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Milestones in tumor vascularization and its therapeutic targeting

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Michele De Palma [1,2,3] & Douglas Hanahan [1,2,3,4]

Research into the mechanisms and manifestations of solid tumor vascularization was launched more than 50 years ago with the proposition and experimental demonstrations that angiogenesis is instrumental for tumor growth and was, therefore, a promising therapeutic target. The biological knowledge and therapeutic insights forthcoming have been remarkable, punctuated by new concepts, many of which were not foreseen in the early decades. This article presents a perspective on tumor vascularization and its therapeutic targeting but does not portray a historical timeline. Rather, we highlight eight conceptual milestones, integrating initial discoveries and recent progress and posing open questions for the future.

The vascular system is composed of blood vessels (BVs) made of tube-forming endothelial cells (ECs) and periendothelial support cells[1]. Like organs, tumors establish a vascular network that supplies oxygen and nutrients and satisfies the metabolic needs of proliferating cancer cells. Tumor vascularization is achieved primarily through angiogenesis, the process involving the sprouting and growth of new BVs from a preexisting vascular network. Owing to incessant and deregulated proangiogenic signaling, tumor BVs (TBVs) frequently manifest a chaotic architecture characterized by excessive branching, abundant dilatations, constrictions and dead ends, discontinuous EC lining, aberrant basement membranes and reduced pericyte coverage. These features are associated with defective BV maturation and functionality, leading to incoherent perfusion, fluid leakage and microhemorrhaging[1]. However, increasing evidence indicates that tumors can also vascularize through angiogenesis-independent mechanisms, most prominently by co-option of the normal tissue vasculature through perivascular cancer cell growth. In these cases, the TBVs display a more coherent and organized architecture[2].

This Perspective focuses on the mechanisms of tumor vascularization and its therapeutic targeting, conceived in the form of eight conceptual milestones (Fig. 1). The related lymphatic vascular system that drains fluid from tissues and tumors through immune-sensing lymph nodes is beyond our scope and has been reviewed elsewhere[3].

Associating angiogenesis with tumor progression

Tumors have long been known to vascularize by attracting and remodeling BVs[4,5]. The use of transparent windows in the early twentieth century revealed that implanting neoplastic tissue in experimental animals triggered more robust proliferative vascular reactions than nonneoplastic tissue. Moreover, tumor grafts failing to induce such vascular responses could not grow[6,7]. This angiogenic response demonstrably involved hitherto unidentified, diffusible proangiogenic factors released by the tumor[5,8]. In 1971, it was postulated that drugs capable of inhibiting tumor vascularization would provide therapeutic benefit[9,10], spawning the modern field of angiogenesis. In subsequent decades, it was experimentally validated that inhibiting angiogenic signaling indeed impairs the vascularization and growth of experimental tumors[11,12] and provides clinical benefit to patients with cancer[13–15]. However, despite early predictions of curative potential[16], clinical outcomes typically only involved delayed time to progression, with modest overall survival benefits in selected cancer types[17,18].

The induction of tumor angiogenesis (the 'angiogenic switch') was found to represent a discrete and requisite step in the multistage development of certain tumor types[19–21], leading to its incorporation as a qualitatively distinct hallmark of cancer[22]. The occurrence of a discrete angiogenic switch was initially demonstrated in RIP1-Tag2 (rat insulin promoter 1–T antigen 2) mice, a genetically engineered mouse model (GEMM) of pancreatic neuroendocrine tumorigenesis[23]. In these mice, a subset of oncogene-induced hyperplastic islets progress into 'angiogenic islets' and then solid tumors with an activated vasculature characterized by EC proliferation, capillary dilatation and sprouting, and frequent blood islands consequent to microhemorrhaging[19]. A fraction of the neoplastic islets were found capable of inducing the migration, proliferation and tube formation of cocultured ECs ex vivo,

[1]Swiss Institute for Experimental Cancer Research (ISREC), School of Life Sciences, Swiss Federal Institute of Technology in Lausanne (EPFL), Lausanne, Switzerland. [2]Agora Cancer Research Center, Lausanne, Switzerland. [3]Swiss Cancer Center Léman (SCCL), Lausanne, Switzerland. [4]Ludwig Institute for Cancer Research, Lausanne Branch, Lausanne, Switzerland. e-mail: michele.depalma@epfl.ch; douglas.hanahan@epfl.ch

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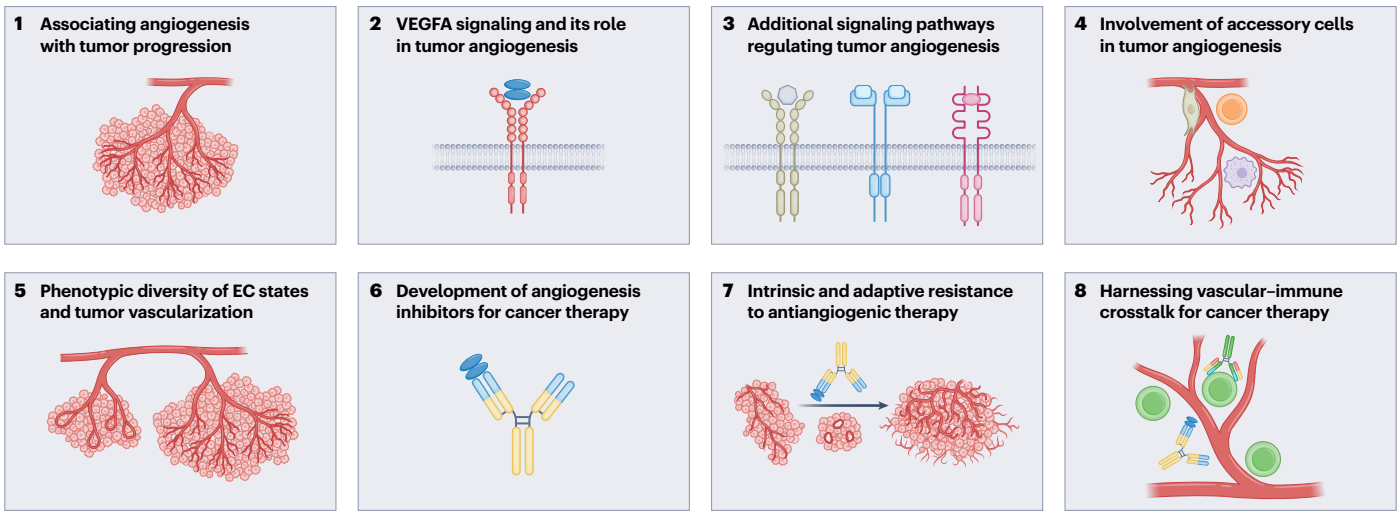

Fig. 1 | Eight conceptual milestones for tumor vascularization and its therapeutic targeting. (1) Associating angiogenesis with tumor progression; (2) VEGFA signaling and its role in tumor angiogenesis; (3) additional signaling pathways regulating tumor angiogenesis; (4) involvement of accessory cells in tumor angiogenesis; (5) phenotypic diversity of EC states and tumor vascularization; (6) development of angiogenesis inhibitors for cancer therapy; (7) intrinsic and adaptive resistance to antiangiogenic therapy; and (8) harnessing vascular–immune crosstalk for cancer therapy.

indicative of a distinctive proangiogenic state[19]. Further evidence was provided in other cancer types[24–27]. For example, activated BVs subtending increasingly aberrant epithelia were evident in the non-invasive stages of ductal carcinoma in situ in human breast cancer[26] and intraepithelial dysplasia in human cervical cancer[27]. Induction of angiogenesis may thus precede and allow the malignant progression of different tumor types[20–22]. The angiogenic switch was initially thought to depend on the de novo synthesis of proangiogenic factors[19]. However, a 'balance hypothesis' has since been proposed, suggesting that a biochemical equilibrium maintains BVs in a quiescent state. This equilibrium is disrupted during the angiogenic switch consequent to increased expression or bioavailability of angiogenesis inducers and/or reduced expression or bioavailability of angiogenesis inhibitors[20,21].

Perhaps counterintuitively, microvessel density in some human cancers is lower than in corresponding normal tissue[28]. Both reduced oxygen consumption by cancer cells and their tolerance of hypoxic conditions may allow for increased intervessel distance in tumors compared to their normal tissue counterparts. Various parameters may influence microvessel density in tumors, including the mode of vascularization (angiogenesis versus co-option), the metabolic and proliferative phenotypes of cancer cells, the diversity and expression level of angiogenic regulators, and the biophysical properties of the tumor-associated stroma (for example, stiffness and interstitial pressure), all of which vary according to tumor type, anatomical site and stage of malignant progression[29]. As a result, microvessel density does not reliably indicate the dependence of a tumor on angiogenesis[28].

