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Hypoxia Inducible Factor-1: The guardian working in hypoxia

by

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Abbreviation List:

AHR	Aryl Hydrocarbon Receptor
ARNT	AHR Nuclear Translocator gene
bHLH	Basic Helix-Loop-Helix
Epo	Erythropoietin
HIF-1	Hypoxia-Inducible Factor-1
HRE	Hypoxia Responsive Element
PAS	Per-Arnt-Sim Domain
ROS	Reactive Oxygen Species

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Abstract

Hypoxia-inducible factor 1 (HIF-1) is an important transcription factor that is expressed upon hypoxia. HIF-1 is a heterodimer of HIF-1α subunit and HIF-1β subunit. HIF-1α is unique to HIF-1 whereas HIF-1β is also known as aryl hydrocarbon receptor (AhR) nuclear translocator (ARNT) gene product. HIF-1β can dimerize with AhR to form AhR complex and can also dimerize with HIF-1α to form HIF-1. HIF-1α subunit and HIF-1β subunit both have basic helix-loop-helix (bHLH) and PAS (*Per-Arnt-Sim*) domain, which are important for DNA binding and dimerization. These two domains are located at the amino-terminal half of each protein. The carboxyl-terminal half of HIF-1α as well as of HIF-1β is important for transactivation and regulation. This paper is focusing on the biochemical and structural properties of HIF-1.

Introduction

All organisms, from bacteria to humans, possess mechanisms to maintain oxygen (O_2) homeostasis that are essential for survival. Hyperoxia (high O_2 concentration) can results in the generation of reactive oxygen species (ROS) and potentially lethal damage to membranes and DNA [1], whereas hypoxia (low O_2 concentration) results in a failure to generate sufficient ATP to maintain essential cellular functions [2]. Normal tissue displays an oxygen gradient (from 2% to 5%) across a distance of 400 μm from a blood supply. On the contrary, tumor cells reveal significant hypoxia: cells adjacent to capillaries display a mean oxygen concentration of 2%, and cells located 200 μm from the nearest capillary display a mean oxygen concentration of 0.2% [3]. Under hypoxic conditions, the production of lactate and other acids via metabolism increases. Therefore, tumor cells live in an acidic environment in contrast to normal tissue as shown in Figure 1 [4]. The survival of tumor cells partly depends on the ability to adapt to hypoxia by recruiting new bloodvessels through angiogenic factors. Hypoxia and hypoglycemia stimulate the expression of vascular endothelial growth factor (VEGF) among other angiogenic factors. VEGF induces the formation of new microvessels to deliver nutrients and expand tumor mass, which we know is tumor angiogenesis [5].

Human tumors can survive under extreme hypoxia, which indicates that their ability to adapt to hypoxic conditions plays a critical role in tumor progression. Generally, cells respond to hypoxic conditions by altering patterns of gene expression. Among the first gene products that are stimulated to over-express at the onset of hypoxia is hypoxia-inducible factor-1 (HIF-1) [6]. Thus, HIF-1 is highlighted on the stage of hypoxia responses. HIF-1 is a transcription factor, which mediates crucial homeostatic responses to reduced O_2 availability in mammals by inducing

angiogenesis, glycolysis and erythropoiesis [7]. We are going to discuss some biochemical properties and structures of HIF-1.

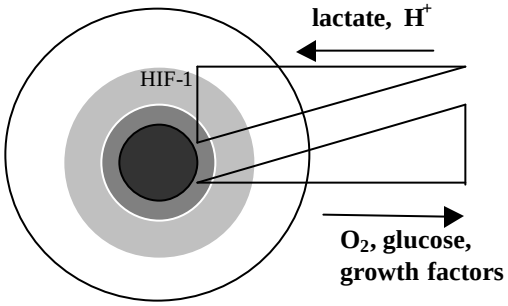

Figure 1. Schematic representation of pH and nutrients across from a tumor's diameter. It shows a core of dead cells (shown in black) surrounded by layers of living cells [4]. The different gray colors represent the pH, O_2 and nutrients gradients. HIF-1 is like a guardian working under the hypoxic conditions, providing cell the ability to survive under hostile environment.

