Forum Review Article

Proinflammatory Stem Cell Signaling in Cardiac Ischemia

Jeremy L. Herrmann,² Troy A. Markel,² Aaron M. Abarbanell,² Brent R. Weil,² Meijing Wang,²
Yue Wang,² Jiangning Tan,² and Daniel R. Meldrum^{1,2,3,4}

Abstract

Cardiovascular disease remains a leading cause of mortality in developed nations, despite continued advancement in modern therapy. Progenitor and stem cell-based therapy is a novel treatment for cardiovascular disease, and modest benefits in cardiac recovery have been achieved in small clinical trials. This therapeutic modality remains challenged by limitations of low donor-cell survival rates, transient recovery of cardiac function, and the technical difficulty of applying directed cell therapy. Understanding the signaling mechanisms involved in the stem cell response to ischemia has revealed opportunities to modify directly aspects of these pathways to improve their cardioprotective abilities. This review highlights general considerations of stem cell therapy for cardiac disease, reviews the major proinflammatory signaling pathways of mesenchymal stem cells, and reviews *ex vivo* modifications of stem cells based on these pathways. *Antioxid. Redox Signal.* 11, 1883–1896.

Introduction

CARDIOVASCULAR DISEASE (CVD) remains a leading cause of death in developed countries. In the United States, CVD resulted in 1 in 2.8 deaths in 2004. CVD causes more deaths annually in the United States than the next four causes combined, including cancer, accidents, Alzheimer disease, and HIV/AIDS (113). Although medical, percutaneous, and surgical interventions for CVD have improved over the past two decades, the limits of their therapeutic efficacy are evident in these statistics. Moreover, a significant population of patients with chronic ischemic heart disease may not be suitable candidates for revascularization procedures. The need for improved intervention for these patients as well as those with acute cardiac ischemia led to the development of cell-based therapy with progenitor cells.

Various progenitor cell types have been studied in animal models, and several small human trials have been conducted for both acute and chronic cardiac ischemia (22). These studies have shown at least modest benefit with stem cell transplantation for cardiac ischemia, but the magnitude and durability of this therapy have yet to be fully defined. Proposed mechanisms for progenitor cell-mediated cardioprotection include local production of growth factors, decreased inflammatory

cytokine production, promotion of cell survival, neovascularization, and decreased negative myocardial remodeling (30). Low survival rates of transplanted cells, as well as the desire to optimize this therapy, have sparked new interest in delineating proinflammatory signaling pathways involved in cardiac ischemia with regard to stem cells. In addition, many efforts to modify aspects of these pathways to enhance their protective effects are under way. This review discusses general considerations of stem cell therapy for cardiac ischemia, the major signaling pathways relevant to these stem cells, *in vitro* and *in vivo* modifications of these pathways, and implications for future study.

Stem Cells and Cardiovascular Disease

Stem cells are undifferentiated cells that have the potential for long-term self-renewal and the ability to differentiate into multiple cell types (pluripotency). Compared with embryonic stem cells, adult stem cells have more-limited ability to differentiate and for self-renewal. However, adult stem cells are less politically opposed, are less oncogenic, and may be less capable of inducing an immune response, as evidenced by their use in bone marrow transplantation (91). Consequently, adult stem cells have been more widely studied in the treatment of

¹Clarian Cardiovascular Surgery and the Departments of ²Surgery, ³Cellular and Integrative Physiology, and the ⁴Center for Immunobiology, Indiana University School of Medicine, Indianapolis, Indiana.

ischemic heart disease through a variety of delivery methods (3, 23).

To date, human clinical studies have only used adult autologous bone marrow–derived progenitor cells. These progenitor populations include hematopoietic and nonhematopoietic (or mesenchymal) stem cells. Hematopoietic stem cells (HSCs) are precursors to blood cell lineages and exhibit extensive self-renewal capacity. They typically express CD34 and CD133 cell-surface markers (27,158); however, other populations of primitive CD34⁺ HSC expression may exist (50, 102, 120). HSCs may differentiate into cardiomyocytes (34, 162) or fuse with donor cardiomyocytes *in vivo* (5, 101).

Mesenchymal stem cells possess the ability to differentiate into several nonhematopoietic cell types, particularly of adipocytic, chondrocytic, and osteocytic lineages (108). Compared with HSCs, they are easier to expand in culture after isolation. MSCs lack expression of CD31, CD34, and CD 45 but do express CD44, CD90, CD105, CD106, and CD166 (55). They are able to mobilize to damaged myocardium during ischemia, differentiate into cardiomyocytes (69, 114, 154), and fuse with cardiomyocytes to effect functional recovery (101). Studies of their cardioprotective effects have demonstrated a strong role for the paracrine release of growth factors and other cytokines (24). MSCs have unique immunologic properties, including the lack of MHC class II molecule expression and the ability to modulate local inflammatory responses, particularly lymphocyte activation, thereby making them useful for allogeneic cell transfers (63, 115). Their ease of culture, paracrine signaling, differentiation, and immunologic properties make them ideal candidates for cell-based therapy for myocardial ischemia.

More recently, the existence of resident native cardiac stem cells (CSCs) has been demonstrated through cell surface-marker identification. These CSCs may lack significant immunogenicity, and their transfer may yield greater functional benefit, based on their native morphology (8, 9). In addition, CSCs may also exhibit cellular senescence similar to that of cardiomyocytes, as evidenced by increased p16^{INK4a} and telomeric shortening with aging (140).

Signaling Pathways

A primary component of stem cell–mediated cardioprotection is the paracrine release of growth factors in response to cytokines generated during ischemia and tissue injury (22). The primary pathways involved in proinflammatory MSC signaling include the nuclear factor kappa-B (NF- κ B), mitogen-activated protein kinase (MAPK) family, Jak/STAT, and Akt/PI3K pathways (25, 48, 145, 148). Understanding the key components of these signaling pathways may allow targeted therapeutic interventions to promote antiinflammatory pathways while mitigating proinflammatory signals.

Nuclear factor kappa-B (NF- κ B)

The NF- κ B family of proteins consists of five transcription factors including NF- κ B1 (p50 and 105), NF- κ B2 (p52), c-Rel, RelA (p65), and RelB (47). They regulate activation of numerous genes involved in inflammatory, immunoregulatory, survival, carcinogenic, and metastatic processes (14, 46, 57, 104). After signal activation, the protein subunits homodimerize to form transcriptionally active (p50:p65) or repressive (p50:p50) units (Fig. 1) (14, 57). The I κ B proteins (I κ B α ,

I κ B β , and I κ B ϵ) bind and sequester the transcription-factor units in the cytoplasm in the inactivated state. Signal activation induces phosphorylation of I κ B α by I κ B kinase (IKK). I κ B β is then ubiquitinated by I κ B ubiquitin ligase and degraded within the proteasome (152). This exposes the nuclear-localization signals on the p50:p65 heterodimer. Once p65 is phosphorylated, the heterodimer translocates to the nucleus, binds specific DNA sequences, and induces transcription of various genes, including cyclin D1, cyclooxygenase-2, and matrix metalloproteinases (12, 134). The IKK complex consists of two catalytic subunits (I κ K α and I κ K β) with a noncatalytic regulatory subunit (NF- κ B essential modulator, or NEMO) (126). I κ K α and I κ K β share similar structures; however, the TNF-induced destruction of I κ B α is mediated by I κ K β and NEMO (14).

A key role of NF- κ B is facilitating cellular proliferation in response to cytokine signaling. Widera and colleagues (152) demonstrated this in adult neural stem cells stimulated by TNF. Specifically, NF- κ B targets expression of cyclin D1, which is necessary for progression from the G₁ to S phase in the cell cycle (53, 60). Phosphorylation of the retinoblastoma gene occurs after cyclin D1 binds its cyclin-dependent kinase, thereby releasing the transcription factor E2F, which facilitates expression of S-phase–related genes (60).

Mitogen-activated Protein Kinases

The mitogen-activated protein kinase (MAPK) family consists of a group of highly conserved protein kinases in eukaryotic organisms. Through their catalyzation of substrate phosphorylation, they regulate numerous cellular functions, such as gene expression, cell-cycle progression/apoptosis, and metabolism, in response to various stimuli (65). The basic MAPK pathway consists of three kinases sequentially activated through phosphorylation (Fig. 2). MAPK kinases (MAP2Ks) phosphorylate MAPK and are themselves phosphorylated by MAPK kinase kinases (MAP3Ks). Each step is counter-regulated by MAPK phosphatases, which dephosphorylate and inactivate the protein kinases. Numerous MAP3Ks exist and allow more specific responses to stimuli by the cell (65). Three subgroups of terminal MAPKs exist in multicellular organisms and include the p38 enzymes, c-Jun NH₂-terminal kinases (JNKs), and extracellular signal-regulated kinases (ERKs) (65).

p38 MAPK

The four p38 kinase isoforms are designated by α , β , γ , and δ . The p38 α kinase is expressed in most cell types and generally regulates inflammatory cytokine expression (65). In the heart, p38 MAPK mediates VEGF and other growth-factor expression by transplanted murine ESCs (121) and MSCs (146). Additionally, it mediates increased caspase activation and production of proinflammatory cytokines in the heart after ischemia (147). Its activity is inversely correlated with cardiac development: p38 MAPK overexpression blocks fetal cardiomyocyte proliferation, whereas inhibition allows proliferation of adult cardiomyocytes in rats (38). p38 MAPK appears to regulate numerous cell-cycle proteins including cdc25B, cdc2, cyclin B, and cyclin D (Fig. 2) (38, 124). Thus, it may be possible to enhance MSC function or proliferation or both during *ex vivo* expansion by regulating p38 MAPK expression.

FIG. 1. TNF-induced NF- κ B pathway. Signal activation induces phosphorylation of $I\kappa\beta$ by the IKK complex. $I\kappa\beta$ is then ubiquitinated and degraded in the proteasome. p50:p65 are released and translocate to the nucleus after phosphorylation. Expression of multiple genes is upregulated by p50:p65.