Vascular endothelial growth factor A signaling and its role in tumor angiogenesis

The discovery of 'tumor angiogenesis factors' spanned several decades, culminating in the identification of signaling molecules capable of inducing angiogenesis[5]. The prototype was a secreted protein initially identified both as a vascular permeability factor and as a vascular endothelial growth factor (VEGF)[30–33], eventually named VEGFA. Its discovery led to the identification of transmembrane receptors for VEGFA expressed on blood ECs and other cell types[34,35]. VEGFA proved to be induced in tumors[36,37], and functional validation of its involvement in tumor angiogenesis came from VEGFA neutralization studies in tumor-bearing mice[11] and genetic knockouts of the *Vegfa* gene in transplantable tumors[12] and GEMMs of cancer[38].

VEGFA is a secreted homodimeric protein required for embryonic development of the vascular system[1] and is the fundamental VEGF family member operative in both physiological and tumor angiogenesis[34]. Transcription of the *VEGFA* gene is regulated by various mechanisms[34]. In normoxic cells, hypoxia-inducible factor 1α (HIF1α) is hydroxylated by oxygen-sensing prolyl hydroxylases and targeted for degradation by the von Hippel–Lindau (VHL) protein. In the hypoxic tumor microenvironment (TME), HIF1α becomes stabilized to enhance *VEGFA* transcription in both cancer cells[37] and tumor-associated cells[39–41]. The HIF pathway can also be activated through sustained receptor tyrosine kinase signaling, genetic alterations in the *VHL* gene and the activity of other transcription factors[34]. Several VEGFA isoforms—of which $VEGFA_{165}$ is the most abundant and biologically important—are then expressed through alternative mRNA splicing[34]. The bioavailability and activity of VEGFA partly depend on proteolytic remodeling of the extracellular matrix (ECM), to which secreted $VEGFA_{165}$ binds and becomes sequestered[34,42,43].

The proangiogenic and vascular-modulatory functions of VEGFA are primarily mediated by VEGF receptor 2 (VEGFR2; also known as kinase insert domain receptor (KDR)) expressed in blood ECs[34,35]. Ligand binding triggers VEGFR2 homodimerization and transphosphorylation. Activated VEGFR2 transmits signals both through the PLCγ (phospholipase Cγ)–MEK–ERK pathway, which promotes EC proliferation, and the PI3K–AKT–mTOR pathway, which is crucial for EC survival. Moreover, VEGFR2 activates the protein kinase SRC at EC junctions, leading to the phosphorylation and internalization of vascular endothelial cadherin (CDH5) and disruption of paracellular junctions, thus increasing vascular permeability. VEGFA also binds a second receptor, VEGFR1 (also known as Fms-related tyrosine kinase 1 (FLT1)), on ECs. However, the weak tyrosine kinase activity of VEGFR1 and its high affinity for VEGFA suggest that it is a decoy receptor that reduces the bioavailability of VEGFA for binding to VEGFR2, thereby limiting EC proliferation and angiogenesis. Furthermore, ECs can release a soluble form of VEGFR1 that traps VEGFA in the extracellular milieu. VEGFR2 can also heterodimerize with other VEGFRs (for example, VEGFR1 and VEGFR3) and form complexes with coreceptors, such as neuropilins (NRPs), modulating VEGFR2 signaling in vascular ECs[34,35].

Initially thought to be restricted to ECs, the VEGFRs are also expressed in non-EC types[44]. VEGFR1-expressing monocytes and macrophages are proangiogenic, and their frequency in blood or

liver metastases correlates with worse outcomes in patients with colorectal cancer[45]. Additionally, VEGFR1 mediates monocyte chemoattraction toward sources of VEGFA[46] and thus to prospective sites of angiogenesis. Subpopulations of T cells can also express VEGFR1 or VEGFR2, particularly upon activation and in certain cancers[44]. Moreover, some cancer cells express VEGFRs, with responses to VEGFA exhibiting complex and even contrasting effects on tumor progression, invasion and metastasis[47,48]. These varied outcomes can also be modulated by distinct VEGFA coreceptors, such as NRP1 and NRP2, and by heterocomplexes between VEGFR2 and other receptor tyrosine kinases[47,48].

The mechanisms of VEGFA-induced tumor angiogenesis have been partly inferred from models of physiological angiogenesis, such as embryonic and retinal development[1,34,35]. In sprouting angiogenesis, VEGFA gradients stochastically induce specialized 'tip ECs' at the leading edge of vascular sprouts. These cells use ECM-degrading enzymes and filopodia to guide sprout elongation toward VEGFA. Behind the tip ECs, proliferative 'stalk ECs' elongate the sprout and deposit basal membrane constituents. Tip ECs, which do not proliferate, prevent adjacent ECs from acquiring tip cell states through a paracrine mechanism involving the delta-like ligand 4 (DLL4)–Notch pathway[1,34,35]. Genetic or pharmacological DLL4 blockade increases tip EC formation and results in excessive vessel sprouting and a dysfunctional vasculature[49,50].

In RIP1-Tag2 mice, VEGFA is expressed in normal and premalignant pancreatic islets before the angiogenic switch, where it is largely sequestered in the ECM and remains inactive[43]. Activation of extracellular proteases, including matrix metalloproteinase 9 (MMP9), releases VEGFA from the ECM to enable its interaction with VEGFR2 on ECs, thereby triggering the angiogenic switch and sustaining angiogenesis during the subsequent stages of tumor progression[21,42,43]. VEGFA or VEGFR2 blockade inhibits angiogenesis in mouse tumor models by reducing the density, branching and permeability of TBVs[1,11,12,38,51]. This emphasizes the pivotal role of the VEGFA–VEGFR2 pathway in tumor angiogenesis[1,34,35], with potential modulatory contributions from other VEGFRs, such as VEGFR3 and its ligands (VEGFC and VEGFD)[52], and coreceptors such as NRP1 (ref. 53). VEGFA/VEGFR2 inhibition not only impairs sprouting angiogenesis but also prunes newly formed TBVs[54–57]. Mature TBVs are, however, more resilient owing to the protective role of endothelium-associated pericytes[58,59]. While inhibition of the VEGFA–VEGFR2 pathway impairs the angiogenic switch in incipient neoplasia[38] and suppresses angiogenic sprouting in various transplant tumor models[51,60], its effects are generally more nuanced and often transient in GEMMs of advanced-stage cancer[2,61], mirroring clinical observations[17,18,61].

Additional signaling pathways regulating tumor angiogenesis

Angiogenesis bioassays[62] have revealed other signaling moieties capable of stimulating and modulating BV growth. The roster includes growth factors, cytokines, proteases and ECM glycoproteins, as well as lipids and nucleic acids[29], as exemplified below.

Fibroblast growth factor 1 (FGF1), one of the first EC mitogens isolated[63,64], is part of a large family of structurally related FGFs. FGFs interact with high-affinity receptors (FGFR1–FGFR4), along with heparin/heparan sulfate proteoglycans as coreceptors, to promote angiogenesis[65,66]. Unconventional secretion of FGF2 was associated with the onset of angiogenesis in a GEMM of fibrosarcoma[67], and blocking multiple FGFs with a soluble FGFR (FGF trap) had antiangiogenic effects in mouse tumor models[68]. FGF signaling can cooperate with VEGFA-induced angiogenesis and supplant it in the context of VEGFA blockade[69–71]. FGFRs are widely expressed, including in cancer cells and cancer-associated fibroblasts (CAFs), such that FGF signaling may have other tumor-promoting effects. Indeed, intratumoral FGF2 levels correlate with clinical outcomes but not with microvessel density in various human cancer types[65,66].

The platelet-derived growth factor (PDGF) family comprises four heparin-binding growth factors that signal through the PDGFRα and PDGFRβ receptors expressed in various cell types[72]. In cancer, PDGF subunit B (PDGFB) can induce VEGFA expression[73] and support angiogenesis by affecting stromal and immune cells[74]. EC-derived PDGFB recruits PDGFRβ-expressing pericytes that stabilize nascent TBVs[59]. Conversely, PDGFR inhibitors disrupt EC–pericyte interactions, enhancing the sensitivity of TBVs to VEGFA/VEGFR inhibitors[58]. Accordingly, simultaneous inhibition of VEGFRs and PDGFRs shows clinical efficacy in some human cancers[75,76].

