional effects of histone methylation are affected by both the position of the modified residues and number of methyl groups. Histone methyltransferases (HMTs) transfer methyl groups from S-adenosylmethionine (SAM) to either lysine or arginine residues, whereas histone demethylases (HDMs) remove methyl groups. The HMTs and HDMs specifically catalyze particular lysine or arginine residues.

4.1. Lysine methylation

Methylation of lysine residues on histones was first identified in the 1960s. Histone lysines can have four states of methylation at different lysine sites. Histones H2B lysine 5 (H2BK5), H3K4, H3K9, H4K20, H3K27, H3K36, and H3K79 are subject to unmethylated, mono-methylation (me1), di-methylation (me2), or tri-methylation (me3) on the ε-amino groups of lysine residues. These lysine methylations change the chromatin structure and regulate gene transcription. Histone lysine methylation is a reversible modification and is maintained by the balance lysine methyltransferases (KMTs) and lysine demethylases (KDMs). The KMTs recruit SAM as a cofactor and catalyze the addition of methyl groups to lysine residues through the SET domain. The KMTs are grouped into the SET domain-containing enzyme families (KMT1–3 and KMT5–7), the KMT4/DOT1 family, and others. The KDMs include the flavin adenine dinucleotide- (FAD-) dependent monoamine oxidase family (KDM1/LSD), the Jumonji C domain-containing demethylase (JMJD) families (KDM2–6), and others. Methylation of H3K4, H3K36, and H3K79 usually correlate with gene activation, whereas methylation of H3K9, H3K20, H3K27, and H3K56 are associated with transcriptional silencing.

4.1.1. Transcriptional activation and lysine methylation

H3K4: H3K4 methylation (H3K4me) is present in euchromatic regions and is usually associated with transcriptional activation. H3K4me3 occurs principally at the 5′ end of actively transcribed genes, near the transcription start site (TSS). H3K4me2 is located throughout genes, but frequently found towards the middle of the coding region of transcribed genes, and H3K4me1 is more abundant at the 3′ ends [41]. H3K4me2 marks can be present at both active and inactive euchromatic genes, whereas H3K4me3 is present exclusively at active genes. H3K4me favors transcriptional activation by facilitating H3 acetylation and the recruitment of RNA polymerase II, but it also antagonizes gene repression by preventing the binding of nucleosome remodeling and deacetylase co-repressor complexes, such as NuRD, and interfering with substrate recognition by the variegation 3–9 (SUV39H) methyltransferases [42]. The balance between KMTs and KDMs is an important dynamics for H3K4me and the regulation of gene transcription. More than ten H3K4 KMTs have been identified, including the mixed-lineage leukemia (MLL)1–4 proteins, along with Set 1a and Set 1b, Ash1L, Set7/9, and also the

SET and MYND domain-containing enzymes (SMYD) family members (SMYD1 and SMYD3). SMYD are involved in many cellular processes, including tumorigenesis and invasiveness. For example, SMYD3 is a novel histone H3K4-specific N-lysine di- and tri-methyltransferase, and highly characteristic active transcription. SMYD3 exerts its effects on initiation, invasion and metastasis of diverse tumors (e.g., esophageal squamous cell carcinoma (ESCC), gastric cancer, HCC, cholangiocarcinoma, breast cancer, prostate cancer, and leukemia). SMYD3 stimulates EZR and LOXL2 transcription to enhance proliferation, migration and invasion by directly binding to sequences of the promoter regions of these target genes [43]. SMYD3 is also capable of increasing cell migration through MMP-9 expression [44]. The MLL proteins (Trithorax homologs in Drosophila) are important for the regulation of developmental genes such as the Hox cluster, and deficiency of MLL1 or MLL2 causes embryonic lethality [45]. MLL1 coordinates with HIF1α and regulates hypoxia-induced HOTAIR expression to facilitate tumorigenesis [46]. In addition, MLL1 interacts with β-catenin to promote cervical carcinoma cell tumorigenesis and metastasis [47]. KMT2B/MLL2 is highly expressed in ESCC and promotes tumor progression by inducing EMT [48]. Another member of MLL family, MLL3 is reportedly mutated in multiple cancers. MLL3 regulates many migration-related genes and downregulation of MLL3 has a profound impact on the progression of ESCC [49]. Furthermore, Kim et al. [50] showed that KMT2D/MLL4 expression is associated with poor survival in breast cancer and regulates tumor proliferation and invasiveness.

Histone lysine methylation is a reversible process. H3K4 is demethylated by the KDM1 family (LSD1 and LSD2), the KDM2 family (FBXL10 and FBXL11), and the KDM5 family (JARID1A, JARID1B, JARID1C, and JARID1D) as well as JARID2 and NO66. The LSD subgroup of KDMs specifically targets the mono- and dimethylated lysines. This group demethylates substrates through a flavin adenine dinucleotide-dependent oxidative reaction, producing lysine and formaldehyde. KDM1A/LSD1 was the first H3K4 lysine-specific demethylase to be identified. We and others demonstrated that SNAIL recruits LSD1 to epithelial gene promoters with demethylation of H3K4me2 and subsequent silencing of target genes to enhance tumor metastasis [51]. SLUG also interacts with LSD1 to facilitate tumor metastasis [52]. In addition, both SNAIL and SLUG recruit LSD1 and bind to a series of E-boxes located within the BRCA1 promoter to repress BRCA1 expression. LSD1 overexpression promoted metastasis whereas knockdown of LSD1 inhibited tumor spread, suggesting that LSD1 is a key regulator of ESCC metastasis [53]. LSD1 and LSD2 act differently in the regulation of gene transcription and chromatin remodeling. However, both of KDM1A and KDM1B are overexpressed in invasive breast carcinoma, and depletion results in high levels of H3K4me1–2. The KDM5/JARID1 family is frequently found in the promoter region of transcriptionally active genes, and results in repressed expression of the target genes. KDM5A is highly expressed in ovarian cancer tissues and facilitates EMT and metastasis [54]. KDM5A promotes an increase in TNC expression, which augments breast cancer cell invasion and metastasis [55]. Reports indicate that, in gastric cancer cell, KDM5A is induced by TGF-β1 and recruited by p-SMAD3 to silence the *E-cadherin* promoter and promote tumor progression [56]. KDM5B plays a role in cell differentiation, stem cell self-renewal and other developmental progresses. Recent studies showed that KDM5B expression was increased in breast, bladder, lung, prostate and many other tumors and promote tumor initiation, invasion and metastasis. Mechanistically, KDM5B exerts its function through modulation of H3K4me3 at the PTEN gene promoter [57].

KDM5C/JARID1C is overexpressed in breast cancer, and its expression is significantly associated with metastasis. This demethylase modulates the status of H3K4 methylation in the breast cancer metastasis suppressor-1 (BRMS1) promoter, and thereby controls the expression of BRMS1 to inhibit tumor progression. Accordingly, the expression of KDM5C and BRMS1 are inversely correlated in human breast cancer [58].

H3K36: Because the level of H3K36me3 is high at the promoter site in active genes, H3K36me3 is involved in active transcription. In contrast, the H3K36me1 signal has a low association with active promoters. H3K36 is methylated by the KMT3 family (SETD2 and NSD1) as well as by NSD2, NSD3, SMYD1, SMYD2, SMYD3, SMYD4, and SMYD5. SETD2 plays a tumor suppressor role in tumor metastasis. Interestingly, SETD2 is frequently either deleted or mutated [59]. In contrast, H3K36 is demethylated by the KDM2 family (FBXL10 and FBXL11), the KDM4/JMJD family (JMJD2A, JMJD2B, and JMJD2C), and NO66. KDM2A expression is increased in breast cancer and associated with poor clinical outcomes [60]. KDM2A promotes lung tumorigenesis by epigenetically enhancing ERK1/2 signaling through demethylation of H3K36 [61]. In addition, KDM8/JMJD5 also demethylates H3K36me2, and overexpression of JMJD5 promotes cell invasion and is significantly correlated with clinical stage, histological grade and lymph node metastasis [62].

H3K79: H3K79me3 is associated with active transcription in yeast, whereas it is localized at both active and silent promoters in humans. H3K79me1 and H3K79me2 do not have any association with either active or silent promoters. H3K79 is methylated by the KMT4 family (DOT1L) and demethylated by PHF8 [63]. Methylation of H3K79 has been implicated in cell cycle regulation and the DNA damage response [63]. Disruption of this methylation can lead to cancers, making DOT1L a potential therapeutic target for cancers such as leukemia [64]. More recently, DOT1L has been implicated in the stimulation of proliferation, self-renewal, and metastatic potential of breast cancer cells [65]. DOTL1 cooperates with c-Myc-p300 complex to epigenetically activate EMT regulators in breast cancer progression. Clinically, DOTL1 expression is associated with poorer survival and aggressiveness of breast cancer [12]. PHF8 is highly expressed in metastatic prostate tissues and plays an important role in controlling invasion and metastasis [66]. PHF8 also interacts with β-catenin, and binds to the promoter region of vimentin, leading to the promotion of gastric cancer progression and metastasis [67].

4.1.2. Transcriptional repression and lysine methylation

H3K9: The methylation of H3K9 (H3K9me) was the first mechanism of gene repression to be linked to KMT. Studies in *Drosophila* showed that the gene *Su(var)39*, later shown to encode a H3K9 HMT, had an important role in the regulation of position-effect variegation [68] and similar enzymes were subsequently discovered in humans (SUV39H1/H2, G9a and Riz1 among others) [69]. H3K9 methylation is important for chromatin condensation and heterochromatin formation. H3K9me is recognized and bound by heterochromatin protein 1 (HP1), which recruits SUV39H, to reinforce the silencing process. H3K9 methylation plays a critical role in the formation of transcriptionally silent heterochromatin and the stable inheritance of the heterochromatin state. H3K9me1 and H3K9me2 are associated with euchromatic gene repression, whereas H3K9me3 is associated with stably silenced heterochromatin. H3K9me2 marks contribute to the maintenance of gene repression

