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Epigenetic Regulatory Mutations and Epigenetic Therapy For Multiple Myeloma

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Abstract

Purpose of review—Next generation sequencing and large scale analysis of patient specimens has created a more complete picture of Multiple Myeloma (MM) revealing that epigenetic deregulation is a prominent factor in MM pathogenesis.

Recent findings—Over half of MM patients have mutations in genes encoding epigenetic modifier enzymes. The DNA methylation profile of MM is related to the stage of the disease and certain classes of mutations in epigenetic modifiers are more prevalent upon disease relapse, suggesting a role in disease progression. Many small molecules targeting regulators of epigenetic machinery have been developed and clinical trials are underway for some of these in MM.

Summary—Recent findings suggest that epigenetic targeting drugs could be an important strategy to cure MM. Combining these agents along with other strategies to affect the MM cell such as IMiDs and proteasome inhibitors may enhance efficacy of combination regimens in MM.

Keywords

Epigenetics; histone acetylation; myeloma; DNA methylation

Introduction

Multiple myeloma (MM) is an incurable disease representing 10% of hematological malignancies and about 1% of all cancer worldwide. Most cases of MM are preceded by a premalignant state known as monoclonal gammopathy of undetermined significance (MGUS). In the later stages of the disease, the malignant plasma cells become independent of the bone marrow by acquiring secondary genetic events providing a fitness advantage, leading to tumor progression. These secondary events allow malignant plasma cells to circulate in peripheral blood or to migrate to other tissues, which leads to plasma cell leukemia (PCL) or extramedullary plasmacytomas. Malignant progression events of MM include activation of *MYC*, the MAPK pathway (*FGFR3*, *KRAS* and *NRAS*), the NF- κ B

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Conflict of interest

There are no conflicts of interest.

pathway and deregulation of cell cycle (CCND1), plasma cell differentiation (IRF4 and PRDM1) and DNA repair pathway (ATM, ATR, p53) (1).

Recently developed agents such as proteasome inhibitors, and immunomodulatory drugs (IMiDs), along with stem cell transplantation have improved the outcome of MM patients and have extended median survival to nearly 7 years, but over time most patients relapse and become refractory to therapy. Recent findings suggest that epigenetic targeting drugs could be a relevant strategy to treat MM.

Recent large throughput analysis of MM patient samples at diagnosis (UK NCRI Myeloma XI study) and at relapse (University of Arkansas for Medical Sciences) revealed that more than half of the patients had mutations in epigenetic enzymes. Mutations cover a large variety of epigenetic modifying enzymes but none of them are present in a large proportion of patients (Figure 1). Furthermore, the comparison with targeted sequencing data from 156 previously relapsed cases demonstrated an increase in the frequency of some of these mutations suggesting a role for epigenetic changes in MM progression (2, 3). Although somatic mutations in many epigenetic modifiers are relatively rare, a large proportion of MM patients present copy number variations (CNV) in these genes (Figure 1).

Epigenetics refers to any somatic inheritable change in gene function occurring without alteration of the DNA sequence. Most epigenetic mechanisms occur at the level of chromatin. Chromatin is organized into nucleosomes, each composed of 146 bp of DNA wrapped around an octamer of four core histones (H3–H4 tetramer and two H2A–H2B dimers) (4). Histones undergo a diverse array of modifications playing an important role in gene expression. This review will focus exclusively on the modifications and mediators of those modifications, which are most relevant to MM.

Histone proteins

The linker histone H1 stabilizes the nucleosome and facilitates folding of nucleosome arrays into higher-order structures in heterochromatin (5). Studies in embryonic stem cells showed that H1 can induce compaction at specific loci by regulating DNA methylation (6, 7) and by binding H3K27me (6). Histone linker mutations are found in about 6% of MM patients mainly in HIST1H1E but also in HIST1H1B, C and D, and mutations cluster in the globular DNA-binding domain (8–10). Similar to MM, mutations in the globular domain of histone H1 are common in follicular lymphoma (FL), suggesting that they may be driver mutations (11, 12). These mutations are predicted to disrupt protein function and specifically, one mutation was demonstrated to impair histone H1 association with chromatin. Lastly, some mutations of histone H2A have been reported in MM patients, but the consequences of these mutations are not clear.

