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Review

The Critical Role of Hypoxia in Cancer Progression: a Mini-review of Recent Literature

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Hypoxia-inducible factor (HIF)-1α has been extensively studied as a cancer-progression protein. It plays a central role in tumor cell proliferation, invasion/metastasis, the epithelial-mesenchymal transition (EMT), angiogenesis, and glucose metabolism. HIF-1α-related proteins, including vascular endothelial growth factor, matrix metalloproteinase-9, lysyl oxidase, Snail, and Glut1, play a variety of roles that promote the aggressive nature of tumors. HIF-1α is expressed under both hypoxic and normoxic conditions in certain types of cancer. In this article, we review the reported properties of this protein in the context of hypoxia and related cellular events. We also briefly describe the transcription and translation of HIF-1α.

Key words: hypoxia-inducible factor-1α, hypoxia, cancer, oncogene, epithelial-mesenchymal transition

1. HIF-1α

Hypoxia is a critical microenvironmental factor that has been shown to induce tumor invasion and metastasis. Hypoxia has profound effects on metabolism, angiogenesis, innate immunity, and stemness induction. The effects of hypoxia are usually mediated by hypoxia-inducible factors (HIFs), i.e., HIF-1α and HIF-2α. HIFs heterodimerize with a common partner, HIF-1β, to regulate downstream target gene expression through a hypoxia-response element (HRE). The expression of HIF-1α protein is regulated by several factors; the most well-known of these regulatory mechanisms is degradation by the presence of Von Hippel Lindow [VHL] under normoxic conditions. On the other hand, under hypoxic conditions, HIF-1α escapes from the VHL machinery and facilitates the transcription of many target genes. Studies in our laboratory have focused on the regulation of HIF-1α via post-transcriptional modifications, particularly in the noncoding regions (i.e., untranslated region, UTR). Experiments investigating the involvement of the 5′and/or 3′ UTRs in U87MG (malignant glioma), HeLa (uterine cervix), and H1299 (lung) cells revealed that these regions significantly regulate HIF-1α translation; specifically, the 5′ UTR is responsible for the upregulation of HIF-1α translation, while the 3′ UTR is responsible for the downregulation of mRNA translation[1]. Interestingly, malignant glioma cells exhibit more than 10-fold higher transactivation capability compared to uterine cervical cells. This is consistent with our clinical observations that malignant glioma responds poorly to radiotherapy, while uterine cervical cancer is highly radiosensitive. Notably, all of these experiments in our laboratory were performed at normal oxygen levels.

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Thus, these results indicate that modification of HIF-1α translation is an important determinant of radiosensitivity.

Another interesting regulatory mechanism affecting HIF-1α expression is microRNA (miRNA). For example, *miR-210*, an miRNA expressed under hypoxic conditions, has been shown to enhance radioresistance[2]. Additionally, *miR-210* suppresses cell viability, induces cell arrest in the G_0/G_1 phase, and increases the rate of apoptosis in hypoxic human hepatoma cell lines (HepG2, SMMC-7721, and HuH7). When treated with anti-*miR-210*, colony formation assays demonstrated a 2-fold increase in radioresistance compared to untreated cells. Since miRNA binds to the 3′ UTR of transcripts, we hypothesized that UTR sequence-specific binding proteins may regulate HIF-1α protein translation. Some candidate proteins have been identified (unpublished data).

