Regulation of Gene Expression by HIF-1α Protein

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Abstract - Hypoxia-inducible factor-1 (HIF-1) mediates the cellular adaptation to low oxygen concentration in the surrounding environment and nutrient depletion and orchestrates a crucial role in physiological and pathological processes. The stability and activity of HIF-1α are performed by various post-translational modifications like hydroxylation, acetylation, and phosphorylation. Being oxygen- transcriptional regulator, HIF-1α undergoes interaction with several protein factors including PHD, pVHL, ARD-1, and p300/CBP. It has been presumed that HIF-1alpha is not only regulated by the oxygen tension, but also by other stimuli, such as transition metals, nitric oxide, reactive oxygen species, growth factors, and mechanical stresses. Hydroxylation of proline and acetylation of lysine within the polypeptide segment called as Oxygen Dependent Degradation Domain (ODDD) induces the association of pVHL and HIF-1α under normoxic conditions. Here, we gather and enumerate the oxygen-dependent and oxygen -independent regulation of HIF-1alpha protein.

Keywords – HIF-1α Protein, PHD,pVHL, Oxygen Dependent Regulation, Oxygen Independent Regulation, Normoxia

I. INTRODUCTION

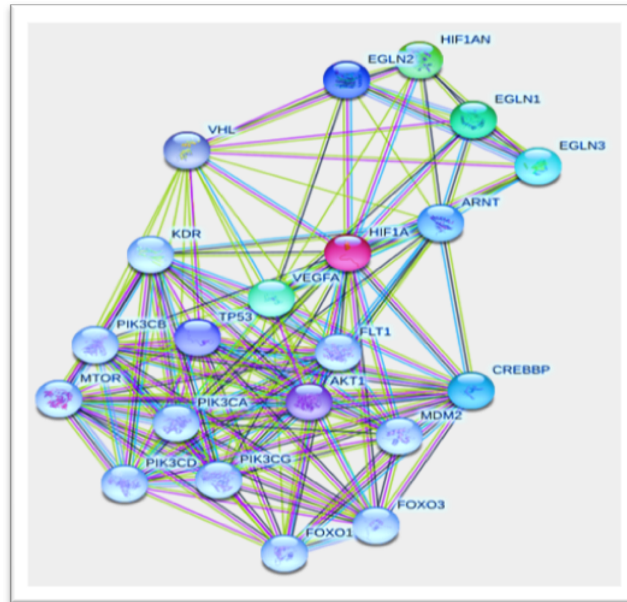
Hypoxia can be roughly defined as the decrease in oxygen availability below normal tissue levels. In order to accomplish aerobic metabolism along with energy generation, mammalian cells are required to maintain proper oxygen haemostasis. Oxygen being an essential component acts as an electron sink to be reduced by four electrons and produce water in the final step of oxidative phosphorylation. During cancer, cardiac-related issues and chronic obstructive pulmonary disorders (COPD), cellular oxygen level is hampered for which the cells attain the status of hypoxic (low oxygen levels). Hypoxia is a usual event in many types of solid tumors, where tumour cells undergo rapid proliferation and gives rise to large solid masses, leading to obstruction and compression of the blood vessels surrounding these masses. As a result, these abnormal blood vessels show anomalous function and bring about the poor oxygen supply to the target tumour cells. At this juncture, organisms and cells escape from these oxygen depleted conditions by activating several adaptive mechanisms. Those hypoxic adaptations at the organism levels includes hyperventilation, enhanced production of blood cells, angiogenesis or formation of new blood vessels which results to the increased rate of delivery of oxygen from the atmosphere to tissues. At the cellular levels, there exists a transition from oxidative phosphorylation to anaerobic glycolysis causing increment in glucose uptake and expression of stress proteins helping in cell survival or apoptosis. Earlier, HIF-1 was discovered by Semenza and co-workers in 1991 during their studies on erythropoietin (EPO) gene, a gene responsible for encoding the erythropoietin hormone for red blood cells production. Before the discovery of HIF-1, Hypoxia Responsive Element (HRE) had been identified in 3' enhancer region of the erythropoietin gene and its transcription is being up-regulated by more than 100-fold by severe hypoxic conditions. HIF-1 alpha shows stabilization only at O2 concentration below 5%. For this outstanding discovery of how cells can sense and adapt to changing oxygen availability, Gregg L.Semenza along with William Kaelin, Peter Ratcliffe, was awarded The Nobel Prize in Physiology or Medicine in 2019.

II. HIF-1α- THE PROTEIN

2.1. *Structure of HIF-1α Protein: -*

Oxygen sensor HIF-1α is 826-amino acid protein where N-terminal portion comprises of a basic domain, a helix-loop-helix domain, and a PAS Domain which are subjected to dimerization with HIF-1β and association with the HRE DNA Core Recognition Sequence (5′-RCGTG-3′). [1, p. 582] Under hypoxic conditions, it stimulates the activation of transcription of over 40 genes including erythropoietin, glucose transporters, glycolytic enzymes, vascular endothelial growth factor, HILPDA, and other genes whose protein products enhance oxygen delivery or promotes metabolic adaptation to hypoxia.

Fig 1. Human HIF-1α Protein Interaction (https://www.sinobiological.com/resource/hif-1-alpha/proteins)



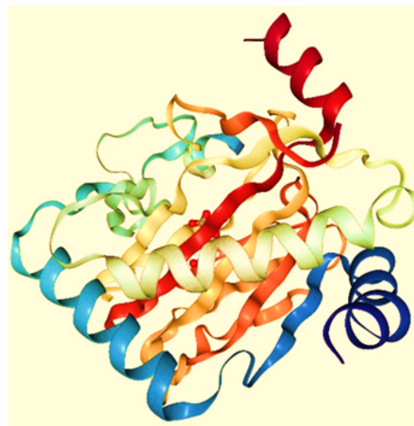


Fig 2. Secondary Structure of HUMAN HIF-1α Protein by X-Ray Diffraction Analysis, Resolution- 1.8290Å. HIF prolyl hydroxylase 2 (PHD2-R281C/P317C) cross-linked to HIF-1alpha NODD-L397C/D412C and N-oxalylglycine (NOG) (complex-1). PDB id- 5L9V (https://www.sinobiological.com/resource/hif-1-alpha/proteins)

2.2. *Position of HIF-1α Protein: -*

HIF-1 alpha protein is present on the 14th Chromosome in human beings. The cytogenic band is given by 14q23.2.

