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REVIEW ARTICLE

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# The multiple roles of tumour stroma

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**Abstract** Since the work of Judah Folkman in the 1970s demonstrating the importance of vascularization on tumour growth the many roles played by tumour stroma have been demonstrated. Vascular endothelial growth factor/vascular permeability factor appears to be the main in vivo inducer of both stromal blood vessels and other components of the tumour stroma. Its action is probably mediated through its strong and long-lasting effect on microvascular permeability leading to fibrin extravasation and organisation ("tumours are wounds that do not heal"). During tumour invasion, stromal fibroblasts participate in the degradation of the extracellular matrix (ECM) by secreting matrix degrading proteases as well as their downstream-activators. Stroma derived factors such as scatter factor/hepatocyte growth factor as well as interactions between neoplastic cells and the ECM can play a role in both tumour cell migration and proliferation. The ECM may also act as a reservoir for growth factors. A novel transcription factor encoded by the c-*ets* 1 proto-oncogene is likely to be involved in the transcriptional regulation of both tumour vascularization and invasion. This contribution summarizes recent developments in the tumour stroma field.

**Key words** Tumour stroma · Roles

## Introduction

The extracellular matrix (ECM) appears during evolution with the onset of multicellular life. Studies in lower animals such as sponges, sea urchins and cnidarians, have shown the remarkable conservation of collagen, proteoglycans and adhesion molecules such as fibronectin, tenascin or laminin (for review see [55]). This intercellular medium affects fundamental cell functions such as cell adhesion, proliferation and differentiation.

The idea that tumour stroma may have an impact on the biological behaviour of neoplasms is as old as the first histological observations of tumours [36]. However, for a long time only the neoplastic cells were the focus of interest in cancer research and the stroma was rather considered a reactive component without major significance. Based on the work of Judah Folkman in the 1970s which showed the importance of vascularization in tumour growth, this view has been abandonned. The tumour stroma is now considered to be an integral part of a neoplasm with a major role in several steps in the metastatic cascade. This review shows some of the interactions which exist between tumour cells and their stromal microenvironment and how they affect the behaviour of neoplasms.

## The crucial role of tumour stroma in tumour vascularization

The induction of the outgrowth of new blood vessels from existing ones, assuring tumour nutrition, was the first established role for tumour stroma. In the 1970s Judah Folkman showed that tumour spheroids in the culture dish cannot grow beyond a size of a few millimetres because they lack vascular support [49]. Hence most tumours in vivo must induce their own vascular supply in order to grow beyond this size. Stromal blood vessels not only ensure tumour nutrition and waste removal but are also points of entry for metastatically-competent tumour cells [116]. Tumour vascularization is nevertheless not a strict prerequisite for growth as demonstrated by the propagation of tumour cells on serous membranes or the ventricular ependyma, without significant induction of new blood vessels. According to current models, the stromal outgrowth of capillaries during angiogenesis passes through several sequential steps. These comprise a degradation of pre-existing vessel basement membranes, proliferation and directed migration of endotheli-

Dedicated to Prof. G. Dhom on the occasion of his 75th birthday

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al cells towards an angiogenic stimulus with the final differentiation of mature capillary loops establishing a functional circulation [49]. A number of angiogenic factors are known which can affect one or more of these processes in cultured endothelial cells or elicit the formation of complete blood vessels in in vivo models such as the rabbit cornea or the chicken chorioallantoic membrane. They are considered to be secreted by tumour cells or macrophages attracted to the tumour site, or to be released from the ECM after degradation during tumour invasion.

