Microenvironmental regulation of tumour angiogenesis

Michele De Palma[1], Daniela Biziato[1] and Tatiana V. Petrova[2]

Abstract | Tumours display considerable variation in the patterning and properties of angiogenic blood vessels, as well as in their responses to anti-angiogenic therapy. Angiogenic programming of neoplastic tissue is a multidimensional process regulated by cancer cells in concert with a variety of tumour-associated stromal cells and their bioactive products, which encompass cytokines and growth factors, the extracellular matrix and secreted microvesicles. In this Review, we discuss the extrinsic regulation of angiogenesis by the tumour microenvironment, highlighting potential vulnerabilities that could be targeted to improve the applicability and reach of anti-angiogenic cancer therapies.

Hypoxia
The condition of low oxygen availability. In tumours, hypoxia is observed in cancer cells that reside more than 70–150 μm away from a perfused blood vessel.

Pro-angiogenic factors
Biological molecules that stimulate endothelial cell proliferation and angiogenesis.

Anti-angiogenic factors
Biological molecules that block angiogenesis or promote the regression of angiogenic blood vessels.

[1]*The Swiss Institute for Experimental Cancer Research (ISREC), School of Life Sciences, École Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland.*
[2]*Department of Fundamental Oncology, Ludwig Institute for Cancer Research and Division of Experimental Pathology, University of Lausanne and University of Lausanne Hospital, 1066 Lausanne, Switzerland.*

Correspondence to M.D.P. and T.V.P.
michele.depalma@epfl.ch; tatiana.petrova@unil.ch

doi:10.1038/nrc.2017.51
Published online 14 Jul 2017

Like normal organs, tumours need to establish a blood supply to satisfy their demand for oxygen and nutrients and accomplish other metabolic functions[1]. This is achieved primarily through angiogenesis, the process whereby new blood vessels develop from a pre-existing vascular network. Hypoxia is a key driver of tumour angiogenesis[2]. Hypoxic cancer cells secrete vascular endothelial growth factor A (VEGFA), which initiates tumour angiogenesis by engaging VEGF receptor 2 (VEGFR2) expressed on the endothelial cells (ECs) of neighbouring blood vessels[1]. Gradients of soluble VEGFA induce the formation of motile ECs, called tip cells, which break down the surrounding extracellular matrix (ECM) and lead the growth of new vascular sprouts towards VEGFA. This process requires the participation of additional signalling molecules, including delta ligand-like 4 (DLL4) and angiopoietin 2 (ANGPT2), which, respectively, control the tip-cell phenotype and destabilize EC junctions[1].

In pre-malignant stages of epithelial tumours (for example, hyperplasia and carcinoma *in situ*), a basal lamina separates the tumour from the vascularized peritumoural tissues, so blood vessels rarely infiltrate these early lesions[3,4]. In malignant tumours, cancer cells acquire invasive behaviours and induce a stromal response involving robust angiogenesis[5]. Therefore, tumour progression from a benign to a malignant stage is typically associated with an angiogenic switch — the triggering and development of a vascular network that is actively growing and infiltrative[5] (FIG. 1). However, considerable variation exists in the patterns of tumour vascularization, which reflect differences in the tumour type, grade and stage (for example, primary versus metastatic), the anatomical site, the stromal cell composition and the spatiotemporal expression of pro-angiogenic factors and anti-angiogenic factors[4,6–10].

Owing to excessive and sustained pro-angiogenic signalling[5], tumour-associated blood vessels (TABVs) typically acquire an aberrant morphology, characterized by excessive branching, abundant and abnormal bulges and blind ends, discontinuous EC lining, and defective basement membrane and pericyte coverage. These features are all indicative of — or conducive to — impaired vascular maturation, poor vessel functionality and incoherent tumour perfusion[1,5,11]. Furthermore, the ECs of TABVs display structural and molecular traits that distinguish them from their counterparts in normal organs (BOX 1).

Although the cancer cells can be an important source of VEGFA and other pro-angiogenic mediators[12], recruited leukocytes increase VEGFA bioavailability and signalling during the angiogenic switch[13]. Furthermore, many signals that emanate from various tumour-associated stromal cells (TASCs), and the ECM in which they are embedded[14], sustain angiogenesis after the angiogenic switch through the subsequent phases of tumour progression (TABLE 1). In this Review, we discuss the extrinsic regulation of angiogenesis by the tumour microenvironment (TME), with the premise that harnessing such regulation may be instrumental in developing more effective anticancer therapies targeting angiogenesis and beyond.

Regulation of angiogenesis by TASCs

The abundance and composition of TASCs vary considerably between tumours and in their diverse microenvironments[15–18]. TASCs can be classified into two main categories on the basis of their origin. Tumour-infiltrating cells of haematopoietic origin are recruited from the bone marrow to the tumour via the systemic circulation and comprise diverse leukocyte types and

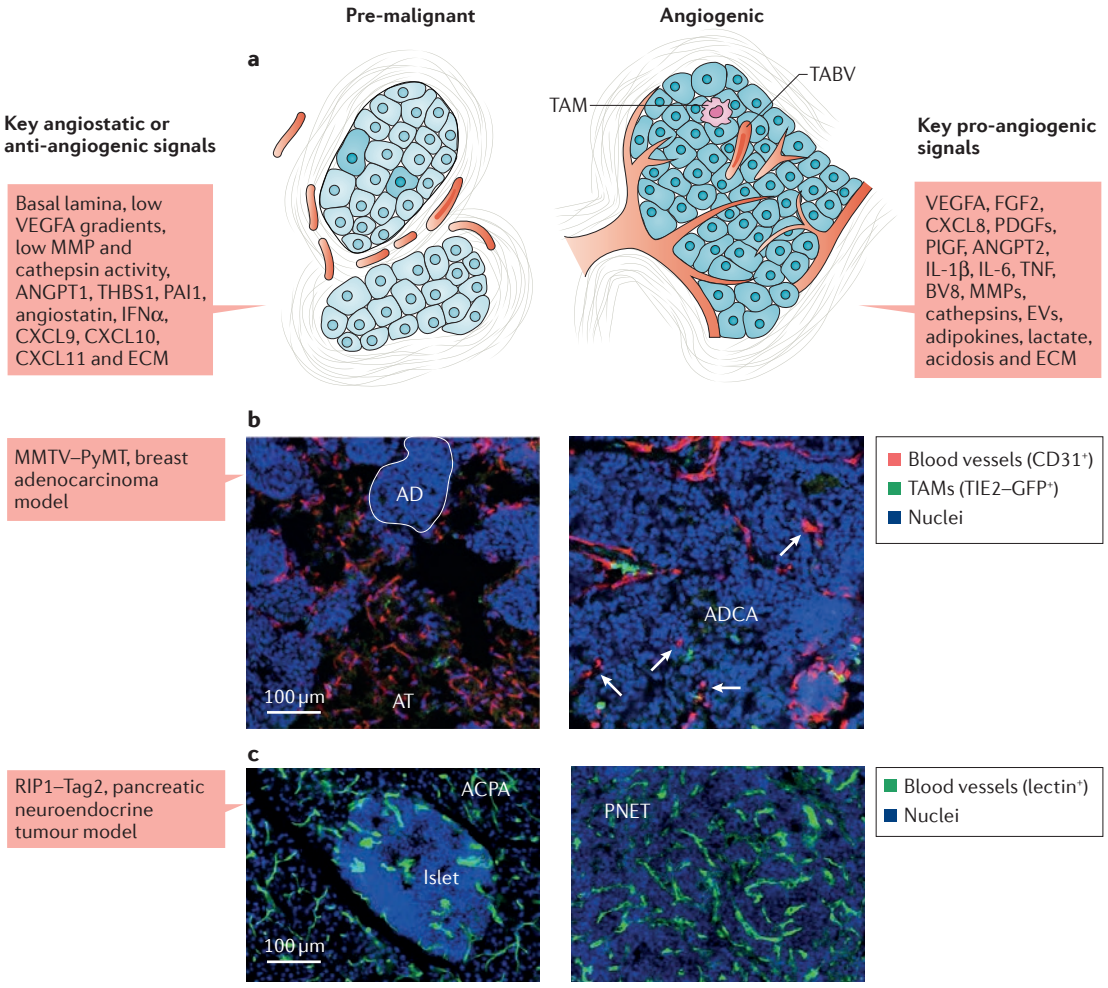

Figure 1 | **Angiogenesis during malignant progression.** Early-stage (pre-malignant) tumours typically display scant or no intra-tumoural vascularization, although a vascularized stroma surrounds the tumours and may adjoin parenchymal tumour domains (part **a**, left panel). In malignant tumours, the cancer cells acquire invasive behaviours and induce a stromal response involving robust intra-tumoural angiogenesis, along with leukocyte infiltration, fibroblast proliferation and extracellular matrix (ECM) deposition (part **a**, right panel). In pre-malignant lesions, a basal lamina separates the tumour from the surrounding tissues; this, together with angiostatic signals conveyed by some ECM components, and the relatively low levels of pro-angiogenic factors, prevents intra-tumoural vascularization or constrains it into a quiescent state. In malignant lesions, angiogenesis is largely controlled through the actions on vascular endothelial cells (ECs) of multiple pro-angiogenic mediators, which include growth factors, cytokines, various ECM proteins, ECM-remodelling enzymes, as well as extracellular vesicles (EVs) and by-products of deregulated tumour metabolism. Panels in **b** show early (left) or late (right) mammary tumours of mouse mammary tumour virus–polyoma middle T antigen (MMTV–PyMT) transgenic mice. Avascular adenomas (AD; left) develop from mammary glands embedded in vascularized adipose tissue (AT). In progressing adenocarcinomas (ADCA; right), blood vessels (arrows) infiltrate the tumours, along with pro-angiogenic tumour-associated macrophages (TAMs; green). In RIP1–Tag2 transgenic mice (part **c**), hyperplastic islets of Langerhans (islet; left) present a quiescent vascular network, which becomes angiogenic and plethoric when the islets progress into pancreatic neuroendocrine tumour (PNET). ACPA, acinar pancreas; ANGPT, angiopoietin; CXCL, CXC-chemokine ligand; EV, extracellular vesicle; FGF2, fibroblast growth factor 2; GFP, green fluorescent protein; IFNα, interferon-α; IL, interleukin; MMP, matrix metalloproteinase; PAI1, plasminogen activator inhibitor 1; PDGF, platelet-derived growth factor; PlGF, placental growth factor; TABV, tumour-associated blood vessel; THBS1, thrombospondin 1; TNF, tumour necrosis factor; VEGFA, vascular endothelial growth factor A. Images in panel **b** were adapted with permission from REF. 215, Elsevier.

Pericyte
Cell that enwraps and promotes the survival of endothelial cells, stabilizing small blood vessels.

Tumour microenvironment
(TME). The complex and dynamic ensemble of cancer cells, tumour-associated stromal cells (TASCs; comprising primarily leukocytes, fibroblasts and vascular cells) and their extracellular products.

Progenitors
Undifferentiated cells capable of producing lineage-committed cellular progeny.

subtypes, such as monocytes and macrophages, neutrophils, lymphocytes, as well as their immature precursors. There are also reports of non-haematopoietic, bone marrow-derived endothelial or mesenchymal progenitors contributing to tumour angiogenesis[19]. Tissue-resident cells are also recruited, including vascular cells (ECs and pericytes), fibroblasts, adipocytes, but also some tissue-resident leukocytes such as mast cells and macrophages. We discuss below the main TASC types involved in the regulation of tumour angiogenesis.

Macrophages. In mouse cancer models, macrophages largely derive from circulating monocytes that extravasate to tumours in response to various chemoattractants, including

Box 1 | **Features of tumour endothelial cells**

Morphology. Tumour endothelial cells (ECs) are structurally abnormal. They generally present excessive fenestrations, uneven surfaces and intra-luminal projections, and loosened intercellular junctions, and can also form multi-layered endothelia. These features favour vascular leakage and may limit blood flow[1,11,224].

Gene expression. The vascular ECs of different tissues and organs show distinct gene expression profiles[224]. In analogy, tumour ECs may display considerable inter- and intra-tumoural molecular heterogeneity[224,225]. Both gene expression profiling and the use of phage-display peptide libraries identified several tumour-type or stage-specific vascular markers (termed 'tumour endothelial markers' or 'vascular zip codes') in mouse models of cancer. The targeting of such tumour EC-specific markers may facilitate the selective delivery of therapeutic agents to tumour-associated blood vessels (TABVs)[226,227].

Proliferative signalling. Tumour ECs display increased proliferative, migratory and tube-formation capabilities in response to growth factors and cytokines, compared with non-tumour ECs[1,224]. Furthermore, they are resistant to senescence and can grow *ex vivo* in serum-free conditions. The upregulation of growth factor and cytokine receptors (for example, vascular endothelial growth factor receptors (VEGFRs)) by tumour ECs may account for such abilities[224]. Tumour ECs, but not normal ECs, may express epidermal growth factor receptor (EGFR) and proliferate in response to EGF[228]. They also show constitutive activation of PI3K–AKT signalling, which promotes cell survival and resistance to apoptosis[225].

Metabolism. Quiescent ECs display relatively high glycolysis rates[1,160]. However, tumour ECs are hyper-glycolytic and largely use aerobic glycolysis to address their energy requirements[160].

Drug resistance. There is evidence for tumour ECs being more resistant than normal ECs to various cytotoxic drugs[229]. For example, tumour ECs were shown to acquire resistance to the cytotoxic agent paclitaxel through the upregulation of the ATP-dependent efflux pump, P-glycoprotein 1 (PGY1; also known as ABCB1), which is induced by VEGFA signalling[230].

Genetic abnormalities. Gene and chromosomal abnormalities, including aneuploidy, supernumerary centrosomes and translocations, have been documented in subpopulations of tumour ECs of both mouse and human origin[225,231]. Tumour ECs may accumulate genetic mutations through several routes. They produce substantial amounts of reactive oxygen species (ROS) in response to cycles of anoxia–reoxygenation (oxidative stress), and are directly exposed to ROS released by tumour-infiltrating inflammatory cells and cancer cells. Furthermore, hypoxia represses the cellular DNA repair machinery. Both processes, coupled to the high proliferation rate of tumour ECs, can be directly mutagenic and also promote genetic instability in tumour ECs[232]. Alternatively, genetic alterations in tumour ECs might result from the direct *trans*-differentiation of cancer cells or, possibly, cancer stem cells into ECs[233,234].

chemokines, pro-inflammatory signalling molecules and damage-associated molecular patterns (DAMPs)[18,20]. Upon their extravasation, monocytes differentiate and mature into tumour-associated macrophages (TAMs) under the influence of colony-stimulating factor 1 (CSF1; also known as M-CSF). T cell cytokines and various tumour-derived factors further sculpt the macrophage phenotype, inducing TAMs to acquire substantial molecular and functional heterogeneity, both within and across distinct cancer types[18,20].

High macrophage numbers are frequently associated with increased vascular density in human tumours[21–23]. Accordingly, macrophages exert a pro-angiogenic role in mouse cancer models. Mouse mammary tumour virus–polyoma middle T antigen (MMTV–PyMT) transgenic mice that had been rendered macrophage deficient through *Csf1* inactivation displayed decreased vascularization in the mammary tumours[24]. Likewise, the broad elimination of TAMs by clodronate liposomes or CSF1 receptor (CSF1R) antibodies decreased angiogenesis in various tumour models[25–27]. However, the pro-angiogenic capacity of TAMs may depend on their activation state, which is modulated by the cytokine milieu to which they are exposed, and the specific TME in which they reside[18,28]. In some developmental processes, such as the remodelling of retinal blood vessels, macrophages may acquire anti-angiogenic or angiostatic functions[29]. However, there is currently little evidence for TAMs having vascular-inhibitory roles in tumours.

TAMs secrete growth factors and inflammatory cytokines that support angiogenesis by promoting EC survival, activation and proliferation (FIG. 2). TAMs are an important source of VEGFA in both mouse and human tumours[26,30–32]. The conditional elimination of *Vegfa* in myeloid cells (including TAMs) delays the angiogenic switch and attenuates the abnormal features of TABVs in mouse cancer models[31]. Furthermore, *Vegfa* deficiency in TAMs limits their ability to restore angiogenesis and to support the relapse of transplanted tumours after chemotherapy[32]. TAM-derived VEGFA also enhances vascular permeability, thereby facilitating cancer cell intravasation and metastasis[33]. Additional pro-angiogenic factors produced by TAMs include two VEGF-family members, placental growth factor (PlGF) and VEGFC, tumour necrosis factor (TNF), interleukin-1β (IL-1β) and IL-6, CXC-chemokine ligand 8 (CXCL8; also known as IL-8) and fibroblast growth factor 2 (FGF2); the angiogenic responses evoked by these cytokines have been reviewed elsewhere[28,29,34]. TAMs also express members of the WNT family. The genetic deletion of *Wnt7b* in TAMs reduced the expression of mitogenic WNT–β-catenin target genes in tumour ECs and decreased the vascular density in MMTV–PyMT mouse mammary carcinomas[35].

