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Original Article

Molecular imaging of stem cells for the treatment of acute myocardial infarction

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Abstract: Stem cell therapy has a unique potential and promises hope for the treatment of acute myocardial infarction. Preclinical studies have identified barriers to clinical translation, one of which involves the monitoring of transplanted cells and the elucidation of their fates *in vivo*. Molecular imaging may help the solutions for these challenges. In this review, we illustrate the mechanisms by which molecular imaging enables insights into and the development of stem cell therapy.

Keywords: Molecular imaging, stem cell therapy, acute myocardial infarction

Introduction

The morbidity and mortality of acute myocardial infarction (AMI) is increasing, and stem cell therapy holds unique potential and promises hope for the treatment of AMI. A meta-analysis of 50 studies enrolling 2625 patients showed that transplantation of adult bone marrow cells improved left ventricle function, infarct size, myocardial remodeling, and reduced the incidence of death, recurrent myocardial infarction, and stent thrombosis [1]. Another meta-analysis of 22 randomized controlled trials, however, found that intracoronary infusion of marrow-derived mononuclear cells did not enhance cardiac function according to magnetic resonance imaging parameters nor did it improve clinical outcomes [2]. The therapeutic benefits of stem cell therapy remain controversial; in addition, the biological behaviors, such as the localization, proliferation, migration, differentiation, and functional mechanism, of transplanted stem cells require future elucidation.

Molecular imaging provides noninvasive, real-time tracking of transplanted stem cells *in vivo* and has answered some of the above questions. In this review, we illustrate how molecular imaging enables the development of stem cell therapy and refer to important recent findings.

Imaging modalities and labeling approaches

Imaging modalities, including fluorescence imaging (FI), bioluminescence imaging (BLI), ultrasound (US), computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), and single photon emission computed tomography (SPECT), have been validated and are in widespread use; each imaging modality has respective strengths and limitations [3]. There is no best modality for spatial resolution, sensitivity, and specificity, leading to a trend of multi-modular imaging to combine the advantages of various modalities.

The two common methods of labeling stem cells with specific imaging probes are direct labeling and reporter gene labeling (**Table 1**). Direct labeling is performed by incubating stem cells with contrast agents, which are trapped intracellularly by active/passive transport or phagocytosis. Examples include near infrared fluorescent dyes for FI, super-paramagnetic iron oxide (SPIO) and ultra-small super-paramagnetic iron oxide (USPIO) for MRI, ^{18}F-FDG for PET, and ^{99m}Tc-HMPAO for SPECT [4]. This approach is quick, simple and highly efficient. These molecular probes do not proliferate along with stem cells, leading to false negatives; further, they could be engulfed by macrophages or remain in the extracellular matrix after cell

Table 1. Comparison of direct labeling and reporter gene labeling

Label approach	Operation	Probes	Imaging Modality	Advantages	Disadvantages
Direct labeling	Incubate stem cells with probe agents	Fluorescent dyes	FI	Quick, simple, and highly efficient	Unable to reflect longitudinal cell number and viability
		SPIO, USPIO	MRI		
		^{18}F-FDG, ^{18}F-RDG	PET		
		^{99m}Tc-HMPAO	SPECT		
		Microbubbles	US		
		Quantum dots	CT		
Reporter gene labeling	Transfect or transduce stem cells with reporter genes	GFP	FI	Correspond to cell number and viability; individualized design	Potential to induce gene mutagenesis
		Fluc	BLI		
		ferritin	MRI		
		HSV1-tk	PET		
		NIS	SPECT		

