[Frontiers in Bioscience E4, 1871-1887, January 1, 2012]

Tumor-stroma interactions in tumorigenesis: lessons from stem cell biology

Franziska Briest[1], Alexander Berndt[2], Joachim Clement[3], Kerstin Junker[4], Ferdinand von Eggeling[5], Susanne Grimm[1], Karlheinz Friedrich[1]

[1]Institute of Biochemistry II, Jena University Hospital, Germany, [2]Institute of Pathology, Jena University Hospital, Germany, [3]Clinic for Internal Medicine II, [4]Clinic of Urology, [5]Institute of Human Genetics and Anthropology, Jena University Hospital, Germany

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1. ABSTRACT

Research in recent years has accumulated a wealth of novel insight into mechanisms by which tumor cells interact with activated fibroblasts, endothelial cells, inflammatory and immune cells and the extracellular matrix. Cancer and stromal cells co-evolve throughout tumorigenesis. As a result, the tumor stroma is now regarded as an essential contributor to tumor establishment, progression and dissemination. Moreover, the formation of suitable stroma niches has emerged as a prime determinant of metastasis. Notably, malignant tumors adopt numerous mechanisms that are also operative in embryonic and adult stem cell biology. Tumor sites show functional characteristics with striking similarities to stem cell niches. This review summarizes the current view of disease-relevant communication between tumor cells and the tumor stroma and relates it to interactions of stem cells and their respective niches. Progress in understanding the pivotal role of the microenvironment in both tumor and stem cell biology renders the tumor stroma an interesting potential future target for specific cancer therapies.

2. INTRODUCTION

Healthy epithelial cells communicate with adjacent mesenchymal cells and macromolecular structures that provide stability, nutrient supply and immune defense. Mutual interactions are specified by surface proteins and solute signaling molecules. The mesenchymal microenvironment consists of a cellular component including fibroblasts, adipocytes, immune cells, glial cells, smooth muscle cells und endothelial cells, as well as of a scaffold part mainly made up of extracellular matrix proteins such as collagens, proteoglycans and glycoproteins. Solute substances such as cytokines, growth factors, enzymes and nutrients are involved in maintenance and development of the microenvironment. Within adenomatous organs and inside carcinomas, the connective tissue with its cellular components is termed stroma. Mechanical stability of the stroma is provided by fibroblasts which produce collagen I, III and V as well as fibronectins, common proteins of the extracellular matrix (ECM). The constitution of the stroma is not static, in fact tissue structure and the ECM are continuously modified

through adaptive, well regulated processes. Degradation of the ECM and of basal laminal proteins is likewise controlled by fibroblasts via secretion of matrix-metalloproteinases (MMPs). The dynamic nature of these events is essential to assure cell migration throughout processes like embryogenesis, wound healing, vascularization and neurogenesis (1).

Homeostasis of the microenvironment ensures adequate anchorage, nutrient supply and signaling not only for differentiated and organ-specific epithelial cell types. Quiescent cells such as stem and progenitor cells also benefit from interactions with appropriate niches which provide optimal chemical and physical conditions to favor maintenance as well as differentiation, proliferation and migration (2).

Even during cancerogenesis, the interactions between a transformed epithelial cell and stroma cells persist and a co-development of cancer cells and cancer associated cells takes place. This phenomenon may lead to a modulation of cellular functions and to grossly altered phenotypes of both tumor and stroma cells.

Interestingly, in the course of tumor development and spreading, cancer cells recapitulate and exploit various mechanisms established by stem cells. For instance, they alter characteristics of interaction with their respective environment by acquisition of a mesenchymal phenotype which shows similarities to stem cells with regard to surface expression patterns. Epithelial mesenchymal transition (EMT) facilitates immune escape and migration and enhances susceptibility to stem cell guiding molecules that attract circulating tumor cells to potential metastatic sites.

3. ROLES OF THE STROMA IN IMMUNE ESCAPE OF TUMOR AND STEM CELLS

The role of the immune system in cancerogenesis and cancer progression, invasion and metastasis appears as a paradox. Two opposing theories have been put forward, which are both supported by experimental data and contribute to the current view of how the immune system responds to tumor development:
One the one hand, the role of the immune system in fighting cancer cells has been described as cancer immune surveillance since the early 20th century (3). Immune effector cells of the innate immune system such as natural killer (NK) and T cells are attracted by cytokines originating from fibroblasts, tumor cells and macrophages (4). They in turn produce pro-inflammatory cytokines such as interleukin (IL)-12 and interferon (IFN)-gamma and, thus, activate dendritic cells that stimulate the immune response by antigen presentation (5). $CD8^+$ T cells act by IFN-gamma-mediated angiostasis and by targeting tumor antigen-bearing tumor and stroma cells (6,7).

A competing classical theory, the cancer immune escape theory, postulates that the inflammatory environment might also stimulate growth of transformed cells (8). Many investigators hold that chronic inflammation can create a tumor promoting environment. Examples for such mechanisms are the development of gastric cancer upon *Helicobacter pylori* stomach infection or the occurrence of bladder cancer as a consequence of schistosomiasis (9). A potential functional connection between inflammation and tumor progression is provided by transcription factor NF-kappaB (10, 11). The tumor stroma generates an inflammatory, hypoxic environment. Macrophages, NK cells, T cells and dendritic cells respond by production of a multitude of cytokines. They activate NF-kappaB, which in turn promotes expression of further pro-inflammatory proteins such as tumor necrosis factor (TNF)-alpha and IL-17. Moreover, NF-kappaB initiates the expression of anti-apoptotic (e.g. Bcl-x_L, cIAP-1 and cIAP-2) and mitogenic proteins (e.g. c-myc, cyclin D1, cyclooxygenase-2) (12-16). TNF-alpha does not only activate NF-kappaB, but is also able to initiate transformation directly by inducing the formation of DNA damaging reactive oxygen species (ROS) (17, 18).

NF-kappaB, a prevalent transmitter of proliferation signals also plays a role in pro-survival signaling and niche cell maintenance of stem cells. In the human hematopoietic stem cell niche it represents a potent, radiation induced pro-survival factor for human osteoblasts (19). Brain inflammation resulting in TNF-mediated activation of the canonical NF-kappaB pathway promotes proliferation and differentiation of neuronal stem cell (20,21).

