Pathogenesis of Myeloma

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Abstract

Multiple myeloma (MM) is a neoplasm of post–germinal center, terminally differentiated B cells. It is characterized by a multifocal proliferation of clonal, long-lived plasma cells within the bone marrow (BM) and associated skeletal destruction, serum monoclonal gammopathy, immune suppression, and end-organ sequelae. MM is preceded by an age-progressive premalignant condition termed monoclonal gammopathy of undetermined significance. Unlike the genomes of most hematological malignancies, and similar to those of solid-tissue neoplasms, MM genomes are typified by numerous structural and numerical chromosomal aberrations as well as mutations in a number of oncogenes and tumor-suppressor genes, some of which have been linked to disease pathogenesis and clinical behavior. Recent studies have also defined the importance of interactions between the MM cells and their BM microenvironment, dysregulation in signaling pathways and in a specialized subpopulation of cells within the tumor (termed myeloma cancer stem cells) for tumor cell growth and survival, and the development of resistance to therapy.

Long-lived plasma cells (PCs): post–germinal center (GC) PCs that undergo immunoglobulin heavy-chain–switch recombination and home to the bone marrow, where they reside as terminally differentiated, nonproliferating cells

MULTIPLE MYELOMA IS A TUMOR OF LONG-LIVED PLASMA CELLS THAT HOME TO THE BONE MARROW

B cell development culminates with the production of plasma cells (PCs), which are terminally differentiated, nondividing cells whose function is to secrete antigen-specific antibodies (1). After T cell–independent antigenic exposure and in combination with other signals, naïve B cells in the splenic marginal zone and circulating mature follicular B cells undergo proliferation and differentiation into low-affinity immunoglobulin M–secreting PCs. PCs formed in this early extrafollicular response do not have somatically mutated immunoglobulin genes and are short lived, undergoing apoptosis in situ. Subsequently, antigen and antigen-specific T helper cells cause naïve follicular B cells to undergo proliferation, multiple rounds of somatic hypermutation of immunoglobulin H (IgH) and immunoglobulin L (IgL) V(D)J sequences, affinity maturation, and class-switch recombination of immunoglobulin in a germinal center (GC) reaction (**Figure 1**) (1, 2). This reaction ultimately produces memory B cells and terminally differentiated, nonproliferating, long-lived PCs that secrete high-affinity antibody with predominantly switched isotype (3). These PCs typically home to the bone marrow (BM), where they receive survival signals such as interleukin (IL)-6 from stromal cells (SCs), and live for many months to years

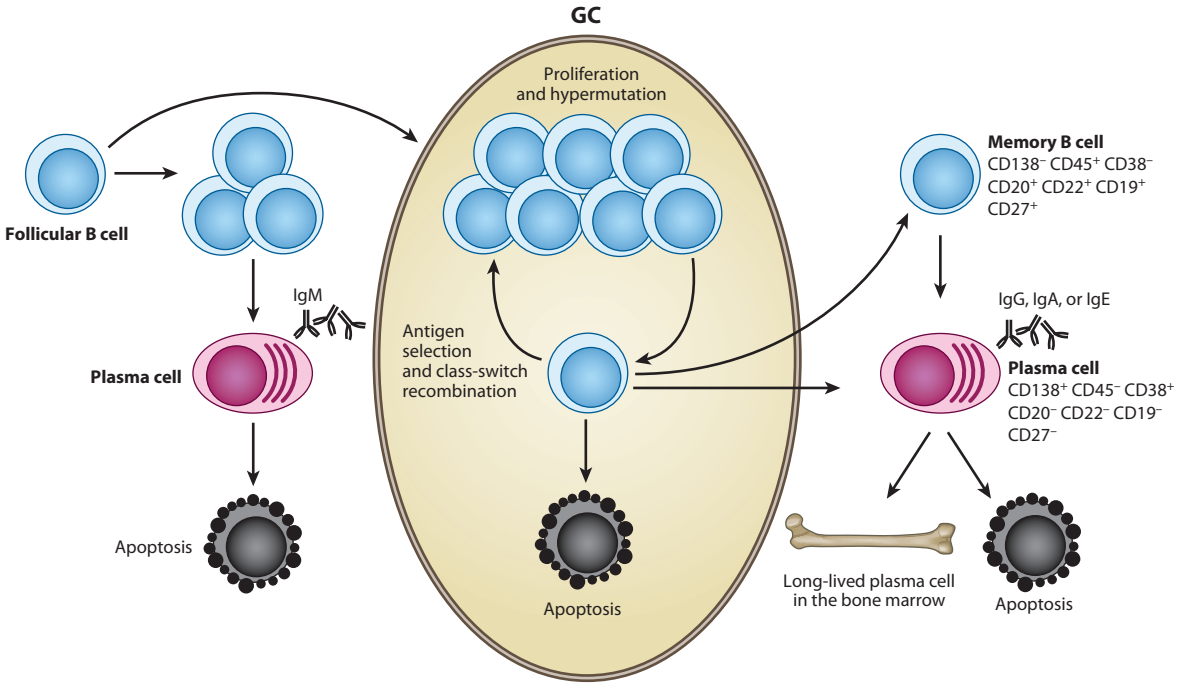

Figure 1

Formation of long-lived plasma cells and the origin of multiple myeloma (MM) cells. After their initial encounter with antigen, naïve follicular B cells differentiate into short-lived plasma cells, undergoing apoptosis in situ. Some activated follicular B cells form a germinal center (GC), where they undergo somatic hypermutation, antigen selection, and immunoglobulin (Ig) heavy-chain-switch recombination. Post-GC plasma cells progress through a memory B cell stage in the primary response or develop directly from GC B cells. Plasma cells that arise from a GC reaction become long lived if they find survival niches, which are located mainly in the bone marrow. The immunoglobulin gene sequences in MM plasma cells are somatically hypermutated and remain constant throughout the clinical course, suggesting that the disease arises from a post-GC B cell. Recent studies indicate that MM cancer stem cells lack CD138 and express the memory B cell markers CD19 and CD27. Adapted from Reference 1.

(4, 5). Although PCs can be generated from pre-GC B cells, multiple myeloma (MM) [and its precursor, monoclonal gammopathy of undetermined significance (MGUS)] is exclusively a post-GC tumor that has phenotypic features of long-lived PCs and is usually distributed at multiple sites in the BM (6, 7). The immunoglobulin gene sequences in MM PCs are somatically hypermutated and remain constant throughout the clinical course, suggesting that the disease arises from post-GC B cells (8, 9).

ORIGIN OF THE MULTIPLE MYELOMA CELL

As in other human cancers, increasing evidence indicates that the initiation, relapse, and myeloma progression are driven by a rare population of cells termed myeloma cancer stem cells. Although PCs phenotypically characterize the disease, recent studies have suggested that they lack significant proliferative capacity; instead, PCs arise from clonogenic cells that resemble memory B cells (10, 11). Studies of the growth fraction of MM PCs both in vitro and in vivo have found that the majority of PCs are quiescent, especially at diagnosis, which suggests that tumor growth is restricted to a specialized subpopulation of cells (12). Initial evidence indicating that MM PCs may be functionally heterogeneous was derived from studies of mouse myeloma cells (13). In these studies, the ability to form tumor colonies in vivo or in vitro was restricted to a minority of cells: roughly 1 in 1,000 to 4,000. Moreover, cells from the splenic tumor colonies were serially transplanted into secondary recipients, demonstrating that injected cells are capable of self-renewal (13). Later studies demonstrated that human myeloma cells are also capable of colony formation and that clonogenic growth occurs at a frequency of 1 in 100 to 100,000 cells (10, 14). Furthermore, MM PCs characterized by surface expression of CD138 are incapable of significant clonogenic growth; however, $CD138^-$ expressing typical B cell surface antigens (CD45, CD22, and CD19) produce tumor colonies that can be serially passaged (15). More recently, investigators developed a novel in vitro three-dimensional stromal culture system that recapitulates both cellular and extracellular features of the BM, and MM growth in this in vitro assay also appears to arise from clonotypic B cells (16).

The data derived from in vivo assays performed to identify MM cells with clonogenic potential are conflicting. For instance, use of the severe combined immune-deficient human (SCID-hu) model, in which human fetal bone fragments are implanted subcutaneously into SCID mice to recreate a humanized microenvironment, shows that mature $CD38^+CD45^-$ human MM cells can generate disease that resembles human MM, including circulating M protein, hypercalcemia, and resorption of the human bone fragments (17). In contrast, $CD38^-CD45^+$ peripheral blood B cells could not engraft, which suggests that only mature PCs are clonogenic. However, in several subsequent studies human clonotypic B cells engrafted and generated MM following intracardiac or direct intraosseous injection into nonobese diabetic (NOD)/SCID mice (18). Similarly, $CD138^+$ PCs failed to engraft NOD/SCID mice following vein tail injection (10, 19), whereas $CD45^+$ peripheral blood cells lacking CD38 and expressing the memory B cell markers CD19 and CD27 serially engrafted mice and gave rise to clonotypic $CD38^+$ PCs that were functionally capable of producing circulating M protein (**Figure 1**). The reasons for these discrepancies are unclear, but they probably represent intrinsic differences between the animal models used in these in vivo experiments. Nonetheless, anti-CD20 therapy has been tested in MM on the basis of studies demonstrating clonogenic $CD20^+$ precursor B cells. Anti-CD20 therapy with rituximab elicits a partial response in only 10% of $CD20^+$ patients with MM (20).

