10

The Roles of Tumor Endothelial Cells in Cancer Metastasis

Kyoko Hida[1] • Nako Maishi[1] • Ryo Takeda[1,2] • Yasuhiro Hida[3]

[1]Vascular Biology and Molecular Pathology, Hokkaido University Graduate School of Dental Medicine, Sapporo, Japan; [2]Department of Oral Diagnosis and Medicine, Hokkaido University Graduate School of Dental Medicine, Sapporo, Japan; [3]Department of Cardiovascular and Thoracic Surgery, Hokkaido University Faculty of Medicine, Sapporo, Japan

Author for correspondence: Kyoko Hida, Vascular Biology and Molecular Pathology, Hokkaido University Graduate School of Dental Medicine, Sapporo, Japan. E-mail: khida@den.hokudai.ac.jp

Cite this chapter as: Hida K, Maishi N, Takeda R, Hida Y. The Roles of Tumor Endothelial Cells in Cancer Metastasis. In: Sergi CM, editor. *Metastasis*. Brisbane (AU): Exon Publications. Online first 2022 Mar 13.

Doi: https://doi.org/10.36255/exon-publications.metastasis.endothelial-cells

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.

In: Consolato M. Sergi, editor. *Metastasis*. Exon Publications, Brisbane, Australia. ISBN: 978-0-6453320-2-5. Doi: https://doi.org/10.36255/exon-publications.metastasis

Copyright: The Authors.

License: This open access article is licenced under Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC 4.0) https://creativecommons.org/licenses/by-nc/4.0/

Keywords: chromosomal abnormality in tumor endothelial cells; endothelial cells in cancer metastasis; stem-like tumor endothelial cells; tumor blood vessels; tumor endothelial cells

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

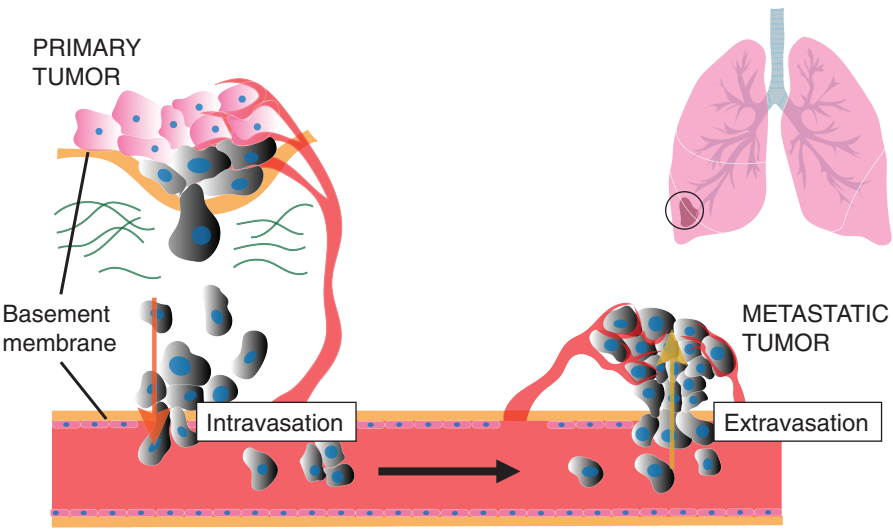

Figure 1. Tumor metastasis process and tumor blood vessels. Tumor cells invade extracellular matrix, intravasate into the bloodstream, disseminate in the circulation and reach distant organs, and extravasate and invade into the parenchyma of distant tissues.

