FRIENDS OR FOES — BIPOLAR EFFECTS OF THE TUMOUR STROMA IN CANCER

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Abstract | The restricted view of tumour progression as a multistep process defined by the accumulation of mutations in cancer cells has largely ignored the substantial contribution of the tumour microenvironment to malignancy. Even though the seed and soil hypothesis of Paget dates to 1889, it has been less than two decades since researchers have included the tumour microenvironment in their analyses of tumour progression. What have we recently learned from studying tumour–stroma interactions, and will this help to define new targets for therapy?

PAGET'S SEED AND SOIL HYPOTHESIS
The English surgeon Stephen Paget compared tumour cells with the seed of plants, in that they are both "carried in all directions; but they can only live and grow if they fall on congenial soil". Similarly, he argued that metastatic cells must thrive only where conditions are in some way favourable.

TUMOUR STROMA
Compartment providing the connective-tissue framework of the tumour. It includes fibroblasts, immune and inflammatory cells, fat cells and blood-vessel cells.

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Based on observations in the early twentieth century that some cancers run in families, researchers began to look for genetic alterations that might underlie cancer pathogenesis. Over the following decades, enormous advances were made in identifying the molecular determinants of carcinogenesis, and tumorigenesis became recognized as a multistep process during which cancer cells accumulate multiple and consecutive genetic alterations[1]. These cancer-cell- and genome-centred models have led to the identification and characterization of many oncogenes and tumour-suppressor genes. However, they have largely ignored the heterogeneous and structurally complex nature of the tissue, or 'organ', called the tumour. Even though PAGET'S SEED AND SOIL HYPOTHESIS dates back to 1889 (REF. 2), the molecular determinants of the seed are still much better understood than those of the soil[3].

It is only recently that tumour progression has been recognized as the product of an evolving crosstalk between different cell types within the tumour and its surrounding supporting tissue, or TUMOUR STROMA (FIG. 1)[4]. Whereas genetically abnormal cells define the tumour compartment itself, the epithelial parenchyma of carcinomas, the surrounding and interwoven stroma, provides the connective-tissue framework of the tumour tissue. This framework includes a specific type of EXTRACELLULAR MATRIX (ECM) — the tumour matrix — as well as cellular components such as fibroblasts, immune and inflammatory cells, and blood-vessel cells. As its constitution resembles that of the granulation tissue formed during wound healing, Hal Dvorak even defined a tumour as "a wound that never heals"[5].

The relative amount of stroma and its composition vary considerably from tumour to tumour and do not correlate with the degree of tumour malignancy[6]. But the interactive signalling between tumour and stroma contributes to the formation of a complex multicellular organ. In a manner similar to the development and function of normal organs, which occurs through reciprocal communication between different cell types, the interaction between cancer cells and their microenvironment can largely determine the phenotype of the tumour. For example, recent studies have shown that the establishment of human breast tumour xenografts in mice depends on the presence of human tumour-derived stromal fibroblasts[7].

Cancer cells themselves can alter their adjacent stroma to form a permissive and supportive environment for tumour progression. Morphological evidence for this 'reactive' tumour stroma has long been described in pathology textbooks[8] as 'DESMOPLASIA', which consists of fibroblast-like cells and specific ECM components, as well as inflammatory and immune cells. ANGIOGENESIS is another process involved in formation of the desmoplasia, as newly formed blood and lymph vessels support tumour growth and spread[9] (FIGS 1,2).

Summary

- Cancer cells can alter their adjacent stroma to form a permissive and supportive environment for tumour progression — this is known as the 'reactive' tumour stroma.
- Cancer cells produce a range of growth factors and proteases that modify their stromal environment.
- These factors disrupt normal tissue homeostasis and act in a paracrine manner to induce angiogenesis and inflammation, as well as activation of surrounding stromal cell types such as fibroblasts, smooth-muscle cells and adipocytes, leading to the secretion of additional growth factors and proteases.
- Activated fibroblasts in the stroma promote tumour progression by secreting growth factors and pro-migratory extracellular-matrix (ECM) components, as well as upregulating the expression of serine proteases and matrix metalloproteinases that degrade and remodel the ECM.
- The induction of inflammation in the tumour stroma also results in production of a range of factors that promote tumour progression.
- Angiogenesis promotes not only tumour growth, but also progression from a pre-malignant to a malignant and invasive tumour phenotype.
- The tumour stroma can have a more direct role in tumorigenesis, by acting as a mutagen.
- By 'normalizing' the tumour stroma, it is possible to slow or reverse tumour progression.

Cancer cells modulate their stromal environment

Cancer cells usually go about generating a supportive microenvironment by producing stroma-modulating growth factors. These include basic fibroblast growth factor (bFGF), members of the vascular endothelial growth factor (VEGF) family, platelet-derived growth factor (PDGF), epidermal growth factor receptor (EGFR) ligands, interleukins, colony-stimulating factors, transforming growth factor-β (TGFβ) and others. These factors disrupt normal tissue homeostasis, similar to the processes of wound healing[10], and act in a paracrine manner to induce stromal reactions such as angiogenesis[11] and the inflammatory response[12]. The factors also activate surrounding stromal cell types, such as fibroblasts, smooth-muscle cells[13] and adipocytes[14], leading to the secretion of additional growth factors and proteases (FIG. 3).

Concomitant with the altered growth-factor expression, and often induced by their autocrine effect on the tumour cells, cancer cells also start to produce proteolytic enzymes[15,16]. These remodel the ECM and the BASEMENT MEMBRANE to allow for a pro-migratory and pro-invasive

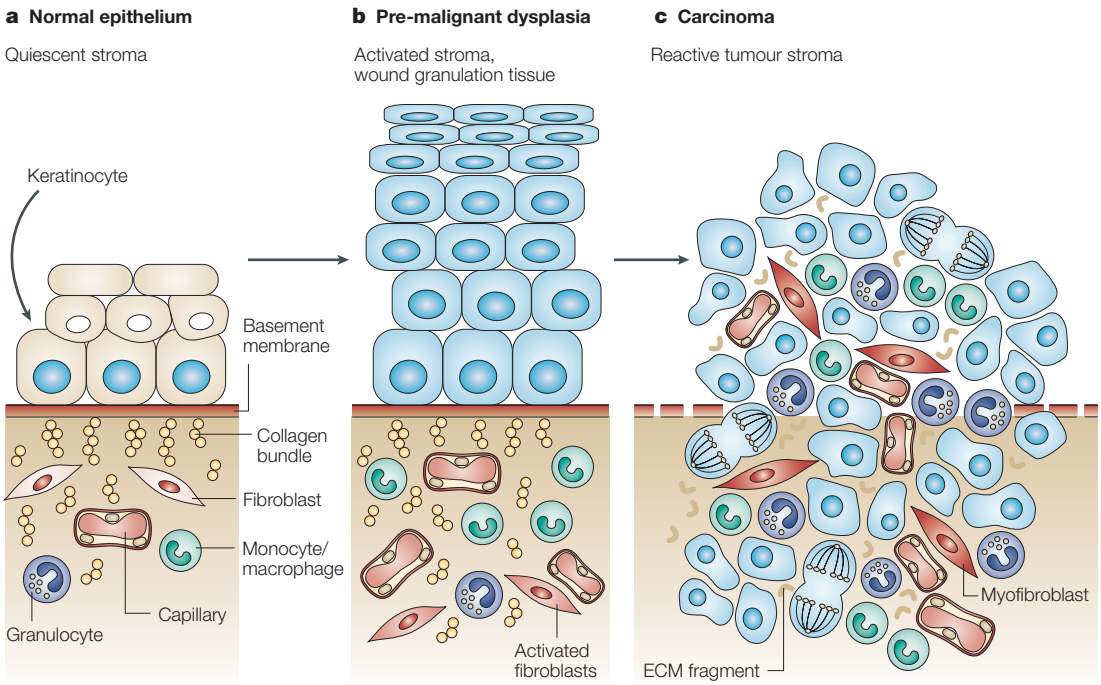


Figure 1 | **Tumour stage depends on stromal activation. a** | A normal well-differentiated stratified epithelium, made up of cells such as keratinocytes in the epidermis, is separated by a well-delineated basement membrane from the dermal or stromal compartment. This stromal compartment normally contains collagen bundles that surround resting fibroblasts, mature blood vessels encircled by an uninterrupted basement membrane (capillary), and a few resident leukocytes (monocytes and macrophages). **b** | During transition to pre-malignant dysplasia, differentiation of epithelial cells is disturbed, resulting in a hyperplastic epithelium (accumulation of blue cells). The basement membrane remains intact, separating the epithelium from a stromal compartment, which contains intact collagen bundles. Fibroblasts, however, become activated, and the number of macrophages increases. The transient angiogenesis that occurs initially during establishment of the transplant is followed by vessel maturation, resulting in a vasculature similar to the one seen with normal epithelia. **c** | Progression to a carcinoma is associated with proliferation of epithelial cells (mitotic cells) along with the development of an activated tumour stroma. In this case, extracellular-matrix (ECM) components such as collagen bundles are degraded, because of increased turnover. The number of inflammatory cells increases and fibroblasts differentiate into myofibroblasts, resulting in their expression of growth factors, matrix components and degrading proteases. Angiogenesis is maintained, resulting in a high number of leaky tumour vessels. Following activation of a tumour stroma with persistent angiogenesis, invasion by tumour cells begins through the degraded basement membrane, and blood vessels infiltrate the tumour tissue.

EXTRACELLULAR MATRIX (ECM)
Complex three-dimensional network of macromolecular protein fibres as well as non-fibrous proteoglycans that is present between clusters of cells in the stroma of all tissues. The ECM provides architectural structure and strength and contextual information for cellular communication, adhesion and migration.