2. Radiosensitivity

Hypoxia is an important contributor to tumor radioresistance. Some studies have clarified that HIF-1α is responsible for decreased radiosensitivity. Ayrapetov *et al*. have shown that dimethyoxalyglycine (DMOG), which elevates HIF-1α expression, has a radioprotective effect[3]. They used MCF-7, a breast cancer cell line, and demonstrated that treatment with DMOG induced an increase in radioresistance of about 2-fold at 2, 4, and 8 Gy in colony formation assays. Forristal *et al*. also indicated that daily injection of DMOG to C57Bl/6 mice results in a statistically significant recovery of hematopoietic cell counts via HIF-1α stabilization after total body irradiation with 9 Gy[4]. Of the proposed radioresistant roles of HIF-α, the most intriguing is that HIF-1α may affect tumor cell clonogenicity following irradiation by altering cellular metabolism. HIF-1α governs the expression of host proteins involved in glycolysis and serves an important role in maintaining energy levels during hypoxia[5, 6], a state that may result in increased clonogenicity after irradiation[7]. Xing *et al*. provided clinical evidence of the potential involvement of HIF-1α in radioresistance. They performed immunohistochemical analysis of HIF-1α in tissues from 69 liver cancer patients and found poor responses to external beam radiotherapy (EBRT) in those with strong HIF-1α staining[8]. Additionally, they suggested that the mechanism of radiation tolerance may be related to the total amount of HIF-1α protein in the cytoplasm or to the localization of HIF-1α. Because HIF-1α regulates the transcription of so many genes, the mechanism mediating radioresistance may not be explained by any one factor. Therefore, bioinformatics analyses may be necessary to fully elucidate the mechanisms involved in this process.

3. Invasiveness

Lysyl oxidase (LOX) family proteins and matrix metalloproteinases (MMPs) are well-studied proteins involved in cell motility. LOX has been identified as an important regulator of hypoxia-induced tumor progression via an HIF-1α-dependent mechanism in numerous cancer types[9-11]. LOX is a copper-dependent amine oxidase that catalyzes the cross-linking of collagen and elastin in the extracellular matrix (ECM), thereby increasing insoluble matrix deposition and tensile strength[12]. In fact, Baker *et al*. demonstrated that LOX promotes extracellular stiffness[13]. Additionally, the LOX gene has been shown to have an HRE in its promoter region[9]. Chromatin immunoprecipitation (ChIP) using rabbit polyclonal antiserum to HIF-1α clearly showed that LOXL2 is also under the influence of HIF-1α[11]. Indeed, HIF-1α binding activity was increased by 3.1-fold compared to the value before ChIP. Such HIF-1α regulation of LOXL2 has also been described by other researchers[12]. Cancer cells that express higher LOX or LOXL2 protein have a more invasive nature[11, 14]. Brekman and Neufeld demonstrated that the number of cells that invaded into collagen gel was significantly reduced by transfection with short-hairpin RNA (shRNA) targeting LOXL2[14]. More recently, LOX has been shown to be related to the depth of invasion in oral carcinoma cells[15]. Furthermore, in breast cancer, LOXL2 promotes invasion by regulating the expression and activity of the extracellular tissue protein inhibitor of metalloproteinase-1 and MMP-9[16]. LOX is also required for the cross-linking of collagen and elastin. However, the reason why high expression of LOX results in a higher invasive nature is not fully understood. Extracellular tissue comprises a wide variety of molecules (i.e., the microenvironment), and the disruption of this microenvironment may promote the invasion of cancer cells. Another interesting protein, E-cadherin, has been shown to be involved in mediating the connections between cells. In an experiment using fluorescent staining, a renal cell carcinoma cell line exhibited clear E-cadherin expression in normoxia, but no expression during hypoxia[11]. The authors demonstrated quantitatively that E-cadherin expression is directly controlled by LOX or LOXL2. Additionally, decreased function or expression of E-cadherin promotes the epithelial-mesenchymal transition (EMT) and metastasis[17, 18]. This finding is supported by other studies[19, 20]. LOX also target Snail, another target molecule involved in invasion. Through a LOXL2-mediated event, Snail is stabilized as tumors progress. Moreover, LOXL2 promotes malignant transformation through Snail-dependent pathways[11].