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

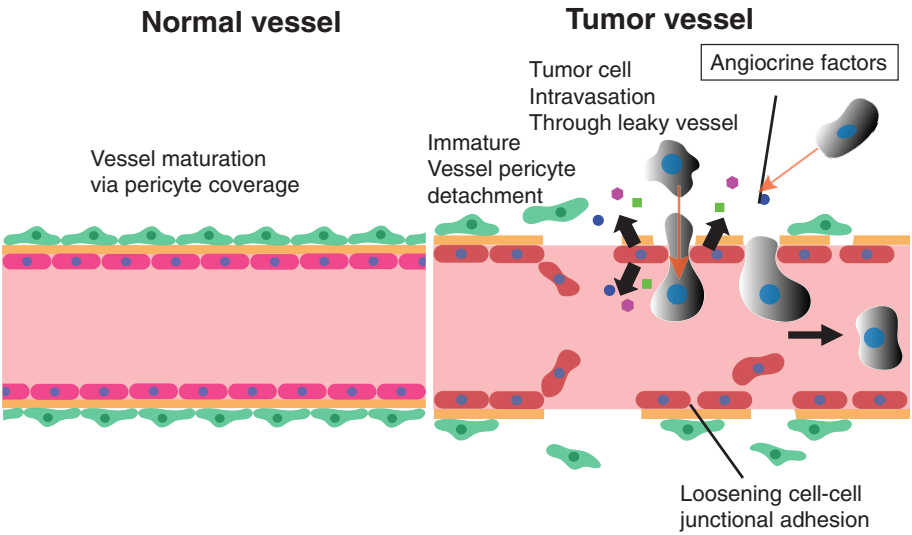

Figure 2. The structure of normal and tumor blood vessels. Normal endothelial cells are uniform, forming a continuous monolayer in normal blood vessels. On the other hand, there are often gaps between adjacent tumor endothelial cells. Transcellular fenestrations also have been observed in tumor endothelial 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.

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

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). Transdifferntiation 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. $ALDH^{high}$ TECs produce longer tubular networks in matrigel than $ALDH^{low}$ TECs (50). The $ALDH^{high}$ TECs are resistant to the chemotherapeutic drug 5-Fluorouracil (5-FU), with upregulation of stemness-related genes, compared with $ALDH^{low}$ TECs (53). Furthermore, $ALDH^{high}$ 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

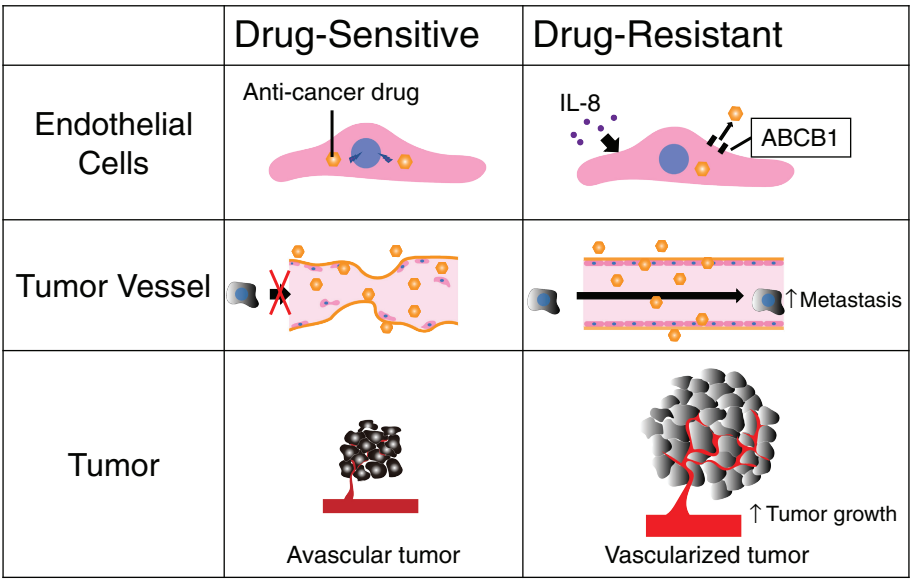

Figure 3. Drug resistance in tumor endothelial cells. During cancer therapy, a drug transporter, ABCB1 is upregulated in tumor endothelial cells. These resistant tumor endothelial cells also sustainably support tumor cells and provide the route for distant metastasis.

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 juxtracrine 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.

Acknowledgment: This research was partially supported funding from by JSPS Grants-in-Aid for Scientific Research on Integrated analysis and regulation of cellular diversity Innovative Areas to K.H. (JP18H05092), JSPS Grants-in-Aid for Scientific Research to N.M. (JP18K09715), Y.H. (JP18H02891), and K.H. (JP18H02996), Grants from Japan Agency for Medical Research and Development (AMED) to K.H. (JP19ck0106406h0002).

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

Copyright and Permission Statement: The authors confirm that the materials included in this chapter do not violate copyright laws. Where relevant, appropriate permissions have been obtained from the original copyright holder(s), and all original sources have been appropriately acknowledged or referenced.

