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Angiogenesis in life, disease and medicine

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The growth of blood vessels (a process known as angiogenesis) is essential for organ growth and repair. An imbalance in this process contributes to numerous malignant, inflammatory, ischaemic, infectious and immune disorders. Recently, the first anti-angiogenic agents have been approved for the treatment of cancer and blindness. Angiogenesis research will probably change the face of medicine in the next decades, with more than 500 million people worldwide predicted to benefit from pro- or anti-angiogenesis treatments.

Blood vessels arose during evolution to carry oxygen to distant organs. Not surprisingly, these vessels are crucial for organ growth in the embryo and repair of wounded tissue in the adult. But an imbalance in the growth of blood vessels contributes to the pathogenesis of numerous disorders. In less than 15 years, an explosion of interest in angiogenesis research has generated the necessary insights to develop the first clinically approved anti-angiogenic agents.

Here I discuss some key mechanisms of angiogenesis and opportunities to develop further novel therapeutic strategies that target this process, to minimize the adverse effects of these treatments and to avoid resistance to this novel medicine.

The discovery of blood and lymph vessels

In primitive animals, such as the worm *Caenorhabditis elegans* and the fruitfly *Drosophila melanogaster*, oxygen is capable of diffusing throughout their small body to all cells. In other species, which developed later in evolution and grew to larger sizes, a vascular network distributes oxygen in the blood to distant cells. The Ancient Greek physician Galen originally proposed that the blood does not circulate but is locally regenerated by the body when its supplies are consumed. Only in 1628 did William Harvey discover that the heart pumps the blood around the body through arteries and that veins return the blood to the heart. A few decades later in 1661, Marcello Malphighi identified the capillaries as the smallest vessels that close the circulatory loop between arteries and veins (Fig. 1a). Around the same time, Caspar Aselius discovered another type of vessel, the lymphatic vessel. Because of the blood pressure, blood plasma continuously leaks from the capillaries, and lymph vessels return this fluid back to the blood circulation. Although blood vessels arose earlier in evolution, lymph vessels are only present in amphibians onwards[1] (Fig. 1b).

The first vessels in life

In the embryo, blood vessels provide the growing organs with the necessary oxygen to develop. Apart from their nutritive function, vessels also provide instructive trophic signals to promote organ morphogenesis (see the review by Coultas, Chawengsaksophak and Rossant in this issue, p. 957). Blood vessels arise from endothelial precursors, which share an origin with haematopoietic progenitors. This close link between the blood and blood vascular systems remains important for angiogenesis throughout life, even in disease (see below). These progenitors assemble into a primitive vascular labyrinth of small capillaries — a process known as vasculogenesis (Fig. 1c). Interestingly, already at this stage capillaries have acquired an arterial and venous cell fate, indicating that vascular-cell specification is genetically programmed and not only determined by haemodynamic force (see p. 937).

During the angiogenesis phase, the vascular plexus progressively expands by means of vessel sprouting and remodels into a highly organized and stereotyped vascular network of larger vessels ramifying into smaller ones (Fig. 1c). Nascent endothelial-cell (EC) channels become covered by pericytes (PCs) and smooth muscle cells (SMCs), which provide strength and allow regulation of vessel perfusion, a process termed arteriogenesis. As reviewed by Alitalo, Tammela and Petrova in this issue (p. 946), the lymphatic system develops differently, as most lymphatics transdifferentiate from veins.

Over the past 15 years, genetic studies in mice, zebrafish and tadpoles have provided insights into the key mechanisms and molecular players that regulate the growth of blood vessels (angiogenesis) or lymph vessels (lymphangiogenesis) in the embryo (see p. 937 and p. 946). For instance, members of the Notch family drive the arterial gene programme, whereas the orphan receptor COUP-TFII regulates venous specification. The homeobox gene *Prox-1*, by contrast, is a master switch of lymphatic commitment. VEGF and its homologue VEGF-C are key regulators of vascular and lymphatic EC sprouting, respectively, whereas platelet-derived growth factor (PDGF)-BB and angiopoietin-1 recruit mural cells around endothelial channels. The formation of vessels is a complex process, requiring a finely tuned balance between numerous stimulatory and inhibitory signals, such as integrins, angiopoietins, chemokines, junctional molecules, oxygen sensors, endogenous inhibitors and many others[2]. An exciting recent development is the discovery of the links between vessels and nerves and, in particular, how axon-guidance signals such as Ephrins, Semaphorins, Netrins and Slits allow vessels to navigate to their targets or control vessel morphogenesis[3]. Angiogenic signals also guide axons and affect neurons in health and disease, as reviewed by Greenberg in this issue (p. 954).

