Cancer-associated fibroblast regulation of tumor neo-angiogenesis as a therapeutic target in cancer (Review)

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Abstract. Adequate blood supply is essential for tumor survival, growth and metastasis. The tumor microenvironment (TME) is dynamic and complex, comprising cancer cells, cancer-associated stromal cells and their extracellular products. The TME serves an important role in tumor progression. Cancer-associated fibroblasts (CAFs) are the principal component of stromal cells within the TME, and contribute to tumor neo-angiogenesis by altering the proteome and degradome. The present paper reviews previous studies of the molecular signaling pathways by which CAFs promote tumor neo-angiogenesis and highlights therapeutic response targets. Also discussed are potential strategies for antitumor neo-angiogenesis to improve tumor treatment efficacy.

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Abbreviations: CAF, cancer-associated fibroblast; TME, tumor microenvironment; VM, vasculogenic mimicry; ECM, extracellular matrix; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; PI3K, phosphoinositide 3-kinase; MMP, matrix metalloproteinase; Ln-5γ2, laminin 5γ2; EphA2, ephrin type A receptor 2; FAK, focal adhesion kinase; α-SMA, α-smooth muscle actin; FAP, fibroblast activation protein; SDF-1, stromal cell-derived factor-1; HUVEC, human umbilical vein endothelial cell; MT1-MMP, membrane type 1-MMP; ROS, reactive oxygen species; EMT, epithelial-mesenchymal transition; GPER, G-protein-coupled estrogen receptor; HIF-1α, hypoxia-inducible factor-1α; EC, endothelial cell; AGM, angiomodulin; EPCs, endothelial progenitor cells; CXCR-4, CXC chemokine receptor type 4; TGF-β, transforming growth factor-β; COX-2, cyclo-oxygenase-2; TGF-βR, TGF-β receptor; VE-cad, vascular endothelial cadherin; HGF, hepatocyte growth factor; PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor; FGF, fibroblast growth factor; AM, adrenomedullin; CLIC3, chloride intracellular channel protein 3; PGs, prostaglandins; CLL, chronic lymphoid leukemia; FGFR, FGF receptor; eng, endoglin; VNR, vinorelbine

Key words: neoplasm, cancer-associated fibroblasts, tumor microenvironment, neo-angiogenesis, signaling pathway, target therapy

Contents

1. Introduction

Metastatic growth relies on the microcirculation to provide adequate nutrients; as such, tumors require extra vascular formation by way of angiogenesis and vascular mimicry (VM) (1-4). Highly aggressive cancers are dependent on angiogenesis and/or VM, making them potential targets for future therapies (5-7). However, to date, anti-angiogenic or anti-VM therapies have elicited only modest effects, suggesting that this approach alone may be ineffective (8,9).

It has been suggested that the tumor microenvironment (TME), which contains various stromal cells as well as the extracellular matrix (ECM), is critical to tumorigenesis, tumor neo-angiogenesis and cancer progression (10,11). Cancer-associated fibroblasts (CAFs), which are the primary stromal cells within the TME, may contribute to tumor neo-angiogenesis via proteomic and degradomic alterations (12,13). As such, understanding how CAFs and tumor neo-angiogenesis interact may be beneficial for developing novel tumor treatments. In the present review, blood supply, TME, CAFs and other research focused on CAF regulation of tumor neo-angiogenesis are discussed. Also reviewed are the underlying molecular signaling pathways and potential anti-angiogenic and anti-VM therapeutic targets.

2. Blood supply for tumors

Tumor angiogenesis is essential for providing adequate nutrition for tumorigenesis and tumor progression; without angiogenesis, tumors are unable to grow and metastasize. Angiogenesis is associated with the ECM, which provides structural support and delivers molecular signals during all stages of tumor angiogenesis (5,6,11). Drugs that are able to block angiogenesis-dependent tumor growth are of great interest, particularly the use of cleaved proteins, monoclonal antibodies, synthetic small molecules and natural products. A number of angiogenic inhibitors, including bevacizumab [a vascular endothelial growth factor (VEGF) inhibitor], sorafenib, sunitinib [a VEGF receptor (VEGFR) and platelet-derived growth factor receptor (PDGFR) inhibitor], erlotinib (an epidermal growth factor receptor inhibitor), thrombospondin-1 [a VEGF and fibroblast growth factor (FGF) inhibitor], TNP-470 [a methionine aminopeptidase-2 inhibitor], SU-5416, endostatin (a VEGFR inhibitor), celastrol and angiostatin, have been reported to have antitumor and anti-angiogenic activities (6,14-17). However, previous studies suggest that anti-angiogenic therapy has limited benefits and that blocking angiogenesis alone may not be effective (8,9). As such, combination treatments may provide improved anti-angiogenic and antitumor activities (6,18,19).

