Review

Role of hypoxia-inducible factor in cell survival during myocardial ischemia–reperfusion

G Loor¹ and PT Schumacker^{*,2}

Hypoxia-inducible factor (HIF) is the principal transcription factor involved in the regulation of transcriptional responses to hypoxia. During hypoxia, HIF- α levels accumulate and trigger an increase in expression of genes involved in glycolysis, glucose metabolism, mitochondrial function, cell survival, apoptosis, and resistance to oxidative stress. In this regard, HIF activation plays an essential role in triggering cellular protection and metabolic alterations from the consequences of oxygen deprivation. This suggests that HIF activation should confer protection against ischemia–reperfusion (I/R) injury, although this protection might require HIF activation before the onset of lethal ischemia. Studies using enhanced expression of HIF-1 α suggest that its upregulation may be a beneficial therapeutic modality in the treatment or prevention of ischemic injury. HIF-regulated gene expression may mediate the late phase of preconditioning, and constitutive HIF activity may influence the expression of genes that are required for the cell to be able to respond to acute preconditioning. This article reviews the current literature on the role of HIF in balancing protection and cell death in the face of ischemia and I/R injury.

Cell Death and Differentiation (2008) 15, 686–690; doi:10.1038/cdd.2008.13; published online 8 February 2008

The cardiovascular system supplies the tissues of the body with blood, which delivers oxygen and nutrients that are required for cell survival. Restriction of regional blood flow results in ischemia, which causes the cellular oxygen tension to decrease and the carbon dioxide tension to rise. Ischemia also deprives cells of metabolic nutrients such as glucose. Cells can survive under these conditions for short periods, but when the duration of the ischemia exceeds a critical threshold, the combined effects of tissue hypoxia and acidosis activate cell death by necrosis and/or apoptosis. Factors that contribute to the initiation of cell death during ischemia include the lack of O₂, tissue acidosis, increased generation of reactive oxygen species (ROS), disruption of calcium homeostasis, depletion of glycogen stores, mitochondrial injury, and oxidative damage to DNA. The duration of the ischemic stress is an important determinant of the resulting tissue damage, in which short periods of ischemia are well tolerated while prolonged periods induce significant cell death. Clinically, the treatment of choice for ischemia involves re-establishing blood flow to the ischemic tissue. However, reperfusion of ischemic tissue paradoxically triggers the morphological appearance of tissue injury, presumably by initiating events that contribute to the activation of cell-death pathways. This has led to the concept of ischemia–reperfusion (I/R) injury as a sequence of events that contribute to cell death.^{1–3}

Empirically, it has been observed that different cell types show widely differing sensitivity to ischemia. Within a given cell type, the vulnerability to I/R-induced cell death can be modified by ischemic preconditioning (IPC), a phenomenon whereby repeated exposures to brief, nonlethal ischemia confer protection against a later ischemic insult.^{4–9} While IPC can induce immediate protection that presumably does not involve *de novo* expression of new genes, the same stimulus also induces a 'late window' of protection that requires transcriptional activation.¹⁰ In this regard, increasing evidence suggests that cell survival during I/R can be influenced by the expression of genes that promote glycolysis, suppress ROS production, limit mitochondrial metabolism, and inhibit pro-apoptotic protein expression. Hypoxia-inducible factor (HIF) is a set of transcription factors that regulate the expression of nearly 200 genes that can affect the cellular adaptive responses to hypoxia and/or ischemia.^{11–15} This review will examine the activation of HIF in response to hypoxia, and examine the evidence suggesting the potential benefits or deficits arising from HIF activation in I/R injury.

Regulation of HIF During Hypoxia

The hypoxia-inducible factor transcription factors function as master regulators of gene expression in response to

¹Department of Surgery, University of Chicago, Chicago, IL, USA and ²Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

*Corresponding author: PT Schumacker, Department of Pediatrics, 303 E Chicago Avenue, Ward Bldg 12-191, Chicago, IL 60611, USA. Tel: +1 312 503 1475; Fax: +1 312 503 2441; E-mail: p-schumacker@northwestern.edu

Keywords: hypoxia; oxidant stress; preconditioning; apoptosis

Abbreviations: DMOG, dimethylxalglycine; HIF(- α), hypoxia-inducible factor (α subunit); I/R, ischemia–reperfusion; IPC, ischemic preconditioning; ODD, oxygen-dependent degradation; PHD, prolyl hydroxylase; ROS, reactive oxygen species; VEGF, vascular endothelial growth factor; vHL, von Hippel-Lindau

