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Biochemical Pharmacology 64 (2002) 889–892

Biochemical Pharmacology

Is HIF-1α a pro- or an anti-apoptotic protein?☆

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Received 9 January 2002; accepted 26 February 2002

Abstract

Hypoxia-inducible factor-1 (HIF-1) is the major transcription factor specifically activated by hypoxia. It induces the expression of different genes whose products play an adaptive role for hypoxic cells and tissues. Besides these protective responses, HIF-1 and/or hypoxia have also been shown to be either anti-apoptotic or pro-apoptotic, according to the cell type and experimental conditions. More severe or prolonged hypoxia rather induces apoptosis that is, at least in part, initiated by the direct association of HIF-1α and p53 and p53-induced gene expression. On the other hand, HIF-1α dimerized with ARNT, as an active transcription factor, can protect cells from apoptosis induced by several conditions. This review is aimed to describe the different mechanisms that account for these opposite effects of HIF-1α.

Keywords: Hypoxia; Apoptosis; Pro-apoptotic; Anti-apoptotic; HIF-1α

1. Introduction

Chronic hypoxia induces the expression of different genes whose products augment non-oxidative synthesis of ATP, increase the oxygen-carrying capacity of blood and multiply the number of capillaries irrigating the hypoxic tissue. All these responses are considered to have physiological role because they are adaptive, easily reversible and elicited with moderate levels of hypoxia. Although there is no strict demarcation between physiological and extreme hypoxia, these adaptive responses must be distinguished from anoxia or extreme low O_2, which induces cell death.

2. Hypoxia-inducible factor-1

HIF-1 is the major transcription factor responsible for specific induction of genes in hypoxia. HIF-1 can also be activated by cytokines, hormones and NO. HIF-1 is composed of two subunits belonging to the bHLH-PAS family: HIF-1α and aryl hydrocarbon receptor nuclear translocator (ARNT). To activate transcription of target genes, HIF-1α dimerizes with ARNT and binds to consensus sequences (hypoxia responsive element; HRE) in the promoter or enhancer of these genes. Proteins encoded by such genes are vascular endothelial growth factor (VEGF), erythropoietin, glucose tranporter-1, glycolytic enzymes and tyrosine hydroxylase [1]. In normoxia, Von Hippel Lindau protein (pVHL) organizes the assembly of a complex that activates the E3 ubiquitin ligase which then ubiquitinylates HIF-1α, targeting its degradation. Recent data showed that the interaction between HIF-1α and pVHL is regulated through hydroxylation of two proline residues of HIF-1α by a prolyl hydroxylase enzyme. In the absence of oxygen, this enzyme is no longer active: the unmodified prolyl-HIF-1α does no longer interact with pVHL and accumulates [2,3].

Subsequent modifications (reduction and phosphorylation) are then required for enhancing the transcriptional activity of HIF-1. On the other hand, ARNT is constitutively expressed in the nucleus and acts as a common

☆This text presents results of the Belgian Program on Interuniversity Poles of Attraction initiated by the Belgian State, Prime Minister's Office, Science Policy Programming. The scientific responsibility is assumed by its authors.

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Abbreviations: ARNT, aryl hydrocarbon receptor nuclear translocator; HIF-1, hypoxia-inducible factor-1; pVHL, Von Hippel Lindau protein; VEGF, vascular endothelial growth factor.

0006-2952/02/$ – see front matter
PII: S0006-2952(02)01155-3

subunit of multiple bHLH-PAS transcription factors. Thus, the hypoxic induction and modifications of HIF-1α determine the HIF-1 transcriptional activity [1,4].

3. Apoptosis

Programmed cell death or apoptosis describes the common pathway leading to "physiological" death as observed in development or to eliminate pathogen-invaded cells. These dying cells share many morphological features, which are distinct from the features observed in cells undergoing pathological, necrotic cell death. Membrane blebbing, phosphatidylserine flip-flop, protein fragmentation, chromatin condensation, DNA fragmentation and cell shrinkage are hallmarks of apoptotic cells.

Various situations can lead to apoptosis and the signal transduction pathways initiated are different but most of them seem to converge to mitochondria which serve as an "integrator" of these signals. Finally, caspases, the central executioners, are activated and bring about most of the changes that characterize apoptotic cell death (for a review, [5]).

Caspases possess an active-site cysteine and cleave substrates at Asp–X bonds. They are synthesized as enzymatically inactive zymogens and get activated by proteolytic cleavage by caspases themselves, resulting in an amplifying cascade. They are specifically activated in apoptotic cells and caspase-mediated cleavage of specific substrates explains most of the typical features of apoptosis [6]. Activation of caspase-activated Dnase (CAD) through caspase 3-meditaed cleavage of the inhibitory subunit is responsible for the typical DNA fragmentation [7]. Cleavage of nuclear lamins is required for nuclear shrinking and loss of overall cell shape is probably caused by the cleavage of cytoskeletal proteins such as fodrin and gelsolin. Two pathways can initiate the caspase activation cascade. Caspase 8 is the key initiator caspase in the death receptor pathway ("extrinsic" pathway). It is probably autocatalytically cleaved through induced proximity triggered by receptor trimerization upon ligand binding. On the other hand, caspase 9 activation occurs upon release from mitochondria and association with Apaf-1 and cytochrome *c* ("intrinsic" pathway).