in differentiated tissues in large genomic regions known as 'large organized chromatin K9–modifications (LOCKs)', and require the activity of the methyltransferase G9a [70]. H3K9me is methylated by the KMT1 family (SUV39H1, SUV39H2, G9a, GLP, SETDB1, and SETDB2). H3K9 is demethylated by the KDM1 family (LSD1), the KDM3 family (JMJD1A, JMJD1B and JMJD1C), and the KDM4 family (JMJD2A, JMJD2B, JMJD2C, and JMJD2D) as well as PHF8 and KDM7A/JHDM1D. SUV39H1 generates H3K9me3, and is involved in breast carcinogenesis. In addition, we found that SUV39H1 cooperates with SNAIL to repress the expression of E-cadherin. Knockdown of SUV39H1 blocked the formation of H3K9me3 and DNA methylation and inhibited cell migration, invasion and metastasis of BLBC [71]. Furthermore, we demonstrated that knocking down G9a resulted in suppression of H3K9 methylation and inhibition of tumor cell migration or invasion [72]. Mechanically, we found that G9a interacted with SNAIL and is critical for SNAIL-mediated E-cadherin repression in human breast cancer. Consistent with our research, Huang et al. [73] demonstrated that knocking down G9a or pharmacological inhibition of its activity suppressed tumor cell growth, colony formation, invasion and migration in non-small-cell lung cancer cells (NSCLC). G9a is also associated with an increased expression in lung cancer [74]. SETDB1 is the most significantly up-regulated epigenetic regulator in human HCCs and prostate cancer [75, 76]. Knockdown of SETDB1 decreases cell migration and invasion and reduces EMT and CSC properties [77]. SETDB1 indirectly up-regulates STAT3 expression and induces TWIST. KDM3A catalyzes the demethylation of H3K9 associated with transcriptional repression, resulting in the derepression and activation of genes involved with invasion and metastasis [78]. Global gene expression profiling demonstrated KDM3A regulates genes and pathways that augment cell migration and metastasis. KDM3A promotes both migration *in vitro* and metastasis *in vivo* by targeting melanoma cell adhesion molecule (MCAM) [79]. Surprisingly, increased expression of KDM3B correlates with improved clinical outcomes [80]. Accordingly, JMJD1B and JMJD2B are associated with PRL-3, a gene crucial to metastasis in colorectal cancer (CRC). However, JMJD1B seems to be a candidate tumor suppressor while JMJD2B seems to be a potential oncoprotein for CRC metastasis and progression [81]. With respect to the breast cancer, KDM4A is a regulator of cancer cell growth and metastasis, which correlates with breast cancer progression, and is associated with the attenuation of the tumor suppressor ARHI [82]. KDM4B is physically associated with β-catenin and binds to the promoter of the β-catenin target gene *vimentin* to increase its transcription by inducing H3K9 demethylation [83]. Inhibition of JMJD2B attenuates migration and invasion of gastric cancer cells *in vitro* and metastasis *in vivo*. KDM4C expression correlates significantly with genes driving metabolic alterations in breast cancer; the mechanism involves an interaction between KDM4C and HIF1α, which is recruited to a subset of genes involved in metabolic remodeling and metastasis [84].

H4K20: H4K20 methylation is also associated with repressed chromatin. A recent genome-wide analysis demonstrated that H4K20me3 was associated with heterochromatin and played a pivotal role in chromatin integrity. In addition, loss of histone H4K20me3 predicts poor prognosis in breast cancer and is associated with invasive activity. On the other hand, H4K20me1 is located in the promoters or coding regions of active genes and co-localizes with H3K9me1, which suggest that H4K20me1 is associated with transcriptional activity. H4K20 is

methylated by the KMT5 family (PR-Set7, SUV4-20H1, and SUV4-20H2) and the KMT7 family (SET7/9). KDMs that catalyze H4K20 demethylation have not been reported. Moreover, ectopic expression of SUV420H1 and SUV420H2 in breast cancer cells suppressed cell invasiveness, whereas knockdown of SUV420H2 activated invasion by normal mammary epithelial-cell *in vitro* [85]. Through its repressive H4K20me3 mark, SUV420H2 silences several key drivers of the epithelial state. Knockdown of SUV420H2 elicited MET on a molecular and functional level. An analysis of human pancreatic cancer biopsies suggests that high levels of SUV420H2 correlate with a loss of epithelial characteristics and progressively invasive cancer [86]. SET8 (also known as PR-Set7/9, SETD8, KMT5A), a member of the SET domain-containing methyltransferase family that specifically target H4K20 for monomethylation, physically interacts with TWIST to promote EMT and invasion by breast cancer cells [87]. Interestingly, SET8 acts as a dual epigenetic modifier on the promoters of E-cadherin and N-cadherin through its H4K20 monomethylation activity [88]. These bipolar roles of SET8 in EMT were also found in prostate cancer, which were mediated by ZEB1 [89]. A recent report indicates that the activation of the Shh pathway is required for EMT in NSCLCs [90]. SET7-mediated Gli3 methylations contribute to the tumor growth and metastasis in NSCLCs *in vitro* and *in vivo* [91].

H3K27: Another important repressive mark is H3K27 methylation which plays an essential role in embryogenesis, cell differentiation and organogenesis. H3K27me3 is associated with constitutive heterochromatin and maintenance of gene repression during early development. According to a genome-wide analysis, the levels of H3K27me2 and H3K27me3 are elevated in silent promoters and reduced in both active promoters and genic regions, whereas the level of H3K27me1 is high in promoters engaged in active transcription, especially downstream of the TSS [92, 93]. In embryonic stem cells (ESCs), H3K27 methylation usually overrides the effect of H3K4me3 in bivalent regions, maintaining them in a repressed state. Upon differentiation, these regions become exclusively marked by either of these modifications, leading to gene activation or repression [92]. H3K27 methylation is catalyzed by the polycomb repressive complex 2 (PRC2), which is composed mainly of suppressor of zeste 12 (SUZ12), embryonic ectoderm development (EED) and EZH2. H3K27 is demethylated by the KDM6 family (KDM6A/UTX and KDM6B/JMJD3), as well as UTY and JHDM1D [94, 95]. EZH2 is overexpressed in prostate and breast cancers and correlates with poor prognosis. Interestingly, EZH2 is essential for CSC self-renewal, and these CSCs provide the seeds for metastatic dispersal and differentiate into tumor-associated endothelial cells. Pre-clinical studies showed that EZH2 can silence several anti-metastatic genes (e.g., E-cadherin and tissue inhibitors of metalloproteinases), thereby favoring cell invasion and anchorage-independent growth. Accordingly, Tiwari and colleagues delineated an elegant pathway wherein TGF-β induces EZH2 expression to elicit EMT programs and metastasis of breast cancers by reprogramming the epigenome [96]. EZH2 represses TIMP2 transcription, which leads to increased activity of MMP-2 and MMP-9 and the invasive capacity of BLBC cells [97]. In pancreatic cancer cells, SNAIL recruits PRC to the E-cadherin promoter by binding to SUZ12 [98]. Increased KDM6A expression is associated with poor prognosis, along with derepression/activation of genetic programs that induce cell proliferation, luminal to basal-like transition, and metastasis. Furthermore, the function of KDM6A correlates with the activity of the MLL4, and increased expression of these epigenetic enzymes correlates with poor survival outcomes in breast cancer [50]. UTX interacts with the

MLL4 complex to activate several pro-metastatic genes including MMP9 and SIX1, leading to increased EMT and metastasis of breast cancer [50]. In colon cancer, KDM6A not only demethylates H3K27me3 at the E-cadherin promoter but also recruits CBP to the E-cadherin promoter, resulting in increased H3K27ac [99]. However, it was also reported that KDM6A inhibited EMT by epigenetic repression of EMT genes in cooperation with LSD1 and HDAC1 [100]. Therefore, the role of KDM6A an EMT suppressor or enhancer requires further investigation. KDM6B expression is also increased in invasive breast carcinomas and enforcing KDM6B overexpression induces EMT, invasive migration, stem cell-like traits, and metastatic properties. The mechanism involves demethylation associated with increased SNAIL or SLUG expression mediating the EMT [101]. Interestingly, KDM6B also modulates the tumor microenvironment and promotes melanoma progression and metastasis through upregulation several targets of NF-κB and BMP signaling, including stanniocalcin 1 (STC1) and chemokine (C-C motif) ligand 2 (CCL2) [102].

In summary, histone lysine methylation modulates chromatin accessibility, transcriptional status, and control of tumor suppressor and oncogene expression in aberrant cell metastasis. Dynamic regulation of the either permissive or repressive histone methylation at different genomic loci and through different molecular mechanisms facilitates the dynamic EMT process.

4.2. Arginine methylation

Histone arginine methylation also occurs in many arginine sites, histone H3 arginine 2 (H3R2), H3R8, H3R17, H3R26, and H4R3 undergo monomethylation (me1), symmetrical dimethylation (me2s), or asymmetrical dimethylation (me2a) on the guanidinyl groups of arginine residues. The N-arginine methyltransferases (PRMTs) are a class of enzymes that transfer a methyl group from SAM to the guanidino nitrogen of arginine. PRMTs generate three arginine methylation forms: monomethylarginine (MMA), asymmetric dimethylarginine (aDMA), and symmetric dimethylarginine (sDMA). Human PRMTs are composed of nine members that are categorized into three groups based on the type of arginine methylation reaction each member catalyzes. Type I is comprised of PRMT1, PRMT2, PRMT3, CARM1/PRMT4, PRMT6, and PRMT8; these catalyze both mono-methyl and asymmetric dimethyl arginine reactions. The type II group is made up of two members, PRMT5 and PRMT9, which catalyze both mono-methyl arginine and symmetric dimethyl arginine. Finally, PRMT7 is, at this point, considered the only bona fide type III methyltransferase and can generate only mono-methyl arginines. Many studies demonstrated that PRMTs regulate a wide range of genetic programs and cellular processes including cell cycle, RNA splicing and differentiation. Although the consequence of lysine methylation is relatively well studied, the role of PRMT action in tumorgenesis is poorly understood. Here, we provide a description of these PRMTs regarding tumor metastasis.

PRMT1: PRMT1 has been extensively studied in many fields. Its activity is responsible for a substantial percentage of methylated arginine residues and modulates a wide range of cell types. Specifically, asymmetric dimethylation on H4R3 by PRMT1 is involved in transcriptional activation, thereby driving oncogenic pathways. PRMT1 is an important regulator of EMT, cancer cell migration, and invasion. PRMT1 can generate H4R3me2a on the promoter region of ZEB1 and TWIST, which play a critical role in EMT [103, 104]. Furthermore, PRMT1

is overexpressed in melanoma; silencing PRMT1 significantly suppresses tumor growth and metastatic ability by targeting activated leukocyte cell adhesion molecule (ALCAM) [105]. Similarly, downregulation of PRMT1 inhibits cell migration and invasion in HCC and oral squamous cell carcinoma (OSCC) [106, 107]. Because of complex alternative splicing in the 5′ region of its pre-mRNA, there are seven distinct PRMT1 isoforms [108]. Each of these isoforms, named PRMT1v1-v7, has distinct characteristics in terms of expression. PRMT1v1 is the most abundantly expressed isoform and likely represents the isoform that is described as PRMT1 in most reports. The expression of alternatively spliced PRMT1 (PRMT1v2) isoform, which is generated through inclusion of alternative exon 2, is significantly altered in breast cancer and promotes invasiveness. The RNA binding protein RALY regulates the PRMT1v2 isoform and promotes metastatic potential [109].

