HIF-mediated endothelial response during cancer progression

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Abstract Tumour growth at primary or secondary extravasation sites leads to localised regions of reduced oxygen tension (hypoxia) in cells both within and surrounding the tumour. Although the angiogenic response of the tumour cell to hypoxia has been widely examined, the effect of hypoxia on other cell types within the tumour microenvironment is less clear. The endothelium is highly responsive to local hypoxia and regulates tumour cell dissemination and ultimately metastatic success through differential regulation of hypoxia-inducible transcription factors (HIFs). The endothelial response to hypoxia particularly mediates key processes that regulate tumour vascularisation and cancer progression, including proliferation, migration, adherence, and vascular permeability. This article describes current understanding of the HIF-mediated endothelial response to hypoxia during cancer progression. Endothelial HIF signalling regulates tumour growth and metastasis and is therefore an attractive putative target for treatments that inhibit cancer progression.

Keywords Cancer · Endothelial · Hypoxia · Metastasis

Introduction

Solid tumours and metastases are characterised by accelerated proliferation rates and insufficient oxygen delivery from the host vasculature, which lead to localised regions of hypoxia in cells within and surrounding the tumour. Hypoxia stimulates new vessel formation in an attempt to

restore blood flow and facilitate continued tumour growth. The remodelling response that follows hypoxia is controlled primarily by the hypoxia-inducible transcription factors (HIFs), which consist of an oxygen-regulated α subunit and a constitutively expressed β subunit. Hypoxia and HIF activation are strongly associated with increased malignancy, decreased therapeutic response, and unfavourable clinical outcome [1]. The endothelium is ideally placed to respond to changes in local oxygenation and mediate physiological and pathological vascular response to hypoxia. The effect of endothelial hypoxia on tumour growth and metastasis is therefore crucial and this article describes the endothelial HIF-mediated response that occurs during cancer progression.

Oxygen-dependent regulation of endothelial HIF

Tumour and endothelial hypoxia stimulate cellular responses that mediate tumour progression and metastasis. These hypoxia and HIF-mediated effects on tumour growth and metastasis are time, isoform, and cell type dependent [2, 3]. The hypoxia-dependent α subunit of HIF [1] is highly expressed in breast tumour and adjacent endothelial regions [4], and endothelial HIF signalling regulates vascular development in mouse embryos [5] and metastasis in mouse models of breast cancer [2]. Endothelial cells also form the barrier between primary tumours and secondary sites of extravasation and are therefore ideally located to respond to local hypoxia. The endothelium can regulate the remodelling response to hypoxia primarily via the activation of HIF1 and 2. HIF1 α is hydroxylated under normoxia by prolyl hydroxylase domain (PHD) enzymes, which allows binding with the E3 ubiquitin ligase complex and subsequent proteosomal degradation (Fig. 1). Also during normoxia, HIF1 α

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Fig. 1 Oxygen-dependent degradation of HIF1 α . **a** Under normoxia PHDs hydroxylate prolyl residues 402 and 564, and FIH hydroxylates asparaginyl residue 803. **b** This allows binding of the VHL complex, which targets HIF1 α for proteosomal degradation, and prevents interaction of HIF1 α with coactivators p300/CBP

hydroxylation by factor inhibiting HIF (FIH) prevents interaction of HIF1 α with its transcriptional co-activators p300 and Creb binding protein (CBP). During hypoxia, however, PHD and FIH substrate availability is limited, which reduces their activity and allows HIF1 α to accumulate. Stable HIF1 α translocates to the nucleus, where it dimerises with the constitutively expressed HIF1 β subunit and forms the active HIF1 complex. Binding of HIF1 to the hypoxia-responsive element (HRE) induces transcriptional upregulation of a catalogue of target genes that modulate tumour vascularisation and growth. A greater understanding of the mechanisms that regulate the remodelling response to endothelial hypoxia during cancer progression may ultimately lead to the development of novel adjunctive cancer treatments.

