Targeting the Scar Forming Fibroblasts with CRISPR/Cas9

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Abstract

CRISPR/Cas9 (clustered regularly interspaced palindromic repeats/CRISPR-associated protein 9)-based DNA editing has revolutionized the process of genome editing by manipulating the germline in Zygotes. However, its application in postnatal gene editing remains difficult due to delivery challenges of the CRISPR/Cas9 components. Prior experiments in mice have reported that embryonic mesenchymal precursors expressing Engrailed-1 (*En1*) generate skin fibroblasts are responsible for most of the scar tissue deposition after skin injury. Thus, developing a distinct method that targets gene expression specifically in *En1*lineage derived fibroblasts has substantial implications in the development of potential therapies for scar free wound healing. Here, we report the generation of a transgenic mouse line that expresses Cas9 exclusively in the *En1* lineage of dermal fibroblasts by crossing mice expressing Cre recombinase after the *En1* promoter with homozygous R26-loxP-STOP-loxP-3xFLAGCas9-eGFP mice to generate *En1* lineage-specific Cas9 mice, which labels all Cas9 fibroblasts with enhanced green fluorescent protein (eGFP) (Figure1). To investigate whether our Cas9-expressing mice could be used to perform efficient gene editing, we used Adeno-Associated Virus (AAV) to deliver single-guide RNA that targets the *Ctnnd1* locus exclusively in En1 lineages of dermal fibroblasts. Intradermal injection of postnatal En1-Cas9 transgenic mice with AAV encoding sgRNA against *Ctnnd1* resulted in robust editing of the *Ctnnd1* locus. This method will be useful for skin disease modeling and provides a means of rapidly editing the profibrotic genes of interest and potential gene therapies in the context of scar free wound healing in future.

Figure

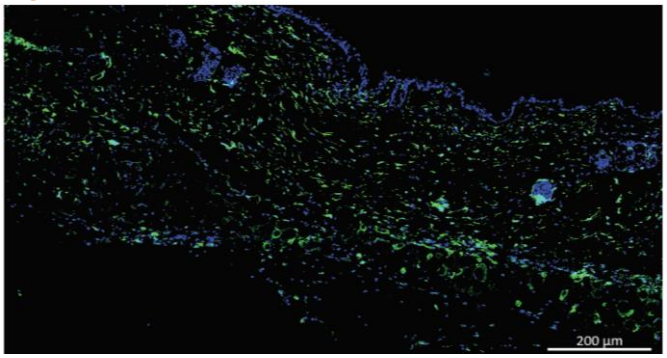


Figure 1: En1-Cas9 mice show robust expression of eGFP on wound bed after 7days post injury, indicating strong Cas9 expression in En1 lineage fibroblasts.

References

1. Rinkevich,Y (2015) Identification and isolation of a dermal lineage with intrinsic fibrogenic potential. Science 348, aaa2151

2. Jiang, D.(2018) Two succeeding fibroblastic lineages drive dermal development and the transition from regeneration to scarring. Nature Cell Biology 20:422–431

3. Platt RJ (2014) CRISPR-Cas9 Knockin Mice for Genome Editing and Cancer Modeling. Cell 159(2):440-55



Biography

Vijayanand Rajendran studied Masters in Biomolecular Sciences at the University of Groningen, The Netherlands and continued his Ph.D. in the field of Skin Regeneration at Yuval Rinkevich lab, Ludwig Maximilian University of Munich, Germany. As a part of his Ph.D. studies, he uses CRISPR-Cas9 system *invivo* to functionally characterize the profibrotic genes to better understanding the mechanism of scarring.

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