Published in final edited form as:

Nat Rev Cancer. 2014 June ; 14(6): 430–439. doi:10.1038/nrc3726.

Hypoxia and the extracellular matrix: drivers of tumour metastasis

Daniele M. Gilkes,

Vascular Program, Institute for Cell Engineering, and McKusick-Nathans, Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA

Johns Hopkins Physical Sciences-Oncology Center, The Johns Hopkins University, Baltimore, Maryland 21218, USA

Gregg L. Semenza, and

Vascular Program, Institute for Cell Engineering, and McKusick-Nathans, Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA

Johns Hopkins Physical Sciences-Oncology Center, The Johns Hopkins University, Baltimore, Maryland 21218, USA

Departments of Pediatrics, Oncology, Medicine, Radiation Oncology and Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA

Denis Wirtz

Johns Hopkins Physical Sciences-Oncology Center, The Johns Hopkins University, Baltimore, Maryland 21218, USA

Department of Chemical and Biomolecular Engineering, The Johns Hopkins University, Baltimore, Maryland 21218, USA

Departments of Oncology and Pathology and Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA

Abstract

Of the deaths attributed to cancer, 90% are due to metastasis, and treatments that prevent or cure metastasis remain elusive. Emerging data indicate that hypoxia and the extracellular matrix (ECM) might have crucial roles in metastasis. During tumour evolution, changes in the composition and the overall content of the ECM reflect both its biophysical and biological properties and these strongly influence tumour and stromal cell properties, such as proliferation and motility. Originally thought of as independent contributors to metastatic spread, recent studies have established a direct link between hypoxia and the composition and the organization of the ECM, which suggests a new model in which multiple microenvironmental signals might converge to synergistically influence metastatic outcome.

Rapid cancer cell proliferation, combined with structural and functional abnormalities in tumour blood vessels, results in regions within solid tumours that have reduced oxygen availability¹. Oxygen availability decreases as the distance from the nearest blood vessel increases. Intratumoural hypoxia is associated with disorganized vascular networks with intercapillary distances that are greater than the diffusion distance of oxygen (which is ~100–200 μm , depending on the local oxygen concentration in the blood and the rates of oxygen consumption)². The direct measurement of the tumour partial pressure of oxygen (PO_2) using Eppendorf microelectrodes (which remains the gold standard for determining oxygen levels) has revealed that patients whose primary tumours are poorly oxygenated (those with $PO_2 < 10$ mmHg) have an increased risk of metastasis and mortality^{1,3,4}. The best understood mechanism of how cancer cells adapt to a hypoxic environment is through the transcriptional activity of hypoxia-inducible factors (hypoxia-inducible factor 1 (HIF1) and HIF2; see BOX 1)⁵. The role of hypoxia and HIFs in reprogramming cancer cells by regulating the expression of multiple genes involved in angiogenesis, by regulating the metabolism of glucose and by regulating cancer cell invasion and metastasis has been extensively reviewed elsewhere^{6–10}. Recent reviews also highlight the importance of hypoxia in recruiting the stromal cell components of the tumour microenvironment^{11,12}. In this Opinion article, we focus on how hypoxia affects extracellular matrix (ECM) deposition, remodelling and degradation, which might potentiate cancer metastasis. Central to this emerging paradigm are three crucial findings: the ECM is a dynamic structure that influences tumour progression^{13–18}; multiple cell types, including cancer cells, contribute to ECM production^{19–23}; and the remodelled ECM within regions of intratumoural hypoxia could be a pathway rather than an obstacle for cancer metastasis^{24–27}.

The ECM and cancer

The ECM is composed of approximately 300 proteins that regulate tissue homeostasis, organ development, inflammation and disease¹⁹. The major constituents of the ECM are fibrous proteins (such as collagens, elastins, fibronectins and laminins) and proteoglycans (such as chondroitin sulphate, heparan sulphate, keratan sulphate and hyaluronic acid) that are locally secreted and assembled into an organized mesh, which forms the structural framework for most tissues²⁸. Molecular approaches aiming to correlate clinical outcomes with specific gene expression patterns within the primary tumour have highlighted genes that encode tumour-associated ECM components^{19,29–34}. An increased expression of genes encoding proteins that mediate ECM remodelling has been associated with increased mortality in patients with breast, lung and gastric cancers^{35,36}. These studies corroborate histological findings that show an excessive ECM deposition (also termed fibrosis) within solid tumours^{37–43}.

The most well-recognized ECM alteration that occurs in the tumour tissue is increased collagen deposition^{44–53}. Collagens are the most abundant ECM components, constituting up to 90% of the ECM and 30% of the total protein in humans, and they provide the structural integrity and the tensile strength of human tissues and organs⁵⁴. In the context of cancer biology, collagens regulate the physical and the biochemical properties of the tumour microenvironment, which modulate cancer cell polarity, migration and signalling^{17,55–58}. Collagen I deposition and cancer metastasis have been causally linked using mice

engineered to express a collagenase-resistant $\alpha 1$ chain of type I collagen (*Colla1^{tm1jae}* mice)⁵⁹. *Colla1^{tm1jae}* mice were crossed with mouse mammary tumour virus promoter-driven polyoma middle T antigen (*MMTV-PyMT*) transgenic mice to model increased type I collagen deposition during the progression of human breast cancer^{59–61}. *Colla1^{tm1jae}; MMTV-PyMT* bitransgenic mice had a threefold increase in the incidence of tumour formation and metastasis compared with their wild-type littermates⁶⁰. Furthermore, histological studies of human breast carcinomas have shown that fibrosis is localized to hypoxic regions within tumours and correlates with immunostaining of the HIF1 target gene product carbonic anhydrase IX (CAIX)^{51,52}. Highly fibrotic tumours also have the highest CAIX immune reactivity, which can independently predict patient relapse rate and shorter disease-free survival^{51,53}. In this Opinion article, we discuss emerging data that has provided experimental evidence linking the mechanisms of hypoxia-induced collagen deposition and remodelling to those of invasion and metastasis.

Tumour ECM synthesis and degradation

The current view of tumour fibrosis suggests that recruited and resident fibroblasts and myofibroblasts within the primary tumour are mediators of tumour fibrosis. These cells are activated by proteins that are secreted by cancer cells, most notably by transforming growth factor- β (TGF β), which stimulates the synthesis of ECM proteins and the remodelling of the ECM by proteases produced by cancer-associated fibroblasts⁶². Fibroblasts that are isolated from the site of a healing wound or from fibrotic tissues secrete higher levels of normal ECM constituents and proliferate more than their normal counterparts that are isolated from healthy organs, which is an explanation for the increase in matrix deposition that occurs within a tumour⁶³. Although it is well accepted that invasive carcinoma is often associated with increased ECM deposition in tumours⁶⁴, there is also evidence for an increased deposition of ECM in hypoxic tumour regions. Recent studies have uncovered mechanisms of tumour fibrosis that specifically occur under hypoxic conditions and that involve not only fibroblasts but also other cell types, including cancer cells^{20,21,65–68}.

Hypoxia induces increased collagen gene expression

Hypoxia and HIF1 have been implicated in renal, liver and adipose tissue fibrosis^{69–71}. Dermal, cardiac and renal fibroblasts cultured under hypoxic conditions show increased type I procollagen $\alpha 1$ mRNA levels^{72–74}. Furthermore, increased levels of type I, II and IV procollagen mRNA are present in the peripheral lung parenchyma and pulmonary artery of rats that have been exposed to hypoxia⁷⁵. However, studies that describe the regulation of collagen gene expression in hypoxic cancer cells *in vitro* and during cancer progression *in vivo* are lacking. By contrast, the dramatic effect of hypoxia on the post-translational modification of collagen is a matter of considerable investigation, as described below.

HIF1 regulates the expression of intracellular collagen-modifying enzymes

Collagen biogenesis originates with gene transcription and is followed by the translation of mRNA into procollagen (pro- α -chains) (FIG. 1). At least 28 collagen subtypes, which are encoded by 42 genes that generate 42 distinct pro- α -chains, have been identified in vertebrates⁷⁶. Within the endoplasmic reticulum, the pro- α -chains undergo multiple post-

translational modifications, which include the hydroxylation of proline and lysine residues, followed by the glycosylation of hydroxylysine residues⁷⁶. The modification of proline to 4-hydroxyproline is essential for the thermal stability of the collagen triple helix⁷⁷.

Procollagen α -chains that are not hydroxylated are improperly folded, which leads to proteolytic degradation and to reduced collagen deposition^{76,78}. Three isoforms of the prolyl 4-hydroxylase α -subunit (P4HA) have been identified (P4HA isoform 1 (P4HA1), P4HA2 and P4HA3) that form A₂B₂ tetramers with P4HB, which results in the generation of P4H1 (from P4HA1), P4H2 (from P4HA2) and P4H3 (from P4HA3) holoenzymes^{79,80}. Three procollagen-lysine 2-oxyglutarate 5-dioxygenase genes (*PLOD1*, *PLOD2* and *PLOD3*) encode enzymes that mediate collagen lysine hydroxylation. Collagen crosslinks that are derived from hydroxylated lysine residues compared with non-hydroxylated lysine residues have increased stability, which leads to increased tissue stiffness⁸¹. Thus, stiff tissues, such as bones, cartilage and tendons, contain a higher percentage of hydroxylated lysine residues in collagen compared with soft tissues, such as the skin⁸¹.

HIF1 regulates the expression of P4HA1, P4HA2, *PLOD1* and *PLOD2* in cancer cells, fibroblasts, chondrocytes and endothelial cells^{20,21,67,82–86}. Abrogating the expression of HIF1 α , P4HA1 or P4HA2 through the stable transfection of cells with short hairpin RNA (shRNA) vectors inhibits collagen deposition from both breast cancer cells and fibroblasts *in vitro*^{21,82}. Reducing the levels of HIF1 α , P4HA1 or P4HA2 *in vivo* results in decreased fibrosis and decreased tissue stiffness in orthotopic tumours that are formed by the injection of human breast cancer cells into the mammary fat pads of immunodeficient mice^{21,68}. Decreasing the levels of HIF2 α expression in breast cancer cells had no effect²¹. Importantly, P4HA1 or P4HA2 knockdown inhibited the spontaneous metastasis of breast cancer cells to the lungs and to the lymph nodes of mice by reducing the formation of collagen fibres, which are required for cancer cell adhesion, spreading and invasion^{21,68}. In contrast to P4HA1 and P4HA2, the depletion of *PLOD2* in breast cancer cells did not suppress collagen deposition *in vitro* or *in vivo*, but reduced tumour stiffness by reducing fibrillar collagen content²⁰. *PLOD2* knockdown also significantly impaired the invasion of cancer cells into the adjacent normal tissue of the mouse mammary fat pad, reduced the number of circulating tumour cells and prevented the spontaneous metastasis of breast cancer cells to the lungs and to the lymph nodes of mice²⁰. In murine models of sarcoma, abrogating HIF1-dependent *PLOD2* expression disrupted collagen modification, cell migration, and pulmonary metastasis⁶⁷. Taken together, the studies described above indicate that hypoxia might regulate ECM deposition by multiple cell types within the tumour microenvironment^{20,21,67,68,82,83,86}. In addition to the marked effects of collagen prolyl and lysyl hydroxylase expression in experimental mouse models of metastasis, *P4HA1*, *P4HA2* and *PLOD2* expression have also been suggested as biomarkers for human cancer progression in several independent studies (BOX 2).

