Robust and Scalable Production of Multipotent Cardiac Progenitor Cells from Human Induced Pluripotent Stem Cells and Their Use in Proliferation and Differentiation Screens

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Abstract

Cardiac progenitor cells (CPCs) represent the earliest stages of mesodermal commitment to the cardiac lineages and directed differentiation of CPCs from pluripotent stem cells has been demonstrated in a number of recent studies. Furthermore, these cells have been shown to be capable of generating other terminal cardiac cell types such as cardiomyocytes, endothelial cells, and vascular smooth muscle cells. The differentiation potential of human pluripotent stem cell-derived CPCs makes them an attractive candidate for drug discovery and regenerative medicine efforts. However, utilization of CPCs for such endeavors requires a robust, scalable, and consistent differentiation methodology.

Here we describe iCell® Cardiac Progenitor Cells, human CPCs generated from peripheral blood mononuclear cell derived iPSCs. iCell CPCs were developed specifically to provide in-vitro access to cardiac development for drug development and regenerative medicine. As such the reagent had to recapitulate native human cardiac development under conditions amenable to high throughput screening (HTS).

iCell Cardiac Progenitor Cells:
- Reproducibly manufactured and cryopreserved at large scale.
- Exhibit cardiac mesodermal markers.
- Can be differentiated into terminal cardiac cell types.
- Amenable to HTS screening in 96 and 384 well plates, yielding high throughput screening (HTS).
- Cardiomyocyte differentiation assay Z' scores > 0.4.
- Provide unprecedented access to cardiac developmental biology for drug discovery and regenerative medicine.

Background

Cardiac Progenitor Cells (CPCs) represent the earliest stages of mesodermal commitment to the cardiac lineages. CPCs can generate cardiomyocytes, endothelial cells, and vascular smooth muscle cells. In addition, CPCs can give rise to highly enriched populations of cardiomyocytes (1,2). The molecular markers used to characterize each intermediate is described here as well.

Figure 1: Cardiac Progenitor Cells (CPCs) represent the earliest stages of mesodermal commitment to the cardiac lineages. CPCs can generate cardiomyocytes, endothelial cells, and vascular smooth muscle cells. In addition, CPCs can give rise to highly enriched populations of cardiomyocytes (1,2). The molecular markers used to characterize each intermediate is described here as well.

Figure 2: Footprint-free episomal reprogramming of human induced pluripotent stem cells (iPSCs). To generate human iPSCs by this method of reprogramming, fresh blood was collected and PBMCs were expanded. Reprogramming plasmids were added using a single transfection in xeno-free conditions amenable to CbMP (3,4).

Characterization

Figure 3: iCell CPC production recapitulates native development. Differentiation conditions of iCell CPCs from iPSCs was optimized for efficient differentiation of CPCs. Daily, during differentiation, cells were isolated and qPCR was conducted to determine the developmental program and CPC emergence.

Figure 4: iCell CPC phenotype is robust across manufacturing batches. Cryopreserved iCell CPCs were thawed and plated as adherent monolayers in defined medium. Two days later, cells were harvested and demonstrated to be KDR+/CD34+(A) flow cytometry and (B) NKKX2.5+/CTNT+ by high content imaging (HCl, 10X). Across different batches, the average characteristic data were: KDR+/CD34+ = 88.3%, NKKX2.5+/CTNT+ = 88.2%, and yield = 9.5 x 10^6 ± 0.3 x 10^6 cells/L.

Figure 5: iCell CPCs exhibit native functionality. iCell CPCs were thawed and plated in T150 flasks as adherent monolayers in defined conditions to promote cardiomyocyte differentiation. Seven (7) days later, cells were harvested and analyzed for CTNT and SMA by flow cytometry (A). In addition, Day 7 cardiomyocyte cultures were harvested and replated into wells of a 96-well plate and analyzed 4 days later for CTNT and NKKX2.5 by HCl (10X) (B).

Figure 6: iCell CPCs retain native developmental potential. iCell CPCs were thawed and cultured in defined medium with the addition of bFGF. Six (6) days after plating, cultures were fixed and stained using Hoechst (blue) and antibodies specific for CD31 (green) and NKKX2.5 (red) to illustrate endothelial cells and cardiomyocytes, respectively. Images were captured using HCl (10X).

Assay Development

Figure 7: iCell CPCs acquire cardiomyocyte markers over time. A. iCell CPCs were thawed and plated into 96-well plates in defined conditions to promote cardiomyocyte differentiation. Cells were fixed at days 3, 4, 6 and 7 followed by staining using Hoechst (blue) and antibodies specific for NKKX2.5 (green) and CTNT (red). Images were captured by HCl (10X). Boxes represent entire wells, duplicate wells are shown for each stain. B. CTNT images were analyzed for % NKKX2.5 and % CTNT over time using the ImageXpress Micro (Molecular Devices).

Figure 8: Robust HTS-amenable proliferation and differentiation assays were developed based on CPC ontology. Native CPCs undergo self-renewal and differentiation to maintain progenitor and differentiated cell populations, respectively. Modulating either of these processes presents an attractive target for drug discovery and regenerative medicine. Robust protocols (Z' > 0.4) were developed with iCell CPCs in 96 or 384 well plates to provide in-vitro access to CPC proliferation and cardiomyocyte differentiation.

Summary

Cardiac progenitor cells (CPCs) are a promising candidate for therapeutic applications due to their ability self-renew and give rise to cells of cardiac lineages including cardiomyocytes. Here we show the robust, scalable and consistent differentiation of iCell Cardiac Progenitor Cells from epidermally derived iPSCs. iCell CPCs are cryopreserved and maintain native characteristics and functionality upon thaw (Fig. 4-6). When plated in conditions which promote cardiomyocyte differentiation, iCell CPCs develop into enriched cultures of cardiomyocytes (Fig. 7). The native ontogeny and potency exhibited by iCell Cardiac Progenitor Cells was captured in robust HTS-amenable assays for self-renewal and cardiomyocyte differentiation; two attractive targets for drug discovery and regenerative medicine that were previously unapproachable.

The routine generation of highly enriched iCell CPCs and development of end-user protocols described here, provide a unique and reliable source of these cells for basic and targeted research applications.

References:

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