Hypoxia-Inducible Factors and Cancer

Jonathan C. Jun, M.D., Aman Rathore, M.B.B.S., Haris Younas, M.B.B.S., Daniele Gilkes, Ph.D., and Vsevolod Y. Polotsky, M.D., Ph.D.

1Division of Pulmonary and Critical Care, Department of Medicine, Johns Hopkins University, Baltimore, MD
2Division of Breast Cancer, Department of Oncology, Johns Hopkins University, Baltimore, MD

Abstract

Purpose Of Review—Hypoxia inducible factors (HIFs) mediate the transcription of hundreds of genes that allow cells to adapt to hypoxic environments. In this review, we summarize the current state of knowledge about mechanisms of HIF activation in cancer, as well as downstream cancer-promoting consequences such as altered substrate metabolism, angiogenesis, and cell differentiation. In addition, we examine the proposed relationship between respiratory-related hypoxia, HIFs, and cancer.

Recent Findings—HIFs are increased in many forms of cancer, and portend a poor prognosis and response to therapy.

Conclusion—HIFs play a critical role in various stages of carcinogenesis. HIF and its transcription targets may be useful as biomarkers of disease and therapeutic targets for cancer.

Keywords
HIF; hypoxia; cancer; metabolism; sleep apnea; VEGF

Introduction

Hypoxia is defined as reduced oxygen availability. Since hypoxia may compromise survival, cells and organisms have evolved several adaptive mechanisms, with many occurring at the transcriptional level. A classic example is an increase in erythropoietin transcription in response to hypoxia to increase hemoglobin. In 1991, Semenza et al. identified a hypoxia inducible enhancer upstream of the human erythropoietin gene in the kidney and livers of transgenic mice rendered functionally hypoxic by anemia. Further studies identified the nuclear factor responsible for the increased transcription, which was named hypoxia
inducible factor (HIF)[1][2]. Hundreds of genes are now known to be transcriptionally regulated by HIF [3]. HIF exists as a heterodimer: a hypoxia-activated α subunit and a constitutively expressed β subunit, also known as aryl hydrocarbon nuclear receptor translocator (ARNT)[4]. There are three isoforms of the α subunit termed HIF-1α, HIF-2α, and HIF-3α. HIF-1α and HIF-2α have been more extensively studied, whereas research on HIF-3α isoforms is relatively scarce. In general, HIF-2α regulates similar genes as HIF-1α, while HIF-3α acts a negative regulator of these genes [5, 6].

**Normal Regulation of Hypoxia Inducible Factors**

Hypoxia regulates HIF-1α through post-translational modification. In the presence of oxygen, prolyl hydroxylase domain (PHD) proteins hydroxylate proline residues on HIF-1α. After hydroxylation, pVHL, the protein product of the von Hippel Lindau tumor suppressor gene, binds and ubiquitinates HIF-1α. Ubiquitinated HIF-1α is then targeted for proteasomal destruction [7]. Iron and 2-oxoglutarate are necessary for PHD activity. In addition, oxygen gradients can impact HIF-1α activity via regulation of Factor Inhibiting HIF (FIH). In the presence of oxygen, FIH hydroxylates HIF-1α at asparagine residues on the C-terminus, thereby blocking the recruitment of p300/CBP coactivators and rendering HIF-1α transcriptionally inactive [8]. During hypoxia, PHD and FIH activity are suppressed, permitting HIF-1α protein to translocate to the nucleus and dimerize with ARNT (also known as HIF-1β). The HIF-1α-ARNT heterodimer then binds to hypoxia response elements with the consensus sequence A/GCGTG on target genes [9].

