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Preconditioning and Stem Cell Survival

Husnain Kh Haider · Muhammad Ashraf

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Abstract The harsh ischemic and cytokine-rich microenvironment in the infarcted myocardium, infiltrated by the inflammatory and immune cells, offers a significant challenge to the transplanted donor stem cells. Massive cell death occurs during transplantation as well as following engraftment which significantly lowers the effectiveness of the heart cell therapy. Various approaches have been adopted to overcome this problem nevertheless with multiple limitations with each of these current approaches. Cellular preconditioning and reprogramming by physical, chemical, genetic, and pharmacological manipulation of the cells has shown promise and "prime" the cells to the "state of readiness" to withstand the rigors of lethal ischemia in vitro as well as posttransplantation. This review summarizes the past and present novel approaches of ischemic preconditioning, pharmacological and genetic manipulation using preconditioning mimetics, recombinant growth factor protein treatment, and reprogramming of stem cells to overexpress survival signaling molecules, microRNAs, and trophic factors for intracrine, autocrine, and paracrine effects on cytoprotection.

Keywords Ischemia · Myocardial Infarction · Preconditioning · Stem Cells

Stem Cell Therapy for Myocardial Regeneration

Myocardial regeneration subsequent to ischemic injury could be accomplished by either transplantation of donor-derived stem or progenitor cells or alternatively by stimulating the endogenous stem cells to more aggressively participate in the repair process. Beginning more than 15 years ago with the use of unipotent myogenic precursor cells from skeletal muscle [57, 58] and terminally differentiated cardiomyocytes [57, 58], heart cell therapy has progressed to the use of multipotent and pluripotent stem and progenitor cells [22, 30, 59, 63, 80, 86, 105, 108, 112, 122]. Despite extensive experimental animal studies and a decade-long clinical assessment of stem and progenitor cells to regenerate the damaged myocardium [25, 34, 36], the choice of donor stem and progenitor cells and transplantation protocols for optimal prognosis still remain the focal issue in the use of stem cells for cardiac repair. Although various cell types have been assessed for their safety, feasibility, and efficacy, the discovery of the presence of resident cardiac stem and progenitor cells and their successful isolation and propagation in vitro for transplantation has significantly moved the field of stem cell research [8, 82]. Embryonic stem cells are promising candidates for cardiac repair. After the recent National Institutes of Health Policy changes regarding the use of embryonic stem cells, investigative efforts for their use in regenerative medicine have been intensified. Embryonic stem cells can provide a clinically relevant and renewable source of cardiomyocytes and endothelial cells for engraftment [2, 118]. Despite their ability to adopt cardiac phenotype, their use is not without technical limitations of undifferentiated propagation in vitro and difficulty of purification of spontaneously differentiated cardiomyocytes from cell culture. Moreover, their teratogenic nature and immunological issues especially with regards to their derivative tissue remain two of the foremost concerns in their clinical application. More recently, strategies have been adopted to achieve tumor-free cardiopoietic programming of embryonic stem cells [15]. The development of induced pluripotent stem cells (iPS cells) from somatic cells is a leap forward in the field of stem cell technology [11]. Since the initial reports, in which iPS cells

H. K. Haider (✉) · M. Ashraf
Department of Pathology and Laboratory Medicine,
University of Cincinnati,
231-Albert,
Sabin Way, OH 45267-0529, USA
e-mail: haiderkh@ucmail.uc.edu

were generated by reprogramming of adult murine and human fibroblasts through retroviral overexpression of four transcription factors including Oct 3/4, Sox2, c-Myc, and Klf4 [109, 120], iPS have been successfully reprogrammed from somatic cells even without the oncogene c-Myc and also by using nonviral and single cassette vectors [44, 88, 106]. These iPS cells are similar to embryonic stem cells in adopting cardiac phenotype, but their use does not raise any ethical or immunological concerns.

Despite the availability of these high-quality cells with near-ideal culture characteristics and ability to adopt cardiac phenotype, the progress in the heart cell therapy has been slowed down by the suboptimal protocols of in vitro processing and handling of the stem cells and for in vivo engraftment in the heart. Accentuated by the harsh microenvironment of the ischemic myocardium, massive death of the transplanted cells postengraftment remains the biggest impediment which significantly lessens the effectiveness of the heart cell therapy. While providing an overview of the massive loss of the donor cells, especially during the acute phase after transplantation, and the possible available strategies to counter this problem, we have discussed in detail our experience in cellular preconditioning to promote stem cell survival in vitro under ischemic conditions and posttransplantation in the infarcted heart.

Acute-Phase Cell Death and Approaches to Enhance Donor Cell Survival

The current transplantation strategies achieve modest engraftment of donor stem cells in the infracted myocardium. One of the major problems in this regard is the rapid and massive loss of donor stem cells after engraftment [79, 90, 94, 114]. The accelerated cell death is multifactorial and is influenced by host inflammatory response mediators, mechanical injury, maladaptation, bouts of ischemia and ischemia–reperfusion, and the origin and quality of the donor cell preparation [40]. However, apoptosis has been implicated as the main contributor in massive loss of donor cells [13, 51]. Apoptosis, a highly orchestrated mechanism of programmed cell death, is initiated by both intrinsic and extrinsic factors. In case of transplanted stem cells, these stimuli include loss of trophic factors, detachment from extracellular matrix, ischemia reperfusion injury, or stimulation of death receptors. In response to these factors, specific signaling pathways are activated and coordinate to execute apoptosis by means of common cell death effector machinery. The extrinsic apoptosis signaling is characterized by ligand binding to the death receptors such as tumor necrosis factor alpha or Fas/CD95 which activates recruitment of adaptor proteins to form death-inducing signaling complex and activation of caspase 8 which ultimately triggers the downstream cascade of caspases [121]. Alternatively, intrinsic initiation of apoptosis involves reduction of the mitochondrial transmembrane potential and concomitant release of apoptogenic factors including cytochrome *c*, endonuclease G, and apoptosis-inducing factor and caspase 3 activation [72]. Taken together, the released cytochrome *c* thus initiates the formation of apoptosome, a complex comprising Apaf-1, cytochrome *c*, deoxyadenosine triphosphate, and pro-caspase-9 to activate caspase 9 which in turn activates pro-caspase-3. Although the two mechanisms appear distinct, a possible crosstalk between the two is responsible for the massive cell death in the infarcted heart.

Antiapoptotic strategies such as gene modification for interleukin 1 (IL-1) expression, use of receptor antagonist, depletion of host C3 complement, and prevention of the humoral immune response by treating host with anti-CD154 before and after cell transplantation have been successfully used for cytoprotection [20, 41, 94]. Similarly, treatment with insulin-like growth factor 1 (IGF-1), vascular endothelial growth factor, β-fibroblast growth factor, and heat shock stimulates cell survival after transplantation. Our experience with cross-species transplantation of human skeletal myoblasts showed that skeletal myoblasts were conditionally immunoprivileged and required transient immunosuppression during early phase of engraftment in the infarcted heart [34, 36]. Similarly, the immunoprivileged status of bone marrow stem cells has been shown in many studies after transplantation into immunocompetent experimental animal models [75, 99]. Put together, these studies underscore the need for strategies which could prevent host immune-response-unrelated factors in order to enhance donor cell survival. In this review, we have discussed in depth the cellular preconditioning approaches to precondition and reprogram the cells for their better survival post transplantation.

The Unrivaled Prosurvival Effects of Preconditioning Approach

Since the inception of the concept that exposure of the heart to brief episodes of ischemia and reperfusion was cytoprotective and protected the heart against a subsequent prolonged ischemia by limiting myocardial cellular damage, preconditioning has been established as the most potent and effective means of cytoprotection [81]. In the later studies, the authors observed significantly attenuated infarct size in the hearts pretreated by multiple 5-min cycles of ischemia with intermittent reperfusion. The phenomenon was termed as ischemic preconditioning and led to the activation of survival signaling in cardiomyocytes. The effect of ischemic preconditioning is described as biphasic,

wherein tolerance to subsequent lethal ischemia/reperfusion is observed within 2–3 h (acute) or between 24 and 72 h (delayed) after the initial sublethal IR injury [17]. The early and late phases of preconditioning shared some common elements in their signaling pathways with the particular involvement of K_{ATP} channel activation via protein kinase C (PKC) signaling. Though without a sharp demarcation, the two phases could be discerned from each other by the fact that the former primarily involved posttranslational modifications of effectors whereas the latter also involved altered gene expression profile and amount of cardioprotective proteins [12, 107]. A recent progress in this regard is the concept of remote ischemic preconditioning in which myocardial preconditioning could be achieved as a result of brief ischemia of an organ or tissue remote from the heart [60]. Although the clinical applicability of the approach is questionable and the underlying mechanism remains unknown, the approach was effective in reducing myocardial infarction in an experimental animal model [50]. More recent studies have shown the activation of Erk1/2 and induction of PKC epsilon and its subcellular redistribution during remote preconditioning [101]. A newer concept related with ischemic preconditioning is postconditioning in which brief episodes of repetitive cycles of ischemia and reperfusion are initiated during reperfusion phase following prolonged ischemic insult to elicit cardiac protection [127]. Since the inception of these initial observations, a plethora of research reports has been published aiming at defining an underlying mechanism(s) responsible for the incredibly potent cardioprotective effects of ischemic preconditioning [16, 32, 37, 116, 119, 124]. However, the signaling cascade triggered by ischemic preconditioning remains largely undefined and is considered to involve multiple signaling pathways. Whereas the earlier studies defined a role for sarcolemma K_{ATP} ($sarcK_{ATP}$) channels in acute-phase ischemic preconditioning in the heart in different experimental animal models [32], later studies have suggested a scheme of events in which mitochondrial K_{ATP} ($mitoK_{ATP}$) channels act as a trigger as well as an end effector in ischemic preconditioning or chemically induced preconditioning [116, 119]. Similarly, a variety of "activators" have also been identified which are released during the course of preconditioning, notable among which are adenosine, catecholamines, bradykinin, opioids, and nitric oxide. These activators are species dependent, and their expression level varies with the intensity and time duration of the preconditioning stimulus [92]. Besides, different protein kinases including Src tyrosine kinases, PKC, phosphatidylinositol-3 kinase, p38 mitogen-activated protein kinase (MAPK), and the JAK/STAT pathway constitute an integral part of preconditioning [16, 18, 37, 96, 124]. In recent years, much research has focused on the central involvement of the $sarcK_{ATP}$ or $mitoK_{ATP}$ channel both as a trigger as well as effector in ischemic preconditioning with equivocal results. Besides activation of the survival signaling pathways, recruitment of stem and progenitor cells into the peripheral circulation and homing into the preconditioned heart has been shown to attenuate infarct size and improved cardiac function [48]. This effect is dependent on upregulated expression of erythropoietin, which induces cell homing by increased stromal-derived factor 1 (SDF-1)/CXCR4 expression and reduces the heart susceptibly to ischemia/reperfusion injury. Additionally, the progenitor cells mobilized into the ischemic myocardium in response to ischemic preconditioning act as reservoirs of cytokines and express an array of potentially cardioprotective molecules including nitric oxide synthase to impart cardioprotection [45]. Further studies have shown upregulation of growth factors in response to ischemic preconditioning [3, 42].