Vessels of disease and death

After birth, angiogenesis still contributes to organ growth but, during adulthood, most blood vessels remain quiescent and angiogenesis occurs only in the cycling ovary and in the placenta during pregnancy. However, ECs retain their remarkable ability of dividing rapidly in response to a physiological stimulus, such as hypoxia for blood vessels and inflammation for lymph vessels[2] (see p. 946). As such, (lymph)angiogenesis is reactivated during wound healing and repair. But in many disorders, this stimulus becomes excessive, and the balance between stimulators and inhibitors is tilted, resulting in a (lymph)angiogenic switch. The best-known conditions in which angiogenesis is

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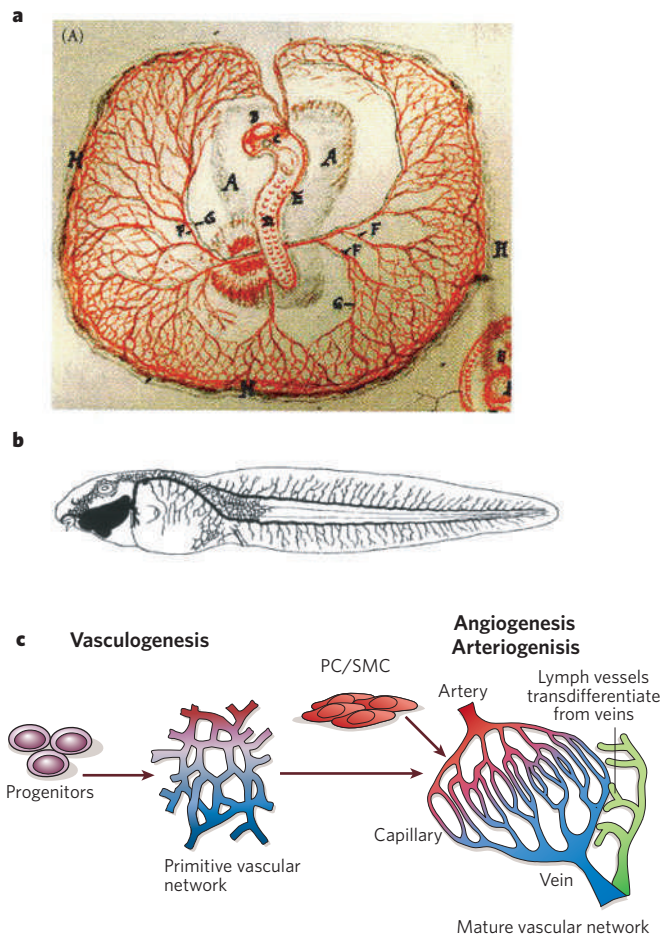


Figure 1 | History and formation of blood and lymph vessels. **a**, Drawing by M. Malphighi (1661), showing the vascular network of arteries, capillaries and veins in a developing chicken embryo (from ref. 26). **b**, Drawing by M. Hoyer displaying the lymphatic network in early tadpoles (from ref. 27). **c**, Development of the vascular systems: during vasculogenesis, endothelial progenitors give rise to a primitive vascular labyrinth of arteries and veins; during subsequent angiogenesis, the network expands, pericytes (PCs) and smooth muscle cells (SMCs) cover nascent endothelial channels, and a stereotypically organized vascular network emerges. Lymph vessels develop via transdifferentiation from veins.

switched on are malignant, ocular and inflammatory disorders, but many additional processes are affected, such as obesity, asthma, diabetes, cirrhosis, multiple sclerosis, endometriosis, AIDS, bacterial infections and autoimmune disease (for a more complete list see Supplementary Table 1). There is even a close link between angiogenesis, neural stem cells and learning.

