



REVIEW

Multiple myeloma: biology of the disease

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ABSTRACT

Multiple myeloma (MM) is a clonal B-cell malignancy characterized by the aberrant expansion of plasma cells within the bone marrow, as well as at extramedullary sites. Decades of scientific research are now beginning to unravel the intricate biology that underlies the pathophysiology of MM. In particular, the roles of cellular differentiation, molecular pathogenesis, and oncogenes involved in the natural history of MM are becoming clearer. This has enabled the identification of specific cytokines, adhesion molecules, and stromal cells that affect MM cell development, disease progression, and treatment responses. This review describes our current understanding regarding the biology of MM, and how this has led to a robust pipeline of novel therapeutic agents with the potential to overcome resistance to existing MM therapies and, therefore, further improve outcomes in patients with MM.

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1. Introduction

Multiple myeloma (MM) is a relatively common clonal B-cell malignancy. The American Cancer Society estimates that 20,180 new cases of MM (11,170 in men and 9,010 in women) will be diagnosed in the United States in 2010, representing a lifetime risk of 1 in 159 (0.63%). MM accounts for 13.4% of all hematologic malignancies diagnosed, 19% of all deaths resulting from hematologic malignancies, and 2% of all cancer deaths. In 2010 there will be an estimated 10,650 deaths in the United States as a result of MM.¹

MM arises in most, if not all, cases from monoclonal gammopathy of undetermined significance (MGUS) or smoldering MM (SMM). MGUS and SMM are characterized by the presence of M-protein and bone marrow (BM) plasmacytosis, and absence of osteolytic lesions, anemia, hypercalcemia, and renal function impairment attributable to the plasma cell (PC) proliferative disorder. The risk of progression to MM or related disorders is substantially different between MGUS and SMM, about 1% per year versus 10–20% per year, respectively.¹

The causes of MGUS/SMM and MM remain largely unknown. Epidemiological studies have shown that MM is associated with older age, with a median age at diagnosis of 66 years; only 10% and 2% of patients are aged younger than 50 years and 40 years, respectively. Individuals with a first-degree relative with MM also have a 3.7-fold higher risk of developing this malignancy than those with unaffected relatives.²

Although the pathogenesis of MM has not been fully elucidated and the role of antigenic stimulation in the pathogenesis of

the disease remains controversial,³ epidemiological data from the United States have demonstrated associations between MM and agricultural workers. These studies demonstrated an increased incidence of lymphohemopoietic cancers associated with lifetime exposure to the chemical alachlor – a commonly used pesticide. There was a 5.66-fold relative risk of MM in the highest exposure category.⁴ Other occupational groups associated with the development of MM include miners, workers exposed to wood dust, and sheet-metal workers.⁵ Families with two or more such affected individuals have been reported, suggesting a possible genetic predisposition.⁶ MM has also been found to occur with a somewhat greater frequency (but less than two-fold) in farmers, paper producers, furniture manufacturers, and wood-workers. Radiation has been linked to risk of developing SMM, although a history of radiation exposure is present in only approximately 1% of patients. Additionally, in the Hiroshima and Nagasaki tumor registries there was no evidence to indicate an increased risk for MM.⁷

Although the exact causes of MM remain elusive, our understanding of the cellular events underlying its development is becoming clearer. This has been facilitated by decades of research and improved scientific techniques that have enabled dissection of the changes that occur during the pathogenesis of MGUS/SMM and MM. These changes include those that take place in the biology and molecular biology of normal B-cell development, as well as in the functioning of adhesion molecules, cytokines, and BM stromal cells (BMSCs).

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2. Biology of MM

With regard to biology, focus has been placed on two distinct aspects of MM: (a) the basis of differentiation between normal and malignant B cells; and (b) the cellular origins of MM.