The outcome of immune reactions inside malignant neoplasias is mostly dependent on the tumor entity. To define reasons why the balance of these opposed processes is shifted in one direction or the other is an important issue of research in cancer immunology.

Tumor recognition by the immune system requires efficient identification of cancer cells. Immune cells, hence, are able to sense mutational alterations and inadequate expression of antigens in an autoimmune manner. In order to establish itself, a tumor must exert mechanisms to circumvent the immune attack. An analogous situation can be found in human embryonic cells which express both maternal and paternal antigens. Embryonic trophoblast cells (ETCs) evade maternal immune response to paternal antigens by shutting off expression of major histocompatibility complex (MHC) II as well as of MHC I. Moreover, they alter expression patterns of human leukocyte antigens (HLAs) and, thus, protect themselves against attacks by maternal NK cells and macrophages (22, 23). Notably, tumor cells also alter their MHC and HLA expression patterns (24-26) and/or lose co-stimulatory molecules such as B7 (CD80) (27, 28) or NKG2D (22, 29).

IL-6 and IL-10 are considered key cytokines of tumor immune escape. By activating janus kinase JAK1 they induce phosphorylation of signal transducers and activators of transcription STAT1 and STAT3. Abundance of tyrosine phosphorylated and, thus, activated STAT1 was shown to correlate with decelerated growth of malignant tumors and favorable prognosis (30). STAT3 activation, in

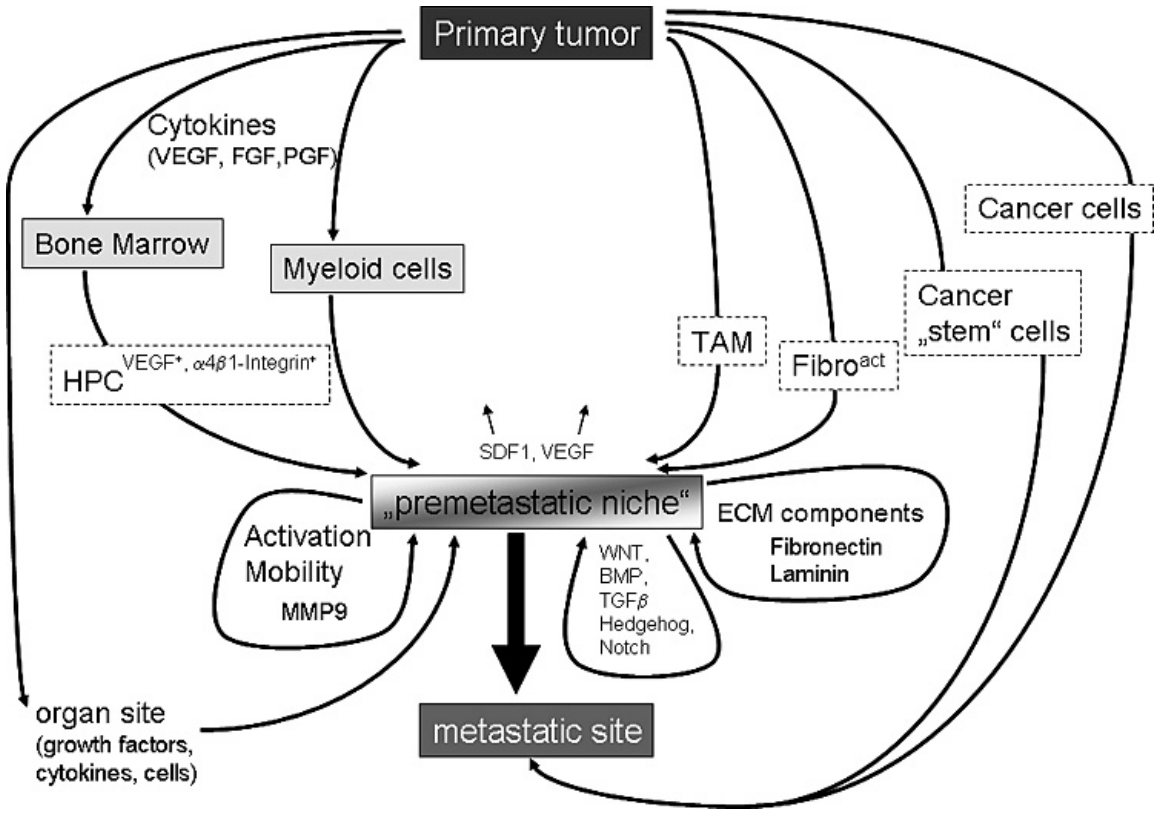

Figure 3. Role of cytokines in cancer and stem cell immune tolerance. Tumor cells and embryonic trophoblast cells (ETCs) evade host immune response by secretion of interleukins and VEGF and, thus, inhibit maturation of dendritic cells (DCs) and activation of natural killer (NK) cells. Immature DCs (iDCs) increase the number of regulatory T cells (T_{regs}), thereby impairing NK cell and $CD8^+$ T cell activity. Moreover, ETCs inhibit macrophage attacks by TNF-alpha secretion, whereas tumor cells promote the development of a cancer associated macrophage phenotype which in turn favors tumor growth.

contrast, is involved in various cancer promoting processes (31). The contribution of STAT3 to the organisms' immune response to the tumor appears to be triggered by initial STAT3 activation in tumor cells. Here it promotes expression of vascular endothelial growth factor (VEGF), IL-6 and IL-10. These factors in turn induce phosphorylation of STAT3 in hematopoietic progenitor cells, resulting in an increased number of both immature and plasmacytoid dendritic cells (iDC, pDC). The ultimate consequence of this sequence of events is enhanced proliferation of regulatory T cells (T_{regs}) in the tumor stroma (32). STAT3-active T_{regs} inhibit proliferation of $CD8^+$ T cells and IFN-gamma secretion via IL-10 and transforming growth factor (TGF)-beta signaling. Immature dendritic cells (DCs) express little MHC II-, CD80 and CD86 surface proteins and are, thus, not able to recruit $CD8^+$-T cells and NK cells (32-34) (Figure 3). Furthermore, STAT3 and its target gene products IL-6 und IL-10 inhibit the expression of immune stimulating factors such as IL-12 and TNF-alpha via the B-raf/mitogen activated protein kinase (MAPK) pathway (35). Cytokine mediated inactivation of DCs and in turn the activation of T_{regs} are crucial events in cancer immune escape (4).

