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Interactions between myeloma cells and bone microenvironment

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Multiple myeloma (MM) is a plasma cell malignancy characterized by the accumulation of malignant plasma cells within the bone marrow (BM). MM cells interact with the microenvironment inducing pathological modifications that in turn support the growth and survival of MM cells. Bone destruction is the hallmark of MM mainly due to the activation of osteoclastic cells and bone resorption together with the inhibition of osteoblast formation induced by MM cells. On the other hand in the last years growing evidences have highlighted that both osteoclasts and osteoblasts may affect MM cells growth and survival being involved in the pathophysiology of MM. The progress in the acknowledge of the biological mechanisms that regulate osteoclast/osteoblast formation and function have allowed to better understand the mechanisms of MM-induced osteolysis and identify new therapeutics targets for the treatment of MM patients.

Several evidences suggest that RANKL/OPG alteration is involved in the induction of osteoclastogenesis and osteoclast activity in MM.[1-3] It has been demonstrated that MM cells induce RANKL expression in stromal/osteoblastic cells and they decrease OPG production by osteoblastic cells inducing an imbalance of RANKL/OPG ratio in favor of RANKL. The cell-to-cell contact is involved in the induction of RANKL in the microenvironment by MM cells through the integrin system VLA-4/VCAM-1 as demonstrated by blocking anti-VLA-4 antibody that completely blunted the induction of RANKL by MM. On the other hand, blocking anti IL-6, IL-1 or TNFα antibodies had no effect on RANKL induction. The role of the VLA-4/VCAM-1 interaction in the activation of osteoclastic cells by myeloma cells has been also reported in a murine model of MM-induced bone disease showing that the blocking of VLA-4 binding of myeloma cells to VCAM-1 on stromal cells decreases the release of bone by resorbing factors by stromal cells and suppresses the development of osteolytic bone lesions.[4] T cells also contribute to the RANKL/OPG imbalance in the bone microenvironment in MM[5-6] because it has been demonstrated that MM cells up-regulate RANKL in T lymphocytes.[5] Different groups have shown that MM patients have an imbalance of the RANKL/OPG ratio in the BM environment confirming the *in vitro* experimental data.[1-3] It has been showed that ex vivo BM specimens obtained from MM patients overexpress RANKL mRNA in comparison with those from healthy donors. Moreover, immunostaining on BM biopsies has demonstrated an increase of the number of RANKL positive stromal cells together with a reduction of OPG expression in trabecular osteoblasts of MM patients with osteolytic lesions as compared to healthy subjects. The imbalance of RANKL/OPG system observed in the BM environment has been confirmed by the finding of high RANKL serum levels and reduced OPG levels in MM patients as compared to normal subjects.[7-8] The critical role of RANKL in MM-induced bone disease has been further confirmed in mouse models.[1,9] The RANKL-RANK-OPG system could be a therapeutical target for myeloma-induced bone disease. Different strategies may be used to block RANKL and its effect on osteoclastic cells as OPG, RANK-FC anti RANKL antibody or modulators of intracellular signaling pathways of its receptor RANK. In MM mouse models it has been demonstrated that RANKL specific inhibitors OPG or RANK-Fc. completely blocked bone destruction with a significant reduction of the number of osteoclastic cells.[1,9] Administration of both RANK-Fc and OPG also caused a marked reduction of tumor burden assessed histologically and serum paraprotein in the serum accompanied by restoration of OPG and RANKL expression within the human xenograft.[1,10] Recombinant OPG coupled to the Fc fragment of immunoglobulin was tried first in clinical trial.[11] Other than OPG the humanized anti-RANKL antibody has been developed for patients. Recently a clinical trial was con-

ducted to investigate the safety, pharmacokinetics and pharmacodynamics of anti-RANKL antibody in patients with MM or bone metastasis from breast cancer.[12]

The histomorphometric studies performed in MM patients have demonstrated that a reduction of the number of osteoblastic cells contributes together with the increase of osteoclast activity to the development of bone lesions.[13] These studies are supported by clinical studies showing that MM patients with bone lesions have a reduction of the osteoblast markers as alkaline phosphatase and osteocalcin together with the increase of the bone resorption markers.[14] In physiological condition, the formation and differentiation of osteoblastic cells from mesenchymal/stromal cells require the activity and function of the transcription factor Runx2/Cbfa1.[15] The potential involvement of Runx2/Cbfa1 in MM induced osteoblast inhibition has been recently highlighted.[16] Experimental data show that human MM cells are able to inhibit osteoblast differentiation in long term BM culture, reducing the number of both fibroblast colony-forming unit (CFU-F) and the colony-forming bone nodule units (CFU-OB) supported by the inhibitory effect observed on the expression of osteoblast differentiation markers as alkaline phosphatase, osteocalcin and collagen I in a short term co-culture system performed between myeloma cells and osteoprogenitor cells This effect seems to be mediated by blocking Runx2/Cbfa1 activity in human osteoprogenitor cells. Indeed, it has been demonstrated that human MM cells inhibit Runx2/Cbfa1 activity in BM osteoprogenitor cells inhibiting the osteoblast differentiation process. The effect of MM cells on Runx2/Cbfa1 activity is mainly mediated by the cell-to-cell contact between MM and osteoprogenitor cells with the involvement of the VLA-4/VCAM-1 interaction as demonstrated by the capacity of neutralizing anti-VLA-4 antibody to reduce the inhibitory effect on Runx2/Cbfa1 activity by MM cells.[16] Clearly, other molecules involved in the myeloma cell adhesion could be implicated in the inhibition of osteoblastogenesis by human myeloma cells. Recent data indicate that the NCAM-NCAM interaction between myeloma cells and stromal/osteoblastic cells may decrease bone matrix production by osteoblastic cells and contribute to the occurrence of bone lesions in MM patients.[17] Soluble factors may contribute to the inhibitory effect of MM cells on osteoblast differentiation and Runx2/Cbfa1 activity as interleukin-7 (IL-7). IL-7 inhibits both CFU-F and CFU-OB formation in human BM culture and reduced Runx2/Cbfa1 activity in human osteoprogenitor cells. The potential involvement of IL-7 has been confirmed by the finding of higher IL-7 plasma levels in MM patients compared to normal subjects and by the capacity of blocking IL-7 to partially blunt the inhibitory effect of MM cells on osteoblast differentiation.[5,16]

