

Tumor Microenvironment: A Review

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Abstract

The microenvironment of a tumor is an integral part of its physiology, structure, and function. It is an essential aspect of the tumor proper, since it supplies a nurturing environment for the malignant process. A fundamental deranged relationship between tumor and stromal cells is essential for tumor cell growth, progression, and development of life threatening metastasis. Non-malignant cells and secreted proteins from tumor and stromal cells are active participants in cancer progression. A growing number of studies demonstrate a positive correlation between angiogenesis, carcinoma-associated fibroblasts, and inflammatory infiltrating cells and poor outcome, thereby emphasizing the clinical relevance of the tumor microenvironment to aggressive tumor progression. Improved understanding of this interaction may provide new and valuable clinical targets for cancer management, as well as risk assessment and prevention. Thus, the dynamic and reciprocal interactions between tumor cells and cells of the tumor microenvironment orchestrate events critical to tumor evolution toward metastasis, and many cellular and molecular elements of the microenvironment are emerging as attractive targets for therapeutic strategies.

Keywords: Tumor microenvironment, Extracellular matrix proteins, matrix metalloproteinases, Angiogenesis, Metastasis.

The complex tissue comprising of extracellular matrix, activated fibroblasts, immune cells, pericytes, adipocytes, epithelial cells, glial cells and vascular and lymphatic endothelial cells and numerous proteins, surrounding a tumor, collectively referred to as the tumor microenvironment. For tumors to become a life threatening entity, they must possess four properties; their ability to move; capacity to degrade tissue matrix (extracellular matrix); ability to survive in the blood; and ability to establish itself in a new tissue environment [1]. In this review, we will discuss the role of various cells and proteins in the tumor microenvironment playing a pivotal role in the tumor infiltration, progression and metastasis.

Component Cells of Tumor Microenvironment

Cellular component of tumor microenvironment include myofibroblasts, fibroblasts, adipocytes, endothelial cells, pericytes, dendritic cells, tumor associated macrophages, immune cells, mast cells and hematopoietic progenitor cells (Table-1).

Myofibroblasts

Myofibroblasts are activated by de novo acquisition of contractile bundles to promote cell migration and to remodel the extracellular matrix (ECM); and secondly, in the presence of mechanical stress and transforming growth factor $\beta 1$ (TGF $\beta 1$), proto-myofibroblasts further differentiate into myofibroblasts that neo-express α -

smooth muscle actin (α -SMA) in stress fibers (Fig.1). α -SMA incorporation into stress fibers that render myofibroblasts highly contractile. Upon injury and loss of tissue homeostasis, inflammatory signals gradually increase extracellular matrix stiffness. Transforming growth factor- β 1 (TGF β 1) in conjunction with the stiff extracellular matrix stimulate proto-myofibroblasts to express and incorporate α -smooth muscle actin (α -SMA) into stress fibers, lead to further extracellular matrix contraction, thereby establishing a mechanical feedback loop. The chemical and mechanical environment created by proto-myofibroblasts and differentiated myofibroblasts support epithelial cell transformation, invasion and tumor growth by secreting matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) including MMP-1, MMP-2, MMP-3, MMP-9, MMP-13, MMP-14[1].

Fibroblasts

Fibroblasts within stroma produce a specific set of proteinases -MMP-1, -2, -3, -9, -11, -13, -14, -19 and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS-5) and tissue inhibitors of metalloproteinases TIMP-1, -2, and -3, into the extracellular space to specifically alter the environment around the tumor and affect multiple events of tumor progression[2].

Adipocytes

Adipose secretory products are collectively referred to as adipokines, considered as contributors to the negative consequences of adipose tissue expansion in cancer, cardiovascular disease, and diabetes. Adipokines such as leptin, regulate the expression and activation of MMPs; for example, it induces the MMP-13 production in glioma cells causing increased migration and tumor invasion. Human adipose tissue-derived stem cells co cultured with cancer cells produce Chemokine (C-C motif) ligand 5

(CCL5), promotes breast cancer cell invasion associated with MMP-9 activity [2].

Few recent studies associates MMPs or TIMPs to the mutual interaction between adipose tissue and the epithelium and further to cancer transformation. MMPs affect the development of the adipose tissue, which further affect the epithelium also. MMPs, especially MMP-3, affect the rate of adipocyte differentiation during mammary gland remodeling during post-lactational involution, when there is programmed cell death of the secretory epithelium takes place along with the repopulation of the mammary fat pad with adipocytes. Fibroblastic adipogenic progenitor cells release very low levels of MMPs or TIMPs. The transcription of a number of MMP and TIMP mRNAs (MMP-2, -3, -13, and -14 and TIMP-1, -2, and -3) takes place in committed preadipocytes. Differentiated adipocytes secrete activated MMP-2[3]. MMP-14 also promotes coordination of adipocyte differentiation, as a 3D-specific modulator of adipogenesis by proteolytically controlling pericellular collagen rigidity [2]. Findings of recent studies have reported a new member of the adipocyte identified as “secretome” (Wdnl1-like family) that enhance MMP-2 activity. Wdnl1-like (a member of the whey acidic protein/four disulfide core [WAP/4-DSC] family, a gene controlling differentiation, in white and brown adipogenesis) participate in remodeling of the extracellular environment in adipogenesis, and in tumor microenvironment where adipocytes acting as a key component of stroma.