Received 04.1.08; accepted 07.1.08; Edited by NS Chandel; published online 08.2.08

hypoxia.¹⁶ HIF is a heterodimer comprised of α and β subunits, which are constitutively expressed.^{17,18} Expression of the β subunit (ARNT) is independent of $[O_2]$, whereas protein stability of the α subunit is regulated in accordance with cellular O_2 levels.^{17,19–21} Under normoxic conditions, the α subunit is degraded through a process involving hydroxylation of conserved proline residues in a region of the peptide referred to as the oxygen-dependent degradation (ODD) domain.^{20,22} A family of 2-oxoglutarate-dependent prolyl-4-hydroxylases (PHDs) is responsible for this event, which requires O_2 , iron, 2-oxoglutarate (α -ketoglutarate), and ascorbate. The hydroxylated proline residues in the ODD domain of HIF- α facilitate recognition by the von Hippel-Lindau (vHL) protein.^{23,24} The vHL protein functions as the E3 ubiquitin ligase that labels the α subunit for degradation by the proteasome.^{23–25} Therefore, only low levels of HIF activity are sustained under normoxia. During hypoxia, the activity of PHDs becomes inhibited, allowing the α subunit to accumulate, heterodimerize with ARNT, and initiate transcription.^{19,26} Other aspects of HIF regulation include hydroxylation of conserved asparagine residues in the C-terminal region of HIF- α , which contributes to the regulation of HIF transcriptional activity.^{27,28} Two isoforms of the α subunit with high sequence homology, HIF-1 α and HIF-2 α , have been identified.²⁹ Germline deletion of either HIF subunit results in embryonic lethality, although differences in the resulting phenotypes suggest that important and unique roles exist for both HIF-1 α and HIF-2 α .^{30–33}

At the simplest level, HIF-dependent expression of glycolytic genes could confer protection by enhancing the capacity for ATP generation by anaerobic glycolysis. However, other mechanisms could also contribute. For example, mice with HIF-2 α germline deletion that survive to birth exhibit multiple organ dysfunctions associated with decreased expression of antioxidant enzymes and evidence of excessive cellular oxidant stress.³⁴ This suggests that HIF-2 might be important in affecting cell survival during I/R through the regulation of cellular antioxidant capacity. Similarly, HIF-dependent genes such as Heme Oxygenase-1 (HO-1) may regulate cell survival in I/R by affecting the response to oxidant stress.³⁵ Other genes regulated by HIF include inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), both of which have been associated with enhanced resistance to ischemia in mice.^{36,37} HIF-dependent genes such as vascular endothelial growth factor (VEGF) are important for regulating local O_2 supply, and may also be important in I/R because they regulate collateral vessel development.^{35,38–41} Hence, the specific mechanisms by which HIF activation might be expected to affect cell survival or death in I/R are not fully understood, but likely involve multiple pathways that regulate antioxidant capacity, angiogenesis, cell death pathways, antiapoptotic pathways, and other diverse functions. Moreover, the gene expression pattern in response to HIF activation is cell type-specific. Hence, the protection against I/R injury conferred by HIF activation in one cell lineage may not be evident in other cell types.

HIF-1 α Activation in Ischemic Myocardium

Coronary artery disease is a major clinical cause of lethal cardiac ischemia.⁴² Several studies have looked at the

hypoxic response of the myocardium to acute or evolving infarctions. Lee *et al.*⁴³ found increased HIF-1 α and VEGF mRNA and protein in ventricular biopsy specimens from patients undergoing coronary bypass surgery who had pathological evidence of infarction or acute ischemia. In rats, widespread induction of HIF-1 α was observed after exposure to systemic hypoxia, whereas localized expression after coronary occlusion occurred primarily at the border of infarcted tissue and persisted for 4 weeks. Upregulation of HIF-dependent proteins such as HO-1 and Glut-1 were also detected in the peri-infarct zone.³⁸ Kido *et al.*³⁹ employed a transgenic model involving constitutive overexpression of HIF-1 α in the myocardium. These mice showed no improvement at 24 h after coronary artery occlusion, but at 4 weeks, they demonstrated an attenuated infarct size and improved cardiac function. Immunohistochemical analysis showed increased expression of HIF-1 α and its downstream targets, VEGF and iNOS, in the peri-infarct regions. In a related study, Date *et al.*⁴⁴ showed that exposing cultured rat cardiomyocytes to 3 h of hypoxia followed by 14 h of normoxia resulted in an increase in VEGF, Glut-1, Glut-4, and iNOS mRNA. Infection with an adenovirus designed to overexpress HIF-1 α caused a similar increase in mRNA expression and a reduction in I/R-induced cell death. These studies suggest that HIF-dependent gene expression could potentially modify cell survival in myocardial I/R (Figure 1).

