Vascular-specific growth factors and blood vessel formation

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A recent explosion in newly discovered vascular growth factors has coincided with exploitation of powerful new genetic approaches for studying vascular development. An emerging rule is that all of these factors must be used in perfect harmony to form functional vessels. These new findings also demand re-evaluation of therapeutic efforts aimed at regulating blood vessel growth in ischaemia, cancer and other pathological settings.

Until recently, vascular endothelial growth factor (VEGF) was the only growth factor proven to be specific and critical for blood vessel formation[1–3]. Other long-known factors, such as the fibroblast growth factors (FGFs), had profound effects in various endothelial cell assays[4]. But such factors were also known to be nonspecific in that they could act on many other cell types, and it was questionable whether the assays used to evaluate them were physiologically relevant. For example, the most widely used assays involved adding putative angiogenic agents to cornea pocket models, or to chick chorioallantoic membranes[5,6]. In such assays, FGFs could robustly induce new vessel growth, but there was limited ability to evaluate the induced vessels functionally, or to determine the relevance of these inductions for normal vascular development.

A recent explosion of newly discovered growth factors acting on the vascular endothelium has coincided with application of powerful new genetic approaches to the problem of vascular development[7,8]. The vascular endothelium-specific growth factors now include five members of the VEGF family, four members of the angiopoietin family, and at least one member of the large ephrin family (Fig. 1). For almost all of these and their receptors, mouse models involving genetic disruption and/or transgenic misexpression have contributed to an understanding of their normal physiological roles, as well as of their pathological capabilities. A rule that is emerging is that all of these factors must be used in perfect harmony, in a complementary and coordinated manner, to form functional vessels[7]. In addition, many other growth factors that are not vascular endothelium-specific are also required for blood vessel formation, such as members of the platelet-derived growth factor or transforming growth factor-β families, although these factors also have critical roles for many other systems as well[8–10]. Furthermore, there are myriad other gene products — ranging from transcription factors to members of the Notch family — that have been shown crucial for vessel formation[8]. In an attempt to do justice to the topic, this review will focus only on the vascular endothelium-specific growth factors, and how they are involved in vessel formation.

The recent explosion in identifying and characterizing physiological regulators of blood vessel growth demands re-evaluation of therapeutic efforts aimed at regulating blood vessel growth — whether it be promoting vascular ingrowth to replenish ischaemic tissue, blocking vessel growth in order to blunt tumours, or repairing damaged and leaky vessels during inflammation or other pathological settings. The privilege of hindsight makes some of the bold, early therapeutic efforts directed towards ischaemic disease, based on random delivery of a single growth factor to grow an entirely new functional network of vessels, now appear somewhat naive and even misguided. On the other hand, recent insights continue to support the notion that blockade of even a single growth factor might limit disease-induced vascular growth, with the most compelling evidence supporting approaches based on blockade of VEGF. Furthermore, recent advances indicate previously unanticipated clinical applications for vascular growth factors, such as the use of angiopoietin-1 (Ang1) for the repair of damaged and leaky vessels.

Vasculogenesis and angiogenic remodelling

Vessel formation can occur by a number of different processes[4]. Early in development, vessel formation occurs by a process referred to as vasculogenesis (Fig. 2, stage A), in which endothelial cells differentiate and proliferate *in situ* within a previously avascular tissue, and then coalesce to form a primitive tubular network. This primary network includes some of the major vessels in the embryo, such as the aorta and major veins, as well as a honeycomb-like plexus connecting these major vessels. Angiogenic remodelling refers to the process by which this initial network is modified — through both pruning and vessel enlargement — to form the interconnecting branching patterns characteristic of the mature vasculature (Fig. 2, stage B). During this time, vessel walls also mature, as endothelial cells integrate tightly with supporting cells (such as smooth muscle cells and pericytes) and surrounding matrix (Fig. 2, stage C). A different process, referred to as angiogenic sprouting, involves the sprouting from existing vessels into a previously avascular tissue. In some cases, it seems as if mature vessels must first be destabilized to allow for subsequent sprouting (Fig. 2, stages D, F); once again, vessels formed by sprouting are initially immature and must further develop. Angiogenic sprouting is responsible for vascularizing certain structures during normal development, such as the neural tube or the retina, and for most new vessel formation in the adult. Destabilization of vessels can also apparently lead to vascular regression (Fig. 2, stage E), as described below.

