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REVIEW

Stem cell death and survival in heart regeneration and repair

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Abstract Cardiovascular diseases are major causes of mortality and morbidity. Cardiomyocyte apoptosis disrupts cardiac function and leads to cardiac decompensation and terminal heart failure. Delineating the regulatory signaling pathways that orchestrate cell survival in the heart has significant therapeutic implications. Cardiac tissue has limited capacity to regenerate and repair. Stem cell therapy is a successful approach for repairing and regenerating ischemic cardiac tissue; however, transplanted cells display very high death percentage, a problem that affects success of tissue regeneration. Stem cells display multipotency or pluripotency and undergo self-renewal, however these events are negatively influenced by upregulation of cell death machinery that induces the significant decrease in survival and differentiation signals upon cardiovascular injury. While efforts to identify cell types and molecular pathways that promote cardiac tissue regeneration have been productive, studies that focus on blocking the extensive cell death after transplantation are limited. The control of cell death includes multiple networks rather than one crucial pathway, which underlies the challenge of identifying the interaction between various cellular and biochemical components. This review is aimed at exploiting the molecular mechanisms by which stem cells resist death signals to develop into mature and healthy cardiac cells. Specifically, we focus on a number of factors that control death and survival of stem cells upon transplantation and ultimately affect cardiac regeneration. We also discuss potential survival enhancing strategies and how they could be meaningful in the design of targeted therapies that improve cardiac function.

Keywords Cell death · Stem cells · Apoptosis · Therapy · Heart · Regeneration

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Introduction

Stem cell therapy has emerged as a protective strategy to improve heart function via repairing the infarcted myocardium and promoting cardiac regeneration [1–3]. Various cell types, including embryonic stem cells (ESC) [4], induced pluripotent stem cells [5], bone marrow and other adult tissue-derived mesenchymal stem cells (MSCs) [6, 7], hematopoietic stem cells (HSCs) [8], and endothelial progenitor cells [9, 10] can transform into cardiac myocytes both in vivo and in vitro. In addition, transplanted cells are useful for production and secretion of survival and angiogenic factors without differentiating into cardiac cell types [11]. Currently, apoptosis appears to be a most predominant form of stem cell death, and is considered a major target for protection of both stem cells and host myocardium. Numerous laboratory investigations and clinical

studies have been conducted to understand the cellular and molecular mechanisms influencing cell loss during cardiac regeneration. In particular, a growing number of recent reports on adult and ESC therapy have made an effort to understand how overcoming cell death during regeneration of cardiac tissue would improve heart function via both autocrine and paracrine mechanisms [12–20]. Stem cell therapy may involve a number of strategies, using various types of donor cells, their genetic modifications, preconditioning and delivery methods as well as ameliorating the damaged myocardium itself. These approaches have been effective in several models, however maintaining stem cell differentiation into human cardiac tissue remains challenging. Here we discuss the regulation of stem cell death and survival as serious obstacles that limit clinical applications of stem cell therapy in the heart.

The strategy of cell transplantation

General requirements for transplantation

Although some investigators have reported evidence of mitotic cardiomyocyte division after infarction [21], the mitotic capacity of cardiac tissue cannot fully restore cellular loss after myocardial infarction (MI) [22]. The goals of cellular cardiomyoplasty are to replace cardiomyocytes lost after ischemia, induce revascularization of the injured region, and prevent deleterious pathological remodeling after MI [23, 24]. Successful cell/tissue transplantation in human patients requires viable cells with good histocompatibility, proper cell proliferation, differentiation and migration along the damaged tissue (Fig. 1). In addition, transplantation efficiency could be enhanced by the ability of stem cells to differentiate, return to quiescence and restore the normal biological and physiological condition of an organ. Transplanted cells are expected to reduce cardiac damage either by participating in the regeneration of the tissue or activating the endogenous regenerative potential of the heart. Nevertheless, over 99 % of MCSs injected into the left ventricular myocardium of mice die within four days after injection [25]. Significant stem cell death also happens with other cell types, indicating the need for understanding of the effect of pro-death stimuli on stem cell activity and repair mechanisms in cardiac pathological conditions [26, 27]. An essential challenge would be to determine the signals controlling reduced stem cell proliferation, limited cell growth and decreased response to beneficial stimuli. The current trend is that a greater quantity of cells would need to be implanted for better therapeutic outcome. Identifying death stimuli that are triggered by harsh conditions after stem cell implantation, as well as developing appropriate transplantation models are important to fully understand molecular networks of cardiac regeneration and to establish effective and long-lasting therapeutic approaches for cardiac patients.

A combination of different tools pointed at different targets might be an effective approach, because enhancement of engraftment, promotion of stem cell adhesion, stimulation of survival pathways, blockade of programmed cell death and angiogenesis induction may have additive effects. Recent studies have used various strategies to enhance stem cell survival under harsh conditions after implantation [28, 29]. Although these procedures support cardiac repair by creating a better environment, the mechanisms of programmed death in these models are not fully understood. Yet, with the current advances in methodology, achieving efficient stem cell therapy requires not only identification of mechanisms that control stem cell survival, but also signals switching off proliferation and differentiation, both spontaneously and induced.

Stem cell resources and selection

A number of lessons have been learned regarding cell type selection and application of cell therapy. Both embryonic and adult stem cell types have been applied to improve regeneration and function of the injured heart [30]. Various sources of stem cells have been considered for cardiac repair; these include skeletal muscle myoblasts, peripheral blood–derived progenitor cells, bone marrow mononuclear cells, umbilical/placental/endometrial stem cells, MSCs, cardiac stem cells, embryonic and induced pluripotent stem cells [20, 31–34]. Carvalho and co-workers have recently proposed the use of amniotic membrane as a potential source of MSCs that could efficiently boost cardiac tissue regeneration [35]. Initial studies were first focused on ESCs, then researchers used autologous adult stem cells which possess almost the same regenerative potential like ESCs beside the advantage that autologous tissue is always better accepted by immune system than any xenograft [36]. On the other hand, cells participating in the repair process are suggested to be host cells that are naturally chemoattracted to the injury site, through occasional host neoangiogenesis, and are not delivered to the damaged area directly by transplantation [37]. Selection of stem cells is often based on certain criteria such as cell survival and proper metabolic activity as found in MSCs when transplanted into ischemic conditions [38]. Therefore, a large number of studies have recently suggested that cell therapy with bone marrow MSCs has great promises in regenerating and repopulating the damaged myocardium, restoring its function and is a safe effective strategy for treating ischemic heart failure [39–41]. Bone marrow-derived MSCs were frequently used to regenerate cardiac ischemic tissues of various animal models and human studies [42–