Angiopoietins (ANGPTs) are cytokines operative in developmental, physiological and pathological vascularization[77]. ANGPT1 and ANGPT2 bind the TIE2 (also known as TEK) receptor (along with TIE1 and integrins as coreceptors) expressed in ECs and subsets of hematopoietic cells[77]. While pericyte-derived ANGPT1 promotes EC survival and quiescence in normal vasculatures, ANGPT2 is elevated by hypoxia and inflammatory stimuli in ECs of many human malignancies, where it facilitates angiogenesis largely through autocrine signaling in VEGFA-stimulated ECs[77,78]. Specific ANGPT2 blockade has antiangiogenic effects encompassing both vascular pruning and normalization[77,79,80], which are enhanced by concomitant VEGFA signaling inhibition[81–85].

Additional growth factors, cytokines and chemokines facilitate tumor angiogenesis by directly stimulating ECs or indirectly influencing cancer cells and tumor-associated cells[29]. These include transforming growth factor-β (TGFβ), tumor necrosis factor (TNF), insulin-like growth factor 1 (IGF1), hepatocyte growth factor (HGF), apelin (APLN), interleukin-1β (IL-1β), IL-6, CXC chemokine ligand 8 (CXCL8) and CXCL12, as well as various adipokines and inflammatory mediators[29]. Their activities are multifaceted, redundant and context dependent. Additionally, secreted proteases, including plasmin, MMPs and cathepsins, regulate the proangiogenic activity of growth factors, such as VEGFA and TGFβ, by converting latent forms into bioactive ones through ECM remodeling[42]. Lipid mediators, noncoding RNAs and other nonproteinaceous molecules also contribute to tumor angiogenesis in concert with VEGFA and other key angiogenic factors[29].

A variety of molecules can induce EC quiescence (angiostasis) or regress angiogenic TBVs[16,20,21,42,86–88]. A fragment of plasminogen, called angiostatin, was among the first to be identified[88]. ECM glycoproteins such as thrombospondin 1 (THBS1) and osteonectin (also known as secreted protein acidic and rich in cysteine (SPARC)) also exhibit antiangiogenic functions[42]. Proteolytic ECM remodeling generates bioactive collagen fragments such as endostatin[87], which limits angiogenesis by competing with EC integrins for interaction with ECM proteins[20,21,29,42]. Endostatin appeared early on to be a promising candidate for antiangiogenic therapy[16], but challenges with its stability and manufacturing costs delayed clinical development[21]. Interferons (IFNs), which are primarily secreted by activated immune cells, can elicit antiproliferative and proapoptotic effects in tumor ECs. IFNα and IFNβ downregulate proangiogenic factors in cancer cells[89,90] and show antiangiogenic properties in mouse tumor models[91–93] and highly angiogenic human cancers[94]. IFNγ directly restrains EC proliferation[95] and instigates angiostatic macrophage programming[96]. Several IFN-inducible factors, such as IL-12, CXCL9, CXCL10 and CXCL11, also exhibit antiangiogenic activity[29]. It is tempting to speculate that some of the antitumoral responses in patients treated with immunotherapies may also involve IFN-dependent effects on the tumor vasculature[95,97,98].

Involvement of accessory cells in tumor angiogenesis

It was initially envisaged that angiogenesis-inducing ligands would be largely expressed by cancer cells as part of their malignant phenotype[12,36–38]. However, in many tumorigenesis pathways, angiogenesis is sustained, at least partly, by accessory cells recruited to form the heterotypic TME[29,99] (Fig. 2). These cells promote tumor

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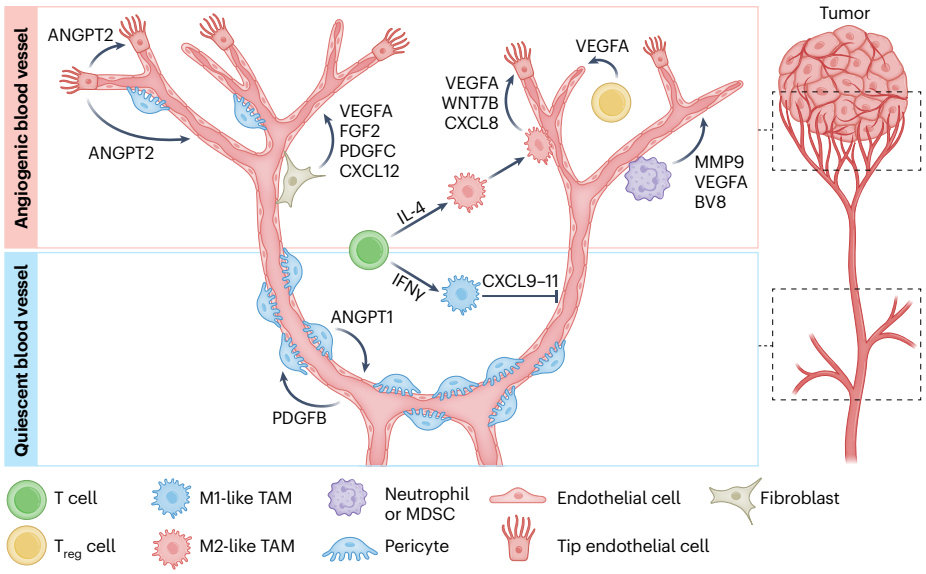

Fig. 2 | Accessory cells in tumor angiogenesis. The schematic illustrates signaling in the TME by accessory cells that either stimulate or inhibit tumor angiogenesis. The stimulators (top) include neutrophils/MDSCs, M2-like TAMs, CAFs and T_{reg} cells that cooperatively promote angiogenesis by secreting proangiogenic growth factors (for example, VEGFA, FGF2, CXCL8, CXCL12, Wnt family member 7B (WNT7B), BV8 and PDGFC). Neutrophils/MDSCs and TAMs can also release bioactive MMP9, which liberates VEGFA sequestered and latent in the ECM. Autocrine ANGPT2 signaling in ECs disrupts pericyte–EC interactions to enable VEGFA-dependent angiogenesis. T cells can modulate angiogenesis indirectly, for example, by programming TAMs to either M2-like (through IL-4) or M1-like (through IFNγ) states. Conversely (bottom), ECs recruit pericytes through PDGFB to stabilize newly formed BVs; in turn, pericytes secrete ANGPT1, which promotes EC survival and quiescence. M1-like TAMs can inhibit angiogenesis through the secretion of CXCL9, CXCL10 and CXCL11, which may act directly on TBVs and also recruit and activate T cells. The multifaceted effects of cancer cells on the programming of these accessory cells are not shown.

angiogenesis by expressing angiogenesis-inducing ligands, including VEGFA, or proteases that release angiogenesis factors from sequestered latent states[29].

Induction of the angiogenic switch and the persistence of tumor angiogenesis involve the recruitment of hematopoietic and mesenchymal-lineage cells from proximal tissues and the bone marrow[29]. Once embedded in the TME, these accessory cells display altered phenotypes and metabolic states in response to tumor-derived cues, often manifesting tumor-promoting capabilities[99]. Both cancer cells and accessory cells can secrete VEGFA in response to hypoxia. Accordingly, selective blockade of human VEGFA only moderately affects vascularization and tumor growth in xenograft models[11], whereas dual human/mouse-specific blockade achieves more profound effects[51].

Myelomonocytic cells, including monocytes, monocyte-derived macrophages and tissue-resident macrophages—collectively referred to as tumor-associated macrophages (TAMs)—have long been implicated as positive regulators of tumor angiogenesis[100,101]. TAMs undergo proangiogenic programming in response to hypoxia and tumor-derived factors[39,102,103], acting as a source of VEGFA, other proangiogenic factors and ECM-remodeling proteases[29,39,40,42,104–106]. They closely interact with nascent TBVs to promote angiogenesis[79,107–109] and facilitate vascular co-option by invasive cancer cells through their ECM-remodeling capacity[110]. Lineage tracing and targeted cell-elimination studies using a *Tek*-regulated genetic system[107] identified a subset of perivascular TAMs overexpressing CD163, lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1), stabilin 1 (STAB1), mannose receptor C type 1 (MRC1), NRP1, IGF1 and CXCL12 in mammary tumor models[111]. Perivascular TAMs exhibiting different combinations of these markers have been documented in a variety of mouse and human cancer types[101,112], where they facilitate angiogenesis, vascular permeability and metastasis[45,101,108,109,112–114]. The 'angio-TAMs'—a transcriptionally defined TAM subset with an angiogenic signature involving higher expression of VEGFA, osteopontin (also known as secreted phosphoprotein 1) and versican—was revealed by single-cell RNA sequencing (scRNA-seq) in several human cancer types[115]. Future studies using targeted cell-depletion strategies coupled with spatial transcriptomics may provide insights into the functional relationships between angio-TAMs[115] and the previously defined perivascular TAMs[112].