VM, a newly defined pattern for tumor blood supply, differs from angiogenesis and vasculogenesis as it does not require endothelial cells (ECs), allowing highly aggressive tumor cell behavior and the expression of EC-associated genes to form ECM-rich tubular networks (4). This provides the required microcirculation for tumor growth and is associated with poor prognosis in patients with highly aggressive malignant tumors, such as melanoma and gallbladder cancer (3,7,20-22). The molecular mechanisms underlying tumor VM formation are associated with activation of the phosphoinositide 3-kinase (PI3K)/matrix metalloproteinases (MMPs)/laminin 5γ2 (Ln-5γ2), epithelial cell kinase/ephrin type A receptor 2 (EphA2)/focal adhesion kinase (FAK) and VEGF-α signaling pathways (7). VM formation in human gallbladder was identified to be triggered by activation of the PI3K/MMPs/Ln-5γ2 and EphA2/FAK/paxillin signaling pathways in three-dimensional matrices of GBC-SD cells *in vitro* and GBC-SD nude mouse xenografts *in vivo* (23-26). Anti-angiogenic therapy has been identified to exhibit promising effects on VM in pancreatic cancer, hepatocellular cancer, hepatoblastoma, breast cancer, glioblastoma, gastric cancer and ovarian cancer (27-34). However, owing to individual differences, these studies have not been applied clinically or, if they have, have had little effect. Considering the number of cells involved in angiogenesis and VM, as well as the different molecular regulation mechanisms, further understanding of the underlying molecular mechanisms of tumor microcirculation is required in order to investigate joint targets and develop novel drugs that target angiogenesis and VM.

3. Tumor microenvironment and CAFs

The majority of human tumors originate from cancer epithelial cells, and for years tumors were considered to be transformed cells with cell-autonomous hyperproliferative and invasive properties. On this basis, treatments were targeted at the tumor itself. However, with the emergence of drug resistance and anti-angiogenic tolerance, tumor occurrence and development are associated with not only the tumor itself, but also with adjacent activated stromal cells and the associated chemokine and cytokine production (10,11). Studies have indicated that tumor progression is associated with the microenvironment of the tumor-host interface, which comprises tumor and stromal cells, as well as genetic mutations and the unlimited proliferation of tumor cells. Cancer-associated stromal cells, including inflammatory cells, vascular cells and CAFs, have a complex tumor-stromal interaction (10,11).

CAFs, which include activated fibroblasts or myofibroblasts around tumor epithelial cells, are the most important host stromal cells in the TME and regulate the microenvironment balance at the tumor-host interface via cell-to-cell contact, soluble factor secretion, ECM modification and promotion of malignant transformation of epithelial cells (10,12,13). Unlike normal fibroblasts, CAFs express α-smooth muscle actin (α-SMA), fibroblast activation protein (FAP) and fibroblast-specific protein-1; they have different gene expression profiles compared with normal fibroblasts (10,12,13,35). CAFs mediate paracrine or autocrine factors between tumor and stromal cells to influence TME and affect tumor dormancy or growth, invasion, angiogenesis and therapeutic resistance (10,12,13,35-39), all of which are associated with poor prognosis in patients with cancer (40,41). Madar *et al* (42) proposed a novel description of CAFs to illustrate that they are not a single cell type, rather comprising various activated cells. Research indicates that CAF inhibition prolongs the survival of patients with pancreatic cancer compared with chemotherapy alone, and that anti-CAFs prevent tumor progression prior to tumor invasion (43-45).

CAFs have a stable genome, are not prone to antigen loss, are tolerant of chemotherapy, are heterogeneous and account for between 50 and 90% of solid tumors, as stromal cells are rich targets and have complex interactions with tumor cells. Therefore, CAFs and their markers may be effective targets of antitumor therapy and drug design (42,43). However, crosstalk and interactions between CAFs and tumor cells and the underlying molecular mechanisms are not fully understood. It has been identified that stromal cell-derived factor-1 (SDF-1)/CXC chemokine 12 (CXC12) promotes angiogenesis in breast cancer (35) and VEGF secreted by CAFs promotes tumor angiogenesis (46). The use of conditioned media and human umbilical vein endothelial cells (HUVECs) in co-culture has suggested that cholangiocarcinoma cells in hepatic stellate dual-conditioned medium had the most marked HUVEC lumen formation ability (47). Furthermore, tumor cells stimulate fibroblasts to produce angiogenic factors with indirect tumor-stromal cell interaction patterns (48) and CAFs are the principal secretors of MMP-2, membrane type 1-MMP (MT1-MMP) and VEGF. PI3K is involved in VM formation by MMP-2 and MT1-MMP, whereas activated MMP-2 and MT1-MMP degrade Ln-5γ2 into the pre-migratory fragments γ2 and γ2x, which are enriched around tumor cells to promote tumor cell invasion and VM formation. As such, antibodies against MMP-2 and MT1-MMP, PI3K inhibitors and Ln-5γ2 target short interfering RNA are able to inhibit VM formation (7). In melanoma cells, VEGF and reactive oxygen species (ROS) regulate cell formation in the lumen-like structure, an effect that is reversed by antioxidants (49). Zinc

finger E-box-binding homologous box (ZEB1) promotes VM formation in colon cancer via epithelial-mesenchymal transition (EMT) (50). Improving our understanding of the integrated mechanisms by which CAFs modulate angiogenesis and VM in human tumors is key to identifying potential novel therapeutic targets for human tumors.