REGULATION OF LONG-LIVED PLASMA CELLS BY TRANSCRIPTIONAL MECHANISM

Although our understanding of how PC formation is regulated is improving (**Figure 2**)

MM: multiple myeloma

MGUS: monoclonal gammopathy of undetermined significance

Post-GC B cells: follicular B cells that have undergone rounds of proliferation as well as affinity maturation, somatic hypermutation, and class-switch recombination of immunoglobulin in a GC

Myeloma progression: refers to driving genetic and epigenetic events that promote the evolution of MGUS and the progression to MM, which is the result of evolving cross talk between MM cells and the BM microenvironment

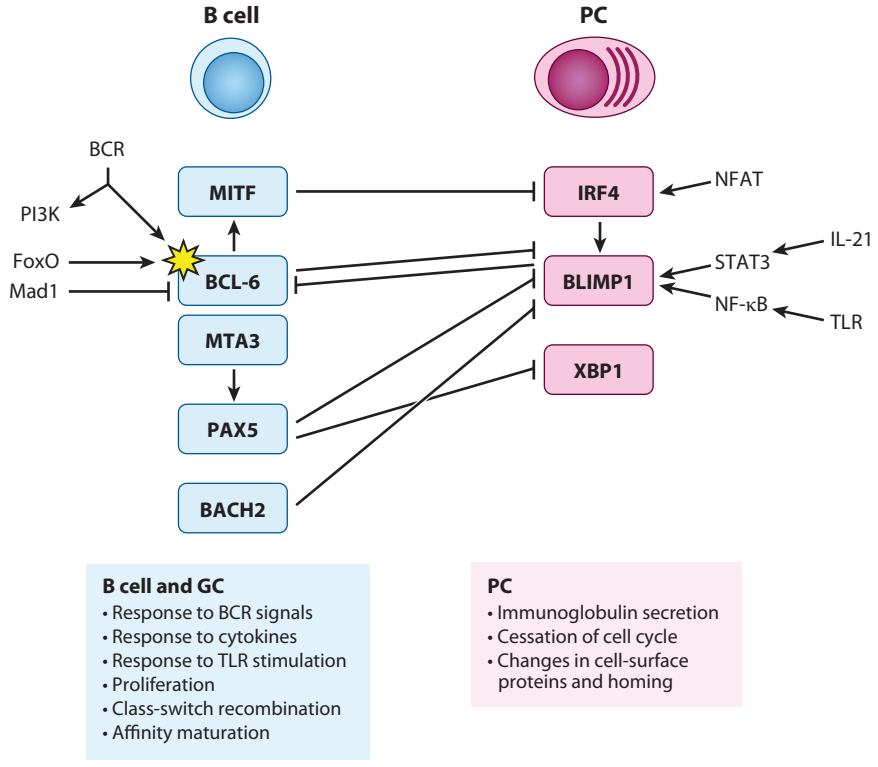

Figure 2

Transcriptional control of plasma cell (PC) development. (*Left*) In B cells, several transcription factors, including MITF (microphthalmia-associated transcription factor), BCL-6 (B cell lymphoma 6), MTA3 (metastasis-associated 1 family, member 3), PAX-5 (paired box protein 1), and BACH2 (BTB and CNC homology 1, basic leucine-zipper transcription factor 2), inhibit plasmacytic development by repressing BLIMP1 (B lymphocyte–induced maturation protein 1), XBP1 (X-box-binding protein 1), and IRF4 (interferon-regulatory factor 4). (*Right*) In PCs, BLIMP1 represses B cell expression programs. This mutual negative regulation prevents the unelicited formation of PCs in the germinal center (GC) and prevents the reversion of PCs to a B cell stage. Arrows and bars indicate positive and negative regulation, respectively, and the star indicates protein degradation. MITF, BCL-6, MTA3, PAX-5, and BACH2 also regulate the expression of genes that are required for B cell and GC functions. BLIMP1, XBP1, and IRF4 induce the expression of genes that are required for PCs. Abbreviations: BCR, B cell receptor; IL-21, interleukin-21; NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor κB; PI3K, phosphatidylinositol 3-kinase; TLR, Toll-like receptor. Adapted from References 1 and 2.

(1, 2), little is known about the transcriptional regulatory mechanisms involved in the maintenance of long-lived PCs. Understanding of these mechanisms may allow the rational design of therapeutic agents to block the activity of PCs in MM.

Aiolos is a member of the Ikaros family of zinc finger–containing transcriptional regulators required for the appearance of high-affinity PCs in the BM (1). In *Aiolos*$^{-/-}$ mice, memory B cells of high affinity form and give rise to short-lived PCs, but these cells did not migrate to the BM. Although BLIMP1 (B lymphocyte–induced maturation protein 1), XBP1 (X-box-binding protein 1), and BCL-6 (B cell lymphoma 6) are expressed normally in *Aiolos*$^{-/-}$ mice, the crucial target gene(s) required for migration to or retention of PCs in the BM or for their response to survival signals remain unidentified. Recent studies have revealed that

the lack of Aiolos accelerates premature B cell apoptosis, a process that is mediated by B cell receptor signaling via elevation in cytochrome *c* (21). Further analysis of these mice should provide important insights into the mechanisms of survival of long-lived PCs in the BM.

Recent studies have shown that continued expression of the transcription factors BLIMP1 and XBP1 is required to maintain PCs. For instance, ectopic expression of BCL-6 and MTA3 in transformed human PC lines is associated with reduced levels of BLIMP1 and XBP1 and induced reversion of the PC phenotype (22). The abundant expression of XBP1 in MM cell lines and MM primary tumors indicates this transcription factor's probable role in MM pathogenesis. Indeed, proteasome inhibitors target the activity of XBP1 and induce MM cell apoptosis, which is associated with increased endoplasmic-reticulum stress, lack of XBP1, and the subsequent failure to maintain the secretory program (23). Importantly, overexpression of XBP1 in transgenic mice causes plasmacytosis and bone lytic lesions. Despite recapitulation of some aspects of human disease, specific genetic lesions that result in the overexpression of XBP1 have not been described, and the true fidelity of this model remains to be determined (24). Use of a mouse model in which a gene encoding the transcriptional repressor BLIMP1 can be inducibly deleted has shown that BLIMP1 is required not only for the formation of PCs but also for their maintenance as long-lived Ig-secreting cells in the BM (25). BLIMP1 is known as the master regulator of PC differentiation because it directly represses transcription factors, which in turn regulate several important programs. For instance, BLIMP1 represses c-Myc, BCL-6, and Pax-5; the latter is required for B cell identity, GC function, and repression of XBP1 (1, 2). It is intriguing, in light of the role of BLIMP1 in suppressing c-Myc expression, that several mouse models displaying plasmacytosis have been derived from the aberrant expression of c-Myc. In one model, the coexpression of Bcl-XL and c-Myc in B cells results in polyclonal PC expansion that later progresses to monoclonal plasmablastic malignancy (26). In a separate model, c-Myc overexpression in post-GC B cells results in PC expansion (27). Interestingly, a recent report demonstrated that differentiation of MM cells can be induced by 2-methoxyestradiol, which requires BLIMP1 not only to upregulate XBP1 expression but also to inhibit c-Myc activity (28). These two signal transduction pathways work independently of one another as well as synergistically to promote the differentiation of MM cells.

How or why changes in different transcription factors cause the dedifferentiation or death of PCs remains unclear. However, the studies discussed in this section emphasize that maintenance of PC fate requires the continuous expression of BLIMP1 and XBP1, as well as the continued absence of PAX-5, BCL-6, and MAT3. This knowledge is likely to provide targets for developing drugs that can block the function of long-lived PCs in MM. A recent report indicates that interference with IRF4 (interferon-regulatory factor 4) expression is lethal to MM cells regardless of their genetic etiology, making IRF4 an "Achilles' heel" that may be exploited therapeutically (29, 30).

