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Hypoxia-Inducible Factors and Cancer

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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

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Compliance with Ethics Guidelines

Conflict of interest

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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- 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 spice 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 up-regulates 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 ketoglutarate 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.

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References

Papers of particular interest, published recently, have been highlighted as:

• Of importance

•• Of major importance

1. Semenza GL, Nejfelt MK, Chi SM, Antonarakis SE. Hypoxia-inducible nuclear factors bind to an enhancer element located 3′ to the human erythropoietin gene. Proceedings of the National Academy of Sciences of the United States of America. 1991; 88(13):5680–5684. [PubMed: 2062846]
2. Semenza GL, Wang GL. A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. Molecular and cellular biology. 1992; 12(12):5447–5454. [PubMed: 1448077]
3. Liu W, Shen S-M, Zhao X-Y, Chen G-Q. Targeted genes and interacting proteins of hypoxia inducible factor-1. International Journal of Biochemistry and Molecular Biology. 2012; 3(2):165–178. [PubMed: 22773957]

4. Wang GL, Jiang BH, Rue EA, Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci U S A. 1995; 92(12):5510–5514. [PubMed: 7539918]

5. Wang V, Davis DA, Haque M, Huang LE, Yarchoan R. Differential gene up-regulation by hypoxia-inducible factor-1alpha and hypoxia-inducible factor-2alpha in HEK293T cells. Cancer research. 2005; 65(8):3299–3306. [PubMed: 15833863]

6. Heikkilä M, Pasanen A, Kivirikko KI, Myllyharju J. Roles of the human hypoxia-inducible factor (HIF)-3α variants in the hypoxia response. Cellular and Molecular Life Sciences. 2011; 68(23): 3885–3901. [PubMed: 21479871]

7. Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, Wykoff CC, Pugh CW, Maher ER, Ratcliffe PJ. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature. 1999; 399(6733):271–275. [PubMed: 10353251]

8. Mahon PC, Hirota K, Semenza GL. FIH-1: a novel protein that interacts with HIF-1alpha and VHL to mediate repression of HIF-1 transcriptional activity. Genes & development. 2001; 15(20):2675–2686. [PubMed: 11641274]

9. Jiang BH, Rue E, Wang GL, Roe R, Semenza GL. Dimerization, DNA binding, and transactivation properties of hypoxia-inducible factor 1. The Journal of biological chemistry. 1996; 271(30):17771–17778. [PubMed: 8663540]

10. He Q, Gao Z, Yin J, Zhang J, Yun Z, Ye J. Regulation of HIF-1{alpha} activity in adipose tissue by obesity-associated factors: adipogenesis, insulin, and hypoxia. American journal of physiology Endocrinology and metabolism. 2011; 300(5):E877–885. [PubMed: 21343542]

11. Dery MA, Michaud MD, Richard DE. Hypoxia-inducible factor 1: regulation by hypoxic and non-hypoxic activators. The international journal of biochemistry & cell biology. 2005; 37(3):535–540. [PubMed: 15618010]

12. Stasinopoulos I, O'Brien DR, Bhujwalla ZM. Inflammation, but not hypoxia, mediated HIF-1α activation depends on COX-2. Cancer biology & therapy. 2009; 8(1):31–35. [PubMed: 19390242]

13•. Hubbi ME, Gilkes DM, Hu H, Kshitiz, Ahmed I, Semenza GL. Cyclin-dependent kinases regulate lysosomal degradation of hypoxia-inducible factor 1alpha to promote cell-cycle progression. Proc Natl Acad Sci U S A. 2014; 111(32):E3325–3334. This study shows that Cdk 1 and 2 physically and functionally interact with HIF-1α, inhibiting or promoting its degradation by lysosomes. [PubMed: 25071185]

14. Herzog J, Ehrlich SM, Pfitzer L, Liebl J, Frohlich T, Arnold GJ, Mikulits W, Haider C, Vollmar AM, Zahler S. Cyclin-dependent kinase 5 stabilizes hypoxia-inducible factor-1alpha: a novel approach for inhibiting angiogenesis in hepatocellular carcinoma. Oncotarget. 2016; 7(19):27108–27121. [PubMed: 27027353]

15. Liu M, Wang D, Li N. MicroRNA-20b Downregulates HIF-1alpha and Inhibits the Proliferation and Invasion of Osteosarcoma Cells. Oncology research. 2016; 23(5):257–266. [PubMed: 27098149]

16. Wang W, Zhang E, Lin C. MicroRNAs in tumor angiogenesis. Life Sciences. 2015; 136:28–35. [PubMed: 26144623]

17. Kulshreshtha R, Ferracin M, Wojcik SE, Garzon R, Alder H, Agosto-Perez FJ, Davuluri R, Liu CG, Croce CM, Negrini M, et al. A microRNA signature of hypoxia. Molecular and cellular biology. 2007; 27(5):1859–1867. [PubMed: 17194750]

18. Movafagh S, Crook S, Vo K. Regulation of hypoxia-inducible factor-1a by reactive oxygen species: new developments in an old debate. Journal of cellular biochemistry. 2015; 116(5):696–703. [PubMed: 25546605]