DNA methylation

DNA methylation has long been associated with transcriptional silencing. As in other malignancies, methylation of promoter and CpG islands linked to tumor suppressor genes is found in MM and some genes become aberrantly silenced by hypermethylation in disease development and progression from MGUS to MM (3, 13–18). Among the genes

downregulated in association with hypermethylation are cell cycle regulators including p15 (19), p53 (20) and an activator of p53, ZNF545 (21). The methylation frequency of *p15*, *p16*, *p53*, and the *DAPK* tumor suppressor increases from MGUS to MM (22).

New analysis of DNA methylation based on next generation sequencing indicate a much more complex picture with DNA methylation occurring outside of CpG islands (23). Studies have observed a relationship between MM stage and methylation patterns. In general, global hypomethylation along with a more active chromatin is seen in MM compared to normal plasma cells (13, 24, 25) and associated with poor outcome (26). Hypomethylated sites consist of repetitive sequence, intergenic regions and gene bodies (27). Increased methylation is progressively acquired during evolution and relapse of MM (28, 29). The hypermethylated sites in MM are localized outside CpG islands within intronic sequences representing B-cell specific enhancer regions (23). They are associated with methylated sites in normal stem cells that gradually become demethylated during normal B-cell differentiation (23, 30). These data suggest that MM progression may occur in part, through dedifferentiation by downregulation of B-cell specific genes.

DNA methylation itself is mediated by a family of DNA methyltransferases (DNMTs), including DNMT1, DNMT3A, DNMT3B, DNMT2 and DNMT3L (31). DNA methylation patterns are established during early development by DNMT3A and DNMT3B then propagated by the methyltransferase DNMT1, which reproduces patterns of methylated between cell generations. While it is unclear how and to what extent active DNA demethylation occurs, recent research suggests that hydroxymethylation of 5-methylcytosine to 5-hydroxymethylcytosine may be an intermediate for the removal of methylated cytosine (32), since DNMT1 does not recognize and propagate 5-hydroxymethylcytosine. Alterations of regulators of DNA methylation have been noted in MM. DNMT3A is aberrantly methylated early in MGUS and myeloma (29). DNMT3A mutations were significantly enriched at relapse in MM patients and found at a site frequently mutated in AML, E477K (2). These data are consistent with the finding that one of the most recurrent DNMT3A mutations impairs sensing and repair of DNA, resulting in chemo-resistance and increased mutagenesis (33).

Hypomethylating agent can reactivate the expression of some genes and kill MM cells (18) or restore sensitivity to other drugs (34). Therefore many clinical trials are undergoing with DNA hypomethylating agents like the pyrimidine nucleoside analogs 5-azacytidine (Aza, Vidaza) and 5-aza-2'-deoxycytidine (Decitabine, Dacogen) in combination with other agent. It is surprising to find only one ongoing clinical trial with DNA hypomethylating agent in MM as single agent (table 1), given the accumulating evidence of DNA hypermethylation in advanced stage and relapse MM.

Chromatin remodeling

Activation and/or repression of transcription require the movement of nucleosomes on DNA, and are performed by ATP-dependent chromatin remodeling complexes. The most frequently mutated/deleted subunits of chromatin remodeling complexes in MM are CHD4 and CHD2, which are members of the transcriptionally repressive complex NuRD

(nucleosome remodeling and HDAC). Of note, CHD4 can regulate DNA damage response and repair (35–37). Additionally, subunits of the ATPase and helicase complex SWItch/Sucrose NonFermentable (SWI/SNF) complex are altered in MM. Significantly, ARID1A, ARID2, ARID4A and ARID5B, subunits responsible for interaction with DNA, are mutated in about 5% of newly diagnosed patients and gene copy loss is commonly observed reaching almost 40% MM cases. Mutations in the ATPase subunit SMARCA4 of SWI/SNF are enriched in relapse MM patients but not found in *de novo* MM, suggesting SMARCA4 may participate in development of resistance.

