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TRAF6 Activation in Multiple Myeloma: A Potential Therapeutic Target

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Abstract

Multiple myeloma (MM) is an incurable B-lymphocyte malignancy. New therapeutic options have become available during the past several years; however nearly all patients acquire resistance to currently available therapeutic agents. Mechanisms contributing to the pathogenesis and chemoresistance of MM include genetic abnormalities, chromosomal translocations, gene mutations, the interaction between MM cells and the bone marrow microenvironment, and defects in the apoptotic signaling pathways. Survival signaling pathways associated with the pathogenesis of MM and bone marrow stromal cells play crucial roles in promoting growth, survival, adhesion, immortalization, angiogenesis, and drug resistance. The receptor activator of nuclear factor-kappa B/receptor activator of nuclear factor-kappa B ligand/tumor necrosis factor receptor-associated factor (RANK/RANKL-TRAF6) signal pathway mediates osteolytic bone lesions through the activation of the NF- κ B and Janus kinase/signal transducer and activator of transcription (JNK) pathways in osteoclast precursor cells and thus contributes to the main clinical manifestations of bone disease. TRAF6 has also been identified as a ligase for Akt ubiquitination and membrane recruitment and its phosphorylation on growth factor stimulation. The inhibition of TRAF6 by silencing RNA or by decoy peptides decreases MM tumor cell proliferation and increases apoptosis as well as bone resorption. Some proteasome inhibitors and benzoxadiazole derivatives showed inhibitory effects on the activity and function of TRAF6. Overall, we propose that TRAF6 may be considered as a potential therapeutic target for the treatment of MM.

Keywords

MM; Osteolytic bone lesions; RANK/RANKL; TRAF6; TRAF6 decoy peptides

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Introduction

Multiple myeloma (MM) is a malignant neoplasm of bone marrow plasma B cells that results in increased levels of monoclonal protein and suppressed levels of other immunoglobulin proteins. It accounts for 1% of all cancers, 10% of all hematologic malignancies, and 20% of deaths related to cancers of the blood and bone marrow.^{1,2} Besides age, several risk factors are associated with MM such as sex, black race, and monoclonal gammopathy of undetermined significance.³ Despite significant advances in the treatment of MM, it still has high morbidity and mortality rates as reflected by an overall median survival of 4 to 7 years.⁴

Clinically, MM is characterized by osteolytic lesions, anemia and other cytopenias, renal failure, hypercalcemia, recurrent infection, and peripheral neuropathy. Among these conditions, bone disease is the most clinically significant clinical manifestation. It is present in up to 60% of patients with MM at the time of diagnosis and can manifest as lytic bone lesions, vertebral body compression fractures, or osteopenia/osteoporosis.^{5,6}

Therapeutic Approaches

Treatment for MM varies from the use of conventional drugs alone to the use of a combination of novel and conventional drugs. In general, combination therapies have proved more effective in preventing relapses than conventional drugs or new drugs alone. Each drug or combination of drugs is administered based on the patient's age and relapse condition.^{7,8} Autologous stem cell transplantation (ASCT) may be used in combination with induction therapies in patients younger than 65 years of age who do not present with renal, heart, lung, or liver complications. High dose dense therapy with ASCT is the standard care for eligible newly diagnosed patients with MM. For elderly patients and those not eligible for high dose density therapy and ASCT, the introduction of new drugs has improved therapeutic effects.⁹ Maintenance therapy has proved effective in delaying the progression of disease.

The use of conventional drugs to treat MM has been superseded by newer drugs that produce higher patient response rates.¹⁰ The 3 main new drugs that have been used to combat MM are bortezomib, thalidomide, and lenalidomide. Bortezomib treats myeloma with its proteasome inhibitory activity by targeting the 26S proteasome, which plays a role in protein degradation in cells.¹¹ Bortezomib also prevents the activation of NF- κ B and thus the production of interleukin (IL)-6; these effects inhibit cell proliferation and can induce apoptosis of the tumor cells. Bortezomib participates in the activation of osteoblast cells that directly combat the high level of bone resorption characteristic of MM.¹² The second-generation proteasome inhibitor, carfilzomib is a peptide ketoepoxide proteasome inhibitor and binds solely to the proteasome. This leads to greater specificity in therapeutic treatments. Carfilzomib also causes lower levels of peripheral neuropathy as a side effect than does thalidomide or lenalidomide.¹³