FIG. 2. Overview of mitogen-activated protein kinase pathways. After stimulation with ligand binding, a series of substrate phosphorylations occur with associated kinase activation. Signal amplification and cross-activation of the various kinases occur because of variable kinase specificities.

JNK

The JNKs are stress-activated protein kinases (SAPKs) encoded by three genes: *JNK 1/SAPK α* , *JNK2/SAPK β* , and *JNK3/SAPK γ* . JNK1 and JNK2 are widely distributed, whereas JNK3 is primarily limited to the brain (109). They are activated by inflammatory cytokines, growth factors, and cellular stresses and facilitate the inflammatory response through regulation of cytokine production, apoptosis, and metabolism (65, 77). These processes are mediated by transcription factors phosphorylated by JNK, including c-Jun, ATF-2, p53, and nuclear factor of activated T cells (NFAT) (Fig. 2) (109). The transcription complex AP-1 contains c-Jun and helps regulate gene expression and apoptosis (65, 141). JNK2 binds c-Jun in the unstimulated state to facilitate c-Jun degradation *via* ubiquitination (4, 109, 116). MEK4 (MKK4) and MEK7 (MKK7) are the primary MEKs for JNK and may also activate the p38 pathway (43).

ERK

ERK 1 and 2 are the primary ERK isoforms expressed by most differentiated cells and are activated by cytokines, growth factors, ligands for G protein-coupled receptors, and cellular stresses. They facilitate several cellular processes, including proliferation, differentiation, and cell motility (65, 109). The MAP3Ks specific to the ERK 1/2 pathway include the Raf isoforms: A-Raf, B-Raf, and C-Raf (Fig. 2). After signal activation, the Raf enzymes are activated through a combination of binding small G proteins of the Ras family and subsequent phosphorylation. MEK 1 and 2 are the MAPKs specific to ERK 1/2 (109).

After signal activation, ERKs 1/2 translocate to the nucleus where they affect gene expression through phosphorylation or stabilization of other nuclear proteins (109). MEKs 1/2, with a nuclear export sequence, regulate ERK nuclear localization by binding it in the dephosphorylated state and harboring it in the cytoplasm (2). Activated ERK 2 may enter the nucleus *via* an active transport process, whereas both active and inactive forms may enter by a passive process (92, 110). ERKs 1/2 are inactivated on dephosphorylation of either the phospho-threonine or phospho-tyrosine residues in the activation loop. Protein phosphatase 2A (PP2A) dephosphorylates the C-Raf inhibitory site, which allows the Raf protein to be activated by Ras (31). Much of ERK 1/2 binds cytoplasmic microtubules where they affect polymerization (112).

Jak/STAT

The Janus kinase (Jak) and signal transducer and activator of transcription (STAT) pathway facilitates signaling by the IL-6 family of cytokines. These include IL-6, cardiotrophin-1 (CT-1), leukemia inhibitory factor (LIF), and IL-27(136). IL-6 binds its membrane-bound receptor (IL-6R or gp80), whereas CT-1 and LIF bind the LIF receptor. After receptor ligand binding, the signaling subunit gp130 dimerizes to form a homodimer, which serves as the common signaling link for these cytokines (Fig. 3). The gp130 molecule transduces the signal through interaction with the tyrosine kinases known as the Janus kinases (Jak 1, Jak2, and Tyk2) (88, 129). Expression of IL-6R α may also be downregulated after ligand interaction *via* an endocytotic mechanism mediated by gp130 (137, 163).

The Jaks phosphorylate tyrosine residues on gp130, thereby allowing it to bind the signal transducers and activators of

transcription (STAT) proteins (81, 96, 129). Dimerization of gp130 also may crossactivate the ERK1/2 pathway (157). After phosphorylation, the STAT proteins translocate to the nucleus, where they influence gene expression by binding DNA elements of IL-6 target genes (58, 151). In particular, STAT3 is a direct transcriptional activator of VEGF and facilitates VEGF release by MSC in response to hypoxia and TNF (149).

Negative feedback of the Jak/STAT pathway occurs through multiple regulatory proteins. The Src homology domain 2-containing protein tyrosine phosphatase (SHP2) attenuates IL-6 signaling *via* a tyrosine kinase mechanism. SHP2 is recruited to the phosphotyrosine-759 residue of activated gp130 and is then tyrosine phosphorylated (72, 80, 123, 133). The SHP2 molecule bears two N-terminal Src homology 2 (SH2) domains and a phosphotyrosine phosphatase (PTP) domain at the C-terminus. When SHP2 is activated through phosphorylation of its tyrosine residues, the phosphorylated residues interact with the SH2 domains and disinhibit the PTP domain (87).

The suppressor of cytokine signaling (SOCS) family includes CIS and SOCS1–7 (59). They are rapidly expressed in response to cytokine signaling *via* Jak/STAT. These proteins contain an N-terminal SH2 domain, an extended SH2 (ESS) domain, a kinase inhibitory region, and a SOCS-box homology domain at the C-terminus (37, 97, 119, 130). Jak activity is inhibited by SOCS1 and, to a lesser extent, by SOCS3. The SOCS proteins inhibit Jak-mediated signaling *via* their SH2 domain, which binds tyrosine-phosphorylated residues in the Jak activation loop (98, 119). SOCS3 regulates IL-6 signaling through binding of its SH2 domain to the phosphotyrosine 759 residue in the SHP2 domain of gp130 (11, 98). Although SOCS3 and SHP2 bind to the same gp130 domain, they independently negatively regulate IL-6 signaling (80). SOCS3 has also been shown to be a negative regulator of LIF signaling *via* gp130 in neural precursor cells (36). Interestingly, TNF may inhibit IL-6 signaling in macrophages by upregulating SOCS *via* p38 MAPK (13). SOCS proteins contain a SOCS-box motif that interacts with elongins B and C. Binding of the elongins targets the SOCS proteins and their substrates for destruction by E3 ubiquitin ligases and proteasomes (67, 160).

The STAT proteins are negatively regulated by the protein inhibitor of activated STAT (PIAS) family of proteins. PIAS1 and PIAS3 specifically inhibit STAT1 and STAT3, respectively. Even though the PIAS proteins lack phosphotyrosine-binding domains, their interaction with STAT requires tyrosine-phosphorylation of STAT. The phosphorylation of STAT may induce a conformational change necessary for binding by PIAS (21, 83). STAT proteins may also be negatively regulated by the ubiquitin-proteasome pathway (73, 84) and in the nucleus by a STAT-inactivating phosphatase (56, 155).

Akt/PI3K

The phosphatidylinositol 3-OH kinase (PI3K)-dependent pathways participate in survival signaling in many cell types (33, 75). Akt, or protein kinase B, is a serine/threonine protein kinase that is central to the PI3K pathway. Akt has been implicated in multiple regulatory processes, including cell growth, proliferation, metabolism, apoptosis, contractility, and coronary angiogenesis, and the mammalian genome contains three Akt/PKB genes: *Akt1/PKB α* , *Akt2/PKB β* , and *Akt3/PKB γ* (27). Much of the initial understanding of its

FIG. 3. The Jak/STAT signaling pathway. Signal activation occurs through binding of IL-6, CT-1, or LIF. Increased gene expression occurs after STAT is phosphorylated, dimerizes, and translocates to the nucleus. Inhibitory steps include the SOCS, SHP2, PIAS, and elongin proteins.

mechanism dealt with its role in IGF-1 signaling (78, 125). Given its direct role in survival signaling, Akt has received particular interest in the *ex vivo* modification of MSCs to improve their survival on transplantation into hostile ischemic environments (see later).

PI3K is activated in response to signal binding by growth factors and generates second messengers, including phosphatidylinositol (3,4)-bisphosphate (PIP₂) and phosphatidylinositol (3,4,5)-trisphosphate (PIP₃) (Fig. 4) (68). The PI3K family is antagonized by the phosphatase PTEN, which converts PIP₃ to PIP₂ (17). The PI3K second messengers recruit molecules bearing a PH domain, including Akt and phosphoinositide-dependent protein kinase-1 (PDK-1) to the membrane (131). Akt is then activated by two sequential phosphorylation events. First, PDK1 phosphorylates a Thr308 residue within the catalytic domain. Next, a Ser473 residue in the hydrophobic motif is phosphorylated by mTORC2, the mammalian target of rapamycin (mTOR)–Rictor complex (51, 118). Ultimately, Akt inhibits apoptosis by phosphorylating and inhibiting BAD, FOXO transcription factors, and IκKβ (76). It also regulates cell-cycle progression, cell size, and metabolism (particularly glucose metabolism) through phosphorylation of its downstream substrates (15, 76).

The TOR proteins are serine/threonine protein kinases of the PIKK family. They play a central role in cell-growth reg-

ulation and primarily facilitate signaling by amino acids and growth factors (61). The mTORC1 complex also contains the regulatory components GβL/LST8, and raptor (51, 71, 100). The mTOR1 complex is inhibited by the rapamycin/FKBP12 (FK506-binding protein) complex, which binds to the FKBP12-rapamycin-binding (FRB) domain within the kinase domain of mTOR (39). The mTORC2 complex is composed of the mTOR, GβL/LST8, Sin1, and rictor proteins (51, 118). The mTORC2 complex is rapamycin insensitive, although its assembly may be inhibited with prolonged (>24 h) exposure to rapamycin (117). Its mSin1 isoform phosphorylates the Ser473 residue in the hydrophobic motif of Akt/PKB (42).

