

Role of the tumor microenvironment in tumor progression and the clinical applications (Review)

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Abstract. Oncogene activation and tumor-suppressor gene inactivation are considered as the main causes driving the transformation of normal somatic cells into malignant tumor cells. Cancer cells are the driving force of tumor development and progression. Yet, cancer cells are unable to accomplish this alone. The tumor microenvironment is also considered to play an active role rather than simply acting as a by-stander in tumor progression. Through different pathways, tumor cells efficiently recruit stromal cells, which in turn, provide tumor cell growth signals, intermediate metabolites, and provide a suitable environment for tumor progression as well as metastasis. Through reciprocal communication, cancer cells and the microenvironment act in collusion leading to high proliferation and metastatic capability. Understanding the role of the tumor microenvironment in tumor progression provides us with novel approaches through which to target the tumor microenvironment for efficient anticancer treatment. In this review, we summarize the mechanisms involved in the recruitment of stromal cells by tumor cells to the primary tumor site and highlight the role of the tumor microenvironment in the regulation of tumor progression. We further discuss the potential approaches for cancer therapy.

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1. Introduction

Tumorigenesis is a complicated and multistep process, in which successive mutations in oncogenes and tumor-suppressor genes virtually result in enhanced proliferation and resistance to cell death. Most human tumor types share various hallmarks, which include sustainment of proliferative signals, evasion of growth suppressors, resistance to cell death, replicative immortality, induction of angiogenesis, activation of invasion and metastasis, energy metabolism, evasion of immune destruction, genome instability and mutation, and tumor-promoting inflammation (1,2). During the past decades of cancer research, our focus on cancer research has shifted from the malignant cancer cell itself to the tumor microenvironment and the complex interactions. The tumor microenvironment, which consists of resident fibroblasts, endothelial cells, pericytes, leukocytes and extracellular matrix, also contributes to the progression of cancer (3).

Studies have provided some evidence that human tumors are more than a mass of accumulating malignant cancer cells. Actually, tumor cells can efficiently recruit stromal cells (4), immune cells (5) and vascular cells (6) by secreting stimulatory growth factors, chemokines and cytokines. In turn, these recruited cells release growth-promoting signals and intermediate metabolites as well as remodel tissue structure to build the microenvironment. The reciprocal communication between cancer cells and the microenvironment eventually leads to enhanced proliferation and metastatic capability, and finally death.

As the tumor microenvironment actively participates in tumor progression and metastasis rather than acting as a by-stander, therapeutic strategies targeting the tumor microenvironment hold great potential. It is known that non-tumor cells are presumably and genetically more stable than tumor cells, thus, therapies targeting the tumor microenvironment are less likely to cause adaptive mutations and rapid metastasis. Yet, considering the complex interactions (stromal cells can both promote and inhibit tumor cell growth), therapies targeting the

tumor microenvironment for cancer therapy should be highly selective. Therefore, further studies must provide new insight into the tumor microenvironment for better cancer therapeutic strategies. We will review how tumor cells recruit stromal cells to the primary tumor site and build the microenvironment. Moreover, we will highlight the role of the tumor microenvironment in the regulation of tumor progression and discuss the potential value for cancer therapy.

2. Constituents of the tumor microenvironment as accomplices in tumor progression

A tumor is a highly complex tissue composed of neoplastic and stromal cells. It is widely known that stromal cells contain a variety of mesenchymal cells, particularly fibroblasts, myofibroblasts, endothelial cells, pericytes and inflammatory cells associated with the immune system. Accumulating evidence has confirmed that tumor cells must recruit and reprogram the surrounding normal cells to serve as contributors to tumor progression. Tumor cells and the supporting normal cells form an organ-like structure and make concerted efforts for rapid proliferation, local invasion and metastases. These normal cells in the tumor microenvironment mainly consist of fibroblasts, immune cells and vascular cells. These cells are recruited to the primary tumor site and build the tumor microenvironment for tumor progression in soluble paracrine signals (Fig. 1).

Fibroblasts are recruited to the tumor microenvironment. Among the supporting cells, fibroblasts represent the majority of the stromal cells in various types of human cancers. Initially, activated fibroblasts inhibit the early stages of tumor progression (7), and this effect is carried out through simple gap junctions between fibroblasts and IL-6 production (8,9). Fibroblasts can then be modulated by tumor cells and develop into cancer-associated fibroblasts (CAFs), which are identified by expression of different biomarks, such as α -smooth muscle actin, vimentin, desmin and fibroblast-activation protein. Although research has made great contributions in this field, the original source of CAFs remains controversial. CAFs are critically involved in promoting growth and angiogenesis, remodeling of the extracellular matrix (ECM) and directing cell-cell interaction (10). Clinical and experimental data indicate that tumor cells secrete a high level of transforming growth factor β (TGF- β), which is strongly chemotactic for fibroblasts and transdifferentiates fibroblasts into CAFs (11,12). The main source of CAFs is thought to be derived from normal fibroblasts through genetic alteration. It has been observed that expression of genes in fibroblasts may be altered via point mutation, loss of heterozygosis, and the number of gene copy changes. The mutation or inactivation of phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase (PTEN) and p53 is frequently detected in CAFs around the primary tumor lesion (13). However, the evidence for genetic alterations as a factor to induce CAFs is still unconvincing. Normal dermal fibroblasts can also be orchestrated indirectly dependent on immune cells by carcinoma cells to express pro-inflammatory genes (14). Except for normal fibroblasts, CAFs are thought to be generated from epithelial cells, endothelial cells and, interestingly, cancer cells (Fig. 1). The myofibroblast, an essential cell type, participates in wound healing (15)

and was also found to be a major source of CAFs (16). Laminin, which critically contributes to cell attachment and differentiation, is downregulated in myofibroblasts in cancer regions, providing an additional evidence that CAFs can be directly differentiated from myofibroblasts (17). In addition, vascular cells such as vascular smooth muscle cells show similar markers and morphology with myofibroblasts, providing another probability that CAFs may be derived from mural cells (18). Platelet-derived growth factor (PDGF) can also indirectly recruit myofibroblasts by stimulation of TGF- β release from macrophages (19). Another potential source of CAFs is human bone marrow-derived mesenchymal stem cells (hMSCs). hMSCs, which are thought to be multipotent cells, are present in adult marrow and have the potential to differentiate into lineages of mesenchymal tissues (20). Under hypoxic conditions, tumor cells secrete IL-6 and activate both Stat3 and MAPK signaling pathways to enhance the migratory potential of hMSCs (21,22). The recruited hMSCs have the potential to develop into CAFs. Notable, surrounding normal epithelial cells can be another source of CAFs by undergoing epithelial-to-mesenchymal transition (EMT) in response to stimuli from the microenvironment. A previous study reported that proliferating endothelial cells induced by TGF- β can undergo a phenotypic conversion into fibroblast-like cells (23). Another recent study confirmed that EndMT (endothelial-to-mesenchymal transition) frequently appears in a variety of cancers. Zeisberg *et al* found that endothelial cells are a source of CAFs by undergoing EndMT at the invasive front of tumors in transgenic mice (24). This suggests that EndMT is an important process for the accumulation of CAFs. Interestingly, CAFs can also be derived from cancer cells directly which shows dangerous signaling. Cancer cells are obstinate and are not eradicated easily. A previous study revealed that under the proper conditions, breast tumor cells can transdifferentiate into myoepithelial cells and finally become myofibroblasts, which are the ancestors of CAFs (25). Meanwhile, recent genetic analysis found that CAFs isolated from human breast tumor biopsies were indeed derived from epithelial cancer cells (26). However, genetic alterations present in both CAFs and cancer cells are not identical, suggesting that only a small part of stromal cells and cancer cells may share a common origin (27). Therefore, it is worthwhile to consider the consequences of tumor cell-derived CAFs in tumor progression, the indirect action of nonmalignant CAFs on associated tumor cells as a mechanism of facilitating tumor growth. The current paradigm would appear then to be that some CAFs encourage their neighbors to become more malignant rather than performing this function themselves.