Histone methylation mediated by SET domain methyltransferases

Histones undergo a diverse array of modifications mediating transition of chromatin between open (active) and closed (repressed) states. Histone methylation is involved in both activation and repression of transcription. Various histone lysine methyltransferases (HMT) that contain the catalytic SET domain are be mutated or deregulated in MM including NSD2, NSD1, SET2D, SETB1 and KMT2A/B/C/D (Figure 1).

The majority of publications, including strong structural studies, indicate that the NSD family enzymes target histone H3 for methylation at lysine 36 (H3K36me), a mark associated with transcriptional activation (38–41). The NSD family is composed of NSD1, 2 and 3 and has been thoroughly reviewed elsewhere (42). NSD1 mutations are found exclusively in relapsed MM patients. While no studies on NSD1 in MM exist, this gene is often hypermethylated in other cancers (43, 44). Mutations in the NSD1 gene are the main cause of the congenital SOTOS syndrome (45, 46), associated with an elevated risk of developing cancer (47) and a specific DNA methylation signature (48).

The most studied epigenetic deregulation in MM originates from a t(4;14) translocation, leading to elevated levels of the NSD2 protein (also called MMSET/WHSC1). This cytogenetic subtype represents about 15% of newly diagnosed MM patients and is associated with early relapse and shorter overall survival. This translocation results in striking up-regulation of the NSD2 protein and accumulation of dimethylated histone 3 lysine tail residue 36 (H3K36me2) broadly across the genome and a global reduction of H3K27me3 a mark added by the SET lysine methyltransferase EZH2. The displacement of EZH2 then yields aberrant activation of oncogenic loci (49, 50). Although the majority of the genome loses H3K27 methylation in the presence of high levels of NSD2, specific loci display elevated H3K27 methylation and EZH2 binding, leading to transcriptional repression of some genes that may play a role in MM pathogenesis (51).

In addition to regulating gene transcription, NSD2 has been reported to regulate the DNA damage response (41). Recently, Shah *et al.* demonstrated that MM cells with low NSD2 expression have delayed restoration of chromatin structure after exposure to agents inducing DNA double-strand breaks (DSB). Conversely, high expressing NSD2 cells had a proliferative advantage and increased survival when exposed to DNA-damaging drugs (52). Several studies showed that the SET2D methyltransferase, which creates the H3K36me3 mark found in gene bodies also participates in DNA repair pathways including DSB repair by homologous recombination (53–55), NHEJ repair pathway (56, 57) and

mismatch repair (58). Mutation in SETD2 is enriched in relapse patients reaching 2.56% frequency. The functional impact of SETD2 mutations in MM has not yet been resolved.

Recently, overexpression of EZH2 was observed in malignant plasma cells and associated with poor prognosis in MM (59, 60). As well, because t(4;14) MM is accompanied with a global reduction of the mark added by EZH2, H3K27me (49, 50), EZH2 inhibitors (EZH2i) have been effective at targeting MM cells with elevated levels of NSD2 (51, 61–64). Similarly, KDM6A loss in MM led to repression of a specific gene set that can be re-expressed by EZH2 inhibition, leading to cell death (65). EZH2 inhibitor sensitivity in MM has been attributed to downregulation of MM oncogenes such as c-MYC, XBP-1, IRF-4, PRDM1 (64), and upregulation of epithelial tumor suppressor genes like CDH1 (63).