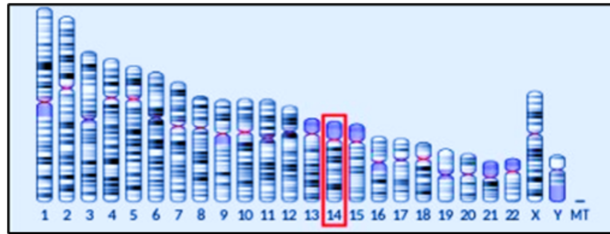


Fig 3. Position of HIF-1 alpha in the human genome
(http://may2017.archive.ensembl.org/Homo_sapiens/Gene/Summary?db=core;g=ENSG00000100644)

2.3. *Motifs and Domains of HIF-1α Protein: -*

Hypoxia-inducible factor (HIF) Transcriptional Complex is the mastermind of hypoxia sensor in the cell [2]. In general, hypoxic cells are at risk of stress-induced insults including oxidative DNA damage, DNA strand breaks, and genetic aberrations, which can restrain growth and ultimately result in cell death. The HIFs belong to members of a family of structurally related basic-helix-loop- helix (bHLH) containing proteins. [1] The prototype of the family is HIF-1. [1] The heterodimeric HIF1 is the combination of – Oxygen labile subunit, HIF1A, the Alpha Subunit, and the Oxygen Insensitive subunit- Aryl Hydrocarbon Receptor Nuclear Translocator (Arnt), the beta subunit. HIF-1α protein is made up of four functional domains- a bHLH domain and a PER-ARNT-SIM (PAS)Domain (whose main function is dimerization and DNA binding) and an Oxygen-dependent degradation domain (ODDD) (mainly involved in targeting the proteasome and degradation) and two transactivation domains (N-TAD and C-TAD) required fortranscriptional activation [2]. HIF-1β possesses bHLH, PAS and transactivation domains. [1] The PAS domain is categorized into two subdomains- PAS-A (228-298 amino acids) and PAS-B (228-298 amino acids). Nuclear Localization Signals (NLSs) are located at the N-terminal (17-74 amino acids) and C-terminal (718-721 amino acids) ends of HIF-1alpha protein. All the PAS Domains are made up of two internal homology units of approximately 50 amino acids- the A and B repeats, each of which has an invariant HXXD Motif (H, histidine; X, any amino acid; D, aspartate). The C-terminal NLS plays a significant role in the accomplishment of hypoxia-induced nuclear import of HIF-1alpha protein, whereas the N-terminal end has no distinct role. The C-terminal end consists of two PEST-like motifs having 499-518 and 581-600 amino acid residues. The PEST motif has a sequence infested with Proline (P), Glutamic Acid (E), Serine (S), and Threonine (T) [1]. Generally, this motif is found in many proteins having a half-life of fewer than 2 hours. So, proteins with the PEST motif become the target for rapid intracellular degradation [4]. That's why it can be said that HIF-1α under normoxic conditions is a very unstable protein with a transient half-life of fewer than 10 mins. The ODDD controls the degradation ofHIF-1 alpha protein. So, the deletion of the entire ODDD makes HIF-1 alpha stable in the absence of hypoxia signalling. Proline residues at 402 and 564 located within the ODD Domain of HIF-1alpha protein are hydroxylated [3]. This hydroxylation leads to its interaction with the von Hippel–Lindau tumour suppressor protein (pVHL), the recognition component of an E3 Ubiquitin Ligase. As a result, HIF-1α undergoes ubiquitination and subsequent degradation by the 26S proteasome. Three evolutionarily conserved HIF prolyl hydroxylases which control the hydroxylation process are – PHD1 (EGLN2), PHD2 (EGLN1), and PHD3 (EGLN3) [1]. Availability of oxygen, iron, 2-Oxoglutarate, and Ascorbate regulates the activity of these prolyl hydroxylases. The activity of HIF-1 alpha is regulated by the cellular oxygen concentration and on the other hand, HIF-1 beta shows constitutive expression.

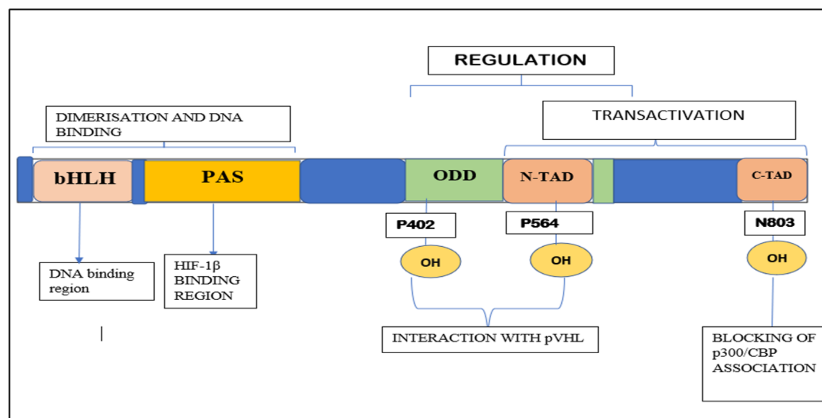


Fig 4. Schematic Representation of HIF-1α and its Functional Domains [2]

