Lin28: Time for Tissue Repair

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Embryos and juveniles in many organisms repair tissue injuries better than adults. In this issue, Shyh-Chang et al. find that postnatal activation of *Lin28a,* a gene typically active in embryonic development, promotes better than normal tissue repair in mice, including following ear and digit injuries.

Regenerative feats abound in the animal kingdom. Regeneration in mammals, however, is typically less impressive than that observed in a variety of amphibians, teleost fish, and invertebrates such as planarians and *Hydra*. Extensive tissue turnover and wound healing is ubiquitous in essentially all long-lived animals, including for skin, blood, and intestine in humans and mice. By contrast, most mammals fail to regenerate missing parts, such as limbs or fingers, and repair of large wounds is limited and often associated with extensive scarring. A manuscript by Shyh-Chang et al. in this issue of *Cell* explores enhancement of tissue repair in mice by activation of a factor normally active only early in life (Shyh-Chang et al., 2013).

There is some naturally existing regeneration in mouse, as *Mus musculus* can regenerate digit tips (Borgens, 1982). Furthermore, in some genetic backgrounds or in related mammalian species, tissue repair is better than that typically observed in humans and *Mus musculus*, suggesting that the capacity for repair might, in principle, be greater than what is typically observed. For example, the *MRL* mouse displays enhanced wound repair exemplified by its capacity to fill in ear punches (Clark et al., 1998), and the African spiny mouse (*Acomys*) can regenerate large regions of skin, including the majority of its dorsal surface (Seifert et al., 2012). In addition to these instances of mammalian regenerative tissue repair, embryos and juveniles in many animals can display more robust repair than in the adult, including in mammals (Deuchar, 1976; Illingworth, 1974; Poss, 2010). For example, heart repair can occur better in 1-day-old than in 7-day-old neonatal mice (Porrello et al., 2011). This raises the question of whether reactivating particular developmental factors might have positive impacts on postnatal wound repair and regeneration.

lin-28 is a gene identified in *C. elegans* that regulates developmental timing (Ambros and Horvitz, 1984). *lin-28* encodes an RNA-binding protein that represses the *let-7* microRNA (miRNA) to inhibit larval progression. Increased *lin-28* function in *C. elegans* delays larval developmental progression, whereas loss of function of *lin-28* causes precocious developmental progression. In mammals, *Lin28a* is expressed in embryonic stem cells and early embryos, with mature *let-7* family miRNAs expressed subsequently. *Lin-28a* overexpression in mice delays puberty and increases growth, raising the possibility that *Lin28a* might regulate developmental stage features in mice as well (Zhu et al., 2010).

Given the connection between juvenile states and tissue repair capacity, and *Lin28* genes and developmental timing, Shyh-Chang et al. investigated the impact of *Lin28a* overexpression on tissue repair in postnatal mice. The strain used (“iLin28a Tg”) had constitutive low-level *Lin28a* expression (above the wild-type state) and doxycycline-inducible *Lin28a.* For many biological processes, the wild-type is tough to beat by increasing or decreasing dosage of genes. However, mice with *Lin28a* overexpressed outstripped the wild-type in tissue repair by many measures. First, shaved iLin28a Tg mice restored their hair more rapidly. Postnatal hair growth involves synchronized follicle cycles of growth (anagen) and rest (telogen) phases. This hair regrowth phenotype appears to be explained by prolonged anagen phases in iLin28a Tg mice and could, in principle, simply reflect timing of these follicle stages rather than enhanced tissue repair per se. Shyh-Chang next investigated repair from injuries requiring production of mesenchyme, connective tissue, skin, and bone. iLin28a Tg mice showed enhanced digit regrowth following amputation at day 2 of life at the distal finger (interphalangeal) joint. Crossing into the MRL mouse genetic background showed modest further enhancement. Next, Shyh-Chang assessed repair following pinnal injury (ear hole punches). iLin28a Tg mice did not disappoint, closing the holes further than in the wild-type and with degree of closure increasing with *Lin28a* dosage (Figure 1).