HIF-1α is also regulated in an oxygen-independent manner. First, HIF-1α can be activated by hormones and inflammatory cytokines. For example, insulin activates HIF-1α via the phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) signaling pathway [10][11]. IL-1β induction of the cyclooxygenase 2 (COX-2) pathway, which catalyzes the conversion of arachidonic acid to lipid mediators including prostanoids, increased HIF-1α without hypoxia [12]. Second, Cyclin-Dependent Kinases (CDKs) also modulate HIF-1α activity. For example, CDK1 over-expression blocks lysosomal degradation, whereas CDK2 activity promotes lysosomal degradation of HIF-1α [13]. CDK5 increases HIF-1α levels and pharmacological or genetic inhibition of CDK5 decreases HIF-1α protein levels [14]. Third, MicroRNAs (miRNA), a group of single-stranded, noncoding regulatory RNAs may target HIF-1α, variably increasing or decreasing its transcription. For example, miR-20b suppresses HIF-1α and vascular endothelial growth factor (VEGF) in osteosarcoma cells; low levels of miR-20b in these cells may therefore be a stimulus for activating HIF-1α [15][16]. Interestingly, the relation between HIF-1α and miRNA is bidirectional, as HIF-1α has been shown to bind to miRNA promoters under hypoxic conditions [17]. Fourth, intracellular reactive oxygen species (ROS), produced under both hypoxic and normoxic conditions, or during mitochondrial respiration, can result in HIF-1α activation. However, even under normoxic conditions oxidizing agents stabilize HIF-1α. Some of the proposed pathways linking ROS to HIF-1α involve phosphorylation or miRNA’s as intermediate steps [18]. Finally, HIF-1α can be stabilized under seemingly normoxic conditions that actually cause intracellular hypoxia. For example, Lee et al. showed that a high fat diet acutely stabilizes HIF-1α in adipocytes from fatty acid-induced mitochondrial uncoupling. [19, 20].
HIF Upregulation in Cancer: Causes

Emerging evidence suggests that HIF-1α plays a role in the pathogenesis of cancer. HIF-1α can be stabilized in the hypoxic core of rapidly expanding, poorly vascularized solid tumors where the partial pressure of oxygen may be <10 mmHg [21]. As little as three hours of hypoxia in vitro stabilizes HIF-1α in cancer cells [22]. Stabilization of HIFs in this setting leads to changes in glycolysis, nutrient uptake, waste handling, angiogenesis, apoptosis, and cell migration that may promote tumor survival and metastasis [23–26]. HIF-1α can also be activated by non-hypoxic pathways as described above. For example, mutations in the von Hippel-Lindau gene cause constitutive upregulation of HIF-1α and HIF-2α [27] leading to tumors in renal, cerebellum, retina, and adrenal tissue [28]. High levels of HIF-1α in VHL syndrome leads to over-expression of growth factors such as vascular endothelial growth factor (VEGF), platelet-derived growth factor-β and transforming growth factor-β which activate downstream receptor tyrosine kinases. Mutations in oncogenic genes such as p53, Rb, Bcl2, Myc, ARF, and Ras have also been shown to stabilize HIF-1α [29]. Inflammation from the aforementioned COX-2 pathway induces HIF-1α activity and has been reported in breast cancer [12]. As yet further evidence that hypoxia is not necessary for HIF-1α activity in cancer, HIF-1α mRNA is elevated in pre-neoplastic breast, colon and prostate lesions [30] and remains elevated when the cells are cultured in normoxic conditions [31].

HIF Upregulation in Cancer: Consequences

Regardless of whether HIFs are stabilized by hypoxia-dependent or independent pathways, they are associated with poor outcomes in several types of cancer [32, 33]. In the discussion below, we discuss known, potentially pro-carcinogenic effects of HIF-1α in terms of cell division, angiogenesis, metabolism, and stem cell formation.

