Wnt Antagonism in Multiple Myeloma: A Potential Cause of Uncoupled Bone Remodeling

Roger N. Pearse

Abstract  Bone disease in patients with multiple myeloma (MM) is characterized by uncoupled bone remodeling, evident as enhanced osteolytic resorption and decreased rather than increased bone formation. MM-triggered osteolysis follows deregulation of the receptor activator of nuclear factor κB ligand (RANKL)/osteoprotegerin cytokine axis. Inhibition of bone formation may result from the ability of MM to inhibit the function of Wnts, secreted glycoproteins critical to osteoblast development. Recent studies show how these processes may be linked.

Uncoupled Bone Remodeling in Myeloma

In contrast to bone metastases in other malignancies, multiple myeloma (MM) causes bone destruction without reciprocal osteoblast activation (1). Although the recruitment of both osteoclasts and osteoblasts is an early marker of MM marrow infiltration, histomorphometric analysis of bone biopsy specimens from patients with advanced MM show osteoclast accumulation at bone-resorbing surfaces with no evidence of bone regeneration within the skeletal lesions (2–4). Consistent with this histologic picture of osteoclast activation and osteoblast inhibition, patients with MM frequently exhibit increased levels of bone resorption markers, including the carboxyl-terminal telopeptide of type I collagen and tartrate-resistant acid phosphatase, without an increase in osteocalcin and alkaline phosphatase, biochemical indicators of osteoid production (5–8).

Osteoclast Activation by Myeloma: Deregulation of the RANKL/Osteoprotegerin Cytokine Axis

Osteoclast activity is stimulated by osteoclast-activating factors (OAFs) produced by both MM cells and cells of the microenvironment in response to MM infiltration (9). A number of OAFs contribute to MM-triggered osteoclastogenesis, including tumor necrosis factor (TNF), interleukin (IL)-1, IL-6, IL-11, and the chemokines macrophage inflammatory protein-1α, macrophage inflammatory protein-1β, and stromal derived factor-1α (10, 11). However, the critical event in MM-triggered osteoclastogenesis seems to be the deregulation of the RANKL/osteoprotegerin cytokine axis.

RANKL (receptor activator of nuclear factor κB ligand, TRANCE (TNF-related activation-induced cytokine), osteoprotegerin ligand, osteoclast differentiation factor, and TNF super family member 11) and its decoy receptor osteoprotegerin (osteoclast inhibitory factor and TNF receptor super family member 11b) control the generation of activated osteoclasts from monocyctic precursors (12–18). RANKL is expressed on the surface of activated T cells, marrow stromal cells, and osteoblasts as a 45-kDa transmembrane protein, and in solution as a 31-kDa product of metalloproteinase cleavage (19–21). RANKL triggers the development and activation of osteoclasts by binding to its functional receptor, RANK (receptor activator of nuclear factor κB, TNF receptor super family member 11a), expressed on osteoclasts and their precursors as an integral membrane protein (19, 22). Osteoprotegerin is secreted by stromal cells, including osteoblasts, as a soluble 110 kDa disulfide-linked homodimer (15, 23). Mice that lack either RANKL or RANK, or that overexpress osteoprotegerin, develop osteopetrosis due to decreased osteoclast activity (15, 17, 18, 22). Conversely, mice that lack osteoprotegerin exhibit profound osteoporosis as a consequence of unopposed RANKL activity (16, 24).

MM triggers the increased expression of RANKL and decreased expression of its inhibitor osteoprotegerin (25, 26). This deregulation of RANKL/osteoprotegerin homeostasis is evident within the bone marrow (BM) microenvironment and is reflected systemically as increased soluble RANKL and decreased osteoprotegerin within the plasma of patients with MM (27–29). The importance of this deregulation is highlighted by (a) the strong negative prognostic significance of high soluble RANKL-low osteoprotegerin plasma concentrations and (b) the ability of RANKL antagonists to block both MM-associated osteolysis and MM tumor progression (25, 28, 30–32).