REFERENCES

1. Folkman J. Anti-angiogenesis: new concept for therapy of solid tumors. Ann Surg. 1972;175(3):409–16. https://doi.org/10.1097/00000658-197203000-00014
2. Folkman J. Tumor angiogenesis: therapeutic implications. N Engl J Med. 1971;285(21):1182–6. https://doi.org/10.1056/NEJM197111182852108
3. Culy C. Bevacizumab: antiangiogenic cancer therapy. Drugs Today (Barc). 2005;41(1):23–36. https://doi.org/10.1358/dot.2005.41.1.875776
4. Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science. 1983;219(4587):983–5. https://doi.org/10.1126/science.6823562
5. Jain RK. Normalizing tumor vasculature with anti-angiogenic therapy: a new paradigm for combination therapy. Nat Med. 2001;7(9):987–9. https://doi.org/10.1038/nm0901-987
6. Ebos JM, Kerbel RS. Antiangiogenic therapy: impact on invasion, disease progression, and metastasis. Nat Rev Clin Oncol. 2011;8(4):210–21. https://doi.org/10.1038/nrclinonc.2011.21

7. van Beijnum JR, Nowak-Sliwinska P, Huijbers EJ, Thijssen VL, Griffioen AW. The great escape; the hallmarks of resistance to antiangiogenic therapy. Pharmacol Rev. 2015;67(2):441–61. https://doi.org/10.1124/pr.114.010215
8. Akiyama K, Ohga N, Hida Y, Kawamoto T, Sadamoto Y, Ishikawa S, et al. Tumor Endothelial Cells Acquire Drug Resistance by MDR1 Up-Regulation via VEGF Signaling in Tumor Microenvironment. The American Journal of Pathology. 2012;180(3):1283–93. https://doi.org/10.1016/j.ajpath.2011.11.029
9. HIDA K, Kikuchi H, Maishi N, Hida Y. ATP-binding cassette transporters in tumor endothelial cells and resistance to metronomic chemotherapy. Cancer Letters. 2017;400:1–6. https://doi.org/10.1016/j.canlet.2017.02.006
10. Ohga N, Ishikawa S, Maishi N, Akiyama K, Hida Y, Kawamoto T, et al. Heterogeneity of Tumor Endothelial Cells. The American Journal of Pathology. 2012;180(3):1294–307. https://doi.org/10.1016/j.ajpath.2011.11.035
11. Maishi N, Annan DA, Kikuchi H, Hida Y, Hida K. Tumor Endothelial Heterogeneity in Cancer Progression. Cancers (Basel). 2019;11(10). https://doi.org/10.3390/cancers11101511
12. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med. 1995;1(1):27–31. https://doi.org/10.1038/nm0195-27
13. Annan DA, Kikuchi H, Maishi N, Hida Y, Hida K. Tumor Endothelial Cell-A Biological Tool for Translational Cancer Research. Int J Mol Sci. 2020;21(9). https://doi.org/10.3390/ijms21093238
14. McDonald DM, Choyke PL. Imaging of angiogenesis: from microscope to clinic. Nat Med. 2003;9(6):713–25. https://doi.org/10.1038/nm0603-713