4. Molecular signaling pathways by which CAFs promote tumor neo-angiogenesis

Cells and non-cellular components are required for tumor neo-angiogenesis, and diverse molecular signaling pathways are involved (Table I).

VEGF/VEGF receptor (VEGFR). VEGF, which signals via its cognate VEGFR, is required for angiogenesis under normal conditions and in cancer (51). It has been identified that activated stromal cells secrete specific molecules that promote the expression of VEGF from tumor cells, and that certain factors secreted by CAFs interact with VEGF to increase tumor angiogenesis and invasiveness. Esophageal squamous cell carcinoma is a highly angiogenic tumor type; biochemical studies have identified that the VEGF signaling pathway derived from activated stromal fibroblasts induces capillary formation in this disease (52). A study involving lung squamous cancer cells revealed that podoplanin downregulated VEGF-C and decreased angiogenic and lymphangiogenic metastases (53). Expression of podoplanin, which promotes breast cancer angiogenesis and lymphangiogenesis (54), is often reported in CAFs, and podoplanin has been identified to stimulate angiogenesis and lymphangiogenesis in invasive ductal carcinoma of the breast via upregulation of VEGF-C rather than VEGF-A or VEGF-D in cancer cells (55,56). Galectin-1, a member of the galectin family of β-galactoside-binding proteins, is involved in cancer cell invasion and tumor angiogenesis (57-59). Galectin-1 has been reported to be upregulated in CAFs of gastric cancer cells (60), and it has been demonstrated that endogenous CAF-derived galectin-1 is essential for accelerating angiogenesis by enhancing VEGF expression in gastric cancer and VEGFR2 phosphorylation in epithelial cells (ECs) (61). The interaction between angiomodulin (AGM) and VEGF also facilitates angiogenesis (62). AGM has been reported to be overexpressed by CAFs in breast, colon, lung and uterus carcinomas (63), and so is considered to be a marker of cancer vasculature permeability (64).

Oxidative stress is known to promote tumor angiogenesis. G-protein-coupled estrogen receptor (GPER), hypoxia-inducible factor-1α (HIF-1α) and ROS are involved in the activation of fibroblasts and upregulation of VEGF expression. GPER knockdown eliminates VEGF expression in activated CAFs under hypoxic conditions, and it has been identified that breast CAFs promote hypoxia-dependent tumor angiogenesis in a HIF-1α/GPER-dependent manner by mediating the expression of VEGF (65,66). A study of p53-deficient colorectal xenograft tumor cells revealed that functional loss of p53 significantly increased tube formation and enhanced neovascularization in a VEGF-dependent manner *in vitro* and *in vivo* by upregulating ROS to activate stromal fibroblasts (67). Similarly, Jo *et al* (68) reported that oxidative stress stimulates angiogenesis in hepatocellular carcinoma via the protein kinase B/VEGF pathway.

It has been identified that endothelial progenitor cells (EPCs) are often located near microvessels in nasopharyngeal carcinoma, and that VEGF-A is overexpressed in stromal and tumor cells, indicating that CAFs may enhance angiogenesis in a VEGF-A- and SDF-1-dependent manner by recruiting EPCs from the bone marrow into tumor stroma (69).

In conclusion, VEGF is an important factor that promotes tumor angiogenesis by interacting with the secretome of CAFs. This suggests that anti-VEGF therapy should target VEGF itself as well as VEGF-coupled molecules. Such therapies may have marked anti-angiogenic effects.

SDF-1 (CXC12)/CXC chemokine receptor type 4 (CXCR-4). SDF-1, or CXC12, is a homeostatic chemokine that signals via CXCR-4, a G-protein-coupled receptor that is primarily secreted by hematopoietic progenitor cells and EPCs within injured sites. The SDF-1/CXCR-4 signaling axis has been reported to serve a role in recruiting EPCs to tumor stroma (70). A co-implantation tumor xenograft model revealed that CAFs were essential for angiogenesis-associated tumor progression in invasive human breast carcinoma because of their ability to secrete SDF-1 and recruit EPCs to the tumor site via CXCR-4, which is expressed by carcinoma cells (35). The same functional role of SDF1 was reported in nasopharyngeal carcinoma (69).

