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Invited Review

HIGHLIGHTED TOPIC | *Oxygen Sensing in Health and Disease*

O_2-regulated gene expression: transcriptional control of cardiorespiratory physiology by HIF-1

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Semenza, Gregg L. O_2-regulated gene expression: transcriptional control of cardiorespiratory physiology by HIF-1. *J Appl Physiol* 96: 1173–1177, 2004; 10.1152/japplphysiol.00770.2003.—The cardiovascular and respiratory systems play key roles in O_2 homeostasis. Physiological responses to hypoxia involve changes in gene expression that are mediated by the transcriptional activator hypoxia-inducible factor (HIF)-1. Analysis of mice heterozygous for a knockout allele at the locus encoding the O_2-regulated HIF-1α or HIF-2α subunit has revealed that these proteins are required for multiple physiological responses to chronic hypoxia, including erythrocytosis and pulmonary vascular remodeling. In mice with partial HIF-2α deficiency, hypoxia-induced expression of endothelin-1 and norepinephrine is dramatically impaired, and the mice fail to develop pulmonary hypertension after 4 wk of exposure to 10% O_2. In mice with partial HIF-1α deficiency, the ability of the carotid body to sense and/or respond to acute or chronic hypoxia is lost. In wild-type mice, brief episodes of intermittent hypoxia are sufficient to induce production of erythropoietin (EPO), which protects the heart against apoptosis after ischemia-reperfusion, whereas in mice with partial HIF-1α deficiency, intermittent hypoxia does not induce EPO production or cardiac protection. Parenteral administration of EPO to rodents is sufficient to induce dramatic protection against ischemia-reperfusion injury in the heart. Thus HIF-1 mediates critical physiological responses to hypoxia, and the elucidation of these homeostatic mechanisms may lead to novel therapies for the most common causes of mortality in the US population.

hypoxia; pulmonary hypertension; erythropoietin; hypoxia-inducible factor

THE FUNCTION OF THE CARDIORESPIRATORY system is to deliver O_2 sufficient for each cell of the body to maintain normal metabolism. When O_2 delivery is impaired, the resulting hypoxia activates homeostatic mechanisms in the cardiovascular and respiratory systems. When hypoxia persists for prolonged periods of time (i.e., more than a few minutes), the response involves changes in gene expression. Several transcription factors have been shown to mediate physiological responses to hypoxia. Early growth response-1 and nuclear factor interleukin-6 appear to play important roles in mediating inflammatory and thrombotic responses to hypoxia in the lung (47–49). The activation of these transcription factors in response to hypoxia occurs only in restricted cell types, and the molecular mechanisms are not well established. In contrast, hypoxia-inducible factor (HIF)-1 functions as a global regulator of O_2 homeostasis. HIF-1 activity is induced by hypoxia in all nucleated cell types via a novel posttranslational mechanism and plays critical roles in the responses of the cardiovascular and respiratory systems to hypoxia.

The purification of HIF-1 revealed that it was a heterodimeric protein composed of HIF-1α and HIF-1β subunits (44). Nucleotide sequence analysis (43) revealed that both proteins contained basic helix-loop-helix-PAS domains [which were first identified in the proteins PER, aryl hydrocarbon nuclear translocator (ARNT), and SIM] and that HIF-1β was identical to ARNT, which was originally identified as a dimerization partner of the aryl hydrocarbon receptor. Subsequently, database searches led to the identification of a protein with sequence similarity to HIF-1α that was designated HIF-2α (14, 16, 17, 41). HIF-2α was shown to dimerize with HIF-1β and bind to the same DNA sequence as heterodimers containing HIF-1α and HIF-1β. Several other basic helix-loop-helix-PAS proteins have been identified that can dimerize with members of the ARNT family [HIF-1β (ARNT), ARNT2, and BMAL1 (ARNT3)], such as the neuronal PAS domain proteins NPAS1 and NPAS2 (52). However, these proteins do not mediate hypoxia-inducible gene expression.

The molecular basis for hypoxia-induced expression of HIF-1 is the O_2-dependent hydroxylation of proline residues in HIF-1α or HIF-2α, which promotes binding of the von Hippel-Lindau tumor suppressor protein, the recognition component of an E3 ubiquitin-protein ligase that targets the proteins for proteasomal degradation (15, 19, 21, 51). In addition, the O_2-dependent hydroxylation of an asparagine residue in HIF-1α and HIF-2α blocks the binding of the coactivator proteins CREB binding protein and p300 (24). Thus the expression and activity of HIF-1α and HIF-2α are O_2 regulated (Fig. 1).