A number of findings point to vascular entothelial growth factor vascular permeability factor (VEGF/VPF) as a major inducer of tumour vascularization. VEGF has initially been demonstrated in culture medium conditioned by tumour cells and in tumour ascites fluid and been shown to increase microvascular permeability strongly [131]. It is a disulphide-linked dimeric glycoprotein with molecular weights between 34 and 46 kDa [27, 28, 98, 130]. Four different polypeptides, 121–206 amino acids long, can be generated through alternative splicing of the VEGF/VPF primary transcript [71, 144]. Two different endothelial receptors, VEGF receptor-1 (Flt-1) and 2 (KDR/Flk-1) are known, both of which are tyrosine kinases with a similar domain structure [33, 95, 96, 141]. Signal transduction by VEGF/VPF within endothelial cells involves an increase in the intracellular calcium ion concentration and the formation of inositol triphosphate (IP3) probably through an activation of phospholipase C [15]. VEGF/VPF stimulates both the proliferation and the migration of endothelial cells in vitro and induces a strong angiogenic response in the chick chlorioallantoic membrane [44, 91]. It is actively secreted by a variety of human tumour cell lines [132]. In vivo, tumour cells of human breast and gastrointestinal carcinomas as well as of glioblastomas have been shown to upregulate VEGF/VPF mRNA while both of its receptors are expressed by adjacent microvascular endothelial cells [17, 18, 108, 109]. Experimental findings further support a role of VEGF/VPF for tumour vascularization. The vascularization and growth of glioblastomas implanted into nude mice can be inhibited by antibodies against VEGF or by introducing a dominant negative mutant of the VEGF-receptor Flk-1 into endothelial cells [83, 95].

Hypoxia is considered to be the main inducer of VEGF-production and secretion during tumour growth as inferred from the particularly strong expression of VEGF transcripts by neoplastic cells directly adjacent to hypoxic necroses [18, 41, 135].

The strong and persisting histamine-like effect of VEGF/VPF on microvascular permeability is thought to play a role in both the induction of new blood vessels and the recruitment of other components of the tumour stroma. It causes an extravasation of plasma proteins including fibrinogen which, after cross-linkage to fibrin, attracts endothelial and other stromal cells in a sequence similar to the continuous formation of granulation tissue [16–19, 40–42].

For other angiogenic factors, such as the fibroblast growth factors (a and bFGF), angiogenin or platelet-derived growth factor (PDGF) which stimulate the migration or proliferation of endothelial cells in vitro but can also affect other cell types in vivo, a role for tumour vascularization is less well established. In a transgenic mouse model of a fibrosarcoma it has been shown that the secretion of bFGF by the tumour cells correlates with the degree of angiogenesis [81]. The secretion mechanism for a and bFGF is not yet known as both lack a signal peptide. However, when A 31 fibroblasts are transfected with a bFGF-gene fused to a signal sequence and injected into nude mice bFGF is secreted and highly vascularized tumours develop [70]. A comprehensive analysis of the distribution of a and bFGF and their receptors in common human tumours has not been carried out. FGF-receptor 1, one of the high affinity receptors for a and bFGF [106] has nevertheless been demonstrated within the microvasculature of malignant melanomas [79].

Angiogenin is another potent stimulator of angiogenesis in the rabbit cornea and has first been isolated from the conditioned medium of a human adenocarcinoma cell line [46]. It is a single chain polypeptide with a molecular weight of 14.4 kDa and has 35% sequence homology with a family of pancreatic ribonucleases (for review see [51]). In endothelial cells angiogenin activates phospholipase C with a transient increase in the intracellular levels of 1,2-diacylglycerol and IP3 [10]. Its in vivo role during tumour vascularization is not well established.

Both the PDFG-B chain which is mitogenic for endothelial cells in vitro [7] and its receptor have been demonstrated within stromal capillaries of human glioblastomas [69].

Transforming growth factor β (TGFβ) and tumour necrosis factor α (TNFα) represent indirect angiogenic factors which inhibit endothelial cell proliferation in vitro

**Fig. 1a, b** Transcripts of c-*ets* 1 within the stromal capillaries of a renal clear cell carcinoma as demonstrated by in situ hybridization. Darkfield illumination and epifluorescence view of the nuclei stained with Hoechst 33258 (**a**). H&E-stained neighbouring section (**b**). *Bar*=100 μm ▶

**Fig. 2** Northern blot analysis of c-*ets* 1 mRNAs in cultured aortic bovine arch (1a–3a) and in bovine brain capillary (1b–3b) endothelial cells. RNA has been extracted 4 days (1a and 1b) and 2 days (2a and 2b) before cell confluence and 4 days after confluence (3a and 3b). There is a marked downregulation of the c-*ets* 1 transcripts (6.8 kb) after cell confluence

