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Tumor stroma as targets for cancer therapy

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Abstract

Cancer is not only composed malignant epithelial component but also stromal components such as fibroblasts, endothelial cells, and inflammatory cells, by which an appropriate tumor microenvironment (TME) is formed to promote tumorigenesis, progression, and metastasis. As the most abundant component in the TME, cancer-associated fibroblasts (CAFs) are involved in multifaceted mechanistic details including remodeling the extracellular matrix, suppressing immune responses, and secreting growth factors and cytokines that mediate signaling pathways to extensively affect tumor cell growth and invasiveness, differentiation, angiogenesis, and chronic inflammatory milieu. Today, more and more therapeutic strategies are purposefully designed to target the TME as well as tumor cells. This review will focus on the role of CAFs in tumor development and the novel strategies to target this component to inhibit the tumor growth.

Keywords

Tumor microenvironment; Cancer-associated fibroblasts; Extracellular matrix; Cancer signaling; Cancer therapy

1. Introduction

Despite continued development of surgical techniques and meticulously designed chemotherapy or radiotherapy regimens, cancer remains the second leading cause of deaths after cardiovascular disease. The American Cancer Society estimates that in 2012, cancer will affect more than 1,600,000 patients and result in nearly 580,000 deaths (Siegel et al., 2012). Cancer has long been believed to be a cell-autonomous process, in which consecutive mutations in oncogenes and tumor suppressors lead to infinite tumor cell proliferation (Kenny et al., 2007). Understandably, most research focuses on these tumor cells. However, mounting evidence suggests that tumor genesis and progression are determined not only by

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Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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tumor cells, but also by a favorable tumor microenvironment (TME) (Hu et al., 2009; Tyan et al., 2011), which supports the “seed [tumor cells] and soil [tumor stroma]” hypothesis proposed by Paget more than 100 years ago (Paget, 1989).

Tumor is considered a complicated “organ” with dysregulated growth (Bissell & Radisky, 2001) that consists of a highly heterogeneous population of cell including tumor cells, fibroblasts, inflammatory cells (e.g., lymphocytes and macrophages), endothelial cells, pericytes, and smooth muscle cells. Existing data clearly reveal that the TME differs distinctly from the corresponding normal stroma (Fig. 1). Rather than a “bystander”, the TME acts as an important “accomplice” of tumorigenesis and development.

Evidence suggests that there is close link between tumor cells and their TME: the basement membrane (BM) is degraded and the extracellular matrix (ECM) is overproduced; epithelial to mesenchymal transition (EMT) may occur; activated fibroblasts and inflammatory cells release plentiful growth factors, cytokines, and ECM-degrading proteases; newly formed vasculatures provide nutritional support to tumor cells (Petersen et al., 2003; Radisky & Radisky, 2007). All of the above changes in turn act on tumor cells and stroma to further alter and facilitate tumor progression and metastasis (Stuelten et al., 2010). On the basis of these data, it has been suggested that targeting the TME is a promising therapeutic approaches for diverse tumor types and disease stages (Chuaysri et al., 2009). The targets are also rapidly expanding from endothelial cells to other stromal components including fibroblasts, inflammatory cells, and the ECM.

In this review, we focus in particular on the role of the major cellular component in tumor milieu, cancer-associated fibroblasts (CAFs). We then discuss several crucial signaling pathways between the tumor and CAFs. Finally, we highlight the current advances in the development of novel therapeutic strategies against CAFs. Alone or in combination with conventional therapies, some target agents have been approved by Food and Drug Administration (FDA), but many more are currently being explored.

2. Cancer-associated fibroblasts (CAFs)

Fibroblasts represent the principal cellular component in the TME. Normal fibroblasts are in an inactive quiescent state and are embedded within the ECM consisting of collagen type I, laminin, fibronectin, and proteoglycans (Bremnes et al., 2011); they play a role in suppressing inappropriate or preneoplastic epithelial proliferation (Schauer et al., 2011). Once they are recruited, activated, and accumulated to the tumor area, these cells which are called CAFs, myofibroblasts, reactive stromal fibroblasts, or tumor-associated fibroblasts, have distinctly different morphological and functional features from their normal counterparts (Orimo et al., 2005). CAFs have higher proliferative activity (Kalluri & Zeisberg, 2006), persistently exist in the tumor milieu, and cannot be removed by apoptosis (Cirri & Chiarugi, 2012).

2.1. Origin and recognition

It is increasingly recognized that CAFs is a heterogeneous cell group with various sources. The locally resident stromal fibroblasts are considered a major source of CAFs and this process is called *fibroblast to myofibroblast transdifferentiation* (FMT) (Untergasser et al., 2005); it is activated by growth factors such as transforming growth factor- β (TGF- β), platelet-derived growth factor (PDGF), and basic fibroblast growth factor (bFGF) released by the tumor cells (Ronnov-Jessen et al., 1995; Anderberg et al., 2009; Sumida et al., 2011). Bone marrow-derived mesenchymal stem cells (MSCs) serve as another important origin for CAFs, contributing approximately 25% to the CAF population in a mouse model of pancreatic tumor (Direkze et al., 2004). The EMT is a complex and fundamental process that

leads to epithelial cells losing their polarization and specialized junction, reorganizing cytoskeleton, and ultimately, acquiring morphological and functional features of mesenchymal-like cells (Cannito et al., 2010). Growing evidence suggests that CAFs are derived from malignant epithelial cells via EMT (Petersen et al., 2003) and endothelial cells via another unique process of transdifferentiation, endothelial to mesenchymal transition. A transgenic mouse model showed that up to 40% of CAFs arose from endothelial cells at the invasive front of tumors (Zeisberg et al., 2007). CAFs also originate from other cell types including smooth muscle cells, pericytes, adipocytes, or inflammatory cells (Fig. 2) (Xouri & Christian, 2010).

To date, CAF recognition depends primarily on their morphology and specific biomarkers. CAFs possess typical morphological features: spindle shape, large euchromatic nuclei, prominent nucleoli, rough endoplasmic reticulum, cytoplasmic myofilaments, Golgi apparatus, and gap junctions (Worthley et al., 2010). They usually show positive expression of alpha-smooth muscle actin (α -SMA), vimentin, fibroblast activation protein (FAP), S100A4 protein/fibroblast specific protein-1, PDGF receptor (PDGFR) α/β , and paladin 4Ig (Ronty et al., 2006; Sugimoto et al., 2006; Paulsson et al., 2009; Hua et al., 2011), and negative expression of epithelial (e.g., cytokeratin), endothelial (e.g., CD31 and CD34) and fully differentiated smooth muscle (e.g., smoothelin) markers (Gonda et al., 2010). Among them, α -SMA is the most widely used CAF marker, although none of these markers were unique to CAFs. Hence, it may be necessary to use a combination of markers to recognize CAFs.

2.2. Genetic and epigenetic alterations

CAFs cultured *in vitro* partially retain their biological properties, even when lacking coexisting malignant epithelial cells (Olumi et al., 1999). Despite relatively little is known about the mechanism, some studies propose that the answer might lie in the genetic or epigenetic alterations of CAFs (Ostman & Augsten, 2009).

In analyses of laser-captured-microdissected stroma adjacent to carcinoma, both loss of heterozygosity (LOH) and/or genetic mutations were found in the tissues of breast (Patocs et al., 2007), head and neck (Weber et al., 2007), and ovarian cancer (Tuhkanen et al., 2006). Most strikingly, certain stroma-specific loci (such as D4S2417, D3S360, and D19S55) are even closely correlated with some important clinicopathological characteristics, including tumor size and regional nodal metastases (Weber et al., 2007). A series of studies showed that CAFs frequently carry *TP53* and *PTEN* mutations (Kurose et al., 2002; Lafkas et al., 2008). Analogously, stromal p53 mutational status is associated with regional lymph node metastases in human sporadic breast cancer (Patocs et al., 2007), and resistance to cytotoxic chemotherapy (Lafkas et al., 2008). The inactivation of *Pten* gene in stromal fibroblasts from mouse mammary glands promoted the initiation, progression, and malignant transformation of mammary epithelial tumors (Trimboli et al., 2009). However, several studies published the controversial results that genetic alterations are extremely rare in breast, ovarian and pancreas carcinomas samples (Qiu et al., 2008; Walter et al., 2008). Some researchers propose that the reasons for the differences may due to sources of specimens, the accuracy of microdissected cells, inherent difference, and detection techniques (Qiu et al., 2008; Worthley et al., 2010).