Emerging model of vascular formation

Recent insights have led to a model of vascular formation that attempts to incorporate the known vascular-specific growth

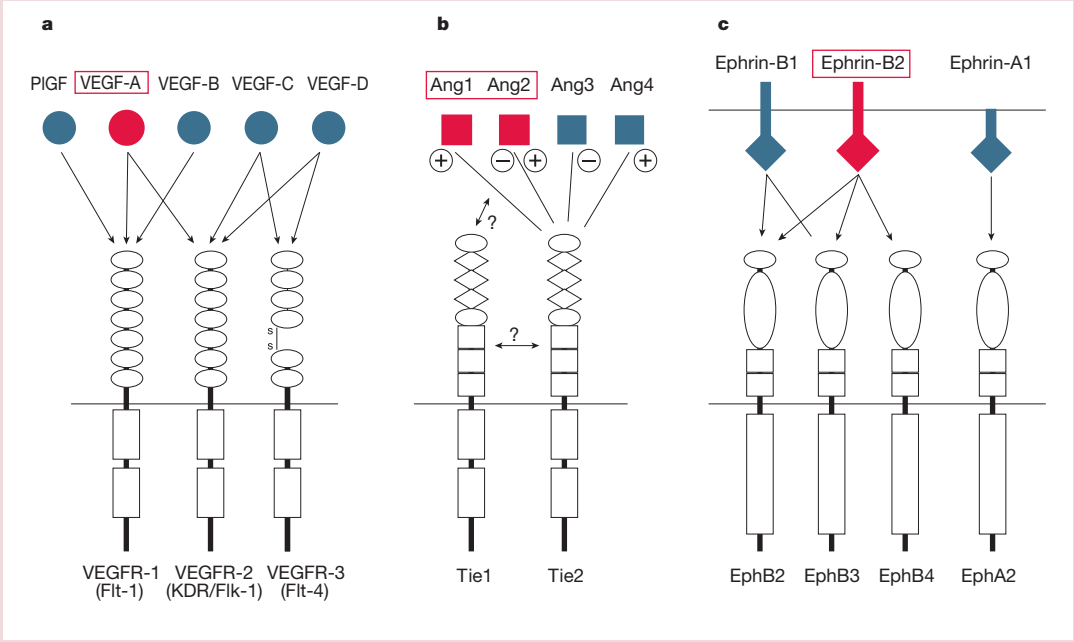

Figure 1 Schematic representation of three families of vascular growth factors and their receptor interactions. **a**, VEGFs; **b**, angiopoietins; **c**, ephrins. The four factors that are discussed in detail in this review are highlighted in red. In **b**, '+' or '–' indicates whether the particular angiopoietin activates or blocks the Tie2 receptor, whereas '?' indicates that a potential interaction has not yet been confirmed experimentally. In **c**, only those members of the large ephrin ligand family (and only their counterpart Eph receptors) that have been implicated in vascular growth are shown.

factors[7,11–14], and the details of this model will be a major subject of this review. According to this model, the first characterized vascular-specific growth factor, VEGF, maintains its position as the most critical driver of vascular formation, as it is required to initiate the formation of immature vessels by vasculogenesis or angiogenic sprouting (Fig. 2, stages A, F), during development as well as in the adult. Ang1 and ephrin-B2 are subsequently required for further remodelling and maturation of this initially immature vasculature (Fig. 2, stages B, C), with ephrin-B2 being particularly important in distinguishing developing arterial and venous vessels, as will be discussed in more detail below.

Following vessel maturation, Ang1 seems to continue to be important in maintaining the quiescence and stability of the mature vasculature (Fig. 2, stage C). Disruption of this stabilizing signal coincides with reinitiation of vascular remodelling in the adult — as occurs in the adult female reproductive system or in tumours (Fig. 2, stage D, and see below). Such de-stabilization seems to involve the autocrine induction — by the endothelium to be remodelled — of a natural antagonist of Ang1, termed Ang2 (Fig. 2, stage D). VEGFs, angiopoietins and ephrin-B2 apparently recapitulate their developmental roles during vascular remodelling in the adult, and administration of individual factors to the adult allows them to reprise these roles but not to trigger the entire process (see below). Thus VEGF administration can initiate vessel formation in adult animals, but by itself promotes formation of only leaky, immature and unstable vessels. In contrast, Ang1 administration seemingly further stabilizes and protects the adult vasculature, making it resistant to the damage and leak induced by VEGF or inflammatory challenges. Altogether, it is becoming clear that precise understanding of the normal developmental roles of the VEGFs, the angiopoietins and the ephrins will greatly aid in understanding how to manipulate these growth factor systems for therapeutic benefit.

