HYPOXIA — A KEY REGULATORY FACTOR IN TUMOUR GROWTH

Adrian L. Harris

Cells undergo a variety of biological responses when placed in hypoxic conditions, including activation of signalling pathways that regulate proliferation, angiogenesis and death. Cancer cells have adapted these pathways, allowing tumours to survive and even grow under hypoxic conditions, and tumour hypoxia is associated with poor prognosis and resistance to radiation therapy. Many elements of the hypoxia-response pathway are therefore good candidates for therapeutic targeting.

ERYTHROPOIETIN
A renal hormone that is induced by anaemia and that activates haemoglobin synthesis by bone-marrow red-cell precursors.

Molecular Oncology Laboratories, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DS, UK.
e-mail: L.Richards@icrf.icnet.uk
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Hypoxia — a reduction in the normal level of tissue oxygen tension — occurs during acute and chronic vascular disease, pulmonary disease and cancer. It produces cell death if severe or prolonged. Tumours become hypoxic because new blood vessels they develop are aberrant and have poor blood flow. Although hypoxia is toxic to both cancer cells and normal cells, cancer cells undergo genetic and adaptive changes that allow them to survive and even proliferate in a hypoxic environment. These processes contribute to the malignant phenotype and to aggressive tumour behaviour.

It was first reported nearly 50 years ago that human tumours grew as cords around blood vessels[1]. Tumour cells that were located more than 180 μm away from blood vessels were observed to necrose; this is similar to the calculated distance that oxygen diffuses as it passes from the capillary to cells before it is completely metabolized. Therefore, it seemed that uncontrolled proliferation caused tumours to outgrow their blood (and therefore their oxygen) supply. This limit in oxygen diffusion was termed 'chronic hypoxia'.

Another type of hypoxia — known as 'acute' or 'perfusion-limited' hypoxia — occurs when aberrant blood vessels are shut down, which can also cause blood flow to be reversed[2]. Closed vessels can be reopened, leading to reperfusion of hypoxic tissue with oxygenated blood. This leads to an increase in free-radical concentrations, tissue damage and activation of stress-response genes — a process known as 'reoxygenation injury'[3]. Hypoxia was initially studied because of its effects on responses to radiotherapy — radiation treatment requires free radicals from oxygen to destroy target cells, and cells in hypoxic areas were found to be resistant to radiation-induced cell death. Tumour cells within the hypoxic areas were observed to survive and continue proliferating, in contrast to those in perfusion-limited areas[4].

Direct evidence of hypoxia in human cancers has been shown most convincingly by the pioneering work of Peter Vaupel and colleagues, who studied the tumour oxygen supply using oxygen electrodes[5,6]. They showed that low oxygen tension in tumours was associated with increased metastasis and poor survival in patients suffering from squamous tumours of the head and neck, cervical or breast cancers. More efficient, less invasive methods to measure hypoxia *in vivo* are currently being developed (ONLINE TABLE 1).

Cells undergo a variety of biological responses in response to hypoxic conditions. The earliest recognized pathway was that hypoxic cells undergo a shift from aerobic to anaerobic metabolism[7]. Hypoxia also induces ERYTHROPOIETIN (EPO) production in renal cells (to increase haemoglobin production) and tyrosine hydroxylase synthesis in neural cells (involved in catecholamine production). One of the most well-studied hypoxia responses is production of growth factors that induce angiogenesis (new blood vessel formation). But how do cells sense hypoxia and what signalling pathways mediate these cellular responses?

CEREBELLAR HAEMANGIOGBLASTOMAS
Non-malignant proliferations of vascular stromal cells in the central nervous system.

Box 1 | Genes induced by hypoxia

Oxygen transport and iron metabolism

• Ceruloplasmin | erythropoietin | ferritin light chain | heme oxygenase-1 | transferrin | transferrin receptor

Angiogenesis

• Adrenomedullin | angiopoietin-2 | cyclooxygenase-2 | endothelin-1 and -2 | fibroblast growth factor-3 | hepatocyte growth factor | histone deacetylase | monocyte chemotactic protein-1 | nitric oxide synthase | osteopontin | placental growth factor | Tie-2 (an angiopoietin receptor) | transforming growth factor (TGF)-α, TGF-β1, TGF-β3 | vascular endothelial growth factor (VEGF)-A | VEGF receptor-1

Glycolysis and glucose uptake

• Aldolase-A | enolase-1 |glucose transporter-1, -3 (GLUT1, GLUT3) | glyceraldehyde-3-phosphate dehydrogenase | hexokinase-1; hexokinase-2 | lactate dehydrogenase-A | phosfructokinase-C | phosfructokinase-L | phosphoglycerate kinase-1 | pyruvate kinase-M

Transcription factors

• Annexin V | BCL-interacting killer (BIK) | cyclin G2 | differentiated embryo-chondrocyte expressed gene 1 (DEC1) | FOS | heat-shock factor | hypoxia-inducible factor (HIF)-1α; HIF-2α | insulin-like growth factor (IGF) binding protein-1, -2, -3 | JUN | KIP1 | lipocortin | nuclear factor-κB (NF-κB) | NIP3 | NIX | transgelin | transglutaminase-2 | WAF1

Metabolism/pH/neurotransmitters

• Acetoacetyl CoA thiolase | adenylate kinase-3 | aminopeptidase-A | carbonic anhydrase-9, -12 | phosphoribosyl pyrophosphate synthetase | spermidine N1-acetyltransferase | tyrosine hydroxylase | α-adrenergic receptor

Growth factors/cytokines

• IGF-2 | interleukin-6 | interleukin-8 | intestinal trefoil factor | macrophage inhibitory factor | platelet-derived growth factor-B | staniocalcin