On the contrary, there is a common view on CAFs' epigenetic alterations. The specific patterns of epigenetic modifications through DNA methylation are identified in the CAFs adjacent to breast or prostate cancer (Hu et al., 2005; Richardson et al., 2007). Gene expression of CAFs can be silenced through the methylation of specific sites in the promoter region, such as the promoters of glutathione S-transferase 1 and retinoic acid receptor b2 (Hanson et al., 2006; Rodriguez-Canales et al., 2007). Global loss of methylation has long

been recognized as a feature of human cancer epithelial cells. Some data highlight the fact that DNA hypomethylation is also present in CAFs and may enhance genetic instability resembling its role in cancer cells (Jiang et al., 2008). The epigenetic alterations in tumor stroma and cancer cells may be associated with specific biological characteristics of HER-2/neu+ breast cancers, such as high aggressiveness and resistance to anti-hormonal therapy (Fiegl et al., 2006).

2.3. Future Challenges

Despite an increasing understanding of CAFs, some detailed mechanisms associated with their origin, function, and genetic alteration remain unclear. It is unclear regarding all the different cell origin of CAFs; it is also unclear whether different original CAFs play different roles in tumor progression or are involved in different signaling pathways, whether CAFs in various tumor sites (e.g. at the invasive front versus in the center) have different functions, and whether genetic alteration is an inevitable result of CAF activation. Fortunately, the current applications of experimental methods to reconstruct the TME *in vitro* by the commercial three-dimensional co-culture matrix device and *in vivo* by co-implantation cancer cells and CAFs in mouse xenograft model may be in part helpful for solving the above-mentioned issues in the future.

3. Functional roles of CAFs in cancer progression

CAFs play a critical role in cancer-stromal interaction. They contribute to the growth and invasion of tumor cells through remodeling of the ECM, suppression of immune responses, and secretion of various soluble factors (growth factors and cytokines), to name a few. In many cancer types, fibroblasts with high α -SMA (Tsujino et al., 2007; Chuaysri et al., 2009), FAP (Henry et al., 2007), or PDGFR β (Paulsson et al., 2009) expression help cancer cells exhibit an aggressive malignant behavior (lymph node metastasis, larger tumor size, and high histopathological grade) and are associated with a poor prognosis.

3.1. The ECM remodeling

The tumor milieu consists of a three-dimensional, protein-rich ECM and stromal cells. The main structural components of the ECM include collagens, laminin, fibronectin, tenascins, integrins, thrombospondin (TSP), and matrix-degrading proteases and their inhibitors such as matrix metalloproteinases/tissue inhibitor of matrix metalloproteinase (MMPs/TIMP) and urokinase plasminogen activator/plasminogen activator inhibitor (uPA/PAI) (Hiscox et al., 2011). The functional roles of these ECM components in cancer are shown in Table 1. Indeed, the composition of the ECM is often altered via soluble factors released by cancer and/or stromal cells. Selective degradation of the ECM is both a process of tissue remodeling and a characteristic of tumor development and invasion (Radisky et al., 2002).

3.1.1. Promoting production of ECM proteins—It is generally accepted that CAFs are the major cells responsible for synthesis, degradation, and modification of the ECM (Gaggioli et al., 2007). Activated CAFs can increase production of ECM proteins, such as collagens (type I, III, and V), integrins, fibronectin, tenascin C, laminin, and TSP (Bremnes et al., 2011). Integrins are cell surface adhesion receptor proteins and the key contributors of tumor progression by mediating cell-matrix and cell-cell adhesion. The force-mediated matrix remodeling is dependent on integrin α 3 and α 5 subunits (Gaggioli et al., 2007). Integrin α 5 β 1 regulates MMPs expression and activity (Maity et al., 2011). In CAFs, integrin α 5 β 1 is upregulated and plays an important role in CAF-driven tumorigenesis and metastasis by increasing cell motility (Coulson-Thomas et al., 2010). Furthermore, integrins (e.g., α v β 3 and α v β 5) can mediate activated endothelial cells to contact with ECM proteins (Buerkle et al., 2002), offer docking sites for endothelial cells, endothelial stem cells, and

inflammatory cells to locate at the site of angiogenesis, and activate signals for endothelial cell migration and survival (Carter, 2010).

Compared with their normal counterparts, most solid tumors usually exhibit extensive proliferation of stromal fibroblast and deposition of ECM components, which form a fibrotic state known as desmoplastic or “reactive” stroma that is associated with poor patient prognosis (Sis et al., 2005; Nystrom et al., 2011) and has significant reference value for diagnosing cancer through image examination (Itoh et al., 2006; Gokalp et al., 2012). As the most abundant ECM proteins in the stroma, collagens contribute to the tensile strength and structural integrity (Kolacna et al., 2007). The covalent cross-linking collagens and elastin are modulated by lysyl oxidase (LOX), an enzyme secreted primarily by fibroblasts (Xiao & Ge, 2012). Inhibition of LOX significantly reduces cross-linked fibrillar collagen (a major component of desmoplastic stroma and fibrosis), fibroblast activation, endothelial cell recruitment, and production of growth factors and cytokines (Barry-Hamilton et al., 2010).

3.1.2. Releasing proteases—In addition to release a range of ECM proteins, CAFs also produce proteases, such as various MMPs, uPA, and FAP, which collectively influence cancer progression by degrading and remodeling the ECM in tumors tissues.

MMPs, which belong to a group of zinc-dependent endopeptidases, play the most important roles in degradation and remodeling of the ECM, which in turn affect tumor invasion and metastasis (Augsten et al., 2010). The TIMPs control MMP activities and therefore minimize matrix degradation. There are more than 20 known human MMPs, which are classified into diverse subtypes according to their structure and substrate specificity. The different stromal and cancer cells produce various sets of MMPs. It is a well-accepted opinion that CAFs are the major source of MMPs, including MMP-1, -2, -3, -9, -11, -13 and -14/membrane type 1 MMP (Heppner et al., 1996; Singer et al., 2002; Bisson et al., 2003). MMP-1 and MMP-13 can degrade collagens; MMP-2 and MMP-9 are gelatinases; MMP-3 and MMP-11 belong to the stromelysins; and MMP-14 is one of the membrane-type MMPs (Bourboulia & Stetler-Stevenson, 2010). Some MMPs have synergistic effects during the remodeling of the tumor-associated BM (Taniwaki et al., 2007). Besides that, MMPs can cleave cell adhesion molecules such as cadherins (Ribeiro et al., 2010) and CD44 (Kajita et al., 2001), proteolytically activate TGF- β (Yu & Stamenkovic, 2000), leading to an increased tumor invasion and angiogenesis. MMPs are also involved in the modulation of the inflammatory response by regulating cytokines and chemokines, such as tumor necrosis factor- α (TNF- α), interleukin (IL)-1 β , stromal-derived factor-1 (SDF-1), and monocyte chemoattractant protein-1 (MCP-1), promoting cancer progression (Manicone & McGuire, 2008).

The uPA system consists of the serine protease uPA, a cellular membrane binding protein (uPA receptor, uPAR) and two endogenous PAIs (PAI-1 and PAI-2). The components of the uPA system are able to induce ECM degradation, facilitate angiogenesis, and lead to tumor invasion and metastasis (Duffy, 2004; Dass et al., 2008). The expression of uPA and uPAR by cancer cells or stromal cells (e.g., CAFs and macrophages) depends on cancer type. For example, uPA is expressed primarily by fibroblasts in breast, ovarian, and colon cancer, whereas cancer cells express both uPA and uPAR in skin squamous cell carcinoma (Rasanen & Vaheri, 2010). The uPA is localized predominantly in CAFs at the invasive front of tumors (Pyke et al., 1991), activating plasmin to plasminogen and further enabling the activation of various proteases (Andreasen et al., 2000). Elevated levels of uPA and PAI-1 are associated with an aggressive course (Steiner et al., 2008; Jelisavac-Cosic et al., 2011). In 2007, uPA and PAI-1, as the first-level-of evidence-1 cancer biomarkers, were recommended by the American Society of Clinical Oncology and other national guidelines

to assess the risk of patients with breast cancer experiencing disease recurrence and to predict response to systemic adjuvant chemotherapy (Schmitt et al., 2010).