The tuberous sclerosis complex (TSC1/TSC2 complex) functions as an upstream negative regulator of mTORC1 (45). TSC1 is required to stabilize TSC2 and prevent its ubiquitination (10). TSC2 is a GTPase-activating protein GAP that hydrolyzes guanosine triphosphate (GTP) to guanosine diphosphate (GDP) on Rheb (Ras homolog enriched in brain). It is through the conversion to Rheb-GDP that mTORC1 signaling is downregulated (64). Rheb appears to be required for amino acid signaling by mTORC1. In conditions of low amino acids or growth factors, FKBP38 binds mTOR and downregulates mTORC1 activity. When amino acids or growth factors are present, Rheb-GTP releases mTOR and upregulates mTOR activity. FKBP38 associates with mTOR through

FIG. 4. The Akt signaling pathway. Growth factor signaling results in activation of PI3K and conversion of PIP2 to PIP3. Proteins containing a PH domain are recruited to the membrane and participate in phosphorylation reactions. PTEN, PP2A, and Rheb-GDP negatively regulate the pathway. PIP2, phosphatidylinositol (3,4)-bisphosphate; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; PH, pleckstrin homology domain; HM, C-terminal hydrophobic motif; 4E-BP, inhibitory eIF4E-binding proteins; S6K, ribosomal S6 kinases; PP2A, protein phosphatase 2A.

the FRB domain (89). Akt-mediated phosphorylation of TSC2 inhibits the TSC complex activity (61). TSC1 is suppressed through phosphorylation of the Ser 487 and Ser 511 residues by I κ B β after TNF signaling (79).

mTORC1 has two classes of substrates: the inhibitory eIF4E-binding proteins (eukaryotic translation initiation factor 4E-binding proteins, or 4E-BP) 1 and 2 and the ribosomal S6 kinases (S6K) 1 and 2. The 4E-BPs in the unphosphorylated state inhibit translation. mTOR phosphorylates 4E-BP, which releases eIF4E, a cytoplasmic cap-binding protein that mediates binding of the protein complex eIF4G to the 5' mRNA cap (111). Phosphorylation of 4E-BP is facilitated by the Ser/Arg-rich protein, SF2/ASF (95).

S6K is a ribosomal kinase and belongs to the same kinase family as Akt. It is activated through phosphorylation of its Thr389 residue by mTOR (106). After activation, S6K phosphorylates its downstream targets including the 4E-BPs. Once phosphorylated, the 4E-BPs release eIF4E, and protein synthesis ensues (40). Akt is negatively regulated through dephosphorylation by protein phosphatases: Protein phosphatase 2A (PP2A) dephosphorylates Thr308, whereas Ser473 is dephosphorylated by the PH domain leucine-rich repeat protein phosphatase (PHLPP) (6, 16).

Ex Vivo Mesenchymal Stem Cell Modification

The interest in optimizing stem cells for therapeutic applications for cardiac ischemia stems from results of early clinical trials involving administration of bone marrow-derived mononuclear progenitor cells. Several trials with generally small numbers of patients demonstrated the relative safety of intracoronary stem cell injection for acute myocardial infarction (1). They also showed modest but significant benefits with regard to improved left ventricular ejection fraction (LVEF), decreased infarct size, and reduced frequency of recurrent ischemic events or need for intervention. Trials of stem cell therapy for chronic postinfarction heart failure also

demonstrated improvements in natriuretic peptide levels, exertional capacity, and mortality at longer than 1 year of follow-up (7, 107). However, the benefit, at least in the acute setting, may be limited, as the LVEF recovery did not persist at 18 months of follow-up in the BOOST study (94). The limited benefits of progenitor cell transplantation may in part be due to low survival rates of donor cells, which may be as low as 1% after transplantation (139). Methods of improving specifically MSC survival and efficacy after transplantation have been explored through *in vitro* and *in vivo* ischemia models, and many approaches directly involve modifying aspects of stem cell signaling. These methods include exposing MSCs to hypoxic or growth factor-supplemented conditions or genetically modifying them to overexpress specific proteins before transplantation.

Preconditioning

Preconditioning of MSCs *in vitro* stimulates a nonspecific activation, which, through upregulation of certain signaling pathways, may result in increased tolerance of the transplanted cells to the ischemic environment. The advantage of this technique is the relative ease of application in the laboratory setting. Some of the earliest work with stem cell modification involved preconditioning MSCs with hypoxia, with resultant increased cellular resistance to, and decreased apoptosis with, subsequent ischemic events, with associated increased activation of the Akt pathway, increased VEGF production, and increased Bcl-2 expression (142). In addition, MSCs exposed to anoxia have shown increased Akt activity and cytoprotection of cocultured cardiomyocytes *in vitro* via paracrine signaling (143).

Exposing MSCs to exogenous growth factors while in culture may also enhance their survival and function after transplantation. Stromal cell-derived factor 1 (SDF-1) is a CXC chemokine whose receptor is CXCR4. SDF-1 is transiently overexpressed by ischemic myocardium and may

augment increased MSC homing to ischemic tissue (19). Rat MSCs preconditioned with SDF-1 showed improved survival and engraftment along with greater recovery of cardiac function after ischemia (105). MSCs preconditioned with fibroblast growth factor-2 (FGF-2), IGF-1, and bone morphogenetic protein-2 demonstrated greater protection of cocultured cardiomyocytes through increased gap-junction formation *in vitro* and decreased infarct size, and improved cardiac function after cardiac ischemia in rats *in vivo* (54).

Growth-factor overexpression

As growth factors and other cytokines secreted by MSCs in response to ischemia have been elucidated, the targeted overexpression of these molecules by MSCs has further enhanced their cardioprotective abilities. Growth-factor overexpression may be accomplished by transfecting the cells with plasmids or other viral vectors bearing the gene of interest. These techniques offer the advantage of achieving a more-specific modification; however, transfection efficiencies and duration of effect are typically variable.

VEGF is a protective growth factor that promotes neovascularization (18). Rat and murine MSCs overexpressing VEGF have shown improved transplanted cell survival, increased capillary density, decreased infarct size, and increased functional cardiac recovery after transplantation of these cells after ischemia (93, 150). These effects appear to be greater with delivery of MSCs overexpressing VEGF than with delivery of unmodified MSCs or VEGF-containing vectors alone (44). Increased vascular density of the ischemic border size appeared to persist at 6 months after transplantation (70). FGF-2 mediates multiple cellular processes, including proliferation, differentiation, survival/apoptosis, and motility, and in myocardium, its main receptor, FGFR-1, mediates these functions through a tyrosine kinase-initiated pathway (29). FGF-2 overexpression by rat MSCs resulted in increased *Bcl2* expression, improved survival under hypoxic stress, increased retention in the myocardium, and improved neovascularization (127).

Angiopoietin-1 (Ang-1) is a growth factor that facilitates vascular development by promoting remodeling of leakage-resistant vascular networks (138). Ang-1 protects MSCs against hypoxia-induced apoptosis, and this process may be mediated by PI3K/Akt activation (85). Ang-1 has been shown to enhance cardiomyocyte survival in rat hearts *in vitro* (26). Transplantation of MSCs overexpressing Ang-1 after acute MI in rats resulted in enhanced angiogenesis and arteriogenesis as well as improved cardiac remodeling compared with MSCs expressing green fluorescent protein; however, improvement in cardiac function was similar between MSC groups (132).

Hepatocyte growth factor (HGF) is a mesenchymal cytokine that promotes cellular proliferation, motility, survival, angiogenesis, and differentiation in multiple cell types (41). MSCs express a tyrosine kinase HGF receptor, and intracellular signaling occurs *via* the ERK1/2, p38 MAPK, and Akt pathways (12, 41). *In vitro* exposure to HGF may promote differentiation of murine MSCs into cardiomyocytes, and long-term HGF exposure may induce migration and actually inhibit proliferation (41). Transplanted MSCs overexpressing HGF improved infarct size, increased capillary density, improved cardiac function, and developed a morphology similar to that of cardiomyocytes in rats (32, 52).

Heme oxygenase-1 (HO-1; heat-shock protein 32) is a stress-inducible enzyme that facilitates heme degradation to carbon monoxide, iron, and biliverdin (103). In addition, HO-1 protects endothelial cells from apoptosis, mediates vasorelaxation, attenuates vascular inflammation, and facilitates angiogenesis and vasculogenesis (86). HO-1 upregulation induced by lipopolysaccharide appears to result in increased NF- κ B and p38 MAPK activity (153). Transgenic mice overexpressing HO-1 in the heart demonstrated decreased cardiomyocyte apoptosis and improved ventricular functional recovery after ischemia (144), and murine MSCs transfected to overexpress HO-1 showed improved resistance to I/R injury as well as functional recovery in ischemic murine hearts (135).

In addition to preconditioning of MSCs, SDF-1 has been targeted for overexpression in MSCs. These MSCs improved cardiomyocyte survival and vascular densities after transplantation after myocardial infarction (161). They also exhibited improved MSC homing and cardiac functional recovery, but demonstrated a possible increased risk for ventricular arrhythmias (28). Transplanted skeletal myoblasts overexpressing SDF-1 showed increased mobilization and homing of MSCs and CSCs to areas of ischemia (35). Likewise, MSCs and CD34⁺ progenitors overexpressing CXCR4 showed improved localization to ischemic territories, survival, and recovery of cardiac function (20, 66, 159).

Overexpression of multiple growth factors may offer additive benefits to MSC survival and function. Rat MSCs cotransfected with VEGF and IGF-1 improved donor cell survival and functional recovery compared with transfection of either growth factor alone (156). Furthermore, MSCs overexpressing the combination of VEGF, FGF, and IGF-1 demonstrated the greatest improvements in cardiac functional recovery and cell survival after transmyocardial revascularization of infarcted rat hearts than with overexpression of any single growth factor alone (128).

Survival Signaling Upregulation

Donor cell survival may be improved by upregulating key elements of survival pathways before transplantation, including Bcl-2 and Akt. Bcl-2 is an antiapoptotic mitochondrial membrane protein that regulates mitochondrial metabolism, particularly calcium metabolism (62, 122) and has been shown to suppress apoptosis of ventricular myocytes *via* NF- κ B signaling (74). MSCs overexpressing Bcl-2 through nonviral transfection decreased cardiomyocyte apoptosis and increased VEGF production *in vitro* while reducing infarct size and improving functional recovery *in vivo* (82).