DESMOPLASIA
The growth of fibrous or connective tissue that is induced by tumours and is characterized by activated fibroblasts, recruited inflammatory and immune cells, and angiogenic blood vessels.

ANGIOGENESIS
The formation of new blood vessels from pre-existing ones.

BASEMENT MEMBRANE
Amorphous, dense, sheet-like, proteinaceous structure that is 50–100 nm thick and that separates epithelial and stromal tissues, and delineates the endothelial lining of vessels.

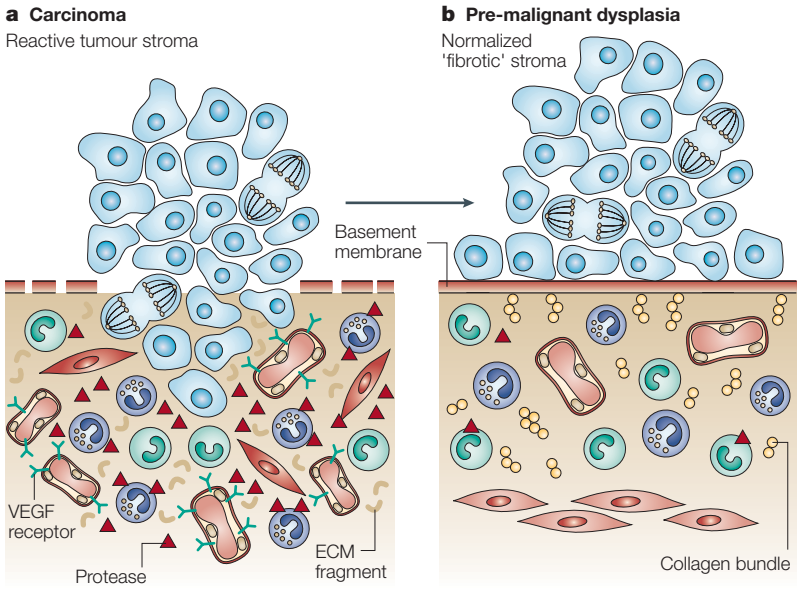


Figure 2 | **Reversion of tumour phenotype by stromal normalization. a** | The reactive tumour stroma is characterized by the presence of proliferating endothelial cells, blood-vessel cells that express the receptor for vascular endothelial growth factor (VEGF), fragments of extracellular-matrix (ECM) molecules, active proteases, activated fibroblasts and a broken basement membrane. **b** | By blocking angiogenesis, the activated stromal compartment of malignant carcinomas is normalized, blood vessels mature and acquire an intact basement membrane, and fibroblast activation is downregulated, although not inhibited. As a consequence of the downregulation of matrix-degrading proteases and reduced ECM turnover in the stroma, an intact basement-membrane zone is re-established and stromal collagen bundles are reformed. As a result of this normalized stromal compartment, the malignant and invasive growth of the carcinoma reverts to a pre-malignant dysplastic phenotype.

environment. In addition, degradation of ECM molecules exposes cryptic protein domains and generates specific new molecule fragments that can have pro-migratory as well as pro- and anti-angiogenic functions[17]. In the remodelled ECM, matrix metalloproteinases (MMPs)[18] activate cell-surface and ECM-bound growth factors[19,20] that contribute to the extensive crosstalk between the microenvironment and the cancer cells (FIG. 1).

The expression of these tumour-cell-derived proteases is frequently modulated by the stromal microenvironment. In the HaCaT model of human skin carcinoma, increased protease expression was observed in benign and malignant (invasive) RAS-transformed (HaCaT-RAS) cell cultures, compared with non-tumorigenic HaCaT cells[21]. However, benign and malignant tumour cells showed no difference in their levels of protease expression. Only when cultured in the presence of stromal fibroblasts did malignancy correlate with increased expression of MMP1 and MMP9 — malignant, but not benign, tumour cells upregulated MMP1 and MMP9 production following co-culture with stromal cells[22]. Furthermore, transplantation of benign tumour cells to mice abrogated MMP1 expression, whereas transplantation increased MMP1 expression in malignant tumour cells. Malignant tumour cells also induced production of interstitial collagenase by the host stroma, whereas benign cells did not[23]. This type of reciprocal upregulation of proteases between cancer and stromal fibroblasts indicates the amount of extensive crosstalk that occurs, which is likely to be mediated by soluble factors secreted by the cancer cells and also activated by the tumour-cell-derived proteases.

MYOFIBROBLASTS
Activated fibroblasts that express α-smooth-muscle actin, specific growth factors and proteases. They are similar to the carcinoma-associated fibroblasts present in the tumour stroma.

Tumour-activated fibroblasts

TGFβ and PDGF are two growth factors that are secreted by a range of tumour cells[13] and are known to mediate the interaction of cancer cells with stromal fibroblasts. Yet the specific role of TGFβ in tumour progression is still controversial. On the one hand, inhibition of TGFβ signalling in stromal fibroblasts by blockade of TGFβ receptor II was shown to induce progression to malignancy in epithelial cells[24]. On the other hand, overexpression of TGFβ in skin papillomas of a transgenic mouse model is associated with progression to metastatic tumours — an effect that might be mediated by both autocrine and paracrine signalling[25]. Together with PDGF, TGFβ was shown to be the main inducer of desmoplasia, through its effect on stromal fibroblasts[26]. One way that this might occur is through TGFβ-induced chemotaxis of fibroblasts and their transdifferentiation into activated smooth-muscle reactive fibroblasts, termed MYOFIBROBLASTS[27–30]. PDGF can also induce myofibroblast proliferation and differentiation[31].

Myofibroblasts in the tumour stroma, also known as carcinoma-associated fibroblasts (CAFs), are large spindle-shaped mesenchymal cells that share characteristics with smooth-muscle cells and fibroblasts. They are identified immunocytochemically based on a combination of different markers, such as the expression of α-smooth-muscle actin, vimentin, desmin and fibroblast activation protein (FAP) [32,33]. FAP is a cell-surface-bound serine protease of reactive tumour stromal fibroblasts that is found in epithelial cancers and in granulation tissue during wound healing[34]. The presence of CAFs in the activated tumour stroma has been observed in many cancer types, such as breast cancer[35], prostate cancer[36] and skin cancer[37]. Their presence was proposed to precede the onset of invasion[13] and to contribute to tumour growth and progression (FIG. 1). Cunha and colleagues demonstrated that the *in vivo* combination of normal human prostatic epithelial cells with CAFs led to limited tumour growth that resembled prostatic intraepithelial neoplasia, and that grafting CAFs with immortalized prostatic epithelial cells that were non-tumorigenic but expressed the SV40T antigen resulted in the formation of malignant tumours[36,38]. So, oncogenic signals from the CAFs seem to stimulate the progression of a non-tumorigenic population of epithelial cells to a tumorigenic one.

Additional studies have reported a similar tumour-promoting effect of stromal fibroblasts in human squamous-cell carcinomas (SCCs) of the skin. When non-tumorigenic immortal human keratinocytes (HaCaT) were transfected with a PDGF expression vector and transplanted into mice, they induced a transient activation of the stroma, resulting in benign tumour growth, whereas non-PDGF-expressing cells remained non-tumorigenic. This effect of PDGF is strictly paracrine, as HaCaT keratinocytes do not express the PDGF receptor, and is accompanied by the

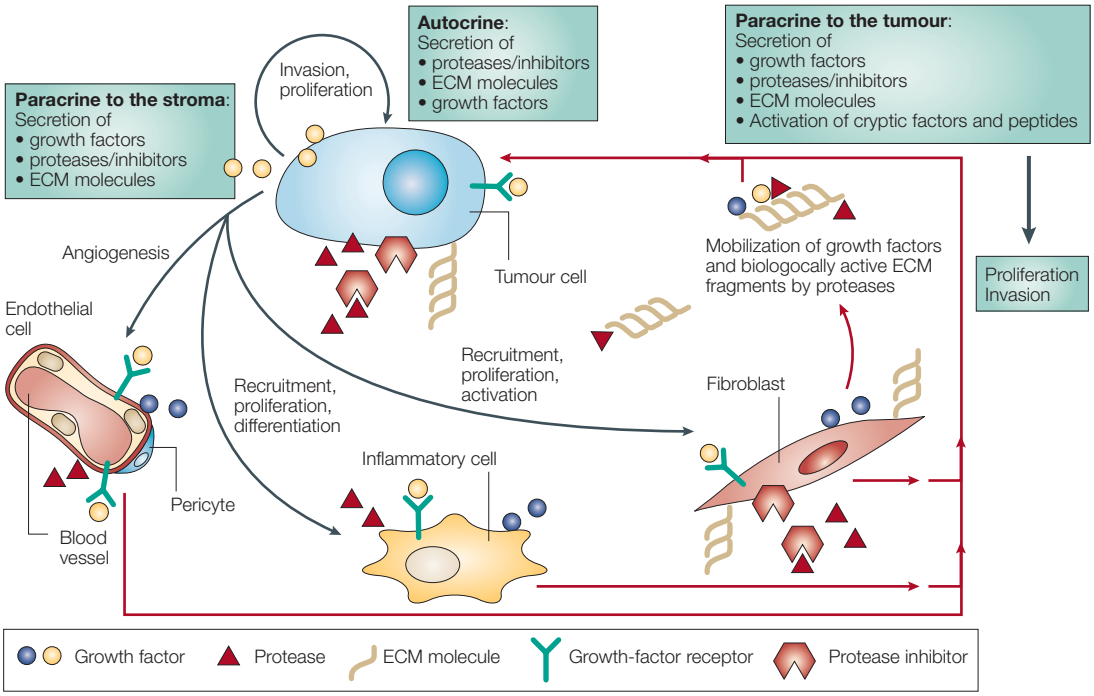


Figure 3 | **Crosstalk between tumour cells and their activated stromal surroundings.** Tumour cells activate their stromal environment by secreting growth factors and proteases, which can act in autocrine and paracrine manners. Concomitantly, they initiate the secretion of specific pro-migratory and -invasive extracellular-matrix (ECM) components, along with their respective receptors, and reduce the expression of protease inhibitors. The imbalance between proteases and their inhibitors then leads to the degradation of ECM components, resulting in the mobilization of matrix-bound growth factors and the generation of reactive ECM fragments. Together with the tumour-derived growth factors, these molecules then induce angiogenesis as well as recruit and activate stromal inflammatory cells and fibroblasts. Fibroblasts secrete further growth factors and proteases to amplify these signals in the cascade that results in the establishment of an activated stroma that promotes malignant tumour growth.