Granulocytic myeloid cells, including mast cells and neutrophils, are known sources of proangiogenic factors in tumors[29,116]. Mast cells secrete pro-MMPs and other proteases, including chymase and tryptase, which activate pro-MMPs[117]. They also release macrophage-attracting cytokines that indirectly promote tumor angiogenesis by recruiting TAMs[118]. Upon activation, human neutrophils deploy granules containing VEGFA[119] and pro-MMP9 unencumbered by tissue inhibitors of metalloproteinases[120], thus facilitating MMP9 activation and VEGFA mobilization. Neutrophils undergo an aberrant maturation trajectory in tumor-bearing mice and patients with cancer, acquiring proangiogenic functions; in cancer, they are sometimes referred to as myeloid-derived suppressor cells (MDSCs)[121]. Neutrophils serve as sources of VEGFA, MMP9, prokineticin 2 (PROK2, also known as BV8), FGF2 and other proangiogenic factors[43,60,116,120,122–127]. They contribute to the angiogenic switch in RIP1-Tag2 mice, partly through MMP9-mediated VEGFA mobilization, but have no discernable effects on the maintenance of the angiogenic phenotype in more advanced tumor stages[43,123,124]. Indeed, neutrophils support tumor angiogenesis in concert with other accessory cells, especially TAMs, and eliminating one cell type experimentally can trigger compensatory responses by others[128,129]. Diverse developmental states exist among tumor-associated neutrophils, each with nuanced angiogenic capacities[116,130]. For example, $SiglecF^{high}$ neutrophils display upregulated expression of angiogenesis and ECM-remodeling genes in a lung adenocarcinoma model[131]. Conversely, IFNβ signaling may abate neutrophil expression of proangiogenic factors[122] and rewire neutrophil granulopoiesis toward an antitumoral and vascular-damaging phenotype[116,132].

Lymphocytes and natural killer (NK) cells also modulate tumor angiogenesis[29]. For example, immunosuppressive regulatory T (T_{reg}) cells increased VEGFA bioavailability in a mouse model of ovarian cancer[133]. Tumor-induced suppression of NK cells and T cells may

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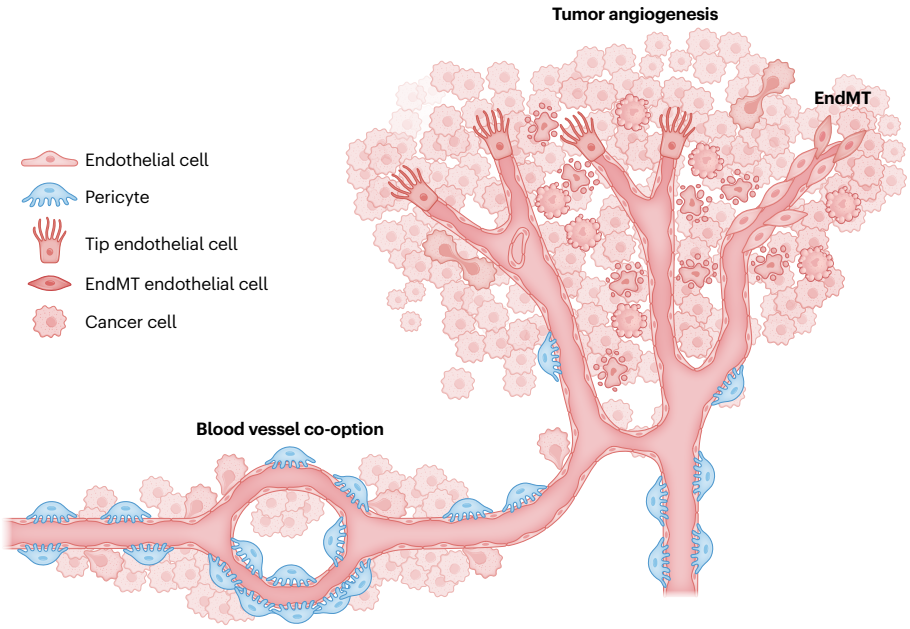

Fig. 3 | Diversity of tumor vascularization: the angiogenic switch and vascular co-option. The schematic illustrates the phenotypic diversity of ECs in TBVs. Tumors may induce sprouting angiogenesis (top), which is primarily induced by VEGFA. Specialized ECs, called tip ECs, sense VEGFA gradients and direct vessel elongation toward sources of VEGFA, which is sustained by proliferative ECs called stalk cells. In angiogenic BVs, some ECs may undergo EndMT (right), a phenotypic state involving proliferative, secretory and profibrotic capabilities, which contributes to vascular dysfunction, inflammation and fibrosis. Cancer cells may also access the vasculature without inducing angiogenesis (left) through a process termed vascular co-option or perivascular invasion. This mode of tumor vascularization has been observed in both primary and metastatic tumors and may be exacerbated during VEGFA signaling blockade. In conceptualizing the progression of multistage tumorigenesis, these alternative phenotypic states can be viewed as reflecting an angiogenic switch and a perivascular invasive switch in cancer cells.

lead to dysfunctional phenotypes with associated proangiogenic activity[29,134]. However, lymphocytes and NK cells are most commonly characterized as antiangiogenic[29]. Indeed, their activation and IFNγ production can elicit antiangiogenic responses in tumors[95,97,98].

CAFs contribute to generating a reactive and angiogenic stroma that perpetuates tumor-promoting responses in solid tumors[135]. CAFs have been shown to stimulate the malignant progression of preneoplastic tissue by altering the biology of the epithelium and surrounding vasculature in individuals predisposed to breast cancer due to *BRCA1* mutations[136]. ScRNA-seq has uncovered substantial CAF heterogeneity in both mouse and human tumors[137]. In MMTV-PyMT (mouse mammary tumor virus–polyoma middle tumor antigen) mammary tumors, three distinct CAF subpopulations were identified, with one, called vascular CAFs, being highly enriched in genes linked to vascular development and angiogenesis[138]. CAFs produce various proangiogenic factors[29,41,109,135,139,140], and the CAF secretome also indirectly enhances tumor angiogenesis by attracting proangiogenic myeloid cells from the systemic circulation through chemokines such as CXCL8 and CXCL12 (refs. 29,135,141). Beyond CAFs, other mesenchymal cells also exhibit proangiogenic activity. Tumors that arise within or near adipocyte-rich tissues, such as breast and ovarian cancers or bone metastases, are exposed to adipocyte-derived factors—collectively termed adipokines—that have proangiogenic functions[29,142].

It should be emphasized that most of the above-discussed studies used mouse tumor models as a platform for mechanistic investigations. Although TAM and CAF numbers positively correlate with vascular density in several human cancer types[143,144], this association does not necessarily imply a causative role. Interrogating the vascular-modulatory functions of tumor-associated cells in patients with cancer remains challenging owing to the limited availability of pre- and posttreatment biopsy data and, perhaps more critically, a lack of drugs selectively targeting specific accessory cell types in the TME[29].

Phenotypic diversity of EC states and tumor vascularization

Classical angiogenesis bioassays[62] initially suggested that solid tumors would be vascularized by phenotypically homogeneous—albeit aberrant—capillary ECs[145]. However, advances in molecular genetics, single-cell analysis and imaging technologies have revealed that tumor ECs exhibit diverse and dynamic states[146,147], including abnormally proliferating[145], senescent[148], transdifferentiated[149] and immune-modulatory[44,150,151] ECs. Tumors can also co-opt quiescent BVs from surrounding tissues[2] (Fig. 3). The regionally variable stimulation of the endothelium by a plethora of vascular regulatory factors produced by cancer cells and recruited accessory cells in distinct TMEs probably results in states of phenotypic plasticity and heterogeneity pertinent to understanding the complicated responses to antiangiogenic therapies.

A recent review of scRNA-seq datasets has cataloged a constellation of EC states varying across different tumor types and studies[147], probably reflecting the existence of multifunctional, heterogeneous EC phenotypes. Certain EC clusters appear to be conserved across multiple cancer types. For example, the expression of the plasmalemma vesicle-associated protein (*PLVAP*) gene, induced by VEGFA signaling, identifies potentially angiogenic, metabolically active and immunosuppressive EC clusters. Conversely, EC clusters expressing the atypical chemokine receptor 1 (*ACKR1*) gene may have proinflammatory and immunostimulatory functions[147]. These findings, while currently descriptive, may help identify more selective and potentially effective therapeutic targets in the tumor endothelium.