It has been reported that the SDF-1/CXCR-4 axis derived from CAFs boosts tumor angiogenesis or VM formation; however, its intrinsic regulatory mechanisms have not been fully elucidated. It has been suggested that fibroblast-derived SDF-1 enhances the invasiveness of pancreatic cancer cells, whereas SDF-1 promotes angiogenic responses in synergy with CXC chemokine ligand (CXCL)8 (71). In mouse xenografts of hepatocellular carcinomas, CAFs could form capillary-like structures, namely VM, via paracrine transforming growth factor-β (TGF-β) and SDF-1 (72). In human fetal lung fibroblast (MRC-5) and human lung cancer (95D) cell lines, the SDF-1/CXCR-4 axis participated in HUVEC and 95D cell migration, as well as HUVEC tube formation and angiogenesis, but not in HUVEC or 95D proliferation (73). An *in vitro* tube formation assay of tongue cancer tissues revealed that tumor necrosis factor α indirectly enhances cancer angiogenesis by increasing activated fibroblast-derived SDF-1 (74). Furthermore, cyclo-oxygenase-2 (COX-2) has been reported to increase tumor stromal formation and angiogenesis by regulating the pro-angiogenic microenvironment via SDF-1/CXCR-4 signaling (75). These results suggest a significant role for the stromal SDF-1/CXCR-4 axis in promoting tumor growth and enhancing angiogenesis, in part via EPC recruitment.

TGF-β/TGF-β receptor (TGF-βR). TGF-β is a pleiotropic growth factor that is expressed by cancer and stromal cells. TGF-β/TGF-βR signaling is required for advanced carcinogenesis via EMT induction, angiogenesis and modification of the stromal compartment (76,77). It has been identified that TGF-β and hypoxia work synergistically to regulate VEGF expression in mRNA biosynthesis (78). However, extracellular TGF-β signaling pathways that trigger angiogenesis are not well-understood. It has been reported that TGF-β-primed myofibroblast secretion upregulates VEGF, which in turn

Table I. CAF regulation of tumor neo-angiogenesis.

Pro-angiogenic factor	Receptor	Underlying molecular mechanism with tumor angiogenesis	(Refs.)
VEGF	VEGFR	CAFs promoted tumor angiogenesis by mediating the expression of VEGF i) through its secretome or ii) in a HIF-1α/GPER-dependent manner	(54-56,60-67)
SDF-1	CXCR-4	SDF-1/CXCR-4 axis derived from CAFs enhances angiogenesis partly through EPC recruitment	(35,70-75)
TGF-β	TGF-βR	TGF-β signaling pathway from CAFs triggers angiogenesis by i) upregulating VEGF expression or ii) interacting with other molecules and promotes VM formation	(52,72,82-86)
HGF	c-MET	Upregulation of HGF from CAF involves promotion of tumor angiogenesis through interaction with VEGF or TGF-β_1	(84,88-89,95-96)
PDGF	PDGFR	PDGF signaling from CAFs may not merely stimulate tumor angiogenesis, but may mediate resistance to anti-angiogenic therapy	(99,103)
FAP	No specific receptor	FAP expressed by CAF promotes angiogenesis by affecting the balance of pro-angiogenic and anti-angiogenic mediators	(105-109)
MMPs	No specific receptor	MMP from CAFs provides suitable conditions for tumor angiogenesis by regulating the degradation and remodeling of the ECM	(113,114)

CAF, cancer-associated fibroblast; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; HIF-1α, hypoxia-inducible factor 1α; GPER, G-protein-coupled estrogen receptor; SDF-1, stromal cell-derived factor 1; CXCR-4, CXC chemokine receptor type 4; EPC, endothelial progenitor cell; TGF-β, transforming growth factor-β; TGF-βR, TGF-β receptor; VM, vasculogenic mimicry; HGF, hepatocyte growth factor; PDGF, platelet-derived growth factor; PDGFR, PDGF receptor; FAP, fibroblast activation protein; MMP, matrix metalloproteinase; ECM, extracellular matrix.

stimulates vascular formation by ECs co-cultured in a three-dimensional assay (52). VM has been identified to be an important complement in the tumor microcirculation (4,22,79) as it is a tubular structure that is formed by tumor cells to transport nutrients, and molecules including vascular endothelial cadherin (VE-cad), MMPs and laminin have been reported to be critical for VM formation (7,80,81). TGF-β_1 derived from CAFs was identified to be a central molecular regulator of mesenchymal stem cells, as well as having a tumor-promoting function in prostate carcinoma progression (82). Furthermore, co-implantation of CAFs with tumor cells in mouse xenografts has been reported to promote the formation of capillary-like structures in hepatocellular carcinomas. A gene knockdown assay and gain- and loss-of-function assays revealed that CAFs secrete TGF-β and SDF-1, which activate VE-cad, MMP2 and Ln-5γ2 expression via TGF-βR1 and CXCR-4 in tumor cells to promote the formation of VM. MicroRNA-101 subsequently inhibits VM formation by suppressing the TGF-β and SDF-1 signaling pathways (72).