Cell division

Severe hypoxia causes mitotic arrest, halting replication at the G1/S phase [34]. In some experimental settings, this arrest was mediated by HIF-1α [35–37]. Hubbi et al. showed that HIF-1α binds to the minichromosome maintenance complex, interfering with DNA helicase activity [38]. Constitutive HIF-1α elevation induced cell cycle arrest via inhibition of c-Myc, leading to net increase in p21, a Cdk inhibitor that serves as a cell cycle checkpoint [39]. However, the role of HIF-1α in mediating hypoxia-induced cell cycle arrest is heterogeneous. Box et al. subjected several cell lines to hypoxia and did not find a consistent pattern of CDK expression or HIF-1α that predicted which cells arrested. In fact, HIF-1α in some arrested cell lines actually decreased [40]. If HIF-1α mediates cell cycle arrest in hypoxia, it might be expected to mitigate rather than propagate cancer. However, cell cycle arrest may confer resistance to chemotherapies directed towards rapidly dividing cells, or prevent cancer cells from depleting their own energy supply. Furthermore, as noted earlier, HIF-1α may be stabilized in normoxia, where cell cycle arrest is not occurring. Other studies suggest an increase in growth and/or survival signaling factors under hypoxic conditions that could enhance cell proliferation and survival of cancer cells [41]. Taken together, this suggests that role of hypoxia may be highly context dependent and could vary within and between different tumor types.
Angiogenesis

Signals such as hypoxia, mechanical stress, and inflammatory cytokines trigger release of proangiogenic factors [42]. Hypoxia is one of the strongest signals for angiogenesis in tumors, leading to the formation of a vascular network required to maintain nutrient and oxygen delivery. In fact, a tumor cannot grow beyond a critical size or metastasize until it acquires the ability to form new blood vessels [59]. This “angiogenic switch” takes place when HIF-1α activates the transcription of factors such as VEGF, angiopoietin 2, stromal-derived factor 1, cyclooxygenase 2 and stem cell factor [26, 43, 44]. In cancer, VEGF is one of the most important angiogenic factors. Targeted deletion of VEGF in embryonic stem cells dramatically reduced tumor growth in mice [44]. Jensen et al showed increased HIF-1α and VEGF levels in glioma cells; inhibition of HIF-1α by transfection of dominant-negative HIF-1α or siRNA reduced VEGF secretion and cell growth [45]. Despite these promising in vitro results, early clinical studies of VEGF inhibitors have been disappointing [46–49], and may reflect the concurrent presence of VEGF splice variants that operate as suppressors of angiogenesis [50, 51] or alternatively the formation of pericytes that protect the newly formed endothelium from being targeted [52, 53]. It is even speculated that inhibition of angiogenesis may paradoxically aggravate tumor hypoxia [47].