In other diseases, such as ischaemic heart disease or preeclampsia, the angiogenic switch is insufficient, causing EC dysfunction, vessel malformation or regression, or preventing revascularization, healing and regeneration (for a more complete list see Supplementary Table 2). Besides its vascular activity, VEGF is also trophic for nerve cells, lung epithelial cells and cardiac muscle fibres, further explaining why insufficient VEGF levels contribute to neurodegeneration[4], respiratory distress and, possibly, cardiac failure (Supplementary Table 2). Angiogenesis has been implicated in more than 70 disorders so far, and the list is ever growing. In this issue, Gariano and Gardner, and Ferrara and Kerbel discuss the key signals of (lymph)angiogenesis in pathological conditions (see pp. 960 and 967. See also p. 946). Interestingly, some molecules such as PlGF (a homologue of VEGF) have a role in angiogenesis in disease without affecting quiescent vessels in healthy organs, making them attractive therapeutic targets for the development of safe anti-angiogenic drugs[5].

Angiogenesis as a promising medicine

Over the past decade, intensive efforts have been undertaken to develop therapeutic strategies to promote revascularization of ischaemic tissues or to inhibit angiogenesis in cancer, ocular, joint or skin disorders. Unfortunately, clinical trials testing the pro-angiogenic potential of VEGF or fibroblast growth factor (FGF) have not had the expected results[6]. Although part of this failure is attributable to suboptimal delivery strategies, stimulating the growth of durable and functional vessels is a more formidable challenge than previously anticipated. Novel strategies, involving transplantation of bone-marrow-derived cells or the delivery of molecules capable of stimulating the growth not only of distal capillaries but also of proximal collateral conduit vessels, may be required in the future[6]. Stimulating lymphangiogenesis is also emerging as a novel treatment of lymphoedema (see p. 946).

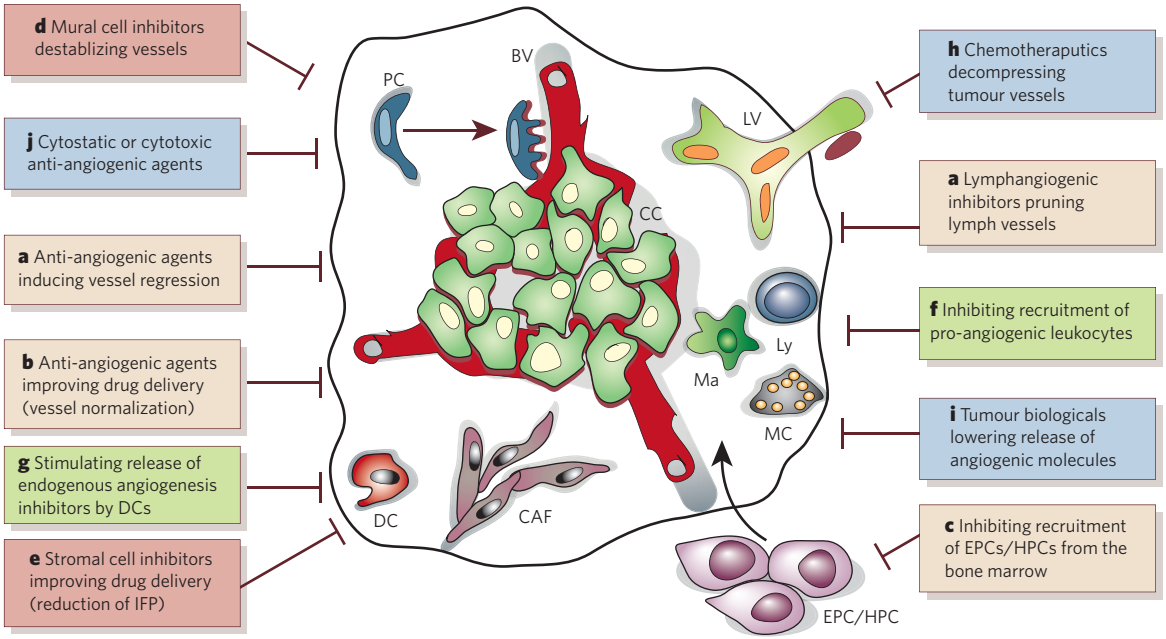
Angiogenesis does not initiate malignancy but promotes tumour progression and metastasis. Unlike tumour cells, ECs are genomically stable and were therefore originally considered to be ideal therapeutic targets that would not become resistant to anti-angiogenic therapy. Most previous efforts have thus been focused on developing anti-angiogenic agents that primarily target ECs. Several reviews in this issue and a forthcoming review by Jain and colleagues[7] provide an update on the clinical use of the first two FDA-approved VEGF antagonists in ocular and maligant disease and discuss opportunities to inhibit lymphangiogenesis in cancer. The recent clinical experience with VEGF inhibitors has provided a number of important, but puzzling, insights and raised various outstanding questions. First, the anti-VEGF antibody Avastin (Genentech) only provides an overall survival benefit in colorectal, breast and lung cancer patients when combined with conventional chemotherapy. It is still not entirely clear why anti-VEGF monotherapy was ineffective in humans, whereas it was effective in rodents. Second, monotherapy with the multi-targeted receptor tyrosine kinase inhibitors (RTKIs) Sorafenib (Bayer and Onyx) or Sutent (Pfizer), which target ECs as well as cancer, and probably also stromal and haematopoietic cells, demonstrates clinical benefit in certain cancers. But does this imply that future anti-angiogenic strategies should target both ECs and non-EC types? Third, despite its ability to block all three VEGF receptors, Vatalanib (Novartis and Schering AG) does not substantially enhance the benefit of conventional chemotherapy. How can this discrepancy with Avastin be explained? Fourth, despite promising success, cancer patients receiving a single class of angiogenesis inhibitors, even in combination with chemotherapy, still die. Does this suggest that the anti-angiogenic strategy is insufficient or does it evoke resistance and, if so, how can we avoid resistance? Can we develop more reliable biomarkers to monitor the efficacy of anti-angiogenic therapy[7] (p. 967)? Fifth, adverse effects have been reported. What are the molecular mechanisms of these effects? For reasons of brevity, I highlight here only a few key issues. Taking the stand that targeting ECs or the principal angiogenic factor VEGF alone may not (ever) suffice to eradicate malignant tumours, I discuss here alternative options to complement the current VEGF-based therapies with strategies that target other angiogenic factors or target, in combination, other non-EC types that indirectly affect angiogenesis (Box 1). In addition, possible mechanisms of the adverse effects and resistance to anti-angiogenic therapy will be highlighted (Box 2).