Immunohistochemical data have revealed increased angiogenesis in mice co-injected with TGF-β_1-pretreated Mc38-luc cells (colon adenocarcinoma cell lines from murine origin) and CAFs, whereas tumor angiogenesis was significantly decreased in mice treated with the TGF-β_1-inhibitory peptide P17 (83). Similar results were observed in esophageal squamous epithelial cells (84) and gastric tumor cells (85). Reverse transcription-polymerase chain reaction and western blot analyses demonstrated that the expression of angiogenic factor genes (α-SMA and VEGF) was significantly increased in ovarian cancer cells treated with CAF condition medium. α-SMA and VEGF downregulation in the intervention group indicated that the effects of CAF angiogenesis are dependent on the TGF-β signaling pathway (86). However, the quantification of cluster of differentiation (CD)31(+) vessels in breast cancer tissues via immunostaining indicated no significant differences in angiogenesis between TGF-β^- xenograft and control groups, indicating that TGF-β^- fibroblasts stimulate tumor growth independently of angiogenesis (87).

Further studies are required to explain the inconsistencies in experimental results. Owing to the pleiotropy of TGF-β, few studies have investigated the molecular mechanisms by which it promotes tumor angiogenesis.

Hepatocyte growth factor (HGF)/c-MET. Angiogenesis begins with local degradation of the basement membrane surrounding capillaries, following which ECs undergo migration and morphogenesis, leading to new capillary structures. It has been identified that the pro-angiogenic properties of ECs were promoted by HGF upregulation (88), and there is crosstalk between the HGF signaling pathway and VEGF-A in ECs (89).

Diverse stromal cells in the TME release HGF, whereas cancer cells express its receptor c-MET (90). Conditioned medium genetic knockdown and overexpression studies

have revealed that the HGF/c-MET axis serves a role in promoting tumorigenesis and invasion in various types of human cancer (91-94). HGF acts as a significant angiogenic growth factor in the development of esophageal squamous cell carcinoma, and an *in vitro* study revealed that HGF stimulates VEGF expression to enhance angiogenesis and tumor cell invasion and migration (95,96). Microvessel density analysis in esophageal squamous cell carcinoma tissues has revealed that CAF-secreted TGF-β_1 and HGF are associated with the proliferation of epithelial cells and angiogenesis (84). As such, increasing our understanding of HGF upregulation in tumor tissues and its interaction with VEGF or TGF-β_1 may be beneficial.

Platelet-derived growth factor (PDGF)/PDGFR. PDGF is a dimeric protein with several variants: PDGF-AA, PDGF-BB, PDGF-AB, PDGF-CC and PDGF-DD (97). Different tumors may have different PDGF/PDGFR expression patterns, and PDGF can be expressed by cancer cells or CAFs (98,99). PDGFR signaling has been reported to be associated with the progression of carcinoma (100) and the efficacy of chemotherapy (101,102). In a cervical squamous cell carcinoma transgenic mouse model, treatment with PDGFR kinase inhibitor suppressed FGF2 and FGF7 expression in tumor stromal fibroblasts, which decreased neo-angiogenesis and tumor growth (103). Increased PDGF-C expression in the stromal cells of chemoresistant tumors contributes to increased neo-angiogenesis during anti-VEGF treatment (99). Together, the results of these studies suggest that CAFs may mediate resistance to anti-angiogenic therapy as well as stimulating tumor angiogenesis.

FAP. FAP is a type II cell-surface-bound transmembrane glycoprotein that is expressed in activated stromal fibroblasts (104). An *in vivo* study of FAP-deficient mouse models indicated a pro-angiogenic effect of FAP (105). Furthermore, increased VEGF expression is associated with FAP upregulation in human pancreatic adenocarcinoma (106), suggesting that CAF-driven angiogenesis may be regulated by FAP. A recent study by Koczorowska *et al* (107) utilized FAP loss- and gain-of-function systems to investigate the effects of FAP on the biological function and secreted proteome of CAFs. The results revealed that increased FAP activity led to a decrease in the anti-angiogenic protein pigment epithelium-derived factor (PEDF) and an increase in pro-angiogenic proteins angiopoietin-1 and VEGF-C in the secretome of CAFs; this suggests that FAP serves a pro-angiogenic function by affecting the balance of pro- and anti-angiogenic mediators. A previous study revealed that FAP-activated protoxin selectively killed FAP-expressing cells and inhibited tumor growth in xenograft models (108), and a FAP-targeted DNA vaccine shifted immune polarization from tumor-promoting to suppression of recruitment of tumor-associated inflammatory cells. This decreased metastasis, angiogenesis and lymphangiogenesis in a murine breast cancer model (109). Together, these previous studies indicate that FAP is associated with the secretion of pro-angiogenic or anti-vascular proteins. It may therefore be beneficial to determine the mechanisms responsible for maintaining the balance of FAP regulatory proteins.