Mitochondria sequester a potent cocktail of pro-apoptotic proteins including various caspases, cytochrome *c*, Apaf-1, AIF and SMAC/diablo which are inhibitors of caspase inhibitors (IAP). Exactly how these proteins are released from mitochondria is not yet known but Bcl-2 family members are intimately involved in the regulation of this process. Bcl-2 and Bcl_{XL} block cytochrome *c* release while Bax, Bad, Bak or Bid induce it [8]. It has been suggested that Bcl-2 proteins might act by inserting into the outer mitochondria membrane, where they could form channels either by oligomerization or by interaction with other proteins like voltage dependent anion channel (VDAC) [9].

Several pro-apoptotic pathways converge to mitochondria [10]. It is notably the case for the translocation of Bax from the cytosol to the mitochondria triggered either by cleaved Bid coming from death receptor-induced caspase 8 activation or by its overexpression through the activation of p53 for example by DNA damages. Serum deprivation also results in the translocation into mitochondria of a pro-apoptotic Bcl-2 family member, dephosphorylated Bad. Finally, signals coming from altered organelles also talk to mitochondria, inducing mitochondrial membrane permeabilization [11].

Apoptosis is thus a tightly regulated endogenous cell death program requiring the coordinated activation of signaling pathways and executioner proteases.

4. Hypoxia as an inducer of apoptosis

When cells are exposed to chronic or extreme hypoxia, the protective adaptive mechanism initiated by HIF-1 is not sufficient, resulting in cell apoptosis. Surprisingly, HIF-1 seem also to be involved in initiating apoptosis as it is in triggering the adaptive response. At least two mechanisms have been unraveled by which HIF-1 may induce apoptosis.

First, hypoxia has been shown to increase the expression of Nip3, a pro-apoptotic member of the Bcl-2 family. This does not occur in HIF-1α deficient cells while Nip3 is constitutively expressed in normoxia in cells defective for pVHL [12]. Moreover, Bruick [13] demonstrated the presence of a function HRE in the Nip3 promoter that is responsive to hypoxia and to forced expression of HIF-1α.

Second, hypoxia induces the stabilization of p53 protein [14]. The p53 tumor suppressor protein is a potent transcription factor that can activate target genes that initiate cell death (e.g. *Bax*) or cause growth arrest (e.g. *p21*) in response to stress or DNA damage. At rest, p53 is targeted for proteasome degradation by MDM2. Phosphorylation of p53, for example by ATM, interferes with the ability to bind MDM2, thereby enhancing p53 protein level. In addition, phosphorylation in the transactivation domain increases p53 transcriptional activity [15]. p53 stabilization in hypoxia is parallel to HIF-1α accumulation and is dependent on the presence of this protein since it is not observed in cells deficient for HIF-1α but not on the transcriptional activity of HIF-1 since it is still observed in cells deficient for ARNT [16]. Moreover, a direct association between p53 and HIF-1α was detected in hypoxic cells by co-immunoprecipitation [16].

The direct interaction between p53 and HIF-1α not only results in p53 stabilization but also in an inhibition of HIF-1-dependent transcription. Competition between p53 and HIF-1 for the coactivator p300 [17] as well as MDM2-dependent degradation of HIF-1α *via* the formation of a complex containing p53, HIF-1α and MDM-2 [18] may explain this effect. Stabilization of p53 in hypoxia results

in the increased expression of p53 target genes such a *p21* and in the induction of apoptosis. Both are abrogated in HIF-1α deficient cells [19].

The HIF-1α dependent p53 mediated induction of apoptosis in hypoxia may have various physiological consequences. In this way, Carmeliet *et al.* [19] showed that tumors from HIF-1α−/− ES cells developed more rapidly than wild type tumors because of a lower rate of apoptosis within the tumor. Conversely, loss of p53 in tumor cells enhances HIF-1α levels and augments HIF-1 dependent transcriptional activation of VEGF in response to hypoxia. This amplification of HIF-1 dependent responses to hypoxia may contribute to the angiogenic switch during tumorigenesis, hence favoring tumor growth [18]. Interaction between p53 and HIF-1α is also responsible for promoting delayed neuronal death in models of CNS ischemia, through the enhanced expression of pro-apoptotic genes by p53 [20,21].

