The Roles of Tumor Endothelial Cells in Cancer Metastasis

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Abstract: Tumor metastasis is the main cause of cancer-related deaths. Tumor metastasis is orchestrated by a complex network of biological events. One such event is the formation of new blood vessels, termed as tumor angiogenesis. Tumor angiogenesis is essential for tumor progression. Without tumor angiogenesis, most solid tumors remain dormant. Apart from supplying tumors with nutrients and oxygen, tumor blood vessels provide a route for metastasis. Endothelial cells are key players in the formation of neovessels. Tumor endothelial cells that line tumor blood vessels differ from normal endothelial cells in many aspects. Tumor endothelial cells are irregular monolayers, have a higher expression of proangiogenic factors, and impaired endothelial barrier function when compared with their normal counterparts. The basement membrane thickness of tumor blood vessels is uneven and the association between pericytes and tumor endothelial cells is weak, leading to vascular leakiness. In this chapter, we highlight the role of endothelial cells in the initial steps of tumor metastasis.
INTRODUCTION

Cancer metastasis is the main cause of cancer death. Hematogenous metastasis is still a major issue in cancer therapy, despite improvements in the development and implementation of targeted therapies. Tumor cells induce neovascularization. Without angiogenesis, most solid tumors remain dormant (1). Tumor cells intravasate from primary tumor into blood circulation and extravasate into distant organ from blood stream (Figure 1). Thus, blood vessels support tumor cell metastasis by providing a route from primary tumors to distant organs. The concept of tumor antiangiogenesis was proposed by Dr. Folkman in his 1971 landmark report (2). Folkman suggested that tumors are dependent on angiogenesis for their progression and that the inhibition of angiogenesis may restrict tumor growth (1). To this end, angiogenesis inhibitors (AIs)—for example, bevacizumab, a humanized anti-vascular endothelial growth factor (VEGF) antibody (3)—have been widely used since its approval by the FDA in 2004.

VEGF is highly expressed in tumor endothelial cells (TECs), and because VEGF is a known factor to enhance permeability of blood vessels (4), tumor blood vessels typically are leaky and immature structures. AIs not only block angiogenesis but also normalize blood vessel integrity and improve the delivery of oxygen and drugs (5). In addition, vascular normalization by AIs increases the

Figure 1. Tumor metastasis process and tumor blood vessels. Tumor cells invade extracellular matrix, intravasate into the bloodstream, disseminate in the circulation and reach distant organs, and extravasate and invade into the parenchyma of distant tissues.

Keywords: chromosomal abnormality in tumor endothelial cells; endothelial cells in cancer metastasis; stem-like tumor endothelial cells; tumor blood vessels; tumor endothelial cells
effectiveness of immunotherapy because immune cells are delivered into tumor tissues via blood vessels. However, the clinical benefits of AIs are limited (6) because of the development of resistance to AIs (7). TECs that line the inner surfaces of tumor blood vessels are the primary targets of AIs. Several reports have demonstrated that TECs are abnormal, and their abnormality is one of the causes of resistance to antiangiogenic therapy (8, 9). In addition, TECs are highly heterogeneous (10, 11). In this chapter, we overview the abnormality and diversity of TECs, offering new perspectives on treatment strategies that can target TECs.

**ABNORMALITY OF TUMOR BLOOD VESSELS**

It is well known that tumor blood vessels differ morphologically from normal blood vessels. Abnormalities in tumor blood vessels may be due to an imbalance of angiogenic factors and their inhibitors (11–13). Vasculature in normal tissues has an organized hierarchical structure that supports efficient blood supply. However, tumor vasculature demonstrates unorganized patterns (14). Tumor blood vessels consist of irregular monolayers of endothelial cells (ECs) and do not have an endothelial barrier function (15). Basement membrane thickness is uneven. Unlike normal blood vessels, the association between pericytes and ECs are loose in tumor blood vessels (16). These abnormalities result in vascular leakiness, resulting in an increase of interstitial fluid pressure in the tumor tissue, causing vessel collapse (17). Consequently, blood flow in tumor vasculature is generally random. This is one of the reasons why tumor is usually hypoxic regardless of the high vascularization. This causes insufficient delivery of anti-cancer drugs and immune cells that attack cancer cells (18).