19. Lee YS, Kim JW, Osborne O, Oh DY, Sasik R, Schenk S, Chen A, Chung H, Murphy A, Watkins SM, et al. Increased adipocyte O2 consumption triggers HIF-1alpha, causing inflammation and insulin resistance in obesity. Cell. 2014; 157(6):1339–1352. [PubMed: 24906151]

20. Wang GL, Jiang BH, Semenza GL. Effect of protein kinase and phosphatase inhibitors on expression of hypoxia-inducible factor 1. Biochemical and biophysical research communications. 1995; 216(2):669–675. [PubMed: 7488163]

21. Helmlinger G, Yuan F, Dellian M, Jain RK. Interstitial pH and pO2 gradients in solid tumors in vivo: high-resolution measurements reveal a lack of correlation. Nature medicine. 1997; 3(2):177–182.
22. Zhao M, Zhang Y, Zhang H, Wang S, Zhang M, Chen X, Wang H, Zeng G, Chen X, Liu G, et al. Hypoxia-induced cell stemness leads to drug resistance and poor prognosis in lung adenocarcinoma. Lung cancer. 2015; 87(2):98–106. [PubMed: 25512094]
23. Luo D, Wang Z, Wu J, Jiang C, Wu J. The role of hypoxia inducible factor-1 in hepatocellular carcinoma. BioMed research international. 2014; 2014:409272. [PubMed: 25101278]
24. Parks SK, Cormerais Y, Marchiq I, Pouyssegur J. Hypoxia optimises tumour growth by controlling nutrient import and acidic metabolite export. Molecular aspects of medicine. 2016; 47–48:3–14.
25•. Xie J, Xiao Y, Zhu XY, Ning ZY, Xu HF, Wu HM. Hypoxia regulates stemness of breast cancer MDA-MB-231 cells. Medical oncology. 2016; 33(5):42. This study shows effects of hypoxia on stemness transformation in MDA-MB-231 cells in breast cancer. [PubMed: 27038472]
26. Nagaraju GP, Bramhachari PV, Raghu G, El-Rayes BF. Hypoxia inducible factor-1alpha: Its role in colorectal carcinogenesis and metastasis. Cancer letters. 2015; 366(1):11–18. [PubMed: 26116902]
27. Krieg M, Haas R, Brauch H, Acker T, Flamme I, Plate KH. Up-regulation of hypoxia-inducible factors HIF-1alpha and HIF-2alpha under normoxic conditions in renal carcinoma cells by von Hippel-Lindau tumor suppressor gene loss of function. Oncogene. 2000; 19(48):5435–5443. [PubMed: 11114720]
28. Latif F, Tory K, Gnarra J, Yao M, Duh FM, Orcutt ML, Stackhouse T, Kuzmin I, Modi W, Geil L, et al. Identification of the von Hippel-Lindau disease tumor suppressor gene. Science. 1993; 260(5112):1317–1320. [PubMed: 8493574]
29. Evan GI, Vousden KH. Proliferation, cell cycle and apoptosis in cancer. Nature. 2001; 411(6835): 342–348. [PubMed: 11357141]
30. Zhong H, De Marzo AM, Laughner E, Lim M, Hilton DA, Zagzag D, Buechler P, Isaacs WB, Semenza GL, Simons JW. Overexpression of hypoxia-inducible factor 1alpha in common human cancers and their metastases. Cancer research. 1999; 59(22):5830–5835. [PubMed: 10582706]
31. Zhong H, Agani F, Baccala AA, Laughner E, Rioseco-Camacho N, Isaacs WB, Simons JW, Semenza GL. Increased expression of hypoxia inducible factor-1alpha in rat and human prostate cancer. Cancer research. 1998; 58(23):5280–5284. [PubMed: 9850048]
32. Chen L, Shi Y, Yuan J, Han Y, Qin R, Wu Q, Jia B, Wei B, Wei L, Dai G, et al. HIF-1 alpha overexpression correlates with poor overall survival and disease-free survival in gastric cancer patients post-gastrectomy. PloS one. 2014; 9(3):e90678. [PubMed: 24614305]
33. Zheng SS, Chen XH, Yin X, Zhang BH. Prognostic significance of HIF-1alpha expression in hepatocellular carcinoma: a meta-analysis. PloS one. 2013; 8(6):e65753. [PubMed: 23799043]
34. Amellem O, Pettersen EO. Cell inactivation and cell cycle inhibition as induced by extreme hypoxia: the possible role of cell cycle arrest as a protection against hypoxia-induced lethal damage. Cell proliferation. 1991; 24(2):127–141. [PubMed: 2009318]
35. Goda N, Ryan HE, Khadivi B, McNulty W, Rickert RC, Johnson RS. Hypoxia-inducible factor 1alpha is essential for cell cycle arrest during hypoxia. Molecular and cellular biology. 2003; 23(1):359–369. [PubMed: 12482987]
36. Gardner LB, Li Q, Park MS, Flanagan WM, Semenza GL, Dang CV. Hypoxia inhibits G1/S transition through regulation of p27 expression. The Journal of biological chemistry. 2001; 276(11):7919–7926. [PubMed: 11112789]
37. Krtolica A, Krucher NA, Ludlow JW. Hypoxia-induced pRB hypophosphorylation results from downregulation of CDK and upregulation of PP1 activities. Oncogene. 1998; 17(18):2295–2304. [PubMed: 9811460]
38. Hubbi ME, Kshitiz, Gilkes DM, Rey S, Wong CC, Luo W, Kim DH, Dang CV, Levchenko A, Semenza GL. A nontranscriptional role for HIF-1alpha as a direct inhibitor of DNA replication. Science signaling. 2013; 6(262):ra10. [PubMed: 23405012]
39. Koshiji M, Kageyama Y, Pete EA, Horikawa I, Barrett JC, Huang LE. HIF-1alpha induces cell cycle arrest by functionally counteracting Myc. The EMBO journal. 2004; 23(9):1949–1956. [PubMed: 15071503]