Glucose metabolism

Long before the discovery of HIF-1α, Otto Warburg in 1927 observed that cancer cells produce high levels of lactate even in the presence of abundant oxygen [54]. He attributed this unusual form of aerobic glycolysis to mitochondrial injury. This glycolytic shift has since been observed in dozens of cancers where rates of glycolysis may be 200 times higher than in non-cancer cells [55]. This has led some to refer to cancer cells as “addicted to glucose” [56], an attribute that enables detection of some tumors with labelled glucose positron emission tomography (PET) imaging. It is now understood that HIF-1α orchestrates several key steps leading to the Warburg effect. First, HIF-1α stimulates glucose uptake necessary to compensate for the relative inefficiency of glycolysis, by upregulating glucose membrane transporters, GLUT1 and GLUT3 [57–59]. GLUT1 levels are a marker of more aggressive tumors in thyroid, breast, and endometrial cancer [60, 61]. Secondly, HIF-1α upregulates glycolytic enzymes such as hexokinases and phosphoglycerate kinase 1 [62]. Third, HIF-1α inhibits mitochondrial respiration by activating the transcription of pyruvate dehydrogenase kinase (PDK), which in turn phosphorylates and inactivates pyruvate dehydrogenase (PDH). PDH catalyzes the conversion of pyruvate to acetyl-CoA, a rate-limiting step of entry into the TCA cycle [55, 63]. Shunting of pyruvate to lactate also reduces mitochondrial ROS, potentially protecting the cell from oxidative stress [64]. Although TCA flux is reduced, HIF-1α increases the efficiency of electron transfer from complex IV to oxygen, by orchestrating an isoform switch from COX4-1 to COX 4-2 [65]. Fourth, HIF-1α decreases mitochondria by increasing expression of BNIP3, a protein involved in autophagy [66]. Fifth, HIF-1α upregulates lactate dehydrogenase A to promote lactate production and regenerate NAD⁺ [67]. Sixth, HIF-1α increases transcription of lactate transporters, including monocarboxylate transporter 4 and NHE1 exchanger present on tumor cell membranes [68, 69] to cope with intracellular lactic acidosis [56]. Elevation of these transporters is associated with poor prognosis in lung and stomach cancer [70, 71]. HIF-1α also buffers pH by upregulating carbonic anhydrase 9 and 12, whose protein
products catalyze hydration of CO$_2$ into bicarbonate. These carbonic anhydrases were first noted to be upregulated by defective VHL in renal cell carcinoma [72] and later found to be controlled by hypoxia in a HIF-1α-dependent manner [73]. Exported lactate may be taken up by other cells such as skeletal muscle and used for aerobic metabolism or converted back to glucose in the liver (Cori cycle) potentially leading to energy wasting and cachexia [74, 75]. A recent paper by Chen et al serves as a complete example of the HIF-1α–glycolysis–cancer axis. Their lab observed that miRNA-18b negatively correlated with malignant melanoma tumor thickness and stage. They provided evidence of microRNA-18b binding to the HIF-1α 3′-UTR, while ectopic expression of this microRNA inhibited glycolysis and cell proliferation. [76, 77]. Hence HIF-1α coordinates multiple steps in glycolysis from glucose transport to lactate efflux allowing cancer cells satisfy their “addiction to glucose”. These adaptations serve the dual purpose of generating ATP rapidly, and directing the TCA cycle towards anabolic functions.

**Fatty acid metabolism**

Fatty acids are energy substrates, components of plasma membranes, and important signaling molecules. Most cells import fatty acids from dietary sources. Some cell types, such as hepatocytes and adipocytes can synthesize fatty acids de novo from carbohydrate-derived acetyl-coA, catalyzed by fatty acid synthase (FAS). It is now appreciated that cancer cells exhibit aberrant lipid metabolism characterized by increased fatty acid synthesis and transport and reduced fatty acid oxidation. First, fatty acid synthesis is upregulated in several cancer types. For example, oncogenic antigen-519 (OA-519) was first identified as a negative prognostic marker in breast cancer; later, peptide sequencing revealed OA-519 to be a FAS and labeled acetate studies confirmed high rates of fatty acid synthesis in OA-519 enriched cells. Furthermore, the fatty acid synthesis inhibitor Cerulenin inhibited growth of these cells [78]. The mechanism by which FAS increases may be through phosphorylation of HIF-1α and upregulation of sterol regulatory-element binding protein (SREBP)-1 [79]. Hypoxic cells can also switch between carbohydrate and amino acid (glutamine) precursors for fatty acid synthesis, via HIF-1α-mediated proteolysis of keto glutarate dehydrogenase [80] [81]. This reductive carboxylation of glutamine spares glucose in hypoxic cancer cells and allows synthesis of macromolecules from TCA intermediates when mitochondrial mutations inhibit glucose oxidation (described below). Secondly, HIF-1α induces expression of fatty acid binding proteins (FABPs) which are involved in fatty acid transport. In human glioblastoma cells, Bensaad et al showed that HIF-1α was necessary for induction of FABP3 and FABP7 leading to lipid droplet accumulations. In fact, fatty acid synthesis was suppressed in their experiments, revealing heterogeneous cellular responses to hypoxia. They also demonstrated that failure to sequester fatty acids in lipid droplets led to oxidative damage, suggesting the teleological basis for this phenotype in cancer cells [82]. Third, HIF-1α decreases fatty acid oxidation. Some pathways of this inhibition intersect with glucose metabolism (e.g. reductions in mitochondria), while others are specific to fatty acid catabolism. For instance, Huang et al demonstrated that HIF-1α inhibits medium and long-chain acyl-CoA dehydrogenases (MCAD and LCAD) which catalyze initial steps of β-oxidation. LCAD inhibition reduced ROS production and inhibited the tumor suppressor, phosphatase and tensin homolog (PTEN), such that the net effect of HIF-1α was to increase cell proliferation. Clinically, reduced LCAD expression in liver cancer cells was associated
with increased mortality [82, 83]. Hence, many cancer cells exhibit an increase in fatty acid synthesis and transport with reduced oxidation leading to intracellular lipid accumulations whose significance is still being investigated.