PRMT2: PRMT2 is also reported to be overexpressed in breast cancer [110]. PRMT2 interacts with many NRs, including ERα and ERβ *in vitro* [111]. Interestingly, the activation of these receptors within cells has both distinct and in some cases opposing effects, which suggests that the functional role(s) PRMT2 are quite diverse. Recently, four alternatively spliced PRMT2 isoforms (PRMT2L2, PRMT2α, β, and γ) in addition to the original PRMT2 isoform were identified [112]. Several splice variants (*i.e.*, PRMT2-α, $-\beta$, $-\gamma$) were identified as induced in breast cancer, particularly in ER, PR-positive breast cancer [110]. PRMT2 directly binds and enhances estrogen-mediated transactivation of ERα, and enhances the promoter activity of the downstream target gene SNAIL. These findings suggest that the increased PRMT2 expression is associated with breast aggressiveness and metastasis [110].

PRMT4: PRMT4, more commonly known as coactivator-associated arginine methyltransferase 1 (CARM1), is involved in the regulation of a number of cellular processes including transcription, pre-mRNA splicing and cell cycle progression. The expression of CARM1 is dysregulated in colorectal, prostate and breast cancer. CARM1 methylates the chromatin-remodeling SWI/SNF core subunit, BAF155 in breast cancer [113]. The methylation of arginine 1064 residue of BAF155 is associated with breast cancer recurrence and metastasis, indicating that CARM1 plays an important role in tumorigenic activity through BAF155. Accordingly, CARM1-induced tumorigenic effects and its expression is increased in invasive breast cancer, and correlates with a high tumor grade [114]. Interestingly, the *CARM1* gene also transcribes four isoforms: the primary isoform CARM1 (CARM1v1) and three alternative isoforms, v2, v3 and v4 [115]. Whether these isoform are responsible for the methylation of distinct substrates and their individual functions requires further study.

PRMT5: PRMT5 is a type II enzyme that generates symmetric dimethylarginine (sDMA). The PRMT5 symmetrically methylates H3R8 site and functions in gene silencing. H3R8me2s strongly associates with H4R3me2s, because both modifications are catalyzed by PRMT5. However, acetylation of H3K9 and H3K14 prevents H3R8 methylation. PRMT5 also acts as a novel cofactor of SHARPIN (Shank-associated RH domain interacting protein), which plays a central role in controlling lung cancer cell metastasis. SHARPIN-PRMT5 is essential for the monomethylation of histones at key metastasis-related genes [116]. PRMT5 has another distinct function; PRMT5 coordinates with multiple Mediator complex subunits to dimethylate H4R3 at the promoter regions of immune response genes and C/EBPβ target

genes [117]. Conversely, PRMT5 methylation of histone H3R2 recruits WDR5 and the MLL complex, stimulating H3K4 methylation and euchromatin maintenance [118]. In the context of cancer metastasis, PRMT5 is involved in TGF-β-WDR77 signaling, which induces cancer cell invasion [119]; this report indicates PRMT5 interacts with the WDR77 complex to catalyze arginine methylation. With respect to the acquisition of EMT via TGF-β signaling, epigenetic PRMT5-WDR77 activity is necessary for tumor invasion and metastasis. Furthermore, PRMT5 appears to be recruited by AJUBA to SNAIL and functions as a co-repressor. PRMT5, AJUBA and SNAIL form ternary complex to repress E-cadherin, concomitant with increase arginine methylation at the locus [120]. PRMT5 also modulates metastasis by methylating KLF4. Methylation blocks ubiquitylation of KLF4 by the von Hippel–Lindau tumor suppressor, and as a result, arginine methylation of KLF4 via PRMT5 increases the level of KLF4 protein and increases the probability of breast carcinogenesis [121].

PRMT6: PRMT6 primarily catalyzes asymmetric dimethylation of H3R2. H3R2me2a counter-correlates with the methylation of H3K4, which suggests that H3R2me2a is a repressive marker. However, PRMT6 also methylates H3K4 since both H3R2me2a and H3K4me3 markers are likely to coexist. Furthermore, genome-wide analyses indicate that both H3R2me1 and H3R2me2a are associated with active genes [93]. Thus, the data on the H3R2me2a marker are contradictory, and further studies are required to resolve this issue. There is also emerging evidence of an oncogenic role of PRMT6 in cancer. Overexpression of PRMT6 is associated with several cancer types, including breast, cervix, prostate, and lung cancers, indicating that PRMT6 might play an important role for the onset, incidence, and metastasis of cancer [122]. Furthermore, Dowhan et al. [123] demonstrated a PRMT6-dependent signature that influences long-term survival in patients with breast cancer.

PRMT7: The oncogenic role of PRMT7 has been emerging over the past few years. There are two isoforms, PRMT7α and β, which are active and have slightly different methylation profiles and locations. PRMT7α localizes to the cytoplasm and nucleus, whereas PRMT7β is exclusively cytoplasmic. R531 of PRMT7 is self-methylated and loss of PRMT7 automethylation leads to a reduced recruitment to the E-cadherin promoter by YY1, which consequently derepresses E-cadherin expression by decreasing the H4R3me2's level [124]. In terms of the functional role of PRMT7, this methytransferase is highly expressed in breast cancer and induces EMT by inhibiting E-cadherin. Baldwin et al. [125] also showed that PRMT7 promotes a well-known metastasis mediator, MMP9 and induces breast cancer cell invasion. Importantly, a gene expression analysis of independent data sets of more than 1200 breast tumors identified PRMT7 expression as significantly increased. In addition, this gene is located 16q22, where the chromosomal region was correlated with an increased metastatic potential of breast cancer [126].

PRMT9: PRMT9 and PRMT5 are the only known mammalian enzymes capable of forming sDMA residues as type II PRMTs. However, the specificity of these enzymes for their substrates is distinct and not redundant. Interestingly Yang et al. [127] showed that PRMT9 is also nonhistone methyltransferase. For example, it methylates the arginine 508 site of the alternative splicing factor SAP145. Given that alternative splicing is of paramount importance in RNA processing, PRMT9 might play a key role in many cellular programs including cancer biology. Recent reports demonstrate that overexpression of PRMT9 strongly promotes HCC invasion and metastasis through EMT by regulating SNAIL expression via activation of the PI3K/Akt/GSK-3β/SNAIL signaling pathway [128].

Many HMTs and HDMs inhibitors have been developed and evaluated in clinical trials, such as chaetocin, BIX-01294, BIX-01338, UNC0638 and DZNep. Chaetocin, a natural fungal substance, is the first inhibitor of an HMT, which targets SUV39H1 without high selectively [129]. Treatment with Chaetocin induces expression of E-cadherin while reducing H3K9me3 but does not produce a global H3K9 methylation on its promoter in multiple tumor cells [130]. By the contrast, BIX-01294 specifically reduces the dimethylation of H3K9me2 through an inhibition of the enzymatic activities of G9a and GLP [131]. Treatment of BIX-01294 activates E-cadherin expression and reverse EMT phenotypes in a variety of cancer cells, and is accompanied by reduced H3K9me2 and increased H3K9 acetylation on the E-cadherin promoter [132]. Another G9a/GLP inhibitor, UNC0638, was developed with higher potency and selectively [133]. UNC0638 treatment not only resulted in lower global H3K9me2 levels but also markedly reduced the abundance of H3K9me2 marks at promoters of known G9a-regulated endogenous genes. UNC0638 treatment activates E-cadherin expression and reverses EMT in PANC-1 pancreatic cancer cells and triple negative breast cancer (TNBC) and suppresses migration and invasion [134]. Because of the importance of H3K27 methylation in cancer, several highly specific EZH2 inhibitors have been developed, such as GSK2816126 and EPZ-6438, which are currently being evaluated in clinical trials for lymphoma and solid tumor/lymphoma respectively [135]. Another EZH2 inhibitor, 3-deazaneplanocin A (DZNep), selectively inhibits H3K27me3 and H4K20me3 [136]. DZNep dampens TGF-β-induced EMT signals and reduces tumor metastasis in pancreatic cancer and colon cancer [136, 137]. We found that Parnate, an LSD1 inhibitor, activates E-cadherin expression and suppresses motility and invasiveness in breast cancer cells [51]. Two highly specific LSD1 inhibitors, GSK2879552 and ORY-1001 are employed to clinical trials for the treatment of small cell lung cancer and acute leukemia [135]. Several inhibitors targeting HDMs also have been developed as well. For example, JIB-04, a specific inhibitor targeting the JMJC-domain, inhibits the activity of H3K4 and H4K9 and attenuates lung cancer cell proliferation [138]. The first reported small molecule PRMT inhibitors, including AMI-1 and AMI-5 were identified through virtual screening and high throughput screening [139]. AMI-1 was reported as type I PRMT and PRMT5 inhibitor [140]. AMI-1 inhibits proliferation and decreases cell migratory activity of CRC cells *in vitro* and in xenograft mouse models [141].

5. Histone modification readers

Sometimes, histone modifications can directly regulate the chromatin dynamic. However, in most cases, the modifications are recognized by proteins containing distinct recognition domains, which act as "readers" and bind to different histone modifications. For example, bromodomain acts as lysine acetylation "readers" of modified histones that mediate signaling transduction changes in gene regulatory networks. In the human genome, there are 61 bromodomains found within 46 proteins that can be divided into eight families based on structure/sequence similarity. Among them, bromodomain and the extra-terminal domain (BET) family recognize acetylated lysine residues in histones H3 and H4. BRD4 is a member of the BET family that carries two bromodomains. Recently, our studies revealed that the di-acetylated TWIST, mediated by Tip60, recruits BRD4 and related

transcriptional components to the super-enhancer of its targeted genes during tumor progression in BLBC [20]. In addition, pharmacologic inhibition of BRD4 with the BET-specific bromodomain inhibitors, JQ1 and MS417, effectively reduces WNT5A expression and suppresses invasion, CSC-like properties and tumorigenicity of breast cancer cells *in vitro* and *in vivo* [20]. Given the extensive cancer-related functions of BRD4 and the proof-of-concept demonstrated by disruption of the BRD4–acetyl-lysine interactions as a therapeutic target, significant efforts have thus been made to develop BRD4 inhibitors from both pharmaceutical and academic settings. BRD4 inhibitors have several chemical classifications including azepines, 3,5-dimethylisoxazoles, pyridones, triazolopyrazines, tetrahydroquinolines (THQs), 4-acyl pyrroles and 2-thiazolidinones [142]. BET inhibitor treatment results in AMIGO2 silencing and changes in PTK7 proteolytic processing, and thus inhibit melanoma metastasis [143].

