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Chapter 1

Introductory Chapter: Primary Concept of Hypoxia and Anoxia

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1. Introduction

Hypoxia is a pathological condition classified as generalized hypoxia and tissue hypoxia. It is well known that arterial oxygen saturation is a part of the normal physiology. A disparity between oxygen supply and its demand at the cellular level may result in a hypoxic condition. Hypoxia in which there is complete deprivation of oxygen supply is referred to as anoxia. Hypoxia belongs to the most grave factors that can directly impair the function of metabolic pathways in the animal cell. These studies dealt mostly with changes in the structure of pulmonary vessels. Normally, PO2 values of −100 Torr in the alveoli of the lungs to less than 10 Torr in tissues such as the medulla of the kidney and the retina in healthy humans are considered as the range of physiological oxygen levels within the tissues of the body [1]. Hypoxia, which occurs when oxygen levels in the microenvironment of a cell, tissue, or organism are reduced relative to the normal physiological state, is associated with a range of physiological and pathophysiological processes [2].

Hypoxia may limit the energy reserves or scope for augmentation and bustle of an organism, it may cause an organism to alter its behavior and/or it may limit the tolerance of an organism to other environmental challenges [3].

2. Hypoxia pathophysiology

Tissue hypoxia is also associated with a diverse and wide range of pathophysiological processes including (but not limited to) vascular disease, chronic inflammation, and cancer [3]. In vascular

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diseases such as atherosclerosis and stroke, vascular occlusion leads to acute or chronic tissue ischemia with resultant hypoxia [4]. In cancer, the growth of a tumor away from the local blood supply eventually leads to tumor hypoxia [2]. Hypoxia induces ischemia, hemorrhage, and stroke and found to cause systemic inflammation response syndrome (SIRS), multiple organ dysfunctions (MOD), and multiple organ failure [5].

Hypoxia has been shown to lead to increases in intracellular free calcium concentration (Ca^{2+}), 5-lipoxygenase, lipid peroxidation, cyclooxygenase (COX), constitutive nitric oxide synthase (cNOS), leukotriene B4 (LTB4), prostaglandin E2 (PGE2), interleukins, tumor necrosis factor-α (TNF-α), caspases, complement activation, Kruppel-like factor 6 (KLF6), inducible nitric oxide synthase (iNOS), heat shock protein 70 kDa (HSP-70), and hypoxia-inducible factor-1α (HIF-1α). The sequence of their occurrence provides the useful information for studying the mechanisms underlying the hypoxia-induced injury as well as therapeutic targets to prevent or ameliorate the injury [6, 7].

3. Discovery of HIF-1

Before HIF-1 was found, HRE had been identified in the 3′-enhancer region of the erythropoietin gene, whose transcription is up-regulated by more than 100-fold by severe hypoxia. Hypoxia, or

