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(Re-)programming of subtype specific cardiomyocytes

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ABSTRACT

Adult cardiomyocytes (CMs) possess a highly restricted intrinsic regenerative potential — a major barrier to the effective treatment of a range of chronic degenerative cardiac disorders characterized by cellular loss and/or irreversible dysfunction and which underlies the majority of deaths in developed countries. Both stem cell programming and direct cell reprogramming hold promise as novel, potentially curative approaches to address this therapeutic challenge. The advent of induced pluripotent stem cells (iPSCs) has introduced a second pluripotent stem cell source besides embryonic stem cells (ESCs), enabling even autologous cardiomyocyte production. In addition, the recent achievement of directly reprogramming somatic cells into cardiomyocytes is likely to become of great importance. In either case, different clinical scenarios will require the generation of highly pure, specific cardiac cellular-subtypes. In this review, we discuss these themes as related to the cardiovascular stem cell and programming field, including a focus on the emergent topic of pacemaker cell generation for the development of biological pacemakers and *in vitro* drug testing.

1. Introduction

The advent of regenerative medicine has opened up new perspectives for so far insoluble clinical problems. Recent progress in understanding the biology of stem cell pluripotency and endogenous repair mechanisms has fostered a deeper understanding of its remarkable therapeutic potential for tissue repair or replacement. Such novel approaches are urgently required to effectively treat the growing burden of disorders characterized by irreversibly damaged or diseased tissue resulting in loss of organ/tissue function associated with a rapidly ageing population. Furthermore, through the production of autologous pluripotent stem cells, regenerative strategies hold promise in providing

Abbreviations: ADSC, adipose tissue-derived mesenchymal stem cell; Alcam, activated leukocyte cell adhesion molecule; AMI, acute myocardial infarction; ANF, natriuretic factor; AP, action potential; ASC, adult stem cell; AV, atrioventricular; AVB, atrioventricular bundle; AVN, atrioventricular node; BB, bundle branch; BCT, bioartificial cardiac tissue; bHLH, basic helix-loop-helix; Bry, Brachyury; CABG, coronary artery bypass graft; $Ca_v1.3$, calcium voltage-gated channel subunit alpha1 D; $Ca_v3.1$, calcium voltage-gated channel subunit alpha1 G; CCS, cardiac conduction system; CF, cardiac fibroblast; CHD, congenital heart disease; CM, cardiomyocyte; CMPC, cardiomyocyte progenitor cell; CMVEC, cardiac microvascular endothelial cell; CPC, cardiac progenitor cell; CS, conduction system; CV, cardiovascular; CVD, cardiovascular disease; Cx30.2, connexin30.2; Cx40, connexin40; Cx43, connexin43; Cx45, connexin45; ECG, electrocardiogram; EMILIN2, elastin microfibril interface 2; EPC, endothelial progenitor cell; EPCS, electric-pulse current stimulation; ESC, embryonic stem cell; FDA, Food and Drug Administration; FGF, fibroblast growth factor; FHF, first (primary) heart field; GF, growth factor; GFP, green fluorescence protein; GO, Gene Ontology; HCN4, hyperpolarization-activated cyclic nucleotide-gated cation channel 4; hPSCreg, Human Pluripotent Stem Cell registry; HTS, high-throughput sequencing; iCM, induced cardiomyocyte; iPSC, induced pluripotent stem cell; iSAB, induced sino-atrial body; Isl1, ISL LIM homeobox 1; JNK, c-Jun N-terminal kinase; LVEF, left ventricular ejection fraction; MAPK, mitogen-activated protein kinase; MB, molecular beacons; MEA, multi-electrode-array; Mlc2v, myosin, light polypeptide 2, regulatory, cardiac, slow; MSC, mesenchymal stem cell; Myh6, myosin, heavy chain 6, cardiac muscle, alpha; Myh7, myosin, heavy polypeptide 7, cardiac muscle, beta; $Na_v1.5$, sodium voltage-gated channel alpha subunit 5; Nkx2-5, NK2 homeobox 5; NPPA, natriuretic peptide A; ODE, ordinary differential equation; PA, polyacrylate; PDMS, polydimethylsiloxane; PLGA, polylactide-co-glycolide; PMC, pacemaker cell; PPT, protein-protein interaction; PSC, pluripotent stem cell; Rarg, retinoic acid receptor, gamma; ROCK, rho-associated, coiled-coil containing protein kinase; Rxra, retinoid X receptor, alpha; SA, sino-atrial; SAN, sinoatrial node; SCD, sudden cardiac death; SCN5A, sodium channel, voltage-gated, type V, alpha subunit; SHF, second heart field; Shox2, short stature homeobox 2; SIRPA, signal-reduced protein alpha; SSS, sick sinus syndrome; Tbx18, T-box 18; Tbx3, T-box 3; TF, transcription factor; THF, tertiary heart field; VCAM1, vascular cell adhesion molecule 1; VCS, ventricular conduction system; VEGF, vascular endothelial growth factor; wt, wild-type.