Thalidomide is an immunomodulatory drug (IMiD). It is believed to play an antiangiogenic role in treatment¹⁴ and prevents cytokine production. Like bortezomib, thalidomide is less effective when used alone than when used in combination with other drugs.¹⁴

Lenalidomide is a second-generation IMiD that acts like thalidomide regarding its mechanism of action but with higher efficiency. Lenalidomide rarely causes significant peripheral neuropathy but still results in venous thromboembolism if combined with dexamethasone or cyclo-phosphamide.¹⁵ The third-generation IMiD pomalidomide is associated with a higher response rate. Combination therapies combine proteasome inhibitors and IMiDs with the conventional alkylating agents, corticosteroids, or

anthracyclines. Combination therapies are more effective against the resistance of MM cancer cells than are either conventional drugs or novel drugs alone.

Other new targeted agents include inhibitors of specific targets and a range of monoclonal antibodies¹⁶ that will probably be most effective when combined with conventional and new agents.

Molecular Mechanisms of the Pathogenesis of MM and Its Resistance

Molecular Mechanisms Involved in the Pathogenesis of MM

Despite the development of effective therapies, MM remains fatal. Drug resistance develops despite the use of novel anti-MM therapies. Mechanisms contributing to the pathogenesis and chemoresistance of MM include chromosomal abnormalities, the interaction of MM cells with their bone marrow microenvironment, defects in apoptotic signaling pathways, and the specialized subpopulation of cells within the tumor (termed *myeloma cancer stem cells*) for tumor cell growth and survival.^{17,18}

The main factors that govern the pathophysiology and malignant growth of MM are genomic instability within the tumor and the interaction between myeloma cells and the bone marrow microenvironment. Chromosomal abnormalities,^{19,20} including hypodiploidy, amplifications—amp(1)(q21), translocations, mutations, and epigenetic dysregulations are found in MM and play key roles in determining tumor progression and drug resistance.^{20,21} Besides molecular alterations of plasma cells, abnormal interactions between plasma cells and bone marrow lead to the activation of signaling pathways that promote the expansion of the malignant clone, stimulate neoangiogenesis and osteoclastogenesis, and protect MM cells from apoptosis.²² Various intracellular pathways are activated through these interactions, including the Ras-Raf-mitogen-activated protein kinase (MAPK), Janus kinase–signal transducer and activator of transcription (JAK-STAT3), phosphatidylinositol 3-kinase (PI3K)-AKT, I B kinase (IKK)/NF- B, Wnt, Notch, insulin-like growth factor (IGF), and pleiotrophin signaling pathways (Figure 1).^{23,24} Interactions among these pathways lead to cell proliferation, survival, resistance to therapy, and dynamic migration and adhesion of MM cells to the bone marrow milieu.²⁵

Molecular Mechanisms of Normal Bone Remodeling and Osteolytic Bone Disease in MM

As discussed earlier, bone disease is the main cause of morbidity in patients with MM and is mainly due to the imbalance of bone remodeling in the bone microenvironment, including increased activation of osteoclasts and impairment of osteoblasts.^{26,27} In normal bone, there is a balanced remodeling sequence: first, osteoclasts resorb bone and then osteoblasts form bone at the same site (Figure 2).^{28–30} The equilibrium between bone resorption and bone formation occurs through adjustment of the ratio RANKL-osteoprotegerin (OPG) to a normal range.^{31,32}

In the bone marrow of patients with MM, the interaction between tumor cells and bone morphogenetic proteins (BMSCs) results in increased osteoclast activity and bone destruction from an asynchronous bone turnover wherein increased osteoclastic bone resorption is not accompanied by a comparable increase in bone formation (Figure 3).^{33,34} These direct interactions between MM cells and bone marrow cells promote stromal cells to secrete increased amounts of cytokines, including IL-6, tumor necrosis factor (TNF)- , transforming growth factor (TGF)- , vascular endothelial growth factor (VEGF), and other factors enhancing the growth of MM cells and inhibiting MM cell-induced apoptosis.¹⁷ MM cells produce osteoclast-activating factors, including IL-1 , TNF- , TGF- , and VEGF, which can induce stromal cells and osteoblasts to produce increased RANKL (membrane bound) with the decreased production of OPG. MM cells also increase the production of

