Summary. The article focuses on the functional impact of tumor-associated fibroblasts (TAF) on its surrounding cells. It intends to cover the recent knowledge on TAF, the phenotype, and expression profile of which have been described in the first part of the review series (Kunz-Schughart and Knuechel, 2002). The present review is subdivided into two main chapters: (1) functional impact of TAF on tumor cells and (2) fibroblast-host cell interactions in tumor tissue. In the first paragraph of chapter (1) about the role of fibroblasts in tumor cell growth and differentiation it is revealed, how strongly cellular interaction is dependent on fibroblast and tumor cell type as well as the spatial ratio between the cells. The variation of cellular behavior depending on quantity of molecules holds also true for the group of ECM molecules, e.g. the balance between MMPs and TIMPs, which provide an interesting therapeutic target in tumor tissue. This is one of the topics addressed in the second paragraph which focuses on tumor cell dissemination. Chapter (2) addresses the relation of TAF to other intra- or peritumoral host cells. The hypoxia-related angiogenesis induction of fibroblasts via growth factor secretion (e.g. VEGF) is considered as important as the immune modulatory properties of fibroblasts on immune cells, such as monocytes/macrophages. These cellular properties can be tested under controlled conditions in three-dimensional heterologous cultures of human cells, providing the chance for systematic modification to assess therapeutic effects in an in vivo like environment.

Key words: Fibroblast, Myofibroblast, Tumor, Endothelium, Angiogenesis, Immune cells, Tumor-associated macrophage, Extracellular matrix

Introduction

Fibroblasts comprise a large part of body tissue, supporting the organization and structural stability of epithelial cells in tissue. They have been investigated thoroughly as an integral part of wound healing and as regulatory cells in developmental processes. The awareness of fibroblasts as a component in epithelial tumor tissue is especially known from the so-called scirrhouss tumor. This pattern of tumor growth is defined by an increase in fibroblast growth and consequent scar-like collagen deposition in tumor tissue (desmoplastic reaction).

The desmoplastic reaction is described most frequently in breast cancer, squamous cell carcinoma e.g. of the head and neck, as well as pancreatic and colorectal carcinoma. In these tumor entities, desmoplasia is associated with tumor progress and poor prognosis. Fibroblasts are the quantitatively most abundant stromal host cell type in desmoplastic tumors and the main source of various ECM components and modulators. These tumor-associated fibroblasts (TAF) were shown to exhibit a spatio-temporal abnormal phenotype, e.g. myofibroblastic and/or fetal-like features, with a multitude of modifications in the expression pattern that critically affect tumor growth and dissemination (for review: Wernert, 1997; Elenbaas and Weinberg, 2001; Tlsty and Heil, 2001). The following text will connect the phenotype of TAF (Fig. 1) to findings of the direct and indirect functional impact on tumor cells.

Functional impact of TAF on tumor cells

Tumor cell growth and differentiation

The phenomenon of histological differentiation of tumor cells induced by embryonic or normal mesenchyme was first described with murine mammary carcinoma cells in vitro (DeCosse et al., 1975) and was revealed in the early 1990s not only for human breast tumor cells cultured in a tumor spheroid-fibroblast monolayer coculture system (Brouty-Boyé et al., 1994)
but also for colorectal (Bosman et al., 1993) and prostatic cancer cells in vitro and in vivo (e.g. Hayashi et al., 1990; Chung, 1991, 1995; Chung et al., 1991; Cunha et al., 1991; Gleave et al., 1991, 1992; Hayashi and Cunha, 1991). The in vivo studies, i.e. coinjection experiments of prostatic cancer cells and normal prostatic or bone marrow fibroblasts into athymic mice, implied a fibroblast-induced histodifferentiation, loss of tumorigenicity and enhanced androgen-sensitivity of tumor cells accompanying an enhanced collagen type I production. Since collagen I was not increased at the invasive front, e.g. in colorectal tumors, it was soon hypothesized that collagen I production in tumor-associated stromal cells is not a protective reaction of the host tissue (e.g. Hewitt et al., 1993). Other experiments using conditioned media from short-term cultures of human fibroblasts demonstrated on the contrary a mitogenic effect on various breast tumor cell lines indicating a paracrine role in the regulation of breast tumor growth (e.g. Cullen et al., 1991; Ryan et al., 1993; Ellis et al., 1994). Ryan and Van Roozendaal and coworkers documented a stimulatory effect of fibroblast-conditioned media regardless of the fibroblast source, e.g. fibroblasts from normal breast, normal skin, breast fibroadenomas, and malignant breast tissue (van Roozendaal et al., 1992; Ryan et al., 1993). However, van Roozendaal described a quantitative difference in the mitogenic stimulus with conditioned media from breast tumor fibroblasts being more effective than that of normal fibroblasts and ER-(estrogen-receptor)positive breast tumor cells (MCF-7 and ZR-75-1) showing a greater response than ER-negative cell lines MDA-MB-231 and Evsa-T (van Roozendaal et al., 1992, 1996). In fact, for Evsa-T cells he reported an infrequent inhibitory effect of fibroblast-conditioned media which is in accordance with other studies applying not only conditioned media but diverse experimental in vitro coculture systems that implied epirubicin and tumor-derived mesenchyme but not normal adult fibroblasts to induce a proliferation boost in mammary carcinoma cells (Armstrong and Rosenau, 1978; Adams et al., 1988a,b; Mukaida et al., 1991; Hofland et al., 1995; Lefebvre et al., 1995; Dong-Le Bourhis et al., 1997). Similarly, Shekhar and coworkers (2001) applied a 3-D coculture model of normal breast epithelial cells MCF10A and preneoplastic MCF10AT1-EIII8 cells and diverse fibroblast types. While normal breast fibroblasts inhibited growth and morphogenesis of both epithelial cell lines by suppressing estrogen-induced effects, tumor-derived fibroblasts conferred morphogenic and mitogenic induction of epithelial cells.