5. HIF-1 as an anti-apoptotic factor?

Hypoxia has also been reported to suppress apoptosis in several experimental conditions. Different possible mechanisms have been suggested in the literature, some of them involving HIF-1 while others are clearly HIF-1 independent. An example of the latter is illustrated by the work of Dong *et al.* [22]. They showed that severe hypoxia could protect kidney proximal tubule cells from staurosporine-induced apoptosis. In parallel, a marked induction of IAP-2 expression was observed that is HIF-1 independent since also present in HIF-1α−/− ES cells. Mcl-1, a Bcl-2 homologue, is another anti-apoptotic protein whose expression is increased in hypoxia. The hypoxia-induced Mcl-1 overexpression observed in neutrophils is dependent on p38 MAPK pathway [23] but the identity of the downstream transcription factor is not determined.

Hypoxia has also been shown to prevent serum deprivation-induced apoptosis of tumor cells which was accompanied with decreased Bax/Bcl-2 ratio, inhibited cytochrome *c* release and reduced caspase 3 activity. Increased VEGF expression in these conditions suggests that VEGF may act as a survival factor. Indeed, anti-VEGF neutralizing antibody blocked the anti-apoptotic effect of hypoxia [24]. VEGF is one of the most important HIF-1 target genes induced in hypoxia but this study did not address whether the increased VEGF secretion observed in their conditions was dependent on this transcription factor. Exogenous VEGF also protect primary cultures of cortical neurons from hypoxia- and glucose deprivation-induced loss of viability [25].

More direct evidence for a protective role of HIF-1 against apoptosis were obtained by Zaman *et al.* [26]. They observed that iron chelators prevented oxidative stress-induced apoptosis in cortical neuron cultures. This protection was associated with enhanced DNA binding activity of HIF-1 and increased expression of HIF-1 target genes such as *enolase*, *p21* and *erythropoietin*. The anti-apoptotic property of HIF-1 was confirmed by the work of Akakura *et al.* [27] who showed that HIF-1α overexpression renders pancreatic cancer cells resistant to apoptosis induced by hypoxia or nutrient deprivation. What remains to be determined is exactly which known or unknown HIF-1 target gene(s) are protective against apoptosis.

6. Conclusion

According to the reports, hypoxia and more specifically HIF-1α exerts pro- or anti-apoptotic effects. The pro-death activity seems to occur for more prolonged or more severe hypoxia and results from the association of HIF-1α with p53. On the other hand, the anti-apoptotic effect is evidenced at level of hypoxia that leads to HIF-1 activation *via* dimerization of HIF-1α and ARNT and adaptive increase in gene transcription. Recently, Suzuki *et al.* [28] elegantly demonstrated that two distinguishable forms of HIF-1α were responsible for these opposite activities. Phosphorylated HIF-1α dimerizes with ARNT while dephosphorylated HIF-1α associates with p53, stabilizes p53 and induces apoptosis *via* Bax overexpression. Fig. 1 schematically summarizes these different results. Apoptosis induction depends on the severity of hypoxia: mild hypoxia is rather protective through the expression of different anti-apoptotic proteins, some of them through HIF-1 transcriptional activity. On the other hand, severe hypoxia leads to cell death at least in part *via* the stabilization of p53 by

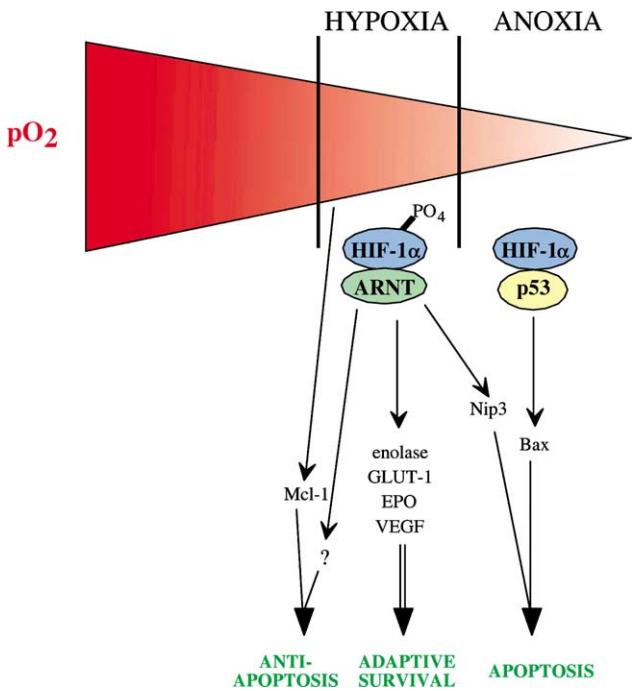

Fig. 1. Schematic representation of the pro- and anti-apoptotic effects of hypoxia.

HIF-1α. p53 mutation occurs in more than 50% of all human cancers [29]. As p53 inhibits HIF-1 transcriptional activity, enhanced angiogenic potential in p53 mutated cells could favor tumor growth in addition to their diminished apoptosis capacity.

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