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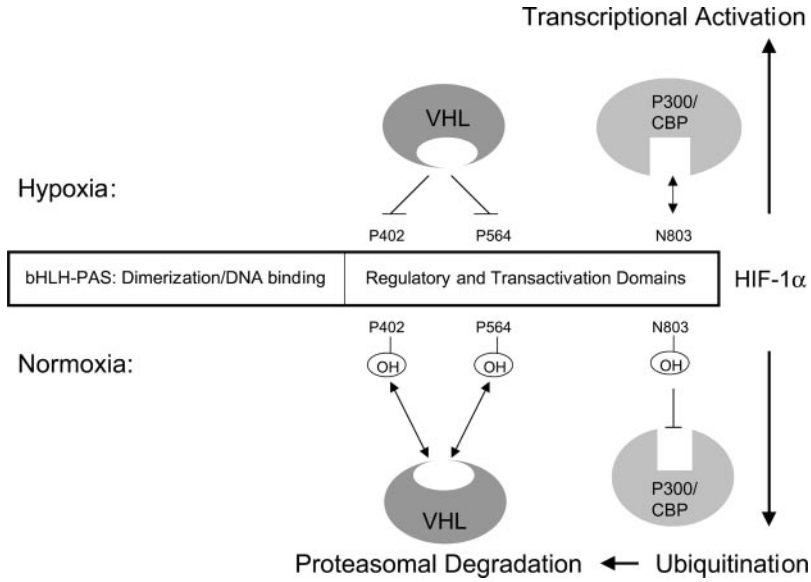

Fig. 1. O_2-dependent regulation of hypoxia-inducible factor (HIF)-1α protein stability and transcriptional activity. The NH_2-terminal half of HIF-1α consists of the basic helix-loop-helix (bHLH)-PAS domain, which mediates dimerization with HIF-1β and DNA binding. The COOH-terminal half consists of domains that regulate protein stability and transcriptional activity. Under normoxic conditions, HIF-1α is hydroxylated on Pro^{402} (P402), Pro^{564} (P564), and Asp^{803} (N803). Hydroxylation of Pro^{402} and Pro^{564} is required for interaction of HIF-1α with von Hippel-Lindau (VHL) tumor suppressor protein, the recognition component of an E3 ubiquitin-protein ligase that targets HIF-1α for degradation by the 26S proteasome. Hydroxylation of Asp^{803} blocks interaction of HIF-1α with the coactivators CREB binding protein (CBP) and p300. Under hypoxic conditions, O_2 becomes rate limiting, and the fraction of HIF-1α that is hydroxylated declines in proportion to P_{O_2}. Thus the cellular O_2 concentration directly regulates expression and activity of HIF-1α. HIF-2α also contains 2 proline residues and 1 asparagine residue that are subject to O_2-dependent hydroxylation.

HIF-1α and HIF-1β are expressed in most cell types, whereas HIF-2α shows a more restricted pattern of expression that includes the developing lung, vasculature, and catecholamine-producing cells. Homozygous targeted inactivation of the mouse *Hif1a* gene encoding HIF-1α resulted in embryonic lethality at midgestation (9, 20, 23, 32). In contrast, the effect of homozygous targeted inactivation of the gene encoding HIF-2α was less severe and more variable in its effects on the developing cardiovascular and respiratory systems (11, 29, 40). Whereas the analysis of homozygous knockout mice demonstrated the critical roles of HIF-1α and HIF-2α in development, analysis of heterozygous knockout mice has demonstrated critical roles for these factors in postnatal physiology.

PULMONARY AND SYSTEMIC RESPONSES TO CHRONIC HYPOXIA

The first study in which this approach was utilized was the analysis of $Hif1a^{+/-}$ and wild-type littermates exposed to 10% O_2 for 1–6 wk. In response to chronic hypoxia, the development of polycythemia was significantly impaired in $Hif1a^{+/-}$ mice compared with wild-type littermates (50). This effect was consistent with the original identification of HIF-1 as an activator of the *EPO* gene, which encodes erythropoietin (EPO), the peptide hormone that regulates red blood cell production (36). The development of hypoxia-induced pulmonary hypertension, as manifested by the presence of right ventricular hypertrophy and increased right ventricular pressure, was also impaired in $Hif1a^{+/-}$ mice (50). In $Hif1a^{+/-}$ mice exposed to 10% O_2 for 3 wk, morphometric analysis of the small pulmonary arterioles that are subject to hypoxia-induced remodeling revealed a reduced number of completely muscularized vessels and reduced medial wall thickness in these vessels (50).