induction of granulation tissue and a transient stimulation of angiogenesis[37]. Further studies have shown that the PDGF-induced stromal activation is mediated by the differentiation of normal dermal fibroblasts to activated fibroblasts that express α-smooth-muscle actin with a phenotype similar to CAFs, which secrete a range of stroma-modulating growth factors such as VEGF and proteases (W. Lederle, M. Skobe, H.J. Stark, M.M. Mueller and N.E.F., unpublished observations).

Activated CAFs could promote tumour progression in several different ways. They secrete pro-migratory ECM components such as tenascin[29]. They also upregulate the expression of serine proteases and MMPs such as urokinase, plasminogen activator (uPA) — a protease that is required for the activation of plasminogen to plasmin — as well as MMP1 and MMP3, which degrade and remodel the ECM[39,40]. This upregulation of MMPs is one of the physiological changes that occur when fibroblasts undergo senescence, which might be an important component of the generation of a pro-oncogenic tissue environment that contributes to the increase in cancer incidence that occurs with age[41]. An additional study showing that MMP11-null fibroblasts do not support *in vivo* growth of breast cancer cells whereas wild-type fibroblasts do supports the role for proteases in the tumour-promoting function of the microenvironment[42]. Additionally CAFs express a range of growth factors and cytokines such as insulin-like growth factor 1 (IGF1) and hepatocyte growth factor (HGF), which promote tumour-cell survival[43] as well as tumour-cell migration and invasion, respectively[29,30]. Finally, CAFs contribute to the generation of a tumour-progression-promoting microenvironment by expressing growth factors like VEGF or MCP1 (REF. 44), which further activate the tumour stroma through the stimulation of angiogenesis and the recruitment of inflammatory cells (FIG. 3).

Inflammation

The abundance of inflammatory and immune cells in the tumour tissue was observed by pathologists as early as the middle of the nineteenth century[45], although the role of these cells in tumour progression has long been a matter of discussion. Whereas immune cells such as natural-killer cells are still believed to have *bona fide* antitumour activity[46], the antitumour effects of inflammatory cells such as monocytes and macrophages, which have been proposed to recognize tumour antigens, has been increasingly disputed. In 1850, Rudolf Virchow was the first to describe a tumour-promoting effect of chronic irritation or inflammation, and numerous scientists have since provided evidence for this phenomenon. Prominent examples include the associations between infection with *Helicobacter pylori* and stomach cancer; human papillomavirus (HPV) and cofactors like *Chlamydia* or herpes simplex virus 2 infection and

cervical cancer; the predisposition of patients with Crohn's disease to colorectal cancer[47,48]; or chronic prostatic inflammation associated with sexually transmitted disease and prostate cancer[49]. All of these disorders cause an inflammatory response that is believed to contribute to tumour development. Even well-established oncogenes like Rous sarcoma virus require wound-induced inflammation to generate second-site tumours[50].

How else can tumours promote inflammation and the recruitmentment of inflammatory cells? Tumour cells overexpress inflammatory cytokines that recruit haematopoietic cells such as lymphocytes, monocytes (macrophages), and neutrophils into the tumour neighbourhood[12,51] (FIG. 3). Expression of the chemoattractant chemokine CC-motive ligand 2 (CCL2) correlates with poor prognosis in breast, cervical and bladder cancer[51,52]. Colony-stimulating factor 1 (CSF1), the main growth and differentiation factor for mononuclear phagocytes such as macrophages, is widely overexpressed in tumours of the breast, uterus, ovary and prostate[53]. In a reverse approach, CSF1-null mice were used to analyse the contribution of the inflammatory cells to tumour progression in a model of mammary tumours induced by tissue-restricted expression of the polyoma middle T antigen. Loss of CSF1 prevented the accumulation of macrophages in the tumour vicinity and slowed tumour progression, by inhibiting its metastatic ability. The metastatic phenotype could be restored by overexpression of CSF1 in the epithelial tumour cells[54].

Recent studies have demonstrated the contribution of the haematopoietic growth factors granulocyte colony-stimulating factor (G-CSF) and granulocyte–macrophage colony-stimulating factor (GM-CSF) to tumour progression in a broad spectrum of human tumours, including SCCs of the skin and the head and neck, meningiomas and gliomas[55–57]. G-CSF and GM-CSF contribute to *in vivo* tumour progression through the recruitment of monocytes, macrophages and neutrophils into the tumour vicinity. Concomitant with the increase in the number of stromal inflammatory cells and following a similar time course, these growth factors were also observed to induce angiogenesis, which might have been mediated by the recruitment of ENDOTHELIAL PROGENITOR CELLS to the tumour tissue. Inflammatory cells — specifically neutrophils — were also observed to contribute to angiogenesis, by remodelling the ECM through the secretion of MMP9 and MMP13 (REF. 58).

A similar finding was reported by Coussens and co-workers for host-derived mast cells. In a transgenic model of HPV-induced skin carcinogenesis, tumour-infiltrating mast cells were found to contribute to carcinogenesis by activating MMP9, which flips the ANGIOGENIC SWITCH that is important for tumour development. Pre-malignant angiogenesis was ablated in mast-cell-deficient (KITw/KITw) mice, and MMP9-null mice showed a significant delay in tumour formation that resulted in considerably less, though more aggressive, tumours[59]. By cleaving ECM components, MMP9 releases sequestered growth factors such as VEGF, which then contribute to angiogenesis.

The role of tumour-infiltrating macrophages in promoting tumour progression was recently reviewed[51]. These cells express a range of proteases, such as uPA and MMPs that remodel the ECM, generating reactive cleavage products of ECM molecules and releasing and activating pro-angiogenic factors such as VEGF and others. Additionally, monocytes, macrophages, mast cells and other leukocytes express angiogenic growth factors including VEGF, angiopoietin 1, bFGF, TGFβ, PDGF, tumour-necrosis factor-α and others[60]. The ability of these molecules to promote and sustain tumour angiogenesis is one of the contributions of the stroma to tumour progression (FIG. 3).

Tumour angiogenesis

Based on observations made nearly 100 years ago that blood vessels grow around tumours[61], along with the pioneering work of Judah Folkman, it is now widely recognized that tumours must induce angiogenesis to grow beyond a size of 1–2 mm in diameter, as well as to metastasize to distant organ sites[9]. Following the onset of angiogenesis, dormant tumours, which are frequently observed in individuals during autopsies, enter the vascular phase and begin exponential growth[62]. There are several excellent reviews that summarize the process of angiogenesis, its regulation by growth factors and proteases, and its contribution to tumour growth and progression[11,60,63]. Targeting the tumour vasculature has become an attractive possibility as a form of anticancer therapy[64] — for further information, see the National Cancer Institute (NCI) web site entitled 'Angiogenesis Inhibitors in Clinical Trials' in the online links box.

Accumulating evidence indicates that angiogenesis promotes not only tumour growth, but also progression from a pre-malignant to a malignant and invasive tumour phenotype. Examples of this include astrocytomas, which do not require initial neovascularization for growth, but acquire their blood supply by co-opting existing normal brain blood vessels. They grow along these blood vessels, acquiring an invasive character that allows them to enlarge as much as some angiogenic tumours[65]. However, following progression to highly malignant (grade IV) glioblastoma multiforme, they become hypoxic and necrotic, partly due to vessel regression and increased tumour-cell proliferation[66]. These hypoxic conditions eventually induce the formation of new blood vessels, which is primarily stimulated by the most prominent hypoxia-induced angiogenic factor VEGF, and make the onset of persistent angiogenesis one of the hallmarks that distinguishes grade IV glioblastomas from lower-grade astrocytomas[65].