HIF-1 α Attenuates Ischemia–reperfusion Injury Through Induction of iNOS

Hypoxia-inducible factor-1 may confer protection against I/R injury by triggering the activation of specific protective genes. iNOS is a known downstream target of HIF-1, and has been shown to be cardioprotective in acute and chronic heart disease.^{45–48} In one study, siRNA silencing of PHD expression in murine microvascular endothelial cells produced a time- and dose-dependent increase in HIF-1 α protein stabilization and a corresponding increase in iNOS mRNA message.⁴⁹ Treatment of intact murine hearts with the siRNA

Figure 1 Schematic overview of the influence of HIF-1 on cell survival and cell death pathways

construct produced a decrease in infarct size and cardiac dysfunction following global I/R. The improved functional recovery was lost in iNOS^{-/-} mice.⁴⁹ In a similar study using a Langendorf perfusion model, Xi *et al.*⁵⁰ showed a reduction in infarct size after selectively activating HIF-1 α using cobalt chloride (CoCl₂). An increase in DNA-binding activity of HIF-1 α was noted within 1 h after CoCl₂ injection. The infarct-limiting effect of CoCl₂ was absent in iNOS knockout mice, again demonstrating the importance of iNOS in the HIF cardioprotective pathway.

Role of HO-1 in Mediating the Protective Effect of HIF-1 Against Ischemia-Reperfusion Injury

Heme Oxygenase-1 expression is increased by HIF-1 α activation.⁴¹ HO-1 degrades heme and generates carbon monoxide and bilirubin, both of which may have antioxidant and/or anti-inflammatory effects. Hearts from transgenic mice overexpressing cardiac-specific HO-1 show a significant reduction in infarct size following I/R.⁵¹ Plasmid systems that upregulate HO-1 expression during hypoxia are also cardioprotective.⁵² Several studies investigating the protective effects of HIF-1 have focused on the role of HO-1 in this response. Zhu *et al.*⁵³ studied protection against I/R in the retina, and found that repeated exposure to hypoxia over 12 days led to sustained increases in HIF-1 α and HO-1 protein expression, which was associated with significant protection. Pachori *et al.*⁵⁴ used a plasmid with a promoter containing the hypoxia-responsive element to induce overexpression of HO-1 during ischemia in mice. Cardiac, hind-limb, or portal vein injection of this construct caused a reduction in I/R injury to the heart, hind limb, and liver, respectively. In another study, rabbits were treated with dimethylxalglycine (DMOG), a PHD inhibitor, to stabilize HIF-1 α during normoxia. These animals demonstrated an increase in myocardial HO-1 expression along with a reduction in infarct size and improved functional recovery after I/R.⁴¹ Interestingly, plasma IL-8 and myocardial myeloperoxidase activity were also decreased. In human microvascular endothelial cells (HMEC), TNF- α -dependent IL-8 promoter activity and protein secretion decreased with increasing concentrations of DMOG in a dose-dependent manner. A decrease in TNF- α -induced migration of polymorphonuclear cells across HMEC-1 monolayers was also noted.⁴¹ These findings suggest that HIF activation attenuates ischemic injury through an anti-inflammatory effect, possibly due to HO-1 expression. This is consistent with the data showing a downregulation of Kit ligand and complement factor H, but inconsistent with the studies showing HIF-dependent upregulation of inflammatory mediators such as MHC I, complement, and interferon in mouse embryonic fibroblasts and cardiac cells exposed to hypoxia.^{55,56}

Role of HIF in the Early and Late Phases of Preconditioning Protection

When a coronary artery is permanently occluded, a core region of necrosis develops because cells cannot survive when deprived of oxygen and glucose for a prolonged period. However, cells in the peri-infarct region have the potential for

rescue because they may still receive some support from adjacent tissue regions via diffusion. During I/R injury, cells are deprived of oxygen and glucose for a limited time, although the peripheral tissue regions may still benefit from nutrient diffusion from surrounding tissue. Reperfusion of the ischemic region restores nutrient supply, but may trigger cell damage by promoting the generation of reperfusion oxidant stress. In either case, the onset of tissue ischemia in patients is often rapid and unanticipated.

