1. Introduction

Despite significant advances in the medical management of heart failure, ischemic insult is still a major cause of death in developed countries [1]. Because the adult human heart has limited intrinsic regenerative capacity, the myocardial loss after a myocardial infarct is irreversible and is subsequently replaced by non-contractile scar tissue, often initiating congestive heart failure [2,3]. Recently, new therapeutic approaches such as gene-, growth factor-, and cell-based therapies have been emerged to improve simple replacement of the myocardium [4].

Specifically, cell-based therapies have been intensively studied in the last few years because of the potential benefits in patients having various heart diseases (e.g., acute myocardial injury, stable coronary artery disease and heart failure) [5]. Several different approaches have been used for cell delivery, such as intracoronary infusion, intravenous infusion, direct injection into endomyocardium and epicardial administration. But many studies in animals have involved transplanting cells into the border region of the infarcted myocardium to i) increase or preserve the number of cardiomyocytes; ii) improve vascular supply; and iii) augment the contractile function of the injured myocardium. As a result, cardiac cell therapy has been proposed as a potential treatment option for end-stage heart failures [6-8]. Currently, several different types of regenerating cells, especially stem cells, have been investigated for their therapeutic capacity to proliferate and differentiate into...
Modification of mesenchymal stem cells for cardiac regeneration

Article highlights.

- The pros and cons of candidate cells for cardiac regeneration.
- Mesenchymal stem cells as the candidate with most potential for cardiac repair.
- A major obstacle to MSC-based therapy: Low viability of engrafted MSCs.
- Anoikis, a potentially large contributor to graft cell death.
- Ex vivo manipulation to enhance anti-death signals.
- Modification of MSCs to increase their adhesiveness.
- Future directions in modification of MSCs for better cardiac repair.

This box summarises key points contained in the article.

functional cardiomyocytes [9-12] because stem cells have properties such as plasticity, the ability to transdifferentiate into multiple lineages, self-renewal and fusion with resident cardiomyocytes, although only in rare cases [13-15].

Among the different types of stem cells, MSCs are recognized as the best potential candidates for cell therapy for heart diseases due to their beneficial properties, such as easy isolation, rapid expansion in vitro and rare formation of teratomas [16]. Indeed, many reports have shown a marked recovery of ventricular function from myocardial infarction after transplantation of MSCs in an animal model [17]. In addition, Chen et al. reported that intra-coronary MSCs improved cardiac function in a human trial [18]. Katritsis et al. also showed that intra-coronary MSCs reduced infarct size in human patients compared with controls. These results demonstrate the safety and feasibility of intra-coronary MSC infusion in post-myocardial infarction patients [19]. Moreover, it seems that intra-myocardial delivery of MSCs during coronary bypass grafting and via catheter-based delivery systems is also safe and feasible [20]. Therefore, MSCs may be utilized as a novel agent to induce regeneration and protection in infarcted myocardium [21,22]. However, MSC-based therapy has the fatal limitation of poor viability of MSCs after cell transplantation [11]. To overcome this limitation, various anti-death strategies have been adopted to improve stem cell survival/number in the infarcted heart. While these approaches have shown promising results, more research is still necessary [23]. Here we review the strategies that have been used to enhance the survival of MSCs after implantation and we discuss the future directions with respect to the cardiac therapeutic potential of modified MSCs in micro-environmental conditions.

2. Overview of cell therapy for cardiac repair

The targets of cell therapy for heart disease are highly dependent on the underlying disease process such as, myocardial ischemia, cardiac dysfunction or a combination of the two. In terms of myocardial ischemia, cell transplantation might be the most effective treatment as long as it can provide a renewable source of proliferating, functional cardiomyocytes and/or contribute to the development of new blood vessels to nourish not only newly formed cardiomyocytes, but also the environment of the ischemic region [24,25]. Indeed, experimental evidence has demonstrated that new endogenous or exogenous cells can be incorporated and become functional within the heart [26,27]. The various cell types used for cardiac repair include unfractionated bone marrow cells (BMCs) and mononuclear cells (BMNCs), MSCs, hematopoietic stem cells (HSCs), endothelial progenitor cells (EPCs), skeletal myoblasts, cardiac progenitor cells, fetal cardiomyocytes, and embryonic stem cells (ESCs) [5,25,28]. Each cell type has its own pros and cons for use in cell-based therapy.