2.1. Differentiation of MM cells

The stages of normal and malignant B-cell differentiation have been described using cell surface phenotype B-cell-restricted and -associated antigens.⁸ Antigenic profiles are useful to identify the stages of malignant B-cell differentiation and to categorize B-cell tumors. For example, MM cells typically express CD138 (syndecan-1), CD38, and monotypic cytoplasmic immunoglobulin (Ig) light chain. Unlike PCs in B-cell lymphomas with plasmacytic differentiation or reactive conditions, myeloma cells rarely express CD19. Most cases of MM also lack other B-cell-associated antigens, such as CD20, CD22, and CD24, although CD20 and CD45 can be positive in a subset of myeloma cases.⁸

Some cell surface antigens that are present in MM are also found on normal PCs (e.g. CD38 and PCA-1), which may suggest that the normal cellular counterpart of MM is the PC. However, some observations do not support this hypothesis. For example, several antigens have been described on the surface of MM cells which, in some cases, react with B cells at stages of differentiation earlier than the PC and also react with non-B cells.^{9–12} Normal PCs have been shown to be CD19⁺ CD56[−], but no MM cells have this phenotype. Also, the core protein of Mucin-1 (MUC-1) glycoprotein antigen is expressed only on MM cells.^{13,14} This observed heterogeneity in cell-surface phenotype has led to controversy as to the cellular origin of MM.

2.2. Cellular origins of MM

The cells that accumulate in the BM of MM patients have PC or plasmablast morphology. However, based on studies using anti-idiotypic antibodies, unique idiotype determinants can identify clones of peripheral blood lymphocytes in patients with macroglobulinemia, MM, MGUS, and chronic lymphocytic leukemia.¹⁵ The presence of idiotype determinants on cytoplasmic μ -containing pre-B cells in MM BM provided further evidence that the oncogenic event driving the development of MM may occur at the pre-B-cell stage.

Interestingly, the identification of B and T cells bearing identical idiotype determinants suggests that target cells for oncogenic transformation could be precursor cells for both B- and T-cell clones.¹⁶ Aneuploid marrow MM cells can express mRNA for cell surface proteins characteristic of myeloid, erythroid, and platelet lineages, further supporting the view that the malignant clone can extend from an early stage of differentiation.¹⁷ Moreover, monoclonal B-lineage cells in the peripheral blood of MM patients, which are late-stage B cells (i.e. low CD19 and CD20, moderate CALLA and PCA-1, with strong CD45RO antigen expression) are continuously progressing toward the PC stage.¹¹

It remains unclear, however, which cell within the malignant clone is “clonogenic” and capable of self-renewal. Some evidence suggests that pre-B and naive B cells migrate from the BM to the lymph node where antigen recognition, selection, and somatic hypermutation occur. The memory-B-cell compartment is thought to contain the cytoplasmic μ -positive precursor cell of MM, which then undergoes Ig class switching in the lymph node.¹⁸ Ig variable (V_H) gene sequence analysis found that MM tumor cells are post-follicular, with the mutated homogeneous clonal sequences indicating no continuing exposure to somatic hypermutation mechanism.¹⁹ V_H gene analysis of IgM from MM cells indicates an origin from a memory cell undergoing isotype

switch events.²⁰ In contrast, mutated heterogeneous sequences in MGUS suggest that tumor cells remain under the influence of the mutator.²¹ Chromosomal aberrations may also play a role in the development of MM, with abnormalities of 14q (the location of IgH) commonly found in MM. As proto-oncogenes are translocated to this region and are overexpressed in B-cell malignancies (such as follicular lymphoma, Burkitt lymphoma, and chronic lymphocytic leukemia), they may also play a role in the oncogenesis of MM. In addition, translocations involving switch regions indicate that the final oncogenic molecular event in MM occurs late in B-cell ontogeny.²¹ CD138[−] cells with a memory B-cell phenotype have been described as clonogenic MM “stem” cells, although this concept needs further validation.²²

Ig production by myeloma PCs is dependent on the unfolded protein response for protein production and folding. X-box binding protein 1 (XBP1) is a crucial molecule in coordinating normal PC differentiation and survival due to its role in regulating the unfolded protein response.^{23,24} In the MRC Myeloma IX study, 253 newly diagnosed patients were analyzed for baseline levels of expression of *IRE1 α* and *XBP1s*: *IRE1 α* and *XBP1* were highly expressed, and patients with low *XBP1s/u* ratios had a significantly better overall survival. In addition, *XBP1s* was an independent prognostic marker, and thalidomide was shown to have a beneficial therapeutic effect in patients with low *XBP1s/u* ratios. This study highlights the importance of XBP1 in myeloma, both as an independent prognostic marker and as a predictor of thalidomide response.²⁵