TAMs often enwrap TABVs, and the intimate association between perivascular TAMs and ECs creates an instructive niche that supports tumour angiogenesis[29,36]. TAMs secrete membrane-bound or soluble proteases that, through ECM degradation, facilitate the infiltrative growth of TABVs and mobilize pro-angiogenic growth factors sequestered in the perivascular ECM[5,14]. Macrophage-derived proteases include matrix metalloproteinases (MMPs; for example, MMP2, MMP9 and MMP12) and serine or cysteine proteinases, such as cathepsins and plasminogen activator[14,37,38]. Genetic or bisphosphonate-mediated inhibition of MMP9 decreased angiogenesis in human tumour xenografts[39] and in a mouse model of human papillomavirus 16 (HPV16)-driven cervical cancer[40]. Likewise, the pharmacological inhibition of cathepsin activity attenuated the vascularization of pancreatic neuroendocrine tumours (PNETs) in RIP1–Tag2 transgenic mice[41]. However, MMPs and cathepsins are expressed by multiple cell types in tumours — not only TAMs but also other leukocytes and cancer cells — and exert broad pro-tumoural functions that can also influence angiogenesis indirectly by regulating diverse parameters of tumour progression[14,37,38].

Conditional cell depletion studies have implicated perivascular TAMs that express the ANGPT receptor TIE2 (also known as TEK) in the promotion of tumour

Damage-associated molecular patterns
(DAMPs). Biological molecules that can initiate an inflammatory response independently of infection.

Clodronate liposomes
A formulation of small lipid vesicles containing a bisphosphonate that is capable of inducing macrophage death upon engulfment.

Angiostatic functions
Properties that promote endothelial cell quiescence and limit angiogenesis.

Table 1 | **Angiogenesis regulators in the TME**

TME component	Main angiogenesis regulators produced by TME component	Effects of TME component on TABVs	Refs
Macrophages	VEGFA, FGF2, CXCL8, CXCL12, PlGF, VEGFC, IL-1β, IL-6, TNF, WNT7B, MMPs and cathepsins	Pro-angiogenic; induce EC proliferation, migration and survival, as well as ECM remodelling, to facilitate sprouting angiogenesis	24,28–37, 40,41,67, 138
	CXCL9, CXCL10, CXCL11 and TNF	Potentially angiostatic under the influence of IFNγ and other T_H1 cytokines	83,85
Neutrophils and MDSCs	VEGFA, FGF2, BV8 and MMP9	Pro-angiogenic; the role is well established during early tumour stages or after therapeutic neutralization of VEGFA	13,54,55,57, 59,64,65
Mast cells	FGF2, VEGFA, TNF, CXCL8, chymase, tryptase and MMP9	Pro-angiogenic during the transition from non-angiogenic to angiogenic tumours	72–74
Eosinophils	VEGFA, FGF2, IL-6, CXCL8 and MMP9	Potentially pro-angiogenic, but relevance for tumour angiogenesis unclear	77
T_H2 cells	IL-4	Potentially pro-angiogenic by stimulating the alternative (M2-like) activation of TAMs	84
T_H1 cells	IFNγ	Potentially angiostatic through the induction of CXCL9, CXCL10 and CXCL11 in TAMs or via direct angiostatic or anti-angiogenic effects on ECs	80–83,85
T_H17 cells	IL-17	Pro-angiogenic by inducing CAFs to release CSF3, which recruits pro-angiogenic neutrophils	219
T_{reg} cells	VEGFA	Pro-angiogenic	86
B cells	VEGFA, FGF2, MMP9 and IgG	Potentially pro-angiogenic, either directly or via IgG-dependent recruitment and activation of myeloid cells	78,79
	Anti-VEGFA or anti-ANGPT2 IgG	Potentially angiostatic through the production of autoantibodies against pro-angiogenic cytokines in the context of immunotherapy	222,223
NK cells	VEGFA	Potentially pro-angiogenic, but relevance for tumour angiogenesis unclear	87,88
Platelets	VEGFA, PDGFB, FGF2 and CXCL12	Pro-angiogenic	90,91,93,95–97
	THBS1, PAI1, endostatin and ANGPT1	Potentially angiostatic	91,92,94
Pericytes	VEGFA, ANGPT1 and ECM components	Promote EC survival and, possibly, proliferation; they may contriute to stabilization of TABVs	100,103
CAFs	VEGFA, PDGFC, FGF2, CXCL12, osteopontin and CSF3	Pro-angiogenic, both directly and indirectly by recruiting myeloid cells and through ECM production	17,117–123
Adipocytes	Adipokines and free fatty acids	Pro-angiogenic and pro-inflammatory; stimulate peri-tumoural angiogenesis	128,130,131, 134
ECM	Periostin, tenascin C, fibronectin, osteopontin and CCN-family proteins	Pro-angiogenic through the storage and concentration of pro-angiogenic factors, and recruitment of pro-angiogenic leukocytes	138,140–147
	THBS1, osteonectin, decorin, proteolytic fragments of type IV and XVIII collagens	Potentially angiostatic	138,139,141
Hypoxia	HIF-inducible genes: *VEGFA*, *CXCL12* and *ANGPT2*	Pro-angiogenic	2
Metabolites	Lactate		153,154,157
	H^+	Pro-angiogenic through increased expression and stabilization of *VEGFA* mRNA	151,152
ROS	Free radicals and non-radical ROS	Potentially pro-angiogenic by enhancing *HIF1* transcription and the expression of pro-angiogenic and pro-inflammatory factors; they also generate pro-angiogenic lipid oxidation products	162,163
Tumour-derived EVs	Various pro-angiogenic and inflammatory mediators, ECM-remodelling enzymes and mitogenic factors for ECs	Potential pro-angiogenic effects mediated via contacts with, or transfer of their cargo to, ECs; relevance for tumour angiogenesis unclear	176,178,182, 187

ANGPT, angiopoietin; CAF, cancer-associated fibroblast; CSF3, colony-stimulating factor 3; CXCL, CXC-chemokine ligand; EC, endothelial cell; ECM, extracellular matrix; EV, extracellular vesicle; FGF2, fibroblast growth factor 2; HIF, hypoxia-inducible factor; IFNγ, interferon-γ; IgG, immunoglobulin G; IL, interleukin; MDSC, myeloid-derived suppressor cell; MMP, matrix metalloproteinase; NK, natural killer; PAI1, plasminogen activator inhibitor 1; PDGF, platelet-derived growth factor; PlGF, placental growth factor; ROS, reactive oxygen species; TABV, tumour-associated blood vessel; TAM, tumour-associated macrophage; T_H, T helper; THBS1, thrombospondin 1; TME, tumour microenvironment; TNF, tumour necrosis factor; T_{reg} cells, regulatory T cells; VEGF, vascular endothelial growth factor.

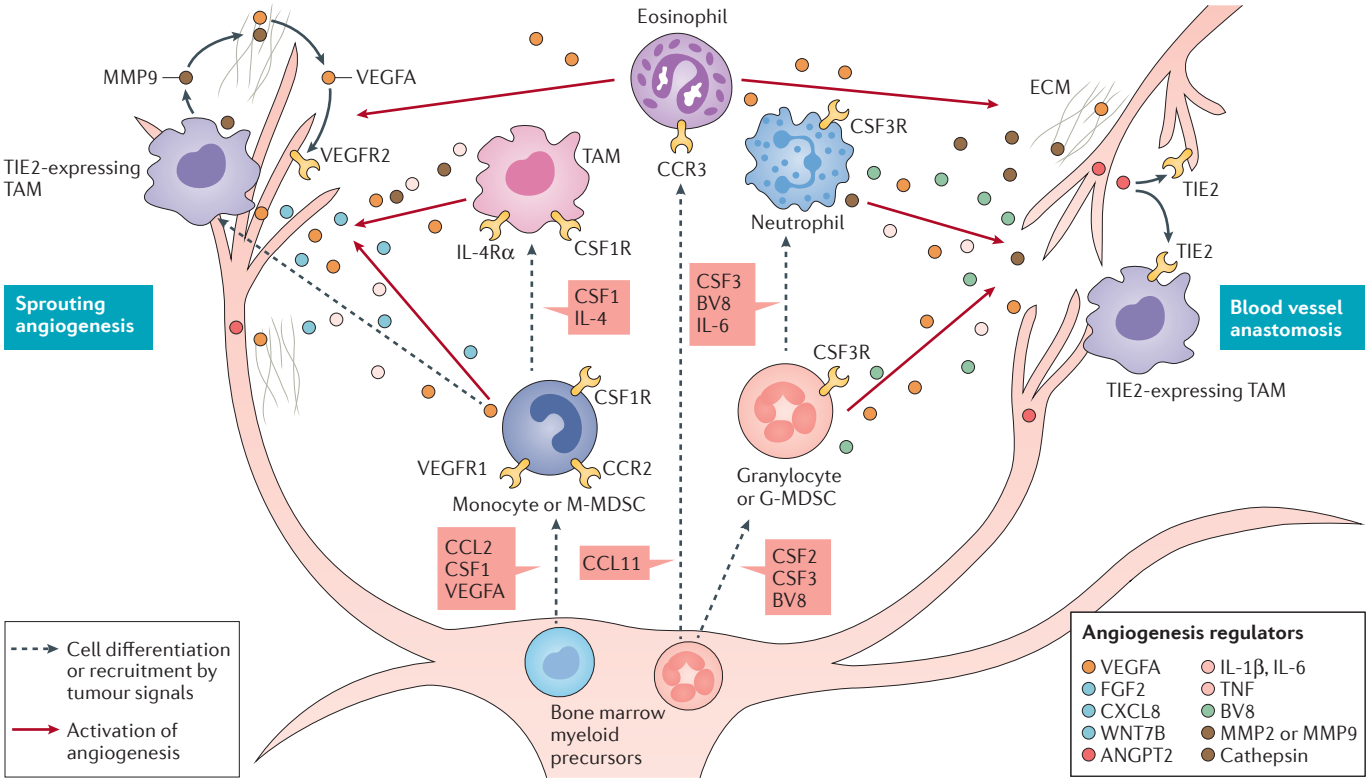

Figure 2 | **Myeloid cell regulation of tumour angiogenesis.** Various tumour-derived myeloid-cell chemoattractants — such as CC-chemokine ligand 2 (CCL2), CCL11, colony-stimulating factor 1 (CSF1), CSF2, CSF3, vascular endothelial growth factor A (VEGFA) and BV8 — recruit immature myeloid cells from the systemic circulation to the tumours. Upon their extravasation, subsets of myeloid cells may differentiate into tumour-associated macrophages (TAMs), neutrophils and eosinophils. Some myeloid cells maintain an immature phenotype in the tumour microenvironment (TME), and are referred to as monocytic myeloid-derived suppressor cells (M-MDSCs) or granulocytic MDSCs (G-MDSCs). Myeloid cells promote tumour angiogenesis by producing pro-angiogenic growth factors such as VEGFA, fibroblast growth factor 2 (FGF2), CXC-chemokine ligand 8 (CXCL8), WNT7B and BV8. They also secrete various pro-inflammatory cytokines, namely interleukin-1β (IL-1β), IL-6 and tumour necrosis factor (TNF), and many proteases, including matrix metalloproteinases (MMPs) and cathepsins, which also have pro-angiogenic roles. Myeloid-cell derived MMP9 mobilizes extracellular matrix (ECM)-bound VEGFA and enables its binding to VEGF receptor 2 (VEGFR2), which is expressed on ECs, triggering angiogenesis. TIE2-expressing TAMs derive from circulating monocytes; they associate with endothelial cells (ECs) and facilitate tumour angiogenesis by providing paracrine pro-angiogenic and tissue-remodelling support to sprouting or anastomosing blood vessels. EC-derived angiopoietin 2 (ANGPT2) supports angiogenesis in an autocrine manner by binding to the TIE2 receptor but also promotes leukocyte extravasation and mediates interactions between angiogenic ECs and TIE2-expressing TAMs. CSF1R, CSF1 receptor; CCR, CC-chemokine receptor; IL-4Rα, IL-4 receptor-α.

angiogenesis[36,42,43]. Accordingly, the abundance of TIE2$^+$ TAMs positively correlates with microvascular density and/or distant metastasis in some types of human cancer[33,44]. Hypoxia-induced expression of CXCL12 (also known as SDF1) and ANGPT2 stimulates the recruitment and perivascular accumulation of TAMs that express the respective cognate receptors CXC-chemokine receptor 4 (CXCR4) and TIE2. These CXCR4$^+$TIE2$^+$ TAMs support angiogenesis in both treatment-naive[43,45] and chemotherapy- or ionizing radiation-treated tumours[32,46–48]. In a transplant sarcoma model, TIE2 increased AKT activation in TAMs and protected them from the pro-apoptotic effects of the chemotherapy drug doxorubicin[49]. Moreover, the genetic inactivation of *Tie2* in TAMs impaired their ability to associate with immature blood vessels and sustain tumour angiogenesis[45] or revascularization after chemotherapy[49] in mouse tumour models. Of note, genetic or pharmacological inhibition of TIE2 in TAMs phenocopies some of the effects of blocking EC-derived ANGPT2 in tumours, suggesting that ANGPT2–TIE2 signalling regulates the pro-angiogenic interactions between perivascular TAMs and nascent TABVs[36,45].

Additional cues may regulate TAM–EC interactions. Notch signalling in macrophages has been implicated in macrophage-assisted pathological angiogenesis[50], but currently its role in tumour angiogenesis is little known. TAMs express vascular guidance molecules, namely semaphorins, some of which modulate EC survival and migration[51]. The physical association between TAMs and TABVs may, therefore, enhance EC survival, activation and migration, to facilitate vascular growth both in untreated tumours and during post-therapy relapse[36]. Finally, macrophages were shown to perform

RIP1–Tag2 transgenic mice
Expression of the SV40 large T antigen (Tag) under the control of the rat insulin promoter (RIP) causes β-cell hyperplasia, which progresses through a series of rate-limiting stages to invasive pancreatic neuroendocrine tumour (PNET).

Vascular guidance
The guided, directional growth of blood vessels.

vascular mimicry[52]. Although macrophages have infiltrative capacity and may transiently develop non-thrombogenic EC-like surfaces[53], it is currently unclear whether, and to what extent, macrophage channels provide a scaffold for subsequent endothelialization of bona fide TABVs.

Neutrophils. Neutrophils are the most abundant granulocytic population in the human blood and generally account for a substantial proportion of the haematopoietic cell infiltrate in experimental and human cancers. CSF3 (also known as G-CSF) is a key regulator of neutrophil production. CSF3 binds to its receptor (CSF3R) expressed on neutrophil precursors to activate the downstream Janus kinase (JAK)–signal transducer and activator of transcription 3 (STAT3) pathway, which promotes neutrophil proliferation and expansion. Recruitment of neutrophils to tumours is in part mediated by CXCL chemokines through the cognate receptors CXCR1 and CXCR2 (REF. 54).

Like macrophages, neutrophils are an important source of pro-angiogenic factors and proteases in the TME[55] (FIG. 2). In mice, STAT3 signalling controls the pro-angiogenic functions of neutrophils and other myeloid cells by activating *Vegfa*, *Fgf2* and *Mmp9* transcription[56]. Human neutrophils contain VEGFA-rich granules that are rapidly deployed on stimulation with TNF[57]. CSF3 induces neutrophils to upregulate the expression of BV8 (also known as prokineticin 2; a hormone-like protein) in a STAT3-dependent manner[58]; in turn, BV8 promotes EC proliferation and angiogenesis in tumours[59]. The pharmacological or genetic blockade of CSF3, CSF3R or BV8 decreases intra-tumoural neutrophils and inhibits tumour angiogenesis and growth[55]. Consistent with its angiostatic functions, type I interferon (IFN) signalling inhibits STAT3 activation and suppresses the production of VEGFA and MMP9 by neutrophils, hence limiting their pro-angiogenic capacity in mouse models of cancer[60].

The pro-angiogenic activity of neutrophils is crucial during the early stages of tumour progression. Neutrophil-derived MMP9 prompts the angiogenic switch in RIP1–Tag2 mice[13,55,61] by facilitating the mobilization of ECM-bound VEGFA and its subsequent binding to VEGFR2 on tumour ECs[13]. Accordingly, neutrophil depletion by GR1 or LY6G antibodies delays the angiogenic switch in both genetically engineered mouse models (GEMMs) of cancer and tumours transplanted in immunocompetent mice[60–62]. Moreover, *Mmp9* deficiency in myeloid cells impairs vascular maturation in transplant tumour models, suggesting that MMP9 also controls late events during tumour angiogenesis[63,64]. The absence of tissue inhibitor of metalloproteinases (TIMPs) in complex with secreted pro-MMP9 is required for the rapid activation and pro-angiogenic capacity of secreted pro-MMP9 (REF. 65). Neutrophils are a key source of TIMP-free pro-MMP9, which greatly exceeds the amount per cell produced by TAMs in transplant tumour models[64]. However, considering their abundance, TAMs may also provide a biologically relevant source of MMP9 in the TME[39,48,61]. Proteases released by activated neutrophils may also function as negative regulators of angiogenesis. For example, proteolysis of plasminogen by MMP9 and/or elastase liberates angiostatin, which limits angiogenesis directly by degrading VEGFA and FGF2, and indirectly by preventing CXCL8-dependent neutrophil recruitment[38].

Immature myeloid cells. In addition to mature macrophages and neutrophils, tumours contain abundant infiltrates of immature myeloid cells, such as deactivated dendritic cells (DCs) and myeloid-derived suppressor cells (MDSCs). The latter comprise myeloid cells at various stages of development and maturation, which can be resolved into monocytic (M-MDSC) and granulocytic (G-MDSC) cell populations[66]. Tumour-derived factors such as CSF3, IL-1β and IL-6, fuel STAT3 activation in MDSCs to promote their expansion, inhibit their full maturation into macrophages or neutrophils and enhance their pro-angiogenic functions in the TME[66]. Although immature DCs and MDSCs display distinctive metabolic properties and immunomodulatory capacities, their pro-angiogenic functions largely overlap with those of mature macrophages and neutrophils[67] (FIG. 2).

