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Chapter 7

The Role of DNA Methylation in the Genetics and Epigenetics of Multiple Myeloma

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Abstract

Multiple myeloma (MM) arises through an accumulation of multiple genetic and epigenetic changes, which play a significant role in tumorigenesis and tumor development. DNA methylation is often found in cancers including MM at the 5-carbon on cytosine residues within CpG islands of genes whose products are associated with the promoter regions of protein-coding genes. This methylation is an epigenetic alteration that leads to heritable changes in gene expression through the recruitment of histone deacetylases and histone methyltransferases. We and other researchers have reported the association of global and regional DNA methylation status with MM. Global DNA hypomethylation is the predominant early change during plasma cell oncogenesis from monoclonal gammopathy of undetermined significance to MM, while regional DNA hypermethylation occurs in tumor relapse and during disease progression. Thus, DNA methylation could be a useful biomarker of MM tumorigenesis and progression. In the current review, we discuss the role of DNA methylation changes; their potential application as epigenetic biomarkers to facilitate risk assessment, diagnosis, prediction of prognosis, and sensitivity to treatment; and epigenetic therapy in MM.

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Introduction

Cancers, including multiple myeloma (MM), arise because of an accumulation of multiple genetic changes, which play a significant role in tumorigenesis and tumor development. In addition to genetics, recent studies revealed the role of epigenetics—heritable information that does not affect DNA sequence—in the pathogenesis of cancers, including MM [1-4]. Among epigenetic changes, DNA methylation and histone modification have been well-studied.

DNA methylation occurs at the 5-carbon on cytosine residues in cytosine-guanine pairs known as CpG dinucleotides. DNA methylation is catalyzed by three DNA methyltransferases, including DNMT1, DNMT3A, and DNMT3B, and is a crucial regulator in different biological processes, such as embryonic development, transcription, chromatin structure, X chromosome inactivation, genomic imprinting, genomic instability, and tumorigenesis [5]. Since transcriptionally active regions of the genome are usually CpG rich, methylation of CpG sites is a critical factor affecting gene transcription. DNA hypermethylation of the large clusters of CpG dinucleotides, referred to as CpG islands, at gene promoters and transcription start sites is an epigenetic alteration that can suppress gene expression through the recruitment of methyl-CpG binding domain proteins, histone deacetylases, and histone methyltransferases, thus causing chromatin condensation [6]. Genome-wide hypomethylation and regional hypermethylation are common events in tumors, including hematological malignancies. In MM, DNA hypomethylation was reported as the predominant early change during tumorigenesis that gradually transforms to regional DNA hypermethylation during disease progression [7-9].

In the current review, we discuss the role of alterations in DNA methylation, potential application of epigenetic biomarkers, and target therapeutics in MM.

2. Molecular Mechanism Involved in Tumorigenesis of MM

MM is a neoplastic plasma cell disorder that is characterized by the clonal proliferation of malignant plasma cells in the bone marrow, the presence of monoclonal immunoglobulin in the serum and/or urine in most cases, and associated organ dysfunction, including lytic bone lesions, compromised immunity, anemia, renal failure, and hypercalcemia [10-12]. Recent studies have shown that MM is consistently preceded by a premalignant stage of clonal plasma cell proliferation, termed monoclonal gammopathy of undetermined significance (MGUS) [13,14]. Approximately 1% of MGUS cases evolve to MM per year [15].

MM advances through a multistep transformation process of specific events, including somatic mutations, chromosomal copy-number changes, and non-random chromosomal translocations such as immunoglobulin gene rearrangements involved in cyclin D; furthermore, epigenetic changes drive progression from MGUS, to symptomatic MM, and ultimately to recurrent myeloma, including extramedullary disease and, in some cases, plasma cell leukemia [4,12,16,17] (Figure 1).