Till now, only MMP expressed by adipose tissue, directly affecting cancer progression is MMP-11. MMP-11 expression invades the surrounding environment and prevents adipogenesis by decreasing preadipocyte differentiation and revert back mature adipocyte differentiation. Adipocyte dedifferentiation cause

accumulation of nonmalignant peritumoral fibroblast-like cells, which promote cancer cell survival and tumor progression [2].

Endothelial Cells

Tumor-associated endothelial cells play a very important role in tumor development and progression. On one side, they form tumor-associated (angiogenic) vessels through sprouting from locally preexisting vessels or recruitment of bone marrow-derived endothelial progenitor cells, to provide nutritional support to the growing tumor and on the other side, they form interface between circulating blood cells, tumor cells and the extracellular matrix. Hence playing a key role in controlling leukocyte recruitment, tumor cell behavior and metastasis. Hypoxia is a vital parameter to modify the tumor microenvironment and endothelial/tumor cell interactions. Tumor cells release factors that encourage tumor angiogenesis, tumor cell motility and metastasis under hypoxic stress,. Out of these factors, vascular endothelial growth factor (VEGF), is a main angiogenic modulator and have a significant role in control of immune tolerance [4]. Endothelial cells also release few proteases and inhibitors in the tumor microenvironment to control tumor progression. These are MMP-2, -3, -7, -14,-19 and ADAM-15, -17, TIMP-1 and -2 respectively [2].

Pericytes

Pericytes play role directly in the pathogenesis of vascular driven diseases, for example, diabetic retinopathy and neoplasia. Tumor vessels are patently abnormal. They are dilated, tortuous, and hyperpermeable with high interstitial fluid pressure due to global environmental abnormalities in the signaling molecules controlling angiogenesis. The role of pericytes in tumors is unclear. Potentially, pericytes may stabilize blood vessels, reduce endothelial proliferation, maintain capillary diameter, control the blood flow, and endow with endothelial

survival signals through heterotypic contacts and soluble factors [5]. Abnormalities in these cells probably contribute to the relatively poor vascularity and high interstitial fluid pressure in tumors.

Dendritic Cells

Human myeloid dendritic cells (DCs) are the chief producers of interleukin -12 (IL-12). This assist in suppression of tumor neoangiogenesis by myeloid DCs [6], which is required for tumor growth and metastasis [7]. It has been reported that the tumor environment shows the presence of angiogenesis stimulatory DCs, such as plasmacytoid DCs but devoid of angiogenesis-inhibitory myeloid DCs [8]. The reason could be the tumor-derived chemokine (C-X-C motif) ligand 12 (CXCL12), which attracts and protects plasmacytoid DCs in the tumor microenvironment (Fig.2). These cells are competent of inducing vascularization by producing tumor necrosis factor- α (TNF- α) and IL-8[9].

Coukos et al in 2004 reported that vascular DCs are recruited by β -defensin, and induce vasculogenesis under the influence of VEGF in mice. Vascular DCs are also found in ovarian tumors [8]. Hence, DCs are responsible for tumor immune-pathogenesis, and tumor vascularization. Simultaneous accumulation of vascular DCs and absence of antiangiogenic myeloid DCs are required for optimal vascularization of tumors, as demonstrated in ovarian tumours. It has been reported recently, that tumor vascularization and growth are also mediated by DC precursors (Fig.2). These cells are attracted to the tumor site by β -defensins and due to the influence of VEGF-A, they differentiate into endothelial-like cells to express both DC and endothelial cell markers [8]. Dendritic cells of tumor stroma, also have a role in tumor progression and metastasis by secreting proteases like MMP-1, -2, -3, -9 and -19 and their inhibitors like TIMP-1 and TIMP-2[2].

