

# Jekyll and Hyde: the role of the microenvironment on the progression of cancer

Michael Allen and J Louise Jones\*

Centre for Tumour Biology, Barts Cancer Institute, Barts and the London School of Medicine and Dentistry, London, UK

\*Correspondence to: J Louise Jones, Centre for Tumour Biology, Barts Institute of Cancer, Barts and the London School of Medicine and Dentistry, Charterhouse Square, London EC1M 6BQ, UK e-mail: l.j.jones@qmul.ac.uk

## Abstract

It is now recognized that the host microenvironment undergoes extensive change during the evolution and progression of cancer. This involves the generation of cancer-associated fibroblasts (CAFs), which, through release of growth factors and cytokines, lead to enhanced angiogenesis, increased tumour growth and invasion. It has also been demonstrated that CAFs may modulate the cancer stem cell (CSC) phenotype, which has therapeutic implications. The altered fibroblast phenotype also contributes to the development of an altered extracellular matrix (ECM), with synthesis of ECM isoforms rarely found in normal tissues, including tenascin-C isoforms and the fibronectin EDA isoform. There is also emerging evidence of how the tensile strength of the tumour-associated ECM may be modified and lead to altered signalling in tumour cells. The hypoxic environment of the tumour stimulates angiogenesis and also impacts on other aspects of cell signalling, including the c-met pathway and lysyl oxidase-mediated signalling, which can directly promote tumour cell invasion. The inflammatory infiltrate associated with many solid tumours also modulates tumour function, having both anti- and pro-tumour effects. All of these components of the microenvironment provide potential targets for therapeutic attack, with a number of molecules already in clinical trials. It is also becoming evident that characterizing the tumour microenvironment can provide important prognostic and predictive information about tumours, independent of the tumour cell phenotype.

Copyright © 2010 Pathological Society of Great Britain and Ireland. Published by John Wiley & Sons, Ltd.

**Keywords:** microenvironment; extracellular matrix; hypoxia; angiogenesis; inflammation; tumour-associated macrophages

Received 12 August 2010; Revised 1 October 2010; Accepted 1 October 2010

No conflicts of interest were declared.

## Introduction

In recent years the tumour microenvironment has become the focus of intense research, with the understanding that the alterations that occur in the stroma around a tumour might prove useful in prognosis and generate new therapeutic targets. However, the importance of the microenvironment is not a new concept. The idea that stromal cells might promote cancer development was first recognized in 1863 when Rudolph Virchow observed leukocytes in the stroma of neoplastic tissue and hypothesized that malignancy originated at sites of chronic inflammation [1]. This focused only on the inflammation and did not consider the role of other stromal elements, although when Paget put forward his 'seed and soil' hypothesis in 1889 this did encompass all components [2]. It was not until 1982 that Bissell *et al* outlined a modern theory that the microenvironment in which a tumorigenic cell evolves is as critical to its evolution as the genetic mutations that it accrues [3]. This review considers several of the key elements that make up the stromal environment and how changes in these compartments influence cancer development and progression

(Figure 1). We also consider how an understanding of the tumour microenvironment may have predictive and prognostic importance, and provide new avenues for therapeutic attack. Whilst such changes do play a role in lymphoma and leukaemia, this review limits itself to the microenvironment of solid tumours.

## Influence of cancer-associated fibroblasts on tumour behaviour

Fibroblasts are among the most abundant cell type in the microenvironment of solid tumours, being particularly prominent in carcinomas of breast, pancreas, colon and prostate. There is abundant evidence that cancer-associated fibroblasts (CAFs) can contribute to tumour growth and spread, mediated through their release of classical growth factors, such as EGF, TGF $\beta$  and HGF, as well as a range of chemokines shown to influence different aspects of tumour cell behaviour (Figure 2) [4].

Orimo *et al* [5] showed that fibroblasts derived from primary human invasive breast carcinomas significantly enhanced tumour growth in xenograft models

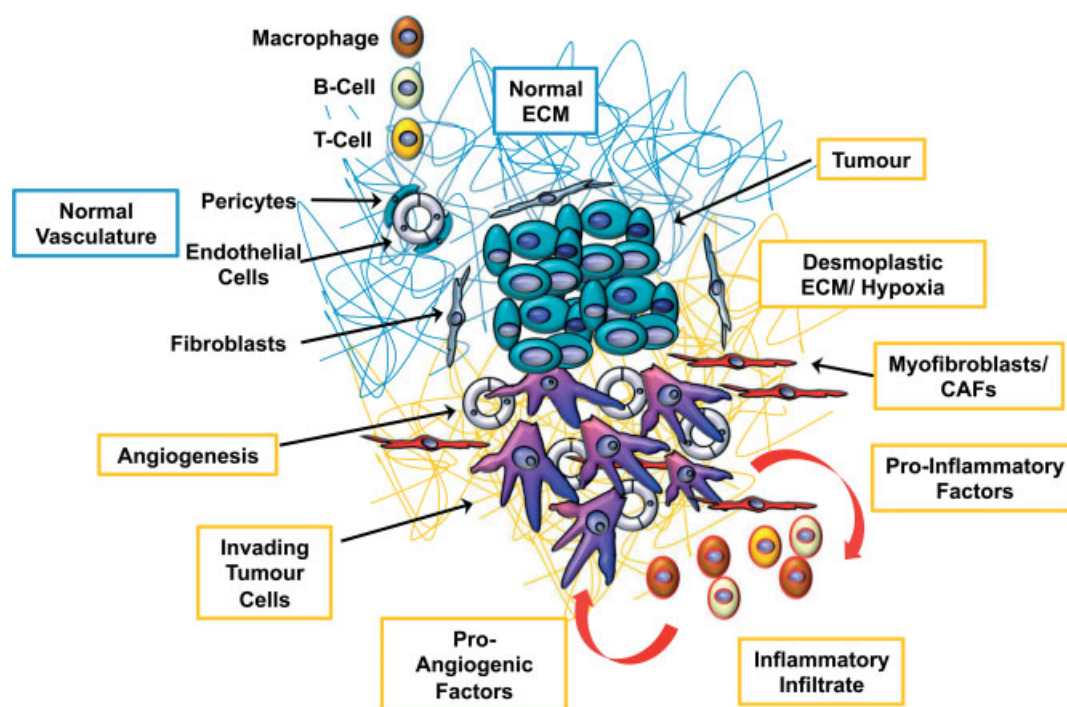


Figure 1. Changes to the normal microenvironment promote tumour invasion. Altered function of carcinoma-associated fibroblasts and induction of an inflammatory infiltrate lead to release of pro-angiogenic factors. Development of a desmoplastic stroma, partly in response to hypoxia, leads to tumour-specific interactions with tumour cell-surface receptors that enhance invasion.

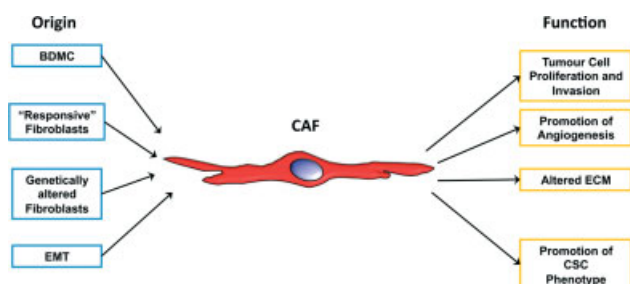


Figure 2. Origins and functions of carcinoma-associated fibroblasts (CAFs). A number of origins have been proposed for CAFs, including bone marrow-derived cells (BMDCs), response of normal fibroblasts to tumour-derived signals, genetic and epigenetic alterations in normal fibroblasts, or epithelial–mesenchymal transition (EMT) of normal or tumour epithelial cells. Their effects on tumour cells include promotion of tumour growth and invasion, stimulation of angiogenesis, generation of an altered extracellular matrix (ECM) and modulation of the cancer stem cell (CSC) phenotype.

compared to their normal counterparts. They demonstrated that these CAFs produced higher levels of stromal-derived factor (SDF)-1, which mediated the recruitment of endothelial progenitor cells into the tumour mass, leading to enhanced angiogenesis as well as directly promoting tumour cell growth via interaction with tumour cell CXCR4. Interestingly, they found that these unique characteristics of CAFs were maintained even in the absence of contact with tumour cells, implying that CAF function is not merely a response to tumour-derived signals [5]. Other stromal-derived growth factors also contribute to the tumour-promoting effect of CAFs. Yang *et al* [6] found

that prostate-derived fibroblasts capable of promoting LNCaP prostate cancer tumorigenesis exhibited significantly higher levels of connective tissue growth factor (CTGF) than non-tumour promoting fibroblasts. Using a xenograft model system they showed that CTGF in tumour-associated stroma induced a significant increase in angiogenesis and enhanced tumour growth. CTGF is potently stimulated by TGF $\beta$  [7], and is over-expressed in the stroma of several cancer types, including breast, pancreatic and oesophageal carcinoma [8–10]. Yang *et al* [6] went on to show that TGF $\beta$ -treatment of non-tumour-promoting fibroblasts led to up-regulation of CTGF and subsequent acquisition of tumour-promoting activity.

In addition to promoting growth of established tumours, there is strong evidence that altered fibroblast signalling may be critical in the initiation of carcinogenesis, in regulating its phenotype and in mediating metastatic spread of tumours.

In a series of now classic experiments by Cunha *et al* [11], the impact of tumour-associated stroma on epithelial behaviour was elegantly demonstrated. The SV40-immortalized but non-tumorigenic prostate epithelial cell line BPH-1 was transplanted into mice with either fibroblasts isolated from primary prostate cancers (i.e. CAFs) or normal prostate fibroblasts. Poorly differentiated carcinomas developed in the presence of CAFs, whilst minimal epithelial cell growth, and no tumour development, was supported in the presence of normal fibroblasts. Interestingly, when BPH-1 cells were grown with rat or mouse urogenital mesenchyme, epithelial cell growth was stimulated but

there was no tumorigenic growth, suggesting that the pro-tumour function of CAFs does not simply relate to enhanced mitogenesis [11]. It is important to note that CAFs did not stimulate tumour development in non-immortalized benign prostate epithelium, suggesting that some epithelial abnormality is necessary to respond to the pro-tumorigenic effects of CAFs. More recently, Bhowmick *et al* [12] generated mice in which the TGF $\beta$ II receptor was selectively ablated in fibroblasts and showed that these mice spontaneously developed neoplastic lesions, including invasive carcinoma of the fore-stomach. They demonstrated that these lesions were associated with expansion of the stromal compartment and enhanced HGF expression [12].

In 2010 Erez *et al* demonstrated that CAFs from dysplastic skin support tumorigenesis by mediating tumour-enhancing inflammation through activation of a pro-inflammatory gene expression signature. They were able to link this effect to NF- $\kappa$ B signalling in the CAF, as when IKK $\beta$  was knocked down there was reduction in recruitment of macrophages and vascularization [13]. These models suggest that altered signalling from fibroblasts can influence early tumour development by disrupting normal epithelial cell–stromal interactions.