MMPs. Fibroblasts are an important source of ECM-degrading proteases, including MMPs, which are zinc-dependent endopeptidases; this suggests that fibroblasts serve a critical role in ECM homeostasis (110,111). Recent reports have revealed that tumor (MMP-1, -2 and -14) and stromal (MMP-9, -13 and -14) MMP expression is associated with tumor progression and is associated with squamous cell carcinoma invasion (112). MMP-13 secreted by activated fibroblasts promotes angiogenesis by releasing ECM-bound VEGF and increasing the invasive capabilities of squamous cell carcinoma cells in a mouse xenograft (113). Zigrino *et al* (114) used MMP-$13^{+/+}$ and null mice to identify that stromal MMP-13 is required for melanoma invasion, angiogenesis and metastasis. In summary, MMP serves an important function in regulating the degradation and remodeling of ECM to provide suitable conditions for tumor angiogenesis.

Other factors. In order to positively participate in neo-angiogenesis, human breast CAFs deposit and secrete more adrenomedullin (AM) compared with normal fibroblasts. AM blockade is able to impair tumor vascular formation and induce apoptosis (115). Notch signaling is an evolutionarily conserved pathway that regulates cell biological characteristics, whose accommodative dysfunction has been implicated in various tumors. It was recently reported that oral squamous cell carcinoma promotes angiogenesis by inducing Notch3 expression in CAFs, whereas other cancer cells have been identified to induce Notch3 expression in CAFs and promote angiogenesis (116). Chloride intracellular channel protein 3 (CLIC3) is a human intracellular Cl^- channel and scaffolding protein that is overexpressed in various tumor types (117-119). An *in vivo* study confirmed that CLIC3, an abundant component of the CAF secretome, increases the invasive abilities of breast cancer cells as well as improving functional vascularization, processes which require active transglutaminase-2 (120). A previous study revealed that cell division cycle 42 effector protein 3 (Cdc42EP3) is required in CAFs to allow for the expression of key angiogenic factors; Cdc42EP3 or SEPT2 depletion in a mammary tumor model significantly decreased the angiogenic potential of CAFs *in vivo* (121). COX-2 and endogenous prostaglandins (PGs) are important determinants of tumor growth and tumor-associated angiogenesis. COX-2 and PGE_2/EP3/EP4 signaling regulate the tumor stromal pro-angiogenic microenvironment and enhance stromal formation via CXCL12/CXCR-4 chemokine systems. Conversely, COX-2 inhibitor has been identified to suppress stromal formation and angiogenesis in a mouse model of Lewis lung carcinoma by inhibiting CXCL12/CXCR-4 *in vivo* (75). The oncogenic potential of chronic lymphoid leukemia (CLL)-derived exosomes has been investigated *in vitro* and *in vivo*. CLL-derived exosomes stimulate stromal cells to induce increased angiogenesis, thus supporting survival and outgrowth (122). FGF-1/-3/FGF receptor (FGFR) 4 signaling derived from CAFs promotes colon cancer cell proliferation and angiogenesis via activating mitogen-activated protein kinase/extracellular-signal-regulated kinase (ERK) kinase/ERK and modulating MMP-7 expression (123). Endoglin (eng) (124), a co-receptor for several members of the TGF-β family, has also been reported to be associated with the process of tumor angiogenesis. In a

