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REVIEW

Hypoxia and cancer

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Abstract A major feature of solid tumours is hypoxia, decreased availability of oxygen, which increases patient treatment resistance and favours tumour progression. How hypoxic conditions are generated in tumour tissues and how cells respond to hypoxia are essential questions in understanding tumour progression and metastasis. Massive tumour-cell proliferation distances cells from the vasculature, leading to a deficiency in the local environment of blood carrying oxygen and nutrients. Such hypoxic conditions induce a molecular response, in both normal and neoplastic cells, that drives the activation of a key transcription factor; the hypoxia-inducible factor. This transcription factor regulates a large panel of genes that are exploited by tumour cells for survival, resistance to treatment and escape from a nutrient-deprived environment. Although now recognized as a major contributor to cancer progression and to treatment failure, the precise role of hypoxia signalling in cancer and in prognosis still needs to be further defined. It is hoped that a better understanding of the mechanisms implicated will lead to alternative and more efficient therapeutic approaches.

Keywords Angiogenesis · Autophagy · Bcl-2/adenovirus EIB 19 kDa-interacting protein 3 · Cancer · Carbonic anhydrase · Hypoxia · Hypoxia-inducible factor · Oxygen-sensor · Tumour metabolism · pH regulation

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Introduction

Cancer is presently a major cause of mortality in developed countries and will become even more so in low-income countries as the global population increases and ages and as improvements in detection are implemented [1]. Although some cancers occur in the young, most are associated with the elderly, and both events represent the accumulation of genetic and epigenetic cell damage [2]. Cancer includes a diverse collection of diseases, from a cellular origin point of view, rather than a single disease, the causes of which are equally as diverse [3]. Aberrant cell-cycle checkpoint control, overactivation of oncogenes and inhibition of tumour-suppressor genes are considered to be primordial in the initiation of tumourigenesis. However, other factors related to the tumour microenvironment are now being recognized as fundamental in tumour progression, increased resistance and metastasis. Hypoxia is one of these factors, the repercussions of which are shared by all cancer types including haematological cancers [4].

The hypoxic tumour phenotype

Robust tumour growth requires the presence of a local vascular network that supplies both oxygen and nutrients to tumour cells. However, a highly proliferating mass of tumour cells develops faster than the vasculature, and tumour cells rapidly meet up with an avascular environment deficient in oxygen, i.e. hypoxic. This is a consequence of the diffusion limit of oxygen within tissues, which has been measured to be around 150 μm [5, 6] (Fig. 1). On histological examination, tumours often show a central core of necrotic cells, which has been suggested to result from a drop in the oxygen availability to conditions of severe hypoxia and glucose

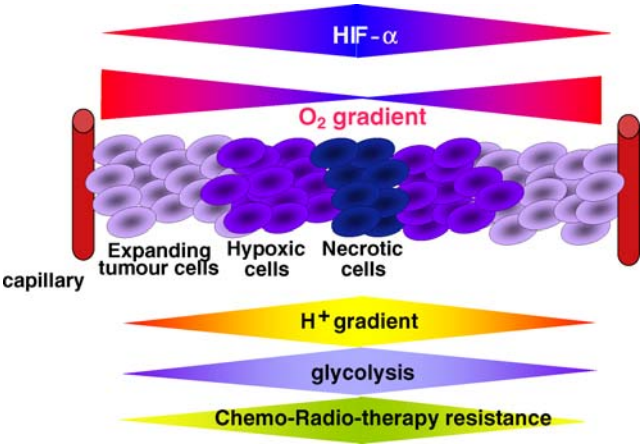

Fig. 1 The characteristics of a hypoxic tumour mass. Blood capillaries carry oxygen to tissues, but since oxygen has a diffusion limit, its concentration decreases as the distance from capillaries increases. Macroscopic examination of solid tumours reveals the presence of expanding tumour cells in proximity to capillaries and a central region of necrotic cells. This gradient of cell viability parallels that of a decreasing gradient of oxygen, which is accompanied by an increase in HIF-1α levels, a decrease in the extracellular pH and an increase in the resistance to radio- and chemo-therapy