A recent study has highlighted a further, potentially important, role for CAFs in the regulation of tumour behaviour—that of modulating the cancer stem cell (CSC) phenotype. In the normal colon, Wnt signalling plays a critical role in maintaining intestinal stem cell and crypt homeostasis [14], whilst disruption of Wnt signalling is recognized as an early event in the development of colorectal cancer: mutation of APC or  $\beta$ -catenin results in accumulation of  $\beta$ -catenin in the nucleus, where it associates with the T cell factor/lymphoid enhancer factor (TCF/LEF) family of transcription factors, leading to sustained transcription of Wnt target genes [15]. Using a TCF/LEF reporter directing the expression of green fluorescent protein (TOP–GFP), Vermeulen *et al* [16] demonstrated that Wnt activity is heterogeneous in colon cancer cells, despite all cells carrying an APC mutation. Furthermore, those cells showing the highest level of Wnt activity, identified by high levels of TOP–GFP, showed up-regulation of stem cell-associated genes and enhanced clonogenic potential. They went on to show that this CSC phenotype could be regulated by tumour-associated myofibroblasts: co-culture of colon cancer cells with myofibroblasts or myofibroblast conditioned medium (CM) resulted in enhanced nuclear  $\beta$ -catenin, increased Wnt activity and enhanced clonogenic activity, with enhanced tumorigenicity when co-injected into mice. This important study indicates that the stem cell phenotype is plastic and is dependent on the tumour microenvironment. The authors showed some evidence to suggest that myofibroblast release of HGF may be involved in this regulation, and suggest that targeting the CSC–microenvironment interface may be the most effective approach to overcome stem cell resistance to current therapies [16].

## The origin of cancer-associated fibroblasts

Cancer-associated fibroblasts (CAFs) form a heterogeneous population, probably related to their diverse origin (Figure 2). Whilst activation of local stromal fibroblasts has traditionally been considered the major source of CAFs [4], experimental models using genetically marked bone marrow-derived cells have demonstrated that these cells can contribute to the tumour-associated stroma and develop markers and functions of CAFs [17]. It has also been suggested that CAFs may be derived from normal or tumour cells that undergo epithelial–mesenchymal transition (EMT) [18], a theory that may explain the identification of identical genetic alterations in tumour epithelium and its associated stroma [19].

The concept of inherent dysfunction of host fibroblasts in patients with cancer remains a controversial area. A number of early reports demonstrating loss of heterozygosity (LOH) in stromal samples suggested that tumour-associated stroma is genetically unstable and may undergo genetic alterations that influence its function [20,21]. More recently, total genome LOH analysis and mutational screening for *TP53* in microdissected breast tumour epithelium and stroma identified somatic *TP53* mutations in the stroma and showed this to be associated with regional lymph node metastasis [22]. This is one of the first studies to suggest the clinical importance of stromal changes in breast cancer. However, this study was performed on DNA from formalin-fixed, paraffin-embedded material, and a limited study on stroma derived from frozen material yielded no evidence of *TP53* mutations [23]. Similarly, a more extensive study of LOH in CAFs derived from breast and ovarian carcinomas suggested that fibroblast LOH was a very rare event and could not explain the CAF phenotype [24]. Further studies are required to settle this controversy.

Other mechanisms by which the altered phenotype of CAFs could be maintained in the absence of a tumour cell population would be via epigenetic modulation of the DNA. Indeed, altered DNA methylation has been identified in fibroblasts from colorectal cancer [25] and breast cancer [26] compared to their normal counterparts, and methylation patterns were associated with altered mRNA levels, suggesting that epigenetic modification could contribute to the CAF phenotype.

Finally, the response of the microenvironment to a tumour may also be influenced by intrinsic host genetic variability. In keeping with this, some reports have indicated that even non-tumour fibroblasts from women with breast cancer differ from those isolated from women without breast cancer [27,28]. Such variability can be generated through single nucleotide polymorphisms (SNPs). Common functional promoter SNPs have been described in many genes, including the matrix metalloproteinase (MMP) enzymes that are involved in modifying the extracellular matrix,



where they have the potential to influence levels of gene expression [29,30]. An analysis of the ability of primary fibroblasts to promote breast cancer cell invasion demonstrated that fibroblasts derived from women with a high-expressing MMP-3 genotype generated significantly more invasion than fibroblasts from women without this genotype, regardless of whether or not the fibroblasts were derived from patients with breast cancer [31]. Similar variations influencing the immune system may also have an effect on tumour development [32]. This suggests that genetic characteristics can influence how the stromal microenvironment responds to a tumour and may influence clinical outcome.

### The extracellular matrix and its remodelling

As well as releasing a range of chemokines and growth factors, CAFs also generate an altered extracellular matrix (ECM) environment. Most solid tumours exhibit a very different profile of ECM proteins in the stroma compared to their normal counterparts, and many of these proteins interact directly with tumour cells, via integrins and other cell surface receptors, to influence functions such as proliferation, apoptosis, migration and differentiation [33]. A number of proteins are consistently up-regulated in solid tumours, including tenascin-C (TNC), fibronectin (FN) and SPARC (secreted protein, acidic and rich in cysteine). Further diversity is introduced through the expression of alternatively spliced variants of some of these proteins, notably TNC and FN. TNC is a multifunctional protein that is expressed at low levels in normal adult tissues but up-regulated in situations associated with cell migration, such as embryogenesis, wound healing and in tumours [34]. It exists as multiple alternatively spliced isoforms, which appear to be expressed in a tumour-specific manner. Whilst the full-length unspliced protein appears to be expressed in both pancreatic and prostate cancer [35,36], a novel isoform containing domain C, which is undetectable in normal tissues and rarely expressed in other tumours, is abundant in high grade gliomas [37]. In both breast [38] and ovarian [39] carcinoma, isoforms containing domains A and D are particularly abundant, and these have been shown to have functional significance, promoting tumour growth and invasion [40]. It is not entirely clear how these tumour-associated isoforms influence tumour cell behaviour, but additional domains can introduce new integrin receptor sites that alter cell signalling [41] or may lead to up-regulation of proteolytic enzymes that can remodel the matrix and enhance invasion [42].

In a similar manner to TNC, alternative splicing of *FN* pre-mRNA, as well as post-translational modification, generates up to 20 variants of this complex protein [43]. In tumours, there is frequent up-regulation of the 'oncofetal' forms of FN that contain extra-domain

(ED)A, EDB and IIICS (type III connecting sequence) sequences [44]. FN-EDA is said to be required for the transduction of TGF $\beta$  signals, and the conversion of fibroblasts to myofibroblasts, a key event in the tumour microenvironment [45], whereas FN-EDB is particularly associated with neovascular structures in many different tumour types [46,47]. Despite this, EDB- (and EDA)-null mice exhibit no defects in physiological or tumour angiogenesis, suggesting that there is functional redundancy, although they remain highly selective markers for the targeting of tumour-associated stroma and vasculature [48].

As well as the composition of the ECM, the mechanical properties of the stroma also have a profound impact on function. The tensile strength or stiffness of the ECM can regulate epithelial cell growth, differentiation and migration [49,50], and reduction of ECM stiffness can repress the malignant behaviour of mammary epithelial cells [49]. One way in which tensile strength can be modulated is via lysyl oxidase (LOX), an enzyme secreted primarily by fibroblasts that serves to cross-link collagens and elastin, increasing the insoluble matrix and contributing to tensile strength [51]. A recent study using the MMTV-Neu mouse model of mammary cancer confirmed enhanced levels of LOX activity, increased collagen cross-linking and elevated ECM stiffness with progression to invasive disease [52]. These stromal changes were associated with increased focal adhesion formation and increased FAK activity, and inhibition of  $\beta$ 1-integrin in this context prevented tumour invasion, suggesting that changes in ECM stiffness regulate epithelial cells through integrin signalling [52]. LOX pre-conditioning and stiffening of the mouse mammary fat pad also resulted in growth and invasion of premalignant mammary cells [52], suggesting that increased ECM stiffness can promote tumorigenesis as well as alter established tumour behaviour. This may offer insight as to how enhanced mammographic density leads to increased relative risk of breast cancer [53].

The ECM undergoes significant remodelling during tumour progressions and this is mediated largely by the extracellular proteinases, particularly the matrix metalloproteinases (MMPs), and the major source of these is from the stromal cells [54]. MMPs have been implicated in the promotion of tumour invasion and metastasis for decades, although enthusiasm for them as a plausible therapeutic target waned following disappointing clinical trials [55]. This has led to a re-examination of MMP function and a greater understanding of the complex roles of these enzymes. It is now evident that some MMPs act as tumour suppressors rather than tumour promoters [56] and, as well as inducing angiogenesis, may in some instances cleave proteins, leading to the generation of anti-angiogenic fragments such as angiostatin [57]. This more detailed understanding of MMP function may facilitate future developments of MMP-targeted therapies.

### Organ-specific microenvironment: the myoepithelial cell

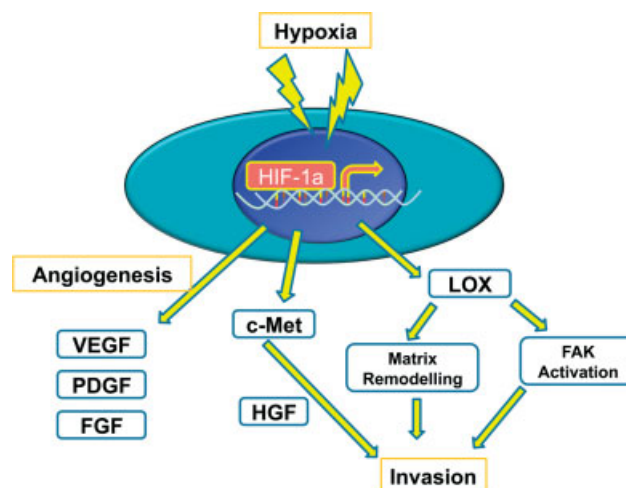
Many of the responses of the microenvironment are common to all organs and tumours, which is one of the features that make this an attractive therapeutic target. However, there are organ-specific differences that are important to understand. This is best illustrated in the breast, where the myoepithelial cell truly acts as the 'Jekyll and Hyde' of the microenvironment. Normal breast myoepithelial cells have been shown to exhibit anti-angiogenic [58], anti-proliferative [59] and anti-invasive [60,61] properties.

However, it is clear that myoepithelial cells change as Ductal Carcinoma in-situ (DCIS) of the breast progresses. These cells show loss of hemidesmosome formation [62] and up-regulation of pro-invasive ECM proteins [38], and Allinen *et al* [63] showed that myoepithelial cells exhibit more dramatic changes than any other cell component between normal and DCIS tissues, suggesting extensive abnormal paracrine interactions in DCIS. Indeed, xenograft studies suggest that dedifferentiation of host myoepithelial cells leads to the transition of *in situ* to invasive disease.