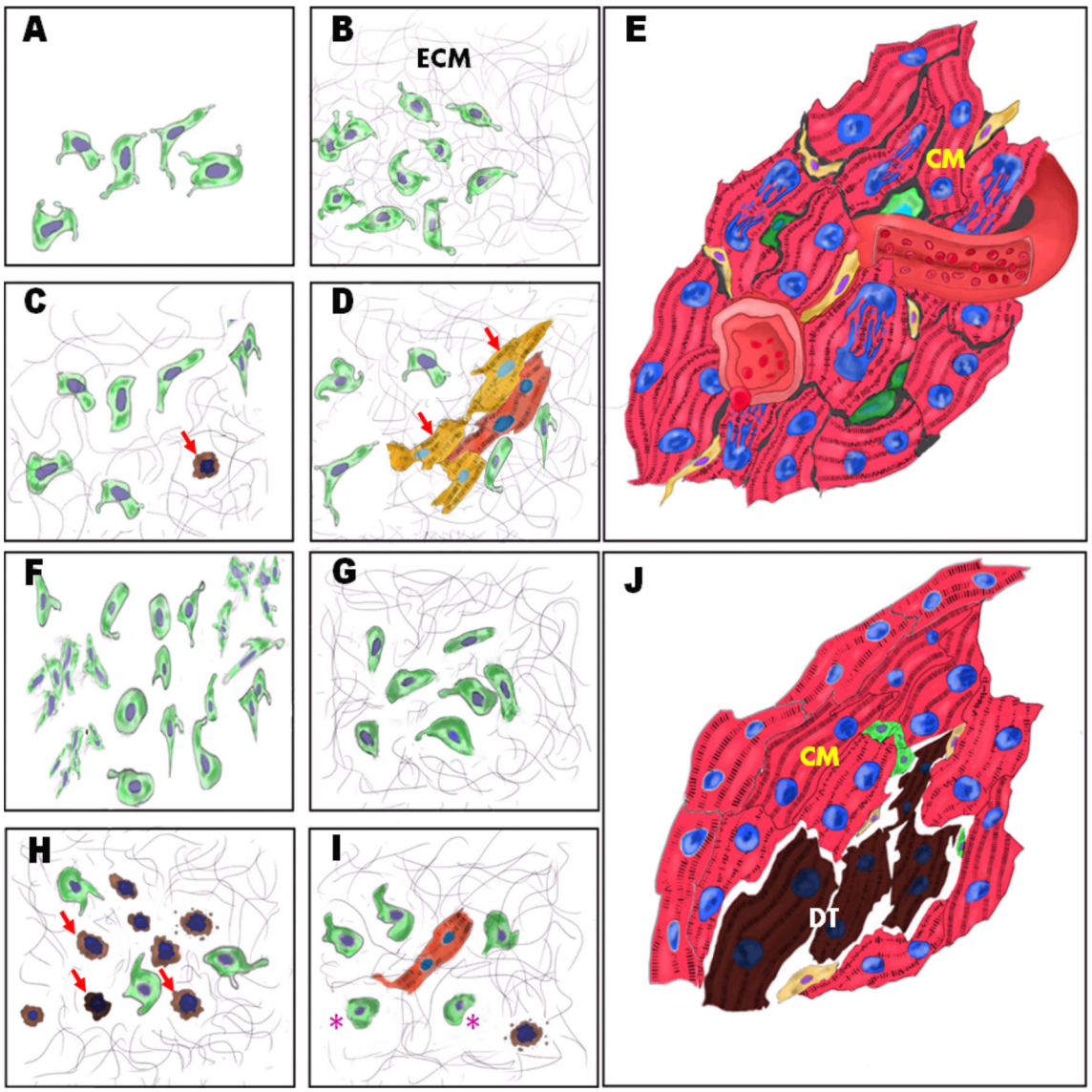

Fig. 1 Illustration showing effects of improved (**a**–**e**) vs current (**f**–**j**) stem cell therapeutic strategies on cell survival and cardiac regeneration. Under improved biological conditions, less numbers of stem cells: (**a**) are required and are expected to undergo efficient proliferation (**b**), exhibit less numbers of dying cells (**c** *arrow*), differentiate into cardiomyocytes (**d** *arrow*), maintain good contact with the ECM as well as with the healthy cardiomyocytes (**d**) and ultimately provide successful cardiac tissue regeneration (**e**). On the contrary, current strategies of stem cell therapy in the heart suffer from inefficient transplantation of large numbers of cells (**f**) that face harsh microenvironments which arrest their proliferation (**g**), force most of them to die (**h** *arrows*), partly due to lack of contact with the ECM as well as with cardiomyocytes (**i**), and this leads to failure of proper cardiac repair (**j**). *ECM* extracellular matrix, *CM* cardiomyocyte, *DT* damaged tissue. *Asterisks* indicate lack of stem cell contact with the ECM

49]. An advantageous aspect of autologous MSCs is that they can be easily obtained from adult patients, they are less likely to cause immune problems like ESC [36] and they can differentiate into functional cardiomyocytes, however poor cell adhesion and increased apoptosis decrease their engraftment [27, 50]. Thus, enhancement of cell survival is essential to help better selection of stem cell and to maximize the benefits of cell therapy for heart diseases. With a better clarification of the factors that determine which stem cells live or die we would likely select only the best stem cells that are the healthiest to use.