HIFs regulate extracellular collagen-modifying enzymes

Following enzymatic modification of type I collagen by hydroxylation, two α 1(I)-chains and one α 2(I)-chain associate to form a triple helix that is secreted into the extracellular space (FIG. 1). Collagen peptidases cleave the carboxy- and amino-terminal peptides, and type I collagen fibrils form spontaneously, are covalently crosslinked on hydroxylysine and lysine

residues and form structurally stable collagen I fibres^{76,87}. Fibrillar collagens, such as type I collagen, establish the interstitial matrix and contribute to tissue stiffness with extensive post-translational modifications that increase tensile strength⁵⁴. Non-fibrillar collagens, such as type IV collagen, constitute a key component of the basement membrane, which is a compact sheet-like structure that functions as a barrier to separate tumour cells from the adjacent stroma⁸⁸.

Collagen crosslinking is extracellularly initiated by the lysyl oxidase (LOX) family of secreted enzymes that oxidatively deaminate lysine or hydroxylysine collagen residues⁸⁷. Three LOX enzymes — LOX, LOX-like protein 2 (LOXL2) and LOXL4 — are important hypoxia-induced and HIF-regulated target gene products that are involved in collagen crosslinking and tumour fibrosis^{65,89–93}. In addition to collagen crosslinking within the primary tumour, secreted LOX has been shown to localize within the lungs and to remodel existing collagen to establish a premetastatic niche containing bone marrow-derived cells (BMDCs), which facilitates colonization of the niche by cancer cells in murine models of breast cancer^{90–91}. LOX family members are upregulated to varying levels and in different combinations in human breast cancers⁹³. Similarly, breast cancer cell lines show different patterns of LOX family member expression in response to hypoxia, but in each case the expression is HIF dependent⁸⁹. Consideration of the specific LOX family members that are induced by hypoxia is therefore essential to prevent collagen remodelling, BMDC recruitment and metastasis in the lungs of tumour-bearing mice^{89,90}. The pharmacological inhibition of LOX by β -aminopropionitrile (β APN) has been reported to inhibit metastasis in experimental mouse models; however, β APN might not inhibit the activity of all LOX family members⁹³, which suggests that HIFs or pan-LOX inhibitors could represent broader targets than currently available drugs or antibodies that target only a subset of LOX and LOXL proteins.

HIF1 and HIF2 can regulate ECM degradation

In addition to collagen deposition, collagen degradation also contributes to ECM remodelling and is mediated by several families of proteinases that have been suggested to promote cancer cell invasion; for example, the matrix metalloproteinases (MMPs) are a family of zinc-dependent enzymes that are divided into several subgroups (collagenases, gelatinases, stromelysins and cell membrane-bound MMPs) with different substrate specificities. Hypoxia is associated with an increase in the expression and the activity of type IV collagen-degrading enzymes (MMP2 and MMP9) *in vitro*^{94–96}. *MMP2* and *MMP9* are upregulated by hypoxia in breast and colon cancer cells via a HIF1-dependent mechanism^{94–96}, whereas membrane-bound membrane-type 1 MMP (MT1-MMP; also known as MMP14) is upregulated in a HIF2-dependent manner^{94,97}. In addition to collagen degradation by MMPs, hypoxic cancer cells also show increased proteolytic activity as a result of HIF-dependent increases in their expression of urokinase plasminogen activator surface receptor^{98,99} (PLAUR). PLAUR promotes cell invasion by altering the interactions between integrins and the ECM. When PLAUR expression levels are depleted by the expression of shRNAs, cells with reduced levels of PLAUR are incapable of intravasation¹⁰⁰. Thus, HIFs activate a transcriptional programme that results in the degradation of the basement membrane while simultaneously increasing the *de novo*

synthesis of fibrillar collagens to function as a physical pathway for tumour invasion (FIG. 2).

Growth factors and ECM deposition

Tumours have long been described as ‘wounds that won’t heal’ (REF. 101). Similarly, hypoxia is known to have a role in both normal and pathological wound healing. In normal cutaneous wounds, HIF1 is important for appropriate angiogenic responses, for mobilization of circulating angiogenic cells, such as endothelial precursor cells and mesenchymal stem cells (MSCs), and for normal wound contraction¹⁰². Partial reduction of HIF1 α expression is consequently sufficient to impair wound healing¹⁰³. During wound healing, angiogenesis and ECM deposition occur in parallel¹⁰⁴; therefore, it is not surprising that some of the same factors that stimulate angiogenesis also promote fibrosis. Hypoxia-induced angiogenic growth factor production has been well established¹⁰⁵. HIF1 has been shown to bind to a *cis*-acting hypoxia-response element in the genes that encode vascular endothelial growth factor (VEGF), stromal cell-derived factor 1 (SDF1; also known as CXCL12), angiopoietin 2 (ANG2), platelet-derived growth factor B (PDGFB), placental growth factor (PGF), connective tissue growth factor (CTGF) and stem cell factor (SCF)^{106–114} and can also indirectly promote fibroblast growth factor 2 (FGF2)¹¹⁵ production in a variety of cell types (TABLE 1).

Although well-known for their influence on tumour angiogenesis, many of these growth factors also contribute to fibrosis^{116–118}, potentially by attracting fibroblasts to the primary tumour and/or by activating resident fibroblasts. Experimental evidence indicates that the recruitment of fibroblasts or myofibroblasts to sites of pathological fibrosis is driven by hypoxia^{119–121}. Similarly, hypoxia increases the recruitment of bone marrow-derived MSCs in murine models of breast cancer, which results in increased lymphatic and vascular metastasis^{112,122}; for example, VEGF — which is released by hypoxic cancer cells but more often by endothelial cells, fibroblasts and inflammatory cells — has been implicated in fibrosis because of its role in stromal cell activation and because it leads to the production of an ECM that is rich in fibronectin and type I collagen⁶³. VEGF also induces microvascular permeability, which in turn mediates an influx of fibroblasts, inflammatory cells and endothelial cells to the primary tumour¹²³.

Hypoxia and macrophage recruitment

Hypoxia-induced growth factor secretion in the primary tumour also promotes the accumulation of macrophages, which rapidly respond to the hypoxic microenvironment by altering their gene expression patterns^{124–126}. The importance of hypoxia in stimulating macrophage infiltration during wound healing has been shown in heterozygous HIF1 α -deficient mice, which show considerable delays in myeloid cell infiltration¹²⁷. Recent studies highlight a potential mechanism of macrophage recruitment into hypoxic regions involving the release of semaphorin 3A by hypoxic cancer cells, which functions as an attractant for macrophages that express neuropilin 1 (NRP1)¹²⁸. Once in the region of hypoxia, macrophages stimulate fibrosis by producing growth factors such as TGF α , TGF β 1, VEGF, FGF, PDGF, tumour necrosis factor- α (TNF α), interleukin-1 (IL-1) and

IL-8, which can attract additional macrophages and mesenchymal cells, such as fibroblasts and endothelial cells, and can further activate stromal cells¹²⁹. Macrophages also directly promote the process of cancer cell intravasation into nearby blood vessels¹³⁰. In addition, they contribute to ECM turnover by secreting MMPs, which suggests that the identification of a specific macrophage subpopulation and/or soluble mediator that preferentially promotes or degrades the ECM might be an important determinant of the extent of fibrosis within a tumour¹²⁹. Taken together, the studies described above suggest that hypoxic signalling engages multiple cell types that contribute to ECM remodelling within the tumour microenvironment (FIG. 3).

Physical properties of tumour ECM

The physical properties of the tumour ECM refer to its stiffness, topography, porosity and solubility¹³¹. The physical properties of the tumour-associated ECM are not only fundamentally different from the ECM of normal tissues but are also continuously remodelled^{17,132}, which reflects the dynamic changes that occur in the tumour microenvironment, including changes in oxygen availability.

Tumour Stiffness

Tumour stroma is typically stiffer than normal stroma; for example, breast tumours can be ten times stiffer than normal breast tissue^{57,133} and expression of collagen-modifying enzymes, such as P4HA1, P4HA2, PLOD2 and LOX, that can be induced by hypoxia promote tumour stiffness^{20,21,58,65,66,82}. Stiffening of the ECM causes a reciprocal increase in the traction forces that are exerted by a cell^{134,135}. Intracellular contraction in response to ECM stiffening results in an increase in the stiffness of the actin cytoskeleton and an increase in cell migration^{57,58,136–138}. Increased tumour stiffness might regulate tumour progression in several ways; for example, increasing matrix stiffness increases RHO-generated cytoskeletal tension to promote focal adhesion assembly and to increase growth factor-dependent ERK activation^{123,135}. Moreover, matrix stiffness facilitates integrin clustering, leading to the activation of focal adhesion kinase 1 (FAK1), which in turn activates the MAP/ERK kinase (MEK; also known as MAP2K)–ERK pathway and leads to increased cell survival, migration, invasion and proliferation^{57,138,139}. Depletion of FAK1 in mouse tumour models inhibits local invasion and metastasis, which indicates that FAK1 activation might be an important mediator of stiffness-induced tumour metastasis^{140–142}. Interestingly, matrix stiffening can lead to a feed-forward signalling mechanism that further increases matrix stiffening; for example, YAP1 is required for matrix stiffening by cancer-associated fibroblasts during tumour progression¹⁴³. Conversely, stiff matrices and the contractile actin cytoskeleton further increase YAP1 activation¹⁴³.

Tumour topography

In addition to the changes in matrix stiffness that occur during tumour progression, the topography of the ECM is also highly dynamic. For example, invasive breast cancers often contain type I collagen fibres that are oriented perpendicular to the tumour margin at the invasive front, in contrast to the non-oriented fibrils that are often seen in less aggressive breast cancers^{25,26,144,145}. Straightened and aligned collagen fibres are found at sites of

breast cancer invasion — a histological pattern that is termed tumour-associated collagen signature 3 (REF. 146), which is associated with decreased patient survival²⁵. Similarly to breast cancer, in early melanomas, collagen is localized to the periphery of the tumour^{144,145,147}. By contrast, metastatic melanomas have a less compact ECM structure with no barrier between the cancer cells and the adjacent normal tissue. Highly aligned collagen fibres within a tumour might not just be predictive of the metastatic potency of the tumour but may also be causative given the finding that cancer cells preferentially invade along straightened and aligned collagen bundles^{24,26,148}.