The luminal diameter of pulmonary arterioles is reduced by depolarization of the vascular smooth muscle cells, resulting in vessel constriction, and by hypertrophy of these same cells. To analyze these physiological responses, pulmonary arteriolar smooth muscle cells (PASMCs) were isolated from mice exposed to room air (21% O_2) or 10% O_2 for 3 wk. The hypoxia-induced depolarization of membrane potential that was demonstrated in PASMCs from wild-type mice was significantly blunted in cells from hypoxic $Hif1a^{+/-}$ mice (37). The hypoxia-induced depolarization of PASMCs from wild-type mice exposed to chronic hypoxia is due to a reduction in the activity of voltage-gated K^+ channels. In contrast, PASMCs from hypoxic $Hif1a^{+/-}$ mice showed virtually no reduction in voltage-gated K^+ channel activity (37). Analysis of capacitance, an electrophysiological measure of cell volume, demonstrated that the hypertrophy of PASMCs from hypoxic wild-type mice was also lost in PASMCs from $Hif1a^{+/-}$ mice (37). Thus partial HIF-1α deficiency had significant effects on critical physiological responses of the pulmonary vasculature to chronic hypoxia.

The same experimental paradigm was subsequently applied to the analysis of mice heterozygous for a knockout allele at the locus encoding HIF-2α. In this line, homozygous null mice died soon after birth of respiratory distress syndrome due to deficient surfactant production (11). The heterozygous null mice developed normally, and when they were exposed to 10% O_2 for 4 wk, these mice did not develop right ventricular hypertension or hypertrophy and showed no evidence of pulmonary vascular remodeling (5). Expression of endothelin-1 (ET-1) is induced in the lungs of mice exposed to hypoxia (25). ET-1 promotes constriction and remodeling of pulmonary arterioles, and inhibitors of the endothelin type A (ET_A) receptor block the development of hypoxic pulmonary hypertension (10, 12, 42). *Et1* gene expression was induced in the lungs of wild-type mice exposed to hypoxia for 6 days but not in the lungs of heterozygous null mice. These results are consistent with the presence of an HIF-1 binding site in the *Et1* gene promoter (18) and suggest that this gene is specifically activated by heterodimers containing HIF-2α.

In humans, high-altitude hypoxia induces increased plasma levels of norepinephrine (7), a potent vasoconstrictor. Complete HIF-2α deficiency resulted in deficient fetal production of catecholamines such as norepinephrine (40). Heterozygotes had normal plasma norepinephrine levels under normoxic conditions, but 4 wk of exposure to 10% O_2 increased plasma

norepinephrine levels only 3.5-fold in heterozygotes compared with 12-fold in wild-type mice (5). These results are consistent with HIF-1 regulation of the gene encoding tyrosine hydroxylase, the rate-limiting enzyme in catecholamine biosynthesis (33).

Whereas partial HIF-2α deficiency resulted in a more dramatic impairment of hypoxia-induced pulmonary vascular remodeling than did partial HIF-1α deficiency, only the latter was associated with impaired development of hypoxia-induced polycythemia (5, 50). Finally, partial HIF-1α deficiency was associated with increased weight loss in response to chronic hypoxia (50), whereas weight loss was decreased in mice with partial HIF-2α deficiency relative to wild-type littermates (5). These data indicate that HIF-1α and HIF-2α play distinct, critical roles in mediating physiological responses to chronic hypoxia. One interesting possibility is that HIF-2α plays a critical role in pulmonary arterial endothelial cells (e.g., in stimulating ET-1 production), whereas HIF-1α plays an important role in PASMCs (Fig. 2).

CAROTID BODY-MEDIATED HYPOXIC VENTILATORY RESPONSES

An essential adaptation to acute and chronic hypoxia is an increase in ventilation that depends on the activity of peripheral chemoreceptors, particularly those within the carotid body, which detect changes in arterial O_2 concentration and relay sensory information to the brain stem neurons that regulate breathing (30). *Hif1a*$^{+/-}$ mice and wild-type littermates showed similar increases in respiratory rate, tidal volume, and minute ventilation in response to acute hypoxia (reduction in fraction of inspired O_2 from 1.00 to 0.12) or hypercarbia (5% CO_2) (22). Chronic hypoxia induces ventilatory adaptation as manifested by an augmented response to subsequent acute hypoxia, an effect that is mediated by the carotid body (30). Wild-type mice showed a significantly increased ventilatory response to 12% O_2 after, compared with before, exposure to chronic hypobaric hypoxia (0.4 atm for 3 days). In contrast, *Hif1a*$^{+/-}$ mice showed a significantly decreased acute hypoxic ventilatory response after, compared with before, exposure to chronic hypoxia (22). The Dejours test, which uses the magnitude of the transitory ventilatory decline in response to a brief hyperoxic exposure as an index of peripheral chemoreceptor, especially carotid body, sensitivity, was performed. This response was markedly blunted in *Hif1a*$^{+/-}$ mice.

