

HYPOXIA-INDUCIBLE FACTOR-1, A NEW POSSIBLE IMPORTANT FACTOR IN NEOPLASIA

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Hypoxia-inducible factor-1 (HIF-1) is one of the major factors responsible for the activation of compensation processes during cell hypoxia. Hypoxia-inducible factor-1 is a dimeric protein complex and serves as a transcription factor regulator for many target genes. Under normoxic conditions it is constitutively produced and degraded by the ubiquitin-proteasome system. But under hypoxic conditions HIF-1 becomes stabilized. The expression of HIF-1 increases vascularization of the ischaemic area and regulates anaerobic metabolism. The same processes were observed in neoplastic tissues. Hypoxia-inducible factor-1 and its participation in neoplasia, the inhibition and stimulation of its transcriptional activity, attracts the attention of many scientists as the understanding of its function in tumour progression could be used in the future for preparation of new antineoplastic therapies.

Key words: hypoxia-inducible factor-1, neoplasia

Introduction

Before algae were created in the evolutionary process, the atmosphere did not contain free oxygen. There was an anaerobic environment. However, about two thousand million years ago oxygen freed in photosynthesis by cyanobacteria started to "pollute" the mainly nitric atmosphere. The constant and long-lasting process of evolution led to the selection of organisms better prepared to adapt to progressive ongoing changes in the gaseous environment. Nowadays, the vast majority of multicellular organisms are dependent on oxygen. As a further consequence of evolution there arose a need to create regulating systems sensitive to a deficit of oxygen. As previously described, the fall in concentration of oxygen in a cell below 2% activates processes which are regulated by proteins called hypoxia-inducible factors, and among them HIF-1 (hypoxia-inducible factor-1) is one of the key elements [1, 2].

Hypoxia-inducible factor-1 structure

Hypoxia-inducible factor-1 was identified in the early 1990s by Semenza and Wang in Hep3B cells

when their studies were concentrated on research for enhancer of the erythropoietin gene. After publication of many research data, it is now known that HIF-1 is a basic protein formed by two subunits, namely HIF-1 α and HIF-1 β (Fig. 1). These subunits belong to the transcription factors called PAS (Per, ARNT, Sim). Hypoxia-inducible factor-1 β is known as the ARNT protein (the aryl hydrocarbon receptor nuclear translocator). Subunits have a "b" (basic) domain which is responsible for binding heterodimeric HIF-1 with DNA at a specific HRE (hypoxia response element) region characterized by the conservative sequence 5'-CGTG-3'. Subunits also contain the domain called HLH (helix-loop-helix), which is responsible for the dimerization. And finally, there is the PAS domain, which is characteristic for the whole group of transcription factors. In some published studies it was shown that HIF-1 α subunit contains two transactivation domains which are found next to the C end of the HIF-1 α molecule. These subunits are called TAD-C and TAD-N. Additionally, between them there is an inhibitor domain (ID). Another domain called ODD (oxygen-dependent degradation domain) plays the most important role in the re-

gulation of HIF-1 α stability. Ubiquitination of ODD in normoxic conditions leads to degradation of HIF-1 α in the proteasome. The HIF-1 family of proteins also consists of tissue specific forms such as HIF-2 α and HIF-3 α . Those factors also are heterodimerized with HIF-1 β and play an analogous function as HIF-1 α [2-6].

Hypoxia-inducible factor-1 degradation

Under normoxic conditions, the half-life of HIF-1 α in cell cytoplasm is about 5 minutes. In normoxic conditions HIF-1 α does not become activated, because the protein is rapidly degraded by the E3 ubiquitin ligase. Enzymes from the 4-proline hydroxylases group lead to hydroxylation of two prolines (P402 and P654) mapped within the ODD region. Those HIF-1 α modifying hydroxylases belong to oxygen- and iron-dependent dioxygenases. The enzymatically modified part of HIF-1 α links to tumour suppressor protein pVHL (von Hippel-Lindau). Finally, all aforementioned processes lead to a subsequent catalytic effect on ubiquitination of the TAD-N domain, and then direct HIF-1 α on the way of degradation by the ubiquitin-proteasome system [2, 5-8].