In analogy, healthy embryonic cytotrophoblasts (ETCs) also recruit T_{regs}, resulting in a mildly inflammatory environment to control the decidual immune response (23). ETCs induce expression of a T helper 2 (T_{H2})-associated cytokine pattern characterized by increased secretion of IL-4, IL-10 und TGF-beta (36), thereby inhibiting macrophage function. Furthermore, they produce VEGF (23), block DC maturation and ultimately trigger T_{reg} activation (32) (Figure 3). ETCs and many tumors also share other immune-suppressive functions. Both cell types express FasL and TRAIL which trigger apoptosis in lymphocytes and both secrete indolamine-2,3-dioxygenase (IDO), thereby suppressing lymphocyte tryptophan metabolism and activity (23).

Interestingly, transcription factor STAT3 is crucially involved in the activities of both tumor and stem cells and their respective interactions with stroma and immune system. In tumors, STAT3 signaling mediates the release of angiogenetic factors such as VEGF, basic fibroblast growth factor (bFGF) and MMP-9 from myeloid-derived suppressor cells (MDSC) and tumor associated macrophages (37) and leads to the recruitment of circulating endothelial progenitor cells (38). IL-6 and TGF-beta co-activate STAT3 in T_{H17} cells. Thus, these cytokines activate T_{H17} proliferation and expression of IL-21, which in turn stimulates IL-23 receptor and IL-17 expression in an autocrine manner (39). This course of events results in

Table 1. Characteristics of tumor-stroma interactions and analogous processes in stem cell biology. For details see text.

	TUMOR CELLS	EMBRYONIC CELLS
Inflammation	TNF-alpha secretion: Survival and proliferation by NF-kappaB mediated expression of anti-apoptotic and mitogenic factors (12-16)	NF-kappaB as survival factor of niche cells and TNF-alpha mediated neuronal SC proliferation (20,21)
Surface properties during immune escape	Altered HLA/ MHC pattern (24-26) Loss of co-stimulators (22,27-29)	Altered HLA/ MHC pattern (22,23)
Cytokine mediated immune escape	IL-6/-10 induced inhibition of DC maturation and Treg induction (32-34) STAT3 as key orchestrator for various mechanisms of immune escape and tumor progression (31,32-35,37-43)	IL-4/-10 induced inhibition of DC maturation and Treg induction (23,32) STAT3 mediated aberrant APC maturation inhibits CD4+ T Cell response (45-47)
Triggering of lymphocyte apoptosis	FasL/ TRAIL Surface expression induce apoptosis of lymphocytes (23)	FasL/ TRAIL Surface expression induce apoptosis of lymphycytes (23)
IDO expression	Secretion of IDO compromises lymphocyte metabolism (23)	Secretion of IDO compromises lymphocyte metabolism (23)
Supportive cells	TAFs induce ECM remodeling (62-66), trigger neoangiogenesis (60,67-70), promote proliferation (74,75,77-80) TAEs induce proliferation (49,50)	Fibroblast-like osteoblasts control cell number (2,84-86,89,90) and induce ECM remodeling (87,88) Endothelial cells determine proliferation and differentiation of HSPCs and embryonic stem cells (93,94)
SDF-1	Various cancer types express CXCR-4 (156) Guidance to metastatic niches and stimulation of growth, motility and survival (156,158-161)	Various stem cell types express CXCR-4 (146-151) Control of stem cell homing, motility and proliferation (139,140,149,158)
Cell phenotype	De-differentiated mesenchymal phenotype of migrating tumor cells and malignant cells near invasive front; EMT (97-109)	Stem/ progenitor cells show an un-/low differentiated phenotype
Construction of ECM	Oncofetal remodeling of ECM proteins enhances neoangiogenesis and cancer cell motility (66,110,115-128)	Embryonic/ oncofetal development of the ECM facilitates stem cell migration throughout embryogenesis, inflammation and wound healing (115-118,122)
"Target" microenvironment	Premetastatic niche provides protection, vascularization, mitogenic signaling (61,102,134,169,170) Frequently high levels of SDF-1 (156,158-161)	Stem cell niche provides anchorage, nutrient supply, proliferation/ maintenance signaling (2,82,88,95) Many types of stem cell niche show high levels of SDF-1 (141-143)

increased expression of MMP-9 and correlates with tumor angiogenesis and growth (40-42). STAT3 signaling in cancer is not only activated by cytokine receptor-associated kinases, but also by receptor tyrosine kinase receptors such as the EGF receptor, c-met, receptors for insulin-like growth factor-1 (IGF-1) and platelet-derived growth factor (PDGF), and by cytosolic tyrosine kinases such as src and abl. Notably, STAT3 function has both a strong immunosuppressive and angiogenetic component.

Recently, STAT3 was also identified as a determinator of cancer cell interaction with cells of the naïve immune response since it regulates the expression of surface receptors recognized by natural killer cells (43). Cancer initiating cells may play a pivotal role in the suppression of an immune attack on the disease by STAT3-mediated processes: Brain cancer initiating cells inhibit T cell responses by producing immunosuppressive cytokines, promoting differentiation of T cells into T_{regs} and by killing T cells through apoptosis via the immunosuppressive protein B7-H1 (44).

STAT3 is not only a pivotal connector in cytokine mediated cancer immune control, but also a key orchestrator of contact-dependent immune suppression by mesenchymal stem cells (MSCs), an aspect with importance for craft-versus-host disease. MSCs constrain T cell responsiveness by IL-10 induction in a STAT3 dependent manner (45, 46), comparable to the ETCs immune suppression. By triggering increased STAT3 activity, MSCs induce aberrant antigen-presenting cell (APC) maturation and, thus, indirectly inhibit $CD4^+$ T cell response (46). This effect is based on cell-cell interaction between stem cell and APC rather than on soluble factors (47).