deprivation resulting in cell death. A number of methods have been developed to measure the oxygen concentration in tissues including chemical markers such as pimonidazole hydrochloride or EF5, oxygen miocroelectrodes or optical partial pressure of oxygen-measuring devices. Such hypoxic zones have been postulated to have a reduced response to radiotherapy due to a decrease in oxygen-free radicals that are required to produce enough DNA damage to give cell death [7]. In addition, cells of these regions are considered to be chemotherapy-resistant due to limited delivery of drugs via the circulation. Hypoxic tumours also show an extracellular pH (pHe) that is lower than that of corresponding normal tissue [8]. The acidotic nature is the consequence of a modification in the metabolism of tumour cells, in particular that of glucose [9] (Fig. 1).

Hypoxia-inducible factor, the molecular key to hypoxia

Hypoxia activates an alpha/beta heterodimeric transcription factor termed appropriately the hypoxia-inducible factor (HIF). Activation resides in the inhibition of posttranslational hydroxylation of the alpha subunit that permits stabilization, heterodimerisation and binding to hypoxia-response elements (HRE) in target genes. The details of the mechanisms of regulation of the stability and activity of HIF-α have been extensively reviewed by us [10–12] and others [13–16]. Suffice it to say that posttranslational hydroxylation by oxygen-dependent oxygenases, prolyl hydroxylase domain proteins and factor inhibiting HIF (FIH) destabilize and inactivate, respectively, HIF-α. The former, by favouring von Hippel-Lindau (VHL) E3 ubiquitin ligase-mediated proteasomal degradation, and the latter, by inhibiting interaction with co-activators such as p300/CBP.

The HIF-mediated cellular response

Non-hydroxylated, active HIF-α/β targets about 1–2% of the human genome leading to induction or repression of genes with subsequent up- or down-regulation of expression, respectively, of the corresponding gene products. A broad range of genes that are implicated in events such as angiogenesis, cell survival/death, metabolism, pH regulation, adhesion, extracellular matrix remodeling, migration and metastasis are targeted [12, 15, 17, 18]. The functional consequences of enhanced expression of a small selection of some of these gene products are discussed below (Fig. 2).

Angiogenesis

HIF-mediated expression of gene products including the vascular endothelial growth factor-A (VEGF-A) and angiopoïetin-2 (Ang-2) allow tumour cells to turn around the hypoxic situation by inducing regrowth of the vascular network, a phenomenon termed angiogenesis [19]. Thereby an oxygenated and nutritional environment is reestablished for maintenance of growth. However, the neo-vessels formed are often distorted and irregular and thus less efficient in oxygen, nutrient transport and drug delivery.

Cell survival or death

Thus, hypoxia initiates a cascade of events that allows tumour cells to continue to proliferate; however, if too severe, hypoxia can also lead to cell death as shown by the presence in tumours of a central necrotic zone. In fact, it can be envisaged that highly variable levels of hypoxia accompany the dynamics of spatiotemporal development of the tumour mass so that a multitude of tumour cell responses are manifested (Fig. 1). Interplay between FIH and the transcriptional activation domains of HIF-1α, based on the degree of oxygen dependence of FIH for activity, has been proposed to select for different gene profiles that determine cell fate [20]. Gene-profile selectivity may also arise from differential action of the three HIF-α subunits and, within the context, may promote cell proliferation or death [21, 22]. The genes *bnip3*, Bcl-2/adenovirus EIB 19 kDa-interacting protein 3, and *bnip3L* (*bnip3-like*), the products of which are members of the BH3-only protein family of cell death factors, are highly induced in hypoxia.

