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Genetic Engineering and Stem Cells: Combinatorial Approaches for Cardiac Cell Therapy

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Every year, almost 1 million people in the United States suffer a myocardial infarction, and those who survive are likely to join the nearly 5.3 million people living with congestive heart failure [1]. The irreplaceable loss of cardiomyocytes during infarction creates an electromechanically dysfunctional tissue substrate that is incapable of supporting a healthy body and prone to life-threatening arrhythmias. Although recent studies have proved that the heart has endogenous repair mechanisms, including the recruitment and maturation of resident stem cells [2], the natural regenerative ability of cardiac tissue is apparently insufficient to prevent the occurrence of heart failure [3], [4]. Therefore, the transplantation of exogenous cells into the damaged myocardium (cellular cardiomyoplasty) has been actively pursued as a method to improve compromised heart function.

A large number of animal studies and recent clinical trials have shown that the implantation of adult stem cells (including myoblasts [5], bone marrow [6], mesenchymal or marrow stromal [7], and resident cardiac stem cells [8]) as well as embryonic stem cells [9] into the infarcted heart can yield significant functional benefits. However, obstacles inherent to the multipotent and proliferative nature of stem cells and the potential risks associated with the application of exogenous cells into the heart may reduce the translational potential of this research. The use of genetic engineering to induce or alter specific protein expression in stem cells has already facilitated research in this field and may, additionally, offer a potential route for designing more efficient cell sources for cardiac repair. As the feasibility of stem cell genetic manipulation has already been proved and as genetic techniques continue to advance [10]–[13], the combinatorial approach for cardiac cell therapy is promising. This review will thus describe the applications of genetic engineering to improve the isolation, selection, and differentiation of stem cells prior to implantation as well as strategies to promote the retention, mobilization, survival, integration, and tracking of stem cells after implantation.

Enhancing the Selection of Cardiogenic Stem Cells

Although the pluripotent nature of stem cells enables diverse regenerative capabilities, specific applications of these cells require defined differentiation. For example, bone marrow-derived mesenchymal stem cells (MSCs) possess the capability to become muscle, fat, bone, or cartilage, but only the first of these four cell types repopulating the injured heart would be beneficial [14]. Furthermore, the injection of highly proliferative uncommitted embryonic stem cells may lead to tumor formation [15]. Thus, selecting and isolating a pure population of stem cells exhibiting cardiogenic characteristics is crucial to the efficacy and safety of cardiac cell therapies. Currently, the stem cells committed to myocardial lineage are genetically selected using different reporter systems linked to the endogenous activation and expression of cardiogenic or myogenic genes.

Cardiomyocytes from human embryonic stem cell-derived embryoid bodies account for less than 10% of the total cell population [16]. Strategies to link the expression of fluorescent proteins to cardiomyogenic promoters delivered as genetically engineered vectors into stem cells have facilitated the successful purification of cardiomyocytes (>95%) through

fluorescence-activated cell-sorting (FACS) techniques [17]. Importantly, genetically engineered expression of fluorescent proteins, such as enhanced green fluorescent proteins (EGFPs), typically does not impair the proliferation, differentiation, or cardiac regeneration capabilities of stem cells [18]. In addition to the use of fluorescent reporters, selection based on genetically acquired resistance to antibiotics driven by the activation of cardiogenic promoters has enabled up to 99% in vitro purification of mouse embryonic stem cell-derived cardiomyocytes [19], [20]. Reporter expression also can be customized through the selection of the associated promoter such that genetically engineered stem cells become selectable at early cardiogenic transcription stages [21]–[25] during cardiomyogenic activation [26]–[28] or throughout the entire life of the cell [10]. For example, selection for early mesodermal (*Brachyury* or *T*) and cardiovascular transcription factors (*Isl1* and *Nkx2-5*) in mouse embryonic stem cells enables the simultaneous derivation of multiple heart cell types, including cardiomyocytes and smooth muscle cells [23] or cardiomyocytes, smooth muscle, and endothelial cells [21], [22]. Specific cardiac muscle cells, such as ventricular myocytes, can be isolated using vectors incorporating the myosin light chain-2v promoter [26], whereas atrial and nodal cells may be preferentially isolated under the control of the ubiquitous cardiac alpha myosin heavy chain promoter [29].