The conflicting in-vitro data were reflected in in-vivo studies. It was for example shown that coinoculation of MCF-7 breast tumor cells with fibroblasts promoted retrieval and growth of subsequent MCF-7 tumors in nude mice (Horgan et al., 1987; Noel et al., 1993a; Foidart et al., 1994). Coinjection of tumor cells and matrigel and repeated injection of fibroblast-conditioned medium at the site of tumor cell inoculum induced similar effects (Noel et al., 1993a,b). In contrast, MCF-7 tumorigenicity and tumor growth were not modified by the presence of normal or tumor-derived fibroblasts in a study performed by Brouty-Boye and Raux (1993). They could also show that the incidence of MDA-MB-231 tumors in nude mice increased for low cell inocula with either normal or malignant mammary tissue fibroblasts. However, at higher cell inoculations the appearance of MDA-MB-231 tumors was delayed in the case of normal fibroblasts and unaffected by tumor-derived fibroblasts. This led to the hypothesis of tumor-fibroblast interactions to critically depend on fibroblast and tumor cell type but also on the proportion of coinoculated cells. A tumor-promoting effect of TAF is supported by the more recent in-vivo and in-vitro observation of Olumi et al. (1998) who demonstrated tumor-derived fibroblasts to suppress tumor cell apoptosis. Although being inconsistent, the literature data clearly imply the source of fibroblast (normal, embryonic, tumor-derived) as a critical determinant in tumor cell response. Both cell-cell and cell-matrix interactions as well as paracrine factors are found to be involved in this dynamic process.

TAF-derived paracrine factors that may affect tumor cell proliferation and differentiation include diverse peptide growth factors such as PDGF-AA and -BB, IGF-I and II, EGF, and TGF-α. TGF-β produced by TAF is supposed to inhibit proliferative activity in epithelial cells. However, with respect to carcinoma cells there is growing evidence that at later stages of cancer progression TGF-β rather contributes to tumor growth, since 1.: many tumor cells become resistant to TGF-β growth inhibition; 2.: TGF-β decreases the immune responses by potentially altering migration, proliferation, and/or maturation of immune cells; and 3.: TGF-β potentially supports EC maturation in the neovasculature (see below) (for review: Gold, 1999).

Tumor cell characteristics are directly and indirectly affected by TAF-derived ECM and ECM-modulating molecules. Some peptide growth factors are sequestrated by proteolytic cleavage from the ECM or from the
molecules are not only implicated in invasion processes that involve proteolytic, bioactive ECM fragments, such as collagen type I which is overexpressed in TAF-derived ECM, as well as by mechanical forces. In contrast to other ECM molecules, TN appears in the interstitial tissue, they can profoundly affect tumor cell migration and metastasis. This was first shown for colon cancers (Armstrong and Armstrong, 2000). Also, FN has a complex pattern of alternative splicing at the mRNA level. The oncofetal isoforms ED-A and ED-B are derived by alternative splicing and contain an extra FN type-III repeat. Another oncofetal FN variant results from translational de novo O-linked glycosylation of a specific threonine residue in the C-terminal region of the FN molecule (Kosmehl et al., 1996; Matsuura et al., 1988). All of these modifications alter the conformation of the FN molecule and thus affect the binding affinity to other ECM substrates, paracrine factors including peptide growth factors, and to carcinoma cells as well as stromal cell types. In ED-A FN transfected CHO cells, a reduced integrin-binding affinity of the RGD site, which in particular but not exclusively binds to integrin α5β1, was shown. This affinity-loss was associated with a promotion of cell spreading and migration (Manabe et al., 1997).