15. Hashizume H, Baluk P, Morikawa S, McLean JW, Thurston G, Roberge S, et al. Openings between defective endothelial cells explain tumor vessel leakiness. Am J Pathol. 2000;156(4):1363–80. https://doi.org/10.1016/S0002-9440(10)65006-7
16. Kalluri R. Basement membranes: structure, assembly and role in tumour angiogenesis. Nat Rev Cancer. 2003;3(6):422–33. https://doi.org/10.1038/nrc1094
17. McDonald DM, Baluk P. Significance of blood vessel leakiness in cancer. Cancer Res. 2002;62(18):5381–5.
18. Jain RK. Normalization of Tumor Vasculature: An Emerging Concept in Antiangiogenic Therapy. Science. 2005;307(5706):58–62. https://doi.org/10.1126/science.1104819
19. Jiang J, Tang YL, Liang XH. EMT: a new vision of hypoxia promoting cancer progression. Cancer Biol Ther. 2011;11(8):714–23. https://doi.org/10.4161/cbt.11.8.15274
20. Chang YS, di Tomaso E, McDonald DM, Jones R, Jain RK, Munn LL. Mosaic blood vessels in tumors: frequency of cancer cells in contact with flowing blood. Proc Natl Acad Sci U S A. 2000;97(26):14608–13. https://doi.org/10.1073/pnas.97.26.14608
21. Bussolati B, Deambrosis I, Russo S, Deregibus MC, Camussi G. Altered angiogenesis and survival in human tumor-derived endothelial cells. The FASEB Journal. 2003:1–3. https://doi.org/10.1096/fj.02-0557fje
22. Matsuda K, Ohga N, Hida Y, Muraki C, Tsuchiya K, Kurosu T, et al. Isolated tumor endothelial cells maintain specific character during long-term culture. Biochem Biophys Res Commun. 2010;394(4):947–54. https://doi.org/10.1016/j.bbrc.2010.03.089
23. Alessandri G, Chirivi RG, Fiorentini S, Dossi R, Bonardelli S, Giulini SM, et al. Phenotypic and functional characteristics of tumour-derived microvascular endothelial cells. Clin Exp Metastasis. 1999;17(8):655–62. https://doi.org/10.1023/A:1006738901839
24. Yamamoto K, Ohga N, Hida Y, Maishi N, Kawamoto T, Kitayama K, et al. Biglycan is a specific marker and an autocrine angiogenic factor of tumour endothelial cells. British journal of cancer. 2012;106(6):1214–23. https://doi.org/10.1038/bjc.2012.59
25. HIDA K, Maishi N, Kawamoto T, Akiyama K, Ohga N, Hida Y, et al. Tumor endothelial cells express high pentraxin 3 levels. Pathology International. 2016;66(12):687–94. https://doi.org/10.1111/pin.12474
26. Dudley AC. Tumor endothelial cells. Cold Spring Harb Perspect Med. 2012;2(3):a006536. https://doi.org/10.1101/cshperspect.a006536
27. Hida K, Hida Y, Shindoh M. Understanding tumor endothelial cell abnormalities to develop ideal anti-angiogenic therapies. Cancer Sci. 2008;99(3):459–66. https://doi.org/10.1111/j.1349-7006.2007.00704.x