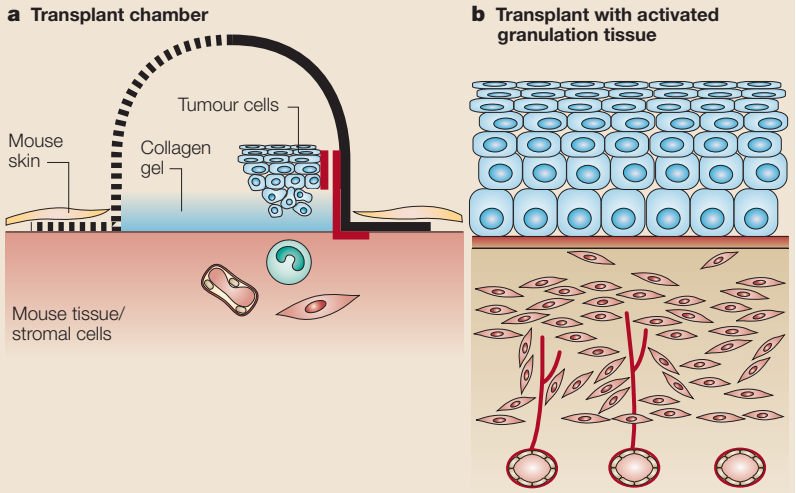
Experiments in the HaCaT skin carcinogenesis model revealed a similar correlation between persistent angiogenesis and tumour malignancy. A useful *in vivo* surface transplantation model has allowed for analysis of the kinetics of tumour–stroma interactions during different stages of malignancy[67] (BOX 1). In this model, researchers can discriminate between pre-malignant and malignant epithelial tumour cells based on their phenotype and their dynamics of stromal activation and angiogenesis induction[68]. When malignant cells are

ENDOTHELIAL PROGENITOR CELLS
Undifferentiated cells that reside in the adult bone marrow or circulate in the blood (circulating progenitors) that can be recruited to the sites of ongoing angiogenesis, where they differentiate and mature into endothelial cells. They are identified by co-expression of haematopoietic stem-cell markers (CD34, AC133) and vascular endothelial cell markers (VEGFR2, TIE2)

ANGIOGENIC SWITCH
Transition of tumours from an avascular state to the active recruitment of blood vessels into the tumour.

Box 1 | **Matrix-inserted surface transplantation model**

A useful *in vivo* surface transplantation model has been developed to allow researchers to analyse the kinetics of tumour–stroma interactions during different stages of malignancy. In this model, epithelial tumour cells are pre-cultured *in vitro* on a collagen (type I) gel mounted between two concentric teflon rings. This chamber is transplanted onto the back muscle fascia of mice (see figure, panel a), resulting in rapid initiation of tumour-cell proliferation. In this system, the collagen gel prevents immediate contact between grafted tumour cells and host cells, while still allowing the interaction through diffusible factors. This approach provides significant advantages in analysing tumour–stroma interactions. In contrast to the transplantation of subcutaneous tumours, which only allows for the analysis of the stromal compartment as an end point observation, this surface transplantation system permits the study of very early steps in the tumour–stroma interaction. Researchers can compare the effects of transplanting normal or pre-malignant cells onto a fully activated carcinoma-promoting stroma. The system therefore presents a highly sensitive tool for observing the early steps of the host stroma response to the tumour signals. For example, researchers can observe the ability of tumour cells to induce vascularization of activated granulation tissue (see figure, panel b) during the beginning of stromal-cell infiltration into the collagen, and measure the kinetics of the replacement of the normal extracellular matrix by the newly formed tumour stroma.



transplanted, stromal activation and angiogenesis persist, whereas both of these are downregulated after initial activation when benign or pre-malignant tumour cells are transplanted. This 'transient' angiogenic response resembles the transient activation of angiogenesis and granulation-tissue formation that occurs during wound healing. The differential dynamics of angiogenesis induction in benign versus malignant transplants and its persistence in the malignant tumours depended on the regulated expression of VEGF receptor 2 (VEGFR2) by endothelial cells in the tumour stroma, which was induced by both tumour types initially, but downregulated within a few weeks by the benign tumour. By contrast, VEGF was continuously expressed by malignant and benign tumour cells after transplantation, independently of the kinetics of angiogenesis[69]. Interestingly, during malignant tumour growth, stromal activation was an early event, with rapid progression of blood vessels and stromal cells towards the tumour cells and their eventual infiltration into the malignant tumour tissue. This preceded tumour-cell invasion into the surrounding host tissue — an observation that is in agreement with studies performed in other clinical and experimental tumour systems[63,68,70]. So, it seems that the activation of stromal cells and the infiltration of blood vessels into the expanding tumour mass is a prerequisite for tumour invasion.

As tumour invasion depends on angiogenesis and persistent expression of VEGFR2, does inactivation of VEGFR2-mediated signalling not only block angiogenesis, but also tumour invasion? Intraperitoneal injection of an anti-VEGFR2 antibody (DC101) into mice after transplantation of both well-differentiated as well as highly malignant, metastasizing keratinocytes, resulted in inhibition of both angiogenesis and tumour invasion. More importantly VEGFR2 blockade induced a phenotypic shift from a highly malignant to a pre-malignant, non-invasive tumour phenotype[69]. Interestingly, this was associated with the remodelling of the activated stroma into a stabilized connective tissue characterized by a normalized tumour stroma border with restoration of mature epithelial basement-membrane structures including HEMIDESMOSOMES at the basal pole of tumour cells and the accumulation of collagen bundles in the vicinity of the tumour tissue. This normalization of the tumour–stroma border zone is most likely due to a downregulation of matrix-degrading proteases such as MMP9 and MMP13 in the stroma, leading to a reduced turnover of crucial basement-membrane constituents like laminin, fibronectin, and collagen type I and IV, thereby enabling their accumulation and structural organization (FIG. 2) (S. Vosseler, unpublished observations).

Researchers recognized the connection between interstitial-ECM- and vascular-basement-membrane-like material during angiogenesis as early as 1938. In a camera lucida study of physiological neovascularization, Clark *et al.* demonstrated that growing vessel sprouts became functional capillary tubes when the perivascular matrix changed to a tissue substance that resembled a 'soft gel'[71]. The vascular basement membrane is an important structural and functional component of vessels. It marks the border between the endothelial cells that populate its inner surface and the pericytes, which are specialized smooth-muscle cells that reside outside the small vessel.

The extracellular matrix

As reviewed by Kalluri[17], there is mounting evidence that ECM and basement-membrane components such as fibronectin, collagen type IV and thrombospondin-1 (TSP1) provide both pro- and anti-angiogenic signals, depending on their structural integrity and assembly. Just as ECM signalling through integrins can induce the malignant phenotype in breast carcinomas and SCCs[72,73], signalling during angiogenesis is also mediated by the binding of ECM components to different integrins. Fibronectin and collagen type IV were shown to have pro-angiogenic effects, through their ability to bind $\alpha_5\beta_1$-integrin and $\alpha_1\beta_1$-integrin (fibronectin), as well as $\alpha_2\beta_1$-integrin (collagen type IV) [74]. On the other hand, degradation of basement-membrane proteins by MMPs blocks signalling through these integrins and therefore inhibits angiogenesis[75].

HEMISDESMOSOME
Specialized junction between an epithelial cell and its basal lamina that mediates their interactions.

TSP1, an ECM protein that is usually found in the provisional wound matrix and in tumour stroma, functions as an inhibitor of angiogenesis. Overexpression of TSP1 by a malignant and invasive human skin SCC cell line prevents blood vessels from penetrating into these tumours *in vivo*, resulting in a transient delay in tumour invasion. Although angiogenesis was not initially inhibited, allowing the accumulation of blood vessels in the surrounding stroma, the accumulation of the overexpressed TSP1 in a capsular layer around the tumour functioned as a barrier to prevent the infiltration of blood vessels into the tumour. Blood-vessel penetration and, as a consequence, tumour invasion and expansion could be re-established by downregulation of TSP1 expression using antisense oligonucleotides[76].

Recent studies indicate that the anti-angiogenic effect of TSP1 is also mediated by integrins[77] and can be prevented through degradation of the TSP1 matrix by MMPs (P. Boukamp, personal communication). Similarly, the formation of a discrete capsular layer around hepatocellular carcinomas is associated with reduced blood-vessel infiltration into the tumour mass and therefore with a decrease of tumour invasiveness[78]. So, the protein composition of the ECM, the structural integrity of ECM proteins and the activity of degrading proteases all seem to have a marked impact on tumour vascularization and can subsequently facilitate or even induce the process of tumour invasion and expansion. This was also confirmed by the lack of tumour vascularization observed after transplantation of mouse skin carcinomas into mice that were null for plasminogen activator inhibitor 1 (PAI1). PAI1 is an endogenous protease inhibitor that counteracts the activation of plasminogen by uPA. In these mice, the balance of uPA and PAI1 was deregulated in the stromal tissue, shifting the balance towards an activated protease that might have generated an excess of anti-angiogenic ECM degradation products. As a consequence, tumour vascularization was abolished and the grafted mouse skin carcinoma cells failed to invade the stroma of the PAI1-deficient host. Re-expression of PAI1 in the knockout animals confirmed that the absence of PAI1 was responsible for this vascular deficiency, as tumour vascularization and invasion were restored[79].

Protease activity uncovers or releases cryptic sites that function to modulate the proliferation, migration and apoptosis of endothelial cells[17,80]. One example for these proteolytic fragments is angiostatin, an amino-terminal fragment of plasminogen that is generated by MMP degradation (MMP3, MMP7, MMP9, MMP12)[81,82]. Angiostatin is a potent inhibitor of endothelial-cell proliferation and angiogenesis. Other inhibitors of angiogenesis include endostatin (a proteolytic fragment of type XVIII collagen), and tumstatin, which is generated from collagen type IV[83,84]. Although endostatin inhibits proliferation and migration of endothelial cells in response to VEGF[85,86], tumstatin was shown to induce the apoptosis of proliferating endothelial cells[87].

On the other hand, overexpression of membrane-bound membrane type 1 MMP (MT1-MMP) in melanoma cells is associated with increased tumour vascularization and tumour growth. MT1-MMP contributes to tumour angiogenesis through various mechanisms, including activation of $\alpha_v\beta_3$-integrin (which protects endothelial cells from apoptosis), fibrinolytic activity, the activation of MMP2 and the transcriptional regulation of VEGF expression[88]. So, altering the protease levels seems to have a paradoxical effect on tumour invasion and angiogenesis. Proteases can be pro-angiogenic, by releasing reactive ECM fragments and activating ECM-bound angiogenic growth factors such as VEGF, bFGF and others[12]. At the same time, unbalanced protease expression can also provide anti-angiogenic signals, such as by generating endogenous angiogenesis inhibitors through the proteolytic modification of ECM components in the matrix (FIG. 3).

