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NEWS

Bidirectional Signaling in Bone

A new study pinpoints a role for ephrin/Eph signaling in crosstalk between osteoblasts and osteoclasts

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New research from a team of scientists in Japan has found that ephrin signaling, a bidirectional communication mechanism with proven roles in a variety of biological processes, including angiogenesis and skeletal patterning, also serves a key function in bone remodeling (1;2). Specifically, experiments demonstrate that osteoblasts signal to osteoclasts to inhibit osteoclast differentiation, and osteoclasts signal to osteoblasts to stimulate osteoblast differentiation, through the binding of ephrin ligands on the surface of osteoclasts to ephrin receptors on the surface of osteoblasts. The new work is the first to provide a detailed molecular mechanism, supported by *in vivo* evidence from transgenic animals, of cross-talk between the cells that resorb bone, and those that make it.

"We've known for many years that the osteoblast lineage communicates with the hemopoetic lineage to regulate osteoclast formation. This research provides the first evidence specifying a particular set of molecules that might be involved in the short-term communication traffic going in the other direction, from osteoclasts to osteoblasts," says T. John Martin, an emeritus professor at the University of Melbourne. "Osteoclasts signal to osteoblasts — that is the message of this new research," agrees Morten Karsdal, head of pharmacology at Nordic Bioscience in Herlev, Denmark.

In addition to elucidating a role for bidirectional signaling in bone, the new research appears likely to affect the way bone scientists think about the coupling of bone resorption to bone formation. Most exciting of all, the work enhances the tantalizing possibility, long awaited by the bone field, of a drug that not only inhibits bone resorption, but that also exerts a simultaneous, bone-building, anabolic effect.

The Research

The investigators first became interested in ephrin signaling based on microarray data from previous studies on knockout mice missing c-fos, a transcription factor whose absence prevents osteoclast differentiation. The scientists introduced another transcription factor, NFATc1, into these mice to see if the defects in osteoclasts could be remedied, and monitored changes in the activation of osteoclast marker genes. "We found that many genes were upregulated, and the gene for ephrinB2 was one of them," says Koichi Matsuo, senior author of the study and an associate professor at Keio University School of Medicine in Tokyo. The bidirectional signaling capability of ephrins intrigued Matsuo and his team, so they decided to investigate the role of ephrinB2, the cell-surface protein encoded by the *Efnb2* gene whose activity was detected in the microarray analysis.

Initial experiments confirmed the presence of both ephrinB2 on osteoclasts and of EphB4, the receptor for ephrin B2, on osteoblasts. To study potential signaling from EphB4 on osteoblasts to ephrinB2 on osteoclasts, known as reverse signaling, the researchers co-cultured bone marrow cells with stromal cells expressing EphB4 and found that osteoclast differentiation was inhibited. Co-cultures of bone marrow cells

overexpressing ephrinB2 produced similar results. In addition to gain-of-function experiments, loss-of-function experiments also demonstrated a role for ephrin signaling: co-cultures of stromal cells and osteoclast precursor cells in which *Efnb2* transcripts had been knocked down with RNA interference technology displayed increased numbers of osteoclasts.

The researchers then looked to examine potential forward signaling from ephrinB2 on osteoclasts to EphB4 on osteoblasts. They found that the addition of ephrinB2 to osteoblastogenic cultures stimulated osteoblast differentiation. The team also generated transgenic mice overexpressing EphB4 in osteoblasts and found that these animals exhibited increased bone mass, greater bone mineral density at the femur, and increases in bone volume, osteoid thickness, mineralizing surface and bone formation rate, compared to control animals. Finally, numerous measures of osteoclast function demonstrated the suppression of these bone-resorbing cells in the transgenic animals. All in all, the results from the forward and reverse signaling experiments showed that osteoblasts send an inhibitory signal that blocks osteoclast differentiation, and osteoclasts send a stimulatory signal that enhances osteoblast differentiation, all through ephrin/Eph interactions.

"The paradigm is new here: there is bidirectional talk between these two cells," says Patrick Ross, a professor in the department of pathology and immunology at Washington University School of Medicine in St. Louis and an expert on cellular signaling in the osteoclast. "Everybody understood that osteoblasts talk to osteoclast precursors and to osteoclasts. Nobody suspected a direct paracrine interaction from osteoclasts to osteoblasts, and that's why this is an important paper."

A New View of Coupling?

The researchers are careful to note that if coupling is viewed as a positive correlation between bone resorption and bone formation, then their results offer evidence of something different, since they found a negative correlation: osteoclast resorption was decreased, while bone formation was increased. They note, though, that in order for coupling to occur, a bone formation phase must follow a bone resorption phase, and in this sense, their theory is consistent with coupling, since ephrin signaling can contribute to this phase change. "When we think about the transition from one phase to another—from resorption to reversal and bone formation—our model can fit beautifully with the coupling theory," Dr. Matsuo emphasizes.