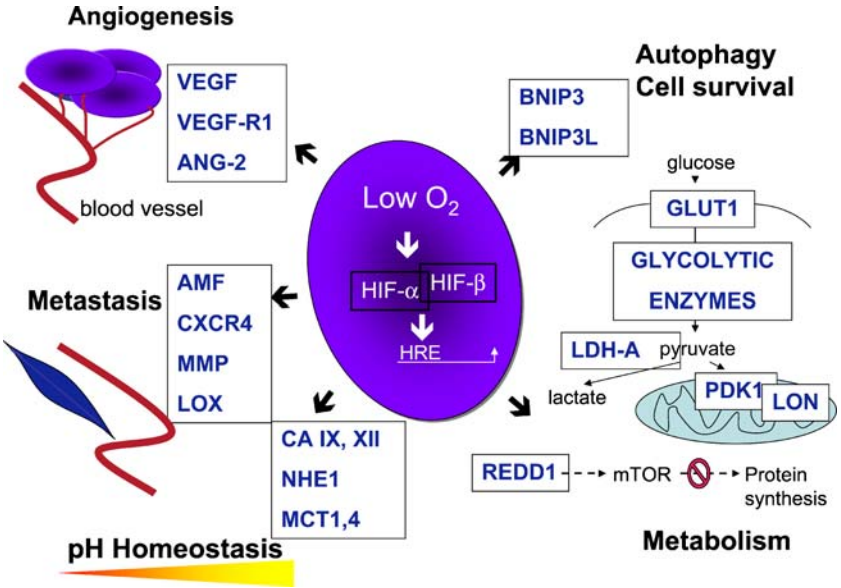

Fig. 2 HIF-induced gene products and their function. The α/β heterodimer HIF bound to hypoxia-response elements (*HRE*) in target genes mediates the expression of a vast array of proteins implicated in functions such as angiogenesis, cell survival/death, metabolism, pH homeostasis and metastasis. A small selection of proteins (*boxed*, *in blue*) is shown and include: *AMF* autocrine motility factor; *ANG-2* angiopoïetin-2; *BNIP3* Bcl-2/adenovirus EIB 19 kDa-interacting protein 3; *BNIP3L* Bcl-2/adenovirus EIB 19 kDa-interacting protein 3 like; *CA IX*, *XII* carbonic anhydrase; *CXCR4* cytokine (C-X-C motif) receptor 4; *GLUT1* glucose transporter 1; *LDH-A* lactate dehydrogenase-A; *LON*, a mitochondrial protease; *LOX* lysyl oxidase; *MCT1*, 4, monocarboxylate transporter; *MMP* matrix metalloproteinase; *NOXA* pro-apoptotic member of Bcl-2 protein family; *PDK1* pyruvate dehydrogenase kinase 1; *REDD1/RTP801*; *VEGF* vascular endothelial growth factor; *VEGF-R1*, vascular endothelial growth factor receptor

Although many studies have pointed at the pro-apoptotic features of these two gene products, these findings are largely controversial. We propose instead that the BH3 domains of BNIP3 and BNIP3L belong to another class, like the BH3 domain of Beclin1, that do not induce cell death but survival by triggering autophagy [12, 23, 24]. Macroautophagy is a process that allows cells to recycle intracellular organelles such as ribosomes and mitochondria for nutritional and protective purposes [25]. Catabolism of organelle components provides nutrient-depleted cells with a source of lipids, amino acids and sugars, and autophagy of mitochondria may protect cells from harmful reactive oxygen species.

Metabolism

A substantial number of genes involved in cellular metabolism, in particular those of glucose, are HIF-mediated. It has been known for many years that cancer cells divert pyruvate metabolism away from mitochondrial oxidative phosphorylation (OXPHOS) toward cytoplasmic conversion of pyruvate to lactic acid [11]. Although this latter simplified pathway produces less adenosine triphosphate (ATP) per molecule of glucose, cells compensate for a reduced yield in ATP production by increasing both the uptake of glucose and the flux in conversion of glucose to pyruvate, i.e. glycolysis. This is made possible through an increase in HIF-mediated expression of both glucose transporters and enzymes of the glycolytic pathway, giving tumours a "glycolytic" phenotype. Diversion of pyruvate toward lactate and away from OXPHOS is also promoted through increased HIF-mediated expression of two key enzymes; lactate dehydrogenase A (LDH-A) [26] and pyruvate dehydrogenase kinase 1 (PDK1) [27, 28]. LDH-A is the enzyme responsible for conversion of pyruvate to lactate, and PDK1 is an inhibitor of pyruvate dehydrogenase that feeds pyruvate into the tricarboxylic acid cycle and thus toward OXPHOS. Thereby, HIF not only channels glucose towards glycolysis by repressing mitochondrial respiration but it also optimizes low levels of respiration by regulating the ratio of isoforms of cytochrome c oxidase, components of the electron transport chain [29]. This strategy not only makes respiration more efficient but may also protect cells from oxidative damage under hypoxic conditions. Metabolic regulation via HIF also brings into play products of tumour suppressors and oncogenes such as p53, c-Myc, Ras and Akt [11, 21, 30].