Mutagenic effects of the stroma

So far, we have focused on the mechanisms by which the stromal environment supports tumour growth and promotes invasion through the stimulation of cancer-cell proliferation, migration and invasion, and the activation of angiogenesis. Yet there is increasing evidence that the tumour stroma can have a more direct role in tumorigenesis, by acting as a mutagen. As previously described, transplantation of immortalized human prostate cells along with CAFs leads to the formation of massive malignant tumours. Remarkably, the isolation of populations of pure human epithelial cells from these tumours yielded cells that were subsequently able to form tumours without the support of connected CAFs. So, oncogenic signals from the CAFs, and potentially also from other stromal cells, had stimulated the progression of a non-tumorigenic cell population to a tumorigenic one. This progression was associated with changes in gene expression, as well as with specific stable genetic alterations[89,90].

Similarly, subcutaneous injection of benign tumorigenic SCC cells and subsequent *ex vivo* re-cultivation of the tumour cells resulted in the progression to more aggressive and eventually metastatic tumours. This enhanced malignant phenotype was stably maintained in the re-cultured tumour cells and was associated with the altered expression of a range of growth factors, such as G-CSF and GM-CSF[91]. In these cases, the tumour microenvironment seems to have had an active mutagenic role in promoting transformation. One mechanism that might underlie these effects includes the generation of an adverse, low pH environment, leading to production of reactive oxygen species that can act as local mutagens[92].

Many cancers can also arise as a consequence of inherited (familial) or acquired genetic mutations in stromal cells. For example in patients with juvenile polyposis, deletions in chromosome 10 and 18 have been detected in stromal cells, but not in epithelial cells[93]. Similarly, Moinfar and colleagues reported that distinct genetic alterations and loss of heterozygosity were observed in stromal cells adjacent to primary breast tumours[94]. Schor and colleagues described a tumour-like phenotype of fibroblasts isolated from the healthy relatives of patients with familial breast disease[95,96]. This

Table 1 | **Cancer clinical trials designed to target the tumour stroma**

Target	Approach	Clinical trial	Outcome/status
Endothelial cells	Inhibition of VEGF signalling	Phase III for colon carcinoma, lung carcinoma and renal-cell carcinoma; Phase II for lung carcinoma and renal-cell carcinoma	Improved survival for colon carcinoma[110]; Phase II studies for lung and renal-cell carcinoma show slightly improved survival[111,112], and Phase III trials for these are ongoing*‡
	Inhibition of endothelial-cell proliferation by TNP 470, a fumagillin analogue	Phase I/II for lung carcinoma and advanced solid tumours	Drug well tolerated; some patients with partial response[111]
	Induction of apoptosis in proliferating endothelial cells with tubulin-binding agents	Phase I for thyroid cancer	Ongoing*
Inflammatory cells	NSAIDs	Phase II for colon carcinoma	Ongoing with first beneficial results[113]
ECM components	Local injection of radiolabelled antibodies against tenascin	Phase I and II for glioma	Increased survival[114]
ECM/basement-membrane signalling	Antibodies against integrin	Phase I and II for lymphoma, melanoma and glioblastoma	Ongoing*
ECM integrity	MMP inhibitors	Phase I, II, III	Initial results were negative[59]; new components and combinations are in Phase I*
ECM fragments	Injection of endostatin	Phase I	Ongoing[111]

*For further information, see the National Cancer Institute's Angiogenesis Inhibitors in Clinical Trials web page in the online links box. ‡For further information, see the Special Project Angiogenesis web page in the online links box. ECM, extracellular matrix; MMP, matrix metalloproteinase; NSAIDs, non-steroidal anti-inflammatory drugs; VEGF, vascular endothelial growth factor.

finding is in agreement with studies indicating that *NF1* heterozygosity in stromal fibroblasts, mast cells and PERINEURAL CELLS is essential for the development of neurofibroma[97].

Experimentally, this concept has been beautifully confirmed by findings that induction of mutations in the mammary stromal compartment by γ-irradiation or treatment with chemical mutagens promotes tumour formation. Long-overlooked studies from the 1950s reported increased tumour formation after carcinogen-treated stroma was transplanted with untreated skin epithelial cells[98]. More recently, the effects of carcinogen treatment on stromal cells were analysed in murine mammary tissue. Injection of unirradiated mammary epithelial cells into irradiated epithelial-cell-free (cleared) mammary fad pads resulted in neoplastic progression of the epithelial cells[99]. Similarly, Maffini and co-workers demonstrated that treatment of cleared mammary fad pads with the carcinogen *N*-nitroso-methyl urea (NMU) generated a stromal environment that was characterized by the acquisition of genetic mutations and allowed the development of epithelial tumours from transplanted normal mammary epithelial cells. Taking this study even further, they showed that treatment of the epithelial cells with NMU was not sufficient to induce tumour formation in an untreated stromal background, but, rather, required the grafting onto NMU-treated stroma[100].

PERINEURAL CELLS
The outermost layer of stromal cells that surround peripheral nerves.

Normalizing the stroma

There is abundant evidence that an abnormal stromal context contributes to, or is even required for, tumour formation and progression. 'Normalization' of the stromal environment should therefore be able to slow or even reverse tumour progression. The potential of a normal context to suppress a tumorigenic phenotype was first reported by Illmensee and Mintz, who showed that malignant mouse teratocarcinoma cells, grown over many transplant generations as ascites tumours *in vivo,* could develop into normal tissues and generate normal mice when injected into developing blastocysts[101]. Bissell and colleagues demonstrated that the presence of a reconstituted physiological basement membrane induces pre-malignant breast epithelial cells to undergo growth arrest and form polarized alveolar structures, as normal epithelial cells would[72]. This normalization is in part mediated by integrins, as blockade of signalling by β_1-integrin reverts tumorigenesis despite maintained genetic abnormalities in the epithelial cells[102]. Similarly, induction of granulation-tissue formation by transplantation of a hyaluronan-based scaffold before the transplantation of malignant keratinocytes into nude mice resulted in the formation of a fibrotic-type stroma that blocked formation of invasive tumours. This inhibition coincided with lack of tumour vascularization and was possibly caused by re-establishment of normal ECM and basement-membrane structures (M. Willhauck, H.J. Stark, N. Mirancea and N.E.F., unpublished observations). Finally, inhibition of angiogenesis — another approach to normalizing the tumour microenvironment — can result in a phenotypic reversion of a malignant and invasive to a non-invasive pre-malignant tumour phenotype[69,103] (FIG. 2).

Taken together, these observations indicate that the tumour microenvironment is a potential therapeutic

target (TABLES 1,2). Advantages to targeting the stroma include the fact that these cells are not as genetically unstable as cancer cells, and are therefore less likely to develop drug resistance[104,105]. There have already been several exciting success stories in the clinical targeting of tumour stroma (TABLE 1). Inhibition of inflammatory cells and cytokines by treatment with non-steroidal anti-inflammatory drugs (NSAIDs) has been shown to lower the risk for colon and breast cancer, and might help to prevent lung, oesophageal and stomach cancers[106]; for further information, see the NCI web site entitled 'Epidemiology: Nonsteroidal Anti-Inflammatory Drugs

Table 2 | **Potential therapeutic targets in the tumour stroma**

Stromal element	Alteration	Clinical and preclinical trial results
Stromal cells		
Endothelial cells	Blocking endothelial-cell proliferation (such as through inhibition of VEGF)	Induces endothelial-cell apoptosis, inhibits angiogenesis (TABLE 1) and numerous experimental systems[64]
Tumour-associated fibroblasts	Inhibiting fibroblast proliferation and activation (such as through inhibition of TGFβ signalling)	Promotes tumour progression; TGFβ overexpression, however, can promote malignancy in certain tumour types; tested in preclinical models of colon carcinoma, prostate and forestomach cancer[13,24]
Macrophages	Inhibiting macrophage recruitment by blocking recruitment factors	Reduces tumour malignancy in preclinical models of breast cancer[51,54]
Mast cells	Inhibiting mast-cell recruitment, such as by blocking recruitment factors	Reduces SCC skin tumour malignancy in mast-cell-deficient mice[12,59]
ECM molecules		
Thrombospondin	Overexpression	Inhibits tumour invasion in experimental models for skin SCC and other tumours[76,77]
Tenacin	Inhibition with radiolabelled inhibitory antibodies; inhibition of expression; blockade of tenascin binding inhibits tumour-cell migration	Prolonged patient survival in clinical trials of patients with gliomas (TABLE 2)[114]; inhibits angiogenesis and tumour-cell migration in preclinical models of melanoma and breast cancer[115,116]
Fibronectin	Inhibitory antibodies that target extradomain B	Inhibits angiogenesis in various tumour models[117]
Decorin	Adenovirus-mediated expression	Suppresses tumorigenicity of colon and squamous carcinoma models[118]
Hyaluronate	Expression; degradation by hyaluronidase	Promotes motility of tumour cells[119]; reduces tumour growth in preclinical models of melanoma and breast cancer[120]
ECM cleavage products	Generation of endostatin, angiostatin, tumstatin and others	Inhibits angiogenesis in various solid tumour models[17,87] and clinical trials (TABLE 2)
Matrix-degrading proteases and inhibitors		
MMPs	Inhibition	Inhibits invasion and angiogenesis, but can also be pro-angiogenic, by inhibiting the generation of reactive ECM fragments; negative results in clinical trials (TABLE 2) involving different treatment combinations[20]
ADAMs	Inhibition	Blocks release of growth factors from the ECM; increased expression observed in human tumour samples and preclinical tumour models[80]
Serpin/PAI1	Altering expression levels	Enzymes are pro-angiogenic at high (therapeutic) concentrations and anti-angiogenic at low (physiological) concentrations in preclinical models of skin SCC and other tumour models[59,80]
TIMPs	Expression	Suppresses tumour invasion and metastasis in tumour models, yet high expression levels correlate with poor prognosis in some human tumour types; expression studies performed in clinical samples and preclinical models[80,88]
Regulatory molecules		
Integrins	Inhibiting signalling	Blocks malignant progression and angiogenesis in clinical trials (TABLE 2) and preclinical models for breast and ovarian cancer[72–74]
Growth factors, cytokines produced by tumour cells (VEGF, PDGF, G-CSF, GM-CSF and others) or stromal cells (TGFβ, CSF1, HGF and IGF1 and others)	Inhibiting signalling	Inhibits tumour progression in preclinical models for colon carcinoma, breast, prostate and forestomach cancer[9,13,24,29,30,54] as well as skin SCC[55,68]
Inflammation-associated growth factors and chemokines	Inhibition	Blocks inflammation, which is associated with poor patient prognosis in clinical and preclincal models[45,51]

