Characterizing activation states of retinal stem cells for endogenous repair

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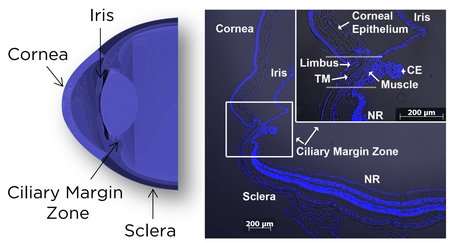
STATEMENT OF THE PROBLEM

Work at our lab has shown that there are about 10 000 retinal stem cells (RSCs) present in the ciliary marginal zone of the human eye at any given age. They are capable of differentiating to all seven retinal cell types. Though they are quiescent in vivo, they become active in vitro and proliferate extensively.

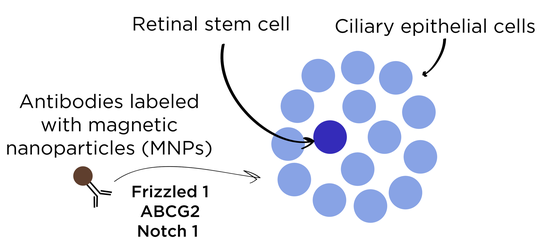
HYPOTHESIS

We can achieve endogenous activation of resident RSCs in vivo by understanding the molecular mechanisms regulating their quiescence.

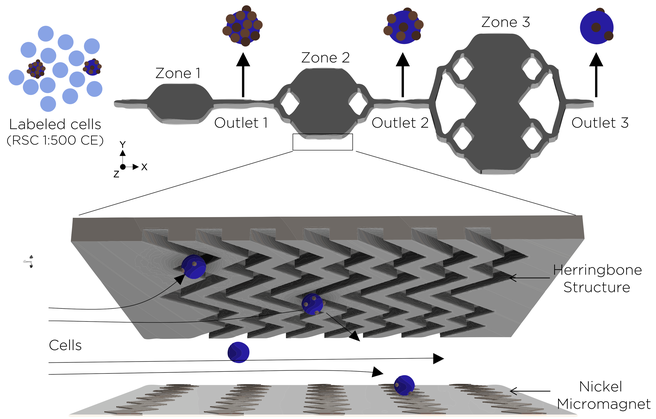
PART I: DISSECTION



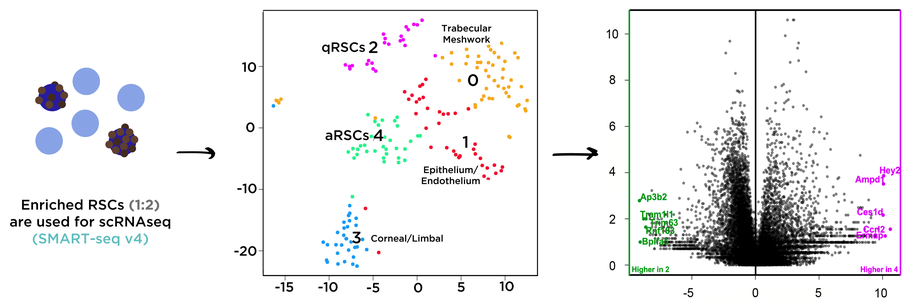
PART II: MAGNETIC LABELLING



PART III: MICROFLUIDIC CELL SORTING



PART IV: FINDING MOLECULAR MARKERS



Two clusters of RSCs were identified and their respective markers were determined. More sphere-forming cells are found in C4 than C2, suggesting a difference in state.

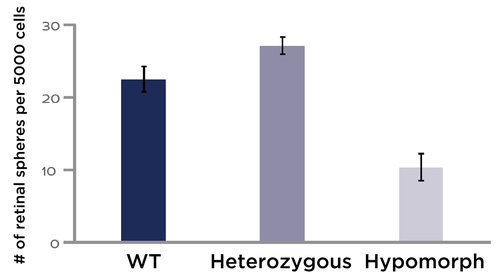
4 differential surface markers & 6 transcription factors identified

NEXT STEPS

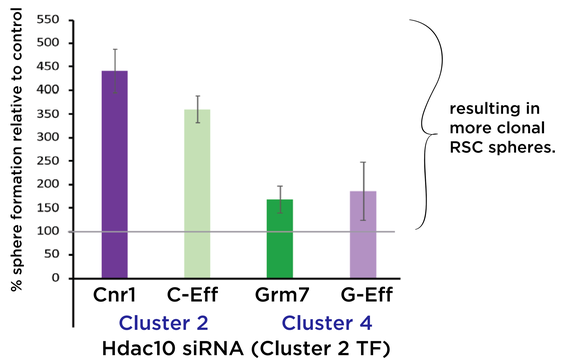
Future work will explore 1) the potential involvement of other differentially expressed genes in state regulation using the same siRNA assays, and 2) the specific mechanisms by which Hdac10 are involved in the regulation of quiescence.

PART V: INVESTIGATING ENRICHED GENES

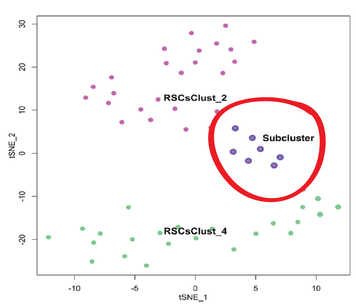
Creb1 hypomorphic mice have a 50% reduction in the absolute number of RSCs *in vitro.*



RNA knock-down of Hdac10 shifted the quiescent RSC population to a more active state...



PART VI: IDENTIFICATION OF THE "PRIMED" STATE



Re-analysis of only the two RSCs clusters revealed the appearance of a third significantly separate sub-cluster of RSCs, which may be a transitional population between the more quiescent and the poised to proliferate RSCs.

SIGNIFICANCE

If successful, our experiments will define quiescent, primed, and activated states of RSCs. Apart from the importance of this knowledge to advance our understanding of retinal development, this data will play a critical role in our efforts to achieve controlled endogenous regeneration of retinal tissue in blind individuals.