Endothelial response to hypoxia during cancer progression

Endothelial HIF signalling upregulates inflammatory cytokines and growth factors that mediate *tumour vascularisation and growth* [6]. Hypoxic activation of endothelium also increases cell adhesion, coagulant properties, and endothelial permeability, which are crucial for the

processes of intravasation and extravasation that together result in *metastasis* [2, 7].

Tumour vascularisation and growth

Diffusion distances from tumour cells to the vasculature increase as these cells rapidly proliferate and the tumour develops. The resulting reduction in regional blood flow leads to localised hypoxia, which in turn stimulates neo-vascularisation that is essential to provide adequate oxygenation of proliferating tumour cells and support tumour growth. Endothelial cells establish this vascular network and these cell types are essential, given that one endothelial cell regulates the survival of approximately 50–100 tumour cells [8]. The transcriptome of endothelial cells in tumour compared with normal adjacent tissue is also substantially different [9–11] and cell proliferation is approximately 20 to 2000-fold greater [12]. Tumour endothelium is therefore an attractive target for anti-angiogenic treatments that impair tumour growth [13]. Hypoxia increases endothelial cell migration, tube formation [14], and capillary density [15] in a time-dependent manner. These *acute* or *chronic responses* are controlled via HIF-induced production of growth factors and cytokines that regulate vascular remodelling and tumour growth.

Acute response

Acute hypoxia rapidly activates endothelial release of a variety of inflammatory cytokines, which increases recruitment and adherence of activated neutrophils to the endothelial wall [16]. Endothelial hypoxia initiates inflammation via upregulation of P- and E-selectins, which regulate inflammatory cell adhesion and migration [6]. E-selectin is hypoxia-inducible and increases leucocyte adhesion to the endothelium [17]. Hypoxic upregulation of intercellular adhesion molecule (ICAM) 1 and vascular cell adhesion molecule (VCAM) 1 in endothelial cells also increases neutrophil adherence to the endothelium [18]. Interleukin (IL) 1 is expressed by hypoxic endothelial cells, which further stimulates ICAM1 [17, 19], and in turn further increases leucocyte recruitment and adhesion [20]. Endothelial cells under hypoxia also release IL8, which similarly increases neutrophil recruitment and activation [21]. Endothelial hypoxia also leads to upregulation of extracellular matrix proteins that regulate the degradation of surrounding tissue and therefore facilitate vascular remodelling. These include matrix metalloproteinase 9 (MMP9) [22], which degrades type IV collagen, and other matrix proteins [23] that together facilitate neovascularisation.

Chronic response

Sustained endothelial hypoxia stimulates upregulation of a catalogue of other HIF-mediated angiogenic growth factors that promote vascularisation. These include but are not limited to platelet-derived growth factor (PDGF), placental growth factor (PLGF), insulin-like growth factor (IGF), basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), thrombospondin, and monocyte chemotactic protein (MCP) 1 [22]. PDGF is produced by endothelial cells and enhances smooth muscle cell proliferation, which is essential for neovascularisation [22]. PDGF can also induce endothelial expression of other angiogenic growth factors such as VEGF [24]. PLGF is another member of the VEGF family that is produced by endothelial cells. PLGF can promote tumour vascularisation by; (1) stimulating the growth, migration, and survival of endothelial cells directly via VEGF receptor (VEGFR) 1 [25]; (2) recruiting monocytes; (3) mobilising endothelial progenitors [26]; and (4) increasing the expression of other angiogenic factors including bFGF, PDGF, and VEGF [27–29]. FGF [30] and IGF [31] are also angiogenic mitogens with important roles in vessel growth. VEGF is another highly potent angiogenic growth factor produced by endothelial cells [32]. Tumour vascularisation and growth may be enhanced by endothelial VEGF in a number of ways. VEGF: (1) stimulates angiogenesis locally by increasing endothelial proliferation and migration [33]; (2)

upregulates urokinase- and tissue-type plasminogen activator (uPA and tPA) activity in endothelial cells, which increases local fibrinolysis [34]; (3) increases endothelial cell survival or mobilises endothelial progenitors [35, 36]; (4) recruits monocytes and neutrophils [37]; and (5) activates monocytes to produce growth factors that are important for neovascularisation [38]. Thrombospondin is an endothelial cell-expressed matrix-associated protein [39], which may regulate angiogenesis via affecting platelet aggregation [40] and endothelial cell adhesion, proliferation, and migration [41]. Endothelial expression of the potent chemoattractant for monocytes, MCP1, is also enhanced by hypoxia [42].