Monoclonal antibody (mAb) therapies are an active area of research in MM. CS1 has been characterized as a novel MM antigen. CS1 mRNA and protein were highly expressed in CD138-purified primary tumor cells from the majority of MM patients (more than 97%) with low levels of circulating CS1 detectable in MM patient sera; this was not seen in healthy donors. HuLuc63, a humanized anti-CS1 mAb, for treating human MM is under investigation. HuLuc63 was shown to inhibit MM cell binding to BMSCs and induced antibody-dependent cellular cytotoxicity against MM cells in a dose-dependent and CS1-specific manner.²⁶

The antitumor effect of murine/human chimeric CD138-specific mAb nBT062 conjugated with highly cytotoxic maytansinoid derivatives has been investigated against MM cells *in vitro* and *in vivo*. Anti-CD138 immunoconjugates significantly inhibited growth of MM cell lines and primary tumor cells from MM patients, without cytotoxicity against peripheral blood mononuclear cells from healthy volunteers. Importantly, nBT062-SPDB-DM4 and nBT062-SPP-DM1 significantly inhibited MM tumor growth *in vivo* and prolonged host survival in the xenograft and severe combined immunodeficient mouse model with mice bearing fetal bone grafts (SCID-hu mice).²⁷

3. Molecular pathogenesis of MM

Identification of the oncogenic events that lead to the development of malignant clones in MM is the basis of molecular biologic research. Much of the progress in this field has been based on karyotypic analyses and the identification of genetic translocations. Gene microarray profiling has also recently been utilized to characterize the changes associated with the progression from normal PCs to MGUS to MM. Data from such studies have shown that the mRNA profiles of MGUS and MM cells are similar, and distinct from that of normal PCs.²⁸ Hence, these studies not only enhance the understanding of basic pathophysiology, but may also identify novel therapeutic targets in MM.

3.1. Karyotypic analyses and genetic translocations in MM

The malignant PCs in MM are localized to the BM in close association with BMSCs. MM cells are long-lived, with a very low

labeling index (1–2%) and rearranged Ig genes that are extensively somatically hyper-mutated in a manner compatible with antigen selection.¹⁹ Thus, it appears that the crucial oncogenic events in MM cells occur either after antigen selection or do not interfere with most of the normal differentiation process involved in generating a long-lived PC. However, MM cells have a significantly lower rate of Ig secretion than normal PCs.

By conventional analyses, karyotypic abnormalities are detected in MM at a frequency of 30–50% in large studies of MM tumors. The frequency and extent of karyotypic abnormalities correlates with the stage, prognosis, and response to therapy. In this way, approximately 20% of abnormalities are present in MM patients with stage I disease, 60% in stage III patients, and >80% in patients with extramedullary tumors.^{29–31} It is noteworthy that this analysis is dependent on obtaining reliable metaphase preparations and greatly under-represents the extent of DNA alterations in these infrequently dividing cell populations.

Using interphase fluorescence *in situ* hybridization (FISH) analysis, two studies reported that at least one chromosome is trisomic in 96% and 89% of MM tumor samples, respectively.^{32,33}

Although conventional karyotypes are not routinely reported for MGUS, it appears that a substantial fraction of MGUS PCs are similarly aneuploid. By using FISH analysis, the incidence of trisomy for at least one chromosome was 43% and 53% in two studies of MGUS cells; in the former, 61% of the cells had an aneuploid DNA content by image analysis.^{32,34}

The characteristic abnormalities in MM are monosomy 13 and trisomies of chromosomes 3, 5, 7, 9, 11, 15, and 19. Non-random structural abnormalities most frequently involve chromosome 1 with no apparent locus specificity. Abnormalities affecting the 14q32 IgH locus occur in 20–40% of patients; the 11q13 Bcl-1 locus is involved in around 20% of patients, but mostly translocated to 14q32; a 13q14 interstitial deletion is present in 15% of patients; and 8q24 is present in about 10% of patients, with about half of these involved in a translocation. Similar translocations have been reported in MGUS and MM, including t(4;14)(p16.3;q32) and t(14;16)(q32;q23), without any obvious clinical or biologic correlation.³⁵