Histone methylation provides docking sites and is recognized by specific reader proteins that contain a methyllysine binding protein, which has emerged as a focus of epigenetic research due to its critical role in gene regulation and oncogenesis. This reader harbors specific motifs, including Chromodomain (CD), MBT, WD40 repeat, PHD finger, PWWP, Tudor and Ankyrin repeat. Methyllysine binding proteins distinguish methylation marks on different residues as well as different methylation states on the same residue and in turn mediate distinct downstream functions [144]. CD-containing HP1 proteins were the first identified methyl-lysine binding proteins and recognize methylated-H3K9 (methyl-H3K9) [145]. HP1α was down-regulated in metastatic cells of colon cancer and thyroid carcinomas relative to non-metastatic cells, indicating HP1α may be directly involved in the silencing of genes that potentiate cancer cell invasive potential and metastasis. Recent evidence implicate HP1α in EMT. The association of HP1α to major satellite repeat sequences located in pericentric heterochromatin decreased during the initial steps of TGF-β-induced EMT in a SNAIL/LOXL2-dependent manner [146]. In addition, HP1α post-translational modifications could participate in the heterochromatin dynamics associated with EMT. In a different set of modifications, four MBT-repeats domain of SFMBT1 recognize H3K4me2/3 and form a stable complex with LSD1. SFMBT1 is essential for SNAIL-dependent recruitment of LSD1 to chromatin, demethylation of H3K4me2, transcriptional repression of epithelial markers, and induction of EMT by TGF-β [147]. H3K4me2/3 is also recognized by the WD40 repeat domain of WDR5, which is also important for the assembly and activity of the SET1 protein complex catalyzing H3K4me3 [148]. Under hypoxic conditions, WDR5 is induced, interacts with HDAC3 and further recruits SET1 complex to activate mesenchymal gene expression to promote EMT [149]. Furthermore, the PRC2 component, EED, also contains a WD40 repeat that recognizes H3K27me3. EED recruits PRC2 to chromatin with pre-existing H3K27me3 to spread the same methylation into adjacent regions [150]. Intriguingly, G9a and GLP itself contain a methyl-lysine binding module (the ankyrin repeat domains), which generates and reads the same epigenetic mark [151]. Several small molecule compounds targeting the lysine methylation reader domain have been developed, including UNC1215 and UNC3866 that block the methyl-lysine binding mediated by the MBT domain-containing protein L3MBTL3, and the CD-containing protein CBX4/7 respectively [152, 153]. However, whether these inhibitors reverse EMT and tumor progression remains unknown.

6. Coordinated histone modification regulation

Because different chromatin modifying enzymes coexist in the same protein complex, and because diverse catalyzed modifications have been implicated in regulating the same set of genes, it is likely that these processes act in concert to orchestrate transcriptional regulation during EMT. For example, HDAC1/2, G9a/GLP, LSD1, HP1 and ZEB1/2 were co-purified in the CtBP1 co-repressor complex [154, 155]. ZEB1/2 could first target the complex to E-cadherin promoter to initiate repression. Next, HADC1/2 would deacetylate histones while the primed H3K9 was methylated by G9a/GLP. Meanwhile, LSD1, which removes H3K4me1/2, whereby the un-methylated H3K4 could also prevent H3K9 from re-acetylation [156, 157]. An affinity purification of Flag-TWIST identified several components of the NuRD chromatin remodeling complex. Among them, TWIST directly interacts with Mi2β, MTA2 and RbAp46 and likely targets the NuRD complex for histone deacetylation and chromatin remodeling on E-cadherin promoter. Together, these epigenetic events lead to gene silencing and promote EMT and breast cancer metastasis [158]. In addition, TWIST was also co-purified with SET8, BRCA1-associated protein (BRAP), NF-kB subunit RelA, PPP2CA and HES6 in MCF7 breast cancer cells [88]. SET8 interacts with TWIST. However, SET8 and TWIST are functionally interdependent in promoting EMT. SET8 mediates E-cadherin repression and N-cadherin activation simultaneously via its H4K20 monomethylation to promote cell invasion and EMT. However, the molecular mechanism that underlies the same repressive protein complex that contributes to opposite functions on different genomic loci remains an open question. Our recent study found that TWIST is diacetylated by Tip60, which was further recognized by BRD4, thereby constructing an activated TWIST/BRD4/P-TEFβ/RNA-Pol II complex at the WNT5A promoter and enhancer to promote EMT and breast cancer cell metastasis [20]. In breast cancer cells, the UTX-MLL4 forms a complex with LSD1/HDAC1/DNMT1 on the promoter of several EMT-TFs and decreases H3K4mes and H3 acetylation. UTX facilitates epigenetic silencing of EMT-TFs by inducing competition between MLL4 and the H3K4 demethylase LSD1, which results in inhibition of EMT and CSC-like properties [100].

MPP8, another methy-H3K9 binding protein, bridges DNMT3A and G9a/GLP to assemble a repressive trimeric protein complex on chromatin by binding to different methyl-lysines. MPP8 also couples H3K9 methylation and DNA methylation to silence epithelial genes and EMT [159, 160]. Interestingly, MPP8 also cooperates with the SIRT1 in this process through a physical interaction [161]. SIRT1 and MPP8 reciprocally promote each other's function and coordinate epithelial gene silencing and EMT. SIRT1 antagonizes PCAF-catalyzed MPP8-K439 acetylation to protect MPP8 from ubiquitin-proteasome-mediated proteolysis. Conversely, MPP8 recruits SIRT1 for H4K16 deacetylation after binding to methyl-H3K9 on target promoters. Therefore, MPP8 not only promote DNA-methylation but also H4K16 deacetylation to fine-tune the transcriptional regulation of EMT.

7. Conclusions and perspectives

Increasing evidences show that aberrant profiles of histone modifications contribute to a dysregulation those results in the metastatic cascade. The biochemically reversible nature

of histone modifications provides a platform for rapid changes in a variety of epithelia and mesenchymal genes during EMT and MET. In concert with different ETM-TFs and oncogenic signaling, pleiotropic histone modifications form a sophisticated and regulated network to coordinate the plasticity and dynamic change required for EMT.

Recent research identifies the critical role of histone modifications in metastasis, but leaves many important, open questions. First, do tumor microenvironmental signals trigger the formation of histone modification enzyme complexes present on different EMT-TFs? Whether these extrinsic signals affect enzyme activity indirectly through intracellular signaling pathways or directly through the EMT-TFs remains to be determined. Second, how do these EMT-TFs form distinct complexes that coordinate the epigenetic regulation of gene expression programs during EMT? Third, EMT is usually activated only transiently and partially. Therefore, which and how do different histone modifying enzymes and the catalyzed modifications contribute to these dynamic changes? Finally, what consequences do epigenetic instabilities have on cancer cell fitness? Do these activities increase plasticity and/or lead to vulnerabilities that it could influence the metastasis?

We know that histone modification enzymes are highly correlated with tumor progression and a poor clinical outcome. Therefore, these enzymes can serve not only as effective biomarkers for earlier diagnosis, but also present multiple therapeutic opportunities. Over the last decade, considerable progress has been made in the discovery and development of potent and selective small molecule inhibitors targeting specific histone modifiers. Many of these molecules are currently under extensive preclinical testing or being evaluated in clinical trials. These inhibitors show great potential as clinically useful drugs. Additionally, inhibitors to specific histone modifying enzymes could serve as useful chemical probes to characterize the function of different epigenetic pathways in EMT *in vivo* as well as many other important pathological diseases.

In all, advances in our understanding of the landscape of histone modifications in metastasis will provide a better sense of the molecular mechanisms associated with metastasis and thus help speed the development of new therapeutic strategies and biomarkers for metastasis.

Acknowledgements

We thank Dr. Cathy Anthony for the critical editing of this manuscript. Our research was supported by the Shared Resources of the University of Kentucky Markey Cancer Center (P30CA177558). Our research was also supported by grants from American Cancer Society Research Scholar Award (RSG13187) and NIH (P20GM121327 and CA230758) (to Y Wu).

Conflict of interest

The authors have declared that no conflict of interests exists.

Author details

Zhaoping Qiu[1,2], Jianlin Wang[1,2] and Yadi Wu[1,2]*

*Address all correspondence to: yadi.wu@uky.edu

1 Department of Pharmacology and Nutritional Sciences, University of Kentucky School of Medicine, Lexington, KY, USA

2 Department of Markey Cancer Center, University of Kentucky School of Medicine, Lexington, KY, USA

References

[1] Van't Veer LJ, Weigelt B. Road map to metastasis. Nature Medicine. 2003;**9**:999-1000

[2] Chambers AF, Groom AC, MacDonald IC. Dissemination and growth of cancer cells in metastatic sites. Nature Reviews. Cancer. 2002;**2**:563-572. DOI: 10.1038/nrc865

[3] Pantel K, Brakenhoff RH. Dissecting the metastatic cascade. Nature Reviews. Cancer. 2004;**4**:448-456. DOI: 10.1038/nrc1370

[4] Thiery JP. Epithelial-mesenchymal transitions in tumor progression. Nature Reviews. Cancer. 2002;**2**:442-454

[5] Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. Cell. 2009;**139**:871-890. DOI: S0092-8674(09)01419-6 [pii] 10.1016/j.cell.2009.11.007

[6] Brabletz T, Kalluri R, Nieto MA, Weinberg RA. EMT in cancer. Nature Reviews. Cancer. 2018;**18**:128-134. DOI: 10.1038/nrc.2017.118

[7] Strahl BD, Allis CD. The language of covalent histone modifications. Nature. 2000;**403**:41-45. DOI: 10.1038/47412

[8] Tessarz P, Kouzarides T. Histone core modifications regulating nucleosome structure and dynamics. Nature Reviews. Molecular Cell Biology. 2014;**15**:703-708. DOI: 10.1038/nrm3890

[9] Jenuwein T, Allis CD. Translating the histone code. Science. 2001;**293**:1074-1080. DOI: 10.1126/science.1063127

[10] Wang Z, Zang C, Rosenfeld JA, Schones DE, Barski A, Cuddapah S, et al. Combinatorial patterns of histone acetylations and methylations in the human genome. Nature Genetics. 2008;**40**:897-903. DOI: 10.1038/ng.154

[11] de Ruijter AJ, van Gennip AH, Caron HN, Kemp S, van Kuilenburg AB. Histone deacetylases (HDACs): Characterization of the classical HDAC family. The Biochemical Journal. 2003;**370**:737-749. DOI: 10.1042/BJ20021321

[12] Cho MH, Park JH, Choi HJ, Park MK, Won HY, Park YJ, et al. DOT1L cooperates with the c-Myc-p300 complex to epigenetically derepress CDH1 transcription factors in breast cancer progression. Nature Communications. 2015;**6**:7821. DOI: 10.1038/ncomms8821

[13] Dhasarathy A, Phadke D, Mav D, Shah RR, Wade PA. The transcription factors Snail and Slug activate the transforming growth factor-beta signaling pathway in breast cancer. PLoS One. 2011;**6**:e26514. DOI: 10.1371/journal.pone.0026514

[14] Liu YN, Lee WW, Wang CY, Chao TH, Chen Y, Chen JH. Regulatory mechanisms controlling human E-cadherin gene expression. Oncogene. 2005;**24**:8277-8290. DOI: 10.1038/sj.onc.1208991

[15] Tuo H, Zheng X, Tu K, Zhou Z, Yao Y, Liu Q. Expression of PCAF in hepatocellular carcinoma and its clinical significance. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi. 2013;**29**:297-300

