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Review

Steering signal transduction pathway towards cardiac lineage from human pluripotent stem cells: A review

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ABSTRACT

In humans injured myocardium cannot avert the onset and progression of ventricular dysfunction because of limited regenerative ability of myocytes. Although limited renaissance of cardiomyocytes has been reported in human infarcted hearts, it is generally accredited that non-functional fibrous tissue replaces the dead myocardium. High cardiovascular morbidity and dearth of donor hearts warrant a constant hunt for radically different approach to treat heart failure. Pluripotent stem (PS) cells possess the ability to produce functional cardiomyocytes for clinical applications and drug development, which may provide the answer to this problem. Although progress has been made in differentiating human PS cells into cardiomyocytes, however, the in vitro differentiation of pluripotent cells into cardiomyocytes involves a poorly defined, inefficient and relatively non-selective process. A thorough understanding of signaling pathways would tender a roadmap for the streamlined development of in vitro cardiac differentiation strategies. The ability to obtain unlimited numbers of human cardiomyocytes would improve development of cell-based therapies for cardiovascular diseases, facilitate the study of cardiovascular biology and improve the early stages of drug discovery. Here in this review, we highlight the interacting endogenous cellular signals and their modulators involved in directing the human PSCs towards cardiac differentiation.

Contents

1. Introduction

For decades now, we have known that human pluripotent stem cells can give rise to spontaneously beating cardiomyocytes in vitro, a phenomenon known as cardiomyogenesis [1]. Kehat et al. in 2001 first reported the spontaneous differentiation of hESCs into cardiomyocytes. Since then, extensive efforts have been made to control the process of cardiomyogenesis [2].

Abbreviations: hESCs, human Embryonic Stem Cells; hPSCs, human Pluripotent Stem Cells; EB, Embryoid Body; NKX2.5, NK2 transcription factor related, locus 5; TAK, Mitogen-activated protein kinase kinase kinase; AP1, Activator Protein 1; WIF-1, Wnt-Inhibitory factor-1; cTnT, Cardiac Troponin T; PTCH, Patched; SMO, Smoothened; αMHC, Alpha myosin heavy chain.

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Early reports capitalizing on spontaneous in vitro cardiomyogenesis cited rather low differentiation efficiency of 8.1% [2]; while more recent efforts using directed in vitro cardiomyogenesis cited more encouraging efficiencies ranging from 30% to 98% [3–10]. To a certain extent, this improvement comes from enhanced understanding of the molecular mechanism governing differentiation of hPSCs to cardiomyocytes.

A lot of our understanding about human cardiomyogenesis in vitro comes from studies done in the murine system. While broadly comparable, in vitro cardiomyogenesis in humans proves to be more challenging than its murine counterpart [11].

Through detailed ultra-structural, immuno-histochemical, molecular, as well as electrophysiological studies; in-vitro cardiomyogenesis in the murine and human models has been demonstrated to recapitulate the in vivo developmental stages (Fig. 1) [11]. Despite the apparent difference of in vitro and in vivo environments, it might be worthwhile to refer to the in vivo system to gain a better understanding of the different cardiomyogenesis stages in vitro. The knowledge about this phenomenon would be instrumental in designing a robust in vitro differentiation protocol which would result in high cardiomyogenesis efficiencies.

In vivo cardiogenesis in mammals starts prior to gastrulation, whereby a number of cells destined to be heart cells are scattered throughout the pre-gastrula epiblast [1]. Nodal expression in the proximal epiblast then starts gastrulation by inducing BMP4 expression in the extra-embryonic ectoderm adjacent to the epiblast [12]. BMP4 expression then activates canonical Wnt signaling back in the proximal epiblast.

Soon after, Wnt expression is restricted to the posterior epiblast only as the anterior visceral endoderm expresses DKK1 (Wnt antagonist), LEFTY1 and CER1 (Nodal antagonists). Active Wnt proteins at the posterior epiblast activate canonical Wnt signaling which in turn induces early cardiomyocyte precursors to go through epithelial–mesenchymal transition (EMT). These cells ingress and migrate through the Primitive Streak (PS) antero-laterally, to form mesodermal cells expressing markers such as T (*Brachyury*) and MIXL1.

Cardiac cells originate from the mesoderm and it has been shown that cardiac differentiation inducing signals are to a large extent of endodermal origin [13].

As the cells migrate, BRACHYURY induces the expression of the basic helix–loop–helix (bHLH) transcription factor mesoderm posterior 1 (MESP1) which further specifies mesodermal cells towards cardiac mesodermal lineage [14]. MESP1 has been termed the "master regulator" of cardiac lineage specification, more specifically in cardiac mesodermal cell specification or cardiovascular lineage commitment [15,16]. At this juncture, cells are in cardiac mesodermal cell stage.