Tumor Associated Macrophages

Within the microenvironment of a tumor, TAMs play a major role in tumor cell development, survival and growth. TAMs are a heterogeneous cell population depending upon oxygen availability (hypoxia vs. normoxia) and tumor progression (small vs advanced tumors) [4, 5]; they are M1 macrophages and M2 macrophages. In early stage of tumor development Type 1 macrophages (M1) infiltrate, get activated in response to inflammatory mediators, and release pro-inflammatory cytokines and chemokines, such as CXCL19 and CXCL10, in order to attract and encourage helper T lymphocyte 1 (Th1), Th17 and natural killer (NK) cell development and differentiation (Fig.3). On contrary, in more advanced tumors or in hypoxic areas of the tumor microenvironment TAMs differentiate more to type 2 macrophage(M2) cell, releasing factors to encourage Th2 differentiation and recruitment [5]. M2-like TAMs express a distinct set of cytokines and chemokines including CCL17, CCL22 and CCL24 favoring regulatory T cell (Treg) recruitment and development. These TAMs cells can be pro-inflammatory with M1-type cells promoting cell growth and recruitment through the production of chemokines and cytokines such as IL-6, TNF- α , IL-23 and IL-12. On the other hand, promote tumor development and immune-regulation with M2-like TAMs inhibiting anti-cancer immunity through the production of transforming growth factor β 1 (TGF- β 1) and IL-10 [5]. Other proteases secreted by tumor associated macrophages in the microenvironment include MMP-1, -2, -7, -9, -12, -14, ADAM-9, -15, -17 and ADAM -4 and inhibitors include TIMP-1, -2, -3 which are involved in tumor progression[2].

Immune Cells

Chronic inflammation, may promote the growth and progression of cancer. Within the immune system,

cytotoxic CD8+ and CD4+ Th1 cells, and their released cytokine interferon- γ (IFN- γ), function as the major anti-tumor immune effector cells, whereas tumor associated macrophages (TAM) or myeloid-derived suppressive cells (MDSC) and their derived cytokines IL-6, TNF, IL-1B and IL-23 act as dominant tumor-promoting forces (Fig.4). However, the exact roles played by Th17 cells and CD4+, CD25+ and Foxp3+ regulatory T lymphocytes and immunoregulatory cytokines such as TGF- β in tumor development and survival is still not very clear. These immune cells and the cellular factors produced by them, such as immunosuppressive and inflammatory cytokines, act as both promoting or discouraging factor in cancer development [5]. The infiltration of neutrophils in the tumor stroma usually associates with poor prognosis [9]. Neutrophils,, can judge the concentration gradient of chemokines such as CXCL1, a homolog of CXCL8 in mice, forms complexes with the heparin sulfate proteoglycan and syndecan-1, on interstitial cell surfaces. MMPs coordinate the recruitment of leukocytes as an essential component of tumor-associated inflammation. Such as MMP-7 play a role in indirectly modulating the bioactivity of CXCL1 by dissociating syndecan-1 from cell surfaces and releasing chemotactic complexes of syndecan-1 and CXCL1 [10]. This effectively leads to the formation of a concentration gradient of soluble chemotactic CXCL1-syndecan-1 complexes (Fig.5). In a similar way, N-terminal processing of neutrophil-attracting CXCL8/interleukin-8 (IL-8) released by MMP-9 cause 10-fold rise in chemotactic activity of neutrophils compared to full length CXCL8. The classical function of MMPs, the degradation of ECM, may have secondary role on the immune system, because some of the proteolytic fragments of MMP-processed ECM components possess chemotactic properties. Loss-of-function or mutations of MMP-8 have demonstrated to contribute to increased

susceptibility of skin adenocarcinoma and melanoma formation in humans [11]. Expression of MMP-8 in tumor cells, also involved in tightening their adhesion to the ECM and therefore may directly prevent metastatic behavior [12].

Mast Cells

Mast cells support tumor development by: (a) imbalancing the normal stroma-epithelium communication (b) promotes tumor angiogenesis (c) secreting growth factors and (d) causing a state of immunosuppression [13]. Mast cells migrates and accumulates to the sites of tumor growth in response to several chemoattractants such as regulated and normal T cell expressed and secreted (RANTES) or monocyte chemotactic protein-1 (MCP-1), are usually associated with poor prognosis [14, 15]. They have been constantly associated with tumor angiogenesis [16] through heparin-like molecules that also promote metastasis due to their anti-clotting properties. They also secrete IL-8 which act as an angiogenesis factor, a tumor cell chemotactic factor and a tumor mitogen [17]. Mast cells release some growth factors, such as epidermal growth factor (EGF), platelet derived growth factor (PDGF), nerve growth factor (NGF), and stem cell factor (SCF) [18]. Mast cells along with stress, are also able to disrupt the blood-brain-barrier (BBB) promoting brain metastases [19]. For the progression of tumor, mast cells within the tumor microenvironment, also release certain kinds of proteases and protease inhibitors. The proteases include MMP-2 and -9, chymase and tryptase where as inhibitors include TIMP-1[2].

Hematopoietic Progenitor Cells

Vascular endothelial cell growth factor receptor-1+ (VEGFR-1+) hematopoietic progenitor cells (HPCs) are closely associated with forming vasculature. It has been reported that VEGFR-1+ HPCs provide vessel stability and promote tumor progression. HPCs promote

angiogenesis is by paracrine release of angiogenic factors, which enhance the recruitment and incorporation of endothelial progenitor cells (EPC) to new tumor vessels [20]. Activated HPCs also release angiogenic factors such as VEGF-A, PDGF, angiopoietins, and brain-derived neurotrophic factor (BDNF), which enhance new vessel formation and stability [21]. In addition, these cells express proteases and inhibitors, released into the extracellular space and influence multiple events of tumor progression. These proteases are MMP-2, -9 and -14 and inhibitors include TIMP-1, -2 [2].