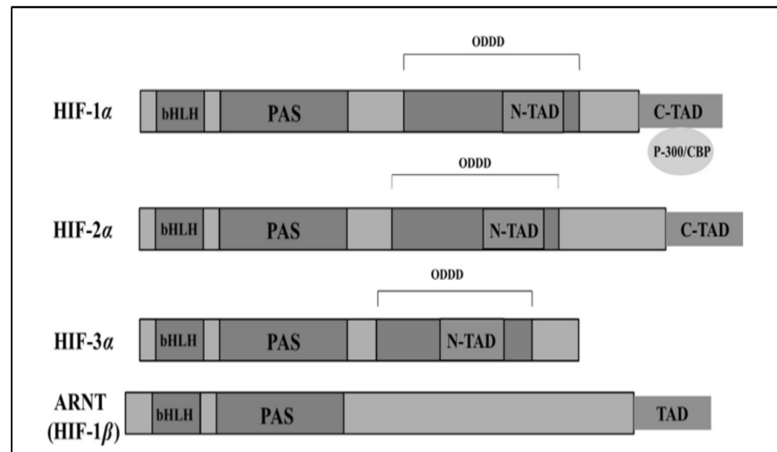


Fig 5. Functional Domains (bHLH, TAD, PAS) of proteins belonging to the bHLH-PAS family [3].

There are three closely related isoforms of HIF-α - HIF-1α, HIF- 2α, and HIF-3α, respectively. There exists a noticeable degree of amino acid sequence similarity between HIF-2α and HIF-1α i.e., 48% sequence similarity [3]. This identity resemblanceindicates their common capability of heterodimerization with HIF-1β and binding with HREs (Hypoxia Responsive Elements). HIF-1α and HIF-2α show variability in distributions in the body. HIF-1αshows ubiquitous expression in the body, but HIF-2α expression is restricted within specific tissues. After the discovery of the inhibitory PAS (IPAS), a spliced variant of HIF-3), a spliced variant of HIF-3α then HIF-3α came into the limelight. This domain is devoid of intrinsic transactivation activity in comparison to the C-TAD of HIF-1α and HIF-2α, rather it acts as a dominant-negative regulator over HIF-1/DNA binding ability. IPAS hinders the interaction of HIF-1α to HIF-1β because IPAS dimerizes HIF-1α. This IPAS/ HIF-1α complex does not bind to the hypoxia-responsive element. Thus, IPAS fails the HIF mediated target gene expression and inhibits transcriptional activation of HIF-1α.

III. HIF-1α- REGULATION

3.1. Oxygen Dependent Regulation of HIF-1α Protein: -

During normoxia, HIF-1α protein is subjected to rapid degradation by pVHL-mediated ubiquitin-proteasome pathway, on the contrary, hypoxia blocks the degradation and emphasizes the accumulation of HIF-1α protein [5]. Targeting of HIF-1α is mediated by the hydroxylation of specific prolyl residues along with the acetylation of Lysine residues (LYS532) by N-acetyltransferase ARD1 (ADP Ribosylation factor domain) [4]. During aerobic conditions, HIF-1α gets hydroxylated by Dioxygenases known as Prolyl Hydroxylases (PHD1, PHD2, and PHD3) located at two conserved proline residues (Pro402 and Pro564) present with oxygen degradation (ODDD) domain and each site possesses a conserved LXXLAP motif [4]. This dioxygenase requires molecular oxygen, 2-oxoG, and ascorbate as co-substrates [5]. Compounds like cobalt chloride and iron chelators like dipyridyl and 2-oxoG analogs – dimethyloxalylglycine (DMOG) inhibit PHD activity and can mimic hypoxia causing HIF-1α stabilization [3]. So, Prolyl Hydroxylase domain-containing proteins (PHDs) are called a bridge between oxygen sensing and HIF-α stabilization. Later on, it has been found that PHD2 plays a pivotal role in controlling the HIF-1α levels by using small interfering RNA (siRNA) techniques. Owing to the blockage in ubiquitination and degradation, HIF-1α gets accumulated and is translocated to the nucleus for dimerization with the HIF-1β by bHLH part of the PAS Domain to yield the HIF-1complex. Hydroxylation of proline residues activates a ubiquitination reaction by the binding of von Hippel-Lindau Tumour Suppressor (pVHL) containing E3-ubiquitin ligase to the HIF-α-ODDD Domain [5]. This E3-ubiquitin ligase is made up of elongin-B, elongin-C, cullin-2, and ring box-1- this complex promotes the polyubiquitylation and proteasomal degradation of HIF- α along with the participation of an E2-ubiquitin-conjugating enzyme, EPF-UCP [4]. Several studies revealed that SSAT2 (Spermidine/Spermine-N-acetyltransferase 2) associates with elongin C and pVHL to promote ubiquitylation of HIF-1α [4]. Overexpression of SSAT2 causes the reduction in HIF-1α Levels whereas the knockdown of SSAT2 enhances the levels of HIF-1α during hypoxia as well as normoxia

[5]. HIF-1α undergoes de-ubiquitylation by a pVHL-interacting de-ubiquitylating enzyme called as VDU-2 (also called USP20) to protect itself from proteasomal degradation [4]. VDU-2 in turn undergoes ubiquitylation and degradation by the pVHL E3 ligase complex [4]. Since pVHL can ubiquitylate both HIF-1α and VDU-2, cellular HIF-1α levels can be assessed by a balance between pVHL mediated ubiquitylation and VDU-2 mediated deubiquitylation [4, 6]. Signalling proteins like SUMO1 (Small Ubiquitin-like Modifier-1) and RSUME (RWD-containing SUMOylating enhancer) can regulate the levels of HIF-1α by SUMOylation of Lys (391) and Lys (477) present within ODDD [4, 6].

