ABSTRACT

Ischemic heart disease, also known as the coronary artery disease (CAD), constitutes a challenge for contemporary medicine and become a target for regenerative medicine. The level of cardiac progenitor cells in the adult heart is insufficient to regenerate the post-infarcted myocardium while adult somatic stem cells of bone morrow demonstrated a limited plasticity. The iPSC technology might become a breakthrough due to possible differentiation of autologous cardiomyocytes. Nevertheless, obtained this way iPSC-CM-like cells demonstrate fetal phenotype which inclines to further research in this direct.

One of the factors supporting a cell maturation is an *in vitro* culture duration. In this dissertation it was demonstrated process of acquiring adult features of cardiomyocytes on the basis of two *in vitro* culture time-points spanning early stages of cardiac development (day 20 and 40 of culture). The following studies were performed in two main phases.

In the first stage a genetic reprogramming was conducted using human skeletal myoblasts inducing pluripotency stem cell line 194 which was generated using genetic construct with integration-free Sendai virus transduction. This was subsequently verified for its pluripotency:

a) elevated expression of pluripotent gene markers was detected compared with muscle initial priming cell suspension, namely *OCT4*, *SOX2*, *c-MYC;*

b) marker proteins for pluripotency was confirmed, by expression of TRA 1-60, TRA 1-81, SSEA-4 and then nuclear ones OCT4, SOX2 and c-MYC;

c) a normal male karyotype was proven;

d) undirected *in vitro* differentiation assay by embryoid bodies was conducted and derivatives of three germ layers were subsequently identified: AFP, SMA and TUJ1 proteins;

e) pluripotency potential was shown *in vivo via* 194 iPSC line administration subcutaneously into mice and histological analysis confirmed structures evidencing three germ layers.

In the second stage validated 194 iPSC line underwent successful cardiac differentiation using the protocol with BMP4 protein and chemical modulators of WNT pathway: CHIR99021, IWR-1 and *Cardiomyocyte Differentiation Kit*. Better efficiency and reproducibility has prompted to use the latter method of differentiation.

Differentiated cardiac myocytes in laboratory *in vitro* conditions on day 40^{th} of culture were more similar to adult myocardium than on day 20^{th}. Hence, cardiac cells cultured until this time-point may be a better choice for applying in research. This has been demonstrated as follows:

a) cardiac protein markers persisted: NKX2.5, cardiac troponin T, connexin 43 and α-MHC;

b) progressive cellular hypertrophy with statistically relevant increase in the circumference and cell surface as well as binucleated cells proportion;

c) along with the cell growth it was formed an uniformly distributed regular mitochondrial network in which functional organelles with generated membrane potential were getting elongated;

d) sarcomere alignment was shown as well as gradual development of the contractile apparatus based on sarcomere staining with α-actinin (Z lines);

e) statistically significant elevation of gene expression similar to human myocardial ventricular cells: *CX43*, *β-MHC* upregulation and *α-MHC* decrease (MHC isoform switch), *TNNI3*, *KCNJ2* and *SERCA 2A* at a similar levels comparable to mature myocardium;

f) more efficient intracellular calcium turnover when measuring based on fluorescent indicator which reflected productive contractile physiology – results suggested increased calcium pool in the SR during the contraction process and elevated Ca^{2+} levels in the cytoplasm, faster calcium release and uptake, more prominent response to the beta-adrenergic receptor stimulus.

Collected data confirmed the other studies indicating still immature phenotype of differentiated cardiomyocytes, iPSC-CMs. The set of proposed here assays can be a ground for further experiments with other iPSC-CM lines or when selecting other biochemical or biophysical cues promoting myogenic cardiac maturation. Moreover, obtained in this manner CM-like cells after 40 days of *in vitro* culture may be used as help for heart disease modelling, new drug screening, cardiotoxicity analysis and in further cell therapeutic approaches for failing heart.