With regards to MMP, a Chinese group found that membrane type 2 (MT2)-MMP, a member of the MMP

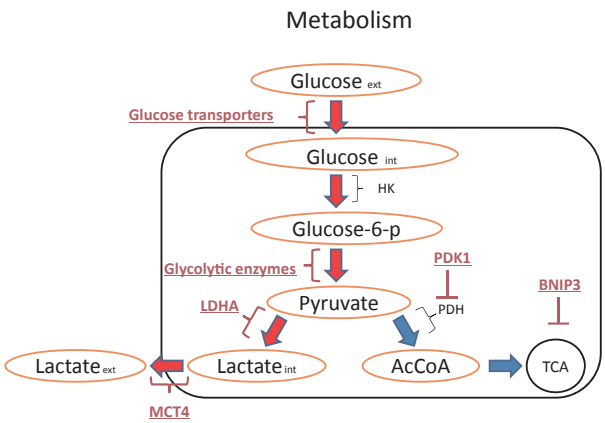

Fig. 1. HIF-1 α induces glucose transporters, glicolytic enzymes, LDHA, and MCT4[38]. It also promotes production of lactate from glucose. In addition, HIF-1α activates PDK1 and BNIP3[39], both of which block the convertion of pyruvate to acetyl-CoA for entering into the TCA cycle.

family, was a novel hypoxia-responsive gene and was upregulated by HIF-1α under hypoxic conditions[21]. They found the mRNA and protein levels of MT2-MMP were significantly increased in pancreatic cancer cells. Degradation of the extracellular matrix plays an important role in tumor metastasis. MMPs have been primarily associated with matrix remodeling, a necessary event during invasion. Other MMP proteins have also been shown to be involved in matrix remodeling. For example, in the esophageal cancer cell line Eca109, Jing *et al.* demonstrated that there was a significant increase in expression of MMP-2 mRNA and protein under hypoxic conditions[22]. Using transwell experiments, they showed that cells transfected with HIF-1α siRNA had significantly reduced motility. Surprisingly, injection of siRNA-transfected cells into nude mice resulted in a more than 50% reduction in lymph node metastases compared to the control. MMP-9 is also upregulated by HIF-α [23, 24]. Pre-operative, short-course radiotherapy has been shown to result in decreased local recurrence rates (e.g., in rectal cancer) and, combined with optimal surgery, improves patient survival. Although radiotherapy[25-27] has proven benefits, a few study groups have reported increased expression and activation of MMPs following radiotherapy[28, 29]. Notably, QRsP cells (mouse fibrosarcoma cells) surviving exposure to 10 Gy exhibited a more than 20-fold increase in the expression of *MMP-13* and *MMP-3* mRNAs in an experiment conducted in our laboratory[30]. Several MMPs are also upregulated in H1299 and A549 cells (both lung adenocarcinoma cell lines) surviving exposure to 10 Gy (unpublished data). Therefore, it is possible that radiotherapy may result in the creation of more aggressive cancer cells. Finally, an interesting report by Suzuki *et al.* demonstrated that MMP-2 accumulated in the leading edge of moving cancer cells (HeLa cells), further supporting the role of MMPs in cancer cell motility[31].

In summary, HIF-1α upregulates a variety of LOX family and MMP proteins, promoting the invasion of cancer cells into surrounding tissues. From the next chapter, we describe glucose metabolism, EMT, and angiogenesis in relation to HIF-α in detail.

4. Glucose Metabolism

Tumor cells require more oxygen than normal tissues due to their rapid proferation. As a result, they are constantly fighting for survival because they are in low-oxygen and low-nutritional states. In contrast, many clinical studies have shown that cancer cells have the potential to proliferate under hypoxia conditions. The transcription factor HIF-1α is intimately involved in this pathway. In the text, we describe the progress of glucose metabolism in tumor cells.