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truly patient-specific therapies for structural and functional repair in disease.

Cardiovascular disease (CVD) is the leading cause of death worldwide (accounting for 31.3% in 2015) and is projected to rise further (WHO 2017). CVD encompasses a range of chronic disease states, including ischemic, rheumatic and hypertensive heart disease, in addition to extracardiac disorders such as stroke. Heart failure represents the final common phenotype resulting from a diverse range of inherited and acquired cardiac insults and affects ~26 million individuals worldwide [1]. Individuals with severe heart failure have a dismal prognosis with a worse 5-year adjusted mortality than many cancers [2]. To date, allogeneic heart transplantation remains the only available treatment option for patients with end-stage heart failure who are symptomatic despite optimal medical and device (cardiac resynchronization) therapy [3,4]. Despite advances in surgical technique, perioperative management and immunomodulation, a major limitation to its wider application is donor organ scarcity: in Europe in 2015, only 604 donor organs were successfully engrafted, while 1140 patients are on the active Eurotransplant waiting list [5]. An additional 209 recipients died before they could undergo heart transplantation [5]. Even for those transplanted, while symptomatic improvement and survival are in general markedly improved, outcomes (median ~11 year survival) are limited by long-term complications, in part associated with immunosuppression, including malignancy, infection, renal dysfunction and allograft vasculopathy [6]. In view of such limitations, highly innovative approaches are under exploration with the ultimate goal of establishing safe, durable cellular replacement and repair of injured or diseased myocardium, in addition to *in vitro* disease modeling and drug development applications [7–9]. A key requirement for these approaches is to ensure highly reliable and robust generation of fully functional cardiomyocytes with physiological properties as close as possible to their natural counterparts. Partially or terminally differentiated cells offer a relevant alternative to somatic stem cell transplantation, given that the latter are still a matter of controversial debate regarding their moderate therapeutic outcomes [10,11]. Pluripotent stem cells (PSC) and their derivatives offer an attractive source for both cell replacement and studying key cellular and molecular processes involved in cardiovascular disease. Equally, resident cells (*e.g.* fibroblasts) may also represent a readily accessible source of cells to study cell fate transition not only within, but even across, germ layers.

2. Tissue regeneration and repair for cardiovascular disease

Normal cardiac function and physiological homeostasis is achieved through the complex interaction of a diverse range of cell types broadly constituting myocyte, vascular and stromal compartments. Even among specific cell types, such as cardiomyocytes (CM), there exist different phenotypes (*e.g.* sinoatrial, atrial, nodal, Purkinje and ventricular). Disease processes do not affect all these cell types uniformly, with relatively greater impact on specific tissue components such as fibrosis or vascular insufficiency.

The human heart does exhibit some regenerative potential, albeit very low, with an annual cardiomyocyte turnover rate of 1% at age 25 years, reducing further to 0.45% by 75 years [12]. As a corollary, adult human cardiomyocytes are long-lived cells, such that <50% will be replaced over a life-span of 75 years. In contrast, the proportion of CM situated in mitosis and cytokinesis is highest in infancy and contributes to developmental growth, suggesting significant cardiac regenerative potential in children and adolescents [13]. Other studies, including data from animal models, have highlighted that CMs, upon transition from the mononucleate to a mature binucleate state, exit the cell cycle and lose their proliferative potential during a short postnatal period [14–16]. In the setting of common CVD such as acute myocardial infarction (MI), leading to the abrupt loss of up to ~1 billion CM, this intrinsic regeneration potential is vastly inadequate, resulting in structural (*i.e.* scar) rather than functional (*i.e.* contractile) repair, and potentially to progressive deleterious ventricular remodeling and post-MI heart failure. However, the identification of adult CM repopulation raises the possibility that either normally resident cell populations such as cardiac progenitor cells (CPCs), or pre-existing CM may represent sources for myocardial repair post-injury [13,17,18].