The importance of endothelial HIF signalling during tumour vascularisation and growth has been highlighted using mice with endothelium-specific HIF α knockout generated by bone marrow reconstitution of mice lacking HIF1 α or HIF2 α in myeloid and endothelial cells driven by the Tie2 promoter [43] or mice with endothelial cell-specific deletion of HIF2 α under the control of the VE-cadherin promoter [44]. Endothelial loss of HIF1 α in mouse models of breast cancer dramatically impairs angiogenic potential as shown by reductions in: (1) VEGF expression; (2) proliferation; (3) migration; (4) extracellular matrix penetration; (5) tube formation; (6) wound healing; (7) tumour vascularisation; and (8) tumour size [43]. Endothelial HIF2 α deletion increased cell migration and invasion in vitro and non-functional vessel formation and growth in an autochthonous skin tumour model [44]; while knockout of endothelial HIF2 α reduced tumour angiogenesis and growth in a subcutaneous xenograft model [45]. These findings demonstrate the crucial requirement for endothelial HIF signalling during tumour vascularisation and growth and identify endothelial HIF1 α and HIF2 α as potential targets for cell-directed anti-angiogenic cancer treatments.

Metastasis

In addition to supporting tumour growth, the tumour vasculature provides the primary entry route for tumour cells into the circulation [46], and up to 2 million tumour cells enter the circulation per cubic centimetre of primary tumour [47]. Tumour endothelial cells produce many HIF-mediated factors that regulate angiogenesis in the primary tumour and sites of metastatic dissemination, and highly vascularised tumours give rise to greater tumour cell entry into the blood [46]. Given that tumour cell entry into the blood is proportional to metastasis, it follows that greater tumour vascularisation leads to enhanced metastasis [46]. Endothelial HIF signalling is also a crucial mediator of metastasis, given that endothelial cells provide the barrier across which tumour cells travel from the primary tumour into the circulation and from the bloodstream into

surrounding tissue. Endothelial HIF signalling can mediate these processes of intravasation and extravasation via effects on *coagulant function* and *vascular permeability*.

Coagulant function

Normoxic endothelial cells suppress coagulation and inflammation, and in doing so maintain a local fibrinolytic and anti-thrombotic state [48]. Conversely, stressors such as mechanical disturbance by tumour formation and tumour hypoxia activate the endothelium and this leads to upregulation of HIF-mediated coagulant and thrombotic factors including platelet-activating factor (PAF), tissue factor (TF), and plasminogen activator inhibitor (PAI) 1 [48, 49]. Hypoxic activation of endothelial cells could promote metastasis by: (1) increasing PAF-induced activation of platelets, which promote tumour cell survival at the site of metastasis [50]; and (2) increasing angiogenesis via TF-induced VEGF expression [51]. Hypoxia also reduces endothelial expression of the anti-coagulant thrombomodulin and increases endothelial cell coagulant activity [6, 7, 52]. Circulating tumour cells arrest in the capillary bed and adhere to the endothelium during extravasation. This vascular blockage is likely to result in localised hypoxia of tumour and endothelial cells. Endothelial hypoxia stimulates thrombus formation [53], which is itself a hypoxic stimulus that leads to upregulation of HIF1 α , and a variety of HIF target genes that mediate tumour development and metastasis [54]. Thrombosis could support extravasation furthermore by: (1) providing a fibrin scaffold for tumour cell migration and binding of angiogenic factors such as VEGF [55], which together enhances metastatic success [56, 57]; and (2) protecting arrested tumour cells from endothelial detachment [58] and elimination by natural killer cells [59, 60]. Conversely, release of coagulant factors from hypoxic tumour endothelium may partly explain the strong association between cancer progression and thrombosis [61].