Under hypoxic conditions, tumor cells convert glucose to lactate. This system does not consume oxygen. At the beginning of this process, tumor cells take up glucose. HIF-1α induces expression of glucose transporter-1 (GLUT1), the main glucose transporter responsible for glucose uptake in cancer cells[33, 34]. Next, hexokinase (HK) phosphorylates glucose converting it into glucose-6-phosphate (G6P) and preventing its diffusion into the next extracellular space by passing through the cell membrane. Pyruvate is then produced from G6P by various glycolytic enzymes.

Tumor cells use an aerobic metabolic pathway under normoxic conditions. In this case, they produce acetyl-CoA from pyruvate, and then turn acetyl-CoA into tricarboxylic acid (TCA). Oxygen is used through the oxidative phosphorylation (OXPHOS) process to stimulate the production of acetyl-CoA[35]. In this case, pyruvate dehydrogenase (PDH) promotes the convention of pyruvate into acetyl CoA in tumor cells. However, under hypoxic conditions, HIF-1α induces pyruvate dehydrogenase kinase 1 (PDK1) in order to suppress PDH activity[36]. In addition, through BNIP3, which is involved in selective autophagy of mitochondria, HIF1-α inhibits the TCA cycle[37-39]. Through these mechanisms, HIF-1α prevents the OXPHOS pathway from using oxygen.

In order to convert lactate to pyruvate, HIF-1α induces lactate dehy-drogenase-A (LDHA). Finally, by inducing H^+/monocarboxylate transporter 4 (MCT4), HIF-1α promotes the export of lactate from tumore cells[37].

Thus, HIF-1α contributes significantly to this glucose metabolic system under hypoxic conditions, as. illustrated in Figure 1.

HIF-1α induces glucose transporters, glicolytic enzymes, LDHA, and MCT4[38]. It also promotes production of lactate from glucose. In addition, HIF-1α

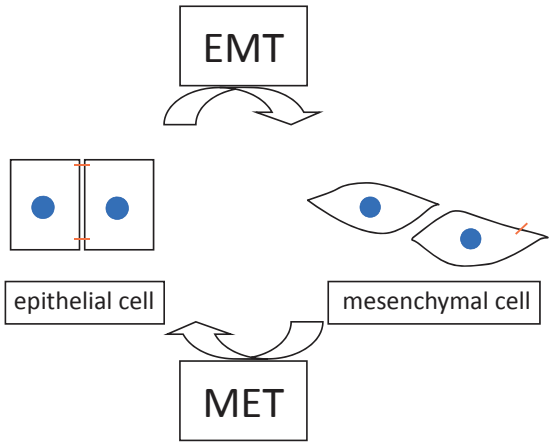

Fig. 2. The EMT and MET are mutually reversible.

activates PDK1 and BNIP3[39], both of which block the convertion of pyruvate to acetyl-CoA for entering into the TCA cycle.

5. Epithelial-mesenchymal transition (EMT)

The EMT, proposed by Hay *et al*. in the early 1980s, comprises a set of morphological changes during which epithelial cells lose their polarity and convert to mesenchymal phenotypes. The EMT is associated with fibrosis, organ formation, neural crest cell motility, and gastrulation in early embryonic development[40]. Moreover since the acquisition of the EMT leads to accumulation of the extracellular matrix and increased motility, the EMT plays an important role in the invasion and metastasis of tumor cells[41-44]. Several mesenchymal markers and epithelial markers have been proposed as target molecules whose expression mediates the changing phenotype of cells undergoing the EMT[41, 42, 45, 46]. These mesenchymal markers include N-cadherin, vimentin, fibronectin, and alpha-SMA. Epithelial markers include E-cadherin, desmolakin, and plakoglobin. The EMT is caused by the downregulation of epithelial markers and the upregulation of mesenchymal markers. Reversal of these changes in expression induces the mesenchymal-epithelial transition (MET). E-cadherin is a typical adhesion molecule in epithelial cells. It contributes to cell-cell adhesion, and loss of E-cadherin expression allows cells to lose their polarity. Since E-cadherin expression is reduced in the EMT, E-cadherin is a representative indicator of the EMT[47]. Additionally, studies have shown that the expression of E-cadherin and the malignancy of tumors are inversely correlated. The transcriptional regulation of E-cadherin is mediated by transcription factors, such as Snail (SNAIL1), Slug (SNAIL2), SIP1, TWIST, and E2A (E47/E12)[43, 44, 48-54].