Hypoxia-inducible factor-1 stabilization, activation and function

HIF-1 α is stabilized in hypoxia as well as after inhibition of 4-proline hydroxylase activity, and then is accumulated in cell cytoplasm. There are also other

known stabilizers of HIF-1 α such as Hsp90 (heat shock protein 90) and Jab1 (Jun activation domain-binding protein 1). Moreover, there was also described a positive influence of NLS (nucleus localization signal) on stabilization of HIF-1 α after its translocation into the cell nucleus. Additionally, stabilization is achieved by dimerization of HIF-1 α and HIF-1 β units. Finally, HIF-1 is linked to DNA through the HSB (HIF-1 binding site) region which is found within HRE. In the cell nucleus there is activation of the TAD-C domain. HIF-1 α is activated by its phosphorylation. Then linking to CBP/p300 (CREB binding protein) as a transcription co-factor occurs, and in this process increases the transactivation capacity of HIF-1 α . The transactivation capacity of HIF-1 α could be blocked by FIH-1 protein (factor inhibiting HIF-1). The latter element is dioxygenase which catalyses Asn803 hydroxylase within the TAD-C domain. After this phenomenon, binding of HIF-1 α with CBP/p300 and its further activation decrease [2, 5, 6, 9, 10].

There was found a correlation between level of expression of HIF-1 α and level of p53 expression. During prolonged hypoxia competition between p53 and HIF-1 α for binding to p300 cofactor occurs. The tumour suppressor gene pVHL was found as another regulator of HIF-1 α activation. In cells with impaired expression of pVHL there is no degradation of HIF-1 α in a normoxic environment. Moreover, decrease in expression or lack of phosphatase and tensin homologue deleted on chromosome ten (PTEN) is related to increased expression of genes regulated by HIF-1 α . Furthermore, it was already proved that

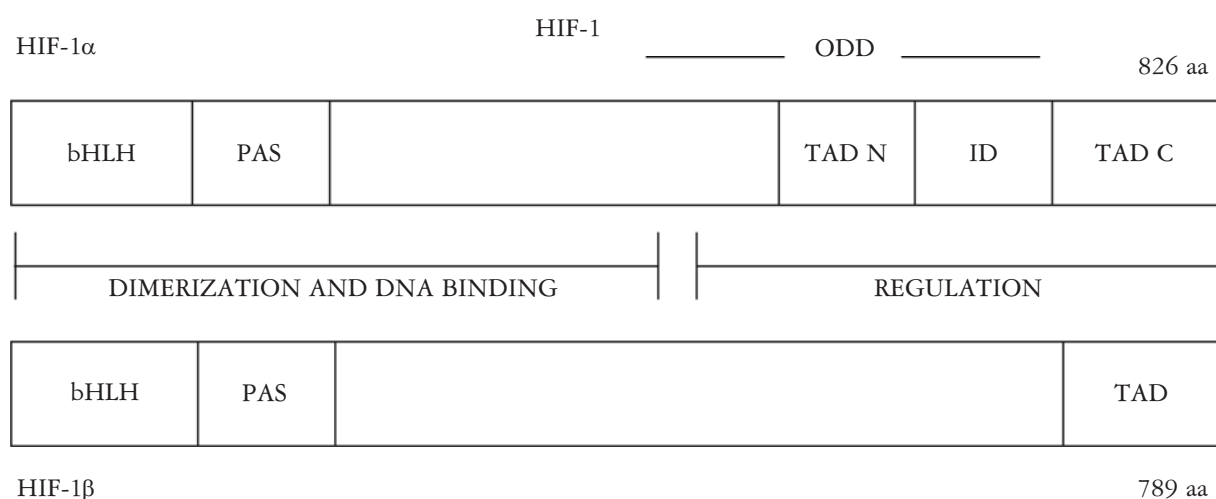


Fig. 1. Structure of HIF-1. Hypoxia-inducible factor-1 is composed of two subunits: HIF-1 α and HIF-1 β . Dimerization of HIF-1 is mediated by HLH and PAS domains. DNA binding involves b region. Transcription is modulated by TAD domain. Between amino acids 401 and 603 with HIF-1 α is ODD domain which controls degradation of the particle under normoxia

changes of activity of such genes as BCR/ABL and HER2/neu lead to increased activity of HIF-1. There were identified numerous growth factors which control HIF-1 α levels in normo- and hypoxia. Among such factors there are EGF, IGF-1, insulin, and bFGF. Additionally, beside the aforementioned proteins, oxygen free radicals play a role in HIF-1 α activation. In this last mentioned group, the main component is H₂O₂, as inside the cell it increases transcriptional activity of genes regulated by the HRE sequence. Also nitric oxide (NO) was recognized as another regulator of HIF-1 activity. But the effect of intracellular concentration of NO on HIF-1 α expression remains unclear. However, it was found that lower NO concentration induces HIF-1 α expression. Some authors even found that heavy metal ions could also have an influence on mechanisms that regulate HIF-1 α function. It could be through an indirect mechanism of inhibition of HIF-1 α degradation. On the other hand, a "redox" cellular environment stabilizes HIF-1 α and increases its phosphorylation and capacity of DNA linkage [2, 6, 8].

