Public release date: 2-Sep-2008

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Researchers offer first direct proof of how osteoarthritis destroys cartilage

Goal: preventative medicine for the leading cause of US disability

A team of orthopaedic researchers has found definitive, genetic proof of how the most common form of arthritis destroys joint cartilage in nearly 21 million aging Americans, according to a study published online Sept. 2 in the *Journal of Bone and Mineral Research.* The findings serve as an important foundation for the design of new treatments for osteoarthritis (OA), researchers said.

OA gradually destroys all cartilage in joints while forming scar tissue and painful bony growths. Advanced cases bring deformity and severe pain as patients loose the protective cushion between bones in weight-bearing joints like knees and hips. Until the late 1980s, OA was regarded as part of growing old. Since then, studies have revealed that biochemical changes contribute to the disease that might be reversed by drugs. Current medications, NSAIDs and Cox 2 inhibitors, are used to reduce symptoms in patients with mild cases, and joint replacement surgery for severe cases. Few options exist for those in between.

Going into the current study, little was known about the cellular and molecular events that cause cartilage to break down in osteoarthritic joints. Past studies had suggested that higher levels of a key signaling protein, beta-catenin, were connected to osteoarthritis, but there was no direct evidence to confirm it, or to suggest its role. The current study provides both.

Researchers genetically engineered adult mice to have high levels of beta-catenin, and saw that they lost most of their articular cartilage, the protective layer that covers the ends of bones within joints. The mice also developed the same bony growths and microfractures seen in the joints of human OA patients. A companion experiment on human cartilage cells taken from patients with severe arthritis also confirmed that their beta-catenin levels were much higher than normal.

"We have created study the first model in a living animal that shows exactly how osteoarthritis causes damage," said Di Chen, M.D., Ph.D., associate professor in the Department of Orthopaedics at the University of Rochester Medical Center, and lead author of the study. "That of course puts us in position to interfere with the processes that contribute to the damage in a new and powerful way."

Study Details

Research teams from Oxford, and from Leiden University in The Netherlands, published the results of gene-mapping studies in 2004 and 2005 that found people with an extremely rare genetic mutation were much more likely to develop osteoarthritis. The mutation was in the frzb (Frisbee) gene, known to code for a protein called sFRP3 that normally keeps beta-catenin levels in check. This link between the frzb mutation, beta-catenin and osteoarthritis was still a hot topic last November at the annual meeting of American College of Rheumatology in Boston. When Chen heard about it from returning colleagues, he joined the race to provide the first direct, genetic evidence in a live, adult mouse that raising beta-catenin levels creates the same effects as osteoarthritis in aging human joints.

To win the race, Chen's team had to overcome a stubborn obstacle. A standard method for determining the function of a protein like beta catenin is to remove the gene that codes for that protein from the embryo of a mouse, and then to observe the biochemical consequences of that removal in the new breed. In many cases, however, the same genes that direct healthy function in adults also control the development of the animal from an embryo into a fetus. Attempts to "knock down" the action of such genes in the embryo are fatal, and long before researchers can study the effect of changes in gene expression that come with age. Maintaining precise levels of beta-catenin, for instance, is vital to the healthy development of bones and cartilage in the fetus.

Chen and colleagues solved the problem by engineering and crossing lines of transgenic mice. They created a mouse with a built-in genetic system that could increase the levels of beta-catenin, but only in response to a specific drug trigger in an aging adult (versus in the womb), and only in a specific cell type (articular cartilage cells). The newly designed beta-catenin conditional activation (cAct) mice represented the first proper tool to study the effect in a live animal, and offered the first direct evidence of a pathway hinted at in the gene mapping studies.

Researchers administered tamoxifen, the chosen drug trigger, to turn up production of beta-catenin production in three- and six-month-old conditional activation mice. Researchers then examined the articular cartilage tissues two months later to look for structural and morphological changes. They found severe destruction in the articular cartilage of eight-month-old beta-catenin cAct mice. Even at the molecular level, the joints of the study mice mimicked those seen in human OA patients. Processes underway meant to heal the joint only added to disease by mistakenly forming bone where cartilage should be and by causing misguided cell growth. Control mice without high levels of beta-catenin expression experienced no damage to their cartilage.

Further analysis found that too much beta-catenin signaled for higher production of an enzyme, matrix metalloproteinase 13 (MMP-13), known to preferentially break down and destroy the type 2 collagen that makes up 90 percent of articular cartilage.

Secondly, higher beta-catenin levels were found to bring about a nearly sixfold increase in expression versus controls of the gene for bone morphogenic protein 2 (BMP-2), which encourages the differentiation of cartilage into bone. In the womb, bone develops in a two-step process: stem cells become cartilage and cartilage is replaced by bone, a process tightly controlled by signaling molecules that include beta-catenin. The same process occurs when bones heal in adults. While the transition of cartilage cells into bone is natural, it is not meant to occur in joints, where cartilage is prevented from becoming bone to maintain a cushion. In addition, higher BMP-2 levels have also been associated by past studies with the formation of painful, bony growths called osteophytes in osteoarthritic joints.

While the original gene mapping studies provided clues about the causes of osteoarthritis, they created mystery as well. The frzb gene mutation found to cause a rise in beta-catenin is extremely rare, but tens of millions of people develop osteoarthritis as they age. Something beside the frzb mutation must be causing most cases. One theory has it that the mechanical force created by the weight of the body on joints over time is converted into ever stronger biochemical signals for more beta-catenin. While the force applied to joints cannot be reduced (except by weight loss), destructive signals sent in response to that force might be shut down by future drugs.

Another theory proceeds from the fact that patients with injuries to the meniscus, the sponge-like layer of cartilage that sits between the bones of the knee, are much more likely to develop osteoarthritis in the ensuing years. Could the deteriorating meniscus be signaling nearby articular cartilage to raise beta-catenin levels?

Chen's team has studies underway looking at whether meniscal injuries or biochemical reactions to mechanical force cause beta-catenin levels to rise. Other studies are already examining exactly how beta-catenin signaling changes levels of BMP-2 and MMP-13 in articular cartilage cells.

Along with Chen, Mei Zhu, Qiuqian Wu, Mo Chen, Chao Xie, Randy Rosier, Regis O'Keefe and Michael Zuscik led the work within the Department of Orthopaedics and Center within the University of Rochester School of Medicine and Dentistry. Dezhi Tang led the effort at the Spine Research Institute at Shanghai University of Traditional Chinese Medicine in Shanghai, China, as did Suyang Hao in the Department of Pathology at the University of Massachusetts Memorial Medical Center. The work was supported by the National Institutes of Health.

"The first step was to prove that beta-catenin is central to OA development, and I think we have done that," Chen said. "Now we are seeking to confirm that mechanical loading and mensical injury create higher levels of beta-catenin in osteoarthritic joints, and that this in turn causes cartilage destruction and too fast differentiation of cartilage into bone."

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