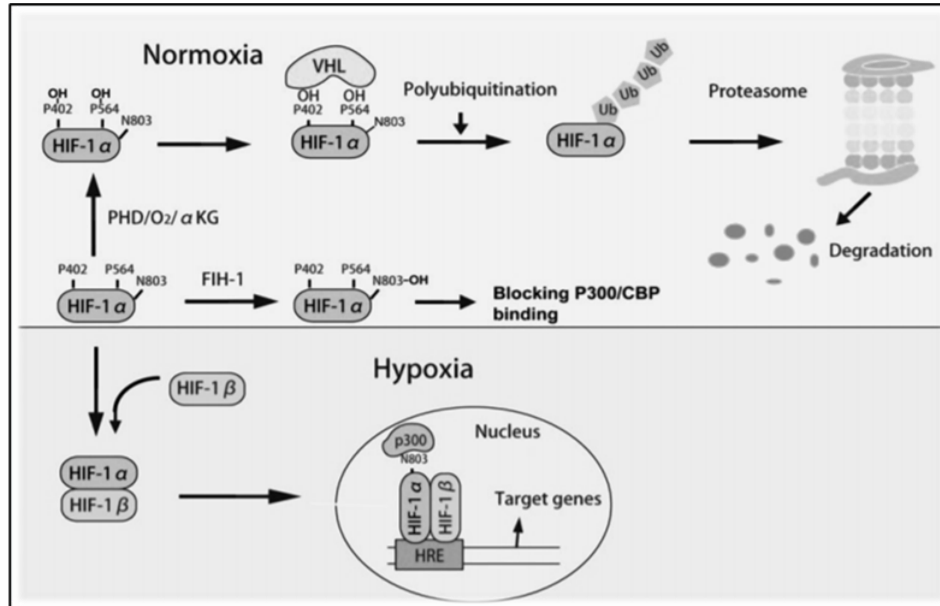


Fig.6- Oxygen Dependent Pathway of HIF-1α Protein [4]

3.2. Oxygen Independent Regulation of HIF-1α Protein: -

TRANSITION METALS - Transition metals like Cobalt and nickel can influence HIF1α activation without the direct participation of oxygen at any stage. They can stabilise the protein under normoxic conditions by inserting into the heme moiety of a putative oxygen sensor protein instead of iron which then locks it in a deoxygenated state [19]. Furthermore, they have been found to be ferrochelatase substrates that get incorporated into heme [20]. Since this enzyme supervises iron insertion into protoporphyrin IX, cobalt/nickel protoporphyrin IX's poorer O2 binding affinity tends to mimic a hypoxic environment thereby triggering HIF1α activation irrespective of the overall oxygen quantity [21]. Zinc also has a similar function for protoporphyrin IX, as demonstrated by a recent report announcing zinc's HIF1α stabilising quality [22]. A mechanism called 'replacement hypothesis' (Figure A) proposes that by PHD activity inhibition through Fe2+ replacement with transition metals at PHD active site allows HIF1α stabilization [23]. The activities of transition metals were first seen in EPO assays as it is produced in liver and kidneys in hypoxic conditions. Upregulation of EPO is also done by Co2+and Ni2+, that was later discovered to occur since Ni2+ enters cells by DMT1(divalent metal ion transporter1); the main Fe2+ trans porter. Hence the intracellular Fe2+ depletion by interfering with the iron homeostasis in turn affects Fe-related activities of PHDs (Prolyl hydroxylase domain) [24]. Other metals like Co2+ transport is also conducted by DMT1. Ni2+ is able to decrease the binding of pVHL to HIF-1α, resulting in a decrease in PHD activity[25]. Ferrous ion, a cofactor of PHD, is coordinated by two histidine residues and a carboxylated residue in PDH[26]. If this metal binding is not tight, however, other metal ions could substitute for the ferrous ion bound to PDH, and inhibits its enzymatic action which might stabilize HIF-1α even under normoxic conditions.

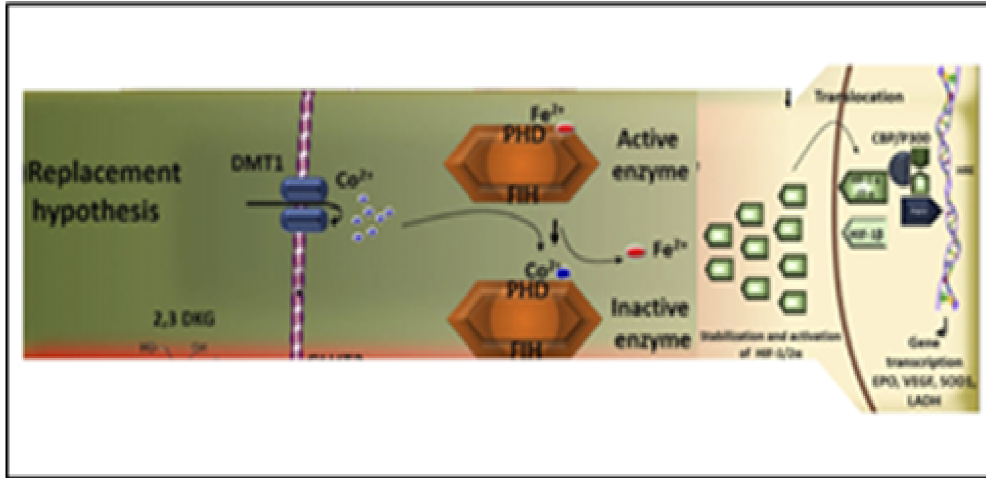


Fig.7- Stabilization mechanism of HIF-1α. Replacement Hypothesis- Cobalt could be delivered to the cells via the divalent metal transporter DMT1 Cited From [23].

NITRIC OXIDE - Induction of iNOS whose transcription is enhanced by HIF-1, leads to nitric oxide (NO) production stimulated by hypoxia [27] [28]. On the other hand, hypoxia-inducible genes like erythropoietin and vascular endothelial growth factor genes' HIF-1 mediated induction is also affected by NO [29]. Therefore, NO's effect on HIF-1α regulation depends on the nitric oxide concentration involved. NO has been shown to stabilize HIF-1α under both normoxia and hypoxia [30] [31].At iNOS induced 1μM concentration of endogenous NO, HIF -1α accumulation and DNA binding activity is enhanced in all all-oxygenic conditions. Conversely, lower (<400nM) concentrations of endogenous NO inhibit stabilization of HIF-1α under hypoxia[32].