FAP, a membrane-bound serine protease of the prolyl oligopeptidase family with unique post-prolyl endopeptidase activity (Kelly, 2005), selectively expresses in CAFs of nearly all epithelial carcinoma (Bremnes et al., 2011). It may directly degrade the ECM or indirectly be involved in the activation/modification of other ECM-proteases/protease inhibitors, thus facilitating the formation of a tumor-permissive milieu (Liu et al., 2012). FAP modulates protein levels of tenascin C and collagen I as well as increases organization of fibronectin and collagen fiber. FAP-dependent alterations in structure and composition of the ECM promote directionality and velocity of tumor cells (Lee et al., 2011). In synchrony with MMP-1, FAP can cleave partially degraded or denatured collagen I (Christiansen et al., 2007). Moreover, FAP associates with integrin $\alpha 3\beta 1$, MMP-2, membrane type 1 MMP, and uPA to increase tumor cell invasion by their synergistic effects on ECM-degradation and adhesion (Liu et al., 2012). It is understandable that FAP α expression is associated with tumor distant recurrence and poor recurrence-free and overall survival in rectal cancer patients (Saigusa et al., 2011).

3.2. Immunosuppressive effects

In general, CAFs may directly affect antitumor immune response by modulating the function of inflammatory cells, and indirectly impact immune response through recruitment of inflammatory cells (e.g., monocytes and macrophage) (Silzle et al., 2003).

Tenascin C, one of the immuno-modulating ECM molecules released by CAFs, can inhibit the migration of monocytes into tumor cell areas and adhesion of T lymphocytes to fibronectin depending on integrin $\alpha 5\beta 1$ and/or $\alpha 4\beta 1$ (Hauzenberger et al., 1999; Loike et al., 2001). TSP-1 is another matricellular glycoprotein with immuno-modulating activity. It can affect antigen-presenting cells (monocytes, macrophages, and dendritic cells) and T lymphocytes, involved in tumor-mediated immunosuppression by interaction with immune cells or activation of growth factors (e.g. TGF- β) to deposit in the ECM (Silzle et al., 2004). Evidence for the role of CAFs in the T-cell regulatory process is somewhat contradictory. Nazareth et al. (2007) reported that CAFs from lung cancers probably affect tumor-associated T-cell function, whereas Haniffa et al. (2007) found that normal fibroblasts isolated from skin also can inhibit T-cell proliferation and activation. In subsequent studies, Balsamo et al. (2009) documented CAFs derived from metastatic melanomas show strong immunosuppressive activity by interfering with natural killer cell functions including cytotoxicity and cytokine production. Kraman et al. (2010) presented that a subset of FAP-expressing stromal cells (all stained with α -SMA) are an immunosuppressive TME component. The depletion of FAP $^{+}$ cells causes rapid hypoxic death of cancer and stromal cells by an interferon- γ and TNF- α -dependent mechanism. Taken together, these observations advocate that CAFs play an important role in suppressing immune response.

3.3. Growth factor and cytokine networks

CAFs secrete various growth factors and cytokines, such as TGF- β , hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), PDGF, SDF-1, cyclooxygenase-2 (COX-2), epidermal growth factor, MCP-1, and interleukins (IL-1, IL-6 and IL-8) in an autocrine and/or paracrine manner, which directly promote growth of the adjacent cancer cells and angiogenesis (Kalluri & Zeisberg, 2006) or indirectly promote *de novo* angiogenesis and metastatic spread by recruiting infiltration of immune cells (Gerber et al., 2009). The functional roles of several important signaling pathways (e.g., TGF- β /Smad, HGF/Met, and PDGF/PDGFR) are shown in Table 2.

3.3.1. TGF- β signaling—It is a well-known phenomenon that TGF- β acts in a dual role: a tumor suppressor in the early stage and a tumor promoter in the advanced stage (Ijichi et al., 2011). The source of TGF- β is not only the cancer epithelial cells but also stromal cells including CAFs through complex autocrine and paracrine loops. There is evidence that TGF- β -mediated signaling between the tumor cells and fibroblasts is bidirectional, involves a distinct combination of signaling cascades, and leads to different molecular outcomes (Stuelten et al., 2010). TGF- β increases its endogenous production via T β R-Smad signaling in stromal fibroblasts, thereby facilitating the generation of an autocrine signaling loop in a positive-feedback manner (Kojima et al., 2010).

TGF- β liberated from tumor cells is responsible for the FMT process (Ronnov-Jessen & Petersen, 1993), which is dependent on cell adhesion and integrin signaling via focal adhesion kinase (Thannickal et al., 2003). It participates in the EMT process through the cooperation with MAPK signaling and stimulating production of PDGF (van Zijl et al., 2009). *In vitro* TGF- β derived from the fibroblasts enhanced the migration and dissemination of tumor cells through the activation of Smad, TAK1-mediated p38, and JNK signaling cascades (Stuelten et al., 2010). TGF- β signaling in stromal cells upregulates not only the expression of bFGF (Story et al., 1996), which is involved in FMT, but also the synthesis and release of connective tissue growth factor, which can increase tumor cell growth, intravasation, and metastatic dissemination by promoting CAFs proliferation (Mazzocca et al., 2010). Moreover, TGF- β is associated with ECM degradation (Hagedorn et al., 2001), enhancement of tumor cell invasiveness (Giannelli et al., 2002), angiogenesis (Mazzocca et al., 2009), and immunosuppressive effect (Schlingensiepen et al., 2011).

3.3.2. HGF/Met signaling—HGF, also known as scatter factor, is a heterodimeric protein. Its sole functional receptor is a transmembrane tyrosine kinase (TK), Met (Tokunou et al., 2001). In the tumor tissues, the dysregulated activation of the HGF/Met axis is due to *Met* gene amplification and mutation (Smolen et al., 2006; Krishnaswamy et al., 2009) and aberrant HGF production (Navab et al., 2009). Although most cancer cells do not produce HGF, they still can have crosstalk with CAFs by secreting HGF-inducers, such as IL-1 β , bFGF, PDGF, TGF- α , and prostaglandin E₂ (PGE₂), subsequently inducing CAFs to secrete HGF (Matsumoto & Nakamura, 2006). Intriguingly, CAFs produce HGF to act on cancer epithelial cells by a paracrine mechanism as well as themselves by an autocrine process, which in turn benefits tumor progression (Tokunou et al., 2001).

The activation of HGF/Met pathways significantly alters the motility of tumor cells (Maulik et al., 2002). HGF protects tumor cells from apoptosis, resulting in resistance to chemotherapy (Provencal et al., 2010). It regulates the production of uPA and uPAR, involving the breakdown of ECM (Nishimura et al., 2003). HGF and chemical hypoxia cooperatively promote VEGF secretion, leading to tumor angiogenesis (Koga et al., 2007). HGF and its signaling also promotes carcinogenesis and EMT (Ogunwobi & Liu, 2011), and contributes to peritoneal dissemination of gastric cancer cells (Toiyama et al., 2012).

3.3.3. PDGF/VEGF superfamily in angiogenesis—CAFs are the major source of VEGF, although VEGF is produced by both cancer epithelial cells and CAFs. Cancer-stromal interaction can increase the production of VEGF (Koshida et al., 2006). CAFs-derived PDGF has been shown to be a key mediator in stimulating VEGF production (Crawford et al., 2009).

The VEGF family is a group of secreted glycoproteins including VEGF A-D, which bind to three transmembrane TK receptors, VEGFR 1-3 (Andersen et al., 2009). VEGFs and their receptors have emerged as paramount mediators of angiogenesis and lymphangiogenesis (Ohta et al., 1999; Cao et al., 2004), involving not only endothelial proliferation, survival

and migration but also the formation of vascular structures (Biselli-Chicote et al., 2012). Additionally, they have vascular permeability activity, leading to increase interstitial fluid pressure in tumor stroma that is associated with chemotherapeutic resistance (Salnikov et al., 2006).

Conceivably, PDGF/PDGFR signaling is involved in angiogenesis in several ways. First, PDGFs recruit bone-marrow progenitor cells; induce them to differentiate into endothelial cells or smooth muscle cells; and promote endothelial/smooth muscle cell growth, and/or migration (Li et al., 2005). Second, PDGF-B derived by endothelial cells recruit pericytes to the vessel wall. In turn, pericytes secrete angiopoietin-1 to promote survival and sprouting of endothelial cells, and maintain endothelial stability, leading to vascular maturation (Augsten et al., 2010; Fuxe et al., 2011). Third, PDGFs indirectly induce angiogenesis by recruitment of VEGF-producing stromal fibroblasts (Dong et al., 2004). Blockade of this signaling can decrease stromal reaction, microvessel areas, and number of pericytes (Sumida et al., 2011). In addition, PDGF/PDGFR signaling can induce EMT (Jechlinger et al., 2006), recruit and activate different subsets of CAFs, and increase interstitial fluid pressure (Anderberg et al., 2009; Sumida et al., 2011).