MIP-1, further inducing osteoclast formation and enhancing adhesive interactions between myeloma cells and stromal cells.³⁴ Osteoclasts secrete matrix metalloproteinases (MMPs), TGF, IGF-1, fibroblast growth factor (FGF), bone morphogenetic proteins (BMPs), platelet-derived growth factor (PDGF) and other proteins, which contribute to the formation of osteolytic lesions and metastasis as well as MM cell proliferation and survival. Osteoblast cells and stromal cells also secrete macrophage colony-stimulating factor (M-CSF) and promote osteoclast formation through binding of this factor to the receptor C-fms on the surface of osteoclast precursor cells. Consequently, MM cells produce some factors such as IL-3 and dickkopf-related protein 1 (DKK1) that suppress osteoblast differentiation and runt-related transcription factor 2 (RUNX-2) activity, and these further enhance the imbalance between bone formation and resorption.³⁴ In addition to osteoclastogenesis, the direct interaction between MM cells and other bone marrow cells along with secreted chemokines, activate pleiotropic signaling pathways that mediate growth, survival, migration, angiogenesis, and drug resistance of MM cells.

TRAF6 Expression and Activity in MM

Tumor Necrosis Factor Receptor and Tumor Necrosis Factor Receptor–Associated Factor Families

TNF receptor (TNFR) superfamily signaling pathways that govern many diverse physiologic and cellular responses—including cellular proliferation, differentiation and apoptosis—are associated with osteolytic bone disease (OBD) in MM. Among these pathways, the receptor activator of nuclear factor-kappa B (RANK) and RANK ligand (RANKL) pathways regulate bone remodeling, mammary gland development, and lymph node organogenesis and play crucial roles in OBD.^{35,36} Ligands of the TNF family interact through a distinct set of specific receptors that lack enzymatic activity and therefore are dependent on the association of adapter molecules.³⁷

TRAFs form a family of cytoplasmic adapter proteins that mediate intracellular signal transduction of receptors of the TNF and toll-like receptor (TLR)/IL-1R (TIR) superfamilies.³⁸ The linkage of the activation of these receptors to downstream signaling events culminates in the regulation of gene transcription. TRAFs exert indispensable functions in a wide array of physiologic and pathologic processes—in particular, various aspects of adaptive and innate immunity, inflammation, and tissue homeostasis. Most of the TRAFs participate in the activation of the transcription factor NF- κ B and members of the MAPK family, including the MAPK, c-Jun N-terminal kinase (JNK), and p38 pathways.^{37,39}

TRAF6 Structure and Function

The TRAF proteins are composed primarily of 4 domains, an amino-terminal really interesting new gene (RING)-finger, multiple zinc finger repeats (TRAF Zn-finger) that are involved in effector functions (essential for the activation of downstream signaling cascades), the N (TRAF-N) (coiled-coil domain), and the C-terminal (TRAF-C) domains required for TRAF trimerization (TRAF-N) and receptor selectivity (TRAF-C) (Figure 4).⁴⁰ Of the 6 TRAFs described to date, TRAF6 shows the least homology to the prototypical TRAF domain sequence and has the most divergent TRAF-C domain.⁴¹ Furthermore, unlike other TRAFs, which only mediate signaling from the TNFR superfamily, TRAF6 also participates in the signal transduction from the IL-1R/TLR superfamily.⁴²

TRAF6 is a convergence point for many diverse signals, both upstream and downstream, and can regulate an adverse array of physiologic processes, including adaptive immunity, innate immunity, bone metabolism, and the development of several tissues, including lymph nodes, mammary glands, skin, and the central nervous system.³⁸

TRAF6 in Malignancy

Activation of NF- κ B is involved in inflammation, innate and acquired immunity, bone remodeling, generation of skin appendices, and cancer development and progression.⁴³ NF- κ B is activated in cells that become malignant tumors and in cells that constitute the tumor microenvironment. The microenvironment cells include macrophages, dendritic cells, neutrophils, mast cells, T cells, and B cells. In those types of cells, the TLR-TRAF6-NF- κ B pathway seems to play a major role. NF- κ B activation results in the production of cytokines, which in turn induce NF- κ B activation in premalignant cells, leading to expression of genes associated with abnormal growth, and ultimately in the development of many cancers. Furthermore, NF- κ B activation is involved in bone metastasis.⁴³ Activation of the RANK-TRAF6-NF- κ B pathway promotes osteoclastogenesis and releases various growth factors stored in bone, which results in the creation of a microenvironment suitable for proliferation and colonization of cancer cells within the bone. Therefore TRAF6-mediated NF- κ B activation in the RANK and TLR pathways plays a crucial role in the development and maintenance of cancer cells, especially cancers that metastasize to the bone such as breast, prostate, lung, and liver cancers.