Intramyocardial injection of MSCs overexpressing Akt into the perinfarct border zone in rat hearts after coronary artery ligation resulted in decreased cardiac remodeling and improved cardiac recovery compared with MSCs not overexpressing Akt (90). The MSCs also appeared to differentiate into cardiomyocytes 2 weeks after infusion. MSCs overexpressing Akt plus reporter genes showed improved MSC engraftment and cardiac recovery after coronary artery ligation in mice; however, although cellular fusion between MSCs and cardiomyocytes was observed as early as 3 days after infusion, it occurred with low frequency and was unaffected by Akt overexpression (99). Conditioned media from Akt-overexpressing MSCs exposed to hypoxia reduced hypoxia-related cardiomyocyte apoptosis compared with conditioned

media from hypoxic MSCs without Akt overexpression *in vitro* (49). When infused into isolated rat hearts, the conditioned media from Akt-overexpressing MSCs reduced infarct size and improved functional recovery. The cardioprotective benefits observed after infusion of conditioned media alone support the role of paracrine signaling by MSCs in the setting of cardiac ischemia.

Conclusions

Progenitor cells hold great potential for treating both acute and chronic ischemic heart disease. Understanding the signaling mechanisms involved in the reaction of these cells to the ischemic state will enable us to bolster their efficacy by modifying their ability to home to ischemic tissue, secrete protective paracrine factors, and incorporate into the myocardium. These studies in animal models have revealed the variety of modifications possible, particularly with regard to upregulating growth-factor production or signaling mechanisms involved in survival and proliferation. The greatest benefit, though, may be in combining modifications to create an ultraprotective stem cell. Further work to elucidate other components of these signaling pathways that could serve as targets of directed modifications (e.g., cell-surface receptors) is ongoing. A challenge to this therapeutic modality includes understanding the effects one modification may have on interconnected pathways to predict results of these therapies. Moreover, developing efficient strategies of translating these techniques into effective clinical practice will be essential for establishing their clinical utility. Despite these challenges, progenitor cell-based therapy for myocardial ischemia remains a promising possibility.

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Abbreviations

4E-BP, eukaryotic translation initiation factor 4E-binding proteins; Ang-1, angiopoietin-1; CVD, cardiovascular disease; CSC, cardiac stem cell; CT-1, cardiotrophin-1; eIF4E, eukaryotic translation-initiation factor 4E; ERK, extracellular signal-regulated kinases; FGF, fibroblast growth factor; FGFR-1, FGF receptor-1; FKBP, FK506-binding protein; FRB, FKBP12-rapamycin binding; GDP, guanine diphosphate; GTP, guanine triphosphate; HGF, hepatocyte growth factor; HO-1, heme oxygenase-1; HSCs, hematopoietic stem cells; IGF, insulin-like growth factor; IKK, I κ B kinase; Jak, Janus kinase; iNOS, inducible nitric oxide synthase; I/R, ischemia/reperfusion; JNK, c-Jun NH₂-terminal kinases; LIF, leukemia inhibitory factor; LIFR, leukemia inhibitory factor receptor; LVEF, left ventricular ejection fraction; MSCs, mesenchymal stem cells; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; MEK, MAP-ERK kinase; MUK, MAPK-upstream protein kinase; NEMO, NF- κ B essential modulator; NFAT, nuclear factor of activated T cells; PDK-1, phosphoinositide-dependent protein kinase-1; PH, pleckstrin homology; PHLPP, PH domain leucine-rich re-

peat protein phosphatase; PIAS, protein inhibitor of activated STAT; PI3K, phosphatidylinositol 3-kinase; PIKK, phosphatidylinositol 3-kinase-related kinase; PIP2, phosphatidylinositol (3,4)-bisphosphate; PIP3, phosphatidylinositol (3,4,5)-trisphosphate (PIP3); PKB, protein kinase B; PP2A, protein phosphatase 2A; PTP, phosphotyrosine phosphatase; SAPK, stress-activated protein kinases; Rheb, Ras homologue enriched in brain; S6K, ribosomal S6 kinases; SDF-1, stromal cell-derived factor-1; SHP2, Src homology domain 2 protein tyrosine phosphatase; SOCS, suppression of cytokine signaling; src, sarcoma; STAT, signal transducer and activator of transcription; TNF, tumor necrosis factor; TRADD, TNF receptor 1-associated death domain; TRAF2, TNF receptor-associated factor 2; TSC, tuberous sclerosis complex; VEGF, vascular endothelial growth factor.

References

1. Abdel-Latif A, Bolli R, Tleyjeh IM, Montori VM, Perin EC, Hornung CA, Zuba-Surma EK, Al-Mallah M, and Dawn B. Adult bone marrow-derived cells for cardiac repair: a systematic review and meta-analysis. *Arch Intern Med* 167: 989–997, 2007.
2. Adachi M, Fukuda M, and Nishida E. Nuclear export of MAP kinase (ERK) involves a MAP kinase kinase (MEK)-dependent active transport mechanism. *J Cell Biol* 148: 849–856, 2000.
3. Al-Radi OO, Rao V, Li RK, Yau T, and Weisel RD. Cardiac cell transplantation: closer to bedside. *Ann Thorac Surg* 75: S674–S677, 2003.
4. Alarcon-Vargas D, and Ronai Z. c-Jun-NH₂ kinase (JNK) contributes to the regulation of c-Myc protein stability. *J Biol Chem* 279: 5008–5016, 2004.
5. Alvarez-Dolado M, Pardal R, Garcia-Verdugo JM, Fike JR, Lee HO, Pfeffer K, Lois C, Morrison SJ, and Alvarez-Buylla A. Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. *Nature* 425: 968–973, 2003.
6. Andjelkovic M, Jakubowicz T, Cron P, Ming XF, Han JW, and Hemmings BA. Activation and phosphorylation of a pleckstrin homology domain containing protein kinase (RAC-PK/PKB) promoted by serum and protein phosphatase inhibitors. *Proc Natl Acad Sci U S A* 93: 5699–5704, 1996.
7. Assmus B, Fischer-Rasokat U, Honold J, Seeger FH, Fichtlscherer S, Tonn T, Seifried E, Schachinger V, Dimmeler S, and Zeiher AM. Transcatheter transplantation of functionally competent BMCs is associated with a decrease in natriuretic peptide serum levels and improved survival of patients with chronic postinfarction heart failure: results of the TOPCARE-CHD Registry. *Circ Res* 100: 1234–1241, 2007.
8. Barile L, Messina E, Giacomello A, and Marban E. Endogenous cardiac stem cells. *Prog Cardiovasc Dis* 50: 31–48, 2007.
9. Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, Kasahara H, Rota M, Musso E, Urbanek K, Leri A, Kajstura J, Nadal-Ginard B, and Anversa P. Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell* 114: 763–776, 2003.
10. Benvenuto G, Li S, Brown SJ, Braverman R, Vass WC, Cheadle JP, Halley DJ, Sampson JR, Wienecke R, and DeClue JE. The tuberous sclerosis-1 (TSC1) gene product hamartin suppresses cell growth and augments the expression of the TSC2 product tuberin by inhibiting its ubiquitination. *Oncogene* 19: 6306–6316, 2000.