Stress-response pathways

• 150-kDa ORP (oxygen-regulated protein) | glucose-related protein | growth arrest- and DNA damage-induced gene (GADD153) | human apurinic apyrimidinic site endonuclease (HAP-1) | thioredoxin

Cell adhesion, extracellular matrix, cytoskeleton and proteases/coagulation

• CD99 | collagen-5α1 | Ku70 | Ku80 | low-density lipoprotein receptor-related protein | metalloproteinases | matrix metalloproteinase-13 | neuronal cell-adhesion molecule L1 (L1CAM) | plasminogen activator inhibitor-1 | proline-4 hydroxylase | tissue factor (TF) | urokinase receptor | vimentin | α-integrin

Summary

- Hypoxia is a reduction in the normal level of tissue oxygen tension, and occurs during acute and chronic vascular disease, pulmonary disease and cancer. It induces a transcription programme that promotes an aggressive tumour phenotype.
- Hypoxia is associated with resistance to radiation therapy and chemotherapy, but is also associated with poor outcome regardless of treatment modality, indicating that it might be an important therapeutic target.
- Hypoxia-inducible factor-1α (HIF-1α) is a key transcription factor that is induced by hypoxia and regulated by a proline hydroxylase.
- Pathways that are regulated by hypoxia include angiogenesis, glycolysis, growth-factor signalling, immortalization, genetic instability, tissue invasion and metastasis, apoptosis and pH regulation.
- Most of the hypoxia-induced pathways promote tumour growth, but apoptosis is also induced by hypoxia. The balance of these pathways might be critical for the effects of hypoxia on tumour growth.
- Drugs that inhibit HIF-1α expression antagonize HIF-1α interaction with CBP/p300 or block downstream function of genes such as vascular endothelial growth factor and cyclooxygenase-2 have potentially important roles in tumour therapy. Hypoxia can also be used to activate therapeutic gene delivery to specific areas of tissue.

The HIF-1 pathway

One way that cells respond to reduced oxygen levels is through hypoxia-inducible transcription factor 1 (HIF-1). HIF-1 is a heterodimer that consists of the hypoxic response factor HIF-1α and the constitutively expressed aryl hydrocarbon receptor nuclear translocator (ARNT) (also known as HIF-1β). In the absence of oxygen, HIF-1 binds to hypoxia-response elements (HREs), thereby activating the expression of numerous hypoxia-response genes, such as the pro-angiogenic growth factor vascular endothelial growth factor (VEGF)[8] (BOX 1; FIG. 1). The redox active apurinic/apyrimidinic endonuclease-1 has been shown to keep HIF-1α in a reduced state[9] that is necessary for its transcriptional function[10].

In the presence of oxygen, HIF-1α is bound to the tumour suppressor Von Hippel–Lindau (VHL) protein. This interaction causes HIF-1α to become ubiquitylated and targeted to the proteasome, where it is degraded[11–14]. Mutations in VHL that are associated with renal cancer and CEREBELLAR HAEMANGIOGBLASTOMAS prevent this ubiquit-ylation, resulting in an accumulation of HIF-1α and continuous activation of hypoxia-response genes[15,16].

Recently, two groups have reported that a prolyl hydroxylase (a tetramer containing two hydroxylase units and two protein disulphide isomerase subunits) is part of the mechanism by which cells sense hypoxia and regulate HIF-1α expression[17,18]. The enzyme, which requires oxygen, ferrous iron and 2-oxoglutarate for

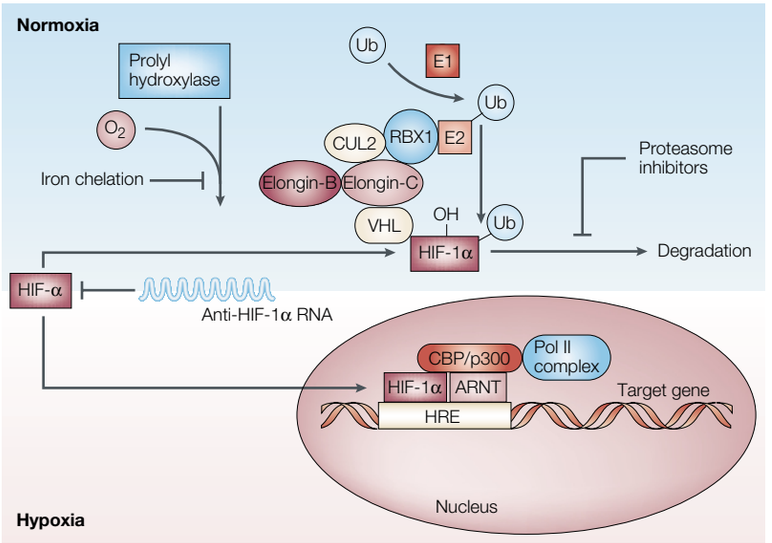

Figure 1 | **HIF-1 pathway.** In the presence of oxygen (O_2), prolyl hydroxylase post-translationally modifies hypoxia-inducible transcription factor (HIF)-1α, allowing it to interact with the von Hippel–Lindau (VHL) complex. Prolyl hydroxylase contains an iron moiety, so iron chelation inhibits this activity. VHL is part of a larger complex that includes elongin-B, elongin-C, CUL2 (REF. 16), RBX1 and a ubiquitin-conjugating enzyme (E2). This complex, together with a ubiquitin-activating enzyme (E1), mediates the ubiquitylation (Ub) of HIF-1α[95,96]. The Ub modification targets HIF-1α for degradation, which can be blocked by proteasome inhibitors. In the absence of oxygen, prolyl hydroxylase cannot modify HIF-1α, and the protein remains stable. Stabilized HIF-1α is translocated to the nucleus, where it interacts with cofactors such as aryl hydrocarbon receptor nuclear translocator (ARNT), CBP/p300 and the DNA polymerase II (Pol II) complex to bind to hypoxia-responsive element (HREs) and activate transcription of target genes[97]. ARNT2 (REF. 98) and MOP3 (REF. 99) are other proteins that have been shown to heterodimerize with HIF-1α (not shown). A natural HIF-1α antisense mRNA has been found in renal cancer[100].

activity, covalently modifies HIF-1α[19], converting it to a hydroxylated form. This form of HIF-1α can then interact with the VHL protein. Under hypoxic conditions, however, this post-translational modification no longer occurs. HIF-1α remains stable and can upregulate expression of its target genes.