These results provided evidence for impaired carotid body function. Because chemoreceptors innervated by the vagus nerve contribute to hypoxic ventilatory responses, especially under conditions of impaired carotid body function, ventilatory responses were monitored in wild-type and *Hif1a*$^{+/-}$ littermates exposed to hypoxia before and after bilateral vagotomy. Whereas hypoxia-induced increases in respiratory rate and integrated phrenic nerve activity were not impaired by vagotomy in wild-type mice, these responses were dramatically reduced in *Hif1a*$^{+/-}$ mice, indicating that these mice depend on chemoreceptors other than the carotid body for O_2 sensing (22).

Remarkably, isolated carotid bodies from *Hif1a*$^{+/-}$ mice superfused with 12% O_2 showed little increase in carotid sinus nerve activity. In contrast, carotid bodies of both genotypes showed a dramatic increase in neural activity when exposed to sodium cyanide, demonstrating that partial HIF-1α deficiency resulted in a dramatic and specific loss of O_2 sensing in the carotid body (22). Immunohistochemical analysis of carotid body sections demonstrated normal expression of chromogranin A and tyrosine hydroxylase, two markers of the glomus cells that mediate O_2 sensing. Morphometric analysis of carotid bodies from wild-type and *Hif1a*$^{+/-}$ littermates revealed no difference in the number of glomus cells per section, mean glomus cell volume, glomic volume, total volume per body, or the ratio of glomic volume to total volume (22).

These data indicate that partial HIF-1α deficiency specifically impairs the ability of the carotid body to sense and/or respond to hypoxia. Because HIF-2α is also expressed in the carotid body (40), these results suggest that HIF-1α plays unique roles in the carotid body. Presumably, target genes that are transactivated only by heterodimers containing HIF-1α encode proteins that play critical roles in carotid body O_2 sensing. In contrast, the functioning of noncarotid peripheral chemoreceptors is less impaired by partial HIF-1α deficiency, indicating differences in the genetic mechanisms underlying the physiological functioning of these different chemoreceptors. Carotid sinus nerve transection initially abolishes acute hypoxic ventilatory responses but subsequently leads to a central reorganization of the chemoreflex pathway and recovery of the response (3, 26, 31). In *Hif1a*$^{+/-}$ mice, a functional denervation of the carotid sinus nerves may lead to similar physiological compensation.

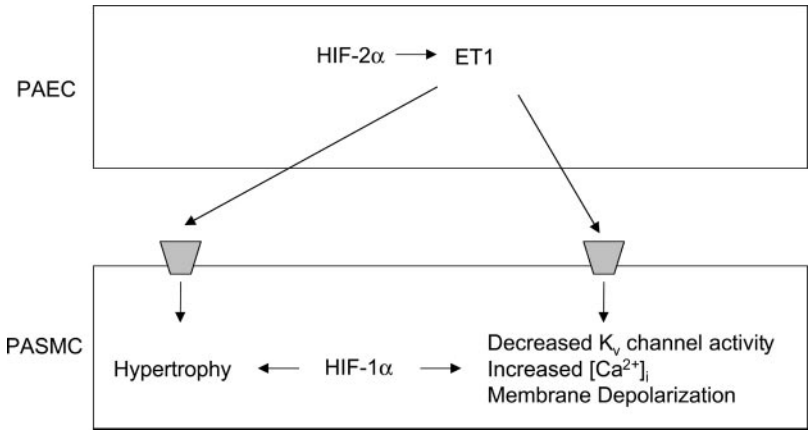

Fig. 2. Involvement of HIF-1α and HIF-2α in physiological responses of pulmonary arterioles to chronic hypoxia. Hypoxia-induced production of endothelin 1 (ET-1) within the pulmonary arteriolar endothelial cell (PAEC) is dependent on the presence of normal levels of HIF-2α. ET-1 is secreted by PAEC, binds to endothelin type A receptors located on the pulmonary arteriolar smooth muscle cell (PASMC), and stimulates membrane depolarization and hypertrophy. Hypoxia also directly stimulates membrane depolarization and hypertrophy of PASMCs, and these effects may require the presence of normal levels of HIF-1α. $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; K_v channel, voltage-gated K^+ channel.