Methods for cell delivery to myocardium

Successful regeneration of the injured heart does not only depend on the cell type and number of injected cells, but is also determined by the route and site of cell delivery as well as the number of injections [51]. The route of stem

cell delivery into the cardiac tissue is considered a major determinant of stem cell survival in the diseased heart [52]. There are variable effects by which these routes can influence cell viability and ultimately impact the success of transplantation. Effects of delivery routes on stem cell survival can be seen in intramyocardial injection route that influences cell survival via inducing mechanical injury, inflammation and islet-like donor cell clusters. On the other hand, intracoronary injection route may also impact stem cell survival by causing poor initial cell retention in the myocardial tissue, however it causes minimal inflammation [53]. In addition, intravenous injection does not allow proper cell recruitment into the heart, consequently providing inadequate cell number; however combined intravenous and intrathymic injection has been shown to prolong survival of cardiac allograft [54]. Although, epicardial placement of 'cell sheets' allows successful cell engraftment, defective integration into cardiac tissue appears to be a major problem that may influence long term cell viability. It has been shown that epicardial placement of MSC-sheets generated using temperature-responsive dishes can greatly enhance donor MSC survival and improve therapeutic effects in an acute MI model, compared to intramyocardial route [55]. Recently, Menasche and colleagues have used human ESC and found that epicardially delivered scaffold-based system is more efficient than hand-held multiple intramyocardial injections as it leads to better cell engraftment and improved function [56]. Furthermore, transplantation of MSCs via the endocardium has been shown to enhance cell retention and improve ejection fraction compared to intravascular infusion method [57].

Pro-death environment in damaged myocardium

Acutely or chronically injured myocardium represents harsh environment not only for the transplanted cells but also for its own cardiomyocytes and residing cardiac stem cells. It is well established that cardiac damage leads to the permanent loss of cardiomyocytes and subsequent left ventricular pathological alterations that ultimately lead to heart failure. Resisting the pro-death environment in the myocardial tissue is an obstacle that represents a great challenge for patient recovery and for clinical trials using stem cell treatment. Although poor viability of cell transplants in the damaged tissue is also well known and described, there are two main aspects which should be attributed to the problem of poor survival of transplanted cells during therapy. The first one is that damaged tissue becomes repellent, i.e. acidic, hypoxic, glucose deprived and under inflammation. The second issue is that transplantable cells may be not prepared to withstand such harsh environment. Local extracellular environment at the site of cell delivery influences not only cell death and survival, but also cellular integration into damaged myocardium. Intramyocardial injection itself is an additional cause of cell death of the heart tissue. Harsh transplantation microenvironment in the injured myocardium can be caused by many factors including acidosis, increased calcium and oxidative stress, inflammatory cells and cytokines, hypoxia, or unbalanced supply of nutrition [58, 59]. These conditions are toxic to the cell transplants and may influence adhesion, survival, migration and integration, and thereby perish the outcome of cell therapy.

In order to overcome the harsh conditions in the infarcted myocardium, different strategies can be used to optimize the host myocardium for proper cell survival (Fig. 2) [60]. Moreover, synergistic therapies including pharmacologic agents have been offered as an effective approach to antagonize harsh conditions and boost cardiac regeneration. Free radical scavengers, anti-inflammatory therapy, co-delivery of extracellular matrix molecules or combinatorial use of these approaches would be main measures towards improvement of the microenvironment for transplantable cells. Microenvironment of transplanted stem cell could also be improved by modulation of immune response, prevention of trophic factor withdrawal and hypoxia [61]. Extensive death of stem cells upon transplantation can be explained by the fact that the current models still do not completely represent the native microenvironment as many experiments prior to transplantation are conducted in vitro with pure cell cultures. It will be necessary to conduct studies that create a microenvironment that mimics the environment of natural tissue in order to provide the required survival signals. Such improvements can dictate stem cell fate by regulating growth, proliferation, and differentiation of cells at the site of regeneration.

Reduction of blood supply

In spite of many potential advantages, cells transplanted into the ischemic myocardium are subjected to immediate hypoxia due to impaired vascularization, and thus undergo significant cell death which represents a major obstacle in a clinical setting. Insufficient blood supply in the diseased heart causes deprivation of nutrients and impacts stem cell survival and growth. Therefore, overcoming hypoxia and ensuring proper oxygenation are critical challenges for blocking death of transplanted stem cells [62]. There are several approaches used for improving blood supply including neovascularization of the graft by means of co-transplantation of endothelial cells, angiogenic pretreatment performed with cell implantation, transplantation of genetically engineered cells expressing pro-angiogenic

