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NEWS

Roles for Cilia in Bone Cells?

New results sharpen the focus on mechanosensory mechanisms

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New research from an interdisciplinary team of kidney and bone specialists has demonstrated the presence of cilia on osteoblasts and osteocytes, and has linked polycystin-1, a protein that associates with cilia and allows them to sense fluid flow in the kidney, with skeletal development in mutant mice (1). When considered along with research by other groups, the new work may herald a new area of investigation in bone biology research. However, whether the study of cilia and bone becomes a truly fruitful avenue of exploration will depend on whether convincing links between cilia and bone function can be demonstrated, particularly *in vivo*, in knockout animals missing cilia and cilia-associated proteins specifically from bone cells.

The Research

Cilia have long been known to occupy the cell surfaces of almost every kind of vertebrate cell, and there has been increasing interest in these hair-like projections amongst biologists (2). However, there had been scant evidence for the presence of cilia in bone cells. In the current study, immunofluorescence, scanning electron microscopy and whole mount immunostaining were used to demonstrate the presence of cilia-like structures in osteoblast and osteocyte cell lines and in primary osteocytes and osteoblasts from the calvarial bones of mice. The investigators also demonstrated the expression of *Pkd1*, the gene that codes for polycystin-1; *Pkd2*, which codes for the related polycystin-2; and the genes encoding polaris and Kif3a, two proteins required for proper cilia formation.

"I don't think there should be any more controversy in the field now whether or not there are cilia on osteocytes," says Lynda Bonewald, a co-author of the current study who identified cilia in the cell lines and primary cells. "What this paper does is show that primary cilia exist in bone cells," agrees Christopher Jacobs, senior author of research presented at the recent ASBMR meeting in Philadelphia that also identified the presence of cilia in osteoblasts and osteocytes from mouse tibial bones.

After confirming the presence of cilia, the researchers, led by senior author L. Darryl Quarles, examined knockout mice they had created with an inactivating mutation in *Pkd1*. They were intrigued by the similarity between the phenotype of these mice and that of mice deficient in the master regulatory gene *Runx2*. Embryos from *Pkd1* homozygous mice exhibited delays in endochondral and intramembranous bone formation, and these changes were associated with decreases in the expression of *Runx2*.

The investigators also found changes in adult mice: heterozygous *Pkd1* mutants were osteopenic, exhibiting a bone mineral density decrease of 9% at three months of age, compared to wild-type animals. Further studies ascribed these bone defects to defective osteoblast function. In addition, osteoblasts taken from the embryos of homozygous mutants also exhibited defects, including significant decreases in *Runx2* mRNA and protein levels, as well as in levels of osteoblast differentiation markers like osterix and osteocalcin. Finally, gain-of-function experiments, where the C-terminal end of the polycystin-1 protein was overexpressed in osteoblasts, restored *Runx2* activity in the osteoblasts of

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homozygous mutants to levels seen in wild-type osteoblasts.

While these results are suggestive, the authors are very careful to note that whether cilia and polycystins have functionally important roles in bone remains to be proven. "While the presence of cilia and the polycystin complex in osteoblasts and osteocytes and the finding of bone abnormalities in mice with mutant polycystin-1 are compelling, in order to definitively assess the specific function of the cilium and polycystin complex as direct regulators of bone function, we need to perform studies that selectively delete polycystin-1 or polycystin-2 or ciliogenic proteins in osteocytes," Dr. Quarles stresses. "These are the critical genetic models that will allow us to define the importance of these molecules and ultimately their role in mechanosensing in bone," says Quarles, the Summerfield Endowed Professor of Nephrology, and director of The Kidney Institute, at the University of Kansas Medical Center.

While Dr. Quarles and his colleagues are working on these kinds of studies, other groups have been conducting similar studies of their own. In fact, there is already compelling evidence, from studies on cartilage-forming chondrocytes, that some of the ciliogenic proteins that give rise to cilia have key roles in the skeletal system. Indeed, in research presented at the last ASBMR meeting, a group led by Rosa Serra removed cilia from chondrocytes by deleting Kif3a, as well as polaris. The changes the group observed were striking.

"The mice develop normally, but then develop a postnatal dwarfism. Between birth and one month of age, the growth plate prematurely closes, due to a defect in chondrocyte proliferation and accelerated hypertrophic differentiation of the chondrocytes," says Serra, an associate professor of cell biology at the University of Alabama at Birmingham. "Interestingly, the orientation of the chondrocytes is altered," Dr. Serra continues. "Normally, the cells of the growth plate are aligned in very organized, neat rows. That organization is completely lost in these mice."

A group led by Bjorn Olsen, a professor of cell biology at Harvard Medical School, is also pursuing evidence for functional roles for polycystins and cilia in the skeletal system. His team is examining polycystin-1 knockout mice missing the protein in specific skeletal tissues, as well as knockouts missing specific ciliogenic proteins.

A Mechanosensory Role?

