Lineage tracing of vasculogenic fibroblasts in vivo and their significance in the rescue of diabetic ischemic tissue

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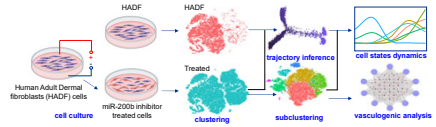
Abstract

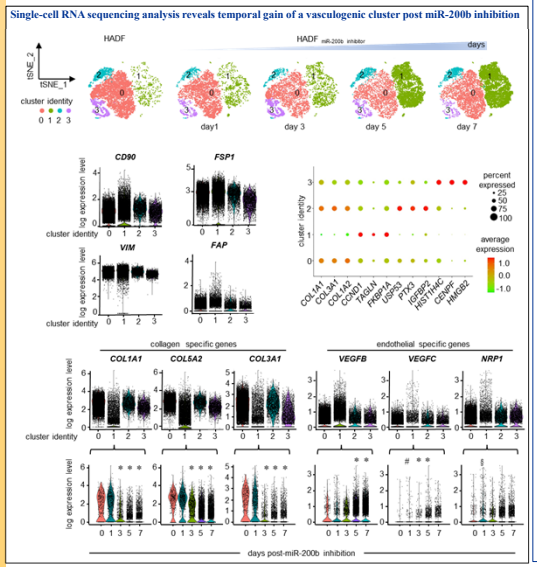
Our recent work reported on the identification of the vasculogenic fibroblast (VF) that is capable of generating new blood vessels during tissue repair (Pal et al. Nat Com, 2023). While these cells are physiologic and injury-inducible, the generation of VF is blunted under conditions of diabetes. Such barrier may be overcome by the inhibition of fibroblast miR-200b using tissue nanotransfection technology (PMIDs: 28785092, 34837085, 35819852, 36380587). To understand the mechanisms underlying VF function and significance, in this work we developed miR-200b-429fl/fl COL1A2creER GT(ROSA)mT/mG mice. In these mice, miR-200b regions in fibroblasts (COL1A2+ cells) are knocked out in response to tamoxifen. To gain insight into the significance of VF in vivo, ischemic hind limb studies were performed. Tamoxifen treatment not only led to Cre excision of miR-200b-429 in these mice, but also induced the constitutive expression of ROSA driven tdTomato in fibroblasts with onset of permanent GFP expression. Significant lowering of miR-200b was noted in laser capture dissected dermal fibroblast tissue elements from the wound-edge of tamoxifen treated mice (n=4). Tamoxifen treatment led to significantly enhanced perfusion in animals subjected to ischemic injury as determined by laser speckle perfusion imaging (Perimed Inc.) (n=8). This rescue was associated with increased abundance of GFP+CD31+ VF (n=5) which accounted for 26% of all CD31+ vascular cells in the laser captured blood vessels (n=9). Thus, targeted lowering of miR-200b abundance within fibroblasts resulted in substantial augmentation of VF in vivo which was associated with improved blood flow.

Study Design



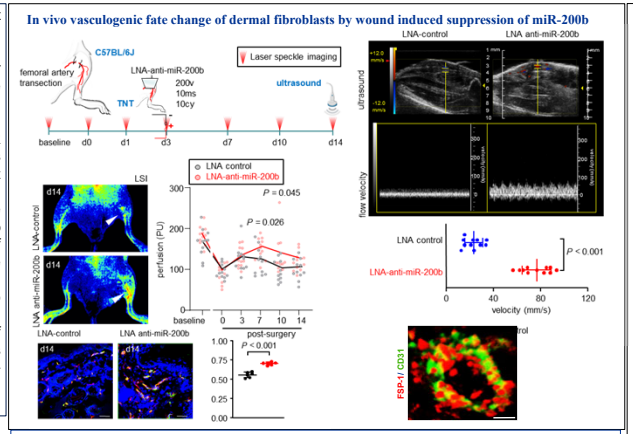
Results

Single-cell RNA sequencing analysis reveals temporal gain of a vasculogenic cluster post miR-200b inhibition

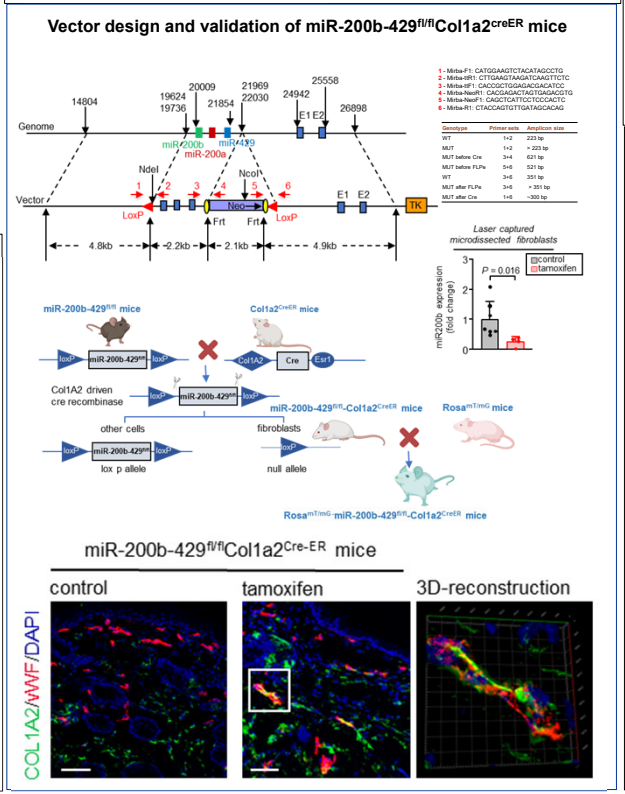


Results

In vivo vasculogenic fate change of dermal fibroblasts by wound induced suppression of miR-200b