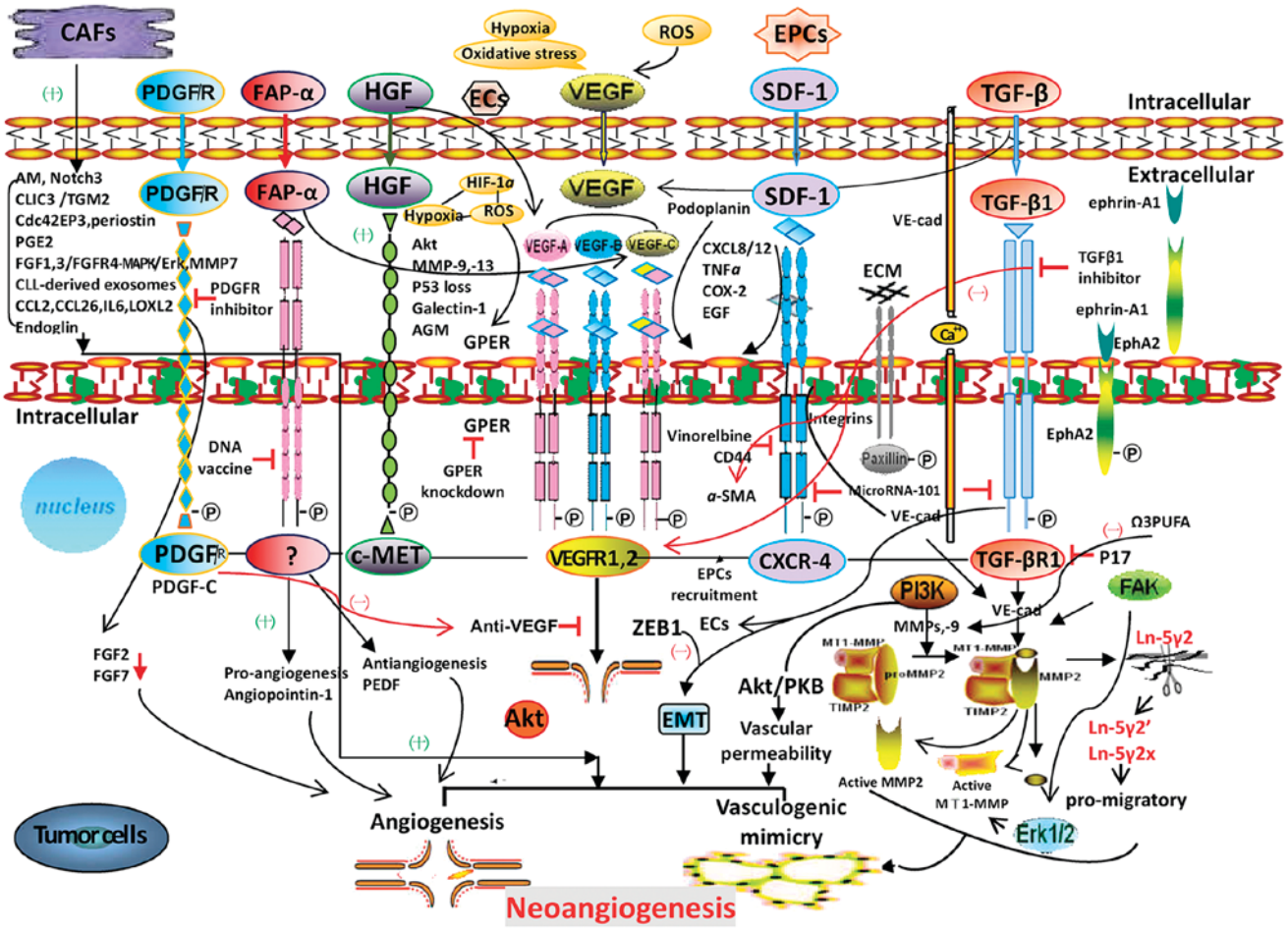

Figure 1. Molecular signaling pathways involved in CAFs regulation of tumor neo-angiogenesis and potential therapeutic targets for tumor angiogenesis and VM. T-shapes and (-) indicate inhibition; (+) indicates promoting. CAFs, cancer-associated fibroblasts; ECM, extracellular matrix; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; PI3K, phosphoinositide 3-kinase; Ln-5γ2, laminin 5γ2; MMP, matrix metalloproteinase; MT1-MMP, membrane type 1-MMP; TIMP, tissue inhibitor of metalloproteinase; EphA2, ephrin type A receptor 2; α-SMA, α-smooth muscle actin; FAP, fibroblast activation protein; ROS, reactive oxygen species; ZEB1, zinc finger E-box-binding homologous box; GPER, G-protein-coupled estrogen receptor; HIF-1α, hypoxia-inducible factor-1α; AGM, angiomodulin; ECs, endothelial cells; EPCs, endothelial progenitor cells; SDF-1, stromal cell-derived factor-1; CXCR-4, CXC chemokine receptor type 4; TNFα, tumor necrosis factor α; COX-2, cyclo-oxygenase-2; EGF, epidermal growth factor; TGF-β, transforming growth factor-β; TGF-βR, TGF-β receptor; VE-cad, vascular endothelial cadherin; HGF, hepatocyte growth factor; PDGF, platelet-derived growth factor; PDGFR, PDGF receptor; PEDF, pigment epithelium-derived factor; AM, adrenomedullin; CLIC3, chloride intracellular channel protein 3; TGM2, transglutaminase-2; CLL, chronic lymphoid leukemia; FGF, fibroblast growth factor; FGFR, FGF receptor; MAPK, mitogen-activated protein kinase; IL6, interleukin 6; LOXL2, lysyl oxidase-like 2; Ω3PUFA, Ω-3 polyunsaturated fatty acids; Akt/PKB, protein kinase B; CCL, CC chemokine ligand; CXCL, CXC chemokine ligand; Cdc42EP3, cell division cycle 42 effector protein 3; PGE2, prostaglandin E_2; Erk, extracellular-signal-regulated kinase; FAK, focal adhesion kinase; CD44, cluster of differentiation 44; P, phospho.

transgenic adenocarcinoma mouse prostate (TRAMP) mouse model, increased vascularization occurred was observed in $eng^{+/+}$ mice compared with in $eng^{+/-}$ mice, suggesting that eng is required for multiple aspects of CAF function (125).