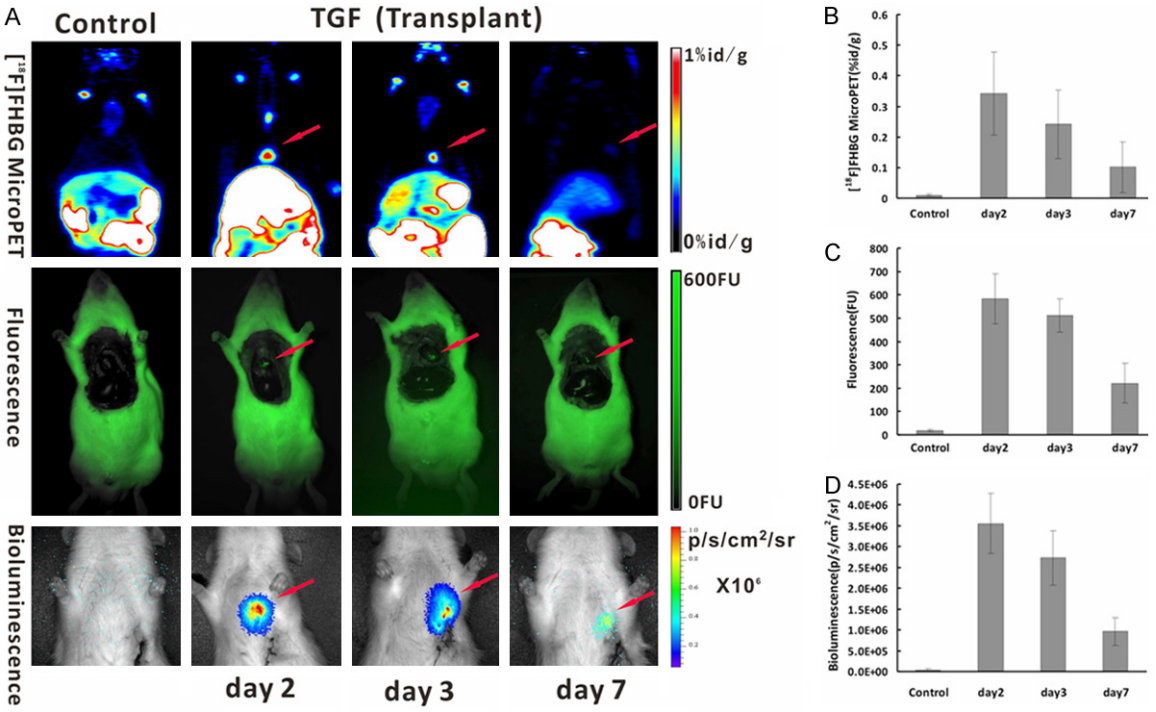

Figure 1. Multimodality molecular imaging in acute myocardial infarction rats after transplanted Ad5-TGF-transfected BMSCs into myocardium. (A) From images of microPET (upper row), Fluorescence (middle row) and Bioluminescence (lower row) imaging, signals in the heart region could be clearly seen in different imaging modalities (indicated by red arrows) at day 2, 3 and 7 after transplantation of Ad5-TGF-transfected BMSCs into the myocardium. Semi-Quantitative analysis results obtained by ROI analysis of the region of heart from ^{18}F-FHBG microPET (B), Fluorescence (C) and Bioluminescence (D) imaging shows that a significant difference could be seen between the experimental group with transplanted Ad5-TGF-BMSCs and the control group with transplanted uninfected BMSCs ($P < 0.05$) in all different imaging modalities. Ad5-TGF: adenovirus carrying herpes simplex virus type 1 thymidine kinase (HSV1-tk), enhanced green fluorescence protein (eGFP), and firefly luciferase (FLuc). BMSCs: bone marrow mesenchymal stem cells. ROI: region of interest [11].

death, leading to false positives [5]. As a consequence, direct labeling is suitable for short-time cell tracking instead of longitudinal tracking because the signal detection cannot reflect real-time cell number and viability.

Reporter gene labeling is performed by transfecting or transducing stem cells with an artificial DNA sequence, which would overproduce the imaging probes or act with specific substrates. Examples include green fluorescent