Another pathway related to nutrient availability, which is modified by HIF, is that of mammalian target of rapamycin (mTOR). On the one hand, growth factors and nutrients potentiate the mTOR pathway in conveying signals of growth and survival through increased protein synthesis, and on the other hand, energy depletion and hypoxia suppress mTOR, saving on energy-consuming protein

synthesis, allowing for cellular adaptation and subsequent survival [12].

Regulation of pH

One of the consequences of the predilection of cancer cells for cytoplasmic glucose metabolism in producing lactic acid is acidosis, a decrease in the extracellular pH [31]. This acidosis, generated by the increased production of carbonic and lactic acids, is exacerbated by the limiting vasculature. Despite a low pHe, the intracellular pH (pHi) of tumour cells is maintained at a relatively normal pH or even slightly more alkaline pH, which is reported to result from HIF-mediated up-regulation and activation of a number of membrane located transporters, exchanges, pumps and ecto-enzymes that are implicated in pH homeostasis. Among these are the growth factor activatable and amiloride-sensitive Na^+/H^+ Exchanger (NHE-1) [32–34] and the H^+/lactate cotransporter (monocarboxylate transporter, MCT1 and MCT4) [35]. In addition, one of the most highly HIF-induced proteins, carbonic anhydrase IX (CA IX), an enzyme that catalyzes the reversible conversion of CO_2 to carbonic acid (Fig. 3), has been reported to regulate the pHe [36], and we propose that it may contribute to an increase in the pHi through $C1^-/HCO_3^-$ exchanger uptake of HCO_3^-. Coupled interaction between MCT1 [37] and CA II and between different CA isoforms and proteins of the superfamily of bicarbonate transporters, including the anion exchangers [38, 39] or sodium bicarbonate co-transporter (NBCs) proteins [39] has been reported. Such interplay would allow tumour cells to maintain a more alkaline pH for subsequent cell growth [40].

Metastasis

Substantial data points toward hypoxic promotion of the invasive potential of tumour cells. HIF activation is associated with loss of E-cadherin, a component of adherens junctions that acts as a suppressor of invasion and metastasis [41]. In this context, it is interesting that TWIST1, a regulator of epithelial-mesenchymal transition [42], is induced in hypoxia [43]. In addition, cells that survive acidosis not only develop a growth advantage but also become more aggressive and invasive [6, 44]. This occurs in part through the activation of HIF-up-regulated proteins implicated in matrix remodeling, such as lysyl oxydase (LOX) [12, 45], metalloproteases that disrupt cell–cell and cell–matrix (ECM) interactions [46]. HIF also activates other genes known to be involved in metastasis and invasion such as the *c-met* proto-oncogene, the chemokine receptor CXCR4 and the autocrine motility factor (AMF) [41, 47].

Clinical significance of hypoxia, HIF and HIF downstream gene products in prognosis

Since hypoxia in tumours and internalization of high levels of glucose into tumours are considered to be indicators of more aggressive tumours, and thus of poor patient prognosis [48], attempts to detect these characteristics in patients' tumours have been developed using positron emission tomography (PET). Hypoxic zones are detected after injection of [fluorine-18] misonidazole (FMISO), while tumours that capture glucose can be detected after injection of [fluorine-18] deoxyglucose (FDG), a non-

