Multiple myeloma and bone disease: pathogenesis and current therapeutic approaches

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Abstract
Multiple myeloma is a haematologic malignancy caused by clonal expansion of malignant plasma cells and associated with bone disease and hypercalcaemia. Myeloma cells are in close proximity to sites of active bone resorption and the interactions between those cells, osteoblasts and osteoclasts, are crucial not only for the bone distraction but for the proliferation of bone marrow cells as well. Recent studies have revealed that numerous regulating factors of osteoblast and osteoclast activity interfere with the pathogenesis of multiple myeloma’s bone disease and that the understanding of the pathophysiological pathways involved is the first step towards discovering novel potential therapeutic approaches.

Hippokratia 2010; 14 (2): 76-81

Key words: multiple myeloma, bone disease, hypercalcaemia, osteoblasts, osteoclasts

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Multiple myeloma (MM) is a haematologic malignancy that is caused by clonal expansion of malignant plasma cells. It was first described in 1848 and today represents 10-15% of all malignant hematological diseases. Clinical manifestations of multiple myeloma are variable and derive either directly from the neoplastic infiltration of bone marrow (osteoporosis, pathologic fracture, bone pain, anaemia) or indirectly from aberrant function of humoral immunity and from secretion of clonal protein.

MM bone disease is associated with excessive tumor-induced, osteoclast-mediated bone destruction. During the past decades our understanding of the pathogenesis of myeloma bone disease has significantly improved. In particular, key mediators of the osteoclastic bone resorption in myeloma have been identified, including receptor activator of nuclear factor κ B ligand (RANKL), macrophage inflammatory protein-1a (MIP-1α), stromal derived factor-1 alpha (SDF-1α), transforming growth factor beta (TGF-β), dickkopf homolog 1 (Dkk1) and secreted frizzled-related proteins (sFRPs). Many of these factors seem to be disregulated in patients with MM, and probably participate in bone disease either alone or concomitantly (Figure 1).

Pathophysiology of increased osteoclast-mediated bone destruction in MM
Lymphotoxin, interleukins and tumor necrosis factor-alpha (TNF-α), represent osteoclast activating factors, secreted by myeloma cells, although it remains unclear whether these factors are also produced by accessory cells involved in cell-cell interactions. The involvement of these factors in the development of myeloma bone disease is well established in vitro; however their role in vivo remains obscure. Nowadays, it is well known that the interactions between myeloma cells and bone marrow stromal cells play a crucial role in the progression of myeloma bone disease as well as in the disequilibrium and dysregulation of the factors involved in its aetiopathogenesis.

The RANK/RANKL/OPG system
Receptor activator of nuclear factor κ B (RANK), receptor activator of nuclear factor κ B ligand (RANKL) and osteoprotegerin (OPG), represent members of the TNF receptor family that play an important role in bone metabolism affecting osteoclast formation and activity.

RANKL is a polypeptide of 217 aminoacids which is expressed on the surface of both osteoblasts and bone marrow stromal cells. The interaction between RANKL...
and RANK, a specific receptor present on osteoclast progenitors and mature osteoclasts, stimulates osteoclast proliferation and bone resorption. On the other hand, OPG is a soluble receptor of 100-110 kD, secreted by osteoblasts and bone marrow stromal cells, that exerts the exact opposite biological activity. Thus, through binding of RANKL, this molecule antagonizes the action of RANK resulting in the inhibition of osteoclast-mediated bone destruction. The role of these molecules has been confirmed in various in vivo studies where, RANK and RANKL knockout mice presented extensive osteopetrosis, while in OPG knockout mouse models severe osteoporosis with fractures was reported.

The ratio of RANKL and OPG in patients with MM is markedly disturbed, with an increase in the expression of osteoclastogenic RANKL and decrease in the production of osteoprotective factor OPG. The increase in the RANKL/OPG ratio was well established in various studies where the co-culture of bone marrow stromal cells and myeloma cells induced the RANKL expression by both cell types, while the OPG expression was concomitantly downregulated by bone marrow stromal cells only, illustrating the importance of cell-cell interactions in the pathogenesis of myeloma bone disease. In a recent study, the RANKL/OPG ratio has been correlated with markers of bone resorption, confirming its potential use as a prognostic factor of both the severity of myeloma bone disease and the survival of MM.

