A Digest on the Role of the Tumor Microenvironment in Gastrointestinal Cancers

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Abstract Experimental studies and analyses of clinical material have convincingly demonstrated that tumor formation and progression occurs through a concerted action of malignant cells and the surrounding microenvironment of the tumor stroma. The tumor microenvironment is comprised of various cell types like fibroblasts, immune cells, vascular cells and bone-marrow-derived cells embedded in the extracellular matrix. This review, focusing on recent findings in the context of gastrointestinal tumors, introduces the different stromal cell types and delineates their contributions to cancer initiation, growth and metastasis. By selected examples we also present how the tumor microenvironment is emerging as a promising target for therapeutic intervention.

Keywords Tumor microenvironment · Tumor stroma · Therapy

Introduction

The tumor microenvironment is composed of various interdependent cell types such as cancer-associated fibroblasts (CAFs), endothelial and lymphatic cells, pericytes, immune cells and bone-marrow-derived cells (BMDC), embedded in the extracellular matrix (ECM). The ECM of tumors has a unique composition determined by the concerted action of cancer cells and stromal cells (Fig. 1).

The seminal review by Gatenby and Gillies (2008) presented the microenvironment as an “education place” for

evolving cancer cells [1]. According to this model for tumor development the normal microenvironment prevents the outgrowth of pre-malignant cells and transformed cells. These cells start to appear when they have overcome several protective barriers provided by the microenvironment. The transformation is associated with a differential protein expression profile, including increased expression of normally low-expressed proteins (e.g. PDGF, VEGF) and the induction of alternate protein family members (e.g. integrins, laminins) which initiates a non-physiological molecular crosstalk between the transformed epithelial cell and the surrounding stroma. Consequently, another set of molecules is produced by the activated stroma under the influence of transformed cells. These factors have either short-ranging effects acting on neighboring cells (e.g. mitogens, proteases, cytokines) or are released in the blood and lymphatic vascular system (e.g. chemokines) and mobilize other cell types from distant sites such as inflammatory cells and BMDC. This reciprocal interaction of cells generates interdependent cell types and gradually impairs the integrity and barrier function of the microenvironment which culminates in its permanent remodeling. Moreover, the altered stroma forces transformed epithelial cells to adapt to the changing conditions and selects for a more malignant phenotype characterized by anchorage-independent growth, increased proliferation, migration and invasion. Finally, the disruption of physical barriers like the basement membrane by enhanced proteolytic activity, a property of the tumor microenvironment, brings stromal and cancer cells in close contact; fueling the malignancy of the latter. Eventually, cancer cells educated from this selection process gain the ability to metastasize [1].

In this review we first introduce the different constituents of the tumor microenvironment. This is followed by some examples of tumor-promoting mechanisms exerted by the

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Fig. 1 Fueling tumorigenesis and cancer cell spreading. The close interaction of cancer cells with its microenvironment—composed of cancer-associated fibroblasts (CAF), immune cells, endothelial and lymphatic cells—results in the production and release of a multitude of soluble factors and constituents of the extracellular matrix (ECM). These molecules in turn have mitogenic, pro-migratory and pro-invasive or chemoattractant properties thereby stimulating e.g. cancer cell growth, invasion and metastasis, vessel formation or the recruitment of immune cells and precursor cells such as bone-marrow-derived cells (BMDC). The figure highlights more recently described molecules in different GI cancer

tumor stroma. Finally, we provide examples how the better understanding of the tumor microenvironment is being translated into the development of novel therapeutic approaches. Some efforts have been made to select examples from gastrointestinal (GI) cancer.

For a more detailed discussion of the various aspects of cancer cell/stroma interplay in gastrointestinal tumors we refer the reader to other reviews by Tahara (2008) [2], Kitadai (2009) [3] and Yashiro and Hirakawa (2010) [4] in this issue of *Cancer Microenvironment*, and to a more recent review on microenvironmental effects on metastasis by Gout and Huot (2008) [5].