3.3.4. Pathways involving chronic inflammatory response—CAFs exhibit a specific proinflammatory signature responsible for its tumor-promoting behavior (Erez et al., 2010). Based on the study of human prostate CAFs, Augsten et al. (2009) found that CAFs-derived orphan chemokine CXCL14 promotes tumor growth by stimulating fibroblast growth and migration, attracting monocytes infiltration, and enhancing angiogenesis. The upregulation of cytokines IL-6, IL-8, and IL-1 β is associated with the inflammatory network, promoting tumorigenesis, angiogenesis, and metastasis (Schauer et al., 2011). Co-cultured with CAFs, oral squamous carcinoma cells increase invasiveness due to upregulation of the chemokine MCP-3 in CAFs that induced by IL-1 α released from cancer cells (Jung et al., 2010). In colorectal cancer, IL-8 is correlated to tumor size, depth of infiltration, the disease stage, liver metastases (Terada et al., 2005), and increase proliferation and migration of cancer cells (Itoh et al., 2005). IL-8 expression in CAFs is highly induced by proinflammatory TNF- α through activation of nuclear factor kappa B (NF- κ B) (Mueller et al., 2007). Colorectal liver metastasis may be supported by CAFs to generate a prometastatic microenvironment through inflammatory activation of IL-6 and MCP-1, similarly and strongly induced by TNF- α (Mueller et al., 2010).

Our laboratory also has determined that another stromal-activating chemokine, growth-regulated oncogene- α —overexpressing ovarian fibroblasts with inhibited p53, can significantly increase ovarian cancer cell proliferation and tumorigenic growth in a mouse xenograft model (Yang et al., 2006). Recently, we provided evidence that elevated expression of IL-1 β and cognate receptor IL-1R1 in ovarian CAFs predicts reduced overall survival duration in patients. Knockdown of p53 expression in ovarian fibroblasts significantly enhanced the expression and secretion of IL-8, growth regulated oncogene- α , IL-6, IL-1 β , and VEGF; significantly increased *in vivo* mouse xenograft ovarian cancer tumor growth; and entirely depended on interaction with NF- κ B (Schauer et al., submitted for publication).

SDF-1/CXCR4 signaling contributes to chemoresistance by inducing the activation of focal adhesion kinase, ERK, and Akt pathways, enhancing transcriptional activities of β -catenin and NF- κ B, and expressing antiapoptotic proteins such as Bcl-2, BCL-xL, survivin (Singh et al., 2010). It also mediates actin polymerization and pseudopodia formation, subsequently induces chemotactic and invasive responses (Muller et al., 2001). Furthermore, SDF-1/CXCR4 signaling may promote the outgrowth of new blood vessels by recruiting circulating endothelial progenitor cells into the tumor stroma (Orimo et al., 2005) or inducing the

secretion of MMPs and proangiogenic factors (e.g., VEGF and IL-8) (Verbeke et al., 2011). It enhances adhesion of circulating tumor cells to vascular endothelial cells, producing prometastatic effects within the intravascular microenvironment (Cardones et al., 2003; Song et al., 2009). CXCR7 (another SDF-1 receptor) can increase the expression of IL-8 and VEGF and act on CD44 and cadherin-11, contributing to angiogenesis and tumor invasion (Wang et al., 2008). In parallel with this, the upregulated expression of CXCR7 by IL-8 promotes tumor growth via EGFR activation (Singh & Lokeshwar, 2011). Furthermore, during tumor progression, preexisting stromal fibroblasts acquire increased cross-communicated TGF- β and SDF-1 autocrine signaling loops, which are probably triggered initially by TGF- β released by cancer epithelial cells, act in a positive-feedback manner, and self-stimulate to maintain their transdifferentiation as myofibroblasts (Kojima et al., 2010).

Recently, epidemiologic studies suggested that long-term daily use of nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin, can reduce the risk of death from cancer (Rothwell et al., 2011). As a result, COX-2 become a potential therapeutic target in cancer treatment and prevention (Lorusso & Ruegg, 2008). COX-2 is a key mediator of inflammatory stimuli and derived by both stromal (e.g., monocytes, macrophages, endothelial cells, and fibroblasts) and epithelial cells. Induced by growth factors, cytokines, and hormones, COX-2 expression is markedly increased at the inflammatory sites (Cathcart et al., 2012). The COX-2/PGE₂ axis indirectly regulates tumor stromal formation and/or angiogenesis via SDF-1/CXCR4 signaling, VEGF, and bFGF, or directly modulates endothelial cell proliferation involving angiogenesis (Saji et al., 2004; Katoh et al., 2010). Blockade of COX-2 can decrease the invasiveness of cancer cells acquired through tumor-stromal interactions (Sato et al., 2004).

4. Therapeutic strategies designed to target the TME

Traditionally, the main treatment of patients with cancer is chemotherapy with cytotoxic agents, radiotherapy, debulking surgery, or any combination of these three methods to reduce tumor cells. With increasing understanding that alterations of the tumor milieu also play pivotal roles in tumorigenesis and progression, it is believed that targeting therapy against the TME, particularly CAFs, open up the new opportunities for cancer therapy. In general, these novel perspective treatment strategies can be summarized as follows: targeting stromal components (such as the ECM and CAFs), and interfering with growth factor/cytokine-mediated signaling involved in CAFs (Fig. 3).

4.1. The ECM antagonists

The advantage of integrin's function as cellular glue and signaling molecule enables it to become a target for cancer therapy. Cilengitide (EMD 121974), a $\alpha_v\beta_3$ and $\alpha_v\beta_5$ antagonist, appeared to prolong survival in a phase II randomized clinical trial when combined with standard radiochemotherapy in patients with newly diagnosed glioblastoma regardless of methylguanine-DNA methyltransferase (MGMT) methylation status (Nabors et al., 2012). A phase III trial is ongoing to further assess the efficacy and safety of cilengitide combination therapy for newly diagnosed glioblastoma with MGMT methylation (ClinicalTrials.gov numbers, NCT00689221, status of clinical trial uses term as defined at ClinicalTrials.gov.). In contrast, the prospects for anti-integrin monoclonal antibody (mAb) are hardly optimistic. Vitaxin (MEDI-522) and volociximab (M200) are two mAbs that have specificity for the integrins $\alpha_v\beta_3$ and $\alpha_5\beta_1$, respectively (Posey et al., 2001; Ricart et al., 2008). Despite the fact that they are well tolerated, insufficient clinical activity (Hersey et al., 2010; Bell-McGuinn et al., 2011) cannot warrant their further studies. Subsequent phase II studies have been terminated (NCT00684996, NCT00369395, NCT00100685, and NCT00516841). Nonetheless, the known promising results in clinical trials still attract a

broad interest in anti-integrin therapies, and hopefully an increasing number of target agents will be developed.

Thus far, the strategies to target the uPA system comprise inhibiting uPA/uPAR expression and activity, or blocking uPA-uPAR interaction. In preclinical studies, inhibition of uPA/uPAR with antibodies or small interfering RNA (siRNA) has caused a significant restraint in tumor cell growth and invasion (Kargiotis et al., 2008; Duriseti et al., 2010). WILEX AG develops two small synthetic molecules specifically against uPA, the intravenous drug WX-UK1 and its oral pro-drug WX-671/Mesupron, which can generate pharmacologically active WX-UK1 by reductively converting the hydroxyamidino function to the amidino function (Meyer et al., 2008). Data from a number of early clinical monotherapy and combination therapy studies show that these drugs are safe and well tolerated without serious adverse events (Goldstein, 2008; Meyer et al., 2008). A combination of WX-UK1 with capecitabine has exhibited therapeutic benefit, including prolonged stable disease and partial responses (Schmitt et al., 2011). Currently, phase II trial with WX-671 and gemcitabine are being conducted in patients with advanced pancreatic cancer (NCT00499265). Accordingly, another noncompetitive peptide-antagonist of the uPA-uPAR interaction called A6 has been confirmed as safe without dose-limiting toxicities in phase I trials for patients with advanced ovarian cancer (Berkenblit et al., 2005). The results from a phase II, randomized, double-blind, placebo-controlled trial of subcutaneous A6 in patients with asymptomatic CA125 progression after first-line chemotherapy for ovarian cancer suggest that A6 therapy shows extending time to clinical disease progression (Ghamande et al., 2008).

