Published in final edited form as:
Autophagy. 2008 May 16; 4(4): 534–536.

Mitochondrial autophagy: life and breath of the cell

Gregg L. Semenza
Vascular Program, Institute for Cell Engineering; Departments of Pediatrics, Medicine, Oncology, Radiation Oncology; and McKusick-Nathans Institute of Genetic Medicine; The Johns Hopkins University School of Medicine; Baltimore, MD 21205

Abstract

Homeostatic responses to reduced O_2 availability are regulated by the transcriptional activator hypoxia-inducible factor 1 (HIF-1) in all metazoan species. An essential adaptation to sustained hypoxia is an active repression of mitochondrial respiration. In mouse embryo fibroblasts, HIF-1 induces expression of BNIP3, which triggers selective mitochondrial autophagy. When exposed to hypoxia, HIF-1-deficient cells do not induce BNIP3 or autophagy, do not decrease mitochondrial mass or downregulate respiration, and die within 72 hours due to toxic levels of reactive oxygen species. These studies indicate that mitochondrial autophagy represents an adaptive metabolic response to hypoxia that is necessary to maintain redox homeostasis and cell survival.

Keywords

mitochondrial autophagy; HIF-1; hypoxia; respiration; reactive oxygen species

Hypoxia-inducible factor 1 (HIF-1) is a transcription factor that serves as a master regulator of oxygen homeostasis in all metazoan organisms[1] from humans[2] to *C. elegans*.[3] HIF-1 controls both O_2 delivery, through regulation of ventilation[4] and vascularization,[5] as well as O_2 utilization, through regulation of glucose and energy metabolism.[6–12] HIF-1 is a heterodimer of O_2-regulated HIF-1α and constitutively expressed HIF-1β subunits.[2] The regulation of HIF-1α involves the hydroxylation of key proline and asparagine residues by dioxygenases that utilize O_2 and α-ketoglutarate as substrates.[13–15] Hydroxylation of Pro-402 and/or Pro-564 (in human HIF-1α) is required for the binding of the von Hippel-Lindau protein (VHL), which is the recognition component of an E3-ubiquitin ligase that targets HIF-1α for proteasomal degradation. Hydroxylation of Asn-803 blocks the binding of the coactivators p300 and CBP to the transactivation domain of HIF-1α. Under hypoxic conditions, the hydroxylases are inhibited, resulting in stabilization and activation of HIF-1α.[13–15]

When cultured under hypoxic conditions for 48 hours, wild-type (WT) mouse embryo fibroblasts (MEFs) markedly reduce their O_2 consumption and ATP production, whereas HIF-1α-null MEFs do not.[10] Thus, HIF-1 actively represses mitochondrial respiration when MEFs are exposed to hypoxic conditions, which increases their reliance on glycolysis, a pathway that produces 18-fold less ATP per mole of glucose. Why?

KO MEFs die if exposed to hypoxia O_2 for 72 hours, whereas WT MEFs survive and even proliferate, albeit at a reduced rate compared to that observed under standard tissue culture

Correspondence to: G. L. Semenza; Broadway Research Building, Suite 671; 733 North Broadway; Baltimore, MD 21205; FAX: 443-287-5618; Email: gsemenza@jhmi.edu.

Addendum to: Zhang H, Bosch-Marce M, Shimoda LA, Tan YS, Baek JH, Wesley JB, Gonzalez FJ, Semenza GL. Mitochondrial autophagy is a HIF-1-dependent adaptive metabolic response to hypoxia. J Biol Chem 2008 Feb 15; [Epub ahead of print].

conditions (20% O_2).[8] The KO MEFs undergo apoptosis that is triggered by increased levels of reactive oxygen species (ROS), in contrast to WT cells, in which ROS levels are decreased after 48–72 hours at 0.5–1% O_2.[8,10] KO cell survival is increased by treating the cells with the superoxide scavenger MnTMPyP.[10] How do WT cells respond to hypoxia in order to prevent ROS-induced cell death?

