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Histone methylation in myelodysplastic syndromes

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Abstract

Histone methylation is a type of epigenetic modification that is critical for the regulation of gene expression. Numerous studies have demonstrated that abnormalities of this newly characterized epigenetic modification are involved in the development of multiple diseases, including cancer. There is also emerging evidence for a link between histone methylation and the pathogenesis of myeloid neoplasms, including myelodysplastic syndromes (MDS). This article provides an overview of recent progress in the studies of histone methylation in myeloid malignancies, with an emphasis on MDS. We cover each type of histone methylation modification and their regulatory mechanisms, as well as their abnormalities in MDS or potential connections to MDS. We also summarize the recent progress in the development of inhibitors targeting histone methylation and their applications as potential therapeutic agents.

Keywords

histone demethylase inhibitor; histone methylation; histone methyltransferase; myelodysplastic syndrome

Myelodysplastic syndromes (MDS) are a very complex group of myeloid neoplasms with diverse molecular backgrounds and natural histories [1]. With a median age of presentation being 70–75 years, the incidence of MDS in the USA and other western societies increases as the population ages [2]. Besides age, risk factors for MDS also include exposure to chemicals, cigarette smoking and prior exposure to chemotherapy or radiation therapy [3]. Indeed, cases of therapy-related MDS (tMDS) have an extremely poor prognosis and tend to be refractory to conventional therapeutic interventions. Overall, MDS is becoming one of the most common forms of myeloid neoplasms in older individuals [2].

Myelodysplastic syndromes have profound heterogeneity in genetic backgrounds, cytogenetic features, morphologic presentation and clinical course. Due to these

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complexities, the diagnosis and prognosis of MDS is extremely heterogeneous. At the present time, diagnosis of MDS is usually based on the 2008 WHO classification system [4]. Prognosis of MDS is calculated using a number of scoring systems, with the International Prognostic Scoring System (IPSS) as the one used most frequently [5]. This system divides MDS patients into four different subsets: low, intermediate-1, intermediate-2 and high risk.

Although there is an approximately 25% of incidence of transforming to AML [6], most patients with lower risk MDS (low or intermediate-1) die from causes intrinsic to MDS and not from transformation to acute myelogenous leukemia (AML) [7]. In addition, while karyotype is one of the most important criteria in different classification systems for MDS, with a large portion of MDS patients displaying clonal chromosome abnormalities, such as 5q-deletions and monosomy 7 [8]. In sharp contrast to AML, balanced chromosomal structural abnormalities, including translocations and inversions, are rarely detected in MDS. Overall, these observations suggest that there are unique pathophysiological mechanisms for MDS that are different from AML.

Besides cytogenetic changes, other known genetic lesions identified in MDS include copy number changes (genetic amplifications or deletions) and mutations that alter the sequence or expression of individual genes. These newly identified lesions in MDS have been summarized by Bejar *et al.* in a recent review, which include mutations within individual genes such as *TP53*, *TET2*, *ASXL1*, *RUNX1*, *IDH1* and *IDH2*, as well as members involved in tyrosine kinase pathway [9]. In addition, it has been demonstrated that MDS is also characterized by frequent epigenetic abnormalities, resulting in aberrant gene expression [10].

Overview of epigenetic regulation and histone methylation in MDS

The term epigenetic modification refers to different forms of biochemical modification of DNA or histone proteins (core histone H2A, H2B, H3 and H4, as well as the linker histone H1) in chromatin that affect gene expression without altering the DNA sequence [11]. While the importance of epigenetic regulation has been demonstrated in different types of malignancies [10,12–14], cancer is now viewed as the result of the acquisition of both genetic and epigenetic abnormalities that cause aberrant gene expression. More importantly, from a cancer therapeutic perspective, in contrast to genetic alterations, epigenetic abnormalities can be reversed, allowing for potential therapeutic interventions.

Among different types of epigenetic modifications, DNA methylation and histone acetylation have been well characterized in various neoplasms, including MDS [10]. Aberrant DNA CpG methylation is very common in MDS [15,16] and aberrant expression as well as genetic lesions of genes encoding DNA methylation regulators has also been reported in MDS. These include overexpression of DNA methyltransferase 1 (DNMT1) and DNMT3A [17], as well as mutations on the gene encoding TET2, an enzyme involved in cytosine demethylation [18,19]. Two DNA hypomethylating agents, 5-azacitidine and 5-aza-2′-deoxycitidine, are approved in the USA for patients with MDS and have shown activity in patients with higher disease risk [20]. For histone acetylation, among the various structurally different, naturally occurring as well as synthetic histone deacetylase inhibitors (HDACis), vorinostat (SAHA), LBH589, depsipeptide, MGCD-0103 and several others have shown clinical activity in trials involving MDS [21–24].