The human TN-C hexamer contains three subunits of 190, 200, and 230 kDa, each consisting of 14.5 EGF-like repeats, 15 units similar to the FN type III homology repeat and a sequence homologous to the globular domain of the β and γ chains of fibrinogen at the C-terminus. Alternative splicing of several FN-III domains leads to small and large splice variants with a distinct binding affinity to FN and a differential incorporation into the ECM. Formation of homo- and heterotypic hexamers further contributes to the functional diversity of TN-C (for review: Jones and Jones, 2000). TN-C is expressed non-ubiquitously with a highly diversified but precise, temporop-spatial pattern associated with transient morphogenetic tissue interactions in developmental processes; in normal adult human tissue, TN-C is variably present for example in smooth muscle cells and vessel walls, and in tonsils (for review: Vollmer, 1994; Chiquet-Ehrismann, 1995; Jones and Jones, 2000). It is highly regulated by a variety of growth factors, hormones, cytokines, vasoactive peptides and ECM proteins as well as by mechanical forces. In contrast to other ECM molecules, TN appears in the interstitial stromal ECM rather than in basement membranes. The
role of TN-C in tumor growth and dissemination has been issue of controversial discussion as it was initially believed to inhibit cancer growth by creating cell boundaries, suppressing cell migration, and down-regulating tumor cell proliferation: contrary to this, it was suggested to stimulate cell movement and subsequent metastatic spread due to its anti-adhesive properties. TN-C is considered an adhesion-modulating molecule since it counteracts the cell adhesion activity of FN (Chiquet-Ehrismann, 1993; Schenck and Chiquet-Ehrismann, 1994; Chiquet-Ehrismann et al., 1995). But TN-C not only interacts with other ECM molecules, it also binds to diverse integrins (αvβ3, α2β1, αvβ6, αxβ1, αxβ1, α9β1) that are expressed on epithelial (tumor) cells, fibroblasts and/or ECs (for review: Jones and Jones, 2000). Interactions were further documented with annexin II, a calcium-dependent, phospholipid-binding protein that is found not only intracellularly but also extracellularly as both soluble and membrane-bound molecule (for review: Chung et al., 1996; Siever and Erickson, 1997). Annexin II heterotetramers are frequently present on the surface of tumor cells interacting with serine proteases plasminogen/plasmin and tissue-type plasminogen activator (tPA) and with the cysteine protease cathepsin B that are all upregulated in a variety of tumors and are involved in ECM degradation (for review: Mai et al., 2000).

Increased expression of HA by TAF does not necessarily account for reduced cell migration. In an experimental in-vivo system with HA-transfected tumor cells it was shown for example that enhanced production of HA, in particular of high molecular mass HA, promotes anchorage-independent growth and tumorigenicity (Kosaki et al., 1999). TAF seem to primarily overexpress high molecular mass HA and mediated fragmentation of these immobilized HA variants by hyaluronidases that are overexpressed in many tumors facilitates tumor invasion.

ECM proteases and their mediators directly determine the transitory adhesion of moving cells to components of the ECM including malignant, endothelial, and immune cells and therefore crucially affect tumor microenvironment, tumor cell invasion/metastasis and angiogenesis (for review: Johansson et al., 2000). In addition, proteolysis of ECM influences cell properties and behavior by modification of cytoskeletal organization and activation of second-messenger and protein-kinase pathways. Proteases with a central role in ECM degradation and cell migration that are overexpressed or activated by tumor-fibroblast interactions include several matrix metalloproteinases (MMPs) such as MMP-2 and MMP-9 (DeClerck, 2000) (see also previous report). Several concise reviews on ECM-degrading enzymes in tumor-associated processes have been published within the last two years (e.g. Curran and Murray, 1999, 2000; Murphy and Gavrilovic, 1999; Bergers and Coussens, 2000; Koblingski et al., 2000). Thus, MMPs and their specific inducers and inhibitors produced by tumor cells and by the tumor-associated stroma represent a relevant target for therapeutic intervention (Heath and Grochow, 2000; Johansson et al., 2000; Nelson et al., 2000). TIMPs are a family of currently four low-molecular weight proteins that critically determine ECM homeostasis by control of MMP activity. Their functions include not only the inhibition of active MMPs but also activation of proMMP. Furthermore they were shown to affect cell proliferation and apoptosis and to bind extracellular matrix (for review: Bode et al., 1999; Curran and Murray, 1999, 2000; Brew et al., 2000). In TAF, TIMP-2 expression has been noted. In the context of tumor biology, coexpression of MMPs and TIMPs is controversially discussed. In addition to their multifunctional nature, i.e. inhibition of MMPs associated with anti-invasive properties on the one hand and growth-factor-like activity on the other hand, discrepant results may be due to the fact that TIMP-2, as the main physiological inhibitor of MMP-2, is secreted as soluble factor by some cell types such as macrophages but in complexes with proMMP-2 by others, e.g. fibroblasts. The mechanism of activation of proMMP-2 via MT1-MMP appears to potentially involve formation of a trimolecular receptor complex with TIMP-2 (Butler et al., 1998). Also, an MT1-MMP promoted internalization and degradation of TIMP-2 seems reasonable according to a study by Maquoi et al. (2000) who applied radioactive-labeled TIMP-2 and MT1-MMP-transfected tumor cells to investigate the interaction between MT1-MMP and TIMP-2. The cooperative mechanism of MT1-MMP, MMP-2 and TIMP-2 becomes even more complex with the observations reviewed by Chen and Wang who described a differential co-localization of the three molecules on specialized surface protrusions of invasive cells.

