

N. Giuliani
V. Rizzoli

Hematology and BMT Center,
University of Parma, Italy

Alterations of microenvironment in multiple myeloma patients: interactions between myeloma cells and osteoprogenitor cells



Multiple myeloma (MM) is a plasmacell malignancy characterized by the tight relationship with the bone marrow (BM) microenvironment that has a pivotal role in the regulation of myeloma cell growth and survival through the production of several growth factors and the increase of BM angiogenesis. Bone microenvironment cells as osteoclasts and osteoblasts also support the survival of MM cells. The study of the alterations of the microenvironment and that of the interactions between MM and microenvironment cells has led to identify new therapeutic targets in MM patients and to develop new drugs targeting both MM cells and the microenvironment including endothelial cells, T lymphocytes osteoclast and osteoblast. Particularly in the last years we and other authors have studied the alterations occurring in the bone cells in MM patients, the biological mechanisms involved in the interactions between MM cells and osteoprogenitor/osteoblastic cells and the potential role of osteoblastic cells in the anti-tumoral effect of the new drugs.

As known, several evidences indicate that the impairment of osteoblast and bone formation in MM is mainly due to the block of the osteogenic differentiation process of mesenchymal cells induced by MM cells. It has been

reported that human MM cells were co-cultured with osteoprogenitor cells, MM cells inhibited osteoblast differentiation in long term bone marrow cultures, reducing the number of both early osteoblast precursors, fibroblast colony-forming units (CFU-F), and the more differentiated osteoblast precursor, the colony-forming osteoblast units (CFU-OB), and decreasing the expression of osteoblast differentiation markers, alkaline phosphatase, osteocalcin and collagen I.¹ These *in vitro* evidences have been confirmed *in vivo* in MM patients by the finding that isolated mesenchymal cells show a reduced *ex vivo* osteogenic differentiation² even if these observation was not confirmed by others.³⁻⁴ The inhibitory effect of MM cells on osteoblast differentiation seems to be mediated by the capacity of MM cells to inhibit Runx2 activity of human mesenchymal stem and osteoprogenitor cells.¹ Consistently it has been reported that the number of mesenchymal and osteoblastic cells positive for Runx2 was lower in patients with osteolytic lesions as compared to patients without skeletal involvement.¹ The suppression of Runx2 activity by MM cells is mediated, at least in part, by the cell-to-cell contact between MM and osteoprogenitor cells. This cell-to-cell contact involves interactions

between VLA-4 on MM cells and VCAM-1 on osteoblast progenitors, as demonstrated by the capacity of a neutralizing anti-VLA-4 antibody to reduce the inhibitory effects of MM cells on Runx2/Cbfa1 activity.¹ The role of the cell-to-cell contact via VLA-4/VCAM-1 interaction in the development of bone lesions and osteoclast activation and osteoblast inhibition in MM has been recently demonstrated using *in vivo* mice models.⁵ In addition to VLA-4/VCAM-1, other adhesion molecules appear to be involved in the inhibition of osteoblastogenesis by human MM cells. For example, NCAM-NCAM interactions between MM cells and stromal/osteoblastic cells can decrease bone matrix production by osteoblastic cells, and may contribute to the development of bone lesions in MM patients.⁶ Soluble factors contribute to the inhibitory effects of MM cells on osteoblast differentiation from mesenchymal stem cells and Runx2/Cbfa1 activity.¹ It has been shown that interleukin-7 (IL-7) decreases Runx2/Cbfa1 promoter activity in osteoblastic cells and the expression of osteoblast markers.⁷ Moreover IL-7 can inhibit bone formation *in vivo* in mice.⁷ We have demonstrated that IL-7 inhibited both CFU-F and CFU-OB formation in human bone marrow cultures, and reduced Runx2/Cbfa1 activity in human osteoprogenitor cells.¹ The potential involvement of IL-7 in MM has been supported by the demonstration of higher IL-7 plasma levels in MM patients compared to normal subjects⁸ and by the capacity of blocking antibodies to IL-7 to partially blunt the inhibitory effects of MM cells on osteoblast differentiation.¹ These studies suggest that MM cells block Runx2/Cbfa1 activity and osteoblast differentiation either by cell-to-cell contact or by secreting IL-7, which leads to a reduction in the number of more differentiated osteoblastic cells. This decreased osteoblast activity contributes to the development of MM bone lesions. Other mechanisms could be involved in the inhibition of Runx2