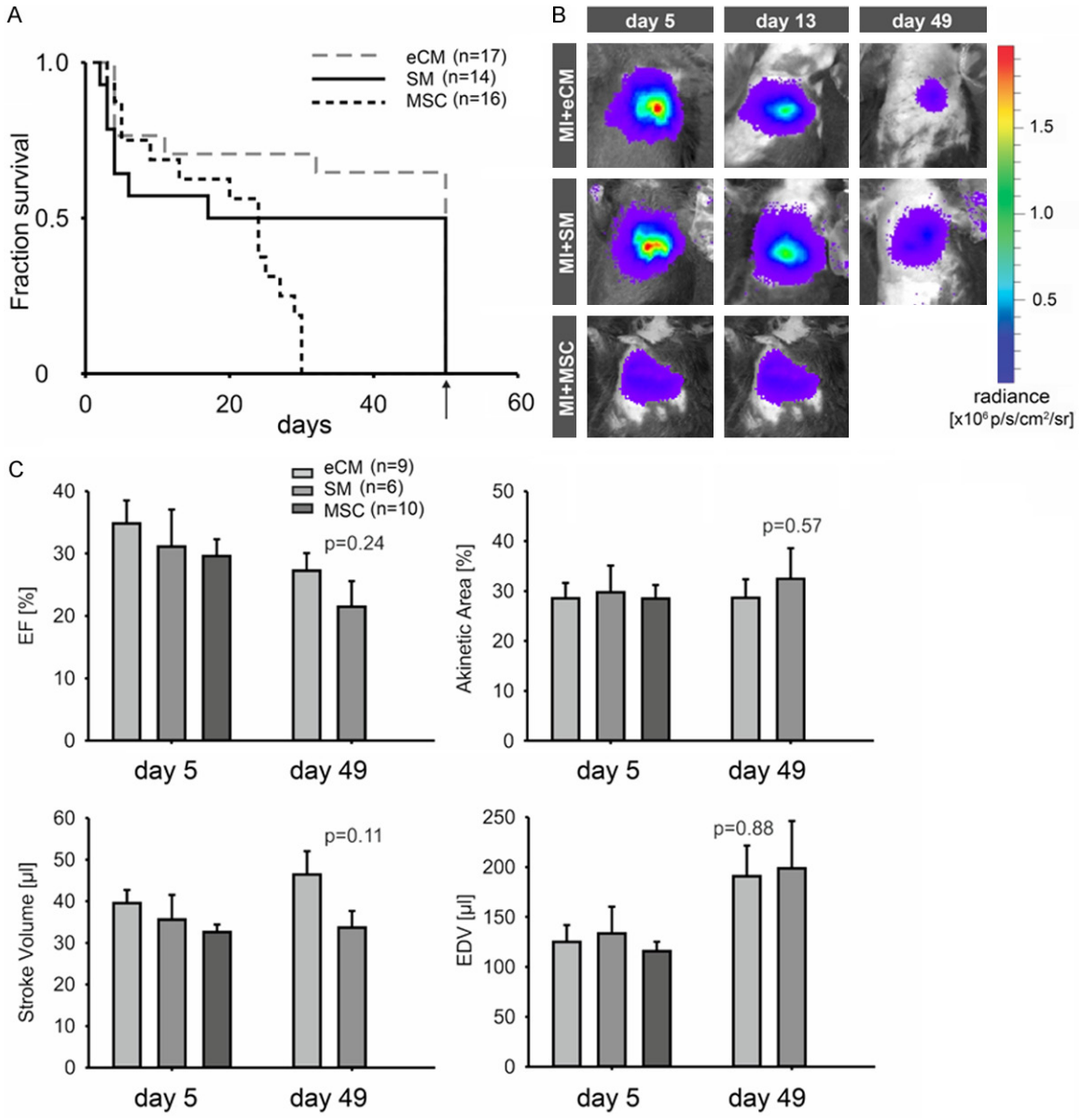

Figure 2. Long-term cell survival and treatment outcome of eCMs, SMs and MSCs. A. Survival curves of mice with myocardial infarction transplanted with eCMs (n = 17), SMs (n = 14) and MSCs (n = 16), followed 7 weeks postoperatively. B. In vivo longitudinal BLI of representative mice at 5, 13 and 49 days after transplantation of eCMs (top) SMs (middle) and MSCs (bottom) to monitor long-term cell survival in infarcted myocardium. C. Longitudinal evaluation of heart function with echocardiography at day 5 and 49 after transplantation of eCMs, SMs and MSCs. eCMs: embryonic cardiomyocytes. SMs: skeletal myoblasts. MSCs: mesenchymal stem cells. MI: myocardial infarction. EF: ejection fraction. EDV: end-diastole volume [17].