In comparison to DNA methylation and histone acetylation, histone methylation has just recently been appreciated as an important epigenetic mechanism of gene regulation, especially after the discovery of the dynamic nature of histone modification and the identification of the family of histone demethylases [25]. Methylation on chromatin histone has been identified on lysine (K) and arginine (R) residues of histone H3 and H4.

Documented methylated lysine residues include H3 lysine 4 (H3K4), H3K9, H3K27, H3K36, H3K79 and H4K20 and each lysine can be mono-, di- and tri-methylated (me1, me2 and me3), respectively [26,27]. Methylation of arginine of histone has been detected on H4 arginine 3 (H4R3), H3R2, H3R8, H3R17 and H3R26, which can be mono-, asymmetric-di- and symmetric-di-methylated [28]. Like other forms of epigenetic modification, histone methylation plays an important role in the regulation of gene transcription as well as the development of disease, by influencing the compaction of chromatin histone tails and the binding of proteins such as transcription factors that recognize histone marks [29,30]. In these methyl histone recognizing/interacting proteins, several metylated histone recognizing domains have been identified. Examples of these domains include the chromo domain in HP1 and CHD1 proteins in recognizing the methylated H3K4 [31]; the Tudor domain in p53BP1 and JMJD2A recognizing H3K9 or H3K4 methylation [32]; the MBT domain in CGI-72 recognizing H3K4 or H4K20 methylation [33]; and the PHD domain used by protein ING2 for gene repression [34]. In contrast to other epigenetic modifications, the effect of histone methylation on transcription (activating vs repressive) is not only dependent on the location of each substrate lysine or arginine, but also on the degree of methylation (mono-, di- or tri-methylation) on each substrate residue. This mechanism provides unique fine-tuning regulation of gene expression. Albeit the subtle differences of the effects on transcription associated within each subtype of methylation, generally, methylation of H3K4, H3K36 and possibly also H3K79, are positively associated with active transcription, whereas H3K9, H3K27 and H4K20 methylation tend to negatively regulate transcription [35]. Histone methylation has also been shown to be dynamically regulated. While the methylated status of histone lysine and arginine is maintained by histone methyltransferases (HMTs), the newly discovered family of histone demethylases (HDMs), particularly histone lysine demethylases (KDMs) [25,27,30,36–37], catalyze the removal of methyl groups.

Inappropriate levels of histone methylation and lesions of histone methylation regulators have both been identified in different types of myeloid neoplasms [38,39]. Evidence has recently emerged that abnormalities of histone methylation may also contribute to the development of MDS [40–42]. To demonstrate the progress in this rapid evolving field, we will discuss the known and potential associations of each type of histone methylation with MDS. We will summarize the aberrant levels of histone methylation on important MDS-related genes, as well as the genetic lesions and functional abnormalities of HMTs, HDMs and their cofactors or interacting proteins. We realize that evidence on the direct involvement of regulation of histone methylation in MDS is still very limited. Molecular research in MDS is hampered owing to the heterogeneity of the disease, the lack of confirmed cell lines or widely available animal models, as well as the limited number of malignant cells that are available. Therefore, in this article, some of the potential roles the discussion of certain types of histone methylation and their regulators in MDS are still hypothetical and sometimes are based on the results achieved from studies performed in other types of myeloid neoplasms, particularly AML.

Abnormalities of histone methylation associated with MDS

Activating histone lysine methylation marks

H3K4 methylation—Methylated H3K4 is mostly concentrated in the proximity of transcription starting sites (TSS) and are associated with activation of transcription [35]. Based on this, our group used chromatin immunoprecipitation (CHIP) combined with deep sequencing techniques to profile the H3K4 trimethylation signature in primary MDS bone marrow cells. With this approach, we have identified a list of aberrantly activated genes associated with differentially high levels of promoter H3K4 trimethylation in MDS [43]. Of interest, a majority of these genes thus identified are involved in NF-κB activation. Further

studies need to be performed to dissect the role of these gene products during the pathogenesis of MDS and to characterize the potential interplay between the regulation of H3K4 trimethylation and NF-κB signaling.

