MULTIPLE MYELOMA

Myeloma bone disease

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Abstract
Bone destruction is a hallmark of multiple myeloma, and recent studies demonstrated a strong interdependence between tumor progression and bone resorption. Increased bone resorption as a major characteristic of multiple myeloma is caused by osteoclast activation and osteoblast inhibition (uncoupling). Myeloma cells alter the local regulation of bone metabolism by increasing the receptor activator of NF-κB ligand (RANKL) and decreasing osteoprotegerin (OPG) expression within the bone marrow microenvironment, thereby stimulating the central pathway for osteoclast formation and activation. In addition, they produce the chemokines MIP-1α, MIP-1β and SDF-1α, which also increase osteoclast activity. Furthermore, myeloma cells suppress osteoblast function by the secretion of osteoblast inhibiting factors, e.g. Dickkopf (DKK)-1. The resulting bone destruction releases several cytokines, which in turn promote myeloma cell growth. Therefore, the inhibition of bone resorption could stop this vicious circle and not only decrease myeloma bone disease, but also the tumor progression. Preclinical studies provided strong evidence that the suppression of the osteoclast activity using bisphosphonates, RANKL blockade or inhibition of MIP-1α or MIP-1β is effective both in reducing myeloma bone disease and tumor growth and therefore may offer an important treatment strategy in multiple myeloma.

Keywords: Myeloma, bone, RANKL

Introduction
Multiple myeloma is a clonal malignancy of terminally differentiated plasma cells. Skeletal complications, including bone pain, osteolytic lesions, pathological fractures and hypercalcemia, are a major cause of morbidity and are found in up to 80% of myeloma patients at presentation [1]. In contrast, these symptoms are rarely seen in other B-cell malignancies. The increased bone turnover has recently been characterized as an important facilitator of proliferation and tumor cell survival in myeloma. Several studies have given growing evidence that blocking the osteolytic process may have an antmyeloma effect. This review will give an overview of novel aspects in myeloma bone disease and show possible developments for novel targets in the therapy of bone destruction in multiple myeloma.

Osteoclast activation
Histomorphometric findings
The main principle of myeloma bone disease is an uncoupled bone remodeling with enhanced osteolytic resorption and decreased bone formation, resulting in prevailing bone destruction. Bone resorption is mediated through osteoclasts, which are derived from the granulocyte-macrophage colony-forming unit and represent differentiated, multinucleated cells. Histomorphometric analysis of bone biopsies from myeloma patients showed osteoclast accumulation only on bone-resorbing surfaces adjacent to myeloma cells, whereas osteoclasts were not increased in bone not invaded by myeloma [2]. Therefore, it has been suggested that osteoclast activity is upregulated by local osteoclast activating factors (OAFs) which are produced by either myeloma cells or cells of the microenvironment [3]. Moreover, ex vivo
cocultures of purified preosteoclasts and plasma cells from myeloma patients showed that myeloma cells recruit preosteoclasts and directly induce their differentiation into mature osteoclasts, which in turn support survival and proliferation of myeloma cells. Several OAFs have been implicated in the pathogenesis of myeloma bone disease. Recently, three major groups of factors have been identified as major osteoclast inducers in multiple myeloma: the receptor activator of NF-κB ligand (RANKL), the chemokines macrophage inflammatory protein (MIP)-1α and MIP-1β, and stromal derived factor-1α (SDF-1α).

**The RANK/RANKL/OPG system**

The receptor activator of NF-κB (RANK)/RANKL/osteoprotegerin (OPG) system has been characterized and described as the final common effector system and the central pathway of osteoclast activation. RANKL (synonym: tumor necrosis factor-related activation induced cytokine, TRANCE) is a member of the tumor necrosis factor (TNF) superfamily. It exists as a cell membrane bound isoform, a secondary soluble variant that is cleaved from the cellular form by metalloproteases and TNF-α converting enzyme (TACE), and a primary secreted isoform. Under normal conditions, RANKL is mainly produced by osteoblastic lineage cells and stromal cells. The receptor for RANKL, RANK, is expressed by osteoclast precursors and mature osteoclasts. Activation of RANK by RANKL results in differentiation, formation, fusion and survival of preosteoclasts. In addition, RANKL directly acts on mature osteoclasts, inducing actin ring formation and activating mature osteoclasts to resorb bone [4]. OPG acts as a soluble neutralizing receptor for RANKL and inhibits osteoclast differentiation and activation. It is secreted by stromal cells and other cell types including osteoblast lineage cells. The biologic effects of RANK, RANKL and OPG have been evaluated by several in vitro and in vivo studies. In animal models, unbalanced expression of these cytokines led to extreme skeletal phenotypes, for example severe osteopetrosis in RANKL knockout mice. In contrast, OPG deficient mice develop osteopenia. In humans, an abnormal RANKL/OPG ratio was found both in benign and malignant bone disease.