ScRNA-seq analysis of the human lung cancer vasculature has identified ECs with features of normal arterial, postcapillary venule and capillary ECs, alongside subpopulations with differentially activated regulatory states, totaling over a dozen distinct phenotypes[152]. Among them, tip ECs display conserved phenotypes in human and mouse lung tumors, suggesting shared mechanisms of VEGFA-induced tip EC

formation. A distinct EC state, termed 'breach' cell, expresses typical tip EC genes along with ECM-remodeling genes. Moreover, the bulk of proliferating ('stalk') ECs could be resolved into several phenotypic states, including 'scavenging' ECs with higher expression of cathepsins and scavenger receptors[152] that may facilitate ECM degradation for vascular invasion in the tumor stroma.

The human lung cancer vasculature exhibits a higher proportion of proliferative ECs compared to the normal lung vasculature[152,153]. This is consistent with earlier studies of breast and colorectal cancer indicating increased EC proliferation in tumors (3–10%) compared to adjacent normal tissue (0.1–1%), with up to 20% of ECs with a proliferative phenotype at the invasive tumor margins[154,155]. Notably, lower frequencies of proliferating ECs were observed in human lung and breast cancer compared to mouse models[152,156], possibly due to a greater reliance on vascular co-option in human tumors[2]. Nonetheless, both human and mouse tumor ECs exhibit higher RNA content, activation of MYC target genes and increased nucleotide metabolism compared to normal tissue ECs, probably indicative of enhanced transcription and proliferation[157]. Metabolic pathway analysis further revealed a shift toward aerobic glycolysis in tumor ECs, which may facilitate rapid ATP production and oxygen transfer to surrounding cells[157].

Tumor ECs can undergo senescence, a potentially reversible cell-cycle arrest state that may precede clearance of dysfunctional cells[148,158]. A meta-analysis of scRNA-seq datasets indicated that tumor ECs can manifest senescent phenotypes involving upregulated expression of inflammatory mediators, chemokines and adhesion molecules that may facilitate recruitment of protumorigenic inflammatory cells[159]. A senescent EC signature negatively correlates with survival and response to immunotherapy in a broad range of cancer types[159]. Additionally, in some pathological conditions, subsets of vascular ECs downregulate the expression of junctional proteins (for example, CDH5) and concurrently develop mesenchymal cell features, a process distinct from cellular senescence and termed endothelial-to-mesenchymal transdifferentiation (EndMT)[149]. During EndMT, ECs acquire proliferative, secretory, thrombogenic and profibrotic phenotypes that contribute to vascular dysfunction, inflammation and tissue fibrosis. In cancer, EndMT may facilitate the generation of a desmoplastic stroma through increased vascular leakage and ECM deposition, thereby promoting cancer cell motility, invasion and metastasis[149]. Accordingly, an EndMT signature is associated with a worse prognosis and therapeutic resistance in pancreatic adenocarcinoma[160].

Another layer of complexity pertains to the immunomodulatory properties of tumor ECs[150,161]. Chronic exposure to VEGFA and other tumor-derived factors renders ECs unresponsive to proinflammatory stimuli such as TNF and IL-1β (ref. 161). This anergic EC state involves the downregulation of T cell adhesion receptors and increased expression of molecules that hinder T cell transmigration[162], leading to reduced T cell recruitment into tumors[150,161]. ScRNA-seq analysis revealed significant downregulation of genes related to antigen presentation, immune-cell chemotaxis and immune-cell trafficking in human lung and mammary tumor ECs compared to healthy tissues[156,157]. Thus, TBVs can be immunosuppressive[150,161]. Nevertheless, solid tumors can occasionally develop high endothelial venules (HEVs), specialized vessels that enable T cell and B cell transmigration in lymphoid organs. In some tumors, HEVs contribute to generating T cell-rich immune aggregates similar to tertiary lymphoid structures, which are associated with antitumor immunity and a more favorable prognosis[151,161]. HEVs, induced by IFNγ, TNF and lymphotoxins produced by activated lymphocytes and NK cells, are often found at the tumor periphery and appear to form independently of angiogenesis, potentially through co-option of postcapillary venules and progressive T cell accrual[151,163].

As noted above, human tumors can vascularize both through sprouting angiogenesis and co-option of preexisting vessels[2]. A third mode of tumor vascularization has been observed in early-stage colorectal carcinogenesis, where crypt hyperplasias vascularize by attracting venous ECs from adjacent, nontransformed epithelial regions in a process that is dependent on APLN but independent of VEGFA[164]. This represents an unconventional mode of neovascularization distinct from both vessel co-option and sprouting angiogenesis, as it involves crypt-ward migration of tube-forming ECs in the absence of EC proliferation; its generality remains to be ascertained.

Although most tumors contain both angiogenic and co-opted BVs, considerable variation is observed, with tumors in highly vascularized organs (for example, lung, brain, liver and lymph node) often displaying moderate-to-high degrees of vascular co-option. For example, the analysis of lung metastases from primary tumors of the breast, colon and kidney found evidence of vessel co-option in approximately 80% of the cases[165]. In low-grade gliomas of the brain, cancer cells co-opt existing vessels without disrupting the blood–brain barrier[2]. However, high-grade gliomas often display mixed angiogenic and co-opted vasculatures. The latter involves 'perivascular cuffing', a process in which cancer cells surround brain capillaries to replace pericytes and astrocytes, thus disrupting the blood–brain barrier[166]. Perivascular spreading and dislodging of pericytes have also been observed in the initial steps of brain metastasis of mouse lung and mammary tumors[167]. The epithelial-to-mesenchymal transition of cancer cells may facilitate perivascular invasion during the initial steps of metastatic colonization[168–170]. Overt metastatic outgrowth, however, is often associated with vascular remodeling and sprouting angiogenesis[2,171,172].

Regardless of the mechanisms involved, co-opted TBVs are distinct from irregular and chaotic angiogenic TBVs[2]. ScRNA-seq analysis of mouse tumors with spontaneous or induced vessel co-option corroborated earlier histological findings that co-opted tumor ECs are largely quiescent, lack both tip and proliferating cells and, perhaps unexpectedly, exhibit transcriptomic profiles reminiscent of normal ECs[110]. Notably, specific protein biomarkers distinguishing co-opted tumor ECs from normal tissue ECs are currently unavailable. Intriguingly, tumor regions with co-opted BVs have occasionally been found to be hypoxic and with upregulated VEGFA expression despite a lack of evidence for angiogenesis[173]. These results suggest that ill-defined mechanisms, possibly involving accessory cells such as angiostatic macrophages[110], render co-opted BVs recalcitrant to hypoxia-induced, VEGFA-mediated proangiogenic signaling, potentially explaining the insensitivity of some tumors to VEGFA pathway inhibitors[169].

Development of angiogenesis inhibitors for cancer therapy

The discovery and functional validation of VEGFA signaling in cancer were concurrent with the pioneering of mechanism-based targeted therapies aimed at oncogenes, which demonstrated the clinical potential of biological therapies for treating cancer[174]. These milestones paved the way for the development, clinical testing and approval of drugs that inhibit tumor angiogenesis by targeting VEGFA/VEGFR and additional signaling pathways[17,18,35].

The VEGFA monoclonal antibody bevacizumab (Avastin) gained initial approval for metastatic colorectal cancer and is now approved for treating other tumor types, including lung, renal, cervical, ovarian and liver cancer, in combination with other agents[13–15,17,18]. Adding bevacizumab to standard-of-care therapy in clinical trials typically produced delayed time to progression with demonstrable, but generally modest, overall survival benefits[17,18]. However, it showed no survival benefits in patients with other tumor types, such as metastatic breast cancer[175]. Distinct VEGF-targeting biologics have also been approved, including ramucirumab (a VEGFR2 monoclonal antibody) for gastric and lung cancer[176,177] and aflibercept (a VEGF trap) for colorectal cancer[178]. Ramucirumab and aflibercept can block multiple VEGF family members, such as placental growth factor (PlGF) and VEGFB, although the combined benefits of neutralizing these factors along with VEGFA remain unclear[18].