The Wnt signaling is also involved in the regulation of osteoblast formation either through its canonical pathway, mediated by β-catenin nuclear translocation, or at least in part through its non-canonical one.[18] Canonical Wnt signaling pathway is activated by Wnt 1/3a, produced in both autocrine and paracrine fashion, and it interacts with Lrp5-6/Fzd receptor complexes.[18] Several molecules negatively regulate canonical Wnt signaling with different mechanisms. Dickkopfs (DKKs) including DKK-1, the secreted frizzled related proteins (sFRPs) as sFRP1-4 and Wnt inhibitory factor (Wif-1) are the main soluble Wnt inhibitors in murine osteoblasts able to block early osteoblast formation inducing death of immature cells but also to stimulate terminal differentiation indicating a dual effect of Wnt signaling in the osteoblastogenesis.[18]

A relationship between Wnt signaling and Runx2 pathways has been also recently shown in murine osteoblasts suggesting that Wnt signaling activation may promotes osteogenesis by directly stimulating Runx2/Cbfa1 gene expression.[19] The potential involvement of Wnt signaling in the suppression of osteoblast formation and function in MM has been recently hypothesized.[20] It has been reported that $CD138^+$ MM cells obtained at the diagnosis from MM patients overexpress DKK-1 as compared to MGUS and normal plasma cells and a tight relationship between DKK1 expression by MM cells and the occurrence of focal lytic bone lesions has been demonstrated in MM patients.[20] On the other hand patients with advanced disease as well as human myeloma cell lines do not express DKK-1 suggesting that DKK-1 mediated bone destruction could occur in the early phase of disease. However, the mechanism by which DKK-1 production by MM cells is related to bone destruction is not completely clear. It has been reported that neutralizing anti-DKK-1 antibody blocks the inhibitory effect of BM plasma of MM patients on BMP-2 induced alkaline phosphatase expression and osteoblast formation by murine mesenchymal cell line but failed to blunt the inhibitory effect on human BM osteoblast formation by MM cells.

Other mechanisms and molecules are involved in the suppression of osteoblast formation in MM. Recent data indicate that IL-3 could be a potential candidate as osteoblast inhibitor in MM patients.[21] In both murine and human system IL-3 inhibits basal and BMP-2 stimulated osteoblast formation in a dose-dependent manner, without affecting cell growth. IL-3 blocks differentiation of pre-osteoblasts to mature osteoblasts *in vitro* at concentrations comparable to those seen in BM plasma from patients with MM. IL-3 levels in BM plasma from patients with MM are increased in approximately 70% of patients compared

to normal controls or MGUS patients and it is mainly produced by T lymphocytes in the microenvironment as recently suggested.[22] Importantly, BM plasma from patients with MM with high levels of IL-3 blocks osteoblast formation in human cultures, and this inhibition is partially reversed by the addition of a neutralizing antibody to human IL-3. The inhibitory effect of IL-3 seems to be increased in the presence of TNFα, a cytokine induced in the MM marrow microenvironment. Interestingly, the effects of IL-3 are indirect and are mediated by $CD45^+/CD11b^+$ monocyte/ macrophages in both human and mouse primary culture systems IL-3 increases the number of $CD45^+$ hematopoietic cells in stromal cell cultures and the depletion of the $CD45^+$ cells abolishes the inhibitory effects of IL-3 on osteoblasts. Accordingly reconstitution of the cultures with $CD45^+$ cells restores the capacity of IL-3 to inhibit osteoblast differentiation.

Other than the block of osteoblast formation several evidences indicate that MM cells may directly act on mature osteoblastic cells. It has been demonstrated that myeloma cells are able to inhibit osteoblast proliferation and up-regulate osteoblast apoptosis in co-culture.[23-24] Moreover osteoblasts obtained from MM patients with extensive bone lesions seem to be highly prone to apoptosis as compared to those without bone lesions.[23] More recently it has been demonstrated that MM cells induce apoptosis in co-culture system with human osteoblastic cells through the partially involvement of the death receptor TRAIL.[24] Besides killing osteoblast human MM cells sensitize osteoblastic cells to cell death mediated by recombinant TRAIL and in turn osteoblastic cells protect MM cells from TRAIL-mediated apoptosis.[24] In line with this evidence it has been demonstrated that osteoblastic cells may inhibit MM cells proliferation[25] suggesting that potential therapies targeting osteoblastic cells and increasing bone formation in MM patients could be associated to a reduction of MM burden.

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