M-MDSCs and macrophages have been thoroughly characterized as both immunosuppressive and pro-angiogenic in cancer[66,68]. T cells extravasate to tumours through a multi-step process that involves binding to cell adhesion molecule (CAM)-family proteins expressed on ECs. Under the influence of myeloid cell-derived VEGFA and FGF2, the ECs of TABVs downregulate the expression and abrogate the clustering of intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion molecule 1 (VCAM1), hence limiting T cell adhesion and extravasation[68–70]. These findings suggest that myeloid cells may impair T cell homing to tumours also through direct effects of their pro-angiogenic products on TABVs[68,70].

Mast cells and eosinophils. Mast cells are tissue-resident granulocytes, the involvement of which in tumour angiogenesis has long been postulated[71]. Mast cells release pro-angiogenic factors, such as FGF2, VEGFA, TNF and CXCL8, along with MMPs, including MMP9; they also produce specific proteases (for example, chymase and tryptase) that activate pro-MMPs[72]. The pro-angiogenic functions of mast cells have been documented in GEMMs of HPV16-driven skin cancer[72], adenomatous polyposis coli (*Apc*)^{Min} intestinal adenoma[73], and MYC-induced PNET[74]. In these cancer models, mast cells surrounded or infiltrated early pre-neoplastic lesions, and their inactivation delayed the angiogenic switch and malignant progression. In PNETs, a mast cell inhibitor could also regress established TABVs by inducing EC apoptosis[74].

Eosinophils represent a minor granulocytic cell infiltrate in experimental mouse tumours[75]. They are mainly recruited to tumours by CC-chemokine ligand 11 (CCL11; also known as eotaxin) through CC-chemokine receptor 3 (CCR3) and preferentially localize to hypoxic areas in tumours[76] (FIG. 2). Eosinophils activated *in vitro* secrete various pro-angiogenic factors through degranulation. They release VEGFA upon stimulation with IL-5, whereas CCL11 and TNF prompt the secretion of FGF2, IL-6, CXCL8 and MMP9, among others[77]. Further

Vascular mimicry
The process whereby vascular-like channels are formed by non-endothelial cells in certain tumours, namely melanomas.

Non-thrombogenic EC-like surfaces
Cellular surfaces capable of preventing the formation of a clot (or thrombus) when in contact with blood.

Type I interferon
A family of secreted proteins with antiviral and immunomodulatory functions, which bind to a common receptor.

Genetically engineered mouse models (GEMMs) of cancer
Transgenic mice in which cancer is initiated and driven by defined genetic alterations, such as the expression of oncogene(s) and/or the inactivation of tumour suppressor gene(s), or both.

Immunocompetent mice
Mice that have an intact immune system. They are permissive to the growth of transplanted tumours with matched genetic background (syngeneic tumours).

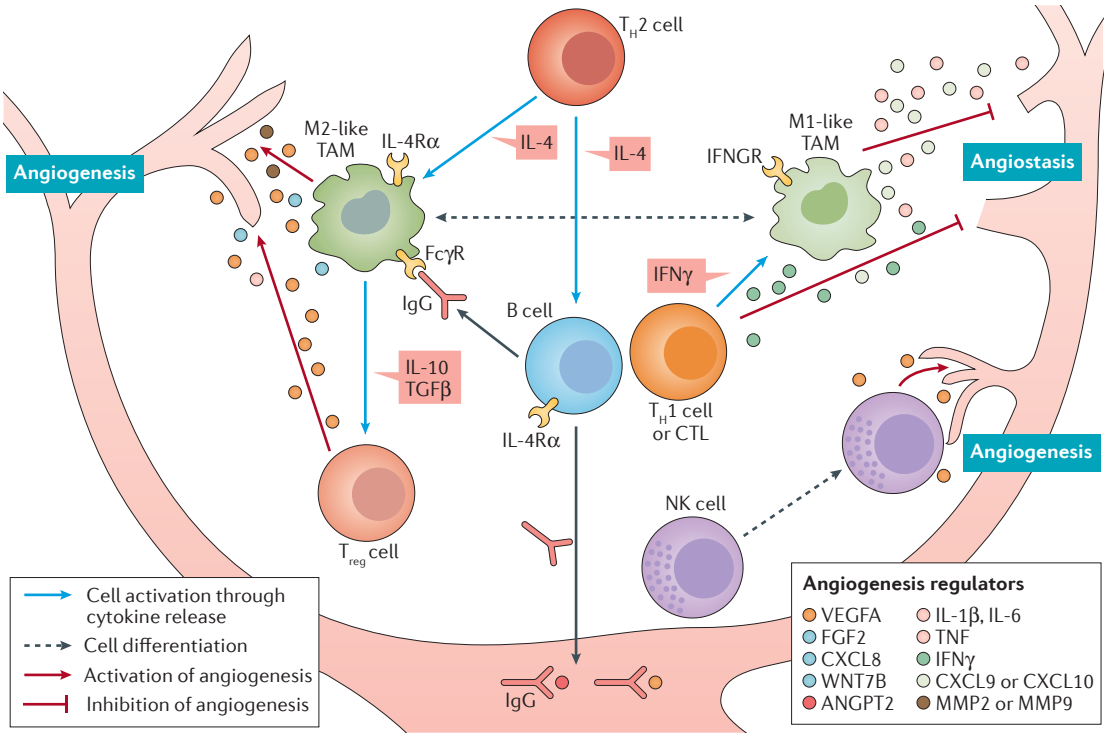

Figure 3 | **Cross-talk between lymphocytes and myeloid cells regulates tumour angiogenesis.** Tumour-infiltrating T helper 2 (T_H2) cells secrete interleukin-4 (IL-4) and promote the differentiation of tumour-infiltrating monocytes and macrophages into pro-angiogenic (M2-like) tumour-associated macrophages (TAMs). Conversely, T_H1 cells and cytotoxic T lymphocytes (CTLs) secrete interferon-γ (IFNγ), which may stimulate monocytes and macrophages to exert angiostatic (M1-like) functions through CXC-chemokine ligand 9 (CXCL9) and CXCL10 production. IFNγ can also inhibit tumour angiogenesis directly by impairing endothelial cell (EC) proliferation. T_H2 cells may activate humoral immunity, prompting B cells to secrete immunoglobulin G (IgG) that can stimulate pro-angiogenic macrophage programming via Fcγ receptor (FcγR) engagement. TAM-derived immunosuppressive cytokines, such as IL-10 and transforming growth factor-β (TGFβ), promote the expansion of regulatory T (T_{reg}) cells that sustain angiogenesis by releasing vascular endothelial growth factor A (VEGFA). The tumour microenvironment (TME) may suppress natural killer (NK) cell cytotoxic activity and induce their upregulation of VEGFA. In the context of cancer vaccines and immune checkpoint blockade, B cells may secrete autoantibodies against pro-angiogenic factors, namely angiopoietin 2 (ANGPT2) and VEGFA, thereby neutralizing their functions and disrupting tumour angiogenesis. FGF2, fibroblast growth factor 2; IFNGR, interferon γ receptor; IL-4Rα, IL-4 receptor-α; MMP, matrix metalloproteinase; TNF, tumour necrosis factor.

studies are necessary to establish the importance of eosinophil-derived pro-angiogenic factors for tumour angiogenesis and revascularization after therapy.

Lymphocytes. Lymphocytes are cells that accomplish antigen-specific immune responses. By modulating myeloid cell activation, B cells and T cells may indirectly control tumour angiogenesis. Furthermore, some lymphocyte-derived cytokines directly influence EC biology in tumours (FIG. 3).

B cells may facilitate angiogenesis in tumours by expressing various pro-angiogenic mediators, including VEGFA, FGF2 and MMP9, in a STAT3-dependent manner[78]. They may also stimulate tumour angiogenesis indirectly through immunoglobulin G (IgG) and by polarizing macrophages. For example, in a GEMM of HPV16-driven skin cancer, deposition of B cell-derived IgG in the pre-malignant skin was shown to recruit and activate pro-tumoural and pro-angiogenic TAMs, which fostered skin carcinogenesis[79]. Pro-angiogenic TAM programming was dependent on activating IgG receptors (FcγRs) expressed on the macrophages. Indeed, mice lacking FcγRs failed to mount a robust angiogenic response and had delayed tumour progression and reduced incidence of squamous cell carcinomas[79].

There is also increasing evidence that T cells modulate tumour angiogenesis, both directly and indirectly. Immunotherapy-elicited $CD4^+$ T helper 1 (T_H1) cells can directly inhibit tumour angiogenesis by enforcing the maturation and/or quiescence of TABVs[80]. This process may involve IFNγ, which restrains EC proliferation and, when overexpressed experimentally, can cause the regression of immature blood vessels[81,82]. T cells may also influence tumour angiogenesis indirectly. For example, $CD4^+$ T_H2 cells secrete IL-4 and stimulate the STAT6-dependent alternative (or M2-like) activation of TAMs, which entails immunosuppressive, tissue-remodelling and pro-angiogenic functions[83,84]. Conversely, IFNγ secreted by $CD4^+$ T_H1 cells or $CD8^+$ cytotoxic T lymphocytes (CTLs) may stimulate TAMs to upregulate the expression of the angiostatic cytokines CXCL9, CXCL10 and CXCL11, in a STAT1-dependent manner[83,85]. There is,

T helper 1 (T_H1) cells
T cells that can stimulate other immune cells, such as macrophages and cytotoxic T lymphocytes, to kill infected or cancer cells. They do so primarily through the release of the T_H1 cytokine interferon-γ.

T_H2 cells
T cells that stimulate B cells to produce immunoglobulins. They do so through the release of T_H2 cytokines, such as interleukin-4.

Cytotoxic T lymphocytes
(CTLs). T cells capable of killing other cells, including cancer cells, generally through the recognition of specific antigens.

however, little evidence for T cells promoting angiostatic or anti-angiogenic TAM reprogramming, at least in treatment-naive, progressing tumours.

In contrast to T_H1 cells and CTLs, immunosuppressive regulatory T (T_{reg}) cells seem to possess pro-angiogenic capacities[68]. T_{reg} cells may facilitate angiogenesis indirectly by suppressing IFNγ-expressing effector T_H1 cells. Furthermore, hypoxia-induced CCL28 recruits T_{reg} cells that express VEGFA, and depletion of T_{reg} cells abates VEGFA levels and angiogenesis in the TME[86]. These observations add weight to the notion that immunosuppressive cell networks involving myeloid cells and T_{reg} cells not only cause subsidence of antitumour immunity, but also function to stimulate tumour angiogenesis[68].

Natural killer cells. Although natural killer (NK) cells have important pro-angiogenic roles in the uterine vasculature, their involvement in tumour angiogenesis is less well understood[87]. The genetic inactivation of *Stat5*, which is required for NK cell-mediated cancer immunosurveillance, upregulates VEGFA in NK cells and enhances angiogenesis in mouse lymphoma models[88]. Deactivated VEGFA-expressing NK cells have been observed in various human cancer types (FIG. 3), suggesting potential associations between NK cell deactivation and angiogenesis in progressing tumours[87].

Platelets. The link between cancer progression and thrombocytosis (increased platelet counts) is well established[89]. Activated platelets are a rich source of pro-angiogenic factors, including VEGFA, platelet-derived growth factors (PDGFs) and FGF2 (FIG. 4). They also contain and deploy angiostatic molecules, such as thrombospondin 1 (THBS1), plasminogen activator inhibitor 1 (PAI1; also known as SERPINE1) and endostatin[90]. Pro-angiogenic and angiostatic molecules are stored in distinct α-granules, which may be selectively released depending on the specific stimulus[91]. However, this concept has been challenged by studies suggesting that platelet secretion is, in fact, a stochastic process[92].

In tumours, platelets are activated at sites of vascular hyperpermeability and plasma leakage by contact with collagen and cancer cells[90,93]. Tumours cause platelet activation, aggregation and degranulation in their vasculature by expressing platelet-activating factors, such as tissue factor (TF), thrombin and ADP. Tumour ECs frequently overexpress TF, and positive correlations between TF expression and microvessel density, or TF and VEGFA expression, have been observed in several cancer types[90,93]. Although disrupting platelet function does not obviously impair tumour angiogenesis[94], the overall outcome of platelet activation and degranulation in tumours appears to be pro-angiogenic[90,93]. In particular, platelet degranulation of VEGFA, CXCL12 and PDGF, may initiate a 'wound-healing' response involving the recruitment and activation of myeloid cells and cancer-associated fibroblasts (CAFs), and increased ECM deposition, which in turn foster tumour angiogenesis[14].

Interestingly, platelets can avidly sequester pro-angiogenic factors in cancer-bearing hosts[95], and platelets isolated from cancer patients indeed contain higher levels of pro-angiogenic factors compared with those from healthy donors[96,97]. In one study, platelets were shown to sequester pro-angiogenic factors from aggressive mouse mammary tumours and to deploy them to indolent tumours to induce angiogenesis and instigate their progression[98]. Platelets can also promote angiogenesis by stimulating the mobilization of myeloid cells from the bone marrow and enhancing their homing to tumours[99]. This may involve deployment to the bone marrow niche of factors that had been sequestered at the tumour site. Provocatively, the shuttling of sequestered myeloid-cell chemoattractants (for example, CXCL12) and pro-angiogenic mediators (for example, VEGFA) by platelets[99] might trigger the coordinate awakening of dormant disseminated cancer cells and thereby induce metastatic outgrowth through the induction of the angiogenic switch. Together, these findings illustrate complex roles for platelets in the regulation of vascular homeostasis and growth in tumours.

Pericytes. Pericytes are cells of mesenchymal origin that enwrap and stabilize capillaries. They are embedded in the basement membrane of small blood vessels and promote survival of ECs, while restraining their proliferation, through the secretion of EC growth factors, MMP inhibitors and various ECM molecules. Pericytes also stabilize EC junctions to limit vascular permeability[100]. At variance with quiescent capillaries, TABVs display uneven and loose pericyte coverage[11,101]. The paucity of stable pericyte–EC interactions in tumours enables sprouting angiogenesis (FIG. 4), but also generates a dysfunctional vascular network characterized by EC hyperplasia, defective cellular junctions and vascular leakiness[11,100,101].

EC-derived PDGFB promotes the recruitment of pericytes to the tumour vasculature[100], whereas the ANGPT–TIE2 system plays fundamental roles in regulating subsequent pericyte–EC interactions[102]. The binding of pericyte-derived ANGPT1 to TIE2 on ECs inhibits EC proliferation, tightens EC junctions and stabilizes newly formed vessels[103]. Furthermore, pericytes express neural cell adhesion molecule 1 (NCAM1) and the NG2 proteoglycan, which contribute to vascular maturation by increasing pericyte recruitment[104,105]. By contrast, angiogenic ECs produce ANGPT2, which competes with ANGPT1 for binding to TIE2, disrupting pericyte–EC interactions and destabilizing the TABVs to enable angiogenesis[102]. Accordingly, genetic or pharmacological inhibition of ANGPT2 or TIE2 activation inhibits tumour angiogenesis and increases pericyte coverage of the surviving blood vessels[45,106–109].

Mounting data suggest that pericytes are heterogeneous cell subpopulations with different developmental origins and diverse gene expression profiles[100]. Two main pericyte subsets have been identified in mice, termed type-1 ($nestin^-NG2^+$) and type-2 ($nestin^+NG2^+$) pericytes[110]. Only type-2 pericytes were found in transplant B16 melanoma and G26-H2 glioma tumour models[110], but it is currently unclear whether other tumour models, such as GEMMs of cancer, also lack type-1 pericytes. Tumour pericytes exert pro-angiogenic functions and display an activated phenotype

Cancer immunosurveillance
The process whereby immune cells, namely lymphocytes and natural killer cells, recognize initiated cancer cells and eliminate them; it may also lead to the selection of less immunogenic cancer cell clones.