Under hypoxic conditions, HIF-1 is induced, binds to the promoter of the *Pdk1* gene, and activates its transcription in WT, but not KO, MEFs.[8,9] *Pdk1* encodes pyruvate dehydrogenase (PDH) kinase 1 (PDK1), which phosphorylates and inactivates PDH, the enzyme that converts pyruvate to acetyl coenzyme A for entry into the mitochondria tricarboxylic acid cycle, which generates reducing equivalents that are passed through the electron transport chain, generating an electrochemical gradient that is used to synthesize ATP. Remarkably, forced overexpression of PDK1 in KO MEFs reduces ROS levels and cell death under conditions of prolonged hypoxia.[8]

In addition to the activity of PDK1 in shunting substrate (pyruvate) away from mitochondria, WT MEFs also reduce their mitochondrial DNA content (relative to nuclear DNA) by more than two-thirds during a 48-hour exposure to 1% O_2.[10] Staining of MEFs with nonyl acridine orange, which binds to cardiolipin in mitochondrial membranes independent of mitochondrial membrane potential, revealed an equally dramatic reduction in mitochondrial mass in hypoxic WT MEFs. In contrast, hypoxia had no effect on mitochondrial DNA or mass in KO MEFs. The remarkably rapid elimination of mitochondria implicated autophagy, yet when the cells were stained with an endoplasmic reticulum-specific dye, no differences were observed between WT and KO MEFs at 1% or 20% O_2.[10] Thus, if autophagy was involved, it must be selective for mitochondria.

Analysis of GFP-LC3 subcellular localization by fluorescence microscopy and LC3 processing by immunoblot assay indicated that autophagy was induced by hypoxia in WT, but not in KO, MEFs.[10] Knockdown of Beclin 1 or Atg5 expression blocked the hypoxia-induced reduction in mitochondrial DNA, mitochondrial mass, O_2 consumption, and ATP production in WT MEFs, confirming that this was due to selective mitochondrial autophagy triggered by HIF-1. But how?

Along with PDK1, one of the most dramatically hypoxia-induced and HIF-1-regulated gene products in MEFs (and other cell types) is BNIP3, which is usually described as a pro-apoptotic member of the Bcl-2 family.[16] In hypoxic WT MEFs, BNIP3 competes with Beclin 1 for binding to Bcl-2, thus increasing the levels of Beclin 1 that are free to induce autophagy.[10] Knockdown of BNIP3, as in the case of Beclin 1 and Atg5, inhibited the hypoxia-induced reduction in mitochondrial DNA, mitochondrial mass, O_2 consumption, and ATP production in WT MEFs. Forced over-expression of BNIP3 in KO MEFs reduced mitochondrial DNA, mitochondrial mass, O_2 consumption, and ATP production in an O_2-independent manner. BNIP3, Beclin 1, or Atg5 loss-of-function increased ROS levels and decreased viability of hypoxic WT MEFs, whereas BNIP3 gain-of-function reduced ROS levels and increased viability of hypoxic KO MEFs.[10] Taken together, these results indicate that HIF-1-mediated induction of BNIP3 triggers mitochondrial autophagy as an adaptive metabolic response to hypoxia.

Whereas increased O_2 concentration (hyperoxia) is known to generate increased ROS,[17] recent studies have revealed that when cells are acutely exposed to hypoxia, the generation of ROS by the mitochondria also increases,[18] which implies that electron transport chain function is optimal at physiological O_2 concentrations and that any deviation from normal results in a reduction in the ability of mitochondria to generate ATP without increasing ROS. Remarkably, the increase of ROS levels under hypoxic conditions is necessary to induce HIF-1 because the

prolyl and asparaginyl hydroxylases, which negatively regulate HIF-1α under normoxic conditions, contain Fe(II) in their catalytic center and are thus inactivated by ROS.[18–20] The induction of HIF-1 then results in adaptive responses that restore oxygen and redox homeostasis.[8,10]