The ECM isolated from wild-type fibroblasts that have been exposed to hypoxia is more highly aligned than the ECM deposited by fibroblasts that have been cultured under ambient conditions, and collagen fibre alignment under hypoxic conditions is abrogated in fibroblasts transfected with shRNA against HIF1 α ⁸². Breast cancer cells that have been plated on ECM produced by hypoxic cells are highly aligned and migrate with directional persistence along ECM fibres, in contrast to cells that have been plated on ECM produced by non-hypoxic cells, which migrate in a random manner²¹. Similarly, in an orthotopic mouse model of breast cancer, aligned ECM fibres are present in the perinecrotic (hypoxic) region of control tumours; by contrast, tumours that are derived from breast cancer cells expressing shRNA against HIF1 α have a disorganized ECM comprised of almost no fibrillar collagen²¹.

One potential mechanism of collagen alignment in hypoxia might involve the activity of the small GTPase RHOA, which interacts with RHO-associated protein kinase 1 (ROCK1) to mediate myosin II phosphorylation, resulting in cell contraction. RHOA-mediated ROCK1 activity is required for caveolin 1-induced cell contraction, which enables cancer cells to align with and potentially to migrate along the pre-existing collagen matrix *in vitro*¹⁴⁹. Experiments *in vivo* also indicate that caveolin 1-dependent regulation of RHOA is required for fibroblasts to produce an aligned matrix¹⁴⁹. In renal clear cell carcinoma, caveolin 1 is a direct transcriptional target of HIF1 and HIF2 (REF. 150). Moreover, hypoxia coordinately regulates the expression of RHOA and ROCK1 through HIF1- and HIF2-dependent transcription in breast cancer cells, which results in increased cell-induced matrix contraction¹⁵¹. An alternative or an additional mechanism of collagen alignment could involve LOX expression. Second harmonic generation (SHG) imaging of mammary glands that have been preconditioned with LOX-expressing fibroblasts shows that they contain more linearized collagen than the mammary glands of control mice⁵⁸. Taken together, these data suggest a model in which hypoxic cells can generate and organize an aligned ECM through multiple mechanisms (FIG. 2).

Biochemical properties of the ECM

The structure of the ECM influences the stability and the bioavailability of growth factors and cytokines¹⁴, many of which are generated under hypoxic conditions (TABLE 1). The balance between ECM-mediated confinement or distribution of growth factors and their concentration will determine their availability to cell surface receptors and consequently will regulate intracellular signalling¹⁴. A highly aligned ECM might more readily establish a chemoattractive gradient that potentiates hypoxic signalling. Conversely, a dense collagen network could function as a sink for growth factors and thereby could reduce their rate of

diffusion. Future studies to determine the ECM arrangement and composition that supports the optimal distribution of growth factors to mediate metastasis might lead to a better understanding of how the ECM influences cancer cell motility and dissemination.

Potential therapeutic interventions

HIF inhibitors

Increased expression of HIF1 α and HIF2 α has been observed in a broad range of human cancers and has been associated (in most but not all cases) with a poor prognosis^{152–154}, which suggests that use of HIF inhibitors has the potential to improve patient survival not only by blocking ECM deposition but also by blocking dozens of other HIF target genes that encode proteins involved in cell survival, angiogenesis, metabolic reprogramming, immortalization, epithelial-to-mesenchymal transition (EMT), stem cell maintenance, resistance to radiation and chemotherapy, invasion and metastasis¹⁵⁵. Although considerable work has been done to characterize the role of HIFs in experimental cancers with regards to tumour incidence and growth¹⁵², the direct requirement for HIFs in metastasis has only recently been shown in both orthotopic models and autochthonous breast tumour models^{156,157}. Conditional knockout models of HIFs have also aided our understanding of how the hypoxic tumour environment affects different cell types to drive cancer progression; for example, loss of either HIF1 α or HIF2 α in mouse vascular endothelial cells has been shown to reduce tumour growth because of impaired angiogenesis^{158,159}. Conversely, haploinsufficiency of prolyl hydroxylase domain-containing protein 2 (PHD2; also known as EGLN1) increases the HIF-driven upregulation of expression of VEGF receptor 1 (VEGFR1) in endothelial cells and decreases intratumoural hypoxia, resulting in decreased HIF1 α expression in cancer cells, which reduces pulmonary metastasis^{160,161}. The studies described above suggest that clinical trials are warranted for HIF inhibitors that show efficacy in preclinical models. It will also be crucial to determine how the activity of each HIF α subunit is affected by the potential inhibitor, given the reported functional differences between HIF1 α and HIF2 α ^{162,163}.

Two inhibitors of HIF1 α accumulation that have shown anticancer effects in preclinical models are the topoisomerase I inhibitor topotecan¹⁵¹ and the cardiac glycoside digoxin^{90,106,157}. In addition to reducing the expression of many HIF target genes, treatment of tumour-bearing mice with digoxin reduces tumour fibrosis, as well as lymph node and lung metastasis^{90,106,157}. A Phase II clinical trial for digoxin is currently being carried out for men with recurrent prostate cancer ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01162135), number: [NCT01162135](https://clinicaltrials.gov/ct2/show/study/NCT01162135)). In addition, a pilot clinical trial of topotecan ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00182676), number: [NCT00182676](https://clinicaltrials.gov/ct2/show/study/NCT00182676)) in patients with advanced cancer and HIF1 α overexpression shown on tumour biopsy was recently reported in which HIF1 α protein levels were undetectable in the post-treatment biopsy samples from four of seven patients who were studied, and decreased tumour blood flow was observed in 70% of patients by contrast-enhanced dynamic magnetic resonance imaging¹⁶⁴. Neither trial assessed treatment-induced changes to the tumour ECM. However, preclinical use of the HIF1 α inhibitor PX-478 or overexpression of a dominant-negative HIF1 α mutant showed that the increased fibrotic response identified in fat pads from mice that were fed a high-fat diet could be effectively prevented by treatment with PX-478. The

preclinical effectiveness of PX-478 has previously been established in tumour models where treatment reduces tumour growth¹⁶⁵ and it will be interesting to determine the effect of PX-478 on tumour fibrosis.

Targeting fibrosis

Blocking collagen hydroxylases or lysyl hydroxylases might also provide a strategy to reduce tumour fibrosis. P4Hs have been regarded as attractive targets for the pharmacological inhibition of collagen accumulation in fibrotic diseases and severe scarring. P4Hs belong to a superfamily of dioxygenases that use oxygen and α -ketoglutarate (also known as 2-oxoglutarate) as substrates. P4Hs are competitively inhibited by α -ketoglutarate analogues, including *N*-oxalylglycine, pyridine 2,4-dicarboxylate and pyridine 2,5-dicarboxylate, coumalic acid and 3,4-ethyl dihydroxybenzoate (EDHB)¹⁶⁶. As these agents are not selective for collagen hydroxylases, it is probable that they will also inhibit the HIF PHDs and will potentially promote HIF expression. Preclinical testing will have to be carried out to determine their potential usefulness in preventing metastasis. Minoxidil has been shown to decrease the expression of PLOD mRNAs and the activity of PLOD proteins and thereby to inhibit fibrosis¹⁶⁷. In a mouse model of sarcoma, minoxidil treatment reduced tumour fibrosis and suppressed lung metastasis⁶⁷.

Studies that target LOX family members have focused on blocking the enzymatic activity of these proteins using competitive inhibitors such as β APN¹⁶⁸ or using neutralizing antibodies, which abrogate lung and liver metastases in xenograft and transgenic mouse models⁹³. D-penicillamine (DPEN), which is a LOXL2 inhibitor, was developed and used to treat rheumatoid arthritis and biliary cirrhosis but it does have some unintended side effects¹⁶⁹. A more selective inhibitory monoclonal antibody (AB0023) against LOXL2 has been developed and was effective in reducing fibrosis in primary and metastatic xenografts as well as in liver and lung fibrosis models in mice¹⁷⁰. The ECM of tumours from mice that had been treated with AB0023 showed a marked reduction in crosslinked collagen compared with results in mice that had been treated with the lysyl oxidase inhibitor β APN¹⁷⁰. Treatment with AB0023 also resulted in a marked reduction in the number of activated fibroblasts and endothelial cells and led to a decreased production of growth factors and cytokines¹⁷⁰. The safety of the humanized version of AB0023, AB0024 (also known as simtuzumab) has been tested in Phase I dose escalation trials in patients with advanced solid tumours¹⁷¹ ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01323933), number: [NCT01323933](https://clinicaltrials.gov/ct2/show/study/NCT01323933)) and with idiopathic pulmonary fibrosis ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01362231), number: [NCT01362231](https://clinicaltrials.gov/ct2/show/study/NCT01362231)). Enrolment for a Phase II clinical trial in patients with idiopathic pulmonary fibrosis ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01759511), number: [NCT01759511](https://clinicaltrials.gov/ct2/show/study/NCT01759511)) has begun. Additional non-selective inhibitors of lysyl oxidases also include *p*-halobenzylamines, ethylenediamine and homocysteine thiolactone^{93,172}.

Conclusions

Although tumours from two different patients might have similar genetic alterations, these tumours will develop in different microenvironmental contexts¹³, which suggests that hypoxia and the ECM are important in contributing to tumour heterogeneity, which might influence metastatic outcome. Hypoxic regions within the tumour microenvironment can

simultaneously relay signals to cancer cells and cells that have been recruited to the local environment directly (for example, by transcriptional reprogramming), through paracrine signalling events and, as highlighted in this Opinion article, by establishing a hypoxia-induced ECM that is fibrotic, stiff and aligned, which are all properties that promote metastatic dissemination^{11,173}. Further studies are needed to investigate the mechanisms by which the hypoxia-induced ECM might have a role in dynamically maintaining and distributing growth factors that provide chemotactic signals to recruit cells to the primary tumour and that promote the intravasation of cancer cells for dissemination to distant organs. Advances in imaging techniques, such as intravital microscopy, have the potential to shed light on this issue and might direct our research to appropriate targeting strategies that will be most beneficial to prevent metastasis¹⁷⁴.

