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COMMENTARIES

The Importance of Notch Signaling in Myeloma Cell-Osteoclast Interactions

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Commentary on: Yin LL. Chondroitin synthase1 is a key molecule in myeloma cell-osteoclast interactions. *J Biol Chem.* 2004 Oct 10.1074/jbc.M409877200.

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.

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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.

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References

1. Pearse RN, Sordillo EM, Yaccoby S, Wong BR, Liau DF, Colman N, Michaeli J, Epstein J, Choi Y. Multiple myeloma disrupts the TRANCE/ osteoprotegerin cytokine axis to trigger bone destruction and promote tumor progression. *Proc Natl Acad Sci U S A*. 2001 Sep 98(20):11581-6.

2. Giuliani N, Bataille R, Mancini C, Lazzaretti M, Barille S. Myeloma cells induce imbalance in the osteoprotegerin/osteoprotegerin ligand system in the human bone marrow environment. *Blood*. 2001 Dec 98(13):3527-33.

3. Hashimoto T, Abe M, Oshima T, Shibata H, Ozaki S, Inoue D, Matsumoto T. Ability of myeloma cells to secrete macrophage inflammatory protein (MIP)-1alpha and MIP-1beta correlates with lytic bone lesions in patients with multiple myeloma. *Br J Haematol*. 2004 Apr 125(1):38-41.

4. Choi SJ, Cruz JC, Craig F, Chung H, Devlin RD, Roodman GD, Alsina M. Macrophage inflammatory protein 1-alpha is a potential osteoclast stimulatory factor in multiple myeloma. *Blood*. 2000 Jul 96(2):671-5.

5. Choi SJ, Oba Y, Gazitt Y, Alsina M, Cruz J, Anderson J, Roodman GD. Antisense inhibition of macrophage inflammatory protein 1-alpha blocks bone destruction in a model of myeloma bone disease. *J Clin Invest*. 2001 Dec 108(12):1833-41.

6. Abe M, Hiura K, Wilde J, Moriyama K, Hashimoto T, Ozaki S, Sakatsuki S, Kosaka M, Kido S, Inoue D, Matsumoto T. Role for macrophage inflammatory protein (MIP)-1alpha and MIP-1beta in the development of osteolytic lesions in multiple myeloma. *Blood*. 2002 Sep 100(6):2195-202.

7. Yaccoby S, Wezeman MJ, Henderson A, Cottler-Fox M, Yi Q, Barlogie B, Epstein J, Johnson CL, Mahaffey SC, Pearse RN, Choi Y. Cancer and the microenvironment: myeloma-osteoclast interactions as a model. *Cancer Res*. 2004 Mar 64(6):2016-23.

8. Abe M, Hiura K, Wilde J, Shioyasono A, Moriyama K, Hashimoto T, Kido S, Oshima T, Shibata H, Ozaki S, Inoue D, Matsumoto T. Osteoclasts enhance myeloma cell growth and survival via cell-cell contact: a vicious cycle between bone destruction and myeloma expansion. *Blood*. 2004 Oct 104(8):2484-91.

9. Croucher PI, Shipman CM, Lippitt J, Perry M, Asosingh K, Hijzen A, Brabbs AC, van Beek EJ, Holen I, Skerry TM, Dunstan CR, Russell GR, Van Camp B, Vanderkerken K. Osteoprotegerin inhibits the development of osteolytic bone disease in multiple myeloma. *Blood*. 2001 Dec 98(13):3534-40.

10. Croucher PI, De Hendrik R, Perry MJ, Hijzen A, Shipman CM, Lippitt J, Green J, Van Marck E, Van Camp B, Vanderkerken K. Zoledronic acid treatment of 5T2MM-bearing mice inhibits the development of myeloma bone disease: evidence for decreased osteolysis, tumor burden and angiogenesis, and increased survival. *J Bone Miner Res*. 2003 Mar 18(3):482-92.

11. Dhodapkar MV, Singh J, Mehta J, Fassas A, Desikan KR, Perlman M, Munshi NC, Barlogie B. Anti-myeloma activity of pamidronate *in vivo*. *Br J Haematol*. 1998 Nov 103(2):530-2.

12. Berenson JR, Lichtenstein A, Porter L, Dimopoulos MA, Bordoni R, George S, Lipton A, Keller A, Ballester O, Kovacs M, Blacklock H,

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Bell R, Simeone JF, Reitsma DJ, Heffernan M, Seaman J, Knight RD. Long-term pamidronate treatment of advanced multiple myeloma patients reduces skeletal events. Myeloma Aredia Study Group. *J Clin Oncol*. 1998 Feb 16(2):593-602.

13. Yin LL. Chondroitin synthase1 is a key molecule in myeloma cell-osteoclast interactions. *J Biol Chem.* 2004 Oct 10.1074/jbc.M409877200.

14. Damiano JS, Cress AE, Hazlehurst LA, Shtil AA, Dalton WS. Cell adhesion mediated drug resistance (CAM-DR): role of integrins and resistance to apoptosis in human myeloma cell lines. *Blood*. 1999 Mar 93(5):1658-67.

15. Grigorieva I, Thomas X, Epstein J. The bone marrow stromal environment is a major factor in myeloma cell resistance to dexamethasone. *Exp Hematol*. 1998 Jul 26(7):597-603.

16. Nefedova Y, Cheng P, Alsina M, Dalton WS, Gabrilovich DI. Involvement of Notch-1 signaling in bone marrow stroma-mediated de novo drug resistance of myeloma and other malignant lymphoid cell lines. *Blood*. 2004 May 103(9):3503-10.

17. Jundt F, Probsting KS, Anagnostopoulos I, Muehlinghaus G, Chatterjee M, Mathas S, Bargou RC, Manz R, Stein H, Dorken B. Jagged1-induced Notch signaling drives proliferation of multiple myeloma cells. *Blood*. 2004 May 103(9):3511-5.

18. Houde C, Li Y, Song L, Barton K, Zhang Q, Godwin J, Nand S, Toor A, Alkan S, Smadja NV, Avet-Loiseau H, Lima CS, Miele L, Coignet LJ. Overexpression of the NOTCH ligand JAG2 in malignant plasma cells from multiple myeloma patients and cell lines. *Blood*. 2004 Dec 104(12):3697-704.

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