Members of the histone-lysine *N*-methyltransferase 2 (KMT2; also known as mixed-lineage leukemia (MLL)) family, methylate histone H3 on lysine 4 (H3K4me), promoting genome accessibility and transcription. Mutations found in MM affect almost all KMT members (KMT2A, B, C, D and E) and are found in about 5% MM patients (66). KMT2 methyltransferase works in complex with the histone acetyltransferase CBP/p300 and members of the SWI/SNF chromatin-remodeling complex to activate transcription (Figure 2). KMT2A and KMT2B are active particularly at promoter regions and add the H3K4me3 mark while KMT2C and KMT2D are localized to enhancers and create the H3K4me1 mark. Unique subunits within each of the KMT2 subgroups may dictate functional specificity since they are involved in recruiting KMT2s to distinct regions in the genome. Many components of KMT2 complexes are mutated in MM and thus, may alter the levels or distribution of H3K4me, deregulating gene expression and contributing to tumor pathogenesis (Figure 2) (66, 67).

Histone de-methylation mediated by JmJc demethylase

The Jumanji (JmJC) class of demethylases utilizes α -ketoglutarate as a co-factor to oxidize and eliminate methyl groups from histones. The demethylase KDM3B and its homologue KDM3A, also altered in MM, demethylate histone H3 lysine 9 (H3K9me). H3K9me is a heritable epigenetic mark essential for formation of heterochromatin. A recent study in yeast showed that inactivation of H3K9 demethylase allowed maintenance of silent chromatin through many mitotic divisions (68). Therefore, disruptive mutations in KDM3B may lead to aberrant inheritance of heterochromatin, and explain the repression of some genes during progression of MM. Additionally the H3K9 methyltransferase EHMT (G9a/KMT1C) and the homologous EHMT1 (GLP/KMT1D) are elevated in MM (69, 70). All these data suggest that some repressive chromatin is maintained during the development of MM, consistent with the DNA hypermethylation observed increasing with progression of MM. By contrast, a recent study showed that KDM3A is overexpressed in MGUS samples compared to normal PC, and was essential for cell proliferation in MM (71).

KDM6A works in complex with KMT2C and D to remove H3K27me mark associated with gene repression (Figure 2). KDM6A may be mutated in up to 7% of MM samples (67), and KDM6A mutations were associated with decreased survival (2, 66, 67). More, CNV

indicates loss of this gene in about 25% MM cases (Figure 1). Thus, deregulation in KDM6A observed in MM may alter H3K4me and H3K27me levels at specific genes.

Drugs targeting histone methylation

H3K36 methyltransferases represent an attractive therapeutic target for some MM subgroups, particularly the t(4;14) subtype. In the last 2 years several groups have attempted to develop inhibitors to the SET-methyltransferase NSD2. One group identified potential inhibitors of NSD2 by screening a recombinant SET methyltransferase domain of NSD2 (72). Another study used molecular modeling simulations with virtual ligand screening and an *in vitro* assay to identify inhibitors targeting the histone binding pocket instead of the SAM, selecting for specificity towards NSD2 and not other NSDs (73). A model that mimics the geometry and charge distribution of the transition state of NSD2 has been developed to guide inhibitor design. However, subsequent *in vitro* or *in vivo* studies have not yet confirmed the potency and selectivity of these NSD inhibitors towards high expressing NSD2 malignancies (72). Since NSD2 play a role in DNA repair, a combination regimen with DNA damaging agents could benefit patient with such tumors.

The convergence of many genetic aberrations in the deregulation of H3K27me in cancer (reviewed in (74)) has led to the development of highly selective inhibitors of EZH2, the PRC2 complex catalytic core (75) (figure 1). These inhibitors directly compete for interaction with the methyl-group donor *S*-adenosyl methionine (SAM). They decrease levels of H3K27me, and reactivate transcription of PRC2/H3K27me repressed genes involved in cell cycle regulation and differentiation (76–79). While these studies have shown promising results, other studies have noted enhanced drug resistance with EZH2i (80). EZH2 oncogenic activity has also been attributed to non-enzymatic functions (81), and therefore inhibitors of its catalytic site may not fully suppress its tumor promoting activity. Since the interaction of EED with EZH2 is essential for activity of the PRC2 complex (82–85), a small molecule was developed to target the alpha-helical domain of EZH2 that binds EED, which disrupts PRC2 complex interaction (79). A recent inhibitor against the H3K27me3 binding pocket of EED has been developed (86).