Biochemical properties of HIF-1

Researchers have been investigating the molecular mechanisms by which reduced oxygen concentration influences the expression of specific genes such as erythropoietin (Epo), the main regulator of erythropoiesis. Although far from completely understanding how cells sense changes in O_2 concentration and transduce this signal to the nucleus, the cloning of a novel transcription factor that is activated by reductions in O_2 (hypoxia) attracted people's attention in 1995. This novel transcription factor, hypoxia inducible factor-1 (HIF-1), was identified to mediate homeostatic responses to hypoxia by transactivates two main classes of genes: those responsible for increased oxygen delivery and those involved in glycolysis [8].

HIF-1 was purified by ion-exchange and DNA-affinity chromatography in 1995 [9]. It was not discovered before possibly because the protein concentration remains at very low level under normoxia (normal oxygen concentration). Biochemical studies showed that HIF-1 is a heterodimer

composed of two subunits: an HIF-1α subunit and an HIF-1β. HIF-1α and HIF-1β are both constitutively expressed inside the cells. But under normoxia, HIF-1α is ubiquitinated and subjected to proteasomal degradation [9]. Therefore, HIF-1α is more important for the expression of HIF-1 and it keeps HIF-1 protein at undetectable level in most cells. Upon hypoxia, the degradation pathway is somehow blocked and HIF-1α is stabilized and translocated to the nucleus. There, it dimerizes with HIF-1β to form HIF-1. However, how the decreased oxygen concentration is sensed and passed along is still under investigations right now.

HIF-1α has 118 negatively charged residues which include Asp and Glu, and 85 positively charged residues which include Arg and Lys. The theoretical pI of HIF-1α is 5.17. The instability index (II) is 55.97, which is classified as unstable (calculated from program ProtParam at http://expasy.cbr.nrc.ca/tools/protparam.html). Experiments showed that the half-life of the HIF-1α protein upon reoxygenation is about 2.5 minutes [8]. This short half life also attributes to the difficult detection of HIF-1 in normoxia.

In purifying this HIF-1 protein, the molar extinction coefficient of the HIF-1α is a very useful parameter. The molar extinction coefficient of a denatured protein can be predicted based on its amino acid composition [10]. Following equation is used for the calculation of the molar extinction coefficient of a protein:

E(Prot) = Numb(Tyr)*Ext(Tyr) + Numb(Trp)*Ext(Trp) + Numb(Cystine)*Ext(Cystine)

E(Prot): molar extinction coefficient of a protein

Numb: number of a particular amino acid present in the protein

Ext: molar extinction coefficient of a particular amino acid

The conditions used for calculation are: pH 6.5, 6.0 M guanidium hydrochloride, 0.02 M phosphate buffer.

For HIF-1α, if assumed that all Cys residues appear as half cystines in this protein, at 280 nm the molar extinction coefficient of HIF-1α is 46070 $M^{-1}cm^{-1}$ and the absorbance is 0.497 at concentration of 1 g/L. If assumed that none of Cys residues appear as half cystines, at 280 nm the molar extinction coefficient of HIF-1α becomes 45230 $M^{-1}cm^{-1}$ and the absorbance is 0.488 with concentration of 1 g/L. With considering all these properties, HIF-1 was successfully purified using ion-exchange and DNA-affinity chromatography by Semenza group [9].

Structure analysis of HIF-1

HIF-1 is a heterodimer, which is composed of an HIF-1α subunit and an HIF-1β subunit [9]. HIF-1β subunit is a 91-94 kDa protein that contains 774/789 amino acids. The 774 and 789 amino acids are isoforms of HIF-1β. It is the protein product of the ARNT gene (Aryl Hydrocarbon receptor [AhR] Nuclear Translocator). ARNT can dimerize not only with AhR protein product in cells subjected to aryl hydrocarbons such as dioxin to form the AhR complex, but also with HIF-1α in cells exposed to hypoxia to form HIF-1 as shown in Figure 2. HIF-1 is a transcriptional factor which we discussed in this paper. AhR complex is also a transcriptional factor which can stimulate the transcription of genes involved in the metabolism of aryl hydrocarbons [11]. Properties about this complex is beyond this paper.