The hallmark genetic lesion in many B-lymphocyte tumors involves dysregulation of an oncogene as a consequence of a translocation involving the IgH locus (14q32.3). Less frequently, variant translocations involve one of the IgL loci (2p12 κ or 22q11 λ). From conventional karyotypic analyses, translocations involving 14q32 appear to occur in 20–40% of MM patients with an abnormal karyotype. The incidence of these translocations is significantly higher in the extramedullary phase of the disease and in cell lines, perhaps because a higher number of metaphase spreads are examined.³⁶

In approximately 30% of these translocations found in MM cells, the partner chromosomal locus is 11q13 (Bcl-1, cyclin D1), but in most cases the partner is not identified (14q32⁺). Transcriptional activation of cyclin D1 has recently been confirmed in some primary tumors, as has cyclin D3 activation associated with t(6;14)(p21;q32) translocation.^{35,37,38} By combining conventional karyotypic analysis with a comprehensive Southern blot assay – which detects translocations involving IgH switch regions – it has become apparent that most MM cell lines have IgH translocations that mainly involve IgH switch regions.²¹

Recent FISH studies have also shown that IgH gene rearrangements are present in 73% of MM patients.³⁹ The apparent oncogene dysregulated by the 4;14 translocation is the fibroblast growth factor receptor 3 (FGFR3) gene. Hence, it is possible that dysregulated expression of FGFR3, as a result of t(4;14), is caused by an FGFR3-mediated signal from FGF produced by stromal cells in the BM microenvironment.⁴⁰ The t(4;14) translocation in MM regulates both FGFR3 and a novel gene, MMSET, resulting

in IgH/MMSET hybrid transcripts.⁴¹ Ectopic expression of FGFR3 promotes MM cell proliferation and prevents apoptosis; its oncogenic potential has been tested in a murine model, in which it transformed hemopoietic cells.⁴²

Although one of the characteristics of cancer is the progressive acquisition of genetic mutations that lead to the activation of oncogenes and/or inactivation of tumor suppressor genes, the role of non-oncogenes in carcinogenesis is now being recognized. MM is addicted to a non-oncogene, the product of which – the interferon regulatory factor (IRF) 4 transcription factor – has a central role for lymphocyte activation and generation of Ig-secreting PCs during the normal immune response. Using a loss-of-function, RNA-interference-based genetic screen, IRF4 inhibition was shown to be toxic to myeloma cell lines. Furthermore, MYC was identified as a direct target of IRF4 in activated B cells and myeloma.⁴³

Although translocation (14;18) occurs at a low frequency (0–15%) in MM patients, overexpression of Bcl-2 is seen in a majority of MM patients and in MM cell lines.^{44,45} In a murine MM cell line, Bcl-XL showed a predominant role in preventing apoptosis in response to cycloheximide treatment or interleukin (IL)-6 withdrawal.⁴⁶ Similarly, overexpression of Bcl-2 or Bcl-XL could prevent apoptosis induced by IL-6 withdrawal in the B-9 IL-6-dependent cell line.⁴⁷ Mcl-1 is overexpressed in MM cells, as a result of upregulation by IL-6, and mediates potent resistance to apoptosis, whereas antisense oligonucleotide to Mcl-1 triggers apoptosis.⁴⁸ Ras mutations occur in about 39% of newly diagnosed MM patients, with the frequency of these mutations increasing with disease progression. Mutations of N- and K-ras are rarely detected in solitary plasmacytoma and MGUS, but occur more frequently in MM (9–30%) and in the majority of terminal disease or PC leukemia patients (63–70%).^{49,50} Activating mutations of the ras oncogenes may also result in growth-factor independence and suppression of apoptosis in MM.