Macrophage Inflammatory proteins (MIPs)

Macrophage Inflammatory protein-1alpha (MIP-1α) is a chemokine produced by myeloma cells, which induces the proliferation and differentiation of osteoclasts, thus affecting bone resorption. The expression of MIP-1α in the serum of patients with MM is upregulated and correlated with both the severity of bone destruction and the overall outcome of the disease itself. MIP-1α is produced in response to several stimuli and through the interaction with several chemokine receptors, such as CCR1 and CCR5, plays its role as a mediator in myeloma bone disease. Recent findings in RANK knockout animal models have shown that the osteoclastogenic effect of MIP-1α is inhibited, suggesting that its action is potentially mediated through RANK/RANKL system.

Additionally there is growing evidence that MIP-1β, a chemokine of the Regulated on Activation Normal T cell Expressed and Secreted (RANTES) family, induces osteoclast proliferation and differentiation and represents a potent mediator of myeloma bone disease.

Vascular endothelial growth factor (VEGF)

It is well established that VEGF plays a crucial role in solid tumors’ neovascularization and that the microvessel density of bone marrow, in patients suffering from MM is correlated with both disease progression and overall outcome. Recent research findings lead to the conclusion that the secretion of this factor from myeloma cells induces osteoclast differentiation and that the blockade of VEGF expression inhibits angiogenesis and bone resorption. All these findings support the hypothesis that VEGF has a role in the pathophysiology of increased osteoclast-mediated bone destruction in MM.

Stromal derived factor-1 alpha/CXC chemokine receptor-4 (SDF-1 alpha/CXCR4)

SDF-1 alpha is a member of the CXC chemokine family and its receptor CXCR4 is expressed on various types of cells, such as malignant cells and osteoclast progenitors. The SDF-1 alpha/CXCR4 complex plays a key role in migration and differentiation of myeloma cells, while there is growing evidence that these molecules increase osteoclast activity and bone resorption. This was related to an overexpression of many genes encoding RANK, RANKL, TRAP, MMP-9, CA-II and Cathepsin-K, which are osteoclast activating factors.

Cell-cell interactions

It is well documented that cell-cell interactions play an important role in the pathogenesis of myeloma bone disease, given the fact that myeloma cells are in close proximity to sites of bone resorption. These interactions involve both bone marrow stromal cells and osteoclasts and they result in osteoclast activation, proliferation and differentiation, either directly or through the upregulation of osteoclastogenic factors such as IL-6 and RANKL. The final outcome is the development and progression of myeloma bone disease.

Pathophysiology of osteoblast inhibition

Although increased osteoclast activity and bone resorption represent the primary pathophysiological pathway in myeloma bone disease, it is well recognized that osteoblasts play a crucial role in bone formation and repair of osteolytic lesions. Thus, a reduction in bone formation due to osteoblast inhibition might be a potential mechanism which results in bone destruction. Dickkopfs (Dkks), secreted frizzled related proteins (sFRPs), IL-3, runt-related transcription factor-2 (Runx-2), hepatocyte growth factor (HGF) and transforming growth factor beta (TGF-β) represent factors which are dysregulated in patients with MM and which may regulate osteoblast function through the inhibition of their formation and differentiation.

Inhibitors of Wnt signaling pathway

The Wnt (the name comes from Wg/wingless and Int genes) signaling pathway has a crucial role in the regulation of skeletal biology and seems to be involved in the pathogenesis of diseases of altered bone mass, such as osteoporosis and MM. This pathway exerts its biological action through the activation or inhibition of various factors, such as glycogen synthase kinase-3 beta, axin, catenin-β and activated protein C and thus controls the formation, differentiation and survival of both precursors and mature osteoblasts. A great number of molecules which antagonize the
functions of Wnt signaling in the extracellular matrix have been identified, such as Dkkks, sFRPs and Wnt inhibitory factor 1 (Wif-1), resulting in the suppression of osteoblast function and the progression of myeloma bone disease\(^{15,16}\).

Dkk1 is a member of the Dickkopf family and is expressed by bone marrow stromal cells, osteoblasts and myeloma cells\(^5\). In vitro studies have demonstrated that this soluble antagonist of the Wnt signaling pathway inhibits bone formation\(^2\). Additionally, the role of this molecule was confirmed in vivo, in studies with transgenic mice overexpressing Dkk1 which developed severe osteopenia, while mutations of low density lipoprotein receptor-related protein 5 (LRP5), that reduce its affinity for Dkk1, were related to increased bone mass. Although the precise role of this molecule in humans remains to be determined, Dkk1 levels were increased in both bone marrow plasma and peripheral blood of patients suffering from MM in relation to the healthy population\(^7\). In addition, Dkk1 was demonstrated to have an inhibitory effect on osteoblast differentiation\(^4\).