Accordingly, development of experimental protocols to robustly generate distinct cardiac cell types and define their specific clinical/preclinical applications is required. We will address the progress made recently with attempts at stem cell and somatic cell-based programming, detailing their therapeutic potential and current stage of development. A major contribution to these has been provided by applying insights gained from the study of cardiovascular developmental biology to which we turn our attention next.

3. Cardiogenesis during development and its regulation

Cardiac development occurs during the early stages of the embryonic phase, and is crucial to ensure adequate nutrient and oxygen supply to, as well as removal of waste from, the growing organism. The mature mammalian heart is highly complex in structure, divided macroscopically into four chambers macroscopically and constituting specific muscle and non-muscle cell types, including left and right atrial CM, left and right ventricular CM, and cells forming the conduction system, sinoatrial pacemaker, vascular smooth muscle, endo- and epicardium [19–23]. The generation of such developmentally diverse cell fates are achieved *via* spatiotemporally stringent molecular regulation, with clear evidence that myocardial cells derive from Brachyury$^+$ (Bry$^+$) mesodermal progenitor cells of the primitive streak during gastrulation through the impact of Wnt signaling [24–26].

Thereafter, two crucial transcription factors (TF) are regarded as cardiovascular fate-determining factors: the bHLH TF MesP1 (mesoderm posterior 1) [27–30] and the surface molecule Flk1 (also known as VEGFR2: vascular endothelial growth factor receptor 2) [31,32]. Further development is achieved from multipotent cardiac progenitor cells [33] and can be distinguished mainly in two origins: i) the first (primary) heart field (FHF) demarcating an Nkx2-5$^+$/Hcn4$^+$ cell population which forms the cardiac crescent [34–41], and ii) the second heart field (SHF) demarcating a Nkx2-5$^+$/Isl1$^+$ cell population derived from the pharyngeal mesoderm and lying medially and posterior to the FHF [41–46]. In avians, a decisive role for the tertiary heart field (THF) in pacemaker development of the sino-atrial (SA) node has also been reported [47,48]. Primary heart field progenitor cells will yield the myocardium of the left ventricle as well as a limited portion of the right ventricle, the right and left atria and large parts of the conduction system (CS), such as the atrioventricular (AV) node and the ventricular CS [20,42]. Multipotent progenitor cells of the SHF will yield myocardium of the right and left atria, the right ventricle and the outflow tract, as well as cardiac vascular smooth muscle and the endocardium [31,46,49]. In addition to these, epicardial progenitor cells give rise to cardiac fibroblasts, vascular smooth muscle, atrial and venous endothelial cells [20,50–52]. Moreover, pro-cardiogenic factors and signaling pathways play a decisive role during development and are distributed from the surrounding endoderm and mesoderm. These include bone morphogenetic proteins [53–57], notch [58], nodal and fibroblast growth factors [59–61], in addition to canonical and non-canonical Wnt/JNK [62–66].

A highly coordinated signaling network determines early cardiac progenitor as well as late specific cell fates, whose disruption can lead to abnormal embryonic development and congenital heart disease (CHD) characterized by malformation of specific cardiac structures [67]. CHD is the most common major congenital defect worldwide with a birth prevalence of between 0.58 and 0.9% [68,69]. Thus, dysregulation of TFs (*e.g.* Nkx2-5 [70–78], Gata4 [77,79–82] or members of the forkhead family [83]) is associated with various abnormalities including atrioventricular block, septal defects or pulmonary stenosis [84–86]. Exemplifying this, smoking-associated cardiac defects have been linked to promotor DNA hypermethylation of Tbx5 and Gata4 caused by maternal nicotine exposure [87]. In contrast, mutations in genes encoding cardiac

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