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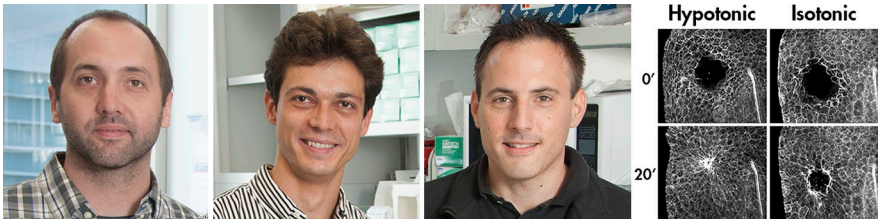
Osmotic gradient is just the tonic for wounded epithelia

Researchers describe how environmental signals trigger wound repair in zebrafish.

Focal Point



PHOTOS COURTESY OF MEMORIAL SLOAN KETTERING CANCER CENTER

(Left to right) William Gault, Balázs Enyedi, and Philipp Niethammer reveal that differences in osmotic pressure between epithelial tissues and their external environment help initiate the tissue's wound response by stimulating ATP release and epithelial cell migration. In a normal, hypotonic environment, zebrafish tailfin epithelium closes a wound within 20 minutes of the initial injury (left). In an isotonic environment, however, cell migration is impaired and the wound remains open (right).

Epithelial layers protect organisms from their external environment, and any damage to these tissues must therefore be repaired as quickly as possible. Wound repair is generally thought to be initiated by intrinsic cues, such as changes in the structure or mechanics of damaged tissues. But Gault et al. reveal that extrinsic signals can also trigger tissue repair. In particular, differences between the osmolarity of zebrafish tissues and their external environment can stimulate wound closure by inducing epithelial cell migration (1).

Zebrafish live in fresh water, which has an osmolarity much lower than that of the animal's interstitial fluids. Philipp Niethammer and colleagues at Memorial Sloan Kettering Cancer Center in New York previously demonstrated that the drop in interstitial osmolarity that occurs when zebrafish tailfins are wounded and exposed to their environment stimulates the recruitment of leukocytes that mediate the tissue's inflammatory response over the following few hours (2). Niethammer wondered whether osmolarity differences might also regulate the more immediate wound response of the tailfin epithelial cells, which quickly act to seal any holes in the tissue. "Because epithelial barriers are so important for protecting the organism, it's essential that wound responses are initiated within seconds of the injury," Niethammer explains.

> "By abrogating the osmolarity difference... the fish didn't know it was injured."

Niethammer and colleagues, led by postdoc William Gault, therefore examined the immediate wound response of zebrafish larvae surrounded by an isotonic, rather than a hypotonic, environment (1). "The sheet of epithelial cells didn't react to the presence of the wound," Niethammer says. "So by abrogating the osmolarity difference between the inside and the outside of the fish, the fish didn't know it was injured."

The zebrafish tailfin epithelium consists of two layers of cells, an inner basal layer attached to the underlying basement membrane and an outer, suprabasal layer that acts as the tissue's permeability barrier. Gault et al. found that, after injury in hypotonic media, suprabasal cells assembled a multicellular actomyosin "purse string" around the wound margin, while the basal cells migrated as a sheet toward the center of the wound, apparently dragging the suprabasal cells along for the ride. In isotonic media, the suprabasal cells still formed a purse string, but the basal cells didn't migrate and therefore failed to close the wound and restore the epithelial barrier. "So there are different wound closure mechanisms in the two layers that have a different dependency on the environmental tonicity," Niethammer explains.

Gault et al. then turned their attention to how osmolarity differences might induce basal cell migration into wounds. Many cell types respond to osmotic shocks by secreting nucleotides such as ATP (3), and ATP has been shown to stimulate epithelial cell migration and wound healing in vitro (4).

The researchers found that ATP was released from zebrafish tailfin wounds in hypotonic, but not isotonic, environments. Moreover, adding exogenous ATP stimulated the migration of basal cells toward wounds even in isotonic media.

"Then we wanted to know what happens if we perturb ATP metabolism inside the tissue," Niethammer says. "To do that, we inhibited the enzymes that break down ATP in the extracellular space." Raising ATP levels by inhibiting these extracellular enzymes pharmacologically, or knocking down a particularly abundant ATPase called ENTPD3, increased the extent and duration of basal cell motility upon wounding. In contrast, lowering ATP levels by adding the soluble ATPase apyrase to wounded tissues decreased cell migration. "So we concluded that ATP is an endogenous wound signal in the zebrafish tailfin," says Niethammer.

This ATP signal is released when wounded tissues experience the lower osmolarity of their external environment. A similar wound detection and response mechanism might operate in mammalian tissues exposed to hypotonic environments, such as the esophageal epithelium. Niethammer, however, still has questions about the mechanism in zebrafish: "How is the ATP released, and how is it sensed by the cells? That's what we're currently working on."

1. Gault, W.J., et al. 2014. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201408049.
2. Enyedi, B., et al. 2013. *Nat. Cell Biol.* 15:1123–1130.
3. Hoffmann, E.K., et al. 2009. *Physiol. Rev.* 89:193–277.
4. Boucher, I., et al. 2010. *Am. J. Physiol. Cell Physiol.* 299:C411–C421.