Inhibition of angiogenesis by targeting endothelial cells

The best-known anti-angiogenic agents of this class are the VEGF inhibitors. The most advanced in the clinic are the anti-VEGF antibody Avastin, a VEGF165 aptamer (Macugen, Eyetech) and various RTKIs, which target VEGFRs and other receptors (see p. 967 and ref. 7). Additional compounds targeting VEGF family members, currently in development, include a VEGF trap (Regeneron) and antibodies against VEGFR-2 or VEGFR-1 (Imclone) and against the VEGFR-1 ligand PlGF (Thrombogenics Ltd and BioInvent International). This class of anti-angiogenic agents not only arrests EC proliferation and prevents vessel growth, but also induces regression of existing vessels by increasing EC death (Box 1). Immature vessels,

Box 1 | Strategies targeting endothelial and non-endothelial cells to inhibit tumour angiogenesis

Tumour angiogenesis has classically been inhibited by anti-angiogenic agents that affect ECs directly. Alternative anti-tumour angiogenesis strategies target other cell types in tumours (mural and stromal cells, haematopoietic cells and tumour cells), which stimulate angiogenesis indirectly. The yellow boxes show agents (such as VEGF inhibitors, metronomic chemotherapy and other compounds) that target endothelial (progenitor) cells (EPCs); they inhibit (lymph)angiogenesis (**a**), induce vessel regression (**a**) and normalization (**b**), and block recruitment of EPCs (**c**). The red boxes show agents (such as PDGF inhibitors) that target mural and stromal cells and destabilize vessels (**d**), reduce the release of pro-angiogenic factors or progenitor cytokines, and lower the interstitial fluid pressure (IFP), which improves drug delivery (**e**). The green boxes indicate agents (such as VEGFR-1 inhibitors, chemokine antagonists and so on) that target haematopoietic cells and reduce the infiltration of pro-angiogenic bone-marrow-derived precursors and mature leukocytes (**c**,**f**), and stimulate the release of endogenous angiogenesis inhibitors in dendritic cells (**g**). The blue boxes show agents targeting cancer cells (chemotherapy, radiation, tumour-cell-targeted biologicals) that improve drug delivery by decompressing tumour vessels (**h**) and decrease the release of (lymph)angiogenic factors (**i**); some anti-angiogenic agents are also cytotoxic for tumour cells (**j**). BV, blood vessel; CAF, carcinoma-activated fibroblast; CC, cancer cell; DC, dendritic cell; LV, lymph vessel; Ly, lymphocyte; Ma, macrophage; PC, pericyte; MC, mast cells.