Hypoxia-inducible factor-1 in inflammation and neoplasia

It is an old dogma that the inflammatory process is triggered, e.g. by a foreign antigen. Then in the natural history of inflammation increased blood inflow is observed. Then increased vascular permeability along with excessive migration of leukocytes through the endothelium occurs. In the acute phase at the site of inflammation accumulation of leukocytes, phagocytic cells and lymphocytes with parallel accumulation of serum proteins is observed. In such an inflammatory environment, HIF-1 α is responsible for the switch of cell metabolism from aerobic to anaerobic. Moreover, inhibition of HIF-1 α synthesis disables the proper function of leukocytes in a low oxygen tension environment. That finally causes inhibition of the inflammatory process cascade. Such a phenomenon could possibly be used in the treatment of chronic inflammation that usually accompanies for instance autoimmunological conditions and neoplastic disease. Moreover, it has already been published that HIF-1 α plays an important role in differentiation of myeloid haematopoietic precursors into monocytes and then even their activation and transformation into macrophages [6, 11, 12].

The response of the organism to hypoxia might include activation of metabolic pathways that do not rely on molecular oxygen. There are also other possible mechanisms, that consist of an increased number of erythrocytes in peripheral blood, or even angiogenesis – in longer observations – which, after

a certain amount of time, finally leads to an increased capillary network at the site of decreased oxygen tension. HIF-1 α is partially responsible for those aforementioned processes. First, it induces transcription of glycolytic enzymes such as hexokinase, aldolase, phosphofruktokinase, pyruvic kinase, enolase, and 3-phosphoglycerine aldehyde dehydrogenase. Additionally it is responsible for activation of glucose transporters, too. Then, cells prepared in such processes are responsible for the metabolism switching to the anaerobic process. Parallely, there occurs induction of transcription of genes that inhibit apoptosis. Among those apoptosis related gene products are: p21, Bcl2/EIB interacting protein 3 and IGF. Finally, HIF-1 α induces production of proteins responsible for oxygen availability in tissue. There are such factors as erythropoietin, transferrin and its receptors, and ceruloplasmin. Moreover, production of factors that stimulate angiogenesis – such as VEGF, its receptor as well as TGF β – could also be controlled by HIF-1 α . Even production of carbonic anhydrase, which regulates intracellular pH, is dependent on HIF-1 [2, 6, 10, 13, 14].

The first researcher who noticed increased blood supply of tumours was Rudolf Virchow. He published his observations in 1893 in the paper entitled "Die Krankhaften Geschwülste". In the conclusion of that publication, he suggested that this phenomenon could be related to disorganization of tumour cells. But the milestone on neoangiogenesis studies was the publication of Folkman's thesis. He assumed that tumour growth as well as formation of metastases depends on development of the vascular network. With such a background, one should also remember that the characteristic feature of solid tumours from the biochemical point of view is hypoxia – which as a normal tumour setting is achieved by abnormalities in its growth/progression. In the vast majority of solid tumours rapid growth of neoplasm is disproportionate to its vascular network formation. Additionally, tumour vessels present structure and function abnormalities. Additionally, there are rather long distances between tumour cells and the vascular lumen, too. And finally, in neoplastic diseases there could develop general anaemia which is induced by neoplasia itself and also by introduced chemotherapy.

A hypoxic environment promotes selection of tumour cells which are resistant to apoptosis but with good capacity for proliferation and division. This is observed in cells which modify their metabolism and possibly are able to stimulate angiogenesis. In the case of malignant neoplasms blood as well as nutrient supply plays a key role in their survival. Progression of primary tumours and formation of distant metastases depend on the network of capillaries and lymphatics, too.