VEGF, its relatives, and their receptors

VEGF was initially defined, characterized and purified for its ability to induce vascular leak and permeability, as well as for its ability to promote vascular endothelial cell proliferation[1,2]. Thus, it was originally termed vascular permeability factor as well as VEGF. Although most research efforts have focused on its growth-promoting ability, recent findings are once again highlighting its potent permeability-inducing effects, and in particular their role in disease. Other members of the VEGF family were identified based on their homology to VEGF[3]. The various members of the VEGF family have overlapping abilities to interact with a set of cell-surface receptors[3] that trigger responses to these factors (Fig. 1a). The main receptors that seem to be involved in initiating signal transduction cascades in response to the VEGFs comprise a family of closely related receptor tyrosine kinases consisting of three members now termed VEGFR-1 (previously known as Flt-1), VEGFR-2 (previously known as KDR or Flk-1) and VEGFR-3 (previously known as Flt-3). In addition, there are a number of accessory receptors such as the neuropilins[15] which seem to be involved primarily in modulating binding to the main receptors, although roles in signalling have not been ruled out.

VEGFR-2 seems to mediate the major growth and permeability actions of VEGF, whereas VEGFR-1 may have a negative role, either by acting as a decoy receptor or by suppressing signalling through VEGFR-2. Thus, mice engineered to lack VEGFR-2 fail to develop a vasculature and have very few endothelial cells[16], whereas mice lacking VEGFR-1 seem to have excess formation of endothelial cells which abnormally coalesce into disorganized tubules[17]. Mice engineered to express only a truncated form of VEGFR-1, lacking its kinase domain, appear rather normal, consistent with the notion that the primary role of VEGFR-1 may be that of a decoy receptor[18]. VEGFR-3 may be important during blood vessel development, but is most unique based on its expression on lymphatic vessels, for which it seems to be critical[19]. The first VEGF relative identified is known as placental growth factor (PlGF), and until recently little was known about its normal function, in part because mice engineered to lack PlGF were overtly normal[8,20]. Recent findings indicate that adult mice lacking PlGF exhibit deficiencies in certain models of adult vascular remodelling, raising the interesting possibility that the activity of PlGF may be limited to these settings[8]. VEGF-C, based on its ability to bind the lymphatic-specific VEGFR-3, seems to be important for lymphatic development, and transgenic overexpression of VEGF-C leads to lymphatic hyperplasia[21]. Mice lacking VEGF-B are overtly normal and fertile, but their hearts are reduced in size, suggesting that VEGF-B may have a role in coronary vascularization and growth[22]. Little is known about the normal physiological role of VEGF-D[3].

VEGF must be well regulated

Compared to its more recently discovered relatives, much more is known about VEGF. It is now quite clear that VEGF is such a potent and critical vascular regulator that its dosage must be exquisitely regulated in spatial, temporal and quantitative manner to avoid vascular disaster. Disruption of both VEGF alleles in mice mimicks

