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# The Landscape of Histone Modification in Cancer Metastasis

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## 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

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## 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  $\beta$ -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 labile 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

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
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  $\epsilon$ -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)<sup>+</sup>-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- $\beta$ ) and WNT co-operated to mediate EMT. TGF- $\beta$  induces the translocation of  $\beta$ -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- $\beta$ -receptor 2 (TGFBR2) expression with an increase of H3K9 acetylation on TGFBR2 promoter to increase TGF- $\beta$  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-2 $\alpha$  is a transcriptional regulator of the *HDAC1* gene, and hypoxia increase HIF-2 $\alpha$  and HDAC1 expression [21]. TGF- $\beta$ -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 ER $\alpha$  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 ER $\alpha$  are co-localized in the cytoplasm of renal cell carcinoma (RCC) cells and HDAC6 enhances cell motility by decreasing acetylated  $\alpha$ -tubulin expression [21]. Loss of  $\alpha$ -Tubulin acetylation by HDAC6 is also associated with TGF- $\beta$ -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<sup>+</sup> 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 Mocetinostat, 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.

## 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  $\epsilon$ -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 HIF1 $\alpha$  and regulates hypoxia-induced HOTAIR expression to facilitate tumorigenesis [46]. In addition, MLL1 interacts with  $\beta$ -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 H3K4me<sub>2</sub> 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 H3K4me<sub>1-2</sub>. 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- $\beta$ 1 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 H3K4me<sub>3</sub> 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  $\beta$ -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 H3K9me<sub>3</sub>, 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 H3K9me<sub>3</sub> 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  $\beta$ -catenin and binds to the promoter of the  $\beta$ -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 HIF1 $\alpha$ , 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 H4K20me<sub>3</sub> was associated with heterochromatin and played a pivotal role in chromatin integrity. In addition, loss of histone H4K20me<sub>3</sub> predicts poor prognosis in breast cancer and is associated with invasive activity. On the other hand, H4K20me<sub>1</sub> is located in the promoters or coding regions of active genes and co-localizes with H3K9me<sub>1</sub>, which suggest that H4K20me<sub>1</sub> is associated with transcriptional activity. H4K20 is

methyated 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 H4K20me<sub>3</sub> 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. H3K27me<sub>3</sub> is associated with constitutive heterochromatin and maintenance of gene repression during early development. According to a genome-wide analysis, the levels of H3K27me<sub>2</sub> and H3K27me<sub>3</sub> are elevated in silent promoters and reduced in both active promoters and genic regions, whereas the level of H3K27me<sub>1</sub> 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 H3K4me<sub>3</sub> 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/JMJ3D3), 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- $\beta$  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 as 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 of several targets of NF- $\kappa$ B 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 guanidiny 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 tumorigenesis 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 ER $\alpha$  and ER $\beta$  *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, PRMT2 $\alpha$ ,  $\beta$ , and  $\gamma$ ) in addition to the original PRMT2 isoform were identified [112]. Several splice variants (*i.e.*, PRMT2- $\alpha$ , - $\beta$ , - $\gamma$ ) were identified as induced in breast cancer, particularly in ER, PR-positive breast cancer [110]. PRMT2 directly binds and enhances estrogen-mediated transactivation of ER $\alpha$ , 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/EBP $\beta$  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- $\beta$ -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- $\beta$  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, PRMT7 $\alpha$  and  $\beta$ , which are active and have slightly different methylation profiles and locations. PRMT7 $\alpha$  localizes to the cytoplasm and nucleus, whereas PRMT7 $\beta$  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 methyltransferase 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-3 $\beta$ /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 selectivity [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- $\beta$ -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]. HP1 $\alpha$  was down-regulated in metastatic cells of colon cancer and thyroid carcinomas relative to non-metastatic cells, indicating HP1 $\alpha$  may be directly involved in the silencing of genes that potentiate cancer cell invasive potential and metastasis. Recent evidence implicate HP1 $\alpha$  in EMT. The association of HP1 $\alpha$  to major satellite repeat sequences located in pericentric heterochromatin decreased during the initial steps of TGF- $\beta$ -induced EMT in a SNAIL/LOXL2-dependent manner [146]. In addition, HP1 $\alpha$  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 H3K4me<sub>2/3</sub> and form a stable complex with LSD1. SFMBT1 is essential for SNAIL-dependent recruitment of LSD1 to chromatin, demethylation of H3K4me<sub>2</sub>, transcriptional repression of epithelial markers, and induction of EMT by TGF- $\beta$  [147]. H3K4me<sub>2/3</sub> 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 H3K4me<sub>3</sub> [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 H3K27me<sub>3</sub>. EED recruits PRC2 to chromatin with pre-existing H3K27me<sub>3</sub> 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, HDAC1/2 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 Mi2 $\beta$ , 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- $\kappa$ B 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-TEF $\beta$ /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 methyl-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 EMT-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.

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## Conflict of interest

The authors have declared that no conflict of interests exists.

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