Extracellular Matrix Proteins

Extracellular matrix proteins present in the tumoral microenvironment include Cadherins, Hepatocyte growth factor (HGF), Transforming growth factor- β (TGF- β), Osteopontin, Galectin, Fibulin, Matrix metalloproteinases (MMPs), Platelet derived growth factor (PDGF), Fibronectin, Laminin, and Collagen IV (Table-2).

Cadherins

Cadherins are transmembrane glycoproteins that play a major role in morphogenesis and maintenance of a differentiated phenotype. Selectins belong to this group, also play a role in cellular adhesion in the metastatic cascade. E-Cadherins function in cell-to-cell binding and are bound intracellularly to the actin cytoskeleton of cells via proteins known as β catenins. Disregulation of E-cadherin is associated with increased invasiveness of cells. The ability of a neoplasm to metastasize is inversely proportional to the expression of E-cadherin. Changes in β -catenin also affect E-cadherin function, with loss of cohesiveness when β -catenin expression is reduced. Hence, Collectively the loss of both, the cadherins and β -catenins has been associated with distant metastasis, poor prognosis and reduced survival in patients with malignancies [22].

Hepatocyte Growth Factor

Hepatocyte growth factor (HGF) is a mitogen for epithelial cells that controls cell proliferation, migration, survival, tumor angiogenesis and invasiveness. It is a pleiotropic cytokine that acts on epithelial cells in several organs. Many studies have demonstrated that HGF plays an important role as a paracrine factor in the invasion and metastasis of oral squamous cell carcinoma (OSCC). Hence, an elevated HGF serum level may be a predictive marker for metastasis in these patients. HGF is also a potent mitogen for various tumor cell types and potentially stimulates tumor invasion and metastasis. HGF has also been identified as a fibroblast-derived epithelial morphogen, inducing branching tubular morphogenesis. There is autocrine HGF expression by the tumor cell itself, stromal (fibroblast) derived HGF also act in a paracrine manner, playing a major role in tumor invasiveness and metastasis. HGF expression by fibroblasts is regulated by malignant epithelial cells, for example, cytokine IL-1, TNF and fibroblast growth factor (FGF) secreted by tumor cells can upregulate HGF expression by fibroblasts, thereby leading to the attainment of invasive growth potential by the tumor cells [23]. HGF, also referred as scatter factor, as it promotes cell motility through tyrosine kinase. It dissociates sheets of epithelium into individual cells [22].

Transforming Growth Factor Beta

TGF- β is a secreted protein that plays a dual role in regulating cell proliferation and eliciting normal transformation of fibroblasts. It is a pluripotent cytokine that inhibit tumor growth at the early stage but also promote advanced tumor cell invasiveness and metastasis at the later stage. It is expressed through receptor serine/threonine kinases and the intracellular Smad. TGF- β inhibits proliferation and induces apoptosis in diverse cell types, and the accumulation of loss-of-function

mutations in the TGF- β receptor or Smad genes in various human cancers classify its pathway as a tumor suppressor in humans. TGF- β promotes tumor cell survival, invasiveness and metastasis by targeting fibroblasts, myofibroblasts, and immune cells in the tumor microenvironment [24]. As, the tumor advances in malignant progression, the genome often accumulates mutations in the TGF- β receptor system which turn the cancer cells unresponsive to TGF- β . It affect nonmalignant stromal cells in multiple way, such as evasion of immune surveillance, exploited by tumor and therefore, turning TGF- β into a tumor-promoting factor that leads to increased invasion and metastasis. TGF- β is activated from an inactive pro-form by proteolytic conversion by furin or other proteinases, such as MMP-9, expressed by inflammatory cells. [26]. Similarly, MMP-14 and MMP-2 activate TGF- β 1 proteolytically [2]. MMP-2 and MMP-9, as well as MMP-14, also indirectly alter TGF- β bioactivity by cleaving the ECM component latent TGF- β -binding protein 1 (LTBP-1), therefore solubilizing ECM-bound TGF- β [28]. Tumor cells acquire nonresponsiveness to TGF- β , indicating proteolytic activation of TGF- β by MMPs has tumor-promoting effects by selectively modulating stroma-mediated invasion and metastasis of the tumor.