Bevacizumab rapidly reduces blood flow in human tumors[179–181] by inhibiting sprouting angiogenesis, leading to decreased microvessel density, and suppressing VEGFA-induced nitric oxide production, causing vasoconstriction[182]. Paradoxically, VEGFA blockade can also transiently improve blood flow through 'vascular normalization', shifting the balance from growth to maturation of TBVs[181,183,184]. The consequences of this remodeling are fewer vascular sprouts, increased pericyte coverage, reduced vascular leakage, improved blood perfusion and enhanced drug delivery through the systemic circulation. A theory that accommodates both reduced vascularization and improved blood flow after VEGFA blockade envisions that vascular normalization is a dynamic response governed by the degree and kinetics of VEGFA signaling inhibition[184]. A 'normalization window' has been identified in mouse tumor models, which can be extended by varying the dosing of VEGF pathway inhibitors[184,185]. As a single agent, bevacizumab counteracts VEGFA-induced vascular permeability in human glioblastoma and helps control cerebral edema, thus providing benefits despite its inconsequential effects on tumor progression[186]. Moreover, improved blood flow and drug delivery may contribute to the additive clinical benefits of combining VEGFA inhibition with chemotherapy, for example, in colorectal and lung cancers[14,15]. While the mechanisms by which blocking VEGFA improves tumor response to chemotherapy are incompletely understood[17,18], VEGFA signaling stimulates vascular ECs and TAMs to adopt immunosuppressive phenotypes that limit the efficacy of chemotherapy and other anticancer agents[44,150,187]. Beyond VEGF inhibition, additional strategies can demonstrably normalize TBVs, including enforced expression of semaphorin 3A (SEMA3A)[188], delivery of agonists of the lymphotoxin-β receptor (LTβR)[189], blocking ANGPT2 (refs. 79,80,85) or activating TIE2 (ref. 190), and inhibiting leucine-rich α-2-glycoprotein-1 (LRG1)[191].

Recent scRNA-seq studies have provided new and potentially clinically relevant insights into the effects of VEGFA signaling inhibition in mouse tumor models[152,192]. The analysis of human tumor xenografts revealed reduced numbers of tip ECs after aflibercept treatment, indicating acute inhibition of sprouting angiogenesis[192]. However, stalk-like cells persisted, suggesting limited effects on preexisting TBVs. Another study using the VEGFR2-specific antibody DC101 (a ramucirumab surrogate) in a lung tumor model showed that tip and breach ECs were most sensitive to the treatment, whereas stalk ECs were less affected[152]. Intriguingly, DC101 increased gene signatures linked to mature vascular functions, including an activated postcapillary vein phenotype[152] characteristic of HEVs[151]. Whether these changes represent transient vascular normalization or transition from angiogenesis to vessel co-option remains to be determined.

Several small-molecule receptor tyrosine kinase inhibitors, with broader specificity than VEGFA/VEGFR2-targeted biologics, exhibit antiangiogenic and antitumoral effects[18]. By primarily targeting both VEGFRs and PDGFRs, sorafenib and sunitinib disrupt TBVs by dissociating PDGFR-dependent pericytes from newly formed EC tubes, rendering them more sensitive to VEGFR2 inhibition[58]. These inhibitors are approved as single agents for treating various cancer types, including pancreatic neuroendocrine tumors, gastrointestinal stromal tumors, hepatocellular carcinoma and renal cancer[75,76]. Other inhibitors, including axitinib, apatinib, lenvatinib, cabozantinib, pazopanib and regorafenib, block VEGFRs and other tyrosine kinases, including PDGFRs, KIT, TIE2, FGFRs and cMET. They are approved for colorectal and renal cancer and hepatocellular carcinoma, often in the advanced or metastatic setting[18]. Small-molecule mTOR inhibitors (rapalogs)—temsirolimus and everolimus—produce antiangiogenic effects by interfering with the PI3K–AKT–mTOR pathway[193]. They are approved for the treatment of advanced renal cell cancer, pancreatic neuroendocrine tumors and breast adenocarcinoma. Of note, tumors can rapidly revascularize upon therapy withdrawal using empty basement membrane sleeves left behind by regressed TBVs[56,57]. For example, discontinuation of sunitinib treatment in patients with renal cell carcinoma resulted in brisk tumor revascularization sustained by highly proliferative ECs[194].

As noted above, ANGPTs have been investigated as antiangiogenic targets[77,195]. The ANGPT1/2-targeting peptibody trebananib was the first to enter clinical testing. While three clinical trials in patients with advanced ovarian cancer failed to show benefit[195], neoadjuvant trebananib improved event-free survival in high-risk, early-stage breast cancer[196]. ANGPT1 and ANGPT2 have contrasting roles in tumor angiogenesis[80], so blocking the vascular-normalizing effects of ANGPT1 might limit the benefits of targeting ANGPT2 in some contexts[79,85]. The bispecific VEGFA/ANGPT2 antibody vanucizumab was compared to bevacizumab in combination with chemotherapy in metastatic colorectal cancer. Although vanucizumab was not superior to bevacizumab in the general population[197], a retrospective analysis showed potentially meaningful benefits in patients with higher ANGPT2 levels in plasma and tumors[198], in agreement with preclinical studies[82–84].

The regulation of tumor angiogenesis is complex and multifactorial, and so are the responses to angiogenesis inhibitors[61,199] and their associated toxicities[18]. Its targeting continues to be beneficial in combination therapies, as further elaborated below.

Intrinsic and adaptive resistance to antiangiogenic therapy

The observations that antiangiogenic drugs produced only transitory efficacy in preclinical and clinical trials spurred investigations into the underlying basis for relapse. Several modes of intrinsic and adaptive resistance to antiangiogenic therapy have been revealed in mouse tumor models[61,199]. Moreover, the prevalence of vascular co-option without evident angiogenesis in many human cancers may help explain the relatively modest clinical benefits produced by angiogenesis inhibitors[2].

In early studies, recombinant endostatin could fully regress rapidly growing transplant tumors without evidence of posttreatment recurrence, spearheading the hypothesis that antiangiogenic therapy would not lead to resistance[16]. However, subsequent work in other tumor models has revealed more nuanced antiangiogenic and antitumoral activities of endostatin[200]. Likewise, clinically approved agents that efficiently quench VEGFA signaling generally achieved partial or transient responses in preclinical models, especially GEMMs of cancer, suggestive of intrinsic or acquired/adaptive resistance[2,17,61,199].

Redundancy in proangiogenic signaling can explain findings of unabated or rebound angiogenesis occurring in the face of VEGFA signaling inhibition[61,199]. Compensatory (adaptive) upregulation of proangiogenic growth factors has been well documented in mouse tumor models following anti-VEGFA therapy. Inhibition of angiogenesis by DC101 in RIP1-Tag2 mice led to hypoxic upregulation of FGF2 and tumor revascularization. Combining DC101 with FGF2 blockade extended the temporal duration of the antiangiogenic response and delayed tumor progression[70]. FGFR inhibition also decreased the vascular density and improved tumor response to anti-VEGFA therapy in a mouse model of obesity-associated breast cancer[71]. In patients with metastatic colorectal cancer receiving bevacizumab with chemotherapy, serum FGF2 levels increased above baseline before the radiographic development of resistance[201]. Brivanib, a dual VEGFR/FGFR inhibitor, showed efficacy in RIP1-Tag2 mice following resistance to sorafenib[202]. In patients with advanced hepatocellular carcinoma resistant to sorafenib, brivanib had demonstrable activity in approximately 10% of the patients, although it did not improve overall survival compared to placebo[203]. Besides FGF signaling, ANGPT2 can limit the efficacy of anti-VEGFA therapy in cancer. Dual VEGFA and ANGPT2 inhibition provides additive benefits in tumors that upregulate endothelial ANGPT2 when VEGFA signaling is blocked[83,84]. ANGPT2 may also mediate refractoriness to anti-VEGFA therapy ab initio. Among patients with metastatic colorectal cancer who received bevacizumab combined with chemotherapy, low pretreatment serum levels of ANGPT2 were associated with a better

