

Regulation of Hypoxia-Induced Cell Death and Application of Hypoxic Preconditioning to Stem Cell Transplantation

Keywords: Apoptosis; Hypoxia; Stem cell; Transplantation; Stem cell therapy

Abstract

Hypoxia is a commonly encountered feature of the cellular microenvironment in a number of processes in which programmed cell death (apoptosis) affects disease progression. These diseases include myocardial infarction, stroke, and ischemic acute kidney injury. Bone marrow-derived mesenchymal stem cells (MSCs) are multipotent adult stem cells that are able to differentiate into endothelial cells, vascular smooth muscle cells, or cardiac-like myocytes when transplanted into the ischemic heart. Both animal and clinical studies have substantiated that MSC transplantation can enhance cardiac function through possible angiogenesis and myogenesis after myocardial infarction. However, some studies have failed to monitor the therapeutic effects of MSC transplantation; therefore, there is a need for supplementary research on the use of MSCs and the improvement of transplantation techniques after MI. An important problem in stem cell therapy for ischemic heart diseases is the low survival of transplanted cells in the ischemic and infarcted sites. Most transplanted cells die within 4 days after transplantation into the ischemic heart. Endogenous and environmental factors, such as hypoxia and inflammatory response, may contribute to cell death. Therefore, enhancing implanted cell survival after transplantation is vital for improving the effect and efficiency of stem cell therapy. In this review, we investigate whether hypoxia is responsible for activating apoptosis signaling in transplanted stem cells, and could be a potential target for enhancing the therapeutic effect of stem cells in treating ischemic heart diseases.

Abbreviations

NOS: Nitric Oxide Synthase; PDK: Pyruvate Dehydrogenase Kinase; polyp: Polyphosphate; PTP: Permeability Transition Pore; ROS: Reactive Oxygen Species; SDF-1: Stromal-Derived Factor-1; UCP: Uncoupling Protein; VEGF: Vascular Endothelial Growth Factor

Introduction

Hypoxia occurs when oxygen demand exceeds supply [1]. Hypoxia causes ATP levels to drop and inhibits maintenance of cellular functions; if the condition lasts for sufficient time, cells die. Severe hypoxia results in a high mutation rate, causing point mutations that may be explained by reduced DNA mismatch repair activity resulting from hypoxia-induced decreases in NLH1 and PMS2 concentrations [2]. In addition, hypoxia induces genetic instability by the induction of fragile sites causing gene amplification [3-5]. Therefore, during severe hypoxia or anoxia, the cell initiates a cascade of events that leads to apoptotic cell death, thereby preventing the accumulation of cells with hypoxia-induced mutations [6]. A classic example of an acute hypoxic event is stroke. Although a stroke lesion can lead to both types of cell death (i.e., necrosis and apoptosis), it is typically characterized by a core of necrosis [7,8], which is a passive,



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uncontrolled type of cell death. Similar cascades are thought to occur during myocardial infarction, which is also characterized by a core of necrosis and a border zone in which cells undergo apoptosis [9,10].

Although diverse treatment for cardiac diseases has been developed, heart failure remains a critical disease [11] because regenerative ability after myocardial infarction (MI) is limited [12-14]. Recently, therapeutic approaches have used genes, growth factors, or cells to enhance myocardial substitutes [15]. Given recent progress in restoring the ischemic heart using mesenchymal stem cells (MSCs), understanding the tissue microenvironment will help optimize the therapeutic potential of these cells [16]. However, MSCs transplanted into an infarcted site of the ischemic heart encounter severe conditions such as calcium excess, acidosis, and reactive oxygen species (ROS), which induce apoptotic signaling and inhibit survival [17-20]. Ultimately, ROS production in the injured heart induces apoptosis in both implanted cells and cardiomyocytes [21-23].

Apoptosis regulatory genes have been the subject of much research. Apoptosis is regulated by both activators and repressors. The balance of these molecules determines whether apoptosis is initiated. Recent research has suggested that some oncogenes and tumor suppressor genes control programmed cell death. *Bcl-2*, *p53*, and *c-Myc* are known to be apoptosis regulatory genes [24-26]. In addition, *Bcl-xL*, *Bcl-W*, and *Mcl-1* are well-established apoptosis inhibitors. In contrast, *Bad*, *Bid*, *Bim*, *Bmf*, *Bik*, *Hrk*, *Noxa*, and *Puma* are known to induce apoptosis [27].

In this review, we investigate whether hypoxia-induced apoptosis is a strong inducer of cell death of implanted MSCs in the ischemic heart, and whether it makes a promising target for enhancing the therapeutic effect of MSCs in clinical research.