It is also important to consider that the collagen-modifying enzymes discussed in this Opinion article might have alternative roles in cancer progression that are not limited to fibrosis⁹³. For example, LOX has a role in PDGF and insulin growth factor 1 (IGF1) signalling, but its precise mechanisms of action remain to be elucidated¹⁷⁵. Additional regulators of the collagen hydroxylases and lysyl oxidases remain to be determined; for example, TGFβ1 has been shown to influence LOX expression⁹³. Furthermore, the role of collagen in the regulation of ECM composition and assembly (and vice versa) is also unknown; for example, collagen I-containing fibrils do not form in the absence of fibronectin *in vivo* and fibronectin fibril assembly has a reciprocal requirement for collagen¹⁷⁶. Whether fibronectin–procollagen interactions are established before the molecules are secreted is unknown and suggests that the complex regulation and dynamics of the ECM need to be carefully investigated in order to design strategies that target the ECM. Another important consideration will be the receptors that interact with the ECM molecules. A recent study regarding the fibrillar collagen receptor discoidin domain receptor 2 (DDR2) has shown that DDR2 is required for breast cancer cell invasion and migration *in vitro* and for metastasis *in vivo* by promoting the stabilization of SNAIL1 (REF. 177).

Future preclinical studies are warranted to identify new inhibitors and/or to identify optimal combinations of existing inhibitors that can block hypoxic changes to the ECM while maintaining the integrity of the ECM in healthy tissues. One major obstacle in the field of cancer therapeutics for metastasis is the definition of success. Many agents effectively target tumour growth but fail to prevent metastasis, which is the major cause of cancer mortality. For metastasis inhibitors to be tested in early phase clinical trials patients that do not already have metastatic disease will have to be included in order to have meaningful end points and to establish efficacy in metastasis prevention.

Acknowledgments

D.M.G. is supported by funding from the National Cancer Institute (NCI) (K99CA181352) and is a Susan G. Komen postdoctoral fellow. Cancer research in the Wirtz laboratory is supported by the US National Institutes of Health (grants U54-CA143868 and R01-CA174388). Cancer Research in the Semenza laboratory is supported by the American Cancer Society, NCI grant U54-CA143868, the Department of Defense Breast Cancer Research Program and the Johns Hopkins Institute for Cell Engineering. G.L.S. is the C. Michael Armstrong Professor at the Johns Hopkins University School of Medicine, USA, and an American Cancer Society Research Professor. D.W. is the T.H. Smoot Professor and Vice Provost for Research at Johns Hopkins University.

References

1. Vaupel P, Mayer A, Hockel M. Tumour hypoxia and malignant progression. *Methods Enzymol.* 2004; 381:335–354. [PubMed: 15063685]
2. Sorg BS, Hardee ME, Agarwal N, Moeller BJ, Dewhirst MW. Spectral imaging facilitates visualization and measurements of unstable and abnormal microvascular oxygen transport in tumours. *J Biomed Opt.* 2008; 13:014026. [PubMed: 18315384]
3. Vaupel P. Prognostic potential of the pre-therapeutic tumour oxygenation status. *Adv Exp Med Biol.* 2009; 645:241–246. [PubMed: 19227477]
4. Vaupel P, Hockel M, Mayer A. Detection and characterization of tumour hypoxia using pO₂ histography. *Antioxid Redox Signal.* 2007; 9:1221–1235. [PubMed: 17536958]
5. Semenza GL. Oxygen sensing, hypoxia-inducible factors, and disease pathophysiology. *Annu Rev Pathol.* 2014; 9:47–71. [PubMed: 23937437]
6. Bertout JA, Patel SA, Simon MC. The impact of O₂ availability on human cancer. *Nature Rev Cancer.* 2008; 8:967–975. [PubMed: 18987634]
7. Finger EC, Giaccia AJ. Hypoxia, inflammation, and the tumour microenvironment in metastatic disease. *Cancer Metastasis Rev.* 2010; 29:285–293. [PubMed: 20393783]
8. Semenza GL. Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics. *Oncogene.* 2010; 29:625–634. [PubMed: 19946328]
9. Semenza GL. Molecular mechanisms mediating metastasis of hypoxic breast cancer cells. *Trends Mol Med.* 2012; 18:534–543. [PubMed: 22921864]
10. Semenza GL. Hypoxia-inducible factors: mediators of cancer progression and targets for cancer therapy. *Trends Pharmacol Sci.* 2012; 33:207–214. [PubMed: 22398146]
11. Semenza GL. Cancer-stromal cell interactions mediated by hypoxia-inducible factors promote angiogenesis, lymphangiogenesis, and metastasis. *Oncogene.* 2013; 32:4057–4063. [PubMed: 23222717]
12. Casazza A, et al. Tumour stroma: a complexity dictated by the hypoxic tumour microenvironment. *Oncogene.* 2014; 33:1743–1754. [PubMed: 23604130]
13. Lu P, Weaver VM, Werb Z. The extracellular matrix: a dynamic niche in cancer progression. *J Cell Biol.* 2012; 196:395–406. [PubMed: 22351925]
14. Hynes RO. The extracellular matrix: not just pretty fibrils. *Science.* 2009; 326:1216–1219. [PubMed: 19965464]
15. Bissell MJ, Radisky DC, Rizki A, Weaver VM, Petersen OW. The organizing principle: microenvironmental influences in the normal and malignant breast. *Differentiation.* 2002; 70:537–546. [PubMed: 12492495]
16. Cretu A, Brooks PC. Impact of the non-cellular tumour microenvironment on metastasis: potential therapeutic and imaging opportunities. *J Cell Physiol.* 2007; 213:391–402. [PubMed: 17657728]
17. van Kempen LC, Ruiter DJ, van Muijen GN, Coussens LM. The tumour microenvironment: a critical determinant of neoplastic evolution. *Eur J Cell Biol.* 2003; 82:539–548. [PubMed: 14703010]
18. Lochter A, Bissell MJ. Involvement of extracellular matrix constituents in breast cancer. *Semin Cancer Biol.* 1995; 6:165–173. [PubMed: 7495985]
19. Naba A, et al. The matrisome: *in silico* definition and *in vivo* characterization by proteomics of normal and tumour extracellular matrices. *Mol Cell Proteomics.* 2012; 11:M111.014647. [PubMed: 22159717]
20. Gilkes DM, et al. Procollagen lysyl hydroxylase 2 is essential for hypoxia-induced breast cancer metastasis. *Mol Cancer Res.* 2013; 11:456–466. [PubMed: 23378577]
21. Gilkes DM, et al. Collagen prolyl hydroxylases are essential for breast cancer metastasis. *Cancer Res.* 2013; 73:3285–3296. [PubMed: 23539444]
22. Hielscher AC, Qiu C, Gerecht S. Breast cancer cell-derived matrix supports vascular morphogenesis. *Am J Physiol Cell Physiol.* 2012; 302:C1243–C1256. [PubMed: 22277754]
23. Langness U, Udenfriend S. Collagen biosynthesis in nonfibroblastic cell lines. *Proc Natl Acad Sci USA.* 1974; 71:50–51. [PubMed: 4359330]

24. Wyckoff JB, et al. Direct visualization of macrophage-assisted tumour cell intravasation in mammary tumours. *Cancer Res.* 2007; 67:2649–2656. [PubMed: 17363585]
25. Conklin MW, et al. Aligned collagen is a prognostic signature for survival in human breast carcinoma. *Am J Pathol.* 2011; 178:1221–1232. [PubMed: 21356373]
26. Provenzano PP, et al. Collagen reorganization at the tumour-stromal interface facilitates local invasion. *BMC Med.* 2006; 4:38. [PubMed: 17190588]
27. Provenzano PP, Inman DR, Eliceiri KW, Trier SM, Keely PJ. Contact guidance mediated three-dimensional cell migration is regulated by Rho/ROCK-dependent matrix reorganization. *Biophys J.* 2008; 95:5374–5384. [PubMed: 18775961]
28. Frantz C, Stewart KM, Weaver VM. The extracellular matrix at a glance. *J Cell Sci.* 2010; 123:4195–4200. [PubMed: 21123617]
29. Ramaswamy S, Ross KN, Lander ES, Golub TR. A molecular signature of metastasis in primary solid tumours. *Nature Genet.* 2003; 33:49–54. [PubMed: 12469122]
30. Eckhardt BL, et al. Genomic analysis of a spontaneous model of breast cancer metastasis to bone reveals a role for the extracellular matrix. *Mol Cancer Res.* 2005; 3:1–13. [PubMed: 15671244]
31. Allinen M, et al. Molecular characterization of the tumour microenvironment in breast cancer. *Cancer Cell.* 2004; 6:17–32. [PubMed: 15261139]
32. Finak G, et al. Stromal gene expression predicts clinical outcome in breast cancer. *Nature Med.* 2008; 14:518–527. [PubMed: 18438415]
33. Qiu TH, et al. Global expression profiling identifies signatures of tumour virulence in MMTV-PyMT-transgenic mice: Correlation to human disease. *Cancer Res.* 2004; 64:5973–5981. [PubMed: 15342376]
34. Ma XJ, Dahiya S, Richardson E, Erlander M, Sgroi DC. Gene expression profiling of the tumour microenvironment during breast cancer progression. *Breast Cancer Res.* 2009; 11:R7. [PubMed: 19187537]
35. Chang HY, et al. Robustness, scalability, and integration of a wound-response gene expression signature in predicting breast cancer survival. *Proc Natl Acad Sci USA.* 2005; 102:3738–3743. [PubMed: 15701700]
36. Chang HY, et al. Gene expression signature of fibroblast serum response predicts human cancer progression: similarities between tumours and wounds. *PLoS Biol.* 2004; 2:E7. [PubMed: 14737219]
37. Artinian V, Kvale PA. Cancer and interstitial lung disease. *Curr Opin Pulm Med.* 2004; 10:425–434. [PubMed: 15316443]
38. Bartow SA, Pathak DR, Mettler FA. Radiographic microcalcification and parenchymal patterns as indicators of histologic ‘high-risk’ benign breast disease. *Cancer.* 1990; 66:1721–1725. [PubMed: 2208027]
39. Bataller R, Brenner DA. Liver fibrosis. *J Clin Invest.* 2005; 115:209–218. [PubMed: 15690074]
40. Bissell DM. Chronic liver injury, TGF β , and cancer. *Exp Mol Med.* 2001; 33:179–190. [PubMed: 11795478]
41. Boyd NF, Jensen HM, Cooke G, Han HL. Relationship between mammographic and histological risk factors for breast cancer. *J Natl Cancer Inst.* 1992; 84:1170–1179. [PubMed: 1635085]
42. Boyd NF, et al. Mammographic densities and the prevalence and incidence of histological types of benign breast disease. Reference Pathologists of the Canadian National Breast Screening Study. *Eur J Cancer Prev.* 2000; 9:15–24. [PubMed: 10777006]
43. Boyd NF, et al. Heritability of mammographic density, a risk factor for breast cancer. *N Engl J Med.* 2002; 347:886–894. [PubMed: 12239257]
44. Shapiro FD, Eyre DR. Collagen polymorphism in extracellular matrix of human osteosarcoma. *J Natl Cancer Inst.* 1982; 69:1009–1016. [PubMed: 6957647]
45. Coussens LM, et al. Inflammatory mast cells upregulate angiogenesis during squamous epithelial carcinogenesis. *Genes Dev.* 1999; 13:1382–1397. [PubMed: 10364156]
46. Jussila T, Kaupila S, Risteli L, Risteli J, Stenback F. Collagen formation in extracellular matrix of transplants of human transformed keratinocyte cell lines. *Anticancer Res.* 2002; 22:1705–1711. [PubMed: 12168857]

