

## Selection of cardiomyocytes and molecular analysis of their differentiation ex - vivo

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The adult cardiac muscle lacks ability to regenerate after myocardial infarction leading to heart failure. At the same time the satellite cells, which are present in the adult skeletal muscle are thought to be its progenitors. Their number and ability to proliferate and reconstruct muscle tissue decrease with aging. However, it was shown that adult multi-potent MSC are capable to create some types of tissues *in vitro* in proper conditions. The conditions and molecular mechanisms of proliferation/differentiation switch of MSC into the required lineage are poorly understood. We successfully cultured cardiomyocytes from heart tissue and analyzed their gene expression at different stages of differentiation.

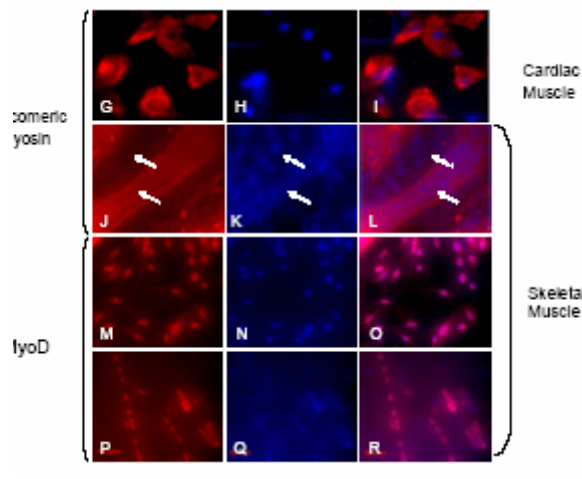


Figure 1: Immuno-staining of ex vivo primary cultured cells are obtained from 1-2 days neonatal rats. Hearts are washed in PBS, minced and then gently agitated in a  $\text{Ca}^{2+}$ - free 0,25% trypsin solution. Collected cells are centrifuged for 5 min at 500xg. The myoblasts from skeletal and heart muscles are diluted with DMEM containing 10% horse serum, 2% chicken embryo extract cultured on 1:1 mixture of collagen-gelatin coated dishes. Cultures are incubated in humidified atmosphere of 5%  $\text{CO}_2$ , 95% air at 37°C. The cultures were stained for myogenic proteins sarcomeric myosin and Myo D.

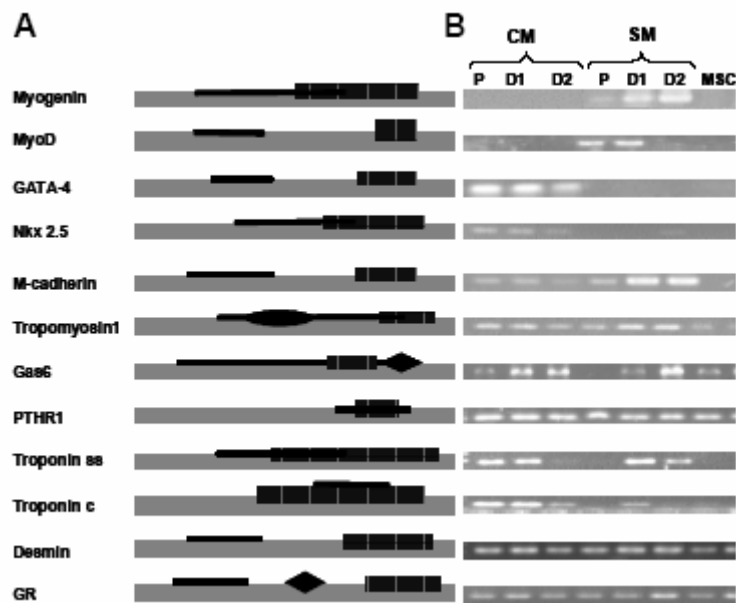


Figure 2: Evaluate the molecular profile of the compared population revealed shared or display distinct gene pattern that specify their lineal diversification.

A. localization of the primers used for PCR.

B. Expression of analyzed genes on cells' at different stages of differentiation. The expression compared between cardiac muscle (CM), skeletal muscle (SM) and mesenchymal stomal cells (MSCs). In summary, we compared myoblasts from cardiac and skeletal muscle for their gene expression to analyze the similarities and differences between these cells types. The profile of these cells allows highlighting the molecular pathways that correlate with the regulation of muscle cells from proliferation to differentiation and thus contributes to our understanding of the regulation of lineage fate decision. Our working hypothesis was to compare the transcriptom profile of different cell types that belong to the same ancestor lineage, namely-the mesenchymal lineage.