### Hypoxia and angiogenesis

Just as normal tissues require a supply of nutrients and the removal of waste products, so do tumours [64]. As tumours grow, areas of nutrient deprivation and oxygen deprivation (hypoxia) arise as a result of an insufficient blood supply [65]. Although a limiting factor for tumour growth, hypoxia also represents a stimulus for invasion and metastasis, and a number of studies have shown that hypoxia is an independent predictor of poor prognosis [66–68]. Hypoxia stimulates hypoxia-inducible family (HIF) proteins, which regulate diverse cellular processes, including metabolism [69], angiogenesis, cell proliferation, apoptosis [70] and tissue remodelling [71]. In addition, hypoxia can down-regulate epithelial E-cadherin and so facilitate EMT [72], and low oxygen tension causes cancer cells to switch to anaerobic metabolism, which greatly increases the genetic instability of the cells (Figure 3) [73,74]. Indeed, in 2001 Jain *et al* developed an *in vivo* microscopy method that demonstrated that VEGF transcription in brain tumours is regulated by the tissue  $pO_2$ . They found that under hypoxic conditions, VEGF-promoter activity increased. This was supported by their *in vitro* experiments [75].

One of the key roles of HIF-1 $\alpha$  in hypoxia is the induction of pro-angiogenic factors, including VEGF, angiopoietin-2, PDGF and FGF [76–79], and down-regulation of anti-angiogenic factors, such as thrombospondin [80]. Angiogenesis is required if a tumour is to progress past a certain size and the microenvironment plays an important role in dictating when the 'angiogenic switch' will occur. This angiogenic switch



**Figure 3.** Effect of hypoxia on tumour cell behaviour: Hypoxia leads to up-regulation of HIF-1 $\alpha$ , which leads to an angiogenic switch, enhancing expression of pro-angiogenic factors such as VEGF, PDGF and FGF, with down-regulation of anti-angiogenic thrombospondin. HIF-1 $\alpha$  also up-regulates *c-met* (leading to enhanced sensitivity to HGF), promotes EMT and increases lysyl oxidase (LOX) activity, which alters the extracellular matrix and activates tumour cell FAK, all of which lead to enhanced tumour cell invasion.

separates cancer development from small (1–2 mm) lesions, which are dormant, to an exponential growth phase [77,81,82].

Whereas the major focus on hypoxia has been its role in enhancing angiogenesis (for reviews, see [78,83]), recently a number of angiogenesis-independent mechanisms for hypoxia-induced tumour progression have been described [84,85]. In one key study, using a series of cell lines derived from breast, lung, cervical and ovarian cancers, among others, Pennacchietti *et al* showed that HIF-1 $\alpha$  binds to the *c-Met* promoter, leading to over-expression of *c-Met* and enhanced sensitivity to HGF. This leads to an 'invasive switch' in the tumour cells, increasing degradation of the ECM and allowing tumour cells to move freely towards more oxygen-rich areas [85]. This has therapeutic implications, since targeting angiogenesis alone may not be sufficient, and indeed may even aggravate, this invasive response to hypoxia.

HIF-1 $\alpha$  can also activate Wnt/ $\beta$ -catenin signalling, as demonstrated in prostate cancer, promoting a more motile and invasive phenotype [86]. Lysyl oxidase (LOX) is also regulated by hypoxia and HIF-1 $\alpha$  [84]. LOX has complex functions, being reported to have both tumour suppressor and tumour promoter properties [51,87,88]. It is clear, however, that LOX contributes to the enhanced invasive properties of hypoxic tumour cells. This is demonstrated by the inhibition of hypoxia-induced invasion achieved by LOX shRNA expression in breast, cervical, head and neck, pancreatic, colon and lung cancer cells [89], and expression of LOX is significantly associated with reduced distant metastasis-free survival and overall survival in ER-negative breast cancers, and in head and neck cancers [90,91]. It appears that LOX enhances

invasion both through matrix remodelling and by directly influencing actin polymerization and FAK activation [92]. The central role of LOX in mediating tumour invasion makes it an attractive therapeutic target but, given its divergent roles, it has been suggested that therapeutic strategies should be focused on ablating the extracellular functions of LOX [84,93].

### The inflammatory microenvironment

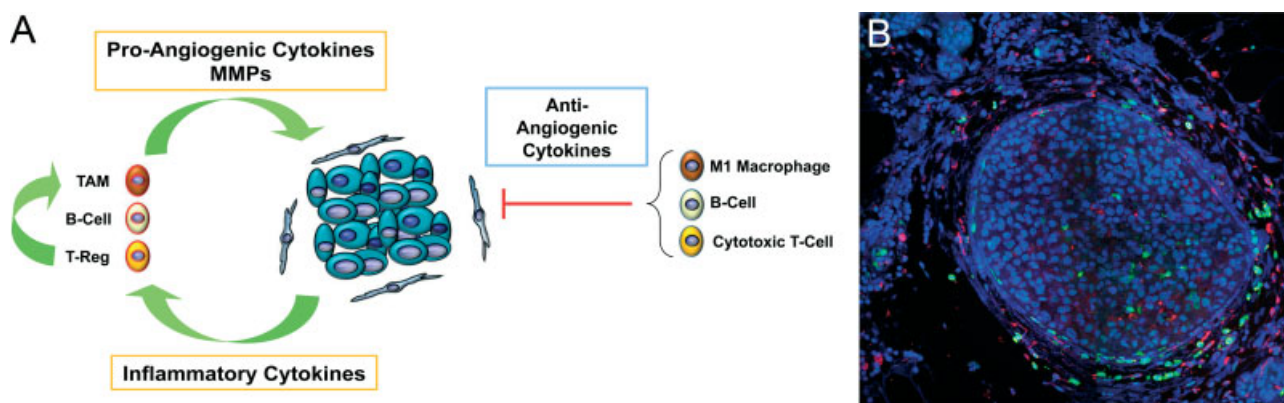
Inflammation has recently been proposed to be the seventh hallmark of cancer [94]. An inflammatory component is present in many solid tumours, and indeed inflammation predisposes to cancer at a number of sites [94,95]. Macrophages form a major inflammatory population in most tumours and are important determinants of the inflammatory milieu. Transgenic mice and human studies have demonstrated the close link between macrophages and tumour progression [96–98]. An important study in which mice, which spontaneously develop mammary tumours (PyMT-MMTV), were crossed with mice that lacked the macrophage growth factor colony stimulating factor (CSF-1), demonstrated that these mice developed tumours much more slowly and had fewer metastases than the control mice [96,99]. This was associated with delayed angiogenesis, which was re-established when the macrophages were restored. This work led to the description of tumour-associated macrophages (TAMs). These are abundant in most forms of solid tumour and display a characteristic phenotype. TAMs produce many tumour-promoting factors, eg EGF and VEGF [100,101], release cytokines and enzymes that promote invasion, angiogenesis and metastasis, eg VEGF and MMP9 [102,103] and down-regulate expression of anti-angiogenic factors, eg IL-12 (Figure 4A) [104].

Broadly speaking, macrophages can be defined as classically (M1) or alternatively (M2) activated [88]. In

normal immunological responses, classical activation is involved with Th1 cytokine responses to pathogens, eg IFN $\gamma$  and LPS. M1 macrophages are characterized by high levels of MHCII, IL-12 and TNF $\alpha$  and production of nitric oxide (NO) and reactive oxygen species (ROS). M2 macrophage differentiation is associated with Th2 cytokines (eg IL-4 and IL-13) resulting from wounding or humoral responses [105]. M2 macrophages can be identified using a number of markers, since they express arginase, mannose receptor, high-level IL-10, low-level MHC II and IL-12 (Figure 4B). Other macrophage populations exist which are not so easily classified, leading to the concept of a spectrum of macrophage phenotypes with a range of functions [106]. TAMs have been suggested to be biased towards an M2 phenotype but contain elements of both M1 and M2.

Hypoxia also plays a key role in determining the phenotype of infiltrating monocytes. TAMs are recruited to sites of hypoxia and necrosis [100,107,108] where, possibly due to high levels of endothelial monocyte-activating polypeptide (EMAP) II, endothelin II produced by the tumour cells [109] and necrotic debris [110], they become trapped and immobilized [111]. TAMs up-regulate HIF1/2 [112,113] in response to the hypoxic environment, leading to expression of HIF-responsive genes *VEGF* and *Tie-2* [114], providing TAMs with a potent pro-angiogenic phenotype. Tie-2 binds angiopoietin-2 (Ang-2), which is found at high levels in breast and other tumours [115], and this causes down-regulation of anti-angiogenic IL-12 and tumoricidal TNF $\alpha$  [116]. Thus, in a complex network, the microenvironment controls macrophage phenotype, which then impacts on tumour cell behaviour.

Other components of the inflammatory infiltrate also modulate tumour behaviour. T-regulatory (T-reg) cells were first identified in 1971 by Gershon and Kondo [117], followed by reports of T cells suppressing the anti-tumour immune response [118], and then by identification of CD4<sup>+</sup> T cells that suppressed autologous



**Figure 4.** (A) Effect of inflammatory cell infiltrate on tumour progression. The inflammatory response, through M1 macrophages, B cells and cytotoxic T cells, exert tumour-suppressor functions. Tumour-derived cytokines can modify the inflammatory infiltrate, polarizing it towards pro-angiogenic and pro-tumour properties, with induction of T-reg cells and the M2 tumour-associated m(TAM) phenotype. (B) Infiltration of tumour-associated macrophages around breast ductal carcinoma *in situ* (DCIS); a duct containing DCIS is surrounded by an inflammatory infiltrate, the majority of which stains positively for arginase (green) and the pan-macrophage marker CD68 (red), indicating an M2 pro-tumour phenotype. Nuclear stain is DAPI.



cytotoxic anti-tumour immune response [119]. These T cells are now classified as CD4<sup>+</sup>, CD25<sup>+</sup>, FoxP3<sup>+</sup> and are thought to protect the host from autoimmune disease by suppressing self-reactive cells and therefore also blocking anti-tumour responses.

The importance of T-regs was demonstrated when unfractionated cells from tumour-draining lymph nodes, taken 9 days after tumour challenge, gave complete rejection of established tumours. However, transfer of even a four-fold higher number of cells harvested at day 12 did not prevent tumour progression, owing to the presence of tumour-induced suppressor T cells, generated in the intervening 3 days, counteracting the anti-tumour response [120]. Further, it has been shown that intra-tumoral depletion of CD4<sup>+</sup> T cells can lead to the eradication of established tumours and the development of long-term anti-tumour memory [121]. It has also been suggested, in a pancreatic model, that the cancer promotes the accrual of T-regs to suppress the anti-tumour response [122].

It is clear that the inflammatory infiltrate associated with solid tumours can exert pro- and anti-tumour functions and, further, that this cellular compartment is highly targetable. It is essential now to establish, and harness, the mechanisms promoting an anti-tumour response and ablate the inflammatory pro-tumour activity.

### Clinical exploitation of the tumour microenvironment

Considerable experimental evidence points to a significant role for the microenvironment in the modulation of tumour development, growth and spread, and it is important to consider how this knowledge can be harnessed for clinical benefit. There are two broad categories in which knowledge of the tumour microenvironment can be exploited, which are discussed below.