MESP1 then triggers the expression of cardiac mesodermal markers which include NKX2.5, TBX5, HAND1/2, GATA4, MEF2C, MYOCD, FOXH1 and ISL1 [15,17]. In mice, NKX2.5 is important for the interpretation of patterning signals within the primitive heart tube and is likely to act in tandem with TBX5 during formation of both the atrial and left ventricular compartments [18]. The cardiac mesodermal cells then further differentiate to give rise to the First Heart Field (FHF) progenitor cells, Second Heart Field (SHF) progenitor cells, endocardium as well as the pro-epicardial mesenchyme. The differentiation of FHF progenitor cells precedes the SHF and this is under the control of active BMP4 signaling. Meanwhile, the progenitor cells of SHF are still proliferative, being regulated by active Hedgehog signaling and down-regulation of canonical Wnt signaling [17]. FGF and Notch signaling come into play once heart fields have been established, controlling proliferation and fate determination of cardiac progenitor cells (CPCs) [17]. Specifically, Notch induces the expression of WNT5a, BMP6 and SFRP1, which altogether serve to increase the proliferation of cardiac progenitor cells (CPCs) [19].

Differentiation of CPCs towards immature cardiomyocytes is initiated when they stop proliferating and this is governed by the down-regulation

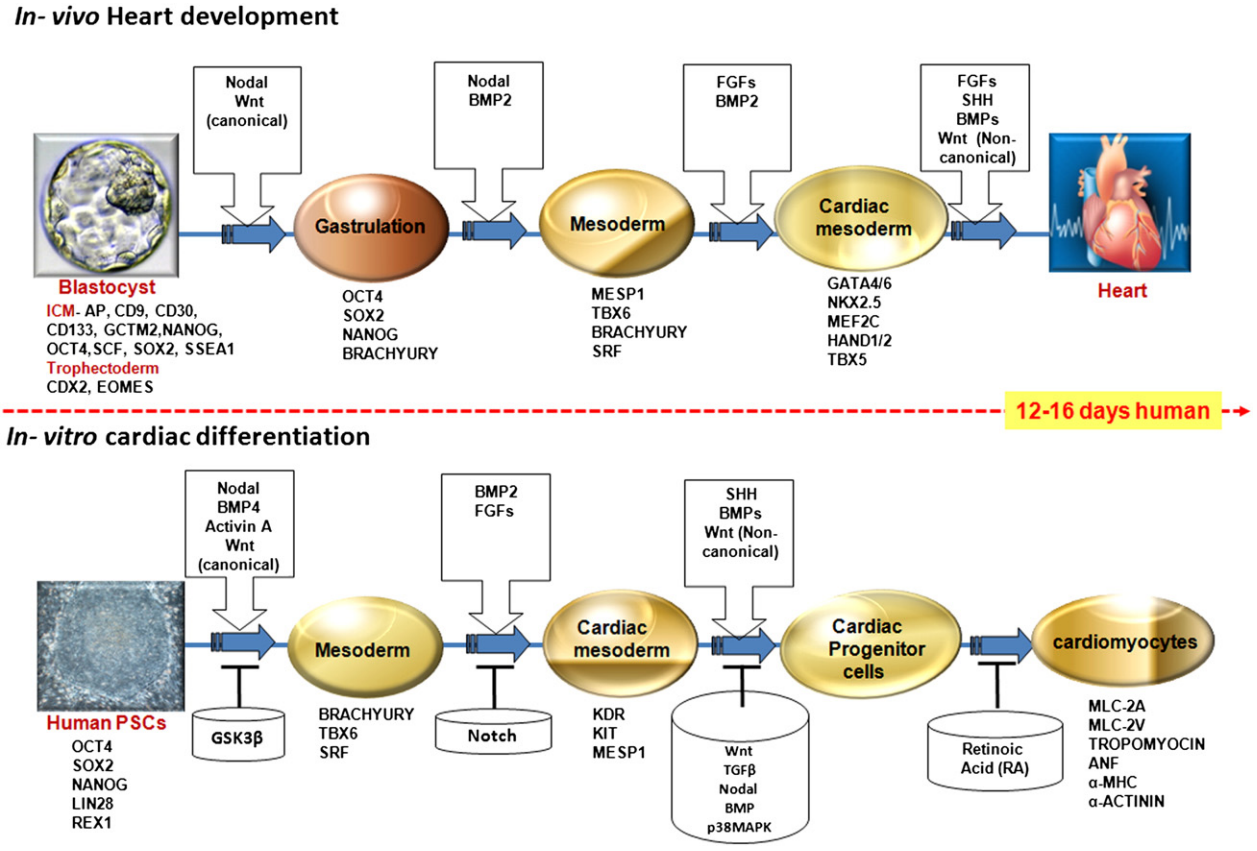

Fig. 1. Stages in cardiomyogenesis in humans with associated markers and signaling pathways: in-vitro cardiomyogenesis in Human model has been demonstrated to recapitulate the in vivo developmental stages.