ESCs derived from the inner mass of the blastocyst have shown the potential to completely regenerate the myocardium [29]. Recent reports have demonstrated that ESCs can be differentiated into cardiac precursor cells, providing an opportunity to induce myocyte development [30,31]. However, the use of ESCs can be limited by ethical concerns, immunological incompatibility, and/or the formation of teratomas [29].

It was reported in 2001 that bone marrow cells can be used to replace cardiac myocytes in the infarcted hearts of an animal model [17]. This finding led to clinical trials of autologous bone marrow intracoronary infusions [17]. Despite the accumulation of data from several clinical trials, the effects of these trials are still controversial because it is not clear whether autologous bone marrow reduces the infarct size, increases ejection fraction or leads to transfer of cells capable of engraftment and differentiation into cardiac myocytes [32].

Skeletal myoblasts are progenitor cells residing within the skeletal muscle, and they can be isolated by skeletal muscle biopsies and expanded in vitro [33]. These cells were the first cell type used in clinical trials, but they were not feasible for use in treating chronic myocardial dysfunction [34]. A recent report also showed no significant benefit in patients with ischemic cardiomyopathy [35].

Although EPCs, identified by the CD34 antigen, have shown the greatest potential for angiogenesis [36], their function and availability in the blood are diminished with atherosclerosis and age [37].

Resident cardiac stem cells have been identified from resident cardiac precursor cells having the capacity to differentiate into cardiac myocytes and vascular elements. In addition, they could be isolated from surgical or endomyocardial biopsies from humans and clonally expanded in vitro [38]. The use of these cells in treatment strategies should be further examined in future studies.

Spermatogonial stem cells have been isolated from adult mouse testes. These cells are pluripotent stem cells that have the capacity to differentiate into cardiac cells [39,40]. Confirmation of this finding in human trials may overcome the ethical and immunological problems associated with the use of embryonic stem cells.
As described above, various cell types can be used in the cell-based therapy for human heart diseases, but there is controversy surrounding the use of each cell type. Thus further examination is necessary for use of any of these cell types in clinical cardiovascular applications, and many questions related to actual clinical practices remain unanswered (e.g., optimal cell type, dose, mode of administration, mechanism of action, safety and efficacy, etc.) [24].

2.1 Mesenchymal stem cells

Mesenchymal stem cells (MSCs), which reside in the bone marrow non-hematopoietic tissues, were first described by Friedenstein at the Gamaleya Institute in Moscow [41]. Since individual MSC isolates have slightly different cell characteristics depending on the isolation and culture methods used, MSCs are currently defined by their adhesiveness to the surface of cell culture dishes and by the absence of hematopoietic markers [42]. Because MSCs can be easily isolated and expanded in culture, they can be administered immediately, without waiting weeks or months for adequate numbers of cells to be achieved by cell culture. MSCs retain their growth and multilineage potential over several passages, although they do lack immortality [42,43]. In the past 10 years, it has been well documented that MSCs have the ability to differentiate into various cell types, including osteoblasts, chondrocytes, myocytes, marrow stromal cells, tendon-ligament fibroblasts, adipocytes and other mesenchymal phenotypes [44]. This multilineage transdifferentiation potential suggests that MSCs would be a good source of cells to treat different diseases. In addition, MSCs also have apparent immunoprivilege, because MSCs express low levels of MHC II compared with MHC I [20]. They display local immunosuppressive properties that permit successful transplantation into an allogenic setting. Indeed, experiments in non-human primates have shown that allogenic MSCs were not rejected, showed similar effects to the autologous MSCs and were detected at nine months after transplantation in the recipient [45-47].

Overall, these studies have suggested that MSCs are an attractive candidate cell type for gene delivery, cell transplantation and tissue engineering applications. Despite the benefits of MSCs, MSC-based therapy is limited in clinical application. That limitation is due to the poor viability of the transplanted cells in the myocardium. It has been reported that only ~ 5% of transplanted MSCs can survive for 14 days in the infarcted porcine heart [48]. Toma et al. also reported that the survival rate of transplanted human MSCs in an uninjured mouse heart is less than 0.5% at 4 days post transplantation [11]. Similar results were obtained from studies using different cell types. For instance, about 7% of skeletal myoblasts, 15% of smooth muscle cells, and 6% of unfractionated bone marrow cells survived at 3 days, 1 week and 3 days, respectively, in the infarcted animal hearts [49-51]. Consequently, cell viability is likely to be the common barrier for any therapeutic regenerative cell-based approach in the infarcted heart.