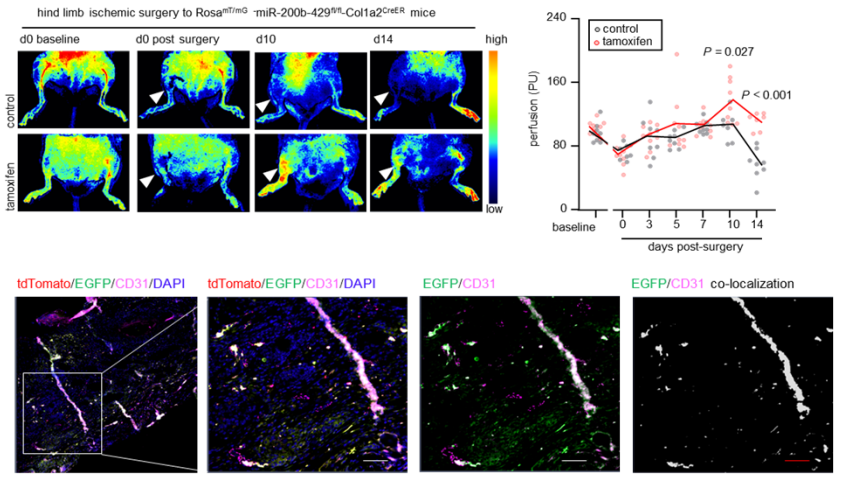


Vector design and validation of miR-200b-429$^{fl/fl}$Col1a2^{creER} mice

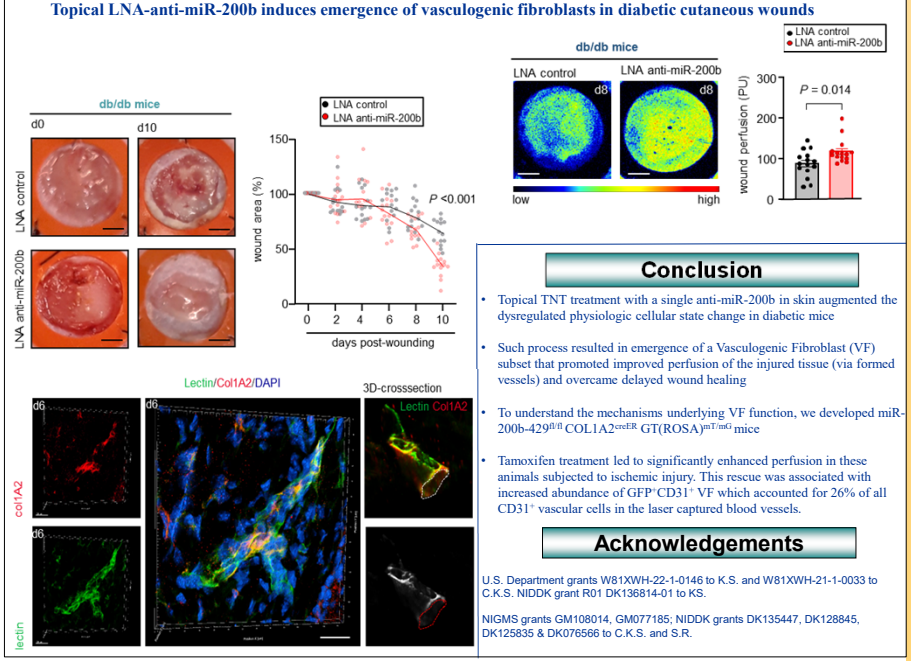


Results

Lineage tracing of vasculogenic fibroblasts in vivo and their significance using miR-200b-429fl/fl COL1A2creER GT(ROSA)mT/mG mice



Topical LNA-anti-miR-200b induces emergence of vasculogenic fibroblasts in diabetic cutaneous wounds



Conclusion

- Topical TNT treatment with a single anti-miR-200b in skin augmented the dysregulated physiologic cellular state change in diabetic mice
- Such process resulted in emergence of a Vasculogenic Fibroblast (VF) subset that promoted improved perfusion of the injured tissue (via formed vessels) and overcame delayed wound healing
- To understand the mechanisms underlying VF function, we developed miR-200b-429$^{fl/fl}$ COL1A2^{creER} GT(ROSA)$^{mT/mG}$ mice
- Tamoxifen treatment led to significantly enhanced perfusion in these animals subjected to ischemic injury. This rescue was associated with increased abundance of GFP^{+}CD31^{+} VF which accounted for 26% of all CD31^{+} vascular cells in the laser captured blood vessels.

Acknowledgements

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