devoid of pericytes, are most susceptible. In addition, VEGF inhibitors suppress the mobilization of endothelial progenitor cells (EPCs) from the bone marrow. Anti-VEGF treatment also improves cytotoxic drug delivery by normalizing the chaotic pattern and abnormal architecture of tumour vessels, and reducing vascular permeability and the interstitial fluid pressure, explaining why this antibody acts as a chemosensitizer and increases the efficacy of chemotherapeutics[8]. Besides these anti-EC activities, VEGF inhibitors are cytotoxic for some malignant cells, activate the anti-tumour immune attack and suppress the pro-angiogenic activity of haematopoietic cells (see below; Box 1). Chemotherapeutics, which target dividing cells, may also inhibit EC growth when delivered in metronomic regimens (that is, a continuous low dose)[7,9] (see p. 967).

Additional anti-angiogenic agents are currently being evaluated. However, the potential to combine VEGF antagonists with other inhibitors of distinct angiogenic targets remains largely untested in the clinic, despite emerging evidence that many more angiogenic factors besides VEGF contribute to the angiogenic switch in tumours, especially in the advanced stage. Nonetheless, such anti-angiogenic combination therapy might very well increase the efficacy of and decrease the resistance to angiogenesis inhibition.

Angiogenesis inhibition by targeting mural and stromal cells

Vessels in tumours are covered by PCs[10]. These mural cells differentiate from pools of c-Kit$^+$Sca-1$^+$VEGFR-1$^+$ perivascular progenitor cells, which are mobilized from the bone marrow in response to PDGF-BB[11]. By locally releasing VEGF (an EC survival factor) and angiopoietin-1 (which tightens vessels by means of a matrix and cell–cell contacts), these mural cells promote vessel stabilization. When PDGFs are overexpressed, tumour vessels are covered by more mural cells and tumour growth is accelerated[10]. Conversely, when PDGFRβ signaling is inhibited, fewer PCs are recruited, tumour vessels are dilated and EC apoptosis is increased. Combined administration of RTKIs, targeting VEGFRs and PDGFRβ, increases the anti-angiogenic effect, even in the often-intractable late stage of solid tumours[12]. PDGRβ inhibitors also destabilize the larger SMC-covered vessels, which supply bulk flow to tumours and render them more susceptible to EC-specific inhibitors (Box 1).

In 1889, Paget proposed that 'seeds' of tumour cells form metastatic deposits only if they land in appropriate 'soils'. The reactive tumour stroma is not simply an innocent bystander but an active contributor to tumour progression. Unlike normal tissue, the tumour stroma contains inflammatory infiltrates, an increased microvessel density and dysfunctional lymphatics, a different and more dense extracellular matrix (ECM) and carcinoma-activated fibroblasts (CAFs). CAFs accelerate tumour growth and may increase their malignancy; they also affect the tumour vasculature in many ways. Indeed, CAFs express PDGFRβ and are recruited to the tumour, proliferate and release angiogenic factors such as VEGF and PlGF in response to PDGF-BB[10]. As well as suppressing angiogenesis, PDGF-BB antagonists lower the interstitial fluid pressure and improve drug delivery through the tumour vasculature (Box 1). Although the precise reason *in vivo* remains speculative, CAFs exert a tension on microfibrillar networks *in vitro* and, when stimulated by PDGF-BB, contract the interstitial matrix, thereby compressing tumour vessels[13]. Stromal

fibroblasts also recruit EPCs by releasing stromal-cell-derived factor 1α (SDF-1α)[14]. Inhibiting this chemokine also inhibits tumour growth (Box 1). Unanswered questions are whether the stroma also renders ECs resistant to chemotherapeutics through cell-adhesion-mediated drug resistance (CAM-DR)[15] and whether stromal cells provide niches for cancer (or endothelial) stem cells.