3. Cause of cell death in the infarcted region

The cause of death of implanted MSCs may begin during the preparation step. This is called ‘anoikis’, where MSCs, which are normally grown attached, are prepared in suspension in order to be injected [52]. Anoikis is a Greek word for homelessness and is defined in cell biology as programmed cell death induced by loss of matrix attachments [53]. In spite of its unique definition, anoikis is essentially an apoptotic process because all the features that characterize apoptosis, including nuclear fragmentation and membrane blebbing, are observed during anoikis [54]. A potentially large contributor to graft cell death in cell-based cardiac repair is anoikis. Obviously, the first stress the cells encounter during the engraftment process is the lack of matrix support. Once MSCs are injected into the infarcted region, they encounter the harsh conditions coupled with the loss of survival signals because of inadequate interaction between cells and matrix (e.g., the deprivation of nutrients and oxygen [55], and inflammation [56]). In general, adhesion to structural glycoproteins of the extracellular matrix (ECM) is necessary for survival of differentiated adherent cells in the cardiovascular system, including endothelial cells, smooth muscle cells, fibroblasts, and cardiac myocytes [57-59]. Adhesion of cells to the matrix, predominantly via integrin molecules, generates an endogenous tensile stress within the cells, called tensile stress [60], and repression of apoptotic signals [61], whereas detachment has the opposite effect. This physiological cellular process plays an important role in differentiation, survival and growth of MSCs. In this respect, the reactive oxygen species (ROS) may intensify the anoikis signals in transplanted MSCs. It is well known that ROS hinder cell adhesion and induces detachment of cells [62,63]. Even though ROS are formed as a natural component of oxygen respiration, ROS are also dramatically increased in ischemic hearts [64]. Indeed, co-injection of skeletal myoblasts with ROS scavenger (superoxide dismutase) increases the graft survival by twofold [49]. Moreover, ROS can induce an inflammatory response as well as hinder cell adhesion, leading to cell death [65].

The grafted cells may experience ischemic conditions devoid of nutrients and oxygen, and inflammation [53,55]. Since myocardial infarction is caused by the abrupt occlusion of one or more of the blood vessels supplying blood to the heart, this state obviously reduces the supply of nutrients and oxygen to the heart muscle as well as to grafted MSCs [53,55]. In addition, it has been reported that the myocardial injury generates a strong inflammatory response comprised of neutrophils and macrophages [66]. These inflammatory cells produce oxygen-derived free radicals and inflammatory cytokines that can cause the death of grafted cells, and can also initiate signaling pathways that induce caspase activation, leading to apoptosis. However, MSCs might be beneficial in the allogenic settings because they have immunomodulatory effects on inflammatory cells as mentioned.

In the last few years, ex vivo manipulation of MSCs has been used to overcome several of the above-mentioned problems.
Indeed, genetic modifications may enhance survival, metabolic characteristics, proliferative capacity and directed differentiation of MSCs. In addition, cells can be engineered to produce secreted paracrine molecules for gene therapy, which may result in further therapeutic benefits.

4. Ex vivo manipulation of MSCs

There are various strategies for manipulating MSCs in order to overcome the low cellular survival and transdifferentiation potency of MSCs after implantation (Table 1). These approaches can be categorized as follows: i) pretreatment with growth factors or cytokines; ii) preconditioning such as hypoxia; and iii) genetic modifications to overexpress anti-death signals. We will review the former two approaches in brief, and focus on genetic modifications in more detail. The role of growth and differentiation factors including fibroblast growth factor (FGF)-2, IGF-1 and Bone morphogenic protein (BMP)-2, in expanding the stem cells and facilitating their engraftment into cardiac tissue has recently been studied in an effort to improve efficacy of MSC implantation [67]. It has been shown that pretreatment with IGF-1 reprograms

