Investigating the role of Tbx5 in the adult cardiac regeneration

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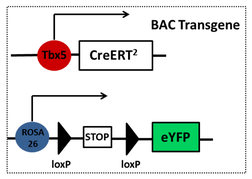
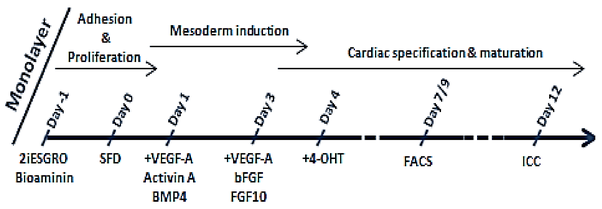
Aim of work Heart failure is the major cause of death in the industrialized countries, representing 30% of all global deaths. Injury to the adult mammalian cardiac muscle, often lead to a heart attack due to irreversible loss of cardiomyocytes (CM), creating an unmet need for identifying idle cardiac regenerative mechanisms. Expression of the embryonic transcription factor Tbx5 is hallmark of first heart field (FHF) progenitors and paramount for differentiation towards a cardiomyocyte fate. In addition, murine and human CPC can be isolated through the expression of Pdgfra, Gfra2 and Kdr surface markers. To further characterize Tbx5-expressing cells, we utilized an *in vitro* differentiation regime to isolate FHF CPC based on the expression of these markers using *Tbx5^{CRE}/R26R$^{eYFP/eYFP}$ in vitro* and *in vivo* models. T-distributed stochastic neighbor embedding (t-SNE) analysis of single-cell RNA-seq data, showed that *in vitro* Tbx5-expressing cells transcriptomically mimic cardiac embryonic CPC development. Our data reveal an over-expression of Tbx5 in the adult injured heart mostly observed around injury sites. Those tbx5-expressing cells could be an effective target for studies involving heart repair and regeneration, inclined to pharmacological modulation in patients with ischemic heart disease.

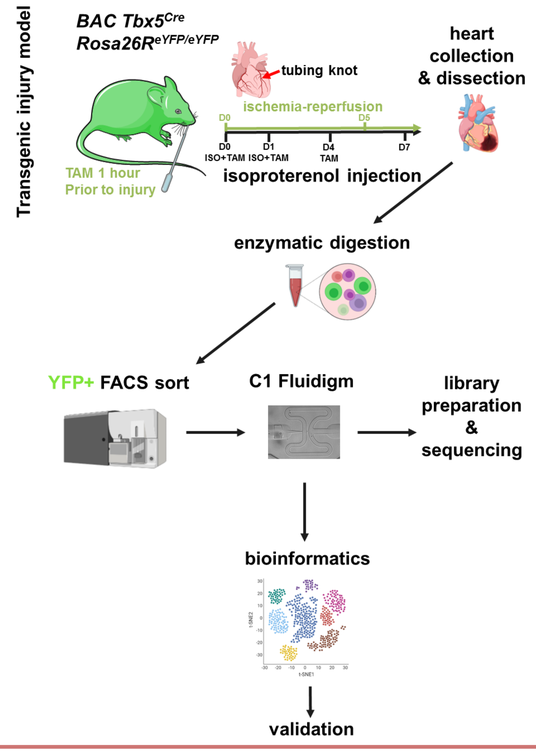
Methodology

- Using the mESC line *Tbx5^{CRE}/R26R$^{eYFP/eYFP}$* we aimed to characterize *in vitro* acquired Tbx5-expressing CPC.





- Using two adult heart injury murine models we aimed to show that Tbx5-expressing cells exist in the injured adult mammalian heart.



Results

1. mESC differentiation (*Tbx5^{CRE}/R26R$^{eYFP/eYFP}$*) into early CPC and CM-like cells

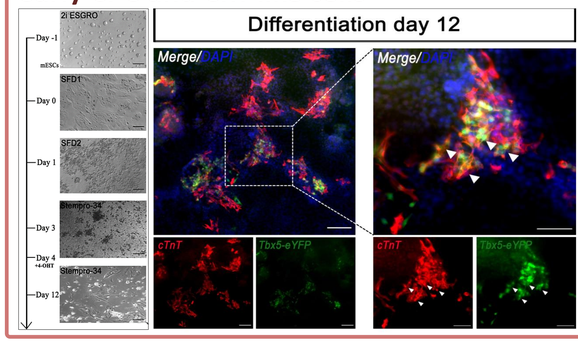
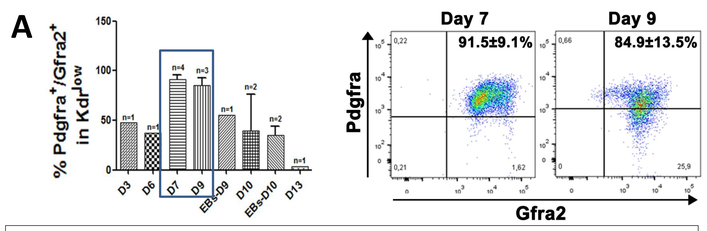


Figure 1: eYFP tags mESC-derived FHF CPC destined to become CM-like cells. White arrowheads: cTnT^{+} cells that expressed Tbx5, Scale bar: 100μm