Once CAFs are stimulated, they can secrete stromal cell-derived factor 1 (SDF-1), which recruits circulating endothelial progenitor cells (EPCs) into the tumor mass to stimulate angiogenesis (28). Importantly, a recent report shed new light on the roles of miRNAs in tumor microenvironment. Downregulation of miR-320 and upregulation of ETS2 (v-ets erythroblastosis virus E26 oncogene homolog 2, one of the direct targets of miR-320), were found to contribute to tumor angiogenesis and tumor-cell invasion in PTEN-deleted stromal fibroblasts (29). Another report revealed that CAFs mediate tamoxifen resistance through IL-6-induced degradation of ER- α in luminal breast cancer (30). This study demonstrated

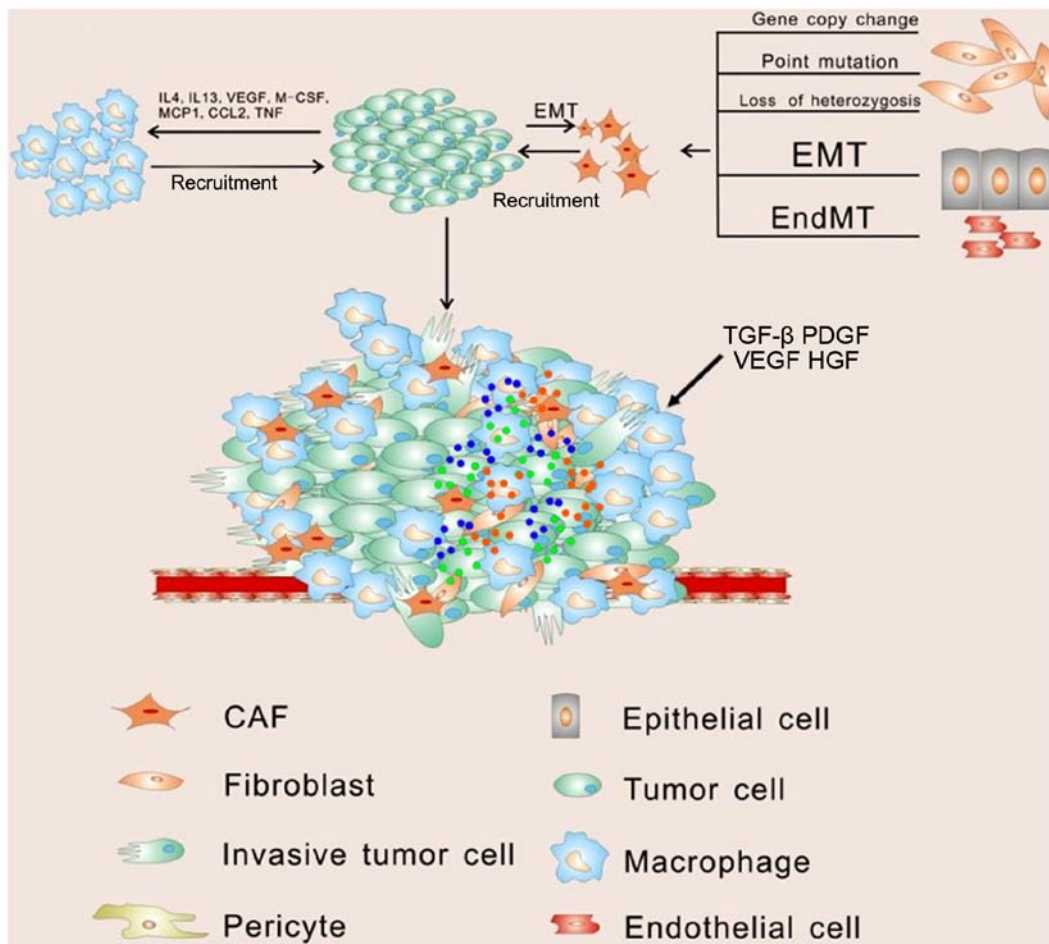


Figure 1. Formation of the tumor microenvironment. Construction of the tumor microenvironment and the detailed processes involved in the recruitment of various cell types are shown. The tumor cells recruit cancer-associated fibroblasts (CAFs), epithelial cells, fibroblasts, pericytes, macrophages and endothelial cells to the primary tumor site. VEGF, vascular endothelial growth factor; CCL2, chemokine chemokine (C-C motif) ligand 2; TNF, tumor necrosis factor; M-CSF, macrophage-colony stimulating factor; MCP-1, monocyte chemotactic protein 1; TGF-β, transforming growth factor β; PDGF, platelet-derived growth factor; HGF, hepatocyte growth factor; EMT, epithelial-to-mesenchymal transition; EndMT, endothelial-to-mesenchymal transition.

that CAFs also play a role in drug resistance. Studies designed to ascertain how CAFs provide a suitable tumor microenvironment may facilitate the development of new therapeutic strategies against tumor progression.

Immune cells are recruited to the tumor microenvironment. Oncogenic mutations and transcription factor activation induce high levels of inflammatory mediators, including cytokines and chemokines. Chemokines and cytokines are critical autocrine and paracrine factors in tumor development, which are secreted into the tumor microenvironment to recruit and activate various inflammatory cells. In turn, these 'educated' inflammatory cells produce more inflammatory signals and form a cancer-related inflammatory microenvironment to induce cancer cell evasion from immune destruction. Finally these inflammatory cells promote tumor progression. Among these immune cells, macrophages represent the majority and play leading roles in cancer-related inflammation. Macrophages can polarize into two different types of macrophages upon different stimulation. Classically activated macrophages (M1), following exposure to interferon, have antitumor activity and elicit tissue destructive reactions. However in response to IL-4 or IL-13, macrophages undergo alternative activation (M2) (31). Tumor-associated

macrophages (TAMs) closely resemble alternative (M2) macrophages, which produce high amounts of interleukin IL-10. Moreover, these cells exhibit anti-inflammatory and tissue repair functions (32). Vascular endothelial growth factor (VEGF), macrophage-colony stimulating factor (M-CSF) and monocyte chemotactic protein 1 (MCP-1) produced by tumor cells efficiently recruit macrophages to the tumor microenvironment by promoting migration and survival (33). Interestingly, a low level of MCP-1 induces modest monocyte infiltration resulting in tumor formation, whereas a high level is associated with massive monocyte/macrophage infiltration into the tumor mass, leading to tumor destruction (34). Experimental evidence suggests that signaling molecules produced by both tumor cells and these macrophages, work together to activate integrin $\alpha 4 \beta 1$, with subsequent stimulation of myeloid cells entering the tumor microenvironment (35). Among these signaling molecules, chemokine and chemokine receptors make up a complex network, which influence the development of primary tumors and metastases (36). Recent data showed that tumor cells and host organ-derived chemokine chemokine (C-C motif) ligand 2 (CCL2) recruit inflammatory monocytes, which differentiate into macrophages and facilitate efficient tumor cell metastasis seeding and growth in distant meta-