Stem Cell Preconditioning and the Underlying Mechanism

As discussed earlier, apoptosis has been implicated as one of the major factors affecting survival of the donor stem cells in the infarcted heart postengraftment [33]. The pioneering work from our laboratory to take advantage of the potent cytoprotective effects of preconditioning in the heart stem cell therapy significantly improved stem cell resistance to oxidant stress in vitro and postengraftment in the infarcted heart [87]. Moreover, the preconditioned cells elicited improved myogenic differentiation potential and paracrine activity in the infarcted heart postengraftment. As a result, we also observed mobilization of myogenic cells which homed into the infarcted myocardium to participate in the repair process (Fig. 1). Since the publication of this report, various research groups have reported the effectiveness of anoxic and hypoxic preconditioning to improve the survival of donor stem cells [61, 97, 111]. Most of these studies, however, do not follow the classical concept of cyclical exposure to the brief episodes of hypoxia or anoxia to induce preconditioning effect. Rather, in most cases, a single long-term exposure to low oxygen presence has been employed as a trigger to initiate survival signaling followed by lethal hypoxia or anoxia. For example, Hu and colleagues reported that low oxygen culture conditions (0.5% oxygen for 24 h) triggered activation of survival signaling pathways in bone marrow mesenchymal stem cells before their engraftment in vivo [43]. The hypoxia-treated cells showed significantly improved survival post-engraftment in the infarcted heart during acute phase of myocardial infarction. Hypoxia also increased proliferation rate and differentiation along the different mesenchymal lineages and modulates the paracrine activity of mesenchymal stem cells [24].

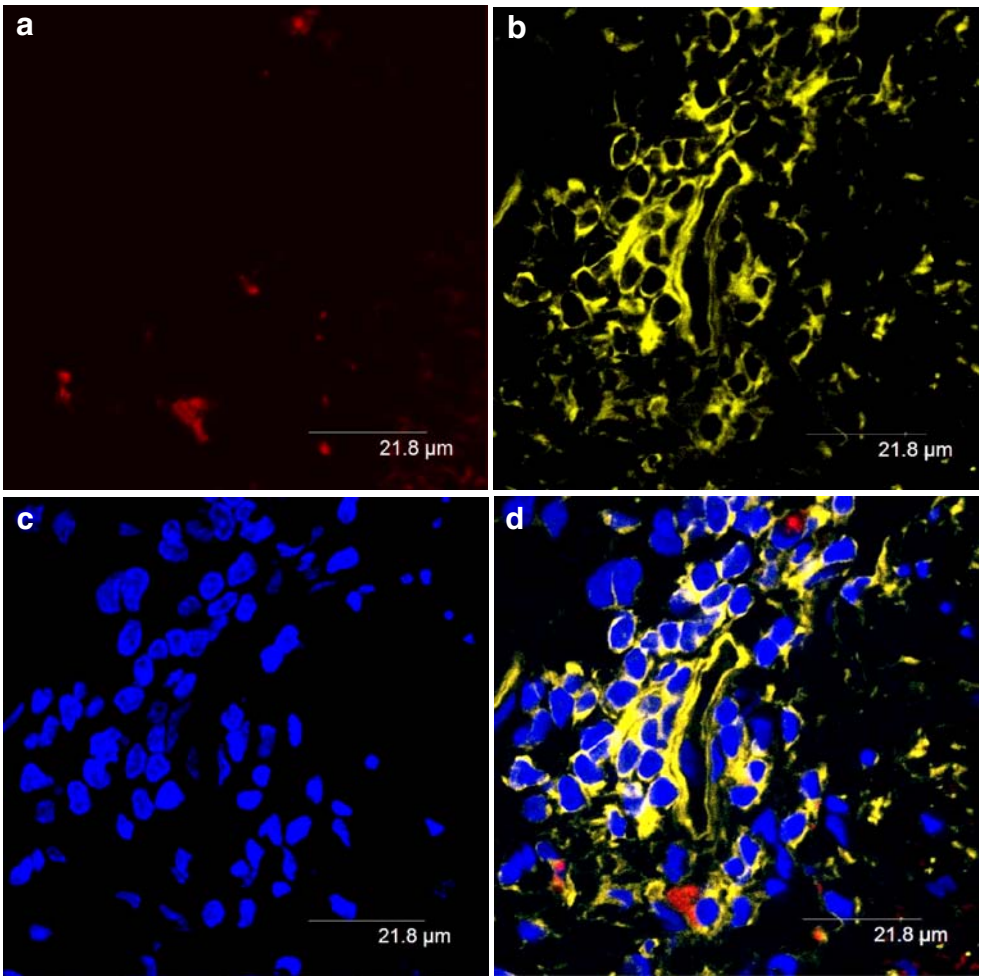

Fig. 1 **a**–**d** Confocal images of the rat heart tissue immunostained for myosin heavy chain (*yellow fluorescence*) postengraftment of pharmacologically preconditioned skeletal myoblasts. The cells were labeled with cell tracker dye PKH26 (*red fluorescence*). Besides myogenic differentiation of the transplanted cells, we also observed extensive presence of myosin-heavy-chain-positive cells mobilized into the transplanted region in the infarcted myocardium. The nuclei were visualized by DAPI staining (original magnification=×63)

The effectiveness of hypoxic preconditioning was ascribed to stabilization and nuclear translocation of hypoxia-inducible factor 1α (HIF-1α), a heterodimeric master switch of hypoxia-responsive array of growth factors which enabled the cells to survive under low oxygen conditions. Besides having prosurvival and cytoprotective effects, hypoxic preconditioning also supports the cells to maintain their stemness and promote their differentiation and proliferation potential postengraftment [7, 38, 97, 113]. It is therefore desirable to precondition the donor cells before engraftment in the ischemic tissues.

In order to validate our hypothesis that intermittent ischemia/reperfusion would be more effective to precondition stem and progenitor cells as compared to one-time longer-term continuous exposure to hypoxia of the same duration and intensity, in a recent study, we demonstrated that pretreatment of the skeletal myoblasts by multiple short duration cycles of ischemia/reperfusion improved their resistance to subsequent episode of lethal ischemia (Fig. 2). Lactate dehydrogenase (LDH) leakage was significantly higher in the nonpreconditioned cells as compared to the ones pretreated with multiple cycles of ischemia/reperfusion. Similarly, LDH leakage from the cells subjected to lethal ischemia was inversely related with the number of ischemia/reperfusion cycles used during preconditioning of the cells. These observations were supported by visualization of higher number of terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-positive cells with hypercontracted and beaded morphology of skeletal myoblasts exposed to lethal ischemia without preconditioning. More interestingly, the degree of cytoprotection was dependent on the number of ischemia/reperfusion cycles. This rationale was also applicable to stem cells other than skeletal myoblasts. We observed that ischemic preconditioning of mesenchymal stem cells with two cycles of 30-min ischemia/reperfusion better supported their survival under subsequent lethal anoxia and also following engraftment in the infarcted heart as compared to nonpreconditioned cells or the cells treated with one cycle of 30-min ischemia/reperfusion [53].