**Fig. 3a, b** Expression of c-*ets* 1 mRNA within stromal fibroblasts directly adjacent to the invasive formations of a poorly differentiated human colon carcinoma. Darkfield (**a**) and brightfield illumination (**b**) of a May-Grünwald-Giemsa-stained section. *Bar*=100 μm

**Fig. 4** Northern blot analysis of c-*ets* 1, collagenase 1, stomelysin 1, urokinase-type plasminogen activator and plasminogen activator inhibitor 1 transcripts after induction by PMA and different cytokines in cultured human foreskin fibroblasts. Note the induction of both c-*ets* 1 and that of proteases mRNAs by PMA, fibroblast growth factor b and tumour necrosis factor α. (*C* control, *TGFβ* transforming growth factor β, *PDGF* platelet-derived growth factor, *GAPDH* glyceraldehyde-3-phosphate-dehydrogenase)

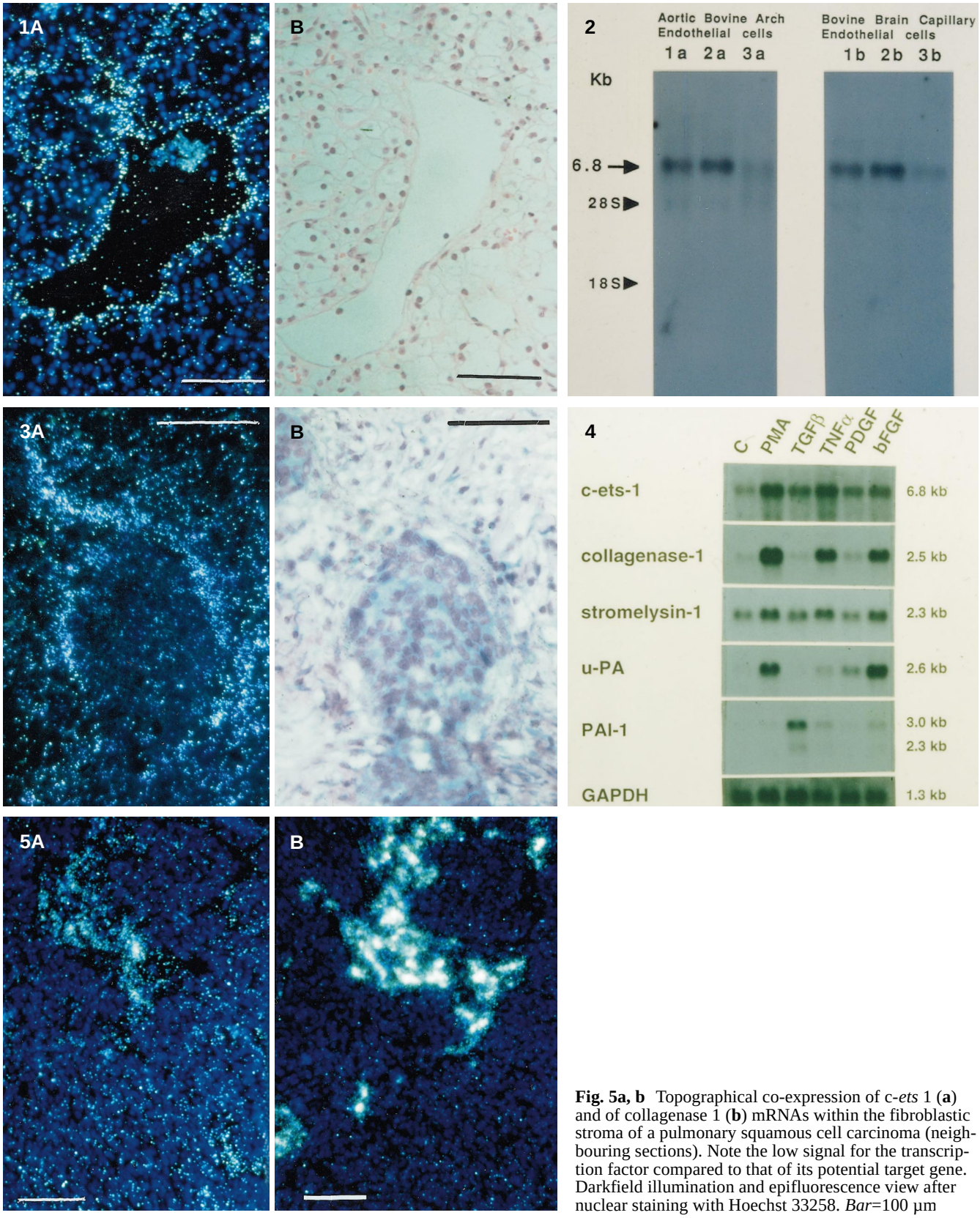