For NO donors, normoxic HIF-1α is accumulated faster but not stably at lower concentrations of 100μM and congregation at higher (1mM) concentration is comparatively slower but more stable. Enhanced accumulation and DNA binding activity of HIF-1α was experimentally shown with 500μM of an NO-donor, S-nitro-N-acetyl penicillamine (SNAP) in A-172 and Hep3B cellsunder normoxia. In contrast, it was shown that under hypoxia, high concentrations of NO donors inhibit HIF-1α accumulation and DNA binding activity. Thus, the current evidence shows moderate to high (500μM to 1mM) concentrations of NO donors induce HIF-1α accumulation and DNA binding activity under normoxia, but inhibit the same responses under hypoxia. With respect to endogenous NO, high concentrations (1μM) seem to stabilize HIF-1α under both normoxia and hypoxia while lower concentrations (<400nM) inhibit hypoxic stabilization of HIF-1α. It has been proposed that S-nitrosation contributes to HIF-1α stabilization in a concentration dependent manner. HIF-1α accumulation is apparently induced by GSNO, which can donate nitrosium. GSNO also stimulates S-nitrosation in all free 15 thiol groups of HIF-1α. It is shown to inhibit the interaction of pVHL with HIF-1α ODDD in the presence of PHD1, PHD2, and PHD3 under normoxia as well.

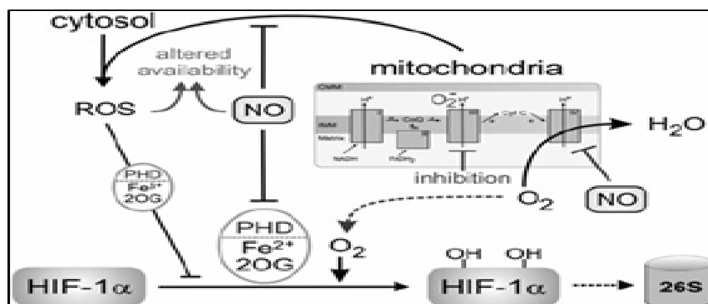


Fig.8-ROS and NO in the stability control of HIF-1α. PHD catalyzed hydroxylation of HIF-1α is under the control of NO and

REACTIVE OXYGEN SPECIES -Mitochondrial electron transport chain has been found to play a crucial role in HIF-1α stabilization since oxygen is required at the final step for transfer of electrons during respiration. When Oxygen levels are decreased, 2 electrons are accumulated in the mitochondria and univalently reduce the existing Oxygen molecules to SO (-O) radicals[34]; which when generated within the mitochondrial respiratory compartments during hypoxic conditions ultimately enhances HIF-1α levels. Further studies have shown that the ROS generated during hypoxic conditions from mitochondrial complex III is responsible for HIF- 1α stabilization through the suppression of Rieske iron-Sulphur proteins in the complex (Figure 5). This hypothesis was backed up by the application of Antimycin A which increases complex III ROS production ultimately enhances HIF -1α accumulation [35]. Later however this hypothesis was negated as being the sole reason for HIF -1α stability in mitochondrial electron transport chain under hypoxia. General mitochondrial ETC inhibitors along with Antimycin A, at concentrations that can inhibit it ETC show a loss of HIF-1α stabilization under hypoxic conditions in recent studies.

Thus, the new model of mitochondrial oxygen sensing indicates the reduced mitochondrial ETC activity results in lesser Oxygen consumption and corresponding increment of cytosolic oxygen levels[36]. This avails more Oxygen to PHD ultimately causing HIF- 1α degradation depending upon the transport chain overall and not exclusively on ROS production. However, under normoxic conditions, ROS like hydrogen peroxide addition can sufficiently stabilize HIF-1α. Studies, including use of catalase (which breaks down hydrogen peroxide) which showed stability inhibition, use of siRNA of Manganese Superoxide Dismutase (Mn-SOD) showing increased HIF-1α levels by preventing pVHL-HIF binding and inhibiting PHD activity, all support this notion.

GROWTH FACTORS - HIF-1α is found to be significantly present in tumours and also, directly influence metastasis and tumour progression. HIF-1 induces angiogenesis promoting one products expression like vascular endothelial growth factor, basic fibroblast growth factor, angiopoietin 2, anadrenomedullin during development of tumours. Not only that, HIF-1 can also allow the tumours to survive under low O2 conditions inducing expression of those genes which promote anaerobic ATP synthesis (glucose transporters), glycolytic enzymes (hexokinase 1 and 3, lactate dehydrogenase A, phosphofructokinase L) and survival factors (Insulin-like growth factor 2) [38]. Intratumoral hypoxic conditions of solid tumours activate (by growth factors) phosphatidylinositol-3-kinase (PI3K) which influences HIF-1α expression in them at a translational level (Fig-C). In prostate cancer (cancer where HIF-1α is well expressed even in normoxia) studies, growth factors like interleukin 1, insulin, epidermal growth factor interacts with their receptors which activate their receptor tyrosine kinases. This in turnstimulates PI3K, its target serine/threonine kinase AKT (or protein kinase B) and downstream component mammalian target of rapamycin (m-TOR). This mTOR stimulates HIF-1α expression under normoxia through eukaryotic translation initiation target serine/threonine kinase AKT (or protein kinase B) and downstream component mammalian target of rapamycin (m-TOR). This mTOR stimulates HIF-1α expression under normoxia through eukaryotic translation initiation 4E (eIF-4E) binding protein’s (4E-BP1) phosphorylation. The 2 components’ integrity is broken down, enabling enhanced HIF-1α translation due to inhibition of cap-dependent mRNA translation. Also, mTOR phosphorylates p70 S6 kinase (S6K) which in turn stimulates phosphorylation of its substrate, the ribosomal protein S6, and induces protein translation. Conversely, negative regulation occurs by PTEN tumour suppressor protein, whichdephosphorylates the products of the PI3K. Hence the activation of this pathway prominently impacts the normoxic HIF-1α expression in tumors. A similar mechanism occurs for human epidermal growth factor receptor 2 (HER2) and breast cancer cells. RAS/RAF/MEK/ERK kinase cascade can be promoted by the activation of RAS by various growth factors. Phosphorylation 4E-BP1, S6K, and MAP kinase interacting kinase (MNK) through the activation of ERK. eIF-4E can be directly phosphorylated by MNK. Thus, all these mechanisms show their influence on the increased HIF -1α mRNA translation. At the transcriptional level, ERK phosphorylates the co-activator CBP/ p300 which increases HIF-1α/p300 complex formation, thus regulating its transcriptional activation.