It is interesting that 2-oxoglutarate is involved in HIF-1α regulation, because there is evidence that mitochondria, which produce this molecule, might also be involved in oxygen sensing, possibly by releasing free radicals that modify HIF-1α[20]. The role of the mitochondria in the hypoxia response, however, is controversial and has not been confirmed[21,22]. The regulation of HIF-1α by prolyl hydroxylase also explains previous observations that desferrioxamine, an iron chelator, can activate HIF-1α. Desferrioxamine inhibits 2-oxoglutarate's prolyl hydroxylase activity, leading to stabilization of HIF-1α. There are now three known prolyl hydroxylases that modify HIF, and further research is required to determine the expression pattern, mechanisms of regulation and role of this enzyme in cancer pathogenesis[23,24].

HIF-1 in embryonic and tumour development

HIF-1 is required for normal embryogenesis, because mice lacking Hif-1α or its homologue Hif-2α (EPAS) both die *in utero*. Hif-1α knockout embryos die at an early stage and undergo abnormal vascular development[25]. Hif-2α knockout embryos die because of adrenal insufficiency, although they can survive with adrenal catecholamine replacement therapy[26]. Another study reported that Hif-2α-null embryos show vascular disorganization throughout the yolk sac and embryo proper[27]. The differences between these two models is not yet clearly understood.

The HIF-1 complex is also involved in tumorigenesis. Mouse hepatoma cell lines that express mutated forms of ARNT form much smaller tumours that express only low levels of VEGF and do not become highly vascularized[28]. ARNT, however, also interacts with other transcription factors, such as the aryl hydrocarbon receptor, so these results are not definitively due to loss of HIF-1 function.

Several studies have associated HIF-1α expression with human cancer progression. Histological analyses have shown that an increased level of intracellular HIF-1α is associated with poor prognosis and resistance to therapy in head and neck cancer, ovarian cancer and oesophageal cancer[29,30]. HIF-1α levels increased in the cytoplasm and the nucleus of cells stained in various solid tumours[29]. In a separate study, HIF-1α was overexpressed in colon, breast, gastric, lung, skin, ovarian, pancreatic, prostate and renal carcinomas, and associated with cell proliferation[30]. HIF-1α mRNA is also upregulated early during wound healing and experimental skin carcinogenesis[31].

HIF-2α expression is also increased in some cancer cell types, such as renal cancer cells and cerebellar haemangioblastomas[32]. Although there are fewer studies on the role of HIF-2α in cancer development, some indicate that this protein is mainly expressed in the stromal macrophages rather than in the epithelial cancer cells[29]. Stromal cells might mediate a different response to hypoxia compared with normal epithelial or cancer cells.

Hypoxia-regulated pathways

Hypoxia-inducible genes regulate several biological processes, including cell proliferation, angiogenesis, metabolism, apoptosis, immortalization and migration (BOX 1). Cancer cells have a variety of mechanisms to take advantage of some of these responses (for example, angiogenesis induction), and to evade others (for example, apoptosis). Many of the known oncogenic signalling pathways[33] overlap with hypoxia-induced pathways (TABLE 1; FIG. 2). Expression profiling studies have highlighted many of the genes that are regulated by hypoxia and by HIF-1α[34–37]. They include angiogenic factors, proliferation and cell-adhesion genes.

Proliferation. Hypoxia induces expression of various growth factors that are known to promote cell proliferation. This proliferation is normally involved in initiating cell migration and regeneration after acute or chronic hypoxia damage. HIF-1α induces production of growth factors such as transforming growth factor-β and platelet-derived growth factor[34–37] (BOX 1). The p42/p44 mitogen-activated protein kinases,

Table 1 | **Factors that regulate HIF-1α expression or function**

Pathway	Action on HIF-1α	References
Ligands of tyrosine kinase receptors (EGF, IGF1 and IGF2, insulin, PDGF)	Increase expression	104
Ligands of other receptors (thrombin, angiotensin)	Increase protein	105
Amplified receptor ERBB2	Increases translation	106
Kaposi's sarcoma virus G-protein-coupled receptor	Increases transcriptional activity	107
p42/p44 MAPK	Phosphorylates HIF-1α	38
RAS expression	Increases protein	49
Small G-protein RAC1	Increases protein	108
PTEN inhibition	Increases protein	41
v-SRC	Increases HIF-1α mRNA	109
Diacyl glycerol kinase	Increases protein	110
Hepatitis B virus protein X	Increases VEGF transcription	111

EGF, epidermal growth factor; HIF, hypoxia-inducible transcription factor; IGF, insulin-like growth factor; MAPK, mitogen-activated protein kinase; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor.

which regulate cell proliferation in response to extracellular growth factors, have been shown to phosphorylate HIF-1α and activate transcription of HIF-1 target genes[38]. This pathway has also been shown to activate HIF-2α[39].