**Fig. 5a, b** Topographical co-expression of c-*ets* 1 (**a**) and of collagenase 1 (**b**) mRNAs within the fibroblastic stroma of a pulmonary squamous cell carcinoma (neighbouring sections). Note the low signal for the transcription factor compared to that of its potential target gene. Darkfield illumination and epifluorescence view after nuclear staining with Hoechst 33258. *Bar*=100 µm

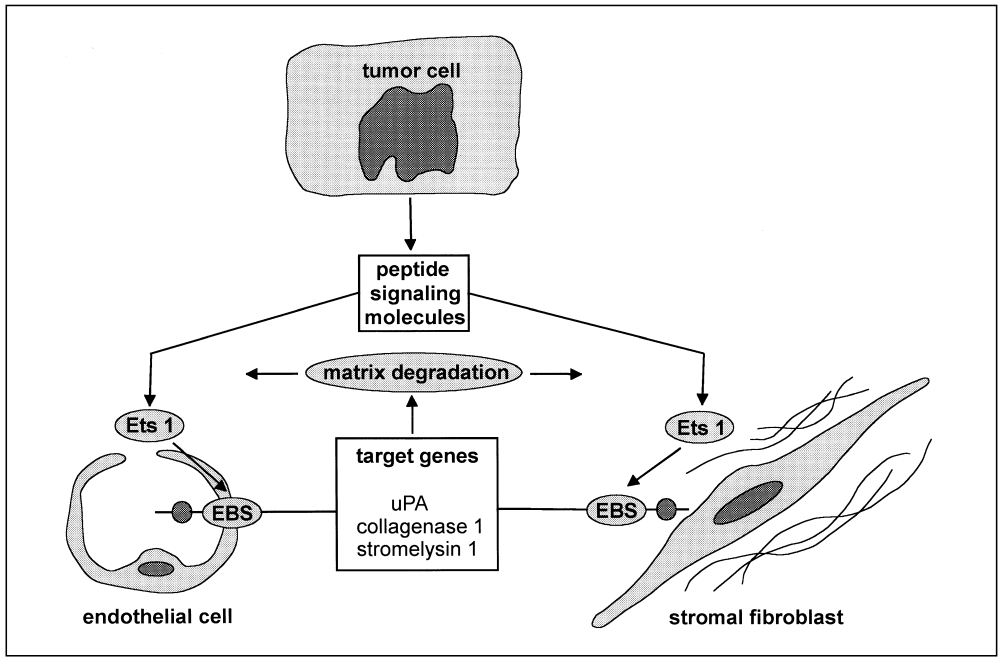


**Fig. 6** The possible mode of induction of the Ets 1 transcription factor within tumour stroma and its putative in vivo target genes. Neoplastic cells secrete peptide signaling molecules which induce Ets 1 within neighbouring stromal endothelial cells and fibroblasts. Ets 1 proteins bind to specific sequences in the promoter regions of genes encoding matrix-degrading proteases and activate their transcription. (*EBS* Ets-binding sites within promoters)

[52, 139] but which are angiogenic in vivo [156]. They are thought to recruit cell types such as macrophages to release angiogenic peptides.