Inhibition of angiogenesis by targeting haematopoietic cells

In 1863, Virchow postulated that inflammation stimulates the progression of cancer. Tumour cells produce various cytokines and chemokines that attract macrophages, dendritic cells, mast cells, T cells and haematopoietic progenitors. Tumour-derived VEGF and PlGF also recruit and stimulate the survival of some of these cells. Apart from releasing mitogenic and survival factors for tumour and stromal cells, stimulating DNA damage, facilitating invasion by means of ECM remodelling and evading the host defence, inflammatory cells also stimulate (lymph)angiogenesis in tumours[16] (Box 1). For instance, tumour-associated macrophages (TAMs) accumulate in hypoxic tumour regions and produce (lymph)angiogenic factors such as VEGF, VEGF-C and VEGF-D. Tie2-expressing monocytes (TEMs), mast cells and platelets also release pro-angiogenic factors[17]. Certain leukocyte-attracting chemokines such as IL-8 directly stimulate EC growth; inhibiting this chemokine retards tumour growth. Blocking the signals that promote leukocyte infiltration and survival may thus inhibit tumour angiogenesis.

In the embryo, haematopoietic stem cells (HSCs) migrate into avascular areas and attract sprouting ECs by releasing angiogenic factors, such as angiopoietin-1 (ref. 18). In the adult, bone-marrow-derived haematopoietic cells expressing markers such as Sca-1, c-Kit, CXCR4 and/or VEGFR-1 become recruited, often together with EPCs, to tumours or ischaemic tissues in response to VEGF and PlGF[14,19,20]. These angio-competent cells extravasate around nascent vessels, where they are retained by SDF-1α, and stimulate growth of resident vessels by releasing angiogenic factors such as VEGF, PlGF and angiopoietin-2 (Box 1). In other cases, these cells function as haemangioblasts, producing both haematopoietic and endothelial progenitors that give rise to new blood vessels. Moreover, in response to P1GF released by tumour cells, VEGFR-1$^+$ haematopoietic bone-marrow progenitors home to tumour-specific premetastatic sites, where they recruit tumour cells and EPCs; anti-VEGFR-1 antibodies prevent the forma-

Box 2 | Mechanism of acquired resistance to anti-angiogenic agents

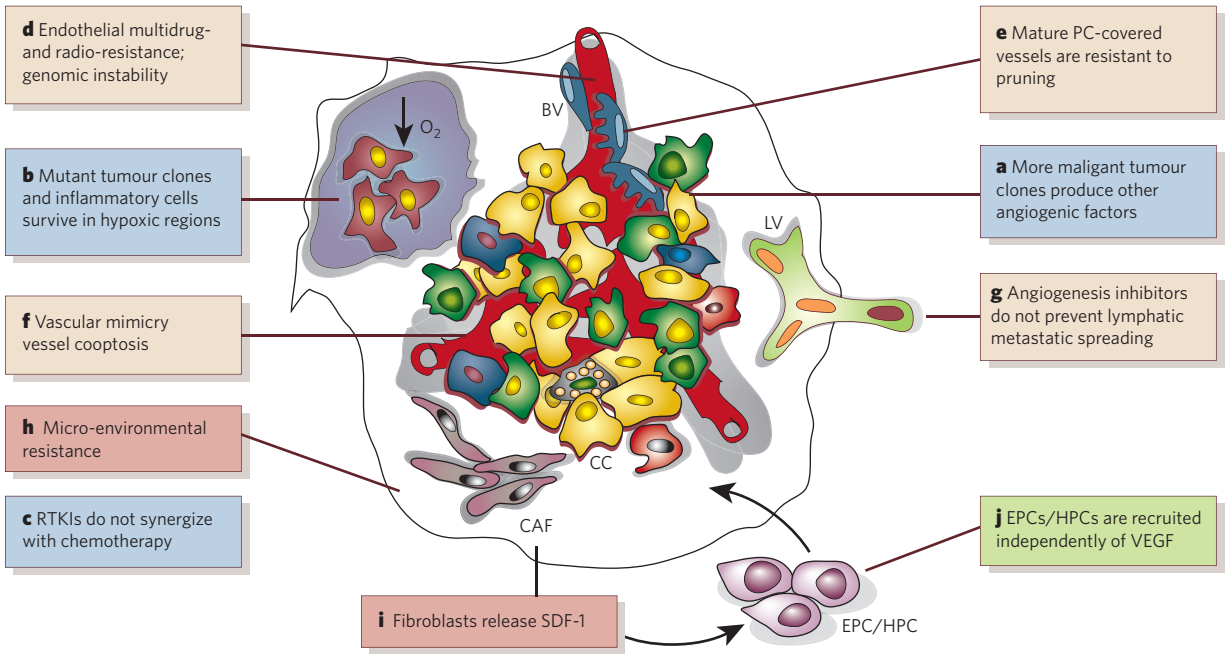
Despite the promising successes of anti-VEGF therapy, cancer patients still die. Emerging evidence suggests that this may be due, at least in part, to acquired resistance to anti-angiogenic agents. Several possible mechanisms are highlighted.