Inducing Apoptosis under Hypoxia

Hypoxia induces necrosis and apoptosis [28,29]. Hypoxia-induced apoptosis is related to increased mitochondrial permeability and release of cytochrome c into the cytoplasm, is caspase-9

dependent but caspase-8 independent, and mainly occurs by way of the intrinsic pathway [30]. However, many cell types endure long periods of hypoxic stress, often requiring additional stressors such as serum deprivation or acidosis for cell death to occur [31-33]. The ability to withstand apoptosis in settings of hypoxic stress is the result of a well-developed cellular response, a phenomenon that is important in many disease processes. The ability to adjust to varying levels of oxygen in the cellular environment is generally attributable to hypoxia-inducible factor-1 (HIF-1). Hypoxia also affects other transcription factors, such as HIF-2, nuclear factor kappaB (NF-κB), and p53, which play significant roles in the regulation of the apoptotic pathway. In many complex disease processes, the change in hypoxic phenotype has a larger effect on cell survival than the direct induction of apoptosis.

Apoptotic Pathways

Extrinsic pathway

Apoptosis is triggered by two signaling pathways. The extrinsic pathway is activated by binding of pro-apoptotic ligands to death receptors on the cell surface. Receptors such as CD95 or tumor necrotic factor receptor 1 (TNF-R1) induce formation of death-inducing signaling complexes (DISCs) comprised of death receptors, TNF receptor-associated death domain (TRADD)-containing proteins, Fas-associated death domain (FADD) adaptor proteins, and initiator caspases. Activation of the death receptor complex following ligand binding promotes interactions between pro-caspase-8 molecules within the DISC, which subsequently activate caspase-3 and induce apoptosis [34-36]. TNF-R1 signaling also promotes cell survival via NF-κB activation, which is stimulated by the recruitment of the TNF receptor associated factor 2 (TRAF2) to ligand-bound receptors. Interestingly, TRADD may serve as a platform for the assembly of TRAF2 or FADD, which determine the subsequent activation of either the NF-κB-induced survival pathway or the caspase-dependent pro-apoptotic pathway, respectively. In contrast, tumor necrotic factor receptor 1 (TNF-R2) does not include a TRADD motif; thus, TRAF2 can directly interact with NF-κB [37,38]. TNF-R2 also enhances cell survival by stimulating mitogen-activated protein kinases (MAPK).

Intrinsic pathway

In contrast to the extrinsic pathway, the intrinsic apoptotic pathway involves non-receptor-mediated intracellular signals. These signals are activated by cell stresses such as cell shrinkage, DNA fragmentation, and cytokine deficiency. The intrinsic pathway can also be activated in response to toxins such as chemotherapeutic agents. Intrinsic apoptosis is controlled by the Bcl-2 family of proteins, which are located on the mitochondrial membrane [39]. Members of the Bcl-2 family are classified according to their anti- or pro-apoptotic function. The anti-apoptotic members of this family include Bcl-2, Bcl-xL, Bcl-W, and Mcl-1. In contrast, Bax, Bak, and Bok are pro-apoptotic and Bid, Noxa, Bad, Bim, Bik, Bmf, Hrk, and Puma can induce the expression of apoptotic members of the family. Under normal conditions, the mitochondrial membrane is polarized such that cytochrome c and ROS are confined within the mitochondrial walls. Pro-apoptotic proteins of the Bcl-2 family stimulate the mitochondrial permeability transport pore and induce cytochrome c release. Once cytochrome c is in the cytoplasm, it binds apoptotic protease activating factor 1 and procaspase-9 to form an “apoptosome”. In the presence of ATP, this complex induces

proteolytic cleavage of procaspase-3 to activated caspase-3. Bax is a well-known pro-apoptotic protein. During apoptosis, Mcl-1 levels are significantly decreased via proteasome degradation. Decreasing levels of Mcl-1 release Bax from the Bak hetero-complex [40,41]. Activated Bax and Bak act as ion channels and connector proteins, allowing for the intermediate release of cytochrome c. In contrast, the anti-apoptotic protein Bcl-2 can inhibit Bax or Bak by enhancing the stability of the mitochondrial membrane or by reducing the transport of pro-apoptotic proteins. Bad and Bid inhibit anti-apoptotic Bcl-2 protein activity and induce pro-apoptotic protein function. Figure 1 shows a schematic of both the extrinsic and intrinsic pathways.