static sites of the lung (37). CCL2 can also increase prostate tumor growth and bone metastasis through macrophage and osteoclast recruitment (38). These studies have made great contributions to our understanding of the microphage recruitment to the tumor site (Fig. 1). Cancer cells that overexpress chemokine (C-X-C motif) ligand 2 (CXCL1/2) can also attract CD11b⁺Gr1⁺ myeloid cells into the primary tumor site, which produce chemokines, including S100A8/9, that enhance cancer cell survival (39). CCL21, expressed by melanoma tumors, shifts the host immune response from immunogenic to tolerogenic, and facilitates tumor progression (40). Other soluble factors, such as prostaglandin E2 (PGE2) and TGF- β , also play an active role in facilitating tumor progression by limiting natural killer (NK) cells (41). Tumor necrosis factor (TNF) signaling can drive myeloid-derived suppressor cell accumulation, and promote tumor cell escape from the immune system (42). In a spontaneous murine model of patent ductus arteriosus (PDA), Bayne *et al* demonstrated that tumor-derived granulocyte-macrophage colony-stimulating factor (GM-CSF) drives the accumulation of Gr-1⁺CD11b⁺ myeloid cells as part of the cancer-associated inflammatory reaction, which in turn suppresses antitumor T cell immunity and promotes tumor growth (43). In particular, CXCL12 can regulate cancer cell survival, proliferation and migration, and, indirectly, via angiogenesis or recruiting immune cells to affect tumor progression (44). These 'educated' immune cells work together with local tumor cells and CAFs to produce more inflammatory factors forming an inflammatory microenvironment and protecting tumor cells from immune destruction. Finally, this cooperation promotes tumor progression and metastasis. However, studies on the relationship between inflammation and cancer are sparse. Progress in the identification of inflammation-dependent mechanisms that affect tumor cell survival, trafficking and chemo-attractive functions are valuable to new drug development. Understanding of the biological and molecular mechanisms of carcinogenesis may provide more opportunities for clinical therapy.

Vascular cells are recruited to the tumor microenvironment. Tumors require the formation of a complex vascular network to meet the metabolic and nutritional needs for growth. Recent evidence suggests that endothelial cells and pericytes, which play essential roles in the 'turn on' of the angiogenic switch (45,46), can also be modulated by tumor cells. Several studies indicate that VEGF is highly expressed in a variety of human tumors, including lung (47), breast (48), ovarian (49), bladder and kidney (50). VEGF elicits a pronounced angiogenic response in a variety of *in vivo* models. VEGF directly activates enterochromaffin cells (Ecs) through mitogenic and promigratory effects, and also mobilizes endothelial progenitor cells (EPCs) from the bone marrow, modulates EPC kinetics and promotes EPC differentiation (51). Interestingly, miR-126 regulates endothelial recruitment and metastatic colonization through IGFBP2, PTPN1 and MERTK targeting (52). Protein kinase C (PKC) inhibition plays a crucial role in the extracellular signal-regulated kinase (ERK) phosphorylation that mediates proliferation of pulmonary vascular endothelial cells (53). Another important signaling pathway PI3K/AKT/mTOR (phosphatidylinositol 3-kinase/AKT/mammalian target of rapamycin) is activated in most human cancers and is closely

related with the production of VEGF. It has been reported that VEGF secretion is increased both in hypoxia-inducible factor 1 (HIF-1)-dependent and -independent manners in response to PI3K/AKT activation (54). Activation of mTOR was detected in head and neck squamous cell carcinoma (HNSCC) patients with metastasis and inhibition of mTOR decreased vascular formation and lymph node metastasis (55,56). Inhibitors targeting signaling and molecules involved in angiogenesis may be a viable strategy for the treatment of cancer.

3. Factors mediating the 'prison break' of tumor cells

Growth-promoting signals. Growth-promoting signals in the microenvironment play a critical role in both normal and pathological tissues. Normal tissues require that growth factors be induced from a quiescent state into an active proliferation state. They tightly control the production and the release of growth-promoting signals that instruct themselves or the entry of other cells into the cell growth and division cycle. At the same time, growth-promoting signals contribute to cancer-sustaining proliferation, which has been confirmed as a hallmark of cancer. Cancer cells obtain growth signals through autocrine and paracrine pathways. Analyzing previous research, we conclude that tumor stromal cells provide cancer cells with growth-promoting signals, including growth factors, cytokines and chemokines. Table I lists these various tumor-promoting molecules.

Growth factors, secreted by stromal cells into the microenvironment, promote tumor progression via stimulation of cellular growth, proliferation and cellular differentiation. For instance, TGF β is known to enhance EMT and invasiveness in primary carcinomas (57). Blocking the TGF β signaling pathway can reduce intravasation and metastatic seeding in the lung as well as bone (58-62). A recent report by Labelle *et al* suggests that platelets secrete TGF β -1 to activate the TGF β /Smad pathway in tumor cells, and enhance invasiveness and metastasis (63). Hepatocyte growth factor (HGF), which was originally cloned as a mitogenic protein in hepatocytes (64), can specifically activate MET receptor tyrosine kinase as well as stimulate mitogenesis, cell motility and matrix invasion (65,66). Two studies found that the higher a patient's HGF level, the less likely he/she was to remain in remission. The study found that stromal cells secreted HGF resulting in activation of the MET, reactivation of the mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3-OH kinase (PI3K)-AKT signaling pathways. These cellular signaling changes in tumor cells immediately induce resistance to RAF inhibition and confer resistance to BRAF inhibitor in BRAF-mutant melanoma cells (67,68). In addition to chemical inhibitors for the inhibition of growth factors, antibodies that target receptors have been developed. Cetuximab, an EGFR monoclonal antibody, acts as an efficient antitumor drug in many types of cancer. The efficiency of cetuximab against chemo- or radio-resistant HNSCC was demonstrated (69). Other growth factors secreted by stromal cells can also promote cancer cell growth. For instance, VEGF, PDGF, bFGF and IGF1 can facilitate tumor progression by stimulating angiogenesis (Table I).

In addition to growth factors, chemokines are also important for enhancing tumor growth. Chemokines are chemotactic cytokines, which are induced by inflammatory cytokines,

Table I. Summary of tumor-promoting molecules.

Name	Main function	References
OPN	Tumor metastasis, protection from apoptosis, induction of tumor-associated inflammatory cells	(204-207)
Galectin-3	Neoplastic transformation, tumor metastasis	(208,209)
VEGF	Stimulates angiogenesis, regulates vascular permeability	(210)
EGF	Promotes cancer growth, contributes to aggressive behavior	(75,211)
TGFβ	Enhances EMT and invasiveness, regulates inflammation	(57,212)
HGF	Angiogenesis, tumorigenesis, tissue regeneration, tumor metastasis	(65,66,213)
Histamine	Increases vascular permeability, pro-inflammatory	(214)
TP	Angiogenesis, chemotherapy activation, promotes tumor growth	(215,216)
BDNF	Tumorigenesis	(217)
P-selectin	Promotes tumor growth, tumor metastasis, pro-inflammatory	(218)
LPA	Survival, cell proliferation, migration, tumor metastasis	(219)
S1P	Survival, vascular permeability, cell invasion	(220)
Prothrombin	Tumor metastasis, tumor progression	(221)
PDGF	Angiogenesis, enhances stromal cell survival, proliferation and migration	(213)
bFGF	Angiogenesis, mitogenic, tumor progression	(213)
SERPINE1	Angiogenesis, tumor invasion	(222)
IGF1	Angiogenesis, mitogenic, tumor progression	(213)
ANGPT1	Angiogenesis, tumor progression	(223)
CCL2	Tumor growth and progression, promotes cancer growth, tumor metastasis, tumor macrophage infiltration	(76-78,224)
CCL3	Angiogenesis, tumor metastasis	(225)
CCL5	Tumor growth and progression, recruits leukocytes during inflammation	(224,226)
CCL6	Tumorigenesis, tumor metastasis	(227)
CXCL8	Angiogenesis, leukocyte chemoattractant, pro-inflammatory	(228)
CCL18	Tumor progression, tumor metastasis	(161)
CCL21	Tumor progression, tumor survival and invasion	(40)
CCL22	Tumor progression, cell migration, tumor metastasis	(229)
CXCL1	Promotes cancer growth, angiogenesis, cancer chemoresistance, tumor metastasis	(39,74,230,231)
CXCL2	Tumor growth and progression, angiogenesis, cancer chemoresistance, tumor metastasis	(230-233)
CXCL3	Tumor growth and progression, angiogenesis, tumor metastasis	(224,230,231,234)
CXCL5	Angiogenesis, tumor metastasis	(230,231,235,236)
CXCL6	Angiogenesis, tumor metastasis	(230,231,237,238)
CXCL7	Angiogenesis, tumor metastasis	(230,231,239)
CXCL8	Tumor growth and progression, angiogenesis, tumor metastasis	(224,231)
CXCL12	Tumor progression, tumor invasion and metastasis	(240-242)