Snail is a zinc-finger transcription factor that is known to be essential for mesoderm formation in *Drosophila*. Snail is part of a family that includes the protein Scratch as well as the Snail isoforms 1–3. The involvement of Snail proteins in the EMT has been demonstrated by experiments with loss of Slug function in chicken embryos. Subsequently, Snail has been shown to be involved in the EMT through 3 mechanisms: 1) overexpressed Snail strongly represses the transcription of E-cadherin, 2) Snail expression increases during the induction of the EMT, and 3) Snail directly binds to the E-cadherin promoter. Therefore, Snail is a typical inducer of the EMT[43]. Snail expression is regulated by the nuclear factor-kappaB (NF-κB) and extracellular signal-regulated kinase (ERK) pathways. Furthermore, as we will discuss later, HIF-1α also affects Snail expression. In addition, HIF-1α functions in maintaining stem cells that have developed resistance to chemotherapy and radiotherapy in the context of Snail overexpression. Therefore, through a variety of mechanisms, Snail expression has been shown to increase the 5-year survival rate in patients with several types of tumors[55].

The importance of the microenvironment in the EMT process in tumor cells has recently become clear. Among the factors comprising the cellular microenvironment, changes in oxygen levels and activation of signal transduction pathways related to hypoxia via HIF are important mechanisms mediating tumor metastasis. With regard to the EMT, EMT and MET switching depends on oxygen tension. The EMT is induced under hypoxic conditions (O_2 pressure is 1% or less), while the MET is induced under normoxic conditions[56]. When the EMT occurs in hypoxic tumor cells, Snail mediates the expression of E-cadherin. According to studies by Xhang *et al*.[56], E-cadherin is suppressed by HIF-1α expression and is negatively correlated with the expression of vimentin and N-cadherin. Therefore, HIF-1α expression is positively correlated with the expression of vimentin and N-cadherin. HREs exist at bp -541 and -651 on the Snail promoter, and the HRE at bp -541 in particular plays an important role in HIF-1α-mediated transcription of Snail. Other studies have also demonstrated that HIF-1α promotes Snail expression[57, 58]. Moreover, HIF has been reported to directly regulate Snail expression in mice, and Snail regulation via HIF-2α induces the EMT in melanoma[59, 60].

During the MET, cells regain properties of epithelial cells and lose their mesenchymal phenotype obtained through the EMT. This phenomenon occurs in locations where tumor cells have migrated. The MET also contributes to the final steps of the metastasis mechanism. Since HIF-1α plays a central role in inducing the EMT and the HIF-1α/Snail/EMT signaling pathway under hypoxic conditions, it is important to fully elucidate the details of these molecular pathways.

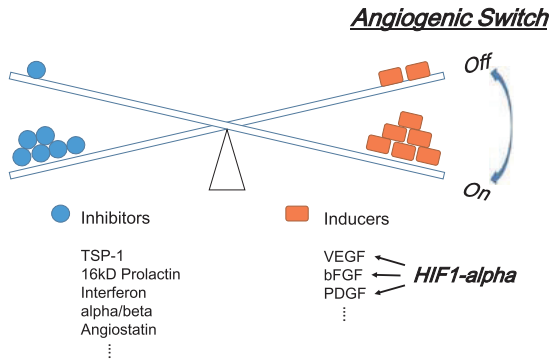

Fig. 3. This chart illustrates the angiogenic switch. Angiogenesis is dependent on the balance between inducers and inhibitors, similar to the movement of a seesaw.