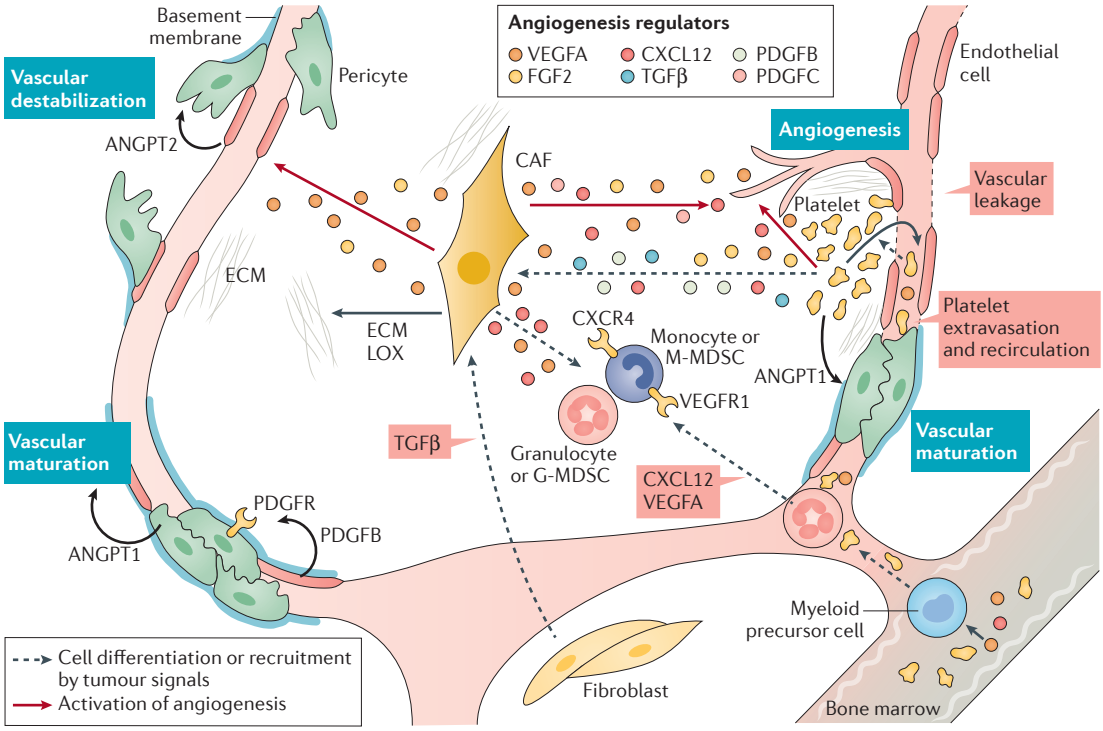

Figure 4 | **Chronic wound-healing response promotes tumour angiogenesis.** Under the influence of transforming growth factor-β (TGFβ) and other tumour-derived factors, peri-tumoural fibroblasts differentiate into cancer-associated fibroblasts (CAFs), which secrete various components of the tumour extracellular matrix (ECM) and induce the crosslinking of collagen fibres through lysyl oxidase (LOX) activity. Moreover, CAFs stimulate angiogenesis by secreting pro-angiogenic growth factors, such as vascular endothelial growth factor A (VEGFA), fibroblast growth factor 2 (FGF2), CXC-chemokine ligand 12 (CXCL12) and platelet-derived growth factor C (PDGFC). The loose association of pericytes with tumour-associated blood vessels (TABVs) favours chronic vascular leakage in tumours. This process is enhanced by autocrine angiopoietin 2 (ANGPT2) signalling and is inhibited by ANGPT1 and PDGFB, which promote vascular maturation when VEGFA and ANGPT2 levels are low. Platelet extravasation and degranulation at sites of vascular leakage liberates numerous pro-angiogenic mediators and proteases, as well as cytokines and growth factors that support the proliferation and activation of CAFs, such as PDGFB and TGFβ. Platelets may also sequester different tumour-derived factors, for example CXCL12 and VEGFA, in the tumour microenvironment (TME) and deploy them to the bone marrow haematopoietic niche to enhance myelopoiesis and myeloid-cell mobilization. CXCR4, CXC-chemokine receptor 4; G-MDSC, granulocytic myeloid-derived suppressor cell; M-MDSC, monocytic myeloid-derived suppressor cell; VEGFR1, VEGF receptor 1.

characterized by increased expression of α-smooth muscle actin (αSMA; also known as ACTA2), regulator of G-protein signalling-5 (RGS5) and endosialin, and reduced levels of desmin and contractile proteins, compared with normal-tissue pericytes[70,100,110]. The acute elimination of tumour pericytes, for instance, by PDGF receptor (PDGFR) signalling inhibition or suicide gene-based cell depletion approaches, disrupts angiogenesis in both transplant and GEMMs of cancer[111,112]. Furthermore, kinase inhibitors that concomitantly block the VEGFRs and PDGFRs, such as sunitinib and sorafenib, induce more pervasive and sustained TABV regression than pure VEGFR inhibitors[113,114]. These findings indicate that pericytes provide crucial pro-survival cues to angiogenic TABVs.

Cancer-associated fibroblasts. CAFs have a key role in producing a reactive stroma that frequently perpetuates a tumour-promoting, tissue-repair response in solid tumours[17]. CAFs largely derive from tissue-resident fibroblasts that, under the influence of transforming growth factor-β (TGFβ), acquire traits of functional hyperactivation, including enhanced proliferation and motility, along with robust ECM biosynthesis and deposition capacity. Indeed, CAFs secrete enzymes, such as lysyl oxidases (LOXs) and hydroxylases, which catalyse the crosslinking of collagens to elastin and other ECM molecules. By controlling the biomechanical properties of the tumour stroma, including stiffness, elasticity and interstitial fluid pressure, CAFs indirectly modulate vascularization and blood flow in tumours[115].

CAFs have well-established pro-angiogenic functions in tumours (FIG. 4). They often colocalize with TABVs in human cancers, and co-implantation of CAFs and cancer cells enhances angiogenesis, decreases cancer cell dormancy and accelerates tumour growth in mice[116,117]. CAFs are a major source of tumour VEGFA[118,119], but can also support tumour angiogenesis in a VEGFA-independent manner[120]. CAF-derived PDGFC sustains angiogenesis by further stimulating CAFs to secrete pro-angiogenic growth factors, such as FGF2 and osteopontin[121–123]. The CAF secretome potentiates

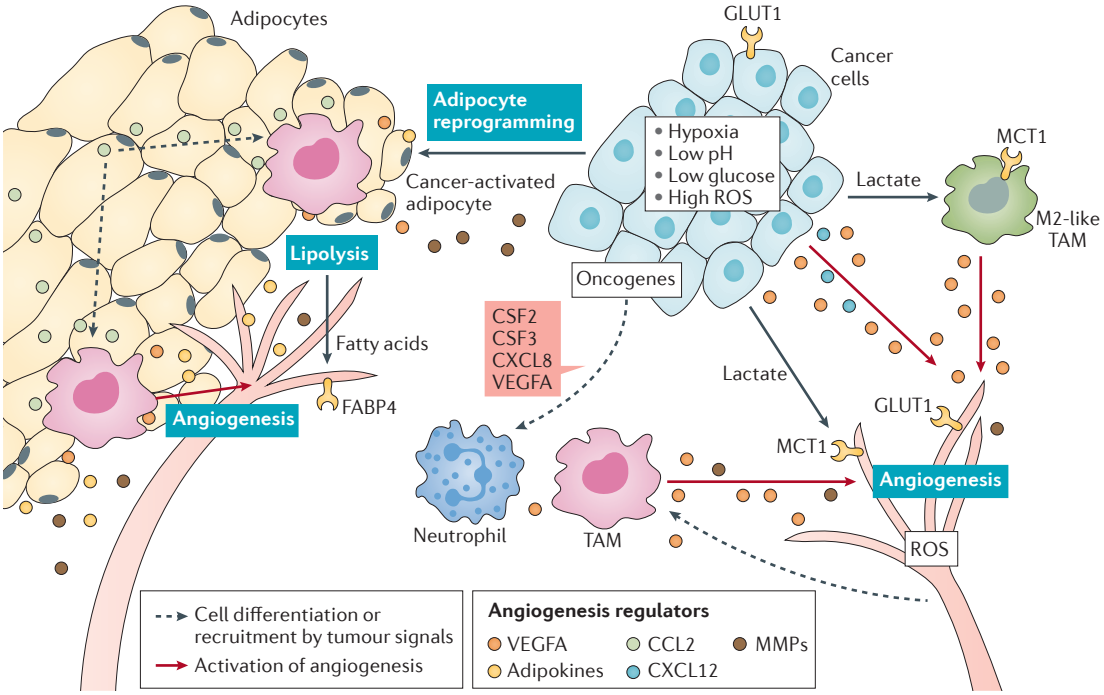

Figure 5 | **Metabolic regulation of tumour angiogenesis.** Under the influence of tumour-derived factors, peri-tumoural adipocytes increase their lipolytic activity and liberate free fatty acids that, upon internalization by vascular endothelial cells (ECs) through fatty acid-binding protein 4 (FABP4), increase the rate of β-oxidation in the tumour-associated blood vessels (TABVs) to sustain angiogenesis. Cancer-activated adipocytes also liberate adipokines, a heterogeneous assortment of growth factors, cytokines and hormones that promote tumour angiogenesis. Adipokines potentially relevant to tumour angiogenesis include leptin, adiponectin, resistin, visfatin, oestrogens, tumour necrosis factor (TNF), interleukin-1β (IL-1β) and IL-6, insulin growth factor 1 (IGF1), vascular endothelial growth factor A (VEGFA), fibroblast growth factor 2 (FGF2), hepatocyte growth factor (HGF), angiopoietins (ANGPTs), CC-chemokine ligand 2 (CCL2) and colony-stimulating factor 2 (CSF2) (reviewed in REF. 128). Chronic hypoxic conditions in the tumour microenvironment (TME) promote the expression of hypoxia-inducible factor (HIF)-induced pro-angiogenic mediators, namely VEGFA and CXC-chemokine ligand 12 (CXCL12), which directly stimulate tumour angiogenesis. Furthermore, sustained oncogenic signalling in the cancer cells is associated with the upregulation of various myeloid-cell chemoattractants and activators, such as CSF2, CSF3, CXCL8 and VEGFA. Metabolically active cancer cells secrete lactate, which is internalized by ECs and tumour-associated macrophages (TAMs) through the lactate importer monocarboxylate transporter 1 (MCT1). Lactate stimulates tumour angiogenesis both by acting directly on ECs and indirectly by promoting M2-like TAM programming. Tumour ECs respond to hypoxia and acidosis by upregulating mediators of the glycolytic pathway, such as glucose transporter 1 (GLUT1). Finally, under the influence of VEGFA, the ECs of TABVs produce various reactive oxygen species (ROS), which promote EC proliferation and angiogenesis under conditions of metabolic stress. MMP, matrix metalloproteinase.

tumour angiogenesis also by attracting vascular ECs and recruiting monocytes from the bone marrow, for example, through the CXCL12–CXCR4 axis[17,117]. In melanoma, aged CAFs secrete the WNT antagonist secreted frizzled-related protein 2 (SFRP2), which exacerbates the angiogenic and malignant behaviour of tumours in old individuals[124]. Although CAFs also secrete angiogenesis inhibitors, such as THBS1 (REF. 17), tumours may overcome their angiostatic properties by adaptively increasing the production of pro-angiogenic factors[125].

Adipocytes. Adipose tissue may foster the growth of initiated cancer cells through the promotion of angiogenesis. Indeed, tumour growth was accelerated when cancer cells were implanted in the white or brown adipose tissue of mice, compared with the subcutaneous space[126,127]. The tumours implanted in adipose tissue displayed a more florid vascular network than those implanted subcutaneously, suggesting a potential role for adipocytes in accelerating angiogenesis[127]. Tumours that arise in or in proximity to adipose tissue (for example, breast, ovarian or colon cancers, as well as bone or lymph node metastases) are exposed to a milieu of cytokines, chemokines and hormones, collectively termed adipokines, some of which have well-established pro-angiogenic functions (FIG. 5). Pro-angiogenic adipokines are secreted by adipocytes, infiltrating inflammatory cells and other adipose tissue-associated stromal cells, and may either target vascular ECs directly or recruit vascular-modulatory inflammatory cells[128].

Of note, obesity is associated with increased risk of several cancer types[129]. The adipose tissue of obese individuals is not only enlarged, but also chronically inflamed and adipokine rich. Adipocytes isolated from obese individuals enhanced EC proliferation and migration *in vitro* to a greater extent than adipocytes from non-obese individuals[130]. Furthermore, in a mouse mammary tumour model, pre-existing obesity

facilitated tumour growth by inducing angiogenesis[131]. Both human and mouse mammary adipocytes were shown to recruit and activate macrophages through a CCL2–IL-1β–CXCL12 signalling pathway. In turn, the activated macrophages promoted stromal angiogenesis before the appearance of cancer nodules[131]. Consistent with these findings, leukaemic cells were shown to preferentially thrive in so-called 'milky spots' — aggregates of immune cells embedded in highly vascularized adipose tissue — in an experimental model of peritoneal metastasis[132].

Compared with normal adipose tissue, the peritumoural adipose tissue is highly vascularized and macrophage rich, and produces higher levels of proteases, ECM proteins and various pro-angiogenic adipokines. Moreover, cancer cells reprogramme adjacent adipocytes to acquire an activated phenotype characterized by reduced cell size and sustained lipolysis[126,133]. As a result of increased lipolysis, cancer-associated adipocytes may supply fatty acids to metastatic ovarian cancer cells through the chaperone protein fatty acid-binding protein 4 (FABP4), boosting β-oxidation of fatty acids in cancer cells[134]. Of note, not only cancer cells but also VEGFA-stimulated, angiogenic ECs express high levels of FABP4 (REF. 135). Angiogenic ECs can utilize fatty acid β-oxidation for their growth[136], so cancer-associated adipocytes may directly fuel peri-tumoural angiogenesis.

The extracellular matrix

The ECM is an intricate network of fibrous proteins, glycosaminoglycans and matricellular proteins that provide structural support as well as biochemical and biomechanical cues for cancer cell growth[115,137,138]. Vascular ECs and mural cells produce a specialized ECM, the basement membrane, which is crucial for blood vessel integrity and function. Sprouting angiogenesis involves the degradation of the basement membrane by MMPs produced by activated ECs and recruited myeloid cells, the ensuing formation of a provisional fibrin- and fibronectin-rich ECM that supports EC proliferation and migration, and the ultimate reassembly of a mature basement membrane that, in the context of non-pathological angiogenesis, contributes to EC quiescence and vascular integrity[1,138,139]. Sustained pro-angiogenic signalling in tumours impairs the subsequent steps of vascular morphogenesis, namely the acquisition of a quiescent EC phenotype and the development of an intact and selectively permeable vascular barrier[1].

In tumours, the vascular basement membrane is frequently discontinuous and loosely associated with ECs and pericytes[140], which contributes to increasing vascular leakiness and facilitates cancer cell intravasation and metastasis[1,115]. Furthermore, the composition, topography and ligand density of both the vascular and interstitial ECM are altered in tumours[138]. The ECM may have both pro-angiogenic and vascular-stabilizing roles. It serves as a depot for various pro-angiogenic growth factors, notably VEGFA, FGFs, PDGFB and TGFβ, which are released in their bioactive forms through the proteolytic processing of the ECM by plasmin, MMPs and other proteases[5,138,139]. The breakdown of the ECM may also generate chemoattractants for pro-angiogenic inflammatory cells, such as TAMs[141]. Direct pro-angiogenic activities have been described for many tumour ECM molecules, such as periostin, tenascins, fibronectin, perlecan, osteopontin and CCN-family proteins[142–144]. For example, in the RIP1–Tag2 PNET model, tenascin C sustained angiogenesis by downregulating Dickkopf-related protein 1 (DKK1) and increasing WNT signalling[145]. Conversely, several ECM matricellular proteins, such as THBS1, osteonectin (also known as SPARC) and the proteoglycan decorin, may exert angiostatic functions[146,147]. Sustained ECM remodelling in tumours may also generate biologically active fragments of type IV and XVIII collagens, which limit angiogenesis by competing with intact collagen fibres for interaction with EC integrins[138,139].

The biophysical and mechanical properties of the tumour ECM, such as the altered geometry and increased density and crosslinking of collagen fibres, influence tumour angiogenesis both directly and indirectly[115,138]. In experimental matrices, ECM stiffness and contractility modulate the spatial organization of VEGFA gradients and VEGFR2 expression by ECs[148,149]. The abnormal arrangement of ECM fibres facilitates tumour angiogenesis also by enhancing the migration of ECs and pro-angiogenic TASCs, such as TAMs and CAFs. Indeed, these cells migrate more rapidly on linearized collagen fibres, which are enriched in tumours compared with non-neoplastic tissues[115,150].

Tumour metabolism

The key role of hypoxia in tumour angiogenesis is well established[2]. The transcriptional activity of hypoxia-inducible factor 1 (HIF1) induces the expression of several pro-angiogenic genes, such as *VEGFA*, *VEGFR2*, *DLL4*, *CXCL12* and *ANGPT2*, in both cancer cells and TASCs[2]. Under hypoxic conditions, cancer cells consume glucose and secrete lactate, which generates an acidic TME (FIG. 5). Glucose deprivation and acidosis increase *VEGFA* mRNA stability post-transcriptionally in the cancer cells[151,152]. Also, ECs internalize cancer cell-derived lactate through the lactate importer monocarboxylate transporter 1 (MCT1; also known as SLC16A1), which enhances angiogenesis in a nuclear factor-κB (NF-κB)- and HIF1-dependent manner[153,154].

Similarly to cancer cells, TASCs also respond to hypoxia[2]. Hypoxic conditioning of TASCs modulates the tumour metabolic landscape and angiogenesis. Hypoxia stimulates CAFs to secrete ECM-remodelling enzymes and HIF-inducible pro-angiogenic factors (for example, CXCL12), which facilitate tumour angiogenesis[2]. Macrophages accumulate in hypoxic tumour regions[155] and around nascent (non-perfused) TABVs[29,36,45]. These hypoxic microenvironments fine-tune the activation of TAMs and stimulate pro-angiogenic gene transcription[156]. In analogy, lactate induces pro-angiogenic (M2-like) TAM activation in a HIF1α-dependent manner[157]. Hypoxic TAMs express several HIF-dependent glycolytic genes, suggesting that they preferentially utilize a glycolytic metabolism[158]. However, under the influence of T_H2 cytokines (for example, IL-4), TAMs may tune

Lipolysis
The process whereby triglycerides are resolved into glycerol and free fatty acids through hydrolysis.

β-Oxidation
The process that occurs in the mitochondrion and that uses fatty acids to generate acetyl-CoA, which is essential for producing ATP through oxidative phosphorylation.

down glycolysis and enhance oxidative phosphorylation, a metabolic switch that is associated with the acquisition of immunosuppressive and pro-angiogenic functions[158]. Attenuated glycolysis in TAMs was also shown to facilitate glucose consumption by tumour ECs, leading to their acquisition of robust angiogenic capacities[159]. Despite their proximity to blood oxygen, tumour ECs mainly rely on aerobic glycolysis (rather than oxidative phosphorylation) and fatty acid oxidation for their bioenergetics and biosynthetic needs[160]. Such metabolic reprogramming enables ECs to create new blood vessels while maximizing oxygen transfer to surrounding tissues, limiting the production of reactive oxygen species (ROS), and producing ATP more rapidly than through oxidative metabolism[160].