TRAF6 Activity and Role in the Pathogenesis of MM

The RANKL/RANK Pathway Mediated by TRAF6 Is Critical to Osteoclastogenesis in MM

The structure-based analysis of TRAF6 confirmed that there are distinct differences in peptide binding to TRAF6 and to the other TRAFs, which may provide the specificity of TRAF6 and its biological function. RANK interacts with 5 adapter molecules of the TRAF family, but only TRAF6 is central to the osteoclastogenic process.³⁷ Other receptors, such as CD40, TLR4, and those recognizing IL-1 and IL-17 also bind TRAF6, but unlike RANK, they are not uniquely capable of promoting osteoclast differentiation.^{42,44} Only RANK is able to transmit specific signals leading to osteoclastogenesis.⁴³ CD40 signaling plays a crucial role in B-cell function, and IL-1R/TLRs activate inflammatory and apoptotic signaling pathways through TRAF6. The significance of this factor in RANK signaling has been clearly established based on the phenotype of TRAF6-deficient mice,^{45,46} but the functional significance of RANK interacting with TRAF1, TRAF2, TRAF3, and TRAF5 remains elusive.

TRAF6 Signal Pathways Involved in the Pathogenesis of MM Resistance and OBD

TRAF6 appears to be the dominant adapter for RANK, at least in its osteoclast-related functions, as TRAF6 knockout mice display severe osteopetrosis (abnormal thickening of the bone).⁴² Many RING domain proteins have been shown to function as E3 ubiquitin ligases that mediate polyubiquitination of target proteins, which are subsequently degraded by the 26S proteasome. The conserved RING domain in the N-terminus of TRAF6 is common to many ubiquitin E3 ligases.⁴⁷ Unlike most E3 ligases, the primary function of TRAF6 is not to target proteins for degradation but to activate downstream kinase cascades.⁴⁸ On binding to RANK, the TRAF6-ubiquitin-conjugating enzyme (Ubc13)–Ubc-like protein (Uev1A) complex catalyzes lysine 63 (Lys63)-linked polyubiquitin chains to mediate the activation of TGF- β -activated kinase 1 (TAK1), which further activates IKK in the NF- κ B pathway and phosphorylates MAPK kinase in the JNK-p38 kinase pathway and MEK in the ERK pathway,⁴⁹ thereby indirectly modulating gene transcription through NF- κ B nuclear translocation and the activation of the AP-1 transcription factor (Figure 5).

It was shown that the activation of nuclear factor of activated T cell, cytoplasmic 1 (NFATc1) is critical for RANKL-mediated osteoclast differentiation.^{50,51} NFATc1 expression is dependent on both the TRAF6 and c-Fos pathways,³⁷ but how RANKL induces the expression of c-Fos remains unclear. RANKL-induced recruitment of TRAF6

mobilizes intracellular calcium by an unknown mechanism, which results in the activation of calcineurin and the dephosphorylation of NFATc1.³⁷ After its rapid translocation into the nucleus, NFATc1 induces a number of genes involved in cell differentiation and also regulates itself in the fulfillment of osteoclastogenesis. Furthermore, RANKL also regulates cytoskeleton reorganization during osteoclast differentiation by activating PI3K through a TRAF6-Src complex.⁵² The role of the other TRAFs in RANKL signaling is not well characterized. The cytoplasmic domain of RANK could interact with TRAF1, TRAF2, TRAF3, TRAF5, and TRAF6; however only TRAF2, TRAF5, and TRAF6 are functionally competent to activate signaling pathways.³⁷ TRAF5 is at least important for acute stress-induced osteoclastogenesis and TRAF2 plays only a minor role in RANKL-induced osteoclastogenesis.⁵³

We can see from these findings that activation of NF- κ B in the osteoclast precursor cells, which is mediated through the RANK-TRAF6 pathway, is essential for the differentiation and maturation of osteoclasts and plays a critical role in resorbing bone to generate a microenvironment in which tumor cells can proliferate and colonize. In addition, the NF- κ B signaling pathway is constitutively activated in MM cells and other bone marrow cells and plays a crucial role in the pathogenesis and maintenance of MM. NF- κ B activation in the bone marrow stromal cells promotes the secretion of many NF- κ B-dependent factors, enhancing the growth of the MM cells and inhibiting apoptosis of malignant plasma cells through the TLR-TRAF6-NF- κ B pathway. Through binding to the corresponding receptors expressed on the MM cells, those cytokines can activate multiple signal pathways, including the NF- κ B pathway in MM cells, to promote survival, proliferation, migration, and drug resistance.