6. Angiogenesis

Angiogenesis is defined as the growth of new blood vessels from pretexting vessels in order to supply cells with oxygen and nutrients. Generally, tumor cells are under a state of chronic hypoxia since they continue to proliferate, consuming large amounts of oxygen. Therefore, tumor cells or stroma attempt to stimulate the growth of blood vessels into the tumor tissue in order to maintain oxygen and nutrient levels. In the text below, we will focus on the functions of angiogenic factors and their relationships with angiogenesis.

Folkman (1971) hypothesized that tumor blood vessel formation was dependent on a tumor angiogenic factor (TAF)[61]. However, later studies demonstrated that angiogenic function stimulated by tumor cells is not clinically dependent on only one factor, but instead on a balance between inducer signaling and inhibitor signaling, called the "angiogenic switch" (Fig. 3)[62]. Angiogenesis is activated while inducer signaling is stronger than inhibitor signaling. These inducers are mainly vascular endothelial growth factors (VEGFs), platelet-derived growth factors (PDGFs), and basic fibroblast growth factor (bFGF)[63, 64].

VEGF (or VEGF-A) is a homodimeric glycoprotein mainly involved in the promotion of angiogenesis through endothelial cell growth, embryogenesis, matrix remodeling, and mitogenesis6[65, 66]. VEGF belongs to the VEGF family of proteins, which includes VEGF-B, VEGF-C, VEGF-D, VEGF-E, and PIGF-1 and -2; each of these molecules has a specific function. In the VEGF signaling pathway, VEGF binds to homologous VEGF receptors (VEGFRs): VEGFR-1 (Flt-1), VEGFR-2 (KDR in humans, Flk-1 in mice), and VEGFR-3. These VEGFRs are transmembrane tyrosine kinase receptors[67, 68]. Binding of VEGF to a VEGFR triggers signal transduction by phosphorylation of tyrosine residues in the intracellular tyrosine kinase domain. Binding of VEGF-A, -B, -E, or PlGF to VEGFR-1 or -2 in vascular endothelial cells leads to induce angiogenesis and permeability[66, 69]. In contrast, binding of VEGF-C or -D to VEGFR-3 or occasionally VEGFR-2 in lymphatic endothelial cells leads to the induction of lymphatic proliferation[70].

PDGF is an angiogenic factor composed of 2 polypeptide subchains (A and B)[71]. PDGF is frequently produced by tumor cells and promotes cell proliferation, migration, and control of self-sufficiency in growth signals[72, 73]. PDGF physiological signaling is transduced by a PDGF receptor (PDGFR). PDGF may be involved in the recruitment of tumor fibroblasts and pericytes in a paracrine manner[74], and tumor fibroblasts may secrete angiogenic factors, including VEGF, to sustain tumor angiogenesis by inducing transcription and secretion, similar to the function of HIF-1α[75]. Tumor pericytes may strengthen developing vessels and prevent the occurrence of hemorrhage and edema[64]. Indeed, many studies have demonstrated that PDGF and/or PDGFR are involved in human cancers[76].

The most important consideration in this is that these angiogenic factors are promoted by hypoxia or HIF-1α[63, 77]. This phenomenon has been suggested to occur throughout the body in both in vivo and in vitro studies[78, 79]. Importantly, if a tumor lacks oxygen and nutrient supply, it can only grow to about 1–2 mm (i.e., about 10^6 cells)[64, 80]. However, HIF-1α stimulates the transcription of numerous angiogenic factors, including VEGFR, and consequently promotes the growth of tumors. Therefore, to prevent angiogenic signaling and tumor growth, anti-angiogenic therapy is becoming more widely used in the clinical setting. Some studies have proven that this therapeutic strategy may elicit synergistic effects when anti-angiogenic therapy or radiotherapy is used concurrently[81].

In conclusion, HIF-1α plays a central role in cancer progression. The molecules mentioned in this article are just a few of many HIF-1α-related genes. Thus, suppression of HIF-1α might become a way of future cancer treatment.

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