In comparison to other types of myeloid neoplasms, including AML, lesions of H3K4 methylation regulators identified in MDS are relatively rare. At the present time, there is only indirect evidence suggesting that two H3K4 methylation regulators, the methyltransferase mixed lineage leukemia (MLL) and the demethylases LSD1, might be involved in MDS. MLL is a H3K4 histone methyltransferase belonging to the family of SET domain proteins [44,45]. In MDS, the translocation of *MLL* gene locus 11q23 is very rare, much less frequent than AML. However, MLL partial tandem duplications (MLL-PTDs) have been described in both primary MDS and tMDS [46,47]. Of interest, the incidence of MLL-PTD increases from *de novo* MDS to secondary AML (s-AML) [47], suggesting that MLL may be involved in the progression of MDS to AML. Functionally, MLL has been reported to bind to several *Hox* gene promoters and to play a crucial role in the positive regulation of *Hox* genes, including *HoxA9* and *Meis1*, in the hematopoietic system [48]. While overexpression of *Hox* genes is commonly detected in the bone marrow cells of MDS [49], it might be a reasonable hypothesis that the positive regulation of certain *Hox* genes by MLL-regulated H3K4 methylation could be involved in the development of MDS. However, more clinical as well as experimental evidence is still needed to support this hypothesis.

LSD1 is the first histone demethylase characterized and also the only none JmjC-domain histone lysine demethylase identified so far [36]. LSD1 protein is located in a transcriptional repressive complex containing CoREST, HDAC1 and HDAC2, which has the activity to remove methyl groups from mono- and di-methyl-H3K4 [50]. Inhibition of CoREST and LSD1 perturbs differentiation of erythroid, megakaryocytic and granulocytic cells as well as primary erythroid progenitors [51,52]. This suggests that LSD1 and the H3K4 methylation regulated by this histone demethylase are important for hematopoiesis. While no genetic lesion or abnormal expression of LSD1 has been reported in MDS, several important transcription regulators that are potentially involved in the pathogenesis of MDS are known to be functionally related to LSD1 and LSD1 mediated H3K4 demethylase activity. For instance, GFI1 is a transcription repressor that has been reported to be expressed at a higher level in high-risk MDS than in low-risk patients [53]. The LSD1 related H3K4 demethylase activity has been demonstrated to be recruited by GFI1 and to mediate the gene repressing activity of GFI1 on its target genes *in vivo*. LSD1 depletion derepresses GFI targets in lineage-specific patterns, which is accompanied by enhanced H3K4 methylation at the respective promoter [51]. Another transcription regulator that is required for hematopoiesis and is functionally related to LSD1 is TAL1 [52]. TAL1 is upregulated in Shwachman–Diamond syndrome, an inherited bone marrow disorder characterized by cytopenia and a high propensity to develop MDS [54]. LSD1 and its demethylase activity negatively regulate TAL1-mediated transcription of *P4.2* gene in undifferentiated, but not in differentiated, murine erythroleukemia (MEL) cells [52] and this dynamic interaction between TAL1 and LSD1 may determine the onset of erythroid differentiation programs [52]. LSD1 may also cooperate with ASXL1 in transcriptional repression [55]. ASXL1 has been found to be mutated in MDS and chronic myelomonocytic leukemia (CMML) [56–58]. These connections between the LSD1 mediated H3K4 demethylation and the functions of several transcriptional factors, which are known to be involved in hematopoiesis and/or the development of MDS, suggest that LSD1 may also have a role in MDS pathogenesis. However, direct molecular and clinical evidence are still in need to support this hypothesis.

H3K36 methylation—In general, histone H3K36 methylation is associated with actively transcribed genes [35]. Although no specific gene that carries aberrant level of H3K36 has been documented in MDS, fusion of H3K36 methyltransferase encoding gene *NSD1*/*NSD3*