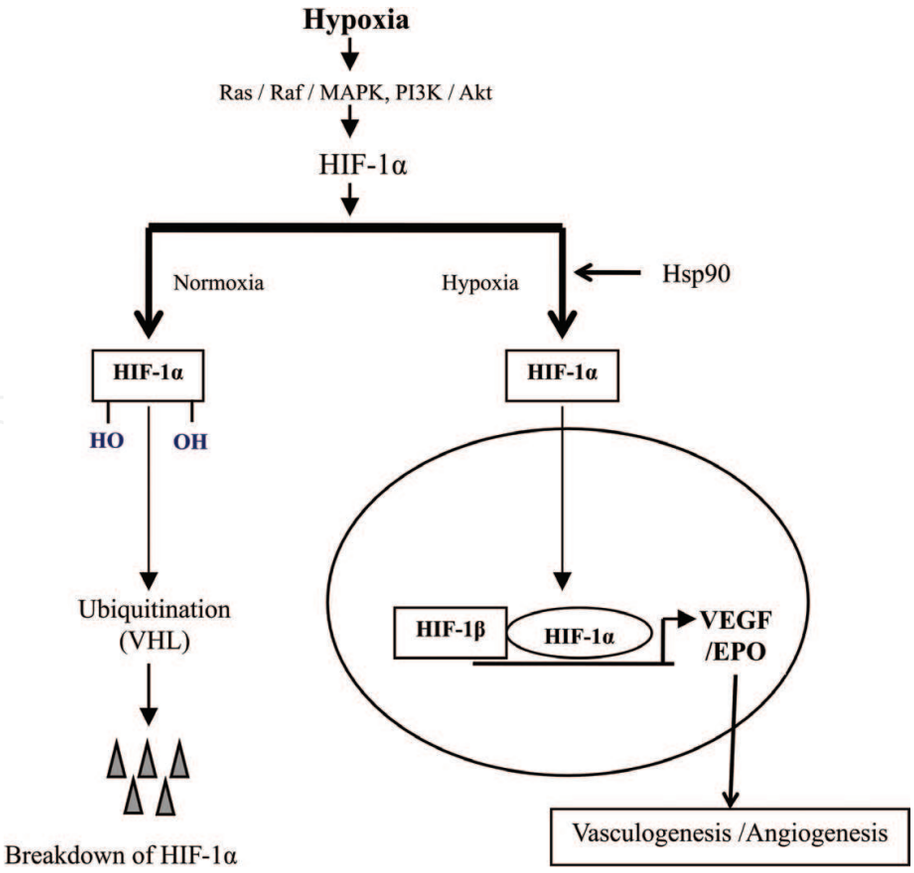

Figure 1. Schematic representation of the cell signaling events leading to ubiquitin-mediated degradation of hypoxia-inducible factor 1α (HIF-1α) and hypoxia-mediated expression of VEGF and EPO protein. VEGF, vascular endothelial growth factor; EPO, erythropoietin; MAPK, mitogen-activated protein kinase.

inadequate oxygenation, causes various responses within the body. Its effects are usually mediated via the activation of hypoxia inducible factor 1 (HIF-1). HIF-1 activation can lead to upregulation of various genes such as erythropoietin and growth factors that help tissues adjust to the decreasing oxygen availability. Another key molecule within this hypoxia-induced response is the presence of nitric oxide [NO]. NO is a ubiquitous gaseous molecule within our body. It is synthesized by nitric oxide synthases (NOS) and its release can be stimulated as a result of inflammatory responses, sympathetic activation and drop in oxygen levels [5]. Increase of HIF-1 translocates to the nucleus, dimerizes with the alpha subunit, and activates the transcription of a number of target genes displaying an HRE shape. Nuclear localization signal (NLS) domains in the alpha and beta subunit confer autonomous translocation into the nucleus 248 [8–10].

One group of HIF-1 target genes is involved in the adaptive response facilitating oxygen delivery to oxygen-deprived tissues. It includes, for example, genes coding for erythropoietin, VEGF-A, and inducible NOS (iNOS) [11, 12] (**Figure 1**).

4. Hypoxia and oxidative stress

During normoxia, about 2–3% of oxygen consumed by the mitochondria is incompletely reduced yielding reactive oxygen species (ROS) [13]. The mitochondrial ROS route to the cytosol and at low to moderate concentrations act as signaling molecules for a number of biological functions like cell growth, differentiation and metabolism and immune functions. Although at high concentrations, they can adversely modify the cell components like lipids, proteins, and DNA. However, the cells are well equipped with antioxidants that are capable of mounting an adequate antioxidant defense against ROS. Oxidative stress results when there is a shift in the balance between the oxidants and antioxidants in favor of oxidants disrupting redox signaling and control and/or inducing molecular damage [14]. In addition to a host of factors, both low and high oxygen levels are capable of inducing increased ROS formation and ultimately oxidative stress. Since oxygen is essential for formation of all ROS several controversies exist, it appears paradoxical for increased ROS formation in low oxygen microenvironment like hypoxia. However, there is enough evidence in support of increased ROS formation and oxidative stress in hypoxia.