Osteopontin

Osteopontin (OPN) is a glycoposphoprotein, involved in physiologic and pathologic processes. It plays a role in various developmental processes, tissue differentiation and in wound repair. OPN is over expressed in many human malignancies and is related with metastasis and poor prognosis in breast cancer patients. The mechanism affecting tumor aggressiveness are not yet completely understood, but several reports indicate that it is involved in processes favouring malignancy such as increased cellular migration and invasion behaviours increased

metastasis, protection from apoptosis, and induction of tumor-associated inflammatory cells. Experimental studies have demonstrated that OPN interact with several factors such as cell surface receptors like integrins and CD44, secreted metalloproteinase inducer, and growth factor/receptor pathways. The interactions of OPN with these factors are the evidence of its role in favouring malignancy [24].

Galectins

The galectins belong to a family of beta-galactoside-binding proteins, characterized by their affinity for beta-galactosides and binds to the carbohydrate portion of cell surface glycoproteins or glycolipids. Galectin-3 is a multifunctional protein, localized and functions in the cytoplasm, cell membrane, nucleus, and the extracellular environment. Galectin-3 expression is associated with neoplastic transformation and progression toward metastasis in breast, colon, stomach and thyroid whereas its down-regulation indicated a considerable decrease in the metastatic potential.

Constantly, galectin-3 binding protein (90 K) has been reported to be strongly expressed in the aggressive cell lines and not or less expressed in the non-tumor cell line of the breast cancer. Its expression is also found to be significant in the secretome of three unrelated cancer cell lines, in breast cancer, melanoma and osteosarcoma [25].

Fibulin-1

Glycoprotein fibulin-1 (FBLN-1), is a cysteine-rich, calcium-binding extracellular matrix and plasma molecule, is abnormally over transcribed in epithelial tumor cell lines and breast surgical specimens. Amplified expression of FBLN-1 has been also demonstrated in human ovarian cancer cells stimulated with estrogens, and resulting ovarian tumor cell invasion and progression. FBLN-1 gets bound to other extracellular matrix proteins, including fibronectin, laminin and nidogen, and

fibrinogen. As FBLN-1 is responsible for laminin polymerization under physiological conditions, its excess in the extracellular matrix may cause disruption of laminin polymers, further modifying the signal mediated by the interaction of tumor cells with extracellular matrix. FBLN-1 might also favour tumor cell extravasation, as this protein increase platelet adhesion by interacting with fibrinogen [26].

Hyaluronic Acid

Hyaluronic acid, an extracellular matrix polysaccharide implicated in wound healing by promoting cell migration through its cell surface receptor, is also found overexpressed in malignant tumors. The concentration of hyaluronic acid in the stroma was reported to be an independent prognostic factor for overall survival. Hyaluronic acid interacts with tumor cells through hyaluran-binding protein receptor for hyaluronic acid-mediated motility (RHAMM) by which it controls ras signaling pathway. Consequently, RHAMM expression has also been associated with lymph node metastasis [27].

Matrix Metalloproteinases

Matrix metalloproteinases (MMPs) belong to a family of zinc-dependent endopeptidases. They participate in several physiological processes including tissue remodeling and organ development, regulation of inflammatory processes, and in diseases such as cancer. MMPs, in malignancy, play a key role in the degradation and remodeling of the ECM, providing the way through the peripheral tissue for invasion and metastasis. MMPs, demonstrate significant consequences on the tumor microenvironment by affecting the growth signals; playing major role in apoptosis, angiogenesis and regulating inflammation [2] (Fig.6).

Unregulated proliferation is a hallmark of cancer cells. This is achieved by two principal ways: by acquiring self-sufficiency in growth-promoting signals or by becoming

resistant to antigrowth signals. MMPs play a major role in disrupting the balance between growth and antigrowth signals in the tumor microenvironment, by influencing the bioavailability of multiple important factors regulating growth. Recent studies have reported a potential role of a disintegrin and metalloproteinases (ADAM proteinases) in the regulation of the EGFR pathway. Such as ADAM-10 stimulates the release of soluble EGF, whereas ADAM-17 is a key converter of pro-forms of other EGFR ligands for example TGF- α and epiregulin. EGFR activation results in the upregulation of MMP-9, which in turn degrades E-cadherin, a controlling agent of many cellular functions including cell-cell adhesion and differentiation. This relation between EGFR, MMP-9, and E-cadherin play a major role in ovarian cancer and metastasis, as activated EGFR and MMP-9 in these specimens are found to be associated with a region of reduced E-cadherin. The cleavage of E-cadherin by MMPs or ADAM proteinases, promote cancer cell proliferation. ADAM-10 also mediates the shedding of E-cadherin, leading to β -catenin translocation to the nucleus, promoting cell proliferation [29].

Evasion from apoptosis, is also an approach to increase the cell numbers and size of tumors. MMPs interfere the induction of apoptosis in malignant cells, involving the cleavage of ligands or receptors that transduce proapoptotic signals [2].