40. Box AH, Demetrick DJ. Cell cycle kinase inhibitor expression and hypoxia-induced cell cycle arrest in human cancer cell lines. Carcinogenesis. 2004; 25(12):2325–2335. [PubMed: 15347600]
41. Franovic A, Gunaratnam L, Smith K, Robert I, Patten D, Lee S. Translational up-regulation of the EGFR by tumor hypoxia provides a nonmutational explanation for its overexpression in human cancer. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104(32):13092–13097. [PubMed: 17670948]
42. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. Nature. 2000; 407(6801):249–257. [PubMed: 11001068]
43. Fang J, Yan L, Shing Y, Moses MA. HIF-1alpha-mediated up-regulation of vascular endothelial growth factor, independent of basic fibroblast growth factor, is important in the switch to the angiogenic phenotype during early tumorigenesis. Cancer research. 2001; 61(15):5731–5735. [PubMed: 11479208]
44. Tsuzuki Y, Fukumura D, Oosthuyse B, Koike C, Carmeliet P, Jain RK. Vascular endothelial growth factor (VEGF) modulation by targeting hypoxia-inducible factor-1alpha--> hypoxia response element--> VEGF cascade differentially regulates vascular response and growth rate in tumors. Cancer research. 2000; 60(22):6248–6252. [PubMed: 11103778]
45. Jensen RL, Ragel BT, Whang K, Gillespie D. Inhibition of hypoxia inducible factor-1alpha (HIF-1alpha) decreases vascular endothelial growth factor (VEGF) secretion and tumor growth in malignant gliomas. Journal of neuro-oncology. 2006; 78(3):233–247. [PubMed: 16612574]
46. Tang CM, Yu J. Hypoxia-inducible factor-1 as a therapeutic target in cancer. Journal of gastroenterology and hepatology. 2013; 28(3):401–405. [PubMed: 23173651]
47. Conley SJ, Gheordunescu E, Kakarala P, Newman B, Korkaya H, Heath AN, Clouthier SG, Wicha MS. Antiangiogenic agents increase breast cancer stem cells via the generation of tumor hypoxia. Proc Natl Acad Sci U S A. 2012; 109(8):2784–2789. [PubMed: 22308314]
48. Hayes DF. Bevacizumab treatment for solid tumors: boon or bust? Jama. 2011; 305(5):506–508. [PubMed: 21285431]
49. Bergers G, Hanahan D. Modes of resistance to anti-angiogenic therapy. Nature reviews Cancer. 2008; 8(8):592–603. [PubMed: 18650835]
50. Woolard J, Wang WY, Bevan HS, Qiu Y, Morbidelli L, Pritchard-Jones RO, Cui TG, Sugiono M, Waine E, Perrin R, et al. VEGF165b, an inhibitory vascular endothelial growth factor splice variant: mechanism of action, in vivo effect on angiogenesis and endogenous protein expression. Cancer research. 2004; 64(21):7822–7835. [PubMed: 15520188]
51. Pritchard-Jones RO, Dunn DB, Qiu Y, Varey AH, Orlando A, Rigby H, Harper SJ, Bates DO. Expression of VEGF(xxx)b, the inhibitory isoforms of VEGF, in malignant melanoma. British journal of cancer. 2007; 97(2):223–230. [PubMed: 17595666]
52. Raza A, Franklin MJ, Dudek AZ. Pericytes and vessel maturation during tumor angiogenesis and metastasis. American journal of hematology. 2010; 85(8):593–598. [PubMed: 20540157]
53. Reinmuth N, Liu W, Jung YD, Ahmad SA, Shaheen RM, Fan F, Bucana CD, McMahon G, Gallick GE, Ellis LM. Induction of VEGF in perivascular cells defines a potential paracrine mechanism for endothelial cell survival. FASEB journal: official publication of the Federation of American Societies for Experimental Biology. 2001; 15(7):1239–1241. [PubMed: 11344100]
54. Warburg O, Wind F, Negelein E. The Metabolism of Tumors in the Body. The Journal of general physiology. 1927; 8(6):519–530. [PubMed: 19872213]
55. Zhang H. HIF-1 suppresses lipid catabolism to promote cancer progression. Molecular & cellular oncology. 2015; 2(4):e980184. [PubMed: 27308514]
56. Marchiq I, Pouyssegur J. Hypoxia, cancer metabolism and the therapeutic benefit of targeting lactate/H(+) symporters. Journal of molecular medicine. 2016; 94(2):155–171. [PubMed: 26099350]
57. Chen C, Pore N, Behrooz A, Ismail-Beigi F, Maity A. Regulation of glut1 mRNA by hypoxia-inducible factor-1. Interaction between H-ras and hypoxia. The Journal of biological chemistry. 2001; 276(12):9519–9525. [PubMed: 11120745]
58. Liu Y, Li YM, Tian RF, Liu WP, Fei Z, Long QF, Wang XA, Zhang X. The expression and significance of HIF-1alpha and GLUT-3 in glioma. Brain research. 2009; 1304:149–154. [PubMed: 19782666]