Fig. 2. Impact of TAF on the matrix metalloproteinase scenario in tumors. All of the factors mentioned may be overexpressed in TAF as a result of tumor cell-fibroblast interaction: MT1-MMP (membrane type 1 matrix metalloproteinase), (pro)MMP1, (pro)MMP2, (pro)MMP3, (pro)MMP-9, uPA (urokinase-type plasminogen activator), uPAR (uPA receptor), Cathepsin B, TIMP-2 (tissue inhibitor of metalloproteinase 2). The net effect of these interactions is an activation of various ECM proteases resulting in matrix degradation and growth factor activation. Tumor growth and dissemination is enhanced via direct and indirect mechanisms including involvement of other host cell types such as endothelial and immune cells.
associated with a distinct function (Chen and Wang, 1999). With regard to proteases involved in the upstream regulation of the MMPs such as uPA and Cathepsin B that are known to be (over)expressed by TAF (Fig. 2) the following observations should be mentioned: for MMP-9 and the urokinase-type plasminogen activator (uPA)-plasminogen-plasmin system there is evidence that the cellular source may determine its activity. While uPAR (urokinase-type plasminogen activator receptor) expression in tumor cells and stromal macrophages is frequently described, Saito et al. (2000) documented an inverse association of liver metastasis of colorectal cancers and stromal expression of MMP-9 or uPAR. To eliminate systemic effects by widely-spread tumor cells, they investigated metachronous hematogenous metastasis of colorectal cancers after surgery and showed that only the number of macrophages was reduced along the invasive margins as compared with non-invasive controls. They concluded that CD68-positive, uPAR+, MMP-9-producing macrophages could be inhibitory to hematogenous metastasis while the presence of uPAR+ fibroblasts and tumor cells that also express MMP-9 rather appeared to be associated with hematogenous metastasis. Cathepsin B which proteolytically activates MMP-1 and MMP-2 as well as uPA also appears to be involved in the downstream regulation of MMPs as it leads to fragmentation of the ECM protease inhibitors TIMPs and PAIs and can promote motility of several tumor cell types (Nieman et al., 1997; Norman et al., 2000; Saed et al., 2001). In vitro experiments with human prostate cancer cells using a 3-D sandwich coculture system indicated that stromal fibroblasts are required to produce capillary-like formation of endothelial cells (ECs) and play an important role in angiogenesis (Janvier et al., 1997). Angiogenesis is mainly initiated by sprouting of endothelial capillaries from existing blood vessels to form an endothelial plexus. After a period of remodeling, the vasculature matures into a stationary state by recruitment of perivascular cells and smooth muscle cells. Vascular maturation is associated with arrest of angiogenesis as manifested by contact inhibition of endothelial cell (EC) proliferation when cocultured with pericytes (PCs) or smooth muscle cells (Oridge and D’Amore, 1987; Nehls et al., 1994). In the immature state the neovasculature is leaky and fragile, and depends on a continuous supply of vascular endothelial growth factor (VEGF) for survival (Benjamin et al., 1998, 1999).

VEGF is actively secreted by a variety of human tumor cell lines and is the major stimulus for both proliferation and migration of ECs during tumor vascularization (for review: Neufeld et al., 1999; Veikkola and Allitalo, 1999) that also stimulates monocyte/endothelial cell interactions and transendothelial migration of monocytes (Heil et al., 2000). Hypoxia is considered the main inducer of VEGF-production and secretion during tumor growth. Here, VEGF production is not only enhanced via activation of the HIF-1α (hypoxia inducible factor alpha) transcription factor pathway (e.g. Forsythe et al., 1996) but the intrinsically labile VEGF mRNA is also stabilized in response to hypoxia (Dibbens et al., 1999). It has become evident that hypoxia as a pathophysiological phenomenon in tumors affects the expression profile not only of the malignant cell population but also of the adjacent stroma. Thus, hypoxia is known to enhance VEGF secretion from various fibroblast types including TAF (Hlatky et al., 1994; Jackson et al., 1997; Pilch et al., 2001). In parallel, hypoxia induces production of angiogenin, another potent EC mitogen (Pilch et al., 2001), but also promotes fibrogenesis by augmenting collagen type I and FN synthesis in parallel to an enhanced expression of TIMP-1 and downregulation of MMP-1 expression (e.g. Falanga et al., 1993; Agocha et al., 1997; Tamamori et al., 1997; Norman et al., 2000; Saed et al., 2001). In spite of inconsistent literature data on MMP-1 expression under hypoxia, it should be emphasized that...
several investigators documented a time-dependent, bidirectional regulation of some of the genes mentioned, i.e. collagen type I is upregulated after short-time exposure (≥ 24 h) to low oxygen and down-regulated under prolonged hypoxic conditions (≤ 48 h) in vitro (Steinbrech et al., 1999a; Yamanaka and Ishikawa, 2000) which may reflect the temporal progress of two dominant processes in tumor stroma – desmoplasia with fibroblast and ECM accumulation and angiogenesis with enhanced EC migration and proliferation.