11. Bergamin E, Wu J, and Hubbard SR. Structural basis for phosphotyrosine recognition by suppressor of cytokine signaling-3. *Structure* 14: 1285–1292, 2006.
12. Bocker W, Docheva D, Prall WC, Egea V, Pappou E, Rossmann O, Popov C, Mutschler W, Ries C, and Schieker M. IKK-2 is required for TNF-alpha-induced invasion and proliferation of human mesenchymal stem cells. *J Mol Med* 86: 1183–1192, 2008.
13. Bode JG, Nimmesgern A, Schmitz J, Schaper F, Schmitt M, Frisch W, Haussinger D, Heinrich PC, and Graeve L. LPS and TNFalpha induce SOCS3 mRNA and inhibit IL-6-induced activation of STAT3 in macrophages. *FEBS Lett* 463: 365–370, 1999.
14. Bonizzi G and Karin M. The two NF-kappaB activation pathways and their role in innate and adaptive immunity. *Trends Immunol* 25: 280–288, 2004.
15. Brazil DP, Yang ZZ, and Hemmings BA. Advances in protein kinase B signalling: AKTion on multiple fronts. *Trends Biochem Sci* 29: 233–242, 2004.
16. Brognard J, Sieracki E, Gao T, and Newton AC. PHLPP and a second isoform, PHLPP2, differentially attenuate the amplitude of Akt signaling by regulating distinct Akt isoforms. *Mol Cell* 25: 917–931, 2007.
17. Cai Z and Semenza GL. PTEN activity is modulated during ischemia and reperfusion: involvement in the induction and decay of preconditioning. *Circ Res* 97: 1351–1359, 2005.
18. Carmeliet P and Collen D. Role of vascular endothelial growth factor and vascular endothelial growth factor receptors in vascular development. *Curr Top Microbiol Immunol* 237: 133–158, 1999.
19. Ceradini DJ, Kulkarni AR, Callaghan MJ, Tepper OM, Bastidas N, Kleinman ME, Capla JM, Galiano RD, Levine JP, and Gurtner GC. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. *Nat Med* 10: 858–864, 2004.
20. Cheng Z, Ou L, Zhou X, Li F, Jia X, Zhang Y, Liu X, Li Y, Ward CA, Melo LG, and Kong D. Targeted migration of mesenchymal stem cells modified with CXCR4 gene to infarcted myocardium improves cardiac performance. *Mol Ther* 16: 571–579, 2008.
21. Chung CD, Liao J, Liu B, Rao X, Jay P, Berta P, and Shuai K. Specific inhibition of Stat3 signal transduction by PIAS3. *Science* 278: 1803–1805, 1997.
22. Crisostomo PR, Markel TA, Wang Y, and Meldrum DR. Surgically relevant aspects of stem cell paracrine effects. *Surgery* 143: 577–581, 2008.
23. Crisostomo PR and Meldrum DR. Stem cell delivery to the heart: clarifying methodology and mechanism. *Crit Care Med* 35: 2654–2656, 2007.
24. Crisostomo PR, Wang M, Markel TA, Lahm T, Abarbanell AM, Herrmann JL, and Meldrum DR. Stem cell mechanisms and paracrine effects: potential in cardiac surgery. *Shock* 28: 375–383, 2007.
25. Crisostomo PR, Wang Y, Markel TA, Wang M, Lahm T, and Meldrum DR. Human mesenchymal stem cells stimulated by TNF-alpha, LPS, or hypoxia produce growth factors by an NF kappa B- but not JNK-dependent mechanism. *Am J Physiol Cell Physiol* 294: C675–C682, 2008.
26. Dallabrida SM, Ismail N, Oberle JR, Himes BE, and Rupnick MA. Angiopoietin-1 promotes cardiac and skeletal myocyte survival through integrins. *Circ Res* 96: e8–e24, 2005.
27. Datta SR, Brunet A, and Greenberg ME. Cellular survival: a play in three Akts. *Genes Dev* 13: 2905–2927, 1999.
28. Deglurkar I, Mal N, Mills WR, Popovic ZB, McCarthy P, Blackstone EH, Laurita KR, and Penn MS. Mechanical and electrical effects of cell-based gene therapy for ischemic cardiomyopathy are independent. *Hum Gene Ther* 17: 1144–1151, 2006.
29. Detillieux KA, Sheikh F, Kardami E, and Cattini PA. Biological activities of fibroblast growth factor-2 in the adult myocardium. *Cardiovasc Res* 57: 8–19, 2003.
30. Dimmeler S, Burchfield J, and Zeiher AM. Cell-based therapy of myocardial infarction. *Arterioscler Thromb Vasc Biol* 28: 208–216, 2008.
31. Dougherty MK, Muller J, Ritt DA, Zhou M, Zhou XZ, Copeland TD, Conrads TP, Veenstra TD, Lu KP, and Morrison DK. Regulation of Raf-1 by direct feedback phosphorylation. *Mol Cell* 17: 215–224, 2005.
32. Duan HF, Wu CT, Wu DL, Lu Y, Liu HJ, Ha XQ, Zhang QW, Wang H, Jia XX, and Wang LS. Treatment of myocardial ischemia with bone marrow-derived mesenchymal stem cells overexpressing hepatocyte growth factor. *Mol Ther* 8: 467–474, 2003.
33. Dudek H, Datta SR, Franke TF, Birnbaum MJ, Yao R, Cooper GM, Segal RA, Kaplan DR, and Greenberg ME. Regulation of neuronal survival by the serine-threonine protein kinase Akt. *Science* 275: 661–665, 1997.
34. Eisenberg LM, Burns L, and Eisenberg CA. Hematopoietic cells from bone marrow have the potential to differentiate into cardiomyocytes *in vitro*. *Anat Rec A Discov Mol Cell Evol Biol* 274: 870–882, 2003.
35. Elmadbouh I, Haider H, Jiang S, Idris NM, Lu G, and Ashraf M. *Ex vivo* delivered stromal cell-derived factor-1alpha promotes stem cell homing and induces angiogenesis in the infarcted myocardium. *J Mol Cell Cardiol* 42: 792–803, 2007.
36. Emery B, Merson TD, Snell C, Young KM, Ernst M, and Kilpatrick TJ. SOCS3 negatively regulates LIF signaling in neural precursor cells. *Mol Cell Neurosci* 31: 739–747, 2006.
37. Endo TA, Masuhara M, Yokouchi M, Suzuki R, Sakamoto H, Mitsui K, Matsumoto A, Tanimura S, Ohtsubo M, Misawa H, Miyaraki T, Leonor N, Taniguchi T, Fujita T, Kanakura Y, Komiya S, and Yoshimura A. A new protein containing an SH2 domain that inhibits JAK kinases. *Nature* 387: 921–924, 1997.
38. Engel FB, Schebesta M, Duong MT, Lu G, Ren S, Madwed JB, Jiang H, Wang Y, and Keating MT. p38 MAP kinase inhibition enables proliferation of adult mammalian cardiomyocytes. *Genes Dev* 19: 1175–1187, 2005.
39. Fingar DC and Blenis J. Target of rapamycin (TOR): an integrator of nutrient and growth factor signals and coordinator of cell growth and cell cycle progression. *Oncogene* 23: 3151–3171, 2004.
40. Fingar DC, Salama S, Tsou C, Harlow E, and Blenis J. Mammalian cell size is controlled by mTOR and its downstream targets S6K1 and 4EBP1/eIF4E. *Genes Dev* 16: 1472–1487, 2002.
41. Forte G, Minieri M, Cossa P, Antenucci D, Sala M, Gnocchi V, Fiaccavento R, Carotenuto F, De Vito P, Baldini PM, Prat M, and Di Nardo P. Hepatocyte growth factor effects on mesenchymal stem cells: proliferation, migration, and differentiation. *Stem Cells* 24: 23–33, 2006.
42. Frias MA, Thoreen CC, Jaffe JD, Schroder W, Sculley T, Carr SA, and Sabatini DM. mSin1 is necessary for Akt/PKB phosphorylation, and its isoforms define three distinct mTORC2s. *Curr Biol* 16: 1865–1870, 2006.
43. Ganiatsas S, Kwee L, Fujiwara Y, Perkins A, Ikeda T, Labow MA, and Zon LI. SEK1 deficiency reveals mitogen-activated

- protein kinase cascade crossregulation and leads to abnormal hepatogenesis. *Proc Natl Acad Sci U S A* 95: 6881–6886, 1998.
44. Gao F, He T, Wang H, Yu S, Yi D, Liu W, and Cai Z. A promising strategy for the treatment of ischemic heart disease: mesenchymal stem cell-mediated vascular endothelial growth factor gene transfer in rats. *Can J Cardiol* 23: 891–898, 2007.
 45. Gao X and Pan D. TSC1 and TSC2 tumor suppressors antagonize insulin signaling in cell growth. *Genes Dev* 15: 1383–1392, 2001.
 46. Garg A and Aggarwal BB. Nuclear transcription factor-kappaB as a target for cancer drug development. *Leukemia* 16: 1053–1068, 2002.
 47. Ghosh S, May MJ, and Kopp EB. NF-kappa B and Rel proteins: evolutionarily conserved mediators of immune responses. *Annu Rev Immunol* 16: 225–260, 1998.
 48. Gnechchi M, He H, Liang OD, Melo LG, Morello F, Mu H, Noiseux N, Zhang L, Pratt RE, Ingwall JS, and Dzau VJ. Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. *Nat Med* 11: 367–368, 2005.
 49. Gnechchi M, He H, Noiseux N, Liang OD, Zhang L, Morello F, Mu H, Melo LG, Pratt RE, Ingwall JS, and Dzau VJ. Evidence supporting paracrine hypothesis for Akt-modified mesenchymal stem cell-mediated cardiac protection and functional improvement. *FASEB J* 20: 661–669, 2006.
 50. Goodell MA, Rosenzweig M, Kim H, Marks DF, DeMaria M, Paradis G, Grupp SA, Sieff CA, Mulligan RC, and Johnson RP. Dye efflux studies suggest that hematopoietic stem cells expressing low or undetectable levels of CD34 antigen exist in multiple species. *Nat Med* 3: 1337–1345, 1997.
 51. Guertin DA, Stevens DM, Thoreen CC, Burds AA, Kalaany NY, Moffat J, Brown M, Fitzgerald KJ, and Sabatini DM. Ablation in mice of the mTORC components raptor, rictor, or mLST8 reveals that mTORC2 is required for signaling to Akt-FOXO and PKCalpha, but not S6K1. *Dev Cell* 11: 859–871, 2006.
 52. Guo Y, He J, Wu J, Yang L, Dai S, Tan X, and Liang L. Locally overexpressing hepatocyte growth factor prevents post-ischemic heart failure by inhibition of apoptosis via calcineurin-mediated pathway and angiogenesis. *Arch Med Res* 39: 179–188, 2008.
 53. Guttridge DC, Albanese C, Reuther JY, Pestell RG, and Baldwin AS Jr. NF-kappaB controls cell growth and differentiation through transcriptional regulation of cyclin D1. *Mol Cell Biol* 19: 5785–5799, 1999.
 54. Hahn JY, Cho HJ, Kang HJ, Kim TS, Kim MH, Chung JH, Bae JW, Oh BH, Park YB, and Kim HS. Pre-treatment of mesenchymal stem cells with a combination of growth factors enhances gap junction formation, cytoprotective effect on cardiomyocytes, and therapeutic efficacy for myocardial infarction. *J Am Coll Cardiol* 51: 933–943, 2008.
 55. Haider H and Ashraf M. Bone marrow stem cell transplantation for cardiac repair. *Am J Physiol Heart Circ Physiol* 288: H2557–H2567, 2005.
 56. Haspel RL and Darnell JE Jr. A nuclear protein tyrosine phosphatase is required for the inactivation of Stat1. *Proc Natl Acad Sci U S A* 96: 10188–10193, 1999.
 57. Hayden MS and Ghosh S. Signaling to NF-kappaB. *Genes Dev* 18: 2195–2224, 2004.
 58. Heinrich PC, Behrmann I, Haan S, Hermanns HM, Muller-Newen G, and Schaper F. Principles of interleukin (IL)-6-type cytokine signalling and its regulation. *Biochem J* 374: 1–20, 2003.
 59. Hilton DJ, Richardson RT, Alexander WS, Viney EM, Willson TA, Sprigg NS, Starr R, Nicholson SE, Metcalf D, and Nicola NA. Twenty proteins containing a C-terminal SOCS box form five structural classes. *Proc Natl Acad Sci U S A* 95: 114–119, 1998.
 60. Hinz M, Krappmann D, Eichten A, Heder A, Scheidereit C, and Strauss M. NF-kappaB function in growth control: regulation of cyclin D1 expression and G0/G1-to-S-phase transition. *Mol Cell Biol* 19: 2690–2698, 1999.
 61. Huang J and Manning BD. The TSC1-TSC2 complex: a molecular switchboard controlling cell growth. *Biochem J* 412: 179–190, 2008.
 62. Ichimiya M, Chang SH, Liu H, Berezsky IK, Trump BF, and Amstad PA. Effect of Bcl-2 on oxidant-induced cell death and intracellular Ca²⁺ mobilization. *Am J Physiol* 275: C832–C839, 1998.
 63. Imanishi Y, Saito A, Komoda H, Kitagawa-Sakakida S, Miyagawa S, Kondoh H, Ichikawa H, and Sawa Y. Allogenic mesenchymal stem cell transplantation has a therapeutic effect in acute myocardial infarction in rats. *J Mol Cell Cardiol* 44: 662–671, 2008.
 64. Inoki K, Li Y, Xu T, and Guan KL. Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. *Genes Dev* 17: 1829–1834, 2003.
 65. Johnson GL and Lapadat R. Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. *Science* 298: 1911–1912, 2002.
 66. Kahn J, Byk T, Jansson-Sjostrand L, Petit I, Shvitiel S, Nagler A, Hardan I, Deutsch V, Gazit Z, Gazit D, Karlsson S, and Lapidot T. Overexpression of CXCR4 on human CD34+ progenitors increases their proliferation, migration, and NOD/SCID repopulation. *Blood* 103: 2942–2949, 2004.
 67. Kamura T, Sato S, Haque D, Liu L, Kaelin WG Jr, Conaway RC, and Conaway JW. The Elongin BC complex interacts with the conserved SOCS-box motif present in members of the SOCS, ras, WD-40 repeat, and ankyrin repeat families. *Genes Dev* 12: 3872–3881, 1998.
 68. Katso R, Okkenhaug K, Ahmadi K, White S, Timms J, and Waterfield MD. Cellular function of phosphoinositide 3-kinases: implications for development, homeostasis, and cancer. *Annu Rev Cell Dev Biol* 17: 615–675, 2001.
 69. Kawada H, Fujita J, Kinjo K, Matsuzaki Y, Tsuma M, Miyatake H, Muguruma Y, Tsuboi K, Itabashi Y, Ikeda Y, Ogawa S, Okano H, Hotta T, Ando K, and Fukuda K. Nonhematopoietic mesenchymal stem cells can be mobilized and differentiate into cardiomyocytes after myocardial infarction. *Blood* 104: 3581–3587, 2004.
 70. Kim C, Li RK, Li G, Zhang Y, Weisel RD, and Yau TM. Effects of cell-based angiogenic gene therapy at 6 months: persistent angiogenesis and absence of oncogenicity. *Ann Thorac Surg* 83: 640–646, 2007.
 71. Kim DH, Sarbassov DD, Ali SM, Latek RR, Guntur KV, Erdjument-Bromage H, Tempst P, and Sabatini DM. GbetaL, a positive regulator of the rapamycin-sensitive pathway required for the nutrient-sensitive interaction between raptor and mTOR. *Mol Cell* 11: 895–904, 2003.
 72. Kim H, Hawley TS, Hawley RG, and Baumann H. Protein tyrosine phosphatase 2 (SHP-2) moderates signaling by gp130 but is not required for the induction of acute-phase plasma protein genes in hepatic cells. *Mol Cell Biol* 18: 1525–1533, 1998.
 73. Kim TK and Maniatis T. Regulation of interferon-gamma-activated STAT1 by the ubiquitin-proteasome pathway. *Science* 273: 1717–1719, 1996.