59. Semenza GL, Roth PH, Fang HM, Wang GL. Transcriptional regulation of genes encoding glycolytic enzymes by hypoxia-inducible factor 1. The Journal of biological chemistry. 1994; 269(38):23757–23763. [PubMed: 8089148]
60. Schonberger J, Ruschoff J, Grimm D, Marienhagen J, Rummele P, Meyringer R, Kossmehl P, Hofstaedter F, Eilles C. Glucose transporter 1 gene expression is related to thyroid neoplasms with an unfavorable prognosis: an immunohistochemical study. Thyroid: official journal of the American Thyroid Association. 2002; 12(9):747–754. [PubMed: 12481939]
61. Krzeslak A, Wojcik-Krowiranda K, Forma E, Jozwiak P, Romanowicz H, Bienkiewicz A, Brys M. Expression of GLUT1 and GLUT3 glucose transporters in endometrial and breast cancers. Pathology oncology research: POR. 2012; 18(3):721–728. [PubMed: 22270867]
62. Firth JD, Ebert BL, Pugh CW, Ratcliffe PJ. Oxygen-regulated control elements in the phosphoglycerate kinase 1 and lactate dehydrogenase A genes: similarities with the erythropoietin 3′ enhancer. Proc Natl Acad Sci U S A. 1994; 91(14):6496–6500. [PubMed: 8022811]
63. Kim JW, Tchernyshyov I, Semenza GL, Dang CV. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. Cell metabolism. 2006; 3(3):177–185. [PubMed: 16517405]
64. Brand KA, Hermfisse U. Aerobic glycolysis by proliferating cells: a protective strategy against reactive oxygen species. FASEB journal: official publication of the Federation of American Societies for Experimental Biology. 1997; 11(5):388–395. [PubMed: 9141507]
65. Fukuda R, Zhang H, Kim JW, Shimoda L, Dang CV, Semenza GL. HIF-1 regulates cytochrome oxidase subunits to optimize efficiency of respiration in hypoxic cells. Cell. 2007; 129(1):111–122. [PubMed: 17418790]
66. Zhang H, Bosch-Marce M, Shimoda LA, Tan YS, Baek JH, Wesley JB, Gonzalez FJ, Semenza GL. Mitochondrial autophagy is an HIF-1-dependent adaptive metabolic response to hypoxia. The Journal of biological chemistry. 2008; 283(16):10892–10903. [PubMed: 18281291]
67. Firth JD, Ebert BL, Ratcliffe PJ. Hypoxic regulation of lactate dehydrogenase A. Interaction between hypoxia-inducible factor 1 and cAMP response elements. The Journal of biological chemistry. 1995; 270(36):21021–21027. [PubMed: 7673128]
68. Ullah MS, Davies AJ, Halestrap AP. The plasma membrane lactate transporter MCT4, but not MCT1, is up-regulated by hypoxia through a HIF-1alpha-dependent mechanism. The Journal of biological chemistry. 2006; 281(14):9030–9037. [PubMed: 16452478]
69. Shimoda LA, Fallon M, Pisarcik S, Wang J, Semenza GL. HIF-1 regulates hypoxic induction of NHE1 expression and alkalinization of intracellular pH in pulmonary arterial myocytes. American journal of physiology Lung cellular and molecular physiology. 2006; 291(5):L941–949. [PubMed: 16766575]
70. Meijer TW, Schuurbiers OC, Kaanders JH, Looijen-Salamon MG, de Geus-Oei LF, Verhagen AF, Lok J, van der Heijden HF, Rademakers SE, Span PN, et al. Differences in metabolism between adeno- and squamous cell non-small cell lung carcinomas: spatial distribution and prognostic value of GLUT1 and MCT4. Lung cancer. 2012; 76(3):316–323. [PubMed: 22153830]
71. Xia J, Huang N, Huang H, Sun L, Dong S, Su J, Zhang J, Wang L, Lin L, Shi M, et al. Voltage-gated sodium channel Nav 1.7 promotes gastric cancer progression through MACC1-mediated upregulation of NHE1. International journal of cancer. 2016
72. Ivanov SV, Kuzmin I, Wei MH, Pack S, Geil L, Johnson BE, Stanbridge EJ, Lerman MI. Down-regulation of transmembrane carbonic anhydrases in renal cell carcinoma cell lines by wild-type von Hippel-Lindau transgenes. Proc Natl Acad Sci U S A. 1998; 95(21):12596–12601. [PubMed: 9770531]
73. Wykoff CC, Beasley NJ, Watson PH, Turner KJ, Pastorek J, Sibtain A, Wilson GD, Turley H, Talks KL, Maxwell PH, et al. Hypoxia-inducible expression of tumor-associated carbonic anhydrases. Cancer research. 2000; 60(24):7075–7083. [PubMed: 11156414]
74. Courtnay R, Ngo DC, Malik N, Ververis K, Tortorella SM, Karagiannis TC. Cancer metabolism and the Warburg effect: the role of HIF-1 and PI3K. Molecular biology reports. 2015; 42(4):841–851. [PubMed: 25689954]
75. Tisdale MJ. Mechanisms of cancer cachexia. Physiological reviews. 2009; 89(2):381–410. [PubMed: 19342610]