Other factors with angiogenic potential in vivo that are produced by fibroblasts either under hypoxic conditions and/or following myofibroblast differentiation include angiogenin, BFGF, TGF-β, TNF-α, and tissue factor (Powell et al., 1999; Norman et al., 2000; Pilch et al., 2001; Rickles et al., 2001; Saed et al., 2001). In an interesting study, Zhao and Eghbali-Webb (2001) showed cardiac fibroblast-conditioned medium containing VEGF, bFGF, PDGF, and TGF-β1 to cause a significant reversal of hypoxia-induced inhibition of DNA synthesis and enhanced expression of Bcl-2 (cell survival-associated protein) in endothelial cells. Thus, while fibroblasts release EC-inhibitory and stimulatory factors, the net effect of fibroblasts in hypoxic areas suggests an endothelial cell protecting and angiogenesis supporting function.

With regard to tumor hypoxia we should also mention the following issues: (1) Via an oxygen sensor, HIF-1 (hypoxia-inducible factor-1), and hypoxia response elements, hypoxia activates a variety of signal transduction pathways including protein kinase C (PKC), protein kinase A (PKA) and tyrosine kinases and regulates expression of a wide range of genes. Hypoxia-induced proteome changes in neoplastic and stromal cells influence tumor propagation and a process of hypoxia-mediated malignant progression also leading to multidrug resistance has been proposed (Höckel and Vaupel, 2001). The first evidence for TAF to be critically affected by hypoxia (Pilch et al., 2001) will hopefully result in an increased effort to gain deeper insight into the regulatory mechanisms relevant in fibroblasts in an oxygen-deficient (tumor) micromilieu in order to establish new ‘fibroblast-based’ therapeutic designs. In fact, candidate genes determining a hypoxic, more aggressive tumor phenotype may also be differentially expressed in TAF or may be supplemented by other tumor-promoting modifications of TAF. As an example, it has been shown recently that hypoxia induces expression of chemokines MCP-1 (monocyte/macrophage chemotactic/chemoattractant protein) and IL-8 in resident human dermal fibroblasts, two factors that mediate chemotaxis of leucocytes (see below) (Galindo et al., 2001). An angiogenic and EC chemotactic potential of MCP-1 has also been documented (Goede et al., 1999; Salcedo et al., 2000). In parallel, the mRNA levels of IL-10 and IFN-γ were shown to increase in fibroblasts under hypoxic conditions; the corresponding cytokines are known to be involved in the regulation of macrophage tumoricidal capacity (Saed et al., 2001). (2) Hypoxia not only directly alters the expression profile of fibroblasts but it may also modulate the effect of other paracrine/autocrine factors on the expression pattern. Agocha et al. (1997), for example, described an inversion of the effect of TGF-β1 and thyroid hormone on production of collagen type I in cardiac fibroblasts in an oxygen-deprived environment; under ambient conditions both TGF-β and thyroid hormone had led to a diminished level of collagen type I. (3) Other effectors can stimulate expression of hypoxia-inducible genes in fibroblasts such as IL-1β (Jackson et al., 1997).

TGF-β is one of the indirect angiogenic factors expressed by TAF and/or activated by the carcinoma cell-fibroblast interaction. This pleiotropic growth factor is not only involved in myofibroblast differentiation processes and capable to induce VEGF production in fibroblasts (Trompezinski et al., 2000) but it is also hypothesized in the recruitment of pericytes (PC) together with smooth muscle cells to mediate angiostasis and survival and to regulate hemodynamics by providing the vessels with the capability of response to vasoreactive signals. During retinal development the pattern of vascular maturation suggests PC and smooth muscle cells to originate from vascular smooth muscle cells and migrate along the growing endothelial cords (Benjamin et al., 1998). However, there are also indications that myofibroblasts provide a source for α-SMA-positive PCs in tumors (Lindahl et al., 1997). Perivascular PC (= Rouget or mural cells) are found under the basement membrane of capillaries with a close structural relationship with EC (Diaz-Flores et al., 1991; Shepro and Morel, 1993; Hirschi and D’Amore, 1996). They represent a heterogenous family of cells with various, tissue-specific functions and have a pluripotent capacity (for review: Egginton et al., 2000). The postulated roles of PC or PC-like cells range from structural support of capillaries, secretion of ECM compounds, provision of an ECM scaffold for EC migration and vessel sprouting, control of vascular permeability, and regulation of capillary perfusion through different mechanisms. In contrast to previous suggestions, Nehls et al. showed that PCs are involved in capillary sprouting in situ and they also provided evidence that at least a fraction of capillary PCs evolve from perivascular, interstitial fibroblasts (e.g. Nehls et al., 1992, 1994). While PC coverage is clearly not the only mechanism of vessel maturation, there is good evidence to suggest that in most organs it does reflect correct function and degree of microvascular maturation (Lindahl et al., 1997, 1998; Benjamin et al., 1998, 1999; Goede et al., 1998). Human tumors, in contrast to xenografts in mice, may contain large fractions of mature vessels. The degree of PC recruitment to the neovascularure in different tumor types reflecting mature vessels varies significantly as shown most recently by Eberhard et al. (2000). Interestingly, the neovascularure in mammaary and colon carcinomas, tumor types often characterized by a marked desmoplastic reaction, had the
highest rate of PC coverage, with as many as 60-70% of mature vessels. While maturation will attenuate the rate of vascular sprouting, it also prevents vascular collapse and regression. Thus, the net effect may not be reduced angiogenesis, but rather a shift towards a more stable vascular bed with better perfusion capacity and loss or reduction in the sensitivity toward antiangiogenic targeting.