**Amino acid metabolism**

Tumor cells gain further survival and proliferation capability by increasing their glutamine supply, and in some instances, changing the metabolic fate of this abundant amino acid. Glutamine is converted to glutamate by glutaminase, then to α-ketoglutarate by glutamate dehydrogenase. Generation of α-ketoglutarate replenishes the TCA cycle when citrate is exported for lipid synthesis. HIF-1α and HIF-2α increase the transport of glutamine and leucine across cell membranes by increasing the expression of their respective transporters [84, 85]. This adaptation increases glutamine availability for use as an energy substrate or as a precursor to de novo fatty acid synthesis. Amino acids become particularly important to cancers with mutations in the TCA cycle or electron transport chain, which render them incapable of citrate formation required for macromolecule synthesis. In this scenario, glutamine is acted upon by mitochondrial and cytosolic isoforms of isocitrate dehydrogenase to form α-ketoglutarate [86]. This pathway is used by renal cell lines deficient in the VHL tumor suppressor protein [81], implicating HIF-1α in this process. The coordinated activation of glutamate transporters and receptors activates the SRC family kinases and downstream signaling pathways that stimulate cancer progression. For example, in hypoxic Hep3b hepatic carcinoma cells, glutamate interacts with AMPA receptors and stimulates MEK-ERK signaling leading to increased proliferation. In melanoma, metabotropic glutamate receptor GRM1 is overexpressed. Overexpression of GRM1 is sufficient to cause neoplastic transformation in murine melanocytes. Wen et al recently demonstrated that this transformation is accompanied by increased angiogenesis and VEGF expression via the Akt-mTOR-HIF-1α pathway [87] and this may be the mechanism by which the GRM1 signaling inhibitor riluzole reduces tumor progression.

**Cancer Stemness**

Cancer stem cells (CSCs) exhibit properties of embryonic stem cells such as self-renewal, pluripotency, and metastatic potential [88, 89]. Although they constitute a minor portion of the total cancer cell population [90, 91], CSCs can repopulate tumors following therapy leading to a more aggressive and resistant phenotype. The precise pathways that confer stemness are still being examined, including JAK/STAT, Wnt/B-catenin, Hedgehog, TGF-beta-Hippo-YAP/TAZ, Notch and Nanog [92–95]. CSCs also exhibit a pronounced shift towards aerobic glycolysis distinct from the remaining tumor bulk [96]. Whether hypoxia promotes stemness, or is simply the milieu in which CSCs exist is not well understood. Xie et al recently showed that culturing a breast cancer cell line under 1% oxygen conditions for 48 hours nearly tripled the proportion of CSCs. The CSCs exhibited suppressed apoptosis, and increased ability to form colonies [25]. However, mechanisms of this transformation, including the role of HIFs, were not assessed in this study. Erler et al previously showed that exposure of human colon cancer cells to hypoxia decreased expression of pro-apoptotic proteins Bid and Bad. Bid was shown to contain a hypoxia response element and its inhibition was dependent on HIF-1α [97]. Cancer cells may also be driven towards a CSC phenotype by surrounding cells. For example, cancer associated fibroblasts play a role in
epithelial mesenchymal transition (EMT) which describes the process by which epithelial cells lose polarity and adhesion to gain migratory and stem cell properties. Giannoni et al showed that when prostate cancer cells were incubated with cancer-derived or in-vitro activated fibroblasts (with TGF-β1 or IL-6 incubation), the cancer cells demonstrated markers of EMT which were also associated with upregulation of COX-2, HIF-1α, and generation of ROS. shRNA directed against NF-KB, COX-2, or HIF-1 prevented EMT [98].

