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Discoveries May Help Scientists Understand Why Disease Turns Soft Tissue Into Bone

Scientists have created a new mouse model that may help researchers explain how a rare disease causes otherwise supple soft tissue and joints to turn into bone.

Fibrodysplasia ossificans progressiva (FOP) is a rare congenital disease that affects the connective tissue, causing muscles, ligaments, and other soft tissues to turn to bone. The process of ossification often occurs following traumatic injury to the soft tissue, and can progress over time to cause nearly all joints of the body to become permanently frozen in place. Affected individuals develop problems speaking, breathing, and eating. There is no treatment, and many people who have FOP die in their twenties or thirties.

Using genetically modified mice, Paul Yu and his colleagues have developed a model that mirrors many aspects of the bone-forming disease. The scientists have shown that treating these mice with a drug that blocks the overactive receptor that causes FOP can reduce abnormal bone growth and preserve joint function. Yu is first author on the paper presenting the results, which is being published on November 30, 2008, in the journal *Nature Medicine*.

"The model of FOP provides a platform for investigating this and other experimental therapies and for understanding how soft tissues can turn into bone in other contexts, such as following joint fractures, burn injuries, or joint replacement surgeries."

- Paul Yu

In 2008, Yu received an Early Career Physician-Scientist Award from the Howard Hughes Medical Institute (HHMI) that provides promising

physician-scientists with the financial support needed to develop their research programs at the beginning of their academic careers. The award is part of a long-time push by HHMI to increase the number researchers who translate basic science discoveries into improved treatment for patients.

Yu, who is at Massachusetts General Hospital and Harvard Medical School in Boston, said the mice harbor a mutation to very similar to those found in humans with the FOP. “The mice appear to develop the lesions only after there is injury or inflammation, which reminiscent of the fact that the human disease frequently flares up in the setting of trauma or viral illness.” Although he is encouraged by this advance, Yu remains cautious about his team's results. The mouse model has some characteristics that are different from the human disease, including having a mutation distinct from that of affected humans, he says, and researchers are still far from testing the drug on humans who have FOP.

Several years ago, scientists linked FOP to overactivity of the bone morphogenetic protein (BMP) signaling pathway. BMP signals help control tissue repair and regulates the differentiation of cells into specialized tissue. BMP signals normally facilitate the development of the skeleton, as well as the specialized structures of organs. Recent studies from Frederick Kaplan and Eileen Shore at the University of Pennsylvania showed that the molecular defect that causes FOP most frequently results from an inherited mutation in the BMP receptor, ALK2. The mutation results in uncontrolled BMP signaling.

At the outset of his medical career, Yu had no idea he would one day study this mysterious disease. As a medical student, he participated in the HHMI Medical Fellows Program, which permitted him to spend a year in the lab of Jeffrey Platt at Duke University Medical School. (Platt is now at the University of Michigan.) There, he studied antibodies that cause rejection in an experimental organ transplantation model. After completing his M.D. and Ph.D. studies, as well as internal medicine and cardiology training, Yu pursued postdoctoral research at Massachusetts General Hospital with his mentor Kenneth Bloch, studying the function of BMPs in the heart and circulatory system. “BMP signaling affects the development of the blood vessels and the heart. In the course of studying that I realized that there weren't very many pharmacologic tools for manipulating BMP signaling,” Yu says. At Harvard, Yu worked with a colleague Charles Hong (now at Vanderbilt University), who identified a chemical that inhibited the function of BMP signals in developing zebrafish.

Yu and Hong realized that not only could these compounds block BMP signals in the heart and circulatory system, they could also be used to treated conditions caused by excessive BMP signaling, such as FOP. The more he read about the disease, the more he began thinking about developing new tools for studying FOP.

To model diseases of overactive BMP signaling, Yu collaborated with Yuji Mishina of the National Institute of Environmental Health Sciences (now at the University of Michigan), who had produced mice harboring a mutant BMP receptor similar to that of humans with FOP. Mishina had already shown that mice that produce this mutant receptor as embryos do not survive to birth. “BMP signals are very important for normal development,” Yu says, “and the effect of overproducing this particular mutant was not tolerated in development.” This is in contrast to humans with FOP, who have no apparent defects at birth.

The team then tried to turn on the mutant *ALK2* gene after birth, using a synthetic hormone to trigger recombination of the mutant gene. That strategy allowed the mice to survive while making mutant protein, but they showed no signs of the bone-forming disease. “The fact that it didn't lead to any ossification really threw us for a loop,” Yu says. “Here we were inducing BMP signals at higher than physiological levels...and the animals did not seem to have bone abnormalities.”

The team had one more idea. For some people with FOP, the disease does not strike until a traumatic injury damages tissues, and the healing process goes awry. For others, viral infections like influenza can ignite an inflammatory response that leads to FOP. So they tried again, this time activating *ALK2* using a virus, which also provoked an immune response. “If we turned on the gene via hormones, we got no inflammation and no ossification. But if we induced it via a virus, we did get inflammation and did get bone. It was very rapid,” Yu says. Within seven days, the mice had early bone and cartilage formation, which made their joints stiff and less mobile. These mice had important similarities to those of FOP, such as the development of marrow within bony lesions.

While they were developing the FOP mouse model, Yu's team was also tweaking the BMP inhibitor that had worked in zebrafish, in collaboration with chemist Greg Cuny and chemical biologist Randall Peterson, who are both at Harvard. “We engaged in a yearlong effort to find a more selective, potent and metabolically stable derivatives. We examined nearly a hundred derivatives and many of them didn't work at all,” Yu says.

Eventually, the researchers identified a derivative of the BMP inhibitor that was potent and stable enough to work in mice. Mutant mice treated with the inhibitor developed much fewer bone lesions than untreated mice. The BMP inhibitor appeared to diminish the overzealous bone-growth instructions from the mutant *ALK2* receptor. “The model of FOP provides a platform for investigating this and other experimental therapies and for understanding how soft tissues can turn into bone in other contexts, such as following joint fractures, burn injuries, or joint replacement surgeries,” Yu explains.

There is still a lot of work to do, Yu says. Completely inhibiting BMP might not work in all cases - for example, it is not known what the long term effects of blocking BMP signals might be, especially in a growing individual. Yu

also wants to find out whether the drug can also inhibit other types of abnormal bone formation or calcification. “As cardiologists, we'd also like to know whether abnormal calcification of blood vessels or heart valves may be blocked with a similar strategy.”