Accounting for the underlying mechanism, most of the previous studies using ischemic preconditioning of cells have reported that HIF-1α serves as the master regulator of genes responsible for survival signaling [43, 85, 125]. Our

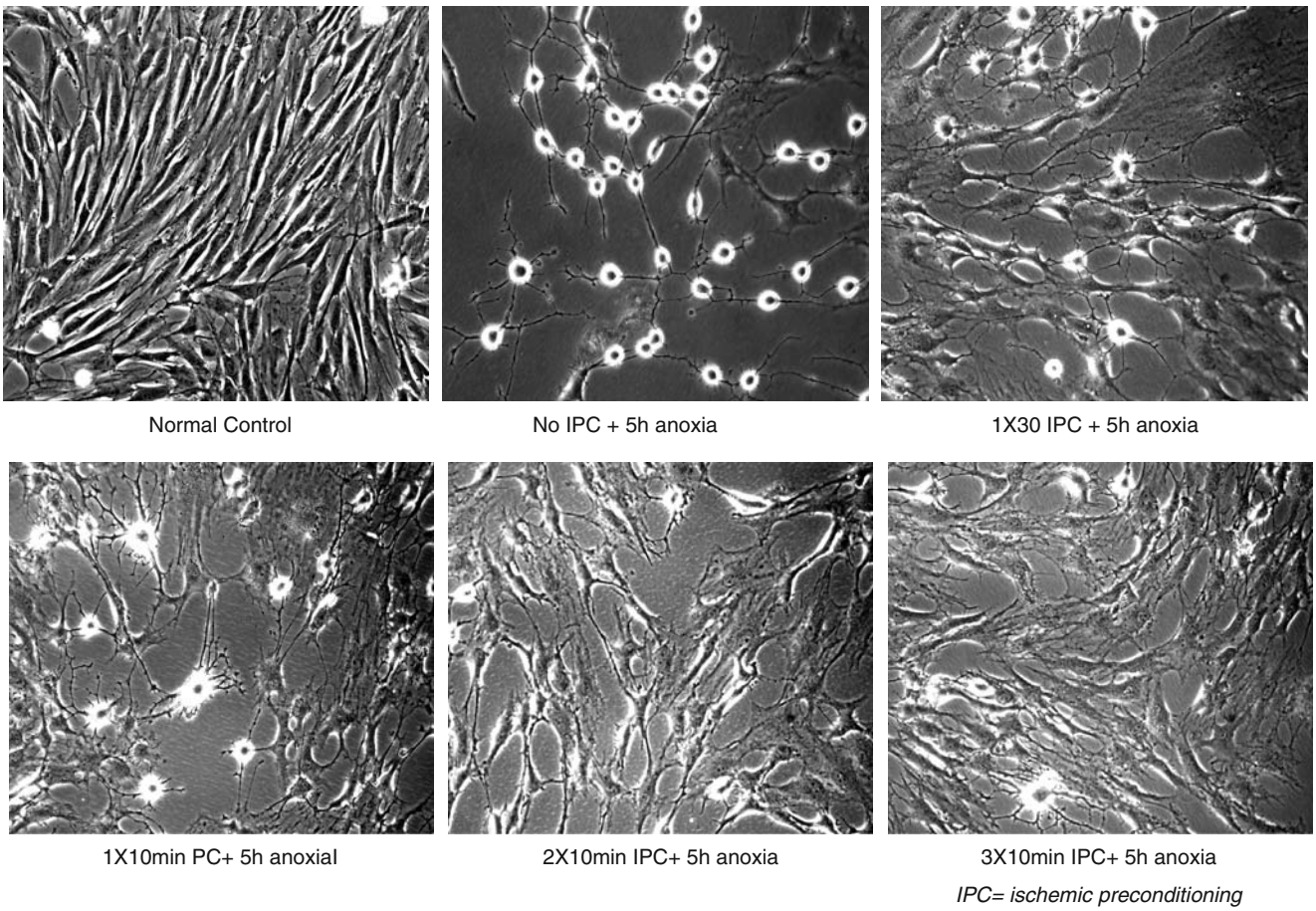

Fig. 2 Effect of ischemic preconditioning on tolerance of skeletal myoblasts to subsequent lethal ischemia. The cells grown as monolayer were subjected to different cycles of ischemic preconditioning (1 × 10-min cycle=10-min anoxia followed by 1-h reoxygenation; 1 × 30-min cycle=30 min anoxia followed by 1-h reoxygenation). Phase-contrast microscopy showed that ischemic preconditioning helped the cells to preserve their structural integrity and showed better tolerance to subsequent lethal ischemia as compared with the nonpreconditioned cells which showed morphological changes such as rounding off and shrinkage of cells (magnification, ×200)

results were in agreement with these conclusions showing significant activation and nuclear translocation of HIF-1α in mesenchymal stem cells preconditioned with two cycles of 30-min ischemia/reperfusion in Akt-dependent manner. In the light of the recently published reports that a select group of HIF-1α-dependent microRNAs (miRs) mechanistically participate in survival signaling [46, 64], we hypothesized that cytoprotective effects of multiple cycles of brief ischemia/reperfusion involved hypoxia-regulated miRs expression downstream of HIF-1α with special emphasis on miR-210 [53]. We observed that induction of HIF-1α in preconditioned mesenchymal stem cells was accompanied by concomitant upregulation of miR-210 which was directly related to the number of cycles of ischemia/reperfusion treatment with two cycles of 30-min ischemia/reperfusion showing maximum level of miR-210 expression. Moreover, inhibition of HIF-1α or that of miR-210 abrogated the cytoprotective effects of ischemic preconditioning. In vitro data on miR-210 which were supported by in vivo studies in a rat model of acute myocardial infarction showed predominantly improved stem cell survival postengraftment. Similarly, participation of miR-210 in endothelial cells functioning in response to hypoxia considerably influenced their migration, capillary network formation, and differentiation capability [31]. Notably, induction of miR-210 occurred as early as 4 h after exposure to hypoxia and persisted for 24 h above baseline levels. Moreover, miR-210 inhibition was associated with increased apoptosis of endothelial cells. A functional link between HIF-1α and miR-210 has also been reported in cancer cells for antiapoptotic [64, 65] effects and adoption of a mutator phenotype to thrive in the adverse microenvironment of tumor [23].

Using real-time polymerase chain reaction (PCR)-based rat apoptotic gene array (SA Biosciences, USA), we identified four possible target genes including CASP8AP2, tumor necrosis factor receptor super family 1a, DNA fragmentation factor-β, and lymphotoxin A which showed more than twofold upregulated expression in miR-210 knockdown cells. In silico target gene analyses and

luciferase reporter assay in preconditioned mesenchymal stem cells further identified FLASH/caspase-8-associated protein 2 (CASP8AP2) as the target gene of miR-210 to improve preconditioned cell survival under lethal anoxia. Induction of FLASH/CASP8AP2 in miR-210 knockdown preconditioned mesenchymal stem cells resulted in increased cell apoptosis. In conclusion, these data added newer dimension to ischemic preconditioning-induced survival signaling in stem cells to signify mechanistic participation of miRs in cytoprotection afforded by ischemic preconditioning via FLASH/CASP8AP2 suppression.

Preconditioning of Stem Cells by Pharmacological Manipulation

The effectiveness of ischemic preconditioning to protect the cells under ischemic stress can also be achieved by pharmacological manipulation of the cells with several mimetics including mitochondrial potassium channel openers [55]. Mitochondrial K^+ channel openers constitute a chemically disparate group of compounds, however, with the common property that these compounds promote influx of K^+ through ATP-sensitive K^+ channels [19, 52, 100]. Diazoxide, a well-known inducer of preconditioning effects by opening of mitoKATP channels, has been widely demonstrated to suppress cell apoptosis [87, 89, 95]. Probing a role for mitoKATP channels in preventing apoptosis of diazoxide-treated cardiac cell under oxidant stress, it was observed that cytoprotective effects of diazoxide were abolished by pretreatment with 5-hydroxydecanoate (a well-known mitoKATP channel opener) [6]. Moreover, the cytoprotective effects of diazoxide were reproduced by pinacidil, another mitoK(ATP) agonist. Although the supporting mechanism of antiapoptosis is not fully defined, it has been shown to involve succinate dehydrogenase inhibition, mitochondrial depolarization, PKC activation, and involvement of a potassium-conductance-independent pathway for cellular protection [62, 95, 110, 115]. We have also shown that cardiac protection afforded by diazoxide treatment involved activation of mitoKATP channels was dependent on Akt translocation from cytosol to mitochondria [5]. The opening of mitoK$_{ATP}$ channels has early-phase effects in altering apoptotic cascade by preventing the release of cytochrome *c* and depolarization. A role for PKC-δ translocation into mitochondria during treatment with diazoxide has also been defined which prompted phosphorylation-dependent activation of mitoK$_{ATP}$ channels. These findings were further supported by our observation that diazoxide-induced cardiac protection was lost after using specific PKC-δ inhibitor [116].

In a recent study, we have reported that diazoxide treatment of mesenchymal stem cells which improved their survival was mediated via activation of nuclear factor (NF)-κB [4]. NF-κB belongs to the family of inducible transcription factors and resides in the cytoplasm in a bound state with the specific inhibitors of NF-κBs in the native unstimulated cells [98]. We observed that diazoxide pretreatment of the cells increased phosphorylation of PI3K, Akt, GSK3β (cytoplasmic), and NF-κB(p65) (nuclear) with concomitant improvement in cell survival. Pretreatment of the cells with Wortmannin, NEMO-binding domain (NBD), or NF-κB (p50) siRNA abolished NF-κB(p65) activity with simultaneous reduction in cell survival. Interestingly, the effect of NF-κB inhibition in terms of reduced viability of preconditioned mesenchymal stem cells was only observed at 24-h time point after preconditioning. Transplantation of preconditioned mesenchymal stem cells promoted their in vivo survival in a rat model of acute myocardial infarction. On the contrary, preconditioned mesenchymal stem cells pretreated with Wortmannin or NF-κB decoy showed poor survival in the infarcted heart. Given the fact that multiple signaling pathways were involved in ischemic preconditioning, pharmacological preconditioning, similar to ischemic preconditioning, "prime" the cells to a "state of readiness" to withstand a subsequent lethal ischemic insult.

Besides initiation of survival signaling, pharmacological manipulation of the stem cells with preconditioning mimetics causes the preconditioned cells to release growth factors and cytokines to act in a paracrine manner to exert cytoprotective and angiomyogenic effects. Such paracrine activity of preconditioned cells adds to "paracrine hypothesis" of preconditioning-induced signaling pathways. We have observed that preconditioned cells show significantly higher release of soluble growth factors including vascular endothelial growth factor, angiopoietin 1, SDF-1α, hepatocyte growth factor, and insulin-like growth factor. Immunohistological studies on the heart tissues for von Willebrand Factor VIII antigen showed significantly higher blood vessel density in preconditioned stem cell transplanted animal group as compared with nonpreconditioned cell transplanted and Dulbecco's modified Eagle's medium-injected animal groups (Fig. 3). The release of these growth factors was NF-κB dependent. Abrogation of NF-κB significantly reduced the paracrine activity of preconditioned mesenchymal stem cells with concomitantly reduced angiogenic activity at 6 weeks after transplantation in the heart.