76. Ryan HE, Lo J, Johnson RS. HIF-1 alpha is required for solid tumor formation and embryonic vascularization. The EMBO journal. 1998; 17(11):3005–3015. [PubMed: 9606183]
77. Chen Y, Zhang Z, Luo C, Chen Z, Zhou J. MicroRNA-18b inhibits the growth of malignant melanoma via inhibition of HIF-1alpha-mediated glycolysis. Oncology reports. 2016; 36(1):471–479. [PubMed: 27220837]
78. Kuhajda FP, Jenner K, Wood FD, Hennigar RA, Jacobs LB, Dick JD, Pasternack GR. Fatty acid synthesis: a potential selective target for antineoplastic therapy. Proceedings of the National Academy of Sciences of the United States of America. 1994; 91(14):6379–6383. [PubMed: 8022791]
79. Furuta E, Pai SK, Zhan R, Bandyopadhyay S, Watabe M, Mo YY, Hirota S, Hosobe S, Tsukada T, Miura K, et al. Fatty acid synthase gene is up-regulated by hypoxia via activation of Akt and sterol regulatory element binding protein-1. Cancer research. 2008; 68(4):1003–1011. [PubMed: 18281474]
80. Sun RC, Denko NC. Hypoxic regulation of glutamine metabolism through HIF1 and SIAH2 supports lipid synthesis that is necessary for tumor growth. Cell metabolism. 2014; 19(2):285–292. [PubMed: 24506869]
81. Metallo CM, Gameiro PA, Bell EL, Mattaini KR, Yang J, Hiller K, Jewell CM, Johnson ZR, Irvine DJ, Guarente L, et al. Reductive glutamine metabolism by IDH1 mediates lipogenesis under hypoxia. Nature. 2011; 481(7381):380–384. [PubMed: 22101433]
82. Bensaad K, Favaro E, Lewis CA, Peck B, Lord S, Collins JM, Pinnick KE, Wigfield S, Buffa FM, Li JL, et al. Fatty acid uptake and lipid storage induced by HIF-1alpha contribute to cell growth and survival after hypoxia-reoxygenation. Cell reports. 2014; 9(1):349–365. [PubMed: 25263561]
83. Huang D, Li T, Li X, Zhang L, Sun L, He X, Zhong X, Jia D, Song L, Semenza GL, et al. HIF-1-mediated suppression of acyl-CoA dehydrogenases and fatty acid oxidation is critical for cancer progression. Cell reports. 2014; 8(6):1930–1942. [PubMed: 25242319]
84. Soh H, Wasa M, Fukuzawa M. Hypoxia upregulates amino acid transport in a human neuroblastoma cell line. Journal of pediatric surgery. 2007; 42(4):608–612. [PubMed: 17448754]
85•. Hu H, Takano N, Xiang L, Gilkes DM, Luo W, Semenza GL. Hypoxia-inducible factors enhance glutamate signaling in cancer cells. Oncotarget. 2014; 5(19):8853–8868. This study demonstrates that HIFs regulate glutamate receptors and transporters, which can activate key signal transduction pathways that promote cancer progression. [PubMed: 25326682]
86. Mullen AR, Wheaton WW, Jin ES, Chen PH, Sullivan LB, Cheng T, Yang Y, Linehan WM, Chandel NS, DeBerardinis RJ. Reductive carboxylation supports growth in tumour cells with defective mitochondria. Nature. 2011; 481(7381):385–388. [PubMed: 22101431]
87. Kelly FJ. Effect of hyperoxic exposure on protein synthesis in the rat. The Biochemical journal. 1988; 249(2):609–612. [PubMed: 2449180]
88. Guan G, Zhang Y, Lu Y, Liu L, Shi D, Wen Y, Yang L, Ma Q, Liu T, Zhu X, et al. The HIF-1α/CXCR4 pathway supports hypoxia-induced metastasis of human osteosarcoma cells. Cancer letters. 2015; 357(1):254–264. [PubMed: 25444927]
89. Jordan CT, Guzman ML, Noble M. Cancer stem cells. The New England journal of medicine. 2006; 355(12):1253–1261. [PubMed: 16990388]
90. Clarke MF. Self-renewal and solid-tumor stem cells. Biology of blood and marrow transplantation: journal of the American Society for Blood and Marrow Transplantation. 2005; 11(2 Suppl 2):14–16.
91. Ajdukovic J. HIF-1 - a big chapter in the cancer tale. Experimental oncology. 2016; 38(1):9–12. [PubMed: 27031712]
92. Hoffmeyer K, Raggioli A, Rudloff S, Anton R, Hierholzer A, Del Valle I, Hein K, Vogt R, Kemler R. Wnt/beta-catenin signaling regulates telomerase in stem cells and cancer cells. Science. 2012; 336(6088):1549–1554. [PubMed: 22723415]
93. Bourguignon LY, Earle C, Wong G, Spevak CC, Krueger K. Stem cell marker (Nanog) and Stat-3 signaling promote MicroRNA-21 expression and chemoresistance in hyaluronan/CD44-activated head and neck squamous cell carcinoma cells. Oncogene. 2012; 31(2):149–160. [PubMed: 21685938]