ADAM, a disintegrin and metalloproteinase; CSF1, colony stimulating factor 1; ECM, extracellular matrix; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; HGF, hepatocyte growth factor; IGF1, insulin-like growth factor 1; MMP, matrix metalloproteinase; PAI1, plasminogen activator inhibitor 1; PDGF, platelet-derived growth factor; SCC, squamous-cell carcinoma; TGFβ, transforming growth factor-β; VEGF, vascular endothelial growth factor.

and Cancer Prevention' in the online links box. Drugs designed to block VEGF signalling have proven successful in the treatment of colorectal cancer[107]. For example, bevacizumab — a blocking monoclonal antibody against VEGF — has been shown in Phase III trials, in a combination with first-line chemotherapy, to significantly prolong the life of patients with colorectal cancer and patients with kidney cancer[121]. Blockade of signalling by the epidermal growth factor receptor ERBB2 (also known as HER2) with a neutralizing antibody has been shown to downregulate tumour-cell-derived pro-angiogenic molecules[108]. Other than its ability to inhibit proliferation of EGFR-expressing cancer cells, this effect on the tumour stroma could be an additional mechanism by which the ERBB2-blocking antibody transtuzumab slows tumour growth in patients with breast cancer[109].

However, there are also some disappointments in targeting the stroma for cancer therapy. Clinical testing of MMP inhibitors has shown no efficacy in patients suffering from advanced stages of cancer, but, rather, has produced severe intolerable side effects and even sometimes worsened the prognosis for the patient[16] (TABLES 1,2). This might be explained by the fact that trials designed for these broad spectrum inhibitors did not take into account the many and contradictory roles that MMPs are believed to have in modulation of cell adhesive functions, integrin signalling, the activation of growth factors and revelation of cryptic sites in the ECM. To overcome these problems, the development of more specific inhibitors is now underway. However, when developing microenvironment-based therapies, we need to keep in mind that targeting just one aspect of the tumour stroma, and doing this in patients with late-stage cancer, is not likely to be successful. Therefore, the aim should be to combine drugs that target different aspects of the activated stroma with cytotoxic therapies that are directed against the tumour cells, thereby treating the tumour as the organ that we now recognize it to be.

1. Hanahan, D. & Weinberg, R. A. The hallmarks of cancer. *Cell* **100**, 57–70 (2000).
2. Paget, S. The distribution of secondary growths in cancer of the breast. *Lancet* **1**, 571–573 (1889).
3. Fidler, I. J. The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. *Nature Rev. Cancer* **3**, 453–458 (2003).
Summarizes our understanding of tumor–stroma interactions, beginning with Paget's seed and soil hypothesis and leading to the most recent findings on the regulatory mechanisms that determine the potential of tumour cells to metastasize.
4. Liotta, L. A. & Kohn, E. C. The microenvironment of the tumour–host interface. *Nature* **411**, 375–379 (2001).
5. Dvorak, H. F. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. *N. Engl. J. Med.* **315**, 1650–1659 (1986).
6. Dvorak, H. F., Senger, D. R. & Dvorak, A. M. Fibrin as a component of the tumor stroma: origins and biological significance. *Cancer Metastasis Rev.* **2**, 41–73 (1983).
7. Kuperwasser, C. *et al.* Reconstruction of functionally normal and malignant human breast tissues in mice. *Proc. Natl Acad. Sci. USA* **101**, 4966–4971 (2004).
8. Willis, R. A. in *Pathology of Tumors* 135–140 (Butterworth, London, 1960).
9. Folkman, J. Fundamental concepts of the angiogenic process. *Curr. Mol. Med.* **3**, 643–651 (2003).
Comprehensive summary of the development of the field of angiogenesis research over the past three decades, introducing fundamental concepts and describing mechanistic insights recently gained.
10. Werner, S. & Grose, R. Regulation of wound healing by growth factors and cytokines. *Physiol. Rev.* **83**, 835–870 (2003).
11. Bergers, G. & Benjamin, L. E. Tumorigenesis and the angiogenic switch. *Nature Rev. Cancer* **3**, 401–410 (2003).
12. Coussens, L. M. & Werb, Z. Inflammation and cancer. *Nature* **420**, 860–867 (2002).
Comprehensive overview of the concept that inflammation is a crucial component of tumour progression.
13. De Wever, O. & Mareel, M. Role of tissue stroma in cancer cell invasion. *J. Pathol.* **200**, 429–447 (2003).
Reviews the role of fibroblasts as a key component of the tumour microenvironment, their activation and their contribution to tumour growth and progression.
14. Manabe, Y., Toda, S., Miyazaki, K. & Sugihara, H. Mature adipocytes, but not preadipocytes, promote the growth of breast carcinoma cells in collagen gel matrix culture through cancer-stromal cell interactions. *J. Pathol.* **201**, 221–228 (2003).
15. Mueller, M. M., Werbowetski, T. & Del Maestro, R. F. Soluble factors involved in glioma invasion. *Acta Neurochir. (Wien)* **145**, 999–1008 (2003).
16. Stetler-Stevenson, W. G. & Yu, A. E. Proteases in invasion: matrix metalloproteinases. *Semin. Cancer Biol.* **11**, 143–152 (2001).
17. Kalluri, R. Basement membranes: structure, assembly and role in tumour angiogenesis. *Nature Rev. Cancer* **3**, 422–433 (2003).
Reviews the role of the basement membrane as a crucial component of the tumour microenvironment whose structure and components regulate the process of tumour angiogenesis.
18. Brinckerhoff, C. E. & Matrisian, L. M. Matrix metalloproteinases: a tail of a frog that became a prince. *Nature Rev. Mol. Cell Biol.* **3**, 207–214 (2002).
19. McCawley, L. J. & Matrisian, L. M. Matrix metalloproteinases: they're not just for matrix anymore! *Curr. Opin. Cell Biol.* **13**, 534–540 (2001).
20. Egeblad, M. & Werb, Z. New functions for the matrix metalloproteinases in cancer progression. *Nature Rev. Cancer* **2**, 161–174 (2002).
21. Bachmeier, B. E., Boukamp, P., Lichtinghagen, R., Fusenig, N. E. & Fink, E. Matrix metalloproteinases-2,-3,-7,-9 and -10, but not MMP-11, are differentially expressed in normal, benign tumorigenic and malignant human keratinocyte cell lines. *Biol. Chem.* **381**, 497–507 (2000).
22. Borchers, A. H. *et al.* Fibroblast-directed expression and localization of 92-kDa type IV collagenase along the tumor-stroma interface in an *in vitro* three-dimensional model of human squamous cell carcinoma. *Mol. Carcinog.* **19**, 258–266 (1997).
23. Airola, K. & Fusenig, N. E. Differential stromal regulation of MMP-1 expression in benign and malignant keratinocytes. *J. Invest. Dermatol.* **116**, 85–92 (2001).
24. Bhowmick, N. A. *et al.* TGF-β signaling in fibroblasts modulates the oncogenic potential of adjacent epithelia. *Science* **303**, 848–851 (2004).
25. Weeks, B. H., He, W., Olson, K. L. & Wang, X. J. Inducible expression of transforming growth factor β1 in papillomas causes rapid metastasis. *Cancer Res.* **61**, 7435–7443 (2001).
26. Lohr, M. *et al.* Transforming growth factor-β1 induces desmoplasia in an experimental model of human pancreatic carcinoma. *Cancer Res.* **61**, 550–555 (2001).
27. Gabbiani, G., Ryan, G. B. & Majne, G. Presence of modified fibroblasts in granulation tissue and their possible role in wound contraction. *Experientia* **27**, 549–550 (1971).
28. Powell, D. W. *et al.* Myofibroblasts. I. Paracrine cells important in health and disease. *Am. J. Physiol.* **277**, C1–C9 (1999).
29. De Wever, O. *et al.* Tenascin-C and SF/HGF produced by myofibroblasts *in vitro* provide the convergent proinvasive signals to human colon cancer cells through RhoA and Rac. *FASEB J.* **18**, 1016–1018 (2004).
30. Lewis, M. P. *et al.* Tumour-derived TGF-β1 modulates myofibroblast differentiation and promotes HGF/SF-dependent invasion of squamous carcinoma cells. *Br. J. Cancer* **90**, 822–832 (2004).
31. Shao, Z. M., Nguyen, M. & Barsky, S. H. Human breast carcinoma desmoplasia is PDGF initiated. *Oncogene* **19**, 4337–4345 (2000).
32. Garin-Chesa, P., Old, L. J. & Rettig, W. J. Cell surface glycoprotein of reactive stromal fibroblasts as a potential antibody target in human epithelial cancers. *Proc. Natl Acad. Sci. USA* **87**, 7235–7239 (1990).
33. Lazard, D. *et al.* Expression of smooth muscle-specific proteins in myoepithelium and stromal myofibroblasts of normal and malignant human breast tissue. *Proc. Natl Acad. Sci. USA* **90**, 999–1003 (1993).
34. Park, J. E. *et al.* Fibroblast activation protein, a dual specificity serine protease expressed in reactive human tumor stromal fibroblasts. *J. Biol. Chem.* **274**, 36505–36512 (1999).
35. Chauhan, H. *et al.* There is more than one kind of myofibroblast: analysis of CD34 expression in benign, *in situ*, and invasive breast lesions. *J. Clin. Pathol.* **56**, 271–276 (2003).
36. Olumi, A. F. *et al.* Carcinoma-associated fibroblasts direct tumor progression of initiated human prostatic epithelium. *Cancer Res.* **59**, 5002–5011 (1999).
37. Skobe, M. & Fusenig, N. E. Tumorigenic conversion of immortal human keratinocytes through stromal cell activation. *Proc. Natl Acad. Sci. USA* **95**, 1050–1055 (1998).
38. Cunha, G. R., Hayward, S. W., Wang, Y. Z. & Ricke, W. A. Role of the stromal microenvironment in carcinogenesis of the prostate. *Int. J. Cancer* **107**, 1–10 (2003).
Discusses the role of stromal–epithelial interactions in normal and malignant prostatic growth and the role that cell–cell interactions and androgen signalling have in determining the malignant phenotype of prostate tumours.
39. Sieuwerts, A. M. *et al.* Urokinase-type-plasminogen-activator (uPA) production by human breast (myo) fibroblasts *in vitro*: influence of transforming growth factor-β_1 (TGFβ_1) compared with factor(s) released by human epithelial-carcinoma cells. *Int. J. Cancer* **76**, 829–835 (1998).
40. Sato, T. *et al.* Tumor-stromal cell contact promotes invasion of human uterine cervical carcinoma cells by augmenting the expression and activation of stromal matrix metalloproteinases. *Gynecol. Oncol.* **92**, 47–56 (2004).
41. Krtolica, A. & Campisi, J. Integrating epithelial cancer, aging stroma and cellular senescence. *Adv. Gerontol.* **11**, 109–116 (2003).
42. Masson, R. *et al. In vivo* evidence that the stromelysin-3 metalloproteinase contributes in a paracrine manner to epithelial cell malignancy. *J. Cell Biol.* **140**, 1535–1541 (1998).
43. Li, G. *et al.* Function and regulation of melanoma-stromal fibroblast interactions: when seeds meet soil. *Oncogene* **22**, 3162–3171 (2003).
44. Orimo, A. *et al.* Cancer-associated myofibroblasts possess various factors to promote endometrial tumor progression. *Clin. Cancer Res.* **7**, 3097–3105 (2001).
45. Balkwill, F. & Mantovani, A. Inflammation and cancer: back to Virchow? *Lancet* **357**, 539–545 (2001).
46. Albertsson, P. A. *et al.* NK cells and the tumour microenvironment: implications for NK-cell function and anti-tumour activity. *Trends Immunol.* **24**, 603–609 (2003).
47. Kornfeld, D., Ekbom, A. & Ihre, T. Is there an excess risk for colorectal cancer in patients with ulcerative colitis and concomitant primary sclerosing cholangitis? A population based study. *Gut* **41**, 522–525 (1997).
48. Castellsague, X., Bosch, F. X. & Munoz, N. Environmental co-factors in HPV carcinogenesis. *Virus Res.* **89**, 191–199 (2002).
49. Nelson, W. G., DeWeese, T. L. & DeMarzo, A. M. The diet, prostate inflammation, and the development of prostate cancer. *Cancer Metastasis Rev.* **21**, 3–16 (2002).
50. Martins-Green, M., Boudreau, N. & Bissell, M. J. Inflammation is responsible for the development of wound-induced tumors in chickens infected with Rous sarcoma virus. *Cancer Res.* **54**, 4334–4341 (1994).