[16] Li Q, Liu Z, Xu M, Xue Y, Yao B, Dou C, et al. PCAF inhibits hepatocellular carcinoma metastasis by inhibition of epithelial-mesenchymal transition by targeting Gli-1. Cancer Letters. 2016;**375**:190-198. DOI: 10.1016/j.canlet.2016.02.053

[17] Wan J, Zhan J, Li S, Ma J, Xu W, Liu C, et al. PCAF-primed EZH2 acetylation regulates its stability and promotes lung adenocarcinoma progression. Nucleic Acids Research. 2015;**43**:3591-3604. DOI: 10.1093/nar/gkv238

[18] Taipale M, Rea S, Richter K, Vilar A, Lichter P, Imhof A, et al. hMOF histone acetyltransferase is required for histone H4 lysine 16 acetylation in mammalian cells. Molecular and Cellular Biology. 2005;**25**:6798-6810. DOI: 10.1128/mcb.25.15.6798-6810.2005

[19] Luo H, Shenoy AK, Li X, Jin Y, Jin L, Cai Q, et al. MOF acetylates the histone demethylase LSD1 to suppress epithelial-to-mesenchymal transition. Cell Reports. 2016;**15**:2665-2678. DOI: 10.1016/j.celrep.2016.05.050

[20] Shi J, Wang Y, Zeng L, Wu Y, Deng J, Zhang Q, et al. Disrupting the interaction of BRD4 with diacetylated Twist suppresses tumorigenesis in basal-like breast cancer. Cancer Cell. 2014;**25**:210-225. DOI: 10.1016/j.ccr.2014.01.028

[21] Ramakrishnan S, Ku S, Ciamporcero E, Miles KM, Attwood K, Chintala S, et al. HDAC 1 and 6 modulate cell invasion and migration in clear cell renal cell carcinoma. BMC Cancer. 2016;**16**:617. DOI: 10.1186/s12885-016-2604-7

[22] von Burstin J, Eser S, Paul MC, Seidler B, Brandl M, Messer M, et al. E-cadherin regulates metastasis of pancreatic cancer in vivo and is suppressed by a SNAIL/HDAC1/HDAC2 repressor complex. Gastroenterology. 2009;**137**:361-71, 371.e1-5. DOI: 10.1053/j.gastro.2009.04.004

[23] Peinado H, Ballestar E, Esteller M, Cano A. Snail mediates E-cadherin repression by the recruitment of the Sin3A/histone deacetylase 1 (HDAC1)/HDAC2 complex. Molecular and Cellular Biology. 2004;**24**:306-319

[24] Tripathi MK, Misra S, Khedkar SV, Hamilton N, Irvin-Wilson C, Sharan C, et al. Regulation of BRCA2 gene expression by the SLUG repressor protein in human breast cells. The Journal of Biological Chemistry. 2005;**280**:17163-17171. DOI: 10.1074/jbc.M501375200

[25] Aghdassi A, Sendler M, Guenther A, Mayerle J, Behn CO, Heidecke CD, et al. Recruitment of histone deacetylases HDAC1 and HDAC2 by the transcriptional repressor ZEB1 downregulates E-cadherin expression in pancreatic cancer. Gut. 2012;**61**:439-448. DOI: 10.1136/gutjnl-2011-300,060

[26] Byles V, Zhu L, Lovaas JD, Chmilewski LK, Wang J, Faller DV, et al. SIRT1 induces EMT by cooperating with EMT transcription factors and enhances prostate cancer cell migration and metastasis. Oncogene. 2012;**31**:4619-4629. DOI: 10.1038/onc.2011.612

[27] Roche J, Nasarre P, Gemmill R, Baldys A, Pontis J, Korch C, et al. Global decrease of histone H3K27 acetylation in ZEB1-induced epithelial to mesenchymal transition in lung cancer cells. Cancers (Basel). 2013;**5**:334-356. DOI: 10.3390/cancers5020334

[28] He J, Shen S, Lu W, Zhou Y, Hou Y, Zhang Y, et al. HDAC1 promoted migration and invasion binding with TCF12 by promoting EMT progress in gallbladder cancer. Oncotarget. 2016;**7**:32754-32764. DOI: 10.18632/oncotarget.8740

[29] Ye Y, Xiao Y, Wang W, Yearsley K, Gao JX, Barsky SH. ERalpha suppresses slug expression directly by transcriptional repression. The Biochemical Journal. 2008;**416**:179-187. DOI: 10.1042/bj20080328

[30] Zhang X, Yuan Z, Zhang Y, Yong S, Salas-Burgos A, Koomen J, et al. HDAC6 modulates cell motility by altering the acetylation level of cortactin. Molecular Cell. 2007;**27**: 197-213. DOI: 10.1016/j.molcel.2007.05.033

[31] Gu S, Liu Y, Zhu B, Ding K, Yao TP, Chen F, et al. Loss of alpha-tubulin acetylation is associated with TGF-beta-induced epithelial-mesenchymal transition. The Journal of Biological Chemistry. 2016;**291**:5396-5405. DOI: 10.1074/jbc.M115.713123

[32] Dokmanovic M, Marks PA. Prospects: histone deacetylase inhibitors. Journal of Cellular Biochemistry. 2005;**96**:293-304. DOI: 10.1002/jcb.20532

[33] Eckschlager T, Plch J, Stiborova M, Hrabeta J. Histone deacetylase inhibitors as anticancer drugs. International Journal of Molecular Sciences. 2017;**18**. DOI: 10.3390/ijms18071414

[34] Trapani D, Esposito A, Criscitiello C, Mazzarella L, Locatelli M, Minchella I, et al. Entinostat for the treatment of breast cancer. Expert Opinion on Investigational Drugs. 2017;**26**:965-971. DOI: 10.1080/13543784.2017.1353077

[35] Damaskos C, Garmpis N, Valsami S, Kontos M, Spartalis E, Kalampokas T, et al. Histone deacetylase inhibitors: An attractive therapeutic strategy against breast cancer. Anticancer Research. 2017;**37**:35-46. DOI: 10.21873/anticanres.11286

[36] Tang HM, Kuay KT, Koh PF, Asad M, Tan TZ, Chung VY, et al. An epithelial marker promoter induction screen identifies histone deacetylase inhibitors to restore epithelial differentiation and abolishes anchorage independence growth in cancers. Cell Death Discovery. 2016;**2**:16041. DOI: 10.1038/cddiscovery.2016.41

[37] Meidhof S, Brabletz S, Lehmann W, Preca BT, Mock K, Ruh M, et al. ZEB1-associated drug resistance in cancer cells is reversed by the class I HDAC inhibitor mocetinostat. EMBO Molecular Medicine. 2015;**7**:831-847. DOI: 10.15252/emmm.201404396

[38] Kristensen LS, Nielsen HM, Hansen LL. Epigenetics and cancer treatment. European Journal of Pharmacology. 2009;**625**:131-142. DOI: 10.1016/j.ejphar.2009.10.011

[39] Jiang GM, Wang HS, Zhang F, Zhang KS, Liu ZC, Fang R, et al. Histone deacetylase inhibitor induction of epithelial-mesenchymal transitions via up-regulation of Snail facilitates cancer progression. Biochimica et Biophysica Acta. 2013;**1833**:663-671. DOI: 10.1016/j.bbamcr.2012.12.002

[40] Kong D, Ahmad A, Bao B, Li Y, Banerjee S, Sarkar FH. Histone deacetylase inhibitors induce epithelial-to-mesenchymal transition in prostate cancer cells. PLoS One. 2012;**7**:e45045. DOI: 10.1371/journal.pone.0045045

[41] Pokholok DK, Harbison CT, Levine S, Cole M, Hannett NM, Lee TI, et al. Genome-wide map of nucleosome acetylation and methylation in yeast. Cell. 2005;**122**:517-527. DOI: 10.1016/j.cell.2005.06.026

[42] Lachner M, Jenuwein T. The many faces of histone lysine methylation. Current Opinion in Cell Biology. 2002;**14**:286-298

[43] Zhu Y, Zhu MX, Zhang XD, Xu XE, Wu ZY, Liao LD, et al. SMYD3 stimulates EZR and LOXL2 transcription to enhance proliferation, migration, and invasion in esophageal squamous cell carcinoma. Human Pathology. 2016;**52**:153-163. DOI: 10.1016/j.humpath.2016.01.012.

[44] Liu Y, Liu H, Luo X, Deng J, Pan Y, Liang H. Overexpression of SMYD3 and matrix metalloproteinase-9 are associated with poor prognosis of patients with gastric cancer. Tumor Biology. 2015;**36**:4377-4386. DOI: 10.1007/s13277-015-3077-z

[45] Yu BD, Hess JL, Horning SE, Brown GA, Korsmeyer SJ. Altered Hox expression and segmental identity in Mll-mutant mice. Nature. 1995;**378**:505-508. DOI: 10.1038/378505a0

[46] Bhan A, Deb P, Shihabeddin N, Ansari KI, Brotto M, Mandal SS. Histone methylase MLL1 coordinates with HIF and regulate lncRNA HOTAIR expression under hypoxia. Gene. 2017;**629**:16-28. DOI: 10.1016/j.gene.2017.07.069

[47] Qiang R, Cai N, Wang X, Wang L, Cui K, Wang X, et al. MLL1 promotes cervical carcinoma cell tumorigenesis and metastasis through interaction with beta-catenin. Onco Targets and Therapy. 2016;**9**:6631-6640. DOI: 10.2147/ott.s114370

[48] Abudureheman A, Ainiwaer J, Hou Z, Niyaz M, Turghun A, Hasim A, et al. High MLL2 expression predicts poor prognosis and promotes tumor progression by inducing EMT in esophageal squamous cell carcinoma. Journal of Cancer Research and Clinical Oncology. 2018. DOI: 10.1007/s00432-018-2625-5

[49] Xia M, Xu L, Leng Y, Gao F, Xia H, Zhang D, et al. Downregulation of MLL3 in esophageal squamous cell carcinoma is required for the growth and metastasis of cancer cells. Tumor Biology. 2015;**36**:605-613. DOI: 10.1007/s13277-014-2616-3

[50] Kim JH, Sharma A, Dhar SS, Lee SH, Gu B, Chan CH, et al. UTX and MLL4 coordinately regulate transcriptional programs for cell proliferation and invasiveness in breast cancer cells. Cancer Research. 2014;**74**:1705-1717. DOI: 10.1158/0008-5472.can-13-1896

[51] Lin Y, Wu Y, Li J, Dong C, Ye X, Chi YI, et al. The SNAG domain of Snail1 functions as a molecular hook for recruiting lysine-specific demethylase 1. The EMBO Journal. 2010;**29**:1803-1816. DOI: 10.1038/emboj.2010.63