94. Espinoza I, Pochampally R, Xing F, Watabe K, Miele L. Notch signaling: targeting cancer stem cells and epithelial-to-mesenchymal transition. OncoTargets and therapy. 2013; 6:1249–1259. [PubMed: 24043949]

95. Ajani JA, Song S, Hochster HS, Steinberg IB. Cancer stem cells: the promise and the potential. Seminars in oncology. 2015; 42(Suppl 1):S3–17.

96. Deshmukh A, Deshpande K, Arfuso F, Newsholme P, Dharmarajan A. Cancer stem cell metabolism: a potential target for cancer therapy. Molecular cancer. 2016; 15(1):69. [PubMed: 27825361]

97. Erler JT, Cawthorne CJ, Williams KJ, Koritzinsky M, Wouters BG, Wilson C, Miller C, Demonacos C, Stratford IJ, Dive C. Hypoxia-mediated down-regulation of Bid and Bax in tumors occurs via hypoxia-inducible factor 1-dependent and -independent mechanisms and contributes to drug resistance. Molecular and cellular biology. 2004; 24(7):2875–2889. [PubMed: 15024076]

98. Giannoni E, Bianchini F, Calorini L, Chiarugi P. Cancer associated fibroblasts exploit reactive oxygen species through a proinflammatory signature leading to epithelial mesenchymal transition and stemness. Antioxidants & redox signaling. 2011; 14(12):2361–2371. [PubMed: 21235356]

99. Semenza GL. Targeting HIF-1 for cancer therapy. Nat Rev Cancer. 2003; 3(10):721–732. [PubMed: 13130303]

100. Semenza GL. Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics. Oncogene. 2010; 29(5):625–634. [PubMed: 19946328]

101. Bos R, van der Groep P, Greijer AE, Shvarts A, Meijer S, Pinedo HM, Semenza GL, van Diest PJ, van der Wall E. Levels of hypoxia-inducible factor-1α independently predict prognosis in patients with lymph node negative breast carcinoma. Cancer. 2003; 97(6):1573–1581. [PubMed: 12627523]

102. Schindl M, Schoppmann SF, Samonigg H, Hausmaninger H, Kwasny W, Gnant M, Jakesz R, Kubista E, Birner P, Oberhuber G. Overexpression of hypoxia-inducible factor 1α is associated with an unfavorable prognosis in lymph node-positive breast cancer. Clin Cancer Res. 2002; 8(6): 1831–1837. [PubMed: 12060624]

103. Charpin C, Secq V, Giusiano S, Carpentier S, Andrac L, Lavaut MN, Allasia C, Bonnier P, Garcia S. A signature predictive of disease outcome in breast carcinomas, identified by quantitative immunocytochemical assays. Int J Cancer. 2009; 124(9):2124–2134. [PubMed: 19142869]

104. Charpin C, Tavassoli F, Secq V, Giusiano S, Villeret J, Garcia S, Birnbaum D, Bonnier P, Lavaut MN, Boubli L, et al. Validation of an immunohistochemical signature predictive of 8-year outcome for patients with breast carcinoma. Int J Cancer. 2012; 131(3):E236–243. [PubMed: 22120430]

105. Helczynska K, Larsson AM, Holmquist Mengelbier L, Bridges E, Fredlund E, Borgquist S, Landberg G, Pahlman S, Jirstrom K. Hypoxia-inducible factor-2α correlates to distant recurrence and poor outcome in invasive breast cancer. Cancer Res. 2008; 68(22):9212–9220. [PubMed: 19010893]

106. Giatromanolaki A, Koukourakis MI, Simopoulos C, Polychronidis A, Gatter KC, Harris AL, Sivridis E. c-erbB-2 related aggressiveness in breast cancer is hypoxia inducible factor-1α dependent. Clin Cancer Res. 2004; 10(23):7972–7977. [PubMed: 15585632]

107. Dales JP, Garcia S, Meunier-Carpentier S, Andrac-Meyer L, Haddad O, Lavaut MN, Allasia C, Bonnier P, Charpin C. Overexpression of hypoxia-inducible factor HIF-1α predicts early relapse in breast cancer: retrospective study in a series of 745 patients. Int J Cancer. 2005; 116(5):734–739. [PubMed: 15849727]