Hypoxia may be a switch to glycolytic metabolism, and an increase in tumor acidosis in some tumors. Hypoxia in tumors further induces tumor aggressiveness through epithelial-mesenchymal transition (EMT), resulting in tumor metastasis (19). Thus, at least theoretically, vascular normalization is beneficial also for anti-metastasis. There is also a dysfunction in the TECs themselves. While normal ECs (NECs) are uniform, forming a continuous monolayer in normal blood vessels, TECs are irregular in shape and size. In addition, there are often gaps between adjacent TECs (20) and transcellular fenestrations also have been observed in TECs. These morphologically abnormal TECs can cause hemorrhage and plasma leakage. Furthermore, loose intercellular adhesion in the tumor blood vessels is one of the mechanisms of tumor cell intravasation (Figure 2).

**PROANGIOGENIC PHENOTYPE IN TUMOR ENDOTHELIAL CELLS**

There are marked phenotypic variations between TECs and NECs. TECs show a higher expression of proangiogenic factors when compared with NECs. For example, VEGFR-1, VEGFR-2, VEGFR-3, VEGF-D, angiopoietin receptor tie-2, and angiopoietin 1 are upregulated in TECs when compared with NECs (21), resulting in a proangiogenic phenotype (22). They also express adhesion
molecules such as ICAM-1, VCAM-1, and E-selectin (23), through which they interact with proinflammatory cells and tumor cells. We have previously demonstrated that TECs show an upregulated expression of secreting factors such as biglycan (24) and pentraxin 3 (PTX3) (25), known as damage-associated molecular pattern (DAMPs), which activate inflammatory signal, via NF-κB without infection. Thus, TECs have been described as “activated” and “chronically inflamed” (26).

Unlike NECs, TECs are highly proliferative (22, 27), self-sustaining, and are less dependent on serum for proliferation (21). FDCP 6 homolog (DEF6) and PTX3, upregulated in TECs, play a role in continuous proliferation (28). TECs are highly migratory than NECs (10, 22, 29). The upregulation of several genes, such as, LOX (30), SBSN (31), and biglycan (24) enhances the migration and tube-forming ability of TECs. Murine TECs maintain their biological characteristics for longer periods in cell culture than NECs (22). Several chemokine receptors CXCR7 (32–34) or PTGIR (35) impart a proangiogenic phenotype to TECs. Furthermore, TECs show altered metabolism. It was recently demonstrated that uncontrolled glycolysis in TECs due to an upregulated expression of glycolysis genes, including the enzyme 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3), contributes to structural deformities observed in tumor blood vessels (36). We have reported that TECs can proliferate even under lactic acidosis which is caused by tumor cell glycolytic metabolism. The pH regulator, carbonic anhydrase 2 (CAII), is involved in resistance to low pH in TECs (13). Moreover, TECs require nucleotide precursors and lipids to maintain

Figure 2. The structure of normal and tumor blood vessels. Normal endothelial cells are uniform, forming a continuous monolayer in normal blood vessels. On the other hand, there are often gaps between adjacent tumor endothelial cells. Transcellular fenestrations also have been observed in tumor endothelial cells. These morphologically abnormal tumor endothelial can cause hemorrhage and plasma leakage. Also, loose intercellular adhesion in the tumor blood vessels is one of the mechanisms of tumor cell intravasation.
their high proliferation. To support these biomolecule demands, TECs express high levels of key enzymes such as D-3-phosphoglycerate dehydrogenase (PHGDH) and phosphoserine aminotransferase 1 (PSAT1) (36) for serine biosynthesis, and fatty acid synthase (FASN) (37) for lipid synthesis. Nucleotide biosynthesis is also enhanced in TECs compared to that in NECs (38). These phenotypes of TECs are beneficial for tumor cells to grow and metastasize.