and *NUP98* have been detected in AML, MDS and tMDS [59,60]. Functionally, fusion protein NUP98–NSD1 activates and maintains a high methylated status of H3K36 on *HoxA9* gene during *in vitro* self-renewal of myeloid stem cells, [59], whereas inhibition of the H3K36-methyltransferase activity of NSD1 prevents the activation of HoxA9 and myeloid progenitor immortalization [61]. These results link deregulated H3K36 methylation to the transcriptional regulation of the *HoxA* locus and potentially to the development of myeloid neoplasms. Whether this chimeric gene products and H3K36 methylation are involved in the pathogenesis of MDS still needs to be investigated. The known histone demethylases that target H3K36 methylation are FBXL10 and FBXL11 [62]. Although no genetic lesion or aberrant expression of these two genes has been reported in MDS, FBXL10 has been found to interact with the leukemia associated with the oncogene BCL6 product in the BCL6-interacting corepressor transcriptional repressing complex [63]. Related to this, high-resolution comparative genomic hybridization combined with FISH analysis has detected the amplification of *BCL6* gene in MDS [64]. Therefore, it would be interesting to examine the interaction between BCL6 and FBXL10 as well as the possible gene silencing mediated by this protein complex in MDS.

H3K79 methylation—Unlike other methylated lysine residues that are located at the N-terminal tail of histones, H3K79 is uniquely located within a loop in the globular domain of histone H3 and is exposed on the nucleosome surface. Although some studies have associated H3K79 methylation with gene activation [35,65], the effect of this histone modification on transcription is still not completely understood.

DOT1L is an H3K79 specific methyltransferase, which unlike most other histone methyltransferases, lacks the SET domain [66,67]. There are two potential associations between DOT1L mediated H3K79 methylation to the pathogenesis of MDS. First, DOTL1 has been found to be involved in the pathobiology of the majority of hematopoietic neoplasms that are characterized by oncogenic fusion proteins including MLL-AF9, MLL-AF10, MLL-ENL, CALM-AF10 and MLL-AF4 [68]. This interaction contributes to the activation of *Hox* family genes such as *HoxA5*, *HoxA9* and *Meis1*, which in many cases, are associated with increase of H3K79 methylation on gene promoters [68,69]. Although these fusion genes are very rarely detected in MDS, one of them, *MLL-AF4*, has been reported in MDS [70]. The second potential association between DOT1L and MDS is based on a mouse model lacking functional DOT1L protein [71]. These mice displayed defects in early erythropoiesis, accompanied by affected expression of *GATA2* and *PU.1* genes and altered H3K79 methylation on their promoters [71]. GATA2 and PU.1 are important transcription factors for the differentiation of hematopoietic progenitors of erythroid and myeloid lineages [72], the two hematopoietic lineages frequently affected in MDS. Of importance, GATA2 has been detected to be aberrantly expressed in MDS [73], whereas PU.1 is known to interact with EVI1 oncogene product [74]. It is also important to know that the MDS–EVI1 fusion gene encodes a PR (PRD1–BF1–RIZ1) domain containing protein which belongs to the SET domain protein superfamily, whereas the EVI1 protein lacking a PR domain is often overexpressed in myeloid neoplasms, including MDS [75].

Repressive histone lysine methylation marks

H3K27 methylation—Numerous studies have demonstrated that methylation on H3K27 correlates with gene repression [35]. In pluripotent embryonic stem cells, the repressive mark H3K27me3 can coexist with and usually dominates, the active mark of H3K4me3 [74]. This so called ‘bivalent domain’ promoter status holds a repressed but poised state for their targets, which are often key differentiation and development regulatory genes. Therefore this bivalent status is thought to enable embryonic stem cells to rapidly execute multiple differentiation programs, including the differentiation toward blood-forming

precursors [76–78]. In AML blast cells, the 'bivalent' promoter status has also been identified for *p15INK4b* tumor suppressor gene, which can be changed to a more repressed status with the loss of H3K4me3 and the exclusive H3K27me3 mark [79]. This change is correlated with DNA methylation [79]. In MDS, gene silencing and hypermethylation of the *p15INK4b* promoter are also both frequent [80,81]. Therefore, although MDS may not share most mechanisms of pathogenesis with AML, it is still possible that in MDS, similar to the silencing of *p15INK4b* in AML, the hypermethylation of *p15INK4b* correlates with a high H3K27me3 level on its promoter in MDS. Status of histone metylation, particularly the level of H3K27 methylation, on *p15INK4b* promoter in MDS patients should be examined. Another tumor suppressor gene that could be epigenetically repressed in MDS is *CTNNA1.* The *CTNNA1* gene is located on chromosome 5q and is frequently repressed in MDS [82]. The correlation between DNA hypermethylation and enrichment of H3K27 methylation has been detected for *CTNNA1* gene in AML cell lines [83]. In MDS, DNA methylation of *CTNNA1* was only found in high-risk, but not low-risk patients, whereas high histone H3K27me3 level has not been detected for this gene in MDS [83,84]. Therefore, although DNA methylation and H3K27 methylation may both contribute to the silencing of key tumor suppressors such as *p15INK4b* and *CTNNA1* in AML, whether a similar mechanism could be true for the same gene silencing in MDS still needs to be examined.