Targeting the HIF pathway in Cancer

HIF-1α overexpression in tumor biopsies is associated with increased patient mortality in human cancers of the bladder, brain, breast, cervix, colon, endometrium, lung, oropharynx, pancreas, skin, and stomach [99, 100]. In breast cancer, increased HIF-1α levels have been demonstrated by immunohistochemistry in biopsies analyzed from both lymph node-negative [101] and lymph node-positive [102] breast cancer patients. Regardless of lymph node status, survival was significantly decreased in those patients with the highest HIF-1α levels in their diagnostic breast cancer biopsies. A recent study, which aimed to standardize immunohistochemical assays to predict outcome among node negative patients, identified a highly predictive signature consisting of 5 markers that included HIF-1α and could predict patient outcome in over 90% of breast cancer patient cases analyzed [103, 104]. HIF-2α has also been correlated to distant recurrence and poor outcome in cancer [105].

The extensive list of HIF target genes provides a molecular basis for the many effects of intratumoral hypoxia on cancer progression, and the reported association between HIF-1α overexpression and adverse outcome for cancer patients [106–112]. The potential target genes regulated by HIF-1 α that may play a role in tumor progression are beginning to be uncovered. One notable challenge is that the specific subset of HIF-1 α target genes that respond to hypoxia differs by cancer type.

Several drugs are being developed which block HIF activity with the goal of inhibiting tumor growth, angiogenesis and/or metastasis in preclinical models [113, 114]. In addition, existing drugs such as digoxin, metformin, or angiotensin-2 receptor blockers can act as non-specific HIF-1α inhibitors and have been used in proof-of-concept studies. Digoxin was identified together with 20 other drugs in a screening library to inhibit HIF-1α gene transcription. Interestingly, several other identified drugs were also cardiac glycosides [115, 116]. Digoxin inhibited HIF-1α and VEGF in non-small cell lung cancer cells cultured in hypoxia, reducing their viability [117, 118]. In a retrospective analysis, patients with prostate cancer taking a non-specific HIF-1α inhibitor such as digoxin, metformin or angiotensin-2 receptor blocker exhibited a lower risk of prostate cancer progression [119]. However, a nonrandomized pilot study of digoxin did not reduce PSA levels over 6 months compared against that of historical controls [120]. In terms of novel therapies directed against the HIF-1α pathway, these are reviewed extensively elsewhere [121–123].

Potential Connections between Respiratory Disorders, HIF, and Cancer

Since hypoxia stabilizes HIF-1α, which in turn is associated with poor prognosis in cancer, it is conceivable that respiratory conditions that reduce tissue oxygen levels could promote
cancer. High altitude may be an illustrative example, since reduced barometric pressure lowers inspired oxygen tension without confounding effects of pulmonary or cardiac disease. Adaptation to high altitude includes HIF mediated responses such as erythropoiesis and reduced mitochondria mass, although few studies have directly measured HIF-1α protein. During an ascent of Mt. Everest, Levett et al found changes in skeletal muscle that would be consistent with HIF activation including decreases in mitochondrial density, PGC-1α, and expression of electron transport chain complexes I and IV. However, muscle HIF-1α protein levels were not elevated, which may reflect degradation during the sampling process [124]. Robach et al. reported a 3 fold increase in HIF-2α mRNA in the skeletal muscle of human subjects living at high altitude associated with increased erythropoietin plasma levels [125]. Interestingly, natural selection may favor reduced HIFs activation at high altitude in Tibetans, averting excessive erythropoiesis – a feature of chronic mountain sickness [126].