MMP inhibitors are promising since they play a role in both epithelial-CAF signaling and ECM remodeling. However, so far, no MMP inhibitors have become a clinical reality for cancer treatment. Several broad-spectrum MMP inhibitors (e.g., marimastat and tanomastat/BAY12-9566) have failed in randomized phase III trials in patients with breast and ovarian cancer because they do not prolong overall and/or progression-free survival or exhibit dose-dependent toxicity (Sparano et al., 2004; Hirte et al., 2006). Future efforts possibly need focus on the development of highly selective MMP inhibitors.

4.2. Anti-CAF therapy

CAFs have been selected as a therapeutic target because of their important roles in tumor progression as well as their genetic stability compared with frequently mutant tumor cells, which may reduce drug resistance (Cirri & Chiarugi, 2012). Anti-CAF therapy possibly has a triple effect via anti-tumor qualities, anti-angiogenesis potencies, and the ability to increase tumor uptake of chemotherapeutic drugs.

It is well established that the tumor-stromal antigen FAP which is expressed selectively in CAFs, can serve as a novel target for the TME against cancer. However, similar to tumor cells, CAFs also have significant individual differences in drug sensitivity. For example, CAF sensitivity to cytotoxic chemotherapy seems to rely on cancer type as well as on the TME (Sonnenberg et al., 2008). It is therefore important to note that FAP inhibition may not halt the tumor cell growth in all cancer types (Liu et al., 2012).

Potential therapeutic strategies include inhibiting FAP enzymatic activity or delivering drugs/toxins to the tumor stroma via antibodies or small-molecular inhibitors. Although a phase I study showed that the biodistribution of ¹³¹I-labelled sibrotuzumab (anti-FAP mAb, F19) selectively accumulated in the tumor area (Scott et al., 2003), a phase II clinical study of unconjugated sibrotuzumab in patients with metastatic colorectal carcinoma demonstrated no clinical activity (Hofheinz et al., 2003). A small-molecule inhibitor Val-boroPro (PT100, talabostat) against multiple cytoplasmic and membrane dipeptidyl peptidases, including FAP, DPP-IV, DPP8, and DPP9, was tested in a clinical phase II trial and also showed

minimal clinical improvement. Nevertheless, these medicines were proven to be well tolerated and may lay the groundwork for future studies (Hofheinz et al., 2003; Narra et al., 2007).

Taking advantage of FAP-restricted tumor expression and of its enzymatic activity, some researchers have directly conjugated anti-FAP mAb with toxins or designed selectively targeted FAP-activated prodrugs/prototoxins to deliver cytotoxic potent agents to the tumor area. In preclinical studies, treatment with mAb FAP5-DM1 (an anti-FAP mAb–maytansinoid conjugate) showed long-lasting inhibition of tumor growth and complete remission in tumor xenograft models without intolerability (Ostermann et al., 2008). The evaluation of FAP-activated doxorubicin prodrug (doxorubicin conjugated with a FAP α -specific dipeptide, Z-Gly-Pro) showed this targeted-delivery strategy was safe and had high antitumor efficiency (Huang et al., 2011). Intratumoral injection of a FAP-activated promelittin prototoxin produced significant growth inhibition of human breast and prostate cancer xenografts with minimal toxicity to the host animals (LeBeau et al., 2009). These promising results may lead to the translation of this strategy into new clinical realities in the future.

Despite a poor understanding of how epigenetic alteration is triggered, there is an increasing awareness that this alteration is an important event during the activation of fibroblasts. Treatment of hepatic stellate cells with DNA methylation inhibitor 5-aza-2'-deoxycytidine can block their transdifferentiation to myofibroblasts *in vitro* (Mann et al., 2007). The epigenetic therapies' effect on the TME and tumor cells may produce the synergistic antitumor response (Place et al., 2011). Therefore, epigenetic interventions are becoming a very attractive candidate for tumor stroma-specific therapies. This strategy has been well presented by Gonda et al. (2010), who emphasize that inhibition of DNA methyltransferase (DNMT1, DNMT3a, and DNMT3b) may offer an opportunity to inhibit CAFs and foresee its prospects.

4.3. Interfering with growth factor/cytokine-mediated crosstalk between CAFs and cancer cells

The signaling between CAFs and epithelial cancer cells offer multiple attractive targets for intervention. Discovering that there are tight interactions between tumor progression and angiogenesis or chronic inflammatory milieu led to the intensive investigation of relevant growth factor/cytokine-mediated signaling pathways as targets for cancer therapy (the currently available data in the latest phase of clinical trials are summarized in Tables 2 and 3). Here are a few signaling pathways that have been actively targeted using a variety of agents:

4.3.1. Disabling TGF- β signaling—TGF- β signaling antagonists include three categories: antisense oligodeoxynucleotide against TGF- β production, small-molecule inhibitors against T β R kinases, and anti-TGF- β /T β R antibodies against ligand-receptor interactions. Each agent in these categories has shown efficacy for halting tumor growth and dissemination in preclinical data (Tzai et al., 1998; Nam et al., 2008; Mazzocca et al., 2009).

Trabedersen (AP 12009), a soluble phosphorothioate antisense oligodeoxynucleotide that is specific for human TGF- β 2 biosynthesis (Jaschinski et al., 2011), has been studied in clinical trials for high-grade glioma, melanoma, pancreatic cancer, and colorectal carcinoma (NCT00431561 and NCT00844064). Trabedersen treatment of patients with recurrent or refractory high-grade glioma led to long-lasting tumor responses and prolonged survival time (Hau et al., 2011). An optimal dose of trabedersen for further clinical development also has been suggested by a randomized and controlled phase IIb study (Bogdahn et al., 2011). These very encouraging results warrant disabling TGF- β pathway as a therapeutic target.

However, due to a lack of patients caused by changes in WHO brain tumor definitions, advances in neurosurgery, and standard of first-line treatment, the pivotal phase III SAPPHERE study in patients with recurrent/refractory anaplastic astrocytoma or secondary glioblastoma (NCT00761280) had to be terminated.

Here, some negative results are worth noting. Recently, in mouse models, long-term LY2109761 (a small-molecule inhibitor of T β RI kinase) exposure led to acquired resistance due to elevated phospho-Smad2 levels. Resultant tumors presented more aggressive and inflammatory features (Connolly et al., 2011). Inhibition of TGF- β also may lead to autoimmune reactions or promote the progression of precancerosis (Massague, 2008). Therefore, it is important to determine how to use TGF- β inhibitors. Only acute administration will likely be required for clinical applications; long-term patient exposure to TGF- β inhibitors should be cautious (Connolly et al., 2011).

4.3.2. Inhibiting the HGF/Met axis—Inhibiting the HGF/Met axis has become one of the hottest approaches in anticancer drug exploitation and is designed with distinct lines such as the following: neutralization using anti-HGF/Met antibodies; knockdown of HGF/Met expression by siRNA and ribozyme; blockade of HGF-Met association by soluble Met, uncleavable single-chain HGF; and suppression of TK by synthetic compounds (Matsumoto & Nakamura, 2006). Owing to effective therapeutic results in the preclinical studies, some inhibitors have been picked up for clinical development.

Among selective inhibitors chosen for potential therapeutic applications, AMG 102 (rilotumumab) and ARQ 197 (tivantinib) have been the most extensively studied. AMG 102 is a humanized mAb against HGF, and several phase II studies (including NCT01039207, NCT01105390, and NCT01113398) will evaluate its tolerance and efficacy as a sole agent or in combination regimens in patients with malignant solid tumors. ARQ 197 is an oral, non-ATP-competitive, small-molecule selective inhibitor of the MET receptor TK (Goldman et al., 2012). Based on clinical data from early clinical trials, two new phase III, randomized, double-blind studies of ARQ 197 plus chemotherapy in locally advanced or metastatic nonsquamous, non-small-cell lung cancer (NSCLC) are in progress (NCT01244191 and NCT01377376). Hopefully, the results will provide a wealth of information on the role of ARQ 197 in treating this type of cancer.

The known data suggest multi-targeted agents also have development potential. Some advanced clinical validations are currently in the pipeline. For example, cabozantinib (XL184) is an antagonist that inhibits VEGFR2, Met and Ret. Phase III clinical trials of chemotherapeutic drug combinations for treatment of patients with advanced thyroid cancer or castration-resistant prostate cancer are recruiting or ongoing (NCT01605227, NCT01522443, and NCT00704730). The safety and efficacy of crizotinib (PF-02341066), a Met and anaplastic lymphoma kinase (ALK) inhibitor, will be observed in a phase IV trial of the patients with NSCLC (NCT01597258).