108. Vleugel MM, Greijer AE, Shvarts A, van der Groep P, van Berkel M, Aarbodem Y, van Tinteren H, Harris AL, van Diest PJ, van der Wall E. Differential prognostic impact of hypoxia induced and diffuse HIF-1α expression in invasive breast cancer. J Clin Pathol. 2005; 58(2):172–177. [PubMed: 15677538]

109. Generali D, Berruti A, Brizzi MP, Campo L, Bonardi S, Wigfield S, Bersiga A, Allevi G, Milani M, Aguggini S, et al. Hypoxia-inducible factor-1α expression predicts a poor response to primary chemoendocrine therapy and disease-free survival in primary human breast cancer. Clin Cancer Res. 2006; 12(15):4562–4568. [PubMed: 16899602]

110. Kronblad A, Jirstrom K, Ryden L, Nordenskjold B, Landberg G. Hypoxia inducible factor-1α is a prognostic marker in premenopausal patients with intermediate to highly differentiated breast cancer but not a predictive marker for tamoxifen response. Int J Cancer. 2006; 118(10):2609–2616. [PubMed: 16381002]

111. Trastour C, Benizri E, Ettore F, Ramaioli A, Chamorey E, Pouyssegur J, Berra E. HIF-1α and CA IX staining in invasive breast carcinomas: prognosis and treatment outcome. Int J Cancer. 2007; 120(7):1451–1458. [PubMed: 17245699]

112. Yamamoto Y, Ibusuki M, Okumura Y, Kawasoe T, Kai K, Iyama K, Iwase H. Hypoxia-inducible factor 1α is closely linked to an aggressive phenotype in breast cancer. Breast Cancer Res Treat. 2008; 110(3):465–475. [PubMed: 17805961]

113•. Xiang L, Gilkes DM, Chaturvedi P, Luo W, Hu H, Takano N, Liang H, Semenza GL. Ganetespib blocks HIF-1 activity and inhibits tumor growth, vascularization, stem cell maintenance, invasion, and metastasis in orthotopic mouse models of triple-negative breast cancer. Journal of molecular medicine. 2014; 92(2):151–164. This study shows that Ganetespib inhibited tumor growth and metastasis in mouse model of triple negative breast cancer by blocking HIF-1 activity. [PubMed: 24248265]

114. Wong CC, Zhang H, Gilkes DM, Chen J, Wei H, Chaturvedi P, Hubbi ME, Semenza GL. Inhibitors of hypoxia-inducible factor 1 block breast cancer metastatic niche formation and lung metastasis. Journal of molecular medicine. 2012; 90(7):803–815. [PubMed: 22231744]

115. Lopez-Lazaro M. Digoxin, HIF-1, and cancer. Proc Natl Acad Sci U S A. 2009; 106(9):E26. author reply E27. [PubMed: 19240208]

116. Zhang H, Qian DZ, Tan YS, Lee K, Gao P, Ren YR, Rey S, Hammers H, Chang D, Pili R, et al. Digoxin and other cardiac glycosides inhibit HIF-1alpha synthesis and block tumor growth. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105(50):19579–19586. [PubMed: 19020076]

117. Wei D, Peng JJ, Gao H, Li H, Li D, Tan Y, Zhang T. Digoxin downregulates NDRG1 and VEGF through the inhibition of HIF-1alpha under hypoxic conditions in human lung adenocarcinoma A549 cells. International journal of molecular sciences. 2013; 14(4):7273–7285. [PubMed: 23549264]

118. Gayed BA, O'Malley KJ, Pilch J, Wang Z. Digoxin inhibits blood vessel density and HIF-1a expression in castration-resistant C4-2 xenograft prostate tumors. Clinical and translational science. 2012; 5(1):39–42. [PubMed: 22376255]

119•. Ranasinghe WK, Sengupta S, Williams S, Chang M, Shulkes A, Bolton DM, Baldwin G, Patel O. The effects of nonspecific HIF1alpha inhibitors on development of castrate resistance and metastases in prostate cancer. Cancer medicine. 2014; 3(2):245–251. This retrospective study showed that patients with prostate cancer on androgen deprivation therapy who were concominantly taking non-specific HIF-1 inhibitors had a reduced risk of developing castrate-resistant prostate cancer. [PubMed: 24464861]

120. Lin J, Zhan T, Duffy D, Hoffman-Censits J, Kilpatrick D, Trabulsi EJ, Lallas CD, Chervoneva I, Limentani K, Kennedy B, et al. A pilot phase II Study of digoxin in patients with recurrent prostate cancer as evident by a rising PSA. American journal of cancer therapy and pharmacology. 2014; 2(1):21–32. [PubMed: 25580468]

121. Xia Y, Choi HK, Lee K. Recent advances in hypoxia-inducible factor (HIF)-1 inhibitors. European journal of medicinal chemistry. 2012; 49:24–40. [PubMed: 22305612]

122. Masoud GN, Li W. HIF-1alpha pathway: role, regulation and intervention for cancer therapy. Acta pharmaceutica Sinica B. 2015; 5(5):378–389. [PubMed: 26579469]

123. Zimna A, Kurpisz M. Hypoxia-Inducible Factor-1 in Physiological and Pathophysiological Angiogenesis: Applications and Therapies. BioMed research international. 2015; 2015:549412. [PubMed: 26146622]