Phosphatidylinositol 3-OH kinase (PI3K) activity is also increased in some cell types under hypoxic conditions[40]. PI3K is one of the key downstream mediators of many tyrosine kinase signalling pathways and is involved in regulating cell proliferation and suppression of apoptosis. The PI3K pathway is inhibited by the phosphoinositide phosphatase PTEN, and mutations in PTEN enhance HIF-1-activated responses[41]. PTEN regulates cell growth and proliferation, and is deleted or mutated in several human cancers, including glioblastoma, endometrial tumours and prostate cancer. So, PTEN mutations might promote tumour growth by synergistically promoting HIF-mediated responses.

HIF-1 also seems to interact with the oncogenic RAS pathway, because loss of HIF-1α negatively affects tumour growth in HRAS-transformed cell lines[42]. However, this negative effect is not due to deficient vascularization. Despite differences in VEGF expression, vascular density is similar in wild-type and HIF-1α-null tumours. This indicates that other pathways downstream of HIF-1 are involved in tumorigenesis — possibly those relating to the anabolic effects of glycolysis[43]. Experiments involving HIF-1α-null embryonic stem-cell-derived tumours have demonstrated opposite results, showing reduced vascularization and increased tumour growth. Another HIF-1-mediated pathway, such as hypoxia-induced apoptosis, might be reduced in these cells[44]. Others have observed that HIF-1α deletion inhibits both cell growth and angiogenesis[45]. The HIF-1-mediated hypoxia response is therefore complex, and different pathways are likely to be activated in different cell types.

The most well-studied HIF-1α-activated growth factors regulate endothelial-cell proliferation and blood-vessel formation. HIF-1 activates transcription of VEGF and one of its receptors, VEGF receptor 1 (VEGFR1/FLT-1). VEGF is a key angiogenic factor that is secreted by cancer cells and normal cells in response to hypoxia. Its receptors — VEGFR1 and VEGFR2 — are primarily expressed on endothelial cells. Hypoxia-induced angiogenesis is blocked by inhibitors of oncogene signalling pathways, such as agents that inhibit RAS, epidermal growth factor receptor, and the receptor tyrosine kinase ERBB2 (HER2/neu). This indicates that there is crosstalk between oncogenic and hypoxia-response pathways (FIG. 2). HIF-1 activation can also lead to reduced expression of anti-angiogenic proteins such as THROMBOSPONDIN-1 and -2 (REFS 46,47).

THROMBOSPONDINS
A multigene family of extracellular proteins that inhibit angiogenesis through several mechanisms, including upregulation of TGF-β and decreasing the cellular response to VEGF.

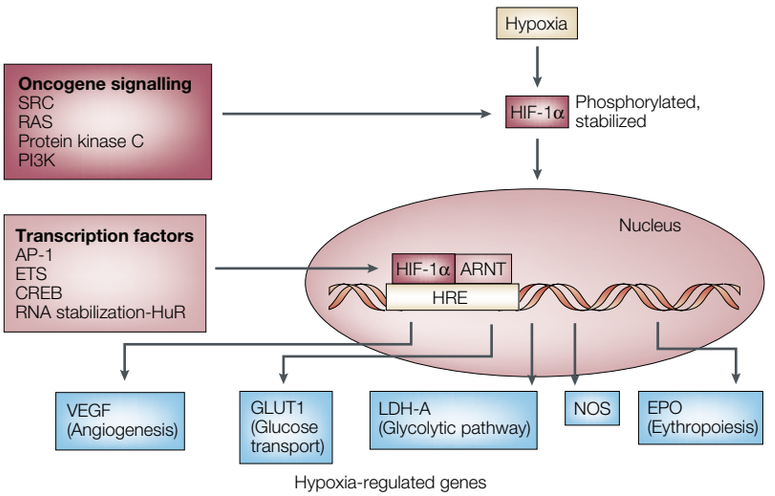

Figure 2 | **Other factors involved in HIF-1 activation of hypoxia-response genes.** Under hypoxic conditions, hypoxia-inducible transcription factor (HIF)-1α is phosphorylated and stabilized through oncogenic signalling pathways involving SRC, RAS, protein kinase C and phosphatidylinositol 3-OH kinase (PI3K). In the nucleus, HIF-1α can also interact with transcription factors such as AP-1, ETS and the cyclic AMP-response-element-binding protein (CREB) to activate transcription. RNA-binding proteins, such as HuR, help to stabilize mRNA[101,102]. HIF-1α-activated genes include vascular endothelial growth factor (VEGF), which promotes angiogenesis; glucose transporter 1 (GLUT1), which activates glucose transport; lactate dehydrogenase (LDH-A), which is involved in the glycolytic pathway; and erythropoietin (EPO), which induces erythropoiesis. HIF-1α also activates transcription of nitric oxide synthase (NOS)[103], which promotes angiogenesis and vasodilation.

Glycolysis. Under hypoxic conditions, cells switch their methods of glucose metabolism from the oxygen-dependent tricarboxylic acid (TCA) cycle to glycolysis, the oxygen-independent metabolic pathway. Hypoxic cancer cells use glycolysis as a primary mechanism of ATP production, and cellular transformation has been associated with induction of glycolysis[7,48]. Glycolysis provides only two ATP molecules for each glucose molecule, in contrast to the TCA cycle, which provides 38 ATP molecules. HIF-1 has been shown to regulate expression of all the enzymes in the glycolytic pathway, as well as expression of the glucose transporters GLUT1 and GLUT3 (REF. 49), which mediate cellular glucose uptake. Recent studies indicate that increased glycolysis is a normal response to proliferation, and that migrating cells also use this pathway as an energy source[43]. The intermediary metabolites of