4.3.3. Stifling angiogenesis—The strategy stifling angiogenesis is regarded as a desirable anticancer approach aimed at multifaceted rationale including enhancement of chemotherapeutic sensitivity and reduction of tumor burden and metastasis (Lin & Sessa, 2004). It has become the first clinical reality among all approaches for targeting the TME owing to some antiangiogenesis drugs receiving FDA approval.

The selective inhibition of the VEGF pathway has been designed for neutralizing VEGF or VEGFR by their respective mAb or for interfering with VEGF-VEGFR binding via decoy receptors (e.g., aflibercept). Bevacizumab (Avastin) is a recombinant humanized anti-VEGF mAb. In combination with chemotherapy or IFN- α , it has been approved for patients with

advanced and/or metastatic colon cancer, renal cell carcinoma (RCC), and NSCLC (Hurwitz et al., 2004; Albiges et al., 2011). Ramucirumab (IMC-1121B) is another fully human mAb that specifically inhibits VEGFR-2 (Krupitskaya & Wakelee, 2009). Several phase III studies of ramucirumab in patients with NSCLC (NCT01168973), digestive tract adenocarcinoma (such as NCT01170663), hepatocellular carcinoma (HCC, NCT01140347), and breast cancer (NCT00703326) are underway. Moreover, aflibercept (VEGF Trap, AVE0005) in combination with chemotherapy has demonstrated significant improvements in progression-free and/or overall survival in patients with advanced NSCLC or metastatic colorectal cancer (Gaya & Tse, 2012) but fails in metastatic pancreatic cancer (NCT00574275).

Specifically inhibiting the PDGF pathway is achieved mainly via human anti-PDGF/PDGFR mAb or highly specific anti-PDGFR small-molecular TK inhibitors (TKIs). IMC-3G3 and MEDI-575 are the fully IgG1 and IgG2 mAb against PDGFR α , respectively. Phase I trials in patients with advanced solid malignancies and lymphomas have revealed that these agents are well tolerated (Shah et al., 2010; Kono et al., 2012). Recently, several phase II clinical trials, using IMC-3G3 or MEDI-575 alone or in combination, recruit for patients with advanced, recurrent, or metastatic tumors including NSCLC (NCT00918203), glioblastoma (NCT00895180 and NCT01268566), and gastrointestinal stromal tumor (GIST, NCT01316263). CR002, a fully mAb directed against PDGF-D, is shown to be safe but has not been studied for its efficacy in cancer patients (Wang et al., 2010). SU101 (leflunomide) is a highly specific anti-PDGFR TKI as well as minimally interacts with EGFR and insulin growth factor-1 receptor (Eckhardt et al., 1999). SU101 monotherapy in patients with hormone-refractory prostate cancer has shown the modest effects (Ko et al., 2001). Phase II/III trials of SU101 combination therapy for stage IV prostate cancer (NCT00004071) and recurrent glioblastoma multiforme (NCT00003293) have been completed, but results have not been described.

Recent studies have shown that some tumors may overcome inhibition of VEGF-mediated angiogenesis through upregulation of PDGF-C (Crawford et al., 2009). In this regard, multiple TKIs, which target VEGFR, PDGFR and the other receptor TK (such as FGFR, FLT-3, and c-Kit), have become a novel class of antitumor drugs. Based on clinical data in the treatment of NSCLC, Subramanian et al. (2010) and Kono et al. (2012) summarized therapeutic effects of these multiple TKIs and discussed their future development.

4.3.4. Neutralizing inflammatory response—The blockade of the SDF-1–CXCR4/CXCR7 axis has been selected as a potential additional target for standard neoadjuvant and adjuvant treatments. Some SDF-1/CXCR4 signaling antagonists, e.g., the anti-CXCR4 drugs plerixafor (AMD3100) and BKT140 (4F-benzoyl-TN14003), the anti-SDF-1 analog CTCE-9908, and the anti-SDF-1 aptamer NOX-A12, have shown significant inhibitory effects of metastasis and of primary tumor growth in mouse tumor models (Tamamura et al., 2003; Smith et al., 2004; Kim et al., 2008). Two phase III clinical trials of plerixafor plus granulocyte colony-stimulating factor (G-CSF) for autologous stem cell mobilization in patients with multiple myeloma or non-Hodgkin lymphoma showed that plerixafor was well tolerated (DiPersio et al., 2009a; DiPersio et al., 2009b). Analogously, BKT140 and NOX-A12 were studied in clinical trials for multiple myeloma and chronic lymphocytic leukemia (NCT01010880, NCT01521533, and NCT01486797). CTCE-9908 has been approved for clinical use in patients with osteosarcoma (Duda et al., 2011).

The accumulation of knowledge on the SDF-1/CXCR7 signaling pathway revealed that CXCR7 also should be used as a therapeutic target. Synchronously targeting CXCR4 and CXCR7 might be more efficient than merely targeting CXCR4 because some shared signaling molecules are blocked (Maksym, et al., 2009). Consequently, various specific

CXCR7 blockers such as small-molecular compounds CCX2066, siRNA, and mAb are already being studied in experimental animal models (Hartmann et al., 2008; Duda et al., 2011). Further studies are needed to translate these inhibitors into the clinic.

Neutralization of the inflammatory response has long been tested with COX-2 inhibitors that are divided as nonselective NSAIDs (e.g., aspirin, ibuprofen, and naproxen) and selective COX-2 inhibitors (COXIBs, e.g., celecoxib and etoricoxib). Many clinical trials of COX-2 inhibitory agents alone or in combination with other drugs are in progress (e.g., NCT01111591, NCT01614301, and NCT00427999). However, various adverse effects have been associated with the use of NSAIDs and COXIBs, such as gastrointestinal complications and inhibition of platelet activation linked with COX-1–specific adverse effects, and high incidence of cardiovascular events associated with prolonged use of selective COX-2 inhibitor. These risks have emerged as important factors to consider during inhibition of COX-2 (Menter et al., 2010). Therefore, although a clinical trial has shown that celecoxib can effectively prevent adenoma formation in patients at high risk of colorectal cancer, it still cannot be recommended routinely for clinical application (Bertagnolli et al., 2006).

On the basis of data from *in vitro* observation, El-Awady et al. (2011) indicated that the combination of celecoxib with chemotherapeutics should be tailored according to tumor type, drugs used, and drug administration schedule. In support of this claim are clinical studies showing that celecoxib combined with carboplatin led to promising clinical activity in patients with recurrent ovarian cancer (Legge et al., 2011); however, in patients with advanced NSCLC, no prolonged survival duration were observed after therapy using celecoxib plus docetaxel and carboplatin (Groen et al., 2011).

5. Conclusions and future direction

Cancer is considered one of the most challenging diseases because of its resistance to treatment. As knowledge accumulates on the crucial roles of the tumor milieu, the consensus is that the TME is an integral part of the tumor that not only provides tumor architectural support but also affects its physiology and function. In particular, CAFs, the most abundant component of TME, play multiple roles in tumor development and therefore offer attractive targets for therapeutic intervention. Numerous studies have confirmed that the TME offer an appropriate “soil” in which to promote tumorigenesis, progression, and metastasis. The various cellular and noncellular components within the microenvironment (e.g., CAFs, integrins, MMPs, endothelial cells, and inflammatory cells) and the signaling pathways as a whole come together in the above-mentioned complicated processes. Purposefully, more and more therapeutic strategies are designed to target the TME as well as tumor cells. Among these targeted agents, some have successfully become a clinical reality and are even used as first-line drugs (such as bevacizumab); others are already in clinical trials, and many more are still in preclinical studies.

Despite this progress, enormous challenges still need to be faced, particularly in how to select the optimal cancer patients and how to establish the optimal treatment schemes. Therefore, well-established biomarker candidates and detection techniques need be developed along with therapeutic strategies targeting CAFs and the other components in TME. Meanwhile, in view of the complicated and close relationship between the TME and tumor cells, the combination of two or more therapeutic agents may be more beneficial to cancer patients than merely targeting single factor. Because we faced with numerous therapeutic agents, better understanding of the TME, more careful drug safety surveillance, and longer-term follow-up for prognostic evaluation can help us make good choices that meet clinical requirements. It is believed that tumor stroma as targets for cancer therapy has paved a wider path for therapeutic intervention of these deadly diseases.