[52] Wu ZQ, Li XY, Hu CY, Ford M, Kleer CG, Weiss SJ. Canonical Wnt signaling regulates Slug activity and links epithelial-mesenchymal transition with epigenetic Breast Cancer 1, Early Onset (BRCA1) repression. Proceedings of the National Academy of Sciences of the United States of America. 2012;**109**:16654-16659. DOI: 10.1073/pnas.1205822109

[53] Alsaqer SF, Tashkandi MM, Kartha VK, Yang YT, Alkheriji Y, Salama A, et al. Inhibition of LSD1 epigenetically attenuates oral cancer growth and metastasis. Oncotarget. 2017; **8**:73372-73,386. DOI: 10.18632/oncotarget.19637

[54] Feng T, Wang Y, Lang Y, Zhang Y. KDM5A promotes proliferation and EMT in ovarian cancer and closely correlates with PTX resistance. Molecular Medicine Reports. 2017;**16**:3573-3580. DOI: 10.3892/mmr.2017.6960

[55] Cao J, Liu Z, Cheung WK, Zhao M, Chen SY, Chan SW, et al. Histone demethylase RBP2 is critical for breast cancer progression and metastasis. Cell Reports. 2014;**6**:868-877. DOI: 10.1016/j.celrep.2014.02.004

[56] Liang X, Zeng J, Wang L, Shen L, Ma X, Li S, et al. Histone demethylase RBP2 promotes malignant progression of gastric cancer through TGF-beta1-(p-Smad3)-RBP2-E-cadherin-Smad3 feedback circuit. Oncotarget. 2015;**6**:17661-17674. DOI: 10.18632/oncotarget.3756

[57] Tang B, Qi G, Tang F, Yuan S, Wang Z, Liang X, et al. JARID1B promotes metastasis and epithelial-mesenchymal transition via PTEN/AKT signaling in hepatocellular carcinoma cells. Oncotarget. 2015;**6**:12723-12739. DOI: 10.18632/oncotarget.3713

[58] Wang Q, Wei J, Su P, Gao P. Histone demethylase JARID1C promotes breast cancer metastasis cells via down regulating BRMS1 expression. Biochemical and Biophysical Research Communications. 2015;**464**:659-666. DOI: 10.1016/j.bbrc.2015.07.049

[59] Wang L, Zehir A, Nafa K, Zhou N, Berger MF, Casanova J, et al. Genomic aberrations frequently alter chromatin regulatory genes in chordoma. Genes, Chromosomes and Cancer. 2016;**55**:591-600. DOI: 10.1002/gcc.22362

[60] Chen JY, Li CF, Chu PY, Lai YS, Chen CH, Jiang SS, et al. Lysine demethylase 2A promotes stemness and angiogenesis of breast cancer by upregulating Jagged1. Oncotarget. 2016;**7**:27689-27710. DOI: 10.18632/oncotarget.8381

[61] Wagner KW, Alam H, Dhar SS, Giri U, Li N, Wei Y, et al. KDM2A promotes lung tumorigenesis by epigenetically enhancing ERK1/2 signaling. The Journal of Clinical Investigation. 2013;**123**:5231-5246. DOI: 10.1172/jci68642

[62] Zhao Z, Sun C, Li F, Han J, Li X, Song Z. Overexpression of histone demethylase JMJD5 promotes metastasis and indicates a poor prognosis in breast cancer. International Journal of Clinical and Experimental Pathology. 2015;**8**:10325-10334

[63] Nguyen AT, Zhang Y. The diverse functions of Dot1 and H3K79 methylation. Genes and Development. 2011;**25**:1345-1358. DOI: 10.1101/gad.2057811

[64] Daigle SR, Olhava EJ, Therkelsen CA, Majer CR, Sneeringer CJ, Song J, et al. Selective killing of mixed lineage leukemia cells by a potent small-molecule DOT1L inhibitor. Cancer Cell. 2011;**20**:53-65. DOI: 10.1016/j.ccr.2011.06.009

[65] Zhang L, Deng L, Chen F, Yao Y, Wu B, Wei L, et al. Inhibition of histone H3K79 methylation selectively inhibits proliferation, self-renewal and metastatic potential of breast cancer. Oncotarget. 2014;**5**:10665-10677. DOI: 10.18632/oncotarget.2496

[66] Bjorkman M, Ostling P, Harma V, Virtanen J, Mpindi JP, Rantala J, et al. Systematic knockdown of epigenetic enzymes identifies a novel histone demethylase PHF8 overexpressed in prostate cancer with an impact on cell proliferation, migration and invasion. Oncogene. 2012;**31**:3444-3456. DOI: 10.1038/onc.2011.512

[67] Li S, Sun A, Liang X, Ma L, Shen L, Li T, et al. Histone demethylase PHF8 promotes progression and metastasis of gastric cancer. American Journal of Cancer Research. 2017; **7**:448-461

[68] Tschiersch B, Hofmann A, Krauss V, Dorn R, Korge G, Reuter G. The protein encoded by the Drosophila position-effect variegation suppressor gene Su(var)3-9 combines domains of antagonistic regulators of homeotic gene complexes. The EMBO Journal. 1994;**13**:3822-3831

[69] Shilatifard A. Molecular implementation and physiological roles for histone H3 lysine 4 (H3K4) methylation. Current Opinion in Cell Biology. 2008;**20**:341-348. DOI: 10.1016/j.ceb.2008.03.019

[70] Wen B, Wu H, Shinkai Y, Irizarry RA, Feinberg AP. Large histone H3 lysine 9 dimethylated chromatin blocks distinguish differentiated from embryonic stem cells. Nature Genetics. 2009;**41**:246-250. DOI: 10.1038/ng.297

[71] Dong C, Wu Y, Wang Y, Wang C, Kang T, Rychahou PG, et al. Interaction with Suv39H1 is critical for Snail-mediated E-cadherin repression in breast cancer. Oncogene. 2013; **32**:1351-1362. DOI: 10.1038/onc.2012.169

[72] Dong C, Wu Y, Yao J, Wang Y, Yu Y, Rychahou PG, et al. G9a interacts with Snail and is critical for Snail-mediated E-cadherin repression in human breast cancer. The Journal of Clinical Investigation. 2012;**122**:1469-1486. DOI: 10.1172/jci57349

[73] Huang T, Zhang P, Li W, Zhao T, Zhang Z, Chen S, et al. G9A promotes tumor cell growth and invasion by silencing CASP1 in non-small-cell lung cancer cells. Cell Death and Disease. 2017;**8**:e2726. DOI: 10.1038/cddis.2017.65

[74] Chen MW, Hua KT, Kao HJ, Chi CC, Wei LH, Johansson G, et al. H3K9 histone methyltransferase G9a promotes lung cancer invasion and metastasis by silencing the cell adhesion molecule Ep-CAM. Cancer Research. 2010;**70**:7830-7840. DOI: 10.1158/0008-5472.can-10-0833

[75] Sun Y, Wei M, Ren SC, Chen R, Xu WD, Wang FB, et al. Histone methyltransferase SETDB1 is required for prostate cancer cell proliferation, migration and invasion. Asian Journal of Andrology. 2014;**16**:319-324. DOI: 10.4103/1008-682x.122812

[76] Wong CM, Wei L, Law CT, Ho DW, Tsang FH, Au SL, et al. Up-regulation of histone methyltransferase SETDB1 by multiple mechanisms in hepatocellular carcinoma promotes cancer metastasis. Hepatology. 2016;**63**:474-487. DOI: 10.1002/hep.28304

[77] Zhang H, Cai K, Wang J, Wang X, Cheng K, Shi F, et al. MiR-7, inhibited indirectly by lincRNA HOTAIR, directly inhibits SETDB1 and reverses the EMT of breast cancer stem cells by downregulating the STAT3 pathway. Stem Cells. 2014;**32**:2858-2868. DOI: 10.1002/stem.1795

[78] Ramadoss S, Guo G, Wang CY. Lysine demethylase KDM3A regulates breast cancer cell invasion and apoptosis by targeting histone and the non-histone protein p53. Oncogene. 2017;**36**:47-59. DOI: 10.1038/onc.2016.174

[79] Sechler M, Parrish JK, Birks DK, Jedlicka P. The histone demethylase KDM3A, and its downstream target MCAM, promote Ewing Sarcoma cell migration and metastasis. Oncogene. 2017;**36**:4150-4160. DOI: 10.1038/onc.2017.44

[80] Paolicchi E, Crea F, Farrar WL, Green JE, Danesi R. Histone lysine demethylases in breast cancer. Critical Reviews in Oncology/Hematology. 2013;**86**:97-103. DOI: 10.1016/j.critrevonc.2012.11.008

[81] Liu Y, Zheng P, Liu Y, Ji T, Liu X, Yao S, et al. An epigenetic role for PRL-3 as a regulator of H3K9 methylation in colorectal cancer. Gut. 2013;**62**:571-581. DOI: 10.1136/gutjnl-2011-301,059

[82] Li LL, Xue AM, Li BX, Shen YW, Li YH, Luo CL, et al. Erratum to: JMJD2A contributes to breast cancer progression through transcriptional repression of the tumor suppressor ARHI. Breast Cancer Research. 2016;**18**:114. DOI: 10.1186/s13058-016-0776-3

[83] Zhao L, Li W, Zang W, Liu Z, Xu X, Yu H, et al. JMJD2B promotes epithelial-mesenchymal transition by cooperating with beta-catenin and enhances gastric cancer metastasis. Clinical Cancer Research. 2013;**19**:6419-6429. DOI: 10.1158/1078-0432.ccr-13-0254

[84] Luo W, Chang R, Zhong J, Pandey A, Semenza GL. Histone demethylase JMJD2C is a coactivator for hypoxia-inducible factor 1 that is required for breast cancer progression. Proceedings of the National Academy of Sciences of the United States of America. 2012;**109**:E3367-E3376. DOI: 10.1073/pnas.1217394109

[85] Yokoyama Y, Matsumoto A, Hieda M, Shinchi Y, Ogihara E, Hamada M, et al. Loss of histone H4K20 trimethylation predicts poor prognosis in breast cancer and is associated with invasive activity. Breast Cancer Research. 2014;**16**:R66. DOI: 10.1186/bcr3681

[86] Viotti M, Wilson C, McCleland M, Koeppen H, Haley B, Jhunjhunwala S, et al. SUV420H2 is an epigenetic regulator of epithelial/mesenchymal states in pancreatic cancer. The Journal of Cell Biology. 2018;**217**:763-777. DOI: 10.1083/jcb.201705031

[87] Fang J, Feng Q, Ketel CS, Wang H, Cao R, Xia L, et al. Purification and functional characterization of SET8, a nucleosomal histone H4-lysine 20-specific methyltransferase. Current Biology. 2002;**12**:1086-1099

[88] Yang F, Sun L, Li Q, Han X, Lei L, Zhang H, et al. SET8 promotes epithelial-mesenchymal transition and confers TWIST dual transcriptional activities. The EMBO Journal. 2012;**31**:110-123. DOI: 10.1038/emboj.2011.364