Tumor angiogenesis is a complicated and multi-factor process. In order to improve our understanding of it, the molecular delivery of each pathway and how multiple pathways are associated with each other require further investigation.

5. Potential therapeutic targets

As angiogenesis is a central process required for the growth of solid tumors, studies to clarify the molecular basis of CAFs-associated tumor angiogenesis have identified a number of potential antitumor agents. Such agents include SU5416 and Z24, which act as inhibitors of VEGFR and FGFR, respectively (126,127). An *in vitro* study of 95D human lung cancer cells revealed that the addition of exogenous SDF-1 attenuates the anti-angiogenic effect of vinorelbine (VNR), suggesting that VNR may influence CAFs to cause SDF-1 and suppress tumor angiogenesis (73). Utilizing the unique enzymatic activity of FAP and its markedly restricted expression in reactive stroma, several tumor treatment strategies have been developed, including the use of small molecules and antibodies to inhibit enzyme activity, as well as immunosuppression to increase intratumoral drug concentrations. Potential mechanisms include decreasing blood vessel density and targeting CAF-associated molecular pathways (128). *In vitro* and *in vivo* experiments indicated that lenvatinib also exerts an anti-angiogenic effect by targeting VEGFR1-3 and FGFR1-4 (129). Ω-3 polyunsaturated fatty acids may suppress MMP-9 expression and tumor angiogenesis (130). Initially, thalidomide was used as an effective antiemetic drug, but its anti-angiogenic effects were gradually identified and

defined. Thalidomide can inhibit angiogenesis by targeting the expression of VEGF, FGF, FDGF, HIF and other molecules. However, clinical studies of thalidomide have not identified consistent antitumor effects (131).

A number of studies have identified that anti-pro-angiogenic agents alone are unable to elicit significant antitumor effects, suggesting that certain factors, such as activin A receptor like type 1 (ALK1), stabilize angiogenesis or convey resistance to anti-angiogenic effects. ALK1 is a transmembrane serine/threonine receptor kinase in the TGF-βR family that is expressed on ECs (132). Intrinsic angiostatic drug resistance and extrinsic mechanisms may serve a role in the resistance of tumor cells to angiostatic therapy (133). Crawford *et al* (99) reported that CAFs from resistant tumors in a murine lymphoma model secreted more compensatory PDGF-C to promote sensitive tumor angiogenesis, even in the presence of anti-VEGF treatment (99). This suggests that tumors are able to activate stromal fibroblasts via different mechanisms. In a glioblastoma model, high PDGF-C expression in CAFs was associated with resistance to anti-VEGF treatment (134). On this basis, therapeutic regimens targeting VEGF and PDGF were attempted in an advanced renal cell carcinoma clinical trial; however, the trial had to be terminated owing to marked toxicity (135). In an animal experiment, treatment with CAFs-inhibitors plus oxaliplatin significantly decreased tumor growth and angiogenesis in colon cancer compared with the use of oxaliplatin or CAF inhibitors alone (136).

Additional factors have been identified to be associated with anti-angiogenesis. Kinugasa *et al* (137) observed that CAFs markedly express CD44 in hypoxic and avascular areas, and that CD44 was markedly increased following treatment with angiogenesis inhibitors *in vitro*. Similarly, periostin, an ECM protein that is principally produced by CAFs, was also hypothesized to be a crucial molecular mediator involved in tumor resistance to anti-angiogenic therapy (138).

These studies indicate that anti-angiogenesis strategies must target angiogenic factors as well as internal molecules that stabilize angiogenesis, while also considering clinical toxicity. CAFs regulate tumor neo-angiogenesis; the molecular signaling pathways by which CAFs may promote tumor neo-angiogenesis and potential therapeutic targets for tumor angiogenesis and VM are depicted in Fig. 1. It is hoped that future studies are able to identify successful therapies utilizing multiple targets and signaling pathways for tumor treatment.

6. Conclusion

The TME and neo-angiogenesis are essential for tumor progression, and so the underlying molecular signaling pathways of CAFs in human tumors are increasingly being investigated. As components of tumor stroma, CAFs may serve as potential therapeutic targets owing to their role in tumor progression. Growth factors and cytokines have also been investigated as potential drug targets for impairing interactions between CAFs and cancer cells. To date, a number of non-clinical studies utilizing anti-angiogenic strategies with multi-molecular combinations have elicited promising results. However, clinical investigations have not yet yielded significant results. Further studies should be performed to identify effective therapeutic targets and treatment options to block tumor metastasis and recurrence.

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The authors declare that they have no competing interests.

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