

## Understanding of the Cellular Structure and Function of Tumor Vessels

JIN Ke-tao<sup>1,2</sup>, ZHU Tie-ming<sup>3</sup>, LAN Huan-rong<sup>4</sup>, GUAN Ming<sup>1</sup>, JIN Zhi-gang<sup>3</sup>

(1. Department of Surgical Oncology, the People's Hospital of Zhuji, Zhuji 311800; 2. Department of Surgical Oncology, the First Affiliated Hospital of College of Medicine, Zhejiang University, Hangzhou 310003; 3. Department of General Surgery, the People's Hospital of Zhuji, Zhuji 311800; 4. Department of Obstetrics and Gynaecology, the Red Cross Hospital of Zhuji, Zhuji 311800)

**Abstract:** A thorough understanding of the cellular structure and function of tumor vessels becomes even more important, as this information is important to interpreting the effects of antiangiogenic agents, with the increasing hope of vascular targeting in cancer. This review intends to integrate our knowledge of the cellular structure and function of tumor vessels into information which could be the basis for developing effective antivascular agents for cancer.

**Key words:** endothelial cells; pericytes; basement membrane; tumor vessels

### INTRODUCTION

Tumor vessels are treated as a clinically important therapeutic target. The abnormalities of tumor vessels provide the potential for targeting these vessels without destroying the normal blood vessels<sup>[1]</sup>. Tumor vessels are considered as dynamic in terms of the formation of new vessels or angiogenesis and the remodeling or "normalization" of existing vessels<sup>[2]</sup>. By now, we have known that tumor can acquire its vasculature by endothelial cell sprouting<sup>[3]</sup>, co-option of pre-existing vessels<sup>[4-7]</sup>, intussusceptive microvascular growth<sup>[8-9]</sup>, postnatal vasculogenesis<sup>[10]</sup>, Glomeruloid angiogenesis<sup>[11-12]</sup>, or vasculogenic mimicry<sup>[13-14]</sup>. Here we should emphasize that these mechanisms, in most cases, are interlinked, and participating simultaneously in physiological and pathological angiogenesis.

Tumor vessels have multiple structural and functional abnormalities compared to normal blood vessels. Their expression of distinctive surface molecules, potential for rapid growth and remodeling, and unusual leakiness, are responsible for mediating spread of tumor cells and maintaining the unusual microenvironment of tumors, and so are key to the efficacy of tumor-targeting therapy<sup>[15-17]</sup>. Like normal blood vessels, tumor vessels consist of endothelial cells, pericytes (mural cells), and basement membrane<sup>[18]</sup>. The endothelial cells, pericytes, and basement membrane of tumor vessels have been studied at the tissue, cellular, and molecular level<sup>[19-29]</sup>.

This review focuses on the cellular structure and function of tumor vessels, and intends to integrate the knowledge of these fields into information for better developing effective antiangiogenic agents in treatment of cancer.

### CELLS INVOLVING IN TUMOR VESSELS

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JIN Ke-tao (1978- ), male, surgeon of Department of Surgical Oncology, the People's Hospital of Zhuji, Ph.D. candidate in Department of Surgical Oncology, the First Affiliated Hospital of College of Medicine, Zhejiang University; research fields: oncology, basic and clinical.