47. Gould VE, Koukoulis GK, Virtanen I. Extracellular matrix proteins and their receptors in the normal, hyperplastic and neoplastic breast. *Cell Differ Dev.* 1990; 32:409–416. [PubMed: 1711920]
48. Zhu GG, et al. Immunohistochemical study of type I collagen and type I pN-collagen in benign and malignant ovarian neoplasms. *Cancer.* 1995; 75:1010–1017. [PubMed: 7842402]
49. Kauppila S, Stenback F, Risteli J, Jukkola A, Risteli L. Aberrant type I and type III collagen gene expression in human breast cancer *in vivo*. *J Pathol.* 1998; 186:262–268. [PubMed: 10211114]
50. Huijbers II, et al. A role for fibrillar collagen deposition and the collagen internalization receptor endo180 in glioma invasion. *PLoS ONE.* 2010; 5:e9808. [PubMed: 20339555]
51. Colpaert CG, et al. The presence of a fibrotic focus in invasive breast carcinoma correlates with the expression of carbonic anhydrase IX and is a marker of hypoxia and poor prognosis. *Breast Cancer Res Treat.* 2003; 81:137–147. [PubMed: 14572156]
52. Trastour C, et al. HIF1 α and CA IX staining in invasive breast carcinomas: prognosis and treatment outcome. *Int J Cancer.* 2007; 120:1451–1458. [PubMed: 17245699]
53. Hasebe T, Tsuda H, Tsubono Y, Imoto S, Mukai K. Fibrotic focus in invasive ductal carcinoma of the breast: a histopathological prognostic parameter for tumour recurrence and tumour death within three years after the initial operation. *Jpn J Cancer Res.* 1997; 88:590–599. [PubMed: 9263537]
54. van der Rest M, Garrone R. Collagen family of proteins. *FASEB J.* 1991; 5:2814–2823. [PubMed: 1916105]
55. Fraley SI, et al. A distinctive role for focal adhesion proteins in three-dimensional cell motility. *Nature Cell Biol.* 2010; 12:598–604. [PubMed: 20473295]
56. Fraley SI, Feng Y, Giri A, Longmore GD, Wirtz D. Dimensional and temporal controls of three-dimensional cell migration by zyxin and binding partners. *Nature Commun.* 2012; 3:719. [PubMed: 22395610]
57. Paszek MJ, et al. Tensional homeostasis and the malignant phenotype. *Cancer Cell.* 2005; 8:241–254. [PubMed: 16169468]
58. Levental KR, et al. Matrix crosslinking forces tumour progression by enhancing integrin signalling. *Cell.* 2009; 139:891–906. [PubMed: 19931152]
59. Liu X, et al. A targeted mutation at the known collagenase cleavage site in mouse type I collagen impairs tissue remodelling. *J Cell Biol.* 1995; 130:227–237. [PubMed: 7790374]
60. Provenzano PP, et al. Collagen density promotes mammary tumour initiation and progression. *BMC Med.* 2008; 6:11. [PubMed: 18442412]
61. Lin EY, et al. Progression to malignancy in the polyoma middle T oncoprotein mouse breast cancer model provides a reliable model for human diseases. *Am J Pathol.* 2003; 163:2113–2126. [PubMed: 14578209]
62. Mao Y, Keller ET, Garfield DH, Shen K, Wang J. Stromal cells in tumour microenvironment and breast cancer. *Cancer Metastasis Rev.* 2013; 32:303–315. [PubMed: 23114846]
63. Kalluri R, Zeisberg M. Fibroblasts in cancer. *Nature Rev Cancer.* 2006; 6:392–401. [PubMed: 16572188]
64. Shekhar MP, Pauley R, Heppner G. Host microenvironment in breast cancer development: extracellular matrix-stromal cell contribution to neoplastic phenotype of epithelial cells in the breast. *Breast Cancer Res.* 2003; 5:130–135. [PubMed: 12793893]
65. Cox TR, et al. LOX-mediated collagen crosslinking is responsible for fibrosis-enhanced metastasis. *Cancer Res.* 2013; 73:1721–1732. [PubMed: 23345161]
66. Erler JT, et al. Lysyl oxidase is essential for hypoxia-induced metastasis. *Nature.* 2006; 440:1222–1226. [PubMed: 16642001]
67. Eisinger-Mathason TS, et al. Hypoxia-dependent modification of collagen networks promotes sarcoma metastasis. *Cancer Discov.* 2013; 3:1190–1205. [PubMed: 23906982]
68. Xiong G, Deng L, Zhu J, Rychahou PG, Xu R. Prolyl-4-hydroxylase α -subunit 2 promotes breast cancer progression and metastasis by regulating collagen deposition. *BMC Cancer.* 2014; 14:1. [PubMed: 24383403]

69. Halberg N, et al. Hypoxia-inducible factor 1 α induces fibrosis and insulin resistance in white adipose tissue. *Mol Cell Biol*. 2009; 29:4467–4483. [PubMed: 19546236]
70. Higgins DF, et al. Hypoxia promotes fibrogenesis *in vivo* via HIF1 stimulation of epithelial-to-mesenchymal transition. *J Clin Invest*. 2007; 117:3810–3820. [PubMed: 18037992]
71. Moon JO, Welch TP, Gonzalez FJ, Copple BL. Reduced liver fibrosis in hypoxia-inducible factor-1 α -deficient mice. *Am J Physiol Gastrointest Liver Physiol*. 2009; 296:G582–G592. [PubMed: 19136383]
72. Falanga V, et al. Low oxygen tension increases mRNA levels of α 1 (I) procollagen in human dermal fibroblasts. *J Cell Physiol*. 1993; 157:408–412. [PubMed: 8227170]
73. Tamamori M, Ito H, Hiroe M, Marumo F, Hata RI. Stimulation of collagen synthesis in rat cardiac fibroblasts by exposure to hypoxic culture conditions and suppression of the effect by natriuretic peptides. *Cell Biol Int*. 1997; 21:175–180. [PubMed: 9151994]
74. Norman JT, Clark IM, Garcia PL. Hypoxia promotes fibrogenesis in human renal fibroblasts. *Kidney Int*. 2000; 58:2351–2366. [PubMed: 11115069]
75. Berg JT, Breen EC, Fu Z, Mathieu-Costello O, West JB. Alveolar hypoxia increases gene expression of extracellular matrix proteins and platelet-derived growth factor-B in lung parenchyma. *Am J Respir Crit Care Med*. 1998; 158:1920–1928. [PubMed: 9847287]
76. Myllyharju J, Kivirikko KI. Collagens, modifying enzymes and their mutations in humans, flies and worms. *Trends Genet*. 2004; 20:33–43. [PubMed: 14698617]
77. Kivirikko KI, Pihlajaniemi T. Collagen hydroxylases and the protein disulphide isomerase subunit of prolyl 4-hydroxylases. *Adv Enzymol Relat Areas Mol Biol*. 1998; 72:325–398. [PubMed: 9559057]
78. Myllyharju J. Prolyl 4-hydroxylases, the key enzymes of collagen biosynthesis. *Matrix Biol*. 2003; 22:15–24. [PubMed: 12714038]
79. Annunen P, et al. Cloning of the human prolyl 4-hydroxylase- α subunit isoform- α (II) and characterization of the type II enzyme tetramer. The- α (I) and- α (II) subunits do not form a mixed- α (I) α (II) β 2 tetramer. *J Biol Chem*. 1997; 272:17342–17348. [PubMed: 9211872]
80. Kukkola L, Hieta R, Kivirikko KI, Myllyharju J. Identification and characterization of a third human, rat, and mouse collagen prolyl 4-hydroxylase isoenzyme. *J Biol Chem*. 2003; 278:47685–47693. [PubMed: 14500733]
81. van der Slot AJ, et al. Increased formation of pyridinoline crosslinks due to higher telopeptide lysyl hydroxylase levels is a general fibrotic phenomenon. *Matrix Biol*. 2004; 23:251–257. [PubMed: 15296939]
82. Gilkes DM, Bajpai S, Chaturvedi P, Wirtz D, Semenza GL. Hypoxia-inducible factor 1 (HIF1) promotes extracellular matrix remodeling under hypoxic conditions by inducing P4HA1, P4HA2, and PLOD2 expression in fibroblasts. *J Biol Chem*. 2013; 288:10819–10829. [PubMed: 23423382]
83. Hofbauer KH, et al. Oxygen tension regulates the expression of a group of procollagen hydroxylases. *Eur J Biochem*. 2003; 270:4515–4522. [PubMed: 14622280]
84. Elvidge GP, et al. Concordant regulation of gene expression by hypoxia and 2-oxoglutarate-dependent dioxygenase inhibition: the role of HIF1 α , HIF2 α , and other pathways. *J Biol Chem*. 2006; 281:15215–15226. [PubMed: 16565084]
85. Aro E, et al. Hypoxia-inducible factor 1 (HIF1) but not HIF2 is essential for hypoxic induction of collagen prolyl 4-hydroxylases in primary newborn mouse epiphyseal growth plate chondrocytes. *J Biol Chem*. 2012; 287:37134–37144. [PubMed: 22930750]
86. Bentovim L, Amarilio R, Zelzer E. HIF1 α is a central regulator of collagen hydroxylation and secretion under hypoxia during bone development. *Development*. 2012; 139:4473–4483. [PubMed: 23095889]
87. Gordon MK, Hahn RA. Collagens. *Cell Tissue Res*. 2010; 339:247–257. [PubMed: 19693541]
88. Tanjore H, Kalluri R. The role of type IV collagen and basement membranes in cancer progression and metastasis. *Am J Pathol*. 2006; 168:715–717. [PubMed: 16507886]
89. Wong CC, et al. Hypoxia-inducible factor 1 is a master regulator of breast cancer metastatic niche formation. *Proc Natl Acad Sci USA*. 2011; 108:16369–16374. [PubMed: 21911388]