The importance of signaling through STAT3 for stem cell development in general is underscored by recent findings showing that STAT3 activation is critical for reprogramming ground state pluripotency (48).

4. SPECIALIZED STROMAL CELLS WITHIN TUMOR MICROENVIRONMENT AND STEM CELL NICHES REGULATE SURVIVAL; PROLIFERATION AND MIGRATION

Transformed epithelial, *i.e.* carcinoma cells and stromal cells co-evolve in the course of tumor development. Thus, occurrence of cancer cells is accompanied by a loss of normal cellular function and a gain of tumor associated activities in the cellular component of the connective tissue, resulting in the formation of the so-called supportive tumor stroma. Several tumor associated stromal cell types have been described.

Tumor associated endothelial cells (TAEs) show enhanced migratory properties and produce large amounts of growth factors and cytokines such as

endothelin-1, IL-6, IL-8, EGF and VEGF, resulting in stimulation of tumor cell proliferation, control of anoikis and motility (49, 50).

Different types of cancer-associated myeloid cells can be found in the tumor stroma. For instance, tumor associated macrophages (TAMs) promote tumor progression and metastasis by stimulating angiogenesis, by suppressing immune responses and by ECM remodeling through proteolytic activity (51).

Tumor associated neutrophils (TANs), reprogrammed by cancer cell derived cytokines such as IL-1 promote tumor growth in the presence of TGF-beta (52). Interestingly, both TAMs and TANs show polarized pro- or antitumor phenotypes depending on local cytokine stimulation (52).

Tumor associated fibroblasts (TAFs) are currently subject of intense research efforts. This subset of activated fibroblasts present in the tumor stroma plays important but ill-defined roles in tumor development and metastasis.

Notably, fibroblasts are also temporarily activatable in non-tumorous tissue. During wound healing, contact of fibroblasts with immune cell adhesion molecules ICAM1 and VCAM1 (53) or stimulation by soluble factors such as FGF2, PDGF, EGF and TGF-beta (54) causes transition to an activated state. The fibroblasts start to produce alpha-smooth muscle actin (SMA), generate stress fibers and gain a phenotype similar to those of smooth muscle cells. By supporting tissue contraction and recruitment of further immune and endothelial cells (55,56) they contribute to wound healing (57,58). During this activated phase, fibroblasts are termed myofibroblasts. Importantly, an analogous, activated myofibroblastic phenotype is also characteristic for fibroblasts of the tumor associated stoma. They differ, however, from "regular" myofibroblasts by the constitutive nature of their activated status. Moreover, TAFs are capable of evading regulatory mechanisms such as apoptosis (1). The origin of TAFs is as yet a matter of debate. Three theories are currently being discussed: (i) TAFs derive from activated residential fibroblasts, (ii) TAFs develop by trans-differentiation of epithelial/endothelial or carcinoma cells by epithelial/endothelial-mesenchymal-transition (EMT), and (iii) TAFs originate from immigration and activation of bone marrow-derived precursor cells (1). Interestingly, it has been demonstrated that TGF-beta stimulation *in vitro* can result in the differentiation of fibroblasts into an activated, alpha-SMA positive cell type (59-61).

TAFs are crucial players during tumor progression. They modify the ECM in many cancer entities by secreting several proteases, such as stromelysin-3 (MMP-11), gelatinase B (MMP-9) and urokinase plasmin activator (uPa). (62-65). Furthermore, they are a source of *de novo* synthesized and deposited oncofetal variants of several ECM adhesion proteins. ECM degradation and oncofetal reorganization leads to the formation of the so-called provisional ECM which is able to promote migration, invasion and metastasis (66). In addition, TAFs favor neoangiogenesis by release of angiogenin, beta-FGF, TGF-beta, TNF-alpha, and "tissue factor" (60, 67-70). In this context, TGF-beta acts indirectly by releasing VEGF from fibroblasts (71) as well as by stimulating maturation of endothelial cells (72). In analogy to wound healing processes, TAFs are furthermore able to recruit endothelial progenitor cells (EPCs) to the tumor site by secretion of the chemokine SDF-1/CXCL12, a ligand of the CXCR-4 receptor (73). SDF-1 likewise promotes proliferation and inhibits apoptosis of CXCR-4-positive cancer cells (74, 75), and it also shows chemorepellent effects on T cells (76). Importantly, TAFs directly stimulate proliferation of tumor cells via growth factors. Among growth stimulatory molecules secreted by TAFs are IGF-I and IGF-II, TGF-beta, PDGF, and hepatocyte growth factor (HGF) (77-80).

Resembling cells of the tumor stroma, different cell types supporting stem cell survival have been functionally described in recent years. Stromal cells can induce phosphorylation of Erk1/2 and Akt1 in mouse neural stem/progenitor cells *in vitro* and, thus, activate proliferation and inhibit apoptosis (81). Furthermore, they control the fate of germ stem cells: In *Drosophila* testis, unpaired (UPD), a protein of niche (hub) cell origin, induces self-renewal of germ cells via JAK-STAT signaling. In *Drosophila* ovary, factors decapentaplegic (DPP) and "glass bottom boat" (GBB) secreted by stromal cap cells suppress germ cell differentiation through SMAD signaling (82).

Inside of mammalian bone marrow niches, a "sophisticated" type of fibroblasts (83), the osteoblasts, provide various factors that regulate number and function of hematopoietic stem and progenitor cells (HSPCs). They secrete osteopontin, an inhibitor of HSPC proliferation (2). Kit ligand expressed by osteoblasts may activate kit receptor on the HSPC surface and, thus, activate survival, proliferation and differentiation in a dependence of signaling through PI3-Kinase, MAPK and JAK-STAT pathways (2, 84-86) Osteoblasts express the notch ligand jagged-1 as well as angiopoietin-1, which cooperatively modulate HSPC maintenance and quiescence (2). Stress induces secretion of osteoblast derived receptor activator of NF-kappaB ligand (RANKL) which in turn activates osteoclasts to release MMP-9 and cathepsin K, thus, mobilizing HSPC to the circulation (87, 88). Several stroma derived cytokines, *e.g.* IL-3, IL-6, IL-11 or G-CSF trigger proliferation of HSPCs (89, 90).

