Identification and isolation of cycling cardiomyocytes for single cell analysis

Authors:
M Baniol¹, J Panula¹, S Reinhardt², EL Graham¹, E Lazar¹, O Bergmann², ¹Karolinska Institute, CMB - Stockholm - Sweden, ²Dresden University of Technology, DFG-Center for Regenerative Therapies Dresden - Dresden - Germany,

Topic(s):
Basic Science - Cardiovascular Development and Anatomy: Stem Cells, Cell Cycle, Cell Senescence, Cell Death

Citation:
Cardiovascular Research (2018) 114 (Supplement 1), S16

Funding Acknowledgements:
Karolinska Institutet, the Swedish Research Council, the Ragnar Söderberg Foundation, Åke Wiberg Foundation, and Jeansson's Foundations

Purpose:
One of the major goals in cardiac regeneration research is to replace lost ventricular tissue with new cardiomyocytes. However, the rate of cycling cardiomyocytes in adult hearts is too low to efficiently compensate for the loss of functional myocardium in heart disease. We hypothesize that cardiomyocyte proliferation is tightly controlled by regulatory mechanisms that can be reactivated to promote endogenous repair after heart injuries. Unequivocal identification of cycling cardiomyocytes is a fundamental requirement for the investigation of these mechanisms.

Methods
We used a preexisting transgenic mouse model based on the FUCCI (Fluorescent Ubiquitination-based Cell Cycle Indicator) system to discriminate cycling cardiomyocytes (S/G2/M phases, green fluorescent nuclei) from non-cycling ones (G0/G1 phases, red fluorescent nuclei). In mice ubiquitously expressing FUCCI constructs, we used the myocyte-specific perinuclear marker Pericentriolar Material 1 (PCM-1) to distinguish cardiomyocyte from non-myocyte cells in the heart tissue. Single cardiomyocyte isolations from FUCCI mice neonatal hearts followed by FACS sorting into 96-well plates were performed to obtain single cell transcriptome of cycling and non-cycling cardiomyocytes at different post-natal stages.

Results:
The presented transgenic strategy proved to be efficient for the identification of cycling cardiomyocytes in neonate, juvenile and adult mouse hearts in vitro and in vivo. The validity of transgenes expression was verified in both systems with immunohistochemistry by co-localization of cardiomyocytes and cell cycle markers with FUCCI fluorescence. The FUCCI system allowed us to confirm previous observations of a gradual decrease in the number of cycling cardiomyocyte during the neonatal period. P0 and P7 cardiomyocyte transcriptome were generated using single cell RNA-sequencing. The cell cycle stage of cardiomyocytes as indicated by their FUCCI fluorescence was highly correlated by their transcriptome profile.

Conclusion
Strategies that allow to unambiguously identify cycling cardiomyocytes in vitro and in vivo provide important tools to study the regulation of cardiomyocyte proliferation. With the FUCCI system we seek to identify novel molecular targets that could promote cardiomyocyte proliferation, by using transcriptome profiling (RNA-seq).
of isolated cycling cardiomyocytes.