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Abbreviations

bFGF	basic fibroblast growth factor
BM	basement membrane
CAF	cancer-associated fibroblast
COX-2	cyclooxygenase-2
COXIBs	COX-2 inhibitors
CXCR	CXC chemokine receptor
ECM	extracellular matrix
EGFR	epidermal growth factor receptor
EMT	epithelial to mesenchymal transition
FAP	fibroblast activation protein
FDA	Food and Drug Administration
FGFR	fibroblast growth factor receptor
FMT	fibroblast to myofibroblast transdifferentiation
G-CSF	granulocyte colony-stimulating factor
HCC	hepatocellular carcinoma
HGF	hepatocyte growth factor
IL	interleukin
LOH	loss of heterozygosity
LOX	lysyl oxidase
mAb	monoclonal antibody
MCP	monocyte chemoattractant protein
MGMT	methylguanine-DNA methyltransferase
MMP	matrix metalloproteinases
MSC	mesenchymal stem cell
NF-κB	nuclear factor Kappa B
NSAIDs	nonsteroidal anti-inflammatory drugs
NSCLC	non-small-cell lung cancer
PAI	plasminogen activator inhibitor
PDGF	platelet-derived growth factor
PDGFR	platelet-derived growth factor receptor

PGE₂	prostaglandin E ₂
RCC	renal cell carcinoma
SDF-1	stromal-derived factor-1
siRNA	small interfering RNA
α-SMA	alpha-smooth muscle actin
TGF-β	transforming growth factor-β
TβR	transforming growth factor-β receptor
TIMP	tissue inhibitor of matrix metalloproteinase
TK	tyrosine kinase
TKI	tyrosine kinase inhibitor
TME	tumor microenvironment
TNF-α	tumor necrosis factor-α
TSP	thrombospondin
uPA	urokinase plasminogen activator
uPAR	urokinase plasminogen activator receptor
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor

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Fig. 1. Differences between normal stroma and malignant tumor microenvironment
 (A) Normal tissue is composed of orderly arranged epithelial cells and supportive stromal cells including quiescent fibroblasts and blood vessels that collectively inhibit inappropriate epithelial proliferation. (B) In malignant tumor tissue, activated cancer-associated fibroblasts and inflammatory cells have close crosstalk to tumor cells via plentiful growth factors and cytokines; newly formed vasculature carries nutritional support to tumor cells.

Fig. 2. Eight possible origins of cancer-associated fibroblasts (CAFs) in the tumor microenvironment

locally resident quiescent fibroblasts via fibroblast to myofibroblast transdifferentiation (FMT), cancer epithelial cells via epithelial to mesenchymal transition (EMT), endothelial cells via endothelial to mesenchymal transition (EndMT), mesenchymal stem cells from the bone marrow, pericytes, smooth muscle cells, adipocytes, and inflammatory cells.

Fig. 3. Several currently therapeutic strategies aim at the tumor microenvironment targeting the fibrotic ECM and cancer-associated fibroblasts, interfering with growth factor/cytokine-mediated signaling, stifling angiogenesis, and neutralizing inflammatory response.

Table 1

The main ECM components, functional roles, and therapeutic approaches in cancer *

ECM components	Functional roles	Agents	Phase	Condition	Intervention	Identifier number (statuses)
Integrins	Tumorigenesis, invasion, angiogenesis, and metastasis via mediating cell-cell and cell-matrix adhesion	Cilengitide (EMD 121974)	III	Glioblastoma with a methylated promoter of MGMT	Cilengitide + radiotherapy + temozolomide	NCT00689221 (active, not recruiting)
		Volociximab (M200)	II	Platinum-resistant, advanced epithelial ovarian or primary peritoneal cancer	Volociximab	NCT00516841 (terminated)
				Stage IV melanoma	Volociximab	NCT00369395 (terminated)
				Metastatic RCC	Volociximab	NCT00100685 (terminated)
				Metastatic pancreatic cancer	Volociximab + gemcitabine	NCT00401570 (completed)
uPA system (uPA, uPAR and PAI)	Angiogenesis, invasion, and metastasis via inducing ECM degradation			Metastatic melanoma without previous chemotherapy	Volociximab + dacarbazine	NCT00099970 (completed)
		Vitaxin (MEDI-522)	II	Metastatic melanoma	MEDI-522	NCT00066196 (completed)
				Metastatic androgen-independent prostate cancer	MEDI-522 + docetaxel + prednisone + zoledronic acid	NCT00072930 (completed)
		A6	II	Refractory/recurrent ovarian epithelial, fallopian tube, or primary peritoneal cancer	A6	NCT00939809 (suspended)
		WX-UK1	I	Advanced malignancies	WX-UK1 + capecitabine	NCT00083525 (completed)
Tenascin C	Tumor growth and invasion via immuno-modulation	WX-671/Mesupron	II	Advanced pancreatic cancer	WX-671 + gemcitabine	NCT00499265 (completed)
		81C6	I/II	Primary/metastatic malignant brain tumor	81C6	NCT00002752 (completed)
		Marimastat	III	SCLC	Marimastat	NCT00003011 (completed)
MMPs/TIMPs	Tumor growth, invasion, angiogenesis via ECM degradation and remodeling, regulation of inflammation			Metastatic breast cancer that is responding/stable after chemotherapy	Marimastat	NCT00003010 (completed)
		Prinomastat (AG3340)	III	Stage III NSCLC	Marimastat	NCT00002911 (completed)
				Metastatic/recurrent NSCLC	Prinomastat + gemcitabine + cisplatin	NCT00004199 (completed)
Thrombospondin-1 (TSP-1)	Tumor invasion via immuno-modulation			Metastatic hormone-refractory prostate cancer	Prinomastat + mitoxantrone + prednisone	NCT00003343 (completed)
		ABT-510/TSP-1 mimetic	II	Advanced RCC	ABT-510	NCT00073125 (completed)
				NSCLC	ABT-510 + carboplatin/taxol	NCT00061646 (terminated)

ECM components	Functional roles	Agents	Phase	Condition	Intervention	Identifier number (status)
				Locally advanced/metastatic soft tissue sarcoma	ABT-510	NCT00061659 (completed)
				Refractory lymphoma	ABT-510	NCT00061672 (completed)

* Based on ClinicalTrials.gov (<http://clinicaltrials.gov/>) accessed on July 23, 2012, with updates based on status as of July 19, 2012. Only the latest phase of clinical trials is chosen.

Table 2
The functional roles of growth factor/cytokine-mediated signaling and therapeutic approaches*

Factors	Functional roles in cancer	Agents	Phase	Condition	Intervention	Identifier number (status)
TGF- β /T β R	Promote tumor progression and metastasis in advanced stage; recruit fibroblasts; induce EMT and FMT; promote proliferation and migration; affect ECM degradation; involved in angiogenesis; support tumor cell survival via evasion of immunity; enhance tumor cell invasiveness	AP12009 (trabectedin)	III	Recurrent/refractory anaplastic astrocytoma or secondary glioblastoma	AP12009	NCT00761280 (terminated)
HGF/Met	Promote tumor cell motility and angiogenesis; lead to resistance to chemotherapy and radiotherapy via protecting tumor cells from apoptosis; involved in ECM degradation; induce EMT	AMG102 (rilotumumab)	II	Advanced RCC	AMG 102	NCT00422019 (active, not recruiting)
				Grade IV malignant glioma	AMG 102 + avastin	NCT01113398 (recruiting)
				Advanced malignant glioma	AMG 102	NCT00427440 (active, not recruiting)
				Advanced gastroesophageal adenocarcinoma	AMG 102 + oxaliplatin + folinic acid + 5-FU	NCT01443065 (recruiting)
				Malignant pleural mesothelioma	AMG 102 + pemetrexed + cisplatin	NCT01105390 (not yet recruiting)
				Refractory/recurrent ovarian epithelial, fallopian tube, and primary peritoneal cancer	AMG 102	NCT01039207 (recruiting)
		Tivantinib (ARQ 197)	III	Locally advanced/metastatic non-squamous, NSCLC	Tivantinib + erlotinib	NCT01244191 (active, recruiting)
				Advanced/metastatic non-squamous, NSCLC with wild-type EGFR	Tivantinib + erlotinib	NCT01377376 (recruiting)
		Cabozantinib (XL184)	III	Metastatic castration-resistant prostate cancer	Cabozantinib	NCT01605227 (recruiting)
				Previously treated symptomatic castration-resistant prostate cancer	Cabozantinib	NCT01522443 (recruiting)
				Advanced medullary thyroid cancer	Cabozantinib	NCT00704730 (active, not recruiting)
		Crizotinib (PF-02341066)	IV	NSCLC	Crizotinib	NCT01597258 (enrolling by invitation)