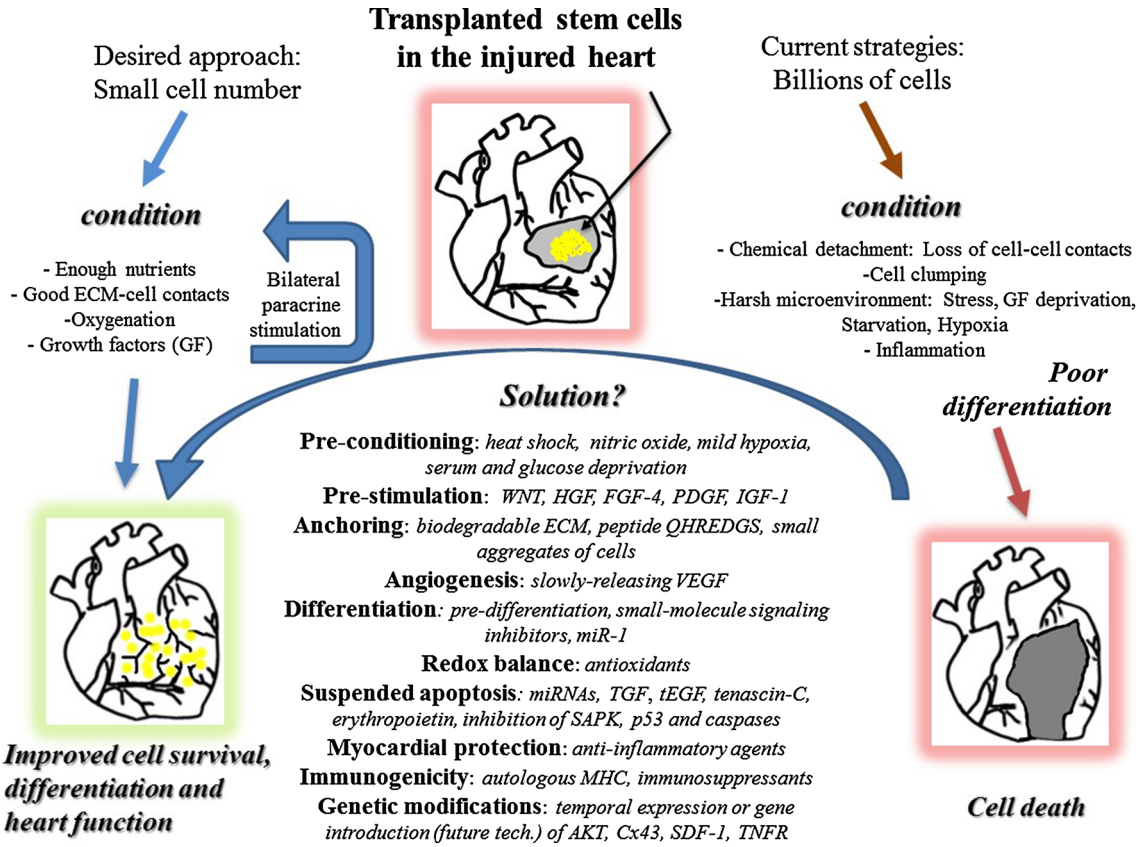

Fig. 2 Schematic representation summarizes major factors affecting stem cell death, survival, differentiation and successful cardiac regeneration. *Left* desired conditions required for stem cell survival and proper cardiac regeneration. *Right* obstacles affecting viability of cells and failure to improve cardiac repair. *ECM* extracellular matrix, *SAPK* stress-activated protein kinases (such as P38 and JNK), *MHC* major histocompatibility complex. Other abbreviations are explained in text

factors, etc. [60]. For example, growth factors such as VEGF and FGF can be added directly into heart tissue or delivered in hydrogels to ensure slow release of these molecules. The use of pro-angiogenic growth factor-loaded hydrogels with stem cells can promote capillary ingrowth and angiogenesis within the myocardium in which stem cells are implanted. It was proposed that hydrogels such as alginate or hyaluronic acid themselves recruit pro-angiogenic cells to improve myocardial regeneration [63]. In vitro techniques such as 3D model of cardiomyocyte spheroids have been successful in providing basic elements for angiogenesis [64]. Enhanced secretion of VEGF and improving blood flow to infarcted myocardium was shown by using MSC spheroids [65]. On the other hand, transplantable MSCs can be hypoxia-prestimulated in such way that survival and angiogenesis mechanisms would be activated and cardiomyocyte apoptosis in the ischemic heart after transplantation would be reduced [66]. Moreover, MSCs are able to induce neovascularization in the injured mouse heart through secretion of placental growth factor [11]. In addition to the above highlighted approaches, recent cell therapeutic studies on myocardial ischemia have applied different protocols using either extra-cardiac blood vessel cell progenitors or stimulating cardiac angiogenesis.

Importance of extracellular contacts

One obvious problem in cell therapy is stem cell withdrawal from their native niche. The stem or progenitor cell niche provides essential extrinsic cues that support cell survival in various tissues including the heart. The stem cell niche is known to regulate self-renewal, differentiation, migration, and proliferation of stem cells; however the regulatory mechanisms are far from being understood. The left ventricular extracellular matrix is known to play an important role in maintaining myocardial geometry and shape after injury [67]. Cardiac regeneration requires proper reorganization of the matrix to protect transplanted stem cells from pro-death factors. In the ischemic heart, the myocardial extracellular matrix composition is significantly altered. For instance, the reserve of collagen type I, which is responsible for the structural support, can decrease from 80 to 40 % after MI [68]. Transplanted stem cells

undergo anoikis (mode of apoptotic cell death) mainly because they lack interaction with the specific extracellular matrix. Lack of stem cell adhesion to the extracellular matrix (ECM) at the site of transplantation is a major pro-apoptotic factor that impacts efficacy of stem cell therapy. This is well explained by in vitro studies that used human pluripotent stem cells (hPSCs) to generate cardiac tissues in a multistep process that leads to functional maturation. Each of these steps is temporally controlled by adhesion signaling that allows both Cell-cell and cell-ECM contacts to ensure proper cell viability, self-renewal and maturation of hPSCs [69]. Several studies have used in vitro models to assess stem cell viability in harsh microenvironments and have revealed that cell-ECM interactions, mediated by integrins are very essential for stem cell survival in these conditions [70]. Although transplantation of undifferentiated hPSC is not a viable option for many years, they gave an opportunity to understand critical in vitro aspects of ECM in stem cell adhesion, apoptosis and routine maintenance. Cell-cell adhesion suppresses cell death by controlling actin-myosin activity. In addition, knockdown of the Cell-cell adhesion protein E-Cadherin activates actomyosin contraction and increases apoptosis in differentiating stem cells [71, 72]. Emerging interesting data of studies using the actin monomer binding peptide Thymosin β4 (Tβ4) have shown successful outcomes that improve extracellular contacts and myocardial regeneration [73].

On the other hand, stem cell survival is often influenced by enzymatic cell detachment (trypsinization). Trypsinization involves proteolytic cleavage of adhesion molecules and membrane receptors that lead to cellular proteome alterations, upregulation of the tumor suppressor p53 and the cell cycle inhibitor p21, and downregulation of anti-apoptotic Bcl-2 protein [74]. Interestingly, cells in suspension normalize very fast when supplied with artificial or biological extracellular matrix to which stem cells readily attach and thereby prevent anoikis induction during or soon after the cell transplantation [75]. Overall, only very few cell types naturally exist unattached, e.g. hematopoietic cells, whereas various other stem cells are very dependent on extracellular contacts and undergo anoikis when held in suspension for more than several hours. To enhance the survival of the transplanted stem cells and improve the composition of ECM in infarcted area we could provide more matrix with the delivered cells in addition to the seeded matrix to reduce stressing the cells during trypsinization and injection (as described above). Even without cells, certain hydrogels may enhance cardiac cell survival by simply supplying extracellular contacts and stimulating anti-apoptotic signaling pathways when injected into pathological area [60]. Another way to avoid anoikis is to transplant cells in aggregates [61] or in mini-tissue patches as cells adhere to each other to respond properly to paracrine survival signals, share nutrients and survive in normal tissue conditions [61, 76, 77]. On the other hand, amniotic membrane can be a source of biocompatible matrix that triggers beneficial effects at the molecular and cellular levels [35]. Interestingly, ECM-initiated survival signaling may be enhanced by direct introduction and overexpression of certain growth factor genes and downstream components of survival signaling pathway, e.g. protein kinase AKT [78]. Advantages, mechanisms and examples of cell transplantations either in aggregates or encapsulated in polymer coating or embedded in biocompatible ECM (hydrogels) have been recently reviewed by Sart and colleagues [79]. Thus, diversity of matrix could be explored in future studies to provide proper adhesion required for retaining the transplanted cells.