The tumor vasculature is derived from sprouting of local blood vessels (angiogenesis). The major MMPs involved in tumor angiogenesis are MMP-2, -9, and -14, and to a lesser extent MMP-1 and -7 [30]. MMP-9 play a discrete role in tumor angiogenesis, by controlling the bioavailability of vascular endothelial growth factor (VEGF), which is considered to be the most potent inducer of tumor angiogenesis. MMP-9, produced by inflammatory cells, provides an angiogenic switch by

making sequestered VEGF bioavailable for its receptor VEGFR2 in pancreatic islet tumors. MMPs also control vascular stability and permeability. Particularly MMP-14, intervene the vascular response to tissue injury and tumor progression by activation of TGF- β [31].

MMP-9, also affect lymphangiogenesis, promoting dissemination of metastases into the lymph [32]. Increased expression of MMP-1, MMP-2 [33] and MMP-3 [34] is linked with lymphatic invasion and lymph node metastases. Inhibition of MMP-2, -9 and -14 attenuates both angiogenesis and lymphangiogenesis.

MMPs play a key role in acute as well as chronic inflammation [35]. They recruit inflammatory cells to the tumor microenvironment, by conversion of Tnf- α /interleukin-8, by production of chemotactic peptides from collagen proline-glycyl-proline peptide (PGP), forming chemotactic gradients by syndecan by secreting soluble gradients of CXCL1 (potent neutrophil attracting chemokine) and by dissociating monocyte chemotactic protein (MCP). TNF- α is one of the most important proinflammatory cytokines. MMPs act as TNF- α converting mediator in specific physiological or pathological conditions. Several tumors produce abundant TNF- α , promoting cancer cell survival in an NF- κ B-dependent manner [36], hence indicating that the conversion of TNF- α by MMPs and ADAM-17 is a vital step in tumor-promoting cascade.

Nonproteolytic functions of MMPs

The hemopexin domain of MMPs is responsible for nonproteolytic function of MMPs. Many members of the MMP family elicit immune or cancer cell migration by mediating chemotaxis without using their proteolytic domain. In an assay by Dufour et al. in 2008, it was demonstrated that the hemopexin domain of MMP-9 is responsible for MMP-9 mediated epithelial cell migration [37]. According to Glasheen et al, the catalytic domain is

essential for all MMP functions, the hemopexin domain is distinctively associated with tissue invasion. The hemopexin domain of MMP-12 (macrophase elastase) plays a major role in its antimicrobial function [38].

Platelet Derived Growth Factor

Platelet derived growth factors (PDGFs), directly or indirectly, stimulate the angiogenic processes. PDGF released by tumor cells directly induces migration and proliferation of endothelial cells and vascular smooth muscle cells (vSMC), suggesting their role in angiogenesis [39]. PDGFs induce transcription and secretion of VEGF (vascular endothelial growth factor) by β -PDGFR (platelet derived growth factor receptor) expressing endothelial cells, signifying an indirect role for PDGF induced angiogenesis [40]. They also intervene the paracrine signaling loop between endothelial cells and vSMC/pericytes during tumor angiogenesis. PDGFs also by act as a potent chemoattractants and mitogens for host mesenchymal cells, speculating its role in cancer metastasis at the preferred organ sites [41].

Fibronectin

Malignant cells donot deposit fibronectin into an insoluble matrix, causing lack of fibronectin. The absence of extracellular matrix, since other matrix components – collagen, laminin and heparin sulfate proteoglycan are concomitantly missing. These cellular characteristics are related with cell migration & invasive potential of malignant cells [41].

Several other extracellular matrix proteins such as laminin, fibronectin and collagen IV stimulate chemotaxis of cancer cells. [23]

Conclusion

Thus, knowledge and control of the tumor microenvironment is becoming as important as the knowledge and control of the transformed cancer cells to better understand the cancer biology and to devise novel

therapeutic approaches. The first step toward this aim is the identification of molecular elements and signaling pathways in the tumor microenvironment for each cancer type. Scientific studies summarized in this review illustrate how the tumor microenvironment contributes to cancer development and progression, as well as the role of their cells and secreted proteins to enhance possible discovery or drug target elucidation.

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List of figures

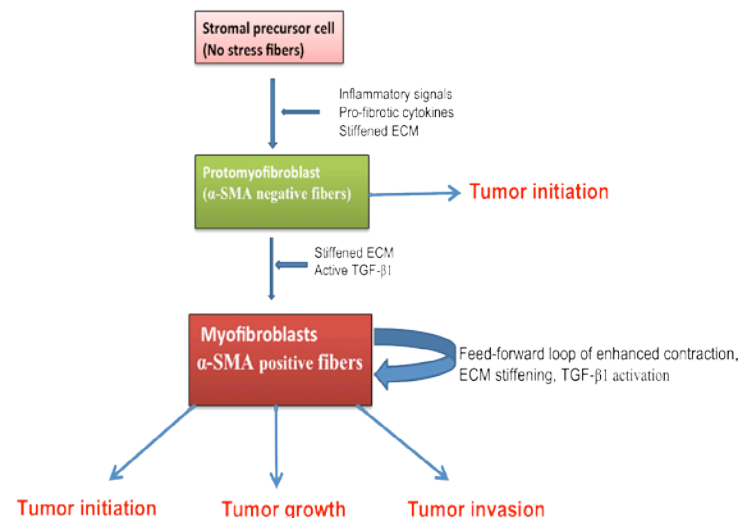


Figure 1 : Role of myofibroblasts in tumor microenvironment – inflammatory signals and pro-fibrotic cytokines lead to formation of protomyofibroblasts with α-SMA negative fibers. This may result tumor initiation directly or differentiate into myofibroblasts with α-SMA positive fibers in the presence of stiffened ECM and active TGF-β1. This further leads to tumor initiation, tumor growth and tumor invasion.

