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Review

The dark side of tumor-associated endothelial cells

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ABSTRACT

Angiogenesis is a hallmark of cancer and a requisite that tumors must achieve to fulfill their metabolic needs of nutrients and oxygen. As a critical step in cancer progression, the 'angiogenic switch' allows tumor cells to survive and grow, and provides them access to vasculature resulting in metastatic progression and dissemination. Tumor-dependent triggering of the angiogenic switch has critical consequences on tumor progression which extends from an increased nutrient supply and relies instead on the ability of the tumor to hijack the host immune response for the generation of a local immunoprivileged microenvironment. Tumor angiogenic-mediated establishment of endothelial anergy is responsible for this process. However, tumor endothelium can also promote immune tolerance by unbalanced expression of co-stimulatory and co-inhibitory molecules and by releasing soluble factors that restrain T cell function and induce apoptosis. In this review, we discuss the molecular properties of the tumor endothelial barrier and endothelial anergy and discuss the main immunosuppressive mechanisms triggered by the tumor endothelium. Lastly, we describe the current anti-angiogenic therapeutic landscape and how targeting tumor angiogenesis can contribute to improve clinical benefits for patients.

1. Introduction

1.1. The angiogenic switch

The proliferation of every normal cell in our body is finely tuned by a network of growth-promoting and inhibitory mechanisms, in the form of soluble factors, physical stress and cell–cell interactions. When homeostatic control succumbs or is hijacked, the cell fails to continue as master of its own destiny within the tissue architecture. Unregulated proliferation and resistance to apoptosis represent two of the first critical events in tumor transformation; afterwards, tumor progressively evolves, driven by genomic instability, which in turn promotes acquisition of new functions and sculpts anti-tumor immunity in a process called "cancer immunoediting" [1,2]. The contribution of the immune system to tumor evolution reflects a double-edged sword that restricts tumor growth in the elimination and equilibrium phases of cancer but eventually succumbs to tumor modulation by supporting cancer progression and metastatic spread during the escape phase. According to this hypothesis, tumors cannot be considered merely a mass of neoplastic and polarized stromal cells but rather a conductor, which exploits physiological immune regulatory mechanisms to generate peripheral and local immune tolerance, establishing a tumor-promoting microenvironment that supports its own growth and ability to metastasize.

Neoplastic cells are characterized by specific hallmarks including undefined proliferation, evasion of growth suppressors, replicative immortality, resistance to cell death, invasive properties and ability to manipulate the local microenvironment by inducing angiogenesis and promoting immune system evasion [3]. Within blood vessel generation, vasculogenesis (assembly of the de novo vasculature assisted by recruitment of endothelial progenitors cells) and angiogenesis (sprouting of new vasculature from already established vessels, a process guided by proliferation of endothelial cells – ECs) are both active during organogenesis. Angiogenesis predominates in the adult and this term was introduced more than 200 years ago by the surgeon John Hunter to describe the growth of new blood vessels during tissue development in adult animals [4]. However, the players involved in angiogenesis and its role in promoting cancer progression were described quite recently by Judah Folkman [5] and then characterized by others [6,7]. Under physiological state, the endothelium is in a quiescent condition, maintained by a finely-tuned homeostatic process, that can be interrupted periodically (*e.g.* during female reproductive cycle), either by a reduction in angiostatic molecules or an increase in angiogenic factors according to the requirements of each body tissue. In each of these cases,