MULTIPLE MYELOMA EVOLUTION AND DIAGNOSTIC CRITERIA

Unlike other hematologic malignancies, MM is preceded by an age-progressive premalignant condition termed MGUS. This condition, which is present in 1% of adults over the age of 25, progresses to MM at a rate of 0.5% to 3% per year (31, 32). A recent study based on more than 77,000 individuals showed that all the patients who eventually developed MM had previously been diagnosed with MGUS (33). Although the probability of progression of MGUS to MM over 25 years of follow-up is 30% (32), little is known about the driving forces that promote the evolution of MGUS and the advancement to MM. Thus, current research is directed to identify biomarkers that render individuals with MGUS and so-called

Table 1 Diagnostic criteria for MGUS, SMM, and MM[a]

MGUS
Serum M protein: 3 g per 100 ml
Bone marrow plasma cells: <10%
No clinical manifestations or other laboratory abnormalities attributable to monoclonal gammopathy
SMM
Serum M protein: ≥3 g per 100 ml
Bone marrow plasma cells: ≥10%
No clinical manifestations or other laboratory abnormalities attributable to monoclonal gammopathy
MM
Serum M protein: 3 g per 100 ml
Bone marrow plasma cells: >10%
Urine light-chain protein: >1 g per day

[a]Abbreviations: MGUS, monoclonal gammopathy of undetermined significance; SMM, smoldering multiple myeloma; MM, multiple myeloma.

smoldering MM likely to progress to active MM, in order to evaluate treatment strategies to inhibit or delay progression. Patients with MGUS have a serum M protein level of less than 3 g per 100 ml and BM PCs of less than 10%; there are no clinical manifestations related to their monoclonal gammopathy. A serum M spike higher than 3 g per 100 ml, a urine light-chain protein greater than 1 g per day, and/or a proportion of BM PCs of 10% or greater are consistent with MM (**Table 1** and **Figure 3**). Patients who fulfill the diagnostic criteria of MM but are asymptomatic are considered to have smoldering MM (**Table 1**). In addition to these clinicopathologic features (34), flow-cytometric immunophenotyping is considered an indispensable tool for the diagnosis, classification, and monitoring of MGUS and MM (35). Active MM is associated with features such as bone lytic lesions, anemia, hypercalcemia, and renal impairment. Upon further progression, MM occurs at extramedullary sites such as blood, pleural fluid, pericardial fluid, and skin. Extramedullary MM is a more aggressive disease. Circulating peripheral blood PCs characterize plasma cell leukemia, which can be categorized as secondary or primary, depending on whether preceding intramedullary myeloma has been recognized.

aCGH: array–comparative genomic hybridization

GEP: gene-expression profiling

ONCOGENOMICS OF MULTIPLE MYELOMA

The MM genome is characterized by a distinctive combination of gains and losses of whole chromosomes, by nonrandom chromosomal translocations that cause dysregulation of the genes at the breakpoints, and by point mutations (**Table 2**). In addition, a constellation of small focal areas of chromosomal amplifications and deletions have recently been identified via high-resolution technologies such as array–comparative genomic hybridization (aCGH) (**Figure 4*a***) and single-nucleotide polymorphism arrays (36–38). Furthermore, gene-expression profiling (GEP) analyses have implicated the Notch and Wnt signaling pathways in PC development (39, 40). The Wnt pathway is of particular interest because several of its components are abnormally expressed in MM cells, especially (*a*) the Frizzled-related protein FRZB, which may be critical in the transition to malignancy (41), and (*b*) Dickkopf-related protein 1 (DKK1), a potent inhibitor of Wnt/β-catenin that is responsible for MM-associated bone disease (**Figure 4*b***) (42). The Wnt signaling pathway can also be activated in MM cells, as we discuss further below (43, 44).

Little characterization of MM at the epigenetic level has been performed to date.

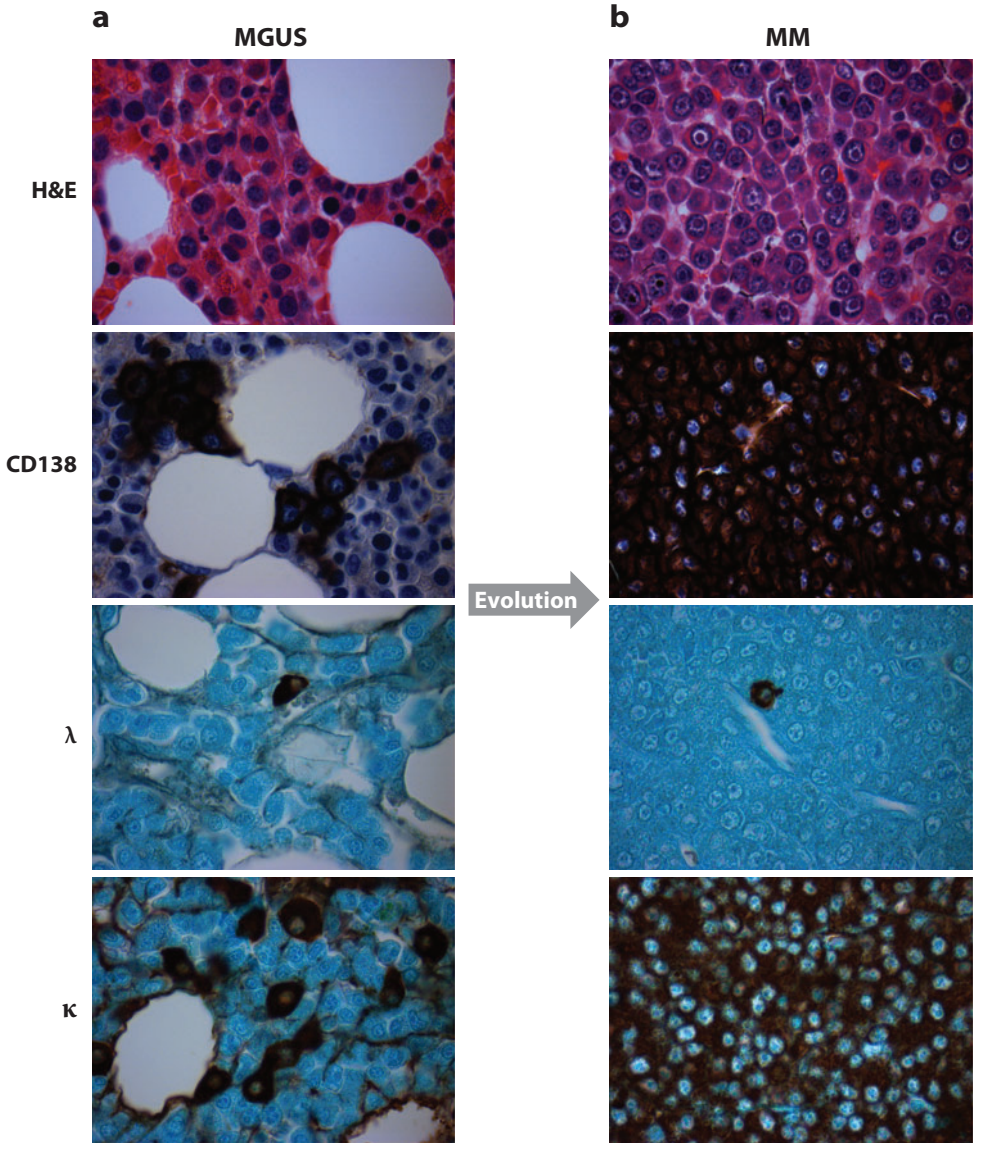

Figure 3

Clinicopathologic characteristic of (*a*) monoclonal gammopathy of undetermined significance (MGUS) and (*b*) multiple myeloma (MM). Unlike other hematologic malignancies, MM is preceded by an age-progressive premalignant condition known as MGUS, in which the burden of plasma cells is higher than normal; however, MGUS occurs in the absence of any clinical signs or symptoms of MM. A recent screening study based on more than 77,000 individuals showed that all the patients who eventually developed MM had previously been diagnosed with MGUS (33). Note the presence of atypical plasma cells (large-sized cells with prominent nucleoli, hyperchromatic chromatin, and binucleation) in the bone marrow of a patient with MM (*right*), compared with bone marrow from the same patient when he had MGUS (*left*). Also note the increased number of clonal plasma cells in MM, as evaluated by immunostaining with anti-CD138 antibodies, and the restricted surface expression of immunoglobulin-κ light chain.

Table 2 Recurrent chromosomal alterations in multiple myeloma[a]

Immunoglobulin translocations		
Locus	**Incidence (%)**	**Oncogene(s)**
17q13	15–20	*Cyclin D1*
6p21	5	*Cyclin D3*
4p16.3	12	*F6FR3*, *MMSET*
16q23	5–10	*c-Maf*
8q24	<10	*c-Myc*
6p25	5	*MUM1*, *IRF4*
20q11	5	*MAFB*
MCR chromosomal gains[b]		
Locus	**Incidence (%)**	**Candidate oncogene(s)**
1q21–1q22	55	*BCL-9*, *IL-6R*
3q27.1–3q27.2	47	*POLR2H*, *EIF4G1*
5p12	44	?
7p12.2	44	?
9q34.11–9q34.3	54	*ABL1*, *ANAPC2*
11q13.4–11q14.1	52	*SPCS2*
15q24.2	44	*IMP3*
19q13.11	47	*PDCD5*
21q22.3	37	*MCM3AP*, *HRMT1L1*
MCR chromosomal losses		
Locus	**Incidence (%)**	**Candidate TSG(s)**
1p13.3–1p12	41	*DENND2D*
8p23.3–8p21.3	28	*DLC1*
10q26.2–10q26.3	18	*PTPRE*
13q34	49	*RFP2*, microRNA 15/16
14q32.13–14q32.2	33	?
16q11.2–16q12.2	31	*CYLD*

[a]The first four translocations are predominantly primary translocations (i.e., they occur as the early, perhaps initiating, event in tumorigenesis). The remaining translocations are rather infrequent and are often secondary translocations (i.e., they occur during tumor progression). Adapted from Reference 6. Chromosomal gains and losses are defined as minimal common regions of DNA gains or losses, as evaluated by array–comparative genomic hybridization (36).
[b]Abbreviations: BCL-9, B cell lymphoma 9; IL-6R, interleukin-6 receptor; MCR, minimal common region; TSG, tumor-suppressor gene.