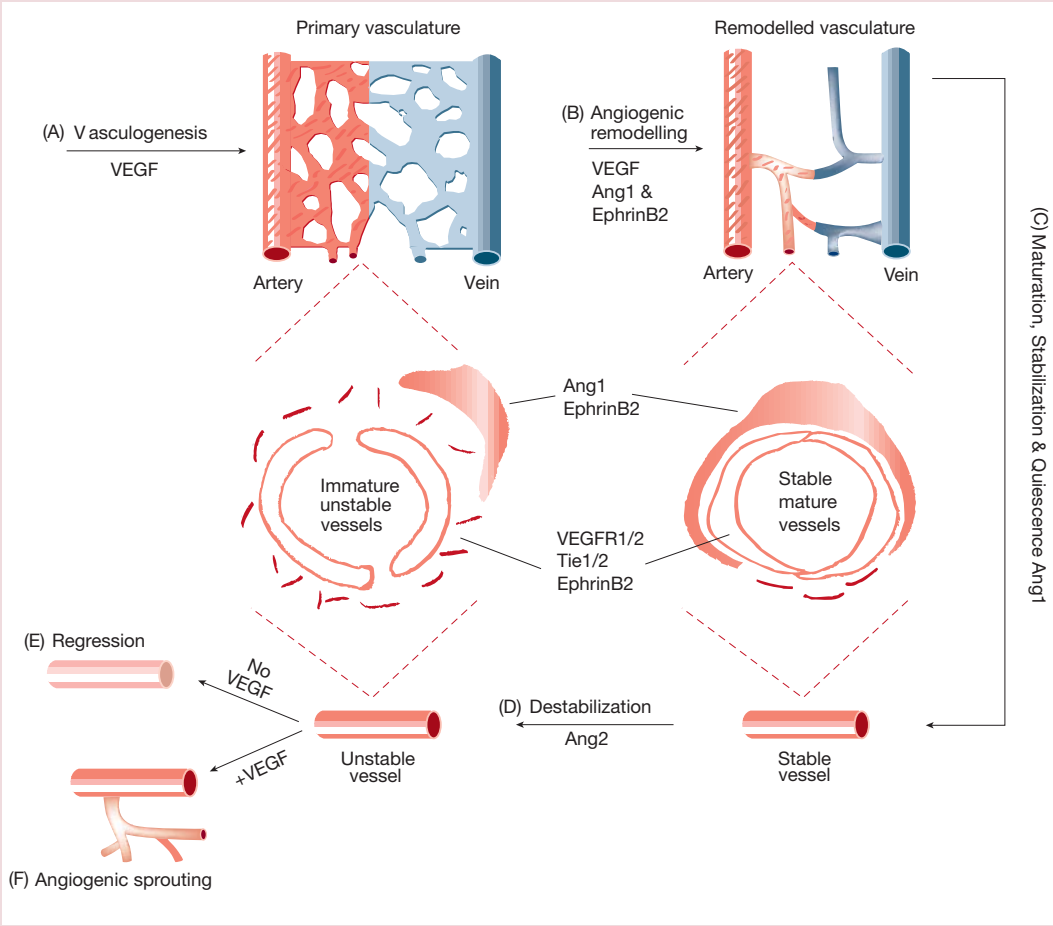

Figure 2 Schematic representation of the roles of VEGF, Ang1, Ang2 and ephrin-B2 during vessel formation. The processes include vasculogenesis (stage A), angiogenic remodelling (B), stabilization and maturation (C), destabilization (D), regression (E) and sprouting (F), as described in detail in the text. An attempt is made to assign the indicated vascular growth factors to the various processes, and to indicate their expression patterns. Although not noted in the figure, expression of ephrin-B2 marks arterial vessels from the earliest developmental times.

knockout of VEGFR-2, resulting in almost complete absence of a vasculature[23,24]. Disruption of even a single VEGF allele in mice leads to embryonic lethality due to severe vascular abnormalities, providing perhaps the only example of embryonic lethality due to a simple half-dosage effect[23,24]. Even more subtle alterations in VEGF expression during embryonic development result in profound abnormalities, leading to embryonic or early post-natal death[25,26]. VEGF continues to be critical during early post-natal growth and development, as evidenced by post-natal VEGF inactivation using Cre-loxP-mediated VEGF gene deletion, or by administration of a soluble VEGF receptor that effectively blocks VEGF action[27]. Although VEGF inactivation is lethal during the first few post-natal weeks, VEGF inactivation in older animals is much less traumatic, seemingly affecting only those structures that continue to undergo vascular remodelling, such as bone growth plates or ovarian corpus lutei[27–29]. Thus, VEGF does not seem to have a continuous maintenance function for much of the adult vasculature.

The most elegant demonstration of the need for exquisite VEGF regulation involves retinal vascularization, which occurs post-natally in rodents. Angiogenic sprouting into the initially avascular and hypoxic rodent retina depends upon its VEGF expression[30–32]. Any perturbation of normal VEGF expression patterns destroys retinal vascularization patterns, with dire results for retinal function; subsequent restoration of VEGF expression does not correct the problem, but rather exacerbates it. A simple way to perturb VEGF expression involves exposing post-natal rodents to a brief period of hyperoxia[31,33,34], which transiently suppresses retinal VEGF, resulting in cessation of vessel growth and even causing vascular regression[31,33,34]. When the rodents are returned to normoxia, the now undervascularized retina becomes hypoxic, causing an abnormal burst of VEGF, which promotes robust new angiogenesis, but of haemorrhagic and leaky vessels growing in totally abnormal patterns that wreak havoc upon the retina. This model reflects the ability of oxygen therapy in premature infants to cause retinopathy of prematurity, and shows the need for precise regulation of VEGF. Similarly, diabetic retinopathy initiates with damage and loss of healthy vessels, followed by retinal hypoxia and resulting VEGF induction, once again leading to an abnormal angiogenic response with leaky and haemorrhagic vessels[35,36]. These findings show that inappropriate induction of VEGF, in the absence of the entire angiogenic programme, leads to formation of immature and leaky vessels that cause disease. These findings also show that tissue hypoxia cannot necessarily induce a useful angiogenic response.