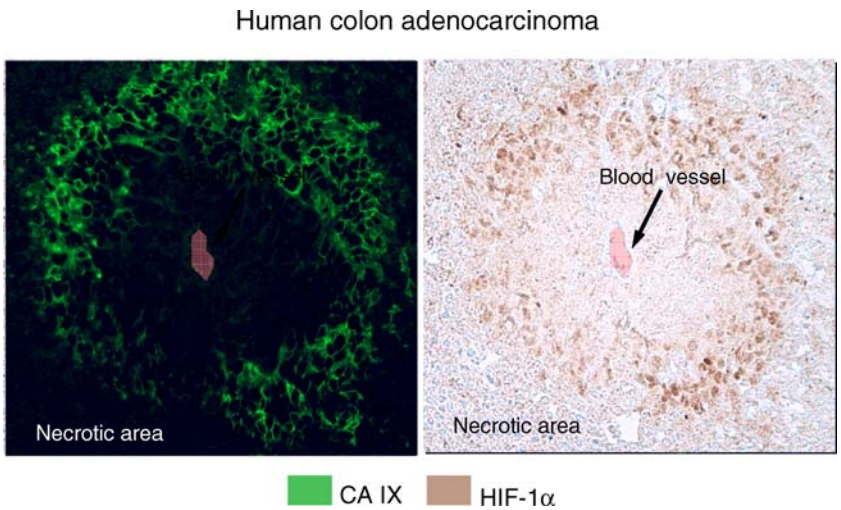

Fig. 3 Tumour expression of carbonic anhydrase IX (CA IX) and hypoxia-inducible factor-1α (HIF-1α). Immunohistological detection of CA IX and HIF-1α colocalized in hypoxic regions of a section of a human colon adenocarcinoma (LS174) grown in *nude* mice (Dayan et al., unpublished data). Note the hypoxic gradient that develops away from the blood vessel and the necrotic area around the most hypoxic ring

metabolizable analogue of glucose (Fig. 4). However, a direct correlation between high glucose uptake and hypoxic regions was not observed [49, 50]. This may result from the fact that only highly hypoxic regions, though not necrotic, are detected with FMISO, so areas where the oxygen concentration is nonetheless sufficiently low for stablization of HIF-α may not be identified. Alternatively, cycles of hypoxia and angiogenic reoxygenation that give rise to a high glucose capture phenotype may have preceded analysis. The recognition that hypoxic areas are radiotherapy resistant has led to a number of strategies to increase the oxygen availability or to deliver radiosensitizing agents [7].

Since HIF-α and HIF-induced proteins such as CA IX, CA XII and Glut1 are highly expressed in renal cell carcinomas (RCC) and in multiple human cancers, their expression has been investigated as markers of tumour aggressiveness and in determining prognosis [22]. RCC is a prototype cancer for understanding the role of HIF in cancer progression since it carries loss-of-function mutations in the *VHL* gene, the product of which is responsible for targeting HIF-α for proteasomal degradation [51]. Thus, in these cancers, HIF-α is stable, and downstream gene products are induced. To better appreciate the implication of HIF in tumour progression and prognosis, immunohistochemical studies have been performed in several other cancer types to detect for both HIF-α and HIF-downstream gene products such as BNIP3, CA IX and XII, Glut1 and VEGF (Figs. 3 and 4). The inherent problem related to the detection of HIF-α in tissue specimens is the short half-life of HIF-α not only in vivo but also when the specimen comes in contact with atmospheric oxygen during surgical removal. In fact, recent studies have established that this was not the case and make these studies reporting levels of HIF-α relevant [52]. It was shown that HIF-1α and CA IX expression correlate with poor prognosis in breast cancer [52]. The longer half-life of the other potential marker proteins may make interpretation difficult as detection may reflect only past events. These markers were shown to correlate for both primary breast tumours and lymph node metastases [53], and further studies have demonstrated reduced survival correlated with CA IX expression in breast cancer [54]. In breast cancer, high BNIP3 expression was associated with good survival outcome in invasive carcinoma but with an increased risk of recurrence and shorter disease-free survival in ductal carcinoma in situ [55], while in non-small lung cancer, high expression was an independent factor for overall survival [56]. Further investigation is required to obtain a better appreciation of the value of HIF or HIF-related marker immunohistochemistry for prognosis.

