Efficient Differentiation of Human Pluripotent Stem Cells Into Contracting Cardiomyocytes

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Abstract

Functionally mature cardiomyocytes produced from a renewable cell source, such as human pluripotent stem cells, are in high demand because of their potential contributions to developmental research, disease modeling, high throughput toxicity drug screening, and clinical therapies. Here we introduce our StemXVivo™ Cardiomyocyte Differentiation Kit (coming soon) which will guide human pluripotent stem cells through the necessary cell fate decisions that recapitulate embryonic development to produce functional cardiomyocytes. Our differentiation protocol has been verified on multiple pluripotent cell lines, including human embryonic stem (ES) cells and human induced pluripotent stem (iPS) cells. The differentiated cardiomyocytes are validated by immunocytochemistry, flow cytometry, and visualization of calcium fluctuations during cellular contraction. We also show that ES-differentiated cardiomyocytes can be used as a tool for investigation, including functional assays for small molecule screening, cardiotoxicity, and cardiac hypertrophy.

StemXVivo Cardiomyocyte Differentiation Kit

- Three-stage directed differentiation followed by an extended maturation and maintenance phase
- Contracting cells are typically observed as early as day 11–13; widespread and synchronous contractions are observed by day 24
- Verified to differentiate multiple human ES and iPS cell lines (in-house and beta-tested in independent academic laboratories)
- Protocol designed to recapitulate embryonic development of cardiomyocytes

Differentiation

Progression of marker expression during kit-differentiated cardiomyocyte development. BG01V human embryonic stem cells were differentiated with the StemXVivo Cardiomyocyte Differentiation Kit and assessed at select time points for stage-specific marker expression. The pluripotency marker Oct-4 (Mouse Anti-Human Oct-4 Monoclonal Antibody; Catalog # MAB7991) is highly expressed during early differentiation (Day 0) and is subsequently downregulated. Expression of the mesoderm marker, Brachyury (Goat Anti-Human/Mouse Brachyury Polyclonal Antibody; Catalog # AF2085), is expressed immediately during differentiation (Day 1). The cardiomyocyte markers NKX2.5 (Goat Anti-Human NKX2.5 Polyclonal Antibody; Catalog # A24444) and Troponin T (Mouse Anti-Human Cardiac Troponin T Monoclonal Antibody; Catalog # MAB1874) are less prevalent in cells during early (Day 0) and intermediate (Day 1) differentiation and become more highly expressed during the latter stages of differentiation (Day 7, Day 25–30). Brachyury and NKX2.5 primary antibodies were visualized with the NorthernLights™ (NL557-conjugated Donkey Anti-Goat IgG Secondary Antibody; Catalog # NL005), Oct-4 and Troponin T were visualized with the NL557-conjugated Donkey Anti-Mouse IgG Secondary Antibody (Catalog # NL007).

Conclusions

- The StemXVivo Cardiomyocyte Differentiation Kit efficiently directs human pluripotent stem cells into functional cardiomyocytes
- Pluripotent stem cells, terminally-differentiated cardiomyocytes, and differentiation intermediates, express stage-appropriate markers throughout the course of the differentiation protocol
- Differentiated cells exhibit function and morphology characteristic of cardiomyocytes
- Differentiated cardiomyocytes can be utilized as a tool for high throughput drug screening

Verification

Cardiomyocyte contraction visualized using the calcium indicator, Fluor-4. BG01V human embryonic stem cells were differentiated into cardiomyocytes with the StemXVivo Cardiomyocyte Differentiation Kit. Cells were then loaded with the calcium indicator, Fluoro-4, which fluoresces upon calcium binding. A) Representative still images of resting and contracting cardiomyocytes, taken from a time-lapse video capturing calcium influx during contraction. B) Rate-interval graph demonstrating cardiomyocyte contraction as measured by recording changes in Fluoro-4 fluorescence intensity. The contraction interval was determined as the time between low Fluoro-4 fluorescence (relaxed cells) and high Fluoro-4 fluorescence (contracted cells).

Investigation

Small molecules affect the rate of cardiomyocyte contraction. Cardiomyocytes were differentiated from the BG01V human embryonic stem cells using the StemXVivo Cardiomyocyte Differentiation Kit and assessed for their ability to contract using the Fluor-4 calcium binding assay. A) Rate-interval graph show fluctuations in Fluoro-4 fluorescence intensity, indicative of cardiomyocyte contraction, under basal conditions (brown; untreated) and following sequential treatment with Isoprenaline (blue; Catalog # 1747), a β-adrenergic agonist, and Propranolol (tan; Catalog # 0624), a β-adrenergic antagonist. B) Table showing drug concentrations and contraction rate of cardiomyocytes during each treatment. The contraction rate of differentiated cardiomyocytes was determined as the number of contractions (contraction-interval plus relaxation interval) per minute.

Conclusions

Differentiated cardiomyocytes respond physiologically to Endothelin-1 induced hypertrophy. Cardiomyocytes differentiated from BG001V human embryonic stem cells were treated with Endothelin-1 (1-1; Catalog # 1140) for 28 hours to induce cardiac hypertrophy. Levels of Pro-Atrial Natriuretic peptide (Pro-ANP) secretion, which is known to be elevated during cardiac hypertrophy, were assessed using ELISA (Coming Soon from R&D Systems). As expected, detection of Pro-ANP increased proportionally with the concentration of Endothelin-1 treatment.