Cycles of hypoxia–reperfusion, high metabolic activity and sustained oncogenic signalling, together induce unbalanced ROS production in tumours[161]. Depending on the exact species, concentration and cellular source, ROS can either promote or inhibit tumour angiogenesis. ROS production in both cancer cells and TASCs cell-autonomously induces VEGFA transcription through HIF1, and also generates lipid oxidation metabolites, such as end-products of docosahexaenoic acid oxidation, which induce tumour angiogenesis in a VEGFA-independent and Toll-like receptor 2 (TLR2)-dependent manner[162,163]. Although ROS production in ECs may sustain tumour angiogenesis[164], excessive ROS levels may blunt EC responsiveness to extracellular VEGFA by increasing VEGFR2 recycling[165], a process that can be bypassed through the induction of anti-oxidative responses[166]. It remains unclear whether ROS generated by cancer cells and TASCs in highly hypoxic and inflamed TMEs can directly penetrate ECs to influence their angiogenic properties.

Finally, there is also experimental evidence for oncogenic drivers to control tumour angiogenesis. Constitutively activated RAS and RAF proteins directly induce the expression of pro-angiogenic factors, such as VEGFA and CXCL8, in cancer cells[167–170]. Furthermore, mutant oncogenes may also elicit pro-angiogenic responses indirectly (for instance, by inducing the expression of myeloid cell chemoattractants[171,172]). However, there is currently little clinical evidence that specific oncogenes, such as mutant KRAS, confer higher sensitivity to anti-angiogenic therapy, for example, in colorectal cancer[173,174].

Tumour-derived extracellular vesicles

Cancer cells and TASCs secrete various vesicles of different sizes, together referred to as extracellular vesicles (EVs), which contain proteins, nucleic acids and lipids that in part reflect the biomolecular composition of the cell of origin[175]. The hypoxic and acidic TME may enhance the production of tumour-derived EVs, and increasing data suggest that tumour-derived EVs can influence vascular function, both locally in tumours and remotely in distant organs through the systemic circulation[29,175,176].

EVs secreted by cancer cells have been shown to contain pro-angiogenic mediators, including VEGFA, CXCL8, IL-6 and FGF2 (REFS 177,178). Of note, the acidic TME may facilitate EV disruption[178], enabling the interaction of pro-angiogenic molecules with cognate receptors expressed on tumour ECs. Cancer cell-derived EVs may also deploy pro-angiogenic ECM-remodelling enzymes, such as urokinase plasminogen activator (uPA), MMP2 and MMP9 (REFS 178,179). Besides cancer cells, several TASC types produce EVs with potential pro-angiogenic functions. These include macrophages[180], platelets[181] and ECs[182,183].

The EV surface displays several tetraspanins[184]. The expression of certain tetraspanins on cancer cell-derived EVs was shown to enhance EV internalization by ECs, which in turn stimulated the transcription of angiogenesis-related genes and promoted EC proliferation and migration[185]. The fusion of tumour EVs with the plasma membrane of ECs may also be conducive to the horizontal transfer of mitogenic RNAs or proteins, which may influence the biology of the recipient ECs and stimulate tumour angiogenesis[182,186–188]. For example, EVs secreted by cancer cells were reported to transfer mutant epidermal growth factor receptor (EGFR) to tumour ECs, inducing mitogenic MAPK and AKT signalling activation[187]. However, although an increasing number of studies document the transfer of functional macromolecules from tumour-derived EVs to TABVs or vascular beds in pre-metastatic sites, the mechanistic underpinning and clinical implications of these phenomena remain poorly understood[175,184].

Distinct organ microenvironments

Heterogeneous vascular morphology and blood vessel patterns are observed across distinct tumour types and in different microenvironments of individual tumours[6–10]. In mouse transplant cancer models, the site of tumour inoculation (for example, orthotopic tumour transplant versus ectopic tumour transplant) can markedly influence angiogenesis along with tumour histopathology, gene expression and several parameters of cancer progression[6,7]. Also, the structure and density of metastasis-associated blood vessels vary considerably according to the location of the metastatic site after dissemination from a primary human tumour[9,10]. Of note, the genetic background of the mouse influences innate and adaptive immune cell biology, which in turn may reverberate on tumour angiogenesis. As a consequence of these many variables, experimental tumours may substantially differ from human tumours in terms of vascular density, functionality and phenotype, as well as responsiveness to anti-angiogenic therapy.

Mouse cancer models typically display fast growth kinetics and a 'pushy' (expansive) growth pattern, which may exacerbate the requirement for angiogenesis and the involvement of pro-angiogenic TASCs. Although sprouting angiogenesis undeniably contributes to human tumour vascularization[5,189], non-angiogenic modes have also been observed, especially in metastatic human cancers. Vascular co-option — the infiltrative growth of cancer cells along pre-existing host vessels — has been documented in tumours that develop in highly vascularized organs, such as the lung, liver, brain and lymph nodes[10,190–192]. Remarkably, the analysis of 164

Glycolysis
The metabolic process that occurs in the cell cytoplasm and that uses glucose to generate pyruvate and the high-energy molecules ATP and NADH; in the presence of oxygen, pyruvate may enter the mitochondrion to sustain oxidative metabolism.

Reactive oxygen species
(ROS). Chemically reactive molecular species that contain oxygen; by reacting with biological molecules, ROS can alter their structure and function.

Oxidative metabolism
The metabolic processes that converge on oxidative phosphorylation to produce ATP.

Extracellular vesicles
(EVs). The heterogeneous assortment of secreted vesicles produced by virtually any cell type through diverse biogenesis processes.

Tetraspanins
A family of transmembrane proteins that organize microdomains enriched in membrane-bound signalling proteins.

Orthotopic tumour transplant
An experimental tumour that results from the injection of cancer cells into the tissue or organ from which the cancer cells were originally derived.

Ectopic tumour transplant
An experimental tumour that results from the injection of cancer cells into an anatomical site that is different from the one from which the cancer cells were originally derived. Generally, ectopic tumours are inoculated in the subcutaneous space.

human lung metastasis specimens derived from primary cancers of the breast, colon or kidney found evidence for vessel co-option in 80% of the cases[190]. Although myeloid cells have been implicated in metastasis-associated angiogenesis in mouse models[193,194], the contribution of TASCs to vessel co-option in both primary and metastatic tumours has been poorly studied, and further work in this area is needed.

Implications for anticancer therapies

As discussed above, the mechanisms involved in the induction and maintenance of the tumour vasculature are diverse and robust, and involve the action of multiple biochemical mediators and cell types with ostensibly redundant pro-angiogenic functions. Accordingly, the pharmacological inhibition of VEGFA signalling inhibits tumour angiogenesis in some but not all mouse cancer models, and it typically does not block tumour progression in mice and humans[189]. By striking contrast, the genetic inactivation of *Vegfa* impairs developmental angiogenesis and is embryonic lethal[1]. These observations support the notion that the regulation of tumour angiogenesis is a multidimensional process that is less dependent on VEGFA signalling than developmental angiogenesis. Furthermore, tumours can rapidly adapt to the neutralization of individual pro-angiogenic growth factors, including VEGFA[189,195], through routes that involve metabolic adaptation and reprogramming[196–201], the enforcement of compensatory pro-angiogenic signals[108,202,203] or the acquisition of angiogenesis-independent modes of tumour growth[190,191,204,205] (FIG. 6a–c).

Myeloid cells, macrophages and neutrophils in particular sustain both VEGFA-dependent and independent angiogenesis in tumours. This is particularly relevant considering the notion that anti-angiogenic drugs provoke the surge, in tumours, of hypoxia-inducible chemoattractants for myeloid cells, which can rescue angiogenesis through VEGFA-independent pathways[48,55,59,195,206,207]. Strategies that selectively impair pro-angiogenic macrophages, for example, perivascular TIE2$^+$ TAMs, may help to disrupt compensatory pro-angiogenic cues[36] while sparing TAM subpopulations that have potential roles in antigen presentation or the production of angiostatic factors in response to T_H1 cytokines[18,83,85]. Blocking ANGPT2 or TIE2 signalling decreases TIE2$^+$ TAMs[208], impedes their association with angiogenic blood vessels[45] and increases the proportion of TAMs that exhibit an M1-like (angiostatic) phenotype[209,210]. However, different myeloid-cell types may contribute to limiting tumour responsiveness to anti-angiogenic therapies[62,67,211]. For example, macrophage or neutrophil elimination in a mouse PNET model did not impede the emergence of resistance to sorafenib, an anti-angiogenic multi-kinase inhibitor, but depleting both cell types improved the therapeutic benefits[62]. These preclinical findings are consistent with initial reports showing that anti-macrophage drugs, for example, CSF1R inhibitors, have limited therapeutic activity in patients with cancer[212,213]. Therefore, broadly targeting myeloid cells may be required for effective ablation of their pro-angiogenic capacity in the context of cancer treatment. A promising targeted approach might be inhibiting the γ-isoform of PI3K (PI3Kγ), which is preferentially expressed in myeloid cells and sustains their immunosuppressive and pro-angiogenic functions[62,214]. Furthermore, TAMs can also be engineered to express biologics that inhibit tumour angiogenesis and reprogramme the TME[215].

Pericytes have emerged as important regulators of tumour angiogenesis and revascularization post-therapy[70]. Interestingly, regression of TABVs in response to anti-VEGFA therapy leaves empty sleeves of basement membrane and pericytes[45,216], which provide a guiding scaffold for rapid tumour revascularization after therapy withdrawal[216]. Co-targeting pericytes and ECs with inhibitors that potently block both PDGFRs and VEGFRs, such as sunitinib, delays tumour revascularization post-therapy compared with selective VEGFR inhibitors[113,114,195] and has clinical efficacy in PNETs[217]. However, there is also evidence for pericytes limiting cancer-cell intravasation and metastasis[104]. The latter observation may explain the propensity of sunitinib to increase metastasis from primary tumours in some cancer models[218]. Because metastatic dissemination from pericyte-depleted tumours may rely, at least in part, on the acute release of ANGPT2 from sensitized ECs, co-targeting ANGPT2 may serve to blunt the pro-metastatic potential of pericyte elimination and to improve the therapeutic benefits[112].

There is increasing evidence that acute vascular pruning by potent angiogenesis inhibitors may exacerbate or even instigate the pro-tumoural capabilities of TASCs[189,195,207]. Notably, interception of VEGFA signalling enhances M2-like TAM polarization[108,206] and pro-angiogenic CAF programming[120]. Moreover, T_H17 cells were shown to produce IL-17 in response to anti-VEGFA therapy; in turn, IL-17 induced tumours to release CSF3, which promoted VEGFA-independent tumour revascularization and regrowth through neutrophil recruitment[219]. Conversely, defined regimens of anti-angiogenic drugs may normalize, rather than regress, TABVs in a process involving the selective pruning of immature capillaries and the concomitant stabilization of perfused vessels[220] (FIG. 6d). Both suboptimal VEGFA neutralization and ANGPT2 inhibition have been reported to normalize TABVs, which may improve chemotherapy delivery, enhance radiosensitivity and facilitate T cell extravasation in tumours[70,209,210,220]. The design of anti-angiogenic treatments needs to incorporate these complexities in order to maximize the therapeutic benefits in cancer patients. This remains a challenging clinical task and open area of preclinical research.

Concluding remarks

Most of the studies discussed in this Review employed mouse cancer models as a platform for mechanistic investigations of molecular or cellular players involved in tumour angiogenesis. The many idiosyncratic details inherent to each tumour model and its underlying biology determine the experimental results and may limit the applicability and relevance of the selected model to human pathology. Interrogating the vascular-modulatory

T_H17 cells
T cells that have roles in protecting organ surfaces, in particular the gut mucosa, from pathogens. They produce interleukin-17 and stimulate B cell-mediated humoral immunity.

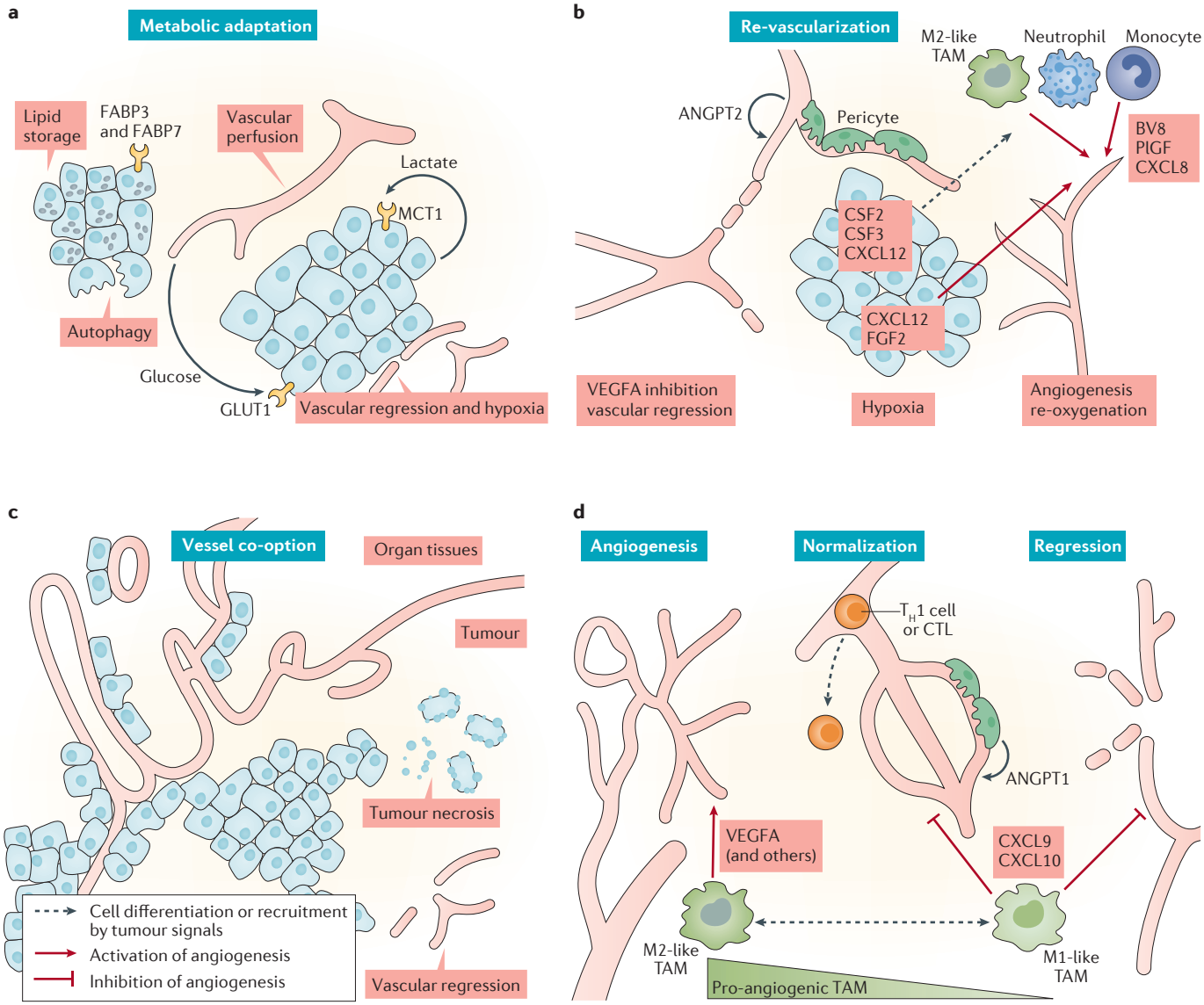

Figure 6 | **Mechanisms of tumour escape from angiogenesis inhibition.** Tumours can adapt to the acute neutralization of key pro-angiogenic growth factors, including vascular endothelial growth factor A (VEGFA). **a** | By acutely disrupting tumour angiogenesis and perfusion, anti-VEGFA therapy can activate metabolic or stress responses in the cancer cells, which enable their survival under hostile conditions of oxygen and nutrient deprivation. Such mechanisms include increased autophagy[196] or the establishment of 'metabolic symbiosis' — the process whereby hypoxic cancer cells in avascular tumour areas import glucose and export lactate, while normoxic cells in proximity to the surviving blood vessels import and catabolize lactate[198–200]. Acute hypoxia may also precondition cancer cells for re-growth after withdrawal of anti-angiogenic therapy by inducing fatty acid uptake and storage through the transporters fatty acid-binding protein 3 (FABP3) and FABP7 (REF. 201). Accordingly, tumour re-growth after anti-angiogenic kinase inhibitors may rely on *de novo* lipogenesis[197]. **b** | Alternative mechanisms of tumour adaptation to VEGFA deprivation include the induction of compensatory pro-angiogenic growth factors, namely fibroblast growth factor 2 (FGF2), angiopoietin 2 (ANGPT2), placental growth factor (PlGF) and BV8, which can rescue angiogenesis in VEGFA-depleted tumours. Furthermore, various anticancer drug regimens provoke the surge, in tumours, of hypoxia-inducible chemoattractants for neutrophils and macrophages, including colony-stimulating factor 2 (CSF2), CSF3 and CXC-chemokine ligand 12 (CXCL12), which recruit angiogenesis-promoting myeloid cells. **c** | Cancer cells may circumvent dependence on angiogenesis by acquiring the ability to hijack the pre-existing vasculature through an infiltrative growth mode called vascular co-option. Cancer-cell growth along existing blood vessels has been implicated in tumour resistance to anti-angiogenic therapy in both preclinical cancer models and patients with colon cancer liver metastases. **d** | Anti-angiogenic drugs may paradoxically improve blood flow by normalizing the tumour-associated blood vessels (TABVs). Vascular normalization can be achieved by attenuating pro-angiogenic signalling in tumours (for example, by chronically reducing VEGFA bioavailability or blocking ANGPT2). Vascular normalisation may reshape the immune cell repertoire of tumours and facilitate antitumour immunity, for example, by improving T cell extravasation or by promoting the conversion of pro-angiogenic (M2-like) into angiostatic (M1-like) tumour-associated macrophages (TAMs). CTL, cytotoxic T lymphocyte; GLUT1, glucose transporter 1; MCT1, monocarboxylate transporter 1; T_H1 cell, T helper 1 cell.

