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Review

Abnormalities of tumor endothelial cells and cancer progression

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ABSTRACT

Tumor growth and metastasis are dependent on angiogenesis, which is the formation of new blood vessels. The newly formed blood vessels around the tumor supply oxygen and nutrients to the tumor, supporting its progression. Moreover, these blood vessels also serve as channels through which tumor cells metastasize to distant organs. The balance between angiogenic stimulators and inhibitors regulates angiogenesis in the tumor microenvironment. Tumor blood vessels, especially the endothelial cells lining tumor blood vessels (tumor endothelial cells [TECs]), are important targets in cancer therapy. As newly formed tumor blood vessels originate from pre-existing normal vessels, tumor blood vessels and TECs have traditionally been considered to be the same as normal ones.

However, tumor blood vessels have a distinctively abnormal phenotype, including morphological alterations. Recently, it has been revealed that TECs constitute a heterogeneous population, exhibiting characteristics that are largely induced by tumor microenvironmental factors. Furthermore, TECs induce cancer progression through metastasis. In this review, we describe recent studies on TEC abnormalities related to cancer progression and consider their therapeutic implications.

Contents

1. Tumor angiogenesis and antiangiogenic therapy

Angiogenesis is the process of new blood vessel formation and is essential for tumor progression. Tumor blood vessels supply the tumor with required oxygen and nutrients, in addition to removing waste products from tumor tissue. Furthermore, tumor blood vessels also provide a gateway for the metastasis of the tumor [1,2]. Antiangiogenic therapy is a relatively novel cancer therapy that was first proposed by Dr. Folkman [1]. The endothelial cells that line tumor blood vessels (tumor endothelial cells [TECs]) have emerged as important targets of angiogenic inhibitors (antiangiogenic therapy) and provide a strategy for cancer treatment; many antiangiogenic drugs have been developed and tested to date [3]. The basis for pursuing this therapy can be summarized as follows: (1) The survival of a large population of tumor cells depends on a few TECs, such that targeting TECs may be more efficient than targeting tumor cells. (2) As TECs exhibit similar characteristics regardless of their tumor of origin, a single, effective antiangiogenic

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drug can be used to treat many forms of cancer. (3) It is considered that TECs in cancer stroma are genetically stable, unlike tumor cells, and therefore do not become drug resistant.

The first antiangiogenic drug, bevacizumab, is a neutralizing antibody against human vascular endothelial growth factor (VEGF) that was approved in 2004 because of the efforts of Dr. Folkman and his collegue [2]. Antiangiogenic drugs (mostly targeting the VEGF/VEGF receptor signaling pathway) have been applied in combination with chemotherapeutic drugs in many cancers. However, although antiangiogenic drugs were considered to be less toxic than other cytotoxic drugs, from recent studies, it is now clear that they may induce severe side effects such as lethal hemoptysis [4,5] and intestinal perforation [6,7]. Accordingly, an important goal in cancer therapy is to develop novel and safer tumor antiangiogenic agents, which in turn depends on a thorough understanding of the biology of TECs.

2. Abnormality of tumor blood vessels

Tumor blood vessels differ in many ways from normal blood vessels. Specifically, unlike normal blood vessels, tumor blood vessels are not organized in a hierarchical branching pattern [8]. The underlying basement membranes of tumor blood vessels vary in thicknesses, and their TECs do not form regular monolayers [9], in contrast to normal blood vessels [10]. In general, tumor blood vessels are often morphologically immature. Pericyte coverage is lower, and they associate with TECs loosely, whereas in normal blood vessels, pericytes and endothelial cells bind to each other tightly [11]. TECs are morphologically irregular, with long cytoplasmic projections extending across the lumen, whereas normal endothelial cells (NECs) are uniform. The endothelial gaps and transcellular fenestrae in the walls of tumor blood vessels result in hemorrhage and plasma leakage.

Consequently, tumor blood vessels are usually leaky. The high interstitial fluid (IFP) pressure in cancer causes vessel collapse and compromised blood flow [12]. These abnormalities of tumor blood vessels have been attributed to the unbalanced expression of angiogenic factors and inhibitors. In addition, these features of tumor vasculature may be one of the reasons why cancers tissues are usually hypoxic, although they are usually highly vascularized. Hypoxia in cancer causes resistance to radiation therapy [13].

Moreover, an abnormal vessel structure allows filling in by adjacent tumor cells and provides a mechanism for tumor cell intravasation, an initial step in metastasis [14].