Previously, it was believed that tumour vessels are formed when the tumour reaches 2 mm in diameter. But in 1999 Holash and co-authors proved that for the initiation of new vascular network formation only 60-80 cells are needed. Newly formed and well functioning vessels could be observed when the tumour was composed of 100 to 300 cells.

The initiation of angiogenesis could be provoked by ischaemia. But on the other hand, it could rely – at least partially – on mutations of oncogenes and suppressor genes. As a result of those overlapping processes neoplastic cells activate specific factors that stimulate divisions and migration of endothelial cells of blood vessels within the close vicinity of the

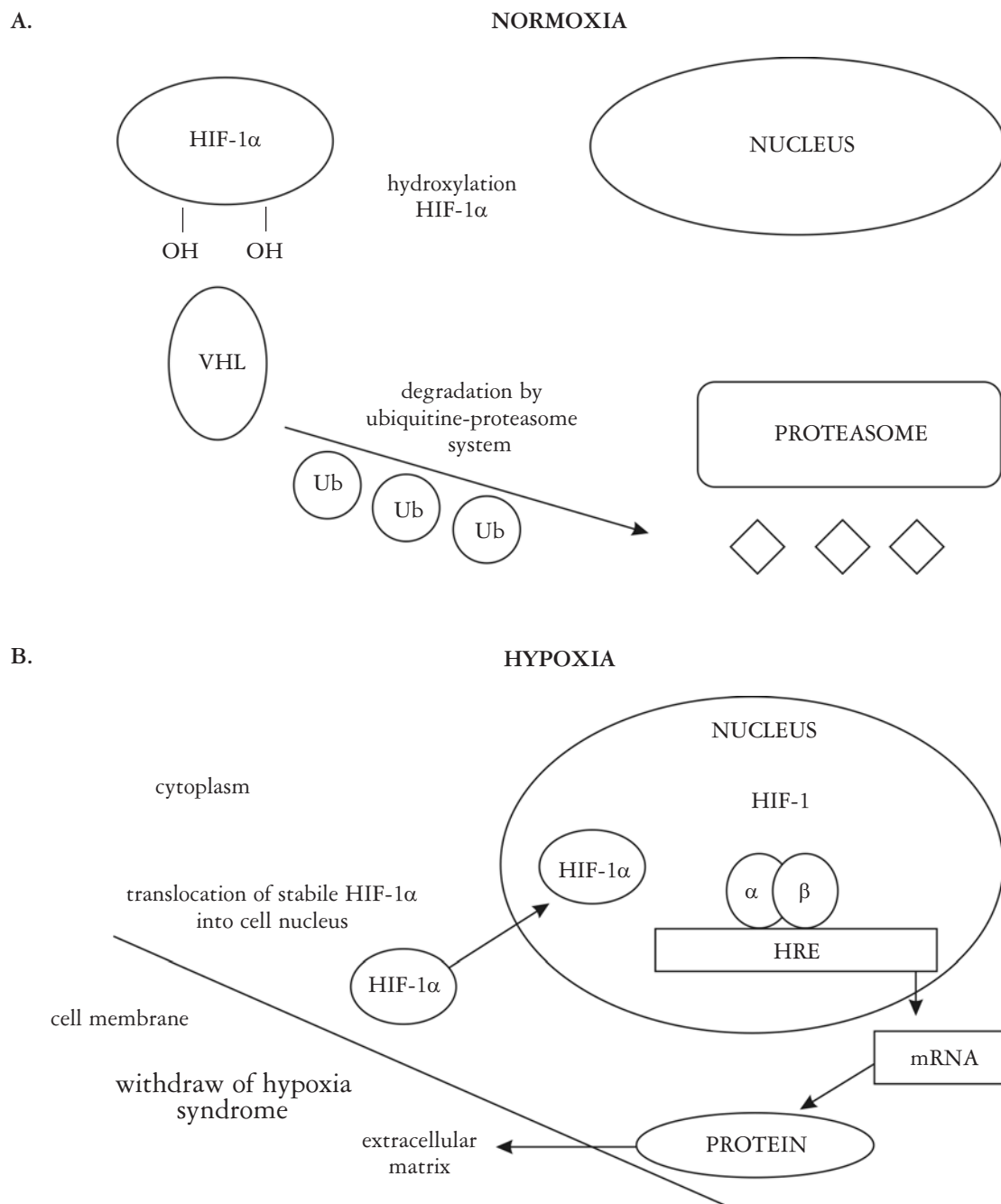


Fig. 2. Mechanism of HIF-1 α regulation. (A) In normoxic environment HIF-1 α is hydroxylated by dioxygenases. Modified HIF-1 α links to VHL protein which catalyses ubiquitination reaction (Ub) and moves HIF-1 α to degradation pathway in proteasome. (B) In hypoxic environment, stable HIF-1 α is translocated to cell nucleus where it is bound to HIF-1 β . Then HIF-1 initiates transcription of target genes by binding to DNA at the HRE (*hypoxia response element*) site. Finally gene expression leads to withdrawal of hypoxia effects. This figure is a modification of a figure from [4]