Immune checkpoint
A ligand–receptor pair that either activates or inhibits a stimulatory signal for lymphocytes, resulting in the activation or suppression of an immune response.

functions of TASCs in patients with cancer is, therefore, needed to understand the extrinsic regulation of human tumour vascularization, in particular in the context of metastatic disease and/or under therapeutic pressure. The clinical testing of an expanding arsenal of drugs targeting specific cellular components of the TME, such as TAMs[85,212,213], pericytes[113,114,195,217] and T cells[210,221–223], may provide clues about the roles played by these cells in the regulation of human tumour angiogenesis. For example, recent clinical studies have revealed unexpected roles for adaptive immune cells in the regulation of human TABVs[221–223]. Tumour vascular destruction and important humoral (IgG-mediated) reactions against ANGPT2 and VEGFA were observed in long-term responding patients who had received a cancer vaccine[222]. Further studies illustrated that tumour regressions after immune checkpoint blockade with antibodies targeting cytotoxic T lymphocyte-associated antigen 4 (CTLA4) or programmed cell-death protein 1 (PD1) were associated with heightened titres of anti-ANGPT2 serum IgG, whereas therapy refractoriness or resistance were associated with higher pre- or on-treatment ANGPT2 serum levels[223]. These provocative findings suggest that tumour responses to immunotherapy may involve, or even require, immune-mediated anti-angiogenic mechanisms. Therefore, if combined with the analysis of the molecular, morphological, and functional properties of TABVs, either on tumour biopsies or through non-invasive imaging tools[27,33,63,220], the clinical deployment of TME-targeted drugs may help to shed new light on the vascular-modulatory functions of TASCs in human cancer.

1. Potente, M., Gerhardt, H. & Carmeliet, P. Basic and therapeutic aspects of angiogenesis. *Cell* **146**, 873–887 (2011).
2. LaGory, E. L. & Giaccia, A. J. The ever-expanding role of HIF in tumour and stromal biology. *Nat. Cell Biol.* **18**, 356–365 (2016).
3. Bossi, P. *et al.* Angiogenesis in colorectal tumors: microvessel quantitation in adenomas and carcinomas with clinicopathological correlations. *Cancer Res.* **55**, 5049–5053 (1995).
4. Bluff, J. E. *et al.* Angiogenesis is associated with the onset of hyperplasia in human ductal breast disease. *Br. J. Cancer* **101**, 666–672 (2009).
5. Bergers, G. & Benjamin, L. E. Tumorigenesis and the angiogenic switch. *Nat. Rev. Cancer* **3**, 401–410 (2003).
6. Fukumura, D., Yuan, F., Monsky, W. L., Chen, Y. & Jain, R. K. Effect of host microenvironment on the microcirculation of human colon adenocarcinoma. *Am. J. Pathol.* **151**, 679–688 (1997).
7. Monsky, W. L. *et al.* Role of host microenvironment in angiogenesis and microvascular functions in human breast cancer xenografts: mammary fat pad versus cranial tumors. *Clin. Cancer Res.* **8**, 1008–1013 (2002).
8. Blouw, B. *et al.* The hypoxic response of tumors is dependent on their microenvironment. *Cancer Cell* **4**, 133–146 (2003).
9. Morrissey, C. *et al.* Differential expression of angiogenesis associated genes in prostate cancer bone, liver and lymph node metastases. *Clin. Exp. Metastasis* **25**, 377–388 (2008).
10. Jubb, A. M. *et al.* Vascular phenotypes in primary non-small cell lung carcinomas and matched brain metastases. *Br. J. Cancer* **104**, 1877–1881 (2011).
11. Morikawa, S. *et al.* Abnormalities in pericytes on blood vessels and endothelial sprouts in tumors. *Am. J. Pathol.* **160**, 985–1000 (2002).
12. Inoue, M., Hager, J. H., Ferrara, N., Gerber, H. P. & Hanahan, D. VEGF-A has a critical, nonredundant role in angiogenic switching and pancreatic beta cell carcinogenesis. *Cancer Cell* **1**, 193–202 (2002).
13. Bergers, G. *et al.* Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. *Nat. Cell Biol.* **2**, 737–744 (2000).
This study implicated myeloid-cell derived MMP9 as a key regulator of the angiogenic switch in a GEMM of cancer.
14. Egeblad, M., Nakasone, E. S. & Werb, Z. Tumors as organs: complex tissues that interface with the entire organism. *Dev. Cell* **18**, 884–901 (2010).
15. De Palma, M. & Hanahan, D. The biology of personalized cancer medicine: facing individual complexities underlying hallmark capabilities. *Mol. Oncol.* **6**, 111–127 (2012).
16. Cortez, E., Roswall, P. & Pietras, K. Functional subsets of mesenchymal cell types in the tumor microenvironment. *Semin. Cancer Biol.* **25**, 3–9 (2014).
17. Kalluri, R. The biology and function of fibroblasts in cancer. *Nat. Rev. Cancer* **16**, 582–598 (2016).
18. Qian, B. Z. & Pollard, J. W. Macrophage diversity enhances tumor progression and metastasis. *Cell* **141**, 39–51 (2010).
19. Fang, S. & Salven, P. Stem cells in tumor angiogenesis. *J. Mol. Cell. Cardiol.* **50**, 290–295 (2011).
20. Lahmar, Q. *et al.* Tissue-resident versus monocyte-derived macrophages in the tumor microenvironment. *Biochim. Biophys. Acta* **1865**, 23–34 (2016).
21. Leek, R. D. *et al.* Association of macrophage infiltration with angiogenesis and prognosis in invasive breast carcinoma. *Cancer Res.* **56**, 4625–4629 (1996).
This report identified a positive correlation between macrophage infiltration and a high vascular grade in human breast cancers.
22. Koh, Y. W., Park, C. S., Yoon, D. H., Suh, C. & Huh, J. CD163 expression was associated with angiogenesis and shortened survival in patients with uniformly treated classical Hodgkin lymphoma. *PLoS ONE* **9**, e870666 (2014).
23. Clear, A. J. *et al.* Increased angiogenic sprouting in poor prognosis FL is associated with elevated numbers of CD163+ macrophages within the immediate sprouting microenvironment. *Blood* **115**, 5053–5056 (2010).
24. Lin, E. Y. *et al.* Macrophages regulate the angiogenic switch in a mouse model of breast cancer. *Cancer Res.* **66**, 11238–11246 (2006).
25. Zeisberger, S. M. *et al.* Clodronate-liposome-mediated depletion of tumour-associated macrophages: a new and highly effective antiangiogenic therapy approach. *Br. J. Cancer* **95**, 272–281 (2006).
26. Priceman, S. J. *et al.* Targeting distinct tumor-infiltrating myeloid cells by inhibiting CSF-1 receptor: combating tumor evasion of antiangiogenic therapy. *Blood* **115**, 1461–1471 (2010).
27. Lohela, M. *et al.* Intravital imaging reveals distinct responses of depleting dynamic tumor-associated macrophage and dendritic cell subpopulations. *Proc. Natl Acad. Sci. USA* **111**, E5086–E5095 (2014).
28. Squadrito, M. L. & De Palma, M. Macrophage regulation of tumor angiogenesis: implications for cancer therapy. *Mol. Aspects Med.* **32**, 123–145 (2011).
29. Baer, C., Squadrito, M. L., Iruela-Arispe, M. L. & De Palma, M. Reciprocal interactions between endothelial cells and macrophages in angiogenic vascular niches. *Exp. Cell Res.* **319**, 1626–1634 (2013).
30. Lewis, J. S., Landers, R. J., Underwood, J. C., Harris, A. L. & Lewis, C. E. Expression of vascular endothelial growth factor by macrophages is up-regulated in poorly vascularized areas of breast carcinomas. *J. Pathol.* **192**, 150–158 (2000).
31. Stockmann, C. *et al.* Deletion of vascular endothelial growth factor in myeloid cells accelerates tumorigenesis. *Nature* **456**, 814–818 (2008).
32. Hughes, R. *et al.* Perivascular M2 macrophages stimulate tumor relapse after chemotherapy. *Cancer Res.* **75**, 3479–3491 (2015).
33. Harney, A. S. *et al.* Real-time imaging reveals local, transient vascular permeability, and tumor cell intravasation stimulated by $TIE2^{hi}$ macrophage-derived VEGFA. *Cancer Discov.* **5**, 932–943 (2015).
34. Murdoch, C., Muthana, M., Coffelt, S. B. & Lewis, C. E. The role of myeloid cells in the promotion of tumour angiogenesis. *Nat. Rev. Cancer* **8**, 618–631 (2008).
35. Yeo, E. J. *et al.* Myeloid WNT7b mediates the angiogenic switch and metastasis in breast cancer. *Cancer Res.* **74**, 2962–2973 (2014).
36. Lewis, C. E., Harney, A. S. & Pollard, J. W. The multifaceted role of perivascular macrophages in tumors. *Cancer Cell* **30**, 18–25 (2016).
37. Olson, O. C. & Joyce, J. A. Cysteine cathepsin proteases: regulators of cancer progression and therapeutic response. *Nat. Rev. Cancer* **15**, 712–729 (2015).
38. Kessenbrock, K., Plaks, V. & Werb, Z. Matrix metalloproteinases: regulators of the tumor microenvironment. *Cell* **141**, 52–67 (2010).
39. Huang, S. *et al.* Contributions of stromal metalloproteinase-9 to angiogenesis and growth of human ovarian carcinoma in mice. *J. Natl Cancer Inst.* **94**, 1134–1142 (2002).
40. Giraudo, E., Inoue, M. & Hanahan, D. An amino-bisphosphonate targets MMP-9-expressing macrophages and angiogenesis to impair cervical carcinogenesis. *J. Clin. Invest.* **114**, 623–633 (2004).
41. Joyce, J. A. *et al.* Cathepsin cysteine proteases are effectors of invasive growth and angiogenesis during multistage tumorigenesis. *Cancer Cell* **5**, 443–453 (2004).
42. De Palma, M., Venneri, M. A., Roca, C. & Naldini, L. Targeting exogenous genes to tumor angiogenesis by transplantation of genetically modified hematopoietic stem cells. *Nat. Med.* **9**, 789–795 (2003).
43. De Palma, M. *et al.* Tie2 identifies a hematopoietic lineage of proangiogenic monocytes required for tumor vessel formation and a mesenchymal population of pericyte progenitors. *Cancer Cell* **8**, 211–226 (2005).
References 42 and 43 illustrated that the conditional elimination of bone marrow-derived, perivascular monocytes was sufficient to impair angiogenesis and delay tumour growth in transplant cancer models.
44. Matsubara, T. *et al.* TIE2-expressing monocytes as a diagnostic marker for hepatocellular carcinoma correlates with angiogenesis. *Hepatology* **57**, 1416–1425 (2013).
45. Mazzieri, R. *et al.* Targeting the ANG2/TIE2 axis inhibits tumor growth and metastasis by impairing angiogenesis and disabling rebounds of proangiogenic myeloid cells. *Cancer Cell* **19**, 512–526 (2011).
46. Kioi, M. *et al.* Inhibition of vasculogenesis, but not angiogenesis, prevents the recurrence of glioblastoma after irradiation in mice. *J. Clin. Invest.* **120**, 694–705 (2010).
47. Kozin, S. V. *et al.* Recruitment of myeloid but not endothelial precursor cells facilitates tumor regrowth after local irradiation. *Cancer Res.* **70**, 5679–5685 (2010).
48. Welford, A. F. *et al.* TIE2-expressing macrophages limit the therapeutic efficacy of the vascular-disrupting agent combretastatin A4 phosphate in mice. *J. Clin. Invest.* **121**, 1969–1973 (2011).
49. Chen, L. *et al.* Tie2 expression on macrophages is required for blood vessel reconstruction and tumor relapse after chemotherapy. *Cancer Res.* **76**, 6828–6838 (2016).