Other paracrine factors involved in the regulation of angiogenesis affecting EC migration and homing are cytokines/chemokines. In particular, CXC chemokines with an ELR motif (Glu-Leu-Arg) at the NH2-terminus such as IL-8, GRO (α, β, γ) (growth related oncogene), NAP-2 (neutrophil activating peptide), or ENA-78 promote angiogenesis while those lacking the ELR motif such as IP-10 are described as inhibitors (Keane and Strieter, 1999; Belperio et al., 2000). IL-8, ENA-78 and some other CXC cytokines may be constitutionally released by resident fibroblasts and myofibroblasts and are enhanced by diverse stimuli (e.g. Andoh et al., 2000; Witowski et al., 2001). Thus, an unbalanced expression of these CXC cytokines in TAF is reasonable to influence tumor angiogenesis.

Some of the soluble molecules affecting tumor angiogenesis may be stored in the TAF-derived ECM. In addition to this indirect function, ECM components and modulators may critically affect tumor vessel formation via diverse mechanisms (Beckner, 1999). As mentioned earlier, HA promotes migration of various cell types in vivo and in vitro under physiological and pathophysiological conditions. TAF produce variable amounts of HA and hyaluronectin. HA action depends on molecular mass, with anti-angiogenetic high-molecular mass HA and small HA oligosaccharides or HA-degrading products stimulating EC migration and proliferation (for review: Rooney et al., 1995). Hyaluronidase-mediated fragmentation of immobilized, high molecular mass HA not only facilitates tumor invasion but the HA-degrading products generated stimulate angiogenesis indicating that both elevated levels of HA and HAase contribute to tumor vascularization. Hyaluronectin impairs the binding of both immobilized HA and HA-derived fragments to EC inhibiting the stimulatory effect of HA in vitro (Trochon et al., 1997) and may be involved in vessel formation and EC maturation. Tokes et al. (1999) found a positive correlation between vascular grade and TN-C in tumor stroma and hypothesized TN-C expression to be associated with EC activation and angiogenesis, a hypothesis that is supported by the observation of a TN-supported EC spreading (Canfield and Schor, 1995) depending on the adherence to the RGD domain and fibrinogen-like terminal knob of TN potentially via integrins α5β1 and α6β3 (Joshi et al., 1993; Striamarao et al., 1993). Also, it has become clear within the last 5 years that the ED-B FN splice variant, being more frequent in the immature tumor vasculature than ED-A FN and absent in mature vessels, is involved in angiogenesis (Kaczmarek et al., 1994; Neri et al., 1997).

Recent studies imply a VEGF-independent, FN-integrin α5β1-mediated pathway contributing to tumor vascularization (Kim et al., 2000). Accordingly, ED-B FN-targeted antiangiogenic therapeutic strategies have been introduced most recently (Neri et al., 1997; Nilsson et al., 2001). Other investigators could not reproduce an ED-B-positive staining of the tumor vascular bed, e.g. in breast and colorectal carcinoma tissue, and thus controversially discuss these therapeutic designs (Midulla et al., 2000). According to their effect on tumor cells, ECM proteases, including MMPs, Cathepsin B, and uPA, also determine EC migration. Protease-mediated cleavage of cell adhesion molecules (E-cadherin) and the development of matrikines such as angiostatin, an endogenous plasminogen cleavage product with antiangiogenic potential shall be mentioned as two regulatory mechanisms (for review: Bergers and Coussens, 2000).