90. Wong CC, et al. Inhibitors of hypoxia-inducible factor 1 block breast cancer metastatic niche formation and lung metastasis. *J Mol Med.* 2012; 90:803–815. [PubMed: 22231744]
91. Erler JT, et al. Hypoxia-induced lysyl oxidase is a critical mediator of bone marrow cell recruitment to form the premetastatic niche. *Cancer Cell.* 2009; 15:35–44. [PubMed: 19111879]
92. Schietke R, et al. The lysyl oxidases LOX and LOXL2 are necessary and sufficient to repress E-cadherin in hypoxia: insights into cellular transformation processes mediated by HIF1. *J Biol Chem.* 2010; 285:6658–6669. [PubMed: 20026874]
93. Barker HE, Cox TR, Erler JT. The rationale for targeting the LOX family in cancer. *Nature Rev Cancer.* 2012; 12:540–552. [PubMed: 22810810]
94. Munoz-Najar UM, Neurath KM, Vumbaca F, Claffey KP. Hypoxia stimulates breast carcinoma cell invasion through MT1-MMP and MMP2 activation. *Oncogene.* 2006; 25:2379–2392. [PubMed: 16369494]
95. Krishnamachary B, et al. Regulation of colon carcinoma cell invasion by hypoxia-inducible factor 1. *Cancer Res.* 2003; 63:1138–1143. [PubMed: 12615733]
96. Choi JY, Jang YS, Min SY, Song JY. Overexpression of MMP9 and HIF1 α in breast cancer cells under hypoxic conditions. *J Breast Cancer.* 2011; 14:88–95. [PubMed: 21847402]
97. Petrella BL, Lohi J, Brinckerhoff CE. Identification of membrane type-1 matrix metalloproteinase as a target of hypoxia-inducible factor 2 α in von Hippel–Lindau renal cell carcinoma. *Oncogene.* 2005; 24:1043–1052. [PubMed: 15592504]
98. Graham CH, Forsdike J, Fitzgerald CJ, Macdonald-Goodfellow S. Hypoxia-mediated stimulation of carcinoma cell invasiveness via upregulation of urokinase receptor expression. *Int J Cancer.* 1999; 80:617–623. [PubMed: 9935166]
99. Buchler P, et al. Transcriptional regulation of urokinase-type plasminogen activator receptor by hypoxia-inducible factor 1 is crucial for invasion of pancreatic and liver cancer. *Neoplasia.* 2009; 11:196–206. [PubMed: 19177204]
100. Kim J, Yu W, Kovalski K, Ossowski L. Requirement for specific proteases in cancer cell intravasation as revealed by a novel semiquantitative PCR-based assay. *Cell.* 1998; 94:353–362. [PubMed: 9708737]
101. Dvorak HF. Tumours: wounds that do not heal. Similarities between tumour stroma generation and wound healing. *N Engl J Med.* 1986; 315:1650–1659. [PubMed: 3537791]
102. Lokmic, Z.; Musyoka, J.; Hewitson, TD.; Darby, IA. International Review of Cell and Molecular Biology. Kwang, WJ., editor. Vol. 296. Academic Press; 2012. p. 139-185.Ch.3
103. Zhang X, et al. Impaired angiogenesis and mobilization of circulating angiogenic cells in HIF-1 α heterozygous-null mice after burn wounding. *Wound Repair Regen.* 2010; 18:193–201. [PubMed: 20163569]
104. Velnar T, Bailey T, Smrkolj V. The wound healing process: an overview of the cellular and molecular mechanisms. *J Int Med Res.* 2009; 37:1528–1542. [PubMed: 19930861]
105. Rey S, Semenza GL. Hypoxia-inducible factor 1-dependent mechanisms of vascularization and vascular remodelling. *Cardiovasc Res.* 2010; 86:236–242. [PubMed: 20164116]
106. Schito L, et al. Hypoxia-inducible factor 1-dependent expression of platelet-derived growth factor B promotes lymphatic metastasis of hypoxic breast cancer cells. *Proc Natl Acad Sci USA.* 2012; 109:E2707–E2716. [PubMed: 23012449]
107. Manalo DJ, et al. Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF1. *Blood.* 2005; 105:659–669. [PubMed: 15374877]
108. Kelly BD, et al. Cell type-specific regulation of angiogenic growth factor gene expression and induction of angiogenesis in nonischemic tissue by a constitutively active form of hypoxia-inducible factor 1. *Circ Res.* 2003; 93:1074–1081. [PubMed: 14576200]
109. Forsythe JA, et al. Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. *Mol Cell Biol.* 1996; 16:4604–4613. [PubMed: 8756616]
110. Ceradini DJ, et al. Progenitor cell trafficking is regulated by hypoxic gradients through HIF1 induction of SDF1. *Nature Med.* 2004; 10:858–864. [PubMed: 15235597]
111. Simon MP, Tournaire R, Pouyssegur J. The angiopoietin-2 gene of endothelial cells is upregulated in hypoxia by a HIF binding site located in its first intron and by the central factors GATA2 and ETS1. *J Cell Physiol.* 2008; 217:809–818. [PubMed: 18720385]

112. Chaturvedi P, et al. Hypoxia-inducible factor-dependent breast cancer-mesenchymal stem cell bidirectional signalling promotes metastasis. *J Clin Invest*. 2013; 123:189–205. [PubMed: 23318994]
113. Kondo S, et al. Connective tissue growth factor increased by hypoxia may initiate angiogenesis in collaboration with matrix metalloproteinases. *Carcinogenesis*. 2002; 23:769–776. [PubMed: 12016149]
114. Higgins DF, et al. Hypoxic induction of CTGF is directly mediated by HIF1. *Am J Physiol Renal Physiol*. 2004; 287:F1223–F1232. [PubMed: 15315937]
115. Le YJ, Corry PM. Hypoxia-induced bFGF gene expression is mediated through the JNK signal transduction pathway. *Mol Cell Biochem*. 1999; 202:1–8. [PubMed: 10705989]
116. Lappi-Blanco E, Soini Y, Kinnula V, Paakko P. VEGF and bFGF are highly expressed in intraluminal fibromyxoid lesions in bronchiolitis obliterans organizing pneumonia. *J Pathol*. 2002; 196:220–227. [PubMed: 11793374]
117. Lasky JA, et al. Connective tissue growth factor mRNA expression is upregulated in bleomycin-induced lung fibrosis. *Am J Physiol*. 1998; 275:L365–371. [PubMed: 9700098]
118. Bonner JC. Regulation of PDGF and its receptors in fibrotic diseases. *Cytokine Growth Factor Rev*. 2004; 15:255–273. [PubMed: 15207816]
119. Phillips RJ, et al. Circulating fibrocytes traffic to the lungs in response to CXCL12 and mediate fibrosis. *J Clin Invest*. 2004; 114:438–446. [PubMed: 15286810]
120. Hayashida K, et al. Bone marrow-derived cells contribute to pulmonary vascular remodeling in hypoxia-induced pulmonary hypertension. *Chest*. 2005; 127:1793–1798. [PubMed: 15888860]
121. Mehrad B, Burdick MD, Strieter RM. Fibrocyte CXCR4 regulation as a therapeutic target in pulmonary fibrosis. *Int J Biochem Cell Biol*. 2009; 41:1708–1718. [PubMed: 19433312]
122. Karnoub AE, et al. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. *Nature*. 2007; 449:557–563. [PubMed: 17914389]
123. Brown LF, et al. Vascular stroma formation in carcinoma *in situ*, invasive carcinoma, and metastatic carcinoma of the breast. *Clin Cancer Res*. 1999; 5:1041–1056. [PubMed: 10353737]
124. Wels J, Kaplan RN, Rafii S, Lyden D. Migratory neighbours and distant invaders: tumour-associated niche cells. *Genes Dev*. 2008; 22:559–574. [PubMed: 18316475]
125. Murdoch C, Giannoudis A, Lewis CE. Mechanisms regulating the recruitment of macrophages into hypoxic areas of tumours and other ischemic tissues. *Blood*. 2004; 104:2224–2234. [PubMed: 15231578]
126. Cramer T, et al. HIF1 α is essential for myeloid cell-mediated inflammation. *Cell*. 2003; 112:645–657. [PubMed: 12628185]
127. Rezvani HR, et al. HIF1 α in epidermis: oxygen sensing, cutaneous angiogenesis, cancer, and non-cancer disorders. *J Invest Dermatol*. 2011; 131:1793–1805. [PubMed: 21633368]
128. Casazza A, et al. Impeding macrophage entry into hypoxic tumour areas by SEMA3A/NRP1 signalling blockade inhibits angiogenesis and restores antitumor immunity. *Cancer Cell*. 2013; 24:695–709. [PubMed: 24332039]
129. Wynn TA, Barron L. Macrophages: master regulators of inflammation and fibrosis. *Semin Liver Dis*. 2010; 30:245–257. [PubMed: 20665377]
130. Joyce JA, Pollard JW. Microenvironmental regulation of metastasis. *Nature Rev Cancer*. 2009; 9:239–252. [PubMed: 19279573]
131. Wirtz D, Konstantopoulos K, Searson PC. The physics of cancer: the role of physical interactions and mechanical forces in metastasis. *Nature Rev Cancer*. 2011; 11:512–522. [PubMed: 21701513]
132. Clarijs R, Ruiter DJ, De Waal RM. Pathophysiological implications of stroma pattern formation in uveal melanoma. *J Cell Physiol*. 2003; 194:267–271. [PubMed: 12548547]
133. Lopez JI, Kang I, You WK, McDonald DM, Weaver VM. *In situ* force mapping of mammary gland transformation. *Integr Biol*. 2011; 3:910–921.
134. Kim DH, et al. Actin cap associated focal adhesions and their distinct role in cellular mechanosensing. *Sci Rep*. 2012; 2:555. [PubMed: 22870384]