EZH2 encodes a H3K27 methyltransferase, which is the catalytic unit of the polycomb repressive complex 2 (PRC2) [85]. The *EZH2* gene is located on 7q36.1, a common locus of chromosomal deletion correlated with a poor prognosis in MDS [86]. Missense mutations of *EZH2* gene that introduce premature truncated forms of this protein have recently been discovered in MDS bone marrow cells [40]. Particularly, most of these mutations cause the alteration or truncation in the SET domain, the essential methyltransferase functional domain of EZH2 [40], suggesting the importance of the histone methylation regulatory activity of EZH2 in the pathogenesis of MDS. However, more recently, overexpression of EZH2 has also been reported in MDS, which is linked to poor prognosis [87]. At the same time, overexpression of EZH2 in breast cancer has been demonstrated to mediate the downregulation of DNA damage repair, which leads to the expansion of tumor initiating cells [88]. Therefore, the exact role of EZH2 in MDS needs to be further characterized.

There are two H3K27 demethylases potentially involved in the development of MDS, which are UTX and JMJD3 [89,90]. Somatic mutations of the *UTX* gene has been reported in CMML and MDS and is associated with increase level of H3K27me3 [41,42]. Another member of the UTX family, JMJD3, is overexpressed during the senescence of mouse embryonic fibroblasts [91] and is accompanied by the recruitment of the protein MLL to the *INK4/ARF* locus, provoking the dissociation of PRC2 and activation of the *INK4/ARF* gene [91]. Related to these results, it has been recently reported that the expression of p16INK4A is upregulated in MDS but not AML [92]. Taken together, these results suggest that during the pathogenesis of MDS, overexpression of JMJD3 could potentially down-regulate the H3K27 methylation of *p16INK4A* promoter, which could cause the deregulation of this gene. However, there is also reports of the silencing and hypermethylation of *p16INK4A* promoter in MDS, particularly in pediatric primary MDS [93]. Therefore, the specific role of *p16INK4A* gene product, as well as the potential regulation of this gene by JMJD3, in the development of MDS still needs further study.

H3K9 methylation—Methylation on H3K9 is a well conserved mark of transcriptional silencing. Although H3K9 methylation has been shown to control DNA methylation in filamentous fungi and plants [94], there is still no direct interplay between H3K9 methylation and DNA methylation documented in mammals. In MDS, the rearrangements of 3q26 are relatively frequent and often lead to the activation of oncogene *EVI1* [95]. In various experimental systems, including mouse models, the activation of EVI1 has been

found to lead to myeloid dysplasia, supporting the contribution of EVI1 to the pathogenesis of MDS [96]. In relation to H3K9 methylation, EVI1 has been shown to interact with H3K9 methyltransferases SUV39H1 and G9a [97]. These interactions are important for bone marrow immortalization [97]. In another study using AML cells, the H3K9 methyltransferase activity of SUV39H1 has been demonstrated to silence *p15INK4B* and *E-cadherin* genes [98], which are two tumor suppressors also frequently silenced in MDS [99,100]. Whether there is also a interaction between SUV39H1/G9a and EVI1 in MDS and if so whether this interaction affects the H3K9 methylation as well as the expression of the tumor suppressors such as p15INK4B and E-cadherin in this disease, are still unclear and therefore require further study.

H4K20 methylation—Methylation on H4K20 is a mark for heterochromatin [35]. H4K20 methylation has also been reported to affect DNA damage checkpoint by recruiting the checkpoint protein 53BP1 [101]. Methyltransferases of H4K20 that have been identified in mammals include SUV420H1/2 [102] and SETD8 [103]. For demethylase, the JmjC domain protein PHF8, has been shown to catalyze the removal of methylation from both H3K9 and H4K20 [104]. Interestingly, the recruitment of PHF8 to chromatin occurs through the interaction between the methyl-lysine recognizing the PHD domain of PHF8 and methylated H3K4 [105], highlighting the mutual regulation between H3K4 and H3K9 or H4K20 methylation.