A number of MM-elaborated cytokines, previously characterized as MM-associated osteoclast-activating factors, trigger RANKL expression by BM stroma, and are likely responsible for the increased expression of RANKL observed in MM (Table 1). The mechanisms behind the reduced expression of osteoprotegerin are less clear. At the protein level, CD138 (syndecan-1), a proteoglycan typically expressed at high levels on the surface...
et al. and Glass et al., discussed below, provide a rationale to uncertain. Similarly, the basis for reduced osteoprotegerin capable of inhibiting RANKL. Thus, the biological significance (33, 34). However, syndecan-bound osteoprotegerin remains interact with the heparin-binding domain of osteoprotegerin of normal and malignant plasma cells, has been shown to interact with the heparin-binding domain of osteoprotegerin (33, 34). However, syndecan-bound osteoprotegerin remains capable of inhibiting RANKL. Thus, the biological significance of osteoprotegerin binding by MM-elaborated syndecan is uncertain. Similarly, the basis for reduced osteoprotegerin transcription in MM is unknown. However, work by Holmen et al. and Glass et al., discussed below, provide a rationale to suggest that inhibition of Wnt signaling may be responsible (35, 36).

### Table 1. Cytokine regulation of RANKL/osteoprotegerin expression

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>RANKL expression</th>
<th>Osteoprotegerin expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostaglandin E₂</td>
<td>↑(mOB)</td>
<td>↓(mOB)</td>
</tr>
<tr>
<td>PTH</td>
<td>↑(mOB)</td>
<td>↓(mOB)</td>
</tr>
<tr>
<td>Cortisol</td>
<td>↑(mMS, hFOB)</td>
<td>↓(mOB)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>↑(hMS, MG-63)</td>
<td>↑(hFOB)</td>
</tr>
<tr>
<td>IL-11</td>
<td>↑(mOB)</td>
<td>↑(hFOB)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>↑(hMS, MG-63)</td>
<td>↑(hFOB)</td>
</tr>
<tr>
<td>Basic fibroblast growth factor</td>
<td>↑(mOB), ↓(ST-2, mOB)</td>
<td>↓(mOB)</td>
</tr>
<tr>
<td>Vitamin D₃</td>
<td>↑(mOB, ST-2)</td>
<td>↓(mOB)</td>
</tr>
<tr>
<td>IL-7</td>
<td>↑(Thymocytes)</td>
<td>↓(mOB)</td>
</tr>
<tr>
<td>IL-17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1α</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transforming growth factor-β</td>
<td>↓(ST-2)</td>
<td>↑(mOB)</td>
</tr>
<tr>
<td>Estradiol</td>
<td>no Δ (hFOB, hOB)</td>
<td></td>
</tr>
<tr>
<td>CaCl₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-β</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD40L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone morphogenic protein 2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**BOLDFACE**: Changes in expression that mimic those seen in MM: increased RANKL or decreased osteoprotegerin. Experimental models used to show changes in expression are in parentheses.

Abbreviations: mOB, primary murine osteoblasts; mMS, primary murine bone marrow stroma; hFOB, primary human fetal osteoblasts; hOB, primary human osteoblasts; hMS, primary human bone marrow stroma; ST-2, immortalized murine marrow fibroblasts; MG-63, immortalized human osteosarcoma; IMR-90, immortalized human fetal lung fibroblast; MC3T3, immortalized murine embryonic fibroblast; DC, primary murine dendritic cells.

of normal and malignant plasma cells, has been shown to interact with the heparin-binding domain of osteoprotegerin (33, 34). However, syndecan-bound osteoprotegerin remains capable of inhibiting RANKL. Thus, the biological significance of osteoprotegerin binding by MM-elaborated syndecan is uncertain. Similarly, the basis for reduced osteoprotegerin transcription in MM is unknown. However, work by Holmen et al. and Glass et al., discussed below, provide a rationale to suggest that inhibition of Wnt signaling may be responsible (35, 36).

### Osteoblast Inhibition by MM: Secretion of Wnt Antagonists

Recent reports document production of soluble inhibitors of Wingless-type (Wnt) ligands by MM (37–39). These reports also present in vitro evidence to suggest that Wnt inhibition is at least partially responsible for MM bone disease (Fig. 1). The strength of this hypothesis is bolstered by multiple lines of evidence showing a critical role for Wnt signaling in bone formation. In vitro, Wnts cooperate with bone morphogenetic proteins to stimulate osteoblast differentiation and function (40, 41). In vivo, Wnts regulate the fate of mesenchymal precursors by determining the commitment to a chondroblast or osteoblast lineage (42–45). Genetic ablation of canonical Wnt signaling in the developing mesenchyme results in chondrocyte accumulation at the expense of osteoblast development. Conversely, constitutive activation of Wnt signaling in the same mesenchymal precursors inhibits chondrocyte differentiation and promotes bone formation. Clinically, a loss of function mutation in the Wnt coreceptor, low-density lipoprotein receptor–related protein 5 (LRP5), is the cause of the osteoporosis-pseudoglioma syndrome (46). By the same token, a gain of function mutation (G171V) in LRP5 results in a familial syndrome characterized by increased osteoblast activity and abnormally high bone mass (47, 48).