In a parallel study, real-time PCR-based growth factor array analysis showed an explicit increase of IL-11 protein levels in the preconditioned skeletal myoblasts which occurred in PI3K/Akt- and p42/44 MAPK–signal transducer and activator of transcription 3 (STAT3)-dependent fashion, thus suggestive of its involvement in survival signaling during preconditioning. IL-11 is a pleiotropic cytokine from IL-6 family and acts via IL-11α receptor to impart cytoprotection albeit with a less understood mechanism. IL-11/IL-11α receptor–ligand inter-

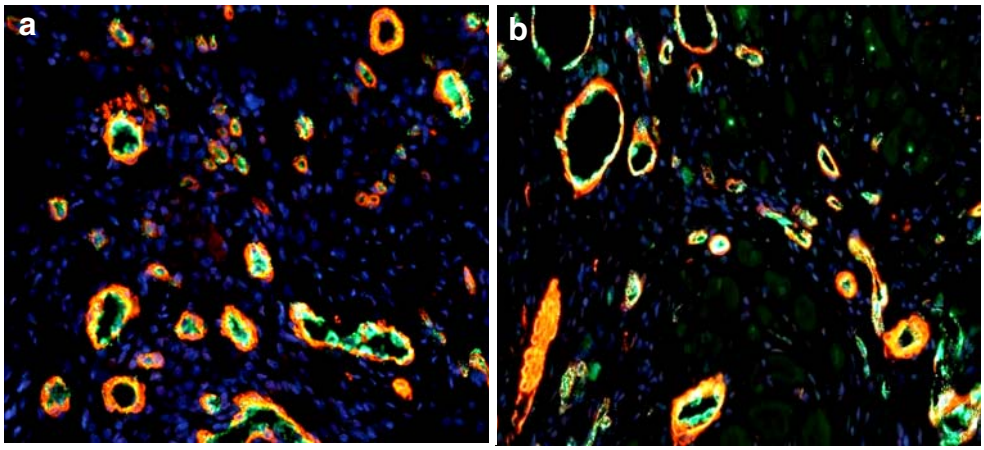

Fig. 3 Double fluorescence immunostaining of the rat heart tissue for von Willebrand Factor VIII (*green fluorescence*) and smooth muscle actin expression (*red fluorescence*) at 6 weeks after transplantation of (**a**) preconditioned and (**b**) nonpreconditioned mesenchymal stem cells. Significantly higher blood vessel density (the number of blood vessels/surface area) was observed in preconditioned cell transplanted animal group as compared to the nonpreconditioned cell transplanted animal group. The nuclei were visualized by DAPI staining (*blue*). (original magnification×40)

action leads to clustering of gp130 with activation of JAK/STAT pathway [74]. Alternatively, STAT3 is phosphorylated by MAPKs which themselves are activated by IL-11/IL-11α interaction [117]. STAT3 which is activated by numerous cytokines, growth factors, and oncogenic proteins is phosphorylated in several cancer specimens and cell lines, leading to cell transformation and tumorigenesis [27]. STAT3 target genes are involved in multiple cellular functions including invasion, cell survival, self-renewal, angiogenesis, and tumor-cell-immune evasion. We therefore proposed that the secreted IL-11 acted in an autocrine and paracrine fashion to promote survival signaling in the preconditioned myoblasts. In a recently concluded study, we have observed that miR-21 was mechanistically involved in preconditioning, and its upregulated expression was observed in Erk42/44-dependent manner. Abrogation of Erk42/44 activity concomitantly reduced miR-21 induction with resultant decrease in cell survival. These are novel findings and clearly demonstrate that preconditioning of cells with diazoxide involves more than just K^+ channel activity and multiple other molecular mechanisms in preconditioning which improve their resistance to oxidant stress. Moreover, pharmacological preconditioning of stem cells before transplantation will be highly effective in overcoming the problem of donor cell death postengraftment.

The Use of Growth Factors in Preconditioning of Stem Cells

Growth factors including cytokines and chemokines are involved in orchestrating the stem-cell-based tissue repair and regeneration. The infarcted heart has a growth-factor-rich microenvironment wherein participation of various growth factors in the repair process is determined by their temporal availability, overlapping pleiotropy, synergistic, and antagonistic interaction. Besides possessing chemotactic abilities, growth factors interact with specific receptors to initiate signaling cascades which are responsible for cell survival, growth, and differentiation characteristics, thus enhancing their therapeutic effectiveness. More recent studies have focused on delivery of recombinant growth factor proteins or gene delivery encoding for the growth factor of interest prior to transplantation of stem cells into the infarcted heart to modify the microenvironment to support donor cell survival. Anticipating the difficulties to maintain optimal and regulated levels of growth factors in the infarcted heart, we included growth factors as the possible candidates for preconditioning. We posit that treatment of stem cells with growth factors prior to engraftment is a simpler and safer strategy to effectively program the cells for better survival and improved proliferation and differentiation characteristics without undesired effects of administering growth factors, the balance of which is delicately maintained in the biological system.

In one of our previous studies, we have shown that presence of CXCR4 receptors on the cells can best be used for preconditioning of the cells with SDF-1 by exploiting SDF-1α/CXCR4 ligand/receptor interaction [91]. The SDF-1α/CXCR4 ligand/receptor system is widely distributed in the biological system and modulates several biological functions through signal transduction pathways including cell growth, proliferation, survival and antiapoptosis, and emigrational and transcriptional activation [21, 29, 69, 104, 126]. We observed that treatment with recombinant SDF-1α protein primed the cells for better survival under anoxic conditions in vitro and after engraftment in the infarcted

heart [91]. The cytoprotective influence of SDF-1α pretreatment involved activation of Akt and was sensitive to the presence of CXCR4 blocker AMD3100. The authors showed the feasibility of chemokine SDF-1 as a preconditioning agent which suppressed apoptosis, enhanced donor cell engraftment, increased vascular density, and improved myocardial function via SDF/CXCR4 signaling. In another study, preconditioning of endothelial progenitor cells by culturing the cells in the medium supplemented with vascular endothelial growth factor 2 (VEGF2) significantly reduced their apoptosis in a dose-dependent manner with concomitant activation of Akt [102]. Similar results were obtained with H9c2 cardiomyoblasts grown on collagen matrix supplemented with VEGF and basic fibroblast growth factor [66, 67]. More recently, growth factor reduced Matrigel supplemented with ZVAD, Bcl-XL BH4, cyclosporine A, IGF-1, and pinacidil which have been used to achieve cytoprotection. The purpose of the mixture of various cytoprotective agents was to address the most prevalent multiple parallel cell death mechanisms [68]. In the same context, growth factor supplements have been added to the stem cell culture to promote their differentiation into a required lineage [14, 83]. For example, $CD133^+$ cells cultured in the presence of $VEGF_{165}$ and brain-derived nerve growth factor adopted myoendothelial phenotype [103]. Several growth factors including bone morphogenetic protein 2 and basic fibroblast growth factor have been implicated as critical components of this early cardiomyogenic-inductive signaling [83].

In our recently concluded study, we have reported that preconditioning of bone-marrow-derived $Sca\text{-}1^+$ cells upregulated connexin 43 which was both cytoprotective as well as helped improved integration with the host myocytes postengraftment [73]. The antiapoptotic effects of IGF-1 were mediated via IGF-1/IGF-1R ligand/receptor interaction which involved sequential activation of PI3K/Akt and MAPK/Erk1/2. Interestingly, knockdown of connexin 43 gene significantly increased cell death in vitro under anoxia in vitro and after engraftment in the infarcted rat heart. Connexin-43-specific siRNA in $^{PC}Sca\text{-}1^+$ induced higher caspase 3 activity under oxygen glucose deprivation with simultaneous loss of preconditioning-induced cytoprotection. The preconditioned cells continued to overexpress connexin 43 after engraftment in the infarcted heart which improved their integration. Immunohistological studies combined with Western blot analysis of cytoplasmic and nuclear fractions of the preconditioned cells showed that preconditioning resulted in translocation of connexin 43 into mitochondria. Confocal imaging of preconditioned $Sca\text{-}1^+$ after immunostaining for connexin 43 and cytochrome *c* showed predominant mitochondrial translocation of connexin 43 in $^{PC}Sca\text{-}1^+$. Mitochondrial subfractionation showed that connexin 43 was mainly translocated onto the inner membrane of mitochondria. To further elucidate the role of upregulated connexin 43 expression in the preconditioned cells, connexin 43 cDNA with a mitochondria targeting sequence (mito-connexin 43) was engineered into pCMV/mito/GFP vector for targeted mitochondrial overexpression of connexin 43 to study cytoprotective role of mito-connexin 43. Our results showed that cells with mito-Cx43 had improved survival under oxygen glucose deprivation as evidenced by LDH and TUNEL assays. Computational analysis revealed a BH3 motif of connexin 43 having a highly conserved pattern of amino acids consistent with Bcl2 family members which regulate cytochrome *c* release. Further studies are in progress to elucidate the role of connexin 43 in cytoprotection. These data clearly demonstrate that IGF-1 preconditioning of stem cells concomitantly met the two fundamental requirements for heart cell therapy, donor cell survival and their engraftment, through upregulated expression of connexin 43. These are significant findings which highlight a dual role for IGF-1 in terms of cytoprotection and cell engraftment.