28. Otsubo T, Hida Y, Ohga N, Sato H, Kai T, Matsuki Y, et al. Identification of novel targets for antiangiogenic therapy by comparing the gene expressions of tumor and normal endothelial cells. Cancer Sci. 2014;105(5):560–7. https://doi.org/10.1111/cas.12394
29. Kurosu T, Ohga N, Hida Y, Maishi N, Akiyama K, Kakuguchi W, et al. HuR keeps an angiogenic switch on by stabilising mRNA of VEGF and COX-2 in tumour endothelium. British journal of cancer. 2011;104(5):819–29. https://doi.org/10.1038/bjc.2011.20
30. Osawa T, Ohga N, Akiyama K, Hida Y, Kitayama K, Kawamoto T, et al. Lysyl oxidase secreted by tumour endothelial cells promotes angiogenesis and metastasis. British journal of cancer. 2013;109(8):2237–47. https://doi.org/10.1038/bjc.2013.535
31. Alam MT, Nagao-Kitamoto H, Ohga N, Akiyama K, Maishi N, Kawamoto T, et al. Suprabasin as a novel tumor endothelial cell marker. Cancer Science. 2014;105(12):1533–40. https://doi.org/10.1111/cas.12549
32. Maishi N, Ohga N, Hida Y, Akiyama K, Kitayama K, Osawa T, et al. CXCR7: A novel tumor endothelial marker in renal cell carcinoma. Pathology International. 2012;62(5):309–17. https://doi.org/10.1111/j.1440-1827.2012.02792.x
33. Yamada K, Maishi N, Akiyama K, Towfik Alam M, Ohga N, Kawamoto T, et al. CXCL12-CXCR7 axis is important for tumor endothelial cell angiogenic property. International Journal of Cancer. 2015;137(12):2825–36. https://doi.org/10.1002/ijc.29655
34. Yanagiya M, Dawood RIH, Maishi N, Hida Y, Torii C, Annan DA, et al. Correlation between endothelial CXCR7 expression and clinicopathological factors in oral squamous cell carcinoma. Pathol Int. 2021;71(6):383–91. https://doi.org/10.1111/pin.13094
35. Osawa T, Ohga N, Hida Y, Kitayama K, Akiyama K, Onodera Y, et al. Prostacyclin receptor in tumor endothelial cells promotes angiogenesis in an autocrine manner. Cancer Sci. 2012;103(6):1038–44. https://doi.org/10.1111/j.1349-7006.2012.02261.x
36. Cantelmo AR, Conradi L-C, Brajic A, Goveia J, Kalucka J, Pircher A, et al. Inhibition of the Glycolytic Activator PFKFB3 in Endothelium Induces Tumor Vessel Normalization, Impairs Metastasis, and Improves Chemotherapy. Cancer Cell. 2016;30(6):1–19. https://doi.org/10.1016/j.ccell.2016.10.006
37. Bruning U, Morales-Rodriguez F, Kalucka J, Goveia J, Taverna F, Queiroz KCS, et al. Impairment of Angiogenesis by Fatty Acid Synthase Inhibition Involves mTOR Malonylation. Cell Metab. 2018;28(6):866–80 e15. https://doi.org/10.1016/j.cmet.2018.07.019
38. Li X, Sun X, Carmeliet P. Hallmarks of Endothelial Cell Metabolism in Health and Disease. Cell Metab. 2019;30(3):414–33. https://doi.org/10.1016/j.cmet.2019.08.011
39. Hida K, Hida Y, Amin DN, Flint AF, Panigrahy D, Morton CC, et al. Tumor-associated endothelial cells with cytogenetic abnormalities. Cancer Res. 2004;64(22):8249–55. https://doi.org/10.1158/0008-5472.CAN-04-1567
40. Akino T, Hida K, Hida Y, Tsuchiya K, Freedman D, Muraki C, et al. Cytogenetic abnormalities of tumor-associated endothelial cells in human malignant tumors. Am J Pathol. 2009;175(6):2657–67. https://doi.org/10.2353/ajpath.2009.090202
41. Taylor SM, Nevis KR, Park HL, Rogers GC, Rogers SL, Cook JG, et al. Angiogenic factor signaling regulates centrosome duplication in endothelial cells of developing blood vessels. Blood. 2010;116(16):3108–17. https://doi.org/10.1182/blood-2010-01-266197
42. Kondoh M, Ohga N, Akiyama K, Hida Y, Maishi N, Towfik Alam M, et al. Hypoxia-induced reactive oxygen species cause chromosomal abnormalities in endothelial cells in the tumor microenvironment. PloS one. 2013;8(11):e80349. https://doi.org/10.1371/journal.pone.0080349
43. Streubel B, Chott A, Huber D, Exner M, Jager U, Wagner O, et al. Lymphoma-specific genetic aberrations in microvascular endothelial cells in B-cell lymphomas. N Engl J Med. 2004;351(3):250–9. https://doi.org/10.1056/NEJMoa033153
44. Lin PP, Gires O, Wang DD, Li L, Wang H. Comprehensive in situ co-detection of aneuploid circulating endothelial and tumor cells. Scientific reports. 2017;7(1):1–10. https://doi.org/10.1038/s41598-017-10763-7
45. Rodriguez FJ, Orr BA, Ligon KL, Eberhart CG. Neoplastic cells are a rare component in human glioblastoma microvasculature. Oncotarget. 2012;3(1):98–106. https://doi.org/10.18632/oncotarget.427