74. Kirshenbaum LA. Bcl-2 intersects the NFkappaB signalling pathway and suppresses apoptosis in ventricular myocytes. *Clin Invest Med* 23: 322–330, 2000.
75. Kulik G, Klippel A, and Weber MJ. Antiapoptotic signaling by the insulin-like growth factor I receptor, phosphatidylinositol 3-kinase, and Akt. *Mol Cell Biol* 17: 1595–1606, 1997.
76. Kuwahara K, Saito Y, Kishimoto I, Miyamoto Y, Harada M, Ogawa E, Hamanaka I, Kajiyama N, Takahashi N, Izumi T, Kawakami R, and Nakao K. Cardiotrophin-1 phosphorylates akt and BAD, and prolongs cell survival via a PI3K-dependent pathway in cardiac myocytes. *J Mol Cell Cardiol* 32: 1385–1394, 2000.
77. Kyriakis JM and Avruch J. Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. *Physiol Rev* 81: 807–869, 2001.
78. Lawlor MA and Alessi DR. PKB/Akt: a key mediator of cell proliferation, survival and insulin responses? *J Cell Sci* 114: 2903–2910, 2001.
79. Lee DF, Kuo HP, Chen CT, Hsu JM, Chou CK, Wei Y, Sun HL, Li LY, Ping B, Huang WC, He X, Hung JY, Lai CC, Ding Q, Su JL, Yang JY, Sahin AA, Hortobagyi GN, Tsai FJ, Tsai CH, and Hung MC. IKK beta suppression of TSC1 links inflammation and tumor angiogenesis via the mTOR pathway. *Cell* 130: 440–455, 2007.
80. Lehmann U, Schmitz J, Weissenbach M, Sobota RM, Hortner M, Friederichs K, Behrmann I, Tsiaris W, Sasaki A, Schneider-Mergener J, Yoshimura A, Neel BG, Heinrich PC, and Schaper F. SHP2 and SOCS3 contribute to Tyr-759-dependent attenuation of interleukin-6 signaling through gp130. *J Biol Chem* 278: 661–671, 2003.
81. Leonard WJ and O'Shea JJ. Jaks and STATs: biological implications. *Annu Rev Immunol* 16: 293–322, 1998.
82. Li W, Ma N, Ong LL, Nesselmann C, Klopsch C, Ladilov Y, Furlani D, Piechaczek C, Moebius JM, Lutzow K, Lendlein A, Stamm C, Li RK, and Steinhoff G. Bcl-2 engineered MSCs inhibited apoptosis and improved heart function. *Stem Cells* 25: 2118–2127, 2007.
83. Liu B, Liao J, Rao X, Kushner SA, Chung CD, Chang DD, and Shuai K. Inhibition of Stat1-mediated gene activation by PIAS1. *Proc Natl Acad Sci U S A* 95: 10626–10631, 1998.
84. Liu D, Scafidi J, Prada AE, Zahedi K, and Davis AE 3rd. Nuclear phosphatases and the proteasome in suppression of STAT1 activity in hepatocytes. *Biochem Biophys Res Commun* 299: 574–580, 2002.
85. Liu XB, Jiang J, Gui C, Hu XY, Xiang MX, and Wang JA. Angiopoietin-1 protects mesenchymal stem cells against serum deprivation and hypoxia-induced apoptosis through the PI3K/Akt pathway. *Acta Pharmacol Sin* 29: 815–822, 2008.
86. Loboda A, Jazwa A, Grochot-Przeczek A, Rutkowski AJ, Cisowski J, Agarwal A, Jozkowicz A, and Dulak J. Heme oxygenase-1 and the vascular bed: from molecular mechanisms to therapeutic opportunities. *Antioxid Redox Signal* 10: 1767–1812, 2008.
87. Lu W, Gong D, Bar-Sagi D, and Cole PA. Site-specific incorporation of a phosphotyrosine mimetic reveals a role for tyrosine phosphorylation of SHP-2 in cell signaling. *Mol Cell* 8: 759–769, 2001.
88. Luttkien C, Wegenka UM, Yuan J, Buschmann J, Schindler C, Ziemiecki A, Harpur AG, Wilks AF, Yasukawa K, Taga T, Kishimoto T, Barbieri G, Pellegrini S, Sendtner M, Heinrich PC, and Horn F. Association of transcription factor APRF and protein kinase Jak1 with the interleukin-6 signal transducer gp130. *Science* 263: 89–92, 1994.
89. Ma D, Bai X, Guo S, and Jiang Y. The switch I region of Rheb is critical for its interaction with FKB38. *J Biol Chem* 283: 25963–25970, 2008.
90. Mangi AA, Noiseux N, Kong D, He H, Rezvani M, Ingwall JS, and Dzau VJ. Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts. *Nat Med* 9: 1195–1201, 2003.
91. Markel TA, Crisostomo PR, Wang M, Herring CM, Lahm T, Meldrum KK, Lillemoe KD, Rescorla FJ, and Meldrum DR. Iron chelation acutely stimulates fetal human intestinal cell production of IL-6 and VEGF while decreasing HGF: the roles of p38, ERK, and JNK MAPK signaling. *Am J Physiol Gastrointest Liver Physiol* 292: G958–G963, 2007.
92. Matsubayashi Y, Fukuda M, and Nishida E. Evidence for existence of a nuclear pore complex-mediated, cytosol-independent pathway of nuclear translocation of ERK MAP kinase in permeabilized cells. *J Biol Chem* 276: 41755–41760, 2006.
93. Matsumoto R, Omura T, Yoshiyama M, Hayashi T, Inamoto S, Koh KR, Ohta K, Izumi Y, Nakamura Y, Akioka K, Kitaura Y, Takeuchi K, and Yoshikawa J. Vascular endothelial growth factor-expressing mesenchymal stem cell transplantation for the treatment of acute myocardial infarction. *Arterioscler Thromb Vasc Biol* 25: 1168–1173, 2005.
94. Meyer GP, Wollert KC, Lotz J, Steffens J, Lippolt P, Fichtner S, Hecker H, Schaefer A, Arseniev L, Hertenstein B, Ganser A, and Drexler H. Intracoronary bone marrow cell transfer after myocardial infarction: eighteen months' follow-up data from the randomized, controlled BOOST (BOne marrow transfer to enhance ST-elevation infarct regeneration) trial. *Circulation* 113: 1287–1294, 2006.
95. Michlewski G, Sanford JR, and Caceres JF. The splicing factor SF2/ASF regulates translation initiation by enhancing phosphorylation of 4E-BP1. *Mol Cell* 30: 179–189, 2008.
96. Murakami M, Hibi M, Nakagawa N, Nakagawa T, Yasukawa K, Yamanishi K, Taga T, and Kishimoto T. IL-6-induced homodimerization of gp130 and associated activation of a tyrosine kinase. *Science* 260: 1808–1810, 1993.
97. Naka T, Narazaki M, Hirata M, Matsumoto T, Minamoto S, Aono A, Nishimoto N, Kajita T, Taga T, Yoshizaki K, Akira S, and Kishimoto T. Structure and function of a new STAT-induced STAT inhibitor. *Nature* 387: 924–929, 1997.
98. Nicholson SE, De Souza D, Fabri LJ, Corbin J, Willson TA, Zhang JG, Silva A, Asimakis M, Farley A, Nash AD, Metcalf D, Hilton DJ, Nicola NA, and Baca M. Suppressor of cytokine signaling-3 preferentially binds to the SHP-2-binding site on the shared cytokine receptor subunit gp130. *Proc Natl Acad Sci U S A* 97: 6493–6498, 2000.
99. Noiseux N, Gnecci M, Lopez-Illasaca M, Zhang L, Solomon SD, Deb A, Dzau VJ, and Pratt RE. Mesenchymal stem cells overexpressing Akt dramatically repair infarcted myocardium and improve cardiac function despite infrequent cellular fusion or differentiation. *Mol Ther* 14: 840–850, 2006.
100. Nojima H, Tokunaga C, Eguchi S, Oshiro N, Hidayat S, Yoshino K, Hara K, Tanaka N, Avruch J, and Yonezawa K. The mammalian target of rapamycin (mTOR) partner, raptor, binds the mTOR substrates p70 S6 kinase and 4E-BP1 through their TOR signaling (TOS) motif. *J Biol Chem* 278: 15461–15464, 2003.
101. Nygren JM, Jovinge S, Breitbach M, Sawen P, Roll W, Hescheler J, Taneera J, Fleischmann BK, and Jacobsen SE.