Two families of soluble Wnt antagonists have been described. Members of the secreted Frizzled-related protein (sFRP) class bind directly to Wnts to prevent receptor engagement, whereas members of the DKK (dickkopf) family bind to Wnt coreceptors LRP5/6 to block the activation of the Wnt receptor complex (Fig. 1; ref. 49). Evaluating 218 primary samples, Tian et al. (38) discovered a significant correlation between MM-associated bone destruction and the expression of DKK1. Tian et al. also showed the abilities of both recombinant human DKK1 and BM plasma taken from MM patients with high DKK1 levels to block the production of alkaline phosphatase by C2C12 mesenchymal cells treated with bone morphogenic protein 2. They also showed that anti-DKK1 antibodies could neutralize the inhibitory effect of BM plasma on C2C12 cells. However, DKK1 was not found in every case of MM bone disease and was noticeably absent from MM clones exhibiting morphologic features suggestive of aggressive behavior. This result predicts additional mediators of osteoblast dysfunction. An earlier screen to distinguish MM-associated transcripts had identified a second Wnt antagonist, sFRP-3 (Frizzled-B; ref. 37). However, sFRP-3 expression has yet to be associated with MM bone disease. Evaluating a limited set of MM cell lines and primary MM samples, Oshima et al. (39) observed the expression of a third Wnt antagonist, sFRP-2. In vitro, recombinant murine sFRP-2 or MM cell line–conditioned medium blocked the production of alkaline phosphatase and the development of mineralized nodules by either MC3T3-E1 or human BM-derived mesenchymal cells treated with bone morphogenic protein 2. Anti-sFRP-2 antibodies neutralized these effects.

These results suggest that Wnt antagonism may be central to the osteoblast defect in MM: DKK1 mediating the osteoblast
inhibition seen in cases of good prognosis MM, and SFRP-2 mediating osteoblast inhibition in clinically aggressive MM.

Additional mechanisms may contribute to the osteoblast defect in MM. Direct contact with MM has been shown to inhibit the generation of osteoblasts from mesenchymal progenitors, possibly through inhibition of Runx2/Cbfα1 (51). MM cells frequently express CD56, secrete IL-3 and IGF-BP4, shed CD126, and trigger stromal production of IL-6, all of which can inhibit osteoblast function (52–56). In addition, MM cells can trigger osteoblast apoptosis through the secretion of Fas ligand and TNF-related apoptosis-inducing ligand (57). However, the relative contribution of each of these mechanisms to MM-associated bone disease is unclear.

**Wnt Regulation of Osteoprotegerin Expression**

Osteoblasts maintain skeletal homeostasis by matching bone resorption with bone formation. Osteoblasts control osteoclastogenesis through balanced expression of RANKL and osteoprotegerin (58). Recent articles by Glass et al. and Holmen et al. describe the participation of Wnt in regulating this balance (35, 36). The authors show that reduced Wnt signaling leads to increased expression of RANKL, decreased expression of osteoprotegerin, heightened osteoclast activity, and osteoporosis—a picture similar to that seen in MM. Given the additional importance of Wnts to osteoblast development, these studies suggest that Wnt antagonism may underlie the effects of MM on both osteoblasts and osteoclasts: a common mechanism to explain the uncoupled bone remodeling seen in MM.

To investigate the role of Wnts in skeletal biology, both Glass et al. and Holmen et al. generated transgenic mice in which mature osteoblasts are either hyporesponsive or hyperresponsive to Wnts. Because of the presence of multiple Wnt ligands (19 in humans) and receptors (10 Frizzled receptors and 2 LRP coreceptors in humans), the authors chose to target two downstream mediators of canonical Wnt signaling: β-catenin, a positive mediator of Wnt signaling, and the adenomatous polyposis coli protein (APC), a negative regulator of Wnt signaling (Fig. 1; ref. 49). β-Catenin is the nexus through which canonical Wnt signaling facilitates the transcription of genes that are regulated by lymphoid enhancer-binding factor 1/T cell–specific transcription factor (LEF/TCF), APC is part of a multiprotein complex that targets β-catenin for degradation. In the absence of Wnt signaling, APC, Axin, and glycogen synthase kinase-3β form a complex that phosphorylates β-catenin at residues S33, S37, and T41. Once phosphorylated, β-catenin is transported to the proteosome for degradation. The binding of Wnt glycoproteins to the Frizzled/LRP receptor complex triggers the phosphorylation of LRP by the serine/threonine kinase CK1ν. Phosphorylated LRP binds Axin, which inhibits the APC/Axin/glycogen synthase kinase complex, thereby preventing the degradation of β-catenin. As a result, β-catenin is allowed to translocate to the nucleus, where it associates with LEF/TCF to facilitate the transcription of their target genes.