Transgenic Overexpression of Growth Factors Preconditions Stem Cells

An alternative strategy to the use of recombinant growth factors to precondition stem cells is to genetically modulate the cells for overexpression of the growth factor/s of interest [28, 29, 78, 123]. The stem and progenitor cells are excellent carriers of therapeutic genes and can be manipulated for therapeutic gene overexpression without compromising their stemness characteristics. Indeed, their genetic modification with genes encoding for growth factor expression accentuates their ability to release these factors. Moreover, these cells serve as a continuous source of growth factors which can act in intracrine, autocrine, and paracrine fashion to exert their effects. In one of our recently published study, we successfully achieved overexpression of IGF-1 in mesenchymal stem cells (~200-fold higher as compared with the native mesenchymal stem cells) by viral vector transduction [35]. The overexpression in the transduced mesenchymal stem cells was sustained for at least 12 days of observation with peak level expression (112 ng/ml by IGF-1-specific ELISA) achieved on day 6 after transduction. The interesting finding of the study was that ex vivo overexpression of IGF-1 resulted in elevated SDF-1α level in the cells which itself is a potent chemoattractant of stem cells. These results implied that genetic manipulation of stem cells not only upregulates the expression of the growth factor for which the transgene has been introduced into the cells; such manipulation has a broader impact in terms of paracrine activity of the genetically manipulated stem cells. Besides, the genetically manipulated mesenchymal stem cells promoted extensive

myogenic response in the infarcted heart (Fig. 4). IGF-1 gene delivery has also been combined with other growth factors and with heart cell therapy to promote donor cell survival, engraftment, and differentiation as a part of the multimodal therapy approach [49, 56, 71]. These studies, however, do not provide much information on the role of IGF-1 to recruit the intrinsically available stem and progenitor cells in the repair process.

Various research groups have also attempted to promote mobilization and homing-in of the intrinsically available stem cells using SDF-1α/CXCR4 ligand/receptor system [39]. A transiently upregulated expression of SDF-1α has been reported in the infarcted heart [1, 93] which requires outside intervention to ensure mobilization process to continue and retain the mobilized cells in the infarcted heart for long enough to ensure their participation in the repair process. Delivery of recombinant SDF-1α protein and plasmid encoding for SDF-1α has been carried out with encouraging results in terms of attenuated infarct size expansion and improved cardiac function [10, 91]. We have combined skeletal myoblast transplantation with gene delivery encoding for SDF-1 transgene [29]. Skeletal myoblasts transfected to overexpress human SDF-1α gene (3:2 *v*/*w* plasmid to lipid ratio, 4-h transfection using FuGene™ 6 in the presence of 125 μM $ZnCl_2$) were transplanted into female rat model of myocardial infarction. On day 4 posttransplantation (in four animals per group), marked expression of SDF-1α/*sry*-gene (p=0.003), total Akt, phospho-Akt, and Bcl2 was observed in animal hearts transplanted with SDF-1α. We also observed significant mobilization of $CD31^+$, C-kit^+, and $CD34^+$ cells which resulted in higher angiogenic response in the animal hearts at 6 weeks postengraftment. Echocardiography showed improved indices of left ventricle contractile function and remodeling in the animal group which received skeletal myoblasts overexpressing SDF-1α. These results highlighted the significance of SDF-1α transgene delivery to the heart to promote stem and progenitor cell migration into

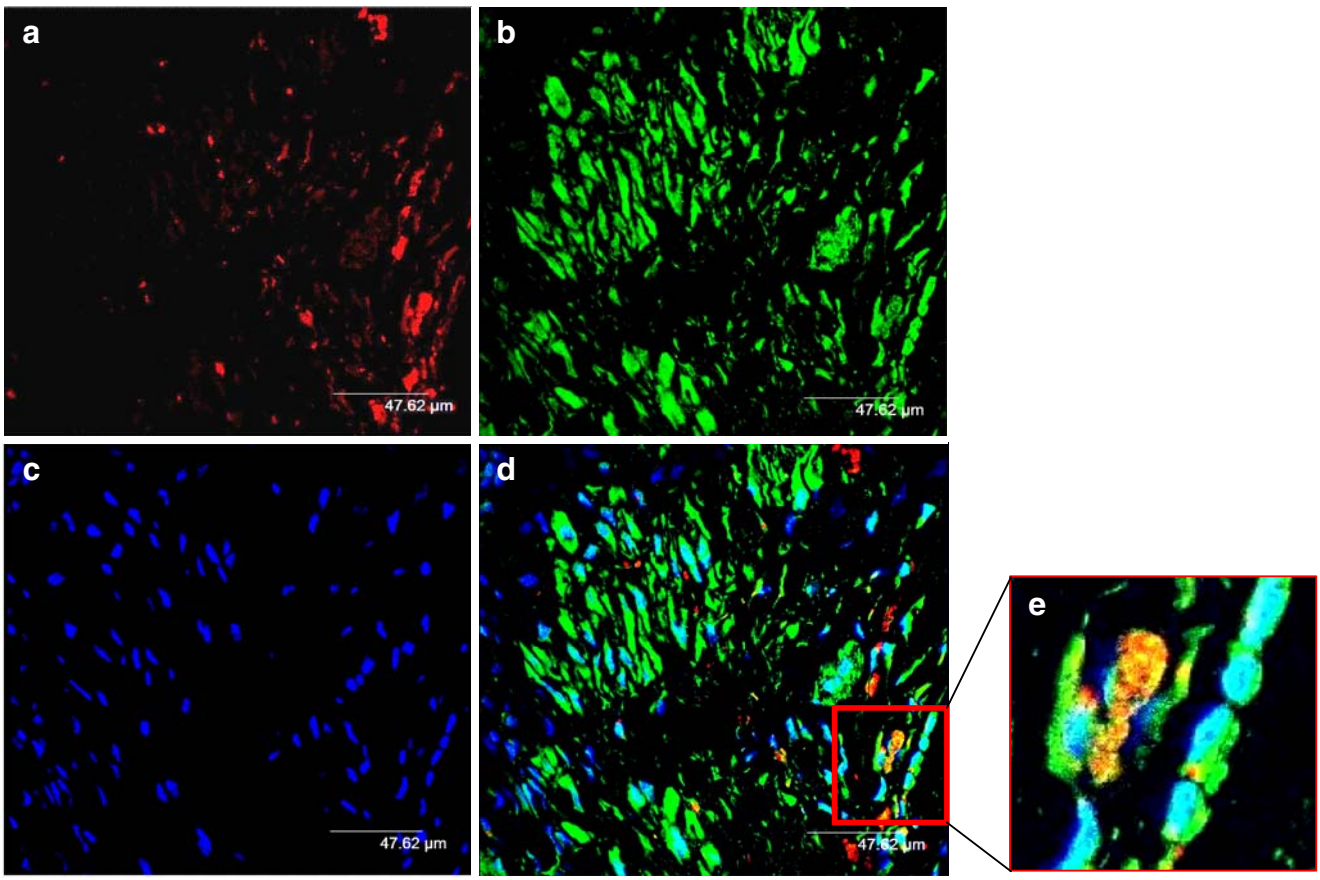

Fig. 4 Immunohistology for myosin heavy chain expression in the rat heart at 6 weeks postengraftment of IGF-1-overexpressing mesenchymal stem cells. The cells were labeled with cell tracker dye PKH-26 (*red fluorescence*; **a**). Confocal imaging revealed extensive neomyogenesis at the site of the cell graft (*green fluorescence*; **b**). DAPI was used for nuclear visualization (*blue fluorescence*; **c**). Merged image (**d**) showed that some of the transplanted cells differentiated to adopt myogenic phenotype as was evident from colocalization of red and green fluorescence. **e** The area in *red box* in merged image **d** has been magnified to show a myosin-heavy-chain-positive neofiber (*green fluorescence*) originating from the transplanted PKH26-labeled preconditioned cells

the infarcted myocardium and activate cell survival signaling and angiomyogenesis.

Combining Growth Factor Expression with Survival Signaling Molecules

The effectiveness of growth factor overexpression for preconditioning effect on stem cells can be enhanced by combining it with overexpression of one or more of the survival signaling molecules. The strategy of transgenic overexpression of prosurvival signaling molecules such as PI3K, Akt, and Bcl2 to prime the cells for better survival under lethal ischemia has shown promising results [66, 67, 70, 76, 77]. A comparison of Bcl-2 overexpression and heat shock treatment for antiapoptotic effects on smooth muscle cells showed significantly reduced cell loss after transplantation and improved cardiac function after myocardial infarction [84]. In one of our previous studies, we have combined transgenic overexpression of Akt and angiopoietin 1 in mesenchymal stem cells to show that concomitant overexpression of Akt and Ang-1 is more effective as compared with overexpression of either one of the factors [47]. Ang-1, a potent modulator of vascular development, promotes endothelial cell survival and interacts with its receptor to activate Akt survival signaling [9, 26, 54]. We observed that co-overexpression of the two genes was cytoprotective for the cells under lethal anoxia. Furthermore, the cells co-overexpressing Akt and Ang-1 were able to engraft better after transplantation in an infarcted rat heart and were able to adopt myogenic and endothelial phenotype.

Concluding Remarks

Stem and progenitor cell survival postengraftment in the heart is influenced by many factors including limited blood supply, deficiency of nutrients, hypoxia, oxidative stress, inflammatory response, and others. The environment is even harsher in the center of the infarcted myocardium where vascular structures are damaged and trophic support is lacking. The death of the transplanted cells further accentuates the harness of the microenvironment by initiating immune and inflammatory response. It is therefore imperative to optimize the cell transplantation conditions by programming and priming the cells before transplantation to withstand the rigors especially during the acute phase after engraftment to allow a time window long enough for the cells to acclimatize and engraft. Cell death is a complex and multifactorial phenomenon and hence requires a multifarious approach to achieve protection of the donor stem cells. A thorough understanding of the cell death mechanisms is therefore prerequisite to define prosurvival strategies. Thus, it would be imperative to prime the cells to a state of "readiness" by stimulation of their survival signaling pathways before their introduction into the ischemic environment. The approach of cellular preconditioning has powerful cytoprotective effects and the signaling pathways involved in cellular preconditioning can be successfully employed for antiapoptotic measures in cell-based therapies.