**CHROMOSOMAL ABNORMALITY AND STEM-LIKE PHENOTYPE IN TECs**

Chromosomal abnormalities have been reported in murine TECs (39), and human renal TECs (40). These include chromosomal aberrations, missing chromosomes, translocations, and abnormal centrosomes characterized by large sizes and excess numbers (39). The hypoxia in tumor microenvironment induces genetic instability and abnormal centrosome structure, resulting in chromosome missegregation (41). We have also found that reperfusion after hypoxia causes chromosome abnormality (42). TECs in B-cell lymphoma were also found to have lymphoma-specific chromosomal translocations (43). More recently, nonhematopoietic aneuploid CD31+ circulating TECs were detected in the peripheral blood of patients with breast cancer, demonstrating that circulating TECs possess chromosomal changes (44). Transdifferentiation from glioblastoma to ECs may be very rare because ECs rarely carry the cancer genetic mutations (45); however, other groups of investigators have demonstrated that transdifferentiation from tumors may occur in other cell types, not ECs (46).

It has been reported that monocyte-derived immature dendritic cells behave as endothelial-like cells in the presence of specific cytokines such as VEGF (47). These variations could lead to TEC diversity. In addition, tumor microenvironment itself can cause TEC diversity. The hypoxic tumor microenvironment induces the expression of “stemness” genes (48). Several studies have identified the upregulated expression of stemness genes such as stem cell antigen-1 (Sca-1) (49), MDR-1 (49) and aldehyde dehydrogenase (ALDH) (50) in TECs. These stem-like cell population is a part of TEC population (51, 52). TECs upregulate ALDH. There are two populations of TECs based ALDH on activity: high, and low. ALDH\textsuperscript{high} TECs produce longer tubular networks in matrigel than ALDH\textsuperscript{low} TECs (50). The ALDH\textsuperscript{high} TECs are resistant to the chemotherapeutic drug 5-Fluorouracil (5-FU), with upregulation of stemness-related genes, compared with ALDH\textsuperscript{low} TECs (53). Furthermore, ALDH\textsuperscript{high} TECs show higher grade of aneuploidy (53). Other reports show that CD133(+) TECs have a higher frequency of aneuploidy than the CD133(-) TECs. This suggests that several TECs originated from progenitor cells may be involved in inducing genetic instability in these cells (54). It has been reported that progenitor-derived TEC which express CD133 are undifferentiated, highly proliferative cells (55).

TECs are indeed different depending on tumor microenvironment (10). Naito et al. reported that vascular-resident stem/progenitor-like ECs, which form a minor population in tumors, contribute to tumor angiogenesis (56). The heterogeneity of TECs has been revealed at the single cell level resolution by recent technological improvement, e.g., single cell RNA sequence.
There are various population of TECs, for example, TECs with highly collagenolytic activity, TECs that attract immune cells, and that with collagen cross-linking activity (52).

**DRUG RESISTANCE IN TECs**

TECs demonstrate drug resistance via high drug efflux (56). The heterogeneity of ECs in tumor tissues may be a mechanism contributing to resistance to anticancer and antiangiogenic therapy. We have shown that the TECs of metastatic melanoma have a higher expression of MDR-1 (8) and ALDH, and are resistant to the drug paclitaxel (53). Another study, using TECs derived from human hepatocellular carcinoma, showed that the CD105+ TECs are more resistant to 5-FU and sorafenib (an antiangiogenic drug) when compared to CD105+ NECs or human umbilical vein ECs (57). IGFBP7 expressed by TECs suppresses IGF1R signaling and the stem-cell-like property of tumor cells. Chemotherapy triggers TECs to suppress IGFBP7, and the upregulation of IGF1 activates the FGF4-FGFR1-ETS2 pathway in TECs and accelerates the conversion of tumor cells to chemoresistant tumor stem-like cells (58). Tumor-derived microvesicles induce EC drug resistance via IL-6 upregulation, suggesting tumor secreting factor cause resistance of TEC to chemotherapy (59). Kikuchi et al. reported that IL-8, induced by anticancer drugs, increases the expression of p-glycoprotein/ABCB1, which is a drug transporter in TECs of human bladder cancers (60) (Figure 3). It has been shown

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*Figure 3. Drug resistance in tumor endothelial cells. During cancer therapy, a drug transporter, ABCB1 is upregulated in tumor endothelial cells. These resistant tumor endothelial cells also sustainably support tumor cells and provide the route for distant metastasis.*
that inhibition of TEC ABCB1 enhanced the therapeutic efficacy of the anti-cancer drug paclitaxel (61) (Figure 3). These resistant TECs also sustainably support tumor cells and provide the route for distant metastasis.