The roles of reactive oxygen species (ROS)

Oxidative stress is known to be a major factor that contributes to a number of intracellular signaling pathways which induce cell death leading to myocardial damage. ROS-associated pathways may influence vital cellular processes including metabolic alterations, cardiomyocyte growth, ECM configuration, ion flux and calcium handling, excitation-contraction coupling, vasomotor tone, gene expression, and mechanisms regulating growth factors and inflammatory cytokines [80]. Oxidative stress in myocardium can be caused by hypoxia as well as by expression of ROS-induced apoptotic factors [81]. Therefore, targeting oxidative stress is believed to be efficient in protecting the damaged myocardium via rescuing cells prone to undergo apoptosis [82]. Upregulation of ROS in stem cells impairs their adhesion, whereas the application of ROS blockers can reverse the disrupted adhesion [59, 81].

Exogenous ROS negatively impacts survival of the transplanted cells, and increased myocardial ROS is known to be a major apoptosis inducer shortly after transplantation [83, 84]. Nevertheless, quiescent stem cells in a hypoxic niche may have decreased ROS due to antioxidant defense mechanisms that protect stem cells from external oxidative stress. Interestingly, upregulation of external ROS has been suggested to contribute to proper maturation of native stem cells [85]. Exposure of cell transplants to hypoxic conditions prior transplantation has been shown to improve their survival and leads to efficient cardiac tissue regeneration [86]. Taken together, delineating the mechanisms by which exogenous and endogenous ROS contribute to apoptosis of both resident and transplanted stem cells will reveal targets and pharmacological compounds required for proper tissue regeneration.

Inflammation

One more factor that directs transplanted cells to die is inflammation. Inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), TGF-β, IL-6 and IL-1β are elevated in infarcted myocardium and may be involved in cell death. This is critical, because cell death could also increase the inflammatory response leading to impairment of cardiac function. Stem cells have been suggested to participate in their own demise by eliciting nonspecific inflammation and activating both mitochondrial and death receptor pathways [87]. Therefore, various approaches are used to increase cell survival in the place of transplantation by down-regulating host immunity. For example, bone marrow MSCs survival can be improved by preventing inflammation soon after transplantation [27, 88–90]. Several measures were shown to modulate inflammation and improve cell survival after transplantation into infarcted myocardium such as targeted inhibition of inflammatory cytokines, co-injection cells with superoxide dismutase (CuZn-SOD), and using IL-1 inhibitor-expressing skeletal myoblasts [91–93].

Moreover, MSCs themselves demonstrated immune-modulatory, anti-inflammatory, and tissue repair properties in a number of studies including preliminary clinical trials. Although there is great controversy concerning the molecular mechanisms involved in the immunosuppressive effect of MSCs, some authors suggested that direct cell-to-cell interactions and soluble factor secretion are involved in immunosuppressive effects in a number of situations [94, 95]. Similarly, MSC spheroids showed enhanced secretion of anti-inflammatory molecule prostaglandin E2 which is thought to be due to local hypoxia in the core of aggregates [79]. Surface modification with biomaterials and bioactive substances and microencapsulation technique was an additional approach used to protect transplanted cells from attack by the host immune system. When embedded within hydrogels or encapsulated in shells, the stem cells demonstrated limited immune response and inflammation [96]. Moreover, additional anti-inflammatory compounds may be added into the gel/shells to augment the effect of protection. For example, IGF-1 was shown not only to attenuate inflammation in post-MI, but also protected transplanted cells against apoptosis [97].

Transplantable cell death/survival signaling

Another critical factor for ensuring proper regeneration and successful restoration of heart function would be transplantable cell resistance to the hostile/toxic factors in the surrounding environment. Thus, beside the methods discussed above (revascularization, ECM supply, ROS scavengers, anti-inflammation techniques), modification of introducible cells is needed to increase their fitness and resistance to apoptotic stimuli generated by the harsh microenvironment in the infarcted heart. In addition, transplantable cells themselves could be conditioned to better survive in harsh conditions. Recent studies have used various strategies to enhance stem cell survival after implantation [28, 29]. Currently, the main factors that underlie the decrease of stem cell survival and differentiation and increase in apoptosis include membrane receptors, proteases, mitochondrial and nuclear proteins, lack of growth factors, loss of telomerase activity and cell–cell signaling. Modifications of cells before transplantation could improves their resistance to harmful agents and enhance their long term viability to efficiently restore cardiac function. Progress in the stem cell biology field suggested new targets, means and methods of protecting stem cells against apoptosis to ensure effective cellular therapy. Further elucidation of apoptosis induction mechanisms in the transplantation area is required to reveal both targets and ways to improve the poor outcome of cell therapy.

Several in vitro models have been used to understand the regulation of stem cell differentiation, survival and apoptosis. Despite potential benefit of these experiments, these models remain controversial, leaving a huge gap in the understanding of the actual control of the biology of stem cells and their future applications in the medical field. This could mainly be explained by the fact that stem cells used by various studies may undergo apoptosis in different ways during repopulation and differentiation in the injured myocardial tissue. For instance, H9C2 cell line is occasionally used to study stem cell apoptosis/survival because they share many of the differentiation features of primary cardiomyoblasts when subjected to reduced serum concentrations at confluence. These studies may suffer from significant limitations because H9C2 cell line shows many differences from primary cells and may thus reveal irrelevant results in apoptotic conditions [98]. In addition, it is important to appreciate the fact that embryonic and adult stem cells may differ in their lifespans. Clarification of the relationship between cellular lifespan and death/survival pathways will be useful for effective stem cell therapy as implication of telomerase activity as a mechanism that suppresses apoptosis and ensures differentiation of the stem cells in the injured heart has recently begun to emerge [25, 99].