OPN, osteopontin; VEGF, vascular endothelial growth factor; EGF, epidermal growth factor; TGFβ, transforming growth factor-β; HGF, hepatocyte growth factor; TP, thymidine phosphorylase; BDNF, brain-derived neurotrophic factor; LPA, lysophosphatidic acid; S1P, sphingosine 1-phosphate; PDGF, platelet-derived growth factor; bFGF, basic fibroblast growth factor; SERPINE1, serpin peptidase inhibitor (also known as plasminogen activator inhibitor-1, PAI1); IGF1, insulin-like growth factor 1; ANGPT1, angiotensinogen 1; CCL, C-C motif chemokine; CXCL5, C-X-C motif chemokine.

growth factors and pathogenic stimuli (70-72). Chemokine signaling plays a major role in cellular transformation, inflammation, and wound healing; as well as tumor growth, angiogenesis, tumorigenesis and metastasis (73) (Table I). Currently, the research of cancer-associated chemokines has mainly focused on CXC chemokines and CC chemokines. Some CXC chemokines promote cancer development mainly by promoting angiogenesis and enhancing tumor metastasis. CXCL1, a small cytokine belonging to the

CXC chemokine family, is overexpressed in 70% of human melanomas and is involved in CRC tumor growth and angiogenesis (74). Overexpression of CXCL1/2 in cancer cells attracts CD11b⁺Gr1⁺ myeloid cells to the primary tumor site, and finally, enhances the viability of cancer cells through S100A8/9 factors (39). The subset of CC chemokine families, which are secreted by stromal cells have multiple functions in the progression of cancer. For example, CCL2 released from stromal cells can promote tumor growth, facilitate macro-

phage infiltration and induce metastasis (75-78). Meanwhile, other molecules, such as osteopontin (OPN), galectin-3 and brain-derived neurotrophic factor (BDNF), are also involved in the tumor microenvironment and promote cancer progression. Furthermore, some molecules in the vasculature, such as LPA, SIP, and prothrombin, play crucial roles in tumor development (Table I).

Small and non-coding RNAs (miRNAs) post-transcriptionally control the translation and stability of mRNAs. These RNAs participate in the regulation of metabolism and tumorigenesis (79-81). RNAs cannot function as extracellular signaling molecules because they are vulnerable to be degraded by ribonucleases (82). But interestingly, recent evidence shows that miRNAs contained in exosomes act as signal transducers and play important roles in the tumor microenvironment acting as a bridge between cancer cells and stromal cells (82-85). Kosaka *et al* showed that miR-143 expression in normal prostate cells was higher and transferred growth-inhibitory signals to prostate cancer cells both *in vitro* and *in vivo*. They highlighted that secretory tumor-suppressive miRNAs may be a death signal from winners to losers in the context of cell competition (85). Macrophages also regulate the invasiveness of breast cancer cells through exosome-mediated delivery of oncogenic miRNAs (86). Notably, tumor-secreted miR-21 and miR-29a also can function by an unexpected mechanism, by binding as ligands to receptors of the Toll-like receptor (TLR) family, murine TLR7 and human TLR8 in immune cells, triggering a TLR-mediated prometastatic inflammatory response that ultimately may lead to tumor growth and metastasis (87). However, the detailed mechanisms of the role of secretory miRNAs in the tumor microenvironment are still poorly understood. Some studies only report that microRNAs can be stable blood-based markers for cancer detection. For example, a significant increase in miR10b, miR34a and miR155 concentrations in the peripheral blood of breast cancer patients and the observed correlation with tumor progression suggest a potential clinical utility of circulating miRNAs as a new class of future biomarkers (88). Serum levels of miR-141 (an miRNA expressed in prostate cancer) can distinguish patients with prostate cancer from healthy controls, and may be used as an important approach for the blood-based detection of human cancer (89). Although the understanding of the role of miRNAs in the tumor microenvironment remains poorly understood, it has been proposed that miRNAs in the tumor microenvironment may potentially serve as paracrine signaling molecules having both tumor-promoting as well as tumor-suppressing effects.

Buffering metabolic stress. Over the past 10 years of cancer research, the reprogramming of energy metabolism has been considered as a hallmark of cancer. Metabolic reprogramming is always considered to be intrinsic to cancer cells, such as oncogene activation, inactivation of tumor-suppressor genes as well as the mutation of glycolytic enzymes (90). Yet, recent research has shifted our focus on the regulation of the tumor microenvironment in tumor metabolism. Compared with normal differentiated cells, cancer cells mainly rely on aerobic glycolysis rather than mitochondrial oxidative phosphorylation to gain the energy needs for rapid proliferation even under normal conditions, which is called the 'Warburg

effect' (91) (Fig. 2). Yet, aerobic glycolysis is less efficient than oxidative phosphorylation for generating ATP; 4 mol ATP per mol glucose compared with 36 mol ATP per mol glucose when under oxidative phosphorylation. To meet the energy needs and high levels of glycolytic intermediates supporting anabolic reactions, tumor cells maintain enhanced glucose uptake through high levels of glucose transporters, lactate dehydrogenase and other glycolytic enzymes (92). Due to the high level of aerobic glycolysis, much lactate is generated by cancer cells, which contributes to an acidic condition, ROS production and MAPK signaling activation (93). High incidence of distant metastasis is related to the suppressed proliferation and cytokine production of human cytotoxic T lymphocytes (94,95). But an elevated level of lactate accumulation in the tumor cells or the microenvironment also leads to an inhibitory effect of glycolysis and restriction of cell growth and proliferation. Tumor cells must secrete lactate into the surroundings via increased expression of lactate transporter monocarboxylate transporters 4 (MCT4). In response, MCT1 expression increases in CAFs resulting in the uptake of tumor-extruded lactate. CAFs increase the expression level of lactate dehydrogenase (LDH-B), resulting in the conversion of the influxed lactate to pyruvate. The pyruvate is shunted to the tricarboxylic acid cycle for ATP generation via oxidative phosphorylation, thereby satisfying the energy needs of the CAFs (96) (Fig. 2A). The released lactate can also be consumed by endothelial cells and stimulate angiogenesis through NF- κ B/IL-8 signaling (97). Interestingly, lactate produced by hypoxic tumor cells may indeed diffuse and be taken up by oxygenated tumor cells (98,99). Sonveaux *et al* suggest that hypoxic tumor cells depend on glucose and glycolysis to produce energy and secrete lactate. The lactate is diffused along its concentration gradient and is taken up by oxygenated tumor cells and used to meet the energy needs (100). A previous study examined the expression of major proteins involved in cellular aerobic and anaerobic metabolism, PDH, PDK1, LDH1, LDH5, MCT1, MCT2 and GLUT1, between normal tissues, cancer cells and tumor-associated stromal cells. The results suggest that the newly formed stroma and vasculature express complementary metabolic pathways, buffering and recycling products of anaerobic metabolism to sustain cancer cell survival (101). Nieman *et al* provide strong evidence that adipocytes promote the initial homing of tumor cells to the omentum through adipokine secretion. Subsequently, adipocytes provide fatty acids to the cancer cells, fueling rapid tumor growth (102) (Fig. 2D). These studies highlight how tumors can survive and grow in hypoxia and an energy crisis. They are capable of organizing the regional stromal cells into a harmoniously collaborating metabolic domain living in 'the same boat'.

