PIEZO de resistance

By Benjamin Boettner, Senior Writer

A pair of recently identified calcium channels—PIEZO1 and PIEZO2—control the response of cartilage to heavy loads and offer new targets for joint injury and osteoarthritis, according to findings from a team at **Duke University**.[1] A channel-blocking peptide protected cartilage cells from injury-induced apoptosis and could lead to a new therapy, but some researchers believe it is premature to take that route without knowing more about the channels' role in cartilage pathology.

The discovery nevertheless adds useful information to the field because although calcium currents have been known to promote both cartilage cell health and death, the channels involved have been elusive.

Earlier this year, the same Duke team showed that calcium influx through the transient receptor potential vanilloid 4 (TRPV4; VRL2) channel has the opposite effect and is involved in keeping cartilage cells—called chondrocytes—healthy.[2]

> **"With our analysis of TRPV4 and the PIEZO proteins, we discovered a yin and yang of cartilage destruction."**
> *—Wolfgang Liedtke, Duke University Medical Center*

In that study, the team showed that TRPV4 currents mediate chondrocyte response to mild mechanical loading and that this process stimulates the synthesis of cartilage-stabilizing extracellular matrix components.

Wolfgang Liedtke, who co-led the team with Duke colleague Farshid Guilak, told *SciBX* that the field has toyed for some time with the notion of directly targeting mechanosensing mechanisms in chondrocytes but has not been able to characterize the relevant proteins.

"With our analysis of TRPV4 and the PIEZO proteins, we discovered a yin and yang of cartilage destruction," he said.

Liedtke is an associate professor of neurology, neurobiology and anesthesiology and Guilak is a professor, vice chair of orthopedic surgery and director of orthopedic research at **Duke University Medical Center**.

Articular cartilage takes the brunt of bearing weight in synovial joints—which include knees, hips, elbows and wrists—but until the Duke team reported its findings, little was known about how cartilage cells conduct calcium to respond to excess loads.

To explore the mechanisms involved, the Duke team started from the basis that chondrocytes undergo apoptotic cell death after acute injury and in certain forms of osteoarthritis (OA)—which leads to continual cell loss because in adults chondrocytes do not divide.

The researchers focused on PIEZO1 (piezo-type mechanosensitive ion channel component 1) and PIEZO2—whose names are derived from the Greek *piezein*, meaning to squeeze or press—because in the last few years the channels have been shown to transduce mechanical signals in several cell types, such as red blood cells and bladder urothelium, and to play a role in touch sensation.

To start, the team showed that both PIEZO channels had high expression in articular cartilage cells from mouse hip and knee joints and in primary chondrocytes from pigs and humans.

To test whether Piezo1 and Piezo2 had altered activity following mechanical loading, the team transfected the two channels individually or together into a mouse neuronal cell line in which they are not normally expressed.

Using an atomic force microscope to exert pressure on the cells and measure the Ca^{2+} currents, the group showed that when coexpressed, the channels responded to pressure with a robust and sustained calcium influx. When expressed alone, neither channel produced a significant response.

The team then applied strong pressure to pig-derived chondrocytes to deform the cells and inhibited PIEZO1 and PIEZO2 using siRNA knockdown. Eliminating the activity of either channel ablated the majority of the calcium conductance, which supported the suggestion that the two channels work in tandem—and possibly in a complex. Blocking channel activity with GsMTx4, a peptide derived from tarantula venom that inhibits PIEZO channels, likewise suppressed the pressure-induced calcium conductance.

Finally, in explants of pig cartilage subjected to high amounts of pressure, application of GsMTx4 prevented chondrocyte death.

The team reported its findings in the *Proceedings of the National Academy of Sciences*. The study was coauthored by Frederick Sachs, a professor of biophysics at the **State University of New York at Buffalo** (SUNY Buffalo), whose group discovered GsMTx4 in 2000.

Liedtke and Guilak told *SciBX* that their next step will be to test whether direct delivery of the peptide to the joint can rescue chondrocyte death in the knee meniscus of traumatized mice—a model of acute joint injury. "It will be important to assess whether intra-articularly injected GsMTx4 can reverse damage after the impact has hit," said Liedtke.