According to numerous previous studies, terminally differentiated cells which are permanently withdrawn from the cell cycle, are usually more resistant to apoptosis than their undifferentiated counterparts. Thus, it is also worth investigating mechanisms by which normal cells acquire resistance to death induction and stress conditions during

differentiation. For example, mouse, rabbit and human differentiating muscle cells display differentiation-induced resistance to genotoxic agents, as well as diminished susceptibility to apoptosis [100–104]. Conversely, some investigators suggested that undifferentiated cells are more resistant to oxidative stress, probably because of a higher level of intracellular redox system, which is characteristic of stem cells [105, 106]. Tissue regeneration and stem cell differentiation is regulated by various protein kinases which transduce intracellular signals to control the activity of key cellular regulatory mechanisms (discussed below). Nevertheless, variability in the level and duration of phosphorylation, the nature of the stimulus and the cross-talk with other signaling pathways await further investigations [107].

Signaling by AKT pathway

Studies of major molecular survival pathways showed great potential for applying PI3K/AKT signaling pathway in providing stem cell protection. This can be exemplified by the findings that overexpression of AKT can block apoptotic cell death of MSCs and thus improve cardiac function [108]. Phosphatidylinositol 3-kinase (PI3K) signaling was demonstrated to control cell survival and is stimulated by insulin-like growth factors (IGFs) via receptor tyrosine kinases (RTKs). The major mediator of this pathway is the AKT kinase which is implicated in stem cell and myocyte survival, myogenesis, in part by promoting the expression of various molecules including the myogenin gene [109–112]. Moreover, both AKT1 and AKT2 proteins and kinase activities are upregulated during myogenic differentiation and are tightly associated with apoptosis-resistance [104, 110, 112, 113]. In particular, AKT signaling in the infarcted heart can block apoptosis, decrease infarct size, and increase left ventricular wall thickness compared to pre-infarction condition [114, 115]. Deeper insight into AKT signaling revealed the importance of its subcellular localization in determining cell death and survival as seen in genetically engineered mice which express AKT in the nucleus and display very strong anti-apoptotic activity without hypertrophic response [116]. In addition, proliferation of myocardial stem and progenitor cell populations in vitro has been shown to be enhanced by myocardial-specific nuclear AKT expression [117, 118]. A central role of PI3K/AKT signaling in cardioprotection has been previously indicated [112, 119, 120]. AKT signaling in MSCs reduces the secretion of proinflammatory molecules and of anti-apoptotic molecule Bcl-2, leading to the enhanced resistance in pathological conditions [79]. Changes in AKT activity have also been demonstrated in osteogenesis, adipogenesis and neurogenesis of muscle-derived stem cells (MDSCs), where AKT phosphorylation correlates with resistance to various toxic treatments that influence survival of the differentiated cells [104]. Furthermore, suppression of PI3K/AKT signaling enhances sensitivity of proliferating MDSCs to daunorubicin induced apoptosis [121]. Although PI3K/AKT signaling pathway is critical for determination of cell survival, a pro-apoptotic role for PI3K/AKT signaling has been suggested [122, 123]. Although a number of investigations have involved AKT as a player in cardiac regeneration [117, 118, 124–127], future cell therapeutic studies involving AKT require careful consideration of important factors that could impact cell survival, including dose of therapy, cell biological features, duration and intensity of AKT activation, implication of autocrine vs paracrine mechanisms and severity of cardiac damage,

Signaling by MAP kinases ERK, JNK and p38

Another family of protein kinases which actively participate in determining regulation of cell survival and cell cycle progression are mitogen-activated protein kinases (MAPKs). ERK1/2 members of this superfamily are known to regulate stem cell differentiation, proliferation and survival [128]. MAPKs have been shown to play critical roles in cardiac physiology and pathology a well as in early cardiogenesis [129–131]. Cardioprotection by MAPK signaling pathway is largely dependent on phosphorylation of key molecules that regulate cardiomyocyte viability and differentiation [132]. Previous studies have indicated that MEK/ERK1/2 pathway protects cardiomyocytes from apoptosis induced by a number of different stimuli that trigger cell death signaling cascades [133–136]. Importantly, activation of ERK1/2 has been shown to contribute to stem cell survival induced by adhesion-based signaling, namely angiopoietin-1 derived peptide QHREDGS [137].

The MAPK stress kinase JNK1/2 signaling is known to induce apoptosis in various mammalian cells, but can also play anti-apoptotic roles. JNK can regulate the mitochondrial pathway of apoptosis by releasing pro-apoptotic molecules such as cytochrome c and AIF, mainly by phosphorylation of the anti-apoptotic protein Bcl-2 [138]. Interestingly, the mitochondrial pathway of apoptosis has been shown to be totally defective in JNK-null fibroblasts [139]. In addition, JNK dominant negative adult cardiac myocytes were resistant to beta-adrenergic receptor-stimulated apoptosis [140]. However, JNK activation was demonstrated to have pro-survival roles in other conditions. For example, activation of JNK blocked ischemia–reperfusion-induced cell death, protected primary myogenic cells subjected to nitric oxide and inhibited cell death of C2 skeletal myoblasts treated with TNF alpha, whereas inhibition of JNK resulted in apoptosis of cultured cardiomyocytes [141–144]. Such a variation of JNK role in cell

survival can be explained by variability in the level and duration of phosphorylation, the nature of the stimulus and the cross-talk with other signaling pathways [107].

p38 MAPK is also involved in myocyte apoptosis both in vivo and in vitro, as well as in the differentiation process. Recently, it has been shown that p38 is required in cell fusion through upregulation of tetraspanin CD53 [145]. It is known that p38 phosphorylates the transcription factor E47 and promotes its interaction with the myogenic regulatory factor MyoD, in addition to its role in enhancing the transcriptional activity of MyoD/E47 complex, resulting in cell differentiation [146]. Interestingly, transgenic mice with dominant-negative upstream p38 kinase MKK6 display reduced MI [147]. In addition, p38-α (±) heterozygote mice were reported to be more resistant to ischemia than wild-type (+/+) mice [128]. Besides, ischemia- or doxorubicin-induced apoptosis of cardiomyocytes in vitro were attenuated by pharmacological p38 inhibitors [148, 149]. These results indicate a pro-apoptotic role for p38 pathway in these models. In contrast, a protective action of p38 has been demonstrated in cardiomyocytes after stimulation with beta-adrenergic receptors [150], and in MDSCs treated with NOC-18 and daunorubicin. Furthermore, inhibition of p38 has been shown to decrease differentiation of MDSCs [151]. Importantly, exogenous ROS, endoplasmic reticulum stress and mitochondrial apoptotic pathways play important roles in apoptosis of MSCs via mechanisms including hydrogen peroxide/p38 (early apoptotic) or JNK-dependent (late apoptotic) death of MSCs [152]. The roles of MAP kinases and MAP kinase BMK/ERK5 in heart development, function and diseases were recently reviewed [130], but the question remains open: how would these kinases benefit patients when there is such a multiplicity in their biological functions?