In addition to glucose, glutamine is the other molecule catabolized in appreciable quantities in most mammalian cell *in vitro* cultures (103); metabolic profiling of the colon and stomach cancer microenvironment by capillary electrophoresis time-of-flight mass spectrometry identified a significant accumulation of all amino acids except glutamine in the tumors (104) (Fig. 2F). CAFs undergo an autophagic program, leading to the generation and secretion of high glutamine levels into the tumor microenvironment to meet the glutamine needs of cancer cells. Cancer cells accumulate glutamine and convert it to glutamate which is further catabolized to α -ketoglutarate,

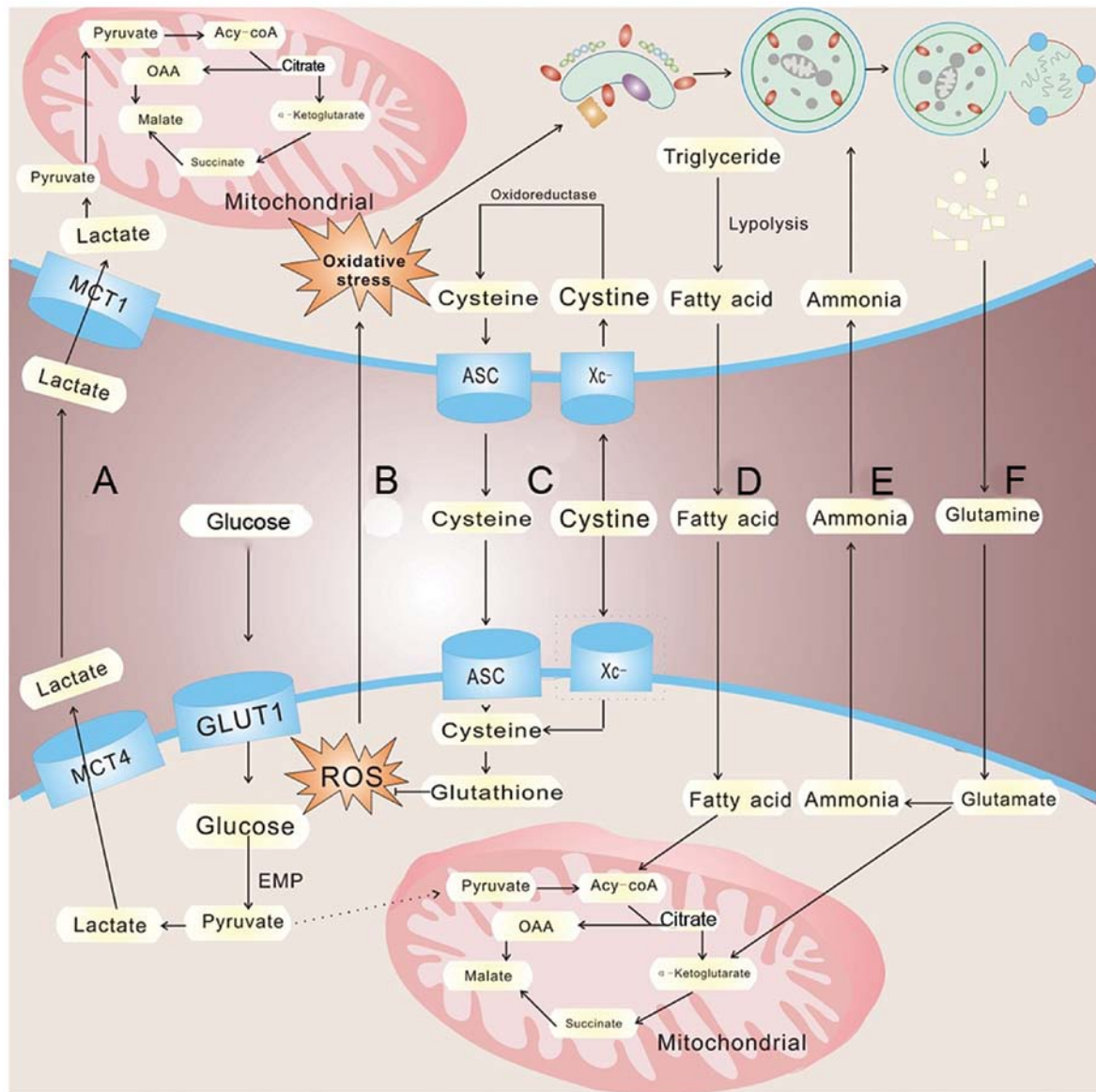


Figure 2. Summary of the role of the tumor microenvironment in the regulation of cancer cell metabolism. (A) Tumor cells, under hypoxic conditions, secrete lactate via MCT4. In response, cancer-associated fibroblasts (CAFs) and oxygenated tumor cells take up the tumor-extruded lactate. (B) Cancer cells induce ROS production in CAFs, leading to the onset of stromal oxidative stress, which in turn, drives autophagy and provides recycled nutrients via catabolism and aerobic glycolysis to feed the appetite of adjacent cancer cells. (C) Tumor stromal cells are able to take up cystine, convert it to the amino acid cysteine, and then secrete it. Tumor cells then use cysteine to produce glutathione, resulting in increased ROS resistance and survival. (D) Adipocytes provide tumor cells with fatty acids supplying the energy needs of rapid tumor growth. (E) Glutamine can be hydrolyzed as ammonia in tumor cells and reused by CAFs. (F) CAFs secrete glutamine into the tumor microenvironment to meet the glutamine needs of the cancer cells. MCT4, monocarboxylate transporter 4; GLUT1: Glucose transporter 1; ASC: Neutral amino acid transporter A; Xc-: Cystine/glutamate transporter; ROS: Reactive Oxygen Species; OAA: Oxaloacetate.

enters the TCA cycle and increases the mitochondrial activity of cancer cells. The by-product, ammonia, freely diffuses into the microenvironment, and then induces autophagy and glutamine production in CAFs, which confirm a cascade between CAFs and cancer cell interactions (105) (Fig. 2E). Not only can glutamine be used in the TCA flux to meet the energy needs of cancer cells, but is also involved in the synthesis of non-essential amino acids alanine, serine, arginine, and proline (106). Jain *et al* suggested a key role for glycine in rapid cancer cell proliferation using metabolic profiling approaches. Interestingly, they found that rapidly proliferating non-transformed cells, including human bronchial epithelial cells and lymphocytes, release rather than consume glycine (107). These reports found that normal cells near tumor cells may provide

glycine for the rapid proliferation of cancer cells. Treatments that target tumors and surrounding cells should be considered, rather than targeting only the tumor cells. This concept may provide us with novel strategies for tumor treatment.

