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Multistep tumorigenesis of multiple myeloma: Its molecular delineation

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Multiple myeloma (MM) is an incurable malignant neoplasm affecting terminally differentiated B-cells. It derives from post-germinal center B-cells and develops as a result of multistep tumorigenic events, since approximately one-third of the cases have a history of preceding monoclonal gammopathy of undetermined significance (MGUS) or smoldering myeloma (SMM). It terminates in the formation of extramedullary invasion or in secondary plasma cell leukemia. To account for this clinical experience, intrinsic chromosomal instability followed by complex chromosomal translocations/deletions has been found to play a crucial role in the development of MM. Representative aberrations include chromosomal rearrangements involving 14q32 loci and deletion at the long arm of chromosome 13. In particular, accumulation of chromosomal translocations involving immunoglobulin heavy chain gene (*IgH*) locus is responsible for the development and characterization of MM. Among them, deregulated expression of *CCND1*, *FGFR3/MMSET* and *c-MAF* is occasionally found in MGUS/SMM, indicating that it is associated with the early development of MM. However, chromosomal rearrangements involving *MUM1*, *c-MYC* and *MAFB* gene loci are rarely found in MGUS/SMM, although they are found in MM in higher frequencies, suggesting that they are relevant to the late progression of MM. On the basis of our study using primary MM samples analyzed by RT/RQ-PCR assay, we have demonstrated that (1) *CCND1*, *FGFR3* and *c-MAF* contribute to the initial development of MM, (2) *c-MYC/MUM1* and *c-MAF* play crucial roles in the progression of *CCND1*⁺ or *c-MAF*⁺ and *FGFR3*⁺ MM, respectively. Moreover, through the identification of target genes regulated by the abovementioned oncogenic transcription factors such as *MUM1* and *MAFB/c-MAF*, we have identified a part of the novel mechanisms, which possibly contribute to the malignant transformation of the MM cells. Contributing to the terminal progression of MM itself are genomic instability and altered methylation of the specific gene promoters. The former results in activation of specific oncogenes such as *RAS* and *FGFR3* or in inactivation of *p53* and the latter in inactivation of tumor suppressor genes including *p16*. Detailed understanding of molecular pathogenesis should help lead to the development of specific molecular targeting therapies against MM.

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A bone disease in multiple myeloma: cellular interplay in the myeloma bone microenvironment

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Multiple myeloma (MM) is characterized by accumulation of monoclonal plasma cells in the bone marrow and progression of devastating lytic bone lesions which cause the most debilitating clinical symptoms including intractable bone pain, disabling multiple fractures and hypercalcemia. Of note, aggressive bone destruction has significantly contributed to its poor prognosis despite the recent development of potent chemotherapeutic regimens. Therefore, elucidation of the molecular mechanisms of bone destruction and tumor progression is essential for the development of effective therapies to improve survival as well as quality of life of MM patients.

Enhanced bone resorption

Interactions between receptor activator of nuclear factor-kappaB (RANK) expressed on the surface of cells of the osteoclastic lineage and RANK ligand expressed on stromal cells play a key role in the formation and activation of osteoclasts (OCs), while osteoprotegerin, a decoy receptor for RANK ligand secreted from various types of cells, inhibits RANK ligand-RANK signaling. MM cells stimulate osteoclastogenesis by triggering a coordinated increase in RANK ligand and decrease in osteoprotegerin in the bone marrow. MM cells reside in the proximity of stromal cells and activate OCs in bone destructive lesions, suggesting involvement of MM cell-derived local factors and/or direct interaction between these cells. We and others have found and reported that osteoclastogenic C-C chemokines macrophage inflammatory protein (MIP)-1alpha and MIP-1beta are secreted by most of primary MM cells from patients

with multiple osteolytic lesions, and potently induce osteoclastogenesis (Blood 100: 2195-2202, 2002). The ability of MM cells to secrete these chemokines correlated well with the extent of MM bone lesions as well as levels of biochemical bone resorption markers in patients with MM (Brit J Haematol 125: 38-41, 2004), suggesting a causal role for these chemokines in the development of lytic bone lesions. In osteoclastogenic cultures with bone cells on dentine slices, MM cells enhanced both formation of OCs and resorption pits. These effects were mostly abrogated by neutralizing antibodies against MIP-1alpha and MIP-1beta in combination, suggesting critical roles for these chemokines. Importantly, the osteoclastogenic activity of MM cells as well as MIP-1alpha and MIP-1beta was completely inhibited by osteoprotegerin, indicating that the MM cell effects are dependent on RANK ligand expression. Indeed, these chemokines induced RANK ligand expression by a stromal cell line, ST-2, in the presence of a suboptimal concentration of 1, 25(OH)₂-dihydroxyvitamin D₃. Furthermore, MM cells express cognate receptors for MIP-1, CCR1 and CCR5. MIP-1 was found to act on MM cells in an autocrine/paracrine fashion to enhance adhesion of MM cells to VCAM-1 on stromal cells as well as to OCs. MM cell adhesion through VLA-4/VCAM-1 further enhances osteoclastogenesis as well as the secretion of MIP-1alpha and MIP-1beta by MM cells, creating a microenvironment with a close cell-cell interaction and high concentration of these chemokines around them. Besides, a portion of MM cells constitutively expressed RANK ligand on their surface, and concomitantly produced VEGF,

a substitute for M-CSF, which is required to induce osteoclastogenesis by RANK ligand, suggesting that they are capable of inducing osteoclastogenesis by themselves. Thus, MM cells potently enhance osteoclastogenesis in the bone marrow microenvironment to cause extensive bone resorption.