[89] Hou L, Li Q, Yu Y, Li M, Zhang D. SET8 induces epithelialmesenchymal transition and enhances prostate cancer cell metastasis by cooperating with ZEB1. Molecular Medicine Reports. 2016;**13**:1681-1688. DOI: 10.3892/mmr.2015.4733

[90] Della Corte CM, Bellevicine C, Vicidomini G, Vitagliano D, Malapelle U, Accardo M, et al. SMO gene amplification and activation of the Hedgehog pathway as novel mechanisms of resistance to anti-epidermal growth factor receptor drugs in human lung cancer. Clinical Cancer Research. 2015;**21**:4686-4697. DOI: 10.1158/1078-0432.ccr-14-3319

[91] Fu L, Wu H, Cheng SY, Gao D, Zhang L, Zhao Y. Set7 mediated Gli3 methylation plays a positive role in the activation of Sonic Hedgehog pathway in mammals. eLife. 2016;**5**. DOI: 10.7554/eLife.15690

[92] Bernstein BE, Meissner A, Lander ES. The mammalian epigenome. Cell. 2007;**128**:669-681. DOI: 10.1016/j.cell.2007.01.033

[93] Barski A, Cuddapah S, Cui K, Roh TY, Schones DE, Wang Z, et al. High-resolution profiling of histone methylations in the human genome. Cell. 2007;**129**:823-837. DOI: 10.1016/j.cell.2007.05.009

[94] Hublitz P, Albert M, Peters AH. Mechanisms of transcriptional repression by histone lysine methylation. The International Journal of Developmental Biology. 2009;**53**:335-354. DOI: 10.1387/ijdb.082717 ph

[95] Xiang Y, Zhu Z, Han G, Lin H, Xu L, Chen CD. JMJD3 is a histone H3K27 demethylase. Cell Research. 2007;**17**:850-857. DOI: 10.1038/cr.2007.83

[96] Tiwari N, Tiwari VK, Waldmeier L, Balwierz PJ, Arnold P, Pachkov M, et al. Sox4 is a master regulator of epithelial-mesenchymal transition by controlling Ezh2 expression and epigenetic reprogramming. Cancer Cell. 2013;**23**:768-783. DOI: 10.1016/j.ccr.2013.04.020

[97] Chien YC, Liu LC, Ye HY, Wu JY, Yu YL. EZH2 promotes migration and invasion of triple-negative breast cancer cells via regulating TIMP2-MMP-2/−9 pathway. American Journal of Cancer Research. 2018;**8**:422-434

[98] Herranz N, Pasini D, Diaz VM, Franci C, Gutierrez A, Dave N, et al. Polycomb complex 2 is required for E-cadherin repression by the Snail1 transcription factor. Molecular and Cellular Biology. 2008;**28**:4772-4781. DOI: 10.1128/MCB.00323-08

[99] Zha L, Cao Q, Cui X, Li F, Liang H, Xue B, et al. Epigenetic regulation of E-cadherin expression by the histone demethylase UTX in colon cancer cells. Medical Oncology. 2016;**33**:21. DOI: 10.1007/s12032-016-0734-z

[100] Choi HJ, Park JH, Park M, Won HY, Joo HS, Lee CH, et al. UTX inhibits EMT-induced breast CSC properties by epigenetic repression of EMT genes in cooperation with LSD1 and HDAC1. EMBO Reports. 2015;**16**:1288-1298. DOI: 10.15252/embr.201540244

[101] Tang B, Qi G, Tang F, Yuan S, Wang Z, Liang X, et al. Aberrant JMJD3 expression upregulates slug to promote migration, invasion, and stem cell-like behaviors in hepatocellular carcinoma. Cancer Research. 2016;**76**:6520-6532. DOI: 10.1158/0008-5472.can-15-3029

[102] Park WY, Hong BJ, Lee J, Choi C, Kim MY. H3K27 demethylase JMJD3 employs the NF-kappaB and BMP signaling pathways to modulate the tumor microenvironment and promote melanoma progression and metastasis. Cancer Research. 2016;**76**:161-170. DOI: 10.1158/0008-5472.can-15-0536

[103] Gao Y, Zhao Y, Zhang J, Lu Y, Liu X, Geng P, et al. The dual function of PRMT1 in modulating epithelial-mesenchymal transition and cellular senescence in breast cancer cells through regulation of ZEB1. Scientific Reports. 2016;**6**:19874. DOI: 10.1038/srep19874

[104] Avasarala S, Van Scoyk M, Karuppusamy Rathinam MK, Zerayesus S, Zhao X, Zhang W, et al. PRMT1 is a novel regulator of epithelial-mesenchymal-transition in non-small cell lung cancer. The Journal of Biological Chemistry. 2015;**290**:13479-13489. DOI: 10.1074/jbc.M114.636050

[105] Li L, Zhang Z, Ma T, Huo R. PRMT1 regulates tumor growth and metastasis of human melanoma via targeting ALCAM. Molecular Medicine Reports. 2016;**14**:521-528. DOI: 10.3892/mmr.2016.5273

[106] Gou Q, He S, Zhou Z. Protein arginine N-methyltransferase 1 promotes the proliferation and metastasis of hepatocellular carcinoma cells. Tumor Biology. 2017;**39**. DOI: 10.1177/1010428317691419

[107] Zhou W, Yue H, Li C, Chen H, Yuan Y. Protein arginine methyltransferase 1 promoted the growth and migration of cancer cells in esophageal squamous cell carcinoma. Tumor Biology. 2016;**37**:2613-2619. DOI: 10.1007/s13277-015-4098-3

[108] Goulet I, Gauvin G, Boisvenue S, Cote J. Alternative splicing yields protein arginine methyltransferase 1 isoforms with distinct activity, substrate specificity, and subcellular localization. The Journal of Biological Chemistry. 2007;**282**:33009-33021. DOI: 10.1074/jbc.M704349200

[109] Bondy-Chorney E, Baldwin RM, Didillon A, Chabot B, Jasmin BJ, Cote J. RNA binding protein RALY promotes protein arginine methyltransferase 1 alternatively spliced isoform v2 relative expression and metastatic potential in breast cancer cells. The International Journal of Biochemistry and Cell Biology. 2017;**91**:124-135. DOI: 10.1016/j.biocel.2017.07.008

[110] Zhong J, Cao RX, Hong T, Yang J, Zu XY, Xiao XH, et al. Identification and expression analysis of a novel transcript of the human PRMT2 gene resulted from alternative polyadenylation in breast cancer. Gene. 2011;**487**:1-9. DOI: 10.1016/j.gene.2011.06.022

[111] Qi C, Chang J, Zhu Y, Yeldandi AV, Rao SM, Zhu YJ. Identification of protein arginine methyltransferase 2 as a coactivator for estrogen receptor alpha. The Journal of Biological Chemistry. 2002;**277**. DOI: 28624-30. DOI: 10.1074/jbc.M201053200

[112] Zhong J, Cao RX, Zu XY, Hong T, Yang J, Liu L, et al. Identification and characterization of novel spliced variants of PRMT2 in breast carcinoma. The FEBS Journal. 2012;**279**:316-335. DOI: 10.1111/j.1742-4658.2011.08426.x

[113] Wang L, Zhao Z, Meyer MB, Saha S, Yu M, Guo A, et al. CARM1 methylates chromatin remodeling factor BAF155 to enhance tumor progression and metastasis. Cancer Cell. 2014;**25**:21-36. DOI: 10.1016/j.ccr.2013.12.007

[114] Habashy HO, Rakha EA, Ellis IO, Powe DG. The estrogen receptor coactivator CARM1 has an oncogenic effect and is associated with poor prognosis in breast cancer. Breast Cancer Research and Treatment. 2013;**140**:307-316. DOI: 10.1007/s10549-013-2614-y

[115] Ohkura N, Takahashi M, Yaguchi H, Nagamura Y, Tsukada T. Coactivator-associated arginine methyltransferase 1, CARM1, affects pre-mRNA splicing in an isoform-specific manner. The Journal of Biological Chemistry. 2005;**280**:28927-28935. DOI: 10.1074/jbc.M502173200

[116] Fu T, Lv X, Kong Q, Yuan C. A novel SHARPIN-PRMT5-H3R2me1 axis is essential for lung cancer cell invasion. Oncotarget. 2017;**8**:54809-54820. DOI: 10.18632/oncotarget.18957

[117] Tsutsui T, Fukasawa R, Shinmyouzu K, Nakagawa R, Tobe K, Tanaka A, et al. Mediator complex recruits epigenetic regulators via its two cyclin-dependent kinase subunits to repress transcription of immune response genes. The Journal of Biological Chemistry. 2013;**288**:20955-20965. DOI: 10.1074/jbc.M113.486746

[118] Migliori V, Muller J, Phalke S, Low D, Bezzi M, Mok WC, et al. Symmetric dimethylation of H3R2 is a newly identified histone mark that supports euchromatin maintenance. Nature Structural and Molecular Biology. 2012;**19**:136-144. DOI: 10.1038/nsmb.2209

[119] Chen H, Lorton B, Gupta V, Shechter D. A TGFbeta-PRMT5-MEP50 axis regulates cancer cell invasion through histone H3 and H4 arginine methylation coupled transcriptional activation and repression. Oncogene. 2017;**36**:373-386. DOI: 10.1038/onc.2016.205

[120] Hou Z, Peng H, Ayyanathan K, Yan KP, Langer EM, Longmore GD, et al. The LIM protein AJUBA recruits protein arginine methyltransferase 5 to mediate SNAIL-dependent transcriptional repression. Molecular and Cellular Biology. 2008;**28**:3198-3207. DOI: 10.1128/mcb.01435-07

[121] Hu D, Gur M, Zhou Z, Gamper A, Hung MC, Fujita N, et al. Interplay between arginine methylation and ubiquitylation regulates KLF4-mediated genome stability and carcinogenesis. Nature Communications. 2015;**6**:8419. DOI: 10.1038/ncomms9419

[122] Yoshimatsu M, Toyokawa G, Hayami S, Unoki M, Tsunoda T, Field HI, et al. Dysregulation of PRMT1 and PRMT6, Type I arginine methyltransferases, is involved in various types of human cancers. International Journal of Cancer. 2011;**128**:562-573. DOI: 10.1002/ijc.25366

[123] Dowhan DH, Harrison MJ, Eriksson NA, Bailey P, Pearen MA, Fuller PJ, et al. Protein arginine methyltransferase 6-dependent gene expression and splicing: association with

breast cancer outcomes. Endocrine-Related Cancer. 2012;**19**:509-526. DOI: 10.1530/erc-12-0100

[124] Geng P, Zhang Y, Liu X, Zhang N, Liu Y, Liu X, et al. Automethylation of protein arginine methyltransferase 7 and its impact on breast cancer progression. The FASEB Journal. 2017;**31**:2287-2300. DOI: 10.1096/fj.201601196R