<table>
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<tr>
<th>Types</th>
<th>Gene</th>
<th>Host</th>
<th>Efficacy</th>
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<tr>
<td>Gene overexpression</td>
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<td>Transfection</td>
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<td>Rat</td>
<td>Increased capillary density, reduction of infarct size and apoptosis</td>
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<td>FGF-2</td>
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<td>Expression of cardiomyocyte-specific factor and anti-apoptotic genes and new vessel formation</td>
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<td>tTG</td>
<td>Rat</td>
<td>Enhanced adhesion, cell survival and migration</td>
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<td>VEGF</td>
<td>Mouse</td>
<td>Angiomyogenesis and myocardial recovery</td>
<td>Markel et al. [78]</td>
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<td></td>
<td></td>
<td>Rat</td>
<td>Improved myocardial perfusion and in restoration of heart function</td>
<td>Yang et al. [91]</td>
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<td>Viral transduction</td>
<td>Akt</td>
<td>Rat</td>
<td>Decreased myocardial infarction, improvement of cardiac function and cell survival</td>
<td>Mangi et al. [73]</td>
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<td>Akt, Ang-1</td>
<td>Rat</td>
<td>Histological and functional benefit</td>
<td>Shujia et al. [92]</td>
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<td></td>
<td>CXCR4</td>
<td>Rat</td>
<td>Improved cardiac performance, migration and viability</td>
<td>Cheng et al. [93]</td>
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<td></td>
<td>HO-1</td>
<td>Rat</td>
<td>Reduced inflammatory markers and apoptosis and increased cytokine production, microvessel density, and cardiac performance</td>
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<td></td>
<td>ILK</td>
<td>Rat</td>
<td>Strengthened cell adhesion and decreased left ventricle infarct size</td>
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<td></td>
<td>TNFR</td>
<td>Rat</td>
<td>Improved left ventricular function</td>
<td>Bao et al. [96]</td>
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<td></td>
<td>VEGF</td>
<td>Rat</td>
<td>Improved myogenesis and angiogenesis, and decreased infarcted region</td>
<td>Gao et al. [97]</td>
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<td>Matsumoto et al. [98]</td>
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<tr>
<td>Pretreatment</td>
<td>BMP-2, IGF-1, FGF-2</td>
<td>Rat</td>
<td>Reduced apoptosis and infarct size and improved cardiac function</td>
<td>Hahn et al. [67]</td>
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<td></td>
<td>HGF</td>
<td>Rat</td>
<td>Improved anti-apoptosis effect, angiogenesis and heart function</td>
<td>Guo et al. [99]</td>
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<td></td>
<td>IGF-1</td>
<td>Mouse</td>
<td>Enhanced engraftment, differentiation and functional improvement</td>
<td>Kofidis et al. [75]</td>
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<td></td>
<td>SDF-1</td>
<td>Rat</td>
<td>Suppression of apoptosis and enhanced survival, engraftment and vascular density</td>
<td>Pasha et al. [100]</td>
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<td></td>
<td>Hsp70</td>
<td>Rat</td>
<td>Improved myocardial repair and cardiac function</td>
<td>Chang et al. [69]</td>
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<td></td>
<td>Atorvastatin</td>
<td>Swine</td>
<td>Improved survival and effects of transplanted MSCs</td>
<td>Yang et al. [71]</td>
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<td></td>
<td>Estrogen</td>
<td>Rat</td>
<td>Inhibited cardiac remodeling and fibrosis</td>
<td>Ray et al. [70]</td>
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<tr>
<td>Precondition</td>
<td>Hypoxia</td>
<td>Rat</td>
<td>Enhanced cardiac protection and functional improvement</td>
<td>Gnechi et al. [72]</td>
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<td></td>
<td>Heat shock</td>
<td>Rat</td>
<td>Enhanced survival of engraftment</td>
<td>Maurel et al. [101]</td>
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</table>

Indeed, genetic modifications may enhance survival, metabolic characteristics, proliferative capacity and directed differentiation of MSCs. In addition, cells can be engineered to produce secreted paracrine molecules for gene therapy, which may result in further therapeutic benefits.
Sca-1+ for pro-survival signaling and cardiomyogenic differentiation, and Cx-43 is believed to play an important role in this process [68]. The combination of these growth factors might greatly enhance the survival and differentiation of MSCs into cardiomyocytes. We have also demonstrated that transplantation of heat shock protein (Hsp)-70-treated MSCs using the Hph-1 protein transduction domain leads to a decrease in both the fibrotic heart area and the apoptotic MSCs using the Hph-1 protein transduction domain leads to a decrease in both the fibrotic heart area and the apoptotic MSCs [69]. The effects of pharmacological intervention have also been investigated. Estrogen stimulates growth hormone production in bone marrow MSCs and EPCs, and this influences myocardial remodeling [70]. Also, treatment with atorvastatin, a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, enhances cell survival and differentiation into cardiomyocytes, decreases the infarcted area, promotes angiogenesis and reverses the ventricular remodeling processes [71].