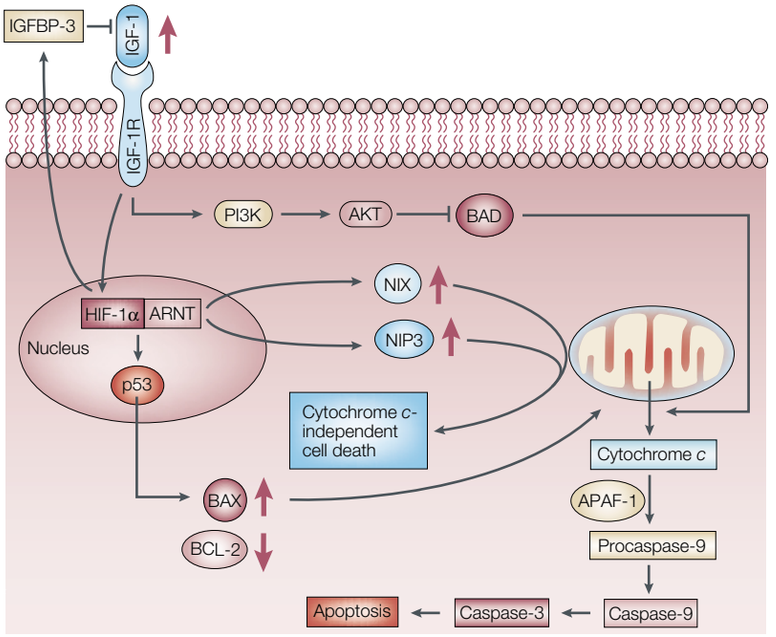

Figure 3 | **Hypoxia regulation of cell-death pathways.** HIF-1 (a complex of HIF-1α and ARNT) activates transcription of many pro-apoptotic genes, some of which are shown here. HIF-1 also interacts with the tumour suppressor p53 to promote p53-dependent apoptosis[57]. HIF-1 activates the transcriptional activity of p53, which leads to transcription of many pro-apoptotic proteins, such as BAX. BAX functions at the mitochondrial membrane to promote release of cytochrome *c*. Cytosolic cytochrome *c* interacts with the apoptotic protease-activating factor-1 (APAF-1), acitvating procaspase-9 conversion to caspase-9. Caspase-9 then activates caspase-3, leading to apoptosis. The pro-apoptotic protein BAD functions at the mitochondrial membrane in a similar manner to BAX, by promoting cytochrome *c* release. BAD can be inhibited by the kinase AKT, which is activated by phosphotidylinositol 3-OH kinase (PI3K), which is induced by insulin-like growth factor-1 (IGF-1) signalling through the IGF-1 receptor (IGF-1R). IGF-1 signalling is therefore anti-apoptotic. HIF-1 activates transcription of the pro-apoptotic protein IGF-binding protein-3 (IGFBP-3), which blocks IGF-1 signalling. HIF-1 also activates expression of NIP3 and NIX[54–56], which induce a mitochondrial-pore permeability transition and cell death through a mechanism that does not involve cytochrome *c* release or caspases. Hypoxia has also been reported to downregulate expression of the anti-apoptotic protein BCL-2 in some cell types[57].

the glycolytic pathway provide the precursors for synthesis of glycine, serine, purines, pyrimidines and phospholipids, all of which are essential for cell growth and maintenance of cells under stress. Tumour cell lines that are deficient in the HIF-1 signalling pathway have lower ATP and glycine concentrations *in vivo* (A.L.H., J. Griffiths and M. Stubbs, unpublished observations).

Immortalization and genetic instability. Hypoxia also affects cellular DNA and chromosomes in ways that could promote transformation. TELOMERASE activity increases when cancer and endothelial cells are placed under hypoxic conditions[50], promoting cellular immortalization. Hypoxia has been shown to induce gene amplification and DNA breaks at FRAGILE SITES[51], and to disrupt repair of DNA damage. Using an assay for repair that is based on host-cell reactivation of ultraviolet-damaged plasmid DNA, cells exposed to hypoxia and low pH have a diminished capacity for DNA repair compared with control cells grown under standard culture conditions[52]. Histone deacetylases (HDACs) — implicated in alteration of chromatin assembly and tumorigenesis — are also activated by hypoxia. A specific HDAC inhibitor, trichostatin A (TSA), reduces hypoxia-induced angiogenesis in the Lewis lung carcinoma model[53].

Apoptosis. Hypoxia induces apoptosis by a number of HIF-1-mediated and -independent pathways (FIG. 3). Hypoxic conditions reduce proliferation and increase apoptosis in wild-type embryonic stem cells, but not in HIF-1α-null cells[44]. HIF-1α activates expression of two pro-apoptotic proteins — NIX and NIP3 — in a wide range of cell lines[54–56]. The mechanism by which NIP3 causes cell death seems to be a combination of both necrosis and apoptosis, termed 'aponecrosis'. NIP3 is a member of the BBL-2 family that localizes to mitochondria, although this is not essential for cell death[55]. NIP3-mediated cell death is independent of APAF-1, caspase activation, cytochrome *c* release, and nuclear translocation of apoptosis-inducing factor. Cells with active NIP3 have phenotypes that are typical of necrosis — early plasma-membrane permeability, mitochondrial damage, extensive cytoplasmic vacuolation and mitochondrial autophagy. NIP3 has been proposed to mediate necrosis-like cell death by opening mitochondrial pores and inducing mitochondrial dysfunction.

HIF-1α has also been shown to promote p53-dependent apoptosis[57] (FIG. 3). When tumour cells with and without *Trp53* (the gene that encodes p53 in mice) mutations are combined and grown *in vivo* in mice, *Trp53*-mutant cells survive better in hypoxic areas than do wild-type cells, indicating that p53 is involved in hypoxia-induced cell death[58]. Under hypoxic conditions, p53 is stabilized to a form that blocks transcription but then requires further modification by phosphorylation to cause apoptosis[59]. HIF-1α has been reported to promote p53-dependent apoptosis, which is mediated by APAF-1 and caspase-9 (REF. 60). Early studies showed that p53 directly interacts with HIF-1α and blocks HIF-1α's ability to activate transcription. Certain mutations in p53 were reported to remove this block[61]. The ability of HIF-1α to interact with p53 depends on the phosphorylation status of HIF-1α. During hypoxia-induced apoptosis, only the phosphorylated HIF-1α binds ARNT[57]. By contrast, the dephosphorylated form of HIF-1α binds p53 and induces apoptosis.

