Why zebrafish can regenerate damaged heart tissue, while other fish species cannot

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Clayton Carey handles a tank containing medaka fish in the Gagnon lab. Credit: Brian Maffly, University of Utah

A heart attack will leave a permanent scar on a human heart, yet other animals, including some fish and amphibians, can clear cardiac scar

tissue and regrow damaged muscle as adults.

Scientists have sought to figure out how special power works in hopes of advancing medical treatments for human cardiac patients, but the great physiological differences between fish and mammals make such inquiries difficult.

So University of Utah biologists, led by assistant professor Jamie Gagnon, tackled the problem by comparing two fish species: zebrafish, which can regenerate its heart, and medaka, which cannot.

A tale of two fish

The team identified a few possible explanations, mostly associated with the immune system, for how zebrafish fix cardiac tissue, according to research published in *Biology Open*.

"We thought by comparing these two fish that have similar heart morphology and live in similar habitats, we could have a better chance of actually finding what the main differences are," said Clayton Carey, a postdoctoral researcher in the Gagnon lab and lead author on the new study.

Gagnon's team wasn't able to solve the mystery—yet—but their study shed new light on the molecular and cellular mechanisms at play in zebrafish's heart regeneration.

"It told us these two hearts that look very similar are actually very different," Gagnon said.

Both members of the teleost family of ray-finned fish, zebrafish (Danio rerio) and medaka (Oryzias latipes) descended from a common ancestor that lived millions of years ago. Both are about 1.5 inches long, inhabit

freshwater and are equipped with two-chamber hearts. Medaka are native to Japan and zebrafish are native to the Ganges River basin.

According to the study, the existence of non-regenerating fish presents an opportunity to contrast the differing responses to injury to identify the cellular features unique to regenerating species. Gagnon suspects heart regeneration is an ancestral trait common to all teleosts.

Understanding the evolutionary path that led to the loss of this ability in some teleost species could offer parallel insights into why mammals cannot regenerate as adults.

With their distinctive horizontal stripes, zebrafish have long been popular as pets in the United States. In the 1970s zebrafish were embraced by biologists as a model organism for studying embryonic development of vertebrates.

Scientists like zebrafish because they can be propagated by the thousands quickly in labs, are easy to study and proved to be extremely hardy.

Zebrafish used in research at the University of Utah. Credit: Brian Maffly, University of Utah

Cold shock to the heart

To conduct their experiments, the Gagnon lab used a device called a cryoprobe to injure the fish hearts in ways that mimic heart attacks in humans, then extracted the hearts after certain time frames to learn how the two species responded differently.

Carey made the cryoprobe from a piece of copper wire, which was cooled in liquid nitrogen to about minus 170 degrees Celsius. Team members cut tiny incisions in the fish's bellies to expose their hearts, then applied the probe for 23 seconds to the edge of the heart.

In 95% of the cases, the fish survived the procedure, although not for long. After three days or 14 days, their hearts were extracted and dissolved into a single-cell solution, which was then subjected to RNA sequencing in search of markers indicating how the fish responded to the injury.

"Zebrafish have this immune response that is typical of what you might see during a viral infection, called an interferon response," Carey said. "That response is completely absent in medaka."

The study documented differences in immune cell recruitment and behavior, epicardial and endothelial cell signaling, and alterations in the structure and makeup of the heart. For example, medaka lack a certain type of muscle cells that are present in zebrafish.

How zebrafish heal damaged cardiac tissue

"My hunch is the ancestor of all animals could regenerate its heart after an injury, and then that's been repeatedly lost in different types of animals," Gagnon said. "I would like to understand why. Why would you lose this great feature that allows you to regenerate your heart after an injury?"

The study indicates the zebrafish's ability to regenerate has something to do with its immune system, but understanding exactly how would take more research. For example, far more macrophages, specialized immune cells, migrated into the wound site in zebrafish than in medaka.

Unlike medaka, the zebrafish form a transient scar that doesn't calcify into rigid tissue.

"What you do with that scar is what matters," Gagnon said. "We think that the interferon response causes these specialized macrophage cells to

come into that wound site and start to promote the growth of new blood vessels."

Over time new muscle replaces the damaged cardiac tissue and the heart heals.

"The more we learn about how animals can regenerate tissues, how those features have been lost in us and other animals, that's going to help us think about our limitations and how we might engineer strategies to help us overcome those," Gagnon said.

"Our hope is that we build this knowledge base in animals that are really accessible and can be studied in incredible detail, then use that knowledge to generate more focused experiments in mammals, and then maybe someday in human patients."

More information: Clayton M. Carey et al, Distinct features of the regenerating heart uncovered through comparative single-cell profiling, *Biology Open* (2024). DOI: 10.1242/bio.060156

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