- Bone marrow-derived hematopoietic cells generate cardiomyocytes at a low frequency through cell fusion, but not transdifferentiation. *Nat Med* 10: 494–501, 2004.
102. Ogawa M, Tajima F, Ito T, Sato T, Laver JH, and Deguchi T. CD34 expression by murine hematopoietic stem cells: developmental changes and kinetic alterations. *Ann N Y Acad Sci* 1938: 139–145, 2001.
 103. Otterbein LE, Soares MP, Yamashita K, and Bach FH. Heme oxygenase-1: unleashing the protective properties of heme. *Trends Immunol* 24: 449–455, 2003.
 104. Pahl HL. Activators and target genes of Rel/NF-kappaB transcription factors. *Oncogene* 18: 6853–6866, 1999.
 105. Pasha Z, Wang Y, Sheikh R, Zhang D, Zhao T, and Ashraf M. Preconditioning enhances cell survival and differentiation of stem cells during transplantation in infarcted myocardium. *Cardiovasc Res* 77: 134–142, 2008.
 106. Pearson RB, Dennis PB, Han JW, Williamson NA, Kozma SC, Wettenhall RE, and Thomas G. The principal target of rapamycin-induced p70s6k inactivation is a novel phosphorylation site within a conserved hydrophobic domain. *EMBO J* 14: 5279–5287, 1995.
 107. Perin EC, Dohmann HF, Borojevic R, Silva SA, Sousa AL, Silva GV, Mesquita CT, Belem L, Vaughn WK, Rangel FO, Assad JA, Carvalho AC, Branco RV, Rossi MI, Dohmann HJ, and Willerson JT. Improved exercise capacity and ischemia 6 and 12 months after transendocardial injection of autologous bone marrow mononuclear cells for ischemic cardiomyopathy. *Circulation* 110(11 suppl 1): II213–II218, 2004.
 108. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, and Marshak DR. Multilineage potential of adult human mesenchymal stem cells. *Science* 284: 143–147, 1999.
 109. Raman M, Chen W, and Cobb MH. Differential regulation and properties of MAPKs. *Oncogene* 26: 3100–3112, 2007.
 110. Ranganathan A, Yazicioglu MN, and Cobb MH. The nuclear localization of ERK2 occurs by mechanisms both independent of and dependent on energy. *J Biol Chem* 281: 15645–15652, 2006.
 111. Reiling JH and Sabatini DM. Stress and mTOR signaling. *Oncogene* 25: 6373–6383, 2006.
 112. Reszka AA, Seger R, Diltz CD, Krebs EG, and Fischer EH. Association of mitogen-activated protein kinase with the microtubule cytoskeleton. *Proc Natl Acad Sci U S A* 92: 8881–8885, 1995.
 113. Rosamond W, Flegal K, Furie K, Go A, Greenlund K, Haase N, Hailpern SM, Ho M, Howard V, Kissela B, Kittner S, Lloyd-Jones D, McDermott M, Meigs J, Moy C, Nichol G, O'Donnell C, Roger V, Sorlie P, Steinberger J, Thom T, Wilson M, and Hong Y. Heart disease and stroke statistics: 2008 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation* 117: e25–e146, 2008.
 114. Rota M, Kajstura J, Hosoda T, Bearzi C, Vitale S, Esposito G, Iaffaldano G, Padin-Iruegas ME, Gonzalez A, Rizzi R, Small N, Muraski J, Alvarez R, Chen X, Urbanek K, Bolli R, Houser SR, Leri A, Sussman MA, and Anversa P. Bone marrow cells adopt the cardiomyogenic fate *in vivo*. *Proc Natl Acad Sci U S A* 104: 17783–17788, 2007.
 115. Ryan JM, Barry FP, Murphy JM, and Mahon BP. Mesenchymal stem cells avoid allogeneic rejection. *J Inflamm (Lond)* 2: 8, 2005.
 116. Sabapathy K, Hochedlinger K, Nam SY, Bauer A, Karin M, and Wagner EF. Distinct roles for JNK1 and JNK2 in regulating JNK activity and c-Jun-dependent cell proliferation. *Mol Cell* 15: 713–725, 2004.
 117. Sarbassov DD, Ali SM, Sengupta S, Sheen JH, Hsu PP, Bagley AF, Markhard AL, and Sabatini DM. Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. *Mol Cell* 22: 159–168, 2006.
 118. Sarbassov DD, Guertin DA, Ali SM, and Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* 307: 1098–1101, 2005.
 119. Sasaki A, Yasukawa H, Suzuki A, Kamizono S, Syoda T, Kinjyo I, Sasaki M, Johnston JA, Yoshimura A. Cytokine-inducible SH2 protein-3 (CIS3/SOCS3) inhibits Janus tyrosine kinase by binding through the N-terminal kinase inhibitory region as well as SH2 domain. *Genes Cells* 4: 339–351, 1999.
 120. Sato T, Laver JH, and Ogawa M. Reversible expression of CD34 by murine hematopoietic stem cells. *Blood* 94: 2548–2554, 1999.
 121. Sauer H, Bekhite MM, Hescheler J, and Wartenberg M. Redox control of angiogenic factors and CD31-positive vessel-like structures in mouse embryonic stem cells after direct current electrical field stimulation. *Exp Cell Res* 304: 380–390, 2005.
 122. Scarabelli TM, Knight R, Stephanou A, Townsend P, Chen-Scarabelli C, Lawrence K, Gottlieb R, Latchman D, and Narula J. Clinical implications of apoptosis in ischemic myocardium. *Curr Probl Cardiol* 31: 181–264, 2006.
 123. Schaper F, Gendo C, Eck M, Schmitz J, Grimm C, Anhu D, Kerr IM, and Heinrich PC. Activation of the protein tyrosine phosphatase SHP2 via the interleukin-6 signal transducing receptor protein gp130 requires tyrosine kinase Jak1 and limits acute-phase protein expression. *Biochem J* 335: 557–565, 1998.
 124. Shi Y and Gaestel M. In the cellular garden of forking paths: how p38 MAPKs signal for downstream assistance. *Biol Chem* 383: 1519–1536, 2002.
 125. Shiojima R and Walsh K. Regulation of cardiac growth and coronary angiogenesis by the Akt/PKB signaling pathway. *Genes Dev* 20: 3347–3365, 2006.
 126. Solt LA, Madge LA, Orange JS, and May MJ. Interleukin-1-induced NF-kappaB activation is NEMO-dependent but does not require IKKbeta. *J Biol Chem* 282: 8724–8733, 2007.
 127. Song H, Kwon K, Lim S, Kang SM, Ko YG, Xu Z, Chung JH, Kim BS, Lee H, Joung B, Park S, Choi D, Jang Y, Chung NS, Yoo KJ, and Hwang KC. Transfection of mesenchymal stem cells with the FGF-2 gene improves their survival under hypoxic conditions. *Mol Cells* 19: 402–407, 2005.
 128. Spiegelstein D, Kim C, Zhang Y, Li G, Weisel RD, Li RK, and Yau TM. Combined transmyocardial revascularization and cell-based angiogenic gene therapy increases transplanted cell survival. *Am J Physiol Heart Circ Physiol* 293: H3311–H3316, 2007.
 129. Stahl N, Boulton TG, Farruggella T, Ip NY, Davis S, Witthuhn BA, Quelle FW, Silvennoinen O, Barbieri G, Pellegrini S, Ihle JN, and Yancopoulos GD. Association and activation of Jak-Tyk kinases by CNTF-LIF-OSM-IL-6 beta receptor components. *Science* 263: 92–95, 1994.
 130. Starr R, Willson TA, Viney EM, Murray LJ, Rayner JR, Jenkins BJ, Gonda TJ, Alexander WS, Metcalf D, Nicola NA and Hilton DJ. A family of cytokine-inducible inhibitors of signalling. *Nature* 387: 917–921, 1997.
 131. Stephens L, Anderson K, Stokoe D, Erdjument-Bromage H, Painter GF, Holmes AB, Gaffney PR, Reese CB, McCormick