activity by MM cells. Recently it has been shown that MM cells suppress the expression of the Runx2 regulating factor TAZ in human mesenchymal stem cells.⁹ IL-3 has been reported as a potential osteoblast inhibitor in MM patients.¹⁰ In both murine and human system, IL-3 inhibited osteoblast formation in a dose-dependent manner, without affecting cell growth. IL-3 blocked differentiation of pre-osteoblasts to mature osteoblasts *in vitro*, at concentrations comparable to those seen in bone marrow plasma from patients with MM. The potential involvement of Wnt signaling in the suppression of osteoblast formation and function in MM has been hypothesized. Primary CD138⁺ MM cells over-express the Wnt inhibitors DKK-1 as compared to plasma cells from MGUS patients and normal plasma cells. Further, using gene expression profiling, they showed a tight relationship between DKK-1 expression by MM cells and the occurrence of focal lytic bone lesions in MM patients. High DKK-1 levels were also observed in bone marrow and peripheral sera in MM patients correlated with the presence of bone lesions.¹¹ Interestingly, patients with advanced disease, as well as human MM cell lines did not express DKK-1, suggesting that DKK-1 may mediate bone destruction in the early phases of disease. MM cells may also produce other Wnt inhibitors, including SFRP-3/FRZB. FRZB is highly expressed by CD138⁺ MM cells from patients as compared to MGUS patients and BM plasma levels are higher in MM patients with bone lesions as compared to those without skeletal involvement.¹² sFRP-2 has been also reported to be produced by some human MM cell lines and by patients with advanced MM bone disease, and can inhibit osteoblast differentiation.¹³ The mechanism by which DKK-1 and the other Wnt inhibitors produced by MM cells is related to bone destruction is not completely understood. Neutralizing anti-DKK-1 antibody can block

the inhibitory effect of bone marrow plasma of MM patients on BMP-2 induced alkaline phosphatase expression and osteoblast formation by a murine mesenchymal cell line¹¹ but failed to block the inhibitory effects of MM cells on human bone marrow osteoblast formation.¹ In addition, only high concentrations of DKK-1 are able to inhibit CFU-F and CFU-OB formation and to block β -catenin signaling in human bone marrow osteoprogenitor cells.¹ Recently we reported that MM cells failed to block the canonical Wnt signaling in human BM osteoblast progenitors but inhibited this pathway in murine system suggesting the block on canonical Wnt pathway does not occur in MM patients.¹² Studies in the SCID-hu mice model of MM have shown that anti-DKK-1 increases bone mineral density and the number of osteocalcin positive osteoblasts compared to control mice.¹⁴ Interestingly, a reduction of the number of osteoclastic cells was also observed, suggesting that Wnt signaling could be involved in the regulation of bone resorption.¹⁴ Consistently, it has been reported that Wnt3a signal pathway activation in the microenvironment and osteoblasts is able to block the development of bone lesions and the growth of MM cells in murine MM models in line with the observation that canonical Wnt pathway stimulate bone formation in mice.¹⁵⁻¹⁶ However it is interesting to note that both anti-DKK-1 antibody and Wnt canonical activation by Wnt3a by lithium¹⁴⁻¹⁶ are able to blunt that inhibitory effect of MM cells on osteoblast formation independently to the production of DKK-1 by MM cells suggesting that the microenvironment rather than MM cells could be the target of anti-DKK-1 antibody therapy. Other mechanisms could be involved in DDK-1 mediated bone destruction in MM. For example, a link between cell adhesion and the Wnt pathway was recently reported. Wnt inhibitors such as DKK-1 are triggered by cell contact and modulate adhesion of leukemia cells to

osteoblasts.¹⁷ Possibly, DKK-1 production by MM cells could be involved in the adhesion of stromal cells and MM cells, which is critical for osteoclast activation and Runx2/Cbfa1 mediated osteoblast inhibition. Furthermore crosstalk between MM cells and the microenvironment can stimulate both DKK-1 and IL-6 production in human bone marrow cultures.¹⁸ Finally, the capacity of DKK-1 to regulate the osteoclast inhibitory factor OPG and RANKL has been also reported in murine osteoblasts.¹⁹