4.1. Hypoxia and mitochondrial ROS generation

Mitochondria have been considered the main source of ROS generation (particularly H_2O_2) during hypoxia. Bell et al. have demonstrated a dose-dependent relationship between ROS and available oxygen levels with an increase in intracellular ROS with increasing severity of hypoxia [15]. During mitochondrial respiration, electrons from NADH and $FADH_2$ are transferred successively through several electron carrier molecules of the electron transport chain (respiratory chain). The electron transport chain consists of series of proteins and organic molecules located in the inner mitochondrial membrane (IMM) and organized as four membrane bound complexes (complex I–IV) which generate a proton gradient across the inner mitochondrial membrane and two mobile carriers—cytochrome c and Ubiquinone (coenzyme Q) and a ATP synthase (complex V, F1F0-ATPase) that uses the proton gradient for ATP synthesis. Complex I (NADH dehydrogenase) and complex II (succinate dehydrogenase) transfer electrons from

NADH and FADH2, respectively, to coenzyme Q (ubiquinone). Ubiquinone can accept one electron to form ubisemiquinone or two electrons to form ubiquinol. Because of its lipid solubility and small size, it is freely mobile within the lipid bilayer of the inner mitochondrial membrane (IMM). Reduced coenzyme Q further feeds the electrons to complex III of the electron transport chain (ETC). Complex III pushes the electrons to complex IV (cytochrome oxidase) via cytochrome c, second mobile carrier of the ETC. At this stage, complex III plays a crucial role of transferring single electrons sequentially to cytochrome c and complex IV since they can accept single electrons at a time unlike complex I and complex II [16]. Complex IV transfers the electrons from cytochrome c to final electron acceptor oxygen which is reduced to water in the process. As the electrons travel through ETC, they move downhill from a higher to lower energy level. The energy released in this downhill movement of electrons is used to pump protons (H^+) (by complexes I, III, and IV) from the mitochondrial matrix to the inter membrane space creating a proton gradient. The generated proton gradient drives formation of energy in the form of ATP from ADP and Pi by ATP synthase. During the ETC, ROS are produced at complex I, II, and III. Complex I and II produce ROS only into the matrix, while complex III produces ROS on both sides of the inner mitochondrial membrane [13]. Complex III is considered as an important source of ROS during hypoxia [17]. Crucial role of complex III in ROS formation in hypoxia necessitates a greater understanding of its role in ETC. Mitochondrial complex III also referred to as cytochrome bc1 complex (cyt bc_1 or bc_1) or ubiquinol cytochrome c oxidoreductase is a dimer with each monomer made up of 11 protein subunits encoded by mitochondrial and nuclear genes [16]. Complex III has three important subunits with known electron transport activity—binuclear Rieske Fe-S protein (2Fe-2S cluster), bis-heme cytochrome b, and cytochrome c_1 [18]. Cytochrome b contains two heme groups. Of the two heme groups, one is low potential heme (b_L) located near the outer surface of the inner mitochondrial membrane and the second high potential heme (b_H) at the center of the membrane about 20 Å from b_L [19]. The complex III has two separate ubiquinol and ubiquinone binding sites—Qo and Qi. Qo is located on the P (positive) side (outer surface) of the inner mitochondrial membrane and is ubiquinol oxidation site. Qi is located on the N (negative) side (close to the matrix) of the inner mitochondrial membrane and is the ubiquinone reduction site [20]. Complex III performs an important function of transfer of single electrons sequentially to cytochrome c and then to cytochrome IV since they can accept single electrons at a time unlike complex I and complex II by a cycle called Q cycle (**Figure 2a** and **b**). Q cycle begins with the binding of first molecule of ubiquinol to Qo site and ubiquinone at Qi site of complex III. The two electrons of ubiquinol follow two separate paths within complex III. One of the electrons from ubiquinol (yielding ubisemiquinone) is transferred to Rieske Iron-Sulfur protein to cytochrome c1 and finally to cytochrome c. The second electron from ubisemiquinone is transferred to cytochrome b and subsequently to ubiquinone bound at the Qi site of complex III converting ubiquinone to ubisemiquinone. Yet another ubiquinol binds at the Qo site with electron following the Rieske Iron-Sulfur, cytochrome c_1, cytochrome c pathway, and the second electron via cytochrome b reduces the ubisemiquinone at the Qi site to regenerate ubiquinol. Thus, one Q cycle involves oxidation of two ubiquinol molecules to ubiquinone with regeneration of one ubiquinol molecule and transfer of four protons from the intermembrane space from the matrix [21].