Stimulation of endothelial cells by angiogenic factors is followed by signal transduction and changes in gene transcription. The Ets 1 transcription factor has evolved as a candidate factor involved in this regulation. It is encoded by the c-*ets* 1 protooncogene, the founder of a new gene family of transcriptional regulators. They all contain a new DNA-binding domain, the so-called *ets*-domain and comprise Ets 1, Ets 2, Erg, Elk 1, Elf 1, Spi 1/PU 1, PEA3 and others [90] (for review see [88, 92]). In vivo, we found c-*ets* 1 actively transcribed in endothelial cells during new blood vessel formation under both physiological and pathological conditions such as vessel formation in the embryo [35, 61, 145, 151], in granulation tissue [151] and during the vascularization of benign and malignant tumours [150, 151] (Fig. 1). When vessel formation terminates the expression of c-*ets* 1 is turned off. This transitory transcription can be simulated in vitro where endothelial cells express c-*ets* 1 mRNA in a preconfluent stage and down-regulate it considerably after confluence (Fig. 2). The in vivo target genes regulated by the Ets 1 proteins during new blood vessel formation are not yet known. Potential candidates comprise the genes encoding different matrix degrading proteases [63, 67, 99, 118, 123, 149] as well as the gene of the α5 integrin chain [12]. The α5β1 integrin is the fibronectin receptor [73] and binding of endothelial cells to fibronectin causes them to grow and to spread [120]. Another transcription factor, SCL or TAL-1, a member of the basic helix-loop-helix family of transcriptional regulators has recently been demonstrated within endothelial cells during new blood vessel formation in mouse embryos [80].

Several inhibitors of angiogenesis are known which diminish the proliferation of endothelial cells in vitro and inhibit the vascularization of experimental tumours such as Lewis lung carcinoma in vivo [102] (for review see [50]). They include the platelet-derived factor 4, the fumagillin-derivative AGM 1470 and angiostatin. All these factors are characterized by low toxicity and can be curative in tumour-bearing animals when combined with cytotoxic drugs. Initial clinical trials with angiogenesis inhibitors are now underway in cancer therapy (for review see [50]).

## Tumour stroma plays a major role in invasion

A second well established role of the tumour stroma is its role in invasion. This has been demonstrated experimentally by the enhancement of invasive properties of carcinoma cells after transplantation into granulation tissue [37, 53]. During invasion stroma is involved in both matrix degradation and tumour cell migration along different components of the ECM.

## Fibroblasts of the tumour stroma help to degrade the extracellular matrix

Whereas normal fibroblasts at different anatomical sites have been shown to exhibit a considerable phenotypic and functional diversity [60, 122, 126] fibroblastic cells of the tumour stroma are less well characterized. One consistent feature of these cells is a myofibroblastic phenotype which is based on the synthesis of α-smooth-muscle actin [60, 122]. Functionally these cells are also capable of producing enzymes involved in the degrada-

