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NEWS

Discovery of New Role for Calcium-Sensing Receptor Strengthens Link Between Formation of Bone and Formation of New Blood Cells

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Researchers at Massachusetts General Hospital, Brigham and Women's Hospital and the Harvard Stem Cell Institute in the Boston area have demonstrated that the calcium-sensing receptor, a protein present on the membranes of many cell types, allows hematopoietic stem cells—the cells that ultimately give rise to new blood cells—to find their way to the proper place in the bone marrow where blood cell production, known as hematopoiesis, takes place (1). In addition to suggesting possible avenues for the improvement of stem cell transplantations, this specification of a novel role for a well-known protein will compel researchers and clinicians to reconsider the relationship between the formation of bone and the formation of new blood cells.

"Bone and bone marrow are very closely linked together. This is something that has been overlooked, but it is clear now that a person's bones do an awful lot for hematopoiesis," says Gregor Adams, lead author of the new study and an instructor in medicine at Massachusetts General Hospital. "What the current study says loud and clear to investigators in the bone field is that bone and blood are a truly integral combination of cells that talk to each other," agrees Laura Calvi, co-author, along with Adams, of an earlier 2003 study that offered the first *in vivo* evidence that osteoblasts, the cells that make bone, regulate blood cell production. "The real take-home message," also agrees Russell Taichman, author of a 2005 review article on the relationship between bone formation and blood formation, "is that, when we study bone in isolation, it's important to remember that the effects on bone have hematopoietic consequences, so we really need to be looking at the whole organ, the marrow and the bone, at the same time."

The first systematic study to provide evidence that osteoblasts might play a role in hematopoiesis was published in 1994 when Taichman, a professor of dentistry at the University of Michigan School of Dentistry, along with colleague Stephen Emerson found that, *in vitro*, osteoblasts produced granulocyte colony-stimulating factor, a growth factor known to stimulate blood cell production. However, nearly a decade elapsed until a similar role for osteoblasts was demonstrated in an animal model. Here, in the 2003 study mentioned above, a team of investigators including Adams and Calvi, an assistant professor of medicine at the University of Rochester School of Medicine and Dentistry, examined a group of mice genetically engineered to overproduce a receptor that stimulates the growth of osteoblasts. They discovered that these mice, who produced increased numbers of osteoblasts, also exhibited increased numbers of hematopoietic stem cells (HSCs) in the bone marrow. They further found that the osteoblasts of these transgenic animals produced high levels of Jagged1, a molecule that binds to a receptor, called Notch-1, that is present on HSCs and is known to regulate the developmental pathway that a variety of cells, including HSCs, follow. The osteoblast then, the researchers concluded, through its associated Jagged1 and the signaling pathway this protein activates, helps to create a niche or specialized microenvironment near the surface of bone that enables HSCs to function properly. The current study builds upon this earlier work and further solidifies a role for bone formation in hematopoiesis. Specifically, it argues that the calcium concentration at the niche enables HSCs to be retained at that niche, and the calcium-sensing receptor

(CaR) is an integral component of the mechanism via which this occurs.

The investigators had two reasons to suspect that the CaR might serve this function. First, they knew that HSCs are found next to bone, the body's major storage source of calcium. They also knew that the CaR is present on other hematopoietic cells. For instance, earlier work demonstrated that the CaR seemed to help monocytes, white blood cells involved in the body's immune response, localize to areas of high calcium concentration present during inflammation and injury. Consequently, Adams and his colleagues wondered whether the CaR in HSCs might play a similar role and allow HSCs to localize to areas of high calcium concentration next to bone. To test this hypothesis, the researchers first verified the presence of the CaR on HSCs. This was itself a formidable achievement, not only because stem cells are difficult to identify, but also because the receptor is produced at much lower levels than in other cells, such as those of the parathyroid glands. Consequently, the next step was to genetically "knock out" this receptor and then to examine the impact. The scientists discovered that while HSCs were present in the circulation and spleen, they were present only in significantly reduced numbers in the bone marrow, which suggested that they were unable to find their way to that particular location.

What factor caused this notable defect in the stem cells? During embryonic development, HSCs migrate from the fetal liver to the bone marrow, so the investigators considered the possibility that the HSCs were not produced properly, or failed to function properly, before they made their journey. However, HSCs from the fetal livers of the transgenic mice were normal in number and function. As a result, transplantation experiments were designed to test whether the defect was one intrinsic to the HSCs themselves that prevented proper localization to the stem cell niche in the bone marrow. In these experiments, the investigators first transplanted fetal liver cells from mice with the CaR into mice whose bone marrow had been destroyed by radiation, and observed that the transplant allowed the irradiated mice to survive. Next, they transplanted fetal liver cells from the transgenic mice missing the CaR into irradiated mice and compared the result. While this transplantation also produced 100% survival, irradiated mice who had received cells from the transgenic animals had fewer HSCs in the bone marrow than mice who had received cells from the normal animals.