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after local basal membrane degradation, ECs change shape and begin proliferating by sensing a gradient of pro-angiogenic signals, invading the surrounding stroma and generating new capillaries. Primary angiogenic mediators include the vascular endothelial growth factor (VEGF) family proteins, fibroblast growth factors (FGF), platelet-derived growth factor (PDGF), placental growth factor (PlGF), angiopoietin (ANG) 2, chemokines and cytokines such as chemokine (C-X-C motif) ligand (CXCL)8, tumor necrosis factor (TNF) α, interleukin (IL) 1β, tumor growth factor (TGF) β, prokineticin (BV8), and matrix metalloproteases (MMPs). Angiogenesis is restricted by several molecules with angiostatic properties such as thrombospondin (TSP) 1, angiostatin, soluble VEGF1, endostatin, vasostatin, calreticulin, tissue inhibitor of metalloproteases (TIMPs), as well as cytokines such as interferon (IFN)γ, IFN-induced cytokines binding CXCR3 (CXCL9/MIG, CXCL10/IP10, CXCL11/IP9) [8], and others [9]. The expression of angiogenic factors is finely tuned by local oxygen levels through hypoxia inducible factor (HIF). This protein is a heterodimer composed by HIF1α and HIF1β able to activate the expression of genes through the binding to hypoxia response element (HRE) sequences placed in their promoter regions. Both subunits are constitutively expressed but HIF1α is quickly hydroxylated under normoxic conditions [10], ubiquitinated and degraded after translation [11]. Once a tumor reaches the size of few millimeters, the simple diffusion of oxygen and nutrients from the surrounding tissue is not sufficient to support cell growth. This results in a condition of low oxygen concentration that stabilizes HIF1α which can then enter the nucleus, dimerize with HIF1β, and trigger the expression of many angiogenic factors (VEGFs, PDGFB, PlGF, ANGPTs) [12], proangiogenic chemokines (stromal cell derived factor 1α - SDF1α/CXCL12 and sphingosine 1 phosphate) and receptors (CXCR4 and sphingosine 1 phosphate receptor) [13], the so called “angiogenic switch”. This process generates chronic endothelium activation, which results in continuous sprouting of new vessels supporting tumor growth [14]. Induction of angiogenesis represents an early event during tumorigenesis [15] for two biological reasons: an elevated proliferation rate requires high consumption of nutrients and oxygen and removal of toxic metabolic products, both duties performed by blood circulation. Furthermore, proliferative switches (*e.g.* RAF and RAS activation) induce activation of the angiogenic program [16,17]. VEGF members include VEGFA, VEGFB, VEGFC, VEGFD and PlGF and exert their angiogenic functions by interacting with the VEGF receptors (VEGFRs). VEGFRs are tyrosine kinase receptors (TKRs) that bind each of these ligands, triggering a tyrosine kinase-based signaling cascade: VEGFR1 is triggered by VEGFB and PlGF to induce haematopoiesis, monocyte migration, and EC metabolism, whereas VEGFR2 is activated following VEGFA binding to induce EC proliferation, survival, angiogenesis and vascular permeability. VEGFC and VEGFD are involved in activating lymphangiogenesis (proliferation of lymphatic endothelial cells – LECs) by triggering VEGFR3 [18]. FGF binds to FGFR and induces the proliferation and migration of ECs [19]. FGFR triggering activates mitogen activated protein (MAPK) and phosphatidylinositol-3 (PI-3) kinases through the adapter protein fibroblast growth factor receptor substrate (FRS2α). The latter also interacts with VEGFR2, inducing extracellular signal-regulated kinase (ERK1/2) mediated VEGFR up-regulation, potentiating VEGF-mediated ECs activation and suggesting a mechanism of cooperation between two pathways [20,21]. In alignment with these findings, FGF inhibition critically affects VEGFA-induced angiogenesis [22].

Another feature of tumor angiogenesis is the presence of polarized ECs, characterized by expression of growth factor receptors (*e.g.* EGFR) [23] and tumor endothelial markers (TEM) [24], up-regulation of angiogenic receptors and constitutive activation of survival PI3K-AKT signaling pathway [25,26], which confer augmented proliferation, migratory and drug resistance capabilities compared to normal ECs. These genetic and phenotypic differences result in morphological, structural and functional abnormalities of tumor-associated blood vessels. The abnormalities include enlarged vessels with severe branching, multilayered and discontinuous EC alignment with defective coverage by pericytes and basement membrane, resulting in a dysfunctional vasculature characterized by low tissue perfusion, leakiness and poor blood flow [27].