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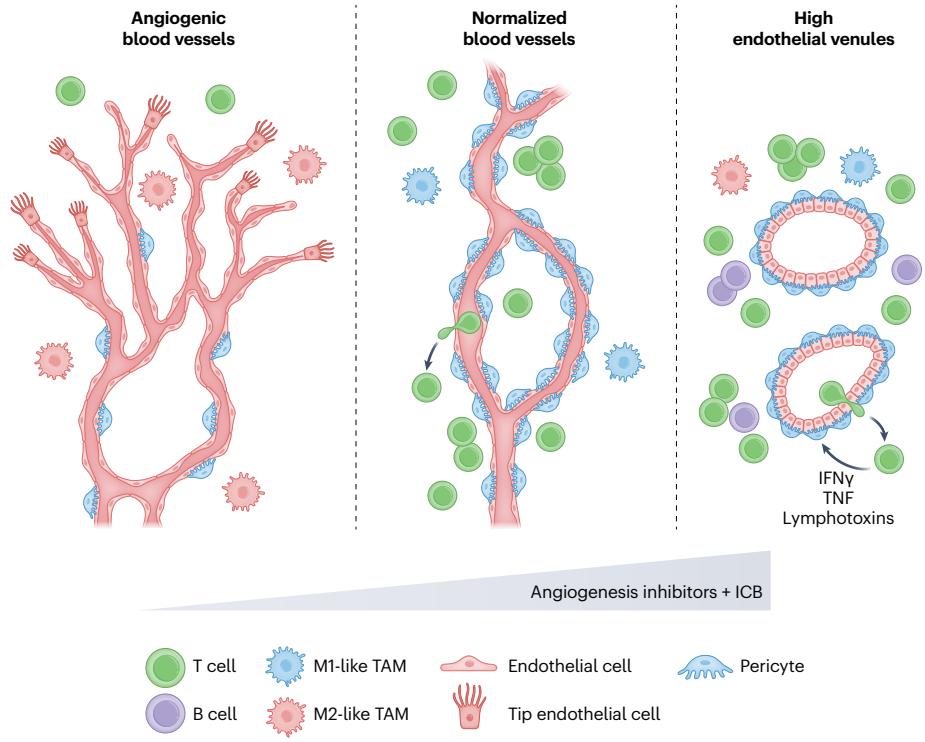

Fig. 4 | Harnessing vascular–immune crosstalk for cancer therapy. Angiogenesis inhibitors—alone or in combination with ICB—can reprogram angiogenic TBVs into 'quasi-normal' states and elicit the formation of HEVs from postcapillary ECs. These reprogrammed BVs facilitate, rather than limit, T cell and B cell infiltration and activation in the TME. Factors produced by de novo-recruited T cells, such as IFNγ, TNF and lymphotoxins, further stabilize T cell-permissive BVs and HEVs, enabling the therapeutic activity of ICB in particular and possibly immunotherapy in general. Combinations of VEGF pathway inhibitors and ICB are producing added therapeutic benefits in an increasing number of preclinical and clinical cancer trials.

response[204]. PDGFs, PROK2, IL-17 and CXCL8 have also been mechanistically implicated in the promotion of VEGFA-independent tumor angiogenesis through direct or indirect effects on the vasculature[61,199]. Additional cytokines and growth factors, such as PlGF, VEGFA, VEGFD, HGF, IL-6 and CXCL12, may serve as predictive biomarkers of response to antiangiogenic therapy, irrespective of their potentially direct involvement in mediating resistance[201,205,206].

A second mode of resistance involves recruitment or in situ reprogramming of accessory cells[61,199]. For example, therapy-induced upregulation of colony-stimulating factor 3 (CSF3), CXCL8, CXCL12 and chemokine ligand 2 (CCL2) fosters tumor infiltration by neutrophils/MDSCs and TAMs, which sustain VEGFA-independent angiogenesis through growth factors and proteases that counterbalance the loss of VEGFA signaling in ECs[60,61,124,129,187,207]. Similarly, CAFs release growth factors that may rescue tumor angiogenesis and growth in the face of VEGFA signaling blockade[208,209]. In VEGFA-depleted tumors of RIP1-Tag2 mice, CAFs upregulate periostin (POSTN), a matricellular protein that attracts and retains proangiogenic TAMs. Genetic inactivation of *Postn* or TAM ablation with a CSF1 receptor (CSF1R) antibody inhibited tumor revascularization and progression during extended VEGFA blockade[210]. These preclinical findings should incentivize clinical testing of combinations of angiogenesis and CSF1R inhibitors in patients with cancer[100].

A third mode of resistance can emerge in treated tumors, whereby cancer cells adapt metabolically to sustain growth despite restricted nutrient and oxygen supply[199]. For example, hepatocellular cancer cells increase autophagy, a prosurvival response mediated by the activation of the AKT–mTOR pathway in response to sorafenib-induced hypoxia[211,212]. Cancer cells in treated tumors may also engage in 'metabolic symbiosis', a process whereby hypoxic cancer cells in avascular tumor areas import glucose and export lactate, whereas normoxic cells near surviving or co-opted TBVs import and use lactate[213–215]. Metabolic reprogramming of cancer cells during antiangiogenic therapy may even exacerbate their malignant behavior in mouse tumor models. For example, sunitinib withdrawal led to accelerated tumor regrowth fueled by a metabolic switch involving increased uptake and metabolism of fatty acids in the cancer cells[216]. Inhibition of fatty acid uptake, storage or metabolism impaired cancer cell survival and tumor regrowth[217,218], suggesting potential for cotargeting with the lipase inhibitor orlistat[216].

A fourth form of resistance involves progression to states of heightened local invasion, whereby cancer cells grow by co-opting the quiescent vasculature of local tissues without the need for neovascularization[165,219–223]. This phenomenon has been clearly documented in human glioblastoma[223,224]. However, a large meta-analysis of phase 3 trials involving more than 4,000 patients with colorectal, breast, renal and pancreatic cancer indicated that disease progression was not accelerated by bevacizumab treatment[225]. Moreover, evidence is still lacking that enhanced cancer cell invasion along co-opted BVs accelerates tumor progression in most patients treated with VEGFA inhibitors[18,199,223,226]. In mouse cancer models, increased perivascular tumor invasion upon VEGFA signaling blockade was facilitated by hypoxia-induced epithelial-to-mesenchymal transition and upregulation of the HGF receptor cMET[227–229]. However, cMET inhibitors have not shown clinical efficacy in tumors that lack activating mutations or amplifications of the *MET* gene, arguing against a pivotal role of cMET in tumor invasion and metastasis[230]. Nevertheless, clear improvements in progression-free survival have not consistently translated into extended overall survival in several phase 3 trials[223], suggesting therapy-induced mechanisms of tumor adaptation.

Harnessing vascular–immune crosstalk for cancer therapy

The emergence of immunotherapies as a new dimension to cancer therapeutics has been tempered by the realization that many solid tumors erect multifaceted barriers to T cell infiltration and function[231].

Among these, the aberrant tumor vasculature hyperstimulated by chronic angiogenic signaling can express immunosuppressive factors that impede T cells seeking to infiltrate tumors[150,161,232]. Concordantly, vascular remodeling by angiogenesis inhibitors can, in some cases, demonstrably attenuate angiogenesis-associated immunosuppression to facilitate the efficacy of antitumor immunity. In preclinical models and clinical trials, angiogenesis inhibitors have been found beneficial in combination with immune checkpoint blockade (ICB), namely, programmed cell death protein 1 (PD-1) or programmed death ligand 1 (PD-L1) antibodies, and other forms of immunotherapy—a treatment modality that we here refer to as antiangiogenic immunotherapy[98] (Fig. 4).

Antiangiogenic therapy has recently witnessed a clinical renaissance thanks to successful combinations of angiogenesis inhibitors and ICB[18,161,231]. The phase 3 KEYNOTE-426 trial demonstrated the superiority of axitinib and pembrolizumab (a PD-1-blocking antibody) over standard-of-care sunitinib in advanced renal cell carcinoma[233]. Other phase 3 trials combining axitinib or bevacizumab with PD-1 or PD-L1 antibodies have shown clinical improvements compared to sunitinib in the same cancer type[234,235]. In phase 3 trials with treatment-naive patients, bevacizumab plus atezolizumab (a PD-L1-blocking antibody) was superior to sorafenib in advanced hepatocellular carcinoma (IMbrave150), and atezolizumab improved clinical response to bevacizumab plus chemotherapy in nonsquamous non-small cell lung cancer (IMpower150)[18,161,236]. As a result of these and other trials, different combinations of angiogenesis inhibitors and ICB have been approved as first- or second-line treatments for advanced renal, liver and lung cancers[18,98,161]. Promising results have also been obtained in other cancer types, such as endometrial and colorectal cancer[237,238]. Interestingly, recombinant endostatin improved tumor response to ICB in a pilot clinical study in patients with pretreated lung cancer[239].