### 1. Endothelial Cells of Tumor Vessels

Tumor angiogenesis is necessary for solid tumor progression and metastasis. Tumor vessels have been shown to differ from normal vessels in morphology. An important concept in tumor angiogenesis is that tumor endothelial cells or tumor-derived endothelial cells are structurally and functionally abnormal<sup>[30]</sup>. Tumor-derived endothelial cells have been characterized by using highly reliable methods, such as immunoelectron microscopy<sup>[31]</sup> and endothelial purification from tumor tissues<sup>[16,32]</sup>. Comparison of molecular profiles of normal endothelial cells and tumor-derived endothelial cells, by micro-array analysis and proteomic mapping, has shown up-regulation of various angiogenesis-related molecules<sup>[33-35]</sup>. These molecules, highly expressed in tumor-derived endothelial cells, although not specific, may be involved in increased proliferation, migration, and permeability of tumor-derived endothelial cells, and involved in matrix remodeling in tumor progression as well<sup>[32]</sup>. Presently, some molecules such as VEGFR-2, CD105 (Endoglin), endothelial protein-disulfide isomerase (EndoPDI), and tumor endothelial markers are noted as representative surface markers of tumor-derived endothelial cells, because they are rarely expressed in the corresponding normal endothelial cells<sup>[36-39]</sup>. Clinical application of antiangiogenic therapy has some targets: membrane proteins specifically expressed in tumor-derived endothelial cells, VEGFR and CD105, for example. Studies in detail on tumor-derived endothelial cells specific molecular markers in each kind of tumor will help us find more effective antiangiogenic therapies of cancer.

### 2. Pericytes of Tumor Vessels

Pericytes, also known as mural cells, are adventitial cells located within the basement membrane of capillaries. Pericytes are generally considered to be contractile cells that stabilize vessel walls and participate in the regulation of blood flow in the microcirculation, because of their multiple cytoplasmic processes, distinctive cytoskeletal elements, and envelopment of endothelial cells<sup>[40-41]</sup>. Pericytes may also influence endothelial permeability, proliferation, survival, migration, and maturation<sup>[42-43]</sup>.

Pericytes in tumors also have multiple structural abnormalities, including a loose association with endothelial cells and cytoplasmic processes that invade the tumor spongy parenchyma, which may make the vessels sensitive to VEGF inhibitors. Similarities of pericytes in spontaneous tumors and implanted tumors suggest that the abnormalities are common in angiogenesis. Pericytes on capillaries were oriented longitudinally along the vessel, had long thin processes, were tightly positioned next to the endothelium, and covered only a small proportion of the vessel surface. Pericytes on venules had an irregular shape, close association with endothelial cells, and covered much of the vessel surface. By comparison, pericytes in the tumors were loosely associated with the endothelium, having processes that extended away from the vessel wall, in some cases overlaid other pericytes, accompanied endothelial sprouts, and even extended beyond the ends of the sprouts<sup>[28]</sup>. Pericytes form sleeves around endothelial sprouts that arise from tumor vessels, may participate in blood vessel growth, and are a potential target in anti-angiogenic therapy<sup>[28]</sup>.

Pericytes were firstly recognized by their distinctive shape and location, and later, they were most commonly identified by molecular markers such as  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA), desmin, or high-molecular weight melanoma-associated antigen (NG2) in tumor vessels<sup>[22,44-45]</sup>. Little is known about the marker variability of pericytes expressed in tumors because most studies have used a single marker, usually  $\alpha$ -SMA or desmin<sup>[46-47]</sup>. Published reports suggest that the amount of pericytes coverage on vessels in different tumors ranges from extensive<sup>[22]</sup> to little or none<sup>[24-25]</sup>. Some of these differences may be explained by differences of pericyte markers expressed in tumors. However, others are likely result from differences in the markers used to identify pericytes or differences in section thickness, in condition that partial pericytes coverage was missed in thin histological

sections.

### **3. Basement Membrane of Tumor Vessels**

The basement membrane of tumor vessels has attracted refreshed attention as a source of angiogenic and antiangiogenic molecules, a site of growth factor binding, a participant in angiogenesis, and a potential target in cancer therapy<sup>[18]</sup>. The identification of basement membrane as a source of angiogenic and antiangiogenic factors and as a potential diagnostic or therapeutic target in cancer further increases the importance of this component of the wall of tumor vessels<sup>[48-51]</sup>.

