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Heterogeneity of tumor endothelial cells and drug delivery☆

Kyoko Hida [a,*], Nako Maishi [a], Yu Sakurai [b], Yasuhiro Hida [c], Hideyoshi Harashima [b]

[a] Vascular Biology, Frontier Research Unit, Institute for Genetic Medicine, Hokkaido University, N15, W7, Kita-ku, Sapporo 060-0815, Japan
[b] Laboratory of Innovative Nanomedicine, Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan
[c] Department of Cardiovascular and Thoracic Surgery, Hokkaido University Graduate School of Medicine, Sapporo, Japan

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ABSTRACT

To date anti-angiogenic therapy has been used for cancer therapy widely, yielding promising results. However, it has been elucidated that current anti-angiogenic drug has several issues to be solved, such as side-effects and drug resistance. It has been reported that tumor endothelial cells (TECs) differ from normal counterparts. In addition, it was shown that the TECs are heterogeneous according to the malignancy status of tumor. The development of novel strategy for targeting tumor vasculature is required. Recently, we have developed an active targeting system, which targets TECs specifically. In this review, we will discuss how TECs in tumor vasculature are heterogeneous and offer new perspectives on a drug delivery system, which can target heterogeneous tumor blood vessels from a viewpoint of personalized medicine.

Contents

1. Introduction

Tumor blood vessels provide nutrition and oxygen, eliminate waste from tumor tissue, and they play a role for gatekeepers for tumor cells to metastasize to distant organs, such as lung, liver and brain [1,2]. Since Folkman proposed the concept that tumor growth was dependent on angiogenesis (tumor angiogenesis) and tumor blood vessels have been recognized as an important target for cancer therapy (anti-angiogenic therapy) [3], many anti-angiogenic drugs have been discovered and tested, yielding promising results [1]. Angiogenesis is regulated by the

Abbreviations: TECs, Tumor endothelial cells; NECs, Normal endothelial cells; VEGF, Vascular endothelial growth factor; DT, Diphtheria toxin; EPR, Enhanced permeability and retention; EMT, Epithelial mesenchymal transition; HUVEC, Human umbilical vein endothelial cells; HMVEC, Microvascular endothelial cells; HB-EGF, Heparin binding EGF-like growth factor; EGF, Epidermal growth factor; HM-TECs, Highly metastatic tumor; LM-TECs, Low metastatic tumors; NGR, Asparagine–glycine–arginine; RGD, Arginine–glycine–aspartic acid; MEND, Multi-functional envelope nanodevice; cRGD, Cyclic RGD; siRNA, Small interfering RNA.

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* Corresponding author. Tel.: +81 11 706 4315; fax: +81 11 706 4325.
E-mail address: khida@igm.hokudai.ac.jp (K. Hida).

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balance between the angiogenic and the endogenous anti-angiogenic factors that are released by tumor and host cells in the relevant microenvironment [4]. Tumor blood vessels differ morphologically and phenotypically from normal blood vessels [5,6].

We previously reported that tumor endothelial cells (TECs) differ from normal endothelial cells (NECs) in characteristics such as cell proliferation, migration ability, gene expression profile, and responses to growth factors and several drugs [7–14].

Recent studies have reported that current anti-angiogenic drugs [mostly inhibitors of vascular endothelial growth factor (VEGF) signaling], sometimes, cause severe side-effects because of damage to NECs. Furthermore, resistance to anti-angiogenic drugs has been reported, in part caused by the heterogeneity of tumor blood vessels [15]. Because the functions of TECs are important in metastasis, we have investigated differences between the TECs of tumors with differing malignancy. We recently investigated phenotypic differences between TECs isolated from high and low metastatic tumors in order to analyze the correlation between TEC characteristics and malignancy status of the tumor [16]. In addition, we have developed an active targeting system, which specifically targets TECs [17,18]. In this review, we will discuss how TECs in tumor vasculature are heterogeneous and offer new perspectives on a drug delivery system, which can target tumor blood vessels from a viewpoint of personalized medicine.

2. Understanding of heterogeneity of tumor endothelial cells (TECs) for personalized anti-angiogenic therapy

2.1. Tumor angiogenesis

Angiogenesis, the process of growth of new blood vessels, is necessary for tumor progression and metastasis [3,19].

Thus, attempts to target TECs with angiogenic inhibitors (anti-angiogenic therapy) have been an important strategy for cancer therapy, and many anti-angiogenic drugs have been discovered and tested to date [1].