In analogy to cancer cells, HSPCs express the chemokine receptor CXCR-4 and are, thus, susceptible to guidance by SDF-1/CXCL12 gradients produced by bone marrow stromal cells (91, 92). This effect is crucial for stem cell homing (see chapter Error! Reference source not found.). Not only endosteal cells regulate HSPC maintenance. Perivascular cells like megakaryocytes and reticular cells secrete angiopoetin and SDF-1 and, thus, regulate quiescence of HSPCs (88).

Endothelial cells play an important role in cancer cell, but also in stem cell associated environment. Appropriate activation of Akt- and MAPK signalling

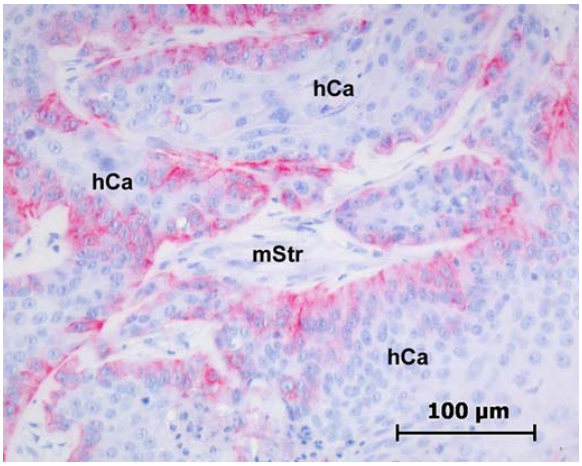

Figure 1. Focally occurring epithelial-mesenchymal-transition in a xenograft of a human oral squamous cell carcinoma cell line (hCa) transplanted onto a nude mouse. Staining was performed with an antibody to human vimentin devoid of reactivity with mouse vimentin (clone Vim 3B4, Dakocytomation, Hamburg, Germany). Note the vimentin-positive carcinoma cells (red) in the vicinity of the mouse stroma compartment (mStr). (bar = 100µm)

pathways renders endothelial cells competent of balancing self-renewal and differentiation of HSPCs by stimulation via angiocrine factors such as FGF-2, IGFBP-2, DLL-1, DHH-30 and BMP-4 (93). Endothelial cells can determine differentiation of embryonic stem cells to cardiomyocytes through signalling by ephrin type-B receptor-4 (EphB4) (94).

Finally, stromal niche cells produce ECM molecules which provide anchorage and modulation of growth factors by proteolytic processing and thereby generating functional components of the stem cell environment (95). Consequently, the ECM itself also participates in stem cell differentiation. For instance, laminin 332, collagen 1 or vitronectin trigger osteogenic differentiation of human mesenchymal stem cells via ERK 1/2 signaling (96).

5. SECONDARY TUMOR FORMARION BY CANCER CELLS INVOLVES THE ACQUISITION OF STEM CELL LIKE ADHESION PROTEIN PATTERNS AND AN EMBRYONAL MATRIX COMPOSTION

Cancer cells are subjected to strong selection pressure when the tumor volume increases. Factors such as the presence of immune cells and reactive oxygen species, hypoxia, nutrient deficiency, and low pH select for a more aggressive cell phenotype. At a certain time point, malignant tumor cells start to migrate through tissue borders such as the basal membrane into the surrounding normal tissue compartment and invade blood and/or lymph vessels. For this purpose, cell-cell contacts, in carcinomas primarily provided by E-cadherin, are disconnected and downregulated (97). This process is initiated and influenced on different levels. The tumor microenvironment can promote mutations in the E-cadherin gene *cdh1* itself or can modify promoter methylation (98). Furthermore, c*dh1* transcription can be inhibited by snail protein, an efficient repressor of E-cadherin. Snail is expressed in a NF-kappaB- dependent manner and is regulated by glycogen synthase kinase (GSK)-3beta (99). Stabilization of snail through modification by lysyloxidase 2 and 3, which in turn is induced by hypoxia inducible Factor (HIF)-1alpha, results in decreased E-cadherin expression. The ultimate consequence of this sequence of events is the induction of EMT (100-101). The dependence of snail expression on HIF-1alpha and NF-kappaB underscores the functional relation of tumor cell behavior to the inflammatory and hypoxic tumor microenvironment.

On the protein level, activities of E-cadherin and catenins are modified by phosphorylation. Inhibitory protein phosphorylation is dependent on cytosolic tyrosine kinases (*e.g.* src), which may become activated by stroma cell derived factors such as EGF-, FGF-, IGF-I- and HGF through their respective receptors (102). Phosphorylated E-cadherin and catenin is ubiquitinylated by the E3-ligase hakai, resulting in subsequent degradation (103). In addition, cleavage of E-cadherin induces the activation of Tcf/ Lef- dependent genes like cyclin D1, c-myc or fra-1 via wnt-signaling by release of beta-catenin. The detached E-cadherin p120ctn fragment inhibits rhoA and activates rac1/cdc42- dependent modification of the actin cytoskeleton, thus evoking a phenotype with enhanced motility (104). The extracellular domain of E-cadherin is cleaved by MMPs and cell-cell contacts are disconnected. The loss of E-cadherin function is a crucial step during EMT. Throughout this process, tumor cells gain a mesenchymal phenotype characterized by *de novo* expression of N-cadherin and vimentin as well as by modification of integrin-mediated intermolecular contacts within the ECM (Figure 1). N-cadherin interacts directly with the FGF receptor and interferes with its internalization (105). This effect leads to constitutive activation of FGF-dependent signaling, *e.g.* persistent activity of mitogenic ras-MAPK- and phosphoinositid-3 kinase (PI-3K)-mediated survival pathways, src- related signal transduction and enhanced MMP-9 expression (106,107). TGF-beta-driven signaling is of key importance in EMT. Downstream reactions driven by the TGF-beta receptor comprise the initiation of signaling pathways around MAP kinase and PI-3 kinase, and also trigger smads and co-smads, which in turn downregulate epithelial proteins such as E-cadherin and certain keratins. In contrast, mesenchymal proteins such as vimentin and fibronectin are upregulated (108). Furthermore, rho becomes degraded via activation of PAR6 and smurf, ultimately promoting disconnection of tight junctions among epithelial cells (109).The migratory behavior of tumor cells does not simply depend on alterations of the cell's phenotype, but is also determined by local conditions. Remodeling of the ECM including the basal membrane structure is a critical requirement for overcoming the epithelial/mesenchymal border and for the invasive and metastatic process. Matrix remodeling includes proteolytic matrix protein degradation or modification as well as *de novo* synthesis and structural reorganization of adhesion protein variants also seen during embryogenesis. This leads to the formation of a so-called