#### Prognostic and predictive value

Bergamaschi *et al* [123] were one of the first groups to classify a cancer subtype on the basis of ECM-related gene profiles. They generated four ECM-gene classes of breast cancer, with one group, characterized by high levels of protease inhibitors, being associated with significantly improved prognosis, and another group, characterized by high-level expression of integrins, metalloproteinases and SPARC, exhibiting poor prognosis and significantly reduced survival. Finak *et al* [124] carried out Laser Capture Microdissection (LCM) of stroma from breast cancers and matched normal tissue in order to characterize stromal gene expression patterns. They identified a 26-gene stromal-derived prognostic predictor (SDPP) that could stratify patients into good and poor prognostic categories independently of classical prognostic factors, including tumour grade, size, LN status and *ER*, *PR* and *Her2* status. The

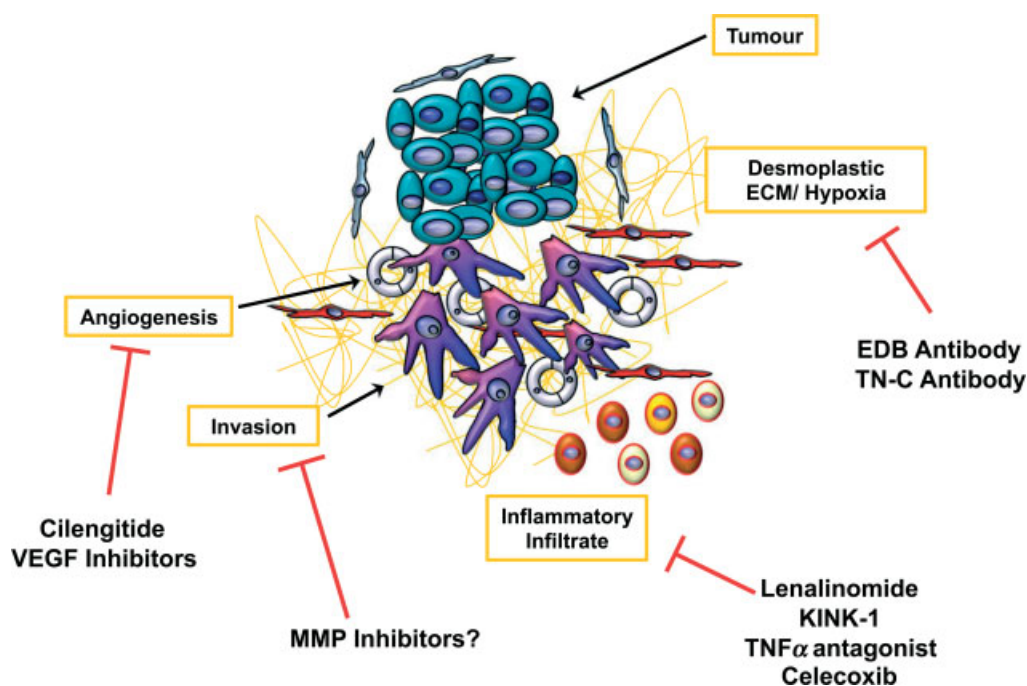
SDPP was also a more powerful predictor of prognosis than other prognostic signatures derived from whole-tumour samples [124], underlining the critical role of the stroma in determining disease response and outcome. The discriminating genes reflect underlying biological processes including angiogenesis, hypoxic response, tumour-associated macrophage responses, as well as regulators of Wnt signalling and development. Whilst many of these biological processes have previously been related to poor prognosis in different cancers, their individual prognostic power is relatively weak; it is the integration of these pathways that provides a powerful, independent prognostic predictor.

In a similar approach in the prostate cancer CR2-Tag mouse model, Bacac *et al* identified a stromal response signature, rich in lysosomal proteases, ADAM-TS and matrix metalloproteinases, reflecting high levels of stromal remodelling [125]. When this signature was evaluated in datasets from human prostate cancer patients, up-regulation of human orthologues of these genes was significantly associated with reduced disease-free survival. Furthermore, the same signature could discriminate between good and poor prognosis in a series of breast cancer patients, but not in lung, gastric or renal cell carcinoma, suggesting an organ-specific pattern of response in the microenvironment.

As well as providing prognostic information, it appears that characteristics of the tumour microenvironment can predict response to treatment. A recently reported prospective gene array study on patients with colorectal cancer identified up-regulation of a TNF family ligand gene called *APRIL* in a subset of patients treated with 5-fluorouracil (5FU), and in an independent cohort they showed that stromal *APRIL* expression was associated with significantly reduced survival [126]. Interestingly, *APRIL* expression had no relationship with prognosis in those patients not treated with 5FU, suggesting that stromal *APRIL* expression acts as a predictive marker for chemoresistance.

Similarly, Farmer *et al* [127] used a bioinformatics approach to identify a gene expression signature that might predict response to neoadjuvant chemotherapy in breast cancer. They identified nine gene clusters, each represented by a single metagene, and showed that only the stromal gene cluster predicted response to chemotherapy. The signature included genes indicative of a reactive stroma, such as *fibronectin*, *SPARC*, *PDGFR* and MMPs. As with the *APRIL* gene [127], this signature was of no prognostic value in those patients not treated with chemotherapy, suggesting that it represents a distinct biological feature of the tumour.

Although it is not entirely clear how the stromal environment might confer resistance to chemotherapy, previous work showing that interaction of tumour cells with fibronectin can enhance tumour survival [128,129], and that engagement of integrins with ECM leads to resistance to apoptosis [130], indicate potential mechanisms. Overall, these studies suggest that anti-stroma agents may have a place in overcoming resistance to chemotherapy.



**Figure 5.** Treatment strategies for targeting the microenvironment. The altered extracellular matrix (ECM) could be targeted using humanized monoclonal antibodies towards tumour-associated ECM isoforms such as fibronectin (FN), EDB and tenascin-C domain C (TNC). The inflammatory infiltrate may be targeted using TNF $\alpha$  antagonists, inhibitors of COX-2, such as Celecoxib, inhibitors of the NF- $\kappa$ B pathway, such as KINK-1, and broader immunomodulatory drugs, such as lenalidomide. Numerous drugs have been developed targeting angiogenesis, including VEGF inhibitors and the  $\alpha$ v $\beta$ 3 integrin antagonist cilengitide. Despite the disappointing results of early MMP inhibitors, more specific MMP inhibitors may still have therapeutic benefit.

### Therapeutic targeting

The biological, prognostic and predictive evidence suggests that the complex tumour microenvironment offers a number of avenues for therapeutic targeting (Figure 5).

The essential requirement of the angiogenic switch for the progression of solid tumours has led to the development of inhibitors as a therapy (Table 1) [131]. The majority of these target the VEGF signalling pathway [132] and can extend progression-free survival in colorectal, lung and breast cancer when used in combination with other chemotherapeutics [133–135]. Angiogenesis is also regulated through integrins  $\alpha$ v $\beta$ 3 and  $\alpha$ v $\beta$ 5, which are expressed on endothelial cells. Inhibitors of these integrins have been shown to suppress tumour growth in preclinical models [136–138]. The mechanism is thought to be through blocking their adhesive functions and hence preventing tumour growth by targeting the tumour cells as well as inhibiting angiogenesis [139,140]. Cilengitide, an  $\alpha$ v $\beta$ 3 inhibitor, has been effective in the treatment of glioma [141]; however, this may be due to glioma tumour cells expressing high levels of  $\alpha$ v integrins, rather than an effect on angiogenesis [142,143]. This hypothesis is further strengthened by the failure of cilengitide to produce results in other clinical settings [144–146]. The reasons for this are unclear but a study by Reynolds *et al* [147] demonstrated that at low doses (0.2–20 nM) cilengitide could enhance the growth of tumours *in vivo* by promoting angiogenesis. In a clinical setting, a large dose of the drug given

at biweekly intervals could lead to plasma levels in patients reaching nanomolar concentrations between administrations, consequently promoting angiogenesis and tumour growth. Thus, further elucidation of the precise biology of angiogenesis is essential to optimize future therapies.

Other aspects of the tumour microenvironment are also being targeted. The expression of 'tumour-specific' ECM proteins has been exploited to target delivery of bioactive molecules to tumours: these ECM components are highly abundant in tumours and are often more stable than antigens located on the cell surface of tumour cells. Radiolabelled antibodies specific to TNC domains A1 and D have been used successfully in the clinic to treat glioma and lymphoma [148]. Using antibody phage technology, a human monoclonal antibody to the C domain of TNC has been generated, and this scFv protein shows a highly selective uptake in gliomas, making it a promising tool for the future [149]. The aim is not necessarily to inhibit the function of the target but to use the target to concentrate delivery of bioactive molecules. This has been particularly successful targeting the FN-EDB [48]. EDB antibodies show specific localization to a range of tumours, including brain, lung and colorectal cancers [150]. The EDB-targeting antibody L19 has been used as a vehicle for TNF $\alpha$  and has been shown to induce necrosis in tumours [151,152]. Recently EDB has been targeted in lymphoma patients, using a radiolabelled antibody 131I-L19SIP. Two patients treated with this antibody showed a sustained partial remission, indicating that a



Table 1. Therapeutic targeting of the microenvironment: examples of small molecules, antibodies and siRNA molecules being used in preclinical studies and phase I–III clinical trials targeting different components of the microenvironment

Micro-environmental target	Molecular target	Molecule	Effect	References
Angiogenesis	$\alpha\beta 3$	Cilengitide	Reduce angiogenesis and increase apoptosis of TEC <i>in vitro</i> . In the clinic they have shown some effect on reducing tumour growth and sensitizing tumours to other treatments	[141,167]
	$\alpha\beta 5$	Abegrin		
	$\alpha 5\beta 1$	Volociximab	Inhibits angiogenesis	[168,169]
	HSP-70	17AAG	Sensitizes endothelial cells to radiotherapy	[170]
	VEGF	Bevacizumab	Neutralize VEGF	[133,171–175]
	PSMA	Antibody (MLN2704)	PSMA expressed on TEC of many solid tumours; delays progression	[176–178]
Signalling inhibitors	CTGF	FG-3019	Blocking antibody decreases tumour growth and metastasis	[179]
		DN-9693	Inhibits VEGF-mediated stabilization of CTGF mRNA	[180]
	FGFR	<i>In vitro</i>	Inhibition of cell proliferation, cell cycle and enhances cell death	[181,182]
	HGF	NK4	Antagonist of HGF	[183]
	PKB/Akt and mTOR	<i>In vitro</i> siRNA	Inhibits FN-induced proliferation	[184]
Cytokine inhibitors	SDF-1/CXCR4	Bryostatin-5	Antagonizes CXCR4-mediated migration and metastasis/inhibits neovascularization	[185,186]
	CD105 (endoglin)	SN6j (Ab)	Inhibits CD105 antagonistic effect on TGF $\beta$ inhibitory functions and induces apoptosis in hypoxia	[187]
ECM degradation inhibitors	FAP $\alpha$ (Seprase)	Sibrotuzumab	Reduced growth and invasion	[188,189]
	Tenascin-C	81C6 ( $^{131}$ I-labelled Ab)	Delays tumour growth, prolonged survival	[190]
		TTA1 (aptamer)	Facilitates delivery of radioactive isotopes	[191]
	uPA/uPAR	A6	Reduces tumour growth, metastasis and angiogenesis	[192]
	MMPs	Marimastat (BB-2516)	<i>In vitro</i> and xenograft models show anti-invasive, metastatic and angiogenic effects	[193,194]
		Rebimastat (BMS-275291)	No conclusive clinical benefit found	[195]
Hypoxia	CAIX	WX-G250	Induces antibody-dependent cellular toxicity	[196,197]

TEC, tumour-associated endothelial cells; PSMA, prostate-specific membrane antigen; CTGF, connective tissue growth factor; SDF-1, stromal-derived factor 1; FGFR, fibroblast growth factor receptor; HGF, hepatocyte growth factor; uPA(R), urokinase plasminogen activator (receptor); VEGF, vascular endothelial growth factor.

therapeutic dose of radioactivity can be delivered to tumours using this approach [153].