protein (GFP) for FI [6], firefly luciferase (Fluc) for BLI [7], herpes simplex virus-type 1 thymidine kinase (HSV1-tk) or its mutant variants for PET [8], sodium iodide symporter (NIS) for SPECT [9], and ferritin and its receptor for MRI [10]. Once a stable expression is established, the intensity of detected signal corresponds to the number and viability of cells. Fusion reporter genes assist in multi-modular imaging (**Figure 1**) [11], and a specific promoter or kinase helps identify the differentiation of stem cells [12]. Caution is required for safety, because transfection and transduction have the potential to induce gene mutagenesis. Several clinical studies have successfully applied HSV1-tk to assess gene therapy for

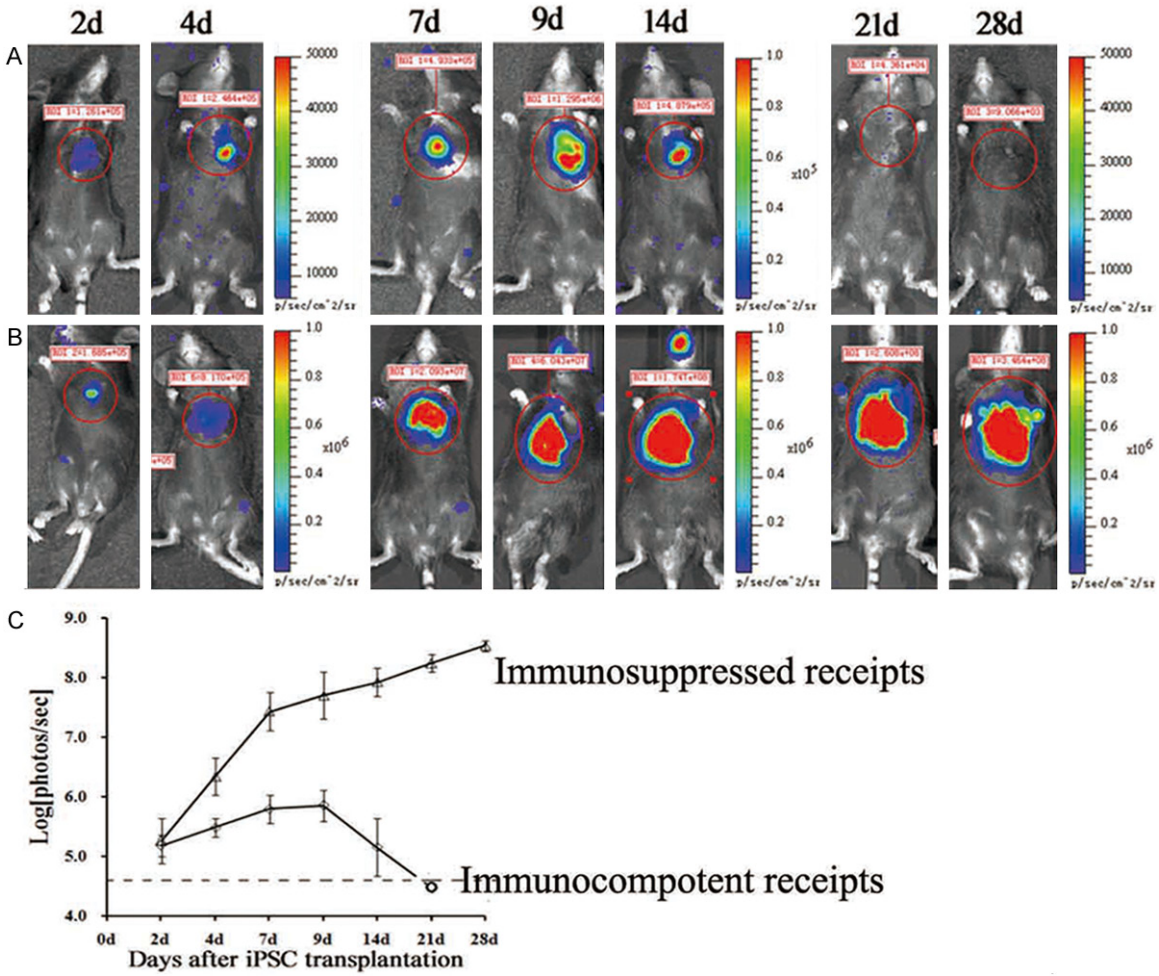

Figure 3. Noninvasive BLI of cardiac transplanted iPSC-CMs. A. Imaging of intramyocardial transplanted iPSC-CMs in immunocompetent allogenic recipients; B. Imaging of intramyocardial transplanted iPSC-CMs in immunosuppressed allogenic receipts; C. The *in vivo* BLI signals of iPSC-CMs in immunocompetent/immunosuppressed allogenic recipients (n = 6/group). BLI: bioluminescence imaging. iPSCs-CM: induced pluripotent stem cells differentiate into cardiomyocytes [19].

brain gliomas and other tumors [13], adding its prospect to the field of cardiovascular diseases.

Longitudinal retention and cell delivery

To achieve sufficient benefits, transplanted stem cells must localize and survive in the target tissue. Direct imaging of *in vivo* cell proliferation requires the utilization of the radiotracers involved in the pathway of DNA synthesis and has not been performed [14]. With reporter gene labeling, a substantial amount of dynamic tracking and semi-quantitative analysis is conducted to monitor the overall survival of stem cells (**Figure 1**). Liu et al. labeled human cardiac progenitor cells (hCPCs) with Fluc and HSV1-tk or its mutant variants that were tracked in a murine myocardial infarction model over a 4-week time period; early cell engraftment assessed by PET was found to predict subsequent cardiac functional recovery, implying a dose-effect relationship [15]. Templin et al. labeled human iPSCs with NIS and performed long-term imaging of the viability and tissue distribution of cellular grafts with novel 3-D NOGA mapping [16]. Equipped with molecular imaging, numerous improvements have been made to track transplanted cells and clarify their outcomes.

Most of the existing studies demonstrated a substantial decline in surviving embryonic stem cells (ESCs) [17], mesenchymal stem cells