Cellular metabolism is critical for the generation of energy in biological systems, however, as a result of electron transfer reactions, reactive oxygen species (ROS) are generated in aerobic cells. ROS and cellular oxidant stress have long been associated with cancer (108-111). Previous evidence suggests that cancer cells normally produce a higher ROS level than normal cells (112). Hypoxia, mitochondrial dysfunction, and inflammation, ionizing radiation, chemotherapeutic agents, hyperthermia, inhibition of antioxidant enzymes, or depletion of cellular reductants such as NADPH and glutathione, can

all lead to the accumulation of ROS in cancer cells (113,114). Although a low level of ROS is easily managed by cancer cells, abnormally high levels of ROS induce oxidative stress. A low level of ROS promotes cell proliferation and differentiation, while a high level of ROS can cause oxidative damage to lipids, proteins, DNA and finally cause cell death (115). Recent evidence highlights the role of ROS in the tumor microenvironment and provides new insight into metabolic associations between cancer cells and non-malignant neighbors in the stroma. Accumulated ROS are dispersed from cancer cells to adjacent fibroblasts (116), leading to the oxidative stress of stromal cells, which, in turn, drives autophagy via HiF1 induction and NF- κ B activation; meanwhile providing recycled nutrients via catabolism and aerobic glycolysis to feed the appetite of adjacent cancer cells. In addition, stromal ROS production induced by cancer cells leads to local DNA damage as well as DNA damage in adjacent cancer cells via a 'bystander effect'. As a consequence, stromal ROS promote aneuploidy and genomic instability in cancer cells, driving tumor-stroma coevolution. These studies proposed a simple solution to the autophagy paradox [both promote cell death and survival (117-119)], which is called 'The Autophagic Tumor Stroma Model of Cancer' (120-126). In this simplistic model, it is proposed that autophagy acts as a tumor suppressor when it occurs in epithelial cancer cells; conversely, autophagy acts as a tumor promoter when it occurs in CAFs. Zhang *et al* provide another mechanism of tumor-stroma interactions to avoid ROS accumulation in cancer cells. They showed that bone marrow stromal cells can express a high level of Xc- transporter and effectively take up cystine to synthesize GSH (127). It is known that a high level of cellular GSH can both release oxidative stress and promote cell survival. Metastatic stress and ROS both are crucial for tumor survival and growth. It is important for us to understand how tumor cells conquer the energy crisis and oxidative stress. When we discuss invasion and metastasis, it must be remembered that tumor cells have accomplices.

4. Processes mediating the 'prison break' of tumor cells

Induction of angiogenesis. Like normal tissues, tumors need to sustain a nutrient supply and evacuate metabolic wastes. Blood vessels nourish nearly every organ of the body, and deviations from normal vessel growth can contribute to numerous diseases. Angiogenesis allows tumors to obtain nutrients and evacuate metabolic waste with no difficulty (128). Tumor-associated angiogenesis is currently known as a hallmark of cancer. It is now widely accepted that the 'angiogenic switch' is under the tight control of pro-angiogenic molecules and anti-angiogenic molecules, and the 'angiogenic switch' is on only when the net balance between pro-angiogenic molecules and anti-angiogenic molecules is tipped in favor of angiogenesis (128-130). Mounting evidence suggests that stromal cells in the tumor microenvironment play critical roles in switching on and sustaining chronic angiogenesis in many tumor types. Among the various types of stromal cells, TAMs are one of the most important cell types involved in promoting tumor-associated angiogenesis. Leek *et al* found that the number of TAMs is positively correlated with tumor angiogenesis in breast carcinomas (131). Subsequent studies have confirmed such a

link in a wide array of tumor types. TAMs can induce angiogenesis through different pathways, which can be divided into three categories. First, TAMs release pro-angiogenic factors directly activating endothelial cells. In early 1984, TAMs were demonstrated to be potent stimulators of neovascularization and endothelial cell proliferation, and that depletion of macrophages from tumor cell suspensions significantly decreased their angiogenic potential (132). VEGF, bFGF, TGF- α/β , EGF and IL-1 β secreted by macrophages control tumor angiogenesis (133-138). These factors induce endothelial cell activation and differentiation into tumor neovessels. Recently, Chen *et al* confirmed that M2 phenotype macrophages can promote angiogenesis in a paracrine manner via the endothelial nitric oxide synthase (eNOS) signaling pathway (139). Additionally, TIE2-expressing macrophages (TEMs) can directly interact with ECs, is important for tumor angiogenesis and can be targeted to induce effective antitumor responses (140). Secondly, TAMs recruit other pro-angiogenic cells. TAMs can attract mononuclear macrophages (MONO) and TEMs into the tumor microenvironment. Recruited TEMs can also recruit endothelial and myeloid progenitors capable of directly incorporating into the tumor vasculature (141). Thirdly, TAMs modulate ECM. Matrix metalloproteinases, which are ECM remodeling enzymes, regulate signaling pathways that control cell growth, inflammation and angiogenesis. Mounting evidence suggests that TAMs also produce and secrete MMP2, MMP7, MMP9 and MMP12 to the tumor microenvironment (142-144). These MMPs can interact with ECM and increase the bioavailability of pro-angiogenic factors, including VEGFA and bFGF and promote angiogenesis.

Other cells, such as CAFs, can also promote angiogenesis. Guo *et al* showed that myofibroblasts express VEGFA and other angiogenic factors leading to the promotion of angiogenesis (145). Additionally, ovarian cancer-associated fibroblasts not only promote angiogenesis, but also lymph angiogenesis (146).

Activation of invasion and metastasis. Tumor metastasis always consists of a series of discrete biological processes that move tumor cells from the primary neoplasm to a distant organ. This process involves local invasion, intravasation, survival in the circulation, arrest at a distant organ site, extravasation, micrometastasis formation, and then metastatic colonization, and finally clinically detectable macroscopic metastases are formed (147-149) (Fig. 3). These events have been considered to be induced by genetic and/or epigenetic alterations within tumor cells, but accumulating evidence supports that tumor metastasis is also mediated by tumor stromal cells. Recent publications have confirmed that the tumor microenvironment contributes to every stage of tumor metastasis. Once the tumor cells need to escape from the primary tumor, they must interact with preexisting host basement membranes and the ECM (Fig. 3A). In early 1986, Liotta *et al* proposed a three-step hypothesis describing the sequence of biochemical events during tumor cell local invasion: i) tumor cell attachment via cell surface receptors which specifically bind to components of the matrix, ii) secretion of hydrolytic enzymes and iii) tumor cell locomotion into the region of the matrix (150,151). MMP-7 expressed by breast epithelial cancer cells not only cleaves matrix components