Constituents of the Tumor Microenvironment

Cancer-Associated Fibroblasts

Fibroblasts in solid tumors, often termed cancer-associated fibroblasts (CAFs), acquire a specific phenotype and display increased proliferation as compared to normal fibroblasts [6]. CAFs express various proteins characteristic for an activated phenotype (e.g. α SMA, FAP, FSP growth and angiogenic factors) [7]. By producing factors that act on the malignant cells, or on other cell types of the microenvironment CAFs contribute to a tumor-permissive neighborhood [8, 9]. CAFs comprise a heterogeneous population of cells. Analyses have revealed different types of fibroblasts in different tumor types, within a given tumor type and even in individual tumors [10–12].

It is likely that CAFs are derived from different sources. Local fibroblasts are a source of CAFs in many settings [13].

Another suggested origin are bone marrow-derived precursors [14–16]. It has also been proposed that CAFs are derived from malignant cells through epithelial-mesenchymal transition (EMT; see below), and eventually also from endothelial cells [17, 18]. These ideas still require further validation in clinical samples. Possibly, the different sources for CAFs might be an explanation for the heterogeneity of this stromal cell population. The observed heterogeneity can also be a reflection of the high grade of plasticity that cells attain when exposed to the tumor microenvironment.

A functional significance of the CAF heterogeneity is suggested by analyses of clinical material. For example, CAF abundance, determined by α SMA or FAP staining, is associated with bad prognosis in colorectal cancer [19, 20].

Immune Cells

Inflammation has been recognized as a critical contribution to tumor development and progression in different types of cancer including cancers of the gastrointestinal tract. Inflammatory responses lead to the recruitment of various immune cells like leukocytes, neutrophils, mast cells and macrophages [21–23]. Among these the latter are one of the best studied mediators of pro-tumorigenic effects of the tumor microenvironment.

Macrophages constitute in some tumor types the major part of the immune cells in the tumor stroma and are often referred to as tumor-associated macrophages (TAMs) [24]. Two phenotypically different sets of macrophages have been described; M1 and M2. The polarization of macrophages can be induced in vitro by stimulating these cells with different cytokines. The M1 type is induced by e.g.

IFN- γ while IL-4, IL-10 and IL-13 lead to the M2 type. The two populations display a different cytokine and chemokine expression profile. The M1 phenotype is generally considered to initiate an anti-tumor response by activating the immune system as well as producing reactive oxygen species (ROS), nitric oxide (NO) and tumor necrosis factor (TNF). In contrast, the M2 phenotype displays immunosuppressive and tumor promoting functions [25]. M2 macrophages also exert pro-metastatic functions by production of angiogenic factors (VEGF, CXCL8) and factors involved in extracellular matrix breakdown [22]. Recently, TAM-derived TNF α was also shown to promote gastrointestinal tumorigenesis by Wnt pathway activation [26, 27].

Infiltration of TAMs is in many cancers (skin, breast and cervix) correlated with bad prognosis [28–30]. However, it has also been reported that a higher abundance of macrophages in colorectal cancer correlates with a more favorable prognosis [31]. These apparently contradicting data might be clarified with specific M1 or M2 markers.

Tumor-associated dendritic cells produce elevated levels of CXCL9 that is associated with an increased abundance of CXCR3 positive T cells in gastric cancer [32]. Dendritic cell expression of TIR8 also appears to have an important role in the inflammatory response of the GI-tract. TIR8 acts as a decoy receptor, inhibiting signaling via members of the IL1R/TLR superfamily, and TIR8 knock out mice display a higher susceptibility to intestinal inflammation and colitis associated cancer [33, 34].

The role of neutrophils in tumors is not fully understood yet. However, as with TAMs two neutrophil populations called TAN (tumor-associated neutrophils) N1 and N2, with opposing roles in tumors, have been recently postulated to exist. In this context, TGF- β present in the tumor microenvironment drives the polarization of neutrophils from the anti-tumorigenic type N1 towards a pro-tumorigenic N2 phenotype. The N1 TAN population is characterized by an increased cytotoxicity towards tumor cells in vitro and shows higher expression levels of pro-inflammatory cytokines and chemokines but lower expression of arginase [35]. An increased number of neutrophils has been observed in colon and gastric human tumours [36, 37]. Moreover, experimental studies in models of colorectal and pancreatic cancer have shown that neutrophils promote tumorigenesis by stimulation of angiogenesis [38, 39].