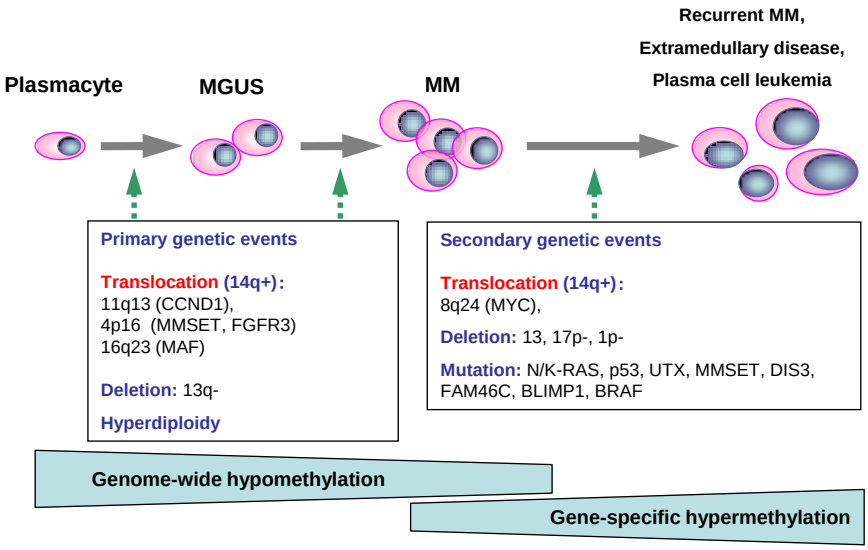

Figure 1. Multiple myeloma (MM) advances through a multistep transformation process due to specific events. These events include somatic mutations, chromosomal copy-number changes, and non-random chromosomal translocations, such as immunoglobulin gene rearrangements involved in cyclin D. Further, epigenetic changes drive progression from monoclonal gammopathy of undetermined significance (MGUS), to symptomatic MM, and ultimately to recurrent myeloma, including extramedullary disease and plasma cell leukemia. Genome-wide hypomethylation occurs during the events indicated by a blue wedge, and gene-specific hypermethylation occurs during the events indicated by a blue wedge.

Recently, several studies using high-throughput sequencing technologies demonstrated heterogeneity of MM genomic evolution and subclonal structure [18-22]. Chapman et al., reported that the analysis of somatic mutations by tumor-genome sequencing in MM cases revealed that, of the numerous genes mutated, identified genes are specifically involved in NFκB activation, protein homeostasis, and histone methylation, which are processes consistent with MM biology [18]. However, the key steps in MM oncogenesis remain unclear [3,4,23,24]. Recent findings also revealed that epigenetics, including DNA methylation and histone modification, is also important in MM pathogenesis. Global methylation analyses in MM have revealed the role of DNA methylation in MM pathogenesis and progress.

2. DNA Hypomethylation in MM

Genome-wide DNA hypomethylation is a common epigenetic alteration in cancer cells. Low levels of DNA methylation in cancer cells is substantially due to the loss of methylation at repetitive sequences such as long interspersed nuclear element-1 (LINE-1; a kind of a retrotransposon), which accounts for 17% of the human genome [19,25]. The following mechanisms have been suggested for DNA hypomethylation in tumorigenesis and tumor development: increased instability of the genome and reactivation of transposable elements (transposons) that can move in DNA [26-28]. Importantly, we and others reported that global methylation levels of DNA repetitive sequences, including LINE-1, progressively decline during the development of MM from MGUS to aggressive myeloma such as plasma cell

leukemia [8,9]. We also reported that there is a significant inverse correlation between the degree of genomic loss and LINE-1 methylation levels, and MM cases with LINE-1 hypomethylation had a significantly poor prognosis [9]. Regarding the pathogenesis of MM plasma cells, microarray data examining genome-wide differences in CpG methylation patterns revealed that genome-wide hypomethylation occurs at the transition from MGUS to MM [7].

3. DNA Hypermethylation in MM

DNA hypermethylation at gene promoters and transcription start sites is an epigenetic alteration that suppresses gene expression. Global DNA hypomethylation is the predominant early change during plasma cell oncogenesis from MGUS to MM, while regional DNA hypermethylation occurs in tumor relapse and during disease progression [7]. We and others studied regional DNA hypermethylation in MM and identified certain key genes as targets for epigenetic inactivation (Table 1).

Table 1. Epigenetically silenced genes in multiple myeloma (MM)

Gene	Chromosome	Function	Frequency of DNA hypermethylation of patient MM samples (n > 50)
CDKN2A (*p16*)	9p21.3	Cell cycle	34% [29]
DAPK1	9q21.33	Apoptosis	52.7% [35]
BNIP3	10q26.3	Apoptosis	5% [36]
RASD1	17p11.2	Cell growth	6–8% [33,40]
SPARC	5q33.1	Cell-extracellular matrix interaction	8–18.2% [36,40]
CD38	4p15	Ectoenzyme	45.9% [40]
GPX3	5q23	Glutathione peroxidase	7.5% [40]
NCAM1 (*CD56*)	11q23.1	Cell adhesion	5% [40]
PDK4	7q21.3	Regulation of metabolism	15.1% [40]
RBP1	3q23	carrier protein involved in the transport of retinol	16.3% [40]
TGFBI	5q31	Inhibition of cell adhesion	18.2% [40]

These genes include the following: cell-cycle regulators, such as cyclin-dependent kinase inhibitor 2A (*CDKN2A*) [29] and 2B (*CDKN2B*) [30] and checkpoint with fork head and ring finger domains (*CHFR*) [31]; genes involved in cell signaling, such as Ras association

(RalGDS/AF-6) domain family member 1 (*RASSF1*) [32], RAS, dexamethasone-induced 1 (*RASD1*) [33], and transforming growth factor, beta receptor II (*TGFBR2*) [34]; genes involved in apoptosis, such as death-associated protein kinase 1 (*DAPK1*) [35] and BCL2/adenovirus E1B 19kDa interacting protein 3 (*BNIP3*) [36,37]; genes involved in antigen presentation, such as class II, major histocompatibility complex, transactivator (*CIITA*) [38]; genes involved in cell-extracellular matrix interaction, such as secreted protein, acidic, cysteine-rich (*SPARC*) [36]; and genes involved in polycomb repressive complexes, such as enhancer of zeste, drosophila, homolog 2 (*EZH2*) [39].