50. Dou, G. R. *et al.* Myeloid-specific blockade of Notch signaling attenuates choroidal neovascularization through compromised macrophage infiltration and polarization in mice. *Sci. Rep.* **6**, 28617 (2016).
51. Tamagnone, L. Emerging role of semaphorins as major regulatory signals and potential therapeutic targets in cancer. *Cancer Cell* **22**, 145–152 (2012).
52. Barnett, F. H. *et al.* Macrophages form functional vascular mimicry channels *in vivo*. *Sci. Rep.* **6**, 36659 (2016).
53. Moldovan, N. I., Goldschmidt-Clermont, P. J., Parker-Thornburg, J., Shapiro, S. D. & Kolattukudy, P. E. Contribution of monocytes/macrophages to compensatory neovascularization: the drilling of metalloelastase-positive tunnels in ischemic myocardium. *Circ. Res.* **87**, 378–384 (2000).
54. Coffelt, S. B., Wellenstein, M. D. & de Visser, K. E. Neutrophils in cancer: neutral no more. *Nat. Rev. Cancer* **16**, 431–446 (2016).
55. Liang, W. & Ferrara, N. The complex role of neutrophils in tumor angiogenesis and metastasis. *Cancer Immunol. Res.* **4**, 83–91 (2016).
56. Kujawski, M. *et al.* Stat3 mediates myeloid cell-dependent tumor angiogenesis in mice. *J. Clin. Invest.* **118**, 3367–3377 (2008).
57. Gaudry, M. *et al.* Intracellular pool of vascular endothelial growth factor in human neutrophils. *Blood* **90**, 4153–4161 (1997).
58. Qu, X., Zhuang, G., Yu, L., Meng, G. & Ferrara, N. Induction of Bv8 expression by granulocyte colony-stimulating factor in CD11b + $Gr1^{+}$ cells: key role of Stat3 signaling. *J. Biol. Chem.* **287**, 19574–19584 (2012).
59. Shojaei, F. *et al.* Bv8 regulates myeloid-cell-dependent tumour angiogenesis. *Nature* **450**, 825–831 (2007).
60. Jablonska, J., Leschner, S., Westphal, K., Lienenklaus, S. & Weiss, S. Neutrophils responsive to endogenous IFN-beta regulate tumor angiogenesis and growth in a mouse tumor model. *J. Clin. Invest.* **120**, 1151–1164 (2010).
61. Nozawa, H., Chiu, C. & Hanahan, D. Infiltrating neutrophils mediate the initial angiogenic switch in a mouse model of multistage carcinogenesis. *Proc. Natl Acad. Sci. USA* **103**, 12493–12498 (2006).
This study highlighted the contribution of neutrophils to angiogenesis in the early stages of tumour progression in a mouse model of PNET.
62. Rivera, L. B. *et al.* Intratumoral myeloid cells regulate responsiveness and resistance to antiangiogenic therapy. *Cell Rep.* **11**, 577–591 (2015).
63. Nakasone, E. S. *et al.* Imaging tumor-stroma interactions during chemotherapy reveals contributions of the microenvironment to resistance. *Cancer Cell* **21**, 488–503 (2012).
64. Deryugina, E. I. *et al.* Tissue-infiltrating neutrophils constitute the major *in vivo* source of angiogenesis-inducing MMP-9 in the tumor microenvironment. *Neoplasia* **16**, 771–788 (2014).
65. Ardi, V. C. *et al.* Neutrophil MMP-9 proenzyme, unencumbered by TIMP-1, undergoes efficient activation *in vivo* and catalytically induces angiogenesis via a basic fibroblast growth factor (FGF-2)/FGFR-2 pathway. *J. Biol. Chem.* **284**, 25854–25866 (2009).
66. Kumar, V., Patel, S., Tcyganov, E. & Gabrilovich, D. I. The nature of myeloid-derived suppressor cells in the tumor microenvironment. *Trends Immunol.* **37**, 208–220 (2016).
67. Coffelt, S. B. *et al.* Elusive identities and overlapping phenotypes of proangiogenic myeloid cells in tumors. *Am. J. Pathol.* **176**, 1564–1576 (2010).
68. Motz, G. T. & Coukos, G. The parallel lives of angiogenesis and immunosuppression: cancer and other tales. *Nat. Rev. Immunol.* **11**, 702–711 (2011).
69. Dirkx, A. E. *et al.* Tumor angiogenesis modulates leukocyte-vessel wall interactions *in vivo* by reducing endothelial adhesion molecule expression. *Cancer Res.* **63**, 2322–2329 (2003).
70. Johansson, A., Hamzah, J. & Ganss, R. More than a scaffold: stromal modulation of tumor immunity. *Biochim. Biophys. Acta* **1865**, 3–13 (2016).
71. Kessler, D. A., Langer, R. S., Pless, N. A. & Folkman, J. Mast cells and tumor angiogenesis. *Int. J. Cancer* **18**, 703–709 (1976).
72. Coussens, L. M. *et al.* Inflammatory mast cells up-regulate angiogenesis during squamous epithelial carcinogenesis. *Genes Dev.* **13**, 1382–1397 (1999).
73. Gounaris, E. *et al.* Mast cells are an essential hematopoietic component for polyp development. *Proc. Natl Acad. Sci. USA* **104**, 19977–19982 (2007).
74. Soucek, L. *et al.* Mast cells are required for angiogenesis and macroscopic expansion of Myc-induced pancreatic islet tumors. *Nat. Med.* **13**, 1211–1218 (2007).
References 72–74 revealed unexpected roles for mast cells as pro-angiogenic cells in different GEMMs of cancer.
75. Movahedi, K. *et al.* Different tumor microenvironments contain functionally distinct subsets of macrophages derived from Ly6C(high) monocytes. *Cancer Res.* **70**, 5728–5739 (2010).
76. Cormier, S. A. *et al.* Pivotal advance: eosinophil infiltration of solid tumors is an early and persistent inflammatory host response. *J. Leukoc. Biol.* **79**, 1131–1139 (2006).
77. Davis, B. P. & Rothenberg, M. E. Eosinophils and cancer. *Cancer Immunol. Res.* **2**, 1–8 (2014).
78. Yang, C. *et al.* B cells promote tumor progression via STAT3 regulated-angiogenesis. *PLoS ONE* **8**, e64159 (2013).
79. Andreu, P. *et al.* FcRgamma activation regulates inflammation-associated squamous carcinogenesis. *Cancer Cell* **17**, 121–134 (2010).
This study illustrated a role for B cells and secreted IgG in promoting pro-angiogenic myeloid cell programming in a mouse model of skin cancer.
80. Tian, L. *et al.* Mutual regulation of tumour vessel normalization and immunostimulatory reprogramming. *Nature* **544**, 250–254 (2017).
81. Fathallah-Shaykh, H. M., Zhao, L. J., Kafrouni, A. I., Smith, G. M. & Forman, J. Gene transfer of IFN-gamma into established brain tumors represses growth by antiangiogenesis. *J. Immunol.* **164**, 217–222 (2000).
82. Kammertoens, T. *et al.* Tumour ischaemia by interferon-gamma resembles physiological blood vessel regression. *Nature* **545**, 98–102 (2017).
References 80–82 showed that T cells regulate the tumour-associated vasculature by limiting angiogenesis.
83. Biswas, S. K. & Mantovani, A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. *Nat. Immunol.* **11**, 889–896 (2010).
84. DeNardo, D. G. *et al.* $CD4^{+}$ T cells regulate pulmonary metastasis of mammary carcinomas by enhancing protumor properties of macrophages. *Cancer Cell* **16**, 91–102 (2009).
85. Baer, C. *et al.* Suppression of microRNA activity amplifies IFN-gamma-induced macrophage activation and promotes anti-tumour immunity. *Nat. Cell Biol.* **18**, 790–802 (2016).
86. Facciabene, A. *et al.* Tumour hypoxia promotes tolerance and angiogenesis via CCL28 and T_{reg} cells. *Nature* **475**, 226–230 (2011).
87. Bruno, A., Ferlazzo, G., Albini, A. & Noonan, D. M. A think tank of TINK/TANKs: tumor-infiltrating/tumor-associated natural killer cells in tumor progression and angiogenesis. *J. Natl Cancer Inst.* **106**, dju200 (2014).
88. Gotthardt, D. *et al.* STAT5 Is a key regulator in NK cells and acts as a molecular switch from tumor surveillance to tumor promotion. *Cancer Discov.* **6**, 414–429 (2016).
89. Sharma, D., Brummel-Ziedins, K. E., Bouchard, B. A. & Holmes, C. E. Platelets in tumor progression: a host factor that offers multiple potential targets in the treatment of cancer. *J. Cell. Physiol.* **229**, 1005–1015 (2014).
90. Sabrkhany, S., Griffioen, A. W. & Oude Egbrink, M. G. The role of blood platelets in tumor angiogenesis. *Biochim. Biophys. Acta* **1815**, 189–196 (2011).
91. Chatterjee, M. *et al.* Distinct platelet packaging, release, and surface expression of proangiogenic and antiangiogenic factors on different platelet stimuli. *Blood* **117**, 3907–3911 (2011).
92. Jonnalagadda, D., Izu, L. T. & Whiteheart, S. W. Platelet secretion is kinetically heterogeneous in an agonist-responsive manner. *Blood* **120**, 5209–5216 (2012).
93. Yan, M., Lesyk, G., Radziwon-Balicka, A. & Jurasz, P. Pharmacological regulation of platelet factors that influence tumor angiogenesis. *Semin. Oncol.* **41**, 370–377 (2014).
94. Ho-Tin-Noe, B., Goerge, T., Cifuni, S. M., Duerschmied, D. & Wagner, D. D. Platelet granule secretion continuously prevents intratumor hemorrhage. *Cancer Res.* **68**, 6851–6858 (2008).
95. Klement, G. L. *et al.* Platelets actively sequester angiogenesis regulators. *Blood* **113**, 2835–2842 (2009).
96. Peterson, J. E. *et al.* VEGF, PF4 and PDGF are elevated in platelets of colorectal cancer patients. *Angiogenesis* **15**, 265–273 (2012).
97. Salven, P., Orpana, A. & Joensuu, H. Leukocytes and platelets of patients with cancer contain high levels of vascular endothelial growth factor. *Clin. Cancer Res.* **5**, 487–491 (1999).
References 95–97 showed that platelets could sequester, and become a reservoir of, pro-angiogenic mediators in patients with cancer.
98. Kuznetsov, H. S. *et al.* Identification of luminal breast cancers that establish a tumor-supportive macroenvironment defined by proangiogenic platelets and bone marrow-derived cells. *Cancer Discov.* **2**, 1150–1165 (2012).
99. Feng, W. *et al.* A novel role for platelet secretion in angiogenesis: mediating bone marrow-derived cell mobilization and homing. *Blood* **117**, 3893–3902 (2011).
100. Armulik, A., Genove, G. & Betsholtz, C. Pericytes: developmental, physiological, and pathological perspectives, problems, and promises. *Dev. Cell* **21**, 193–215 (2011).
101. Eberhard, A. *et al.* Heterogeneity of angiogenesis and blood vessel maturation in human tumors: implications for antiangiogenic tumor therapies. *Cancer Res.* **60**, 1388–1393 (2000).
102. Saharinen, P., Eklund, L. & Alitalo, K. Therapeutic targeting of the angiopoietin-TIE pathway. *Nat. Rev. Drug Discov.* http://dx.doi.org/10.1038/nrd.2016.278 (2017).
103. Thurston, G. *et al.* Leakage-resistant blood vessels in mice transgenically overexpressing angiopoietin-1. *Science* **286**, 2511–2514 (1999).
104. Xian, X. *et al.* Pericytes limit tumor cell metastasis. *J. Clin. Invest.* **116**, 642–651 (2006).
105. You, W. K., Yotsumoto, F., Sakimura, K., Adams, R. H. & Stallcup, W. B. NG2 proteoglycan promotes tumor vascularization via integrin-dependent effects on pericyte function. *Angiogenesis* **17**, 61–76 (2014).
106. Nasarre, P. *et al.* Host-derived angiopoietin-2 affects early stages of tumor development and vessel maturation but is dispensable for later stages of tumor growth. *Cancer Res.* **69**, 1324–1333 (2009).
107. Holopainen, T. *et al.* Effects of angiopoietin-2-blocking antibody on endothelial cell-cell junctions and lung metastasis. *J. Natl Cancer Inst.* **104**, 461–475 (2012).
108. Rigamonti, N. *et al.* Role of angiopoietin-2 in adaptive tumor resistance to VEGF signaling blockade. *Cell Rep.* **8**, 696–706 (2014).
109. Park, J. S. *et al.* Normalization of tumor vessels by Tie2 activation and Ang2 inhibition enhances drug delivery and produces a favorable tumor microenvironment. *Cancer Cell* **30**, 953–967 (2016).
110. Birbrair, A. *et al.* Type-2 pericytes participate in normal and tumoral angiogenesis. *Am. J. Physiol. Cell Physiol.* **307**, C25–C38 (2014).
111. Sennino, B. *et al.* Sequential loss of tumor vessel pericytes and endothelial cells after inhibition of platelet-derived growth factor B by selective aptamer AX102. *Cancer Res.* **67**, 7358–7367 (2007).
112. Keskin, D. *et al.* Targeting vascular pericytes in hypoxic tumors increases lung metastasis via angiopoietin-2. *Cell Rep.* **10**, 1066–1081 (2015).
113. Erber, R. *et al.* Combined inhibition of VEGF and PDGF signaling enforces tumor vessel regression by interfering with pericyte-mediated endothelial cell survival mechanisms. *FASEB J.* **18**, 338–340 (2004).
114. Bergers, G., Song, S., Meyer-Morse, N., Bergsland, E. & Hanahan, D. Benefits of targeting both pericytes and endothelial cells in the tumor vasculature with kinase inhibitors. *J. Clin. Invest.* **111**, 1287–1295 (2003).
References 113 and 114 highlighted the anti-tumoural benefits of co-targeting pericytes and ECs in mouse cancer models, paving the way to the clinical testing of non-selective VEGFR and PDGFR inhibitors in PNETs and other cancer types.
115. Egeblad, M., Rasch, M. G. & Weaver, V. M. Dynamic interplay between the collagen scaffold and tumor evolution. *Curr. Opin. Cell Biol.* **22**, 697–706 (2010).
116. Tuxhorn, J. A., McAlhany, S. J., Dang, T. D., Ayala, G. E. & Rowley, D. R. Stromal cells promote angiogenesis and growth of human prostate tumors in a differential reactive stroma (DRS) xenograft model. *Cancer Res.* **62**, 3298–3307 (2002).
117. Orimo, A. *et al.* Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. *Cell* **121**, 335–348 (2005).

118. Gerber, H. P., Kowalski, J., Sherman, D., Eberhard, D. A. & Ferrara, N. Complete inhibition of rhabdomyosarcoma xenograft growth and neovascularization requires blockade of both tumor and host vascular endothelial growth factor. *Cancer Res.* **60**, 6253–6258 (2000).
119. Fukumura, D. *et al.* Tumor induction of VEGF promoter activity in stromal cells. *Cell* **94**, 715–725 (1998).
References 117–119 implicated CAFs as an important source of VEGFA and myeloid-cell chemoattractants in mouse tumour models.
120. Crawford, Y. *et al.* PDGF-C mediates the angiogenic and tumorigenic properties of fibroblasts associated with tumors refractory to anti-VEGF treatment. *Cancer Cell* **15**, 21–34 (2009).
121. Pietras, K., Pahler, J., Bergers, G. & Hanahan, D. Functions of paracrine PDGF signaling in the proangiogenic tumor stroma revealed by pharmacological targeting. *PLoS Med.* **5**, e19 (2008).
122. Anderberg, C. *et al.* Paracrine signaling by platelet-derived growth factor-CC promotes tumor growth by recruitment of cancer-associated fibroblasts. *Cancer Res.* **69**, 369–378 (2009).
123. Hou, X. *et al.* PDGF-CC blockade inhibits pathological angiogenesis by acting on multiple cellular and molecular targets. *Proc. Natl Acad. Sci. USA* **107**, 12216–12221 (2010).
124. Kaur, A. *et al.* sFRP2 in the aged microenvironment drives melanoma metastasis and therapy resistance. *Nature* **532**, 250–254 (2016).
125. Filleur, S. *et al. In vivo* mechanisms by which tumors producing thrombospondin 1 bypass its inhibitory effects. *Genes Dev.* **15**, 1373–1382 (2001).
126. Dirat, B. *et al.* Cancer-associated adipocytes exhibit an activated phenotype and contribute to breast cancer invasion. *Cancer Res.* **71**, 2455–2465 (2011).
127. Lim, S., Hosaka, K., Nakamura, M. & Cao, Y. Co-option of pre-existing vascular beds in adipose tissue controls tumor growth rates and angiogenesis. *Oncotarget* **7**, 38282–38291 (2016).
128. Cao, Y. Angiogenesis and vascular functions in modulation of obesity, adipose metabolism, and insulin sensitivity. *Cell Metab.* **18**, 478–489 (2013).
129. Renehan, A. G., Zwahlen, M. & Egger, M. Adiposity and cancer risk: new mechanistic insights from epidemiology. *Nat. Rev. Cancer* **15**, 484–498 (2015).
130. Bougaret, L. *et al.* Supernatants of adipocytes from obese versus normal weight women and breast cancer cells: *in vitro* impact on angiogenesis. *J. Cell. Physiol.* **232**, 1808–1816 (2016).
131. Arendt, L. M. *et al.* Obesity promotes breast cancer by CCL2-mediated macrophage recruitment and angiogenesis. *Cancer Res.* **73**, 6080–6093 (2013).
132. Hagiwara, A. *et al.* Milky spots as the implantation site for malignant cells in peritoneal dissemination in mice. *Cancer Res.* **53**, 687–692 (1993).
133. Wagner, M. *et al.* Inflamed tumor-associated adipose tissue is a depot for macrophages that stimulate tumor growth and angiogenesis. *Angiogenesis* **15**, 481–495 (2012).
134. Nieman, K. M. *et al.* Adipocytes promote ovarian cancer metastasis and provide energy for rapid tumor growth. *Nat. Med.* **17**, 1498–1503 (2011).
References 133 and 134 showed that cancer cells could reprogramme peri-tumoural adipocytes to sustain tumour inflammation, angiogenesis and growth.
135. Harjes, U., Bridges, E., McIntyre, A., Fielding, B. A. & Harris, A. L. Fatty acid-binding protein 4, a point of convergence for angiogenic and metabolic signaling pathways in endothelial cells. *J. Biol. Chem.* **289**, 23168–23176 (2014).
136. Schoors, S. *et al.* Fatty acid carbon is essential for dNTP synthesis in endothelial cells. *Nature* **520**, 192–197 (2015).
137. Dvorak, H. F. Tumor stroma, tumor bood vessels, and antiangiogenesis therapy. *Cancer J.* **21**, 237–243 (2015).
138. Bonnans, C., Chou, J. & Werb, Z. Remodelling the extracellular matrix in development and disease. *Nat. Rev. Mol. Cell Biol.* **15**, 786–8014 (2014).
139. Arroyo, A. G. & Iruela-Arispe, M. L. Extracellular matrix, inflammation, and the angiogenic response. *Cardiovasc. Res.* **86**, 226–235 (2010).
140. Baluk, P., Morikawa, S., Haskell, A., Mancuso, M. & McDonald, D. M. Abnormalities of basement membrane on blood vessels and endothelial sprouts in tumors. *Am. J. Pathol.* **163**, 1801–1815 (2003).
141. Sorokin, L. The impact of the extracellular matrix on inflammation. *Nat. Rev. Immunol.* **10**, 712–723 (2010).
142. Shao, R. *et al.* Acquired expression of periostin by human breast cancers promotes tumor angiogenesis through up-regulation of vascular endothelial growth factor receptor 2 expression. *Mol. Cell. Biol.* **24**, 3992–4003 (2004).
143. Van Obberghen-Schilling, E. *et al.* Fibronectin and tenascin-C: accomplices in vascular morphogenesis during development and tumor growth. *Int. J. Dev. Biol.* **55**, 511–525 (2011).
144. Klenotic, P. A., Zhang, C. & Lin, Z. Emerging roles of CCN proteins in vascular development and pathology. *J. Cell Commun. Signal.* **10**, 251–257 (2016).
145. Saupe, F. *et al.* Tenascin-C downregulates wnt inhibitor dickkopf-1, promoting tumorigenesis in a neuroendocrine tumor model. *Cell Rep.* **5**, 482–492 (2013).
146. Lawler, P. R. & Lawler, J. Molecular basis for the regulation of angiogenesis by thrombospondin-1 and -2. *Cold Spring Harb. Perspect. Med.* **2**, a006627 (2012).
147. Rivera, L. B., Bradshaw, A. D. & Brekken, R. A. The regulatory function of SPARC in vascular biology. *Cell. Mol. Life Sci.* **68**, 3165–3173 (2011).
148. Mammoto, A. *et al.* A mechanosensitive transcriptional mechanism that controls angiogenesis. *Nature* **457**, 1103–1108 (2009).
149. Rivron, N. C. *et al.* Tissue deformation spatially modulates VEGF signaling and angiogenesis. *Proc. Natl Acad. Sci. USA* **109**, 6886–6891 (2012).
150. Mason, B. N., Starchenko, A., Williams, R. M., Bonassar, L. J. & Reinhart-King, C. A. Tuning three-dimensional collagen matrix stiffness independently of collagen concentration modulates endothelial cell behavior. *Acta Biomater.* **9**, 4635–4644 (2013).
151. Yun, H., Lee, M., Kim, S. S. & Ha, J. Glucose deprivation increases mRNA stability of vascular endothelial growth factor through activation of AMP-activated protein kinase in DU145 prostate carcinoma. *J. Biol. Chem.* **280**, 9963–9972 (2005).
152. Shi, Q. *et al.* Regulation of vascular endothelial growth factor expression by acidosis in human cancer cells. *Oncogene* **20**, 3751–3756 (2001).
153. Vegran, F., Boidot, R., Michiels, C., Sonveaux, P. & Feron, O. Lactate influx through the endothelial cell monocarboxylate transporter MCT1 supports an NF-kappaB/IL-8 pathway that drives tumor angiogenesis. *Cancer Res.* **71**, 2550–2560 (2011).
154. Sonveaux, P. *et al.* Targeting the lactate transporter MCT1 in endothelial cells inhibits lactate-induced HIF-1 activation and tumor angiogenesis. *PLoS ONE* **7**, e33418 (2012).
155. Casazza, A. *et al.* Impeding macrophage entry into hypoxic tumor areas by Sema3A/Nrp1 signaling blockade inhibits angiogenesis and restores antitumor immunity. *Cancer Cell* **24**, 695–709 (2013).
156. Laoui, D. *et al.* Tumor hypoxia does not drive differentiation of tumor-associated macrophages but rather fine-tunes the M2-like macrophage population. *Cancer Res.* **74**, 24–30 (2014).
157. Colegio, O. R. *et al.* Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. *Nature* **513**, 559–563 (2014).
References 155–157 illustrated the effects of tumour hypoxia on the pro-angiogenic macrophage phenotype.
158. Biswas, S. K. Metabolic reprogramming of immune cells in cancer progression. *Immunity* **43**, 435–449 (2015).
159. Wenes, M. *et al.* Macrophage metabolism controls tumor blood vessel morphogenesis and metastasis. *Cell Metab.* **24**, 701–715 (2016).
160. Potente, M. & Carmeliet, P. The link between angiogenesis and endothelial metabolism. *Annu. Rev. Physiol.* **79**, 43–66 (2017).
161. Gorrini, C., Harris, I. S. & Mak, T. W. Modulation of oxidative stress as an anticancer strategy. *Nat. Rev. Drug Discov.* **12**, 931–947 (2013).
162. Xia, C. *et al.* Reactive oxygen species regulate angiogenesis and tumor growth through vascular endothelial growth factor. *Cancer Res.* **67**, 10823–10830 (2007).
163. West, X. Z. *et al.* Oxidative stress induces angiogenesis by activating TLR2 with novel endogenous ligands. *Nature* **467**, 972–976 (2010).
164. Garrido-Urbani, S. *et al.* Targeting vascular NADPH oxidase 1 blocks tumor angiogenesis through a PPARalpha mediated mechanism. *PLoS ONE* **6**, e14665 (2011).
165. Warren, C. M., Ziyad, S., Briot, A., Der, A. & Iruela-Arispe, M. L. A ligand-independent VEGFR2 signaling pathway limits angiogenic responses in diabetes. *Sci. Signal.* **7**, ra1 (2014).
166. Okuno, Y., Nakamura-Ishizu, A., Otsu, K., Suda, T. & Kubota, Y. Pathological neoangiogenesis depends on oxidative stress regulation by ATM. *Nat. Med.* **18**, 1208–1216 (2012).
167. Rak, J. *et al.* Oncogenes and tumor angiogenesis: differential modes of vascular endothelial growth factor up-regulation in ras-transformed epithelial cells and fibroblasts. *Cancer Res.* **60**, 490–498 (2000).
168. Sparmann, A. & Bar-Sagi, D. Ras-induced interleukin-8 expression plays a critical role in tumor growth and angiogenesis. *Cancer Cell* **6**, 447–458 (2004).
This study showed that a mutant RAS oncogene fostered myeloid-cell infiltration and tumour angiogenesis by activating the transcription of *Cxcl8* in cancer cells.
169. Bottos, A. *et al.* Targeting oncogenic serine/threonine-protein kinase BRAF in cancer cells inhibits angiogenesis and abrogates hypoxia. *Proc. Natl Acad. Sci. USA* **109**, E353–E359 (2012).
170. Sharma, A. *et al.* Mutant V599EB-Raf regulates growth and vascular development of malignant melanoma tumors. *Cancer Res.* **65**, 2412–2421 (2005).
171. Phan, V. T. *et al.* Oncogenic RAS pathway activation promotes resistance to anti-VEGF therapy through G-CSF-induced neutrophil recruitment. *Proc. Natl Acad. Sci. USA* **110**, 6079–6084 (2013).
172. Pylayeva-Gupta, Y., Lee, K. E., Hajdu, C. H., Miller, G. & Bar-Sagi, D. Oncogenic Kras-induced GM-CSF production promotes the development of pancreatic neoplasia. *Cancer Cell* **21**, 836–847 (2012).
173. Cremolini, C. *et al.* FOLFOXIRI plus bevacizumab versus FOLFIRI plus bevacizumab as first-line treatment of patients with metastatic colorectal cancer: updated overall survival and molecular subgroup analyses of the open-label, phase 3 TRIBE study. *Lancet Oncol.* **16**, 1306–1315 (2015).
174. Tabernero, J. *et al.* Ramucirumab versus placebo in combination with second-line FOLFIRI in patients with metastatic colorectal carcinoma that progressed during or after first-line therapy with bevacizumab, oxaliplatin, and a fluoropyrimidine (RAISE): a randomised, double-blind, multicentre, phase 3 study. *Lancet Oncol.* **16**, 499–508 (2015).
175. Becker, A. *et al.* Extracellular vesicles in cancer: cell-to-cell mediators of metastasis. *Cancer Cell* **30**, 836–848 (2016).
176. Kholia, S. *et al.* Extracellular vesicles as new players in angiogenesis. *Vascul. Pharmacol.* **86**, 64–70 (2016).
177. Skog, J. *et al.* Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat. Cell Biol.* **10**, 1470–1476 (2008).
178. Taraboletti, G. *et al.* Bioavailability of VEGF in tumor-shed vesicles depends on vesicle burst induced by acidic pH. *Neoplasia* **8**, 96–103 (2006).
179. Graves, L. E. *et al.* Proinvasive properties of ovarian cancer ascites-derived membrane vesicles. *Cancer Res.* **64**, 7045–7049 (2004).
180. Squadrito, M. L. *et al.* Endogenous RNAs modulate microRNA sorting to exosomes and transfer to acceptor cells. *Cell Rep.* **8**, 1432–1446 (2014).
181. Janowska-Wieczorek, A. *et al.* Microvesicles derived from activated platelets induce metastasis and angiogenesis in lung cancer. *Int. J. Cancer* **113**, 752–760 (2005).
182. Sheldon, H. *et al.* New mechanism for Notch signaling to endothelium at a distance by Delta-like 4 incorporation into exosomes. *Blood* **116**, 2385–2394 (2010).
183. Lombardo, G. *et al.* Activated Stat5 trafficking via endothelial cell-derived extracellular vesicles controls IL-3 pro-angiogenic paracrine action. *Sci. Rep.* **6**, 25689 (2016).
184. Tkach, M. & Thery, C. Communication by extracellular vesicles: where we are and where we need to go. *Cell* **164**, 1226–1232 (2016).
185. Nazarenko, I. *et al.* Cell surface tetraspanin Tspan8 contributes to molecular pathways of exosome-induced endothelial cell activation. *Cancer Res.* **70**, 1668–1678 (2010).
186. Zhuang, G. *et al.* Tumour-secreted miR-9 promotes endothelial cell migration and angiogenesis by activating the JAK-STAT pathway. *EMBO J.* **31**, 3513–3523 (2012).
187. Al-Nedawi, K., Meehan, B., Kerbel, R. S., Allison, A. C. & Rak, J. Endothelial expression of autocrine VEGF upon the uptake of tumor-derived microvesicles containing oncogenic EGFR. *Proc. Natl Acad. Sci. USA* **106**, 3794–3799 (2009).

