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Newts' ability to regenerate tissue replicated in mouse cells

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Tissue regeneration a la salamanders and newts seems like it should be the stuff of science fiction. But it happens routinely. Why can't we mammals just re-grow a limb or churn out a few new heart muscle cells as needed? New research suggests there might be a very good reason: Restricting our cells' ability to pop in and out of the cell cycle at will -- a prerequisite for the cell division necessary to make new tissue -- reduces the chances that they'll run amok and form potentially deadly cancers.

Now scientists at the Stanford University School of Medicine have taken a big step toward being able to confer this regenerative capacity on mammalian muscle cells; they accomplished this feat in experiments with laboratory mice in which they blocked the expression of just two tumor-suppressing proteins. The finding may move us closer to future regenerative therapies in humans — surprisingly, by sending us shimmying back down the evolutionary tree.

"Newts regenerate tissues very effectively," said Helen Blau, PhD, the Donald E. and Delia B. Baxter Professor and a member of Stanford's Institute for Stem Cell Biology and Regenerative Medicine. "In contrast, mammals are pathetic. We can regenerate our livers, and that's about it. Until now it's been a mystery as to how they do it."

Blau is the senior author of the research, which will be published in *Cell Stem Cell* on Aug. 6. Kostandin Pajcini, PhD, a former graduate student, and Jason Pomerantz, MD, a former postdoctoral scholar in Blau's laboratory, are primarily responsible for the work and are first author

and co-senior author, respectively.

Although there's been a lot of discussion about using adult or embryonic stem cells to repair or revitalize tissues throughout the body, in this case the researchers weren't studying stem cells. Instead they were investigating whether myocytes, run-of-the mill muscle cells that normally don't divide, can be induced to re-enter the cell cycle and begin proliferating. This is important because most specialized, or differentiated, cells in mammals are locked into a steady state that does not allow cell division. And without cell division, it is not possible to get regeneration.

In contrast, the cells of some types of amphibians are able to replace lost or damaged tissue by entering the cell cycle to give rise to more muscle cells. While doing so, the cells maintain their muscle identity, which prevents them from straying from the beaten path and becoming other, less useful cell types.

Pomerantz and Blau wondered if it could be possible to coax mammalian cells to follow a similar path. To do so, though, they needed to pinpoint what was different between mammalian and salamander cells when it comes to cell cycle control. One aspect involves a class of proteins called tumor suppressors that block inappropriate cell division.

Previous research had shown that a tumor suppressor called retinoblastoma, or Rb, plays an important role in preventing many types of specialized mammalian cells, including those found in muscle, from dividing willy-nilly. But the effect of blocking the expression of Rb in mammalian cells has been inconsistent: In some cases it has allowed the cells to hop back into the cell cycle; in others, it hasn't.

The researchers employed some evolutionary detective work to figure out that another tumor suppressor called ARF might be involved. Like

Rb, ARF works to throw the brakes on the cell cycle in response to internal signals. An examination of the evolutionary tree provided a key clue. They saw that ARF first arose in chickens. It is found in other birds and mammals, but not in animals like salamanders nestled on the lower branches. Tellingly, it's also missing in cell lines that begin cycling when Rb is lost, and it is expressed at lower-than-normal levels in mammalian livers — the only organ that we humans can regenerate.

Based on previous investigators' work with newts, Blau said it "seemed to us that they don't have the same limitations on growth. We hypothesized that maybe, during evolution, humans gained a tumor suppressor not present in lower animals at the expense of regeneration."

Sure enough, Pajcini and Pomerantz found that blocking the expression of both Rb and ARF allowed individual myocytes isolated from mouse muscle to dedifferentiate and begin dividing. When they put the cells back into the mice, they were able to merge with existing muscle fibers — as long as Rb expression was restored. Without Rb the transplanted cells proliferated excessively and disrupted the structure of the original muscle.

"These myocytes have reached the point of no return," said Blau. "They can't just start dividing again. But here we show that temporarily blocking the expression of just two proteins can restore an ancient ability to contribute to mammalian muscle."

The key word here is "temporarily." As is clear from the mouse experiments, blocking the expression of tumor suppressors in mammalian cells can be a tricky gambit. Permanently removing these proteins can lead to uncontrolled cell division. But, a temporary and well-controlled loss — as the researchers devised here — could be a useful therapeutic tool.

The research required some sophisticated technology to separate individual myocytes from one another for study. To do so, Pajcini traveled to Munich to learn how to optimize a technique normally used on cryopreserved and fixed tissue sections — "laser micro-dissection catapulting" — for use with living cells. But the effort paid off when he was able to prove conclusively that once the expression of the two proteins was blocked, individual live cells were, in fact, dividing in culture.

Next, the researchers would like to see if the technique works in other cell types, like those of the pancreas or the heart, and whether they can induce it to happen in tissue at sites of injury. If so, it may be possible to trigger temporary cell proliferation as a means of therapy for a variety of ailments.

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