Although no specific mutation or abnormalities involving H4K20 methyltransferase or demethylase has been identified in MDS, potential involvement of H4K20 methylation in MDS has been implicated in the knockout mouse model of *Arid4a* and *Arid4b* genes [106]. These mice show hematopoietic phenotypes, include ineffective hematopoiesis, followed by transition to CMML-like myelodysplastic/myeloproliferative disorder and then transformation to AML [106]. Of interest, there is increase of histone methylation of H3K4, H3K9 and H4K20 in the bone marrow cells of the knockout mice [106], suggesting the involvement of H4K20 methylation in MDS.

Histone arginine methylation marks

In comparison to histone lysine methylation, reports on characterization of different forms of histone arginine methylation in MDS are more limited. Members of the family of protein arginine methyltransferases (PRMT) in human, which can catalyze the arginine methylation of histone and nonhistone proteins, are PRMT1–11 [107]. Among them, PRMT1, 2, 4, 5 and 6 have been associated with the methylation of histone arginine [107]. Of interest, the potential pathogenetic role of PRMTs in MDS and other myeloid neoplasms may be partially mediated through the interactions between PRMT and histone lysine methyltransferases. For instance, H4R3 methyltransferase PRMT1, an essential regulator enhancing the self-renewal of primary hematopoietic cells, is a component of the oncogenic transcriptional complex containing H3K4 methyltransferase MLL [108]. Another H3R2 methyltransferase PRMT6 has been found to inhibit the recruitment of the H3K4 methytransferase subunit WDR5 to its target and repress the H3K4 trimethylation of the target genes such as *HoxA* genes [109,110]. It should be noted that PRMTs also target nonhistone proteins, which can also contribute to the pathogenesis of MDS. For instance, besides H3R4, PRMT1 also methylates arginine R206 and R210 on RUNX1 protein [111]. RUNX1 is an important transcription factor frequently mutated in MDS [46]. When $CD34^+$ cells differentiate into myeloid cells, the expression of PRMT1 is upregulated, which is accompanied with high arginine methylation on RUNX1 protein [111]. For the removal of methyl unit, as of now only JMJD6 has been demonstrated to be a histone arginine demethylase [112], which removes methylation from H3R2 and H4R3 [112]. No specific defect on JMJD6 has been identified in MDS and other forms of myeloid neoplasms yet.

Drug development targeting histone methylation

Owing to the importance of histone methylation in gene regulation and its involvement in cancer, the discoveries of potential drugs targeting regulators of histone methylation are now being actively pursued. However, there are still quite a few limitations that hamper the development of therapeutic HMT and HDM inhibitors. One major limitation is the lack of crystallographic structural information of HMTs and HDMs, which is usually required to study the interaction between the inhibitors and its targeted proteins. The other important issue is the problem of specificity. For instance, *S*-adenosyl-L-methionine (SAM) and analogues are used as cofactor not only by HMTs, but also by DNA methyltransferases and other enzymes [113]. Similarly, current JmjC domain HDM inhibitors also lack subtype specificities. These make the inhibition HMTs via targeting SAM cofactors and the use of JmjC domain HDM inhibitors problematic in therapeutic applications. Therefore, in comparison to the targeting of DNA methylation and histone deacetylation, development of histone methylation inhibitors is still in its infancy and the number of inhibitors reported to target histone methylation is quite limited. Among the compounds reported as HMT/HDM inhibitors, a few have been demonstrated to have potential activities in myeloid neoplasms.

Inhibitors for HMT

3-deazaneplanocin A (DZNep) is a potent inhibitor of *S*-adenosylhomocysteine (AdoHcy) hydrolase, which in turn causes by-product inhibition of SAM-dependent methyltransferases, including H3K27 methyltransferase EZH2 [114]. DZNep has been demonstrated to cause pharmacologic disruption of EZH2-related histone methylation and associated anticancer activity in several types of cancers, including in AML cell lines and primary AML cells [115]. This is accompanied by the activation of several tumor suppressor genes together with repression of cyline E and HoxA9 [115]. When combined with pan-HDACi panobiostat, DZNep synergistically improved the survival of immunodeficient mice with HL-60 leukemia [115]. These findings suggest that targeting EZH2 mediated H3K27 methylation by DZNep could be a potential effective epigenetic therapy against myeloid neoplasms. However, as motioned above, there is the issue of specificity, since DZNep, as a compound targeting the SAM analogues, may also affect the activities of multiple SAM-dependent methyltransferases other than EZH2. The other complication involved in the potential application of DZNep in MDS is that the definite role of EZH2 in MDS is still unclear, since at the same time of the identification of missense mutation of EZH2 in MDS, this gene has also been found in some cases to be overexpressed in MDS and other types of cancer [87,116].