However, epigenetic changes such as gene methylation (45, 46), acetylation (47), and microRNAs have been described (**Figure 4*c***) (48, 49).

MM can be subdivided into two groups according to the pattern of chromosomal gains and losses (50, 51). Approximately 55–60% of MM primary tumors are characterized by a hyperdiploid karyotype with a number of chromosomes ranging from 48 to 74 (50) and trisomies of odd-numbered chromosomes including 3, 5, 7, 9, 11, 15, 19, and 21 (**Figure 5*a***). The remaining cases make up a nonhyperdiploid group, which includes tumors with a hypodiploid, near-diploid, pseudodiploid, or near-tetraploid chromosome number (i.e., fewer than 48 or more than 74 chromosomes) (50, 52). The mechanisms underlying this pattern have not been elucidated; importantly, ploidy status rarely changes during disease

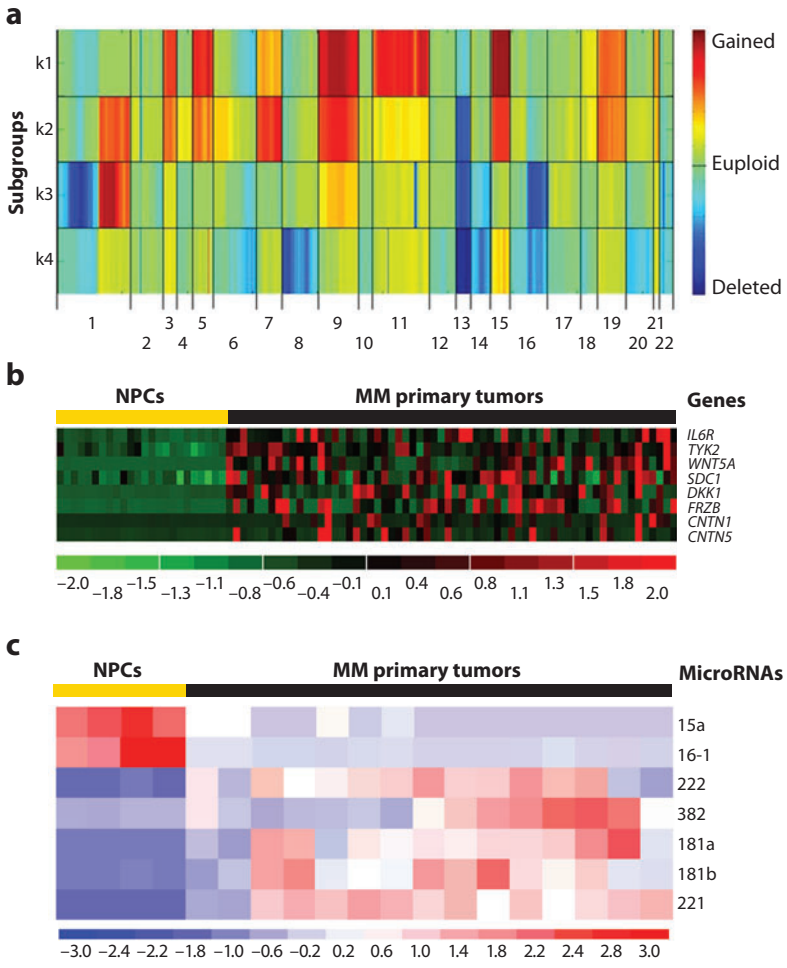

Figure 4

Oncogenomic and epigenetic analysis of multiple myeloma (MM). (*a*) Array–comparative genomic hybridization (aCGH)-based classification and survival of MM patients. Nonnegative matrix factorization (NMF) classification, rank k = 4, identifies four distinct subgroups associated with prognostic outcomes. The aCGH profiles of 67 clinically annotated primary tumors were subjected to NMF analyses. The *y* axis indicates the four subgroups identified by NMF. The *x* axis represents the genomic map position (from chromosome 1 to chromosome X). The colors denote gained (*red*), euploid (*yellow/green*), or deleted (*blue*) chromosome material (36). (*b*) Gene-expression profiling analysis of normal plasma cells (NPCs) and MM primary tumors reveals contrasting patterns of expression and highlights the role of the Wnt pathway in MM pathogenesis. (*c*) MicroRNA profiling studies of NPCs and MM primary tumors reveal contracting patterns of expression, suggesting that microRNAs play a role in MM pathogenesis. Adapted from Reference 49.

progression (53), and patients with hyperdiploid MM tend to have a better prognosis than do those with nonhyperdiploid disease (54, 55). On the basis of recent genomic and GEP studies, the hyperdiploid and nonhyperdiploid groups have been further subclassified. Within the hyperdiploid group, aCGH-based classification (36) has identified a subset of patients who present additional gains on 1q and/or losses of chromosome 13. These patients have a worse prognosis than do patients in the nonhyperdiploid group.

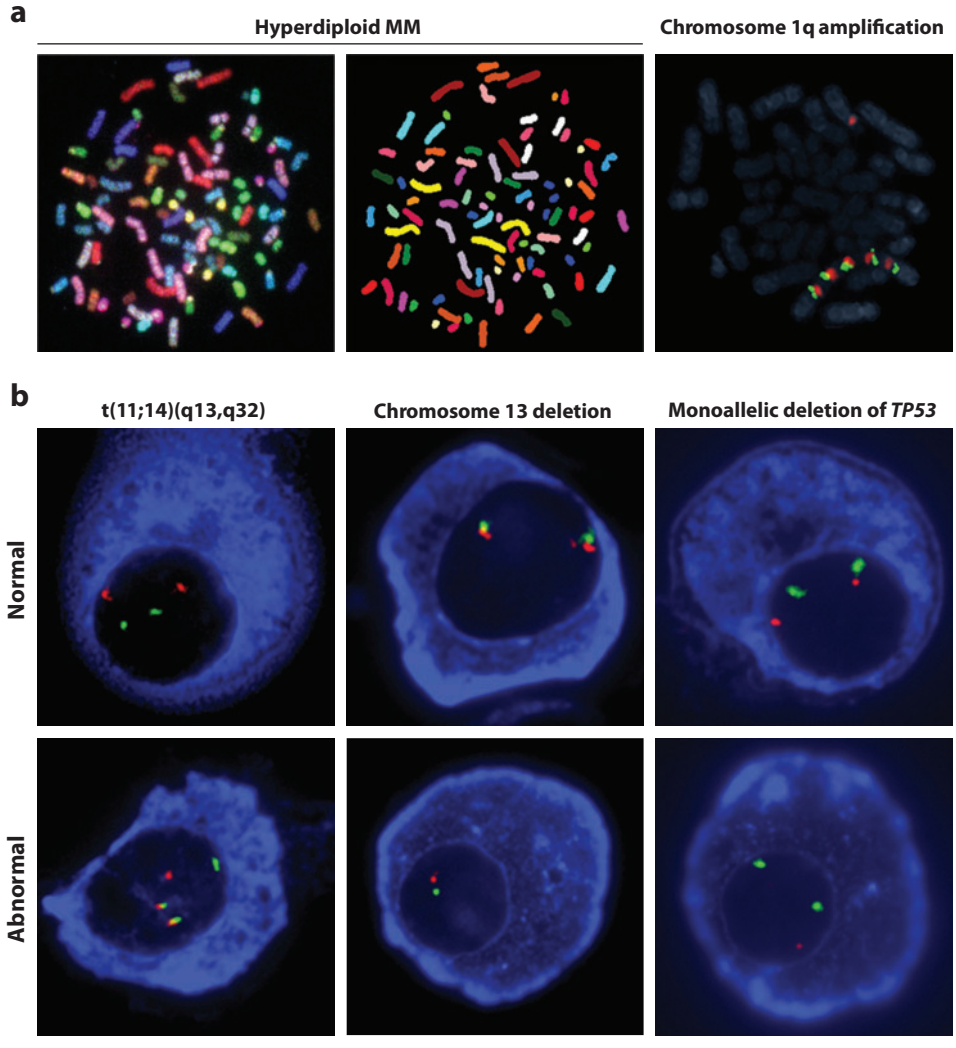

Figure 5

Spectral karyotypic (SKY) and fluorescence in situ hybridization (FISH) analysis of multiple myeloma (MM). (*a*) SKY analysis showing a case of hyperdiploid MM with gain of chromosome 3. (*Left*) SKY image in display colors. (*Middle*) Spectral image in classification colors. (*Right*) Metaphase FISH analysis showing amplification of chromosome 1q. Pictures provided by Dr. Jeffrey R. Sawyer and Alexei Protopopov. (*b*) FISH analysis showing examples of MM with (*left*) *CND1C* translocation t(11;14)(q13;q32), (*middle*) monoallelic deletion of chromosome 13, and (*right*) monoallelic deletion of *TP53*. Pictures provided by Dr. Rafael Fonseca.