Components of the immune system have also been harnessed for therapeutic gain. One of the approaches taken has been to re-establish an anti-tumour inflammatory milieu. TNF $\alpha$  antagonists have been shown to induce stabilization of disease and partial responses in breast and advanced cancer [154,155], and multiple myeloma is treated very successfully with combinations of drugs, including lenalidomide [156,157], which suppresses the production of several inflammatory cytokines [158]. Work by Hagemann *et al* [159],

aimed at reprogramming the macrophage phenotype, has demonstrated that by inhibiting IKK $\beta$  macrophages change from a TAM phenotype to a tumour suppressive phenotype. A novel small molecule of IKK $\beta$  has been developed (KINK-1), which demonstrates the ability to sensitize tumours in mice to doxorubicin and reduce tumour mass and metastases [160]. It remains to be determined whether there is any effect on macrophage phenotype.

A number of drugs in clinical trials for other diseases that target the immune system, such as non-steroidal anti-inflammatory drugs (NSAIDs; eg COX inhibitors

such as celecoxib) for treating arthritis [161,162], have been applied to some tumours, eg malignant melanoma and pancreatic cancer [163,164]. COX-2 inhibitors have also been shown to prevent the recurrence of sporadic [165] and genetically predisposed [166] adenomas.

## Conclusions

It has taken decades for the concept of the microenvironment as an important determinant of tumour behaviour to gain acceptance. Elucidating the nature of the interactions between the tumour and the multiple facets of the microenvironment will allow us to harness this relationship for clinical benefit.

## Author contributions

Both authors contributed equally to the preparation of this review.

## Teaching Materials

PowerPoint slides of the figures from this review are supplied as supporting information in the online version of this article.

## References

- Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet* 2001; **357**: 539–545.
- Paget S. The distribution of secondary growths in cancer of the breast. *Lancet* 1889; **133**: 571–573.
- Bissell MJ, Hall HG, Parry G. How does the extracellular matrix direct gene expression? *J Theor Biol* 1982; **99**: 31–68.
- Kalluri R, Zeisberg M. Fibroblasts in cancer. *Nat Rev Cancer* 2006; **6**: 392–401.
- Orimo A, Gupta PB, Sgroi DC, *et al.* Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. *Cell* 2005; **121**: 335–348.
- Yang F, Tuxhorn JA, Ressler SJ, *et al.* Stromal expression of connective tissue growth factor promotes angiogenesis and prostate cancer tumorigenesis. *Cancer Res* 2005; **65**: 8887–8895.
- Grotendorst GR, Okochi H, Hayashi N. A novel transforming growth factor- $\beta$  response element controls the expression of the connective tissue growth factor gene. *Cell Growth Differ* 1996; **7**: 469–480.
- Frazier KS, Grotendorst GR. Expression of connective tissue growth factor mRNA in the fibrous stroma of mammary tumors. *Int J Biochem Cell Biol* 1997; **29**: 153–161.
- Koliopoulos A, Friess H, di Mola FF, *et al.* Connective tissue growth factor gene expression alters tumor progression in esophageal cancer. *World J Surg* 2002; **26**: 420–427.
- Wenger C, Ellenrieder V, Alber B, *et al.* Expression and differential regulation of connective tissue growth factor in pancreatic cancer cells. *Oncogene* 1999; **18**: 1073–1080.
- Olumi AF, Grossfeld GD, Hayward SW, *et al.* Carcinoma-associated fibroblasts direct tumor progression of initiated human prostatic epithelium. *Cancer Res* 1999; **59**: 5002–5011.
- Bhowmick NA, Chytil A, Plieth D, *et al.* TGF $\beta$  signaling in fibroblasts modulates the oncogenic potential of adjacent epithelia. *Science* 2004; **303**: 848–851.
- Erez N, Truitt M, Olson P, *et al.* Cancer-associated fibroblasts are activated in incipient neoplasia to orchestrate tumor-promoting inflammation in an NF- $\kappa$ B-dependent manner. *Cancer Cell* 2010; **17**: 135–147.
- Korinek V, Barker N, Moerer P, *et al.* Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. *Nat Genet* 1998; **19**: 379–383.
- van de Wetering M, Sancho E, Verweij C, *et al.* The  $\beta$ -catenin/TCF-4 complex imposes a crypt progenitor phenotype on colorectal cancer cells. *Cell* 2002; **111**: 241–250.
- Vermeulen L, De Sousa EMF, van der Heijden M, *et al.* Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. *Nat Cell Biol* 2010; **12**: 468–476.
- Direkze NC, Jeffery R, Hodivala-Dilke K, *et al.* Bone marrow-derived stromal cells express lineage-related messenger RNA species. *Cancer Res* 2006; **66**: 1265–1269.
- Radisky ES, Radisky DC. Stromal induction of breast cancer: inflammation and invasion. *Rev Endocr Metab Disord* 2007; **8**: 279–287.
- Fukino K, Shen L, Patocs A, *et al.* Genomic instability within tumor stroma and clinicopathological characteristics of sporadic primary invasive breast carcinoma. *J Am Med Assoc* 2007; **297**: 2103–2111.
- Kurose K, Hoshaw-Woodard S, Adeyinka A, *et al.* Genetic model of multi-step breast carcinogenesis involving the epithelium and stroma: clues to tumour-microenvironment interactions. *Hum Mol Genet* 2001; **10**: 1907–1913.
- Moinfar F, Beham A, Friedrich G, *et al.* Macro-environment of breast carcinoma: frequent genetic alterations in the normal appearing skins of patients with breast cancer. *Mod Pathol* 2008; **21**: 639–646.
- Patocs A, Zhang L, Xu Y, *et al.* Breast-cancer stromal cells with TP53 mutations and nodal metastases. *N Engl J Med* 2007; **357**: 2543–2551.
- Campbell IG, Qiu W, Polyak K, *et al.* Breast-cancer stromal cells with TP53 mutations. *N Engl J Med* 2008; **358**: 1634–1635; author reply, 1636.
- Qiu W, Hu M, Sridhar A, *et al.* No evidence of clonal somatic genetic alterations in cancer-associated fibroblasts from human breast and ovarian carcinomas. *Nat Genet* 2008; **40**: 650–655.
- Adany R, Iozzo RV. Altered methylation of versican proteoglycan gene in human colon carcinoma. *Biochem Biophys Res Commun* 1990; **171**: 1402–1413.
- Hu M, Yao J, Cai L, *et al.* Distinct epigenetic changes in the stromal cells of breast cancers. *Nat Genet* 2005; **37**: 899–905.
- Hannan MA, Siddiqui Y, Rostom A, *et al.* Evidence of DNA repair/processing defects in cultured skin fibroblasts from breast cancer patients. *Cancer Res* 2001; **61**: 3627–3631.
- Schor SL, Schor AM. Phenotypic and genetic alterations in mammary stroma: implications for tumour progression. *Breast Cancer Res* 2001; **3**: 373–379.
- Ye S. Polymorphism in matrix metalloproteinase gene promoters: implication in regulation of gene expression and susceptibility of various diseases. *Matrix Biol* 2000; **19**: 623–629.
- Hinoda Y, Okayama N, Takano N, *et al.* Association of functional polymorphisms of matrix metalloproteinase (MMP)-1 and MMP-3 genes with colorectal cancer. *Int J Cancer* 2002; **102**: 526–529.