3. Abnormal phenotype of tumor endothelial cells

Phenotypic difference at the molecular and functional levels have been identified using TECs and NECs isolated from tumor and normal tissue, respectively. However, for many years, most studies on tumor angiogenesis were performed using NECs such as human umbilical vein endothelial cells (HUVECs), and few studies had examined the features of isolated TECs. TECs have been isolated by immunomagnetic sorting using antibodies against endothelial cell markers such as CD31 or CD144, but possible contamination by tumor cells and fibroblasts has been a concern for TEC culture. We have eliminated human tumor cells using diphtheria toxin (DT) in a subculture of mouse TECs isolated from a human tumor xenografted into mice [15]. The use of DT was based on the fact that heparin-binding epidermal growth factor (HB-EGF) is a receptor of DT in human but not in mouse cells, and DT is toxic for human cells expressing HB-EGF but not for mouse cells [16]. Nonetheless, obtaining enough TECs to allow their study remains difficult because these cells constitute only a small cell fraction within cancer tissues.

Recent studies have demonstrated molecular differences between TECs and NECs [17,18]. For example, our laboratory has shown that isolated TECs exhibit several abnormalities compared with NECs [15,19], including differences in their responsiveness to growth factors such as EGF [20], adrenomedullin [21], and VEGF [22], all of which contribute to the proangiogenic phenotype of TECs. VEGF stimulates the migration of TECs and enhances their survival in an autocrine manner. This VEGF autocrine loop also contributes to the antiapoptotic phenotype of TECs when compared with NECs [22]. Furthermore, several cytogenetic abnormalities, such as aneuploidy and abnormal centrosomes, have been reported in TECs from mouse tumors [15] and in human renal carcinomas [23]. Studies confirmed that these abnormalities were not due to tumor cell contaminants. Cytogenetic abnormalities indicate genetic instability and drug resistance and likely explain the frequently observed resistance of TECs to chemotherapeutic agents [24,25], including vincristine resistance of TECs from a renal carcinoma [24], 5-fluorouracil and adriamycin resistance of TECs from a hepatocellular carcinoma [25,26], and the tumor-derived VEGF-mediated resistance of TECs to paclitaxel [26]. However, the actual mechanism underlying the drug resistance of TECs remains unknown.

4. Heterogeneity of tumor endothelial cells

Endothelial cells may be morphologically and functionally heterogeneous. For example, the rolling velocity and arrest frequency of leukocytes at NEC junctions is different than that at central areas [27]. Inter-organ differences in NECs have also been reported [28]. In pre-existing blood vessels, stem-like endothelial cells with a proangiogenic phenotype have been identified. For example, Naito et al. showed the presence of endothelial cells expressing ABCB1/p-glycoprotein (P-gp) in residential normal blood vessels and tumor blood vessels [29,30]. Additionally, in rat brain blood vessels, P-gp and endothelial barrier antigen are heterogeneously expressed, particularly at the single-cell level, suggesting a lack of uniformity in the blood-brain barrier [31].

5. TEC heterogeneity

There are many examples of TEC heterogeneity [15,17,24,32]. We have demonstrated that some TECs show upregulated aldehyde dehydrogenase (ALDH) expression. In addition, these $ALDH^{high}$ TECs formed more tubes on matrigel even under serum starvation, suggesting that they are more proangiogenic than $ALDH^{low}$ TECs, although the detailed role of ALDH in angiogenesis requires further study [33].

Within the tumor vasculature, the morphology and pericyte coverage of tumor blood vessels varies depending on tumor type and progression stage [28,34,35]. Furthermore, we have reported that these heterogeneities [36] differ between highly metastatic (HM) and low metastatic (LM) tumors. The blood vessels of HM tumors are more immature, with fewer pericytes, than the blood vessels of LM tumors [36]. These features contribute to the higher hypoxic nature of HM than LM tumors. TECs isolated from HM tumors (HM-TECs) have higher proliferative index, motility, and sensitivity to VEGF; more abundant extracellular matrix (ECM); and greater invasive ability than their LM counterparts (LM-TECs). In addition, several angiogenesis-related genes, such as VEGFR-1, VEGFR-2, and VEGF, and the genes encoding the PI3K/Akt signaling pathway, which is crucial in angiogenesis, are upregulated in HM-TECs [37,38]. The higher invasive potential of HM-TECs, including a higher level of Akt phosphorylation under basal conditions and upregulation of gelatinase/collagenase IV matrix metalloproteases (MMP-2 and MMP-9) subsequent to PI3K/Akt pathway activation,

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