Early-onset reciprocal chromosomal translocations involving the IgH locus at 14q32.3 (56), and less frequently the IgL locus at 2p12,κ or 22q11,λ (57), are also characteristic of the MM genome (**Table 2**). In these primary translocations (58), various genes are juxtaposed to a strong Ig enhancer that dysregulates their mRNA expression. Two translocations directly increase the expression of cyclins: (*a*) t(11;14)(q13;q32), which occurs in 15% to 20% of MM patients, induces cyclin D1 overexpression (**Figure 5*b***) (59); and (*b*) t(6;14)(p21;q32), which is present in 5% of MM cases, increases expression of cyclin D3

(60). Another translocation, t(4;14)(p16.3;q32), is present in approximately 15% of patients (61) and dysregulates the expression of the Wolf-Hirschhorn syndrome candidate 1 gene [*WHSC1*, also known as multiple myeloma set domain (*MMSET*)], which encodes a protein with homology to histone methyltransferases; and the receptor tyrosine kinase fibroblast growth factor receptor 3 (*FGFR3*) gene (62). Finally, t(14;16)(q32;q23) dysregulates the oncogene *MAF*, a basic leucine-zipper transcription factor, in 5% to 10% of patients (61, 63), and t(14;20)(q32;q11) affects another member of this family, *MAFB*, in 5% of cases. These rearrangements generally seem to be mutually exclusive, although in 5% of MGUS and 25% of advanced MM cases two independent translocations may be found in the same patient (64). The oncogenic consequences of these translocations on MM pathogenesis—with the exceptions of those caused by *MAF* and *FGFR3* deregulation—have not yet been extensively explored. MAF promotes MM cell proliferation and increases MM cell adhesion to bone marrow stromal cells (BMSCs) (63). Inhibition of FGFR3 in t(4;14) MM induces PC differentiation and apoptosis (65). However, FGFR3 is not expressed in 25% of patients with t(4;14) MM, which suggests that it may not be universally relevant as a therapeutic target in this group of patients (66). Importantly, these chromosomal translocations more often affect nonhyperdiploid patients (57) and are linked to prognosis. Specifically, t(14;16) and t(4;14) translocations are associated with a poor prognosis (67), whereas patients presenting with t(11;14) translocations have a longer survival time relative to all other genetic subtypes (68).

Expression studies have identified subtypes within the nonhyperdiploid group associated with specific chromosomal translocations (54). Moreover, translocations directly or indirectly affect the expression of cyclins. The fact that cyclin D1 is also consistently dysregulated in the hyperdiploid group, by a yet-to-be-defined mechanism, suggests a major role of D-type cyclin dysregulation in the early pathogenesis of all cases of MM (69). In addition to the hyperdiploid/nonhyperdiploid dichotomy and the presence of chromosomal translocations, gains or losses of specific chromosomal regions occur in all MM patients and are linked to prognosis. These include chromosome 13 monosomy (**Figure 5*b***), loss of the short arm of chromosome 17 (where the tumor-suppressor gene *TP53* resides) or the short arm of chromosome 1 (52), and gains or amplifications of the long arm of chromosome 1 (**Figure 5*a***) (36, 54, 70). The oncogene *MYC* is involved in chromosomal translocations and/or amplifications in up to 45% of patients with advanced MM (6). Deletions of chromosomes 17p and 1p, as well as loss of chromosome 13, are linked to poor prognosis (52, 71), although the prognostic significance of chromosome 13 remains controversial (72). Indeed, chromosome 13 loss portends poor prognosis to conventional low- and high-dose chemotherapy but not to the proteasome inhibitor bortezomib (73). Gains or amplifications of chromosome 1q were also recently proposed as an adverse prognostic factor (36, 54, 70, 71). In particular, in the most comprehensive expression profiling survey of MM patients published to date, Shaughnessy et al. (71) identified 70 genes linked to early disease-related death, thereby providing the first validated classifier for prognosis prediction in uniformly treated MM patients. Strikingly, 30% of these genes were located on chromosome 1, with most of the downregulated genes located on the short arm of chromosome 1 and most of the upregulated genes on 1q. These recent expression profiling findings confirm the critical role of genes located on chromosome 1 in the survival of MM patients.

aCGH (**Table 2**) and single-nucleotide polymorphism surveys, coupled with expression analysis (36–38), recently identified many focal genetic lesions that are believed to be related to MM initiation and progression. Research efforts in this direction have uncovered the high level of molecular heterogeneity of MM and have highlighted the difficulties that will be faced in the identification of common genetic events that could be targeted in all MM

BMSCs: bone marrow stromal cells

patients. Our long-term laboratory efforts have defined the importance of interactions between MM cells and their BM microenvironment for tumor cell growth and survival, as well as the development of resistance to therapy. In designing new approaches for therapy that could be useful in most MM patients independently of the genetic makeup of the tumor cells, a possible therapeutic alternative may be to disrupt the tropism between MM cells and their BM microenvironment.

Tumor-suppressor genes that are mutated in MM include *TP53* (**Figure 5*b***), phosphatase and tensin homolog (*PTEN*), the cyclin-dependent kinase inhibitors *CDKN2A* and *CDKN2C* (6), tumor necrosis factor (TNF) receptor–associated factor 3 (*TRAF3*), and cylindromatosis (*CYLD*) (37). These findings implicate a role for tumor-suppressor genes in MM. Additionally, in approximately 10% of patients with t(4;14) MM, oncogenic, activating mutations of *FGFR3* are present (74). Importantly, two members of the Ras family (*NRAS* and *KRAS*) are mutated at codons 12, 13, and 61 in 40% to 55% of MM patients versus only 5% in MGUS (6, 75), which suggests a major role for activation of the mitogen-activated protein kinase (MAPK) pathway in the progression from MGUS to MM. Indeed, oncogenomics studies have identified only a few differences that distinguish MGUS from MM (76). Both conditions can present either a hyperdiploid karyotype or a hypodiploid karyotype (54) and similar chromosomal translocations that affect the IgH or IgL locus (77). Using GEP arrays, a previous study did not find significant differences between MGUS and MM, whereas a more recent analysis identified a signature of MGUS shared by a subgroup of MM patients with a better prognosis (78). However, a concern about this study is the issue of possible contamination with nonmalignant PCs, which frequently occurs when BM involvement by malignant PCs is low, such as in MGUS. In this regard, in a recent reanalysis of the same data set, in which contamination with nonmalignant cells was carefully excluded, the same pattern could not be reproduced (79). A higher incidence of t(4;14) in MM versus MGUS was reported in some but not all studies (80). At present, therefore, *RAS* mutations are the major genetic difference between MGUS and MM.

The studies that have taken place over the past decade have highlighted several rearrangements that affect the genome of MM cells at different levels. However, a peculiarity in the pathogenesis of MM is the essential role played by the BM microenvironment and by the mutual interactions between the cells residing in this niche and MM cells. Studying the BM compartment has been challenging, but recent work has reinforced the relevance of BM accessory cells and the extracellular matrix (ECM) proteins in MM pathogenesis (81). Oncogenomics studies are crucial to understanding the genetic heterogeneity of MM cells that is likely to influence their interaction with the BM, which in turn induces growth, survival, and drug resistance in MM cells. However, an important issue that remains to be resolved by oncogenomic studies is the biological significance of the identified genetic alterations, particularly mutations and small areas of amplification or deletions. A major caveat of these studies is that they did not use the most appropriate control reference DNA, namely DNA from normal PCs from the same patient. This is a critical issue because MM is a tumor of post-GC B cells (82), and genetic instability of B cells in the GC may be responsible for the genetic alterations that are common to normal PCs and MM cells.