The ubiquitin-proteasome pathway is the major cellular degradative system for several proteins involved in cell proliferation and survival in MM cells. Recently, it has been demonstrated that this pathway may regulate osteoblast differentiation and bone formation *in vitro* and *in vivo* in mice.²⁰ The ubiquitin-proteasome pathway can modulate the expression of BMP-2, which can induce osteoblast differentiation through the Wnt signaling and regulates the proteolytic degradation of the osteoblast transcription factor Runx2/Cbfa1.²¹ Different proteasome inhibitors that bind the catalytic , subunits of the 20S proteasome and block its activity are able to stimulate bone formation in neonatal murine calvarial bones.²⁰ A strong correlation between the capacity of these compounds to inhibit proteasomal activity in osteoblasts and their bone forming activity was also demonstrated.²⁰ Consistent with these *in vitro* observations, the administration of the natural proteasome inhibitors, PS1 and epoximicin, to mice increases bone volume and bone formation rate over 70% after 5 days, indicating a potent stimulatory effect of these drugs on osteoblastic cells.²⁰ These evidences are strongly supported by the *in vivo* observations obtained in MM patients treated with Bortezomib, the first representative of this class of drugs with a potent anti-MM activity. An increase of total alkaline phosphatase and in parallel of bone specific alkaline phosphatase has been reported in MM patients that

respond to the treatment with bortezomib.²²⁻²⁴ Similarly a significant effect on bone remodeling markers has been reported in MM patients treated with bortezomib.²⁵⁻²⁶ Finally, recent our data indicate that bortezomib induce osteoblast phenotype in osteoblast progenitors *in vitro* increasing Runx2/Cbfa1 activity without changing Runx2/Cbfa1 mRNA level and consequently stimulating the expression of osteoblast markers as collagen I and osteocalcin.²⁷ This stimulatory effect was further confirmed by the finding that bortezomib increased bone nodule formation by human osteoprogenitor cells. A similar effect was observed using specific proteasome inhibitors as MG-132 and MG-262 indicating that the pro-osteogenic effect of bortezomib is due to its capacity to block proteasome activity in osteoblast and osteoblast progenitors.²⁷ In line with these observations it has been reported that bortezomib stimulate bone regeneration in mice in part by the modulation of the bone specific transcription factor Runx2/Cbfa1 and it is able to rescue bone loss in a mouse model of osteoporosis.²⁸