31. Holliday DL, Hughes S, Shaw JA, *et al.* Intrinsic genetic characteristics determine tumor-modifying capacity of fibroblasts: matrix metalloproteinase-3 5A/5A genotype enhances breast cancer cell invasion. *Breast Cancer Res* 2007; **9**: R67.
32. Hunter KW, Crawford NP. Germ line polymorphism in metastatic progression. *Cancer Res* 2006; **66**: 1251–1254.
33. Nelson CM, Bissell MJ. Modeling dynamic reciprocity: engineering three-dimensional culture models of breast architecture, function, and neoplastic transformation. *Semin Cancer Biol* 2005; **15**: 342–352.
34. Jones PL, Jones FS. Tenascin-C in development and disease: gene regulation and cell function. *Matrix Biol* 2000; **19**: 581–596.
35. Chen J, Chen Z, Chen M, *et al.* Role of fibrillar Tenascin-C in metastatic pancreatic cancer. *Int J Oncol* 2009; **34**: 1029–1036.
36. Katenkamp K, Berndt A, Hindermann W, *et al.* mRNA expression and protein distribution of the unspliced tenascin-C isoform in prostatic adenocarcinoma. *J Pathol* 2004; **203**: 771–779.
37. Carnemolla B, Castellani P, Ponassi M, *et al.* Identification of a glioblastoma-associated tenascin-C isoform by a high affinity recombinant antibody. *Am J Pathol* 1999; **154**: 1345–1352.
38. Adams M, Jones JL, Walker RA, *et al.* Changes in tenascin-C isoform expression in invasive and preinvasive breast disease. *Cancer Res* 2002; **62**: 3289–3297.
39. Wilson KE, Bartlett JM, Miller EP, *et al.* Regulation and function of the extracellular matrix protein tenascin-C in ovarian cancer cell lines. *Br J Cancer* 1999; **80**: 685–692.
40. Hancox RA, Allen MD, Holliday DL, *et al.* Tumour-associated tenascin-C isoforms promote breast cancer cell invasion and growth by matrix metalloproteinase-dependent and independent mechanisms. *Breast Cancer Res* 2009; **11**: R24.
41. Meiners S, Powell EM, Geller HM. Neurite outgrowth promotion by the alternatively spliced region of tenascin-C is influenced by cell-type specific binding. *Matrix Biol* 1999; **18**: 75–87.
42. Sarkar S, Nuttall RK, Liu S, *et al.* Tenascin-C stimulates glioma cell invasion through matrix metalloproteinase-12. *Cancer Res* 2006; **66**: 11771–11780.
43. White ES, Baralle FE, Muro AF. New insights into form and function of fibronectin splice variants. *J Pathol* 2008; **216**: 1–14.
44. Oyama F, Hirohashi S, Sakamoto M, *et al.* Coordinate oncodevelopmental modulation of alternative splicing of fibronectin pre-messenger RNA at ED-A, ED-B, and CS1 regions in human liver tumors. *Cancer Res* 1993; **53**: 2005–2011.
45. Serini G, Bochaton-Piallat ML, Ropraz P, *et al.* The fibronectin domain ED-A is crucial for myofibroblastic phenotype induction by transforming growth factor- $\beta$ 1. *J Cell Biol* 1998; **142**: 873–881.
46. Kaczmarek J, Castellani P, Nicolo G, *et al.* Distribution of oncofetal fibronectin isoforms in normal, hyperplastic and neoplastic human breast tissues. *Int J Cancer* 1994; **59**: 11–16.
47. Castellani P, Borsi L, Carnemolla B, *et al.* Differentiation between high- and low-grade astrocytoma using a human recombinant antibody to the extra domain-B of fibronectin. *Am J Pathol* 2002; **161**: 1695–1700.
48. Kaspar M, Zardi L, Neri D. Fibronectin as target for tumor therapy. *Int J Cancer* 2006; **118**: 1331–1339.
49. Paszek MJ, Zahir N, Johnson KR, *et al.* Tensional homeostasis and the malignant phenotype. *Cancer Cell* 2005; **8**: 241–254.
50. Engler AJ, Sen S, Sweeney HL, *et al.* Matrix elasticity directs stem cell lineage specification. *Cell* 2006; **126**: 677–689.
51. Kagan HM, Li W. Lysyl oxidase: properties, specificity, and biological roles inside and outside of the cell. *J Cell Biochem* 2003; **88**: 660–672.
52. Levental KR, Yu H, Kass L, *et al.* Matrix crosslinking forces tumor progression by enhancing integrin signaling. *Cell* 2009; **139**: 891–906.
53. Martin LJ, Boyd NF. Mammographic density. Potential mechanisms of breast cancer risk associated with mammographic density: hypotheses based on epidemiological evidence. *Breast Cancer Res* 2008; **10**: 201.
54. Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. *Nat Rev Cancer* 2002; **2**: 161–174.
55. Coussens LM, Fingleton B, Matrisian LM. Matrix metalloproteinase inhibitors and cancer: trials and tribulations. *Science* 2002; **295**: 2387–2392.
56. Gutierrez-Fernandez A, Fueyo A, Folgueras AR, *et al.* Matrix metalloproteinase-8 functions as a metastasis suppressor through modulation of tumor cell adhesion and invasion. *Cancer Res* 2008; **68**: 2755–2763.
57. Houghton AM, Grisolan JL, Baumann ML, *et al.* Macrophage elastase (matrix metalloproteinase-12) suppresses growth of lung metastases. *Cancer Res* 2006; **66**: 6149–6155.
58. Nguyen M, Lee MC, Wang JL, *et al.* The human myoepithelial cell displays a multifaceted anti-angiogenic phenotype. *Oncogene* 2000; **19**: 3449–3459.
59. Shao ZM, Nguyen M, Alpaugh ML, *et al.* The human myoepithelial cell exerts antiproliferative effects on breast carcinoma cells characterized by p21WAF1/CIP1 induction, G<sub>2</sub>/M arrest, and apoptosis. *Exp Cell Res* 1998; **241**: 394–403.
60. Jones JL, Shaw JA, Pringle JH, *et al.* Primary breast myoepithelial cells exert an invasion-suppressor effect on breast cancer cells via paracrine down-regulation of MMP expression in fibroblasts and tumour cells. *J Pathol* 2003; **201**: 562–572.
61. Sternlicht MD, Kedeshian P, Shao ZM, *et al.* The human myoepithelial cell is a natural tumor suppressor. *Clin Cancer Res* 1997; **3**: 1949–1958.
62. Bergstraesser LM, Srinivasan G, Jones JC, *et al.* Expression of hemidesmosomes and component proteins is lost by invasive breast cancer cells. *Am J Pathol* 1995; **147**: 1823–1839.
63. Allinen M, Beroukhi R, Cai L, *et al.* Molecular characterization of the tumor microenvironment in breast cancer. *Cancer Cell* 2004; **6**: 17–32.
64. Papetti M, Herman IM. Mechanisms of normal and tumor-derived angiogenesis. *Am J Physiol Cell Physiol* 2002; **282**: C947–970.
65. Dor Y, Porat R, Keshet E. Vascular endothelial growth factor and vascular adjustments to perturbations in oxygen homeostasis. *Am J Physiol Cell Physiol* 2001; **280**: C1367–1374.
66. Zhong H, De Marzo AM, Laughner E, *et al.* Overexpression of hypoxia-inducible factor 1 $\alpha$  in common human cancers and their metastases. *Cancer Res* 1999; **59**: 5830–5835.
67. Brizel DM, Sibley GS, Prosnitz LR, *et al.* Tumor hypoxia adversely affects the prognosis of carcinoma of the head and neck. *Int J Radiat Oncol Biol Phys* 1997; **38**: 285–289.
68. Sundfor K, Lyng H, Rofstad EK. Tumour hypoxia and vascular density as predictors of metastasis in squamous cell carcinoma of the uterine cervix. *Br J Cancer* 1998; **78**: 822–827.
69. Chen CL, Chu JS, Su WC, *et al.* Hypoxia and metabolic phenotypes during breast carcinogenesis: expression of HIF-1 $\alpha$ , GLUT1, and CAIX. *Virchows Arch* 2010; **457**: 53–61.
70. Carmeliet P, Dor Y, Herbert JM, *et al.* Role of HIF-1 $\alpha$  in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. *Nature* 1998; **394**: 485–490.
71. Canning MT, Postovit LM, Clarke SH, *et al.* Oxygen-mediated regulation of gelatinase and tissue inhibitor of metalloproteinases-1 expression by invasive cells. *Exp Cell Res* 2001; **267**: 88–94.
72. Esteban MA, Tran MG, Harten SK, *et al.* Regulation of E-cadherin expression by VHL and hypoxia-inducible factor. *Cancer Res* 2006; **66**: 3567–3575.
73. Kim JW, Tchernyshyov I, Semenza GL, *et al.* HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch



- required for cellular adaptation to hypoxia. *Cell Metab* 2006; **3**: 177–185.
74. Bindra RS, Schaffer PJ, Meng A, *et al.* Alterations in DNA repair gene expression under hypoxia: elucidating the mechanisms of hypoxia-induced genetic instability. *Ann NY Acad Sci* 2005; **1059**: 184–195.
  75. Fukumura D, Xu L, Chen Y, *et al.* Hypoxia and acidosis independently up-regulate vascular endothelial growth factor transcription in brain tumors *in vivo*. *Cancer Res* 2001; **61**: 6020–6024.
  76. Tsuzuki Y, Fukumura D, Oosthuysen B, *et al.* Vascular endothelial growth factor (VEGF) modulation by targeting hypoxia-inducible factor-1 $\alpha$   $\rightarrow$  hypoxia response element  $\rightarrow$  VEGF cascade differentially regulates vascular response and growth rate in tumors. *Cancer Res* 2000; **60**: 6248–6252.
  77. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* 1996; **86**: 353–364.
  78. Ruan K, Song G, Ouyang G. Role of hypoxia in the hallmarks of human cancer. *J Cell Biochem* 2009; **107**: 1053–1062.
  79. Shweiki D, Itin A, Soffer D, *et al.* Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. *Nature* 1992; **359**: 843–845.
  80. Laderoute KR, Alarcon RM, Brody MD, *et al.* Opposing effects of hypoxia on expression of the angiogenic inhibitor thrombospondin 1 and the angiogenic inducer vascular endothelial growth factor. *Clin Cancer Res* 2000; **6**: 2941–2950.
  81. Baeriswyl V, Christofori G. The angiogenic switch in carcinogenesis. *Semin Cancer Biol* 2009; **19**: 329–337.
  82. Bergers G, Benjamin LE. Tumorigenesis and the angiogenic switch. *Nat Rev Cancer* 2003; **3**: 401–410.
  83. Patel NS, Muneer A, Blick C, *et al.* Targeting vascular endothelial growth factor in renal cell carcinoma. *Tumour Biol* 2009; **30**: 292–299.
  84. Erler JT, Bennewith KL, Nicolau M, *et al.* Lysyl oxidase is essential for hypoxia-induced metastasis. *Nature* 2006; **440**: 1222–1226.
  85. Pennacchietti S, Michieli P, Galluzzo M, *et al.* Hypoxia promotes invasive growth by transcriptional activation of the *met* proto-oncogene. *Cancer Cell* 2003; **3**: 347–361.
  86. Jiang YG, Luo Y, He DL, *et al.* Role of Wnt/ $\beta$ -catenin signaling pathway in epithelial–mesenchymal transition of human prostate cancer induced by hypoxia-inducible factor-1 $\alpha$ . *Int J Urol* 2007; **14**: 1034–1039.
  87. Csiszar K, Fong SF, Ujjalusi A, *et al.* Somatic mutations of the lysyl oxidase gene on chromosome 5q23.1 in colorectal tumors. *Int J Cancer* 2002; **97**: 636–642.
  88. Kaneda A, Wakazono K, Tsukamoto T, *et al.* Lysyl oxidase is a tumor suppressor gene inactivated by methylation and loss of heterozygosity in human gastric cancers. *Cancer Res* 2004; **64**: 6410–6415.
  89. Cairns RA, Khokha R, Hill RP. Molecular mechanisms of tumor invasion and metastasis: an integrated view. *Curr Mol Med* 2003; **3**: 659–671.
  90. Chang HY, Nuyten DS, Sneddon JB, *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.
  91. Chen Y, Shi G, Xia W, *et al.* Identification of hypoxia-regulated proteins in head and neck cancer by proteomic and tissue array profiling. *Cancer Res* 2004; **64**: 7302–7310.
  92. Payne SL, Fogelgren B, Hess AR, *et al.* Lysyl oxidase regulates breast cancer cell migration and adhesion through a hydrogen peroxide-mediated mechanism. *Cancer Res* 2005; **65**: 11429–11436.
  93. Solinas G, Germano G, Mantovani A, *et al.* Tumor-associated macrophages (TAM) as major players of the cancer-related inflammation. *J Leukoc Biol* 2009; **86**: 1065–1073.
  94. Colotta F, Allavena P, Sica A, *et al.* Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. *Carcinogenesis* 2009; **30**: 1073–1081.
  95. Mantovani A, Romero P, Palucka AK, *et al.* Tumour immunity: effector response to tumour and role of the microenvironment. *Lancet* 2008; **371**: 771–783.
  96. Lin EY, Nguyen AV, Russell RG, *et al.* Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. *J Exp Med* 2001; **193**: 727–740.
  97. Qian B, Deng Y, Im JH, *et al.* A distinct macrophage population mediates metastatic breast cancer cell extravasation, establishment and growth. *PLoS One* 2009; **4**: e6562.
  98. Ojalvo LS, Whittaker CA, Condeelis JS, *et al.* Gene expression analysis of macrophages that facilitate tumor invasion supports a role for Wnt-signaling in mediating their activity in primary mammary tumors. *J Immunol* 2010; **184**: 702–712.
  99. Hsu HP, Shan YS, Lai MD, *et al.* Osteopontin-positive infiltrating tumor-associated macrophages in bulky ampullary cancer predict survival. *Cancer Biol Ther* 2010; **10**.
  100. Lewis JS, Landers RJ, Underwood JC, *et al.* Expression of vascular endothelial growth factor by macrophages is up-regulated in poorly vascularized areas of breast carcinomas. *J Pathol* 2000; **192**: 150–158.
  101. O'Sullivan C, Lewis CE, Harris AL, *et al.* Secretion of epidermal growth factor by macrophages associated with breast carcinoma. *Lancet* 1993; **342**: 148–149.
  102. Lewis CE, Pollard JW. Distinct role of macrophages in different tumor microenvironments. *Cancer Res* 2006; **66**: 605–612.
  103. Mantovani A, Schioppa T, Porta C, *et al.* Role of tumor-associated macrophages in tumor progression and invasion. *Cancer Metast Rev* 2006; **25**: 315–322.
  104. Sica A, Saccani A, Bottazzi B, *et al.* Autocrine production of IL-10 mediates defective IL-12 production and NF- $\kappa$ B activation in tumor-associated macrophages. *J Immunol* 2000; **164**: 762–767.
  105. Gordon S. Alternative activation of macrophages. *Nat Rev Immunol* 2003; **3**: 23–35.
  106. Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. *Nat Rev Immunol* 2008; **8**: 958–969.
  107. Leek RD, Landers RJ, Harris AL, *et al.* Necrosis correlates with high vascular density and focal macrophage infiltration in invasive carcinoma of the breast. *Br J Cancer* 1999; **79**: 991–995.
  108. Leek RD, Lewis CE, Whitehouse R, *et al.* Association of macrophage infiltration with angiogenesis and prognosis in invasive breast carcinoma. *Cancer Res* 1996; **56**: 4625–4629.
  109. Murdoch C, Giannoudis A, Lewis CE. Mechanisms regulating the recruitment of macrophages into hypoxic areas of tumors and other ischemic tissues. *Blood* 2004; **104**: 2224–2234.
  110. Lewis CE, Hughes R. Inflammation and breast cancer. Microenvironmental factors regulating macrophage function in breast tumours: hypoxia and angiopoietin-2. *Breast Cancer Res* 2007; **9**: 209.
  111. Turner L, Scotton C, Negus R, *et al.* Hypoxia inhibits macrophage migration. *Eur J Immunol* 1999; **29**: 2280–2287.
  112. Burke B, Giannoudis A, Corke KP, *et al.* Hypoxia-induced gene expression in human macrophages: implications for ischemic tissues and hypoxia-regulated gene therapy. *Am J Pathol* 2003; **163**: 1233–1243.
  113. Burke B, Tang N, Corke KP, *et al.* Expression of HIF-1 $\alpha$  by human macrophages: implications for the use of macrophages in hypoxia-regulated cancer gene therapy. *J Pathol* 2002; **196**: 204–212.