oncofetal matrix environment – a provisional extracellular matrix milieu providing more flexibility and plasticity. Matrix degradation is accomplished to a major extent by matrix metalloproteinases (MMPs). MMPs are predominantly produced by TAFs and TAMs inside the tumor stroma, but also by tumor cells themselves. Following their activation inside the extracellular space, MMPs target structure proteins of the ECM. The basal membrane is primarily degraded by MMP-2 and MMP-9 due to its high content of collagen IV, a preferred substrate of these proteolytic enzymes. MMPs do not only remodel the ECM, but also release latent ECM-sequestered growth factors such as TGF-beta, IGF-I and IGF–II, thereby stimulating further tumor development (110).

Another important aspect of MMP function is localization-dependent activation. Once released into the extracellular space, some MMPs cooperate with adhesion receptors or become processed by membrane associated MMPs (MT-MMPs). The surface protein EMMPRIN (extracellular matrix metalloproteinase inducer; CD147), expressed on many tumor cells is able to direct the activity of MMP-1 to the tumor site by specific association (111). The alpha v beta3 integrin receptor complex orchestrates proteolytic activities in a spatially restricted fashion, since it is associated with MMP-2, MMP-7, MMP-9 as well as with several MT-MMPs (112-113). Activity of MMPs is further controlled by the local presence and activity of specific inhibitors, the tissue inhibitors of metalloproteinases (TIMPs).

Besides MMPs, other proteases and protease regulators play prominent roles in invasion-related ECM remodeling. Plasmin degrades a variety of matrix components like fibronectin or laminin, and activates many MMPs by specific cleavage of the respective zymogens. Enhanced plasmin activity at tumor sites is generated by urokinase-type plasminogen activator (uPA), a potent activator of plasmin. uPA is attracted to cancer cell surfaces by its receptor uPaR (110, 114).

Matrix degradation at the invasive front of tumors is accompanied by *de novo* synthesis of embryonic variants of several adhesion proteins. Well characterized is the reorganization of fibronectin, tenascin-C, collagen and laminin in several carcinoma entities (66). Embryonic or oncofetal isoforms of fibronectin and tenascin-C are generated by alternative splicing. In the case of fibronectin, inclusion of the extra domains A and B (EIIIA, EIIIB) and domains in the IIICS regions leads to the formation of fibronectin variants preferentially expressed during embryogenesis, inflammation and wound healing as well as, notably, in the course of tumor stroma formation (115-118).

Similar to the situation with fibronectin, isoforms of tenascin-C are generated by alternative splicing. Large tenascin-C isoforms are components of embryonic as well as of tumor tissue and are also expressed during inflammation and wound healing (119). Oncofetal remodeling of the fibronectin and tenascin-C matrix occurs also during tumor angioneogenesis. The functional importance of re-expression of oncofetal fibronectin and tenascin-C matrix protein variants is not fully understood. In general, there is an increase in oncofetal fibronectin and tenascin-C synthesis and deposition in the invasive front of several carcinoma entities, accompanied by tumor cell de-differentiation and progressing malignancy grade. Several reports showed that the abundance of EIIIA fibronectins is a prerequisite for both development of the myofibroblastoid phenotype and endothelial cell activation (120). Tenascin-C in general is known as a modulator of cell-matrix adhesion. It could be shown that large tenascin-C promotes tumor cell proliferation and migration, and regulates vessel formation and tumor immunity. It also contributes to genomic instability of tumor cells (121). Importantly, a role of tenascin-C in the formation of stem cell niches is being discussed (122).

Laminin variants are generated by the alternative assembly of different alpha, beta and gamma chains. The resulting heterotrimeric proteins are expressed in a cell type and differentiation dependent manner (123). Laminins are main components of the epithelial and endothelial basal membranes (BM). Reorganization of the tumor basal membrane is a critical prerequisite for cancer cell invasion. The tumor malignancy grade appears to be correlated with a loss of basal membrane laminins and collagen IV ("BM brakes"). The complexity of basal membrane remodeling is further increased by changes in laminin isoform incorporation as well as by enhanced abundance of oncofetal fibronectin and large tenascin-C variants (124). Proteolytic processing of the BM laminin-332 results in protein fragments which promote cancer cell migration and influence the tumor cell phenotype (125).

Both tumor and stromal cells contribute to oncofetal remodeling of the ECM and basal membrane. They interact in the regulation of synthesis as well as in the structural reorganization of matrix proteins, resulting in the development of a tumor-specific matrix scaffold as well as in tumor-specific integrin signaling (126-128).

In summary, combined activities of tumor and stromal cells lead to the formation of an embryonic matrix composition with a higher susceptibility for MMPs and the property of providing migratory signals via integrin signaling and supporting cell phenotype changes. Several reports show that oncofetal fibronectin, tenascin and laminin variants are capable of influencing or regulating the process of EMT, thereby driving the development of an invasive and metastatic carcinoma cell phenotype.