Channeling spider venom

According to Jessica Bertrand, several animal models are available that could help validate—or refute—the findings, but the real breakthrough will be to connect PIEZO channel expression to disease mechanisms. Bertrand is a group leader funded by the Emmy-Noether young investigator program at the **University of Muenster**. Her research focuses on regenerative cartilage biology.

"The most important question for me to be answered now is whether there is a pathology associated with the regulation of PIEZO expression in cartilage," she said.

She added that before moving ahead with the inhibitor, its effects on chondrocyte biology—including how it influences collagen, the joint-specific proteoglycan aggrecan (ACAN; CSPG1) and cell death mechanisms—should be assessed in more detail and over longer

"The most important question for me to be answered now is whether there is a pathology associated with the regulation of PIEZO expression in cartilage."
—Jessica Bertrand, University of Muenster

periods. "If chondrocytes tend to differentiate to a hypertrophic phenotype or dedifferentiate into a fibroblastic phenotype, the substance would be no help," she said.

However, Bertrand agreed that once the team moves to animal models, the meniscus knee joint model of OA is a valid one to pursue. She added that a recently developed blunt impact model and a treadmill model of OA involving mechanical overuse could help stratify the effects of the inhibitor in different forms of OA.

Tonia Vincent told *SciBX* that conditional knockout models involving cartilage-specific deletion of the PIEZO proteins could help validate the channels as therapeutic targets. "If these mice are resistant to osteoarthritis—by surgical joint destabilization, say—then this would be the most convincing evidence that blockade would be efficacious," she said.

Vincent is an honorary clinical research fellow in the Department of Medicine at **Imperial College London** and runs the *in vivo* osteoarthritis program at the **Kennedy Institute of Rheumatology at the University of Oxford**.

Although Liedtke agreed that understanding the basis of PIEZO signaling in inflammation is important, his team is moving ahead with studies on the inhibitor, and he believes it could lead to new therapeutics.

Philip Gottlieb, who works with Sachs, told *SciBX*, "GsMTx4 is the D-enantiomer of a natural tarantula venom peptide identified in screens as an inhibitor of endogenous, and at the time unknown, calcium channels."

He added, "The compound inserts itself at the boundary between the channel and the lipid bilayer of target cells. The D-form shows greater stability than the natural peptide and has no toxicity in our tests. It has all the properties that we need, and we are currently trying to figure out whether it works only on PIEZO1 or on both channel proteins *in vivo*."

Gottlieb is a research associate professor at SUNY Buffalo. Sachs, Gottlieb and two other SUNY Buffalo researchers cofounded **Tonus Therapeutics Inc.** in 2009.

Duke University has not applied for a patent covering the data reported in *PNAS*. However, the team is looking for commercial partners to develop the findings.

The IP for GsMTx4 is owned by SUNY Buffalo and licensed to Tonus. In September, **Akashi Therapeutics Inc.** acquired global rights to GsMTx4 from Tonus and has the compound—now dubbed AT-300—in preclinical development to treat Duchenne muscular dystrophy (DMD).

Boettner, B. *SciBX* 7(46); doi:10.1038/scibx.2014.1368
Published online Dec. 11, 2014

REFERENCES

1. Lee, W. *et al. Proc. Natl. Acad. Sci. USA*; published online Nov. 10, 2014; doi:10.1073/pnas.1414298111
   Contact: Wolfgang B. Liedtke, Duke University Medical Center, Durham, N.C.
   e-mail: wolfgang@neuro.duke.edu
   Contact: Farshid Guilak, same affiliation as above
   e-mail: guilak@duke.edu
2. O'Conor, C.J. *et al. Proc. Natl. Acad. Sci. USA* **111**, 1316–1321 (2014)

COMPANIES AND INSTITUTIONS MENTIONED

Akashi Therapeutics Inc., Cambridge, Mass.
Duke University, Durham, N.C.
Duke University Medical Center, Durham, N.C.
Imperial College London, London, U.K.
Kennedy Institute of Rheumatology at the University of Oxford, Oxford, U.K.
State University of New York at Buffalo, Buffalo, N.Y.
Tonus Therapeutics Inc., Williamsville, N.Y.
University of Muenster, Muenster, Germany