46. Soda Y, Marumoto T, Friedmann-Morvinski D, Soda M, Liu F, Michiue H, et al. Transdifferentiation of glioblastoma cells into vascular endothelial cells. Proceedings of the National Academy of Sciences. 2011;108(11):4274–80. https://doi.org/10.1073/pnas.1016030108
47. Fernandez Pujol B, Lucibello FC, Gehling UM, Lindemann K, Weidner N, Zuzarte ML, et al. Endothelial-like cells derived from human CD14 positive monocytes. Differentiation. 2000;65(5):287–300. https://doi.org/10.1046/j.1432-0436.2000.6550287.x
48. Carnero A, Lleonart M. The hypoxic microenvironment: A determinant of cancer stem cell evolution. Bioessays. 2016;38 Suppl 1:S65–74. https://doi.org/10.1002/bies.201670911
49. Matsuda K, Ohga N, Hida Y, Muraki C, Tsuchiya K, Kurosu T, et al. Isolated tumor endothelial cells maintain specific character during long-term culture. Biochemical and Biophysical Research Communications. 2010;394(4):947–54. https://doi.org/10.1016/j.bbrc.2010.03.089
50. Ohmura-Kakutani H, Akiyama K, Maishi N, Ohga N, Hida Y, Kawamoto T, et al. Identification of Tumor Endothelial Cells with High Aldehyde Dehydrogenase Activity and a Highly Angiogenic Phenotype. PloS one. 2014;9(12):e113910–17. https://doi.org/10.1371/journal.pone.0113910
51. Nagy JA, Dvorak HF. Heterogeneity of the tumor vasculature: the need for new tumor blood vessel type-specific targets. Clin Exp Metastasis. 2012;29(7):657–62. https://doi.org/10.1007/s10585-012-9500-6
52. Goveia J, Rohlenova K, Taverna F, Treps L, Conradi LC, Pircher A, et al. An Integrated Gene Expression Landscape Profiling Approach to Identify Lung Tumor Endothelial Cell Heterogeneity and Angiogenic Candidates. Cancer Cell. 2020;37(1):21–36 e13. https://doi.org/10.1016/j.ccell.2019.12.001
53. HIDA K, Maishi N, Akiyama K, Ohmura-Kakutani H, Torii C, Ohga N, et al. Tumor endothelial cells with high aldehyde dehydrogenase activity show drug resistance. Cancer Science. 2017;108(11):2195–203. https://doi.org/10.1111/cas.13388
54. Akino T, HIDA K, Hida Y, Tsuchiya K, Freedman D, Muraki C, et al. Cytogenetic Abnormalities of Tumor-Associated Endothelial Cells in Human Malignant Tumors. The American Journal of Pathology. 2010;175(6):2657–67. https://doi.org/10.2353/ajpath.2009.090202
55. Rafii S, Lyden D, Benezra R, Hattori K, Heissig B. Vascular and haematopoietic stem cells: novel targets for anti-angiogenesis therapy? Nature Reviews Cancer. 2002;2(11):826–35. https://doi.org/10.1038/nrc925
56. Naito H, Kidoya H, Sakimoto S, Wakabayashi T, Takakura N. Identification and characterization of a resident vascular stem/progenitor cell population in preexisting blood vessels. The EMBO Journal. 2011;31(4):1–14. https://doi.org/10.1038/emboj.2011.465
57. Xiong YQ, Sun HC, Zhang W, Zhu XD, Zhuang PY, Zhang JB, et al. Human Hepatocellular Carcinoma Tumor-derived Endothelial Cells Manifest Increased Angiogenesis Capability and Drug Resistance Compared with Normal Endothelial Cells. Clinical Cancer Research. 2009;15(15):4838–46. https://doi.org/10.1158/1078-0432.CCR-08-2780
58. Cao Z, Scandura JM, Inghirami GG, Shido K, Ding BS, Rafii S. Molecular Checkpoint Decisions Made by Subverted Vascular Niche Transform Indolent Tumor Cells into Chemoresistant Cancer Stem Cells. Cancer Cell. 2017;31(1):110–26. https://doi.org/10.1016/j.ccell.2016.11.010
59. Torii C, Maishi N, Kawamoto T, Morimoto M, Akiyama K, Yoshioka Y, et al. miRNA-1246 in extracellular vesicles secreted from metastatic tumor induces drug resistance in tumor endothelial cells. Scientific reports. 2021;11(1):13502. https://doi.org/10.1038/s41598-021-92879-5
60. Kikuchi H, Maishi N, Annan DA, Towfik Alam M, Ibrahim Hassan Dawood R, Sato M, et al. Chemotherapy-induced IL-8 upregulates MDR1/ABCB1 in tumor blood vessels and results in unfavorable outcome. Cancer Res. 2020. https://doi.org/10.1158/0008-5472.CAN-19-3791
61. Akiyama K, Maishi N, Ohga N, Hida Y, Ohba Y, Alam MT, et al. Inhibition of Multidrug Transporter in Tumor Endothelial Cells Enhances Antiangiogenic Effects of Low-Dose Metronomic Paclitaxel. The American Journal of Pathology. 2015;185(2):572–80. https://doi.org/10.1016/j.ajpath.2014.10.017
62. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. Nat Med. 2013;19(11):1423–37. https://doi.org/10.1038/nm.3394
63. Butler JM, Kobayashi H, Rafii S. Instructive role of the vascular niche in promoting tumour growth and tissue repair by angiocrine factors. Nat Rev Cancer. 2010;10(2):138–46. https://doi.org/10.1038/nrc2791