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References

1. Abbott, J. D., Huang, Y., et al. (2004). Stromal cell-derived factor-1alpha plays a critical role in stem cell recruitment to the heart after myocardial infarction but is not sufficient to induce homing in the absence of injury. *Circulation, 110*(21), 3300–3305.
2. Abdul Kadir, S. H., Ali, N. N., et al. (2009). Embryonic stem cell-derived cardiomyocytes as a model to study fetal arrhythmia related to maternal disease. *Journal of Cellular and Molecular Medicine* (in press)
3. Addya, S., Shiroto, K., et al. (2005). Ischemic preconditioning-mediated cardioprotection is disrupted in heterozygous Flt-1 (VEGFR-1) knockout mice. *Journal of Molecular and Cellular Cardiology, 38*(2), 345–351.
4. Afzal, M. R., Haider, K. H., et al. (2009). Preconditioning promotes survival and angiomyogenic potential of mesenchymal stem cells in the infarcted heart via NF-kappaB signaling. *Antioxidants & redox signalling* (in press)
5. Ahmad, N., Wang, Y., et al. (2006). Cardiac protection by mitoKATP channels is dependent on Akt translocation from cytosol to mitochondria during late preconditioning. *American Journal of Physiology. Heart and Circulatory Physiology, 290*(6), H2402–H2408.
6. Akao, M., Ohler, A., et al. (2001). Mitochondrial ATP-sensitive potassium channels inhibit apoptosis induced by oxidative stress in cardiac cells. *Circulation Research, 88*(12), 1267–1275.
7. Akita, T., Murohara, T., et al. (2003). Hypoxic preconditioning augments efficacy of human endothelial progenitor cells for therapeutic neovascularization. *Laboratory Investigation, 83*(1), 65–73.
8. Anversa, P., Leri, A., et al. (2006). Cardiac regeneration. *Journal of the American College of Cardiology, 47*(9), 1769–1776.
9. Asahara, T., Chen, D., et al. (1998). Tie2 receptor ligands, angiopoietin-1 and angiopoietin-2, modulate VEGF-induced postnatal neovascularization. *Circulation Research, 83*(3), 233–240.
10. Askari, A. T., Unzek, S., et al. (2003). Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. *Lancet, 362*(9385), 697–703.
11. Azarnoush, K., Maurel, A., et al. (2005). Enhancement of the functional benefits of skeletal myoblast transplantation by means of coadministration of hypoxia-inducible factor 1alpha. *Journal of Thoracic and Cardiovascular Surgery, 130*(1), 173–179.
12. Baines, C. P., Pass, J. M., et al. (2001). Protein kinases and kinase-modulated effectors in the late phase of ischemic preconditioning. *Basic Research in Cardiology, 96*(3), 207–218.

13. Baldi, A., Abbate, A., et al. (2002). Apoptosis and post-infarction left ventricular remodeling. *Journal of Molecular and Cellular Cardiology, 34*(2), 165–174.
14. Bartunek, J., Croissant, J. D., et al. (2007). Pretreatment of adult bone marrow mesenchymal stem cells with cardiomyogenic growth factors and repair of the chronically infarcted myocardium. *American Journal of Physiology. Heart and Circulatory Physiology, 292*(2), H1095–H1104.
15. Behfar, A., Perez-Terzic, C., Faustino, R. S., Arrell, D. K., Hodgson, D. M., Yamada, S., et al. (2007). Cardiopoietic programming of embryonic stem cells for tumor-free heart repair. *Journal of Experimental Medicine, 204*, 405–420.
16. Bellis, A., Castaldo, D., et al. (2009). Cross-talk between PKA and Akt protects endothelial cells from apoptosis in the late ischemic preconditioning. *Arteriosclerosis, Thrombosis, and Vascular Biology, 29*(8), 1207–1212.
17. Bolli, R. (2000). The late phase of preconditioning. *Circulation Research, 87*(11), 972–983.
18. Budas, G. R., Churchill, E. N., et al. (2007). Cardioprotective mechanisms of PKC isozyme-selective activators and inhibitors in the treatment of ischemia–reperfusion injury. *Pharmacological Research, 55*(6), 523–536.
19. Busija, D. W., Katakam, P., et al. (2005). Effects of ATP-sensitive potassium channel activators diazoxide and BMS-191095 on membrane potential and reactive oxygen species production in isolated piglet mitochondria. *Brain Research Bulletin, 66*(2), 85–90.
20. Camirand, G., Caron, N. J., et al. (2002). Treatment with anti-CD154 antibody and donor-specific transfusion prevents acute rejection of myoblast transplantation. *Transplantation, 73*(3), 453–461.
21. Chen, M., Xie, H. Q., et al. (2008). Stromal cell-derived factor-1 promotes bone marrow-derived cells differentiation to cardiomyocyte phenotypes in vitro. *Cell Proliferation, 41*(2), 336–347.
22. Crisostomo, P. R., Abarbanell, A. M., et al. (2008). Embryonic stem cells attenuate myocardial dysfunction and inflammation after surgical global ischemia via paracrine actions. *American Journal of Physiology. Heart and Circulatory Physiology, 295* (4), H1726–H1735.
23. Crosby, M. E., Kulshreshtha, R., et al. (2009). MicroRNA regulation of DNA repair gene expression in hypoxic stress. *Cancer Research, 69*(3), 1221–1229.
24. Das, R., Jahr, H., et al. (2009). The role of hypoxia in MSCs: Considerations for regenerative medicine approaches. *Tissue Engineering Part B Reviews* (in press)
25. Dawn, B., Abdel-Latif, A., et al. (2009). Cardiac repair with adult bone marrow-derived cells: The clinical evidence. *Antioxidants & Redox Signalling, 11*, 1865–1882.
26. DeBusk, L. M., Hallahan, D. E., et al. (2004). Akt is a major angiogenic mediator downstream of the Ang1/Tie2 signaling pathway. *Experimental Cell Research, 298*(1), 167–177.
27. Devarajan, E., & Huang, S. (2009). STAT3 as a central regulator of tumor metastases. *Current Molecular Medicine, 9*(5), 626–633.
28. Dzau, V. J., Gnecchi, M., et al. (2005). Enhancing stem cell therapy through genetic modification. *Journal of the American College of Cardiology, 46*(7), 1351–1353.
29. Elmadbouh, I., Haider, H., et al. (2007). Ex vivo delivered stromal cell-derived factor-1alpha promotes stem cell homing and induces angiomyogenesis in the infarcted myocardium. *Journal of Molecular and Cellular Cardiology, 42*(4), 792–803.
30. Farahmand, P., Lai, T. Y., et al. (2008). Skeletal myoblasts preserve remote matrix architecture and global function when implanted early or late after coronary ligation into infarcted or remote myocardium. *Circulation, 118*(14 Suppl), S130–S137.
31. Fasanaro, P., D'Alessandra, Y., et al. (2008). MicroRNA-210 modulates endothelial cell response to hypoxia and inhibits the receptor tyrosine kinase ligand Ephrin-A3. *Journal of Biological Chemistry, 283*(23), 15878–15883.
32. Gross, G. J., & Auchampach, J. A. (1992). Blockade of ATP-sensitive potassium channels prevents myocardial preconditioning in dogs. *Circulation Research, 70*(2), 223–233.
33. Haider, H., & Ashraf, M. (2008). Strategies to promote donor cell survival: Combining preconditioning approach with stem cell transplantation. *Journal of Molecular and Cellular Cardiology, 45*(4), 554–566.
34. Haider, H., Jiang, S. J., et al. (2004). Effectiveness of transient immunosuppression using cyclosporine for xenomyoblast transplantation for cardiac repair. *Transplantation Proceedings, 36*(1), 232–235.
35. Haider, H., Jiang, S., et al. (2008). IGF-1-overexpressing mesenchymal stem cells accelerate bone marrow stem cell mobilization via paracrine activation of SDF-1alpha/CXCR4 signaling to promote myocardial repair. *Circulation Research, 103*(11), 1300–1308.
36. Haider, H., Tan, A. C., et al. (2004). Myoblast transplantation for cardiac repair: A clinical perspective. *Molecular Therapy, 9*(1), 14–23.
37. Hausenloy, D. J., & Yellon, D. M. (2006). Survival kinases in ischemic preconditioning and postconditioning. *Cardiovascular Research, 70*(2), 240–253.
38. Heads, R. J., Yellon, D. M., et al. (1995). Differential cytoprotection against heat stress or hypoxia following expression of specific stress protein genes in myogenic cells. *Journal of Molecular and Cellular Cardiology, 27*(8), 1669–1678.
39. Hiasa, K., Ishibashi, M., et al. (2004). Gene transfer of stromal cell-derived factor-1alpha enhances ischemic vasculogenesis and angiogenesis via vascular endothelial growth factor/endothelial nitric oxide synthase-related pathway: Next-generation chemokine therapy for therapeutic neovascularization. *Circulation, 109* (20), 2454–2461.
40. Hodgetts, S. I., Beilharz, M. W., et al. (2000). Why do cultured transplanted myoblasts die in vivo? DNA quantification shows enhanced survival of donor male myoblasts in host mice depleted of CD4+ and CD8+ cells or Nk1.1+ cells. *Cell Transplantation, 9*(4), 489–502.
41. Hodgetts, S. I., & Grounds, M. D. (2001). Complement and myoblast transfer therapy: Donor myoblast survival is enhanced following depletion of host complement C3 using cobra venom factor, but not in the absence of C5. *Immunology and Cell Biology, 79*(3), 231–239.
42. Hu, X., Dai, S., et al. (2007). Stromal cell derived factor-1 alpha confers protection against myocardial ischemia/reperfusion injury: Role of the cardiac stromal cell derived factor-1 alpha CXCR4 axis. *Circulation, 116*(6), 654–663.
43. Hu, X., Yu, S. P., et al. (2008). Transplantation of hypoxia-preconditioned mesenchymal stem cells improves infarcted heart function via enhanced survival of implanted cells and angiogenesis. *Journal of Thoracic and Cardiovascular Surgery, 135*(4), 799–808.
44. Huangfu, D., Osafune, K., et al. (2008). Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. *Nature Biotechnology, 26*(11), 1269–1275.
45. Ii, M., Nishimura, H., et al. (2005). Endothelial progenitor cells are rapidly recruited to myocardium and mediate protective effect of ischemic preconditioning via "imported" nitric oxide synthase activity. *Circulation, 111*(9), 1114–1120.
46. Ivan, M., Harris, A. L., et al. (2008). Hypoxia response and microRNAs: No longer two separate worlds. *Journal of Cellular and Molecular Medicine, 12*(5A), 1426–1431.

