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Signal regulatory protein alpha (SIRPA) and kinase domain receptor (KDR) are key expression Markers in cardiac specific precursor selection from hADSCs,,

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Abstract

Background

Cardiomyocyte enrichment strategies so far have not yielded scalable cardiac specific cell type. More so, the current data is restricted to embryonic stem cells (ESCs)/induced pluripotent stem cells (iPSCs), wherein the use of viral vectors is fraught with increased risk during clinical use. Herein, we profiled time-dependent gene/protein expression patterns across the cardiac ectoderm, endoderm, and mesoderm for isolating cardiac precursors from human adipose derived stem cells (hADSC). Methods Direct cardiac differentiation of hADSCs was carried out with 5-azacytidine and basic fibroblast growth factor (bFGF) in a one month long culture. The cells were periodically harvested, analyzed for unique persistent markers and their inherent regulation using quantitative polymerase chain reaction (qPCR), flow cytometry, immunoblot and immunocytochemistry assays. The identified markers were super paramagnetic iron oxide nanoparticle (SPION) tagged for segregation by magnetic activated cell sorting (MACS) and further evaluated their differentiation potential and checked for the purity by flow cytometry.

Results

The results demonstrated pronounced up-regulation of mesodermal and mature cardiac lineage markers at three weeks, while there was a down-regulation of pluripotent stem cell markers. This perhaps could be attributed to de-differentiation in maintaining the cardiac phenotype. However, signal regulatory protein alpha (SIRPA) and kinase domain receptor (KDR) persisted all through the culture period of one month, making them the most relevant and reliable cardiac specific markers. Dual labeling of these markers to SPION for cardiomyocyte enrichment by MACS column yielded cardiomyogenic-like cells in differentiation cultures with several functional positive markers. Conclusions Thus, SIRPA and KDR together provide cues in the enhancement and up-scaling of cardiomyocyte production in the cell replacement therapy. Focal points • Benchside Identification of specific cell phenotypic markers to identify cardiac precursors in any tissue source with minimal cell manipulation is a novel process development tool in clinical translation. • Bedside A product developed in a closed system would minimize extraneous contaminants in long term cultures and development of such procedures minimizes culture failure rates from bench side. • Industry This unique identification of cell-specific marker would enable a tissue-specific translational plan and immensely help in the cardiac regeneration. • Government Financial investment and support from the government is vital in the optimization and validation for better health care and would contribute in reducing the disease burden. • Regulatory The stringent regulatory guidelines worldwide on minimal cell manipulation for entering into stem cell based clinical trials preclude the need to develop alternate approaches in product and process developmental technology, which can be easily translated in a clinical setup.

Keywords:

CardiomyocytehADSCSignal regulatory protein alpha (SIRPA)Kinase domain receptor (KDR)