Ischemic preconditioning is a phenomenon whereby brief periods of ischemia render the heart resistant to subsequent periods of lethal I/R. Protection by IPC is initiated by a 'triggering' event that activates an 'effector' process that confers protection.⁵⁷⁻⁵⁹ Protection against I/R-induced cell death is evident during an 'immediate/early window', and again after 24 h during the 'late window' of protection.^{60,61} The mechanism triggering these events is not fully understood, but ROS from mitochondria have been implicated, along with the opening of a mitochondrial ATP-dependent potassium channel (mito K_{ATP}).^{61,62} Some studies suggest that increases in ROS signaling lead to opening of the K_{ATP} channel,⁵⁹ while others suggest that K_{ATP} opening leads to an increase in ROS production, which then mediates subsequent protection.⁶³ Hypoxia itself leads to an increase in mitochondrial ROS production⁶⁴ and subsequent HIF-1 α activation,^{65,66} so it is conceivable that ROS signaling during the triggering phase leads to HIF-dependent gene expression that results in protection.

In the context of ischemia or I/R, one might predict that HIF-1 α activation could confer protection through its effects on downstream targets in affecting vascular tone, angiogenesis, and glycolytic metabolism. Indeed, several HIF-dependent genes have been implicated in the late phase of preconditioning, including iNOS,³⁷ cyclooxygenase-2,^{36,67} and HO-1^{68,69} (Figure 2). However, a direct test of the hypothesis that HIF activation is the common factor linking the expression of these protective genes has not been reported.

Given that HIF acts exclusively as a transcription factor, and that late preconditioning protection requires gene transcription and translation of new proteins, one might predict that HIF-mediated protection would require time between the triggering stimulus and the lethal stress to effect protection. In

Figure 2 Role of HIF-dependent gene expression on regulation of processes affecting cell death in I/R, possibly through the regulation of inducible nitric oxide synthase (iNOS), heme oxygenase-1 (HO-1), cyclooxygenase-2 (COX-2), and antioxidant enzymes

Figure 3 HIF-dependent gene expression and protection from I/R. Under normal conditions, low basal activation of HIF contributes to the constitutive gene expression pattern. This profile affects the sensitivity of the cells to I/R, and their ability to respond to acute preconditioning triggers. Late-phase preconditioning induced by hypoxia, DMOG, CoCl_2 , or other preconditioning triggers may involve enhanced activation of HIF, resulting in increased expression of genes that augment resistance to I/R-induced cell death

this regard, HIF could be viewed as an anticipatory response whose activation would need to precede the lethal ischemic stimulus. Therefore, it would seem unlikely that HIF could be involved in the immediate/early phase of IPC. However, recent evidence indicates that HIF status may affect immediate/early preconditioning as well. Cai *et al.*⁷⁰ subjected wild-type mice, or mice heterozygous with respect to HIF-1 α (HIF-1 $\alpha^{+/-}$) to brief periods of ischemia, followed by 30 min of continuous ischemia. Preconditioning improved infarct size and function after I/R in wild-type animals, but not in HIF-1 $\alpha^{+/-}$ hearts. These studies strongly implicate HIF-1 α in the immediate protection conferred by IPC. However, this response does not necessarily mean that immediate protection is conferred by the acute activation of HIF-dependent genes. Rather, it is possible that a decreased constitutive expression of a set of HIF-regulated genes in the HIF-1 $\alpha^{+/-}$ hearts might have undermined their ability to activate or transmit the signals necessary for triggering protection in response to an acute preconditioning stimulus (Figure 3). For example, Cai *et al.* noted that mitochondrial oxidant signals were inhibited in the heterozygous animals, which could have undermined their ability to trigger preconditioning in response to brief ischemia. Consistent with this interpretation, they found that the hearts responded to preconditioning by adenosine, which triggers protection through a pathway that does not require mitochondrial ROS signaling.⁵⁹

Summary

Ischemia, I/R, and chronic hypoxia are all capable of engaging cellular death pathways leading to tissue injury and organ dysfunction. HIF is the principal regulator of cellular transcriptional responses to hypoxia. During hypoxia, HIF triggers the expression of genes involved in oxygen transport, oxygen utilization, glycolytic metabolism, cell death, cell survival, and other processes that can affect cell survival in ischemia. Prior activation of HIF can confer protection against these insults at the cellular, tissue, and organ level. However, low levels of

HIF remain active even under normoxic conditions, allowing HIF to contribute to the regulation of genes that define the proteomic state of the cell. In this regard, basal HIF activity can affect cellular resistance to acute stresses that do not permit sufficient time to mount a *de novo* transcription–translation response. There is still much to learn about the role of HIF in mediating the cellular and tissue responses to I/R, and an ability to manipulate HIF activity may potentially impact our treatment of ischemic tissue injury.