How does it work? The obvious candidate target of Lin28a action was the *let-7* family of microRNAs, which are normally inhibited by Lin28a. However, whereas overexpressed Lin28a did inhibit production of mature *let-7* microRNAs in postnatal wound repair, *let-7* regulation was not the whole story. For example, inhibition of *let-7* family members with an antimiR did not improve ear hole punch healing. Overexpression of *let-7* did result in worse pinnal repair than in the wild-type, indicating that inhibition of *let-7* might be one component of *Lin28a*’s effects. In addition, numerous mRNAs that encode proteins involved in metabolism were bound by Lin28a and their translation promoted by *Lin28a*. Accordingly, metabolic differences in vivo and in MEFs were apparent following *Lin28a* overexpression. Oxidative phosphorylation and glycolysis were increased in animals overexpressing *Lin28a*, with inhibitor experiments pointing to oxidative

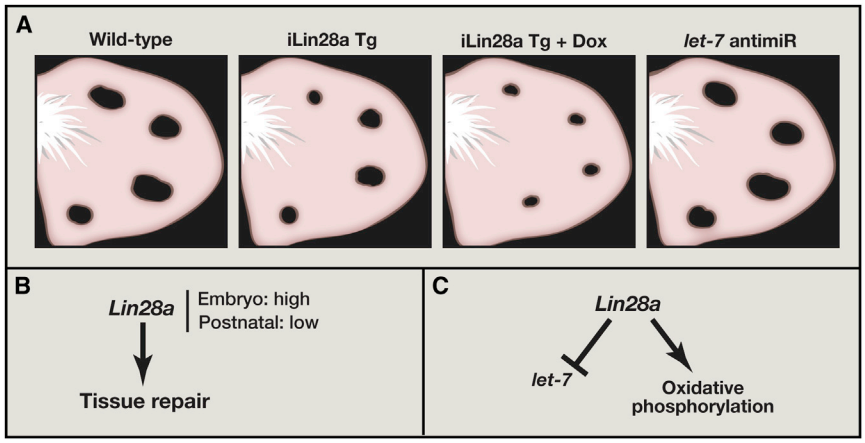

Figure 1. *Lin28a* Can Promote Tissue Repair

(A) Hole punches in mouse ears fail to robustly repair in the wild-type. iLin28a Tg animals express constitutive extra *Lin28a,* and *Lin28a* is further induced by addition of doxycycline (dox). Increasing *Lin28a* levels improved ear tissue repair. By contrast, inhibition of *let-7* with an antimiR failed to improve ear tissue repair. See Shyh-Chang et al., 2013.

(B) *Lin28a* levels are higher in embryos than postnatally, and overactivation of *Lin28a* postnatally can enhance tissue repair.

(C) Model: *Lin28a* promotes tissue repair by inhibiting *let-7*-family microRNAs and, at least in part independently from *let-7*, by enhancing oxidative phosphorylation.

phosphorylation as particularly important for the regenerative repair effects observed. Inhibition of oxidative phosphorylation blocked the effects of *Lin28a* on pinnal repair without significantly impacting repair in the wild-type. Whereas this indicates that normal oxidative phosphorylation is required for the effects of *Lin28a* overexpression, presumably many genes (e.g., for basic cell functioning and cell division) are required for the effects of *Lin28a*, without necessitating them being key mediators of *Lin28a* action. Therefore, it is the combination of this data with Lin28a mRNA-binding data and metabolic analyses that together suggest that increased oxidative phosphorylation might be an important physiological target of *Lin28a* for enhanced repair.

Any genetic manipulation enhancing wound repair or regeneration at developmental stages when it is normally limited is an important advance. Therefore, it is intriguing to find this developmental regulator as a factor that can promote postnatal tissue repair. There are limits to the effects of *Lin28a* with age, however. For example, there was no apparent effect on adult regenerative repair from digit amputation and heart damage. It was somewhat surprising that *let-7* inhibition did not cause the same effect as *Lin28a*, although Shyh-Chang et al. do demonstrate that other Lin28a targets exist. Whereas inhibition of *let-7* microRNAs with an antimiR was effective, in principle, the continuous low-level *Lin28a* expression in iLin28a Tg mice might produce a state that differs from transient *let7* inhibition. Indeed, fetal deletion of *Lin28a* affects postnatal growth, whereas postnatal deletion does not, demonstrating postnatal phenotypes can be influenced by when in life the *Lin28* pathway components are perturbed (Shinoda et al., 2013).

This work raises many intriguing questions for future investigation. For example, what is the *Lin28* activity state in other regenerative animals? Do *Lin28* levels correlate with other instances of waning regenerative abilities with developmental time, such as in *Xenopus*? Is *Lin28* active in mammalian ears that are normally capable of repairing hole punch injuries, such as in rabbits? In general, the direction of identifying juvenile factors that can promote repair postnatally is an attractive one. This work also highlights the merits of comparing metabolic states of regenerative and nonregenerative tissues for a fuller understanding of tissue repair potential. Investigating the effects of manipulation of genes involved in embryo-juvenile-adult transitions could continue to be a fruitful area for understanding and changing the limits on repair.

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