THE ROLE OF TECs IN CANCER PROGRESSION

Tumor stromal cells such as cancer-associated fibroblasts and the immunosuppressive myeloid-derived suppressor cells contribute to tumor progression (62). TECs also play important roles at this process. The enhanced VEGF signaling in TECs causes immature blood vessel formation, through which tumor cells could intravasate easily, as described above. Upregulation of adhesion molecules in TECs is also an advantage for tumor cells to attach to ECs, which lead to extravasation to drive metastatic dissemination. In addition, TECs secrete various inductive factors named “angiocrine factors” (11), which stimulate growth and migration of tumor cells (63). Biglycan has been detected in human lung cancer patients and TEC biglycan level was correlated to poor prognosis of cancer patients (64). TECs produce endothelin-1, bFGF, TGFβ, IL-6, and IL-8 as paracrine mediators of prostate cancer progression (65). Other angiocrine factors, including IL-6, IL-3, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage-CSF (GM-CSF), IL-1, and nitric oxide, stimulate leukemia cancer cell growth. In addition, TEC-derived Jag1 activates Notch2 to promote invasiveness of lymphoma cells (66). CXCR7 upregulated in TECs regulates CXCL12–CXCR4-mediated tumor cell transendothelial migration (67). Platelet-derived growth factor (PDGF) signaling is important for inhibitor of differentiation 4 (ID4)-mediated regulation of ECs and glioma cells by promoting the PDGF–NOS (nitric oxide synthase)–ID4 signaling axis. These effects maintain cancer stemness and promote tumor angiogenesis (68). Moreover, TECs stimulate tumor cell intravasation and metastasis. TEC-Notch1 promotes lung metastasis with neutrophil infiltration. ALK1 expression in TECs is an independent prognostic factor for metastasis of breast cancer (69). The oxygen-sensing prolyl hydroxylase domain protein 2 (PHD2) in TECs is involved in vessel shaping. PHD2 deficiency normalized blood vessels, which led to the reduction of tumor cell intravasation and metastasis (70). We have shown that biglycan, a small leucine-rich repeat proteoglycan, was remarkably upregulated in TECs of metastatic tumors and facilitated the migration of toll-like receptor2/4-expressing tumor cells, which increased circulating tumor cells and lung metastasis (71). Endothelial calcineurin activates the outgrowth of metastases (72). These studies suggested that TECs actively promote tumor progression and metastasis.

In recent years, immune checkpoint inhibitors have become key drugs for antitumor immunity (73). Tumor blood vessels play an important role in delivering immune cells into tissues (74). The abnormalities of TECs suppress T-cell trafficking and function, resulting in immune-suppression (75). VEGF and prostaglandins induce CD95 (FasL) expression on TECs, leading to apoptosis of anticancer CD8+ T cells. Upregulation of CD73 on TECs reduces effector T-cell homing, whereas anti-CD73 antibodies can restore efficacy of antitumor immunotherapy and decrease tumor angiogenesis (76). In addition, PD-L1 is expressed in TECs. Biglycan secreted from TEC induces tumor fibrosis (77), which acts as
a barrier for immune cells to migrate towards tumor cells, which leads tumors’ escape from immunity. Tumor fibrosis also induces tumor cell invasion via integrin signaling, leading tumor progression. Thus, TECs support tumor cell progression in various manners. Vascular normalization is a promising concept in anticancer treatment and can potentially improve the outcome of immunotherapies (78).

CONCLUSION

The functions of ECs in angiogenic blood vessels in tumor tissues are not only to transport nutrients and oxygen for tumor survival and growth, but also to actively promote tumor progression and chemoresistance by expressing various juxtagraine or paracrine factors. Antiangiogenic therapy has been widely used in many types of tumors; however, since it is now clear that TECs are heterogeneous, to understand the complex situation in the tumor microenvironment, companion diagnostics to monitor vascularization is required.


Conflict of Interest: The authors declare no potential conflicts of interest with respect to research, authorship and/or publication of this manuscript.

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