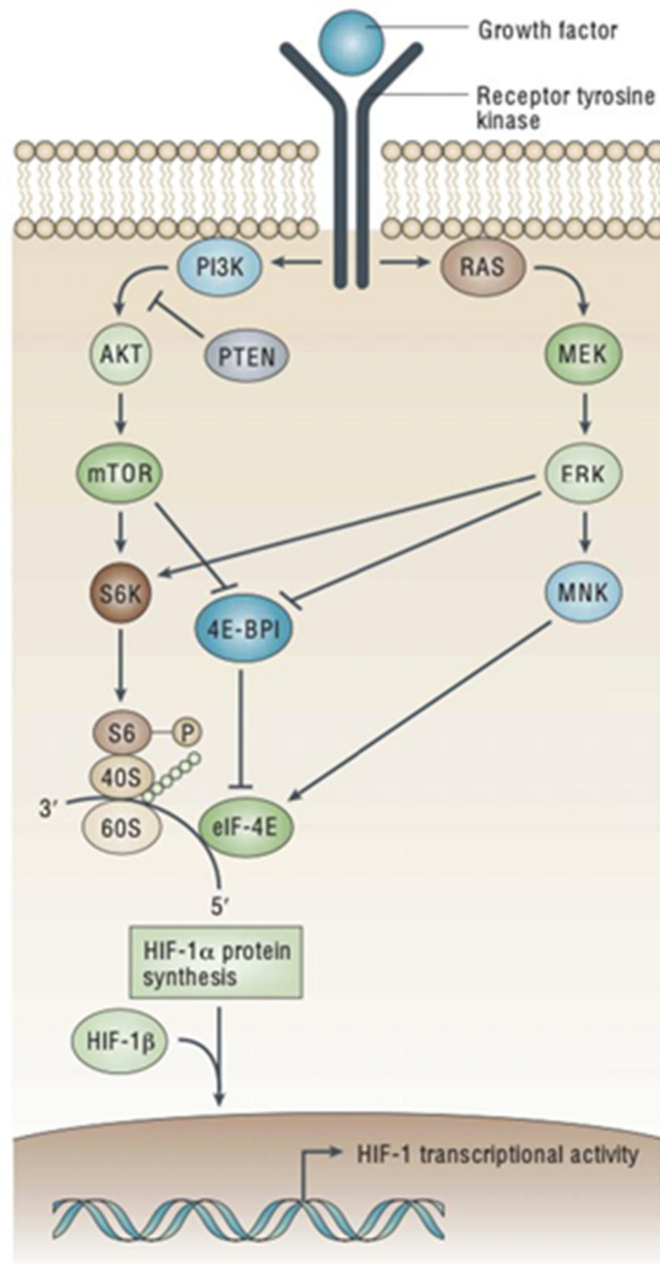


Fig.9- Regulation of HIF-1 Protein Synthesis Cited from Gregg L.Semenza(2003), Targeting HIF-1 for Cancer Therapy (doi;10.1038/nrc1187)

3.3. MECHANISMS FOR REGULATION OF HIF-1α mRNA LEVEL: -

Apart from the regulation by protein stability and half-life, HIF-1α can be regulated by mRNA transcription as well as protein synthesis in response to hypoxia along with stimulation by growth factors, hormones, cytokines, heat shock, irradiation and availability of nutrients respectively. At this juncture, there are three major pro-survival pathways to enhance transcription initiation and translation of HIF-1α- ERK/MAPK, JAK/STAT, and PI3K/Akt/mTOR [8].

In cancer, MAPK signaling via ERK1/2 was mainly associated with regulation of HIF-1 transactivation through phosphorylation of p300/CPB cofactors. JAK/STAT pathway activates Akt-mediated HIF1α transcription via STAT3 [8]. Again, on the other hand, PI3K/Akt/mTOR signaling cascade directly increases the rate of transcription and translation of the HIF-1α gene [8].

Initiation of transcription is considered to be one of the most influential steps for influencing the gene expression level [8]. Generally, it is stimulated by one or more transcription factors that can undergo association with the cognate enhancer sequence located within its target promoter region [8]. It had been reported that each transcription factor such as the ISGF3 complex, which is composed of STAT1/STAT2/ IRF9, STAT3, or NF-κB binds to the promoter region of the HIF-1α gene to activate the transcription initiation [8]. The expression of HIF-1α gene can be positively autoregulated by the CpG demethylation whereas Kozlowski et al. found that the HIF-1α promoter has an HRE which is suppressed by methylation of a CpG dinucleotide located in the core element which in turn enables the binding of the HIF-1α to its own promoter [8]. This results in the auto transactivation of HIF-1α expression. Another negative regulator of the transcription initiation is NRF-1 which binds to the HIF-1α promoter region to repress initiation [8]. Moreover, USP52/PAN2 can stabilize the HIF-1α mRNA by interacting with the 3'-untranslated region (UTR) of HIF-1α mRNA which leads to an increase in HIF-1α protein levels [10, 11].