Consistent with the above findings concerning the devastating consequences of unregulated VEGF expression, several studies have delivered excess VEGF to adult tissues — to adult muscle using retrovirally engineered myoblasts[37], to skin using transgenic or adenoviral delivery[38–41], or to whole animals using acute adenoviral delivery[42] — and found that leaky and haemorrhagic vessels were formed, often associated with an inflammatory response, resulting in pronounced tissue swelling and oedema.

The angiopoietins and their Tie receptors

Despite its requisite role in vascular formation, VEGF must work in concert with other factors. The angiopoietins (Fig. 1b) seem to be some of VEGF's most important partners (Fig. 2). The angiopoietins were discovered as ligands for the Ties, a family of receptor tyrosine kinases that are as selectively expressed within the vascular endothelium (despite expression in some other cells, such as in the haemopoietic lineage) as are the VEGF receptors[43–47]. There are now four definitive members of the angiopoietin family, although Ang3 and Ang4 may represent widely diverged counterparts of the same gene locus in mouse

and man[12,48,49]. All of the known angiopoietins bind primarily to Tie2, and it is unclear whether there are independent ligands for the second Tie receptor, Tie1, or — as currently seems more likely — whether the known angiopoietins can in some way or under some circumstances also engage Tie1, perhaps as a second component in a heteromerized complex. The rest of this review will deal only with Ang1 and Ang2, since little more can be said at this time about Ang3 and Ang4.

Ang1 stabilizes vessel walls

The most important insights into the normal roles of Ang1 and its Tie2 receptor came from the analysis of mice engineered to lack these gene products[11,50,51]. Unlike mouse embryos lacking VEGF or VEGFR-2, embryos lacking Ang1 or Tie2 develop a rather normal primary vasculature. However, this vasculature fails to undergo normal further remodelling. The most prominent defects are in the heart, with problems in the associations between the endocardium and underlying myocardium as well as in trabeculae formation, and also in the remodelling of many vascular beds into large and small vessels. In these vascular beds, as in the heart, ultrastructural analysis indicates that endothelial cells fail to associate appropriately with underlying support cells, which are the cells that provide the Ang1 protein that acts on endothelial Tie2 receptors[11]. This finding led to the suggestion that Ang1 does not supply an instructive signal that actually directs specific vascular remodelling events, but rather has more of a permissive role by optimizing the manner in which endothelial cells integrate with supporting cells, thus allowing them to receive other critical signals from their environment[11].

Transgenic overexpression of Ang1 in skin results in pronounced hypervascularization[40,52]. Although there are modest increases in vessel number, the most marked increase is in vessel size. In contrast, VEGF in similar models primarily increases vessel number[38–40]. These findings indicate that Ang1 might promote circumferential as opposed to sproutive growth. Combining transgenic Ang1 and VEGF leads to unprecedented hypervascularity resulting from increases in both vessel size and number[40]. The vascular patterns induced by the combination are still obviously abnormal morphologically, suggesting that much must be learned about exploiting even this growth factor combination in therapeutic settings so as to grow normal vessels.

In addition to their effects on vascular morphology, transgenic overexpression of Ang1 and VEGF had distinct effects on vascular function and integrity. As had been expected, VEGF led to immature, leaky and haemorrhagic vessels[38–40]. On the other hand, Ang1 led to vessels that were actually resistant to leak, whether the leak was induced by VEGF or inflammatory agents[40]. This resistance seems related to the ability of Ang1 to maximize interactions between endothelial cells and their surrounding support cells and matrix, as the Ang1 vessels were resistant to treatments that normally created holes in the endothelial cell barrier[40]. These findings indicated that Ang1 might counter the effect of VEGF on permeability, raising multiple therapeutic possibilities[40]. There are numerous disease processes — ranging from diabetic retinopathy to inflammation to brain oedema following ischaemic stroke — in which vessels become damaged and leaky, and an agent that could repair the damage and prevent the leak could have enormous therapeutic benefit. Supporting the clinical potential of Ang1, acute adenoviral administration of Ang1 to adult animals showed that Ang1 can indeed protect the adult vasculature from vascular leak, without inducing immediate changes in vascular morphology[42].

Ang2: agonist and antagonist?