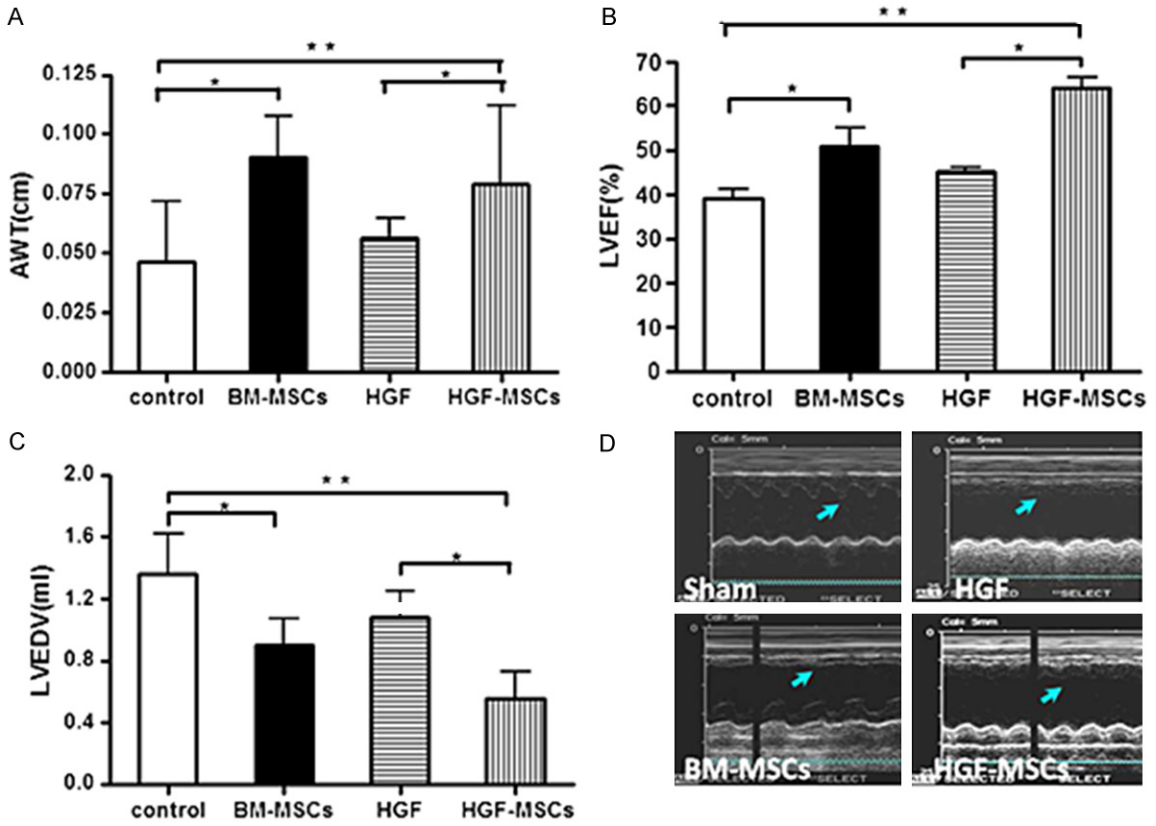

Figure 4. Echocardiography evaluation of murine hearts after MI with or without cells transplantation. (A) The AWT of groups transplanted with HGF-MSCs were thicker than the HGF group (*P < 0.05) or the control group (**P < 0.01). AWT was also showed thicker in BM-MSCs group compared with control group (*P < 0.05). LVEF (B) was higher while LVEDd (C) was lower in HGF-MSCs group compared with HGF group (*P < 0.05) or HGF-MSCs group (**P < 0.01). (D) M-mode echocardiograms are shown at 8 weeks after MI with or without cells injection and best cardiac function was shown in HGF-MSCs group. BM-MSCs: bone marrow-derived mesenchymal stem cells. HGF: hepatocyte growth factor. AWT: anterior wall thickening. LVEF: left ventricular ejection fraction. LVEDV: left ventricular end-diastolic volume [18].

(MSCs) [18], induced pluripotent stem cells (iPSCs) [19] and other stem cells, predominantly within the first week (**Figure 2**). Such limited retention might be due to a hypoxic environment, ischemia-reperfusion injury, inflammatory reaction, immunological rejection (**Figure 3**), cell apoptosis or other underlying causes [20]. Among these causes, cell apoptosis could be imaged with specific probes aimed at the components involved in the cascade. A caspase-3 specific reporter gene, pcFluc-DEVD, was designed, which took effect when caspase-3 intercepted DEVD, yielding an active Fluc protein [21]. Similarly, cyclic HSV1-tk successfully monitored the apoptosis of tumor cells after chemotherapy *in vivo* with PET imaging, which provides better clinical application [22]. Other researchers have shown that stem cells could migrate and disappear into peripheral tissues or form teratomas, highlighting the importance of the homing of stem cells.

A group of studies focused on the timing, routes, and dosage of cell delivery to explore the best protocols. Houtgraaf et al. found that intracoronary infusion of mesenchymal precursor cells directly after infarction reduced the loss of myocardial cells, lowered the infarct size, abrogated adverse remodeling, and improved cardiac function, despite the hypothesis that inflammatory cells recruited to repair an injured myocardium might be hostile to cell viability [23]. A meta-analysis showed that the ideal cell-infusion time was 4-7 days after an infarction, when the chemotactic factors reached at peak [24]. Another meta-analysis indicated more improvement with late injections (> 1 week) [25]. Swijnenburg et al. showed

that the timing of delivery had little effect on the overall intra-myocardial retention and cardiac function preservation in acute and subacute models [26]. Regarding the delivery route, intra-myocardial injection is more effective than intra-coronary injection; however, clinically, it is more difficult to perform and fewer results are obtained [27]. Regarding dosage, most studies implied a dose-dependent effect on CD34+BMCs [28] and other stem cells [29]. Such divergent opinions suggest that continued research is required.