Endothelial Cells and Pericytes

Angiogenesis, the formation of new blood vessels from existing vessels, is an important factor for tumor progression and metastasis. To be able to grow, a tumor must recruit vessels which provide it with oxygen and nutrients, and which are also used to remove waste products. The

tumor vasculature is obviously also a major route for spread of metastasizing cells.

A variety of different factors is involved in the induction, stabilization, migration and branching of newly growing vessels. Angiogenic factors, e.g. VEGF, FGF, CXCL8 stimulate angiogenesis by acting directly on endothelial cells. VEGF-A is the prototypical angiogenic protein that mediates migration, proliferation and cell survival of endothelial cells. VEGF can be produced by cancer cells and CAFs from colon tissue and its expression is clearly increased under co-culture conditions [40]. Other proteins of major importance for vessel formation are angiopoietin-1 (ang-1), Dll4 and members of the ephrin and TGF- β families. Additionally, PDGFs are involved in the recruitment of pericytes [41].

Tumor vessels are mainly formed through angiogenesis e.g. in response to hypoxia that induces VEGF-A expression. However, it is not only the expansion of local endothelial cells which builds up the vessels in tumors. Also bone-marrow-derived endothelial progenitor cells (EPCs) are incorporated into the vessel wall. Critical roles of bone-marrow-derived EPCs are suggested by experiments that demonstrate that absence of these cells reduces or prevents tumor growth in different animal tumor models [42, 43]. It is likely that the dependency of these cells will vary between different tumor types. Further studies are thus warranted to give a more detailed picture of the significance of these cells for tumor angiogenesis in different clinical settings. Some studies have also reported cancer cells integrated in the vessel wall, giving rise to what is commonly called “mosaic vessels” [44]. It has been suggested that the presence of cancer cells in the vasculature might predict tumor aggressiveness and also that these cells might contribute to the leakiness of tumor vessels. Other studies have proposed that the “mosaic” pattern of tumor vessels is derived from variations in marker expression among different subsets of endothelial cells, rather than integration of cancer cells into the vessel wall [45].

The morphology of vessels in tumors is different from normal vessels. They have an irregular and chaotic structure and are often leaky and haemorrhagic [46, 47]. Gene expression analysis of normal and tumor vessels from colorectal tissue have revealed transcriptional differences between normal and tumor endothelial cells [48]. Although endothelial cells are in general considered genetically stable, some studies have suggested that tumor endothelial cells are characterized by genetic instability [49–51].

Pericytes are recruited to the vessel wall in response to endothelial cell-derived PDGF-B or TGF- β and are important for vessel stability and function. In turn, ang-1 secreted by pericytes promotes survival and sprouting of endothelial cells and thereby contributes to vessel maturation [52]. In tumors, pericytes are less tightly attached to the

vessels, have a different shape and express other markers than their normal counterparts [53]. Also, they are often less abundant on tumor vessels than on normal vessels which might contribute to the leakiness of the tumor vasculature. Pericyte coverage has been associated with different aspects of tumor growth. Some experimental data showed that increased pericyte enhance tumor growth [54, 55]. However, pericyte coverage has also been implied as a barrier for metastasis [56].

Analyses using different markers have provided early indications that multiple pericyte subsets exist, which might differ with regard to sensitivity to PDGFR-targeting agents [57, 58].

The potential prognostic significance of pericyte coverage, and of different pericyte subsets, remains poorly characterized, although some studies have demonstrated that reduced pericyte coverage is correlated with metastasis and bad prognosis [59].

