A miRNA Cassette Reprograms Smooth Muscle Cells into Endothelial Cells

A miRNA approach to vascular transdifferentiation

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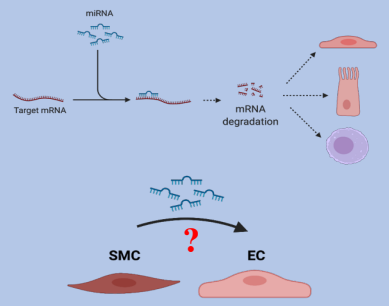
Introduction

MicroRNAs (miRNAs) are non-coding RNAs which repress translation of mRNAs via 3'UTR binding.

Endothelial cells (ECs) grow slowly, whereas smooth muscle cells (SMCs) are more proliferative.

Hypothesis: a miRNA cassette can transform SMCs into ECs, called **induced endothelial cells (iECs)**.



Generating iECs from SMCs

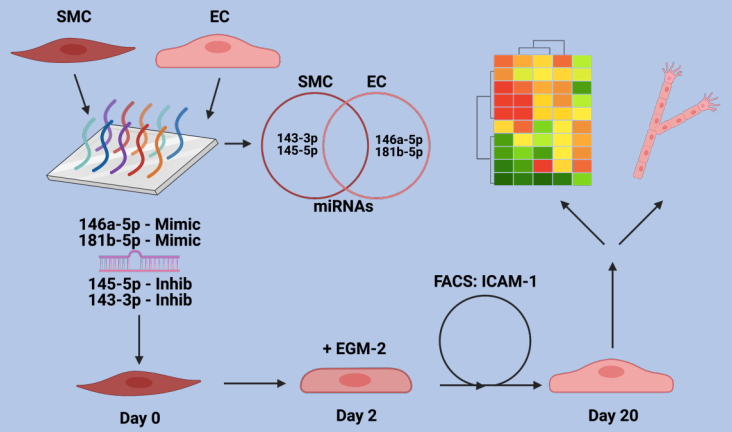


Figure 1: Generation of iECs. Transcriptional profiling of SMCs and ECs identified differential miRNA profiles. Transfection of coronary artery smooth muscle cells (CASMCs) followed by ICAM-1 selection in endothelial growth media. Multi-scale profiling of iECs to validate endothelial-like properties.

iECs express EC markers

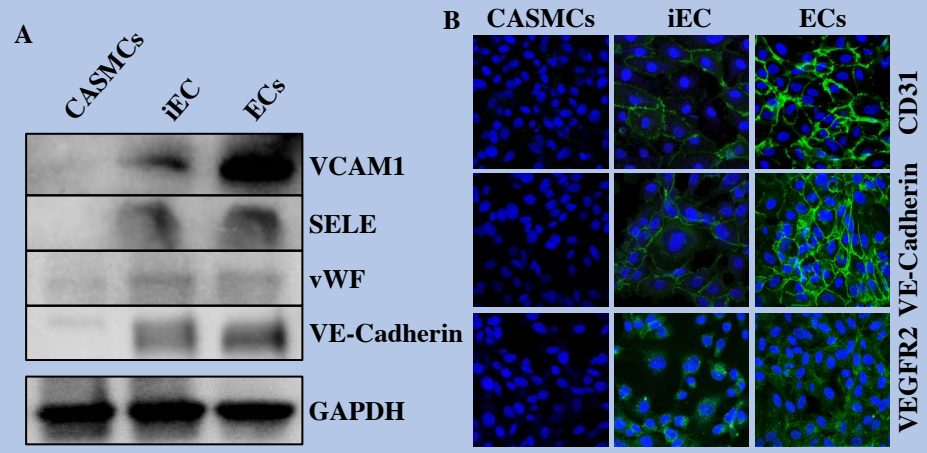


Figure 2: iECs express EC markers. A) Western blots of cell lysates from CASMCs, iECs, and ECs stained against VCAM1, SELE, vWF and VE-Cadherin. B) Confocal micrographs of EC markers demonstrate correct protein localization in iECs.