tion of ECM-components which is one of the prerequisites for invasion. Whereas it has been known for a long time that these enzymes can be secreted by neoplastic cells (for review see [100]), it has become clear only in recent years that a major fraction is also produced by stromal myofibroblasts. Transcripts of the interstitial or type 1 collagenase, the two ($M_r$ 72000 and 92000) type 4 collagenases, stromelysin 1 and 3 as well as of the serine protease urokinase-type plasminogen activator (uPA) implicated in the downstream-activation of these metalloproteinases have all been demonstrated within stromal myofibroblasts of different human tumours such as skin, lung, breast, colon and ovarian cancers [3, 5, 13, 58, 59, 101, 112, 114, 115, 150] (for review see [153–155]). The activity of theses stromal proteases during tumour invasion is tightly controlled. At the transcriptional level cytokines, such as epidermal growth factor (EGF), bFGF and TNFα which can be secreted by tumour cells or stromal cells [6, 140] have been shown to stimulate the transcription of several metalloproteinase genes [56, 77, 150]. The Ets-1 transcription factor has again been implicated in this regulation. Our own data show that c-*ets* 1 is expressed by stromal fibroblasts of different invasive human carcinomas (of the lung, breast, colon and others) in contrast to non-invasive lesions [13, 150]. Moreover, c-*ets* 1 transcripts are frequently found in fibroblasts directly adjacent to invasive tumour formations (Fig. 3) and we have found that the conditioned medium of different human tumour cell lines induced the transcription of c-*ets* 1 within cultured human fibroblasts [57, 150]. Co-transfection experiments and mutational analyses of promoter sequences have shown that the Ets-1 proteins can transactivate the rat stromelysin 1 gene promoter [149] and that Ets-binding sites within the promotors of the genes encoding uPA [99, 118] and collagenase 1 [63] are necessary for the transcriptional activation of these genes. Also both genes encoding the 72 kDa as well as the 92 kDa collagenases contain within their transcriptional regulatory domains binding sites for the Ets-1 proteins [67, 123]. In cultured human fibroblasts we found a correlation between the transcription of c-*ets* 1 and its potential target genes encoding collagenase 1, stromelysin 1 and uPA after stimulation by bFGF, TNFα or phorbol 12-myristate 13-acetate (PMA) [150] (Fig. 4). In vivo we demonstrated a topographical co-localization of transcripts of c-*ets* 1 and of these proteases within the fibroblastic stroma of human lung and colon cancers [13, 150] (Fig. 5). The transcriptional regulation of matrix degrading proteases during tumour invasion may also involve other members of the *ets*-gene family and probably further transcription factors such as AP1 which has also been shown to transactivate different protease genes in vitro [2, 99]. The possible in vivo roles of the Ets 1 proteins for matrix degradation during angiogenesis and tumour invasion are schematically summarized in Fig. 6.

Once synthesized, matrix-degrading proteases are secreted as inactive pro-enzymes which must be locally activated. Recent evidence suggests that the tumour stroma is also involved in this process. The activation is carried out by the uPA/plasmin system of serine proteases [125]. Pro-uPA has been shown to be produced by stromal fibroblasts [113, 150] and its receptor, a membrane-bound glycosylphophatidylinositol-anchored cell surface receptor at which pro-uPA itself is proteolytically activated is expressed on tumour cells or stromal macrophages [9, 113]. Activation of pro-uPA is followed by the proteolytic downstream-activation of plasminogen to plasmin and of the different metalloproteinases which finally degrade the ECM [93, 125].

Finally stroma cell elements are involved in the regulation of matrix degradation by different inhibitors of serine proteases (plasminogen-activator inhibitors 1 and 2, PAI 1 and 2) and of metalloproteinases (tissue type inhibitors of metalloproteinases, TIMP 1 and 2). Such inhibitors can be produced by stromal cells and stored in the ECM [26, 87, 112, 117]. TGFβ stimulates the synthesis of both PAI 1 and TIMP 1 but inhibits that of collagenase 1 within cultured fibroblasts and vascular smooth muscle cells [97, 103, 117] (see also Fig. 4).

## Stroma and tumour cell migration

Active migration of neoplastic cells is a second prerequisite for tumour invasion. It involves both an active locomotion of neoplastic cells and changes in the cells adherence systems with components of the ECM which is the substrate along which tumour cells move.

As a fibroblast-derived mediator of epithelial motility and growth, scatter factor/hepatocyte growth factor (SF/HGF) has received particular attention [138, 159] (for review see [148]). It is a heterodimeric glycoprotein composed of a 60 kDa α and a 30 kDa β chain linked by a disulfide bond. Both chains are translated from a single mRNA. The active form is generated by a cleavage of this precursor chain. After binding to its cell surface receptor, a tyrosine kinase encoded by the c-*met* protooncogene, SF can induce the dissociation of tumour cells into motile cells [94] (for reviews see [11, 142, 148]). When NBT-II bladder carcinoma cells are transfected with an expression vector for SF/HGF they exhibit an increase of invasive properties both in vitro into 3-dimensional collagen gels and upon injection into nude mice [8]. In vivo both SF/HGF and its receptor have been demonstrated immunohistochemically within the stroma of different human tumours such as lung, liver and pancreatic carcinomas [38, 159]. In recent years a SF-like factor (SFL [8]) and a scattering agent produced by monocytes [76] have also been identified.