References

- Giuliani N, Colla S, Morandi F, et al. myeloma cells block RUNX2/CBFA1 activity in human bone marrow osteoblast progenitors and inhibit osteoblast formation and differentiation. *Blood* 2005;106:2472-83.
- Corre J, Mahtouk K, Attal M, et al. Bone marrow mesenchymal stem cells are abnormal in multiple myeloma. *Leukemia* 2007;21:1079-88.
- Garderet L, Mazurier C, Chapel A, et al. Mesenchymal stem cell abnormalities in patients with multiple myeloma. *Leuk Lymphoma* 2007;48:2032-41.
- Arnulf B, Lecourt S, Soulier J, et al. Phenotypic and functional characterization of bone marrow mesenchymal stem cells derived from patients with multiple myeloma. *Leukemia* 2007;21:158-63.
- Mori Y, Shimizu N, Dallas M, et al. Anti-alpha4 integrin antibody suppresses the development of multiple myeloma and associated osteoclastic osteolysis. *Blood* 2004;104:2149-54.
- Ely SA, Knowles DM. Expression of CD56/neural cell adhesion molecule correlates with the presence of lytic bone lesions in multiple myeloma and distinguishes myeloma from monoclonal gammopathy of undetermined significance and lymphomas with plasmacytoid differentiation. *Am J Pathol* 2002;160:1293-9.
- Weitzmann MN, Roggia C, Toraldo G, et al. Increased production of IL-7 uncouples bone formation from bone resorption during estrogen deficiency. *J Clin Invest* 2002;110:1643-50.
- Giuliani N, Colla S, Sala R, et al. Human myeloma cells stimulate the receptor activator of nuclear factor-kappa B ligand (RANKL) in T lymphocytes: a potential role in multiple myeloma bone disease. *Blood* 2002;100:4615-21.
- Li B, Shi M, Li J, et al. Elevated tumor necrosis factor-alpha suppresses TAZ expression and impairs osteogenic potential of Flk-1+ mesenchymal stem cells in patients with multiple myeloma. *Stem Cells Dev* 2007;16:921-30.
- Ehrlich LA, Chung HY, Ghobrial I, et al. IL-3 is a potential inhibitor of osteoblast differentiation in multiple myeloma. *Blood* 2005;106:1407-14.
- Tian E, Zhan F, Walker R, et al. The role of the Wnt-signaling antagonist DKK1 in the development of osteolytic lesions in multiple myeloma. *N Engl J Med* 2003;349:2483-94.
- Giuliani N, Morandi F, Tagliaferri S, et al. Production of wnt inhibitors by myeloma cells: potential effects on canonical wnt pathway in the bone microenvironment. *Cancer Res* 2007;67:7665-74.
- Oshima T, Abe M, Asano J, et al. Myeloma cells suppress bone formation by secreting a soluble Wnt inhibitor, sFRP-2. *Blood* 2005;106:3160-5.
- Yaccoby S, Ling W, Zhan F, et al. Antibody-based inhibition of DKK1 suppresses tumor-induced bone resorption and multiple myeloma growth *in vivo*. *Blood* 2007;109:2106-11.
- Qiang YW, Shaughnessy JD Jr, Yaccoby S. Wnt3a signaling within bone inhibits multiple myeloma bone disease and tumor growth. *Blood* 2008;112:374-82.
- Edwards CM, Edwards JR, Lwin ST, et al. Mundy GR. Increasing Wnt signaling in the bone marrow microenvironment inhibits the development of myeloma bone disease and reduces tumor burden in bone *in vivo*. *Blood* 2008;111:2833-42.
- De Toni F, Racaud-Sultan C, Chicanne G, et al. A crosstalk between the Wnt and the adhesion-dependent signaling pathways governs the chemosensitivity of acute myeloid leukemia. *Oncogene* 2006;25:3113-22.
- Gunn WG, Conley A, Deininger L, et al. A crosstalk between myeloma cells and marrow stromal cells stimulates production of DKK1 and interleukin-6: a potential role in the development of lytic bone disease and tumor progression in multiple myeloma. *Stem Cells* 2006;24:986-91.
- Qiang YW, Chen Y, Stephens O, et al. Myeloma-derived Dickkopf-1 disrupts Wnt-regulated osteoprotegerin and RANKL production by osteoblasts: a potential mechanism underlying osteolytic bone lesions in multiple myeloma. *Blood* 2008;112:196-207.
- Garrett IR, Chen D, Gutierrez G, et al. Selective inhibitors of the osteoblast proteasome stimulate bone formation *in vivo* and *in vitro*. *J Clin Invest* 2003;111:1771-82.
- Zhao M, Qiao M, Oyajobi BO, et al. E3 ubiquitin ligase Smurf1 mediates core-binding factor alpha1/Runx2 degradation and plays a specific role in osteoblast differentiation. *J Biol Chem* 2003;278:27939-44.
- Zangari M, Esseltine D, Lee CK, et al. Response to bortezomib is associated to osteoblastic activation in patients with multiple myeloma. *Br J Haematol* 2005;131:71-3.
- Zangari M, Yaccoby S, Cavallo F, et al. Response to bortezomib and activation of osteoblasts in multiple myeloma. *Clin Lymphoma Myeloma* 2006;7:109-14.
- Shimazaki C, Uchida R, Nakano S, et al. High serum bone-specific alkaline phosphatase level after bortezomib-combined therapy in refractory multiple myeloma: possible role of bortezomib on osteoblast differentiation. *Leukemia* 2005;19:1102-3.
- Heider U, Kaiser M, Muller C, et al. Bortezomib increases

- es osteoblast activity in myeloma patients irrespective of response to treatment. *Eur J Haematol* 2006;77:233-8.
26. Terpos E, Heath DJ, Rahemtulla A, et al. Bortezomib reduces serum dickkopf-1 and receptor activator of nuclear factor-kappaB ligand concentrations and normalises indices of bone remodeling in patients with relapsed multiple myeloma. *Br J Haematol* 2006;135:688-92.
 27. Giuliani N, Morandi F, Tagliaferri S, et al. The proteasome inhibitor bortezomib affects osteoblast differentiation in vitro and in vivo in multiple myeloma patients. *Blood* 2007;110:334-8.
 28. Mukherjee S, Raje N, Schoonmaker JA, et al. Pharmacologic targeting of a stem/progenitor population in vivo is associated with enhanced bone regeneration in mice. *J Clin Invest* 2008;118:491-504.