A traditional concept in anti-angiogenic therapy has been as follows: i) one TEC supports many tumor cells; thus, the targeting of endothelial cells can be a much more effective strategy than the targeting of tumor cells. ii) TECs are the same among all tumor types; hence, an ideal anti-angiogenic drug would be useful in treating all cancers. iii) Until recently, TECs were believed to be genetically stable; therefore, TECs may not acquire drug resistance, unlike tumor cells. However, recent studies suggest that TECs are different from NECs and may also be heterogeneous among organs or tumor types. Drug resistance has been reported in anti-angiogenic therapy [20], although the mechanism involved in resistance to anti-angiogenic therapy is still not fully understood. Also, contrary to the presumption that anti-angiogenic drugs should not be toxic unlike cytotoxic drugs, they have been reported to cause severe side-effects such as lethal hemoptysis [2,21], intestinal perforation [22,23], cardiac ischemia or infarction [24,25] and cerebral ischemia [26].

To develop an ideal tumor anti-angiogenic therapy, it is very important to understand the heterogeneous phenotype of tumor vasculature and TECs.

2.2. Tumor blood vessels and drug delivery

Tumor vasculature plays a key role in delivery of not only oxygen and nutrition but also drugs to tumor tissue. It is well documented that tumor blood vessels differ morphologically from normal blood vessels (Fig. 1). It has been suggested that abnormalities in tumor blood vessels may be attributable to an imbalanced expression of angiogenic factors and inhibitors. Tumor vessels are unorganized whereas normal vasculature shows a hierarchical branching pattern of arteries, capillaries and veins [6]. TECs do not form regular monolayers and do not have a normal endothelial barrier function [27]. Basement membranes have a varied thickness in type IV collagen layers, which are not usually seen in normal vasculature [28]. Pericytes and TECs have abnormally loose associations to each other [29]. These abnormalities result in leakiness of tumor blood vessels. The high interstitial fluid pressure in a tumor causes blood vessel collapse. Thus, tumor blood vessels are often twisted in appearance with uneven vessel diameters due in part to compression of the immature vessel wall [5]. These abnormalities consequently affect blood flow. Indeed, tumor vasculature shows chaotic blood flow. For example, some tumor vessels are not perfused with blood and some vessels have chaotic blood flow that may reverse direction. This is one of the reasons why tumor tissue is usually under hypoxic conditions, even though it is highly vascularized. This can sometimes lead to resistance to radiation therapy [30].

There is dysfunction also in TECs. NECs form a uniform and continuous monolayer, whereas TECs have an irregular shape and size with long and fragile cytoplasmic projections extending across the lumen.

In addition, there are often gaps between adjacent TECs, resulting in the obstruction of tumor cells proximal to these gaps [31].

Similarly, transcellular fenestrae have also been observed in the tumor blood vessel wall.

Taken together, these endothelial gaps and fenestrae in the TECs are perhaps able to cause hemorrhage and plasma leakage, which are common observations in tumors. Furthermore, holes in the tumor blood vessels may be involved in a mechanism for tumor cell intravasation, an initial step of metastasis.

On the other hand, such leaky vasculature offers the advantage of drug delivery because vessel leakiness promotes nanodrug accumulation in the tumor tissue. This is well known as the “enhanced permeability and retention (EPR) effect” [32,33]. The EPR effects have been exploited for passive targeting of drugs into tumors. The most significant angiogenic factor is VEGF, which stimulates angiogenesis and also increases the permeability of blood vessels. Currently, inhibition of VEGF signaling has been a strategy for both anti-angiogenic therapy and vascular normalization, in which anti-angiogenic agents temporarily “normalize” both structure and function of tumor vasculature and more efficiently enable the delivery of oxygen and therapeutic drugs. Vascular normalization by anti-VEGF drugs seems to be an attractive strategy regarding delivery of drugs to tumors but it is hard to predict how long anti-VEGF drugs can be used and when such drugs can be stopped. In some studies, long term anti-VEGF therapy can sometimes cause undesirable ischemic changes, resulting in the cancer becoming more malignant [34] because of epithelial mesenchymal transition (EMT) induced by hypoxia in tumor cells. In addition, it has been reported that the tumor vasculature is heterogeneous in terms of structure and maturity with respect to pericyte coverage, tight junctions, and fenestration. The EPR effect is a concept of a passive drug targeting to tumors and, is dependent on structural differences in tumor vessels. On the other hand, active targeting of the tumor vasculature using anti-angiogenic therapy seems to be independent of these structural differences in blood vessels (Fig. 2). This is because a specific binding between ligand and surface protein on TECs, plays a pivotal role in active-targeting strategy.

To develop novel anti-angiogenic drug regimen with no side-effects and higher efficacy, understanding of the heterogeneity of tumor vasculature is important.

In Section 2.3, we focus on heterogeneity of TECs from the viewpoint of drug delivery, especially active targeting to tumor blood vessels.

2.3. Properties of TECs

Traditionally, there have been assumptions that TEC is the same as NEC because blood vessels branch into tumors from surrounding normal tissue, and thus most studies on tumor angiogenesis have been conducted using NECs such as human umbilical vein endothelial cells (HUVEC) for a long time. However, it has been elucidated that there are phenotypic differences at the molecular and functional levels between TECs and NECs, using isolated endothelial cells where St Croix's