47. Jiang, S., Haider, H., et al. (2006). Supportive interaction between cell survival signaling and angiocompetent factors enhances donor cell survival and promotes angiomyogenesis for cardiac repair. *Circulation Research, 99*(7), 776–784.
48. Kamota, T., Li, T. S., et al. (2009). Ischemic pre-conditioning enhances the mobilization and recruitment of bone marrow stem cells to protect against ischemia/reperfusion injury in the late phase. *Journal of the American College of Cardiology, 53*(19), 1814–1822.
49. Kanemitsu, N., Tambara, K., et al. (2006). Insulin-like growth factor-1 enhances the efficacy of myoblast transplantation with its multiple functions in the chronic myocardial infarction rat model. *Journal of Heart and Lung Transplantation, 25*(10), 1253–1262.
50. Kharbanda, R. K., Mortensen, U. M., et al. (2002). Transient limb ischemia induces remote ischemic preconditioning in vivo. *Circulation, 106*(23), 2881–2883.
51. Khoynezhad, A., Jalali, Z., et al. (2004). Apoptosis: Pathophysiology and therapeutic implications for the cardiac surgeon. *Annals of Thoracic Surgery, 78*(3), 1109–1118.
52. Kicinska, A., & Szewczyk, A. (2003). Protective effects of the potassium channel opener-diazoxide against injury in neonatal rat ventricular myocytes. *General Physiology and Biophysics, 22*(3), 383–395.
53. Kim, H. W., Haider, H. K., et al. (2009). Ischemic preconditioning augments survival of stem cells via MIR-210 expression by targeting caspase-8 associated protein 2. *Journal of Biological Chemistry, 284*, 33161–33168.
54. Kim, I., Kim, H. G., et al. (2000). Angiopoietin-1 induces endothelial cell sprouting through the activation of focal adhesion kinase and plasmin secretion. *Circulation Research, 86*(9), 952–959.
55. Kis, B., Nagy, K., et al. (2004). The mitochondrial K(ATP) channel opener BMS-191095 induces neuronal preconditioning. *NeuroReport, 15*(2), 345–349.
56. Kofidis, T., de Bruin, J. L., et al. (2004). Insulin-like growth factor promotes engraftment, differentiation, and functional improvement after transfer of embryonic stem cells for myocardial restoration. *Stem Cells, 22*(7), 1239–1245.
57. Koh, G. Y., Klug, M. G., et al. (1993). Differentiation and long-term survival of C2C12 myoblast grafts in heart. *Journal of Clinical Investigation, 92*(3), 1548–1554.
58. Koh, G. Y., Soonpaa, M. H., et al. (1993). Long-term survival of AT-1 cardiomyocyte grafts in syngeneic myocardium. *American Journal of Physiology, 264*(5 Pt 2), H1727–H1733.
59. Kolossov, E., Bostani, T., et al. (2006). Engraftment of engineered ES cell-derived cardiomyocytes but not BM cells restores contractile function to the infarcted myocardium. *Journal of Experimental Medicine, 203*(10), 2315–2327.
60. Konstantinov, I. E., Arab, S., et al. (2005). The remote ischemic preconditioning stimulus modifies gene expression in mouse myocardium. *Journal of Thoracic and Cardiovascular Surgery, 130*(5), 1326–1332.
61. Kubo, M., Li, T. S., et al. (2008). Hypoxic preconditioning increases survival and angiogenic potency of peripheral blood mononuclear cells via oxidative stress resistance. *American Journal of Physiology. Heart and Circulatory Physiology, 294*(2), H590–H595.
62. Kudo, M., Wang, Y., et al. (2002). Adenosine A(1) receptor mediates late preconditioning via activation of PKC-delta signaling pathway. *American Journal of Physiology. Heart and Circulatory Physiology, 283*(1), H296–H301.
63. Kudo, M., Wang, Y., et al. (2003). Implantation of bone marrow stem cells reduces the infarction and fibrosis in ischemic mouse heart. *Journal of Molecular and Cellular Cardiology, 35*(9), 1113–1119.
64. Kulshreshtha, R., Davuluri, R. V., et al. (2008). A microRNA component of the hypoxic response. *Cell Death and Differentiation, 15*(4), 667–671.
65. Kulshreshtha, R., Ferracin, M., et al. (2007). Regulation of microRNA expression: the hypoxic component. *Cell Cycle, 6*(12), 1426–1431.
66. Kutschka, I., Chen, I. Y., et al. (2006). Collagen matrices enhance survival of transplanted cardiomyoblasts and contribute to functional improvement of ischemic rat hearts. *Circulation, 114*(1 Suppl), I167–I173.
67. Kutschka, I., Kofidis, T., et al. (2006). Adenoviral human BCL-2 transgene expression attenuates early donor cell death after cardiomyoblast transplantation into ischemic rat hearts. *Circulation, 114*(1 Suppl), I174–I180.
68. Laflamme, M. A., Chen, K. Y., et al. (2007). Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. *Nature Biotechnology, 25*(9), 1015–1024.
69. Lataillade, J. J., Domenech, J., et al. (2004). Stromal cell-derived factor-1 (SDF-1)\CXCR4 couple plays multiple roles on haematopoietic progenitors at the border between the old cytokine and new chemokine worlds: Survival, cell cycling and trafficking. *European Cytokine Network, 15*(3), 177–188.
70. Li, W., Ma, N., et al. (2007). Bcl-2 engineered MSCs inhibited apoptosis and improved heart function. *Stem Cells, 25*(8), 2118–2127.
71. Liu, T. B., Fedak, P. W., et al. (2004). Enhanced IGF-1 expression improves smooth muscle cell engraftment after cell transplantation. *American Journal of Physiology. Heart and Circulatory Physiology, 287*(6), H2840–H2849.
72. Logue, S. E., Gustafsson, A. B., et al. (2005). Ischemia/reperfusion injury at the intersection with cell death. *Journal of Molecular and Cellular Cardiology, 38*(1), 21–33.
73. Lu, G., Haider, H. K., et al. (2009). Sca-1+ stem cell survival and engraftment in the infarcted heart: Dual role for preconditioning-induced connexin-43. *Circulation, 119*(19), 2587–2596.
74. Mahboubi, K., Biedermann, B. C., et al. (2000). IL-11 activates human endothelial cells to resist immune-mediated injury. *Journal of Immunology, 164*(7), 3837–3846.
75. Makkar, R. R., Price, M. J., et al. (2005). Intramyocardial injection of allogenic bone marrow-derived mesenchymal stem cells without immunosuppression preserves cardiac function in a porcine model of myocardial infarction. *Journal of Cardiovascular Pharmacology Therapy, 10*(4), 225–233.
76. Mangi, A. A., Noiseux, N., et al. (2003). Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts. *Nature Medicine, 9*(9), 1195–1201.
77. Matsui, T., Tao, J., et al. (2001). Akt activation preserves cardiac function and prevents injury after transient cardiac ischemia in vivo. *Circulation, 104*(3), 330–335.
78. Matsumoto, R., Omura, T., et al. (2005). Vascular endothelial growth factor-expressing mesenchymal stem cell transplantation for the treatment of acute myocardial infarction. *Arteriosclerosis, Thrombosis, and Vascular Biology, 25*(6), 1168–1173.
79. Muller-Ehmsen, J., Whittaker, P., et al. (2002). Survival and development of neonatal rat cardiomyocytes transplanted into adult myocardium. *Journal of Molecular and Cellular Cardiology, 34*(2), 107–116.
80. Mummery, C., van der Heyden, M. A., et al. (2007). Cardiomyocytes from human and mouse embryonic stem cells. *Methods in Molecular Medicine, 140*, 249–272.
81. Murry, C. E., Jennings, R. B., et al. (1986). Preconditioning with ischemia: A delay of lethal cell injury in ischemic myocardium. *Circulation, 74*(5), 1124–1136.
82. Nadal-Ginard, B., Anversa, P., et al. (2005). Cardiac stem cells and myocardial regeneration. *Novartis Foundation Symposium, 265*, 142–154.