Several compounds have shown inhibition against HMTs that regulate histone methylation on residues other than H3K27. These include the H3K9 methyltransferase inhibitors chaetocin (targeting SUV39H1) [117], BIX-01294 (targeting G9a) [118] as well as the PRMT1 inhibitors such as arginine methyltransferase inhibitor 1 (AMI-1) derivatives [119] and RM65 [120]. Particularly, among these compounds, G9a inhibitor BIX-01294 has shown potential biological effect, which could activate *IP-10* gene, a potential disease gene in idiopathic pulmonary fibrosis [121].

Inhibitors for HDM

A series of inhibitors targeting the non-JmjC domain H3K4 demethylase LSD1 have been synthesized by different groups. Most of these compounds are derivatives of monoamine oxidase (MAO) inhibitors, based on the similar oxidative manner between LSD1 and MAO [122]. Among them, a derivative (14e) of tranylcypromine (PCPA) synthesized by Binda *et al.* has shown to enhance the efficacy of retinoic acid on growth and differentiation of AML cell line NB4 [123].

In comparison to the inhibitors targeting LSD1, reports about the inhibitors against JmjC domain HDMs are quite limited. Several compounds have recently been shown to inhibit the H3K9 demethylase JMJD2A, including a pyridine-2,4-dicarbo-xylin acid derivative, pyridine-2,4-dicarboxylin acid-dimethyl ester [124]. Some of these JMJD2A inhibitors have shown combinational anticancer effects in prostate and colon cancer cell lines when applied with a LSD1 inhibitor NCL-2 [125].

Although some of the HMT/HDM inhibitors described above have shown potential activities in certain myeloid neoplasms such as AML, there is still no report about trials of these compounds in MDS. This could be owing to the currently still limited number of potential inhibitors, the specificity issue of these inhibitors, as well as the complicity of the pathology of MDS, including the lack of representative cell lines and the limited animal models of this disease. Therefore, a potential application of a specific HMT/HDM inhibitor in MDS is dependent on a better characterization of the role of these HMTs/HDMs in the pathogenesis of MDS or at least in a subtype of MDS, in parallel with further development/optimization of the potential HMT/HDM inhibitors. For instance, based on both clinical data and phenotypes of mouse models, EVI1 has been clearly shown to involve in MDS pathogenesis. Recently, H3K9 methyltransferases SUV39H1 and G9a has been demonstrated to interact with EVI1, which could then affect bone marrow immortalization [97]. It is therefore possible that a better characterization of SUV39H1 and/or G9a in MDS, combined with further optimization of their inhibitors such as the above described chaetocin [117] or BIX-01294 [118], may lead to preclinical trials of these compounds on an animal model such as the EVI1 mice which exhibit MDS phenotype, and furthermore, potential applications of these drugs on MDS patients.

Future perspective

There has been a rapid progress towards the understanding about the regulation of histone methylation and its role in human disease. Owing to the complexity of the pathophysiology of MDS, as well as the lack of representative cell lines or animal models, studies addressing histone methylation in MDS are still quite limited and a large portion of the evidence, in regards to the involvement of a specific histone methylation regulator in the pathogenesis of MDS, is still indirect and sometimes based on the predictions from the results in other types of myeloid neoplasms. Despite these limitations, results summarized in this article provide preliminary evidence for the potential involvement of histone methylation in the pathogenesis MDS (Table 1). Therefore, we believe that further investigations of histone methylation in MDS are both important and promising in the years to come. Detailed investigations of histone methylation in MDS should include careful clinical observations about the alterations of a specific HMT, HDM or their cofactor/target in patients, detailed molecular and cellular studies of these gene products in transcription regulation, as well as the generation of appropriate animal models involving specific key histone methylation regulators that could mimic some key features of MDS. Achievements of these clinical and basic studies, together with the progress in the development of the drugs inhibiting HMTs/HDMs, will eventually help us to identify and target essential histone methylation regulators in the treatment of MDS.