Factors	Functional roles in cancer	Agents	Phase	Condition	Intervention	Identifier number (status)
VEGF/VEGFR	Paramount mediators of vascular and lymphatic vessel formation; increase vascular permeability	Ramucirumab (IMC-1121B)	III	NSCLC	Ramucirumab + docetaxel	NCT01168973 (recruiting)
				Metastatic gastric adenocarcinoma	Ramucirumab + paclitaxel	NCT01170663 (recruiting)
				HCC	Ramucirumab	NCT01140347 (recruiting)
				Metastatic colorectal cancer	Ramucirumab + FOLFIRI	NCT01183780 (recruiting)
				Adenocarcinomas of the gastric or gastroesophageal junction	Ramucirumab	NCT00917384 (active, not recruiting)
PDGF/PDGFR	Contribute to angiogenesis and lymphangiogenesis; induce EMT and FMT; recruit toward tumor area; associate with chemo-resistance via regulating interstitial fluid pressure	Aflibercept (VEGF Trap, AVE0005)	III	Her2 ⁺ , unresectable, locally recurrent/metastatic breast cancer	Ramucirumab + docetaxel	NCT00703326 (active, not recruiting)
				Metastatic colorectal cancer after failure of an oxaliplatin-based regimen	Aflibercept + FOLFIRI	NCT00561470 (active, not recruiting)
				Metastatic colorectal cancer previously treated with an oxaliplatin-based regimen	Aflibercept + FOLFIRI	NCT01571284 (recruiting)
		IMC-3G3		Untreated locally advanced/metastatic NSCLC	IMC-3G3 + paclitaxel + carboplatin	NCT00918203 (active, not recruiting)
				Platinum-refractory/resistant advanced ovarian cancer	IMC-3G3 + liposomal doxorubicin	NCT00913835 (active, not recruiting)
SDF-1-CXCR4	Mediate chemoresistance; induce chemotactic and invasive response; promote the outgrowth of new blood vessels; involve in FMT and enhancing tumor cells migration, proliferation		II	Metastatic castration-refractory prostate cancer after disease progression/intolerance on docetaxel-based chemotherapy	IMC-3G3 + mitoxantrone	NCT01204710 (active, not recruiting)
				Recurrent glioblastoma multiforme	IMC-3G3	NCT00895180 (recruiting)
		MEDI-575		Unresectable and/or metastatic GIST	IMC-3G3	NCT01316263 (active, not recruiting)
		SU101 (leflunomide)		Recurrent glioblastoma multiforme	MEDI-575	NCT01268566 (active, not recruiting)
		Plerixafor (AMD3100)		Recurrent glioblastoma multiforme	SU101 + procarbazine	NCT00003293 (completed)
COX-2/PGE ₂	Involve in inflammation and tumorigenesis; stimulate tumor cell proliferation, survival	Plerixafor (AMD3100)	IV	Non-Hodgkin lymphoma	Plerixafor + G-CSF	NCT01164475 (recruiting)
		BKT140		Non-Hodgkin lymphoma; multiple myeloma	Plerixafor + G-CSF	NCT01339572 (recruiting)
		NOX-A12		Multiple myeloma	BKT140	NCT01010880 (completed)
				Recurrent multiple myeloma	NOX-A12	NCT01521533 (recruiting)
		Colecoxib		Recurrent chronic lymphocytic leukemia	NOX-A12	NCT01486797 (recruiting)
COX-2/PGE ₂	Involve in inflammation and tumorigenesis; stimulate tumor cell proliferation, survival	Colecoxib	IV	Biliary-pancreas cancer	Celecoxib	NCT01111591 (recruiting)
		Etoricoxib		Hormone-refractory prostate cancer	Etoricoxib + pioglitazone + dexamethasone + treosulfan + Imatinib	NCT00427999 (active, not recruiting)

Factors	Functional roles in cancer	Agents	Phase	Condition	Intervention	Identifier number (status)
	and motility; regulate tumor stromal formation and angiogenesis; protect tumor cells to evade immune surveillance					

Abbreviations: 5-FU, 5-fluorouracil; FOLFIRI, irinotecan plus leucovorin plus 5-fluorouracil; XELOX, oxaliplatin plus capecitabine; mFOLFOX6, oxaliplatin plus leucovorin plus 5-fluorouracil.

*Based on ClinicalTrials.gov (<http://clinicaltrials.gov/>) accessed on July 23, 2012, with updates based on status as of July 19, 2012. Only the latest phase of ongoing clinical trial is chosen.

Table 3
Multiple TKIs targeting to VEGFR and PDGFR in the latest phase of ongoing clinical trials*

Agents	Targets**	Phase	Condition	Intervention	Identifier number (status)
Sorafenib (BAY43-9006)	VEGFR-2,3 PDGFR- β Raf-1	IV	Advanced HCC	Sorafenib; sorafenib + Nerison/Nerbas fatty ointment	NCT01098760 (active, not recruiting)
			Unresectable and/or metastatic HCC	Sorafenib	NCT01203787 (recruiting)
			Advanced HCC	Sorafenib; sorafenib + hepatectomy; sorafenib + transcatheter hepatic arterial chemoembolization	NCT01409499 (recruiting)
			Advanced RCC	Sorafenib	NCT01353794 (not yet recruiting)
Sunitinib (SU01124)	VEGFR PDGFR c-Kit FLT-3	IV	Metastatic RCC and GIST	Sunitinib	NCT01073644 (recruiting)
			NSCLC, RCC and GIST	Sunitinib	NCT01402089 (not yet recruiting)
			Advanced/metastatic well differentiated PNEC	Sunitinib	NCT01525550 (not yet recruiting)
			Imatinib-resistant/intolerant malignant GIST	Sunitinib	NCT00793871 (active, not recruiting)
			Advanced RCC	Observational study	NCT00836745 (recruiting)
			RCC	Sunitinib	NCT00716625 (enrolling by invitation)
Pazopanib	VEGFR PDGFR c-Kit	IV	GIST	Sunitinib	NCT00716820 (enrolling by invitation)
			Advanced and/or metastatic RCC	Pazopanib	NCT01521715 (recruiting)
Cediranib (Recentin™, AZD2171)	VEGFR PDGFR c-Kit	III	Stage IIIB/IV NSCLC	Cediranib + carboplatin + paclitaxel	NCT00795340 (active, not recruiting)
			Metastatic colorectal cancer	Cediranib + FOLFOX; Cediranib + XELOX	NCT00399035 (active, not recruiting)
			Recurrent glioblastoma	Cediranib; cediranib + lomustine	NCT00777153 (active, not recruiting)
Axitinib (AG-013736)	VEGFR PDGFR c-Kit	III	Metastatic RCC	Axitinib	NCT00920816 (recruiting)
			Metastatic RCC	Axitinib	NCT00678392 (active, not recruiting)
			Clear Cell RCC	Axitinib	NCT01599754 (recruiting)
Motesanib (AMG 706)	VEGFR-1,2 PDGFR c-Kit	III	Advanced non-squamous NSCLC	Motesanib + paclitaxel + carboplatin	NCT00460317 (active, not recruiting)

Agents	Targets**	Phase	Condition	Intervention	Identifier number (status)
BIBF 1120	VEGFR PDGFR FGFR	III	Stage IIIB/recurrent non-squamous NSCLC	BIBF 1120 + pemetrexed	NCT00806819 (active, not recruiting)
			Advanced ovarian cancer	BIBF 1120 + carboplatin + paclitaxel	NCT01015118 (active, not recruiting)
			Stage IIIB/IV or recurrent NSCLC	BIBF 1120 + docetaxel	NCT00805194 (active, not recruiting)
Linifanib (ABT-869)	VEGFR PDGFR- β CSF-1R c-Kit FLT-1	III	Advanced HCC	Linifanib	NCT01009593 (active, not recruiting)

Abbreviations: FLT, FMS-like tyrosine kinase; CSF-1, colony-stimulating factor 1; PNEC, pancreatic neuroendocrine carcinoma; FOLFEX, 5-fluorouracil plus leucovorin plus oxaliplatin; XELOX, capecitabine plus oxaliplatin.

* Based on ClinicalTrials.gov (<http://clinicaltrials.gov/>) accessed on July 23, 2012, with updates based on status as of July 19, 2012.

** Ref. (Subramanian et al., 2010; Kono et al., 2012).