135. Samuel MS, et al. Actomyosin-mediated cellular tension drives increased tissue stiffness and beta-catenin activation to induce epidermal hyperplasia and tumour growth. *Cancer Cell*. 2011; 19:776–791. [PubMed: 21665151]
136. Wozniak MA, Desai R, Solski PA, Der CJ, Keely PJ. ROCK-generated contractility regulates breast epithelial cell differentiation in response to the physical properties of a three-dimensional collagen matrix. *J Cell Biol*. 2003; 163:583–595. [PubMed: 14610060]
137. Sahai E, Marshall CJ. Differing modes of tumour cell invasion have distinct requirements for Rho/ROCK signalling and extracellular proteolysis. *Nature Cell Biol*. 2003; 5:711–719. [PubMed: 12844144]
138. Kim DH, Wirtz D. Focal adhesion size uniquely predicts cell migration. *FASEB J*. 2013; 27:1351–1361. [PubMed: 23254340]
139. Provenzano PP, Keely PJ. The role of focal adhesion kinase in tumour initiation and progression. *Cell Adh Migr*. 2009; 3:347–350. [PubMed: 19690467]
140. Provenzano PP, Inman DR, Eliceiri KW, Beggs HE, Keely PJ. Mammary epithelial-specific disruption of focal adhesion kinase retards tumour formation and metastasis in a transgenic mouse model of human breast cancer. *Am J Pathol*. 2008; 173:1551–1565. [PubMed: 18845837]
141. Lahlou H, et al. Mammary epithelial-specific disruption of the focal adhesion kinase blocks mammary tumour progression. *Proc Natl Acad Sci USA*. 2007; 104:20302–20307. [PubMed: 18056629]
142. Baker AM, Bird D, Lang G, Cox TR, Erler JT. Lysyl oxidase enzymatic function increases stiffness to drive colorectal cancer progression through FAK. *Oncogene*. 2013; 32:1863–1868. [PubMed: 22641216]
143. Calvo F, et al. Mechanotransduction and YAP-dependent matrix remodelling is required for the generation and maintenance of cancer-associated fibroblasts. *Nature Cell Biol*. 2013; 15:637–646. [PubMed: 23708000]
144. Tamimi SO, Ahmed A. Stromal changes in early invasive and non-invasive breast carcinoma: an ultrastructural study. *J Pathol*. 1986; 150:43–49. [PubMed: 3023597]
145. Tamimi SO, Ahmed A. Stromal changes in invasive breast carcinoma: an ultrastructural study. *J Pathol*. 1987; 153:163–170. [PubMed: 3694320]
146. Conklin MW, Keely PJ. Why the stroma matters in breast cancer: insights into breast cancer patient outcomes through the examination of stromal biomarkers. *Cell Adh Migr*. 2012; 6:249–260. [PubMed: 22568982]
147. Ruiter D, Bogenrieder T, Elder D, Herlyn M. Melanoma–stroma interactions: structural and functional aspects. *Lancet Oncol*. 2002; 3:35–43. [PubMed: 11905603]
148. Wang W, et al. Single cell behaviour in metastatic primary mammary tumours correlated with gene expression patterns revealed by molecular profiling. *Cancer Res*. 2002; 62:6278–6288. [PubMed: 12414658]
149. Goetz JG, et al. Biomechanical remodeling of the microenvironment by stromal caveolin 1 favours tumour invasion and metastasis. *Cell*. 2011; 146:148–163. [PubMed: 21729786]
150. Wang Y, et al. Hypoxia promotes ligand-independent EGF receptor signalling via hypoxia-inducible factor-mediated upregulation of caveolin 1. *Proc Natl Acad Sci USA*. 2012; 109:4892–4897. [PubMed: 22411794]
151. Gilkes DM, et al. Hypoxia-inducible factors mediate coordinated RhoA-ROCK1 expression and signalling in breast cancer cells. *Proc Natl Acad Sci USA*. 2014; 111:E384–E393. [PubMed: 24324133]
152. Keith B, Johnson RS, Simon MC. HIF1 α and HIF2 α : sibling rivalry in hypoxic tumour growth and progression. *Nature Rev Cancer*. 2012; 12:9–22. [PubMed: 22169972]
153. Semenza GL. Targeting HIF1 for cancer therapy. *Nature Rev Cancer*. 2003; 3:721–732. [PubMed: 13130303]
154. Shen C, et al. Genetic and functional studies implicate HIF1 α as a 14q kidney cancer suppressor gene. *Cancer Discov*. 2011; 1:222–235. [PubMed: 22037472]
155. Semenza GL. HIF1 mediates metabolic responses to intratumoural hypoxia and oncogenic mutations. *J Clin Invest*. 2013; 123:3664–3671. [PubMed: 23999440]

156. Liao D, Corle C, Seagroves TN, Johnson RS. Hypoxia-inducible factor 1 α is a key regulator of metastasis in a transgenic model of cancer initiation and progression. *Cancer Res.* 2007; 67:563–572. [PubMed: 17234764]
157. Zhang H, et al. HIF1-dependent expression of angiopoietin-like 4 and L1CAM mediates vascular metastasis of hypoxic breast cancer cells to the lungs. *Oncogene.* 2012; 31:1757–1770. [PubMed: 21860410]
158. Tang N, et al. Loss of HIF1 α in endothelial cells disrupts a hypoxia-driven VEGF autocrine loop necessary for tumorigenesis. *Cancer Cell.* 2004; 6:485–495. [PubMed: 15542432]
159. Yamashita T, et al. Hypoxia-inducible transcription factor 2 α in endothelial cells regulates tumour neovascularization through activation of ephrin A1. *J Biol Chem.* 2008; 283:18926–18936. [PubMed: 18434321]
160. Mazzone M, et al. Heterozygous deficiency of PHD2 restores tumour oxygenation and inhibits metastasis via endothelial normalization. *Cell.* 2009; 136:839–851. [PubMed: 19217150]
161. Leite de Oliveira R, et al. Gene-targeting of PHD2 improves tumour response to chemotherapy and prevents side-toxicity. *Cancer Cell.* 2012; 22:263–277. [PubMed: 22897855]
162. Acker T, et al. Genetic evidence for a tumour suppressor role of HIF2 α . *Cancer Cell.* 2005; 8:131–141. [PubMed: 16098466]
163. Mazumdar J, et al. HIF2 α deletion promotes KRAS-driven lung tumour development. *Proc Natl Acad Sci USA.* 2010; 107:14182–14187. [PubMed: 20660313]
164. Kummur S, et al. Multihistology, target-driven pilot trial of oral topotecan as an inhibitor of hypoxia-inducible factor 1 α in advanced solid tumours. *Clin Cancer Res.* 2011; 17:5123–5131. [PubMed: 21673063]
165. Sun K, Tordjman J, Clment K, Scherer Philipp E. Fibrosis and adipose tissue dysfunction. *Cell Metab.* 2013; 18:470–477. [PubMed: 23954640]
166. Rose NR, McDonough MA, King ON, Kawamura A, Schofield CJ. Inhibition of 2-oxoglutarate dependent oxygenases. *Chem Soc Rev.* 2011; 40:4364–4397. [PubMed: 21390379]
167. Murad S, Walker LC, Tajima S, Pinnell SR. Minimum structural requirements for minoxidil inhibition of lysyl hydroxylase in cultured fibroblasts. *Arch Biochem Biophys.* 1994; 308:42–47. [PubMed: 8311472]
168. Tang SS, Trackman PC, Kagan HM. Reaction of aortic lysyl oxidase with β -aminopropionitrile. *J Biol Chem.* 1983; 258:4331–4338. [PubMed: 6131892]
169. Siegel RC. Collagen crosslinking. Effect of D-penicillamine on crosslinking *in vitro*. *J Biol Chem.* 1977; 252:254–259. [PubMed: 13066]
170. Barry-Hamilton V, et al. Allosteric inhibition of lysyl oxidase-like-2 impedes the development of a pathologic microenvironment. *Nature Med.* 2010; 16:1009–1017. [PubMed: 20818376]
171. Zardavas D, Baselga J, Piccart M. Emerging targeted agents in metastatic breast cancer. *Nature Rev Clin Oncol.* 2013; 10:191–210. [PubMed: 23459626]
172. Liu G, Nellaiappan K, Kagan HM. Irreversible inhibition of lysyl oxidase by homocysteine thiolactone and its selenium and oxygen analogues: Implications for homocystinuria. *J Biol Chem.* 1997; 272:32370–32377. [PubMed: 9405445]
173. Gilkes DM, Semenza GL. Role of hypoxia-inducible factors in breast cancer metastasis. *Future Oncol.* 2013; 9:1623–1636. [PubMed: 24156323]
174. Entenberg, D., et al. *Current Protocols in Cell Biology.* Wiley; 2013. p. 19.7.1-19.7.19.Ch. 19
175. Giampuzzi M, et al. Downregulation of lysyl oxidase-induced tumorigenic transformation in NRK-49F cells characterized by constitutive activation of RAS proto-oncogene. *J Biol Chem.* 2001; 276:29226–29232. [PubMed: 11323426]
176. Kadler KE, Hill A, Canty-Laird EG. Collagen fibrillogenesis: fibronectin, integrins, and minor collagens as organizers and nucleators. *Curr Opin Cell Biol.* 2008; 20:495–501. [PubMed: 18640274]
177. Zhang K, et al. The collagen receptor discoidin domain receptor 2 stabilizes SNAIL1 to facilitate breast cancer metastasis. *Nature Cell Biol.* 2013; 15:677–687. [PubMed: 23644467]

178. 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 USA*. 1995; 92:5510–5514. [PubMed: 7539918]
179. Wang GL, Semenza GL. Purification and characterization of hypoxia-inducible factor 1. *J Biol Chem*. 1995; 270:1230–1237. [PubMed: 7836384]
180. Semenza GL, et al. Hypoxia response elements in the aldolase A, enolase 1, and lactate dehydrogenase A gene promoters contain essential binding sites for hypoxia-inducible factor 1. *J Biol Chem*. 1996; 271:32529–32537. [PubMed: 8955077]
181. Kaelin WG Jr, Ratcliffe P. J Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. *Mol Cell*. 2008; 30:393–402. [PubMed: 18498744]
182. Tian H, McKnight SL, Russell DW. Endothelial PAS domain protein 1 (EPAS1), a transcription factor selectively expressed in endothelial cells. *Genes Dev*. 1997; 11:72–82. [PubMed: 9000051]
183. Wiesener MS, et al. Induction of endothelial PAS domain protein-1 by hypoxia: characterization and comparison with hypoxia-inducible factor-1 α . *Blood*. 1998; 92:2260–2268. [PubMed: 9746763]
184. Maxwell PH, et al. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature*. 1999; 399:271–275. [PubMed: 10353251]
185. Zundel W, et al. Loss of PTEN facilitates HIF1-mediated gene expression. *Genes Dev*. 2000; 14:391–396. [PubMed: 10691731]
186. Akakura N, et al. Constitutive expression of hypoxia-inducible factor-1 α renders pancreatic cancer cells resistant to apoptosis induced by hypoxia and nutrient deprivation. *Cancer Res*. 2001; 61:6548–6554. [PubMed: 11522653]
187. Zhong H, et al. Modulation of hypoxia-inducible factor 1 α expression by the epidermal growth factor/phosphatidylinositol 3-kinase/PTEN/AKT/FRAP pathway in human prostate cancer cells: implications for tumour angiogenesis and therapeutics. *Cancer Res*. 2000; 60:1541–1545. [PubMed: 10749120]
188. Rajkumar T, et al. Identification and validation of genes involved in cervical tumorigenesis. *BMC Cancer*. 2011; 11:80. [PubMed: 21338529]
189. Dong S, et al. Histology-based expression profiling yields novel prognostic markers in human glioblastoma. *J Neuropathol Exp Neurol*. 2005; 64:948–955. [PubMed: 16254489]
190. Arao T, et al. ZD6474 inhibits tumour growth and intraperitoneal dissemination in a highly metastatic orthotopic gastric cancer model. *Int J Cancer*. 2006; 118:483–489. [PubMed: 16052530]
191. Bos PD, et al. Genes that mediate breast cancer metastasis to the brain. *Nature*. 2009; 459:1005–1009. [PubMed: 19421193]
192. Kavak E, Unlu M, Nister M, Koman A. Meta-analysis of cancer gene expression signatures reveals new cancer genes, SAGE tags and tumour associated regions of co-regulation. *Nucleic Acids Res*. 2010; 38:7008–7021. [PubMed: 20621981]
193. Chang KP, et al. Identification of PRDX4 and P4HA2 as metastasis-associated proteins in oral cavity squamous cell carcinoma by comparative tissue proteomics of microdissected specimens using iTRAQ technology. *J Proteome Res*. 2011; 10:4935–4947. [PubMed: 21859152]
194. Jarzab B, et al. Gene expression profile of papillary thyroid cancer: sources of variability and diagnostic implications. *Cancer Res*. 2005; 65:1587–1597. [PubMed: 15735049]
195. Brigstock DR. Connective tissue growth factor (CCN2, CTGF) and organ fibrosis: lessons from transgenic animals. *J Cell Commun Signal*. 2010; 4:1–4. [PubMed: 19798591]
196. Vlodavsky I, et al. Extracellular matrix-resident growth factors and enzymes: possible involvement in tumour metastasis and angiogenesis. *Cancer Metastasis Rev*. 1990; 9:203–226. [PubMed: 1705486]
197. Swigris J, Brown K. The role of endothelin 1 in the pathogenesis of idiopathic pulmonary fibrosis. *BioDrugs*. 2010; 24:49–54. [PubMed: 20055532]
198. Kagami S, Border WA, Miller DE, Noble NA. Angiotensin II stimulates extracellular matrix protein synthesis through induction of transforming growth factor- β expression in rat glomerular mesangial cells. *J Clin Invest*. 1994; 93:2431–2437. [PubMed: 8200978]