The Extracellular Matrix

The ECM is made up of different classes of macromolecules including collagens, laminins, fibronectins, proteoglycans and hyaluronan. The basement membrane, which is a specialized part of the ECM, separates the epithelium from the mesenchymal cells, and offers a proliferative barrier. In cancer, the composition of the ECM is often altered by factors produced by cancer and stromal cells. Matrix metalloproteinases (MMPs) are one of the most important factors involved in degradation and remodeling of the ECM, which in turn affect many aspects of tumor development such as cellular interactions and cell dissemination [5]. Another important factor controlling ECM properties in cancer is heparanase, which regulates the integrity of heparan sulfates [60].

ECM-derived molecules modulate the properties of the different tumor-resident cell types. For example cancer cell-derived mucins lead to the induction of the tumor-promoter COX2 in stromal cells [61]. The engagement of ECM receptors, e.g. integrins, also controls the production of tumor-promoting factors. Integrin occupancy affects the epithelial cancer cell phenotype by regulating E-cadherin levels [62, 63]. Furthermore, ECM proteins, such as TGFBI, can enhance the metastatic potential of colon cancer cells by promoting their extravasation [64].

A previously unrecognized but important tumor-promoting property of the ECM is its regulation of tumor stroma stiffness. Increased stiffness induces tension forces leading also to integrin-dependent signaling and cytoskeletal remodeling which enhance cell motility and migration [65].

Finally, the ECM also influences the bioavailability of secreted factors e.g. by serving as a storage reservoir of growth factors [66].

Mechanisms by Which the Tumor Microenvironment Promotes Cancer

It is now well-established that non-malignant cells of the microenvironment provide malignant cells with a set of growth factors, chemokines, and integrin ligands which stimulate cancer cell growth, migration and invasion. Similarly, the non-malignant cells are a rich source of pro-angiogenic factors. More recently it has been recognized that the tumor stroma is also critically involved in buffering the acidic tumor microenvironment and influences drug uptake and sensitivity. Two general aspects of the interplay between the microenvironment and the malignant cells merits to be highlighted: (1) interactions between cell types occur in a reciprocal manner and (2) individual mediators are involved in many different processes.

The paracrine PDGF signaling is a clear example of the reciprocal interaction between cancer cells and stromal cells [67–69]. Whereas the PDGF-receptors α and β are predominantly expressed on fibroblasts and pericytes, cancer and endothelial cells express the corresponding ligands. PDGF ligands, derived from the malignant cells thus leads to the activation of fibroblasts and pericytes that in turn respond by secreting cancer-promoting molecules such as bFGF and HGF [70, 71]. A similar reciprocal interaction has also been described for the hedgehog signaling pathway [72].

An example of a paracrine mediator with multiple and complex functions is TGF- β . In the case of CAFs this factor predominantly acts as a stimulator, whereas it acts as a growth inhibitor for most epithelial cells, but also in some situations as an inducer of epithelial-mesenchymal transition [73]. Furthermore, the complexity of this factor is illustrated by findings that indicate that endothelial cells will show different responses to TGF- β -dependent on the profiles of TGF- β type I and type II receptors that are expressed [74].

The following paragraphs, focusing on more recent findings in the context of GI cancers, introduce some mechanism by which the tumor stroma contributes to the establishment of a tumor-permissive environment.

Signals Stimulating Cancer Cell Growth, Migration, Invasion and Angiogenesis

The different cell types in the microenvironment become stimulated by cancer cell-produced factors such as TGF- β , EGF, TGF- α , PDGFs, FGFs, IGFs, shh and MMPs [69, 72, 75–79]. In turn, stromal cells respond by producing growth factors (e.g. HGF, EGF, VEGF-A, bFGF), chemokines (e.g. CXCL-8; CXCL-12), cytokines (e.g. IL-6, IL-11), proteases (e.g. adam9; MMP, MT-MMP, legumain) and adhesion molecules like integrin 4 [40, 71, 80–82]. These proteins shift cells in an activated state, favor proliferation and

angiogenesis, protect from cell death, support substrate independent growth and contribute to ECM remodeling. They also mediate the recruitment of inflammatory and precursor cells, induce EMT and stimulate invasion and metastasis of cancer cells (Fig. 1) [83–88].

