Epigenetic profiling of cardiac stem cells from the adult mammalian heart

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The epigenetic mechanisms such as DNA methylation and histone modifications controlling cardiac lineage decisions during development, hypertrophic growth in disease, and self-repair by endogenous or exogenous stem cells following infarction are among the most exciting aspects of current cardiovascular biology.

Recently discovered cardiac stem cells (CSCs), may have large impact on maintaining the slow turnover of cardiomyocytes during normal development and lifespan, and to be extremely important in cell therapy for resurrecting damaged myocardium and the heart failure.

Though the molecular mechanisms controlling the heart development were largely explored and shown to involve number of epigenetic events such as CpG island promoter methylation, histone modifications, and chromatin remodeling (Ellison, et. all, 2012), still little consideration has been given to epigenetic state in adult cardiac stem cells.

Previously, we have established *in vitro* individual clones of cardiac Sca1-positive side polulation cardiac stem cells from adult mouse hearts. Surprisingly, although they express many but not all cardiac transcription factors (e.g. GATA4, Mef2a, Nkx2.5) they still remain in undiferrentiated state capable to give rise at least a few cardiac cell linages (Nosega, et al., 2011).

Here, we elucidated the essential epigenetic mechanisms which maintain clonally expanded cardiac stem cells in undifferentiated state in comparison with cardiac myocytes and undifferentiated mouse stem cells. We anylysed 4 individual clones using expression profiling, ChIP-Seq and genomic DNA methylation analysis. Surprisingly, we discovered that clonally expanded cardiac stem cells demonstrate high heterogeity between different clones at the level of gene expression (mRNA profile, protein expression) as well as at the level of gene

promoter methylation and chromatin modifications (Histone 3 Lys4 tri-methylation, Histone 3 Lys27 tri-methylation, Histone 3 Lys36 tri-methylation). Naturally, the difference was even higher when CSCs were compared with adult cardiac myocytes or undifferentiated mouse embrionic stem cells. Although transcription factor such as GATA4 and Nkx2.5 are expressed in bouth cardomyocytes and cardiac stem cell, we clearly distinquished transcription factor binding events in between tested cell types.

The analysis of histone modifications and transcription factors binding events was performed using the ChIP-Seq Illumina platform with consequent application of the statistical software such as R-language, Bowtie, MAQ or BWA and others (Ali et all, 2012).

The obtained results shed light on our understanding of the role of cardiac stem cells in the adult mammalian heart and contribute to the development of the novel therapeutic approaches to regenerate or repair damaged cardiac tissue.

Keywords: Cardiac Sca-1 positive SP cells, epigenetics, ChIP-Seq, RNA-Seq

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