It has been reported that only RANK is able to transmit specific signals leading to osteoclastogenesis.⁴³ Unlike CD40, another TNF superfamily receptor, which contains a single TRAF6 binding site as well as a binding site for TRAF2, TRAF3, or TRAF5 in its cytoplasmic tail,⁵⁴ RANK contains 3 TRAF6-binding sites and 2 sites critical for binding of other TRAF family numbers.⁵⁵ Thus the RANK-TRAF6 signal is more potent than the CD40-TRAF6 signal in terms of NFATc1 activation and induction of osteoclastogenesis. In addition, RANK may harbor a specific domain that facilitates the formation of TRAF6 oligomers and amplifies TRAF6 signaling on ligand stimulation much more efficiently than does CD40.⁵⁵ An inhibitor of RANK-TRAF6 signaling that targets this specific domain could be effective in blocking bone metastasis, osteoclastogenesis, and the development of MM.

TRAF6 as a Therapeutic Target in MM

TRAF6 and Yin Yang 1

The findings above demonstrated that the NF- κ B pathway is activated by TRAF6. It has been reported that NF- κ B also induces Yin Yang 1 (YY1) transcriptionally.⁵⁶ Hence, it may be possible that TRAF6 will also be involved in the regulation of YY1 through NF- κ B as a mechanism of linkage. If this were the case, it is possible to target YY1 to inhibit the progression and resistance of MM to drugs. YY1 is a transcription factor that functions in transcriptional activation, repression, and as an initiator element-binding protein in certain instances. YY1 expression levels have been shown to be elevated in MM. Huerta-Yepez et al used three MM cell lines (RPMI-8226, U266, and MMIS) and normal bone marrow cell lines as a control in several experiments and found high YY1 expression in each of the MM lines and low expression in the healthy bone marrow cells.⁵⁷ We propose that inhibition of YY1 by a small interfering RNA (siRNA) or chemical inhibitors may inhibit, in part, TRAF6-mediated functions in MM.

The Proteasome Inhibitors and Novel Compounds

The RING E3 ligase TRAF6 participates in several signaling pathways controlling immunity, osteoclastogenesis, skin development, and brain functions. Thus, TRAF6 might represent a new target for therapeutic purposes. Inhibition of the ubiquitin ligase activity of TRAF6 could be relevant to the treatment of inflammation and cancers.⁵⁸ Accumulating evidence has shown that some proteasome inhibitors target MM, such as both bortezomib and salinosporamide A; they have been shown to inhibit osteoclast formation and prevent bone resorption by inactivating the 26S and 20S components of the proteasome complex, respectively.⁵⁹ Bortezomib treatment of MM downregulates TRAF6 expression at both the protein and mRNA levels, which leads to a reduction in osteoclast formation.⁶⁰ The specific mechanism behind the proteasome inhibitory effect of bortezomib on TRAF6 remains unknown and more research on this topic may increase the potential of developing TRAF6 as a therapeutic target against MM (Figure 6).

As such, bortezomib represents a novel generation of anticancer agents. Unfortunately, proteasome inhibitors suffer, like most other chemotherapeutic agents, from a narrow therapeutic index. Compounds targeting oncogenic E3 ligases could show a better specificity, a lower toxicity, and possibly a better potency than proteasome inhibitors. Several benzoxadiazole derivatives were shown to inhibit TRAF6 autoubiquitination in vitro ($IC_{50} < 1 \mu M$) in the presence of Uev1A and Ubc13, by enzyme-linked immunoassay and western blot experiments.^{61,62}

Chemical Inhibitors That Directly Target TRAF6 (Peptides, siRNA)

