“Epidermal and dermal remodelling during cutaneous wound healing and scar free regeneration of adult zebrafish”

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Abstract

Adult zebrafish (*Danio rerio*) have the ability to regenerate their skin after injury without any scar formation. In contrast, mammals lose this ability in adulthood, resulting in tissue fibrosis and scarring. In this thesis, investigating the underlying mechanisms for cutaneous wound healing in adult zebrafish and finding out the differences between mammalian scarring and zebrafish scar-free regeneration were the aims.

It has been formerly shown that fish wounds become colonized by innate immune cells and fibroblasts, forming a collagen-rich granulation tissue, which, however, is only transient and fully resolved during later stages. Two aspects were investigated of the interaction between innate immune cells and fibroblasts during zebrafish granulation tissue formation and resolution, one via Fibroblast growth factor (Fgf) signalling, and the other via Lysyl hydroxylase 2 (Lh2), respectively. Global blockage of Fgf signalling leads to failed granulation tissue formation similar to the effects of immune suppression, suggesting that innate immune cells might promote granulation tissue formation by secreting Fgfs to stimulate fibroblast recruitment and/or proliferation. We demonstrated that dermal fibroblasts during cutaneous wound healing were labelled via a transgenic zebrafish line, and wounding-induced proliferation was necessary for fibroblasts to form the granulation tissue. Moreover, transgenic approaches for fibroblast-specific blockage of Fgf signalling revealed that fibroblasts receive Fgf signals to form the granulation tissue.

In mice wounds, profibrotic macrophages release Resistin-like alpha (RELMα), which induces the expression of *Plod2* (LH2) in fibroblasts. LH2, an enzyme that promotes the formation of DHLNL-crosslinks, directs persistent pro-fibrotic collagen cross-links among fibrillar collagens, leading to unresolvable fibrotic tissue. Interestingly, *Relmα* is absent from the zebrafish genome; therefore, we investigated this pathway in zebrafish and performed single-cell RNA sequencing to identify the cellular dynamics in more detail. Our results demonstrated that *plod2* is expressed by wound dermal fibroblasts downstream of TGFβ signalling.

Strikingly, we found that even forced overexpression of *plod2* during zebrafish wound healing, although causing thicker collagen fibres in a small proportion, does not affect granulation tissue resolution, and does not result in scarring. Moreover, loss of Lh2 or gain of mouse *Relmα* functions during cutaneous wound healing did not affect granulation tissue size or resolution. These data suggest that additional downstream signalling pathways are at play in zebrafish to resolve the transient fibrosis. In the long run, these findings might provide novel insights for therapeutic anti-fibrotic approaches in humans.

In addition to dermal remodelling during zebrafish cutaneous wound healing, epidermal homeostasis during wound healing and in unwounded skin were explored. Our results demonstrated that cells in the neoepidermis decrease their proliferation rates to recuperate epidermal homeostasis after wounding. Moreover, dysregulation of Fgf signalling reduces epidermal proliferation rates and decreases the size of the epidermal cells. In this study, we showed for the first time that adult zebrafish extrude their epidermal cells to maintain homeostasis.