Apart from Dkk1, myeloma cells secrete various Wnt signalling antagonists, such as sFRPs (sFRPs 1-4)\(^8\). It was demonstrated that sFRP3 is overexpressed by myeloma cells in patients with MM and along with Dkk1 contributes to the development of osteolytic lesions which occur in myeloma bone disease\(^9\). Furthermore, sFRP2 is also secreted by certain MM cell lines and there is evidence that this factor induces bone resorption and inhibits osteoblast differentiation\(^17\).

**TGF-β**

TGF-β holds a key role in pathophysiological mechanisms in malignant diseases and is secreted by bone matrix during osteoclast mediated bone destruction\(^7\). It represents a multifunctional growth factor which, and in the case of MM, exerts its biological action through the inhibition of osteoblast differentiation\(^4\). In addition, in vitro studies have shown that the inhibition of TGF-β signaling results in the blockade of the inhibitory action of myeloma cells on osteoblast differentiation\(^18\).

**Runx2/Cbfα1**

Runx2 represents a transcription factor, which controls skeletogenesis through the differentiation of mesenchymal stem cells to osteoblasts\(^16\). This was demonstrated with the use of animal models, where Runx2 knockout mice showed a complete lack of osteoblasts and ossification, establishing the importance of this factor in osteoblastogenesis\(^8\). In humans, the co-culture of myeloma cells with osteoblast progenitors, inhibited the formation and differentiation of osteoblasts as well as the expression of osteocalcin, alkaline phosphatase and collagen I genes, which represent biological markers of osteoblast differentiation. The effect of Runx2 on osteoblasts is mediated by interleukin-7 and various cell-cell interactions\(^9\).

**Interleukin-3 (IL-3)**

IL-3 represents a cytokine with a potential inhibitory effect on osteoblast formation and differentiation in patients with MM\(^5\). It was demonstrated, both in human and animal models, that IL-3 has an indirect effect on the differentiation of primary stromal cells to osteoblasts, while it was found that the expression of this factor in the bone marrow of patients with MM is upregulated in relation to healthy controls\(^8,19\). In addition it was found that TNF-α enhances the inhibitory effect of IL-3\(^17\). Furthermore, Lee et al, have found that this cytokine is also involved in osteoclast activation and enhances osteoclast formation in vitro\(^8\). These data suggest that IL-3 exerts a dual function, affecting both osteoclast mediated bone resorption and osteoblast bone formation.

**Hepatocyte growth factor (HGF)**

Myeloma cells secrete HGF and thus its levels are increased in the serum of patients with MM\(^1\). This increase was positively correlated with a poor outcome and negatively correlated with the level of alkaline phosphatase in the serum of the patients\(^7\). These data, in combination with recent findings which showed that HGF inhibits bone morphogenetic protein (BMP) osteoblastogenesis in vitro and suppresses Runx2 gene expression, demonstrate that this growth factor may hold a key role in the pathogenesis of myeloma bone disease as well as in the discovery of targeted pharmacotherapy in the future.

**The ubiquitin-proteasome pathway**

The ubiquitin-proteasome pathway represents a cellular system which degrades various proteins involved in the proliferation and survival processes of myeloma cells\(^20\). Current researches, both in vivo and in vitro, with the use of animal models, suggest that this pathway regulates osteoblast differentiation and osteosynthesis\(^20\). This regulation is based on the modification of the expression of Wnt signalling and transcription factor Runx2\(^2\).

**Current therapeutic approaches in myeloma bone disease**

The optimal therapy for MM bone disease and hypercalcaemia associated with cancer is the effective primary tumor reduction, making direct antitumor treatment necessary. Since MM is a disease without radical treatment, antihypercalcaemic therapy should be considered as an intermediate measure with no ultimate effect on patient’s survival\(^1,21\).

Except for saline hydration and calcitrius, there are measures that affect bone metabolism, such as bisphosphonates. Bisphosphonates are inhibitors of osteoclastic bone resorption and are considered to be effective agents in the treatment of hypercalcemia associated with cancer, osteoporosis and other situations associated with increased bone resorption\(^11\). Glucocorticoids and calcitonin can be used also in combination with bisphosphonates\(^2,21\). Although many pharmacologic agents can be used in the management of myeloma bone disease and associated...
Dexamethasone and/or chemotherapeutic agents\(^2,26\). Bortezomib is the only agent to date proved to have a survival benefit in the settings of relapsed MM\(^2,27\).

