Establishing the Role of CXCR2 in Mammalian Tissue Regeneration

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Introduction

Skin injuries are repaired through scar formation or tissue regeneration. Scar formation is more common in adult mammals and leads to loss of normal tissue architecture and function. Past work from our lab showed that aged mice and topical imiquimod-treated mice, compared to young mice and untreated mice, have improved full-thickness tissue regeneration. Our goal is to study the mechanisms underlying scar formation in order to discover therapeutics that can promote skin regeneration.

Background

- Single-cell RNA sequencing on regenerative and scarring mice models showed differences in neutrophil populations and increased CXCR2-CXCL signaling in scarring mice.
- CXCR2 is a cytokine receptor that mediates immune cell migration and angiogenesis through ligands like CXCL1 and CXCL2.
- CXCR2 is expressed on multiple cell types, including keratinocytes, fibroblasts, and neutrophils.
- Other groups have studied CXCR2 in tissue regeneration, but no consensus has been reached on its precise role.
- To study wound healing, we used two models: Wound-induced hair neogenesis and ear hole closure.
- Recent work from our lab showed that CXCR2 knockout mice have improved ear hole closure compared to wild-type mice.

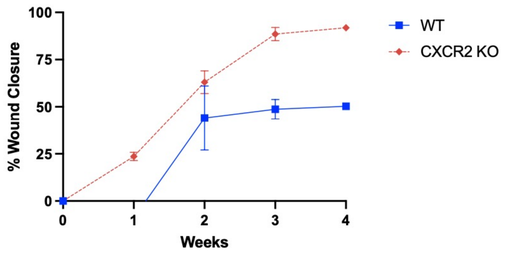

Figure 1. CXCR2 global knockout exhibit improved ear hole closure. Percentage of wound closure in wild-type (blue line) and CXCR2 global knockout mice (red).

Aim

CXCR2 global knockout mice heal wounds with increased tissue regeneration compared to wild-type. We wish to investigate the role of CXCR2 deficiency in promoting tissue regeneration and suppressing skin fibrosis.

1. Which cell type is responsible for the CXCR2 knockout mice's tissue regeneration ability?
2. What is the role of CXCR2-deficient neutrophils in tissue regeneration?

Wound Healing Assays

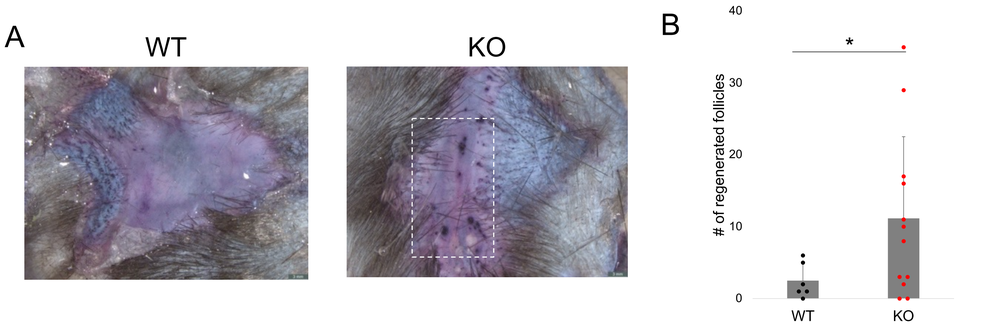

Figure 2. Wound-induced hair neogenesis in CXCR2 global knockout (KO) and wild-type (WT) mice. (A) Representative images of wounds. Area with regenerated follicles shown in white box. (B) Quantification of regenerated follicle numbers. *N* = 6 (WT), *N* = 12 (KO). *N* = biological replicates per group. Data are pooled from 4 independent experiments. $p < 0.05$.