Superoxide can be generated at Qo and Qi sites at complex III by one electron reduction of oxygen to superoxide (**Figure 2a** and **b**) [21]. Ubisemiquinone that is formed repeatedly at Qo and Qi sites

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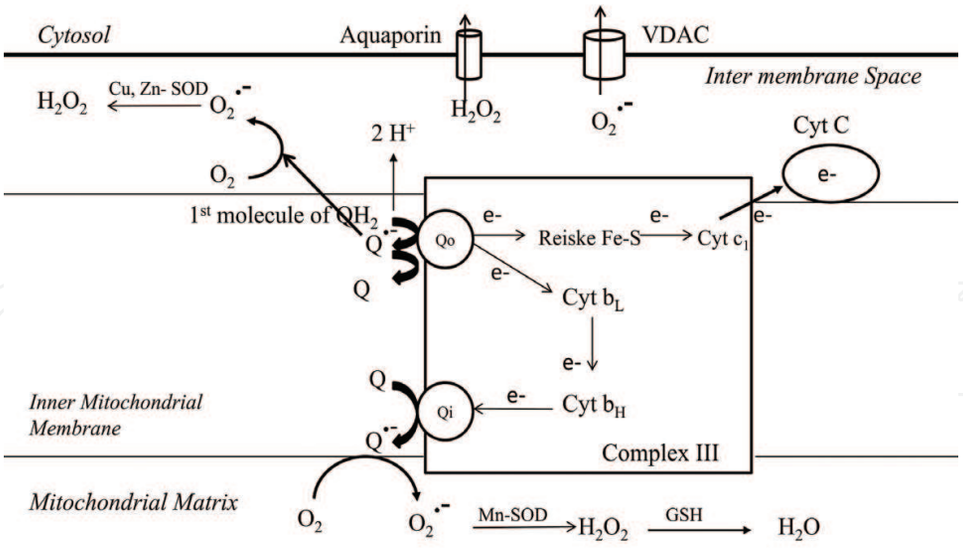

Figure 2. Schematic diagram depicting the Q-cycle at complex III of the mitochondrial electron transport chain and the generation of superoxide and H_2O_2 radicals at Qo and Qi sites of complex III.

of complex III is the site of ROS formation in hypoxia. The molecular oxygen that is lipophilic is dissolved in the hydrophobic environment within the membrane is highly electrophilic and can potentially capture electrons from ubisemiquinone forming superoxide radical [16]. Superoxide generated at Qo site is released into the intermembrane space and that generated at Qi site is

released to the mitochondrial matrix. Superoxide is an important source of H_2O_2 during hypoxia. Superoxide can be dismutated to H_2O_2 in the matrix by Cu, Zn-SOD and in the intermembrane space by Mn-SOD [18]. Hydrogen peroxide can travel to the cytosol via the membrane aquaporin channels [22]. Superoxide may also enter the cytosol through voltage-dependent anion channels (VDACs) [23]. The mechanism by which hypoxia increases ROS generation are poorly understood; however, several hypothesis have been proposed like O_2-dependent structural changes that prolong the lifetime of ubisemiquinone ("semiquinone lifetime" hypothesis), increase in the accessibility of O_2 to a site where single electrons can be captured ("oxygen access" hypothesis) or enhancement of the directional escape of superoxide to the intermembrane space versus matrix compartments ("vectoral transport" hypothesis) [16, 21].