Stem cells share migratory properties with malignant tumor cells. Tumor cell–matrix interactions resemble processes in early ontogenesis where pluri- or omnipotent stem cells have to transmigrate throughout the embryo in order to form tissues and organs at distant sites. During embryonic development, HSPCs resettle from fetal liver to bone marrow. Stem cell release, migration into the blood system, homing, and re-engraftment is a lifelong dynamic process of bone marrow and blood homeostasis (91). HSPCs continuously travel from the bone marrow to

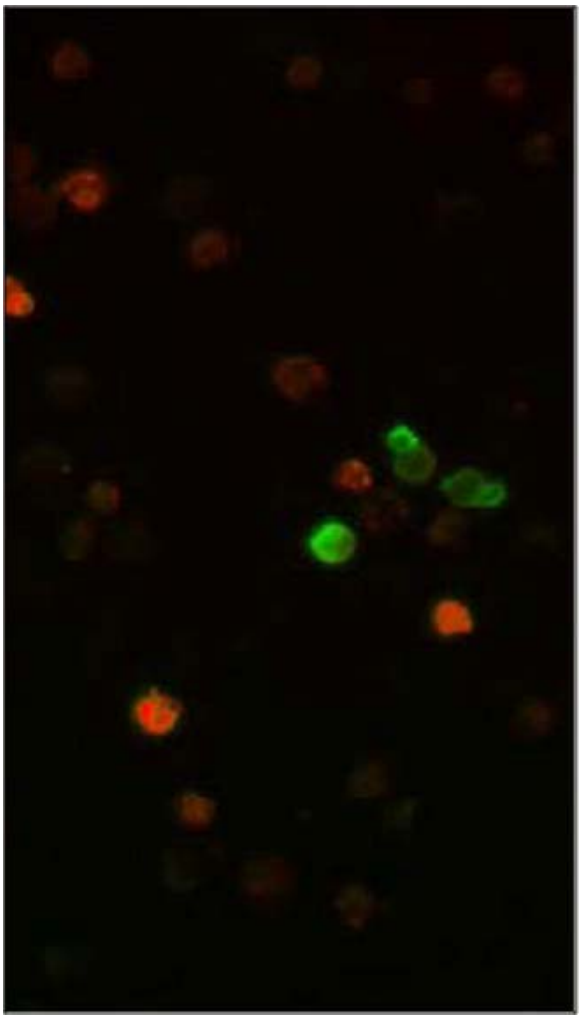

Figure 2. Detection of circulating tumor cells. Disseminated cells from solid tumors can be identified by epithelial markers after enrichment from peripheral blood of cancer patients. Circulating tumor cells are labelled with CD326 (EpCAM)-FITC in green. For control, leukocytes are stained with CD45-phycoerythrin in red.

multiple extra-medullary tissues by blood vasculature. After transiently residing there, they return to the blood via draining lymphatics. Circulation of HSPCs assures the replenishment of specialized, tissue-resident myeloid cells under steady-state conditions and contributes to innate immune response by means of toll-like receptors (TLRs). TLR activation triggers differentiation upon contact with bacterial or viral components such as lipopolysaccharides (LPS) (129).

Stress situations like injury or inflammation as well as administration of granulocyte colony-stimulating factor (G-CSF) can induce massive stem cell mobilization and bone marrow emigration. This process requires the loosening of adhesive interactions between stem cells and the microenvironment, *i.e.* remodeling of niche ECM (87), followed by proliferation of immature leukocytes and their egress to the circulation. Mobilization is accompanied by intense proliferation and differentiation of progenitors as well as by activation of neutrophils and osteoclasts due to accumulation of RANKL and HGF in the endosteum region. Osteoclasts secrete a panel of proteases (MMP-9, elastase, cathepsin G and K, CD26 and MT1-MMP) which degrade SDF-1 and ECM proteins and, thus, promote cell motility and invasion (130, 87). Kit-Ligand is released by cleavage of its membrane anchor and selectins become activated (131). During migration, selectins mediate "rolling", *i.e.* the reversible contact to endothelial cells. Circulating HSPCs express lower levels of adhesion molecules such as V-CAM-1 and the beta1-integrin VLA-4 as well as of beta2-integrin LFA-1 (Leukocyte Function Antigen-1) (132).

6. TUMOR METASTASIS IS DEPENDENT ON MEDIATORS PRIMARILY CONTROLING STEM CELL CIRCULATION AND HOMING

If invasive cells leave the original tumor site and metastasize, they use either the blood or the lymphatic vessel system. Accordingly, the route of metastatic spread is termed hematogenic and lymphogenic (133). Lymphogenically spread cells, however, can also reach the venous circulation due to the fact that lymphatic vessels drain into blood through the thoracic duct. Circulating tumor cells (CTC) originating from a given tumor entity obviously share characteristics which determine preferred routes of dissemination and sites of resettlement (Figure 2). Cells are guided into vessels by chemokines, hence they need to express the respective receptors. The necessity to interact with endothelial cells inside the blood vessels to extravasate at a special destination selects for cells expressing particular surface proteins. Importantly, the conditions at the target tissue determine the ability of the CTC to engraft (134).

A pivotal role in guiding the migration of CTCs is attributed to factors secreted by the stroma. Vessel intravasation due to chemotactic effects of macrophage and fibroblast derived EGF was described for several carcinomas (136-137). Important chemotactic mediators of lymphatic metastasis are the chemokines SCL/CCL21 and CCL2. The melanoma cell line B16, for instance, expresses chemokine receptors CCR7 and CCR8 and, consequently, predominantly undergoes lymphatic dissemination in response to ligand chemokines secreted by lymphatic stromal cells (138).

Remarkably, in a large number of tumors TAFs and stromal cells of distant sites determine the migration of cancer cells by release of SDF-1 (CXCL12). As mentioned before, the chemokine SDF-1 is a key orchestrator for bone marrow homing of HSPCs and other non-hematopoietic tissue-committed stem cells. It also controls inflammation and tissue regeneration processes (139, 140). SDF-1 is produced by bone marrow stromal cells and various epithelial cells of different tissues such as lymph node, muscle, lung, liver, kidney and several regions of the central nervous system (141-143). In bone marrow, SDF-1 is primarily secreted by osteoblasts of the endosteum (144). SDF-1 is the unique ligand to the G-protein coupled CXC receptor 4 (145) and controls migration of $CD34^+$ HSPCs,

pre-B- and T lymphocytes (146). CXCR-4 can also be found on primordial germ cells (147), skeletal muscle satellite progenitor cells (148), neural stem cells (149), liver oval/stem cells (150) and retinal pigment epithelium progenitors (151). Functional consequences of CXCR-4 activation comprise induction of motility, chemotactic response, adhesion and secretion of MMPs and angiopoetic factors (140). SDF-1 functions as a chemoattractant for CXCR-4$^+$ cells and therefore controls homing back circulating cells to tissues bearing high SDF-1 levels. After binding to its receptor, it initiates various signaling cascades in its target cells, among them the activation of focal adhesion components such as pyk-2, FAK, paxilin and pro-survival pathways via MAPK p42/44-ELK-1 and PI-3K-akt-NF-κB (140, 152-154). It also stimulates externalization (rather than *de novo* expression) of LFA-1, VLA-4 and -5 in human HSPCs and, thus, mediates tight reattachment on endothelial layer and re-engraftment of the target tissue (155).