In sum, there are promising key roles for MAPK signaling components in enhancing stem cell therapy via regulation of apoptosis, ensuring differentiation and enhancement of cardiac regeneration. As genetic modifications (mainly used to overexpress certain genes) are hardly allowed in human patients due to possibility of insertional mutagenesis, small-molecule kinase inhibitors are the second very potent tools that regulate cellular response to extrinsic stimuli including apoptotic response, differentiation, survival signaling or even reprogramming [153–155]. Because small molecules are able to penetrate cellular membranes and to inhibit both cytoplasmic and nuclear proteins, many of them are proposed to be used in clinics. This data need to be carefully evaluated prior to applying small-molecule inhibitors of these kinases in clinical trials [130, 156]. Although such compounds perform well in vitro and may seem to be the future of many therapies, not all of them are specific or without off-target effects in vivo [157]. Moreover, it is possible that major players in signal transduction and regulation of apoptosis change their role in determining cell fate during differentiation. This may be crucial when considering small-molecule inhibitor interventions either before or after stem cell transplantations, when suppression of apoptosis is favorable in order to restore cell population in the pathological area.

Other signaling molecules

Many other components of stem cell death and survival signaling pathways have been identified, however much remains to be learned. The cell membrane-embedded molecules integrins play major role in cell fate decision after transplantation. Integrins often mediate cell survival through activation of ERK1/2, Integrin-linked kinase (ILK) signaling [137], partially through NF-kB which in turn regulates secretion growth factors such as VEGF, FGF and BDNF [158]. Among other molecules variously participating in stem cell death regulation there are pro-apoptotic Rho/ROCK (Rho-associated kinase) [72, 159], mTORC1 and SIRT1 which are involved in stress adaptation and protection of cells, as has been demonstrated in animal models and in clinical trials [160], cytokines macrophage migration inhibitory factor (MIF) and GDF-15 [161]. Recent investigations have shown that MSCs produce a set of growth factors and promote cardioprotection via mechanisms involving AKT, PI3K, estrogen receptor-α and STAT3 pathways [53, 162–165]. So far, there is a little information concerning strategies to protect the transplants against both intrinsic and extrinsic cell death, however it is well established that bone marrow-derived MSCs secrete survival growth factors that can work in a paracrine manner on the infarcted heart to prevent cell death and inflammation. It is known that harsh microenvironment often triggers pro-death cytokines (e.g. FasL) to stop cell proliferation and differentiation cascades. Interestingly, MSCs were shown to be extremely sensitive to FasL-induced cell death [166]. Thus, the trials performed to improve the harsh microenvironments in order to increase survival of implanted cells could benefit from targeting Fas-FasL-caspase-8 pathway.

Moreover, mitochondria are important players in apoptosis regulation and are essential organelles for long-term survival of eukaryotic cells [167–169]. Many mitochondrial proteins (including: Bcl-2 protein family, cytochrome c, apoptosis-inducing factor AIF etc.) are regulated upon stress conditions. For example, MSCs are sensitive to hypoxia and serum deprivation signaling via mechanisms modulating mitochondrial integrity and function, possibly independent of caspase-8 [170]. Mitochondrial ATP-sensitive potassium (mitoKATP) channels contribute cardioprotection against hypoxia when their ROS-regulated

opening leads to synthesis of antioxidant molecules [171]. Thus, preventing the intrinsic (mitochondrial) apoptotic pathway and maintaining proper mitochondrial respiration in transplanted cells are critical factors for the success of stem cell therapy.

Improving the cells for transplantation

As described above, microenvironment of transplanted stem cell could be improved by modulation of immune response, trophic factor withdrawal and hypoxia [61]. Treatments directed to improve the properties of transplantable cells include pretreatment (with small molecule inhibitors, peptides, miRNAs, exosomes), preconditioning (using heat shock, hypoxia, exposure to the oxidative stress), genetic modification, and co-transplantation of different cell types.

Pretreatments and preconditioning

Stem cell survival appears to be improved by preconditioning prior to implantation. Successful protection of transplantable cells was demonstrated by using cytokines, antioxidants, nitric oxide, pharmacological inhibitors and other drug treatment, glucose deprivation, administration of growth factors, expression of pro-survival proteins, growth factors, anti-apoptotic proteins, subjecting cells to hypoxia or heat shock [26, 106, 172–176]. One of the major goals of preconditioning is stimulation of anti-apoptotic and inhibition of pro-apoptotic pathways in transplantable cells. Molecular mechanism of cell preconditioning are hoped to modulate cell signaling and metabolism when adapting to mild stress conditions [177]. It involves activation or inhibition of main cell survival/death regulators such as of PI3K/AKT, ERK, mammalian target of rapamycin (mTOR), NADPH oxidase (NOX) signaling molecules and pathways which surely participate in cellular homeostasis in vivo [178, 179]. Similar and miscellaneous mechanisms of cell protection during preconditioning have been observed in a number of studies.

For example, chronic exposure to oxidative stress induces a transient release of ROS from mitochondria, leading to the activation of extracellular signal-regulated kinase ERK which promotes the expression of anti-apoptotic proteins. At lower physiological levels endogenous ROS, such as H_2O_2, can act as an intracellular signaling molecule regulating kinase-driven processes of cell proliferation, migration, anoikis, survival and autophagy [180]. Heat-shocking of cells acts through induction of well-known cytoprotective heat shock proteins (HSPs) which activate PI3K/Akt and ERK signaling, leading to increased expression of anti-oxidants, anti-apoptotic and trophic factors [181, 182]. Similarly, hypoxia upregulates hypoxia-inducible factor (HIF-1) resulting in reduced oxidative phosphorylation, inhibition of tumor suppressor p53, increased expression of VEGF receptors, etc., and activation of Akt which targets anti-apoptotic Bcl-2 and pro-apoptotic caspases [183–185].