The notion that electron transport chain activity is optimized to the physiological PO_2 is supported by the discovery that under hypoxic conditions, HIF-1 mediates a subunit switch in the cytochrome *c* oxidase complex, in which the COX4-1 isoform is replaced by COX4-2.[11] Remarkably, a similar subunit switch had previously been demonstrated in *Saccharomyces cerevisiae*, but through a completely different molecular mechanism,[21] as yeast lack HIF-1. Thus, this appears to be a case of convergent evolution in which a critical problem (how to maintain optimal efficiency of the electron transport chain despites changes in PO_2) was solved independently in yeast and metazoans.

If HIF-1 can trigger mitochondrial destruction, can it also block mitochondrial production? The answer is yes, at least in the clear cell type of renal cell carcinoma, which is characterized by dysregulated, constitutive (O_2-independent) HIF-1 activity due to VHL loss-of-function. In these cells, HIF-1 represses the activity of C-MYC, which would otherwise transactivate the gene encoding PGC-1β, a key mediator of mitochondrial biogenesis.[12] Thus, a single genetic alteration can induce a dramatic reprogramming of metabolism in cancer cells. Whether autophagy also plays an important role in renal carcinoma cells has not yet been determined but seems unlikely because the increase in respiration that is induced by forced expression of VHL in these cells can be completely counteracted by PGC-1β knockdown.

These exciting recent discoveries represent a paradigm shift in our understanding of the O_2-dependent regulation of mitochondrial function and underscore the precise balance that must be maintained between energy and redox homeostasis. These results are not simply an oddity of MEFs exposed to low O_2 concentrations for too long, as mitochondrial DNA content and BNIP3 expression are significantly decreased in the lungs of mice that are heterozygous for the KO allele (and thus partially deficient for HIF-1α), as well as in the lungs of mice with complete deficiency of HIF-1β in endothelial cells (which make up about half of the cells in the lung).[10]

Since these differences were observed in animals maintained in room air, and the lungs are exposed to the highest O_2 concentration of any tissue in the body, it appears that HIF-1-dependent regulation of mitochondria occurs over the entire range of physiological PO_2. This is clearly a dynamic property of cells: just as hypoxia, acting through HIF-1, stimulates the respiratory centers in the CNS to increase the rate at which a person takes each breath,[4] so changes in intracellular PO_2 trigger adjustments in mitochondrial mass and cellular respiration. Thus, mitochondrial autophagy is not just a crude recycling program, but rather it is an important component of the elegant physiological system controlled by HIF-1 to maintain oxygen and redox homeostasis.

REFERENCES

1. Semenza GL. Life with oxygen. Science 2007;318:62–64. [PubMed: 17916722]
2. Wang GL, Jiang BH, Rue EA, Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O_2 tension. Proc Natl Acad Sci U S A 1995;92:5510–5514. [PubMed: 7539918]
3. Jiang H, Guo R, Powell-Coffman JA. The *Caenorhabditis elegans* hif-1 gene encodes a bHLH-PAS protein that is required for adaptation to hypoxia. Proc Natl Acad Sci U S A 2001;98:7916–7921. [PubMed: 11427734]
4. Peng YJ, Yuan G, Ramakrishnan D, Sharma SD, Bosch-Marce M, Kumar GK, Semenza GL, Prabhakar NR. Heterozygous HIF-1α deficiency impairs carotid body-mediated systemic responses and reactive

oxygen species generation in mice exposed to intermittent hypoxia. J Physiol 2006;577:705–716. [PubMed: 16973705]