Function mechanism and cell type

The differentiation of stem cells into cardiac cells and the replacement of ischemic tissues in situ could be hypothesized. Increasingly, iPSCs are being used to complement their embryonic counterparts. Carpenter et al. demonstrated efficient cardiac differentiation of human iPSCs that gave rise to the retention of progenitors within infarcted hearts in rats and reduction of heart remodeling after ischemic damage [30]. Wang et al. generated a fusion gene driven by a murine stem-cell virus promoter and an endothelial-specific promoter. With BLI and immunohistochemical staining, they found that human MSCs differentiated into endothelial cells and were integrated into blood vessels as long as 50 days after an experimental myocardial infarction and that increased angiogenesis and decreased fibrosis were associated with functional cardiac improvement [31]. The relatively poor retention of stem cells poses a challenge to explaining their therapeutic effects.

Another characteristic of stem cells is their paracrine effect. Researchers found that BMSCs took effect by paracrine signaling and immune regulation instead of by differentiation into myocardial or vascular cells and that the cells would not survive long *in vivo* (**Figure 2**) [32]. Fan et al. showed that BMSCs inhibited pro-apoptotic gene (Bax, caspase-3) and inflammatory biomarker (MMP-9, oxidized protein, CD40+ cells) expressions and benefited angiogenesis (VEGF, CD31+ cells, SDF-1alpha, CXCR4) and myocardium preservation (connexin43, troponin-I, cytochrome-C) [33]. Wang et al. also confirmed that angiogenesis and/or the paracrine effect and not myogenesis had responsibility for functional improvement following CD34+ cell therapy because treatment with anti-VEGF rather than anti-α4β1 abolished improvement in cardiac function [34]. In addition, human embryonic stem cell-derived vascular cells (hESC-VCs) could have a paracrine effect on various cytokines to promote angiogenesis, pro-survival and anti-apoptotic effects, accompanied by a pronounced recruitment of endogenous c-kit(+) cells to the injury site [35]. Stem cells such as skeletal myoblasts [36], bone marrow-derived stem cells [37], and cardiosphere-derived stem cells [38] generate sufficient cytokines, chemokines and growth factors to protect myocardial cells, regulate angiogenesis, inhibit post-infarction inflammation and fibrosis, and stimulate the recruitment and differentiation of endogenous stem cells.

Adjuvant treatment

It has been shown that co-therapy with antioxidants [39], immune-suppressants (**Figure 3**) [40], and growth factors [41] could lengthen the survival of stem cells and strengthen their therapeutic effects. Huber et al. applied costimulation-adhesion therapy through a TIM3-dependent mechanism for preventing post-transplantation rejection of human ESC derivatives [42]. Zhang et al. showed that combination therapy with rosuvastatin and adipose-derived mesenchymal stem cells (AD-MSCs) had a synergetic effect on improving the myocardial function after an infarction via the PI3K/Akt and MEK/ERK pathways [43]. Similarly, exendin-4 could improve the survival and therapeutic efficacy of ADSCs through STAT3 activation [44]. To maintain a longer and real-time co-effect, stem cells are modified to self-generate adjuvants (**Figure 4**) [45] or microRNAs [46].

Efforts have been made to design a graft system with improved bioactivity and tissue compatibility. Xu et al. utilized scaffolds mimicking the morphology of the cardiac extracellular matrix to preserve the cardiac differentiation of CDCs [47]. Ban et al. encapsulated murine ESC-derived CMs with PA-RGDS to evade the immune system and supply substrates for angiogenesis [48]. Williams et al. combined hMSCs and hCSCs and illustrated the important biological interactions between them that enhanced cell-based therapeutic responses

[49]. Multidisciplinary collaboration between molecular imaging, biomaterials, bioengineering and other medical or non-medicals sciences offers promise for stem cell therapy.

Conclusion

By taking advantage of their self-renewing, pluripotent characteristics and paracrine effects, stem cells have significant potential for the treatment of AMI. Preclinical studies have identified barriers to clinical translation, one of which is the monitoring of transplanted cells and the elucidation of their fates *in vivo*. Equipped with the recent advances in molecular imaging, researchers are able to obtain better solutions for the questions that remain. Direct imaging of transplanted stem cells has provided remarkable insight into the biological behavior, mechanism of action and therapeutic efficacy of stem cells. An improved understanding of the determinants of success has become crucial, and efforts are being made to prolong cell survive with cell modification and coincident substrate delivery. A bright future is anticipated; however, future studies are required because some conclusions are equivocal.

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Disclosure of conflict of interest

None.

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