The ROS produced can either participate in cell signaling or induce irreversible cellular damage and death [24]. There is substantial evidence to suggest the role of ROS produced at complex III of ETC in hypoxia signaling by stabilizing HIF-1α by preventing its hydroxylation by prolyl hydroxylases in low-oxygen microenvironment. This allows HIF-1α translocation to the nucleus and dimerization with HIF-1β initiating transcription of target genes (**Figure 3**) [17]. Prolyl hydroxylases (PHDs) belong to a family of mixed function oxidases involved in the hydroxylation of proline residues of HIF-1α that signals it for degradation. These enzymes require 2-oxoglutarate (α-ketoglutarate) and oxygen as substrates and non-heme iron as a cofactor [16]. The activities of PHDs have been extremely sensitive to inhibition by ROS although the mechanisms by which the ROS alter the activity of PHDs are unknown. However, the following hypotheses have been put forward: (1) possible oxidation of ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}) forbidding the mandatory binding of ferrous iron to prolyl hydroxylases; (2) recruiting ascorbate for free radical scavenging making it unavailable for

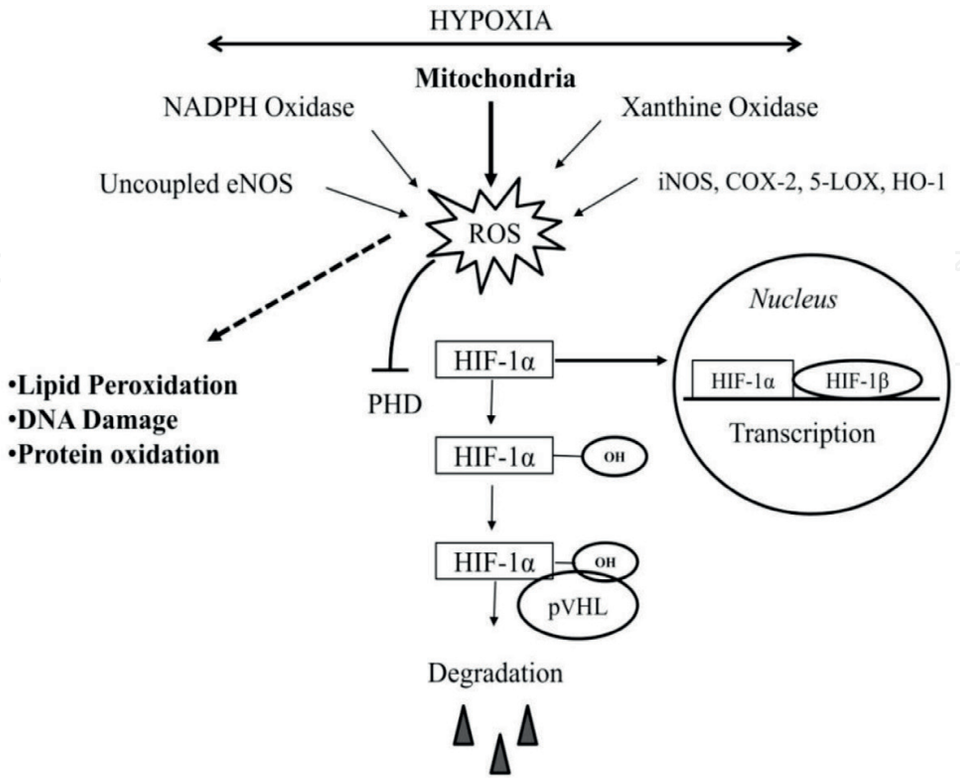

Figure 3. Schematic diagram depicting the various sources of ROD and the biological effects of ROS.