MULTIPLE MYELOMA–BONE MARROW NICHE

It is well established that the physical interaction between MM cells and the BM microenvironment, which can be modeled in vitro and in vivo, plays a crucial role in MM pathogenesis (**Figure 6**) (83–85). The BM microenvironment consists of a variety of ECM proteins (e.g., fibronectin, collagen, laminin, and osteopontin), and cells (e.g., hematopoietic stem cells, progenitor and precursor cells, immune cells, erythroid cells, SCs, endothelial

BM microenvironment: consists of a variety of (*a*) extracellular matrix proteins including fibronectin, collagen, laminin, and osteopontin and (*b*) cells including hematopoietic stem cells, progenitor and precursor cells, immune cells, erythroid cells, stromal cells, endothelial cells, adipocytes, osteoclasts, and osteoblasts

ECM: extracellular matrix

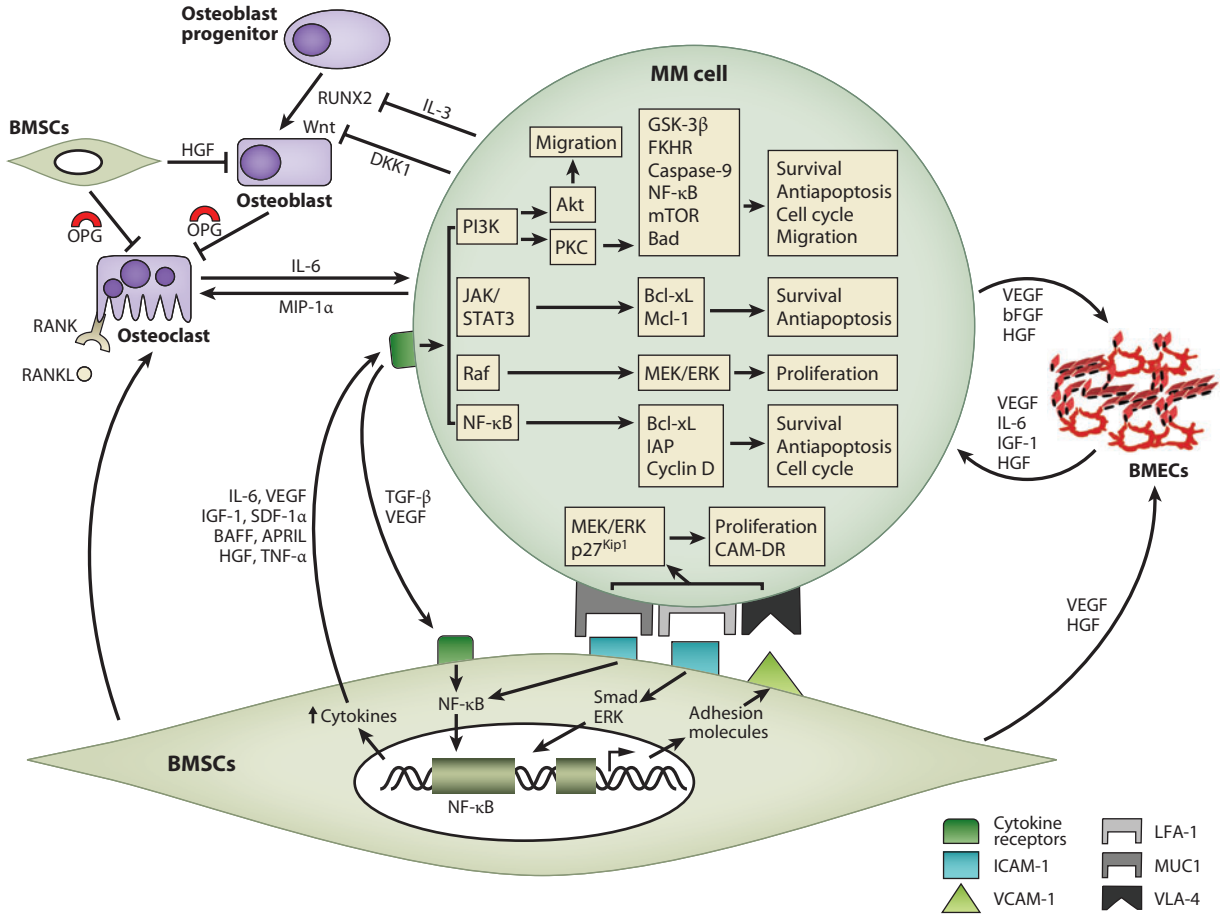

Figure 6

The role of the bone marrow (BM) microenvironment in multiple myeloma (MM) angiogenesis and bone disease. MM cells closely interact with their neighboring cells, such as BM stromal cells (BMSCs), fibroblasts, adipocytes, BM endothelial cells (BMECs), and inflammatory cells, as well as osteoblasts and osteoclasts. Abbreviations: bFGF, basic fibroblast growth factor; CAM-DR, cell adhesion–mediated drug resistance; DKK1, Dickkopf-related protein 1; GSK-3β, glycogen synthase kinase 3β; HGF, hepatocyte growth factor; ICAM-1, intercellular adhesion molecule 1; IGF, insulin-like growth factor 1; IL, interleukin; JAK/STAT3, Janus kinase/signal transducer and activator of transcription 3; LFA-1, leukocyte function–associated antigen 1; MEK/ERK, Ras/Raf/mitogen-activated protein kinase kinase/extracellular signal–regulated kinase; MIP-1α, macrophage inflammatory protein 1α; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor κB; OPG, osteoprotegerin; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; RANK, receptor activator of NF-κB; RANKL, RANK ligand; SDF-1α, SC-derived factor 1α; TGF-β, transforming growth factor β; TNF-α, tumor necrosis factor α; VCAM-1, vascular cell–adhesion molecule 1; VEGF, vascular endothelial growth factor; VLA-4, very late antigen 4. Adapted from Reference 95.

cells, adipocytes, osteoclasts, and osteoblasts). Normally, BM cells secrete paracrine factors including IL-6, insulin-like growth factor 1 (IGF-1), vascular endothelial growth factor (VEGF), B cell–activating factor, FGF, SC-derived factor 1α (SDF-1α), and TNF-α, which are further upregulated following the adhesion of MM cells to the BM milieu (86). Direct interaction between MM cells and BM cells along with secreted chemokines activates pleiotropic signaling pathways that mediate growth, survival, drug resistance, and migration of MM cells (84), as well as BM osteoclastogenesis (87) and angiogenesis (88).

Angiogenesis: the process of new blood vessel formation in the BM from preexisting vasculature through a multistep process comprising perivascular detachment of existing vessels, matrix degradation, migration of endothelial cells, and formation of a functional vascular plexus, which is supported by pericytes and basement membrane constituents

HOMING AND ADHESION OF MULTIPLE MYELOMA CELLS TO THE BONE MARROW

Homing of MM cells to the BM microenvironment is mediated by the chemokine SDF-1α and its receptor CXCR4 on MM cells. Binding of SDF-1α to CXCR4 induces motility and cytoskeletal rearrangement in MM cells; conversely, specific CXCR4 inhibitors and anti-CXCR4 antibodies inhibit migration of MM cells in vitro, which suggests that SDF-1α–CXCR4 interaction is a critical regulator of MM homing (49). Both homotypic and heterotypic adhesion of MM cells to the BM milieu is then mediated through several adhesion molecules. For instance, CD44 (a major hyaluronan), very late antigen 4 (VLA-4, CD49d), VLA-5 (CD49e), leukocyte function–associated antigen 1 (LFA-1, CD11a), neuronal cell–adhesion molecule (NCAM, CD56), intercellular adhesion molecule 1 (ICAM-1, CD54), syndecan 1 (CD138), and MPC-1 (CD49e) mediate the adhesion of MM cells to the BM. VLA-4 expressed on MM cells mediates binding to both the ECM and BMSCs through fibronectin and vascular cell–adhesion molecule 1 (VCAM-1, CD106), respectively (**Figure 6**). The binding to fibronectin upregulates $p27^{Kip1}$ and induces nuclear factor κB (NF-κB) activation in MM cells (89), which confer cell adhesion–mediated drug resistance to conventional chemotherapy (89, 90).

CD138 is a transmembrane heparan sulfate–bearing proteoglycan expressed in most MM cells. Adhesion of MM cells to ECM through the binding of CD138 to type I collagen (91) induces the expression of matrix metalloproteinase 1 (MMP-1), thereby promoting bone resorption and tumor invasion. Elevated serum-soluble CD138 correlates with increased tumor cell mass, decreased MMP-9 activity, and poor prognosis. Importantly, soluble CD138 promotes the growth of MM cells in vivo (92), and a murine/human chimeric CD138-specific antibody has anti-MM activity (93). Therefore, delineating the mechanisms whereby MM cells home and localize in the BM both provides novel biomarkers and identifies potential therapeutic strategies to overcome cell adhesion–mediated drug resistance (**Figure 6**).