Osteoclast-mediated myeloma cell growth and survival

MM almost exclusively develops in the bone marrow, suggesting that the bone marrow microenvironment supports MM cell growth and survival. Among cell components in the bone marrow microenvironment, roles for stromal cells in MM cell growth and survival have been extensively studied. Besides stromal cells, we found that the growth and survival of MM cells are potently enhanced by OCs (Blood in press). The effects of OCs were only partially inhibited by an anti-human IL-6 neutralizing antibody despite the increased production of IL-6 by OCs in co-cultures with MM cells. In addition, human MM cell growth was enhanced even in co-cultures with mouse or rabbit OCs whose IL-6 cannot act on human cells. Furthermore, prevention of cellular contact between MM cells and OCs by membrane filters completely abolished the OC effect. Therefore, OCs may enhance MM cell growth largely through a close cell-cell interaction by elaborating unknown factor(s) other than IL-6. Similar to osteoclastogenesis, angiogenesis is enhanced in the bone marrow in patients with MM, which has drawn considerable attention as a potential therapeutic target. We found that OCs constitutively secrete high levels of an angiogenic noncollagenous matrix protein, osteopontin (OPN), which is known to cooperatively act with VEGF in angiogenesis. Conditioned media (CM) from OCs enhanced vascular tubule formation as potently as those from MM cells. Interestingly, CM from co-cultures of both cells further enhanced it, suggesting cooperative interactions between OCs and MM cells in angiogenesis. Antibodies against OPN or VEGF each alone partially and both in combination almost completely abrogated vascular tubule formation enhanced by CM from the co-cultures. Therefore, OCs enhance

angiogenesis in concert with MM cells, which is largely mediated by cooperative actions of OPN and VEGF derived from OCs and MM cells, respectively. Collectively, OCs may enhance MM progression not only directly but also through enhanced angiogenesis, thereby forming a vicious cycle between bone destruction and MM expansion.

Impaired bone formation

Along with enhanced bone resorption, mineralization is impaired in MM bone lesions, observed as “punched-out” lesions on X-ray examinations. Consistently, analyses of ongoing bone metabolism in MM by biochemical bone markers also suggested an imbalance of bone turnover with enhanced osteoclastic bone resorption and concomitantly suppressed bone formation. These clinical evidence also suggests suppression of bone formation as a contributing factor to a bone loss in MM. Despite recent advances in our understanding of the mechanisms of osteolysis enhanced in MM, little is known about factors responsible for impaired bone formation. A canonical Wingless-type (Wnt) signaling pathway has recently been shown to play a critical role in osteoblast differentiation. Wnt proteins are secreted cysteine-rich glycoproteins and known as regulators of the differentiation of hematopoietic and mesenchymal cells as well as embryonic development. Because CM from MM cells suppress osteoblast differentiation as determined by alkaline phosphatase activity and mineralization and because several secreted Frizzled related protein (sFRP) and dickkopf (DKK) family members are known as soluble Wnt antagonists, we examined the expression of sFRP-1, 2 and 3 and DKK-1 in MM cells. We found that MM cell lines including U266, RPMI8226 and ARH77 secreted only sFRP-2 at protein levels, although sFRP-3 as well as sFRP-2 were expressed in all these cell lines and DKK-1 was expressed only in U266 cells at mRNA levels. Importantly, sFRP-2 mRNA and protein expression was detected in most MM cells from patients with advanced or terminal stages of MM (3/4 and 8/10, respectively). In order to examine a biological role for

sFRP-2, we added recombinant sFRP-2 to MC3T3-E1 osteoblastic cell cultures together with BMP-2. Exogenous sFRP-2 partially suppressed alkaline phosphatase activity but almost completely blocked mineralized nodule formation enhanced by BMP-2. Furthermore, sFRP-2 immunodepletion significantly restored mineralized nodule formation in MC3T3-E1 cells suppressed by ARH77 CM. These results suggest that sFRP-2 alone is able to suppress osteoblast differentiation induced by BMP-2 and that MM cell-derived sFRP-2 is among predominant factors responsible for defective bone formation in MM. More recently, Tian et al. reported that DKK-1, an inhibitor of a LRP5/6 Wnt co-receptor, is secreted from MM cells from the patients with bone lesions (N Engl J Med 349:2483-2494, 2003). In contrast to sFRP-2, DKK-1 was shown to be preferentially expressed in a phenotypically mature type of MM cells, but hardly in plasmablastic or immature types of MM cells. Because MM cell-derived factors such as DKK-1, IGF-BP4 and IL-3 other than sFRP-2 have been implicated as an inhibitor of osteoblast differentiation, sFRP-2 may also act in concert with such other factors to potently suppress bone formation in MM. Thus, MM cells may cause an imbalance of bone turnover by enhancing osteoclastic bone resorption and at the same time suppressing bone formation, which leads to devastating destruction and a rapid loss of bone. Elucidation of MM cell-derived factors responsible for suppression of bone formation may lead to a novel therapeutic approach to prevent devastating bone destruction that cannot be fully ameliorated by antiresortive therapies.