Other TRAF6 inhibitors include decoy peptides (small amino acids) and siRNAs, which restrict TRAF6 activity. The restriction of TRAF6 implies a decline in osteoclast activity. TRAF6-mediated signaling functions were inhibited by peptide interruption of the TRAF6-receptors. Chung et al et al used decoy peptides made from the joining of RANK's TRAF6-receptor sequences with the hydrophobic signal peptide from the Kaposi fibroblast growth factor.⁴⁰ The peptides show specificity for the TRAF6-binding domains. Cells that were treated with the decoy peptides demonstrated a decrease in osteoclast formation.⁶² The TRAF6 decoy peptide functions through 2 pathways. In the first pathway, a high concentration of peptides may outcompete a lower concentration of internal receptors. In the second pathway, the peptides use hydrophobic signaling to interact with the cell membranes and may thus also outcompete a lower level of TRAF6 receptors. Poblens et al have shown that the core motif (RKIPTEDEY) of TRAF6 decoy peptides (T6DP) inhibited RANKL-mediated osteoclastogenesis and bone resorption.⁶³ In contrast, TRAF2/5 decoy peptides appeared to have no impact. Thus disruption of the RANK-TRAF6 interaction may prove useful as a novel target for the development of a small-molecule therapeutic agent for the treatment of bone-related diseases.

We have evaluated different siRNAs for inhibiting the TRAF6-mediated activation of NF- κ B. The results showed that the TRAF6 C-terminal (siTRAF6C) reduced TRAF6 protein expression, substantially interfered with IL-1-induced NF- κ B and c-Jun/AP-1 activation, and significantly reduced myeloma proliferation and enhanced apoptosis in vitro.⁶⁴ Preliminary studies in our laboratory have also shown that blocking TRAF6 can also enhance the anti-MM effects of chemotherapy.⁶⁴ More importantly, marked growth inhibition with siTRAF6C was detected in vivo when these cells were implanted into the bone marrow of irradiated normal mice. Furthermore, our results also demonstrated that total endogenous c-Jun is reduced through either treatment with siTRAF6C or blocking its action using a TRAF6-dominant negative peptide (TRAF6dn).⁶⁵ C-Jun is a component of the transcription factor complex AP-1, which binds and activates transcription at TRE/AP-1 elements to stimulate osteoclast formation. TRAF6 may be an excellent target to block both

myeloma cell and osteoclast cell signaling, which are important for the survival and proliferation of MM cells as well as the formation of osteoclasts. These studies suggest that TRAF6 may be a new molecular target to block the cell signal transduction important for the survival and proliferation of MM cells.

Clinical Implications and Future Directions

The establishment of a clinically applicable TRAF6 inhibitor should provide an agent with the capability to both inhibit MM growth and prevent its most important clinical manifestation— bone loss. Moreover, downstream mediators of TRAF6 signaling, including the NF- κ B pathway, have been shown to render tumor cells resistant to a wide variety of chemotherapeutic agents. These effects can be overcome with inhibitors of NF- κ B, including proteasome inhibitors and other chemotherapeutic drugs. Previous studies in our laboratory have shown that inhibition of NF- κ B can overcome resistance to a variety of chemotherapeutic agents, including alkylating agents such as melphalan and anthracyclines such as doxorubicin. Notably, combinations of treatments that block this transcription factor with chemotherapeutic agents have proved highly effective for the treatment of patients with MM. Despite the initial efficacy of these NF- κ B inhibitors in enhancing and in some cases establishing sensitivity to chemotherapy, patients eventually acquire drug resistance. Thus the need for additional drugs that can overcome chemotherapy resistance and improve the efficacy of currently available agents is critical to improve the outcome for patients with MM. We believe that blocking TRAF6 activity will provide a new target that is capable of also overcoming chemotherapy resistance. Additional findings on the role of TRAF6 in resistance should provide support for the development of new therapeutic agents for use alone or in combination with conventional therapeutic agents for the treatment of MM as well as its associated major clinical manifestation— bone disease.

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Figure 1. Signaling Pathways Associated with Pathogenesis and Resistance of MM. Various Signaling Pathways, Including the Ras/Raf/Mitogen-Activated Protein Kinase (MAPK), Phosphatidylinositol 3-Kinase (PI3K)/Akt, Janus Kinase–Signal Transducer and Activator of Transcription (JAK-STAT), NF- B, and Wnt are Constitutively Activated in MM Cells by Various Cytokines/Growth Factors and the Interactions of MM Cells With Stromal Cells and ECM. These Pathways are Associated With the Proliferation, Survival, Migration, and Drug Resistance of MM Cells.^{17,18,23–25}

Figure 2.