- F, Tempst P, Coadwell J, and Hawkins PT. Protein kinase B kinases that mediate phosphatidylinositol 3,4,5-trisphosphate-dependent activation of protein kinase B. *Science* 279: 710–714, 1998.
132. Sun L, Cui M, Wang Z, Feng X, Mao J, Chen P, Kangtao M, Chen F, and Zhou C. Mesenchymal stem cells modified with angiopoietin-1 improve remodeling in a rat model of acute myocardial infarction. *Biochem Biophys Res Commun* 357: 779–784, 2007.
 133. Symes A, Stahl N, Reeves SA, Farruggella T, Servidei T, Gearan T, Yancopoulos G, and Fink JS. The protein tyrosine phosphatase SHP-2 negatively regulates ciliary neurotrophic factor induction of gene expression. *Curr Biol* 7: 697–700, 1997.
 134. Takada Y, Singh S, and Aggarwal BB. Identification of a p65 peptide that selectively inhibits NF-kappa B activation induced by various inflammatory stimuli and its role in down-regulation of NF-kappaB-mediated gene expression and up-regulation of apoptosis. *J Biol Chem* 279: 15096–15104, 2004.
 135. Tang YL, Tang Y, Zhang YC, Qian K, Shen L, and Phillips MI. Improved graft mesenchymal stem cell survival in ischemic heart with a hypoxia-regulated heme oxygenase-1 vector. *J Am Coll Cardiol* 46: 1339–1350, 2005.
 136. Terrell AM, Crisostomo PR, Wairiuko GM, Wang M, Morrell ED, and Meldrum DR. Jak/STAT/SOCS signaling circuits and associated cytokine-mediated inflammation and hypertrophy in the heart. *Shock* 26: 226–234, 2006.
 137. Thiel S, Behrmann I, Dittich E, Muys L, Tavernier J, Wijdenes J, Heinrich PC, and Graeve L. Internalization of the interleukin 6 signal transducer gp130 does not require activation of the Jak/STAT pathway. *Biochem J* 330: 47–54, 1998.
 138. Thurston G, Suri C, Smith K, McClain J, Sato TN, Yancopoulos GD, and McDonald DM. Leakage-resistant blood vessels in mice transgenically overexpressing angiopoietin-1. *Science* 286: 2511–2514, 1999.
 139. Toma C, Pittenger MF, Cahill KS, Byrne BJ, and Kessler PD. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation* 105: 93–98, 2002.
 140. Torella D, Rota M, Nurzynska D, Musso E, Monsen A, Shiraishi I, Zias E, Walsh K, Rosenzweig A, Sussman MA, Urbanek K, Nadal-Ginard B, Kajstura J, Anversa P, and Leri A. Cardiac stem cell and myocyte aging, heart failure, and insulin-like growth factor-1 overexpression. *Circ Res* 94: 514–524, 2004.
 141. Tournier C, Hess P, Yang DD, Xu J, Turner TK, Nimnual A, Bar-Sagi D, Jones SN, Flavell RA, and Davis RJ. Requirement of JNK for stress-induced activation of the cytochrome c-mediated death pathway. *Science* 288: 870–874, 2000.
 142. Uchiyama T, Engelman RM, Maulik N, and Das DK. Role of Akt signaling in mitochondrial survival pathway triggered by hypoxic preconditioning. *Circulation* 109: 3042–3049, 2004.
 143. Uemura R, Xu M, Ahmad N, and Ashraf M. Bone marrow stem cells prevent left ventricular remodeling of ischemic heart through paracrine signaling. *Circ Res* 98: 1414–1421, 2006.
 144. Vulapalli SR, Chen Z, Chua BH, Wang T, and Liang CS. Cardiospecific overexpression of HO-1 prevents I/R-induced cardiac dysfunction and apoptosis. *Am J Physiol Heart Circ Physiol* 283: H688–H694, 2002.
 145. Wang M, Crisostomo PR, Herring C, Meldrum KK, and Meldrum DR. Human progenitor cells from bone marrow or adipose tissue produce VEGF, HGF, and IGF-I in response to TNF by a p38 MAPK-dependent mechanism. *Am J Physiol Regul Integr Comp Physiol* 291: R880–R884, 2006.
 146. Wang M, Sankula R, Tsai BM, Meldrum KK, Turrentine M, March KL, Brown JW, Dinarello CA, and Meldrum DR. P38 MAPK mediates myocardial proinflammatory cytokine production and endotoxin-induced contractile suppression. *Shock* 21: 170–174, 2004.
 147. Wang M, Tsai BM, Turrentine MW, Mahomed Y, Brown JW, and Meldrum DR. p38 mitogen activated protein kinase mediates both death signaling and functional depression in the heart. *Ann Thorac Surg* 80: 2235–2241, 2005.
 148. Wang M, Zhang W, Crisostomo P, Markel T, Meldrum KK, Fu XY, and Meldrum DR. Endothelial STAT3 plays a critical role in generalized myocardial proinflammatory and proapoptotic signaling. *Am J Physiol Heart Circ Physiol* 293: H2101–H2108, 2007.
 149. Wang M, Zhang W, Crisostomo P, Markel T, Meldrum KK, Fu XY, and Meldrum DR. STAT3 mediates bone marrow mesenchymal stem cell VEGF production. *J Mol Cell Cardiol* 42: 1009–1015, 2007.
 150. Wang Y, Haider HK, Ahmad N, Xu M, Ge R, and Ashraf M. Combining pharmacological mobilization with intramyocardial delivery of bone marrow cells over-expressing VEGF is more effective for cardiac repair. *J Mol Cell Cardiol* 40: 736–745, 2006.
 151. Wegenka UM, Buschmann J, Luttkien C, Heinrich PC, and Horn F. Acute-phase response factor, a nuclear factor binding to acute-phase response elements, is rapidly activated by interleukin-6 at the posttranslational level. *Mol Cell Biol* 13: 276–288, 1993.
 152. Widera D, Mikenberg I, Elvers M, Kaltschmidt C, and Kaltschmidt B. Tumor necrosis factor alpha triggers proliferation of adult neural stem cells via IKK/NF-kappaB signaling. *BMC Neurosci* 7: 64, 2006.
 153. Wijayanti N, Huber S, Samoylenko A, Kietzmann T, and Immenschuh S. Role of NF-kappaB and p38 MAP kinase signaling pathways in the lipopolysaccharide-dependent activation of heme oxygenase-1 gene expression. *Antioxid Redox Signal* 6: 802–810, 2004.
 154. Yamada Y, Yokoyama S, Wang XD, Fukuda N, and Takakura N. Cardiac stem cells in brown adipose tissue express CD133 and induce bone marrow nonhematopoietic cells to differentiate into cardiomyocytes. *Stem Cells* 25: 1326–1333, 2007.
 155. Yamamoto T, Sekine Y, Kashima K, Kubota A, Sato N, Aoki N, and Matsuda T. The nuclear isoform of protein-tyrosine phosphatase TC-PTP regulates interleukin-6-mediated signaling pathway through STAT3 dephosphorylation. *Biochem Biophys Res Commun* 297: 811–817, 2002.
 156. Yau TM, Kim C, Li G, Zhang Y, Weisel RD, and Li RK. Maximizing ventricular function with multimodal cell-based gene therapy. *Circulation* 112(9 suppl): II23–II28, 2005.
 157. Yeoh GC, Ernst M, Rose-John S, Akhurst B, Payne C, Long S, Alexander W, Croker B, Grail D, and Matthews VB. Opposing roles of gp130-mediated STAT-3 and ERK-1/2 signaling in liver progenitor cell migration and proliferation. *Hepatology* 45: 486–494, 2007.
 158. Yin AH, Miraglia S, Zanjani ED, Almeida-Porada G, Ogawa M, Leary AG, Olweus J, Kearney J, and Buck DW. AC133, a novel marker for human hematopoietic stem and progenitor cells. *Blood* 90: 5002–5012, 1997.

159. Zhang D, Fan GC, Zhou X, Zhao T, Pasha Z, Xu M, Zhu Y, Ashraf M, and Wang Y. Over-expression of CXCR4 on mesenchymal stem cells augments myoangiogenesis in the infarcted myocardium. *J Mol Cell Cardiol* 44: 281–292, 2008.
160. Zhang JG, Farley A, Nicholson SE, Willson TA, Zugaro LM, Simpson RJ, Moritz RL, Cary D, Richardson R, Hausmann G, Kile BJ, Keni SB, Alexander WS, Metcalf D, Hilton DJ, Nicola NA, and Baca M. The conserved SOCS box motif in suppressors of cytokine signaling binds to elongins B and C and may couple bound proteins to proteasomal degradation. *Proc Natl Acad Sci U S A* 96: 2071–2076, 1999.
161. Zhang M, Mal N, Kiedrowski M, Chacko M, Askari AT, Popovic ZB, Koc ON, and Penn MS. SDF-1 expression by mesenchymal stem cells results in trophic support of cardiac myocytes after myocardial infarction. *FASEB J* 21: 3197–3207, 2007.
162. Zhang N, Mustin D, Reardon W, Almeida AD, Mozdziak P, Mrug M, Eisenberg LM, and Sedmera D. Blood-borne stem cells differentiate into vascular and cardiac lineages during normal development. *Stem Cells Dev* 15: 17–28, 2006.
163. Zohlnhofer D, Graeve L, Rose-John S, Schooltink H, Dittrich E, and Heinrich PC. The hepatic interleukin-6 receptor: down-regulation of the interleukin-6 binding subunit (gp80) by its ligand. *FEBS Lett* 306: 219–222, 1992.

Address correspondence to:

Daniel R. Meldrum, M.D.

635 Barnhill Drive

Van Nuys Medical Science Bldg. Rm. 2017

Indianapolis, IN 46202

E-mail: dmeldrum@iupui.edu

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