Does high altitude confer increased risk of cancer or cancer death? A comparison of high-altitude counties versus sea-level counties matched for socioeconomic status in the United States showed a reduced age-adjusted cancer mortality rate in the high altitude residents (defined as elevations greater than 2134 m)[127]. The decreased mortality at higher altitude was also seen in older studies that specifically examined only those of Caucasian race[128] or that stratified analysis by extent of urbanization [129]. It is possible that the protective effects of high altitude on cancer mortality are driven by other unmeasured demographic or environmental factors, or that mortality may not be the best means to capture an effect of the HIF pathway on cancer. However, it should also be emphasized that hypoxemia – a relative reduction in oxyhemoglobin saturation – is not tantamount to cellular oxygen insufficiency. This fact is demonstrated by unaltered lactate/pyruvate ratios in human experiments of hypoxic gas breathing [130]. In mice exposed to near-lethal levels of hypoxia, HIF-1α expression was elevated in some tissues only transiently, while in other tissues such as brain and muscle, HIF-1α was detectable in normoxia [131]. Thus, hypoxemia does not reliably induce cellular anaerobic conditions, and tissue hypoxia is neither necessary nor sufficient for persistent activation of HIF-1α.

Obstructive Sleep Apnea, HIF, and Cancer

OSA is a breathing disorder characterized by episodic upper airway obstruction that disrupts ventilation during sleep. Each disruption in breathing lasting over 10 seconds is defined as an apnea while a milder decrease in inspiratory flow lasting over 10 seconds is defined as a hypopnea. Apneas and hypopneas are accompanied by decreases in oxygen saturation, leading to a characteristic pattern of intermittent hypoxia during sleep. Could this pattern of hypoxia stabilize HIF and promote cancer? Evidence for this possibility is mostly indirect in nature. In a prospective Spanish study, the degree of nocturnal hypoxemia from OSA was associated with an increased incidence of cancer over the 4.5 year follow-up period, for patients <65 years old [132]. Gaoatswe et al. showed that patients with OSA have a lower number of invariant natural killer cells than non-OSA controls[133].
Authors speculated that this deficiency in cancer immunity could be a mechanism linking OSA with cancer. Conversely, a large Canadian healthcare database did not identify a relationship between a diagnosis of OSA and cancer incidence over ~8 years [134].

In some experiments, rodents were exposed to intermittent hypoxia as simulation of OSA. Intermittent hypoxia may result in sustained hypoxia in some regions such as adipose tissue [135] resulting in HIF-1α stabilization [136]. Exposure of mice to 4 weeks of intermittent hypoxia accelerated tumor growth of implanted melanoma cells [137] and induced metastasis to the lung [138]. A follow-up experiment suggested crosstalk between tumor cells and a tumor associated macrophages as a potential mechanism [139]. Interestingly, sleep fragmentation without hypoxia promoted tumor growth through macrophage recruitment [139] and suppressed NADPH oxidase activity leading to reduced ROS levels [140]. However, HIF-1α was not measured in these intermittent hypoxia-cancer experiments.

**Conclusion**

HIFs play important roles in regulating oxygen metabolism in health and disease. HIF-1α is often upregulated in cancer, through both hypoxic and non-hypoxic pathways. Thereafter, HIF-1α may promote tumor survival by several overlapping mechanisms. There are intriguing studies suggesting that hypoxemia from OSA may promote cancer, but further research is warranted to confirm these observations and implicate the HIF pathway.

**Acknowledgments**

Jonathan C. Jun is supported by Nutrition Obesity Research Center (NORC) under NIH P30DK072488, American Academy of Sleep Medicine Foundation Junior Faculty Award 106-JF-14, and National Institutes of Health 1K08HL109475

Vsevolod Y. Polotsky is supported by the NIH grants R01 HL128970, R01 HL133100, P50 ES018176 and by the American Sleep Medicine Foundation grant 133-BS-15

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