MULTIPLE MYELOMA–BONE MARROW STROMAL CELL INTERACTIONS

Not only does adhesion of MM cells to BMSCs localize tumor cells in the BM niche, it also has important functional consequences. It induces NF-κB-dependent transcriptional activation and secretion of chemokines by BMSCs. Among the cytokines secreted by BMSCs is IL-6, which enhances MM cell growth, survival, drug resistance, and migration; conversely, inhibition of NF-κB activity abrogates this response (94). However, MM cells localized in the BM milieu secrete cytokines including TNF-α, transforming growth factor β (TGF-β), and VEGF, which further upregulate IL-6 secretion from BMSCs, thereby generating a strong paracrine loop between MM and BMSCs (95). Also, NF-κB mediates the expression of many adhesion molecules expressed on both MM cells and BMSCs, and activation of NF-κB by cell adhesion and cytokines such as TNF-α augments the binding of MM cells to BMSCs, which in turn induces IL-6 transcription and secretion by BMSCs (94). Within the BM milieu, binding of the TNF receptor superfamily member 5, CD40, on MM cells with its ligand CD40L on BM cells upregulates the expression of adhesion molecules (e.g., LFA-1 and VLA-4), which further enhances the adhesion of MM cells to BMSCs as well as IL-6 and VEGF secretion from BMSCs (96). Interestingly, anti-CD40 antibodies (SGN-40 or CHIR-12.12) inhibit MM cell adhesion to fibronectin and BMSCs, thereby decreasing IL-6 and VEGF secretion from BMSCs (97). This finding underscores the potential of disrupting the tropism between MM cells and their BM microenvironment as a therapeutic approach that could be useful in most MM patients, independently of the genetic makeup of the tumor cells.

In addition to NF-κB, several other signaling pathways mediate the pleiotropic proliferative and antiapoptotic response of MM cells upon interaction with the BM microenvironment. These pathways include (*a*) the phosphatidylinositol-3 kinase (PI3K)/Akt pathway, (*b*) the Ras/Raf/MAPK kinase (MEK)/extracellular signal–regulated kinase (ERK) pathway, and (*c*) the Janus kinase 2 (JAK2)/signal transducers and activators of transcription 3 (STAT3) pathway. Downstream effects upon engagement of these pathways include cytoplasmic sequestration of many transcription factors (e.g., FKHRL-1), upregulation of cell cycle–regulatory proteins (e.g., D-type cyclins) and antiapoptotic proteins (e.g., Bcl-2, Bcl-xL, Mcl-1, caspase inhibitors), and increased activity of telomerase. Importantly, all these events promote MM cell growth, survival, and migration, thereby contributing to myeloma progression and constitutive drug resistance (84, 98). Moreover, many of the growth factors secreted by MM cells and BMSCs stimulate osteoclastogenesis [e.g., IL-6, IL-1, VEGF, SDF-1α, macrophage inflammatory protein 1α (MIP-1α)] and angiogenesis (VEGF). Thus, targeting MM-BMSC interactions and related growth factors may provide the basis for the development of novel treatment strategies for MM and associated bone disease.

Moreover, genetic lesions in MM may modulate their ability to interact with the BM milieu. For instance, MM cells with a t(14;16) translocation overexpress the transcription factor MAF, which not only transactivates the cyclin D2 promoter (thereby increasing MM cell proliferation), but also upregulates β7 integrin expression (thereby enhancing tumor cell adhesion to BMSCs) (63). In contrast, multiple trisomies and cyclin D1 are underrepresented or absent in PC leukemias, which do not rely on the BM microenvironment for cell growth and survival (99). These examples highlight the biological significance of the interplay between specific genetic lesions and the signaling pathways that mediate MM-microenvironmental interactions.

MYELOMA-ASSOCIATED ANGIOGENESIS

BMECs: bone marrow endothelial cells

Angiogenesis refers to the formation of new blood vessels from preexisting vasculature. It is a multistep process comprising perivascular detachment of existing vessels, matrix degradation, migration of endothelial cells, and formation of a functional vascular plexus that is supported by pericytes and basement membrane constituents (100). Tumor angiogenesis develops through the same steps but shows a markedly increased proliferative activity of endothelial cells and has significant functional and structural differences in the vascular plexus (101). Several studies have documented the critical role of angiogenesis in MM (102).

Bone marrow endothelial cells (BMECs) secrete SDF-1α, which mediates the initial homing of MM cells to the BM stromal compartment through CXCR4. Adhesion between MM cells and BMECs then upregulates many cytokines with angiogenic activity. Angiogenesis promotes MM cell growth (*a*) by enhancing delivery of oxygen and nutrients and removing catabolites and (*b*) through the associated secretion of growth factors from endothelial cells. MM cells may also constitutively produce, as a result of oncogene activation and/or genetic mutations, factors such as VEGF, basic fibroblast growth factor (bFGF), and MMPs that stimulate BM angiogenesis (103). Conversely, BMECs secrete growth factors including VEGF, IL-6, and IGF-1, which promote MM cell growth in the BM milieu (104). Importantly, growth factors and cytokines such as VEGF and IL-8 allow MM cells to recruit new blood vessels in the BM (**Figure 6**) (105). The BMECs in these new MM-associated vessels further support MM cells through cytokines and direct adhesive interactions. Importantly, these autocrine and/or paracrine loops in the BM milieu may mediate the progression of MM. BMECs from MM patients express not only VEGF and bFGF, but also several other proangiogenic molecules including angiopontin 1, TGF-β, platelet-derived growth factor, hepatocyte growth factor (HGF), and IL-1.

The level of BM angiogenesis, as assessed by grading and/or microvessel density (MVD), is consistently increased in patients with progressive MM compared with those with inactive disease or MGUS (106). In active MM, MVD correlates with the degree of PC proliferation and infiltration and is therefore an adverse prognostic marker. Moreover, a recent analysis in newly diagnosed MM found a correlation between MVD, CD138 in blood and BM, and the level of serum HGF, which further suggests the potential utility of MVD as a new biomarker of MM progression (100, 107). Importantly, BM neovascularization can be targeted by novel agents. Thalidomide, for example, inhibits BMEC-mediated secretion of VEGF, bFGF, and HGF; BMEC proliferation; and capillarogenesis in patients with MM (108).

MYELOMA-ASSOCIATED BONE DISEASE

One of the main features of MM is the development of osteolytic bone lesions. Such lesions are caused by enhanced bone destruction, which results from asynchronous bone turnover wherein increased osteoclastic bone resorption is not accompanied by a comparable increase in bone formation (87, 109). Recent characterization of the interaction between MM cells and osteoclasts and osteoblasts has provided a better understanding of MM bone disease at the molecular level, as we discuss further below (**Figure 6**).

HYPERSTIMULATION OF OSTEOCLASTOGENESIS

Following homing to the BM, MM cells activate osteoclasts (110), which leads to bone resorption, lytic bone lesions, and/or diffuse osteopenia. BMSCs and osteoblasts regulate osteoclastogenesis by producing receptor activator of NF-κB ligand (RANKL) and osteoprotegerin (OPG), respectively (111). Importantly, RANKL expression is associated with osteoclast differentiation and is induced preferentially in immature osteoblasts (112). RANKL binds to RANK on osteoclasts, thereby stimulating their differentiation and activity; OPG functions as a decoy receptor by binding to RANKL and preventing its interaction with RANK, thereby inhibiting its osteoclastogenesis-stimulating activity (**Figure 6**) (113). Blockade of RANKL binding to RANK receptor by OPG, a soluble form of the RANK receptor, inhibits osteoclast maturation and bone reabsorption (114). In contrast, binding of MM cells through α4β1 integrin to VCAM-1 on BMSCs decreases secretion of OPG and increases expression of RANKL, thereby promoting bone reabsorption and osteolysis (115). Importantly, MM cells affect the OPG/RANKL ratio in the BM environment, thereby promoting lytic bone lesions (116).

Another mechanism that contributes to bone destruction in MM occurs through MIP-1α, which is secreted by MM cells (87). MIP-1α is a potent inducer of osteoclast formation that occurs independently of RANKL and enhances both RANKL-stimulated and IL-6-stimulated osteoclast formation (117). MIP-1α binds to CCR1 on osteoclasts and CCR5 on MM cells, thereby blocking CCR1, and CCR5 inhibits osteoclast formation and MM cell adhesion to BMSCs (118). Levels of MIP-1α in the BM plasma of patients with MM are elevated and correlate with the presence of osteolytic bone lesions. Moreover, an antisense oligonucleotide against MIP-1α decreases bone destruction, MM adherence to BMSCs, and MM tumor burden in a mouse model of MM (117).

Importantly, osteoclasts produce several factors that stimulate MM cell growth, including IL-6. This interplay among the activated osteoclasts, SCs, and osteoclast-activating factors perpetuates a vicious cycle of bone destruction. In addition, IL-6 secretion from BMSCs is triggered by MM-BMSC adhesion; it not only induces MM cell proliferation and survival but also stimulates osteoclastogenesis and contributes, along with cytokines such as IL-1β, VEGF, and HGF, to the increased bone resorption observed in MM (119). p38 MAPK in BMSCs upregulates production of IL-11, RANKL, and MIP-1α, which induce

osteoclast formation and activity. Conversely, a p38 MAPK inhibitor can suppress these cytokines, which leads to decreased MM cell proliferation and adhesion, as well as decreased osteoclastogenesis (120). Chondroitin synthase 1, which is upregulated by MM cell–osteoclast interactions, induces Notch signaling and survival of MM cells and therefore represents a novel therapeutic target (121).