Accumulating evidence demonstrates that SWI/SNF inactivated tumors can be targeted by EZH2 inhibitors (81, 87, 88). Recently, EZH2 catalytic and non-catalytic activity has been demonstrated to be essential for the survival of tumors harboring mutations of various subunits of SWI/SNF, such as *ARID1A* and *SMARCA4*, and co-occurrence of RAS mutations abolished this dependence (81). Consistently, clinical activity of EZH2i was observed in patient tumors samples harboring mutations in SWI/SNF complex subunits (66, 89), giving hope for MM patients with SWI/SNF mutations. The idea of targeting EZH2 in MM is new thus, only one trial is recently initiated with EZH2i (GSK2816126) as a single agent in MM, where no results are obtained yet (90) (Table 1).

Histone acetylation

Histone acetylation is regulated by the balanced activities of histone acetyltransferases (HATs) and histone deacetylases (HDACs). In general histone acetylation is associated with

transcriptional activation, and recent investigations revealed that HDAC1, 2, 3 and 6 are highly expressed in MM contributing to the malignant phenotype (91). Conversely, mutations in the HAT CREBBP appear more common in relapsed MM and gene copy loss of CREBBP and EP300 is observed in 10% MM patients (Figure 1). The exact targets of HDACs and HATs in MM are not certain since these enzymes also target more than a thousand non-histone proteins involved in a variety of pathways (92) including some involved in DNA repair and chromatin remodeling as well as proteins outside of the nucleus not directly involved in gene regulation. Among nuclear acetylation protein targets, many are associated with MM pathogenesis such as p53, HSP90, NF- κ B, RUNX3, STAT-3, E2F1 and c-MYC (93).

Drugs targeting histone acetylation

In February 2015, the pan-HDAC inhibitor panobinostat was approved by US Food and Drug Administration (FDA) for treatment of relapse/refractory MM (94–96). The pleiotropic nature of HDACs and HATs, have led to various combination strategies in the clinic. In MM, HDAC6, a cytoplasmic protein, seems to be critical for HDACi sensitivity. HDAC6 is involved in the disposal of unfolded proteins through its ability to bind ubiquitinated proteins (97–99) and to interact with tubulin and dynein motors (100, 101). Because normal and malignant plasma cells are highly secretory, they rely on the unfolded protein response pathway to prevent the aggregation of accumulating misfolded proteins. Preclinical studies showed that HDACi in combination with proteasome inhibitors blocking protein degradation pathways were effective at eliminating MM cells (102–105). Therefore, efforts have been made to develop HDAC6 inhibitors with enhanced specificity (106). One such inhibitor, Ricolinostat (ACY-1215) demonstrated some antitumor activity in combination therapy (107). Beyond Ricolinostat's primary target HDAC6, IKZF1/3 and MYC were downregulated (103). HDACi has not yet demonstrated response as single agent in MM.

Acetylated lysine residues on histone tails can be recognized by the bromodomain and extraterminal domain (BET) family of proteins, which consists of four proteins (BRDT, BRD2, BRD3, BRD4) each containing two discrete bromodomains. These proteins help link promoters and enhancers to facilitate transcriptional initiation and elongation. Inhibitors of the bromodomain protein BRD4 (BET), lead to the downregulation of *MYC* and its target genes critical for the development of MM (108) (109, 110). The upregulation of *MYC*, is seen in up to 50% of MM and it has been associated with the development of MM from MGUS, as well as late disease progression (111). Since there are no drugs that directly target MYC and MYC upregulation is important in MM, several phase I/II clinical trials have been initiated in MM using BET inhibitors GSK 525762 (NCT01943851), CPI-0610 (NCT02157636), and OTX015 (NCT01713582) (Table 1). So far, some durable responses were been observed in DLBCL with OTX015, but results are inconclusive for MM (112).