Tumour-cell-related mechanisms are shown below in blue. **a**, During tumour progression, mutant tumour cell clones (yellow and blue caner cells) may become selected and express more of the same or other angiogenic factors. Tumour cells may also upregulate additional angiogenic factors in response to anti-angiogenic treatment (that is upregulation of P1GF and FGF-2 after VEGF inhibition, for example, of VEGF after EGFR or VEGFR-2 inhibition or or IL-8 after HIF-1 inhibition). **b**, Mutant tumour cell clones (for instance, those lacking p53 or HIF-1) or pro-angiogenic inflammatory cells may survive better in hypoxic tumours after angiogenesis inhibition; their reduced vascular dependence impairs the anti-angiogenic response. **c**, Some RTKIs do not synergize with chemotherapy.

EC-related mechanisms are shown below in yellow. **d**, ECs are chemoprotected by high levels of VEGF and other EC survival factors in tumours , which upregulate anti-apoptotic signals and multidrug-resistance-associated proteins. Hypoxic activation of HIF-1 also renders ECs resistant to irradiation. In rare cases, ECs even exhibit cytogenic abnormalities and may be genomically unstable, but this has only been observed in some human cancers. **e**, Pre-existing supply vessels are covered by SMCs and are not easily pruned by EC-targeted treatment. **f**, A fraction of tumour vessels, lined by malignant cells (vascular mimicry) or co-opted from existing vessels, may be less sensitive to anti-angiogenic treatment. **g**, Tumour cells metastasize through lymph vessels; their growth is not (necessarily) blocked by anti-angiogenic therapy.

Stromal-cell-related mechanisms are shown below in red. **h**, Tissue-specific differences in the micro-environment may determine tumour malignancy (for instance, HIF-1$^{-/-}$ gliomas are more malignant in the brain than the skin). **i**, CAFs produce SDF-1 to recruit EPCs/HPCs .

Bone-marrow-derived cell-related mechanisms are shown below in green. **j**, Tumours recruit pro-angiogenic EPCs, HPCs and inflammatory cells independently of VEGF . PC, pericyte; BV, blood vessel; LV, lymph vessel; CC, cancer cell; CAF, carcinoma-activated fibroblast; SDF-1, stromal-cell-derived factor 1. References and additional (more hypothetical) mechanisms are listed in Supplementary Table 3.



tion of such premetastatic niches[21]. Blocking mobilization of these cells by interfering with SDF-1 and PlGF is thus a novel strategy to reduce tumour angiogenesis, growth and even metastasis. Broad-spectrum RTKIs, which block c-Kit, may also have similar effects. Stimulating the function of dendritic cells (DCs) might also be considered, as these antigen-presenting cells not only mount an anti-tumour immune attack but also release endogenous anti-angiogenic cytokines. Because PlGF and VEGFR-1 suppress DC function[22], inhibiting these molecules offers novel opportunities (Box 1).

