Identification and isolation of a dermal lineage with intrinsic fibrogenic potential

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Philipp Hacker, 19.10.2015
Introduction

- Wound Healing: Inflammation (48 hours after injury)

Source: Wound repair and regeneration
Geoffrey C. Gurtner, Sabine Werner, Yann Barrandon & Michael T. Longaker
Introduction

- Wound Healing: New tissue formation (2-10 days after injury)

Source: *Wound repair and regeneration*
Geoffrey C. Gurtner, Sabine Werner, Yann Barrandon & Michael T. Longaker
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- Wound Healing: Remodelling (1-12 months after repair)

Source: Wound repair and regeneration
Geoffrey C. Gurtner, Sabine Werner, Yann Barrandon & Michael T. Longaker
Aim of the study: To identify and isolate the fibroblast lineage(s) with fibrogenic potential in vivo
Cre/LoxP Mouse Model

FACS – based isolation of Engrailed-1 positive Fibroblasts (EPFs) and Engrailed-1 negative Fibroblasts (ENFs) from dorsal skin samples

Gene expression analysis
I. Engrailed-1 lineage positive (EPFs) vs Engrailed-1 lineage negative (ENFs) fibroblasts – Gene expression analysis
I. Differential gene expression of EPFs and ENFs

Population dynamics
I. Morphologie of EPFs and ENFs

EPFs green (GFP)
ENFs red (RFP)
II. Fibrogenic potential is lineage-restricted
At least two embryonic lineages exist (EPFs and ENFs), but EPFs are the only effectors of connective tissue secretion and formation in vivo during embryonic development, postnatal wound healing, cancer stroma formation and radiation fibrosis of the skin.

II. Fibrogenic potential is lineage-restricted
III. Fibrogenic potential of dermal fibroblasts is cell-intrinsic

- Oral mucosa wounds show more rapid healing and less scarring formation than cutaneous wounds – is this property cell-intrinsic or owed to the environmental conditions of the host tissue?

**Wnt1Cre** transgenic mouse (labels early migratory neural crest populations)

**R26mTmG** transgenic mouse

**Wnt1Cre;R26mTmG** transgenic mouse
III. Fibrogenic potential of dermal fibroblasts is cell-intrinsic

FACS – based isolation of Wnt-1 positive Fibroblasts (WPFs) and Wnt-1 negative Fibroblasts (WNFs) from oral dermis samples
III. Fibrogenic potential of dermal fibroblasts is cell-intrinsic

Wounded oral dermis
III. Fibrogenic potential of dermal fibroblasts is cell-intrinsic
III. Fibrogenic potential of dermal fibroblasts is cell-intrinsic

New approach: Reciprocal transplantation experiments
IV. Ablation of EPF-lineage reduces scarring?

- Different mouse model: **En1Cre;R26mTmG** mice were crossed with **R26tm1(HBEGF)Awai** mice which express simian diptheria toxin receptor (DTR) → EPFs are GFP and DTR positive
IV. Ablation of EPF-lineage reduces scarring?
V. How to isolate EPFs

- FACS-based fibroblast purification from skin lysates of wild-type mice aged 8 weeks
- Cell surface marker screening (including 176 monoclonal antibodies) revealed **CD26** as the marker with the biggest difference between EPFs and ENFs
V. How to isolate EPFs

At baseline ECM expression of EPFs and ENFs is similar, whereas the response to a stimuli and the following upregulation of ECM gene expression is much higher in EPFs.
VI. Inhibition of CD26 reduces cutaneous scarring during wound healing

- CD26 is a surface marker for a fibroblast lineage responsible for ECM deposition
- Diprotin A was used to inhibit CD26
Conclusion/Discussion

- Distinct fibroblast lineages represent unique cell types.
- EPFs are the primary lineage contributing to connective tissue deposition during embryonic development, postnatal wound healing, cancer stroma formation.
- EPFs fibrogenic potential is cell intrinsic.
- CD26 is a marker for the distinct lineage of fibroblasts that contributes the bulk of connective tissue in scarring and other conditions involving fibrosis (EPFs).
- Inhibition of CD26 might be a promising step forward to regulating fibrosis.
Personal Comments/ Critics

- Why did they use Engrailed-1 mice?
- Where do the Engrailed-1 positive fibroblasts come from?
- Mouse model is used in this study – to what extent are the findings applicable in humans?
- CD26 maybe marker for other cells?
- In my opinion a very credible and sophisticated paper
Thank you for your attention!