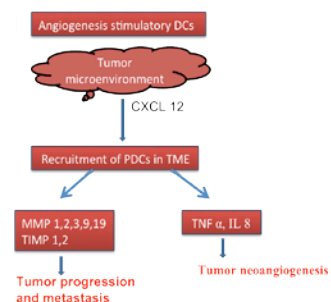


Figure 2: Dendritic cells in tumor microenvironment – plasmacytoid DCs are angiogenesis stimulatory present in

TME. CXCL12 chemokine attracts and recruit plasmacytoid DCs, which induce tumor neoangiogenesis by producing TNFα and IL8 and tumor progression and metastasis by secreting MMPs and TIMPs. Angiogenesis inhibitory myeloid DCs suppress tumor neoangiogenesis by producing IL-12.

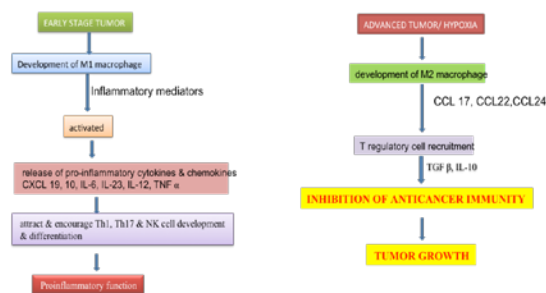


Figure 3: TAMs in tumor microenvironment – In early stage, infiltration of M1 macrophage become activated by inflammatory mediators and secrete proinflammatory cytokines lead to development and differentiation of Th1, Th17 and NK cell with proinflammatory function. In advanced tumor/hypoxia, development of M2 macrophage resulting regulatory Tcell recruitment in the presence of CCL17, 22 and 24, further inhibiting anticancer immunity and tumor growth under influence of TGF-β and IL-10.

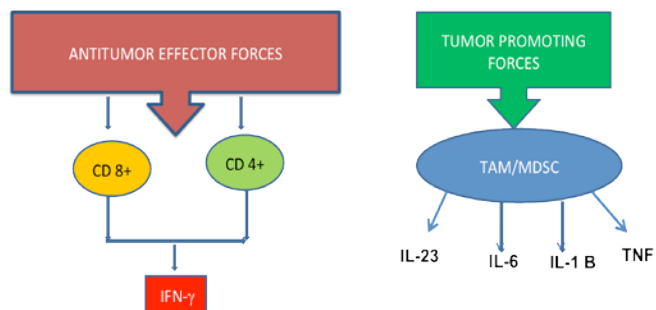


Figure 4: Immune cells in tumor microenvironment – cytotoxic CD8+ and CD4+ T helper lymphocytes along with their produced cytokine IFN-γ have antitumor immune effects and tumor associated macrophages(TAMs) or myeloid derived suppressive cell and their cytokines IL-23, IL-6, IL-1B, TNF have tumor promoting forces in the TME.

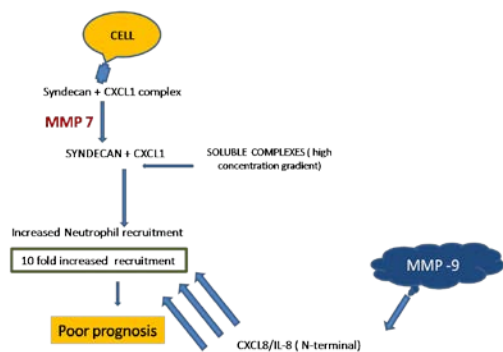


Figure 5: Neutrophils in tumor microenvironment – neutrophils, sense the concentration gradient of CXCL1 and forms complexes with syndecan-1. MMP-7 cleaves syndecan-1 and CXCL 1 which results in generation of

concentration gradient of soluble chemotactic complexes, increasing neutrophil recruitment. Neutrophil recruitment also increased 10 fold by N-terminal processing of neutrophil attracting CXCL8/IL8 by MMP-9. Increased neutrophil recruitment correlates with poor prognosis of the tumor.