124. Levett DZ, Radford EJ, Menassa DA, Graber EF, Morash AJ, Hoppeler H, Clarke K, Martin DS, Ferguson-Smith AC, Montgomery HE, et al. Acclimatization of skeletal muscle mitochondria to high-altitude hypoxia during an ascent of Everest. FASEB journal: official publication of the Federation of American Societies for Experimental Biology. 2012; 26(4):1431–1441. [PubMed: 22186874]

125. van Patot MC, Gassmann M. Hypoxia: adapting to high altitude by mutating EPAS-1, the gene encoding HIF-2alpha. High altitude medicine & biology. 2011; 12(2):157–167. [PubMed: 21718164]

126. Bigham AW, Lee FS. Human high-altitude adaptation: forward genetics meets the HIF pathway. Genes & development. 2014; 28(20):2189–2204. [PubMed: 25319824]

127. Youk AO, Buchanich JM, Fryzek J, Cunningham M, Marsh GM. An ecological study of cancer mortality rates in high altitude counties of the United States. High altitude medicine & biology. 2012; 13(2):98–104. [PubMed: 22724612]

128. Hart J. Cancer mortality for a single race in low versus high elevation counties in the u.s. Dose-response: a publication of International Hormesis Society. 2011; 9(3):348–355. [PubMed: 22013397]

129. Amsel J, Waterbor JW, Oler J, Rosenwaike I, Marshall K. Relationship of site-specific cancer mortality rates to altitude. Carcinogenesis. 1982; 3(5):461–465. [PubMed: 7094209]

130. Read LC, Ballard FJ, Francis GL, Baxter RC, Bagley CJ, Wallace JC. Comparative binding of bovine, human and rat insulin-like growth factors to membrane receptors and to antibodies against human insulin-like growth factor-1. The Biochemical journal. 1986; 233(1):215–221. [PubMed: 3513757]

131. Stroka DM, Burkhardt T, Desbaillets I, Wenger RH, Neil DA, Bauer C, Gassmann M, Candinas D. HIF-1 is expressed in normoxic tissue and displays an organ-specific regulation under systemic hypoxia. FASEB journal: official publication of the Federation of American Societies for Experimental Biology. 2001; 15(13):2445–2453. [PubMed: 11689469]

132•. Martínez-García MA, Campos-Rodriguez F, Durán-Cantolla J, de la Peña M, Masdeu MJ, González M, del Campo F, Serra PC, Valero-Sánchez I, Ferrer MJS, et al. Obstructive sleep apnea is associated with cancer mortality in younger patients. Sleep Medicine. 2014; 15(7):742–748. This prospective cohort study showed that OSA is associated with increased incidence of cancer. [PubMed: 24907033]

133. Gaoatswe G, Kent BD, Corrigan MA, Nolan G, Hogan AE, McNicholas WT, O'Shea D. Invariant Natural Killer T Cell Deficiency and Functional Impairment in Sleep Apnea: Links to Cancer Comorbidity. Sleep. 2015; 38(10):1629–1634. [PubMed: 26414901]

134•. Kendzerska T, Leung RS, Hawker G, Tomlinson G, Gershon AS. Obstructive sleep apnea and the prevalence and incidence of cancer. CMAJ: Canadian Medical Association Journal. 2014; 186(13):985–992. [PubMed: 25096668]

135. Shelton LS, Pensiero MN, Jenkins FJ. Identification and characterization of the herpes simplex virus type 1 protein encoded by the UL37 open reading frame. Journal of virology. 1990; 64(12): 6101–6109. [PubMed: 2173782]

136. Drager LF, Yao Q, Hernandez KL, Shin MK, Bevans-Fonti S, Gay J, Sussan TE, Jun JC, Myers AC, Olivecrona G, et al. Chronic intermittent hypoxia induces atherosclerosis via activation of adipose angiopoietin-like 4. American journal of respiratory and critical care medicine. 2013; 188(2):240–248. [PubMed: 23328524]

137. Almendros I, Montserrat JM, Ramirez J, Torres M, Duran-Cantolla J, Navajas D, Farre R. Intermittent hypoxia enhances cancer progression in a mouse model of sleep apnoea. The European respiratory journal. 2012; 39(1):215–217. [PubMed: 22210813]

138. Szablewski L. Expression of glucose transporters in cancers. Biochimica et biophysica acta. 2013; 1835(2):164–169. [PubMed: 23266512]

139. Almendros I, Wang Y, Becker L, Lennon FE, Zheng J, Coats BR, Schoenfelt KS, Carreras A, Hakim F, Zhang SX, et al. Intermittent Hypoxia-induced Changes in Tumor-associated Macrophages and Tumor Malignancy in a Mouse Model of Sleep Apnea. American Journal of Respiratory and Critical Care Medicine. 2014; 189(5):593–601. [PubMed: 24471484]

140. Zheng J, Almendros I, Wang Y, Zhang SX, Carreras A, Qiao Z, Gozal D. Reduced NADPH oxidase type 2 activity mediates sleep fragmentation-induced effects on TC1 tumors in mice. Oncoimmunology. 2015; 4(2):e976057. [PubMed: 25949873]