Acknowledgements. Supported by NHLBI Grants HL35440 and HL079650, and by the American Heart Association.

- Braunwald E, Kloner RA. Myocardial reperfusion: a double-edged sword? *J Clin Invest* 1985; **76**: 1713–1719.
- Logue SE, Gustafsson AB, Samali A, Gottlieb RA. Ischemia/reperfusion injury at the intersection with cell death. *J Mol Cell Cardiol* 2005; **38**: 21–33.
- Bernardi P, Krauskopf A, Basso E, Petronilli V, Blachly-Dyson E, Di LF *et al.* The mitochondrial permeability transition from *in vitro* artifact to disease target. *FEBS J* 2006; **273**: 2077–2099.
- Bolli R. Cardioprotective function of inducible nitric oxide synthase and role of nitric oxide in myocardial ischemia and preconditioning: an overview of a decade of research. *J Mol Cell Cardiol* 2001; **33**: 1897–1918.
- Hanley PJ, Daut J. KATP channels and preconditioning: A re-examination of the role of mitochondrial KATP channels and an overview of alternative mechanisms. *J Mol Cell Cardiol* 2005; **39**: 17–50.
- Garlid KD, Dos SP, Xie ZJ, Costa ADT, Paucek P. Mitochondrial potassium transport: the role of the mitochondrial ATP-sensitive K⁺ channel in cardiac function and cardioprotection. *Biochim Biophys Acta* 2003; **1606**: 1–21.
- Gross GJ, Peart JN. KATP channels and myocardial preconditioning: an update. *Am J Physiol Heart Circ Physiol* 2003; **285**: H921–H930.
- Gross GJ, Auchampach JA. Role of ATP dependent potassium channels in myocardial ischemia. *Cardiovasc Res* 1992; **26**: 1011–1016.
- Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 1986; **74**: 1124–1136.
- Bolli R. The late phase of preconditioning. *Circ Res* 2000; **87**: 972–983.
- Semenza GL. HIF-1: mediator of physiological and pathophysiological responses to hypoxia. *J Appl Physiol* 2000; **88**: 1474–1480.
- Hu CJ, Wang LY, Chodosh LA, Keith B, Simon MC. Differential roles of hypoxia-inducible factor 1 α (HIF-1 α) and HIF-2 α in hypoxic gene regulation. *Mol Cell Biol* 2003; **23**: 9361–9374.
- Bell EL, Emerling BM, Chandel NS. Mitochondrial regulation of oxygen sensing. *Mitochondrion* 2005; **5**: 322–332.
- Semenza GL. HIF-1, O₂, and the 3 PHDs: how animal cells signal hypoxia to the nucleus. *Cell* 2001; **107**: 1–3.
- Schumacker PT. Hypoxia-inducible factor-1 (HIF-1). *Crit Care Med* 2005; **33** (12 Suppl): S423–S425.
- Mazure NM, Brahimi-Horn MC, Berta MA, Benizri E, Bilton RL, Dayan F *et al.* HIF-1: master and commander of the hypoxic world – a pharmacological approach to its regulation by siRNAs. *Biochem Pharmacol* 2004; **68**: 971–980.
- Wang GL, Jiang B-H, Rue EA, Semenza GL. Hypoxia-inducible factor 1 is a basic-helix–loop–helix-PAS heterodimer regulated by cellular O₂ tension. *Proc Natl Acad Sci USA* 1995; **92**: 5510–5514.
- Wang GL, Semenza GL. Characterization of hypoxia-inducible factor 1 and regulation of DNA binding activity by hypoxia. *J Biol Chem* 1993; **268**: 21513–21518.
- Semenza GL. Signal transduction to hypoxia-inducible factor 1. *Biochem Pharmacol* 2002; **64**: 993–998.
- Huang LE, Arany Z, Livingston DM, Bunn HF. Activation of hypoxia-inducible transcription factor depends primarily upon redox-sensitive stabilization of its α subunit. *J Biol Chem* 1996; **271**: 32253–32259.
- Huang LE, Gu J, Schau M, Bunn HF. Regulation of hypoxia-inducible factor 1 α is mediated by an O₂-dependent degradation domain via the ubiquitin–proteasome pathway. *Proc Natl Acad Sci USA* 1998; **95**: 7987–7992.
- Pugh CW, O'Rourke JF, Nagao M, Glead JM, Ratcliffe PJ. Activation of hypoxia-inducible factor-1; definition of regulatory domains within the α subunit. *J Biol Chem* 1997; **272**: 11205–11214.
- Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ *et al.* Targeting of HIF- α to the von Hippel-Lindau ubiquitylation complex by O₂-regulated prolyl hydroxylation. *Science* 2001; **292**: 468–472.
- Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohn M *et al.* HIF α targeted for VHL-mediated destruction by proline hydroxylation: implications for O₂ sensing. *Science* 2001; **292**: 464–468.

25. Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME *et al.* The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature* 1999; **399**: 271–275.
26. Mole DR, Maxwell PH, Pugh CW, Ratcliffe PJ. Regulation of HIF by the von Hippel-Lindau tumour suppressor: implications for cellular oxygen sensing. *IUBMB Life* 2001; **52**: 43–47.
27. Lando D, Peet DJ, Gorman JJ, Whelan DA, Whitelaw ML, Bruck RK. FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor. *Genes Dev* 2002; **16**: 1466–1471.
28. Bracken CP, Fedele AO, Linke S, Balrak W, Lisy K, Whitelaw ML *et al.* Cell-specific regulation of hypoxia-inducible factor (HIF)-1alpha and HIF-2alpha stabilization and transactivation in a graded oxygen environment. *J Biol Chem* 2006; **281**: 22575–22585.
29. Wiesener MS, Turley H, Allen WE, William C, Eckardt KU, Talks KL *et al.* Induction of endothelial PAS domain protein-1 by hypoxia: characterization and comparison with hypoxia-inducible factor-1alpha. *Blood* 1998; **92**: 2260–2268.
30. Hu CJ, Iyer S, Sataur A, Covello KL, Chodosh LA, Simon MC. Differential regulation of the transcriptional activities of hypoxia-inducible factor 1 alpha (HIF-1alpha) and HIF-2alpha in stem cells. *Mol Cell Biol* 2006; **26**: 3514–3526.
31. Raval RR, Lau KW, Tran MGB, Sowter HM, Mandriota SJ, Li JL *et al.* Contrasting properties of hypoxia-inducible factor 1 (HIF-1) and HIF-2 in von Hippel-Lindau-associated renal cell carcinoma. *Mol Cell Biol* 2005; **25**: 5675–5686.
32. Sowter HM, Raval R, Moore J, Ratcliffe PJ, Harris AL. Predominant role of hypoxia-inducible transcription factor (Hif)-1alpha versus Hif-2alpha in regulation of the transcriptional response to hypoxia. *Cancer Res* 2003; **63**: 6130–6134.
33. Covello KL, Kehler J, Yu HW, Gordan JD, Arsham AM, Hu CJ *et al.* HIF-2alpha regulates Oct-4: effects of hypoxia on stem cell function, embryonic development, and tumor growth. *Genes Dev* 2006; **20**: 557–570.
34. Scortegagna M, Ding K, Oktay Y, Gaur A, Thurmond F, Yan LJ *et al.* Multiple organ pathology, metabolic abnormalities and impaired homeostasis of reactive oxygen species in Epas1^{-/-} mice. *Nat Genet* 2003; **35**: 331–340.
35. Semenza GL. Surviving ischemia: adaptive responses mediated by hypoxia-inducible factor 1. *J Clin Invest* 2000; **106**: 809–812.
36. Bolli R, Shimura K, Tang XL, Kodani E, Xuan YT, Guo Y *et al.* Discovery of a new function of cyclooxygenase (COX)-2: COX-2 is a cardioprotective protein that alleviates ischemia/reperfusion injury and mediates the late phase of preconditioning. *Cardiovasc Res* 2002; **55**: 506–519.
37. Guo Y, Jones WK, Xuan YT, Tang XL, Bao W, Wu WJ *et al.* The late phase of ischemic preconditioning is abrogated by targeted disruption of the inducible NO synthase gene [see comments]. *Proc Natl Acad Sci USA* 1999; **96**: 11507–11512.
38. Jurgensen JS, Rosenberger C, Wiesener MS, Warnecke C, Horstrup JH, Grafe M *et al.* Persistent induction of HIF-1alpha and -2alpha in cardiomyocytes and stromal cells of ischemic myocardium. *FASEB J* 2004; **18**: 1415–1417.
39. Kido M, Du L, Sullivan CC, Li X, Deutsch R, Jamieson SW *et al.* Hypoxia-inducible factor 1-alpha reduces infarction and attenuates progression of cardiac dysfunction after myocardial infarction in the mouse. *J Am Coll Cardiol* 2005; **46**: 2116–2124.
40. Lu MJ, Chang H, Chang CC, Wang BW, Shyu KG. Temporal and spatial expression of hypoxia-inducible factor-1alpha and vascular endothelial growth factor in a rat model of myocardial ischemia with or without reperfusion. *J Formos Med Assoc* 2005; **104**: 707–714.