Recent reports suggested that the RANKL/RANK/OPG system is involved in myeloma bone disease. Myeloma cells break the local RANKL/OPG balance [5,6] by several mechanisms, they increase the availability of RANKL within the bone marrow microenvironment. Different studies have demonstrated that myeloma cells induce RANKL expression by stromal cells through direct cell to cell contact [5,7]. Whether myeloma cells directly express RANKL, has been discussed controversially. Recent data of independent studies performed by several groups showed that human bone marrow myeloma cells express RANKL. In a murine model, Oyajobi et al. demonstrated that interactions between myeloma and stromal cells lead to increased RANKL expression in both cell types. Other authors found RANKL protein and mRNA in murine myeloma cells [8]. The expression of RANKL by human bone marrow myeloma cells was recently demonstrated on protein level by immunocytochemistry [9] and flow cytometry [10]. Expression of RANKL mRNA in plasma cells purified from bone marrow aspirates of myeloma patients could be demonstrated by RT-PCR by several investigators [11–13]. In a study evaluating the clinical impact of RANKL expression, nonmyelomatous plasma cells from controls showed no or only a weak surface expression of RANKL, whereas surface RANKL could be detected on bone marrow plasma cells from all patients with multiple myeloma. According to the bone status determined by conventional radiography, multiple myeloma patients were divided into a group with osteolytic bone lesions and a group without osteolysis. Bone marrow plasma cells derived from patients with osteolytic bone lesions showed a significantly higher level of surface RANKL expression compared to plasma cells from patients without osteolysis (P < 0.01) [11]. Moreover, myeloma cells have been demonstrated to enhance RANKL expression by activated T-cells [14] and endothelial cells. Taken together, myeloma cells enhance the local RANKL availability.

In addition to the stimulatory effects on RANKL, myeloma cells decrease the OPG availability within the bone microenvironment. They lead to a down-regulation of OPG mRNA and protein secretion in osteoblasts and stromal cells. Furthermore, myeloma cells produce and shed syndecan-1 (CD 138), a transmembrane proteoglycan, that binds to the heparin-binding domain of OPG and mediates its internalization and lysosomal degradation [15]. Studies showed reduced serum OPG levels in patients with multiple myeloma, compared to normal controls, and an inverse correlation of the OPG levels with the number of radiographic osteolytic lesions [16]. The combination of these effects results in an increased RANKL/OPG ratio that favors the formation and activation of osteoclasts.

**MIP-1α and MIP-1β**

MIP-1α has been identified as another important factor responsible for osteoclastic bone resorption in myeloma. MIP-1α is a low molecular weight chemokine that belongs to the RANTES (regulated on activation normal T-cell expressed and secreted) family of chemokines. It binds to its receptors CCR1, CCR5 and CCR9 and acts as chemoattractant of phagocytes and osteoclasts. MIP-1α has been
shown to induce late stage of differentiation on human osteoclast progenitors and osteoclast formation in bone marrow cultures. Using an RNase protection assay, Choi et al. [17] identified MIP-1α as an osteoclastogenic factor in multiple myeloma. In their study, MIP-1α mRNA expression was significantly increased in patients with advanced myeloma as compared to normal controls. Moreover, MIP-1α protein levels were elevated in the bone marrow supernatants of 62% of patients with active myeloma, but only in 17% of patients with stable myeloma and not in healthy individuals. Recombinant human MIP-1α as well as bone marrow plasma from patients with myeloma induced osteoclast formation in human bone marrow cultures. This effect could be inhibited by a neutralizing antibody against MIP-1α or transfection of myeloma cells with an antisense construct to MIP-1α. Abe et al. showed that both MIP-1α and MIP-1β are produced and secreted by myeloma cells, and that the secretion of MIP-1 correlates with the ability of myeloma cells to enhance osteoclastic bone resorption [18]. Antibodies against MIP-1α and MIP-1β or their receptor CCR5 could block these effects. The level of MIP-1α expression or secretion was correlated with the severity of myeloma bone disease by several authors.