51. Pollard, J. W. Tumour-educated macrophages promote tumour progression and metastasis. *Nature Rev. Cancer* **4**, 71–78 (2004).
Overview of the evidence from clinical and experimental studies that macrophages promote solid-tumour progression and metastasis by adopting a trophic role that facilitates angiogenesis, matrix breakdown and tumour-cell motility — all of which are elements of the metastatic process.
52. Leek, R. D. & Harris, A. L. Tumor-associated macrophages in breast cancer. *J. Mammary Gland Biol. Neoplasia* **7**, 177–189 (2002).
53. Kacinski, B. M. CSF-1 and its receptor in ovarian, endometrial and breast cancer. *Ann. Med.* **27**, 79–85 (1995).
54. Lin, E. Y., Nguyen, A. V., Russell, R. G. & Pollard, J. W. Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. *J. Exp. Med.* **193**, 727–740 (2001).
55. Mueller, M. M. & Fusenig, N. E. Constitutive expression of G-CSF and GM-CSF in human skin carcinoma cells with functional consequence for tumor progression. *Int. J. Cancer* **83**, 780–789 (1999).
56. Mueller, M. M. *et al.* Autocrine growth regulation by granulocyte colony-stimulating factor and granulocyte macrophage colony-stimulating factor in human gliomas with tumor progression. *Am. J. Pathol.* **155**, 1557–1567 (1999).
57. Braun, B., Lange, M., Oeckler, R. & Mueller, M. M. Expression of G-CSF and GM-CSF in human meningiomas correlates with increased tumor proliferation and vascularization. *J. Neurooncology* **68**, 131–140 (2004).
58. Obermueller, E., Vosseler, S., Fusenig, N. E. & Mueller, M. M. Cooperative auto- and paracrine functions of G-CSF and GM–CSF in the progression of skin carinoma cells. *Cancer Res.* (in the press).
59. DeClerck, Y. A. *et al.* Proteases, extracellular matrix, and cancer: a workshop of the path B study section. *Am. J. Pathol.* **164**, 1131–1139 (2004).
60. Carmeliet, P. & Jain, R. K. Angiogenesis in cancer and other diseases. *Nature* **407**, 249–257 (2000).
61. Goldman, E. The growth of malignant disease in man and the lower animals with special reference to the vascular system. *Lancet* **2**, 1236–1240 (1907).
62. Black, W. C. & Welch, H. G. Advances in diagnostic imaging and overestimations of disease prevalence and the benefits of therapy. *N. Engl. J. Med.* **328**, 1237–1243 (1993).
63. Hanahan, D. & Folkman, J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* **86**, 353–364 (1996).
64. Kerbel, R. & Folkman, J. Clinical translation of angiogenesis inhibitors. *Nature Rev. Cancer* **2**, 727–739 (2002).
Discusses the role of angiogenesis inhibitors as cancer therapeutics and deals with the questions that are associated with their clinical use and their further optimization.
65. Vajkoczy, P. *et al.* Microtumor growth initiates angiogenic sprouting with simultaneous expression of VEGF, VEGF receptor-2, and angiopoietin-2. *J. Clin. Invest.* **109**, 777–785 (2002).
66. Holash, J., Wiegand, S. J. & Yancopoulos, G. D. New model of tumor angiogenesis: dynamic balance between vessel regression and growth mediated by angiopoietins and VEGF. *Oncogene* **18**, 5356–5362 (1999).
67. Fusenig, N. E. *et al.* in *Proteases and their Inhibitors in Cancer Metastasis* (eds Foidart, J. M. & Muschel, R. J.) 205–223 (Kluwer Academic, Dordrecht, The Netherlands, 2002).
68. Mueller, M. M. & Fusenig, N. E. Tumor-stroma interactions directing phenotype and progression of epithelial skin tumor cells. *Differentiation* **70**, 486–497 (2002).
69. Skobe, M., Rockwell, P., Goldstein, N., Vosseler, S. & Fusenig, N. E. Halting angiogenesis suppresses carcinoma cell invasion. *Nature Med.* **3**, 1222–1227 (1997).
70. Smith-McCune, K. K. & Weidner, N. Demonstration and characterization of the angiogenic properties of cervical dysplasia. *Cancer Res.* **54**, 800–804 (1994).
71. Clark, E. R. & Clark, E. L. Microscopic observations on the growth of blood capillaries in the living organisms. *Am. J. Anat.* **64**, 251–264 (1938).
72. Roskelley, C. D. & Bissell, M. J. The dominance of the microenvironment in breast and ovarian cancer. *Semin. Cancer Biol.* **12**, 97–104 (2002).
Summarizes the role of the tumour microenvironment in determining breast cancer phenotypes, with particular emphasis on the suppressive effects of the environment.
73. Andriani, F., Garfield, J., Fusenig, N. E. & Garlick, J. A. Basement membrane proteins promote progression of intraepithelial neoplasia in 3-dimensional models of human stratified epithelium. *Int. J. Cancer* **108**, 348–357 (2004).
74. Jain, R. K. Molecular regulation of vessel maturation. *Nature Med.* **9**, 685–693 (2003).
75. Yang, C. *et al.* Integrin $\alpha1\beta1$ and $\alpha2\beta1$ are the key regulators of hepatocarcinoma cell invasion across the fibrotic matrix microenvironment. *Cancer Res.* **63**, 8312–8317 (2003).
76. Bleuel, K., Popp, S., Fusenig, N. E., Stanbridge, E. J. & Boukamp, P. Tumor suppression in human skin carcinoma cells by chromosome 15 transfer or thrombospondin-1 overexpression through halted tumor vascularization. *Proc. Natl Acad. Sci. USA* **96**, 2065–2070 (1999).
77. Lawler, J. The functions of thrombospondin-1 and-2. *Curr. Opin. Cell Biol.* **12**, 634–640 (2000).
78. Lockwood, D. S. *et al.* Tumor progression in hepatocellular carcinoma: relationship with tumor stroma and parenchymal disease. *J. Gastroenterol. Hepatol.* **18**, 666–672 (2003).
79. Bajou, K. *et al.* Absence of host plasminogen activator inhibitor 1 prevents cancer invasion and vascularization. *Nature Med.* **4**, 923–928 (1998).
80. Noel, A. *et al.* Membrane associated proteases and their inhibitors in tumour angiogenesis. *J. Clin. Pathol.* **57**, 577–584 (2004).
Comprehensive overview on the role of different proteases in ECM degradation — an important mechanism for generating biologically active proteins that regulate processes such as angiogenesis and tumour invasion.
81. Cornelius, L. A. *et al.* Matrix metalloproteinases generate angiostatin: effects on neovascularization. *J. Immunol.* **161**, 6845–6852 (1998).
82. Dong, Z., Kumar, R., Yang, X. & Fidler, I. J. Macrophage-derived metalloelastase is responsible for the generation of angiostatin in Lewis lung carcinoma. *Cell* **88**, 801–810 (1997).
83. O'Reilly, M. S. *et al.* Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. *Cell* **88**, 277–285 (1997).
84. Marneros, A. G. & Olsen, B. R. The role of collagen-derived proteolytic fragments in angiogenesis. *Matrix Biol.* **20**, 337–345 (2001).