TAF and immune cells

In spite of fibroblasts and EC, the peritumoral stroma in desmoplastic carcinomas also contains diverse immune cell types such as cytotoxic T and NK cells as well as macrophages and dendritic cells. According to the expression profile depicted in our previous review, TAF produce a multitude of paracrine immune-modulators including chemokines, cytokines, and peptide growth factors. In addition, fibroblasts may secrete low-molecular weight inflammatory mediators such as prostaglandin E2 (PGE2) (Schrey and Patel, 1995; Kim et al., 1998; Powell et al., 1999) that critically affect immune cell activity. Vancheri et al. (1996), for example, showed that human lung fibroblasts inhibit TNF-α production by LPS-activated human peripheral blood monocytes. Later they documented fibrotic lung fibroblasts to produce less PGE2 with a reduced ability to downregulate LPS-stimulated TNF-α secretion from monocytes than normal fibroblasts (Vancheri et al., 2000).

While TAF have rarely been considered important effectors of antitumor immune responses, fibroblasts are known to take part in immune reactions during tissue damage and injury by modulating local cellular and cytokine milieu, adjusting the kinetics and components of the inflammatory infiltrate to the type of damage, and by modulating the functional status of the immunocompetent cells, as reviewed by Buckley et al. (2001). Lack of deactivation of fibroblasts contributes to persistence of the inflammatory infiltrate and prolonged inflammation. Accordingly, fibroblasts are considered sentinel cells in chronic inflammation as they essentially contribute to leukocyte migration to the site of damage and influence the local immune response (Murakami and Okada, 1997; Smith et al. 1997). It is well known that chronic inflammation and malignancies show some molecular and cellular similarities even with regard to the cytokine profile (Opdenakker and Van Damme, 1992; Balkwill and Mantovani, 2001). In addition, the
data reviewed in our previous report, e.g. with regard to cytokine/chemokine expression profile in TAF, imply a critical impact of TAF on immune cell infiltration, activity, and maturation.

Tumor-associated macrophages (TAM) represent a major component of the leukocytic infiltrate of many solid tumors. They derive from blood monocytes that migrate into tumor-tissue and undergo a process of differentiation determined by the tumor microenvironment. As indicated by several *in-vitro* and *in-vivo* studies, TAM are often characterized by a lack of immunological activity and therefore rather contribute to than limit tumor growth and progression (for review: Mantovani et al., 1992; Elgert et al., 1998). In ductal breast carcinomas a high macrophage content even correlated with poor prognosis and clinical outcome (Leek et al., 1996). In this tumor entity, TAM seem to be predominantly located within the fibroblastic stroma surrounding tumor cell islets (Müller, 1992), as indicated in Fig. 3a. In addition to an abnormal TAM phenotype affected by TAF, the following phenomenon is to be considered: using a regressive rat colorectal tumor model, Lieubeau et al. (1999) revealed that T lymphocytes and monocytes/macrophages are found outside progressive tumors that were surrounded by a sheath of myofibroblasts and concluded that tumor-activated myofibroblasts may prevent physical contact between cancer and immune cells to effectively avoid tumor cell destruction.

Monocyte-to-macrophage differentiation/maturation in tissue is a process modulated by the cooperation of paracrine factors, cell-cell and cell-matrix interactions. Thus, *in-vitro* investigations focusing on the mechanisms leading to or determined by the disturbed differentiation pattern of TAF also require complex model systems. One of these systems is the spheroid coculture model. In MCS of urothelial tumor cell lines and suspensions of monocytes it was shown that the highly invasive bladder cancer cell line J82 downregulated the expression of several maturation-associated surface antigens and inhibited the shift in the cytokine repertoire usually associated with macrophage maturation. This effect was not described in monolayer cocultures (Konur et al., 1996, 1998). However, this model initially introduced by Hauptmann et al. (1993) ignores the potential impact of stromal fibroblasts. The first attempts to evaluate the role of TAF using fibroblast spheroids and monocytes from healthy donors (Fig. 3b) indicate a dominant role of fibroblasts derived from diverse pathological conditions including invasive ductal breast tumors on the recruitment of monocytes and also imply an impact on the maturation process (unpublished data).