Likewise the growth of metastatic tumors is supported by the microenvironment. For instance, CAFs established from colon-derived liver metastases express the cancer cell growth promoting factors cyclooxygenase-2 (COX2) and TGF- β 2 [89].

Many of these cancer-stimulatory pathways controlled by the tumor microenvironment are also discussed in more detail in other recent reviews [8, 9, 22, 23].

The Buffering Capacity of the Tumor Microenvironment

During the course of tumorigenesis the accelerated growth of tumor-residing cells leads to the formation of a hypoxic microenvironment. This condition induces the immigration of vessels into the tumor. But it also forces tumor cells to use alternate, anaerobic metabolic pathways with the consequence of creating an acidic microenvironment.

A recent study by Koukourakis et al. (2006) demonstrated that CAFs display high expression of multiple proteins involved in buffering of the acidic microenvironment produced by the glycolytic malignant cells [90]. Furthermore, the stromal cells use alternative metabolic pathways and thereby contribute to the formation of a “harmoniously collaborating metabolic domain”. Interestingly, these metabolic differences occurred together with similar cell signaling pathway profiles of colon cancer and stromal cells [91].

The Contribution of the Microenvironment to Epithelial-Mesenchymal-Transition

A feature that is frequently seen in colon and other types of cancer is EMT. The most typical feature of EMT is the reduction of E-cadherin protein, caused e.g. by up-regulation of several EMT-promoting transcription factors (Snail1 and 2, TWIST, Zeb1) [92]. Nearly all colon tumors possess a deregulated β -catenin signaling pathway [86, 93]. Detection of an EMT phenotype or nuclear β -catenin accumulation is commonly observed in different carcinomas particularly at the invasive front where stromal cells and parenchymal cells interact [94, 95].

There is growing evidence that the microenvironment is a key player in stimulating EMT thereby enhancing the invasive properties of cancer. This occurs by activation of various signaling pathways such as tyrosine kinase, integrin, Wnt/ β -catenin and TGF- β which control EMT [96]. Conversely, inhibition of phosphatases like SHP2, LAR and PTEN is associated with TGF- β -stimulated EMT [97]. Furthermore,

many inflammation-derived factors have also been implicated in EMT through mechanisms that involve stabilization or activation of NF- κ B and COX2 [98–101].

The Impact of the Tumor Microenvironment on Drug-Uptake and -Sensitivity

Recent research activity has established that the tumor stroma influences drug-uptake and also affects the intrinsic drug sensitivity of malignant cells [102]. Mechanisms whereby the microenvironment affects drug sensitivity include the production of paracrine-acting factors, as well as modulation of cancer cell adhesion through a tumor-specific ECM (as described above).

The particular composition and physical properties of the tumor microenvironment can limit drug-uptake e.g. through an increased interstitial fluid pressure and by presenting a dysfunctional vasculature [103]. Experimental studies have demonstrated that normalization of the tumor vasculature as well as reduction of the interstitial fluid pressure, represents therapeutic opportunities for improving drug delivery [104–108]. Most recently it was also demonstrated that targeting of stromal hedgehog-signaling improved gemcitabine-uptake in a ras-dependent mouse model of pancreas cancer [109].

Novel Therapeutic Approaches Targeting the Microenvironment

The tumor microenvironment is beginning to attract attention as a therapeutic area for mainly three interrelated reasons. First, as discussed above, studies from model systems suggest that the tumor microenvironment plays an essential role in stimulation of tumor growth and progression. Secondly, also emphasized in previous parts, the tumor stroma controls tumor drug-uptake and-sensitivity. Thirdly, data derived from analyses of human tumor tissue demonstrate prognostic impact of stromal markers, supporting the clinical relevance of the over-all notion of the tumor microenvironment as a clinically relevant component of cancer disease [110–114].

The following paragraph gives some examples of studies introducing the microenvironment as a promising target area for therapeutic intervention. For some other relevant topics, such as targeting pro-inflammatory cytokines or chemokines the reader is referred to Schwarz and Wells (2008) [115].