Promoting Cardiogenic Differentiation of Donor Cells

Although genetic selection procedures are instrumental for the isolation of cardiogenic cells as well as the removal of potentially tumorigenic undifferentiated stem cells, the use of selection alone to obtain a sufficient quantity of cells for implantation would initially require a very large amount of stem cells. Therefore, new genetic engineering strategies are being pursued to actively increase the efficiency of stem cell differentiation into cardiac cells or to introduce cardiogenic potential in noncardiac cells. For example, human MSCs genetically engineered to express myocardin, a dynamic cardiomyogenic transcription factor, attained a cardiac phenotype with an efficiency of 90–100% when implanted into infarcted mouse heart. As a result, left ventricular function was increased, and detrimental ventricular remodeling was reduced, when compared with the use of control MSCs [30]. Furthermore, human myocardial scar fibroblasts engineered to overexpress the myocardin gene attained cardiac gap junctions and became capable of transmitting action potentials and repairing conduction blocks within cardiac cocultures [31]. Similarly, forced expression of the muscle-specific transcription factor, MyoD, in primary rat cardiac fibroblasts resulted in the formation of multinucleated myotubes, yielding a potentially abundant myogenic source for cardiac cell therapies [32].

Facilitating Identification, Retention, and Mobilization of Implanted Cells

The ability to assess the structural and functional integration as well as differentiation of implanted cells in the heart requires both in vivo and ex vivo tracking of the location and the fate of the injected cells. Genetic engineering of stem cells to express fluorescent [33], luminescent [34], or colorimetric [35] proteins permits the localization and imaging of live implanted cells in animal studies and the assessment of cell developmental state when the reporter is driven by a differentiation-dependent promoter. For example, in the experiments by Rota et al., bone marrow cells were isolated from genetically engineered mice, and, after implantation into an infarcted mouse myocardium, the donor cells were tracked for their ability to undergo transdifferentiation into functional cardiomyocytes [36]. Specifically, enzymatically dissociated transdifferentiated bone marrow cells were identified by their EGFP label and shown to exhibit cardiac function including shortening upon electrical stimulation, calcium transients, and action potentials. In addition, implanted bone marrow stem cells identified by the expression of EGFP or a *c-myc* nuclear tag driven by a cardiac specific promoter were shown to couple to host cardiomyocytes by gap junction immunostaining, dye transfer, and coordinated calcium transients.

The lack of cell retention at the site of injection [37] and the tendency of intracoronary or intravenously delivered cells to travel to the liver or spleen rather than mobilize at the infarction site [38] have been recognized as important obstacles to successful cardiac cell therapy. Recently, the retention of rat MSCs at the site of injection was improved threefold when the cells were genetically engineered to express tissue transglutaminase (tTG), a fibronectin receptor that promotes integrin-mediated cell adhesion [39]. The resulting improvement of cardiac function highlighted the fact that cell implantation therapies are unlikely to succeed if the donor cells fail to remain at the injury site. Similarly, the migration of MSCs from an intravenous rat tail injection to the site of myocardial infarction was increased more than twice when cells were retrovirally infected to overexpress CXCR4, a stromal-derived factor-1 (SDF-1) receptor [40]. The release of SDF-1 from ischemic myocardial tissue has been shown to attract the circulating progenitor cells expressing CXCR4 to migrate to the injury site and, after binding to SDF-1, participate in endogenous tissue repair [41]. As a result, the accumulation of genetically engineered MSCs within the infarct site translated into significant recovery of left ventricular function, whereas the rats injected with control MSCs exhibited no functional improvement.

Encouraging Survival of Implanted Cells and Regeneration of Host Tissue

Even if the implanted cells are successfully delivered to the site of cardiac injury, their survival within the hostile ischemic and/or inflamed microenvironment is found to be relatively low and unpredictable [42]. Overexpressing antiapoptotic and proangiogenic genes has had the dual benefit of directly promoting the survival of implanted cells as well as the paracrine factor-induced recovery and regeneration of the host myocardium [43]. In particular, the genetically engineered expression of Akt [44], hypoxia inducible factor 1 α (HIF-1 α) [45], fibroblast growth factor-2 (FGF-2) [46], or heme oxygenase-1 (HO-1) [47] within implanted bone marrow-derived stem cells has yielded up to fivefold greater survival relative to that of control cells. Similarly, rat MSCs genetically engineered to express the vasodilator peptide, adrenomedullin, exhibited increased survival upon implantation and induced microvessel formation within the host myocardium [48]. Antiapoptotic and proangiogenic characteristics were also genetically engineered into mouse embryonic stem cells [49] and bone marrow-derived MSCs [50] through the forced expression of the vascular endothelial growth factor (VEGF). In most of the cell implantation studies, the combined efforts of selecting purified stem cell sources for injection and ensuring their safe arrival and survival at the injury site directly correlated with the enhanced recovery of cardiac function.