83. Nakamura, T., & Schneider, M. D. (2003). The way to a human's heart is through the stomach: Visceral endoderm-like cells drive human embryonic stem cells to a cardiac fate. *Circulation, 107* (21), 2638–2639.
84. Nakamura, Y., Yasuda, T., et al. (2006). Enhanced cell transplantation: Preventing apoptosis increases cell survival and ventricular function. *American Journal of Physiology. Heart and Circulatory Physiology, 291*(2), H939–H947.
85. Nanduri, J., Yuan, G., et al. (2008). Transcriptional responses to intermittent hypoxia. *Respiratory Physiology and Neurobiology, 164*(1–2), 277–281.
86. Nelson, T. J., Martinez-Fernandez, A., Yamada, S., Perez-Terzic, C., Ikeda, Y., & Terzic, A. (2009). Repair of acute myocardial infarction by human stemness factors induced pluripotent stem cells. *Circulation, 120*(5), 408–416.
87. Niagara, M. I., Haider, H., et al. (2007). Pharmacologically preconditioned skeletal myoblasts are resistant to oxidative stress and promote angiomyogenesis via release of paracrine factors in the infarcted heart. *Circulation Research, 100*(4), 545–555.
88. Okita, K., Nakagawa, M., et al. (2008). Generation of mouse induced pluripotent stem cells without viral vectors. *Science, 322* (5903), 949–953.
89. O'Sullivan, J. C., Yao, X. L., et al. (2007). Diazoxide, as a postconditioning and delayed preconditioning trigger, increases HSP25 and HSP70 in the central nervous system following combined cerebral stroke and hemorrhagic shock. *Journal of Neurotrauma, 24*(3), 532–546.
90. Pagani, F. D., DerSimonian, H., et al. (2003). Autologous skeletal myoblasts transplanted to ischemia-damaged myocardium in humans. Histological analysis of cell survival and differentiation. *Journal of the American College of Cardiology, 41*(5), 879–888.
91. Pasha, Z., Wang, Y., et al. (2008). Preconditioning enhances cell survival and differentiation of stem cells during transplantation in infarcted myocardium. *Cardiovascular Research, 77*(1), 134–142.
92. Patel, H. H., Gross, E. R., et al. (2005). Sarcolemmal KATP channel triggers delayed ischemic preconditioning in rats. *American Journal of Physiology. Heart and Circulatory Physiology, 288*(1), H445–H447.
93. Pillarisetti, K., & Gupta, S. K. (2001). Cloning and relative expression analysis of rat stromal cell derived factor-1 (SDF-1)1: SDF-1 alpha mRNA is selectively induced in rat model of myocardial infarction. *Inflammation, 25*(5), 293–300.
94. Qu, Z., Balkir, L., et al. (1998). Development of approaches to improve cell survival in myoblast transfer therapy. *Journal of Cell Biology, 142*(5), 1257–1267.
95. Rajapakse, N., Kis, B., et al. (2003). Diazoxide pretreatment induces delayed preconditioning in astrocytes against oxygen glucose deprivation and hydrogen peroxide-induced toxicity. *Journal of Neuroscience Research, 73*(2), 206–214.
96. Ravingerova, T., Matejikova, J., et al. (2007). Differential role of PI3K/Akt pathway in the infarct size limitation and antiarrhythmic protection in the rat heart. *Molecular and Cellular Biochemistry, 297*(1–2), 111–120.
97. Rosova, I., Dao, M., et al. (2008). Hypoxic preconditioning results in increased motility and improved therapeutic potential of human mesenchymal stem cells. *Stem Cells, 26*(8), 2173–2182.
98. Rothwarf, D. M., & Karin, M. (1999). The NF-kappa B activation pathway: A paradigm in information transfer from membrane to nucleus. *Science's STKE: Signal Transduction Knowledge Environment, 1999*(5), RE1.
99. Saito, T., Kuang, J. Q., et al. (2002). Xenotransplant cardiac chimera: Immune tolerance of adult stem cells. *Annals of Thoracic Surgery, 74*(1), 19–24. discussion 24.
100. Sato, T., Li, Y., et al. (2004). Minoxidil opens mitochondrial K (ATP) channels and confers cardioprotection. *British Journal of Pharmacology, 141*(2), 360–366.
101. Shimizu, M., Tropak, M., et al. (2009). Transient limb ischaemia remotely preconditions through a humoral mechanism acting directly on the myocardium: Evidence suggesting cross-species protection. *Clinical Science (London), 117*(5), 191–200.
102. Shintani, S., Kusano, K., et al. (2006). Synergistic effect of combined intramyocardial CD34+ cells and VEGF2 gene therapy after MI. *Nature Clinical Practice. Cardiovascular Medicine, 3*(Suppl 1), S123–S128.
103. Shmelkov, S. V., Meeus, S., et al. (2005). Cytokine preconditioning promotes codifferentiation of human fetal liver CD133+ stem cells into angiomyogenic tissue. *Circulation, 111*(9), 1175–1183.
104. Shyu, W. C., Lin, S. Z., et al. (2008). Stromal cell-derived factor-1 alpha promotes neuroprotection, angiogenesis, and mobilization/homing of bone marrow-derived cells in stroke rats. *Journal of Pharmacology and Experimental Therapeutics, 324*(2), 834–849.
105. Singla, D. K. (2009). Embryonic stem cells in cardiac repair and regeneration. *Antioxidants & Redox Signalling, 11*, 1857–1863.
106. Sommer, C. A., Stadtfeld, M., et al. (2009). Induced pluripotent stem cell generation using a single lentiviral stem cell cassette. *Stem Cells, 27*(3), 543–549.
107. Stein, A. B., Bolli, R., et al. (2007). The late phase of ischemic preconditioning induces a prosurvival genetic program that results in marked attenuation of apoptosis. *Journal of Molecular and Cellular Cardiology, 42*(6), 1075–1085.
108. Strauer, B. E., Ott, G., et al. (2009). Bone marrow cells to improve ventricular function. *Heart, 95*(2), 98–99.
109. Takahashi, K., & Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell, 126*(4), 663–676.
110. Takashi, E., Wang, Y., et al. (1999). Activation of mitochondrial K(ATP) channel elicits late preconditioning against myocardial infarction via protein kinase C signaling pathway. *Circulation Research, 85*(12), 1146–1153.
111. Tang, Y. L., Zhu, W., et al. (2009). Hypoxic preconditioning enhances the benefit of cardiac progenitor cell therapy for treatment of myocardial infarction by inducing CXCR4 expression. *Circulation Research, 104*(10), 1209–1216.
112. Tateishi, K., Takehara, N., et al. (2008). Stemming heart failure with cardiac- or reprogrammed-stem cells. *Journal of Cellular and Molecular Medicine, 12*(6A), 2217–2232.
113. Theus, M. H., Wei, L., et al. (2008). In vitro hypoxic preconditioning of embryonic stem cells as a strategy of promoting cell survival and functional benefits after transplantation into the ischemic rat brain. *Experimental Neurology, 210*(2), 656–670.
114. Toma, C., Pittenger, M. F., et al. (2002). Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation, 105*(1), 93–98.
115. Wang, Y., & Ashraf, M. (1999). Role of protein kinase C in mitochondrial KATP channel-mediated protection against Ca2+ overload injury in rat myocardium. *Circulation Research, 84*(10), 1156–1165.
116. Wang, Y., Takashi, E., et al. (2001). Downregulation of protein kinase C inhibits activation of mitochondrial K(ATP) channels by diazoxide. *Circulation, 104*(1), 85–90.
117. Waxman, A. B., Mahboubi, K., et al. (2003). Interleukin-11 and interleukin-6 protect cultured human endothelial cells from H_2O_2-induced cell death. *American Journal of Respiratory Cell and Molecular Biology, 29*(4), 513–522.
118. Winkler, J., Hescheler, J., et al. (2005). Embryonic stem cells for basic research and potential clinical applications in cardiology. *Biochimica et Biophysica Acta, 1740*(2), 240–248.
119. Xu, M., Wang, Y., et al. (2001). Mitochondrial K(ATP) channel activation reduces anoxic injury by restoring mitochondrial mem-

brane potential. *American Journal of Physiology. Heart and Circulatory Physiology, 281*(3), H1295–H1303.
120. Yamanaka, S. (2007). Strategies and new developments in the generation of patient-specific pluripotent stem cells. *Cell Stem Cell, 1*(1), 39–49.
121. Yamaoka, M., Yamaguchi, S., et al. (2000). Apoptosis in rat cardiac myocytes induced by Fas ligand: Priming for Fas-mediated apoptosis with doxorubicin. *Journal of Molecular and Cellular Cardiology, 32*(6), 881–889.
122. Yao, K., Huang, R., et al. (2008). Administration of intracoronary bone marrow mononuclear cells on chronic myocardial infarction improves diastolic function. *Heart, 94*(9), 1147–1153.
123. Yau, T. M., Kim, C., et al. (2005). Increasing transplanted cell survival with cell-based angiogenic gene therapy. *Annals of Thoracic Surgery, 80*(5), 1779–1786.
124. Yu, H. M., Zhi, J. L., et al. (2006). Role of the JAK–STAT pathway in protection of hydrogen peroxide preconditioning against apoptosis induced by oxidative stress in PC12 cells. *Apoptosis, 11*(6), 931–941.
125. Yuan, G., Nanduri, J., et al. (2008). Induction of HIF-1alpha expression by intermittent hypoxia: involvement of NADPH oxidase, Ca2+ signaling, prolyl hydroxylases, and mTOR. *Journal of Cellular Physiology, 217*(3), 674–685.
126. Zemani, F., Silvestre, J. S., et al. (2008). Ex vivo priming of endothelial progenitor cells with SDF-1 before transplantation could increase their proangiogenic potential. *Arteriosclerosis, Thrombosis, and Vascular Biology, 28*(4), 644–650.
127. Zhao, Z. Q., & Vinten-Johansen, J. (2006). Postconditioning: Reduction of reperfusion-induced injury. *Cardiovascular Research, 70*(2), 200–211.