To reduce Wnt signaling in mature osteoblasts, both groups targeted β-catenin using a murine transgenic line in which the β-catenin coding sequence is flanked with loxP sequences. To specifically target β-catenin in differentiated osteoblasts, these mice were mated to mice expressing Cre recombinase driven by either the α1 (I) collagen promoter or the osteocalcin promoter. The resulting α1col(Δβ cat) and αc1(Δβ cat) mice were markedly osteoporotic, exhibiting increased numbers of osteoclasts and increased serum markers of bone resorption. Surprisingly, osteoblast numbers and function seemed to be normal, in contrast with the reduced number and function of osteoblasts seen when Wnt signaling is targeted in osteoblast precursors.

To enhance Wnt signaling in mature osteoblasts, Glass et al. crossed transgenic mice expressing Cre driven by the α1 (I) collagen promoter with mice in which exon 3 of β-catenin (containing S33, S37, T41, and S45) had been flanked with loxP sequences. Elimination of exon 3 results in a stable β-catenin isoform that is not targeted for proteosome degradation, yet is competent to mediate transcriptional activation of LEF/TCF target genes. The resulting α1col(β catΔ3) mice were osteopetrotic, exhibiting failed tooth eruption and heightened bone mass due to reduced numbers and function of osteoclasts. Again, osteoblast numbers and function seemed to be normal, in contrast with the increased osteoblast number and function seen when Wnt signaling is constitutively active in osteoblast precursors. A similar osteopetrotic phenotype was exhibited by mice with constitutive Wnt signaling in mature osteoblasts.

![Fig. 1. Wnts regulate osteoprotegerin expression by osteoblasts (50). β-Catenin is the nexus through which canonical Wnt signaling facilitates the transcription of genes that are regulated by LEF/TCF enhancers. In the absence of Wnt signaling, APC, Axin, and glycogen synthase kinase-3β form a complex that phosphorylates β-catenin. Once phosphorylated, β-catenin is transported to the proteosome for degradation. Binding of Wnt glycoproteins to the Frizzled/LRP receptor complex triggers the phosphorylation of Disheveled (Dsh) and LRP. Phosphorylated Disheveled and LRP recruit and bind Axin, which disrupts the APC/Axin/glycogen synthase kinase complex, thereby preventing the degradation of β-catenin. As a result, β-catenin is allowed to translocate to the nucleus, where it associates with LEF/TCF to facilitate the transcription of their target genes.](image-url)
due to targeted deletion of the APC gene. To generate these mice, Holmen et al. crossed transgenic mice expressing Cre driven by the osteocalcin promoter in mice with which the APC gene had been flanked with loxP sequences.

These murine models show a consistent effect of Wnts on osteoblast control of osteoclast development: enhanced Wnt signaling leading to reduced osteoclastogenesis and reduced Wnt signaling leading to increased osteoclastogenesis. Insight into this effect has come from transcriptional profiling of osteoblasts derived from α1col(β catΔ3) mice (increased Wnt signaling) and Lrp5−/− mice (reduced Wnt signaling; ref. 36). Osteoblasts with increased Wnt signaling were found to express osteoprotegerin at a high level, whereas osteoblasts with decreased Wnt signaling had a reduced expression of osteoprotegerin. Wnt regulation of osteoprotegerin expression was confirmed using Northern blot, real-time reverse-transcriptase PCR, in situ hybridization, and ELISA on samples taken from α1col(β catΔ3), α1col(Δβ cat), oc(Δβ cat), and oc(ΔAPC) mice (35, 36). In addition, reciprocal regulation of RANKL by Wnts was observed in osteoblasts with reduced Wnt signaling (35). This used to show the reduced expression of RANKL in osteoblasts oc(2.1).

References


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