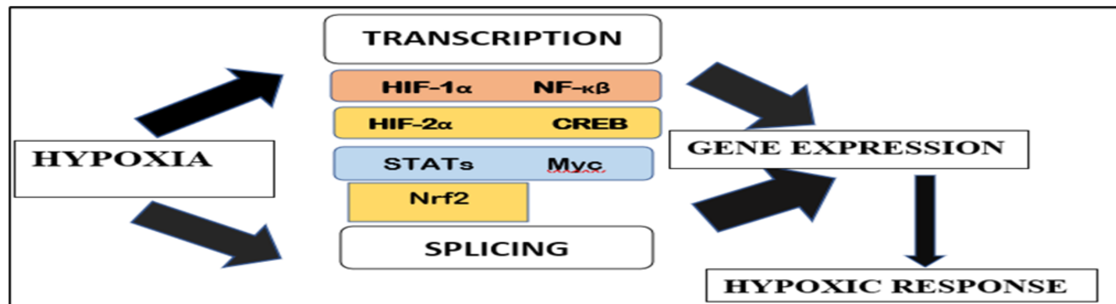


Fig.10. Transcription Regulation of HIF-1 gene [9]

3.3. Post Transcriptional Regulation Under Hypoxia: -

The RNAs formed during transcription are called as primary transcripts. The primary transcript normally undergoes further enzymatic alteration called as post-transcriptional processing. Post-transcriptional processing is required to convert the primary RNAs into functional or active forms. In case of higher eukaryotes, most genes are being interrupted by intervening sequences known as introns. After the event of transcription, the primary transcript termed as pre-mRNA undergoes- end modifications, pre-mRNA splicing, polyadenylation, and editing. Pre-mRNA splicing is mainly concerned with the removal of introns from the pre-mRNAs and ligates the remaining regions to form exons. This is known as Constitutive Splicing where all exons are being ligated without addition or deletion of nucleotides [9]. Mainly there are special signals present at the both ends of introns are – there is a consensus sequence GURRGU (here, R stands for purine) at the 5'end of introns in humans- the first G nucleotide is the site of cleavage for the first step of the splicing reaction and there is CAGconsensus sequence located at 3' end of the introns. Along with this, there is a stretch of pyrimidine residues located 20-30 nucleotides upstream of the 3' splice site and promotes recognition of this site. The excised introns obtained as a result of transesterification reaction are released as a lariat structure where in which the G nucleotide in the 5'splice site is conjugated with a branch point nucleotide by 2'–5'phosphodiester formation [9]. Along with the intronic splicing signals, there are many regulatory elements in exons that can modulate splicing either by enhancing or inhibiting exon recognition such as, such as exon splicing enhancer (ESE) and exon splicing silencer (ESS), respectively [9]. As the name suggests, ESE enhances the recognition of the exon where it is located. On the contrary, ESS inhibits the exon recognition where it resides. Alternative splicing is another way of pre-mRNA Splicing where internal exons are also spliced along with intron resulting in the production of a different proteins and it is of several types- cassette exon type, alternative 5' or 3' splice site usage, mutually exclusive introns, and intron retention [9]. Alternative splicing is under the regulation of additional splicing factors along with spliceosomes. One of the best-characterized splicing factors is serine-arginine-rich (SR) protein having one or two RNA-binding domains (RBDs) and an arginine-serine-rich (RS) domain at the amino-terminus and carboxy-terminus ends respectively [9]. 12 SR proteins have been identified in humans [9]. SR proteins can facilitate exon recognition by binding with ESE by bridging the two sides of the exon on interaction with the U2AF heterodimer and U170K proteins through their RS domain [12]. There exists another group of splicing regulators- Heterogeneous ribonucleoproteins (hnRNPs). 20 proteins have been identified in the hnRNP family which is termed as A1–U harboring varieties of RBDs. Exon skipping is accomplished by hnRNP A1 on binding with high-affinity binding sites of exon.

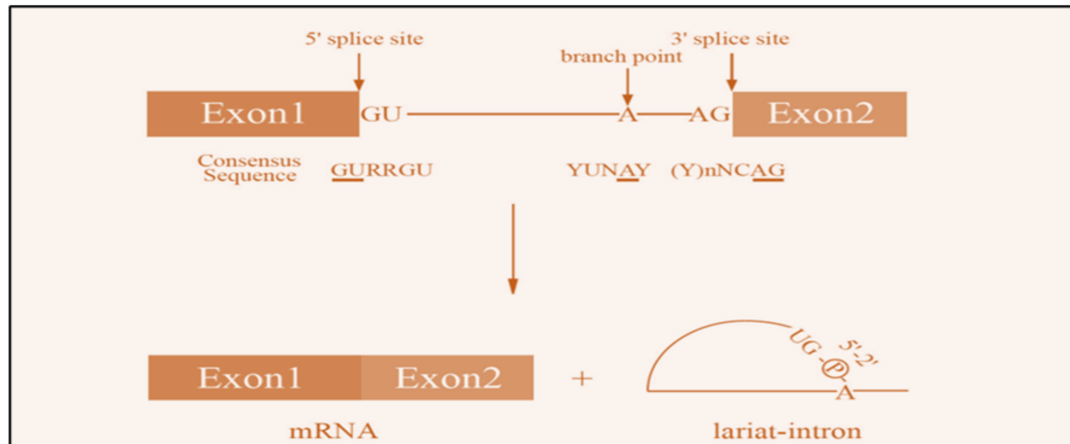


Fig.11.Diagrammatic view of Splicing Reactions and Important Splicing Signals [9]

3.5. Role of Translation in HIF-1α Protein Expression: -

HIF-1α synthesis during hypoxia is mainly regulated at the level of translation rather than transcription. During the hypoxic condition, there is an immediate stoppage of general protein translation as a means of reducing energy consumption from stress. Initiation of translation is mediated by cap-dependent or IRES-dependent mechanisms [13]. The PI3K/Akt Pathway and the binding of YB-1 to the unique secondary structure of 5'UTR of HIF-1α mRNA enhance the initiation of translation [13]. In this context under hypoxic conditions, ATR also stimulates the translation initiation of HIF-1α protein via a cis-acting element in the HIF-1α open reading frame [14]. During hypoxia, translation can be inhibited by the two separate pathways- the first pathway involving the Unfolding Protein Response (UPR) is rapidly activated at meagre oxygen concentration i.e., <1%, andinduces the activation of endoplasmic reticulum kinase- PKR-like ER Kinase (PERK) [16]. This kinase is involved in the phosphorylation of a crucial regulatory component of transcription initiation- eIF2α. The second pathway is under the control of mTOR which under prolonged hypoxic conditions gets inhibited by REDD1(Regulated in Development and DNA-damage responses) and tuberous sclerosis (TSC1–TSC2) complex causing hypo-phosphorylation of 4E-BP1 [17]. Translation elongation also gets suppressed during hypoxia via AMP-activated-protein kinase or mTOR-dependent phosphorylation of eEF2 kinase.