Molecular Mechanism of Normal Bone Remodeling. In Normal Bone, There is a Balanced Remodeling Sequence: the Activities of the Osteoclasts and Osteoblasts are Well Balanced—the Osteoclasts Clear out the Fatigued Bone and the Osteoblasts Begin the Rebuilding of New Bone. The Osteoclast Precursor Cells are Attracted to the Remodeling Sites Followed by Fusion of Activated Preosteoclasts to Form Multinucleated Osteoclasts. Osteoclasts Resorb the Fatigued Bone. During Resorption, the Bone Releases Local Chemotactic Factors From the Osteoclasts Such as Bone Morphogenetic Protein (BMP), Transforming Growth Factor- (TGF-), and Collagen to Inactivate the Formation and Activity of Osteoclasts and Thus Induce the Maturation of Osteoblasts and Attract them to the Resorption Sites. The Osteoblasts Replace the Resorbed Bone and Eventually Form New Mineralized Bone. Many Systemic Hormones and Local Factors Play Important Roles in the Equilibrium Between Bone Resorption and Bone Formation Through Adjustment of the Receptor Activator of NF- B Ligand–Osteoprotegerin (RANKL-OPG) Ratio to a Normal Range. Most Osteotropic Factors, Such as Parathyroid Hormone (PTH), Vitamin D₃, and Thyroxine (T₄) Induce the Formation of Osteoclasts by Increasing the Expression of RANKL in The Marrow Stromal Cells and Osteoblasts. RANKL Binds the Receptor Activator of NF- B (RANK) Receptor on the Osteoclast Precursors and Induces the Formation of Osteoclasts and thus Promotes the Survival of Osteoclasts. In Addition, the Osteoblasts Produce Interleukin (IL)-6, IL-1, Prostaglandin (PG), and Colony-Stimulating Factors (CSFs), Which Induce the Formation of the Osteoclasts. In Addition to RANK, a Decoy Receptor, Osteoprotegerin (OPG), Inhibits Both the Binding of RANKL to RANK and the Differentiation and Resorption of Osteoclasts. TGF- , Insulin-like Growth Factor (IGF) and IGF-1, Fibroblast Growth Factors (FGFs), and Platelet-Derived Growth Factor (PDGF) Promote the Proliferation of Osteoblast Precursors. Type I Collagen, Osteocalcin, IGF-1, and BMP Promote the Differentiation of the Osteoblast Precursors Into Mature Forms. Runt-Related Transcription Factor 2 (RUNX-2) A is Also Critical for the Differentiation of Osteoblasts.^{28–32}

Figure 3.

Involvement of the Microenvironment in the Bone Remodeling in MM. In Patients With Multiple Myeloma (MM), Bone Resorption by the Osteoclasts is Increased and Exceeds Bone Reformation. The Bone Marrow Microenvironment Plays an Important Role in MM Bone Disease and MM Pathogenesis. MM Cells Interact with Neighboring Cells in the Bone Marrow, Such as the Osteoclasts, Osteoblasts, Bone Marrow Stromal Cells (BMSCs), Bone Marrow Endothelial Cells (BMECs), and Inflammatory Cells. Cytokines and Growth Factors are Produced and Secreted by MM and Other Cells Through Cell-Cell Contact and are Regulated Both By Autocrine and Paracrine Loops and Cell-Cell Adhesion. The Interactions Between MM Cells and Neighboring Cells Induces NF- κ B Activation in These Cells and Further Leads to an Increased Ratio of Receptor Activator of NF- κ B ligand–Osteoprotegerin (RANKL-OPG), Osteoclast Differentiation, and Bone Resorption, as well as Increased Growth, Survival, Metastasis, Angiogenesis, and Drug Resistance of MM Cells.^{7,17,18,33,34,66}

Abbreviations: HGF = hepatocyte growth factor; M-CSF = macrophage colony-stimulating factor; VCAM-1 = vascular cell adhesion molecule 1; VLA-4, very late antigen 4.

Figure 4.