41. Ockaili R, Natarajan R, Salloum F, Fisher BJ, Jones D, Fowler III AA *et al.* HIF-1 activation attenuates postischemic myocardial injury: role for heme oxygenase-1 in modulating microvascular chemokine generation. *Am J Physiol Heart Circ Physiol* 2005; **289**: H542–H548.
42. Thom T, Haase N, Rosamond W, Howard VJ, Rumsfeld J, Manolio T *et al.* Heart disease and stroke statistics – 2006 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation* 2006; **113**: e85–e151.
43. Lee SH, Wolf PL, Escudero R, Deutsch R, Jamieson SW, Thistlethwaite PA. Early expression of angiogenesis factors in acute myocardial ischemia and infarction. *N Engl J Med* 2000; **342**: 626–633.
44. Date T, Mochizuki S, Belanger AJ, Yamakawa M, Luo ZG, Vincent KA *et al.* Expression of constitutively stable hybrid hypoxia-inducible factor-1alpha protects cultured rat cardiomyocytes against simulated ischemia-reperfusion injury. *Am J Physiol Cell Physiol* 2005; **288**: C314–C320.
45. Tang XQ, Yu HM, Zhi JL, Cui Y, Tang EH, Feng JQ *et al.* Inducible nitric oxide synthase and cyclooxygenase-2 mediate protection of hydrogen peroxide preconditioning against apoptosis induced by oxidative stress in PC12 cells. *Life Sci* 2006; **79**: 870–876.
46. Bulhak AA, Sjoquist PO, Xu CB, Edvinsson L, Pernow J. Protection against myocardial ischaemia/reperfusion injury by PPAR-alpha activation is related to production of nitric oxide and endothelin-1. *Basic Res Cardiol* 2006; **101**: 244–252.
47. Cuong DV, Kim N, Youm JB, Joo H, Warda M, Lee JW *et al.* Nitric oxide-cGMP-protein kinase G signaling pathway induces anoxic preconditioning through activation of ATP-sensitive K⁺ channels in rat hearts. *Am J Physiol Heart Circ Physiol* 2006; **290**: H1808–H1817.
48. Jones SP, Bolli R. The ubiquitous role of nitric oxide in cardioprotection. *J Mol Cell Cardiol* 2006; **40**: 16–23.
49. Natarajan R, Salloum FN, Fisher BJ, Kukreja RC, Fowler III AA. Hypoxia inducible factor-1 activation by prolyl 4-hydroxylase-2 gene silencing attenuates myocardial ischemia reperfusion injury. *Circ Res* 2006; **98**: 133–140.
50. Xi L, Taher M, Yin C, Salloum F, Kukreja RC. Cobalt chloride induces delayed cardiac preconditioning in mice through selective activation of HIF-1alpha and AP-1 and iNOS signaling. *Am J Physiol Heart Circ Physiol* 2004; **287**: H2369–H2375.
51. Yet SF, Tian R, Layne MD, Wang ZY, Maemura K, Solovyeva M *et al.* Cardiac-specific expression of heme oxygenase-1 protects against ischemia and reperfusion injury in transgenic mice. *Circ Res* 2001; **89**: 168–173.
52. Tang YL, Tang Y, Zhang YC, Agarwal A, Kasahara H, Qian K *et al.* A hypoxia-inducible vigilant vector system for activating therapeutic genes in ischemia. *Gene Therapy* 2005; **12**: 1163–1170.
53. Zhu Y, Zhang Y, Ojwang BA, Brantley Jr MA, Gidday JM. Long-term tolerance to retinal ischemia by repetitive hypoxic preconditioning: role of HIF-1alpha and heme oxygenase-1. *Invest Ophthalmol Vis Sci* 2007; **48**: 1735–1743.
54. Pachori AS, Melo LG, Hart ML, Noiseux N, Zhang L, Morello F *et al.* Hypoxia-regulated therapeutic gene as a preemptive treatment strategy against ischemia/reperfusion tissue injury. *Proc Natl Acad Sci U S A* 2004; **101**: 12282–12287.