These results indicate that the functions of HIF-1α vary with its phosphorylation status and that dephosphorylated HIF-1α might mediate apoptosis by binding and stabilizing p53, or p53 might prevent HIF-1α from activating transcription of anti-apoptotic genes. p53 has also been reported to target HIF-1α to a degradation pathway that involves MDM2 (REF. 62). However, not all these experiments have been reproduced, and HIF-1α functions in both p53-positive and -null cells.

Tumour cells have developed many mechanisms to evade HIF-1-mediated cell death under hypoxic conditions. For example, induction of the anti-apoptotic gene *IAP2* occurs by a HIF-1-independent hypoxia-driven pathway in cultured cancer cells[63]. But when do cancer cells undergo changes that allow them to evade

TELOMERASE
A ribonucleoprotein that maintains telomere length. Telomerase activity is repressed in most normal adult human somatic tissues, limiting replicative capacity. Reactivation of telomerase is believed to be a necessary event for the sustained growth of most human tumours.

FRAGILE SITE
A site in a chromosome that is susceptible to chromosome breakage and fusion with other chromosomes.

the apoptotic programme? Hypoxia occurs in the early stages of tumour development (before metastasis), and is therefore commonly observed in non-invasive tumours such as intraductal breast cancer. The activation of pro-apoptotic genes is therefore also likely to occur during these early stages of tumour development. So there might be early selective pressure on cancer cells to escape hypoxia-induced apoptosis. A similar selection process might occur in later stages of tumour development, promoting the aggressive phenotype that is associated with hypoxia.

pH regulation. The metabolic activities of cancer cells affect the overall pH of tumours. Tumours have been shown to adapt to pH changes and grow at lower pHs than are found in normal tissues, giving the tumour a growth advantage. Many proteases are activated under acidic conditions, promoting tumour invasion of surrounding tissue.

Glycolysis is thought to be the main mechanism by which tumours lower their pH, through generation of lactic acid. CARBONIC ANHYDRASES, which reversibly convert carbon dioxide and water to carbonic acid, might also be involved. The activities of two isoforms — carbonic anhydrase-9 and -12 — were reported to be downregulated by *VHL*[64] and strongly induced by hypoxia in a range of tumor cell lines[65], indicating that their transcription might be regulated by HIF-1. Transcription of carbonic anhydrase-9 is activated by hypoxia and suppressed in normoxia[66]. This enzyme is expressed in perinecrotic areas in a wide range of tumour types, and high expression levels have been associated with poor prognosis[67]. Although it is clear that lactate produced by glycolysis generates an acidic micro-environment in tumours, tumour cells that express defective forms of lactate dehydrogenase still maintain a low extracellular pH[68]. This indicates that carbonic anhydrase activity might also contribute to the tumour's low pH.

Growth inhibitory signals. Normal cells undergo cell-cycle arrest under conditions of severe hypoxia, but are capable of recovering if hypoxia is not prolonged. The cyclin-dependent kinases WAF1 (p21) and KIP1 (p27) might be involved in mediating hypoxia-related growth arrest. KIP1 was shown to be induced by hypoxia, leading to G1/S arrest[69]. A similar study reported that WAF1 and KIP1 regulate cell-cycle re-entry after hypoxic stress, but are not necessary for hypoxia-induced arrest[70]. There is evidence that changes in pH are more important than hypoxia *per se* in cell death[117]. Another mechanism to prevent cells from proliferating under hypoxic conditions is induction of differentiation. INVOLUCRIN is a marker of squamous cell differentiation (and therefore inhibition of proliferation). Involucrin expression has been shown to co-localize with hypoxic areas of squamous-cell cancers[71].

Other hypoxia-response factors

Although most research on hypoxia has concentrated on the HIF-1 pathway, there are several other transcription factors that are activated by hypoxia. These include the cyclic AMP-response-element-binding protein (CREB)[72] and nuclear factor-κB (NF-κB)[73]. These factors seem to act independently of HIF-1, but further research is required to find out how they are regulated.

Hypoxia also induces blood-clot formation. Monocytes cultured under hypoxic conditions upregulate expression of the transcription factor early growth response-1 (EGR-1), causing expression of the cell-surface protein tissue factor (TF), leading to vascular fibrin deposition and blood-clot formation[74]. Mononuclear phagocytes and vascular smooth muscle cells also upregulate TF under hypoxic conditions[75]. Accordingly, increases in coagulation, DEEP-VEIN THROMBOSIS and PULMONARY EMBOLISM THROMBOSIS are recognized features of cancer. Blood clots also induce platelets to release angiogenic factors such as VEGF, promoting revascularization of the clot, but also promoting tumour vascularization. The EGR-1 pathway is also activated during reoxygenation and might therefore be involved in acute intermittent hypoxia.

Metal-transcription factor-1 is another hypoxia-induced transcription factor. It activates expression of placental growth factor (PlGF) — another ligand of VEGFR1 — and metallothionein[76]. PlGF has been shown to synergize with VEGF to promote angiogenesis[77]. By upregulating PlGF, endothelial and cancer cells amplify signalling through the VEGFR1.

So, to continue growing under hypoxic conditions, tumours take advantage of a variety of hypoxia-induced growth-promoting signals, and modify growth-inhibitory signalling events. In any population of cancer cells, different aspects of these pathways are affected, and the subset of cells that ends up with the optimum profile can survive and proliferate.