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Executive summary

- Myelodysplastic syndromes (MDS) are a very complex group of myeloid neoplasms and are becoming one of the most common forms of myeloid neoplasms in older individuals.
- The heterogeneity of MDS makes both diagnosis and molecular studies of this disease complex.

Overview of epigenetic regulation & histone methylation in MDS

- Epigenetic regulations have been demonstrated to play an important role in the pathogenesis of malignancies. Compared with genetic lesions, abnormalities of epigenetic regulation can potentially be reversed by pharmacological intervention.
- Among different epigenetic modifications, DNA methylation and histone acetylation are the best characterized. Development of drugs targeting these two types of epigenetic regulation is more advanced, including in MDS.
- Although both the substrate lysine residues and the degree of methylation affect gene expression, generally, histone methylation on H3K4, H3K36 and possibly also H3K79 is positively associated with active transcription and methylation on H3K9, H3K27 as well as H4K20 tends to negatively regulate transcription.
- Histone methylation is dynamically regulated by histone methyltransferases and histone demethylases.

Abnormalities of activating histone methylation marks in MDS

- Summaries are provided for each type of histone methylation regarding the abnormities that have been identified in, or been potentially linked to, MDS. These include altered histone methylation levels and abnormities of its regulators as well as target genes.

Drug development targeting histone methylation

- Inhibitors targeting histone methyltransferases have been developed and some of them have shown anticancer activity in acute myelogenous leukemia cells.
- Inhibitors targeting histone demethylases, such as non-Jmjc domain histone demethylase LSD1, have been developed and shown to have a biological effect in acute myelogenous leukemia cells, whereas inhibitors targeting Jmjc domain histone demethylases are still quite limited.

Future perspective

- Studies of histone methylation in MDS are important and promising. These will help us to identify key histone methylation regulators as prognostic markers or therapeutic targets for the treatment of MDS.

Table 1

Abnormalities of histone methylation identified in myelodysplastic syndromes: histone methyltransferases, histone demethylases and their associated gene products in myelodysplastic syndromes.

Modified residue	HMT/HDM involved in MDS	Defect of HMT/HDM in MDS	Defect of targets/interacting proteins of HMT/HDM in MDS	Inhibitor
Activating K methylation				
H3K4	MLL (HMT); LSD1 (HDM)	Gene amplification (rare)	HoxA9, Meis1 (possible targets of MLL): overexpressed in MDS LSD1 interacting proteins; GFI1: overexpressed in high-risk MDS; TAL1: upregulated in SDS syndrome; ASXL1: loss of function mutation in MDS	Several MAO inhibitor derivatives
H3K36	NSD1/3 (HMT); JHDM1A/B (HDM)	NUP98–NS1/3 fusion	HoxA9 (possible target of NSD1): overexpressed in MDS; BCL6 (interact with JHDM1a/b): amplification of BCL6 gene in MDS	
H3K79	DOT1L (HMT)		MLL–AF4 fusion (interact with DOT1L): gene fusion in MDS rare); targets of DOT1L: GATA2: overexpressed in MDS; PU.1: interacting with EVI1	
Repressive K methylation				
H3K27	EZH2 (HMT); UTX (HDM); JMJD3 (HDM)	Mutation/overexpression in MDS; mutations in MDS	p16INK4 (target of JMJD3): overexpressed in MDS but not AML	DZNep
H3K9	SUV39H1 (HMT); G9A (HMT)		EVI1 (interacting protein of SUV39H1 and G9a): overexpressed in MDS; p15INK4b and E-cadherin (targets of SUV39H1): silencing in MDS	
H4K20		Level increase in Arid4a/4b KO mice, which have defects that progress into MDS and AML		
R methylation				
H4R3	PRMT1	PRMT1 enhances self-renewal of hematopoietic cells	MLL	AMI-1 derivatives, RM65
H3R2	PRMT6	PRMT6 inhibits MLL mediated H3K4 methylation	Chaetocin, BIX-01294	

AMI-1: Arginine methyltransferase inhibitor 1; AML: Acute myelogenous leukemia; DZNep: 3-deazaneplanocin A; HDM: Histone demethylases; HMT: Histone methyltransferases; K: Lysine residue; KO: Knockout; MAO: Monoamine oxidase; MDS: Myelodysplastic syndromes; MLL: Mixed-lineage leukemia; PRMT: Protein arginine methyltransferases; R: Arginine residue.