Ang2 was cloned based on its homology to Ang1, and displayed similarly high affinity for Tie2, but — depending on the cell examined — Ang2 could either activate or antagonize Tie2 (ref. 12). Transgenic overexpression of Ang2 in the embryonic endothelium resulted in embryonic death due to defects resembling those of Ang1 or Tie2 knockouts, demonstrating that Ang2 could act as a Tie2 antagonist *in vivo*, at least under some circumstances[12]. This possibility became even more intriguing when Ang2 expression profiles were examined. In adult animals, Ang2 was induced in the endothelium of vessels undergoing active remodelling, such as sprouting or regressing vessels in the ovary[12,53], or in tumours[13,14,54,55] (as will be discussed in detail below). These findings, together with the possibility that Ang2 could act as a Tie2 antagonist, led to the hypothesis that Ang2 might provide a key de-stabilizing signal involved in initiating angiogenic remodelling[12–14,55]. That is, based on previous evidence that Ang1 engagement of the Tie2 receptor was constitutive in the adult vasculature and indeed necessary to maintain its quiescence (Fig. 2, stage C), it was proposed that autocrine induction of Ang2 in endothelium blocked this constitutive stabilizing influence of paracrine Ang1, allowing the endothelial cells to revert to a more plastic and destabilized state reminiscent of developing vessels (Fig. 2, stage D). Such destabilized vessels could then be prone to two fates. On the one hand, these destabilized vessels would be prone to regression in the absence of associated growth factors, as also occurs with primitive vessels during development (Fig. 2, stage E). On the other hand, they would be more sensitive to angiogenic changes induced by simultaneously available angiogenic factors such as VEGF, essentially recapitulating an early embryonic situation in which VEGF acts prior to the involvement of Ang1 (Fig. 2, stage F).

This model of Ang2 as a destabilizing signal that reverts vessels to a more plastic and tenuous state, initially developed based on observations in the remodelling ovary[12], is consistent with more recent data in tumours (see below) as well as emerging data from knockout mice lacking Ang2. One of the best characterized settings of post-natal vascular regression and remodelling in mice involves the eye, in which regression of the hyaloid vasculature encasing the lens is coupled to angiogenic sprouting that leads to vascularization of the initially avascular retina, as described above. Neither regression of the hyaloid vasculature nor vascularization of the retina occur in mice lacking Ang2 (S.J.W., R. Tzekova, Q. Wong, N.W.G., C. Suri & G.D.Y., unpublished results). These data show that Ang2 is required for some post-natal vascular remodelling events, and support the notion that Ang2 provides a key role in destabilizing the vasculature in a manner that is necessary for its subsequent remodelling. However, other defects in the Ang2-knockout mice suggest that it may in some cases also have an agonistic role. That is, it is highly expressed in the developing aortic wall, which does not develop properly in mice lacking Ang2. Similarly, lymphatic development is perturbed in these mice.

The ephrins

The Eph receptor tyrosine kinases comprise the largest known family of growth factor receptors (Fig. 1c), and use the similarly numerous ephrins as their ligands[7,56]. The ephrins are unlike ligands for other receptor tyrosine kinases in that they must be tethered to the membrane to activate their Eph receptors[7,57]. Although initially characterized in the nervous system[7,56], recent knockout studies have suggested key roles for ephrin-B2 and its EphB4 receptor during vascular development[58–60]. Mouse embryos lacking ephrin-B2 and EphB4 suffer fatal defects in early angiogenic remodelling that are somewhat reminiscent of those seen in mice lacking Ang1 or Tie2[58–60]. Moreover, ephrin-B2 and EphB4 display remarkably reciprocal distribution patterns during vascular development, with ephrin-B2 marking the endothelium of primordial arterial vessels while EphB4 marks the endothelium of primordial venous vessels[58–60]. These distributions suggested that ephrin-B2 and EphB4 are involved in establishing arterial versus venous identity, perhaps in fusing arterial and venous vessels at their junctions, and that defects in these processes might account for the early lethality observed in mouse embryos lacking these proteins[58–60] (Fig. 2, stage A).