199. Wang Z, et al. Hypoxia-inducible factor 1 α contributes to the profibrotic action of angiotensin II in renal medullary interstitial cells. *Kidney Int.* 2011; 79:300–310. [PubMed: 20881940]
200. Grotendorst GR, Rahmanie H, Duncan MR. Combinatorial signalling pathways determine fibroblast proliferation and myofibroblast differentiation. *FASEB J.* 2004; 18:469–479. [PubMed: 15003992]

Box 1**Regulation of HIFs**

Hypoxia-inducible factors (HIFs) are transcription factors that function as heterodimers, which consist of an oxygen-regulated HIF1 α (or HIF2 α) subunit and a constitutively expressed HIF1 β subunit^{178,179}. HIFs bind to the consensus sequence 5'-RCGTG-3' that is present within or near HIF-regulated genes¹⁸⁰. HIF1 α protein levels are regulated by oxygen-dependent prolyl hydroxylation, which is required for binding of the von Hippel-Lindau (VHL) tumour suppressor protein, leading to ubiquitylation and proteasomal degradation of HIF1 α ¹⁸¹. Hydroxylation of HIF1 α residues Pro402 and Pro564 is catalysed by HIF prolyl hydroxylase domain-containing protein 1 (PHD1), PHD2 and PHD3 in a reaction that is dependent on the presence of cofactors, oxygen and α -ketoglutarate (also known as 2-oxoglutarate). Under low oxygen conditions, HIF1 α hydroxylation, ubiquitylation and degradation are inhibited¹⁵³. HIF2 α , which shares 48% amino acid sequence identity with HIF1 α , is also oxygen-regulated and binds to HIF1 β to form HIF2, which activates the transcription of some, but not all, HIF target genes^{182,183}. Many oncogenic alterations in cancers cells, including loss of function of VHL, PTEN and p53^{184–186}, as well as activation of the PI3K–AKT¹⁸⁷ pathway, also cause an increase in HIF activity. Data obtained from many recent studies that use a range of approaches have revealed unique roles for HIF1 α and HIF2 α in both normal and cancer cells¹⁵².

Box 2**Collagen hydroxylases and cancer**

Procollagen-lysine 2-oxyglutarate 5-dioxygenase 2 (*PLOD2*) was included among the genes shown to be upregulated in gene expression screens of cervical cancer¹⁸⁸, glioblastoma¹⁸⁹ and gastric cancer¹⁹⁰, and was 1 of 17 genes that predicted breast cancer metastasis to the brain¹⁹¹. Gene expression studies also revealed increased *PLOD2* mRNA expression in primary sarcoma samples from patients with metastatic compared to non-metastatic sarcomas⁶⁷. Moreover, human osteosarcoma samples have two to three times more hydroxylysine content than normal bone collagen, which indicates that PLOD activity is increased in these patients⁴⁴. Increased prolyl 4-hydroxylase α -subunit isoform 1 (*P4HA1*) expression was revealed by a meta-analysis that was used to identify genes that are upregulated across many different cancer types¹⁹². P4HA2 was determined to be a metastasis-associated protein in oral cavity squamous cell carcinoma using comparative tissue proteomics¹⁹³. Increased *P4HA2* expression levels also discriminated papillary thyroid cancer from normal thyroid tissue¹⁹⁴. Increased *P4HA1*, *P4HA2* or *PLOD2* mRNA expression is predictive of breast cancer patient survival; the predictive power is improved when the expression of all three genes is evaluated and determined to be greater than the median expression level^{20,21}.

Figure 1. Biosynthesis of fibrillar collagens

The biosynthesis of type I collagen and other fibrillar collagens can be divided into intracellular (parts **a–c**) and extracellular (parts **d–f**) steps. The first intracellular step involves the synthesis of procollagen polypeptides from any of 42 distinct collagen gene transcripts (part **a**). Procollagens are post-translationally modified within the cisternae of the endoplasmic reticulum (ER) by prolyl 4-hydroxylase α -subunit isoform 1 (P4HA1), P4HA2 and P4HA3 and by procollagen-lysine 2-oxyglutarate 5-dioxygenase 1 (PLOD1), PLOD2 and PLOD3 lysyl hydroxylase enzymes (part **b**). Hydroxylase residues can be further modified to galactosyl hydroxylysine and to glucosylgalactosyl hydroxylysine by collagen galactosyltransferase and glucosyltransferase, respectively. The carboxyl termini of three properly hydroxylated procollagen molecules will associate and spontaneously propagate a procollagen triple helix from the carboxyl terminus to the amino terminus. The triple helical procollagen will be transported from the ER to the extracellular space via the Golgi (part **c**). Two metalloproteinases, a procollagen N-terminal proteinase and a procollagen C-terminal proteinase, cleave the non-helical termini (part **d**) and the mature collagen proteins spontaneously aggregate to form a collagen fibril (part **e**). The final step, collagen fibre formation, is initiated by collagen crosslinking, which is catalysed by lysyl oxidase (LOX) family members and occurs via the lysine aldehyde- or hydroxylysine aldehyde-initiated pathway (part **f**). The number and the proportion of the various crosslinks are tissue specific and are regulated by the steric relationship between localized collagen molecules, the type of collagens co-polymerized and the glycosylation and the hydroxylation of the participating amino acid residues. For example, lysine aldehyde-initiated crosslinks are found in soft connective tissue, in contrast to hydroxylysine aldehyde-initiated crosslinks, which are

found in stiff connective tissues. Many non-fibrillar collagens retain a non-collagenous N- or C-terminal, which prevents the spontaneous formation of collagen fibrils, and in these collagens cysteine crosslinks might be the only source of covalent intermolecular bonds. Enzymes highlighted in red are induced under hypoxic conditions. LOXL, LOX-like protein.

Figure 2. Hypoxia promotes ECM remodelling to facilitate metastasis

Extracellular matrix (ECM) remodelling is tightly controlled to maintain tissue integrity. Cancer cells and associated stromal cells that have been exposed to hypoxia are transcriptionally reprogrammed to produce: matrix metalloproteinases (MMPs) and other proteases, which degrade the basement membrane surrounding a tumour (part **a**); aligned collagen fibres within the interstitial matrix, which function as a highway for local invasion, intravasation and metastasis (part **b**); and growth factors, which might be retained in the fibrotic microenvironment and function as chemotactic signals that recruit and activate stromal cells to further promote cancer progression (part **c**).

Figure 3. Hypoxia recruits and reprogrammes cells to produce fibrillar collagen

Hypoxia-induced and hypoxia-inducible factor (HIF)-regulated growth factor secretion by tumour cells promotes the recruitment of macrophages and fibroblasts to hypoxic regions of the primary tumour. Macrophages produce growth factors such as transforming growth factor β 1 (TGF β 1) and platelet-derived growth factor (PDGF) that activate recruited and resident fibroblasts to stimulate collagen deposition. Hypoxic cancer cells also signal to mesenchymal stem cells, which might participate in collagen deposition. HIFs regulate the production of collagen-modifying enzymes, including prolyl 4-hydroxylase α -subunit isoform 1 (P4HA1), P4HA2, procollagen-lysine 2-oxyglutarate 5-dioxygenase 2 (PLOD2), lysyl oxidase (LOX), LOX-like protein 2 (LOXL2) and LOXL4 to facilitate the proper maturation of collagen fibres. Together, these signalling pathways promote the production of a fibrillar collagen network (that is produced by multiple cell types), which increases the ability of cancer cells to invade blood vessels. BMDSCs, bone marrow-derived stem cells; ECM, extracellular matrix.

Table 1

Factors induced by HIFs and their role in fibrosis

Factor induced by HIFs	Role in fibrosis
platelet-derived growth factor	Stimulates the replication, the survival and the migration of myofibroblasts ¹¹⁸
Connective tissue growth factor	Promotes collagen deposition by myofibroblasts ¹⁹⁵
Fibroblast growth factor 2	Promotes the proliferation and the differentiation of endothelial cells, smooth muscle cells and fibroblasts, and stimulates collagen deposition ¹⁹⁶
Endothelin	Promotes fibroblast activation, proliferation and differentiation into myofibroblasts ¹⁹⁷
Angiotensin	Stimulates TGF β production ¹⁹⁸ and promotes collagen I and collagen III deposition ¹⁹⁹
Insulin growth factor 2	Increases connective tissue growth factor-stimulated collagen deposition ²⁰⁰
CXC-chemokine ligand 2	Promotes fibrocyte recruitment ¹¹⁹

HIFs, hypoxia-inducible factors; TGF β , transforming growth factor- β .