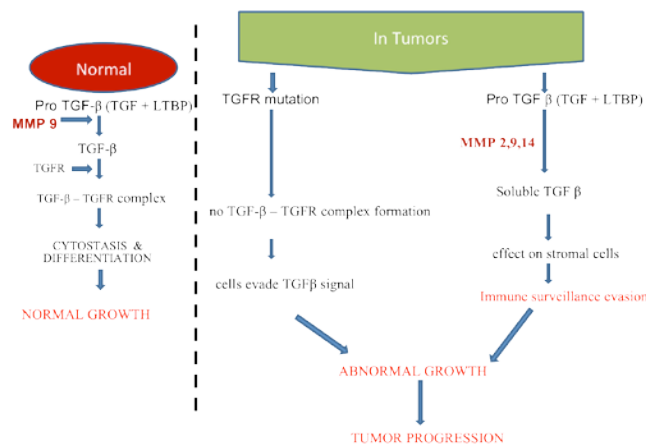


Figure 6: Role of MMPs in TGF β Pathway – In normal, growth proTGF β is converted into TGF β under the influence of MMP-9 and forms complex with TGFR resulting normal cyto-stasis and differentiation. In tumors, one pathway is through mutation in TGFR leading lack of TGF β-TGFR complex evading TGF β signal and causing abnormal growth and tumor progression. Other pathway is through proTGF β leading to formation of soluble TGF β under influence of MMP-2, 9, 14 effecting stromal cells and evasion of immune surveillance leading to abnormal growth and tumor progression.

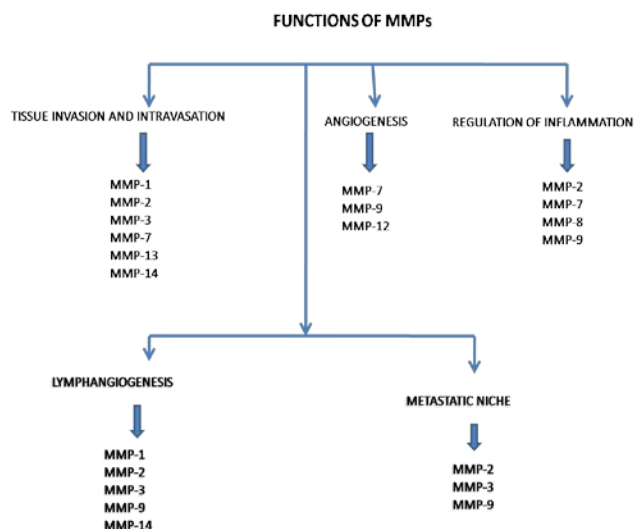


Figure 7: Role of various types of MMPs in tumor-microenvironment – For tumor invasion and intravasation MMP1, 2, 3, 7, 13 and 14, for angiogenesis MMP7, 9 and 12, for regulation of inflammation MMP2, 7, 8 and 9, for lymphangiogenesis MMP1, 2, 3, 9 and 14 and in metastatic niche MMP2, 3 and 9 are involved.

Table.1:- Role of various types of cells present in the tumor-microenvironment.

Cells	Role In TME
1. Myofibroblasts	Tumor initiation, growth and tumor invasion by incorporation of α -smooth muscle actin fibers
2. Adipocytes	Adipokines lead to increased tumor cell migration and tumor invasion by regulating expression and activation of MMPs
3. Tumor endothelial cells	A.) Leucocytes recruitment B.) Tumor cell behaviour and metastasis
4. Pericytes	A.) Stabilise blood vessels B.) Inhibit endothelial cell proliferation C.) Maintain capillary diameter D.) Regulate blood flow E.) Provide endothelial survival signals
5. Dendritic cells	A.) Induction of vascularity B.) Tumor immune-pathogenesis
6. Tumor associated macrophages	A.) Immune regulation B.) Promote cell growth C.) Promote tumor development
7. Immune cells	A. Promote growth and progression of cancer
8. Mast cells	Promote tumor development by A.) Disturbing the normal stroma-epithelial communication B.) Facilitating tumor angiogenesis C.) Releasing growth factors D.) Inducing state of immunosuppression

Table.2:- Role of various extracellular proteins present in the tumor-microenvironment.

ECM Proteins	Role in TME
1. CADHERINS	A. Increased invasiveness of cells and metastasis B. Related to reduced survival and poor prognosis
2. HEPATOCYTE GROWTH FACTOR	Regulates cell proliferation, migration, survival, tumor angiogenesis, invasiveness
3. TRANSFORMING GROWTH FACTOR β	Promotes tumor cell survival, invasiveness and metastasis by targeting fibroblasts, myofibroblast and immune cells in tumor microenvironment
4. OSTEOPONTIN	Increased cellular and invasion behaviours, protection from apoptosis, increased metastasis and induction of tumor-associated inflammatory cells
5. GALECTINS	Neoplastic transformation and progression towards metastasis in breast, colon, stomach and thyroid
6. FIBULIN	Ovarian tumor cell invasion and progression, tumor extravasation
7. HYALURONIC ACID	Lymph node metastasis