Chromosome 13 deletions are present in over half of all cases of MM and are associated with a poor prognosis.^{33,49,50} However, considering these deletions are also associated with MGUS, their role in transformation to MM is at present undefined.^{35,39,51} Altered transcriptional regulation of genes mapping to chromosome 1 may contribute to disease progression in MM. In a study using microarray analysis on tumor cells from 532 newly diagnosed patients with MM, 70 genes with a 30% mapping to chromosome 1 ($p < 0.001$) were linked to early disease-related death; most upregulated genes mapped to chromosome 1q, and downregulated genes mapped to chromosome 1p.⁵²

Understanding of the molecular pathogenesis of MM has resulted in a new proposed classification of MM. A majority of MM tumors have chromosomal abnormalities and are broadly classified into hyperdiploid or non-hyperdiploid tumors. Nearly half of MM tumors are hyperdiploid, the remaining being categorized as non-hyperdiploid, which includes tumors which are hypodiploid, pseudodiploid, or subtetraploid. These non-hyperdiploid tumors have been associated with a poorer prognosis in MM patients. Five recurrent IgH translocations have been seen in MM, including MMSET and FGFR3 (15%), cyclin D3 (3%), cyclin D1 (15%), c-maf (5%), and MAFB (2%), accounting for a prevalence of 40%.⁵³

4. Role of adhesion molecules, cytokines, and BMSCs in MM

The biologic and molecular biologic analysis of MM is beginning to provide us with a clearer picture of how the disease develops. In addition, these analyses have begun to reveal the processes that underpin the MM disease course and patient outcomes. Two factors have been identified as having crucial roles in MM progression: (a) the adhesion molecules expressed by MM cells and their interaction with the BMSC; and (b) the effects of cytokines on the MM cells.

Table 1

Examples of cytokines involved in MM and their effect on MM cells and BMSCs.

Cytokine	Source	Effect(s)
VEGF ^{65,67,68}	MM cells	Augments MM cell growth and angiogenesis
	BMSCs	Induces MM cell migration via PKC signaling
TNF- α ⁵³	Various	Upregulates ICAM-1 and VCAM-1 expression on MM cells and BMSCs
		Induces BMSC IL-6 transcription and secretion
TGF- β ⁶⁹	MM cells	Triggers IL-6 secretion by BMSCs
		Downregulates B cells, T cells, and natural killer cells
IGF-1 ⁶⁰	BMSCs	Augments MM cell growth, survival, and drug resistance
MIP-1 α ^{70,71}	BMSCs	Osteoclast-stimulating factor in MM
BAFF ^{63,64}	BMSCs	B-cell maturation
IL-6 ^{60,66,72–76,80}	BMSCs	MM cell growth, survival, and resistance to dexamethasone-induced apoptosis
IL-15 ⁷⁷	MM cells	Induces autocrine growth
IL-21 ⁷⁸	MM cells	Induces autocrine growth

BAFF: B-cell activating factor; BMSC: bone marrow stromal cells; IGF: insulin-like growth factor; IL: interleukin; MIP: macrophage inflammatory protein; MM: multiple myeloma; TGF: transforming growth factor; TNF: tumor necrosis factor; VEGF: vascular endothelial growth factor.

4.1. Adhesion molecules

Adhesion molecules mediate both homotypic and heterotypic adhesion of tumor cells to either extracellular matrix (ECM) proteins or BMSCs and play a vital role in the pathogenesis of disease progression.⁵³ After class switching in the lymph node, adhesion molecules (e.g. CD44, VLA-4, VLA-5, LFA-1, CD56, syndecan-1, and MPC-1) mediate the homing of MM cells to the BM.^{54,55} Subsequently, MM cells bind to BMSCs and to the ECM, mediated, respectively, by VLA-4 to VCAM-1 and by syndecan to type I collagen plus VLA-4 to fibronectin. Such binding not only localizes tumor cells in the BM microenvironment, but also stimulates IL-6 transcription and secretion from BMSCs with related paracrine growth of MM cells.^{56,57}