114. Venneri MA, De Palma M, Ponzoni M, *et al.* Identification of proangiogenic TIE2-expressing monocytes (TEMs) in human peripheral blood and cancer. *Blood* 2007; **109**: 5276–5285.
115. Tsutsui S, Inoue H, Yasuda K, *et al.* Angiopoietin 2 expression in invasive ductal carcinoma of the breast: its relationship to the VEGF expression and microvessel density. *Breast Cancer Res Treat* 2006; **98**: 261–266.
116. Murdoch C, Tazzyman S, Webster S, *et al.* Expression of Tie-2 by human monocytes and their responses to angiopoietin-2. *J Immunol* 2007; **178**: 7405–7411.
117. Gershon RK, Kondo K. Infectious immunological tolerance. *Immunology* 1971; **21**: 903–914.
118. Berendt MJ, North RJ. T-cell-mediated suppression of anti-tumor immunity. An explanation for progressive growth of an immunogenic tumor. *J Exp Med* 1980; **151**: 69–80.
119. Chakraborty NG, Twardzik DR, Sivanandham M, *et al.* Autologous melanoma-induced activation of regulatory T cells that suppress cytotoxic response. *J Immunol* 1990; **145**: 2359–2364.
120. Peng L, Kjaergaard J, Plautz GE, *et al.* Tumor-induced L-selectin high suppressor T cells mediate potent effector T cell blockade and cause failure of otherwise curative adoptive immunotherapy. *J Immunol* 2002; **169**: 4811–4821.
121. Yu P, Lee Y, Liu W, *et al.* Intratumor depletion of CD4<sup>+</sup> cells unmasks tumor immunogenicity leading to the rejection of late-stage tumors. *J Exp Med* 2005; **201**: 779–791.
122. Linehan DC, Goedegebuure PS. CD25<sup>+</sup> CD4<sup>+</sup> regulatory T-cells in cancer. *Immunol Res* 2005; **32**: 155–168.
123. Bergamaschi A, Tagliabue E, Sorlie T, *et al.* Extracellular matrix signature identifies breast cancer subgroups with different clinical outcome. *J Pathol* 2008; **214**: 357–367.
124. Finak G, Bertos N, Pepin F, *et al.* Stromal gene expression predicts clinical outcome in breast cancer. *Nat Med* 2008; **14**: 518–527.
125. Bacac M, Provero P, Mayran N, *et al.* A mouse stromal response to tumor invasion predicts prostate and breast cancer patient survival. *PLoS One* 2006; **1**: e32.
126. Petty RD, Samuel LM, Murray GI, *et al.* APRIL is a novel clinical chemo-resistance biomarker in colorectal adenocarcinoma identified by gene expression profiling. *BMC Cancer* 2009; **9**: 434.
127. Farmer P, Bonnefoi H, Anderle P, *et al.* A stroma-related gene signature predicts resistance to neoadjuvant chemotherapy in breast cancer. *Nat Med* 2009; **15**: 68–74.
128. Damiano JS, Cress AE, Hazlehurst LA, *et al.* Cell adhesion mediated drug resistance (CAM-DR): role of integrins and resistance to apoptosis in human myeloma cell lines. *Blood* 1999; **93**: 1658–1667.
129. Hazlehurst LA, Valkov N, Wisner L, *et al.* Reduction in drug-induced DNA double-strand breaks associated with  $\beta 1$  integrin-mediated adhesion correlates with drug resistance in U937 cells. *Blood* 2001; **98**: 1897–1903.
130. Weaver VM, Lelievre S, Lakins JN, *et al.*  $\beta 4$  integrin-dependent formation of polarized three-dimensional architecture confers resistance to apoptosis in normal and malignant mammary epithelium. *Cancer Cell* 2002; **2**: 205–216.
131. Kerbel R, Folkman J. Clinical translation of angiogenesis inhibitors. *Nat Rev Cancer* 2002; **2**: 727–739.
132. Ellis LM, Hicklin DJ. VEGF-targeted therapy: mechanisms of anti-tumour activity. *Nat Rev Cancer* 2008; **8**: 579–591.
133. Hurwitz H, Fehrenbacher L, Novotny W, *et al.* Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004; **350**: 2335–2342.
134. Sandler A, Gray R, Perry MC, *et al.* Paclitaxel–carboplatin alone or with bevacizumab for non-small-cell lung cancer. *N Engl J Med* 2006; **355**: 2542–2550.
135. Miller K, Wang M, Gralow J, *et al.* Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. *N Engl J Med* 2007; **357**: 2666–2676.
136. Mitjans F, Meyer T, Fittschen C, *et al.* *In vivo* therapy of malignant melanoma by means of antagonists of  $\alpha v$  integrins. *Int J Cancer* 2000; **87**: 716–723.
137. Buerkle MA, Pahernik SA, Sutter A, *et al.* Inhibition of the  $\alpha v$  integrins with a cyclic RGD peptide impairs angiogenesis, growth and metastasis of solid tumours *in vivo*. *Br J Cancer* 2002; **86**: 788–795.
138. MacDonald TJ, Taga T, Shimada H, *et al.* Preferential susceptibility of brain tumors to the antiangiogenic effects of an  $\alpha v$  integrin antagonist. *Neurosurgery* 2001; **48**: 151–157.
139. Varner JA, Cheresch DA. Integrins and cancer. *Curr Opin Cell Biol* 1996; **8**: 724–730.
140. Hodivala-Dilke KM, Reynolds AR, Reynolds LE. Integrins in angiogenesis: multitasking molecules in a balancing act. *Cell Tissue Res* 2003; **314**: 131–144.
141. Nabors LB, Mikkelsen T, Rosenfeld SS, *et al.* Phase I and correlative biology study of cilengitide in patients with recurrent malignant glioma. *J Clin Oncol* 2007; **25**: 1651–1657.
142. Chatterjee S, Matsumura A, Schradermeier J, *et al.* Human malignant glioma therapy using anti- $\alpha v \beta 3$  integrin agents. *J Neurooncol* 2000; **46**: 135–144.
143. Taga T, Suzuki A, Gonzalez-Gomez I, *et al.*  $\alpha v$ -Integrin antagonist EMD 121974 induces apoptosis in brain tumor cells growing on vitronectin and tenascin. *Int J Cancer* 2002; **98**: 690–697.
144. Eskens FA, Dumez H, Hoekstra R, *et al.* Phase I and pharmacokinetic study of continuous twice weekly intravenous administration of Cilengitide (EMD 121974), a novel inhibitor of the integrins  $\alpha v \beta 3$  and  $\alpha v \beta 5$  in patients with advanced solid tumours. *Eur J Cancer* 2003; **39**: 917–926.
145. Friess H, Langrehr JM, Oettle H, *et al.* A randomized multicenter phase II trial of the angiogenesis inhibitor Cilengitide (EMD 121974) and gemcitabine compared with gemcitabine alone in advanced unresectable pancreatic cancer. *BMC Cancer* 2006; **6**: 285.
146. Hariharan S, Gustafson D, Holden S, *et al.* Assessment of the biological and pharmacological effects of the  $\alpha v \beta 3$  and  $\alpha v \beta 5$  integrin receptor antagonist, cilengitide (EMD 121974), in patients with advanced solid tumors. *Ann Oncol* 2007; **18**: 1400–1407.
147. Reynolds AR, Hart IR, Watson AR, *et al.* Stimulation of tumor growth and angiogenesis by low concentrations of RGD-mimetic integrin inhibitors. *Nat Med* 2009; **15**: 392–400.
148. Reardon DA, Akabani G, Coleman RE, *et al.* Phase II trial of murine (131)I-labeled antitenascin monoclonal antibody 81C6 administered into surgically created resection cavities of patients with newly diagnosed malignant gliomas. *J Clin Oncol* 2002; **20**: 1389–1397.
149. Silacci M, Brack SS, Spath N, *et al.* Human monoclonal antibodies to domain C of tenascin-C selectively target solid tumors *in vivo*. *Protein Eng Des Sel* 2006; **19**: 471–478.
150. Santimaria M, Moscatelli G, Viale GL, *et al.* Immunoscintigraphic detection of the ED-B domain of fibronectin, a marker of angiogenesis, in patients with cancer. *Clin Cancer Res* 2003; **9**: 571–579.
151. Huang X, Molema G, King S, *et al.* Tumor infarction in mice by antibody-directed targeting of tissue factor to tumor vasculature. *Science* 1997; **275**: 547–550.
152. Borsi L, Balza E, Carnemolla B, *et al.* Selective targeted delivery of TNF $\alpha$  to tumor blood vessels. *Blood* 2003; **102**: 4384–4392.
153. Sauer S, Erba PA, Petrini M, *et al.* Expression of the oncofetal ED-B-containing fibronectin isoform in hematologic tumors

