

COMMENTARY

Role of endothelial cells in tumor microenvironment

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To the Editor:

Endothelial cells line the vascular system and play essential roles in regulating tumor initiation, progression, and metastasis. The application of single-cell RNA sequencing has identified distinct lineages of endothelial cells during the spatial-temporal evolution of the tumor microenvironment (TME). Subgroups of endothelial cells exist that either promote or prevent tumor progression from non-invasive to the invasive stage. Mechanisms of functional tumor endothelial cells (TECs) include cytokine secretion, which activates receptors on the tumor cells and/or suppresses antitumor immune reaction via attenuating the cytotoxic responses of the immune cells (Figure 1). There are currently few available methods to detect TECs in the clinical setting; it would be a promising approach to leverage TECs targeted therapy to improve the current treatment regimens for early-stage cancer.

TECs could promote cancer progression by supporting tumor metabolism¹ or secreting paracrine factors.² Recently, by single-cell RNA sequencing (scRNA-seq), we could gain a landscape view of the dynamic gene network in the TECs.³ For example, in a metastatic melanoma study, Tirosh et al. identified that the TECs in the drug-resistant tumors harbored heterogeneous genetic

signal compared to the normal ECs gene expression.⁴ The scRNA-seq analysis aims to discover the unique biomarker or signaling ligands/receptors on the cell population of interest. For example, one scRNA-seq profiling of mouse lung endothelial cells defined a specific lung endothelial cell population,⁵ marked by carbonic anhydrase 4 (CA4), whose expression depends on VEGFA expression AT1-positive cells.⁶ The Notch ligand Dll4 was abundantly expressed by endothelial cells, impacting smooth muscle cells (SMC) and fibroblasts by binding with their Notch1 or Notch3 receptors (Figure 2).⁷ The other endothelial-specific markers include Ephrins⁸ and Slit2,⁹ as well as TEC specific tip cell-specific marker CXCR4, PGF and LXN, etc.¹⁰

The VEGF and Notch signaling pathways in tumor ECs could be more active than in the normal tissue endothelial cells, responsible for the upregulation of angiogenesis.¹¹ The downregulation of gene expression related to immune activation in tumor ECs indicates that tumor ECs would suppress the antitumor immune function. VEGF or Dll4-Notch signaling upregulations to play decisive roles in tumor formation and metastasis, probably through VEGF or Dll4-mediated formation of TEC subgroups (i.e., tip-like, transition, and stalk-like cells) (Figure 1), as evidenced

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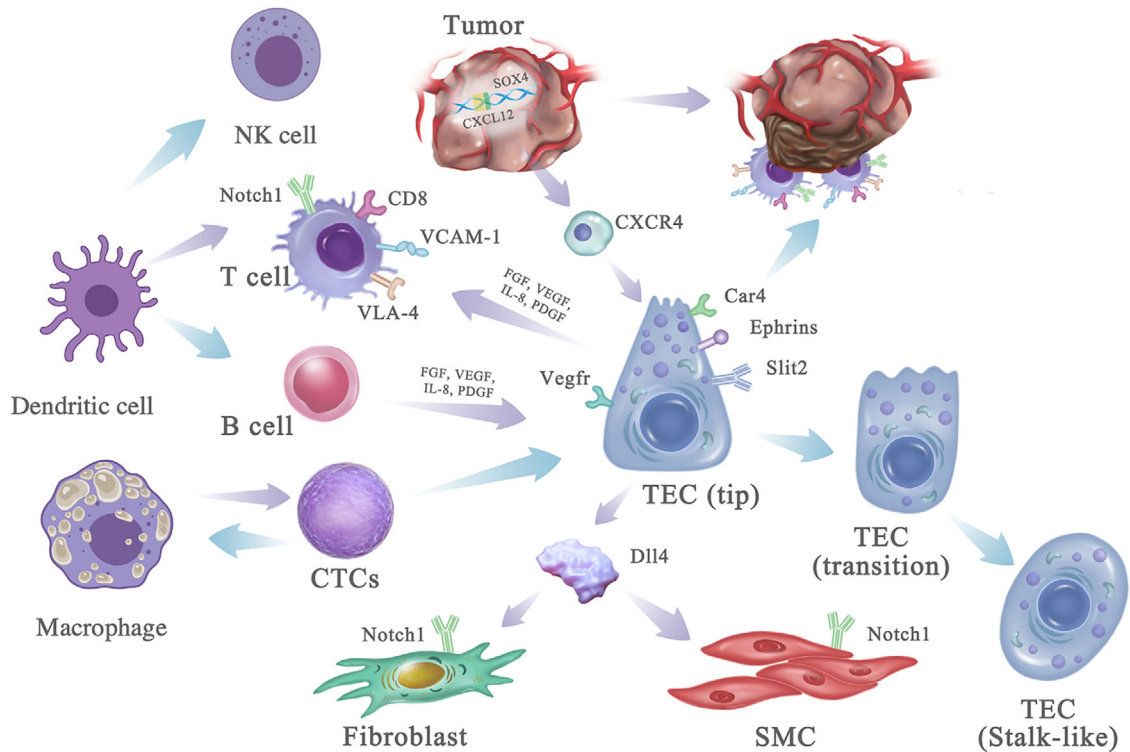


FIGURE 1 Schematic of tumor microenvironment in the early stage of the malignant tumor