During tumour cell migration, changes occur in the adherence systems of cells with the ECM. These cell-matrix interactions are largely mediated through the large family of transmembrane integrin receptors (for reviews see [20, 30, 72, 73]). Currently 14 types of integrin α and 9 types of β chains are known both of which are involved in the recognition of different matrix components and about 20 integrin heterodimers are defined.

The diversity is further increased by splice variants of integrin mRNAs. Many human tumours such as lung, breast, gastric, colon, pancreas, renal and prostate carcinomas have been shown to express different integrin heterodimers [4, 14, 21, 39, 64, 65, 82, 84, 85, 105, 107, 160, 161]. Upon fixation of an integrin to its extracellular ligand the ensuing signalling cascade can affect cell migration [24, 30, 31, 73, 78, 104, 128]. Fixation is followed by the binding of the cytoplasmic domain of the integrin β-chain to a complex of cytoskeletal proteins in focal adhesions (including α-actinin, vinculin, talin, paxillin and tensin) which are linked to actin filaments. A central role is then played by the recruitment and the activation of a novel tyrosine kinase, focal adhesion kinase (FAK) which is autophospharylated at tyrosine 397. This phosphotyrosine peptide motif binds to the SH2 (*src*-homology) domain of pp $60^{src}$ which in turn phosphorylates FAK on tyrosine 925. The SH2 and SH3 domains of several proteins binding to phosphotyrosine motifs (SH2) and short proline-rich sequences (SH3) then allow the formation of supramolecular complexes of signalling proteins which include other tyrosine kinases (like Src and Csk), the adaptor proteins (exclusively composed of SH2 and SH3 domains) Grb2 and Crk and the guanine nucleotide exchange factors mSOS and C3G. The latter convert inactive ras-GDP to active ras-GTP and this activates the ras-mitogen-activated protein kinase pathway of downstream signalling which can finally induce cell migration [24, 104]. An antiserum reacting with several αv integrins has been shown to inhibit adhesion and spreading of a human colon carcinoma cell line (HT29) on fibronectin and vitronectin [89] and an anti-human fibronectin receptor (α5β1 integrin) antibody prevents Ehrlich ascites tumour cells from adhering to fibronectin-coated plates [137]. Integrin-mediated cell adhesion and tyrosine phosphorylation are also involved in SF/HGF-induced cell migration. Squamous cell carcinoma cells stimulated by SF/HGF first form integrin-mediated focal adhesions with the ECM. FAK is then tyrosine phosphorylated together with the c-Met receptor. A gradual loss of tyrosine phosphorylation finally goes along with the disruption of focal adhesions and the beginning of cell migration on various extracellular matrices [94].

Interactions between tumour cell integrins and the ECM can be modified by growth factors and cytokines. Interleukin 1 increases β1-integrin expression in human osteosarcoma cells [34] and nerve growth factor induces the expression of a laminin binding integrin on the PC 12 phaeochromocytoma cell line [119].

An extracellular integrin ligand which has been shown to play a role in cell attachment or detachment during tumor cell migration is tenascin (or cytotactin [22, 43, 100a], for review see [74]). It is composed of six disulphide-linked polypeptides of 190–250 kDa which radiate from a centre and which contain both adhesive and counteradhesive domains [75, 142]. The high molecular weight splice variant of tenascin has been shown to downregulate focal adhesions and to facilitate cell migration (for review see [74]). However, the binding of tenascin to tumour cells via the α9β1 integrin or several subtypes of the alpha V integrin [111, 158] has been found to trigger a transmembrane signal which inhibits cell spreading [68].

CD 44 represents yet another cell-matrix receptor with a potential role in tumour cell migration (for review see [54]). It is a ubiquitous surface molecule and a number of isoforms exist which are generated by alternative splicing of ten "variant" exons and additional modifications by glycosylation. CD 44 is expressed on a variety of cell types and involved in physiological cell migration and lymphocyte activation [134]. It belongs to the family of hyaladherins which show an affinity to hyaluronic acid. Variants of CD 44 have been found to confer metastatic properties to a nonmetastatic rat pancreas carcinoma cell line [62] which can be prevented by a monoclonal antibody to the metastasis-specific domain of CD 44v [129]. In transfection experiments the CD 44H variant has been shown to promote migration of melanoma cells by interacting through its extracellular domain with hyaluronic acid [143]. A number of human carcinomas express the CD 44v variant comprising the exon v6. The expression of this variant has been found to be correlated with poor prognosis in human colorectal and breast cancers [66, 110, 136, 152].