HIF-1α subunit is a 120 kDa protein containing 826 amino acids and it is unique to HIF-1 [12]. It contains basic helix-loop-helix (bHLH) domain and PAS (***P****er*, ***A****rnt*, ***S****im*) domain at the amino-terminal half, which are required for heterodimerization with HIF-1β and binding to DNA. Basic helix-loop-helix (bHLH) domain is one major DNA binding motif commonly found in many transcriptional factors [2]. Proteins containing this motif usually work as homodimer or heterodimer.

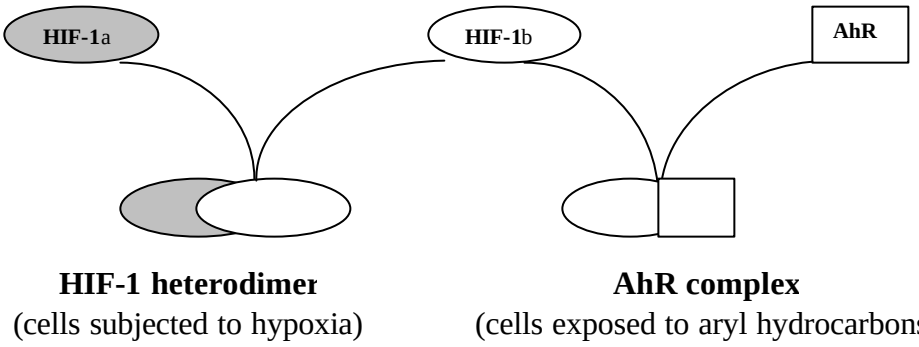

Figure 2. **Relationships of HIF-1**a **, HIF-1**b **and AhR.** ARNT gene product can dimerize with AhR to form AhR complex when the cells are exposed to aryl hydrocarbons, and can also form HIF-1 with HIF-1α when cells are subjected to hypoxia [8].

PAS domain was first found in the transcription factors *Per* and *Sim* in *Drosophila.* All PAS domains contain two direct internal repeats of approximately 50 amino acids, A and B, each of which contains a conserved HXXD motif. H represents the amino acid histidine. X can be any amino acid. D represents the amino acid aspartate [13]. PAS domain locates at the amino-terminal half of the HIF-1α protein and at the same location of the HIF-1β protein. Therefore, the amino-terminal half of the protein determines dimerization and DNA binding activity whereas the carboxyl-terminal half of the protein determines oxygen-regulated stability and transcriptional activity of HIF-1 [14]. The structure of the HIF-1A and HIF-1B gene are shown in Figure 3.

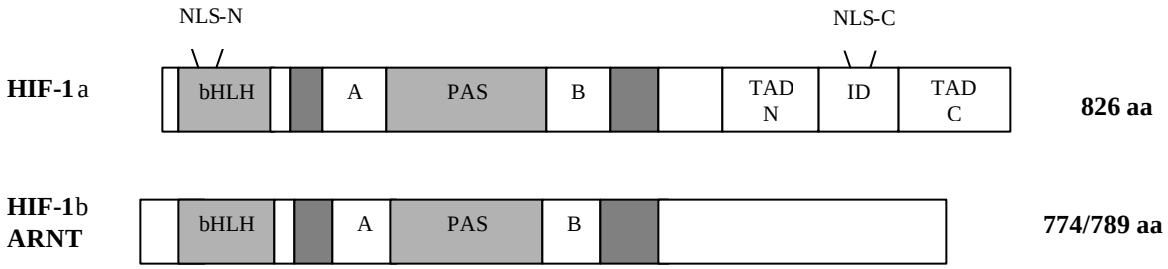

Figure 3: Human HIF1A and HIF1B gene structures. Structural motifs shown: basic helix-loop-helix (bHLH) domain, PAS domain with A and B repeats, amino-terminal (N) and carboxyl-terminal (C) nuclear localization signal (NLS), transactivation domain (TAD) and transcriptional inhibitory domain (ID). Different length of each domain (depicted as box) represents different number of amino acids inside [14].