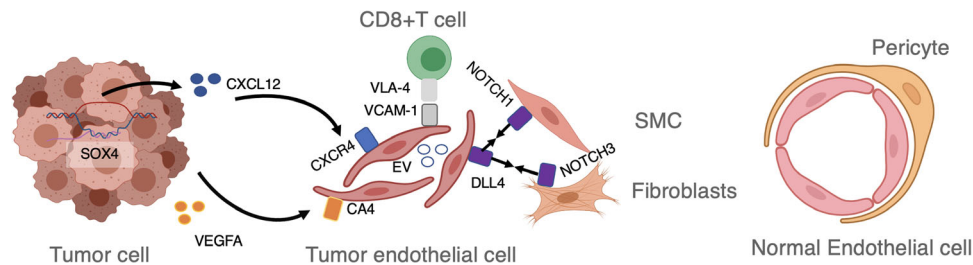


FIGURE 2 Illustration of gene- and regulation-based molecular mechanisms in tumor endothelial microenvironment

by scRNA-seq profiles and tip TEC signature genes are well related with patient survival.¹⁰

scRNA-seq studies can also reveal the impact of tumor cells on TECs. The aberrant gene within tumors could activate the *CXCL12* promoter within tumors, which could bind and activate CXCR4 signaling in endothelial cells, induce neovascularization, and promote distant metastasis in hepatocellular carcinoma cells.^{12,13} The tumor-initiating cells could impact surrounding endothelial cells by transferring oncogenic sequences to TECs via extracellular vehicles (EVs), which subsequently induces micronuclei formation, along with TEC migration and proliferation (Figure 2).^{14,15}

Thus, the identification and validation of TEC-specific markers will improve the current diagnosis for early detection of TMEs. Wang et al. developed an apelin-based syn-Notch receptors binding system to detect the angiogenic

signaling within tumor.¹⁶ Based on scRNA-seq, one subgroup of TEC, tip TECs, correlated with patient survival. Therefore, Tip TECs can be considered a potential marker to evaluate the effectiveness of VEGF blockade therapies.¹⁰ Besides, a higher proportion of endothelial cells can be harvested in clinical core biopsy samples than in surgical resection samples.¹⁷ Due to the technical difficulties of obtaining TECs, one approach is to apply a set of transgenic reporter mice to capture endothelial cells in mouse models. He et al. used this method to apply scRNA-seq on mouse brain and lung endothelial cells and studied the vascular populations and transcriptome during development and disease.¹⁸

In addition to the interaction between tumor cells and TECs, other types of cells in TME (e.g., tumor-associated macrophage and cancer-associated fibroblasts) also engage in cell-cell communications with TECs.¹⁹

Dendritic cells were one of the most abundant cells in the immune microenvironment,²⁰ glioma-associated macrophages have an emerging role in the promotion of tumor proliferation, invasion, and metastasis and also stimulation of neoangiogenesis,^{21,22} the intense interrelation between immune cells and extracellular matrix molecules in building the TME,^{23,24} NK cells are also one important subset of tumor-antagonizing immune cells that mediate the immunosurveillance of tumor.²⁵ The induction of retinoblastoma protein (Rb)-mediated senescence on KRAS mutant pancreatic ductal adenocarcinoma could produce pro-angiogenic factors and promote the tumor vasculature normalization, which in turn enhance drug delivery and efficacy of cytotoxic gemcitabine chemotherapy.^{26,27} Meanwhile, endothelial cell activation could contribute to CD8⁺ T cells' infiltration by induction of VCAM-1, which binds with VLA-4 on CD8⁺ T cells. Similarly, IgG4⁺CD49b⁺CD73⁺ B cells expressing pro-angiogenic cytokine could efficiently promote endothelial cell tubes' formation in the tumor microenvironment.²⁸


Regarding translational therapy, historically, anti-angiogenesis therapy targets tumor vascular network growth, which mainly consists of two mechanisms: (a) angiogenesis, the formation of new blood vessels from existing vessels, and (b) vasculogenesis, the de novo formation of blood vessels from endothelial precursors.²⁹ Many growth factors promote angiogenic output (e.g., FGF, VEGF, IL-8, and PDGF) and endogenous inhibitors also exist to block vessel growth (e.g., thrombospondin, tumstatin, canstatin, endostatin, angiostatin, and interferon-alpha/beta).³⁰ However, the targeted inhibition of the VEGF signaling has not always resulted in favorable outcomes in cancer patients' treatment.³¹ Recently, the combination of anti-PD-L1 and anti-VEGF therapy showed significant benefit in patients with unresectable hepatocellular carcinoma.³² It is hinted that the anti-VEGF neutralizing antibody reversed the immunosuppressive function of TECs and promoted T-cell infiltration in the tumor. This warrants a better understanding of the complex interactions between tumor and TECs.

Also, scRNA-seq of TECs provides the potential to identify more targeted therapy based on the cellular and molecular changes within TECs, and overcome the limitations of anti-angiogenesis therapies. Furthermore, a subgroup of circulating tumor cells (CTCs) population in colorectal cancer patients' blood was TECs³³; the screening of TECs in patients' peripheral blood with different stages of tumor has the potential to serve as a biomarker for early tumor diagnosis. It would be valuable to define the critical regulatory nodes within the TEC gene expression network, explore their expression by multiomics, and, most importantly, to integrate regulatory nodes' expres-

sion with patients' clinical phenomes by way of clinical transomics.^{34,35} With the rapid development of biotechnologies, a deep understanding of the crosstalk between tumor cells and TECs at the early stage of tumorigenesis will help discover more precise and potent biomarkers and improve clinical outcomes for cancer treatment.

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