Among the adhesion molecules expressed by MM cells, syndecan-1 is of key importance. Indeed, this protein is a multifunctional regulator of tumor cell growth and survival as well as of bone cell differentiation. Syndecan-1 is shed from the surface of most MM cells and has several roles in the pathogenesis of MM: it induces apoptosis; can inhibit the growth of MM cells; and mediates decreased osteoclast and increased osteoblast differentiation.⁵⁸ Elevated serum syndecan-1 correlates with increased tumor cell mass, decreased metalloproteinase-9 activity, and poor prognosis.^{58,59}

The effects of syndecan-1 levels on MM prognosis are thought to be a result of several pathways. For example, tumor necrosis factor (TNF)- α upregulates adhesion molecules on MM cells and BMSCs, thereby increasing binding and cell adhesion-mediated (CAM) drug resistance.⁶⁰ Matrix metalloproteinase-1 is also induced by adhesion, which favors bone resorption and tumor invasion.⁶¹ As the disease progresses, the development of PC leukemia is characterized by decreased expression of certain adhesion molecules (e.g. CD56, VLA-5, MPC-1, and syndecan-1), which in turn facilitate tumor cell mobilization. Furthermore, the acquisition of other adhesion molecules on PC leukemia cells, such as CD11b, CD44, and RHAMM, assists transit through the endothelium during egress from the BM.⁶² Importantly, adhesion molecules may play a direct role in the response of MM to therapy. Indeed, MM cells resistant to melphalan and doxorubicin typically overexpress VLA-4, which mediates adherence to ECM proteins such as fibronectin and induces CAM drug-resistance associated with upregulation of p27 in tumor cells.⁶²

Due to the central role of adhesion molecules in the pathogenesis of MM, therapeutic strategies targeting these proteins have been developed and tested in animal models. Anti-ICAM-1 antibodies, for

example, inhibit tumor development in SCID mice.⁶³ Furthermore, a model of MM in SCID-hu mice has provided an *in vivo* model for the evaluation of the homing of human MM cells to human BM ECM proteins and BMSCs, the biologic sequelae of binding, as well as for the testing of novel treatments based on interruption of this process.⁶⁴ Importantly, characterization of the mechanism whereby MM cells home to the host BM and adhere to BMSCs and ECM proteins, as well as the functional sequelae of this binding, has identified targets for novel therapies. Our previous studies, for example, have identified the adhesion molecules mediating MM cell binding to fibronectin and BMSCs, as well as the MM cell growth and survival advantage conferred by this binding.^{54,57,65}

4.2. Cytokines

BMSCs secrete numerous cytokines including IL-6, insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF), and B-cell activating factor (BAFF), which augment MM cell growth, survival, drug resistance, and migration in the BM milieu (Table 1).^{51,54,63–79} BAFF, produced by the BMSCs, specifically osteoclasts, may be of particular importance in MM. This cytokine signals through several receptors including BAFF-R, transmembrane activator calcium-modulator and cyclophilin ligand interactor (TACI), and B-cell maturation antigen (BCMA).^{67,68} The level of TACI gene expression in MM cells is associated with their dependence on the microenvironment, and this signaling cascade has a pro-survival effect on MM cells.

Other cytokines that act to promote the survival of MM cells include TNF- α , transforming growth factor (TGF)- β , macrophage inflammatory protein (MIP)-1 α , IL-15, and IL-21 (Table 1). IL-6 is the major cytokine mediating MM cell growth, survival, and resistance to dexamethasone-induced apoptosis via MAPK and PI3-K/Akt, Jak/STAT, and PI3-K/Akt signaling cascades, respectively. IL-6 is produced by adhesion of MM cells to BMSCs, which triggers its paracrine NF- κ B-dependent transcription and secretion by BMSCs.^{56,57,73,81–86}

Wnts are a family of secreted glycoproteins that bind to frizzled seven-transmembrane span receptors. Intracellularly, the Wnt signaling cascade blocks degradation of β -catenin in proteasomes, thereby leading to accumulation of β -catenin in the cytoplasm. Wnt signaling also regulates various developmental processes and can lead to malignant formation. Studies in MM have shown that the canonical Wnt signaling pathway is activated following treatment with Wnt-3a, which is associated with

the accumulation of β -catenin. Wnt-3a treatment also leads to significant morphological changes in MM cells, accompanied by rearrangement of the actin cytoskeleton.⁸⁷ Derksen and colleagues demonstrated that MM cells overexpress β -catenin, including its N-terminally unphosphorylated form, consistent with active β -catenin/T-cell factor-mediated transcription.⁸⁸ Furthermore, MM cells in BM-biopsy specimens contain detectable dickkopf-1 (DKK1), which is a negative regulator of the Wnt signaling cascade and a target of the β -catenin/TCF pathway.⁸⁹ Elevated DKK1 levels in BM plasma and peripheral blood from patients with MM have been shown to correlate with DKK1 gene-expression patterns and are associated with the presence of focal bone lesions.⁴⁵