Directly Improving Myocardial Function by Promoting Stem Cell Integration

Although functional improvements within an infarcted heart following stem cell injection may be correlated with the broad spectrum of paracrine factors secreted by the donor cells [51], the importance of host–donor electromechanical coupling is undeniable [52]. Ideally, for safe and efficient repair, exogenously implanted cells should be both myogenic and able to architecturally and functionally integrate within the heart syncytium [53]. Initial clinical trials involving the implantation of autologous skeletal myoblasts were associated with arrhythmias, possibly owing to the lack of gap junction formation between differentiated myotubes and host tissue [54]. Implanting skeletal myoblasts derived from transgenic mice that express connexin-43 via a skeletal muscle promoter eliminated the arrhythmias associated with the implantation of wild-type cells [55]. On the other hand, MSCs, which endogenously express gap junctions and functionally couple to host cardiomyocytes, exhibited significantly reduced arrhythmogenesis when compared with skeletal myoblasts [56].

In addition to reconnecting electrical gaps within the diseased myocardium, genetically engineered stem cells hold promise as biological pacemakers for regulating abnormal heart

rhythm [57]. Unlike traditional electronic pacemakers, biological pacemakers are expected to retain their responsiveness to endogenous endocrine signaling or pharmacological interventions. Recently, implanted green fluorescent protein-labeled human embryonic stem cell-derived cardiomyocytes were shown to functionally integrate within the myocardium and actively pace guinea pig ventricles after cryoablation of the atrioventricular (AV) node [18]. Similarly, implanted human MSCs genetically engineered to express “funny” current (HCN2 gene) were able to pace, in a catecholamine-responsive manner, canine ventricles after AV node ablation [58]. Mechanistically, the HCN2 expressing MSCs were not able to actively generate action potentials, but they produced a depolarizing current, which spread to coupled cardiomyocytes, bringing them to the excitation threshold. Customized pacemaking function in the future could be accomplished by engineering targeted mutations within the ion channel genes expressed in the implanted cells [59].

Future Perspectives

The implantation of stem cells to restore lost or impaired heart function is an exciting and promising frontier in biomedical and clinical research. The search for the ideal cell source for cardiac repair has significantly advanced throughout the past decade, but the perfect cell may be one that is genetically designed rather than discovered. As described in this review, genetic engineering is a viable strategy for overcoming potential limitations inherent in stem cell experimentation as well as exogenous cell implantation. With the recent discovery that adult human fibroblasts can be genetically engineered to attain stem cell-like properties (induced pluripotent stem cells) [60], [61], the future of cardiac cell therapy may involve the reprogramming of autologous adult cells into stem cells suitable for cardiogenic differentiation and implantation [62]. Success of this new approach may eliminate the ethical and immunogenic complications intrinsic to the use of embryonic stem cells while providing an abundant cell source for implantation therapies. Nevertheless, while combined efforts in genetic engineering and stem cell biology may create the most effective treatments for heart disease, it is imperative that extensive preimplantation and postimplantation studies be used to establish the associated risks of these technologies. As an alternative, the identification of soluble factors that would produce phenotypic changes similar to those induced by genetic manipulation is currently under pursuit by several research groups.

In conclusion, the current efforts to characterize and improve the effectiveness of stem cells for the treatment of myocardial infarction and heart failure are promising. Further understanding of the genetic mechanisms that govern cardiogenic stem cell differentiation as well as the cellular properties that lead to safe host–donor integration and successful functional repair of damaged heart tissue will provide future opportunities for the use of genetic manipulations in cardiac stem cell research. The studies that use genetic engineering to enhance the selection, survival, and integration of stem cells in cardiac implantation therapies will be vital to the forward momentum of this field.

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