SURVIVAL OF ISCHEMIC CELLS MEDIATED BY EPO

In the heart, survival of tissue subjected to prolonged lethal ischemia can be increased by prior exposure to repeated brief periods of sublethal ischemia induced by transient coronary artery occlusion and reperfusion, a phenomenon known as preconditioning (28). After the preconditioning stimulus, there is an early window of acute protection that lasts 1–2 h followed by a late window of delayed protection that lasts ~12–72 h (45). Systemic exposure of animals to chronic hypoxia also induces protection against myocardial ischemia (1, 39). More recently, short episodes of intermittent or continuous hypoxia have been shown to induce delayed cardiac protection (6, 46).

Wild-type mice were exposed to five cycles of 6% O_2 for 6 min and reoxygenation for 6 min and then allowed to recover for 24 h. The hearts were isolated for Langendorff perfusion and subjected to 30 min of global ischemia followed by 2 h of reperfusion. The hearts of mice subjected to intermittent hypoxia showed a dramatically increased recovery of left ventricular developed pressure and a dramatic reduction in infarct size after ischemia-reperfusion compared with controls (6). In contrast, prior exposure of *Hif1a*$^{+/-}$ mice to intermittent hypoxia had no protective effect on the heart. Immediately after the 1 h of intermittent hypoxia, EPO mRNA expression in the kidneys of wild-type mice was induced more than sixfold, resulting in a greater than ninefold increase in plasma EPO levels 1 h later. In contrast, no significant increase in EPO mRNA expression was detected in the kidneys of *Hif1a*$^{+/-}$ mice immediately after intermittent hypoxia (6). Intraperitoneal administration of a single dose of recombinant human EPO (rhEPO) 24 h before ischemia-reperfusion was sufficient to induce increased functional recovery, as measured by left ventricular developed pressure, and decreased apoptosis, as measured by TdT-mediated dUTP nick end labeling and activated caspase-3 assays (6). Daily rhEPO administration for 7 days was also shown to result in functional improvement and decreased myocyte loss after in situ coronary artery occlusion and reperfusion (8).

Exposure of rats to continuous hypoxia (3 h at 8% O_2) or administration of a chemical inducer of HIF-1 activity (cobalt chloride or desferrioxamine) 24 h before cerebral hypoxia-ischemia also dramatically reduced infarct size (2). The protective effect may be mediated via HIF-1-induced EPO expression, because administration of rhEPO also protects against ischemic brain injury in rodents (4, 38). However, whereas cardiac protection appears to involve the endocrine production of EPO in the kidney, cerebral protection may involve a paracrine mechanism, in which hypoxic astrocytes produce EPO, which binds to EPO receptors on neurons (27).

FUTURE RESEARCH DIRECTIONS

Like a blind man touching an elephant, the studies summarized in this review represent only small pieces of the large and rapidly growing body of data regarding the role of HIF-1 in human physiology and pathophysiology. Other important areas of investigation, such as the role of HIF-1 in angiogenesis and cancer, have been reviewed recently elsewhere (34, 35). The many critical roles of HIF-1 generate excitement that the factor may represent a therapeutic target in several common disease states, but these data also indicate that it may be difficult to achieve desired therapeutic efficacy without incurring unwanted side effects. In this regard, the demonstration that partial deficiency of HIF-1α or HIF-2α does not result in disease but does protect against hypoxia-induced pulmonary hypertension suggests that inhibitors of HIF-1 activity may be of therapeutic utility in patients with chronic lung disease. However, such inhibitors are likely to target both HIF-1α and HIF-2α, and the consequences of combined inhibition are unknown.

The administration of EPO to patients presenting with acute heart attack or stroke may provide a means of reducing infarct size, mortality, and morbidity. Prophylactic use of EPO is less likely to be useful, because the development of polycythemia resulting from chronic administration would itself increase the risk of heart attack and stroke. If encouraging preliminary data (13) are confirmed in larger clinical trials, EPO therapy could have a tremendous impact on public health, given the fact that heart attack and stroke combined represent the most common cause of death in the US population. The studies described in this review illustrate how the combined application of molecular and physiological methods represents a powerful means to elucidate fundamental homeostatic mechanisms that can then be exploited therapeutically.