While optimal culture conditions including normoxia, have been used in laboratories in order to achieve high cell vitality and proliferation rates, the effects of hypoxia preconditioning on MSCs have also been investigated [72]. The effects of hypoxia preconditioning were considered in the context of simulating hypoxia exposure studies in vitro or the microenvironment in vivo in myocardial infarction and hind limb ischemia models. If MSCs are subjected to hypoxia in vitro, the Akt signaling pathway is activated so that cell viability and cell cycle rates are maintained [73]. Moreover, hypoxia is known to induce differential secretion of paracrine molecules from MSCs [72]. Li et al. showed that hypoxia could induce VEGF overexpression and enhance survival rates under hypoxia after B-cell lymphoma (Bcl)-2-gene transfer in rat MSCs in vitro, and they concluded that a hypoxia-regulated, VEGF-mediated cardioprotective effect and induction of functional collateral vessels contribute to the preconditioning effects [72,74].

### 4.1. Genetic modification to enhance anti-death signals

Genetic modification to enhance anti-death signals was first demonstrated by Mangi and his colleagues who found Akt over-expressing MSCs are more resistant to apoptosis in vitro and in vivo. They were also able to show dramatic improvement in cardiac function in a rodent MI model, with almost complete replacement of the infarct scar with functioning myocardium [73]. Interestingly, it has been further shown that Akt-transduction stimulates MSCs to produce paracrine factors such as secreted frizzled-related protein 2, which exerts a beneficial effect on the infarcted heart post-engraftment. IGFB-1, an upstream activator of Akt, has been shown to enhance survival of transplanted neonatal cardiomyocytes and smooth muscle in the injured heart [72.75]. In addition, transfection of MSCs with bFGF showed increased survival and cytoprotection in hypoxic conditions. The transfected cells also caused greater neovascularization compared to non-treated MSCs in a previous study [76]. Tang et al. found that MSCs transduced with haemxygenase, an enzyme that prevents oxidative damage, showed both better engraftment and enhanced cell survival following intra-myocardial delivery relative to non-transduced MSCs [77]. In a recent study, myocardial transplantation of VEGF-transfected MSCs led to greater improvement in both myocardial perfusion and restoration of heart function than either cellular or gene therapy alone [78]. Li et al. demonstrated that overexpression of Bcl-2 inhibits cell death by blocking mitochondrial cytochrome c release, which leads to caspase activation and cell death. Bcl-2 is upregulated in surviving ventricular myocytes after myocardial infarction, and transgenic overexpression of Bcl-2 in the engrafted cells limits myocardial infarct size and improves ventricular function after ischemia/reperfusion [74].

### 4.2. Genetic modification to enhance adhesion

As mentioned above, anoikis is potentially a large contributor to graft cell death in cell-based cardiac repair. In addition to the approach used to augment anti-apoptotic signal molecules, similar strategies have been used to enhance the adhesive properties of MSCs.

Memon et al. found that myoblast cell sheets implanted with intact culture matrix onto the surface of infarcted rat hearts improved cardiac outcomes compared with direct injection of myoblasts. This cell sheet technology also has the advantage of maintaining cell–cell contacts that promote survival [61]. Two recent studies have established that successful engraftment of cardiomyocytes is enhanced by co-delivery of the cells with matrix. Kutschka et al. demonstrated that grafts were larger and ventricular function was improved in infarcted rat hearts when human ESC-derived cardiomyocytes were delivered together with collagen matrices, including Gel Foam and the basement membrane preparation, Matrigel [79]. Laflamme et al. found that Matrigel significantly enhanced survival of hESC-derived cardiomyocytes in infarcted hearts of athymic rats [80]. MSC-derived plasminogen activator inhibitor 1 (PAI-1) does not alter MSC survival through a plasmin-dependent mechanism, but rather directly affects the adhesion of MSCs to their surrounding matrices [81].