Domains in the TRAF6 Protein. The TRAF6 Protein Consists of 4 Parts, Namely, a Really Interesting New Gene (RING) Finger Domain, a Cluster of 5 Zinc Fingers (Zn finger) Domain, a Coiled-Coil Domain (also Called TRAF-N) and a TRAF-C Domain. The RING Finger Domain and Zinc Fingers Domain are Essential for the Activation of Downstream Signaling Cascades. The Zinc Fingers Domain Binds to Specific DNA Sequences and the RING Domain is Believed to Function as an E3 Ubiquitin Ligase and Plays a Role in Autoubiquitination by Catalyzing Covalent Attachment of Lys63 Poly-Ub Chains to Activate Downstream Kinase Cascades Through Interaction With the E2 Ubc13. The RING Finger Domain of TRAF6 Plays an Important Role in the Formation and Activation of Multinucleated Osteoclasts and is Critical for the Full Activation of Janus Kinase (JNK) and p38. In Addition, the RING Domain Has Been Found to be Important In Autodegradation and the Induction of Apoptosis. The TRAF-C Domain Permits Self-Association (Homo- and Heterodimerization of The TRAF Proteins) and Interaction With Receptors and Other Signaling Proteins.^{40-42,67}

Figure 5.

Role of TRAF6 in RANK Signal Transduction Pathway. Receptor Activator of NF- κ B (RANK) Signaling is the Main Signal Pathway Associated With Osteolytic Bone Disease (OBD) In Multiple Myeloma (MM). TRAF6 is Critical in Regulation of RANK-Signaling Events. The N-Terminal Zinc-Binding Domain of Tumor Necrosis Factor Receptor-Associated Factor (TRAF)6, Especially the Really Interesting New Gene (RING) Domain, Appears to Mediate These Downstream Signaling Pathways. Receptor Activator of NF- κ B Ligand (RANKL) Stimulates the Lys63-Linked Polyubiquitination of TRAF6 in the Presence of the Dimeric E2 Enzyme Consisting Of Ubiquitin-Conjugating Enzyme 13 (Ubc13) and Ubiquitin-Conjugating Enzyme Variant (Uev1A). The Lys63-Linked Polyubiquitin Chain on TRAF6 Recruits Transforming Growth Factor- β -Activated Protein Kinase 1-Binding Protein (TAB) to the Complex, which Results in the Autoactivation of TGF- β -Activated Kinase 1 (TAK1) by an Unknown Mechanism. Subsequently, TAK1 Activates Downstream Kinases to Activate the Transcription Factors NF- κ B and AP-1 (Consisting of c-Fos and c-jun). TRAF6 also Mobilizes Intracellular Calcium to Activate Calcineurin, Causing the Dephosphorylation of Nuclear Factor of Activated T Cells Cytoplasmic 1 (NFATc1) and its Translocation Into the Nucleus, where, in Concert with NF- κ B and AP-1, it Leads to the Promotion of the Expression of Key Genes Associated With Osteoclast Differentiation. RANKL Also Stimulates the Src Kinase Pathway (PI3K/Akt) Through TRAF6 to Initiate Cytoskeleton Rearrangements and Actin ring Formation for the Bone-Resorbing Activity of the Osteoclast and Induces Antiapoptosis Activity of MM Cells. AKT also Plays a Key Role in the Phosphorylation of the I κ B Kinase (IKK) Complex to Activate NF- κ B.^{35,37,42,47-53,67}

Figure 6.

Targeting TRAF6 to Inhibit MM Cell Proliferation, Survival, and Reversal of Drug Resistance. Several Potential Therapeutic Approaches that are Aimed at Targeting TRAF6 in MM, Directly and Indirectly, Have Been Considered, With the Objective of Inhibiting Multiple Myeloma (MM) Cell Proliferation, Survival, Antiapoptotic Pathways, and Reversal of Drug Resistance. The Proteasome Inhibitors Bortezomib and Salinosporamide A Inhibit NF- κ B Activity and Other Pathways, Leading to the Inhibition of TRAF6 Expression and Activity. Benzoxadiazole Derivatives Inhibit TRAF6 Autoubiquitination and Inhibit Cell Proliferation In Vitro. Silencing RNA to TRAF6 Inhibits TRAF6-Mediated Downstream Survival Pathways such as the NF- κ B and MAPK Pathways and Sensitizes MM Cells to Cytotoxic Drug-Induced Apoptosis. TRAF6 Decoy Peptides Have Been shown to Compete for TRAF6 biological Activities and Can Block MM-Mediated Osteoclastogenesis and Bone Resorption. Inhibition of TRAF6 with a Dominant Negative Peptide (TRAF6dn) Inhibits Both MM Cell Growth and Osteoclast Formation and also Reduces NF- κ B Activation and c-Jun Levels.^{40,59,60,62–65}

Abbreviation: siRNA = small interfering RNA.