Cell-type Specific CXCR2 Knockout

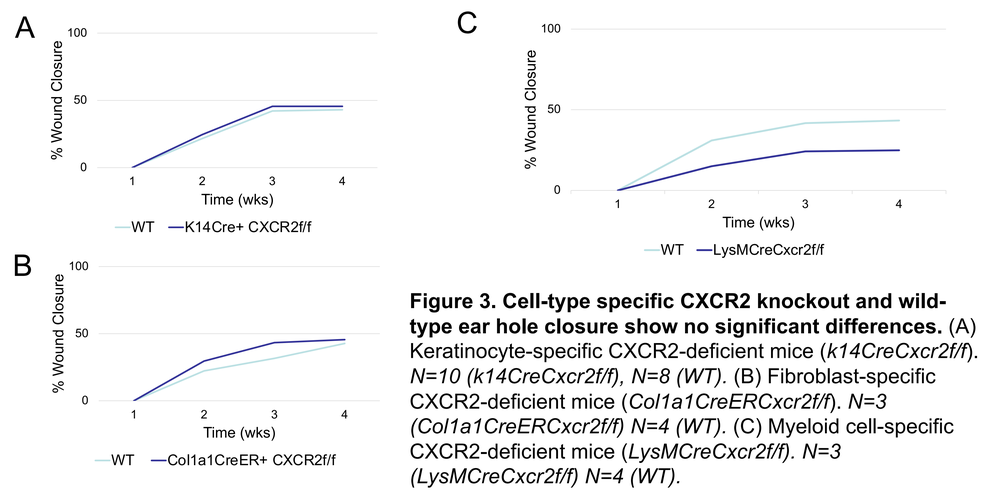

Figure 3. Cell-type specific CXCR2 knockout and wild-type ear hole closure show no significant differences. (A) Keratinocyte-specific CXCR2-deficient mice (*k14CreCxcr2f/f*). *N=10 (k14CreCxcr2f/f), N=8 (WT).* (B) Fibroblast-specific CXCR2-deficient mice (*Col1a1CreERCxcr2f/f*). *N=3 (Col1a1CreERCxcr2f/f) N=4 (WT).* (C) Myeloid cell-specific CXCR2-deficient mice (*LysMCreCxcr2f/f). N=3 (LysMCreCxcr2f/f) N=4 (WT).*

CXCR2 Signaling

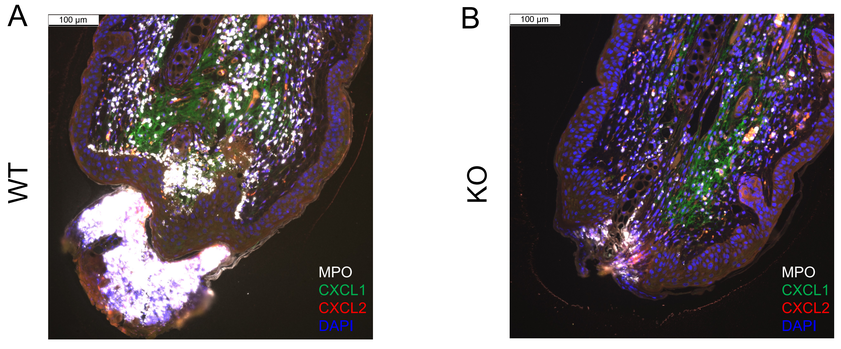

Figure 4. Representative immunofluorescence sections for MPO (white), CXCL1 (green), and CXCL2 (red) and counterstaining by DAPI (blue) in wound edge tissue. (A) Wild-type day 3 post-ear punch. (B) CXCR2 global knockout day 3 post-ear punch.

Neutrophil Recruitment

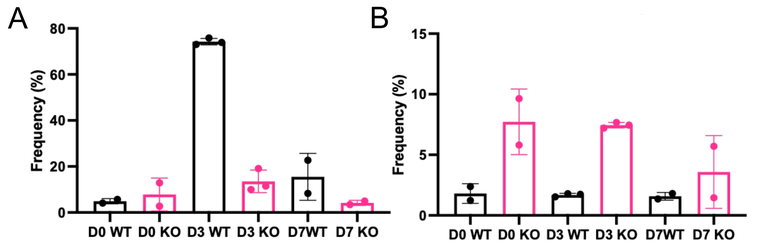

Figure 5. Characterization of neutrophil recruitment at day 0 (D0), day 3 (D3), and day 7 (D7) in CXCR2 global knockout (KO) and wildtype (WT) mice by flow cytometry. (A) Neutrophil frequency in wound edge skin tissue. (B) Neutrophil frequency in spleen. *N* = 2 (WT D0); *N* = 3 (WT D3); *N*= 2 (WT D7); *N* = 2 (KO D0); *N* = 3 (KO D3); *N* = 2 (KO D7).

Conclusions & Future Directions

The next steps focus on using other models to study the effect of CXCR2-deficient neutrophils on regeneration and to determine the mechanism behind improved regeneration.

- Study tissue regeneration using the stented back wound model in CXCR2 global knockout (KO) and wild-type (WT) mice.
- Conduct a bone marrow transplant from a CXCR2 KO mouse into a WT mouse for a more efficient and neutrophil-specific CXCR2 deficiency.
- Perform parabiosis between a CXCR2 KO and WT mouse to study if circulating factors promote scar formation using ear hole closure model.
- Immunofluorescence on CXCR2 KO and WT mice to study the role of NETosis as a potential mechanism for reduced fibrosis in the CXCR2 KO mice.

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