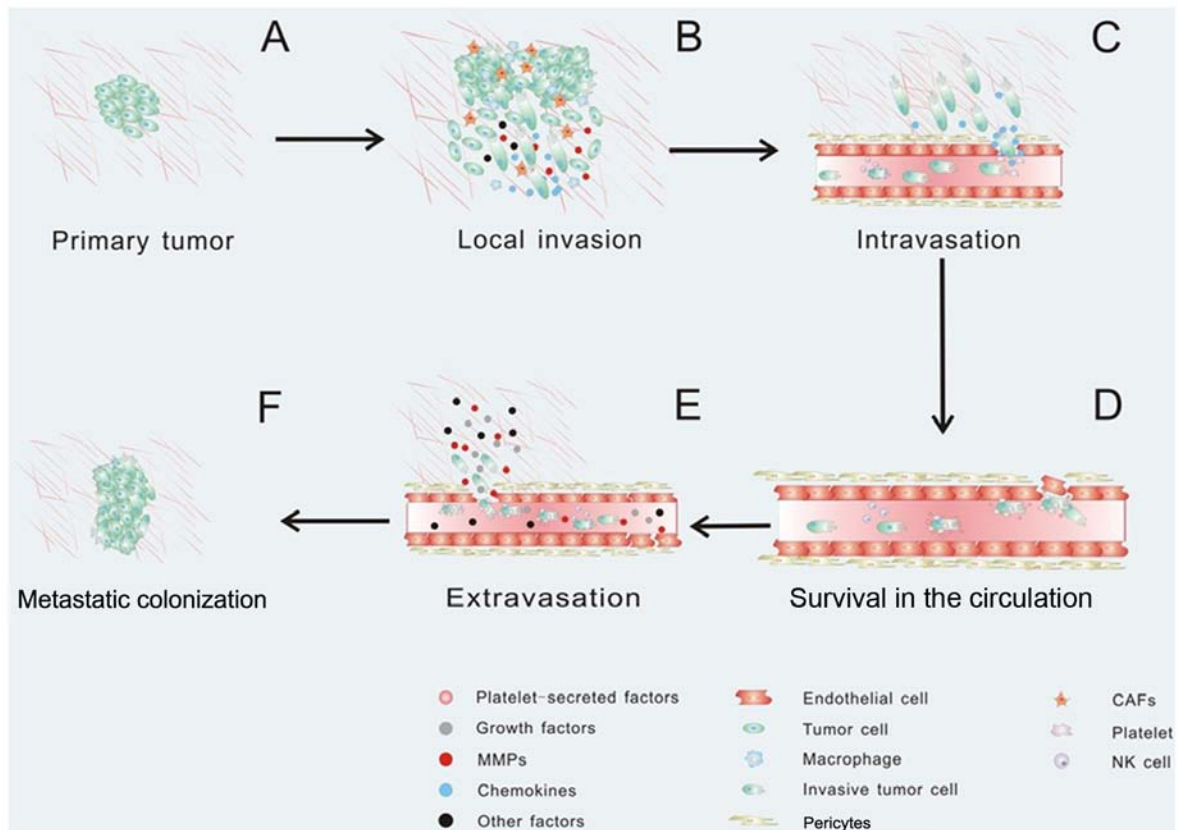


Figure 3. The tumor microenvironment regulates cancer metastasis and contributes to every stage of tumor metastasis. (A-F) A detailed summary of the role of the microenvironment in the metastasis of tumor is provided in the text. In brief, firstly, the primary tumor can invade to the surrounding tissue and then invade into the vascular. Secondly, these successfully invasive tumor cells must survive in the circulation system. Finally, the surviving tumor cells can extravasate to a new site and virtually carry out metastatic colonization. CAFs, cancer-associated fibroblasts; NK cell, natural killer cell; MMPs, metalloproteinases

in the tumor microenvironment, but also cleaves the cell surface adhesion molecule E-cadherin, leading to disruption of basement membrane structures and breast epithelial cell-cell junctions (152,153). It has been confirmed that CAFs and macrophages promote cancer cell invasion by secreting matrix metalloproteinases which cause proteolysis of the ECM leading to the promotion of cancer cell invasion (154-156). Fibroblasts also facilitate tumor cell invasion through force- and protease-mediated ECM remodeling (157-159), and intrinsic fibroblast caveolin-1 enhances tumor cell invasion by force-dependent remodeling of the surrounding environment via Rho GTPase activation (160). Activated macrophages and TAMs produce CCL18 and promote the invasiveness of breast cancer cells via the functional receptor for CCL18, PTPN13 (161) (Fig. 3B). Interestingly, the ECM in the path of the invading cell can be remodeled by invadopodia (162), which are actin-rich membrane protrusions with a matrix degradation activity formed by invasive carcinoma cells (163). Podosomes, which are similar to invadopodia in molecular composition with ventral membrane protrusions and invaginations formed by macrophages and other type of cells (164-166), are proposed to play a role in ECM remodeling and then promote carcinoma cell invasion. For example, v-src-transformed 3Y1 rat fibroblast (3Y1-src) cells cultured on fibronectin degrade the fibronectin mainly at the podosomes, which is thought to underlie the invasive

phenotype of 3Y1-src cells (167). Yamaguchi *et al* showed that macrophage podosomes have a matrix degradation activity and that colony-stimulating factor-1 (CSF-1) regulates the formation and organization of macrophage podosomes (162). These observations highlight the critical role of these specialized protrusive structures, invadopodia and podosomes, in tumor invasion (Fig. 3B). EMT is a hypothesized program of development of biological cells characterized by loss of cell adhesion, repression of E-cadherin expression, and increased cell mobility. EMT is essential for numerous developmental processes including mesoderm formation and neural tube formation and is regulated by many transcription factors, including zinc finger protein snail 1 (SNAIL), SNAIL2, zinc finger E-box-binding homeobox 1 (ZEB1), ZEB2, TWIST, FOXC1 (forkhead box protein 1), FOXC2, TCF3 (transcription factor 3 - also known as E47), and GSC (homeobox protein goosecoid) (168). During tumor cell invasion, tumor cells co-opt EMT and the basement membrane becomes fragmented. The tumor cells can intravasate into lymph or blood vessels, allowing their passive transport to distant organs (169). It has been reported that tumor-associated fibroblasts (TAFs) induce the significant overexpression of FGFR4 in colorectal cancer cell lines and play a critical role in colorectal cancer EMT and metastasis (170). The stromal cells also stimulate EMT and promote tumor cell invasion. Pericytes, associated with endothelial cells, promote tumor angiogenesis, and

promote tumor progression (171,172). By using genetic mouse models or pharmacological inhibitors, Cooke *et al* showed that pericyte depletion suppressed tumor growth but resulted in hypoxia-associated EMT (173). Meanwhile, inflammation plays an important role in inducing EMT. Snail, which plays an essential role in inducing EMT and cancer metastasis by repressing expression of E-cadherin (174-176), can be stabilized by the inflammatory cytokine TNF α through the activation of the NF- κ B pathway (177). It has been revealed that EMT is a dynamic process controlled by an inflammatory microenvironment.