REFERENCES

1. **Baker EJ, Boerboom LE, Olinger GN, and Baker JE.** Tolerance of the developing heart to ischemia: impact of hypoxemia from birth. *Am J Physiol Heart Circ Physiol* 268: H1165–H1173, 1995.
2. **Bergeron M, Gidday JM, Yu AY, Semenza GL, Ferriero DM, and Sharp FR.** Role of hypoxia-inducible factor-1 in hypoxia-induced ischemic tolerance in neonatal rat brain. *Ann Neurol* 48: 285–296, 2000.
3. **Bisgard GE, Forster HV, and Klein JP.** Recovery of peripheral chemoreceptor function after denervation in ponies. *J Appl Physiol* 49: 964–970, 1980.
4. **Brines ML, Ghezzi P, Keenan S, Agnello D, de Lanerolle NC, Cerami C, Itri LM, and Cerami A.** Erythropoietin crosses the blood-brain barrier to protect against experimental brain injury. *Proc Natl Acad Sci USA* 97: 10526–10531, 2000.
5. **Brusselmans K, Compernolle V, Tjwa M, Wiesener MS, Maxwell PH, Collen D, and Carmeliet P.** Heterozygous deficiency of hypoxia-inducible factor-2α protects mice against pulmonary hypertension and right ventricular dysfunction during prolonged hypoxia. *J Clin Invest* 111: 1519–1527, 2003.
6. **Cai Z, Manalo DJ, Wei G, Rodriguez ER, Fox-Talbot K, Lu H, Zweier JL, and Semenza GL.** Hearts from rodents exposed to intermittent hypoxia or erythropoietin are protected against ischemia-reperfusion injury. *Circulation* 108: 79–85, 2003.
7. **Calbet JA.** Chronic hypoxia increases blood pressure and noradrenaline spillover in healthy humans. *J Physiol* 551: 379–386, 2003.
8. **Calvillo L, Latini R, Kajstura J, Leri A, Anversa P, Ghezzi P, Salio M, Cerami A, and Brines M.** Recombinant human erythropoietin protects the myocardium from ischemia-reperfusion injury and promotes beneficial remodeling. *Proc Natl Acad Sci USA* 100: 4802–4806, 2003.
9. **Carmeliet P, Dor Y, Herbert JM, Fukumura D, Brusselmans K, Dewerchin M, Neeman M, Bono F, Abramovitch R, Maxwell P, Koch CJ, Ratcliffe P, Moons L, Jain RK, Collen D, Keshert E, and Keshet E.** Role of HIF-1α in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. *Nature* 394: 485–490, 1998.
10. **Chen SJ, Chen YF, Meng QC, Durand J, Dicarlo VS, and Oparil S.** Endothelin-receptor antagonist bosentan prevents and reverses hypoxic pulmonary hypertension in rats. *J Appl Physiol* 79: 2122–2131, 1995.
11. **Compernolle V, Brusselmans K, Acker T, Hoet P, Tjwa M, Beck H, Plaisance S, Dor Y, Keshet E, Lupu F, Nemery B, Dewerchin M, Van Veldhoven P, Plate K, Moons L, Collen D, and Carmeliet P.** Loss of HIF-2α and inhibition of VEGF impair fetal lung maturation, whereas treatment with VEGF prevents fatal respiratory distress in premature mice. *Nat Med* 8: 702–710, 2002.
12. **DiCarlo VS, Chen SJ, Meng QC, Durand J, Yano M, Chen YF, and Oparil S.** ET_A-receptor antagonist prevents and reverses chronic hypoxia-induced pulmonary hypertension in rat. *Am J Physiol Lung Cell Mol Physiol* 269: L690–L697, 1995.