Hypoxia-targeted therapies

Reducing tissue hypoxia. Correction of hypoxia before radiation therapy has been routine for many years, by using blood transfusion to bring the haemoglobin concentration in patients above 12 g/l — a concentration associated with better response to therapy[78]. EPO is given to patients to protect them from chemotherapy-associated anaemia, as well as to patients suffering from anaemia of chronic disorders. Intriguingly, clinical trial results have indicated that EPO might improve the survival of cancer patients[79]. It is possible that EPO, by restoring tumour oxygenation, allows cancer cells to proliferate and therefore become more sensitive to chemotherapy. EPO might also shut down hypoxia-mediated angiogenesis. Other approaches to improve blood flow and oxygen delivery to tumours include the use of ACCELERATED RADIOTHERAPY WITH CARBOGEN AND NICOTINAMIDE[80]. Inhibitors of RAS also reduce oxygen consumption by tumours and so increase local oxygen concentrations[81].

Hypoxia-activated prodrugs. Another way to exploit hypoxia is to induce tumour-specific toxicity using prodrugs that are only activated under hypoxic conditions[82]. For example, the drug tirapazamine inhibits DNA repair

CARBONIC ANHYDRASES
Enzymes that convert carbon dioxide to carbonic acid and then to protons and bicarbonate ions.

INVOLUCRIN
A cytoskeletal protein in squamous cells that is involved in their terminal differentiation.

DEEP-VEIN THROMBOSIS
The process of clot formation in the venous circulation, usually in the lower limbs or pelvis.

PULMONARY EMBOLISM THROMBOSIS
The occlusion of pulmonary veins by clots dislodged from peripheral deep veins, usually from the lower extremities.

ACCELERATED RADIOTHERAPY WITH CARBOGEN AND NICOTINAMIDE
Experimental technique to improve blood flow and oxygen delivery to tumours.

Table 2 | **Oncogenic signalling inhibitors that also block HIF-1**

Drug	Target	References
Herceptin, Iressa, herbimycin	Tyrosine kinases	112
Calphostin C	Protein kinase C	112
Wortmannin, LY294002	PI3K	113
PD98059	MAPK	114
Rapamycin	FRAP/mTOR	115
Diphenylene iodonium	Redox signalling	87
Mannoheptulose	Glucokinase	116

FRAP, FKBP/rapamycin-associated protein; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol 3-OH kinase.

when activated under hypoxic conditions, and acts synergistically with both radiation therapy and chemotherapy. Tirapazamine, used in combination with platinum therapy, improves survival of patients with non-small-cell lung cancer[83]. Other non-cytotoxic compounds that bind to substrates only under low oxygen conditions can be radiolabelled and used in combination with positron emission tomography or conventional γ-camera imaging to identify and observe hypoxic areas within tumours[84].

HIF-1 and HREs. Because activation of HIF-1 has been associated with a variety of tumours and oncogenic pathways, it is a prime target for anticancer therapies. Therapies are under development to block HIF-1α itself or HIF-1α-interacting proteins. Recent data indicate that HIF-1α antisense therapy might act synergistically with immunotherapy[85]. In a mouse model of thymic T-cell lymphoma, stimulation of specific T-cell responses with a co-activating ligand for T cells induced regression of lymphoma but could not cure mice. *In vivo* delivery of antisense to HIF-1α alone by direct intratumour injection inhibited tumour growth, but combination of the two treatments caused marked tumour regression and a sustained antitumour immune response[85]. A gene-therapy strategy to block the interaction between HIF-1α and its transcriptional co-activator CBP/p300 led to attenuation of hypoxia-inducible gene expression and inhibition of tumour growth in a mouse xenograft model[46].

There are also natural antagonists to HIF-1α, such as p34srj (for serine–glycine-rich junction). This protein is induced by hypoxia and also blocks the interaction of HIF-1α with CBP/p300 (REF. 86). Small-molecule inhibitors of HIF-1α function, such as diphenylene iodonium[87] (TABLE 2), have been reported, although its exact mechanism is not clear.

HREs linked to marker genes or prodrug activation systems can be used to selectively activate therapeutics in hypoxic regions[88,89]. For example, gene-therapy vectors that carry pro-apoptotic or anti-proliferation genes driven by HREs can be selectively targeted to cancer cells in hypoxic regions of the tumour. Anaerobic bacteria provide another method of delivering genes specifically to hypoxic cells[89]. Anaerobic bacteria have shown tumour-specific proliferation in animal models and inhibited tumour growth[90], and clinical trials are underway.

Macrophages are also attracted to areas of hypoxia, and might be developed as a useful mechanism for delivering therapeutic genes to hypoxic areas. These cells can be transfected with adenoviral vectors, and might be used to deliver therapeutic HRE-containing genes to hypoxic areas. Genetically engineered macrophages have been shown to migrate to hypoxic areas in xenografts and upregulate HRE-driven transgenes in a hypoxia-dependent manner[91]. Herpes simplex virus thymidine kinase (HSV-TK) gene expression driven by the *VEGF* promoter was shown to be effective in mediating ganciclovir-induced killing of highly metastatic Lewis lung carcinoma cells under hypoxic conditions *in vivo* (REF. 92).

Future directions

The ability to survive under hypoxic conditions is one of the fundamental physiological differences between tumour cells and normal cells, although the qualitative and quantitative differences in the hypoxia response by both cell types are not known. In both tumour and normal cells, hypoxia activates a complex transcriptome that includes pathways downstream of HIF-1α and other signalling pathways. Many questions need to be answered about how cells sense hypoxia and activate these pathways, and how the pathways are integrated. For example, it is important to learn more about the function of oxygen sensors such as proline hydroxylase. It might be a target of oncogenes and also a potential target for anticancer drugs. There are an increasing number of proteins found to interact with VHL besides HIF-1α, and these might also be important in the response to hypoxia. These include fibronectin and heterogeneous nuclear ribonucleoprotein (hnRNP)[93,94]. Also, most pathology and gene-expression studies are done on primary tumours, and little is known about hypoxia in metastatic tumours.