There is evidence that the effects of MIP-1α are dependent on the RANKL pathway. Both MIP-1α and MIP-1β enhance RANKL expression in stromal cells. In a murine model of myeloma, injection of recombinant MIP-1α produced a strong increase in osteoclast formation in normal mice, but not in RANKL/OPG animals [19].

Other factors

Before the characterization of RANKL and MIP-1α as osteoclast inducing factors, several cytokines have been implicated in the pathogenesis of myeloma bone disease. These factors, e.g. lymphotoxin, TNFα, IL-1β, IL-6 have been found to be overproduced in some myeloma patients and therefore were discussed as potential OAFs. However, currently they can not be regarded as main inducers of osteoclast activation in multiple myeloma.

A recent study by Zannettino et al. investigated the role of the SDF-1α in the pathogenesis of myeloma bone disease [20]. SDF-1α, a chemokine highly expressed by bone vascular endothelial and marrow stromal cells, increases the recruitment and migration of osteoclast precursors by inducing matrix metalloproteinase-9 (MMP-9) activity. SDF-1α acts through binding to its receptor CXCR4, which is expressed on leukocytes, mature dendritic cells and osteoclast precursors. Zannettino et al. found that myeloma cells produce SDF-1α protein. In their study, myeloma patients exhibited elevated plasma levels of SDF-1α as compared to controls, and the level of SDF-1α positively correlated with the presence of bone lesions on radiology. In an in vitro osteoclast-potentiating culture system, SDF-1α increased osteoclast motility and bone-resorbing activity.

Osteoblast inhibition

In contrast to bone metastases in other malignancies, multiple myeloma causes bone destruction without a propositional osteoblastic reaction, resulting in an uncoupling of the normal bone remodelling sequence. The inability of antiresorptive drugs to repair lytic bone lesions suggests that impairment of osteoblast function is an important factor in myeloma bone disease. Serum concentrations of osteocalcin, which reflect the osteoblastic activity, are reduced in patients with advanced disease. Histomorphometric analysis of bone biopsies from patients with overt myeloma showed a reduced number and activity of osteoblasts on bone surfaces adjacent to myeloma cells [2]. In vitro studies revealed that myeloma cells affect the growth and function of human osteoblast-like cells. Both osteoblast growth and function are inhibited when cultured in medium conditioned by myeloma cells, suggesting that this effect is due to osteoblast inhibiting factors that are secreted by myeloma cells [21]. However, the responsible factors remained unclear.

A recent study suggested that Dickkopf (DKK)-1 could be one of these factors [22]. DKK-1 is an inhibitor of the Wnt signaling pathway, which represents a major signaling pathway in osteoblasts. Wnt glycoproteins bind to the Wnt receptor and its coreceptors LRP5/LRP6 and inhibit the degradation of β-catenin, thus leading to its cytoplasmatic accumulation, translocation into the nucleus and stimulation of expression of osteoblastic target genes. In the absence of a Wnt signal, cytoplasmatic β-catenin is phosphorylated and degraded by the proteasome. Extracellular Wnt antagonists prevent ligand–receptor interactions and can be divided into two functional classes. Members of the secreted frizzled-related protein (sFRP) class, for example sFRP3 (synonym FrzB), bind to Wnt proteins, whereas members of the DKK family bind to the LRP5/LRP6 component of the Wnt receptor complex. Using gene-expression profiles of myeloma patients, Tian et al. found an overexpression of the DKK-1 gene in multiple myeloma patients with focal bone lesions.

In addition to this mechanism, malignant plasma cells are able to induce osteoblast apoptosis. Silvestris et al. found a significantly increased expression of Fas ligand (Fas-L) and tumor-necrosis-factor-related apoptosis inducing ligand (TRAIL) in myeloma cells and an overexpression of Fas and death receptor (DR) 4/5 by osteoblastic lineage cells obtained from patients with extensive osteolytic lesions [23]. Further research on the interaction between myeloma cells and...
osteoblasts is needed in order to understand the mechanism of osteoblast inhibition and identify possible therapeutic targets in the treatment of myeloma bone disease.

**Evaluation of bone disease and biochemical markers**

Evaluation of myeloma bone disease requires conventional radiography including X-ray scans of the skull, cervical, thoracic and lumbar spine, ribs, proximal humeri and femora, and pelvis. The more sensitive technique is magnetic resonance imaging (MRI) and can show pathologic results even in patients with normal X-ray scans. However, histomorphometric studies have demonstrated that increased bone resorption can be present even in the absence of radiographic abnormalities. New laboratory parameters that reflect bone metabolism may help to overcome the diagnostic problems in estimating the activity of bone disease.