Transcription Factors

Hypoxia-inducible factor

Hypoxia-inducible factor (HIF) is a transcription factor that allows cells to adapt to low-oxygen conditions. Hypoxic adaptation is mostly mediated through HIF-1α, which is degraded by prolylhydroxylase (PHD) under normoxic conditions [41]. At hypoxic levels, however, PHD enzymes are inactivated and HIF-1α proteins are free to bind to HIF-1α/aryl hydrocarbon nuclear translocator (ARNT) and p300. p300 then allows the binding of HIF with the promoter region of target genes in the nucleus [42,43]. This process allows for enhanced angiogenesis, hematopoiesis, and improved cell survival by enhancing intracellular ATP and oxygen levels and reducing toxic ROS production [41]. In addition, HIF-1α induces cell cycle arrest in order to reduce the cell’s workload [44]. Although HIF-2α is similar to HIF-1α, it differs in many important aspects. First, HIF-2 is not ubiquitously expressed and interacts with individual cofactors. For example, HIF-2 does not regulate genes involved in glycolysis, but is the main regulator of erythropoietin in the adult kidney. Moreover, HIF-2 increases cell proliferation via c-Myc [45,46].

NF-κB

Along with its role in mediating the response of the immune system to infection, NF-κB regulates the expression of cytokines,

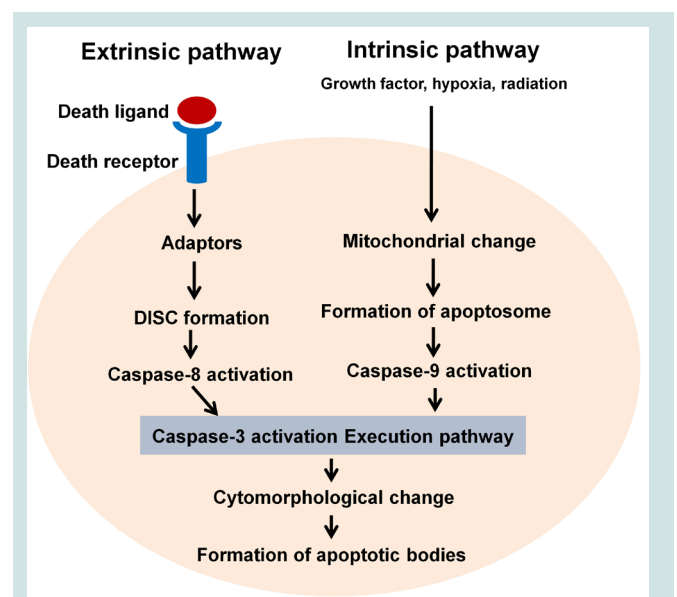


Figure 1: Extrinsic and intrinsic apoptosis pathways. A schematic view of the two main apoptotic pathways: the intrinsic pathway and the extrinsic pathway.

growth factors, and anti-apoptotic factors [47]. When NF- κ B is inactive, its subunits are segregated in the cytoplasm by the inhibitor of NF- κ B (I κ B) proteins, I κ B α and I κ B β . Upon activation, I κ B is phosphorylated, and its degradation leads to release of bound NF- κ B dimers. The free dimers translocate to the nucleus and bind to target promoter or enhancer sites [47]. NF- κ Bs are activated via both canonical and noncanonical pathways; however, hypoxia leads only to NF- κ B activation via the canonical pathway [48]. More specifically, low levels of oxygen stimulate I κ B kinase (IKK), which leads to the phosphorylation and degradation of I κ B, and then to NF- κ B activation and knockdown of PHD enzymes [49].

p53

HIF-1 α is activated under hypoxic conditions, which may have functional significance for the role of the tumor suppressor p53. p53 is involved in the regulation of the cell cycle, DNA repair, and apoptosis. This molecule is induced by pro-apoptotic proteins such as Puma and Noxa. Accumulation of p53 under hypoxic conditions is probably not induced by DNA damage [1,50]. Under hypoxic conditions, p53 interacts with transcriptional corepressors such as mSin3A/histone deacetylases, inducing apoptosis via transrepression of α , β -tubulin [51].

Survival pathways involved in hypoxic preconditioning of stem cells

Because metabolic dysfunction and glutamate excitotoxicity occur in ischemic brain injury, mass apoptosis is induced in always with extra injury from increased ROS, inflammatory responses, activation of apoptotic and other pathological processes. Preconditioning treatments for stem cells have been shown to improve resistance to these insults by increasing anti-apoptotic signals [52,53]. Many survival and defensive molecules, including HIF-1 α [54], trophic/growth factors [55], Akt [56,57], extracellular signal-regulated kinase (ERK) [57], glycogen synthase kinase-3 β (GSK-3 β), matrix metalloproteinase-2 (MMP-2) [19], survivin [58], and Bcl-2 are engaged in reaction to preconditioning stimuli. Selective up-regulation of these molecules binding to improving defensive signaling is adequately controlled in both preconditioned stem cells and the cells nearby to injury sites [59].