Ephrin-B2 continues to selectively mark arteries during later embryonic development as well as in the adult, although this expression extends progressively from the arterial endothelium to the

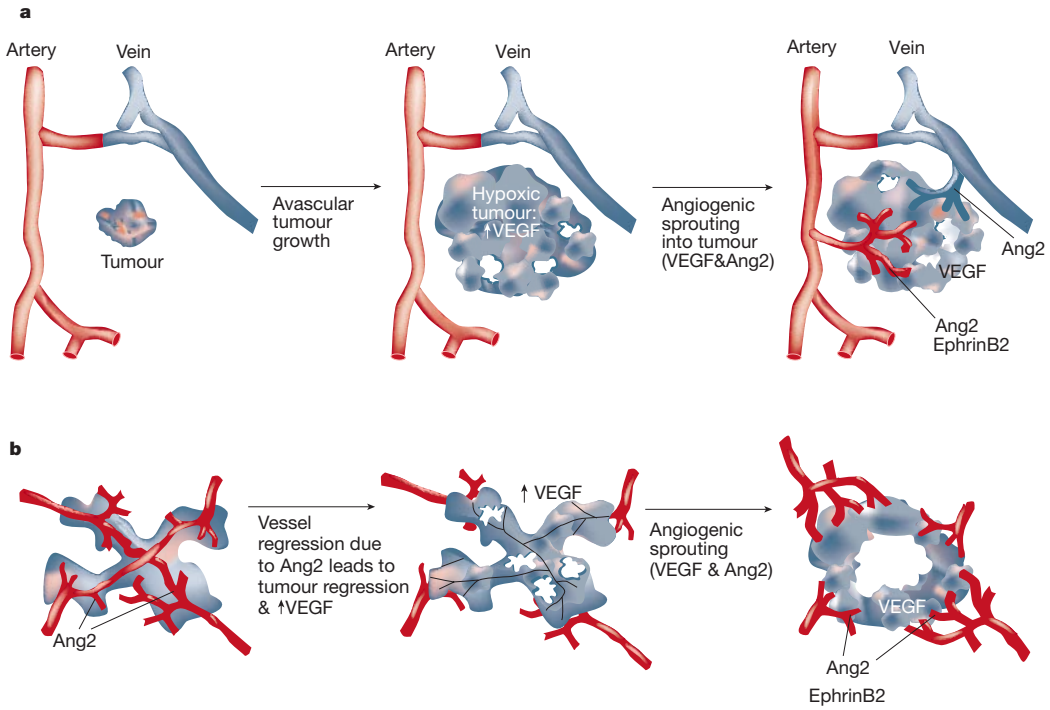

Figure 3 Models of tumour angiogenesis. **a**, Model of avascular tumour initiation contrasted with **b**, tumour initiation involving host vessel co-option. An attempt is made to assign the indicated vascular growth factors to roles in the various indicated steps in tumour development, and to indicate their expression patterns.

surrounding arterial smooth muscle and to pericytes (N.W.G. and G.D.Y., unpublished results; D. Shin and D. J. Anderson, unpublished results). Thus, ephrin-B2 is apparently not only required during the earliest stages of arterial/venous determination, but may continue to be important during the development of arteries, perhaps by regulating interactions between endothelial and smooth muscle cells involved in the formation of arterial muscular walls (Fig. 2, stage B). In adult settings of angiogenesis, as in tumours or in the female reproductive system, the endothelium of new vessels strongly re-expresses ephrin-B2 (N.W.G. and G.D.Y., unpublished results; D. Shin and D. J. Anderson, unpublished results) (Fig. 3a,b). The finding that angiogenic sprouting in the adult and in tumours involves re-expression of the ephrin-B2 arterial marker challenges existing dogma that such sprouting primarily involves venous or uncommitted vessels, and also suggests that ephrin-B2 may be important in these angiogenic settings.

VEGF and Ang2 in tumour angiogenesis

Much has been made of the notion that tumours and metastases initiate as small avascular masses, which only subsequently induce the angiogenic ingrowth that is required to allow further growth of the early tumour[61–63] (Fig. 3a). It is clear that many natural tumours initially arise in this manner, particularly primary epithelial tumours that are initially separated from underlying vessels by a basement membrane that must be broken before tumour cells can access the vasculature. In addition, many artificial model systems forcibly create initially avascular tumours by placing tumour cells in a space that is normally devoid of vessels — such as the subcutaneous space, the cornea pocket or the vitreous or the tumour window — thus requiring angiogenesis to get vessels to the tumour.