Inhibition of angiogenesis by targeting neoplastic cells

Future standard treatment of cancers will involve the use of cytotoxic, radiation or tumour-cell-targeted biological tools to destroy malignant cells. These regimens also inhibit tumour angiogenesis, directly or indirectly (Box 1). Indeed, blood and lymph vessels often collapse under the high compressive mechanical stresses inside tumours. By destroying tumour cells, vessels are decompressed, resulting in increased perfusion and drug delivery. Furthermore, tumour cells release numerous angiogenic molecules and induce the expression of angiogenic receptors in tumour vessels (for instance, EGF induces endothelial growth factor (EGF) receptors and VEGFRs in tumour-associated vessels) (see p. 967). Thus, Herceptin (Genentech), an anti-EGFR antibody used to block the growth of neoplastic epithelial cells, also acts as an anti-angiogenic cocktail by lowering angiogenic factors and upregulating endogenous angiogenesis inhibitors[23]. Many other EGFR inhibitors could have similar anti-angiogenic activities[7]. By producing factors that induce lymph node lymphangiogenesis, primary tumours also prepare their future lymphatic metastatic transport to sentinel lymph nodes[24].

Anti-angiogenic factors may also be cytostatic or cytotoxic for tumour cells (Box 1). Indeed, tumour cells often express receptors for VEGF (VEGFR-1 and Neuropilin1), PDGF, FGF, EGF, stem-cell factor (SCF) and other angiogenic factors[25] (see p. 967). Hence, anti-angiogenic drugs could lead to the direct killing of cancer cells by interfering with survival pathways and/or enhancing sensitivity to other treatments. The potential of the broad-spectrum RTKIs Sorafenib or Sutent to inhibit both EC and tumour-cell division (and possibly also that of other non-ECs) may explain their efficacy as monotherapy for renal cell carcinoma and gastrointestinal stromal tumours, respectively. Of interest, Neuropilin1 mediates VEGF-driven survival and migration of tumour cells but lacks a tyrosine kinase domain, and is thus not inhibited by RTKIs.

Conclusions and future directions

Angiogenesis inhibitors are likely to change the face of medicine in the next decade. Because of VEGF's predominant role in angiogenesis, inhibition of VEGF seems to be necessary but is probably insufficient to permanently halt this process in many disorders. In fact, emerging evidence indicates that inhibition of a single target leads to upregulation of additional angiogenic factors: for instance, PlGF is upregulated after anti-VEGF therapy, VEGF after anti-VEGFR-2 or anti-EGFR administration, and interleukin (IL)-8 after hypoxia-inducible facotor 1 (HIF-1) deletion (see Supplementary Table 3 for more information). Combined treatment of anti-angiogenic agents with distinct complementary mechanisms of action, targeting other angiogenic molecules and/or targeting not only ECs but also other pro-angiogenic cell types, may thus offer advantages of increased efficacy — at least if toxicity is not a concern (see below). Another advantage is that such combinations may give the tumour less chance to escape from anti-angiogenic treatment. Exploring strategies to delay, minimize or even avoid resistance to anti-angiogenic agents might further increase the benefit of anti-angiogenic treatments. A number of known and hypothetical mechanisms of resistance to anti-angiogenesis are listed in Box 2 and Supplementary Table 3.

As anti-angiogenic agents are likely to be delivered earlier and earlier to more and more patients for less advanced, life-threatening disease, probably in combination with additional medications, the safety of anti-angiogenic treatment is a topic of emerging importance. On the basis of pharmacological and genetic studies in mice, inhibition of VEGF-driven angiogenesis might have been expected to cause many more adverse effects (Supplementary Table 4). Fortunately, such toxicity has not been observed in humans, but it may emerge in conditions where the risk is increased by genetic predisposition or pharmacological treatment. Some of the adverse effects of anti-VEGF therapy can be explained by the requirement of threshold levels of VEGF for the survival and maintenance of quiescent vessels in healthy organs (Supplementary Table 4). An attractive, novel class of target thus includes molecules, such as PlGF, that only affect angiogenesis in disease without affecting quiescent vessels in healthy organs[5]. The challenge for the future is to develop such novel anti-angiogenic strategies and to optimize combinatorial treatment regimens to fully exploit the therapeutic potential of angiogenesis inhibition. ■

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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