13. **Ehrenreich H, Hasselblatt M, Dembowski C, Cepek L, Lewczuk P, Stiefel M, Rustenbeck HH, Breiter N, Jacob S, Knerlich F, Bohn M, Poser W, Ruther E, Kochen M, Gefeller O, Gleiter C, Wessel TC, De Ryck M, Itri L, Prange H, Cerami A, Brines M, and Siren AL.** Erythropoietin therapy for acute stroke is both safe and beneficial. *Mol Med* 8: 495–505, 2002.
14. **Ema M, Taya S, Yokotani N, Sogawa K, Matsuda Y, and Fujii-Kuriyama Y.** A novel bHLH-PAS factor with close sequence similarity to hypoxia-inducible factor 1α regulates the VEGF expression and is potentially involved in lung and vascular development. *Proc Natl Acad Sci USA* 94: 4273–4278, 1997.
15. **Epstein AC, Gleadle JM, McNeill LA, Hewitson KS, O'Rourke J, Mole DR, Mukherji M, Metzen E, Wilson MI, Dhanda A, Tian YM, Masson N, Hamilton DL, Jaakkola P, Barstead R, Hodgkin J, Maxwell PH, Pugh CW, Schofield CJ, and Ratcliffe PJ.** *C. elegans* EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. *Cell* 107: 43–54, 2001.
16. **Flamme I, Frohlich T, von Reutern M, Kappel A, Damert A, and Risau W.** HRF, a putative basic helix-loop-helix-PAS-domain transcription factor, is closely related to hypoxia-inducible factor-1α and developmentally expressed in blood vessels. *Mech Dev* 63: 51–60, 1997.
17. **Hogenesch JB, Chan WK, Jackiw VH, Brown RC, Gu YZ, Pray-Grant M, Perdew GH, and Bradfield CA.** Characterization of a subset of the basic-helix-loop-helix-PAS superfamily that interacts with components of the dioxin signaling pathway. *J Biol Chem* 272: 8581–8593, 1997.
18. **Hu J, Discher DJ, Bishopric NH, and Webster KA.** Hypoxia regulates expression of the endothelin-1 gene through a proximal hypoxia-inducible factor-1 binding site on the antisense strand. *Biochem Biophys Res Commun* 245: 894–899, 1998.
19. **Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohh M, Salic A, Asara JM, Lane WS, and Kaelin WG Jr.** HIFα targeted for VHL-mediated destruction by proline hydroxylation: implications for O_2 sensing. *Science* 292: 464–468, 2001.
20. **Iyer NV, Kotch LE, Agani F, Leung SW, Laughner E, Wenger RH, Gassmann M, Gearhart JD, Lawler AM, Yu AY, and Semenza GL.** Cellular and developmental control of O_2 homeostasis by hypoxia-inducible factor 1α. *Genes Dev* 12: 149–162, 1998.
21. **Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, Kriegsheim Av Hebestreit HF, Mukherji M, Schofield CJ, Maxwell PH, Pugh CW, and Ratcliffe PJ.** Targeting of HIF-α to the von Hippel-Lindau ubiquitylation complex by O_2-regulated prolyl hydroxylation. *Science* 292: 468–472, 2001.
22. **Kline DD, Peng YJ, Manalo DJ, Semenza GL, and Prabhakar NR.** Defective carotid body function and impaired ventilatory responses to chronic hypoxia in mice partially deficient for hypoxia-inducible factor 1α. *Proc Natl Acad Sci USA* 99: 821–826, 2002.
23. **Kotch LE, Iyer NV, Laughner E, and Semenza GL.** Defective vascularization of HIF-1α-null embryos is not associated with VEGF deficiency but with mesenchymal cell death. *Dev Biol* 209: 254–267, 1999.
24. **Lando D, Peet DJ, Whelan DA, Gorman JJ, and Whitelaw ML.** Asparagine hydroxylation of the HIF transactivation domain: a hypoxic switch. *Science* 295: 858–861, 2002.
25. **Li H, Chen SJ, Chen YF, Meng QC, Durand J, Oparil S, and Elton TS.** Enhanced endothelin-1 and endothelin receptor gene expression in chronic hypoxia. *J Appl Physiol* 77: 1451–1459, 1994.
26. **Martin-Body RL, Robson GJ, and Sinclair JD.** Restoration of hypoxic respiratory responses in the awake rat after carotid body denervation by sinus nerve section. *J Physiol* 380: 61–73, 1986.
27. **Morishita E, Masuda S, Nagao M, Yasuda Y, and Sasaki R.** Erythropoietin receptor is expressed in rat hippocampal and cerebral cortical neurons, and erythropoietin prevents in vitro glutamate-induced neuronal death. *Neuroscience* 76: 105–116, 1997.
28. **Murry CE, Jennings RB, and Reimer KA.** Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 74: 1124–1136, 1986.
29. **Peng J, Zhang L, Drysdale L, and Fong GH.** The transcription factor EPAS-1/hypoxia-inducible factor 2α plays an important role in vascular remodeling. *Proc Natl Acad Sci USA* 97: 8386–8391, 2000.
30. **Prabhakar NR.** Oxygen sensing by the carotid body chemoreceptors. *J Appl Physiol* 88: 2287–2295, 2000.