Interestingly, many tumors express CXCR-4 in a hypoxia-dependent fashion (156). Sensitivity of tumor cells to SDF-1 is further enhanced by tumor ECM-derived proteins such as fibronectin, uPAR and VCAM-1 (157). Moreover, SDF-1 increases the affinity of cells for these factors by activating and modulating the function of cancer cell Integrins (158). Metastases dissolved from the primary tumor, are attracted along a SDF-1 gradient. High concentrations of SDF-1 in distant lymphoid, lung, bone and liver tissue direct the CTC chemotactically to prospective niches and promote the establishment of secondary tumors (156, 158-161).

The interaction with adhesion molecules is pivotal for organ-specific spreading of cancer cells. “Capturing” of the CTC by endothelial cells inside the vessel is favored by particular CTC surface proteins such as sialyl Lewis X or beta1-integrins which bind to E-selectins or VCAM-1 proteins of the endothelium. After adhesion, the CTCs strengthen adherence to subendothelial basal membrane proteins such as laminin and collagen IV and V by SDF-1 promoted mediation of beta-1 and beta-4 family integrins and by CD44. The interplay of these interactions is a requirement for tissue immigration and, ultimately, the establishment of metastases (162-164).

7. PREMETSTATIC NICHES SHARE FUNCTIONAL PROPERTIES WITH STEM CELL NICHES

The “seed and soil” hypothesis put forward more than a century ago held that distant engraftment sites gain a microenvironment convenient for cancer cells long before the CTC actually arrives (165). Various recent observations are consistent with this view. Myeloid cells and fibroblasts appear to be involved in the formation of the favorable prematastatic niche. In the course of lung metastasis formation, myeloid cells were shown to be directed to the lung upon secretion of pro-inflammatory cytokines such as VEGF and placenta growth factor (PGF) by the primary tumor (166-168). Concurrently, activated fibroblasts migrate to the premetastatic site where they alter their expression levels of SDF-1, fibronectin and VEGF (169). Bone marrow- derived HPCs expressing VEGF and CXCR-4 receptors as well as beta1-integrin, thus, are able to create niches inside prospective target organs prior to CTC arrival. This process is initiated by systemic changes induced by primary tumor formation. Colonized HPCs at the metastatic site produce MMP-9 and thereby facilitate the immigration of additional cells. It is likely that further interactions between metastatic cells and niche cells ultimately result in the generation of a fully-fledged tumor microenvironment convenient for vascularization and protection.

The normal adult stem cell fate is determined by the stem cell niche that provides anchorage and nutrient supply and controls signaling. Many extrinsic factors regulating stem cell number, proliferation and cell fate such as Wnt proteins, BMPs, FGF, hedgehog and notch are generated by the niche cells (170). Pretty much similarly as normal stem cells which need their niches to grow and survive, metastatic tumor cells require a particular niche to develop from a micrometastasis to a well nourished and growing secondary tumor. Both systems, the cancer as well as the stem cell niche are complex and dynamic structures that transform their conditions in cooperation with the hosted cells. Thus, HSPCs are able to engraft different niches in the bone marrow or the spleen sinosoids depending on their level of differentiation, as tumor cells from the same primary lesion can settle as metastases in totally different tissues and organs.

8. CONCLUSION AND OUTLOOK

Processes such as angiogenesis, invasion, metastasis and immune escape are dependent on tumor-adapted cells and their specific signaling properties. Their sphere of influence is not limited to the primary tumor site itself but extents to very distant sites such as potential metastatic target organs. Thus, the microenvironment does not only assure the survival and prosperity of the primary tumor, but also promotes the successful engraftment of metastatic cells. Mechanisms underlying long distance migration of CTCs share many similarities with those controlling stem cell circulations. CTCs are directed from a highly adapted primary tumor microenvironment to well-prepared distant niches by molecules involved in niche-to-niche migration of HSPCs and other types of stem/ progenitor cells. Moreover, they display several stem cell properties regarding immune control, egress of primary site, homing and niche requirements. This fact has frequently been regarded as supportive for the notion of cancer stem cells (158, 163, 171). The cancer stem cell model, however, is highly controversial due to accumulating inconsistent findings, for instance the observation that any individual melanoma cell has the potential to give rise to a secondary tumor (172-174). Considering the emerging importance of the stroma for tumor development, it is obviously not the intrinsic properties that make a CTC show stem cell like characteristics, but rather the environment that exerts multifaceted influence on it.

Importantly, stroma derived factors and their signaling constitute novel promising targets for therapy. Examples are CXCR-4 inhibitors such as AMD3100,

AMD3465 or RCP168 which interrupt the interaction of several solid cancer entities and their metastases (colorectal cancer, glioblastoma, osteosarcoma lung metastases). They were also shown to block crosstalk of some lymphomas (multiple myeloma, AML, CLL) with their microenvironment and to make them more susceptible to chemotherapy (175-180). Interestingly, the same agents can be used for stem cell mobilization in clinical reconstitution of hematopoiesis in autologous and allogeneic transplants. They are also currently being evaluated for their perspectives in the treatment of cardiovascular diseases by stem cell activation (181-183).

Taken together, our increasing knowledge on disease-relevant functions of the tumor stroma is backed up by intriguing findings on the interaction between stem cells and their microenvironment. As a result, numerous novel ways of understanding and fighting malignant neoplasias can be envisaged.

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Key Words: Microenvironment, Tumor associated fibroblast, Stem cell, Tumor stroma, Metastasis, Stem cell niche, Review

Send correspondence to: Karlheinz Friedrich, Jena University Hospital, Institute of Biochemistry II, Nonnenplan 4, D-07743 Jena, Germany, Tel: 49-3641-9396405, Fax: 49-3641-938682, E-mail:khf@mti.uni-jena.de

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