More research is required to determine the ratio of the pro-survival pathways and apoptotic pathways that are activated by cancer cells in response to hypoxia, and how these are regulated. For example, a tumour that overexpresses NIP3 instead of VEGF might undergo slower growth, because cells would undergo high levels of apoptosis and a small amount of angiogenesis. Therapeutics that disrupt HIF function in these cells might only promote tumour growth. It will be important to analyse expression patterns of hypoxia-response genes in human cancer cells by microarray analysis, and relate the gene-expression patterns to levels of apoptosis, angiogenesis and metastasis.

An important issue that is difficult to prove in the clinic is whether hypoxia generates an aggressive tumour phenotype or whether an aggressive tumour phenotype generates hypoxia. In one case, it is possible that tumours that contain activating MYC or RAS mutations have an aggressive phenotype, increasing oxygen consumption and generating hypoxia — these factors would switch on the HIF-1 signalling pathway. Alternatively, hypoxic tumour conditions might activate expression of genes

that promote tumour growth, leading to a more aggressive phenotype. These different possibilities could lead to different therapeutic results. If the HIF pathway is modified, for example, in those tumour types in which the hypoxia was generating the aggressive phenotype, correction of anaemia (reoxygenation) and a blockade of HIF function would reduce tumour growth. Conversely, if an aggressive phenotype is the cause of hypoxia, correcting anaemia might actually enhance tumour growth by providing more oxygen to the tumour cells, and blockade of HIF would have little effect.

The full profile of hypoxia-responsive genes is likely to be known soon, as microarray analysis is underway. It will take much longer, however, to figure out the mechanisms by which hypoxia modulates tumour growth and differentiates the tissue- and cell-specific patterns of response, to understand fully how cancer cells manipulate these pathways to promote their own survival. It is becoming clear that cancer involves the dysregulation of many signalling pathways, and that the greatest therapeutic effects are likely to be achieved by treating patients with several different types of anti-cancer drugs, just as we use combination therapies for infections and hypertension. Those inhibiting the effects of hypoxia are likely to be one component. However, the main hypoxia-response pathways must still be defined. Although — clearly — VEGF is one of them, there are many others to discover.

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Shows that cell death in hypoxia might be mediated by low pH rather than low oxygen.

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Online links

DATABASES

The following terms in this article are linked online to:

CancerNet: http://cancernet.nci.nih.gov/
breast cancer | cervical cancer | colon carcinoma | endometrial tumours | gastric carcinoma | glioblastoma | head and neck tumours | oesophageal cancer | ovarian cancer | pancreatic carcinoma | prostate carcinoma | renal cancer | skin carcinoma

GenBank: http://www.ncbi.nlm.nih.gov/
HSV-TK

LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink/
α-integrin | α-adrenergic receptor | acetoacetyl CoA thiolase | adenylate kinase-3 | adrenomedullin | AKT | angiopoietin-2 | annexin V | APAF-1 | ARNT | ARNT2 | BAD | BAX | carbonic anhydrase-9 | caspase-3 | caspase-9 | CBP | CD99 | ceruloplasmin | collagen-5α1 | CREB | CUL2 | cyclin G2 | cyclooxygenase-2 | cytochrome *c* | DEC1 | EGR-1 | elongin-B | elongin-C | endothelin-1 | endothelin-2 | enolase-1 | epidermal growth factor receptor | ERBB2 | erythtopoietin | ETS | ferritin light chain | fibroblast growth factor-3 | fibronectin | FOS | GADD153 | GLUT1 | GLUT3 | glyceraldehyde-3-phosphate dehydrogenase | HAP-1 | heat-shock factor | heme oxygenase-1 | hepatocyte growth factor | hexokinase-1 | hexokinase-2 | Hif-1α | HIF-1α | Hif-2α | HIF-2α | hnRNP | *IAP2* | IGF-1 | IGF-2 | IGF binding protein-1 | IGF binding protein-2 | IGF binding protein-3 | IGF-1R | interleukin-6 | interleukin-8 | intestinal trefoil factor | JUN | Ku70 | Ku80 | KIP1 | lactate dehydrogenase-A | L1CAM | lipocortin | low-density lipoprotein receptor-related protein | macrophage inhibitory factor | matrix metalloproteinase-13 | MDM2 | metal-regulatory transcription factor-1 | metalloproteinases | metallothionein | monocyte chemotactic protein-1 | MOP3 | NF-κB | NIP3 | nitric oxide synthase | NIX | osteopontin | p300 | p44 mitogen-activated kinase | p53 | phosphoglycerate kinase-1 | phosphoribosyl pyrophosphate synthetase | PI3K | placental growth factor | plasminogen activator inhibitor-1 | platelet-derived growth factor | platelet-derived growth factor-B | proline-4 hydroxylase | PTEN | pyruvate kinase-M | RBX1 | spermidine N1-acetyl transferase | SRC | TGF-α | TGF-β1 | TGF-β3 | thioredoxin | Tie-2 | transferrin | transferrin receptor | transgelin | transglutaminse-2 | tyrosine hydroxylase | urokinase receptor | VEGF | VEGFR1 | VEGFR2 | VHL | vimentin | WAF1

FURTHER INFORMATION

SRI web site on hypoxia in cancer:
http://www.sri.com/pharmdisc/cancer_biology/laderoute.html
Access to this interactive links box is free online.