31. **Roux JC, Peyronnet J, Pascual O, Dalmaz Y, and Pequignot JM.** Ventilatory and central neurochemical reorganisation of O_2 chemoreflex after carotid sinus nerve transection in rat. *J Physiol* 522: 493–501, 2000.
32. **Ryan HE, Lo J, and Johnson RS.** HIF-1α is required for solid tumor formation and embryonic vascularization. *EMBO J* 17: 3005–3015, 1998.
33. **Schnell PO, Ignacak ML, Bauer AL, Striet JB, Paulding WR, and Czyzyk-Krzeska MF.** Regulation of tyrosine hydroxylase promoter activity by the von Hippel-Lindau tumor suppressor protein and hypoxia-inducible transcription factors. *J Neurochem* 85: 483–491, 2003.
34. **Semenza GL.** Angiogenesis in ischemic and neoplastic disorders. *Annu Rev Med* 54: 17–28, 2003.
35. **Semenza GL.** HIF-1 and tumor progression: pathophysiology and therapeutics. *Trends Mol Med* 8 *Suppl*: S62–S67, 2002.
36. **Semenza GL and Wang GL.** A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. *Mol Cell Biol* 12: 5447–5454, 1992.
37. **Shimoda LA, Manalo DJ, Sham JS, Semenza GL, and Sylvester JT.** Partial HIF-1α deficiency impairs pulmonary arterial myocyte electrophysiological responses to hypoxia. *Am J Physiol Lung Cell Mol Physiol* 281: L202–L208, 2001.
38. **Siren AL, Fratelli M, Brines M, Goemans C, Casagrande S, Lewczuk P, Keenan S, Gleiter C, Pasquali C, Capobianco A, Mennini T, Heumann R, Cerami A, Ehrenreich H, and Ghezzi P.** Erythropoietin prevents neuronal apoptosis after cerebral ischemia and metabolic stress. *Proc Natl Acad Sci USA* 98: 4044–4049, 2001.
39. **Tajima M, Katayose D, Bessho M, and Isoyama S.** Acute ischaemic preconditioning and chronic hypoxia independently increase myocardial tolerance to ischaemia. *Cardiovasc Res* 28: 312–319, 1994.
40. **Tian H, Hammer RE, Matsumoto AM, Russell DW, and McKnight SL.** The hypoxia-responsive transcription factor EPAS1 is essential for catecholamine homeostasis and protection against heart failure during embryonic development. *Genes Dev* 12: 3320–3324, 1998.
41. **Tian H, McKnight SL, and Russell DW.** Endothelial PAS domain protein 1 (EPAS1), a transcription factor selectively expressed in endothelial cells. *Genes Dev* 11: 72–82, 1997.
42. **Tjen-ALooi S, Ekman R, Osborn J, and Keith I.** Pulmonary vascular pressure effects by endothelin-1 in normoxia and chronic hypoxia: a longitudinal study. *Am J Physiol Heart Circ Physiol* 271: H2246–H2253, 1996.
43. **Wang GL, Jiang BH, Rue EA, and Semenza GL.** Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O_2 tension. *Proc Natl Acad Sci USA* 92: 5510–5514, 1995.
44. **Wang GL and Semenza GL.** Purification and characterization of hypoxia-inducible factor 1. *J Biol Chem* 270: 1230–1237, 1995.
45. **Williams RS and Benjamin IJ.** Protective responses in the ischemic myocardium. *J Clin Invest* 106: 813–818, 2000.
46. **Xi L, Tekin D, Gursoy E, Salloum F, Levasseur JE, and Kukreja RC.** Evidence that NOS2 acts as a trigger and mediator of late preconditioning induced by acute systemic hypoxia. *Am J Physiol Heart Circ Physiol* 283: H5–H12, 2002.
47. **Yan SF, Fujita T, Lu J, Okada K, Shan Zou Y, Mackman N, Pinsky DJ, and Stern DM.** Egr-1, a master switch coordinating upregulation of divergent gene families underlying ischemic stress. *Nat Med* 6: 1355–1361, 2000.
48. **Yan SF, Lu J, Xu L, Zou YS, Tongers J, Kisiel W, Mackman N, Pinsky DJ, and Stern DM.** Pulmonary expression of early growth response-1: biphasic time course and effect of oxygen concentration. *J Appl Physiol* 88: 2303–2309, 2000.
49. **Yan SF, Zou YS, Mendelsohn M, Gao Y, Naka Y, Du Yan S, Pinsky D, and Stern D.** Nuclear factor interleukin 6 motifs mediate tissue-specific gene transcription in hypoxia. *J Biol Chem* 272: 4287–4294, 1997.
50. **Yu AY, Shimoda LA, Iyer NV, Huso DL, Sun X, McWilliams R, Beaty T, Sham JS, Wiener CM, Sylvester JT, and Semenza GL.** Impaired physiological responses to chronic hypoxia in mice partially deficient for hypoxia-inducible factor 1α. *J Clin Invest* 103: 691–696, 1999.
51. **Yu F, White SB, Zhao Q, and Lee FS.** HIF-1α binding to VHL is regulated by stimulus-sensitive proline hydroxylation. *Proc Natl Acad Sci USA* 98: 9630–9635, 2001.
52. **Zhou YD, Barnard M, Tian H, Li X, Ring HZ, Francke U, Shelton J, Richardson J, Russell DW, and McKnight SL.** Molecular characterization of two mammalian bHLH-PAS domain proteins selectively expressed in the central nervous system. *Proc Natl Acad Sci USA* 94: 713–718, 1997.