[125] Baldwin RM, Haghandish N, Daneshmand M, Amin S, Paris G, Falls TJ, et al. Protein arginine methyltransferase 7 promotes breast cancer cell invasion through the induction of MMP9 expression. Oncotarget. 2015;**6**:3013-3032. DOI: 10.18632/oncotarget.3072

[126] Thomassen M, Tan Q, Kruse TA. Gene expression meta-analysis identifies chromosomal regions and candidate genes involved in breast cancer metastasis. Breast Cancer Research and Treatment. 2009;**113**:239-249. DOI: 10.1007/s10549-008-9927-2

[127] Yang Y, Hadjikyriacou A, Xia Z, Gayatri S, Kim D, Zurita-Lopez C, et al. PRMT9 is a type II methyltransferase that methylates the splicing factor SAP145. Nature Communications. 2015;**6**:6428. DOI: 10.1038/ncomms7428

[128] Jiang H, Zhou Z, Jin S, Xu K, Zhang H, Xu J, et al. PRMT9 promotes hepatocellular carcinoma invasion and metastasis via activating PI3K/Akt/GSK-3beta/Snail signaling. Cancer Science. 2018. DOI: 10.1111/cas.13598

[129] Greiner D, Bonaldi T, Eskeland R, Roemer E, Imhof A. Identification of a specific inhibitor of the histone methyltransferase SU(VAR)3-9. Nature Chemical Biology. 2005;**1**:143-145. DOI: 10.1038/nchembio721

[130] Lakshmikuttyamma A, Scott SA, DeCoteau JF, Geyer CR. Reexpression of epigenetically silenced AML tumor suppressor genes by SUV39H1 inhibition. Oncogene. 2010;**29**:576-588. DOI: 10.1038/onc.2009.361

[131] Kubicek S, O'Sullivan RJ, August EM, Hickey ER, Zhang Q, Teodoro ML, et al. Reversal of H3K9me2 by a small-molecule inhibitor for the G9a histone methyltransferase. Molecular Cell. 2007;**25**:473-481. DOI: 10.1016/j.molcel.2007.01.017

[132] Liu S, Ye D, Guo W, Yu W, He Y, Hu J, et al. G9a is essential for EMT-mediated metastasis and maintenance of cancer stem cell-like characters in head and neck squamous cell carcinoma. Oncotarget. 2015;**6**:6887-6901. DOI: 10.18632/oncotarget.3159

[133] Vedadi M, Barsyte-Lovejoy D, Liu F, Rival-Gervier S, Allali-Hassani A, Labrie V, et al. A chemical probe selectively inhibits G9a and GLP methyltransferase activity in cells. Nature Chemical Biology. 2011;**7**:566-574. DOI: 10.1038/nchembio.599

[134] Liu XR, Zhou LH, Hu JX, Liu LM, Wan HP, Zhang XQ. UNC0638, a G9a inhibitor, suppresses epithelialmesenchymal transitionmediated cellular migration and invasion in triple negative breast cancer. Molecular Medicine Reports. 2018;**17**:2239-2244. DOI: 10.3892/mmr.2017.8190

[135] Finley A, Copeland RA. Small molecule control of chromatin remodeling. Chemistry and Biology. 2014;**21**:1196-1210. DOI: 10.1016/j.chembiol.2014.07.024

[136] Tan J, Yang X, Zhuang L, Jiang X, Chen W, Lee PL, et al. Pharmacologic disruption of Polycomb-repressive complex 2-mediated gene repression selectively induces apoptosis in cancer cells. Genes and Development. 2007;**21**:1050-1063. DOI: 10.1101/gad.1524107

[137] Mody HR, Hung SW, AlSaggar M, Griffin J, Govindarajan R. Inhibition of S-adenosylmethionine-dependent methyltransferase attenuates TGFbeta1-induced EMT and metastasis in pancreatic cancer: Putative roles of miR-663a and miR-4787-5p. Molecular Cancer Research. 2016;**14**:1124-1135. DOI: 10.1158/1541-7786.mcr-16-0083

[138] Wang L, Chang J, Varghese D, Dellinger M, Kumar S, Best AM, et al. A small molecule modulates Jumonji histone demethylase activity and selectively inhibits cancer growth. Nature Communications. 2013;**4**:2035. DOI: 10.1038/ncomms3035

[139] Cheng D, Yadav N, King RW, Swanson MS, Weinstein EJ, Bedford MT. Small molecule regulators of protein arginine methyltransferases. The Journal of Biological Chemistry. 2004;**279**:23892-23899. DOI: 10.1074/jbc.M401853200

[140] Castellano S, Milite C, Ragno R, Simeoni S, Mai A, Limongelli V, et al. Design, synthesis and biological evaluation of carboxy analogues of arginine methyltransferase inhibitor 1 (AMI-1). ChemMedChem. 2010;**5**:398-414. DOI: 10.1002/cmdc.200900459

[141] Zhang B, Dong S, Zhu R, Hu C, Hou J, Li Y, et al. Targeting protein arginine methyltransferase 5 inhibits colorectal cancer growth by decreasing arginine methylation of eIF4E and FGFR3. Oncotarget. 2015;**6**:22799-22811. DOI: 10.18632/oncotarget.4332

[142] Liu Z, Wang P, Chen H, Wold EA, Tian B, Brasier AR, et al. Drug discovery targeting bromodomain-containing protein 4. Journal of Medicinal Chemistry. 2017;**60**:4533-4558. DOI: 10.1021/acs.jmedchem.6b01761

[143] Fontanals-Cirera B, Hasson D, Vardabasso C, Di Micco R, Agrawal P, Chowdhury A, et al. Harnessing BET inhibitor sensitivity reveals AMIGO2 as a melanoma survival gene. Molecular Cell. 2017;**68**:731-744.e9. DOI: 10.1016/j.molcel.2017.11.004

[144] Wagner T, Robaa D, Sippl W, Jung M. Mind the methyl: methyllysine binding proteins in epigenetic regulation. ChemMedChem. 2014;**9**:466-483. DOI: 10.1002/cmdc.201300422

[145] Maison C, Almouzni G. HP1 and the dynamics of heterochromatin maintenance. Nature Reviews. Molecular Cell Biology. 2004;**5**:296-304. DOI: 10.1038/nrm1355

[146] Millanes-Romero A, Herranz N, Perrera V, Iturbide A, Loubat-Casanovas J, Gil J, et al. Regulation of heterochromatin transcription by Snail1/LOXL2 during epithelial-to-mesenchymal transition. Molecular Cell. 2013;**52**:746-757. DOI: 10.1016/j.molcel.2013.10.015

[147] Tang M, Shen H, Jin Y, Lin T, Cai Q, Pinard MA, et al. The malignant brain tumor (MBT) domain protein SFMBT1 is an integral histone reader subunit of the LSD1 demethylase complex for chromatin association and epithelial-to-mesenchymal transition. The Journal of Biological Chemistry. 2013;**288**:27680-27691. DOI: 10.1074/jbc.M113.482349

[148] Wysocka J, Swigut T, Milne TA, Dou Y, Zhang X, Burlingame AL, et al. WDR5 associates with histone H3 methylated at K4 and is essential for H3 K4 methylation and vertebrate development. Cell. 2005;**121**:859-872. DOI: 10.1016/j.cell.2005.03.036

[149] Wu MZ, Tsai YP, Yang MH, Huang CH, Chang SY, Chang CC, et al. Interplay between HDAC3 and WDR5 is essential for hypoxia-induced epithelial-mesenchymal transition. Molecular Cell. 2011;**43**:811-822. DOI: 10.1016/j.molcel.2011.07.012

[150] Margueron R, Justin N, Ohno K, Sharpe ML, Son J, Drury WJ 3rd, et al. Role of the polycomb protein EED in the propagation of repressive histone marks. Nature. 2009; **461**:762-767. DOI: 10.1038/nature08398

[151] Collins RE, Northrop JP, Horton JR, Lee DY, Zhang X, Stallcup MR, et al. The ankyrin repeats of G9a and GLP histone methyltransferases are mono- and dimethyllysine binding modules. Nature Structural and Molecular Biology. 2008;**15**:245-250. DOI: 10.1038/nsmb.1384

[152] James LI, Barsyte-Lovejoy D, Zhong N, Krichevsky L, Korboukh VK, Herold JM, et al. Discovery of a chemical probe for the L3MBTL3 methyllysine reader domain. Nature Chemical Biology. 2013;**9**:184-191. DOI: 10.1038/nchembio.1157

[153] Stuckey JI, Dickson BM, Cheng N, Liu Y, Norris JL, Cholensky SH, et al. A cellular chemical probe targeting the chromodomains of Polycomb repressive complex 1. Nature Chemical Biology. 2016;**12**:180-187. DOI: 10.1038/nchembio.2007

[154] Shi Y, Sawada J, Sui G, Affar el B, Whetstine JR, Lan F, et al. Coordinated histone modifications mediated by a CtBP co-repressor complex. Nature. 2003;**422**:735-738. DOI: 10.1038/nature01550

[155] Shi Y, Lan F, Matson C, Mulligan P, Whetstine JR, Cole PA, et al. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. Cell. 2004;**119**:941-953. DOI: 10.1016/j.cell.2004.12.012

[156] Nishioka K, Chuikov S, Sarma K, Erdjument-Bromage H, Allis CD, Tempst P, et al. Set9, a novel histone H3 methyltransferase that facilitates transcription by precluding histone tail modifications required for heterochromatin formation. Genes and Development. 2002;**16**:479-489. DOI: 10.1101/gad.967202

[157] Wang H, Cao R, Xia L, Erdjument-Bromage H, Borchers C, Tempst P, et al. Purification and functional characterization of a histone H3-lysine 4-specific methyltransferase. Molecular Cell. 2001;**8**:1207-1217

[158] Fu J, Qin L, He T, Qin J, Hong J, Wong J, et al. The TWIST/Mi2/NuRD protein complex and its essential role in cancer metastasis. Cell Research. 2011;**21**:275-289. DOI: 10.1038/cr.2010.118

[159] Chang Y, Sun L, Kokura K, Horton JR, Fukuda M, Espejo A, et al. MPP8 mediates the interactions between DNA methyltransferase Dnmt3a and H3K9 methyltransferase GLP/G9a. Nature Communications. 2011;**2**:533. DOI: 10.1038/ncomms1549

[160] Kokura K, Sun L, Bedford MT, Fang J. Methyl-H3K9-binding protein MPP8 mediates E-cadherin gene silencing and promotes tumor cell motility and invasion. The EMBO Journal. 2010;**29**:3673-3687. DOI: 10.1038/emboj.2010.239

[161] Sun L, Kokura K, Izumi V, Koomen JM, Seto E, Chen J, et al. MPP8 and SIRT1 crosstalk in E-cadherin gene silencing and epithelial-mesenchymal transition. EMBO Reports. 2015;**16**:689-699. DOI: 10.15252/embr.201439792

IntechOpen