reducing ferric iron or probably by preventing direct binding of ascorbate to prolyl hydroxylase; (3) altering the concentrations of 2-oxoglutarate and succinate that might also have an prolyl hydroxylase activity [16, 25, 26]. Paddenberg et al. demonstrated a role of complex II of the electron transport chain in hypoxia-induced ROS generation particularly in the pulmonary vasculature. Complex II is the smallest of the protein complexes of the ETC located on the matrix side of the inner mitochondrial membrane. Under normoxia, this complex oxidizes succinate to fumarate meanwhile transferring two electrons to ubiquinone and reducing it to ubiquinol. During hypoxia, complex II switches its function from succinate dehydrogenase to fumarate reductase changing the direction of electron flow from ubiquinol to fumarate (fumarate accepting as electron acceptor), thereby generating ROS and accumulating succinate [27].

5. Conclusion

Hypoxia exposure induces generation of ROS (reactive oxygen species) and increases expression of p53, NF-kβ, AP-1, MAPK, and HIF-1α. The increase in expression of all these transcription factors leads to either cellular adaptation or cell death. The mechanisms by which mammalian cells adapt to acute and chronic alteration of oxygen tension are extremely important to understand the exact homeostasis regulation to counteract hypoxia-induced cell damage as therapeutic strategy.

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References

[1] Taylor CT. Mitochondria and cellular oxygen sensing in the HIF pathway. Biochemical Journal. 2008;**409**:19-26

[2] Semenza GL. Life with oxygen. Science. 2007;**318**:62-64

[3] Ratcliffe PJ, O'Rourke JF, Maxwell PH, Pugh CW. Oxygen sensing, hypoxia-inducible factor-1 and the regulation of mammalian gene expression. Journal of Experimental Biology. 1998;**201**:1153-1162

[4] Das KK, Das S, Ambekar JG. Hypoxia and oxidative stress: Cell signaling mechanisms and protective role of vitamin C and cilnidipine. In: Catala A, editor. Lipid Peroxidation: Inhibition, Effects and Mechanisms. Chapter 11, ISBN 978-1-53610-506-3. NY: Nova Science Publishers; 2017. pp. 249-262

[5] Kiang JG, Tsen KT. Biology of hypoxia. Chinese Journal of Physiology. 2006;**49**(5):223-233

[6] Kiang JG, Bowman PD, Zhao B, Atkins JL, Tsokos GC. Combat Casualty Care in Ground Based Tactical Situations: Trauma Technology and Emergency Medical Procedures, held in St. Pete Beach, USA, 16-18 August 2004, and published in RTO-MP-HFM-109

[7] Das KK, Saha S. Hypoxia, lead toxicities and oxidative stress: Molecular interactions and antioxidant (vitamin C) defense. Current Signal Transduction Therapy. 2014;**9**:113-122

[8] Semenza GL, Nejfelt MK, Chi SM, Antonarakis SE. Hypoxia-inducible nuclear factors bind to an enhancer element located 3'to the human erythropoietin gene. Proceedings of the National Academy of Sciences of the United States of America. 1991;**88**:5680-5684

[9] 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. Proceedings of the National Academy of Sciences of the United States of America. 1995;**92**:5510-5514

[10] Das KK, Nemegouda SR, Patil SG, Saha S. Possible hypoxia signaling induced alteration of glucose homeostasis in rats exposed to chronic intermittent hypoxia-role of antioxidant (vitamin C) and Ca^{2+} channel blocker (cilnidipine). Current Signal Transduction Therapy. 2016;**11**(1):49-55

[11] Jośko J, Mazurek M. Transcription factors having impact on vascular endothelial growth factor (VEGF) gene expression in angiogenesis. Medical Science Monitor. 2004;**10**(4): RA89-RA98