Conclusion

MM is still considered incurable and treatment of relapsed myeloma remains unsatisfactory. New sequencing studies revealed that like in other malignancies, epigenetic mutations and CNV are common in MM. Furthermore, the increased frequency of mutations in genes

encoding for histone methyltransferases and DNA methylation modifiers in relapse patients suggests that these events may have important functions in disease progression and resistance to therapy. All these findings yield potential avenues for therapy.

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Key points

- Analysis of a large cohort of multiple myeloma patients using next generation sequencing revealed that half of the patients have mutations in epigenetic-modifying enzymes covering a broad range of functions, perhaps converging into common mechanisms to promote tumorigenesis.
- Among mutations found in MM, some are associated with poor survival such as mutations in histone demethylase (KDM6A). Others may be involved in resistance to current therapy, including mutations regulating DNA methylation (DNMT3A), and acetylation (CREBBP).
- Various epigenetic alterations in MM converge to deregulate H3K27 methylation levels and many studies present evidence that EZH2 could be a potential therapeutic target.

SNP data were obtained from the UK NCRI Myeloma XI clinical trial and from the University of Arkansas for Medical Sciences dataset. CNV data were generated as part of the Multiple Myeloma Research Foundation Personalized Medicine Initiatives (<https://research.themmr.org> and www.themmr.org)

Figure 1. Summary of single nucleotide polymorphisms (SNP) and copy number variation (CNV) found in MM patients

*SNP data were obtained from the UK NCRI Myeloma XI clinical trial and from the University of Arkansas for Medical Sciences dataset.

**CNV data were generated as part of the Multiple Myeloma Research Foundation Personalized Medicine Initiatives, which include 804 MM patients (<https://research.themmr.org> and www.themmr.org)

Figure 2. Functions of epigenetic enzymes deregulated in MM

In MM, many component of the KMT2C/D complex are mutated and EZH2 is often overexpressed. KMT2C/D work as a complex that contain the HAT CBP/p300, the chromatin remodeling complex SWI/SNF, and the demethylase KDM6A. All together, they activate transcription by acetylating histones and by removing the repressive mark H3K27me added by EZH2, the catalytic core of the PRC2 complex. KMT2C/D itself, add the H3K4 methylation mark associated with transcription activation.

Table 1

Summary of clinical trials examining epigenetics targeting drugs as single agent in multiple myeloma.

Target	Drug	Phase	Identifier	Status
BET	GSK 525762	I/II	NCT01943851	Recruiting
	CPI-0610	I	NCT02157636	Recruiting
	OTX015	I	NCT01713582	Ongoing
HDAC	Rocilinostat	I/II	NCT01323751	Ongoing
	Tefinostat	I/II	NCT00918333	Ongoing
	Abexinostat	I	NCT01149668	Completed
	CI-994	II	NCT00005624	Completed
	CUDC-907	I	NCT01742988	Recruiting
	Entinostat	I	NCT00020579	Completed
	ITF2357	II	NCT00792506	Terminated
	Belinostat	II	NCT00131261	Completed
	Tefinostat	I	NCT00820508	Completed
	Vorinostat	I	NCT00005634	Completed
	Panobinostat	I/II	NCT00857324	Terminated
	Panobinostat	I	NCT00962507	Completed
	Panobinostat	II	NCT00445068	Terminated
	Panobinostat	I/II	NCT00918333	Ongoing
HMT	GSK2816126	I	NCT02082977	Recruiting
DNMT	Azacitidine	I	NCT00006019	Completed