188. Umezu, T. *et al.* Exosomal miR-135b shed from hypoxic multiple myeloma cells enhances angiogenesis by targeting factor-inhibiting HIF-1. *Blood* **124**, 3748–3757 (2014).
189. Ferrara, N. & Adamis, A. P. Ten years of anti-vascular endothelial growth factor therapy. *Nat. Rev. Drug Discov.* **15**, 385–403 (2016).
190. Bridgeman, V. L. *et al.* Vessel co-option is common in human lung metastases and mediates resistance to anti-angiogenic therapy in preclinical lung metastasis models. *J. Pathol.* **241**, 362–374 (2017).
191. Frentzas, S. *et al.* Vessel co-option mediates resistance to anti-angiogenic therapy in liver metastases. *Nat. Med.* **22**, 1294–1302 (2016).
References 190 and 191 highlighted the potential clinical implications of vascular co-option for resistance to anti-angiogenic therapy in metastatic disease.
192. Pezzella, F. *et al.* Non-small-cell lung carcinoma tumor growth without morphological evidence of neo-angiogenesis. *Am. J. Pathol.* **151**, 1417–1423 (1997).
193. Bonapace, L. *et al.* Cessation of CCL2 inhibition accelerates breast cancer metastasis by promoting angiogenesis. *Nature* **515**, 130–133 (2014).
194. Gordon-Weeks, A. N. *et al.* Neutrophils promote hepatic metastasis growth through fibroblast growth factor 2-dependent angiogenesis in mice. *Hepatology* **65**, 1920–1935 (2017).
195. Bergers, G. & Hanahan, D. Modes of resistance to anti-angiogenic therapy. *Nat. Rev. Cancer* **8**, 592–603 (2008).
196. Hu, Y. L. *et al.* Hypoxia-induced autophagy promotes tumor cell survival and adaptation to antiangiogenic treatment in glioblastoma. *Cancer Res.* **72**, 1773–1783 (2012).
197. Sounni, N. E. *et al.* Blocking lipid synthesis overcomes tumor regrowth and metastasis after antiangiogenic therapy withdrawal. *Cell Metab.* **20**, 280–294 (2014).
198. Allen, E. *et al.* Metabolic symbiosis enables adaptive resistance to anti-angiogenic therapy that is dependent on mTOR signaling. *Cell Rep.* **15**, 1144–1160 (2016).
199. Jimenez-Valerio, G. *et al.* Resistance to antiangiogenic therapies by metabolic symbiosis in renal cell carcinoma PDX models and patients. *Cell Rep.* **15**, 1134–1143 (2016).
200. Pisarsky, L. *et al.* Targeting metabolic symbiosis to overcome resistance to anti-angiogenic therapy. *Cell Rep.* **15**, 1161–1174 (2016).
201. Bensaad, K. *et al.* Fatty acid uptake and lipid storage induced by HIF-1alpha contribute to cell growth and survival after hypoxia-reoxygenation. *Cell Rep.* **9**, 349–365 (2014).
References 196–201 highlighted distinct mechanisms whereby tumours escape the inhibitory effects of anti-angiogenic therapy through metabolic reprogramming.
202. Casanovas, O., Hicklin, D. J., Bergers, G. & Hanahan, D. Drug resistance by evasion of antiangiogenic targeting of VEGF signaling in late-stage pancreatic islet tumors. *Cancer Cell* **8**, 299–309 (2005).
203. Scholz, A. *et al.* Endothelial cell-derived angiopoietin-2 is a therapeutic target in treatment-naive and bevacizumab-resistant glioblastoma. *EMBO Mol. Med.* **8**, 39–57 (2016).
204. Paez-Ribes, M. *et al.* Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. *Cancer Cell* **15**, 220–231 (2009).
205. Rubenstein, J. L. *et al.* Anti-VEGF antibody treatment of glioblastoma prolongs survival but results in increased vascular cooption. *Neoplasia* **2**, 306–314 (2000).
206. Gabrusiewicz, K. *et al.* Anti-vascular endothelial growth factor therapy-induced glioma invasion is associated with accumulation of Tie2-expressing monocytes. *Oncotarget* **5**, 2208–2220 (2014).
207. De Palma, M. & Lewis, C. E. Macrophage regulation of tumor responses to anticancer therapies. *Cancer Cell* **23**, 277–286 (2013).
208. Huang, H. *et al.* Specifically targeting angiopoietin-2 inhibits angiogenesis, Tie2-expressing monocyte infiltration, and tumor growth. *Clin. Cancer Res.* **17**, 1001–1011 (2011).
209. Kloepper, J. *et al.* Ang-2/VEGF bispecific antibody reprograms macrophages and resident microglia to anti-tumor phenotype and prolongs glioblastoma survival. *Proc. Natl Acad. Sci. USA* **113**, 4476–4481 (2016).
210. Schmittnaegel, M. *et al.* Dual angiopoietin-2 and VEGFA inhibition elicits antitumor immunity that is enhanced by PD-1 checkpoint blockade. *Sci. Transl Med.* **9**, aak9670 (2017).
211. Pahler, J. C. *et al.* Plasticity in tumor-promoting inflammation: impairment of macrophage recruitment evokes a compensatory neutrophil response. *Neoplasia* **10**, 329–340 (2008).
This study suggested that the genetic impairment of macrophages could exacerbate the tumour-promoting functions of neutrophils in a mouse model of cervical cancer.
212. von Tresckow, B. *et al.* An open-label, multicenter, phase I/II study of JNJ-40346527, a CSF-1R inhibitor, in patients with relapsed or refractory Hodgkin lymphoma. *Clin. Cancer Res.* **21**, 1843–1850 (2015).
213. Butowski, N. *et al.* Orally administered colony stimulating factor 1 receptor inhibitor PLX3397 in recurrent glioblastoma: an Ivy Foundation Early Phase Clinical Trials Consortium phase II study. *Neuro Oncol.* **18**, 557–564 (2016).
214. Kaneda, M. M. *et al.* PI3Kgamma is a molecular switch that controls immune suppression. *Nature* **539**, 437–4424 (2016).
215. De Palma, M. *et al.* Tumor-targeted interferon-alpha delivery by Tie2-expressing monocytes inhibits tumor growth and metastasis. *Cancer Cell* **14**, 299–311 (2008).
216. Mancuso, M. R. *et al.* Rapid vascular regrowth in tumors after reversal of VEGF inhibition. *J. Clin. Invest.* **116**, 2610–2621 (2006).
217. Raymond, E. *et al.* Sunitinib malate for the treatment of pancreatic neuroendocrine tumors. *N. Engl. J. Med.* **364**, 501–513 (2011).
218. Ebos, J. M. *et al.* Neoadjuvant antiangiogenic therapy reveals contrasts in primary and metastatic tumor efficacy. *EMBO Mol. Med.* **6**, 1561–1576 (2014).
219. Chung, A. S. *et al.* An interleukin-17-mediated paracrine network promotes tumor resistance to anti-angiogenic therapy. *Nat. Med.* **19**, 1114–1123 (2013).
220. Carmeliet, P. & Jain, R. K. Principles and mechanisms of vessel normalization for cancer and other angiogenic diseases. *Nat. Rev. Drug Discov.* **10**, 417–427 (2011).
221. De Palma, M. & Jain, R. K. CD4 + T cell activation and vascular normalization: two sides of the same coin? *Immunity* **46**, 773–775 (2017).
222. Schoenfeld, J. *et al.* Active immunotherapy induces antibody responses that target tumor angiogenesis. *Cancer Res.* **70**, 10150–10160 (2010).
223. Wu, X. *et al.* Angiopoietin-2 as a biomarker and target for immune checkpoint therapy. *Cancer Immunol. Res.* **5**, 17–28 (2017).
References 222 and 223 revealed that the anti-tumoural effects of certain cancer immunotherapies may also involve immune-mediated anti-angiogenic or angiostatic mechanisms.
224. Aird, W. C. Endothelial cell heterogeneity. *Cold Spring Harb. Perspect. Med.* **2**, a006429 (2012).
225. Hida, K., Maishi, N., Torii, C. & Hida, Y. Tumor angiogenesis — characteristics of tumor endothelial cells. *Int. J. Clin. Oncol.* **21**, 206–212 (2016).
226. Chaudhary, A. & St Croix, B. Selective blockade of tumor angiogenesis. *Cell Cycle* **11**, 2253–2259 (2012).
227. Pasqualini, R., Moeller, B. J. & Arap, W. Leveraging molecular heterogeneity of the vascular endothelium for targeted drug delivery and imaging. *Semin. Thromb. Hemost.* **36**, 343–351 (2010).
228. Amin, D. N., Hida, K., Bielenberg, D. R. & Klagsbrun, M. Tumor endothelial cells express epidermal growth factor receptor (EGFR) but not ErbB3 and are responsive to EGF and to EGFR kinase inhibitors. *Cancer Res.* **66**, 2173–2180 (2006).
229. Xiong, Y. Q. *et al.* Human hepatocellular carcinoma tumor-derived endothelial cells manifest increased angiogenesis capability and drug resistance compared with normal endothelial cells. *Clin. Cancer Res.* **15**, 4838–4846 (2009).
230. Akiyama, K. *et al.* Tumor endothelial cells acquire drug resistance by MDR1 up-regulation via VEGF signaling in tumor microenvironment. *Am. J. Pathol.* **180**, 1283–1293 (2012).
231. Hida, K. *et al.* Tumor-associated endothelial cells with cytogenetic abnormalities. *Cancer Res.* **64**, 8249–8255 (2004).
232. Kim, Y. W. & Byzova, T. V. Oxidative stress in angiogenesis and vascular disease. *Blood* **123**, 625–631 (2014).
233. Soda, Y. *et al.* Transdifferentiation of glioblastoma cells into vascular endothelial cells. *Proc. Natl Acad. Sci. USA* **108**, 4274–4280 (2011).
234. Williamson, S. C. *et al.* Vasculogenic mimicry in small cell lung cancer. *Nat. Commun.* **7**, 13322 (2016).

Acknowledgements
Work in the authors' laboratories is mainly supported by the European Research Council (to M.D.P.), the Swiss National Science Foundation (grants 31003A-165963 to M.D.P.; 31003A-156266, 31ER30-160674 and 316030-164119 to T.V.P.), the Swiss Cancer League (grants KFS-3759-08-2015 to M.D.P.; KLS-3406-02-2014 to T.V.P.), the Leenaards Foundation, the San Salvatore Foundation, and the Swiss Bridge Foundation (to M.D.P. and T.V.P.).

Author contributions
M.D.P. conceived and wrote the article and display items. T.V.P. contributed to discussions of the content and writing of the article. M.D.P., D.B. and T.V.P. researched the data for the article. M.D.P., D.B. and T.V.P. reviewed and edited the article before submission.

Competing interests statement
The authors declare competing interests: see Web version for details.

Publisher's note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.