4. Applications of HIF-1α Protein

CANCER THERAPY - As HIF-1α is so fundamentally involved in tumor metastasis, need to manipulate its action pathways to inhibit them is of great therapeutic use. HIF-1α inhibition can usually be achieved through manipulation of one of the following steps: HIF-1α mRNA expression; HIF-1α protein level (protein translation or degradation); HIF-1α/HIF-1β dimerization; HIF-1α-DNA binding (HIF-1α/HRE); or HIF-1α transcriptional activity (CH-1 of p300/C-TAD of HIF-1α) [22]. As was done for pancreatic cancer cells, dominant negative HIF-1α produced from HIF1 stabilization and activation pathway disruption led to reduction of tumorigenicity of those carcinogenic cells through glucose metabolism suspension. Furthermore, the cancer cells were rendered susceptible to apoptosis and hypoxia induced growth inhibition.

Among the therapeutic agents discovered targeting signal transduction pathways many have shown to block HIF-1α function and produce antiangiogenic effects. Some of them are calphostin C (inhibitor a protein kinase C); wortmannin and LY294002 (inhibitors of PI3K); PD98095 (inhibitor of a MAPK); rapamycin [inhibitor of a FKBP1associated protein or mammalian target of rapamycin (FRAP/mTOR)]; diphenylene iodonium (a redox signalling blocker), and manno-heptulose (inhibitor of a glucokinase) [26]. HIF-1 transcriptional activation pathway can be inhibited by molecular agents including YC-1 (Topoisomerase 1 inhibitor), 17-allyl-amino-geldanamycin (Hsp90 inhibitor) by preventing VEGF expression, stunting xenograft growth and vascularization among other beneficiary functions[22]. Among these agents mentioned above, benzoquinone annamycin antibiotic "geldanamycin" disrupts Hsp90 binding to client proteins by competing with its ATP binding site, exposing them to ubiquitination and proteasomal degradation. As PI3K-specific inhibitors, LY294002 and wortmannin limit the synthesis of HIF-1α protein in the prostate carcinoma-derived cell lines PC-3 and DU145 in a dose-dependent fashion[27]. Rapamycin induced mTOR inhibition decreases uptake of glucose on AML cell lines, shift from glycolysis (decrement) to OXPHOS (enhancement) in ALL cell lines[28]. YC-1

downregulates HIF-1α and HIF-2α at the post-translational level by stimulating FIH binding to C-TAD even in hypoxia and function by preventing the recruitment of p300 by C-TAD.

ISCHEMIC DISEASE THERAPY - Opposite to the specified effect needed for cancer therapy, for ischemic disease treatment would require amplification of HIF-1α function for it to be advantageous[21]. Diseases like stroke and heart attack occur as a result of localized hypoxia manifested as cerebral and myocardial ischemia[22]. HIF-1α induced increase of VEGF expression stimulates formation of new vasculature at the trouble site of heart or brain, thus enhancing blood and Oxygen supply there and compensating for the ischemic effects. Under ischemia in brain tissue, HIF-1α stimulates vascular growth and helps ischemic organs survive hypoxia. The macrophage derived peptide PR39 has been shown to stabilize HIF-1 bydecreasing its degradation, leading to accelerated formation of vascular structures in vitro. Direct induction of HIF-1 has been achieved by using the N- or C-terminal of ODDD polypeptides that block VHL-mediated degradation [23].

PSORIASIS - Several clinical and pre-clinical studies show an increase in the expression of HIF-1α in psoriatic skin lesions as well as in circulation [24]. Immunoreactive keratinocytes show increased HIF-1α expression. Agents like dimethyl fumarate and cannabinoids are reported to produce improvement in psoriasis by modulating HIF-1α-dependent signaling pathway.

COVID -19 - Hypoxia is a primary pathophysiologic feature and main cause of mortality in patients with severe COVID-19 and it accompanies all the stages of the disease [25]. “Cytokine storm” which is the simultaneous activation of large number of white blood cells releasing inflammatory cytokines like IL-1β and IL-6 that can eventually lead to multiple organ failure, has been observed to be influenced by hypoxia. By behaving like a DAMP, ATP in extracellular matrix instigates cell injury and death and serves as a potent inflammatory agent, whereas adenosine has potent anti-inflammatory functions through its G-protein coupled receptors [25]. HIF-1α switching to anaerobic glycolysis promotes the accumulation of adenosine, turning on this pathway which allows adaptive immunity to take over the hyperactive innate response. Thus, moderate levels of hypoxia can induce ‘hypoxic conditioning’, a phenomenon having vaccine potential against hypoxia-induced lethal damages on the body like chronic inflammation and cytokine expression. All these factors have made scientists take HIF-1α as a strong candidate for COVID therapies in the future.

IV. CONCLUSION

Due to its involvement in dealing with oxygen, an extremely valuable and essential biochemical element, HIF-1α and its potential as therapeutic agent for diverse disease ranges is a hot topic of interest for the scientific community. As aforementioned in the above sections, interaction of this protein with its surrounding compounds is indeed quite complex and thus would require greater understanding of the precise nature and conditions of its multiple functions and then proceed to utilise it for medicine. Oncogenic and ischemic use of this protein has shown quite promising and sometimes lifesaving results, showcasing exactly how strongly it can influence bodily mechanisms. Hypoxic conditioning is seen as a frontier mode of HIF-1α research, with several studies at their infancy. Hence, HIF-1α can be safely deemed either a superlative friend or ferocious foe depending on its applications down the line.

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