Knockout experiments of this sort may lend further support to the opinion, held by many of the bone specialists who spoke to *BoneKEy*, that cilia may play multiple roles in the skeletal system, just as they do in other systems. However, a specifically mechanosensory role for cilia in bone is among the possibilities generating the most intense interest. The presence of cilia, particularly in osteocytes, is especially exciting to bone researchers interested in how the skeleton responds to mechanical stimulation because the identity of the mechanosensing molecules and mechanisms that allow bone to sense mechanical forces remains a mystery.

Cilia are attractive contenders to play a mechanosensory role because of their ability to sense interstitial fluid flow, which may produce a shear stress on bone that osteocytes can recognize. "The shape and the size of primary cilia — the fact that they stick out from the cell, and that they are actually fairly rigid — makes them good candidates for structures that could respond to fluid flow," says Charles Anderson, who, as a co-author of the ASBMR abstract submitted by Dr. Jacobs and colleagues, confirmed the presence of cilia on osteocytes and osteoblasts and removed the organelles in order to study cell responsiveness to fluid flow. "One of the challenges is that the physical properties of flow are such that flow diminishes close to the cell surface, but if you have something sticking up into the fluid, it would allow the cell to sense flow more effectively than just by sensing it at the surface," says Andersen,

a graduate student in the department of biological sciences at Stanford University.

The idea that cilia might act as fluid flow mechanosensors in osteocytes also receives support from the demonstration of exactly that kind of behavior in kidney epithelial cells, where cilia sense fluid flow through polycystin-1 and polycystin-2. Deleting these proteins specifically in osteocytes, and then testing how animals without them respond to mechanical strain, will go a long way towards confirming a mechanosensory role for cilia in bone, and experiments of this kind are in the works.

However, just because cilia act in a specific way to sense fluid flow in the kidney does not necessarily mean they will function in an identical fashion in bone, says Charles Turner, a professor of biomedical engineering at Indiana University-Purdue University Indianapolis who notes an interesting clue that mechanosensation might work differently in the two tissues. "When there is mechanosensation on a renal epithelial cell in the kidney, that cell remains an epithelial cell — it keeps its phenotype," Dr. Turner observes. "But what we expect mechanosensation to do in bone cells is to tell them to go through a process that allows them to start making bone stronger or more rigid — to change their phenotype. Thus, if cilia are functioning as mechanosensors in bone, they are probably working in a different way than they do in the kidney."

There are further challenges to theories positing a mechanosensory role for cilia in osteocytes. For instance, one conceptual difficulty concerns whether enough space exists to allow cilia on osteocytes to fit in the bone environment in a way that would allow them to act as effective mechanosensors, according to Dr. Turner. "A typical cilium is about 2 to 20 microns in length, and osteocytes sit in a cave of bone that only gives about a micron or less of space between the cell membrane and the bone lacunar surface, which doesn't create any room for a cilium to stick out off the bone surface very far," Dr. Turner notes.

Another conceptual problem is that the part of the osteocyte that seems most suited to detect fluid flow doesn't even appear to contain a cilium. "There is only one cilium per cell, and that cilium is on the cell body, instead of on the dendritic-like projections that come out of osteocytes," says Henry Donahue, a professor of orthopaedics and rehabilitation at Penn State University College of Medicine who studies the effects of fluid flow on cell-cell communication between osteocytes and osteoblasts. "These dendritic processes are in the best position, because of their location in a geometrically defined area of the canaliculi, to detect strain from fluid flow. However, the cilia aren't located there, as far as we know," he points out. Dr. Donahue does note that proteins associated with cilia could still be involved in mechanosensation, if not cilia *per se.*

A further complication is that cilia could be sensing a number of different kinds of forces. A fluid flow force is a type of force often postulated, but it is possible that cilia are acting as chemosensors. In fact, Dr. Quarles notes that there is even some debate on how cilia and polycystins act in the kidney, with some researchers suspecting they function as chemosensors that detect chemicals in the environment. It could even be that cilia serve as vibration sensors, and Dr. Quarles and his colleagues are planning studies to determine more precisely the nature of the force that cilia may recognize.

Cilia could also be acting as mechanosensors in more than one cell type in bone, including the osteoblast. In results presented at ASBMR, Dr. Jacobs and his colleagues used either siRNA knockdown or a chemical removal process to inhibit cilia in osteoblasts and osteocytes, and found defects in both cell types. "Bone cells that have had their cilia removed or interfered with are unable, or less able, to respond to mechanical forces in culture," says Jacobs, an associate professor of mechanical engineering at Stanford University, as well as the director of the Palo Alto DVA Center for Bone and Joint Rehabilitation Research and Development.

The crucial test though, he stresses, is whether similar results can be generated *in vivo*. "If you apply loading to the bone of a mutant mouse with defective polycystin or primary cilia, is that bone less sensitive to mechanical loading? Those will be the key experiments," says Jacobs, who is planning experiments along these lines using knockout animals missing the Kif3a protein that is necessary for cilia formation from osteoblasts and osteocytes.

Excitement about cilia has surged recently in the field of biology. However, only new data to come from experiments in living animals will determine if cilia, once viewed as nothing more than useless, vestigial structures, will ultimately deserve a prominent place in bone biology research as well.

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