The generation of an actively growing solid tumor mass with a high cellular proliferative rate significantly reduces the availability of O_2, especially for the cells found within the inner tumor core. Tumor and stromal cells subsequently release augmented levels of pro-angiogenic factors, especially VEGFA, angiopoietin (ANGPT) 2, and CXCL12, driven by the activation of HIF1 [28,29]. Another consequence of reduced O_2 availability is the metabolic change from oxidative phosphorylation to aerobic glycolysis, which results in dramatically enhanced glucose consumption and elevated accumulation of lactate with consequent acidosis of the tumor microenvironment. These hypoxia-dependent metabolic modifications are associated with drug resistance and support angiogenesis at both the tumor and stromal levels. Within tumor cells, nutrient deprivation and acidosis stabilize *VEGFA* mRNA [30], whereas tumor-derived lactate supports angiogenesis and tumor growth by activating ECs *via* an autocrine NF-κB-CXCL8 pathway that promotes migration and tube formation [31] and polarizes tumor-associated macrophage (TAM) towards a proangiogenic M2-like phenotype [32]. CXCR4 is expressed by many cell types including leukocytes, ECs, epithelial and cancer cells. CXCL12-CXCR4 interaction is directed involved in chemotaxis, invasion and recruitment of ECs to neoangiogenic niches to promote blood vessel sprouting [33]; moreover, the CXCL12-CXCR4 axis fosters angiogenic responses by activating AKT signaling and consequent VEGF synthesis in cancer cells [34]. Thus, blood vessel sprouting within tumors reflects a process in which tumor and stromal cells cooperate and synergize to sustain tumor growth and invasion.

Tumor stroma is composed of two main categories of cells, classified according to their origin surrounded by an extracellular matrix (ECM): bone marrow-derived, tumor infiltrating hematopoietic cells (predominantly leukocytes), and tissue resident cells, such as ECs, pericytes, adipocytes, fibroblasts and resident macrophages. Within the tissue-resident stromal cells, the most important angiogenic players are cancer-associated fibroblasts (CAFs) and pericytes. TGFβ-activated CAFs participate in tumor angiogenesis by directly secreting angiogenic factors such as VEGFA, bFGF, CXCL12 and by modifying the composition and stiffness of the ECM and the local interstitial pressure, collectively contributing to tumor progression [35,36]. Pericytes physically surround the vessels and play a crucial role in regulating endothelial proliferation and in promoting ECs survival and establishment of tight junctions [37]. However, cancer polarized-pericytes may support the survival of tumor blood vessels since their targeting in combination with anti-angiogenic inhibitors improves treatment efficacy compared to each single agent in a preclinical model of pancreatic islet carcinoma [38]. Within the tumor-infiltrating, bone marrow-derived leukocyte component, TAMs, granulocytes and myeloid derived suppressor cells (MDSCs) are the most abundant and representative angiogenic inducers. TAMs can either support or restrict blood vessel sprouting, according to the immune context and specific microenvironment to which they are exposed [39]. M2-polarized TAMs induce angiogenesis by directly releasing high amounts of growth factors such as VEGFA, VEGFC, PlGF, basic fibroblast growth factor (bFGF), platelet derived growth factor β (PDGFβ), cytokines such as IL1β, or by producing membrane-bound or soluble proteases such as cathepsins or MMPs, which mobilize proangiogenic molecules sequestered in the ECM and remodel it supporting EC invasion [40–42]. Moreover, TAMs may indirectly promote angiogenesis by releasing inflammatory cytokines (IL6, CXCL8), which support the recruitment and activation of other myeloid subsets such as granulocytes and MDSCs [40]. Granulocytes and MDSCs are another main source of proangiogenic factors, especially VEGFA, bFGF, MMP9, BV8, whose expression is up-regulated following triggering of CSF3R and STAT3 activation [43]. In support of neutrophil and MDSC proangiogenic role, many researchers have