From this outcome, the researchers concluded that the defect in the HSCs resided only in the HSCs themselves and impacted their ability to find their way to the niche. Since knocking out the CaR can significantly alter the animal's environment—CaR inactivation causes an abnormally high concentration of calcium in the blood, for instance—and because it is always possible that the defect arose because of one of these broader environmental alterations, the results of the transplantation experiments put this concern to rest. "Once the transplantation experiments are performed," Calvi says, "it's very difficult to explain the behavior by the HSCs as an environmental effect. Instead, it's clear that the cells have lost their ability to see a beacon, since when they are put into a normal environment, they cannot find the place where they need to go."

With this general understanding of the nature of the HSC abnormality generated from the earlier experiments, the investigators then tried to pin down the nature of the defect more precisely. Interestingly, fetal liver HSCs from the transgenic mice were normal in many ways. For instance, they produced normal levels of several molecules already known to help HSCs find their way to the bone marrow. However, the HSCs from the transgenic mice did not bind properly to collagen I, an extracellular matrix protein produced by osteoblasts near the bone surface. "Cells that lack the calcium-sensing receptor can get to the bone marrow," Adams says, "but they can't get to the correct location where they are meant to be," because of the difficulty they have in binding to this protein.

The specific manner in which the CaR mediates binding to collagen I remains to be worked out, including the identification of the putative molecule whose production, in response to CaR activation, facilitates the binding. "What is the cell-surface receptor that binds to the collagen?" wonders Edward Brown, co-author of the current study and leader of the team that first cloned and characterized the CaR from bovine parathyroid cells in 1993. "How is it regulated by the calcium-sensing receptor? Is its expression increased on the cell-surface when the calcium-sensing receptor is activated? Is there increased gene transcription for this molecule?" Since, in response to CaR activation, there are known instances of increases in the production of adhesion molecules known as integrins that allow cells to bind to components of the extracellular matrix, and also because there are antibodies that block many of these molecules, a clear experimental avenue to pursue has already emerged. "You could block the adhesion molecule with an antibody and see if it turns a normal stem cell into a calcium-sensing receptor knockout stem cell," says Brown, also a professor of medicine at Harvard Medical School and leader of a group of co-authors for the current study based at Brigham and Women's Hospital. Brown is also interested in studying the precise impact the receptor has on the fate of a stem cell, which can either divide into a new stem cell, differentiate into a more specialized cell, or die. "Does the receptor determine the choices the cell makes between those three possible outcomes?" he asks. Finally, Brown is also interested in investigating the potential role of other molecules that might also stimulate the CaR.

Though further experiments are necessary to elucidate the role of the CaR in stem cell function, the clinical implications of the current study have already materialized. Indeed, there are already agents in use that stimulate or inhibit the CaR. Consequently, it is quite conceivable that these agents could be used for clinical purposes in the stem cell realm: antagonists could be employed to block the CaR and thus to mobilize stem cells from the HSC niche into the circulation. Conversely, CaR agonists could be used to stimulate the receptor and thus to help cells better engraft in the niche during stem cell transplantations. These principles are now being tested in pre-clinical murine models by Adams and his colleagues.

While future studies will address remaining questions, the current study makes the link between the formation of bone and the formation of blood cells difficult to ignore. When considered along with additional evidence of the link from animal models—for instance, transgenic mice that lack the gene for Cbfa1/Runx-2, a transcription factor known to control the differentiation of osteoblasts, not only fail to show bone formation, but also fail to develop bone marrow and consequently exhibit abnormal hematopoiesis—the current study solidifies the idea that osteoblasts help to regulate hematopoiesis. This is a notion that, historically, has been slow to catch on. Indeed, bone researchers have previously focused more narrowly on the origin of osteoclasts, the cells that break down and reabsorb bone, from the monocyte-macrophage blood cell lineage as the "site where bone and bone marrow interact, instead of concentrating more generally on hematopoiesis as a process that occurs under the control of a conversation between bone cells and bone marrow cells," as Brown puts it. The new research on the CaR has just made this bone-blood dialogue much louder.

References

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