64. Morimoto H, Hida Y, Maishi N, Nishihara H, Hatanaka Y, Li C, et al. Biglycan, tumor endothelial cell secreting proteoglycan, as possible biomarker for lung cancer. Thorac Cancer. 2021;12(9):1347–57. https://doi.org/10.1111/1759-7714.13907
65. Pirtskhalaishvili G, Nelson JB. Endothelium-derived factors as paracrine mediators of prostate cancer progression. Prostate. 2000;44(1):77–87. https://doi.org/10.1002/1097-0045(20000615)44:1<77::AID-PROS10>3.0.CO;2-G
66. Cao Z, Ding BS, Guo P, Lee SB, Butler JM, Casey SC, et al. Angiocrine factors deployed by tumor vascular niche induce B cell lymphoma invasiveness and chemoresistance. Cancer Cell. 2014;25(3):350–65. https://doi.org/10.1016/j.ccr.2014.02.005
67. Zabel BA, Wang Y, Lewen S, Berahovich RD, Penfold ME, Zhang P, et al. Elucidation of CXCR7-mediated signaling events and inhibition of CXCR4-mediated tumor cell transendothelial migration by CXCR7 ligands. J Immunol. 2009;183(5):3204–11. https://doi.org/10.4049/jimmunol.0900269
68. Jeon HM, Kim SH, Jin X, Park JB, Kim SH, Joshi K, et al. Crosstalk between glioma-initiating cells and endothelial cells drives tumor progression. Cancer Res. 2014;74(16):4482–92. https://doi.org/10.1158/0008-5472.CAN-13-1597
69. Cunha SI, Bocci M, Lovrot J, Eleftheriou N, Roswall P, Cordero E, et al. Endothelial ALK1 Is a Therapeutic Target to Block Metastatic Dissemination of Breast Cancer. Cancer Res. 2015;75(12):2445–56. https://doi.org/10.1158/0008-5472.CAN-14-3706
70. Mazzone M, Dettori D, de Oliveira RL, Loges S, Schmidt T, Jonckx B, et al. Heterozygous deficiency of PHD2 restores tumor oxygenation and inhibits metastasis via endothelial normalization. Cell. 2009;136(5):839–51. https://doi.org/10.1016/j.cell.2009.01.020
71. Maishi N, Ohba Y, Akiyama K, Ohga N, Hamada J-i, Nagao-Kitamoto H, et al. Tumour endothelial cells in high metastatic tumours promote metastasis via epigenetic dysregulation of biglycan. Scientific reports. 2016;6(1):1–13. https://doi.org/10.1038/srep28039
72. Hendrikx S, Coso S, Prat-Luri B, Wetterwald L, Sabine A, Franco CA, et al. Endothelial Calcineurin Signaling Restrains Metastatic Outgrowth by Regulating Bmp2. Cell Rep. 2019;26(5):1227–41 e6. https://doi.org/10.1016/j.celrep.2019.01.016
73. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012;366(26):2443–54. https://doi.org/10.1056/NEJMoa1200690
74. Vestweber D. How leukocytes cross the vascular endothelium. Nat Rev Immunol. 2015;15(11):692–704. https://doi.org/10.1038/nri3908
75. Johansson-Percival A, He B, Ganss R. Immunomodulation of Tumor Vessels: It Takes Two to Tango. Trends Immunol. 2018;39(10):801–14. https://doi.org/10.1016/j.it.2018.08.001
76. Wang L, Fan J, Thompson LF, Zhang Y, Shin T, Curiel TJ, et al. CD73 has distinct roles in nonhematopoietic and hematopoietic cells to promote tumor growth in mice. The Journal of clinical investigation. 2011;121(6):2371–82. https://doi.org/10.1172/JCI45559
77. Cong L, Maishi N, Annan DA, Young MF, Morimoto H, Morimoto M, et al. Inhibition of stromal biglycan promotes normalization of the tumor microenvironment and enhances chemotherapeutic efficacy. Breast Cancer Res. 2021;23(1):51. https://doi.org/10.1186/s13058-021-01423-w
78. Huang Y, Yuan J, Righi E, Kamoun WS, Ancukiewicz M, Nezivar J, et al. Vascular normalizing doses of antiangiogenic treatment reprogram the immunosuppressive tumor microenvironment and enhance immunotherapy. Proc Natl Acad Sci U S A. 2012;109(43):17561–6. https://doi.org/10.1073/pnas.1215397109