Approved Targeted Drugs Impacting on Tumor Stroma

During the last decade the notion of targeted therapies has been clinically validated. Approximately 15 drugs are now approved that act by, more or less specific, targeting of growth factors, their receptors or down-stream signaling

molecules, e.g. mTOR. It is noteworthy in the context of a discussion of the tumor microenvironment that among these are already drugs that act exclusively or partially by targeting the microenvironment.

Bevacizumab is a monoclonal antibody that binds to VEGF and thereby prevents VEGF receptor signaling, and which is now, based on a set of phase III studies, approved for many common solid tumors including colorectal cancer [116–118]. Although there is still a debate whether the therapeutic effects occur predominantly by “anti-angiogenesis” or “vessel normalization” the use of this drug is a solid example of a therapy that acts by targeting the tumor microenvironment.

Sorafenib and sunitinib belong to the sub-group of multi-kinase-inhibitors and have both shown efficacy e.g. in phase III studies of renal cell cancer [119, 120]. Both drugs block multiple kinases including VEGF- and PDGF-receptors. It is likely that targeting of these receptors, which are expressed predominantly on endothelial cells and CAFs/ pericytes, respectively, contributes to the therapeutic effects of these drugs e.g. in renal cancer.

Finally, anti-stromal effects are also possibly involved in the action of the two mTOR inhibitors, everolimus and temsirolimus, which have shown their efficacy in phase III studies [121, 122].

Experimental Approaches Targeting CAFs

The evidence that CAFs provide signals critical for tumor growth and progression have prompted studies that have tested if prevention of CAF recruitment or expansion could act as novel therapeutic strategies. These studies have relied on targeting of e.g. TGF-beta, PDGF or hedgehog, which all are growth factors implied in CAF growth.

Studies with different types of PDGF inhibitors have demonstrated anti-tumoral effects of such drugs in orthotopic colorectal cancer models as well as in a genetic model of HPV-associated cervical cancer [123, 124]. In the latter study inhibition of PDGF receptor-dependent production of angiogenic factors by CAFs was identified as a major mechanism of action, in addition to general effects on CAF recruitment.

A recent study has also demonstrated potent anti-tumoral effects following prevention of hedgehog-signaling through the fibroblast compartment of experimental tumors, including models of colorectal cancer [72].

Experimental Cell Impermeable Pro-Drugs

The development of cell-impermeable pro-drugs is a novel approach using the fact that certain proteases are highly enriched in the tumor microenvironment compared to normal tissue. The peptide-linked chemotherapeutics become released upon proteolytic cleavage and act on tumor

and stromal cells at the site of processing. Importantly, the tumor-specific expression of the protease diminishes the drug-induced toxicity in other tissues.

Wu et al. demonstrated the benefit of such an initiative by creating a cell-impermeable doxorubicin-based pro-drug [125]. Doxorubicin becomes released by the action of the protease legumain that was shown to be secreted by tumor-associated macrophages and endothelial cells and accumulates in the ECM. The pro-drug blocked efficiently the growth of several subcutaneously growing cancer cell lines even when doxorubicin alone was not effective. Another study demonstrated also potent antitumor activity using a protease-activated pro-drug in xenograft models [38]. Here the activation of the drug is putatively mediated by tumor-infiltrating neutrophils or macrophages. Moreover, when an anti-angiogenic antibody was used together with this pro-drug the anti-tumoral effects of the latter were potentiated in a xenograft model of colon cancer [38].

Experimental Immunotherapy

Stimulating the host immune system against tumors by either raising CD8+ T killer cells or activating CD4+ T cells has been shown to be another promising therapeutic approach, at least in animal settings. Intratumoral injection of an adenoviral vector expressing CCL17 induced the recruitment of macrophages and CD8+ T cells and slowed the progressing growth of established murine colon carcinoma [126].

The efficacy of targeting CAFs using a DNA vaccine was elegantly demonstrated by Loeffler et al. [127]. The vaccine was directed against the fibroblast activation protein (FAP) which is overexpressed by CAFs. Importantly, this approach was effective in suppressing colon cancer cell growth in xenografts and also enhanced drug uptake. A similar approach using an anti murine PDGFR- β DNA vaccine showed also anti-tumor effects by interfering with pericytes and thus inhibiting angiogenesis [128].