Amino-terminal collagen type-I telopeptide (NTx) in urine and carboxy-terminal telopeptide of type-I collagen (ICTP) in serum reflect the osteoclastic activity and bone destruction. In myeloma, ICTP correlates with bone resorption and has a prognostic value. As shown by Jakob et al. serum ICTP levels differ significantly between MGUS and myeloma and increase parallel to the myeloma stage according to Durie and Salmon [24]. Another study on myeloma patients demonstrated that serum levels of ICTP were elevated in patients with abnormal bone MRI but lacking lytic bone lesions in conventional radiography [25]. Tartrate-resistant acid phosphatase isozyme-5b (TRACP-5b) was described as another novel parameter reflecting osteoclast activity. Terpos et al. found increased TRACP-5b serum levels in myeloma patients, and the levels were associated with the radiographically assessed severity of bone disease [26].

**Treatment approaches**

Several in vitro and in vivo experiments as well as clinical observations have shown the importance of the positive feedback between myeloma progression and bone resorption for sustaining the disease process [27,28]. The close association between bone resorptive activity and myeloma progression has been highlighted in an experiment using C57BL/KaLwRij mice. In these animals, ovariectomy induced an accelerated bone remodelling, resulting in reduced trabecular bone volume and increased osteoclast number. Non-ovariectomized mice were used as control. Injection of 5T2MM myeloma cells lead to bone disease in both groups with an increased tumor growth and earlier development of osteolytic lesions in ovariectomized mice, thereby confirming the interdependence of osteoclasts and myeloma cells. Therefore, targeting the osteoclast induced bone disease might interrupt the feedback loop and have an additional antimyeloma effect.

**Bisphosphonates**

Bisphosphonates are currently the most widely used class of antiresorptive drugs in the treatment of myeloma bone disease. They selectively concentrate at the boundary between osteoclast and bone resorption surface. Their main effect is the suppression of osteoclast activity and function. Bisphosphonates are based on a P–C–P structure similar to endogenous pyrophosphate. Two side chains are attached to the carbon, influencing the antiresorptive potency. Nitrogen containing aminobisphosphonates (e.g. pamidronate, ibandronate, alendronate and zoledronic acid) are more potent than the first generation bisphosphonates (e.g. clodronate and etidronate). Several large, randomized, placebo-controlled clinical trials have proved the efficacy of bisphosphonates, i.e. clodronate p.o., pamidronate i.v. and zoledronic acid, in the therapy of myeloma bone disease [29]. Currently, the most potent bisphosphonate is zoledronic acid. Preclinical studies as well as randomized clinical trials (RCTs) showed that zoledronic acid had a superior effect over pamidronate in the treatment of hypercalcemia of malignancy [30]. Long-term follow-up data confirm that zoledronic acid was more effective than pamidronate in reducing the risk of skeletal complications in patients with bone metastases from breast carcinoma and was of similar efficacy in patients with multiple myeloma [31].

Although the main effect of bisphosphonates is the suppression of osteoclast activity, other mechanisms may add to the effects of aminobisphosphonates in multiple myeloma.

**RANKL antagonists**

After the identification of the RANK/ RANKL/ OPG system as the final effector system of osteoclast activation, systemic RANKL blockade has been evaluated in animal models and first clinical trials, using OPG, OPG-Fc fusion protein or RANK-Fc. Treatment of myelomatous SCID-hu hosts with RANK-Fc not only reduced osteoclast formation and myeloma-induced bone resorption, but also resulted in a sustained suppression of paraprotein levels by more than 80%, reflecting a reduced myeloma cell burden. Moreover, studies in the 5T2MM model showed that RANKL blockade by OPG-Fc caused not only inhibition of development of osteolytic bone lesions, but as well a decreased tumor burden and a significant increase in time to morbidity.
The treatment was associated with a decreased number of osteoclasts but had no effect on apoptosis and proliferation of 5T33MM cells in vitro, indicating that the antmyeloma effect of RANKL inhibitors is related to inhibition of osteoclast activity.

In humans, trials with an OPG-Fc fusion protein, with a recombinant osteoprotegerin construct and with a human monoclonal antibody to RANKL were performed. These preliminary observations indicate that targeting the RANK/RANKL/OPG system may inhibit the development of myeloma bone disease and also decreases myeloma growth and may therefore offer a new treatment strategy in multiple myeloma.

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References