In addition to osteoclast-mediated destruction of bone, some tumor cells may be able to directly degrade bone. Some human MM cells acquire the functional properties of osteoclasts and degrade bone (122). The ability of tumor cells to directly degrade bone correlates with expression of αvβ3 integrin; conversely, silencing of β3 integrin inhibits osteolysis in vitro (123). These data suggest that αvβ3 integrin on MM cells, in addition to enhancing MM cell invasiveness (124), may also facilitate osteolysis.

Hyperstimulation of osteoclasts, in addition to promoting bone destruction, also helps drive further tumor progression. Cellular contact between MM cells and osteoclasts causes release of IL-6 and osteopontin, which support MM growth (125). Osteoclasts also release angiogenic factors (including osteopontin) that, in concert with other BM factors, induce increased angiogenesis (126). This link between bone turnover and angiogenesis may be one reason that MM presents as such a highly angiogenic disease, and it may also explain the high rates of relapse and chemoresistance that are characteristic of MM.

INHIBITION OF OSTEOBLAST DIFFERENTIATION

Bone resorption is typically reversed when osteoblasts fill lytic spaces with new bone matrix. This process in MM, however, is inhibited by different factors, and decreased activity of osteoblasts also contributes to osteolytic bone lesions in MM. The formation and differentiation of osteoblastic cells from mesenchymal stem cells depend on the canonical Wnt signaling pathway (127) and require the activity and function of the transcription factor Runx2/Cbfa1 (128). Binding of VLA-4 on MM cells to VCAM-1 on osteoblast progenitors downregulates Runx2/Cbfa1 activity (129). Enhancement of Wnt signaling by lithium chloride treatment or by overexpression of Wnt3a results in bone-inhibited bone destruction and reduced tumor burden in murine models of MM (130, 131). Soluble factors expressed by MM cells, such as DKK1 and IL-3, may also contribute to the inhibitory effects of MM cells on osteoblast differentiation and Runx2/Cbfa1 activity. Interestingly, MM cells interfere with Wnt-mediated bone formation by secreting DKK1, a potent Wnt inhibitor. DKK1 is elevated in the BM and blood of MM patients with osteolytic lesions (**Figure 6**) (42). Serum DKK1 levels correlate with the extent of lytic bone lesions, and patients without bone lesions have lower DKK1 levels than do patients who have bone lesions (132). Transgenic mice that overexpress DKK1 exhibit osteopenia, whereas reduction of DKK1 expression in such mice increases bone mass (133, 134). This finding offers further support for a role of DKK1 in regulating bone loss in vivo. In addition, recombinant human DKK1 or BM plasma containing elevated levels of DKK1 inhibits the differentiation of osteoblast precursor cells in vitro (42), and elevated DKK1 levels in BM plasma and peripheral blood from patients with MM are associated with focal bone lesions. Conversely, a neutralizing anti-DKK1 antibody inhibits bone resorption and tumor cell growth in a mouse model of MM (135, 136). Although these data support targeting DKK1 to MM bone disease, this approach may be harmful to MM patients due to the potential role of the canonical Wnt pathway in promoting MM tumor growth (44).

Because expression of OPG (137) and RANKL (138) is also regulated by Wnt signaling, DKK1 may play a key role in mediating the osteolytic process in the BM microenvironment in MM. In addition, MM cell lines and patient MM cells constitutively produce another soluble Wnt inhibitor, Frizzled-related protein 2, which also significantly suppresses osteoblast differentiation (139).

Although IL-3 has been shown to stimulate osteoclast activity, a recent study reported that IL-3 also inhibits basal osteoblast formation stimulated by bone morphogenetic protein 2 without affecting cell growth, which suggests that IL-3 plays a role in the bone-destructive process in MM by inhibiting osteoblast formation (140). TGF-β from MM cells augments IL-6 secretion from BMSCs (141) and osteoblasts (142), thereby further stimulating osteoclast activity. HGF, which can directly inhibit osteoblastogenesis in vitro, is elevated and inversely correlated with bone-specific alkaline phosphatase, a marker of osteoblast activity, in sera from MM patients. Therefore, multiple cytokines mediate the imbalance of osteoblast/osteoclast activity in MM (143). Importantly, osteoblasts can be activated by proteasome inhibitors (144), suggesting that the novel agents that induce responses even in advanced MM may also induce new bone formation.

IMPACT OF BONE MARROW INTERACTIONS ON MULTIPLE MYELOMA CELL MOLECULAR PROFILES

Constitutive genetic alterations in MM cells are not the sole determinants of the biological behavior of MM cells in their local microenvironment. Instead, the molecular profiles of MM cells show a wide range of inducible alterations at the transcriptional and proteomic levels (81) that mediate the protective effects of the BM microenvironment on MM cells, enhance adhesion of MM cells to BMSCs and BM accessory cells, recruit new blood vessels to provide nutrients and growth factor support for MM cells, and/or trigger increased osteoclastic bone resorption (145). For example, the interaction of MM cells with BMSCs and BM accessory cells upregulates transcripts for cytokines such as IL-6 (146), HGF, and IGF (86); the antiapoptotic protein Mcl-1 (147); and heat shock proteins (Hsps), which regulate the conformation and function of proliferative/antiapoptotic proteins (148). This increased autocrine production of cytokines, along with paracrine cytokine production from the BMSCs, stimulates proliferative/antiapoptotic signaling cascades in MM cells. Upregulation of Mcl-1 or Hsps contributes to increased MM cell survival and drug resistance in the BM milieu. Cytokine-driven signaling events also lead to transcriptional upregulation of adhesion molecules, such as integrins and ECM proteins, and enhances the binding of MM cells to BMSCs, which in turn promotes the viability of MM cells in the BM milieu by activating antiapoptotic signaling cascades (e.g., the FAK/PI3K axis) both directly (via adhesion molecules and cell-cell contact) and indirectly (by increasing the secretion of antiapoptotic cytokines) (149, 150). These inducible molecular alterations in MM cells that interact with their microenvironment also influence MM-associated neoangiogenesis and bone resorption. Finally, constitutive chromosomal translocations, amplifications, deletions, or gene mutations in MM cells modulate the transcriptional, proteomic, and functional sequelae triggered in MM cells that interact with their local BM milieu (81). For example, IL-6 secretion is upregulated by the MM-BMSC interaction and may be particularly important in MM cells that overexpress IL-6 receptor due to amplifications in chromosome 1q21 (**Table 2**, **Figure 6**) (36). These studies suggest that oncogenomics can be used to show MM subtype–specific interactions with the BM milieu.

Interestingly, magnetic beads coated with antibodies against CD138 are broadly used to isolate normal and MM PCs for ex vivo functional and molecular studies, including GEP analysis. Considering the biological role of CD138, it will be important to learn whether its engagement during the processes of purification with anti-CD138 antibodies has any biological consequences on the cells, including in gene expression, given that GEP analysis comparing normal PCs with MM cells is performed following CD138 purification (71, 72).

SUMMARY POINTS

1. Despite recent advances in its treatment, MM remains an incurable disease, and additional therapies are urgently needed. Significant efforts to identify the molecular genetic events driving this malignancy aim to improve early detection and provide novel and more effective therapeutic targets.
2. Genetic and epigenetic studies have uncovered a high level of molecular heterogeneity of MM, which highlights the difficulties that will be faced in identifying common genetic events that could be targeted in all myeloma patients. These studies suggest a need for patient selection or customized therapy.
3. Studies on PC development emphasize that maintenance of the PC fate requires specific interplay between transcriptional factors. This knowledge is likely to provide targets for developing drugs that can block the functions of long-lived PCs and MM cells.
4. Laboratory efforts have defined the importance of interactions between MM cells and their BM microenvironment for tumor cell growth and survival, as well as the development of resistance to therapy, tumor angiogenesis, and myeloma bone disease.
5. MM is preceded by an age-progressive condition termed MGUS, and recent screening studies established that all patients who eventually developed MM had previously been diagnosed with MGUS. This stepwise evolution in MM pathogenesis provides a unique opportunity for the identification of early markers for disease progression.

FUTURE ISSUES

1. Given the molecular heterogeneity of MM cells and the critical role of the BM microenvironment for MM cell growth and survival, future efforts to design new and more effective approaches for therapy in most myeloma patients—independently of the genetic makeup of the tumor cells—should lead to strategies to disrupt the tropism between myeloma cells and their BM microenvironment.
2. The high-resolution views afforded by current genome scanning and sequencing platforms will provide unprecedented opportunities for the discovery of cancer genes of prime disease significance in MM. However, there is a critical need for the development of appropriate biological systems for functional validation (e.g., primary PC lines and xenograft mouse models as well as bona fide genetic mouse models of MM).
3. MGUS and smoldering MM patients represent groups that will benefit from biomarker development to identify the patients who are likely to progress to active myeloma. Protocol treatments directed to target molecules involved in disease progression in these high-risk patients may help prevent or delay disease progression.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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