![Fig. 3. Immunohistochemical detection of monocytes/macrophages in 5 µm sections. (a) Invasive ductal breast tumor (G3) stained for CD68 using DAB for color development and hematoxilin as counterstain. (b) BT474 breast tumor spheroid, (c) spheroids of tumor-derived fibroblasts (TAF) both cocultured with monocyte suspension (10^4 monocytes/spheroid) for 40 h. Cocultures were stained for CD14 using the APAAP-technique and Fast Red for color development. In breast tumors, tumor-associated macrophages are primarily located in the fibroblastic stroma. This behavior is reflected in spheroid culture. Only a few monocytes migrate into tumor spheroids but massive infiltration is seen in spheroids of tumor-derived fibroblasts.](image-url)
In spite of the impact of the ECM and ECM-modulating molecules such as proteases derived from TAF on immune cell migration, one of the paracrine factors that may play an essential role in this scenario shall be mentioned. In breast carcinomas, the C-C cytokine MCP-1 was detected in both tumor and stromal cells (Valkovic et al., 1998). In another study, elevated levels of MCP-1 were shown by in-situ hybridization in extracts of malignant breast tumors and correlated with a higher macrophage content (Ueno et al. 2000). Considering the portion of fibroblasts contributing to the tumor mass in desmoplastic tumors and the multiple stimuli in the tumor environment that may lead to enhanced MCP-1 release from fibroblast (see previous review), TAF have to be interpreted as a relevant source of MCP-1. Interaction between TAF and TAM may further add to the increased MCP-1 level in tumor tissue. Neither normal fibroblasts nor monocytes alone produced significant levels of MCP-1 in vitro, but a significant TNF-α-related increase in MCP-1 expression in both cell populations was documented following cocultivation (Zickus et al., 1998). Thus, MCP-1 is one of the candidate cytokines to primarily originate from tumor-associated stromal cells, in particular TAF, affecting immune cell response. In a dose- and time-dependent manner MCP-1 for example stimulates monocyte attachment to ECM components such as laminin or FN via β2 integrins (Jiang et al., 1994; Penberthy et al., 1995). It is also involved in the regulation of transendothelial chemotaxis of monocytes and T lymphocytes by dynamic activation and deactivation of β1 integrins (α4β1, α5β1) via molecular mechanisms that are currently under investigation (Carr et al., 1996; Weber et al., 1996; Ashida et al., 2001; Cambien et al., 2001). However, MCP-1 not only affects immune cell migration but also exerts further immunomodulatory effects, for example by reducing leukotriene B4 production by macrophages, suppressing CD4+ T cell proliferation, and modulating release of IL-4 and IFN-γ in CD4+ T lymphocytes (Hogaboam et al., 1998; Matsuakawa et al., 1999). The interstitial fibroblast is also capable of regulating its own behavior within the interstitial environment via the expression of chemokines and chemokine receptors. In a mouse model of acute sepsis, endogenous MCP-1 was identified as a factor influencing the cytokine balance in tissues in favor of anti-inflammatory and immune-enhancing cytokines (Matsuakawa et al., 2000). It is unclear, if the related regulatory pathways are still intact in tumor tissues. Interestingly, migration of normal monocytes and macrophages is controlled by MCP-1 but TAM, i.e. macrophages isolated from tumor tissues, may show reduced response to MCP-1, presumably due to a ‘defective’ expression of the MCP-1 receptor CCR2 (Müller et al., 1997; Sica et al., 2000). Together with the data reviewed earlier on the expression profile of TAF (Kunz-Schughart and Knechel, 2002), these observations indicate a net effect of immune dysfunction in invasive tumors with desmoplastic reaction. The enhanced expression of various immune cell deactivating or suppressing factors not only by tumor cells (Elgert et al., 1998) but also by TAF, such as IL-6, IL-10, TGF-β1, PGE2, or M-CSF, is likely to play an additional critical role. Understanding the impact of TAF and TAF-derived factors on the immune reaction may thus lead to the design of novel therapeutic strategies aimed to affect the generation of these immune modulators by TAF.

With regard to the ECM, it is obvious that integrin-matrix interactions, presence or absence of ECM proteases and soluble ECM fragments are involved in adherence and migration of immune cells. Cellular FN is described to affect cytokine expression in monocytes/macrophages, e.g. induction of IL-10 production (Yoshida et al., 1999). FN fragments are implicated in the modulation of monocyte integrin expression and migration (Trial et al., 1999) and the hyaluronan/CD44 system is known to influence lymphocyte rolling (Clark et al., 1996). However, the impact of specific TAF-derived ECM molecules on immune cell activity, such as oncofetal FN splice variants, is not well understood. Tatts and coworkers (1999) presented an interesting study regarding TN-C using an in-vivo mouse model with spontaneously-developing mammary tumors followed by metastatic lung disease. Their data on TN-C-null mice crossed with the tumor-developing mouse strain indicate a very limited role of TN-C in spontaneous tumor development, growth, and metastasis. However, the TN-C null stromal compartment contained significantly more monocytes/macrophages indicating an immunomodulatory activity of TN-C which is in accordance with a previous study showing TN-inhibited monocyte adhesion to FN in parallel to an immuno-suppressive effect on T-cell activation (Ruegg et al., 1989). Again, the awareness that TAF-derived factors including complexed and soluble ECM compounds affect immune cell (dys)function in tumors shall motivate scientists to elucidate the underlying mechanisms in order to delineate new therapeutic targets.

Conclusion

Far from being complete, our present report gives an overview and discusses how TAF directly and indirectly affect tumor growth and propagation in order to stimulate consideration of TAF as a ‘novel’ therapeutic target in desmoplastic tumors. The complex network of effects transduced by TAF and TAF-derived factors on tumor and host cells is highlighted with respect to tumor cell behavior, EC migration, vessel maturation, and immune response. Recapitulating the modified expression profile of TAF as compared with normal fibroblasts, as emphasized in part I of this review article series ‘Tumor-associated fibroblasts (part I): active participants in tumor development and progression?’, some examples are given to stress the potential impact of TAF on tumor progression, i.e. TAF-derived signals resulting in angiogenesis and immune dysfunction.
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