As mentioned above, preclinical studies showed that the ubiquitin-proteasome machinery regulates osteoblast differentiation and bone formation. Several clinical studies suggest that the response to Bortezomib observed in patients with MM was associated with a significant increase in alkaline phosphatase (ALP) and osteocalcin and support the hypothesis that a direct stimulatory effect on bone formation process could occur during Bortezomib treatment\(^2,28\). Additionally, Bortezomib has been shown to inhibit Dkk1 expression in bone and bone-derived cells, suggesting a novel mechanism by which the proteasome inhibitor may modulate bone formation in the bone microenvironment\(^2,29\). Thus, proteasome inhibitor Bortezomib may be effective both for the treatment of MM and the associated bone disease.

**Anti-RANKL therapy**

The increase in our understanding of the mechanisms involved in the development of destructive osteolytic bone disease and the following hypercalcaemia observed in MM leads to the discovery of novel potential therapeutic approaches. As mentioned above, the dysregulation of the RANKL/RANK/OPG system is a crucial mechanism in the development of myeloma bone disease\(^1\). This knowledge has led to the development of RANKL antagonists, such as OPG-Fc and RANKL-Fc, that inhibit the action of RANKL\(^29\). These inhibitors are specific and effective and have been used in several published preclinical studies\(^30\).

A phase 1 trial evaluated the use of AMGN-0007 as a potential therapeutic agent in the treatment of bone disease in patients with MM or breast carcinoma with confirmed lytic bone lesions. AMGN-0007 is a recombinant OPG construct that blocks differentiation and activation of osteoclasts through binding to RANKL\(^31\).

A recent study in postmenopausal women showed that AMG162 is more effective in decreasing bone turnover markers at even lower doses\(^32\). Also two potential concerns with Fc-OPG therapy are cited. The first is the generation of anti-Fc-OPG antibodies, which might react with endogenous Fc-OPG and neutralize its activity. The second concern is that the binding of Fc-OPG to TNF could inhibit the role of this factor in tumor growth\(^32\). Thus, AMG162 has been proposed as safer and more efficacious therapeutic inhibitor of RANKL.

Denosumab, a novel agent which is fully human monoclonal antibody against RANKL is under investigation in clinical trials to date. Initially, this monoclonal antibody was IgG type, known as AMG161. Because of the fact that AMG161 was shown to have a cytotoxic effect on target cells and induced the apoptosis of RANKL-producing cells (osteoblasts, T-cells, stromal cells), it was converted into a noncytotoxic IgG antibody, known as AMG162\(^29\). Studies in postmenopausal women with low bone mineral density (BMD) have shown that Denosum-
ab compared with bisphosphonates could decrease bone resorption and increase bone mineral density\textsuperscript{29}. The duration of suppression of bone turnover is dose-dependent\textsuperscript{15}. A phase 1 trial concerning patients with MM and lytic bone lesions showed that Denosumab was effective in rapid dose-dependent suppression of bone resorption, lasting at least three months and has no direct effect on osteoblast activity\textsuperscript{31}. It should be noted that bisphosphonates and anti-RANKL therapy suppress osteoclastic bone resorption, while the proteosome inhibitors increase osteoblastic bone formation. Denosumab takes precedence over bisphosphonates at certain points. Concerning the adverse events from the use of bisphosphonates, renal toxicity is a potential complication\textsuperscript{31}. A potential advantage of Denosumab is that this factor requires a short period of time to clear over, whereas bisphosphonates’ half-life time is at least five years\textsuperscript{29}. Due to this long-term bone turnover inhibition, bones are fragile and microfractures are accumulated. Another disadvantage of bisphosphonates is the inability to use them in combination with anabolic agents\textsuperscript{29}. Apart from the anti-RANKL therapy, several preclinical studies investigate numerous agents involved in bone metabolism, such as MIP-1α, CCR1, CCR5 and Dkk1\textsuperscript{26,34}.\par

Conclusions

Multiple myeloma is a hematologic malignancy caused by clonal expansion of malignant plasma cells, associated with bone disease and hypercalcemia as its primary metabolic complication. Although the pathogenetic mechanisms of disease have been clarified to a large extent, MM remains an incurable disease with low life expectancy. While there are many therapeutic approaches in myeloma bone disease and subsequent hypercalcemia, none is entirely satisfactory and effective, and the identification of novel therapeutic targets seems to be a definite and imperative need.\par

Conflict of Interest Statement

No conflict of interest was stated.

References