ICB has been shown to prevent early exhaustion of T cells and has demonstrable clinical activity in several cancer types, but these benefits are often limited to patients with tumors containing preexisting T cell infiltrates[231]. Given that angiogenic TBVs typically suppress T cell infiltration, pharmacologically impaired angiogenic signaling may improve T cell trafficking by normalizing TBVs or promoting HEV formation[98,150,161,232]. Bulk and scRNA-seq analyses of ECs from mouse tumors exposed to antiangiogenic agents have revealed upregulation of genes involved in immune-cell chemotaxis, T cell adhesion and trafficking, and antigen presentation[85,152,192,240]. Concurrent PD-1 or PD-L1 blockade sustains the activation of T cells and protects them from the inhibitory effect of PD-L1, which becomes upregulated in cancer cells and accessory cells, including ECs, in response to T cell-derived IFNγ (refs. 85,241). Because IFNγ is angiostatic, it further contributes to sustaining vascular normalization[242,243]. Moreover, PD-L1 blockade reprograms the tumor vasculature, tilting it toward a proinflammatory and antigen-presenting cell-like state that can facilitate T cell recruitment[244]. This feed-forward loop, initiated by antiangiogenic therapy and perpetuated through ICB, improved tumor control in multiple models, including GEMMs of cancer refractory to either monotherapy[85,241,245,246]. Interventions limiting angiogenic signaling, such as VEGFA or ANGPT2 blockade[85,241,245,247–251], targeting TNF-family factors to the tumor vasculature[189,252,253], or enhancing EC–pericyte interactions[191,254]—among other approaches[255–258]—demonstrably enhanced ICB outcomes in mice.

The presence of HEVs in certain human tumors is associated with better responses to ICB[151,161]. Congruently, in preclinical models, the inhibition of angiogenic signaling, especially when combined with ICB, induces peri- and intratumoral HEV formation[151], where circulating T cells preferentially accumulate[259]. T cells extravasating in HEV-rich areas mature into PD-1$^+$TCF1 (T cell factor 1)$^+$CD8$^+$ T cell progenitors that eventually differentiate into T effector cells[163]. In turn, tumors require sustained T cell and NK cell-derived signals, namely IFNγ and lymphotoxins, to maintain HEVs. Given that vascular normalization in response to antiangiogenic therapy facilitates extravasation and perivascular accumulation of T cells[85,98,150,161,232], it seems likely that the so-called normalized TBVs also encompassed HEVs in studies in which HEVs were not assessed.

In some cases, longer-term analysis of clinical trials has revealed more limited efficacy of antiangiogenic immunotherapy than seen in interim reports, and evidence is emerging that certain patient subgroups vary in clinical responses. Thus, in the phase 3 IMmotion151 trial, atezolizumab plus bevacizumab showed trends of improved survival versus sunitinib only in those patients whose renal cell tumors had pretreatment transcriptomic profiles indicative of T effector or proliferative states[260]. In the IMpower150 lung adenocarcinoma trial, subgroup analysis showed no survival gains by atezolizumab plus bevacizumab and chemotherapy, compared to bevacizumab and chemotherapy, in patients whose tumors had wild-type *KRAS* alleles[261]. Moreover, it was unclear whether bevacizumab contributed to clinical response in the general population, although a modest survival advantage was seen in patients with mutant *KRAS* tumors[261]. These clinical data are suggestive of complex mechanisms mediating intrinsic or adaptive resistance to antiangiogenic immunotherapy and echo observations in patients treated with other ICB combinations[231]. Predictive biomarkers of response are emerging that appear to largely overlap with those identified previously for ICB; such biomarkers need to be evaluated in prospective clinical trials. A high neutrophil-to-lymphocyte ratio was predictive of poor response in patients with renal cell carcinoma treated with axitinib plus ICB[262]. A meta-analysis of 30 tumor types, which used transcriptional profiles to stratify patients based on baseline angiogenic and immune activity gene sets, concluded that angiogenic activity and T cell immunity are inversely correlated across tumors[263]. Tumors could be classified into three angio-immune subtypes: high angiogenesis/low T cell activity (C1), low angiogenesis/high T cell activity (C3) and intermediate states (C2). Features in the C3 group included a higher pericyte-to-EC ratio (indicative of more mature or normalized TBVs) and higher inflammation scores, including T effector cell functions. Interestingly, in the Javelin Renal 101 clinical trial, patients with renal cell carcinoma who were categorized into the C3 angio-immune subtype had remarkable responses to the combination of axitinib and avelumab (a PD-L1 antibody)[263,264]. Besides baseline features predictive of response, mechanisms of adaptive resistance to antiangiogenic immunotherapies are currently being elucidated in mouse tumor models. For example, in a preclinical GEMM of lung adenocarcinoma, antiangiogenic therapy facilitated tumor infiltration by immunosuppressive T_{reg} cells, which expressed higher PD-1 levels than other T cell subsets[265]. A PD-1 antibody preferentially bound to and activated the T_{reg} cells, thereby limiting the efficacy of antiangiogenic immunotherapy. Disrupting T_{reg} cell survival through TAM elimination unleashed the efficacy of antiangiogenic immunotherapy[265].

VEGFA can have other immunosuppressive functions in the TME that are independent of its effects on TBVs[44]. VEGFA can promote the recruitment of circulating monocytes that differentiate into immunosuppressive TAMs[46,266], impair dendritic cell maturation[267] and induce T cell exhaustion[268]. VEGFA induces tumor-infiltrating VEGFR2-expressing CD8$^+$ T cells to express inhibitory immune checkpoints through the VEGFR2–PLCγ–calcineurin–NFAT (nuclear factor of activated T cells) pathway, thereby promoting T cell exhaustion[268]. Accordingly, genetic inactivation of *Vegfr2* in T cells relieved T cell exhaustion in a colorectal cancer model[269]. VEGFA can also directly promote T_{reg} cell expansion in tumor-bearing mice and patients with cancer[270]. Collectively, these findings underscore the multifaceted role of VEGFA in the modulation of tumor-associated immunosuppression[44,150] and may help explain the therapeutic benefits of inhibiting angiogenic signaling in combination with therapeutic strategies aimed to stimulate immune responses against cancer[98,161,240,251].

Concluding remarks

Angiogenesis has, on the one hand, been validated as a functional hallmark of cancer and a new drug target and, on the other hand, conceptually expanded in scope to embrace diverse functions well beyond the supply of oxygen and nutrients to tumors. An emerging complexity lies in the heterogeneity of tumor ECs and BVs, revealed in part by single-cell analysis and mechanistic investigations in tumor models, which have illustrated far more phenotypic states than those envisaged in earlier studies. Further delineating the heterogeneity of EC states and vascularization patterns in tumors—and their significance for tumorigenesis and consequentiality for anticancer therapy—stands as an important challenge for the future. Most prominent is the realization that tumor vascularization can also be accomplished through the co-option of quiescent BVs. The potential significance of vascular co-option is reflected in the unanticipated occurrence of resistance to angiogenesis inhibitors, once buoyed by the hope that ECs, being chronically proliferative and yet genetically stable, would not be subject to therapeutic resistance.

A silver lining for angiogenesis inhibitors in cancer therapy is their capability to remodel the angiogenic tumor vasculature into a state of quasi-normality by pruning angiogenic ECs while leaving pericyte-covered ECs in a less dense vascular network more permissive to T cell extravasation. As a result, angiogenesis inhibitors are showing benefits in combination with immunotherapies that bolster T cell function. Because angiogenic scores based on transcriptional profiling may not accurately distinguish angiogenic and co-opted vessels, a key question for the future is whether tumors with varying ratios of angiogenic and nonangiogenic BVs—determined, for example, by spatial transcriptomics—respond differently to antiangiogenic immunotherapy. Co-opted vasculatures are suspected to be largely insensitive to VEGFA pathway inhibitors, such that tumors with a prevalence of quiescent co-opted BVs are not expected to benefit from therapeutic targeting unless inhibition of VEGFR signaling alleviates tumor-associated immunosuppression independently of its effects on TBVs. These broader conceptual horizons for the biology of tumor vascularization and angiogenic signaling solidify their importance both for tumorigenesis and malignant progression and for incorporating mechanism-guided drugs into combinatorial therapeutic strategies that more broadly benefit patients with cancer.

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Competing interests

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and is an inventor on patents on engineered immune cells filed by the Swiss Federal Institute of Technology in Lausanne (EPFL). D.H. has received sponsored research grants from Hoffmann La-Roche and Bristol Myers Squibb; serves on the scientific advisory boards of Pfizer Oncology, Opna Bio, 4D Molecular Therapeutics and Cellestia; and is a founder of Opna Bio, which has licensed an EPFL patent describing the RNA-binding protein FMRP as a new cancer target.

Additional information

Correspondence should be addressed to Michele De Palma or Douglas Hanahan.

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