Central roles of HIF-1 in hypoxic preconditioning

In stem cells, HIF-1 also plays a role in preconditioning and provides an advantage in transplantation therapy (Figure 2). HIF-1 induces the cysteine glutamate interchange system of NSCs by increasing expression of the light-chain subunit xCT [60], which is a limit stage for brain antioxidant glutathione (GSH) production [61]. HIF-1 overexpression in MSCs leads to up-regulation of genes that contribute to cell adhesion, migration, and paracrine effect. Transplantation of these cells into the myocardium of rats after induction of MI improves revival of cardiac functions and angiogenesis [58,62]. Collectively, these results suggest that HIF-1 is a significant mediator of stem cell preconditioning (Figure 2).

Hypoxic Preconditioning Provides Therapeutic Benefits in Stem Cell Therapies Enhanced Cell Survival In Vitro and After Transplantation

The survival rate of implanted cells is a major issue once cells are transplanted into the ischemic heart or brain. The preconditioning factors mentioned here usually confer superior survival of stem cells

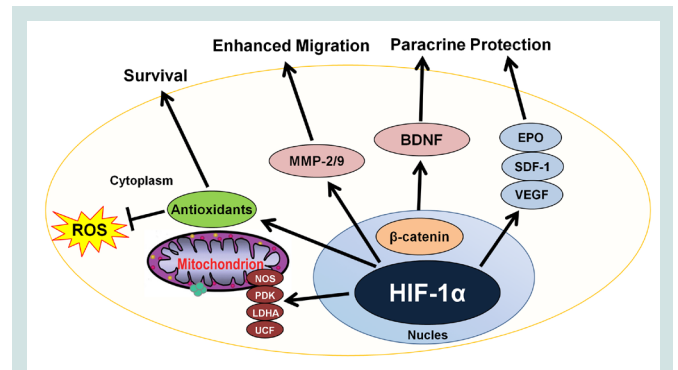


Figure 2: Central roles of HIF-1 signaling pathways in stem cells. Hypoxic preconditioning insults increase HIF-1 expression. HIF-1 then regulates antioxidants, survival signals, and many other genes related to cell adhesion, polarization, migration, and paracrine protection.

and progenitors *in vitro* or after transplantation. Preconditioning using lethal hypoxia and EPO significantly increased the capacity of treated cells to withstand apoptotic and other stresses *in vitro*, as well as in the harsh environment of ischemic infarct sites [17,63,64]. MSCs and embryonic stem cell-derived neural progenitor cells (ES-NPCs) show improved survival following lethal exposure to hypoxic conditions (1% O₂), as well as a 40–50% decrease in apoptosis and caspase activation. Hypoxic preconditioning increased the secretion of EPO and up-regulated the expression of Bcl-2, HIF-1, EPO receptor (EPOR), neurofilament (NF), and synaptophysin in ES-NPCs. The defensive effect was decreased by blocking EPOR, and pretreatment of ES-NPCs with recombinant human EPO mimicked the effects of hypoxic preconditioning. Three days after transplantation into the ischemic rat brain, a 30–40% lower apoptosis was observed in hypoxic preconditioned ES-NPCs as compared to normoxic cells. These surviving ES-NPCs also showed extensive neuronal differentiation in the ischemic brain and improved revival of sensory motor function [64]. A similar survival effect of hypoxia pretreatment has been reported in human ES-NPCs [65]. Previous studies have shown that ES-NPCs have the capacity for peripheral nerve injury recovery [66]. Therefore, improved stem cell survival by preconditioning shows promise for application in cell therapies for tissue repair [67].

Increased regenerative potential of hypoxic-preconditioned stem and progenitor cells

Preconditioned stem cells show several improved regenerative abilities, including improved migration and homing to lesion sites. In bone marrow-derived hemangioblasts, many chemokine and angiogenic genes were up-regulated after hypoxic induction, which accelerated their differentiation toward the endothelial lineage [68]. Hypoxia also improves the differentiation of EPC-like attaching cells, which promote neovascularization [69]. Their function is dependent on secretion of VEGF and increased VEGF2R expression in reaction to hypoxia [70]. Hypoxic-preconditioned MSCs show increased expression of Wnt4, and induced neovascularization was observed in a murine model of hindlimb ischemia [71]. Another important signaling molecule, sonic hedgehog (SHH), may be involved in the EPC-mediated angiogenesis and neovascularization induced by VEGF, SDF-1, and angiopoietin-1 [72]. Hypoxic preconditioning of MSCs showed benefits for transplantation therapy for bone regeneration by restoring osteogenic differentiation [73].

Conclusion

Oxygen levels are important in the general physiology of the cell and in instances where cell physiology contributes to disease progression. A better understanding of the transcriptional response to hypoxia and the particular mechanisms underlying hypoxic apoptosis will determine our success in developing therapies that focus on controlling apoptosis induced by disease conditions.

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