Also, immunotherapy towards tumor-promoting and abundant constituents of the extracellular matrix like MUC1 has also shown to effectively suppress orthotopic and metastatic tumor growth [129].

The use of Combinatorial Approaches

Clinical experience and experimental studies convincingly demonstrate the benefit of combination treatments. The microenvironment-targeting drugs are therefore also likely to ultimately find their best use in combination with other drugs. Some experimental studies have also recently been done in GI cancer models to support this notion [130].

Juan et al. (2009) observed in a colon cancer xenograft model a potentiated anti-tumoral effect when an anti-angiogenic antibody was used together with the prodrug

9-aminocamptothecin glucuronide [38]. The tyrosine kinase inhibitor Imatinib also showed strong combination effects with irinotecan when used in an orthotopic colon cancer model with a PDGF-receptor-dependent tumor stroma [123]. Combination effects were also observed with regard to the incidence of lymph node metastases. In another study three drugs-lenalidomide, sunitinib and low-dose metronomic cyclophosphamide, were selected to target the inflammatory component, the vasculature and the epithelial compartment and potent anti-tumoral effects of the combination were observed [131].

Future Perspectives

In a tumor biology context there are a number of emerging areas where studies on the microenvironment will make significant progress during the upcoming years.

Most studies on interactions between the microenvironment and the malignant cells still focus on the growth and metastasis properties of mature primary tumors. It is predicted that future studies will shift towards analyses of the very early stages of tumor formation, including analyses of microenvironment-derived barrier-and support-functions on cancer stem cells. Similarly, novel studies on the role of local inflammatory and mesenchymal cells in the formation of the proposed pre-metastatic niche are expected [132]. Some early studies already indicate the potential of such analyses [133, 134]. Possibly, these future studies will be able to take advantage of novel systems biology approaches as a supplement to the now dominating paradigm where complex processes are explained in terms of action(s) of single, or few, individual signaling molecules.

The notion that primary tumors exert important systemic effects is receiving renewed attention. Much of this interest is derived from the many studies that suggest a bone-marrow-derived origin of many of the tumor cell types. Other related studies have also made direct links between primary-tumor-derived hypoxia-induced factors, such as lysyl oxidases, which are produced in the primary tumor but exert their pro-metastatic effects by priming distant organs [135]. Future studies are thus awaited that explore how the microenvironment modulates this type of signaling, and also investigate to what extent the microenvironment acts as a direct source of these systemically acting molecules.

Translational studies on the prognostic and response-predictive significance of stroma-derived markers are likely to continue to produce novel findings. Some recent studies, pointing to new opportunities, are studies in breast cancer which demonstrated prognostic and response-predictive relevance of stroma-derived gene signatures [136, 137]. Studies in this area are likely to benefit from an improved "taxonomy" of the cellular constituents of the

microenvironments. Whereas the classification of cells of the immune system has made lots of progress the last decade, the vascular cells and the CAFs still remain very poorly defined. Identification of functionally relevant subsets of these cells is therefore highly warranted.

Novel drugs that act by targeting the microenvironment will hopefully be introduced in the near future. As outlined above, the endothelial cells are now established drug targets through the approval of e.g. bevacizumab. The next years will eventually bring to oncology practice novel drugs targeting immune cells or CAFs. Some categories of drugs in this field where tempered optimism can be suggested include e.g. various inhibitors targeting chemokine, cytokine, hedgehog, PDGF and TGF- β signaling [68, 115, 138, 139]. Similar hopes can be maintained also for the continued development of drugs targeting ECM remodeling, such as heparanase inhibitors, and the integrin antagonists that are under development [60, 140].

Finally, to complete the circle of this overview with a connection to its beginning, it can be speculated that the exploitation of the tumor microenvironment will not only occur through therapeutic intervention with pro-tumorigenic signals, but also through cancer preventive efforts involving support of the barrier-functions of the normal microenvironment.

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