iECs transcriptomes align with ECs over SMCs

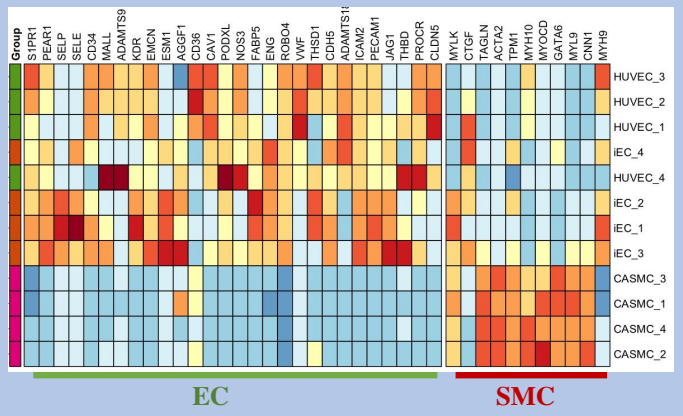


Figure 3: iEC transcriptomes mirror ECs and overexpress EC genes. Transcriptional profiling by hierarchical cluster analysis determined that iEC transcriptomes more closely matches those of ECs. Heatmap clustering analysis confirmed that iECs exhibit robust increases in endothelial pathways.

iECs exhibit EC-like functionality and improve ischemic blood flow

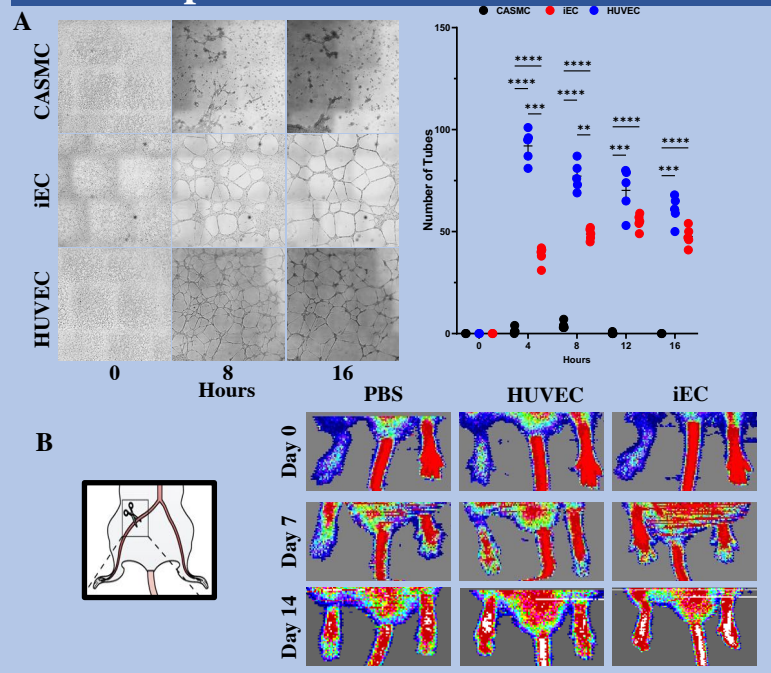


Figure 4: iECs exhibit EC functionality. A) iECs undergo tube formation on Matrigel similar to ECs. B) In a hind limb ischemia model, iECs restore blood flow more quickly than PBS treatment.

Conclusion: miRNAs can transform ECs to iECs

- MicroRNA profiling determined miR-143 and miR-145 ↑ in SMCs, miR-181b and miR-146a ↑ in ECs.
- Transfection of SMCs with miR-143 and miR-145 inhibitors along with miR-181b and miR-146a mimics can transdifferentiate cells
- Generated iECs exhibit endothelial-like transcriptomes, protein profiles, and functionality.