In a previous study, we were able to show that tissue transglutaminase (tTG) over-expression significantly enhanced the adhesion of MSCs. This leads to increased survival of the implanted cells via an integrin-dependent mechanism [60]. Although the adhesion is mainly mediated by integrin α5β3 and the β1 family integrin, α5β1 [82], tTG on the cell surface acts as a coreceptor for fibronectin (Fn) in cell adhesion associated with integrin [83,84]. tTG enhances adhesion by acting as a bridge between integrins and Fn or by mediating the formation of ternary complexes where all three proteins interact with each other [14]. tTG-MSCs also showed a significant increase in spreading and a relatively minor increase in migration. In addition, we observed that phosphorylation of focal-adhesion-related kinases including FAK, Src and PI3K, was significantly increased. We also
confirmed an increase in the phosphorylation level of extracellular signal-regulated kinases, which are major signal mediators of cell proliferation.

We have also demonstrated that integrin-linked kinase (ILK) is required in hypoxic mesenchymal stem cells to strengthen cell adhesion to the ischemic myocardium. ILK is a 59-kDa Ser/Thr kinase that binds to the cytoplasmic domain of β-integrin and participates in the regulation of cell adhesion, growth, shape change, and ECM assembly upstream of the Akt/protein kinase B (PKB) and mitogen-activated protein (MAP) kinase pathways [85-87]. ILK also interacts with the b1-subunit of integrin and plays a crucial role in integrin-mediated cell adhesion and signaling [62,88]. Our data suggest that hypoxic surroundings suppress expression of the ILK protein. We also observed that transfection of the ILK gene enhances phosphorylation of ERK and Akt, which play critical roles in the regulation of adhesion-mediated cell survival signals in hypoxic MSCs. In addition, an increase in the Bcl-2:Bax ratio and an inhibition of caspase-3 activation was detected, indicating that the ILK gene promoted cell adhesion in hypoxic conditions, which helps to prevent apoptosis. ILK also enhances phosphorylation of PKB/Akt, which plays a critical role in the regulation of adhesion-mediated cell survival signals. Transplantation of ILK-MSCs results in a larger decrease in infarct size and a greater improvement in left ventricle function compared with transplantation of naive MSCs. Moreover, improved microvessel density was closely correlated with the presence of ILK-MSCs in the infarcted area.

5. Conclusions

Since transplanted MSCs improve cardiac function in ischemic heart diseases, the outlook for MSC-based therapies is promising although the mechanism of action is not well known. However, the poor viability of the cells after implantation is still a major obstacle in the use of MSCs for cardiac repair. This review summarizes our current understanding of the pretreatment strategies, mainly focusing on genetic modifications, used to enhance survival of MSCs after implantation for cardiac repair. These strategies comprise the genetic augmentation of anti-death signals in MSCs and enhance adhesion by overexpression of tTG or ILK, leading to better recovery and adhesion after implantation. Such adhesion-enhanced MSCs further improved the cardiac function of infarcted myocardium to a greater extent than standard MSCs. Therefore, enhancement of cell adhesion and spreading should be one of the major goals in the development of cell transplantation techniques, including the therapeutic use of progenitor cells. In addition to this enhancement of adhesion, the control of reactive oxygen species may be another option in the pretreatment of MSCs. However, the intra-arterial administration of adhesion-enhanced MSCs needs to be investigated in terms of the retention rate in the infarcted region because enhanced adhesion may compromise the migratory or homing ability of cells [89,90]. Nevertheless, genetic modification of MSCs to improve their therapeutic potential in the treatment of infarcted myocardium appears to be effective. In particular, strategies that enhance adhesion are emerging as some of the most promising methods to increase the survival of implanted MSCs.