55. Chen WJ, Chen HW, Yu SL, Huang CH, Wang TD, Chen JJ *et al.* Gene expression profiles in hypoxic preconditioning using cDNA microarray analysis: altered expression of an angiogenic factor, carcinoembryonic antigen-related cell adhesion molecule 1. *Shock* 2005; **24**: 124–131.
56. Greijer AE, van der Groep P, Kemming D, Shvarts A, Semenza GL, Meijer GA *et al.* Up-regulation of gene expression by hypoxia is mediated predominantly by hypoxia-inducible factor 1 (HIF-1). *J Pathol* 2005; **206**: 291–304.
57. Cohen MV, Yang XM, Liu GS, Heusch G, Downey JM. Acetylcholine, bradykinin, opioids, and phenylephrine, but not adenosine, trigger preconditioning by generating free radicals and opening mitochondrial K(ATP) channels. *Circ Res* 2001; **89**: 273–278.
58. Gross GJ, Fryer RM. Mitochondrial KATP channels – triggers or distal effectors of ischemic or pharmacological preconditioning? *Circ Res* 2000; **87**: 431–433.
59. Lebuffe G, Schumacker PT, Shao ZH, Anderson T, Iwase H, Vanden Hoek TL. ROS and NO trigger early preconditioning: relationship to mitochondrial KATP channel. *Am J Physiol Heart Circ Physiol* 2003; **284**: H299–H308.
60. Bolli R, Bhatti ZA, Tang XL, Qiu Y, Zhang Q, Guo Y *et al.* Evidence that late preconditioning against myocardial stunning in conscious rabbits is triggered by the generation of nitric oxide. *Circ Res* 1997; **81**: 42–52.
61. Sun JZ, Tang XL, Park SW, Qiu Y, Turens JF, Bolli R. Evidence for an essential role of reactive oxygen species in the genesis of late preconditioning against myocardial stunning in conscious pigs. *J Clin Invest* 1996; **97**: 562–576.
62. Vanden Hoek TL, Becker LB, Shao Z, Li C, Schumacker PT. Reactive oxygen species released from mitochondria during brief hypoxia induce preconditioning in cardiomyocytes. *J Biol Chem* 1998; **273**: 18092–18098.
63. Pain T, Yang XM, Critz SD, Yue Y, Nakano A, Liu GS *et al.* Opening of mitochondrial KATP channels triggers the preconditioned state by generating free radicals. *Circ Res* 2000; **87**: 460–466.
64. Duranteau J, Chandel NS, Kulisz A, Shao Z, Schumacker PT. Intracellular signaling by reactive oxygen species during hypoxia in cardiomyocytes. *J Biol Chem* 1998; **273**: 11619–11624.
65. Chandel NS, McClintock DS, Feliciano CE, Wood TM, Melendez JA, Rodriguez AM *et al.* Reactive oxygen species generated at mitochondrial Complex III stabilize HIF-1alpha during hypoxia: A mechanism of O₂ sensing. *J Biol Chem* 2000; **275**: 25130–25138.
66. Guzy RD, Schumacker PT. Oxygen sensing by mitochondria at complex III: the paradox of increased reactive oxygen species during hypoxia. *Exp Physiol* 2006; **91**: 807–819.
67. Wang Y, Kodani E, Wang JX, Zhang SX, Takano H, Tang XL *et al.* Cardioprotection during the final stage of the late phase of ischemic preconditioning is mediated by neuronal NO synthase in concert with cyclooxygenase-2. *Circ Res* 2004; **95**: 84–91.
68. Dawn B, Bolli R. HO-1 induction by HIF-1: a new mechanism for delayed cardioprotection? *Am J Physiol Heart Circ Physiol* 2005; **289**: H522–H524.
69. Yoshida T, Maulik N, Ho YS, Alam J, Das DK. H(mox-1) constitutes an adaptive response to effect antioxidant cardioprotection: A study with transgenic mice heterozygous for targeted disruption of the Heme oxygenase-1 gene. *Circulation* 2001; **103**: 1695–1701.
70. Cai Z, Zhong H, Bosch-Marce M, Fox-Talbot K, Wang L, Wei C *et al.* Complete loss of ischaemic preconditioning-induced cardioprotection in mice with partial deficiency of HIF-1a. *Cardiovasc Res* 2007; **77** (in press).