In summary, both subunits (α and β) belong to the basic helix-loop-helix (bHLH) family containing a PAS domain. Therefore, the HIF-1α and HIF-1β (ARNT) proteins share the following structural motifs in common: (a) The bHLH, or basic helix-loop-helix domain, is the hallmark of an extensive superfamily of transcription factors. The HLH domains mediate protein dimerization, which is necessary for DNA binding mediated by the basic domains, and (b) A PAS domain is also required for dimerization [15].

Biological properties of HIF-1

HIF-1α protein expression, HIF-1β protein expression and HIF-1 DNA binding activity all increase exponentially as cellular oxygen concentration is decreased from 20% to 0.5% as shown in Figure 4. For all three parameters, curves showed a point of inflection at 4 to 5% oxygen concentration. It is revealed that oxygen concentrations in most tissue under normal physiologic conditions are in the range of 2 to 5%, indicating that any decrease in tissue oxygenation would occur along the steep portion of the HIF-1 response curve [16]. And remember that hypoxia is widespread in solid tumors: the mean oxygen concentration in cells adjacent to capillaries is about 2%, and the mean oxygen concentration cells located 200 μm from the nearest capillary is about 0.2% [3]. HIF-1 is highly expressed in this concentration range. At the same time, HIF-1 can stimulate the expression of a large group of genes. Basically, the genes that is upregulated by HIF-1 under hypoxia can be classified into two classes: those in charge of oxygen delivery and those involved in glycolysis [8]. Therefore, tumor adapts to hypoxia in two ways: increasing oxygen delivery and decreasing oxygen consumption. This may partly accounts for the ability of tumors to adapt to hypoxia and the understanding of this process will lead to the fundamental understanding of cacinogenesis.

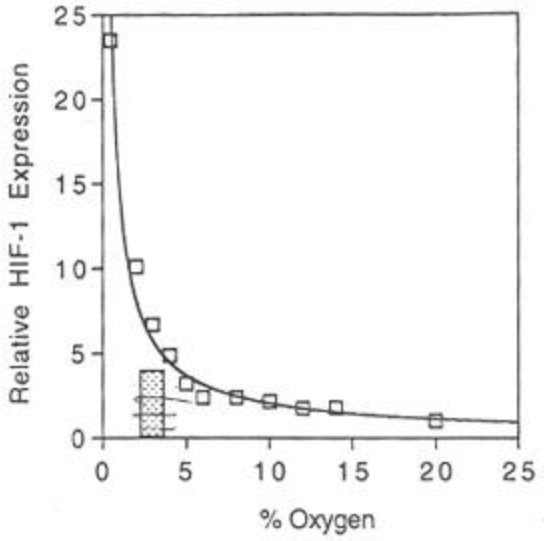

Fig. 4: Relative HIF-1 expression as a function of oxygen concentration. The stippled bar indicates the range of oxygen concentration that have been measured in the hearts of laboratory animals at rest [16].

Summary

Due to the high proliferative rate and limited nutrients, tumor cells live in a very hostile microenvironment. The ability of adapting to the environment is important for the survival of tumor cells. Hypoxia-inducible factor 1 (HIF-1) is an important transcription factor that is working in hypoxia. HIF-1 is a heterodimer of HIF-1α and HIF-1β. HIF-1α is unique to HIF-1 whereas HIF-1β is also known as ARNT gene product. Under normoxia, HIF-1α is expressed but ubiquitinated and subjected to proteasomal degradation. Upon hypoxia, HIF-1α is stabilized and translocated to nucleus. Then it forms a heterodimer with HIF-1β. This accounts for the quick response of the cell to hypoxia. But how the low oxygen concentration is sensed and transduced, and how HIF-1α is regulated is far from clear. And the understanding of these processes can significantly facilitate tumor therapy.

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