Despite all the attention directed towards avascular tumour growth, recent findings[14,55] have refocused attention on previous observations[64–66] that many tumours, and metastases in particular, do not initiate in an avascular manner (Fig. 3b). Rather, tumour cells can initially home in on and grow by co-opting existing host vessels, and thus start off as well-vascularized small tumours[13,14] (Fig. 3b, left). In response to co-option, the host vessels mount a defence — sensing inappropriate co-option, they regress, choking off the tumour and resulting in a secondarily avascular and hypoxic tumour (Fig. 3b, middle). However, successful tumours seem to overcome host vessel regression by inducing robust new angiogenesis (Fig. 3b, right). Ang2 and VEGF inductions correlate remarkably well with the above processes[13,14,55]. That is, soon after tumour co-option, host vessels start expressing high autocrine levels of Ang2; thus Ang2 is one of the earliest tumour markers described, and one of the most general because it marks co-opted vessels and not the tumour cells themselves (Fig. 3b, left). Consistent with the possibility that autocrine Ang2 expression can destabilize vessels (Fig. 2, stage D), the co-opted vessels begin to die by an apoptotic process shortly after expressing Ang2 (Fig. 3b, middle). As vessels die, the tumour becomes secondarily avascular and hypoxic, resulting in marked induction of tumour-derived VEGF (Fig. 3b, middle). These high levels of VEGF correlate with cessation of regression of the destabilized co-opted vessels, and onset of robust new angiogenesis sprouting from these vessels, allowing for tumour survival and further growth (Fig. 3b, right). Thus, in such settings, endothelial Ang2 expression seems to correlate with vessel destabilization, apparently leading to vessel regression in the absence of tumour-derived VEGF, or robust new angiogenesis following induction of tumour-derived VEGF (stage D in Fig. 2, and Fig. 3b). The possibility that tumour vessel Tie2 receptors are blocked continuously by Ang2 and thus have an imbalance towards VEGF may well explain long-standing observations that tumour vessels fail to mature, exhibit poor associations between endothelial cells and their supporting cells, and are characterized by their leaky and haemorrhagic state.

One practical prediction, which applies whether tumour growth initiates avascularly or through co-option, is that anti-VEGF therapy should ultimately blunt tumour growth. Early studies using an anti-VEGF antibody provided the first support for this notion[67]. This

has subsequently been confirmed in many laboratories using numerous approaches ranging from antibodies that bind and block VEGF, to those that bind and block VEGFR-2, to small molecules that block the activity of the VEGF-2 kinase domain, to genetic ablation of VEGF in tumour cells[68]. Thus, blockade of VEGF represents the best validated and most compelling anti-angiogenesis approach described so far.

Perspectives and therapeutic possibilities

There are many critical growth factors involved in the physiological regulation of blood vessel formation, and the actions of these molecular players must be very carefully orchestrated in terms of time, space and dose so as to form a functioning vascular network. The complexity of the process makes ongoing therapeutic efforts aimed at growing new vascular networks to treat ischaemic disease, using random delivery of single agents, appear somewhat naive with the potential to cause more harm (by forming malfunctioning vessels prone to leak and haemorrhage) than good. In their defence, these efforts were initiated years ago when much less was understood about the process of vascular formation. Recent failures of large, well-controlled clinical trials for cardiac ischaemia using delivery of single agents (either VEGF or FGF)[69,70] raises the question of why these trials failed despite claims of success in animal studies and earlier, smaller (and uncontrolled) human trials. As recently discussed[68], this may be due to the failure of animal models to correctly model the human disease, as well as the need for blind approaches in both animal and human studies to overcome investigator bias when measuring subjective endpoints, together with the requirement for placebo controls in settings where there is a marked placebo effect in subjective patient reports of their own condition.

Although the complexities of vascular formation create significant challenges for those trying to grow vessels for therapeutic use, these same complexities may work in favour of therapeutic approaches aimed at blocking vessel growth. That is, blockade of many different molecular players may all result in the blunting of vessel formation. There is no doubt that VEGF is the best-validated target for anti-angiogenesis therapies, based on overwhelming genetic, mechanistic and animal efficacy data. Despite the attention devoted to a number of other putative angiogenic antagonists for use in cancer (for example, endostatin, angiostatin and antithrombin)[71–73], most of these antagonists have yet to be characterized from a mechanistic and genetic point of view. Thus, they lack defined mechanisms of action, and cannot be placed within existing models of molecular angiogenesis using genetic approaches. Also troubling is that these agents seem to work whether they are delivered as properly folded proteins or as denatured aggregates[72].

Recent efforts also indicate as yet unimagined applications for vascular growth factors. For example, the possibility that Ang1 may help prevent or repair damaged and leaky vessels offers therapeutic hope for an assortment of unmet clinical needs, such as in diabetic retinopathy, acute macular degeneration, ischaemia/reperfusion injury (which can occur after strokes and in acute respiratory distress syndrome), or in inflammatory settings[40,42]. The continued discovery and characterization of the molecular factors that regulate vessel formation will lead to additional unexpected therapeutic opportunities, as well as to the refinement of current therapeutic approaches aimed at growing or blocking vessel formation. □

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