Reprogramming of mouse embryonic fibroblasts to cardiomyocytes

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Abstract

Objectives:
During the reprogramming of differentiated cells into induced pluripotent stem cell (iPS) by defined transcription factors, generation of other specific cell types was recently reported (Efe et al., 2011). Our study is aimed to define optimal conditions during reprogramming of mouse embryonic fibroblasts (MEF) leading to cardiogenesis.

Methods:
Using viral transduction of mouse fibroblast derived from different parts of the body (i.e. tail tips, ears, abdominal pars) as well as from the whole body, we delivered transcription factors: Oct4, Sox2, Klf4, and c-Myc, the classical factors frequently used for the generation of iPS cells. We further tested other factors and conditions (BMP-4, Activin, DCA, FGF-2, Wnt inhibitors, VEGF, serum concentration, including different timing of treatment) that could potentially enhance cardiac differentiation. In order to confirm cardiogenesis, we performed immunostaining for specific cardiac markers. Functional characterization of derived cardiomyocytes is being done in order to control cardiac-specific functionality. Thus, development of action potentials, responses to the beta-adrenergic agonists as well as release of calcium from the sarcoplasmic reticulum is being investigated.

Results:
Our preliminary results indicate that within ~10 days from transgene induction and subsequent periodical stimulation by other factors, mouse embryonic fibroblasts (MEFs) are reprogrammed to spontaneously contracting clusters of cardiomyocytes. Process of newly emerging clusters in the fibroblast culture continues over period of several days. Before the detection of the first cardiomyocytes no intermediate iPS stage is observed. Our immunocytological experiments indicate positive signals of differentiated cardiomyocytes for the sarcomeric protein troponin I (TnI) as well as for the cardiac sarcoplasmic protein ryanodine receptor (RyR2) and others.

Conclusions:
Our first results indicate the possibility to activate early cardiac reprogramming from MEFs. In addition, our results could offer optimal conditions to enhance cardiogenesis and platform for derivation of specific and functional mouse cardiomyocytes. Transdifferentiation of MEF has the potential for being a reproducible method for generating cardiomyocytes in a quick and reliable way.