Vascular permeability

Hypoxia increases endothelial intracellular gaps, capillary permeability [6], and molecular transport through endothelial monolayers [7]. These effects could be mediated by VEGF and are likely to facilitate metastasis. VEGF is a vascular permeability factor whose endothelial expression is increased by hypoxia and HIF stabilisation. VEGF increases vascular permeability and this could increase metastasis by: (1) generating gaps between endothelial cells, which enables intravasation of tumour cells [62]; and (2) increasing interstitial fluid pressure, which is associated with increased metastasis [63]. Acute endothelial hypoxia also leads to transcriptional upregulation of other

vasoactive substances involved in modulating vascular tone. These include vasoconstrictors and smooth muscle mitogens such as endothelin 1 and thrombospondin 1 [22]. Chronic endothelial hypoxia increases the expression of vasodilatory factors such as endothelial nitric oxide synthase (eNOS) and HIF directly regulates hypoxic induction of NOS in endothelial cells [64]. The vasodilatory NOS product nitric oxide (NO) acts directly on smooth muscle cells and indirectly facilitates vasodilation by inhibiting the actions of hypoxia-induced vasoconstrictors. Endothelial NO also facilitates tumour vascularisation by: (1) inducing relaxation of adjacent smooth muscle cells [65]; (2) inhibiting platelet aggregation and adhesion [66]; (3) regulating smooth muscle cell and fibroblast mitosis [67, 68]; (4) inhibiting endothelial apoptosis [69]; and (5) promoting endothelial proliferation [70]. Endothelial NO also modulates the hypoxic induction of other vasoactive factors such as endothelin 1 and therefore creates a reciprocal feedback loop [71]. Another positive feedback loop is created between HIF1 α and reactive oxygen species (ROS), whose endothelial production is increased during hypoxia [72], which in turn inhibits PHD activity and therefore increases HIF1 α expression [73–75].

The importance of endothelial HIF/VEGF/NO signalling during metastasis was comprehensively shown in mice with endothelial HIF deletion [2]. Endothelial HIF1 α knockout in mouse models of breast cancer dramatically impairs metastatic progression and reduces: (1) NO synthesis; (2) VEGF expression; (3) tumour cell migration through endothelial layers; and (4) pulmonary metastasis. Conversely, endothelial loss of HIF2 α has the opposite effect in each case. These findings identify endothelial HIF signalling as a crucial regulator of metastasis and demonstrate opposing roles for the HIF isoforms in regulating malignancy. This study highlights the importance of isoform- and cell-specific HIF targeting in the development of novel treatments that aim to suppress metastasis.

Future perspectives

Localised hypoxia occurs in tumours and occlusive thrombi and the endothelial HIF-mediated response is integral to both tumour and thrombus development, but mechanisms that regulate the association between thrombus formation and metastatic success remain unclear [56, 57, 60]. HIF-mediated endothelial response to thrombus formation may induce a catalogue of factors that control cancer progression, including VEGF and NO, which enhance tumour cell transport across endothelial monolayers and increase metastatic success [2]. Conversely, hypoxic endothelial cells within tumours release coagulants and other cytokines that increase thrombus formation,

Fig. 2 Proposed mechanism of regulation of thrombosis-associated cancer progression. Thrombus formation stimulates a HIF-mediated hypoxic response in endothelial cells, which leads to expression of angiogenic factors, and in turn increases tumour growth and

metastasis. Conversely, tumour formation stimulates an endothelial HIF-mediated hypoxic response that leads to expression of coagulants and increased thrombus formation

such as TF and PAI1 [61]. Taken together these events create a positive feedback loop (Fig. 2) that may contribute to the association between cancer progression and thrombosis. Hypoxia and HIF-mediated mechanisms that regulate this positive correlation are currently under scrutiny in our laboratory.

In summary, endothelial HIF regulates cancer progression and is therefore an attractive target for novel adjunctive cancer therapies. The effects of endothelial HIF signalling on metastasis are isoform dependent, however, so these therapies should aim to target the HIF pathway in an isoform-specific manner.

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Conflict of interest The authors declare they have no conflict of interest.

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