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Research update

Harnessing the secretome of cardiac stem cells as therapy for ischemic heart disease ☆

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ABSTRACT

Adult stem cells continue to promise opportunities to repair damaged cardiac tissue. However, precisely how adult stem cells accomplish cardiac repair, especially after ischemic damage, remains controversial. It has been postulated that the clinical benefit of adult stem cells for cardiovascular disease results from the release of cytokines and growth factors by the transplanted cells. Studies in animal models of myocardial infarction have reported that such paracrine factors released from transplanted adult stem cells contribute to improved cardiac function by several processes. These include promoting neovascularization of damaged tissue, reducing inflammation, reducing fibrosis and scar formation, as well as protecting cardiomyocytes from apoptosis. In addition, these factors might also stimulate endogenous repair by activating cardiac stem cells. Interestingly, stem cells discovered to be resident in the heart appear to be functionally superior to extra-cardiac adult stem cells when transplanted for cardiac repair and regeneration. In this review, we discuss the therapeutic potential of cardiac stem cells and how the proteins secreted from these cells might be harnessed to promote repair and regeneration of damaged cardiac tissue. We also highlight how recent controversies about the efficacy of adult stem cells in clinical trials of ischemic heart disease have not dampened enthusiasm for the application of cardiac stem cells and their paracrine factors for cardiac repair: the latter have proved superior to the mesenchymal stem cells used in most clinical trials in the past, some of which appear to have been conducted with sub-optimal rigor.

1. Introduction

Heart disease remains the major cause of death and morbidity worldwide [1]. Despite recent therapeutic advances, current pharmacological and surgical interventions only delay progression of the disease but fail to arrest deterioration toward end-stage heart failure, so that heart transplantation remains the therapeutic option of last resort. Over the last decade, stem cell-based therapies have provided great promise for treatment of damaged heart tissue to address this unmet clinical need. While many preclinical studies reported benefits of stem cell therapy in ischemic heart disease, clinical trials thus far have revealed only modest improvement in outcomes and have not met the high expectations. It is therefore important to probe more deeply the mechanisms underlying the repair processes promoted by stem cells.

Several types of stem cells have been identified and investigated for their cardioreparative effects, and more effective stem cell types for cardiac repair and regeneration are emerging [2]. The discovery of

Abbreviations: bFGF, basic fibroblast growth factor; CDC, cardiosphere-derived cell; CFU-F, colony forming unit-fibroblasts; CSC, cardiac stem cell; ECM, extracellular matrix; EPC, endothelial progenitor cell; ESC, embryonic stem cell; FSTL-1, follistatin like-1; HGF, hepatocyte growth factor; IGF-1, insulin-like growth factor-1; IL-1β, interleukin-1β; iPSC, induced-pluripotent stem cell; MI, myocardial infarction; MMP, metalloproteinase; MSC, mesenchymal stem cell; PDGFR-α, platelet-derived growth factor receptor-α; SDF-1, stromal cell-derived factor-1; Sca-1, stem cell antigen-1; TIMP, tissue inhibitor of metalloproteinase; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor; Wt-1, Wilm's Tumor Gene-1.

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rare populations of stem cells residing in adult human hearts has provided new impetus in the field of myocardial biology. Several types of cardiac stem cell (CSC) have been shown to exhibit greater therapeutic benefits in cardiac repair than extra-cardiac stem cells such as those derived from bone marrow [3]. Transplantation of CSCs has been proposed to improve cardiac performance through differentiation into cardiovascular cells as well as by release of trophic paracrine factors that promote endogenous repair [2,4]. These paracrine factors could be growth factors, cytokines, extracellular vesicles or exosomes and genetic materials such as mRNA and miRNA, which are collectively termed 'stem cell secretomes'. Despite these encouraging findings with CSCs, many important issues remain to be resolved including the optimal cell type, the cell dose, the route of administration, the timing of treatment and defining how exactly they work. Indeed there is no cell therapy to date that can claim undisputed mechanisms responsible for the cardioreparative effects observed. However, all recent studies have focused on paracrine mechanisms that are primarily responsible for the cardioreparative effects of CSCs [5,6]. Herein we review the cardiac repair and regenerative potential of CSCs and their paracrine signaling.

2. Sources of stem cells for cardiac repair and regeneration

Multiple types of stem cell have been utilized for regenerative repair of the heart over almost 2 decades. They are generally categorized into pluripotent stem cells and adult stem cells [2].