Heat shocking, exposure to hypoxia and oxidative stress are the most common pretreatments of cells. Other treatments performed to improve cell survival can target cell death signaling and execution molecules, such as Rho-associated kinase, p38 MAPK, caspase inhibition, transforming growth factor (TGF) treatment, etc. [60]. It has been also reported that extracellular ligands tEGF as well as matrikine tenascin-C can protect MSCs from FasL-induced cell death during transplantation [186, 187]. Recently, many other preconditioning approaches were suggested to improve the outcome of stem cell therapy. For example, new promising results show that Wnts, a family of secreted hydrophobic glycoproteins which are ligands to Frizzled receptors, play a role in neural stem cell self-renewal, differentiation and in engraftment of transplanted myogenic cells in vivo [188, 189].

On the other hand, previous reports have suggested beneficial effect of cell therapy through paracrine function and by using in vitro generated special conditioned medium [12, 37, 190, 191]. For example, human umbilical cord blood mononuclear cell-conditioned media blocked hypoxia-induced apoptotic cell death not only in human coronary artery endothelial cells, but also in cardiomyocytes [192]. The beneficial effects of MSCs also rely on the secretion of paracrine factors and immune-regulatory molecules such as IL-6, IL-10, and indoleamine 2,3-dioxygenase [79]. Given the fact that distinct transplanted cell types show noticeable differences both in their intracellular signaling as well as their effect in the infarcted cardiac area, future studies should find the right composition of any applied cell-free system [193]. Combined treatment directed at multiple cell survival/death pathways simultaneously would be a promising strategy.

Exosomes and microRNAs

Exosomes are cholesterol-rich phospholipid vesicles with abundant miRNAs which are known to post-transcriptionally modulate gene expression and contribute to various pathological and physiological conditions. Recent studies uncovered that MSCs are able to secrete cholesterol-rich phospholipid particles which contain miRNAs [194]. The released exosomes act on the hearts and vessels and exert benificial roles including: cell survival, cardiac regeneration, anti-cardiac remodeling, anti-inflammatory effects, neovascularization and anti-vascular remodeling [195, 196].

Interesting latest reports suggest that microRNAs play significant roles in tissue regeneration [197] where tissue-resident adult stem cell population can be pharmacologically forced to go back into cell cycle and start regenerating wounds [198, 199]. In particular, miRNAs have been demonstrated to be essential regulators of stem cell fate [200]. Modulations of miRNA profiles were seen in MSC apoptosis stimulated by serum deprivation and hypoxic conditions. Many miRNAs have been shown to promote metabolism, viability and differentiation of muscular progenitors into cardiac myocytes under stress and ischemia [201]. Overexpression of miR-20, miR-21 and miR-23a protects from apoptosis of MSCs exposed to hypoxia and serum deprivation [202]. In addition, miR-24 under cardiac-specific Myh6 promoter caused strong protective role in transgenic mice after MI [203]. Recent investigations indicated the role of miR-23a inhibition on TNF-α-induced programmed cell death and regeneration in the damaged myocardium [204, 205]. Moreover, hypoxia-induced miR-210 has been shown to be a major regulator of bone marrow-derived MSC apoptosis via caspase-8-associated protein-2 (Casp8ap2)-dependent pathway and that preconditioned MSCs were well protected against cell death compared to non-preconditioned MSCs [206]. Interestingly, miR-133a is an abundantly expressed microRNA in the cardiac muscle and is downregulated in patients with MI [207]. miR-1 has been shown to block apoptosis and enhance differentiation of ESC cells into cardiac cells [208]. Thus miRNAs encapsulated in exosomes appear to have a good therapeutic potential with or even without MSCs transplantation. Yet, with the current advances in methodology, achieving efficient stem cell therapy requires not only identification of miRNA mechanisms that control stem cell survival, but also their target genes/pathways that switch-off proliferation and control differentiation.

Gene therapy

More than a decade ago, stem cell viability and success of transplantation have been shown to be enhanced by gene modification. Although results of initial clinical trials using genetically engineered stem cell therapy are encouraging, many cell survival aspects need to be understood before this approach is fully applied in cardiac repair. A number of pro-survival proteins or angiogenic factors, including connexin Cx43, tumor necrosis factor receptor TNFR, stromal cell-derived factor-1 SDF-1, signaling kinase AKT1, ILK and growth factors HGF, FGF-4 and VEGF-A have been shown to contribute to repopulation of cells when overexpressed in an exact damaged site [78, 175, 209–211]. Other data showed that anti-apoptotic BCL-2 transgene expression can efficiently attenuate cardiomyoblast death early after transplantation [212], and that ERBB4 (erb-b2 receptor tyrosine kinase 4) overexpression potentiate MSC survival in the infarcted heart [213]. Genetic modification of MSCs with ILK been shown to promote cell survival and function of infarcted myocardium in vitro and in vivo [214]. Furthermore, it was demonstrated that cell adhesion benefit was quite negligible when compared with overexpression of AKT transgenes [78].

Organ engineering

As genetic modification is hardly accepted in clinical trials aiming to improve effectiveness of transplantation, current manipulation of cell signaling should be achieved by transient stimulation/preconditioning approaches [215]. Organ engineering may provide an alternative treatment for end stage cardiac failure when donor hearts are available. Bioengineering of the heart has been performed via procedures applying decellularization-recellularization strategies; however, maintaining sufficient cell viability upon cardiac specific recellularization will be the challenge for upcoming research and should be well understood before bioengineering of the heart is widely used in clinics [216].

Concluding remarks

The involvement of various signal transduction pathways in cell death and survival processes would offer development of new approaches for stem cell-based therapies and tissue engineering but interactions of these pathways remain very complicated. Thus protection from stem cell death requires progress in various fronts including bioinformatics, cell biology, genetics, proteomics, structural biology, and pharmacology. It would be effective to simultaneously and mechanistically target major pathological causes of cell loss like hypoxia-reoxygenation, inflammation and deficiency of native ECM to achieve successful cardiac tissue regeneration. Both pro- and anti-survival networks integrate cardiac environmental cues that regulate stem cell decision to live or die. Importantly, the roles of various regulatory proteins may change during stem cell differentiation and their effects depend on the cell type, stage of development and apoptotic stimuli. In sum, understanding molecular and cellular regulation of stem cell death and survival in the heart will help preventing loss of vast majority of transplanted cells as well as identify target molecules that offer new therapeutic options for patients with heart diseases.

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