The Importance of Notch Signaling in Myeloma Cell-Osteoclast Interactions

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Multiple myeloma develops almost exclusively in bone marrow and generates devastating bone destruction by osteoclasts recruited around multiple myeloma cells. In the development of lytic bone lesions, myeloma cells stimulate osteoclastogenesis by triggering a coordinated increase in receptor activator of NF-κB ligand (RANKL) and a decrease in osteoprotegerin in bone marrow stromal cells (1,2). Osteoclastogenic chemokines macrophage inflammatory protein (MIP)-1α and MIP-1β are secreted from most myeloma cells (3) and play a critical role in the development of lytic bone lesions (4,5). These chemokines act on myeloma cells in an autocrine/paracrine fashion and enhance myeloma cell adhesion to stromal cells through activation of integrins, including VLA-4. The interaction between myeloma and stromal cells then induces RANKL expression of stromal cells, leading to osteoclast differentiation and activation (6).

The almost exclusive development of multiple myeloma in bone marrow suggests that the bone marrow microenvironment supports myeloma cell growth and survival. Osteoclasts induced by myeloma cells are among the major constituents of the bone marrow microenvironment surrounding myeloma cells, and increasing evidence indicates that interactions between osteoclasts and myeloma cells play an important role in myeloma cell expansion in bone marrow. Human blood mononuclear cell-derived osteoclasts have been shown to enhance the growth and survival of myeloma cells more potently than stromal cells in a cell-cell contact-dependent manner (7,8) and to rescue myeloma cells from apoptosis induced by serum depletion or doxorubicin treatment in vitro (8). In addition, administration of bisphosphonate or inhibitors of RANKL, such as RANK-Fc and osteoprotegerin, not only prevents myeloma cell-induced bone destruction, but also interferes with tumor progression in animal models of multiple myeloma (1,9,10). These results have been supported by clinical findings that inhibition of bone resorption by bisphosphonates can reduce the tumor burden without chemotherapy in smoldering myeloma patients (11) and improved survival in patients with advanced stages of multiple myeloma (12). These observations suggest that increased osteoclast number and activity around myeloma cells contributes to aggressiveness and drug resistance of myeloma cells and that there is a vicious cycle between myeloma cells and osteoclasts in the bone marrow microenvironment. Thus, enhancement of osteoclastic bone resorption causes not only destructive bone lesions, but also the development of drug resistance, which further aggravates multiple myeloma expansion in bone marrow. However, the mechanism whereby osteoclasts enhance myeloma cell growth and survival is not fully understood. To address this issue, Yin (13) applied surface enhanced laser desorption/ionization proteomics and examined global changes in secreted proteins after coculturing myeloma cells and osteoclasts.
Using the powerful proteomic approach, Yin (13) found about 30 peaks, with changes after six hours of coculturing the cells; however, the most prominent secreted protein, which was identified as chondroitin synthase 1 (CHSY1), was upregulated by 4.6-fold after coculturing myeloma cells with osteoclasts. In addition, tranfection of small interfering RNA (siRNA) to CHSY1 into osteoclasts resulted in an increase in apoptosis, with a decrease in viability of myeloma cells, suggesting that an increase in CHSY1 contributes to the enhanced survival of myeloma cells in cell-cell interactions between myeloma cells and osteoclasts (13). However, because CHSY1 is a type II membrane protein, the mechanism of its secretion from osteoclasts into extracellular milieu is unclear from the data. CHSY1 has a Fringe-like domain containing a DDD motif, and the DDD motif of Fringe regulates Notch-1 and Notch-2 signaling via its glycosyltransferase activity to add O-linked fucose glycans to Notch epidermal growth factor repeats. Glycosylation by the Fringe DDD motif potentiates Notch-2 signaling, while inhibiting Notch-1 signaling. If the Notch receptor is activated by its ligands Delta and Jagged, a 100-kDa Notch intracellular domain is cleaved and translocated into the nucleus to initiate downstream signaling. Yin (13) further examined the mechanism by which CHSY1 affects myeloma cell survival and found that only Notch-2 was cleaved when myeloma cells were cocultured with osteoclasts and that CHSY1 siRNA inhibited Notch-2 activation. From these observations, Yin (13) hypothesized that CHSY1 secreted from osteoclasts that were in contact with myeloma cells plays an important role in the enhancement of myeloma cell survival in the bone marrow microenvironment by modulating Notch signaling via its Fringe-like domain. Among cell components in the bone marrow, stromal cells have been regarded as a major target of cell-cell interactions with cells of hematopoietic lineage, including myeloma cells. In fact, cell-cell interactions with myeloma cells enhance the production of interleukin 6 (IL-6) from stromal cells, which promotes the proliferation of myeloma cells and protects them from apoptosis induced by anticancer agents (14,15). Because Notch ligands on bone marrow stromal cells bind to Notch receptors expressed on hematopoietic stem cells to control their cell fate and survival, Notch signaling has also been examined in myeloma cell-stromal cell interactions. Nefedova et al. (16) examined Notch expression in myeloma and other malignant lymphoid cell lines and found that Notch-1 to Notch-4 were all expressed in most of the cell lines studied. In contrast to the observations of Yin, Nefedova and colleagues found that only Notch-1 activation resulted in tumor cell protection from melphalan- and mitoxantrone-induced apoptosis. In addition to Notch receptors, Jagged1 and Jagged2 have been overexpressed in myeloma cells (17,18), and Jagged2 binding to its receptor Notch-1 on stromal cells has induced the secretion of IL-6, vascular endothelial growth factor, and insulin-like growth factor 1 from stromal cells in coculture (18). Nonmalignant plasma cells have not been shown to express Jagged2 and have shown low to undetectable levels of Notch (17,18). Although these results are consistent with the notion that Notch signaling plays an important role in the growth and survival of myeloma cells in the bone marrow microenvironment, further studies are needed to clarify the types of Notch (and its ligands) involved, as well as the directions of the signals involved in the enhanced growth and survival of myeloma cells in bone marrow. In addition, the relationship between cell-cell interactions of myeloma cells with stromal cells and osteoclasts remains to be clarified with regard to Notch signaling. Nevertheless, the novel observation by Yin -- that interactions between myeloma cells and osteoclasts stimulate Notch signaling -- raises an important question of how changes in Notch signaling affect myeloma cell growth and survival. The results also provide possible new targets for the treatment of this incurable disease.
References