tumour. Then it is finalized by formation of tumour surrounding and penetrating vessels. Newly formed vessels are responsible for sufficiently fulfilling the tumour's metabolic needs, which include supply of nutrients and removal of metabolic waste products. But there is also the possibility of escape of neoplastic cells from their primary site throughout newly formed capillaries. This leads to formation of distant metastases. Compared with normal vessels, those formed within the tumour are different. The pathological vessels are characterized by abnormal shape, increased permeability, abnormal blood flow, and they are unable to obtain normal morphological maturation [2, 4, 6, 15-19].

Some preneoplastic lesions, as well as the majority of primary and metastatic tumours, reveal increased expression of HIF-1 α . Its overexpression is mainly related to tumour hypoxia. As is already in part explained above, HIF-1 activation favours adaptation of neoplastic cells to hypoxia. Additional mechanisms involved in this process might include accumulation of tumour suppressor gene mutations. So far, genetic abnormalities have been found in such genes as PTEN and pVHL or p53. Moreover, there was also found activation of oncogenes, e.g. Ras, HER2/neu, and Src. Finally, tumour cells equipped with progression mechanisms including one of the aforementioned promoting pathways are capable of expansion and further tumour growth. Moreover, HIF-1 α through inhibition of incorrect paired bases recognition complex expression (MutS α) is mainly responsible for genetic instability of neoplastic cells. This factor induces mutation in the cell genome at an early stage of tumour growth. This is reflected by the usually low expression of non-mutated wild-type p53 gene in tissues involved later by the primary tumour.

Hypoxia-inducible factor-1 α inhibits apoptosis induced by hypoxia and increases the rate of cell proliferation through induction of proteins responsible for cell division and differentiation. Hypoxia-inducible factor-1 α stimulates tumour neo-angiogenesis by induction of transcription of genes for VEGF and Cyr61 (cysteine rich protein 61), which increase the risk of formation of tumour metastases by easier movement of neoplastic cells into blood vessels. The same factor that we mentioned earlier allows for the tumour cells' adaptation to anaerobic metabolism, through increased expression of all genes of the glycolytic cycle, and activates glucose transporters and CAIX, which regulate pH inside the cells [2, 6, 8, 14, 18].

The correlation between increased vascular supply and tumour growth and formation of metastases raised a need for studies on the mechanisms responsible for those phenomena. Such knowledge could serve as a background for the search for substances that might inhibit neoangiogenesis and then inhibit

expansion of neoplastic diseases. Some authors have even published papers on possible anti-angiogenic therapy based on inhibition of tumour vascularity. In some centres worldwide there are ongoing clinical trials on the use of synthetic inhibitors of angiogenesis in the treatment of such cancers as: breast, ovaries, colon, lungs, pancreas, prostate, kidneys, solid tumours of different locations, and even multiforme glioblastoma. Therapeutic anti-angiogenic protocols in oncology usually are based on inhibition of angiogenesis stimulators or on the activation of angiogenesis suppressors. But recently published papers were focused on inhibition of angiogenesis through interfering with molecular mechanisms responsible for formation of blood vessels. But, there were also published papers on paradoxical decreased tumour growth under HIF-1 α overexpression. However, such contrary data do not remove the possibility of use of HIF-1 α as an attractive target for anti-tumour therapy in the future [4, 15, 16, 19].

Closing remarks

In summary, all the available data published so far on structure, functions, target genes and activation pathways of HIF-1 give us a wide range of possibilities to treat diseases such as cancer and ischaemia. Precise recognition of mechanisms that activate transcription factors through HIF-1 might help in the development of new and possibly better antineoplastic therapies.

This paper was partially supported by grant No. N401 082136 from the Ministry of Sciences and Higher Education.

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