5. Bosch-Marce M, Okuyama H, Wesley JB, Sarkar K, Kimura H, Liu YV, Zhang H, Strazza M, Rey S, Savino L, Zhou YF, McDonald KR, Na Y, Vandiver S, Rabi A, Shaked Y, Kerbel R, Lavallee T, Semenza GL. Effects of aging and hypoxia-inducible factor 1 activity on angiogenic cell mobilizationand recovery of perfusion after limb ischemia. Circ Res 2007;101:1310–1318. [PubMed: 17932327]
6. Iyer NV, Kotch LE, Agani F, Leung SW, Laughner E, Wenger RH, Gassmann M, Gearhart JD, Lawler AM, Yu AY, Semenza GL. Cellular and developmental control of O_2 homeostasis by hypoxia-inducible factor 1α. Genes Dev 1998;12:149–162. [PubMed: 9436976]
7. Seagroves TN, Ryan HE, Lu H, Wouters BG, Knapp M, Thibault P, Laderoute K, Johnson RS. Transcription factor HIF-1 is a necessary mediator of the Pasteur effect in mammalian cells. Mol Cell Biol 2001;21:3436–3444. [PubMed: 11313469]
8. Kim JW, Tchernyshyov I, Semenza GL, Dang CV. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. Cell Metab 2006;3:177–185. [PubMed: 16517405]
9. Papandreou I, Cairns RA, Fontana L, Lim AL, Denko NC. HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. Cell Metab 2006;3:187–197. [PubMed: 16517406]
10. Zhang H, Bosch-Marce M, Shimoda LA, Tan YS, Baek JH, Wesley JB, Gonzalez FJ, Semenza GL. Mitochondrial autophagy is a HIF-1-dependent adaptive metabolic response to hypoxia. J Biol Chem. 2008 Feb 15;[Epub ahead of print].
11. Fukuda R, Zhang H, Kim JW, Shimoda L, Dang CV, Semenza GL. HIF-1 regulates cytochrome oxidase subunits to optimize efficiency of respiration in hypoxic cells. Cell 2007;129:111–122. [PubMed: 17418790]
12. Zhang H, Gao P, Fukuda R, Kumar G, Krishnamachary B, Zeller KI, Dang CV, Semenza GL. HIF-1 inhibits mitochondrial biogenesis and cellular respiration in VHL-deficient renal cell carcinoma by repression of C-MYC activity. Cancer Cell 2007;11:407–420. [PubMed: 17482131]
13. Schofield CJ, Ratcliffe PJ. Signalling hypoxia by HIF hydroxylases. Biochem Biophys Res Commun 2005;338:617–626. [PubMed: 16139242]
14. Kaelin WG Jr. The von Hippel-Lindau protein, HIF hydroxylation, and oxygen sensing. Biochem Biophys Res Commun 2005;338:627–638. [PubMed: 16153592]
15. Dann CE III, Bruick RK. Dioxygenases as O_2-dependent regulators of the hypoxic response pathway. Biochem Biophys Res Commun 2005;338:639–647. [PubMed: 16140259]
16. Bruick RK. Expression of the gene encoding the proapoptotic Nip3 protein is induced by hypoxia. Proc Natl Acad Sci U S A 2000;97:9082–9087. [PubMed: 10922063]
17. Turrens JF, Freeman BA, Levitt JG, Crapo JD. The effect of hyperoxia on superoxide production by lung submitochondrial particles. Arch Biochem Biophys 1982;217:401–410. [PubMed: 6291460]
18. 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. [PubMed: 16857720]
19. Knowles HJ, Raval RR, Harris AL, Ratcliffe PJ. Effect of ascorbate on the activity of hypoxia-inducible factor in cancer cells. Cancer Res 2003;63:1764–1768. [PubMed: 12702559]
20. Gao P, Zhang H, Dinavahi R, Li F, Xiang Y, Raman V, Bhujwalla ZM, Felsher DW, Cheng L, Pevsner J, Lee LA, Semenza GL, Dang CV. HIF-dependent antitumorigenic effect of antioxidants in vivo. Cancer Cell 2007;12:230–238. [PubMed: 17785204]
21. Burke PV, Poyton RO. Structure/function of oxygen-regulated isoforms in cytochrome *c* oxidase. J Exp Biol 1998;201:1163–1175. [PubMed: 9510528]