4.3. Adhesion molecules, cytokines, and MM treatment

An important consequence of the adhesion of MM cells to the BM is a change in gene profile, which can lead to several significant biologic effects. These include upregulation of growth, survival, and drug resistance genes in tumor cells; upregulation of adhesion molecules on MM cells and BMSCs; and changes in cytokines in BMSCs using both *in vitro* and *in vivo* models of human MM in mice.^{90,91} For example, the Notch signaling pathway is induced upon the interaction of MM cells with BMSCs, which induces melphalan resistance.⁹² Other studies have shown that MM cell adhesion to fibronectin confers conventional CAM drug resistance, with the induction of p27 and G₁ cell-cycle growth arrest.⁶² Interestingly, novel agents including thalidomide and thalidomide analogs such as the IMiDs® immunomodulatory compound lenalidomide as well as the proteasome inhibitor bortezomib can target both the tumor cell and its BM microenvironment and thereby overcome CAM conventional drug resistance.^{60,93,94}

Thalidomide and lenalidomide have been shown to modulate and potentiate host immune responses against MM. IMiDs® immunomodulatory agents augment cytotoxic T-lymphocyte and NK-cell activity against MM cell lines and autologous MM cells, and are associated with increased IL-2 levels in serum.⁹⁵ Lenalidomide significantly costimulates proliferation of CD3⁺ T cells induced by CD3 ligation, immature dendritic cells, or mature dendritic cells. T-cell proliferation triggered by dendritic cells is abrogated by cytotoxic T-lymphocyte antigen (CTLA)-4-Ig. Lenalidomide overcomes the inhibitory effects of CTLA-4-Ig on Epstein–Barr-virus- and influenza-specific CD4 and CD8 T-cell responses, as measured by cytokine capture and enzyme-linked immunosorbent spot (ELISPOT) assays. Lenalidomide also triggers tyrosine phosphorylation of CD28 on T cells, followed by activation of NF- κ B.⁹⁶ Furthermore, IMiDs® immunomodulatory compounds facilitate the nuclear translocation of nuclear factor of activated T cells (NF-AT)-2 and activator protein-1 via activation of PI3-K/Akt signaling, with resultant IL-2 secretion. IMiDs® immunomodulatory agents enhance both NK-cell cytotoxicity and antibody-dependent cellular cytotoxicity induced by triggering IL-2 production from T cells.⁹⁷ Lenalidomide also inhibits VEGF and IL-6, which are known to downregulate the antigen-presenting function of dendritic cells in MM.⁹⁸ The therapeutic use of IMiDs® immunomodulatory compounds is further supported by these studies defining the molecular mechanisms whereby lenalidomide triggers NK-cell-mediated cytotoxicity against MM cells.

5. Future directions

The past two decades have witnessed major advances in molecular, cytogenetic, and genomic studies that have improved our understanding of MM pathogenesis. Ongoing and future oncogenomic studies are better elucidating MM disease biology, defining prognostic factors, and identifying potential targets and biomarkers for novel therapies. These studies will also identify

mechanisms of sensitivity versus resistance to conventional and novel MM therapies. These studies will ultimately allow for profiling of individual tumor signatures both at time of diagnosis and at relapse, thereby providing the rationale for selection of the most effective single agent or combination novel therapy.

Conflict of interest statement

Anuj Mahindra and Teru Hideshima have no relevant conflicts of interest to declare. Kenneth C. Anderson is on the Advisory Board of Celgene Corporation, Millennium, Novartis, Onyx, and Bristol-Myers Squibb.

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