2. Segregation of FHF and SHF progenitors



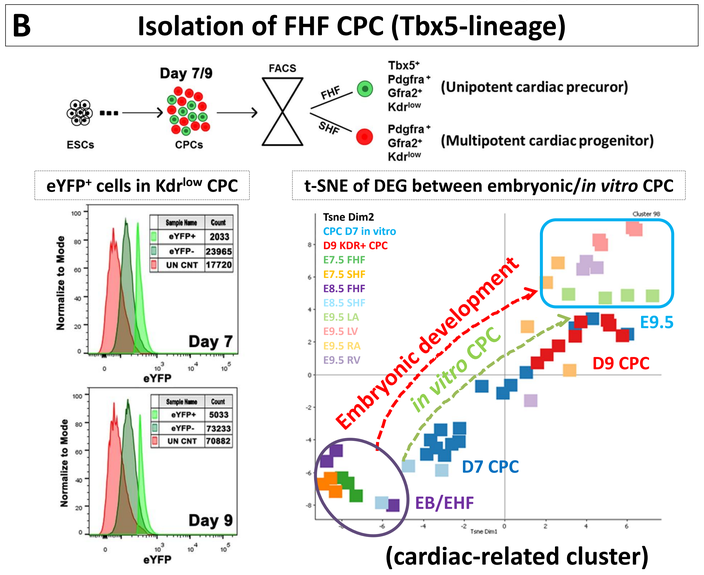
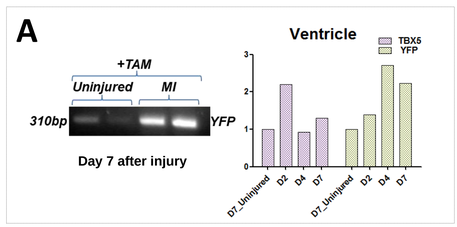
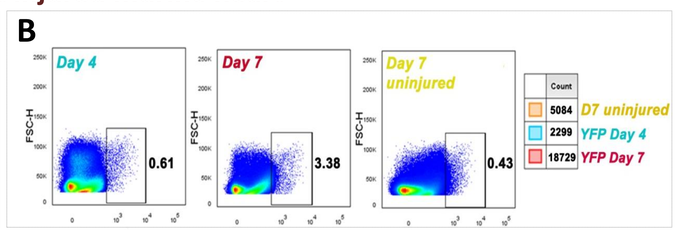


Figure 2: A) *In vitro* differentiation experiments indicate that there is an enrichment of Pdgfra^{+}Gfra2^{+}Kdr^{low} cells in the culture between day 7 and 9 of differentiation. B) Tbx5^{+} CPC are enriched in the Pdgfra^{+}Gfra2^{+}Kdr^{low} population and expand from day 7 to 9. The transcriptome of this CPC population is compared to that from embryonic cardiac development.

3. Tbx5 over-activation in the adult injured murine heart





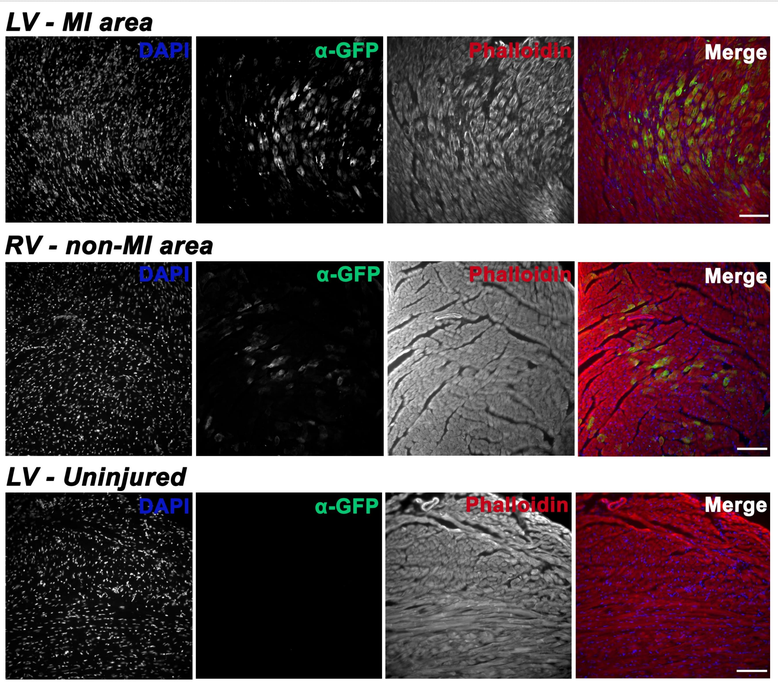


Figure 3: Myocardial infraction (MI) was induced through chemical injury and left artery descending (LAD). Flow-cytometric analysis was performed in order to collect eYFP^{+} cells. A) Tbx5 was over-expressed in the ventricle of the adult injured heart B) Cardiac eYFP^{+} cells were collected from the adult injured heart 2 to 7 days after injury and were compared to control adult uninjured eYFP^{+} cells. C) Immunohistochemical analysis indicated that YFP^{+} cells were mostly present in the injury site, but also in distal cardiac areas. Scale bar: 100μm

Conclusions

- mESC-derived Tbx5-expressing cells are only able to differentiate to CM-like cells, verifying their unipotent character.
- The *in vitro* acquired Tbx5-expressing CPC population, transcriptomically resemble that of embryonic CPC. Therefore, this *in vitro* system could be a useful tool to further characterize the unipotent Tbx5 CPC population and shed light into developmental pathways of the heart.
- Upon MI, the adult heart possess a ventricular cardiac cell population that transiently re-activates the cardiac embryonic transcription factor Tbx5.
- The YFP^{+} cell population in the adult injured heart may tag a CM precursor population that is being examined further (**Highlights from the Young in basic science channel – 28th August**).

Acknowledgements

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