The study also has the potential to have a deeper impact on the way the bone field thinks about the coupling of bone resorption to bone formation. In fact, bone experts view the ephrin signaling theory as compatible with other theories, such as the release of growth factors from the bone matrix as a consequence of resorption, and the release of secreted factors from osteoclasts independent of resorption, that have been proposed to explain the unique relationship between resorption and formation. Consequently, instead of viewing coupling as a single overarching event, perhaps it is best viewed as a number of discrete events operating over different time periods.

"We should probably stop using the term 'coupling,' because it's too all-embracing," says T. John Martin. "There are clearly ways in which resorption can produce factors that can influence the formation process, but there are also likely short-term communication mechanisms between osteoclasts and osteoblasts."

"All of these mechanisms can be operative," says Morten Karsdal, expressing a similar sentiment. "Ephrin signaling might be very important for penetration of osteoclasts into the bone lining cell layer—it's a specific event. Secreted factors released from osteoclasts might be a different event. They all add up to the story of how osteoclasts initiate bone formation," he says.

Karsdal is especially interested in pinning down the nature of those secreted factors. In particular, Karsdal points to osteopetrotic patients, who exhibit a linear correlation

between the number of osteoclasts, which in these individuals do not resorb bone, and the number of osteoblasts. Patients deficient in the proton pump that helps to acidify the space where bone resorption occurs, and whose osteoclasts also fail to resorb bone, also show this correlation, which suggests that the coupling factor is a secreted one independent of resorption.

"The traditional way of thinking that osteoclast resorption of the matrix is necessary for bone formation needs to be modified. Yes, osteoclasts are important, but they do not need to dig down into the bone," Karsdal stresses. "There are factors, independent of those stored in bone matrix, that can be released by the osteoclast," agrees Anna Teti, a professor of histology at the University of L'Aquila in Italy who has also published research on patients with osteopetrosis.

Karsdal sees great promise in acidification inhibitors as pharmaceutical agents that can lead to an uncoupling where bone resorption decreases without the corresponding decrease in bone formation. Like acidification inhibitors, potential manipulation of the ephrin/Eph signaling mechanism unearthed in the new study may also offer, eventually, an avenue to a pharmaceutical uncoupling of resorption and formation, since overexpression of EphB4 in osteoblasts resulted in an inhibition of resorption but also an increase in bone formation. However, while the concept is promising, it is far too early to tell whether manipulating ephrin/Eph signaling will work in practice.

Remaining Questions

Indeed, before a drug can be developed, several fundamental questions about ephrin/Eph signaling in bone must first be addressed. For instance, the mechanism proposed by the researchers requires cell-to-cell contact between osteoblasts and osteoclasts. Consequently, one of the more interesting issues regarding the new theory is whether sufficient, direct physical contact actually occurs between the two cell types during bone remodeling. Furthermore, if there is sufficient cell-cell contact, when does it take place? "Determining the temporal scheme of the ephrin/Eph interaction would be a really exciting area of future research," notes Laurie McCauley, a professor of dentistry at the University of Michigan School of Dentistry. Dr. Matsuo says that his team has conducted studies using electron microscopy to address the issue of cell-cell contact, and believes evidence from these experiments supports the idea that contact is taking place. His team plans further studies to generate more direct imaging of the interaction *in vivo*.

Of course, osteoclasts and osteoblasts are not the only cells to interact in the bone microenvironment, and ephrin signaling may be important in interactions with those other cell types. For instance, Dr. Matsuo notes that because blood endothelial cells also express ephrin/Eph family members, it will be interesting to look at ephrin signaling interactions between those cells, osteoblasts, and osteoclasts. The goal is to generate knockout mice lacking different family members and to assess whether any of these animals exhibit a particular bone phenotype.

Interestingly, in the current study, when the investigators looked at the femurs and tibiae from knockout mice lacking ephrinB2, they found no evidence of any noticeable bone phenotype, as bone volume, osteoblast surface and bone mineral density did not differ significantly from controls. They speculate that other Eph receptors may interact with ephrinB1, another ephrin family member that is also expressed on the surface of osteoclasts. Investigating the possibilities and implications of this kind of signaling redundancy may also become a fruitful path for future studies.

All of these questions could very well keep bone scientists quite busy in the foreseeable future, according to Florent Elefteriou, co-author with Gregory Mundy of a review of the paper in *Cell* and an assistant professor at Vanderbilt University Medical Center in Nashville, Tennessee. "This is likely the very beginning of a new subfield in the bone field," he says.

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