[12] Tjong YW, Li M, Hung MW, Wang K, Fung ML. Nitric oxide deficit in chronic intermittent hypoxia impairs large conductance calcium activated potassium channel activity in rat hippocampal neurons. Free Radical Biology and Medicine. 2008;**44**:547-557

[13] Hamanaka RB, Chandel NS. Mitochondria reactive oxygen species regulate hypoxic signaling. Current Opinion in Cell Biology. 2009;**21**(6):894-899

[14] Jones DP. Redefining oxidative stress. Antioxidants & Redox Signaling. 2006;**9**:1865-1879

[15] Bell EL, Klimova TA, Eisenbart J, Schumacker PT, Chandel NS. Mitochondrial reactive oxygen species trigger hypoxia-inducible factor-dependent extension of the replicative life span during hypoxia. Molecular and Cellular Biology. 2007;**27**(6):5737-5745

[16] Guzy RD, Schumacker PT. Oxygen sensing by mitochondria at complex III: The paradox of increased reactive oxygen species during hypoxia. Experimental Physiology. 2006; **91**(5):807-819

[17] Murphy MP. How mitochondria produce reactive oxygen species. Biochemical Journal. 2009;**417**:1-13

[18] Bell EL, Klimova TA, Eisenbart J, et al. The Qo site of the mitochondrial complex III is required for the transduction of hypoxic signaling via reactive oxygen species production. Journal of Cellular Biology. 2007;**177**(6):1029-1036

[19] Matsuno-Yagi A, Hatefi Y. Ubiquinol-cytochrome c oxidoreductase. The redox reactions of the bis-heme cytochrome b in ubiquinone-sufficient and ubiquinone-deficient systems. Journal of Biological Chemistry. 1996;**271**(11):6164-6171

[20] Tian H, Yu L, Mather MW, Chang-An Y. Flexibility of the neck region of the Rieske iron-sulfur protein is functionally important in the cytochrome bc1 complex. Journal of Biological Chemistry. 1998;**273**(43):27953-27959

[21] Guzy RD, Hoyos B, Robin E, Chen H, Liu L, Mansfield KD, et al. Mitochondrial complex III is required for hypoxia-induces ROS production and cellular oxygen sensing. Cell Metabolism. 2005;**1**(6):401-408

[22] Tafani M, Sansone L, Limana F, Arcangeli T, De Santis E, Polese M, Fini M, Russo MA. The interplay of reactive oxygen species, hypoxia, inflammation, and sirtuins in cancer initiation and progression. Oxidative Medicine and Cellular Longevity. 2016;**2016**:3907147

[23] Chandel NS, McClintock DS, Feliciano CE, Wood TM, Melendez JA, Rodriguez AM, Schumacker PT. Reactive oxygen species generated at mitochondrial complex III stabilize hypoxia-inducible factor-1 alpha during hypoxia; a mechanism of O_2 sensing. Journal of Biological Chemistry. 2000;**275**(33):25130-25138

[24] Solaini G, Baracca A, Lenaz G, Sgarbi G. Hypoxia and mitochondrial oxidative metabolism. Biochimica et Biophysica Acta. 2010;**1797**(6-7):1171-1177

[25] Qutub AA, Popel AS. Reactive oxygen species regulate hypoxia-inducible factor 1alpha differentially in cancer and ischemia. Molecular and Cellular Biology. 2008;**28**(16):5106-5119

[26] Pouysségur J, Mechta-Grigoriou F. Redox regulation of the hypoxia-inducible factor. Journal of Biological Chemistry. 2006;**387**(10/11):1337-1346

[27] Paddenberg R, Ishaq B, Goldenberg A, Faulhammer P, Rose F, Weissmann N, Braun-Dullaeus RC, et al. Essential role of complex II of the respiratory chain in hypoxia induced ROS generation in the pulmonary vasculature. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2003;**284**:L710-L719

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