85. Jin, X. *et al.* Evaluation of endostatin antiangiogenesis gene therapy *in vitro* and *in vivo*. *Cancer Gene Ther.* **8**, 982–989 (2001).
86. Joki, T. *et al.* Continuous release of endostatin from microencapsulated engineered cells for tumor therapy. *Nature Biotechnol.* **19**, 35–39 (2001).
87. Maeshima, Y. *et al.* Distinct antitumor properties of a type IV collagen domain derived from basement membrane. *J. Biol. Chem.* **275**, 21340–21348 (2000).
88. Sounni, N. E., Janssen, M., Foidart, J. M. & Noel, A. Membrane type-1 matrix metalloproteinase and TIMP-2 in tumor angiogenesis. *Matrix Biol.* **22**, 55–61 (2003).
89. Tlsty, T. D. Stromal cells can contribute oncogenic signals. *Semin. Cancer Biol.* **11**, 97–104 (2001).
Reviews the transforming effect of stromal cells on epithelial components and provides insights into the possible regulatory mechanisms that underlie the initiation and progression of malignant cells.
90. Phillips, J. L. *et al.* The consequences of chromosomal aneuploidy on gene expression profiles in a cell line model for prostate carcinogenesis. *Cancer Res.* **61**, 8143–8149 (2001).
91. Mueller, M. M. *et al.* Tumor progression of skin carcinoma cells *in vivo* promoted by clonal selection, mutagenesis, and autocrine growth regulation by granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor. *Am. J. Pathol.* **159**, 1567–1579 (2001).
92. Yuan, J. & Glazer, P. M. Mutagenesis induced by the tumor microenvironment. *Mutat. Res.* **400**, 439–446 (1998).
93. Jacoby, R. F. *et al.* A juvenile polyposis tumor suppressor locus at 10q22 is deleted from nonepithelial cells in the lamina propria. *Gastroenterology* **112**, 1398–1403 (1997).
94. Moinfar, F. *et al.* Concurrent and independent genetic alterations in the stromal and epithelial cells of mammary carcinoma: implications for tumorigenesis. *Cancer Res.* **60**, 2562–2566 (2000).
95. Haggie, J. A., Sellwood, R. A., Howell, A., Birch, J. M. & Schor, S. L. Fibroblasts from relatives of patients with hereditary breast cancer show fetal-like behaviour *in vitro*. *Lancet* **1**, 1455–1457 (1987).
96. Weaver, V. M. & Gilbert, P. Watch thy neighbor: cancer is a communal affair. *J. Cell Sci.* **117**, 1287–1290 (2004).
97. Zhu, Y., Ghosh, P., Charnay, P., Burns, D. K. & Parada, L. F. Neurofibromas in NF1: Schwann cell origin and role of tumor environment. *Science* **296**, 920–922 (2002).
98. Billingham, R., Orr, J. & Woodhouse, D. Transplantation of skin components during chemical carcinogenesis with 20-methylcholantrene. *Br. J. Cancer* **5**, 417–432 (1951).
99. Barcellos-Hoff, M. H. & Ravani, S. A. Irradiated mammary gland stroma promotes the expression of tumorigenic potential by unirradiated epithelial cells. *Cancer Res.* **60**, 1254–1260 (2000).
100. Maffini, M. V., Soto, A. M., Calabro, J. M., Ucci, A. A. & Sonnenschein, C. The stroma as a crucial target in rat mammary gland carcinogenesis. *J. Cell Sci.* **117**, 1495–1502 (2004).
101. Illmensee, K. & Mintz, B. Totipotency and normal differentiation of single teratocarcinoma cells cloned by injection into blastocysts. *Proc. Natl Acad. Sci. USA* **73**, 549–553 (1976).
102. Weaver, V. M. *et al.* Reversion of the malignant phenotype of human breast cells in three-dimensional culture and *in vivo* by integrin blocking antibodies. *J. Cell Biol.* **137**, 231–245 (1997).
103. Vajkoczy, P. *et al.* Targeting angiogenesis inhibits tumor infiltration and expression of the pro-invasive protein SPARC. *Int. J. Cancer* **87**, 261–268 (2000).
104. Sporn, M. B. & Suh, N. Chemoprevention of cancer. *Carcinogenesis* **21**, 525–530 (2000).
105. Kerbel, R. S. A cancer therapy resistant to resistance. *Nature* **390**, 335–336 (1997).
106. Ricchi, P., Zarrilli, R., Di Palma, A. & Acquaviva, A. M. Nonsteroidal anti-inflammatory drugs in colorectal cancer: from prevention to therapy. *Br. J. Cancer* **88**, 803–807 (2003).
107. Salgaller, M. L. Technology evaluation: bevacizumab, Genentech/Roche. *Curr. Opin. Mol. Ther.* **5**, 657–667 (2003).
108. Izumi, Y., Xu, L., di Tomaso, E., Fukumura, D. & Jain, R. K. Tumour biology: herceptin acts as an anti-angiogenic cocktail. *Nature* **416**, 279–280 (2002).
109. Tan, A. R. & Swain, S. M. Ongoing adjuvant trials with trastuzumab in breast cancer. *Semin. Oncol.* **30**, 54–64 (2003).
110. Ferrara, N., Hillan, K. J., Gerber, H. P. & Novotny, W. Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. *Nature Rev. Drug Discov.* **3**, 391–400 (2004).
111. Sridhar, S. S. & Shepherd, F. A. Targeting angiogenesis: a review of angiogenesis inhibitors in the treatment of lung cancer. *Lung Cancer* **42** (Suppl. 1), 81–91 (2003).
112. Yang, J. C. *et al.* A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. *N. Engl. J. Med.* **349**, 427–434 (2003).
113. Koehne, C. H. & Dubois, R. N. COX-2 inhibition and colorectal cancer. *Semin. Oncol.* **31**, 12–21 (2004).
114. Goetz, C. *et al.* Locoregional radioimmunotherapy in selected patients with malignant glioma: experiences, side effects and survival times. *J. Neurooncol.* **62**, 321–328 (2003).
115. Tanaka, K., Hiraiwa, N., Hashimoto, H., Yamazaki, Y. & Kusakabe, M. Tenascin-C regulates angiogenesis in tumor through the regulation of vascular endothelial growth factor expression. *Int. J. Cancer* **108**, 31–40 (2004).
116. Kalembeyi, I. *et al.* Tenascin-C upregulates matrix metalloproteinase-9 in breast cancer cells: direct and synergistic effects with transforming growth factor β1. *Int. J. Cancer* **105**, 53–60 (2003).
117. Ebbinghaus, C., Scheuermann, J., Neri, D. & Elia, G. Diagnostic and therapeutic applications of recombinant antibodies: targeting the extra-domain B of fibronectin, a marker of tumor angiogenesis. *Curr. Pharm. Des.* **10**, 1537–1549 (2004).
118. Reed, C. C., Gauldie, J. & Iozzo, R. V. Suppression of tumorigenicity by adenovirus-mediated gene transfer of decorin. *Oncogene* **21**, 3688–3695 (2002).
119. Ichikawa, T. *et al.* Increased synthesis of hyaluronate enhances motility of human melanoma cells. *J. Invest. Dermatol.* **113**, 935–939 (1999).
120. Shuster, S., Frost, G. I., Csoka, A. B., Formby, B. & Stern, R. Hyaluronidase reduces human breast cancer xenografts in SCID mice. *Int. J. Cancer* **102**, 192–197 (2002).
121. Mass, R. D. *et al.* Bevacizumab in combination with 5-FU/leucovorin improves survival in patients with metastatic colorectal cancer: A combined analysis. *Proc. Am. Soc. Clin. Oncol.* **22**, A3616 (2004).

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