2.1. Pluripotent stem cells

Pluripotent stem cells, including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), can differentiate into each of the three germ lineages (i.e. ectoderm, mesoderm and endoderm). They have unlimited proliferative potential and can differentiate into *bona fide* cardiomyocytes for cardiac repair. A recent hallmark study by Chong et al. using a non-human primate model of myocardial infarction (MI) has demonstrated that intramyocardial injection of 1 billion human cardiomyocytes derived from ESCs was able to remuscularize the infarcted myocardium [7]. However, the risk of teratoma formation, a risk of arrhythmia, long-term immune suppression and ethical considerations remain significant challenges and prevent acceptance of ESCs in regular clinical practice [8,9]. There is also concern about how long the benefits of ESC therapy persist, and whether the minimally vascularized neo-myocardium can be maintained for life is an important issue. The discovery of iPSCs, which are engineered from somatic cells by reprograming through transduction of a few defined pluripotent transcription factors [10], circumvent some of the concerns related to ESCs. The former still hold promise for treating cardiac disease, but many studies have shown inconsistent cardiogenic potential of iPSCs due to their line-to-line variability, along with the lack of evidence for an optimal source of somatic cells and the use of different reprograming techniques with variable differentiation efficiencies [11,12].

2.2. Adult stem cells

Adult stem cells located throughout the body are multipotent stem cells with the ability to self-renew. They include skeletal myoblasts, and progenitor cells derived from bone marrow, adipose tissue, umbilical cord, amniotic fluid, and CSCs [2]. These cells have broad capability to support continued renewal, replacing cells lost as they repair damaged tissues in the body on a daily basis. Many pre-clinical studies have shown that all these adult stem cells are capable of differentiating into cardiomyocytes and vascular cells [13]. They also have the practical advantage after transplantation of not requiring immunosuppression, allowing the application of both autologous and allogeneic cells. Furthermore, adult stem cells, especially mesenchymal stem cells (MSCs), seem capable of modulating the host immune system. Studies have shown that they play important roles in preventing proliferation of activated-T cells and associated production of cytokines, MSCs thus suppress inflammation and exert trophic influences on tissue repair, making them eminently suitable for clinical studies [9,14].

There have been over 100 clinical studies exploring the potential of adult stem cells for repair of damaged or diseased heart tissue [2,15]. However, clinical trials using adult stem cells isolated from extra-cardiac organs and tissues have failed to fulfill the promises of the preclinical studies. Meta-analyses of clinical trials using bone marrow-derived cells to treat heart disease have shown only modest or zero improvement in cardiac function, but the most benefit was apparent in patients who underwent coronary artery bypass grafting surgery [16,17]. Whether the lack of beneficial effect is attributed to the sub-optimal cell sources used in these trials remains uncertain. On a more optimistic note, in recent years the stem cell community has embraced the notion of using organ-specific stem cells for treating organ-specific diseases to optimize therapeutic efficacy, and CSCs are regarded as the 'next-generation' cell source for cardiac repair [2].

3. Cardiac stem cells

Despite the long historical belief that the heart is an organ without capacity to self-renew or regenerate following heart damage, the discovery of CSCs in postnatal hearts has provided a boost to the field of myocardial biology and has raised hope that such cells might be exploited therapeutically as an expandable autologous cell source for cardiac repair and regeneration [18]. Since the functions of stem cells are known to be regulated by various dynamic factors localized in their microenvironment or niche, it is logical that CSCs might be more beneficial than cells from extra-cardiac sources, for they are thereby reintroduced back into their natural milieu. To date, at least nine sub-types of CSC have been identified; these are c-Kit$^+$ cells, cardiosphere-derived cells (CDCs), Sca-1$^+$ cells, cardiac mesoangioblasts, cardiac side population cells, Islet-1$^+$ cells, epicardium-derived progenitor cells, cardiac colony forming unit-fibroblasts (CFU-F) and W8B2$^+$ cells. The origin of these CSCs has been comprehensively reviewed by Chong et al. [19] and will not be discussed further here.

3.1. c-Kit$^+$ CSCs

c-Kit$^+$ CSCs were the first subset of stem cells found to be resident in the adult human heart, identified by Beltrami et al. in 2003 [20]. c-Kit$^+$ CSCs were shown to reside in the atria and apex of adult hearts [21]. Rat and human c-Kit$^+$ CSCs have been reported to differentiate into endothelial and smooth muscle cells as well as cardiomyocytes in vitro [18,22]. Transplantation of c-Kit$^+$ CSC into infarcted animal hearts has been shown to reduce infarct size, attenuate adverse cardiac remodeling and improve cardiac function post MI [22–25]. Transplanted cells showed mitotic activity, displayed cardiac phenotypes and promoted better contractility after infarction than did resident cardiomyocytes [22–25]. However, due to low engraftment (<3%), it is highly unlikely that c-Kit$^+$ cells contributed directly to cardiac repair. Instead, the authors suggested that their paracrine activities might activate intrinsic repair mechanisms, as suggested by the abundance of endogenous c-Kit$^+$ CSCs both in the infarcted region and remote areas of the heart [25].

These encouraging results with c-Kit$^+$ CSCs led to the first randomized and open-labeled SCIPIO (Stem Cell Infusion in Patients with Ischemic Cardiomyopathy) clinical trial (Table 1). Intracoronary infusion of 1 million c-Kit$^+$ CSCs in these patients with

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