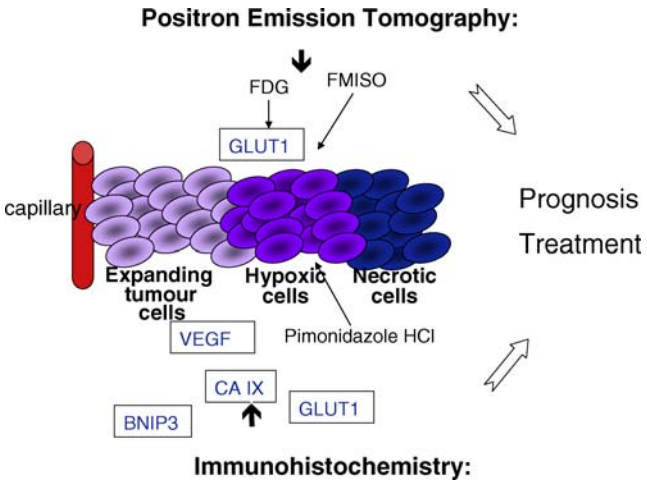

Fig. 4 Clinical significance of hypoxia and HIF in prognosis. Imaging by PET allows detection of hypoxic zones and zones of high glucose uptake in tumours after injection to patients of [fluorine-18] misonidazole (FMISO) or [fluorine-18] deoxyglucose (FDG), respectively. Immunohistochemistry of surgical specimens allows detection of hypoxic regions using primonidazole HCl and detection of HIF-target gene products such as *BNIP3* Bcl-2/adenovirus EIB 19 kDa-interacting protein 3; *CA IX* carbonic anhydrase; *GLUT1* glucose transporter 1; *VEGF* vascular endothelial growth factor. Further studies are required to determine the clinical potential of such imaging technologies in prognosis and treatment of different cancer types

Harnessing phenotype in combating tumour growth

Novel processes that engage perturbations in the tumour microenvironment may prove efficient as cancer therapies. Inhibition of angiogenesis, although not devoid of harmful side effects, is showing potential in treatment of several different types of cancer when in combination with classical chemotherapy [57]. Both intervention at the level of HIF [58] and HIF downstream genes, in addition to those that regulate angiogenesis, merit investigation (Fig. 5). Alternative strategies that target the particularities of the tumour

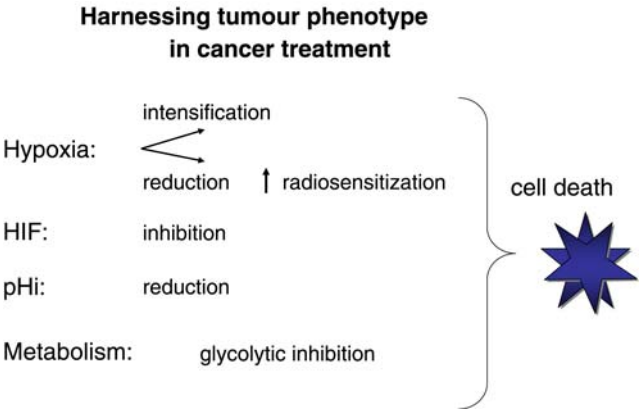

Fig. 5 Potential novel approaches to turning around the hypoxic tumour phenotype. By controlling the hypoxic nature of tumours and the HIF-mediated cellular adaptation or microenvironmental consequences, such as acidosis, novel therapeutic approaches should promote cell death

phenotype may provide tumour-specific agents that hold the advantage of sparing normal tissue [59–61], which is not the case with classical chemotherapy. Such strategies may prove beneficial alone or in combination with presently employed cytotoxic agents [62].

Conclusions

The understanding of how hypoxia drives tumour progression is attracting substantial investigation, and an impressive number of reviews have ensued; however, a lot remains to be done to clarify not only the mechanisms involved but also the implication for diagnosis and treatment. Further investigation into the relevance of HIF-induced gene products as markers of prognosis should follow. The development of anti-angiogenic agents with significant potential as a cancer therapy has led the way in demonstrating that the hypoxic response of tumours can be targeted. Additional targets involved in HIF signalling and in its consequences should also prove beneficial in slowing cancer progression and metastasis.

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