6. Expert opinion

Despite the growing evidence supporting the therapeutic potential of stem cells to treat heart disease, several fundamental issues need to be addressed in rigorously controlled clinical trials, such as what type of cells should be administered, how many cells are needed for myocardial regeneration, and what delivery method is optimal for MSC therapy. These decisions need to be made based on the nature of the injury. Even more importantly, the major problem of extensive cell loss after implantation needs to be solved. In fact, many studies have shown that the majority of cells delivered to the heart die within the first week. The solution of simply transplanting an extremely large number of cells does not seem to be an appropriate strategy. The observed cell death is caused by both the ischemic microenvironment, which is devoid of nutrients and oxygen, and the loss of survival signals due to inadequate cell–cell and cell–matrix interactions. To overcome this problem, three general strategies can be employed. The first is targeting a specific molecular pathway, for example, through expression of an anti-apoptotic protein or blocking a caspase. The second is inducing a broader spectrum cytoprotective state, for example, through heat shock or induction of a hypoxia response. The third is enhancing adhesion of MSCs to the extracellular matrix and/or nearby cells that reside in the ischemic border region. The potential targets for MSC manipulation are listed in Table 2. Investigators may use any one of these three strategies to increase cell survival (e.g., pretreatment with some growth factors or cytokines during expansion, conventional transfection for overexpression, direct protein delivery using protein transduction domains, and viral vectors encoding cytoprotective genes, etc.). In addition, investigators can use a combination of methods to further maximize cell survival. For example, by combining genetic modification and preconditioning of MSCs, one may further enhance cell survival after implantation. This broad spectrum of approaches can be quite powerful in their ability to protect cells after implantation. Despite these strong pro-survival effects, another issue to consider is that the ideal treatment should not induce permanent changes in the graft’s physiology nor induce an undesired host response. Also, the safety of genetically modified MSCs should be verified before they are applied in a clinical setting. At the same time, the combined use of different cell types may be possible in clinical settings since EPCs are the most suitable cell type for induction of vascular regeneration and ESCs and MSCs are the most potent cell types for cardiac regeneration.
The pretreatment of MSCs, particularly through genetic modification, may induce indirect beneficial effects, possibly through paracrine actions. Indeed, paracrine molecules secreted by genetically modified MSCs may have different therapeutic profiles compared with paracrine molecules from naïve MSCs, which actually enhance therapeutic actions. Taking into account that therapeutic factors secreted from stem cells can be as effective as administration of the cell itself, another option may be to administer specific proteins produced by these cells for cardiac therapy. To do this, investigators need to identify the molecules and characterize the mechanisms by which each molecule acts. Overall, the pretreatment of MSCs, particularly through genetic modification, represents an important innovation because use of this approach may resolve issues of cell viability, scalability and immune tolerance, while enhancing function.

**Declaration of interest**

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### Table 2. Potential targets for enhancing survival of MSCs.

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<td>Anoxic preconditioning</td>
<td>Antiapoptotic effect of MSCs</td>
<td>He et al. [102]</td>
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<tr>
<td>Preconditioning with growth factor</td>
<td>Prosurvival signaling and cardiomyogenic differentiation</td>
<td>Lu et al. [68]</td>
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<td>Trophic activity</td>
<td>Non-invasive stem cell therapeutic approach, improvement of ventricular function and attenuation of apoptosis and fibrosis</td>
<td>Shabbir et al. [103]</td>
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<td>Anti-apoptotic inducer</td>
<td>Decreased apoptosis, increased cell viability and induction of survival pathway</td>
<td>Chen et al. [104], Chen et al. [105]</td>
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<td>Paracrine factor</td>
<td>Cardiac repair, endogenous regeneration, cytoprotection and neovascularization</td>
<td>Gnecchi et al. [72]</td>
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Bibliography


37. Schmidt-Lucke C, Rostig L, Rissig L, Fichtlscherer S, et al. Reduced number of circulating endothelial progenitor cells predicts future cardiovascular events: proof of concept for the clinical importance of
endogenous vascular repair. Circulation 2005;111:2981-7


42. Muraglia A, Cancedda R, Quarto R. Clonal mesenchymal progenitors from human bone marrow differentiate in vitro according to a hierarchical model. J Cell Sci 2000;113:1161-6


45. Devine SM, Bartholomew AM, Mahmud N, et al. Mesenchymal stem cells are capable of homing to the bone marrow of non-human primates following systemic infusion. Exp Hematol 2001;29:244-55


60. Song H, Chang BW, Lim S, et al. Sca-1+ side population cells in bone-marrow-derived mesenchymal stem cells expanded from aging murine bone marrow. Exp Hematol 2006;34:1325-30


69. Yang YJ, Qian HY, Huang J, et al. Evidence supporting paracrine hypothesis for Akt-modified mesenchymal stem cell-mediated cardiac protection and functional improvement. FASEB J 2006;20:661-9
Modification of mesenchymal stem cells for cardiac regeneration

95. Song SW, Chang W, Song BW, et al. Integrin-linked kinase is required in hypoxic mesenchymal stem cells for strengthening cell adhesion to ischemic myocardium. Stem Cells 2009;27:1358-65

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