Recently, Kaiser et al., investigated the association between DNA methylation and MM prognosis using a genome-wide DNA methylation array of 159 patients treated in the Medical Research Council Myeloma IX trial [40]. They identified the following 8 epigenetically regulated genes with changes in DNA methylation status that were significantly associated with prognosis: *CD38*, *RASD1*, *SPARC*, glutathione peroxidase 3 (*GPX3*), neural cell adhesion molecule 1 *(NCAM1*), pyruvate dehydrogenase kinase 4 (*PDK4*), retinol-binding protein 1 (*RBP1*), and transforming growth factor, β induced (*TGFBI*). Importantly, multivariate analysis confirmed that *GPX3*, *RBP1*, *SPARC*, and *TGFBI* are associated with survival, and methylation of the genes is independent of established risk factors for MM. Methylation levels of these 4 genes is low in MGUS, and then increasing methylation is associated with more aggressive MM cellular phenotypes. Walker et al., investigated DNA methylation patterns associated with MM subtypes [7]. They found specific profiles with increased hypermethylation in clinically aggressive subtypes, such as plasma cell leukemia, and in the prognostically unfavorable t(4;14) cytogenetic subtype with overexpressed *MMSET*, which encodes a histone methyltransferase. These findings suggest that methylation changes affect disease biology.

Recent reports correlated hypermethylation of promoter-associated CpG islands with silencing of microRNAs (miRNAs), which are small 18–22 nucleotide RNAs that regulate many intracellular functions [41]. Dysregulation of miRNA genes has been implicated in MM. Moreover, several reports of MM described the role of hypermethylation of tumor-suppressor miRNA genes, including *miR-34b/c* [42], *miR-194-2-192* [43], and *miR-203* [44]. Combined genome-wide analysis of miRNA methylation and miRNA expression profiling is warranted to clarify the role of epigenetic regulation of miRNA in MM.

4. DNA Methylation As an Epigenetic Biomarker in MM

The current prognostic factors in MM include cytogenetic aberrations, such as the nonhyperdiploid, cytogenetically detected chromosomal 13q deletion, t(4;14), t(14;16), 1q gain, and del(17p) detected by fluorescence *in situ* hybridization [10]. Novel therapeutics, such as the proteasome inhibitor bortezomib, can partially overcome adverse outcomes conferred by these abnormalities [45]. However, there has been much less progress in the development of predictive biomarkers for specific treatments [46]. To identify predictive biomarkers for the effect of myeloma therapeutics, appropriate clinical trial designs are necessary. Since some novel MM therapeutics in development have specific molecular

targets, the identification of biomarkers that also characterize drug sensitivity is a promising therapeutic strategy [45].

As mentioned above, hypermethylation of *TGFB1*, *SPARC*, *RBP1*, and *GPX3* is associated with significantly shorter overall survival, independent of age, international staging system score, and adverse cytogenetics [40]. Future prospective studies will verify these genes as prognostic MM biomarkers.

We identified *RASD1* as a possible biomarker in MM [33]. *RASD1*, located on chromosome 17p11.2 with frequent loss of heterozygosity in various human tumors, encodes a Ras GTPase with tumor suppressor functions induced by dexamethasone [47,48]. Importantly, MM cells that show *RASD1* methylation are resistant to dexamethasone, and combined treatment with dexamethasone and the hypomethylating agent decitabine (5-aza-2′-deoxycytidine), which inhibits DNA methyltransferase, restores the cytotoxicity of dexamethasone to tumor cells. While the hypermethylation of *RASD1* was observed in approximately 10% of primary MM samples, the methylation levels of *RASD1* were elevated in all of the MM cases that had pair DNAs after repeated antimyeloma therapy, including dexamethasone.

Limited studies have addressed the antitumor effects of the hypomethylating agents decitabine and azacitidine (5-azacytidine) in MM, demonstrating significant *in vitro* antimyeloma activity. The mechanisms involve changes in gene expression and induction of DNA damage [49,50]. Recently, a gene expression-based DNA methylation score was reported, which relates the expression of methylation-regulated genes to predict the efficacy of hypomethylating agents—decitabine and azacitidine—in human MM cell lines and in patient MM cells *in vitro* [51,52]. Phase I/II clinical trials are ongoing to study the side effects and best dose of azacitidine in combination with lenalidomide and dexamethasone in MM; therefore, an investigation regarding association of the methylation score and the response of MM patients could provide promising information [53]. Taken together, these findings suggest the involvement of epigenetic gene silencing in MM progression and drug resistance and the usefulness of demethylation therapy for MM treatment.

Conclusion

In summary, DNA methylation functions in MM tumorigenesis and progression. Several reports have suggested that DNA methylation could be a useful biomarker to predict prognosis and sensitivity to treatment. A further comprehensive analysis using a genome-wide approach with high-throughput sequencing technologies will be necessary to clarify the molecular mechanisms of MM oncogenesis and progression. Epigenetics has become to an essential research area where important challenges should be resolved through further investigations of MM.

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