After local invasion, the tumor cells infiltrate into the vascular spaces and establish direct contact with the blood. In this step, the tumor cells invade across the endothelial basal lamina and migrate between the endothelial cells lining the capillaries that service the tumor (178). Intravasation is always critical and is the rate limiting step of tumor metastasis (148,179). Recent evidence suggests that the tumor microenvironment can promote cancer cell intravasation and metastasis. For instance, CCL2-expressing tumor cells attract monocytes and activate endothelial cells through CCR2, showing that a tumor cell-derived chemokine induces vascular permeability and enables efficient tumor cell intravasation (37,180) (Fig. 3C). In particular, platelets influence vascular integrity and play an important part in tumor metastasis (181). Notably, platelets secrete TGF β 1 to activate the TGF β /Smad pathway, which synergize with the NF- κ B pathway, and enhance invasiveness and promote metastasis (63). When cancer cells enter the circulation system, most of the cancer cells will not survive due to the loss of integrin-dependent adhesion to ECM components causing anoikis, damage incurred by hemodynamic shear forces and the predation by cells by the innate immune system, specifically NK cells (149) (Fig. 3D). The circulating tumor cells can be detected in the bloodstream of patients using microchip technology, immunomagnetic nanoscreening and 2-NBDG fluorescence imaging (182-184). In order to survive in the circulation, tumor cells recruit platelets which, in turn, form a coat to protect them from the innate immune system. Even if tumor cells are NK susceptible and cytotoxic NK cells threaten their life in the blood, platelets are capable of protecting them from cytolysis by forming a physical shield around cancer cells, thereby promoting metastasis (185,186). As platelets become activated, they can release growth factors, chemokines and protease, which can perpetuate the cohesion of heteroaggregates containing tumor cells. Platelets can also support the attachment to the endothelium and thereby contribute to metastasis (181) (Fig. 3D). During circulation, these invasive tumor cells may arrest at any distant organ site. When the new site is ready for metastatic tumor growth, the primary tumors are able to secrete factors to induce cancer cell extravasation. For example, the secreted angiopoietin-like-4 (Angptl4), as well as EREG, COX-2, MMP-1, and MMP-2, are able to disrupt pulmonary vascular endothelial cell-cell junctions to facilitate cancer cell extravasation (59,187). When cancer cells arrive at a secondary site, the microenvironment is phenotypically and functionally distinct from the primary tumor, which may cause some physical barriers. In order to overcome physical barriers at the secondary site, primary tumors can secrete factors that perturb the distant microenvironment (Fig. 3E). For example, the pre-metastatic niche

referred to as interactions between metastatic tumor cells and their stromal cells (188), have been defined as a new concept, which describes the tumor microenvironment playing important roles in the tumor cell survival in the circulation and growth at a secondary site. Primary tumors can secrete growth factors priming certain tissues in the metastatic site for tumor cell engraftment and growth (189). The primary tumor cells secrete pro-inflammatory factors such as VEGF-A, TGF β and TNF α inducing the expression of chemoattractants S100A8 and S100A9 in lung VE-cadherin⁺ endothelial cells and Mac1⁺ myeloid cells (190,191). But the exact mechanism by which these chemoattractants elicit cell accumulation is not known. Hiratsuka *et al* showed that serum amyloid A (SAA)3 induced in pre-metastatic lungs by S100A8 and S100A9 has an important role in the accumulation of myeloid cells and acts as a positive-feedback regulator for chemoattractant secretion. Meanwhile, SAA3 can stimulate NF- κ B signaling in a TLR4-dependent manner and facilitate metastasis (192). More interestingly, Kaplan *et al* found that bone marrow-derived hematopoietic progenitor cells that express vascular endothelial growth factor receptor 1 home to tumor-specific pre-metastatic sites and form cellular clusters before the arrival of tumor cells (193). They first demonstrated that a non-neoplastic cell population can portend a future metastatic site. After extravasation, tumor cells utilize the microenvironment in the metastatic site and form a new tumor microenvironment supporting metastatic tumor growth (Fig. 3F).

5. Therapeutic implication

Cancer cells require an enormous variety of genetic changes to elicit tumorigenesis. The clinical therapy for many types of human cancers has mainly focused on the malignant cancer cell itself, and have made great achievements, yet cancer therapy still remain a great challenge. Currently, the most commonly used radiotherapy and chemotherapy strategies have serious side effects, such as destruction of the patient immune system, and patients rapidly develop therapeutic resistance. As described above, the tumor microenvironment commonly participates in tumor initiation as well as progression in many tumor types, providing us with the hope that therapeutic targeting of these events could be efficient for cancer therapy. Recent publications provide strong evidence that tumor stromal cells forming the tumor microenvironment contribute to chemoresistance. For example, CCR2 null host mice responded better than a control when treated with doxorubicin. This effect was induced because myeloid cells can be recruited to doxorubicin-treated tumors through a stromal CCL2/CCR2 chemokine/chemokine receptor axis leading to chemoresistance (194). Similarly, during treatment with platinum analogs, endogenous mesenchymal stem cells (MSCs) are activated and release polyunsaturated fatty acids which protect cancer cells against a range of chemotherapeutics (195). These outstanding findings show that the tumor microenvironment is a potent administrator of resistance to traditional cytotoxic therapies, mainly chemotherapy and radiotherapy, and reveal potential available targets to enhance chemotherapy efficacy in patients. Multitargeted approaches, in which tumor cells and the tumor microenvironment are simultaneously inhibited, have been developed in recent years. These multitargeted approaches

have many advantages compared with traditional therapies. On the one hand, stromal cells in the tumor microenvironment are presumably genetically stable, while tumor cells are known to be genetically unstable. Therefore, it is less likely for cancer patients to accumulate adaptive mutations as well as rapidly acquire resistance to chemotherapy as well as radiotherapy. On the other hand, the tumor microenvironment has a certain similarity in diverse cancer types, thus one therapeutic target of the tumor microenvironment can be implicated in more than one type of cancer. Based on research of the tumor microenvironment, multiple technologies have been developed for the research of cancer, such as liquid biopsy (196) and *in silico* molecular biology (197). The liquid biopsy is used to analyze tumor DNA in urine for non-muscle invasive bladder cancer patients and provides a non-invasive approach for bladder cancer detection (198). The *in silico* biomarker profiling technology is used to identify GLUT4-specific inhibitors for cancer therapy (199). These results made valuable contributions for the personalized/precision medicine and hold great potential for personalized detection.

Due to these advantages, targeting the tumor microenvironment holds great potential for cancer therapy. There are many tumor-promoting factors in the tumor microenvironment, suggesting that inhibition of these tumor-promoting factors or destroying these signaling pathways can prevent the development of cancer. For example, tumors in a stroma xenograft model treated with the TGF- β inhibitor exhibited a reduction in blood vessels (200). As enhancement of GSH synthesis in CLL cells is possibly a crucial mechanism by which stromal cells facilitate leukemia cell survival and drug resistance through providing cysteine (127), a strategy to destroy stromal protection of CLL cells by inhibiting the transporter to impact the uptake of cystine by stromal cells as well as act in concert with traditional drugs may be an efficient pathway to cure CLL. In addition, the remodeling of ECM can be regulated by many families of matrix-degrading enzymes such as heparanase, chymases, MMPs and tryptases (201). Inhibitors of these enzymes such as PI-88 [an inhibitor for heparanase (202)] may prevent the multistep pathway in the tumor microenvironment in order to treat cancer. However, some fragments of basement membrane collagen generated by MMP have endogenous effects as integrin-mediated suppressors of pathologic angiogenesis as well as tumor growth (203). This fact indicates that the delicate balance between the tumor-inhibitory and tumor-promotion functions of stromal cells should be considered. In addition, the normal function of stromal cells should not be destroyed following therapy. More research is needed to develop more efficient approaches to combat cancer.

6. Conclusion

This review highlights the evidence for the crucial role of the tumor microenvironment in tumor progression and metastasis. As noted, tumor initiation as well as progression are complex and multistep processes in which the tumor microenvironment may contribute to its success. In this dynamic progression, the tumor microenvironment can affect cancer cell proliferation as well as tumor metastasis. For this reason, research on cancer must combine the tumor cell-intrinsic pathway with the extrinsic pathway in the tumor microenvironment. The myste-

rious role of the tumor microenvironment is being deciphered in primary and metastatic tumors, particularly using various new fields such as secreted miRNAs, metabolism, and pre-metastasis. Targeting the tumor microenvironment combined with current clinical approaches holds great potential for developing new efficient therapies. Cancer medicine must move toward a new era of personalized diagnostics and therapeutics that aggressively embraces integrative approaches.

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