The transitory adhesions of moving tumour cells to components of the ECM are thought to be liberated by locally limited proteolysis mediated through systems of membrane bound proteases on tumour cells such as uPA fixed to its membrane bound receptor uPAR [113] or the recently discovered membrane-type matrix metalloproteinase [124, 146].

## The role of stroma in tumour cell proliferation

Tumour stroma has finally been shown to take part in the regulation of neoplastic cell proliferation. Again several mechanisms appear to be involved:

### Cell-matrix interactions can transduce signals which modify tumour cell proliferation

The cell signalling cascade following fixation of tumour cell integrins to their extracellular ligands can not only affect cell migration but also cell proliferation. It has been shown in a human colon carcinoma cell line that the expression of the αv β6 integrin, an epithelial cell fibronectin receptor enhances the proliferative capacity of these cells both in vitro and in vivo in a nude mouse model [1]. However, growth arrest has been reported in transfection experiments of a rectal carcinoma cell line expressing the α6 β4 integrin, a laminin receptor [25].

Integrins can also be involved in growth factor induced tumour cell proliferation. For an embryonic carcinoma cell line, a relationship was demonstrated between FGF-stimulated cell division and attatchment to laminin or fibronectin [127]. Analogously, the responsiveness of

many other cell types to growth factors depends upon their attachment to components of the extracellular matrix via integrins [24].

### Tumour cell proliferation can be modified by stromal cells and stromal cell derived factors

Cells of the urogenital mesenchyme exert a growth inhibitory effect on the rat prostatic Dunning tumour [29] and normal fibroblasts have been shown to inhibit the proliferation of v-*myc* transformed quail myoblasts [86].

A growth promoting activity for the LNCaP prostate carcinoma has been isolated from the conditioned medium of a bone fibroblastic cell line [23].

Fibroblast derived HGF/SF can not only influence cell motility but has also been shown to either stimulate or inhibit tumour cell proliferation in different colon carcinoma cell lines [76]. In cultured fibroblasts the expression of HGF/SF has been found to be regulated by fibroblast density as well as by inhibitory and stimulatory factors present in the conditioned medium of breast carcinoma cell lines [133].

### The ECM can act as a reservoir of growth factors

Because of the negative charge and varying pore sizes of glycosaminoglycans and the GAG-chains of proteoglycans these molecules can finally act as a molecular sieve which controls the storage and traffic of signalling molecules according to their size and charge [121]. Decorin for example is a proteoglycan with a single GAG chain which binds and inactivates TGFβ, a factor involved in a variety of responses of many cell types including growth and differentiation (for review see [45, 142]). In the stroma of human colon carcinomas the decorin gene has been found to be hypomethylated and thereby transcriptionally activated leading to an accumulation of decorin which might thus inactivate TGFβ [74].

Other growth factors such as EGF and a and bFGFs which also affect the proliferation of many cell types can be stored in heparan-sulphate chains of extracellular proteoglycans and may be released by heparinases during tumour invasion [47, 121, 147]. The low-affinity binding of bFGF to cell surface heparan sulphates of syndecans, a family of transmembrane proteoglycans, appears to be a prerequisite for the efficient activation of the high affinity bFGF-receptor by its ligand [48, 157].

## Conclusions

In conclusion the emerging field of stroma research has already uncovered many of the interactions which undoubtedly exist between neoplastic cells and their stromal microenvironment. From these results it is clear that the behaviour of neoplastic cells can no longer be understood without taking into account their active and reciprocal dialogue with their surroundings. Further work along this avenue will better charaterise many other roles of tumour stroma and may also help to develop new strategies for cancer treatment.

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