The basement membrane in tumor vessels has obvious abnormalities, including irregular thickness, multiple layers, loosing association with endothelial cells and pericytes, and reminiscent of degenerating or regenerating blood vessels. Basement membrane also covers most endothelial sprouts. These characters of the vascular basement membrane are in accordance to the dynamic character of endothelial cells and pericytes in tumors<sup>[18]</sup>.

It is supposed that the basement membrane of endothelial cells is degraded during angiogenesis to enable sprout formation and endothelial cell migration. Instead, it is possible that the basement membrane does not disappear during angiogenesis but remodels continuously as endothelial sprouts form and new vessels grow<sup>[52]</sup>. Many morphological studies have reported that the basement membrane of tumor vessels is defective or absent<sup>[53]</sup>. However, other studies suggest that it is present but morphologically abnormal<sup>[54]</sup>. The application of different approaches probably results in these discrepancies. Some reports of defective or absent basement membrane on tumor vessels are based on observations made by transmission electronic microscopy (TEM) or scanning electronic microscopy (SEM)<sup>[55-56]</sup>. The high resolution of these approaches can reveal tiny abnormalities but may miss unstained components and the overall amount of coverage. Light microscopic immunohistochemistry can detect the specific protein components of basement membrane and readily provide an overview despite its lower spatial resolution. This approach is frequently used to visualize basement membrane in tumors<sup>[57-58]</sup>. The challenge is how to distinguish the basement membrane of blood vessels from that associated with tumor cells or other components. Co-localization of markers for basement membrane and endothelial cells helps in this regard. However, the distribution and extent of coverage of basement membrane proteins specifically associated with endothelial cells and pericytes has been examined in relatively few tumors<sup>[54]</sup>.

### **INTERACTIONS AMONG ENDOTHELIAL CELLS, PERICYTES, AND BASEMENT MEMBRANE OF TUMOR VESSELS**

Interactions among endothelial cells, pericytes, and basement membrane in the tumor blood vessel wall have recently come into focus as central processes in the regulation of vascular formation, stabilization, remodeling, and function.

Tumor vessels are heterogeneous in their pericytes coverage and antiangiogenic therapy which is directed against the endothelium appears to result in ablation of the naked endothelial tubes, whereas the pericytes covered stretches are protected<sup>[59]</sup>. This has led to the idea that combinations of antiendothelial and antipericyte agents might act synergistically in antiangiogenic therapy. A research group tested this concept by applying combinations of VEGF- and PDGF-pathway inhibitors in a transgenic model of pancreatic islet tumors. Indeed, they recorded complementary and synergistic antiangiogenic and antitumor effects<sup>[60]</sup>.

It was proposed that the vessel sprouting is produced by endothelial offshoots followed by pericytes coverage. Retraction of the endothelial tube, on the other hand, precedes pericytes regression in the process of vessel

regression<sup>[55]</sup>. The process of vessel sprouting and regression can be concluded as follows<sup>[55]</sup>: (1) Endothelial cell protrusion from a preexistent vessel is a first step of sprouting. Then, the endothelial cells proliferate, become covered by pericytes, and form a tubular structure. The tip of the endothelial cells is exposed without any investment of pericytes. (2) Vessel regression proceeds with retraction of the endothelial tube, and is followed by that of the pericytes. A tapered basement membrane remains to cover this type of vascular tip.

## SUMMARY

Tumors need a vessel network to supply them with blood and nutrition. The formation of tumor vessel needs the cooperation of endothelial cells, pericytes, and basement membrane. Anyway, the data described above are far from complete, but should be rather accentuated that a thorough information of the cellular structure and function of tumor vessels will be surly helpful for developing effective antiangiogenic agents for treatment of cancer. The antiangiogenic strategies must be harmonized with the mechanism of the angiogenesis of tumors. Better understanding of the cellular structure and function of the tumor vessels will certainly help to find novel antiangiogenic agents and novel anti-cancer strategies in combination with standard radio- and chemotherapies.

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(Edited by Jane Chen)