- enables ED-B-targeted 131I-L19SIP radioimmunotherapy in Hodgkin lymphoma patients. *Blood* 2009; **113**: 2265–2274.
154. Brown ER, Charles KA, Hoare SA, *et al.* A clinical study assessing the tolerability and biological effects of infliximab, a TNF $\alpha$  inhibitor, in patients with advanced cancer. *Ann Oncol* 2008; **19**: 1340–1346.
  155. Madhusudan S, Foster M, Muthuramalingam SR, *et al.* A phase II study of etanercept (Enbrel), a tumor necrosis factor- $\alpha$  inhibitor in patients with metastatic breast cancer. *Clin Cancer Res* 2004; **10**: 6528–6534.
  156. Richardson PG, Weller E, Lonial S, *et al.* Lenalidomide, bortezomib, and dexamethasone combination therapy in patients with newly diagnosed multiple myeloma. *Blood* 2010; **116**: 679–686.
  157. Schey SA, Morgan GJ, Ramasamy K, *et al.* The addition of cyclophosphamide to lenalidomide and dexamethasone in multiply relapsed/refractory myeloma patients; a phase I/II study. *Br J Haematol* 2010; **150**: 326–333.
  158. Kotla V, Goel S, Nischal S, *et al.* Mechanism of action of lenalidomide in hematological malignancies. *J Hematol Oncol* 2009; **2**: 36.
  159. Hagemann T, Lawrence T, McNeish I, *et al.* 'Re-educating' tumor-associated macrophages by targeting NF- $\kappa$ B. *J Exp Med* 2008; **205**: 1261–1268.
  160. Schon M, Wienrich BG, Kneitz S, *et al.* KINK-1, a novel small-molecule inhibitor of IKK $\beta$ , and the susceptibility of melanoma cells to antitumoral treatment. *J Natl Cancer Inst* 2008; **100**: 862–875.
  161. Bingham CO III, Smugar SS, Wang H, *et al.* Early response to COX-2 inhibitors as a predictor of overall response in osteoarthritis: pooled results from two identical trials comparing etoricoxib, celecoxib and placebo. *Rheumatology (Oxf)* 2009; **48**: 1122–1127.
  162. Chan FK, Lanas A, Scheiman J, *et al.* Celecoxib versus omeprazole and diclofenac in patients with osteoarthritis and rheumatoid arthritis (CONDOR): a randomised trial. *Lancet* 2010; **376**: 173–179.
  163. Bhatt RS, Merchan J, Parker R, *et al.* A phase 2 pilot trial of low-dose, continuous infusion, or 'metronomic' paclitaxel and oral celecoxib in patients with metastatic melanoma. *Cancer* 2010; **116**: 1751–1756.
  164. Lipton A, Campbell-Baird C, Witters L, *et al.* Phase II trial of gemcitabine, irinotecan, and celecoxib in patients with advanced pancreatic cancer. *J Clin Gastroenterol* 2010; **44**: 286–288.
  165. Bertagnolli MM, Eagle CJ, Zauber AG, *et al.* Five-year efficacy and safety analysis of the Adenoma Prevention with Celecoxib Trial. *Cancer Prev Res* 2009; **2**: 310–321.
  166. Phillips RK, Wallace MH, Lynch PM, *et al.* A randomised, double blind, placebo controlled study of celecoxib, a selective cyclooxygenase 2 inhibitor, on duodenal polyposis in familial adenomatous polyposis. *Gut* 2002; **50**: 857–860.
  167. Mulgrew K, Kinneer K, Yao XT, *et al.* Direct targeting of  $\alpha$ v $\beta$ 3 integrin on tumor cells with a monoclonal antibody, abegrin. *Mol Cancer Ther* 2006; **5**: 3122–3129.
  168. Bhaskar V, Zhang D, Fox M, *et al.* A function blocking anti-mouse integrin  $\alpha$ 5 $\beta$ 1 antibody inhibits angiogenesis and impedes tumor growth *in vivo*. *J Transl Med* 2007; **5**: 61.
  169. Ramakrishnan V, Bhaskar V, Law DA, *et al.* Preclinical evaluation of an anti- $\alpha$ 5 $\beta$ 1 integrin antibody as a novel anti-angiogenic agent. *J Exp Ther Oncol* 2006; **5**: 273–286.
  170. Kabakov AE, Makarova YM, Malyutina YV. Radiosensitization of human vascular endothelial cells through Hsp90 inhibition with 17-N-allilamino-17-demethoxygeldanamycin. *Int J Radiat Oncol Biol Phys* 2008; **71**: 858–865.
  171. Kabbinnar F, Hurwitz HI, Fehrenbacher L, *et al.* Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. *J Clin Oncol* 2003; **21**: 60–65.
  172. Zhu AX, Blaszkowsky LS, Ryan DP, *et al.* Phase II study of gemcitabine and oxaliplatin in combination with bevacizumab in patients with advanced hepatocellular carcinoma. *J Clin Oncol* 2006; **24**: 1898–1903.
  173. Xu L, Duda DG, di Tomaso E, *et al.* Direct evidence that bevacizumab, an anti-VEGF antibody, up-regulates SDF1 $\alpha$ , CXCR4, CXCL6, and neuropilin 1 in tumors from patients with rectal cancer. *Cancer Res* 2009; **69**: 7905–7910.
  174. Willett CG, Duda DG, di Tomaso E, *et al.* Complete pathological response to bevacizumab and chemoradiation in advanced rectal cancer. *Nat Clin Pract Oncol* 2007; **4**: 316–321.
  175. Willett CG, Boucher Y, di Tomaso E, *et al.* Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. *Nat Med* 2004; **10**: 145–147.
  176. Henry MD, Wen S, Silva MD, *et al.* A prostate-specific membrane antigen-targeted monoclonal antibody-chemotherapeutic conjugate designed for the treatment of prostate cancer. *Cancer Res* 2004; **64**: 7995–8001.
  177. Ma D, Hopf CE, Malewicz AD, *et al.* Potent antitumor activity of an auristatin-conjugated, fully human monoclonal antibody to prostate-specific membrane antigen. *Clin Cancer Res* 2006; **12**: 2591–2596.
  178. Wolf P, Gierschner D, Buhler P, *et al.* A recombinant PSMA-specific single-chain immunotoxin has potent and selective toxicity against prostate cancer cells. *Cancer Immunol Immunother* 2006; **55**: 1367–1373.
  179. Dornhofer N, Spong S, Bennewith K, *et al.* Connective tissue growth factor-specific monoclonal antibody therapy inhibits pancreatic tumor growth and metastasis. *Cancer Res* 2006; **66**: 5816–5827.
  180. Kondo S, Tanaka N, Kubota S, *et al.* Novel angiogenic inhibitor DN-9693 that inhibits post-transcriptional induction of connective tissue growth factor (CTGF/CCN2) by vascular endothelial growth factor in human endothelial cells. *Mol Cancer Ther* 2006; **5**: 129–137.
  181. Giulianelli S, Cerliani JP, Lamb CA, *et al.* Carcinoma-associated fibroblasts activate progesterone receptors and induce hormone independent mammary tumor growth: a role for the FGF-2/FGFR-2 axis. *Int J Cancer* 2008; **123**: 2518–2531.
  182. Fischer H, Taylor N, Allerstorfer S, *et al.* Fibroblast growth factor receptor-mediated signals contribute to the malignant phenotype of non-small cell lung cancer cells: therapeutic implications and synergism with epidermal growth factor receptor inhibition. *Mol Cancer Ther* 2008; **7**: 3408–3419.
  183. Ohuchida K, Mizumoto K, Murakami M, *et al.* Radiation to stromal fibroblasts increases invasiveness of pancreatic cancer cells through tumor–stromal interactions. *Cancer Res* 2004; **64**: 3215–3222.
  184. Han S, Khuri FR, Roman J. Fibronectin stimulates non-small cell lung carcinoma cell growth through activation of Akt/mammalian target of rapamycin/S6 kinase and inactivation of LKB1/AMP-activated protein kinase signal pathways. *Cancer Res* 2006; **66**: 315–323.
  185. He X, Fang L, Wang J, *et al.* Bryostatin-5 blocks stromal cell-derived factor-1 induced chemotaxis via desensitization and down-regulation of cell surface CXCR4 receptors. *Cancer Res* 2008; **68**: 8678–8686.
  186. Guleng B, Tateishi K, Ohta M, *et al.* Blockade of the stromal cell-derived factor-1/CXCR4 axis attenuates *in vivo* tumor growth by inhibiting angiogenesis in a vascular endothelial growth factor-independent manner. *Cancer Res* 2005; **65**: 5864–5871.



187. Takahashi N, Haba A, Matsuno F, *et al.* Antiangiogenic therapy of established tumors in human skin/severe combined immunodeficiency mouse chimeras by anti-endoglin (CD105) monoclonal antibodies, and synergy between anti-endoglin antibody and cyclophosphamide. *Cancer Res* 2001; **61**: 7846–7854.
188. Loeffler M, Kruger JA, Niethammer AG, *et al.* Targeting tumor-associated fibroblasts improves cancer chemotherapy by increasing intratumoral drug uptake. *J Clin Invest* 2006; **116**: 1955–1962.
189. Hofheinz RD, al-Batran SE, Hartmann F, *et al.* Stromal antigen targeting by a humanised monoclonal antibody: an early phase II trial of sibtuzumab in patients with metastatic colorectal cancer. *Onkologie* 2003; **26**: 44–48.
190. Reardon DA, Akabani G, Coleman RE, *et al.* Salvage radioimmunotherapy with murine iodine-131-labeled antitenascin monoclonal antibody 81C6 for patients with recurrent primary and metastatic malignant brain tumors: phase II study results. *J Clin Oncol* 2006; **24**: 115–122.
191. Hicke BJ, Stephens AW, Gould T, *et al.* Tumor targeting by an aptamer. *J Nucl Med* 2006; **47**: 668–678.
192. Berkenblit A, Matulonis UA, Kroener JF, *et al.* A6, a urokinase plasminogen activator (uPA)-derived peptide in patients with advanced gynecologic cancer: a phase I trial. *Gynecol Oncol* 2005; **99**: 50–57.
193. Rosenbaum E, Zahurak M, Sinibaldi V, *et al.* Marimastat in the treatment of patients with biochemically relapsed prostate cancer: a prospective randomized, double-blind, phase I/II trial. *Clin Cancer Res* 2005; **11**: 4437–4443.
194. Goffin JR, Anderson IC, Supko JG, *et al.* Phase I trial of the matrix metalloproteinase inhibitor marimastat combined with carboplatin and paclitaxel in patients with advanced non-small cell lung cancer. *Clin Cancer Res* 2005; **11**: 3417–3424.
195. Lara PN Jr, Stadler WM, Longmate J, *et al.* A randomized phase II trial of the matrix metalloproteinase inhibitor BMS-275291 in hormone-refractory prostate cancer patients with bone metastases. *Clin Cancer Res* 2006; **12**: 1556–1563.
196. Siebels M, Rohrmann K, Oberneder R, *et al.* A clinical phase I/II trial with the monoclonal antibody cG250 (RENCAREX((R))) and interferon- $\alpha$ -2a in metastatic renal cell carcinoma patients. *World J Urol* 2010.
197. Bleumer I, Oosterwijk E, Oosterwijk-Wakka JC, *et al.* A clinical trial with chimeric monoclonal antibody WX-G250 and low dose interleukin-2 pulsing scheme for advanced renal cell carcinoma. *J Urol* 2006; **175**: 57–62.