Mechanisms of Tumor Angiogenesis

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Abstract

Tumor angiogenesis, the process by which blood vessels penetrate and grow in the tumor microenvironment, is essential for oxygen and nutrient supply and hence constitutes a key player for the survival of solid neoplasms. Different mechanisms of angiogenesis are developed during tumor progression such as vasculogenesis, sprouting angiogenesis, intussusception, and vasculogenic mimicry. The transition from a quiescent vasculature to an actively growing one follows a series of synchronic events and is finely tuned by a wide array of molecules and positive and negative regulators of angiogenesis. Beginning with blood vessel sprouting and endothelial cell proliferation, followed by vessel navigation, remodeling, stabilization, and maturation, and finishing with blood vessel regression, the main molecular factors involved in the progression of each step are profoundly detailed. When the balance between positive and negative regulators of angiogenesis is shifted toward proangiogenic molecules, the quiescent vasculature becomes activated and initiates the angiogenic state of tumor development. The role of intratumoral hypoxia as a potent activator of the angiogenic switch, its regulation, and a detailed description of normal and aberrant tumor vessels are also provided. The understanding of the foundations of these mechanisms is crucial for an effective therapeutic targeting of the angiogenic process.

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Introduction

Tumor angiogenesis, the process by which blood vessels penetrate and grow in the tumor microenvironment, is essential for oxygen and nutrient supply and, therefore, for survival of solid neoplasms. Taking into account its role in tumor progression and metastasis, angiogenesis is one of the widely acknowledged hallmarks of cancer (Hanahan and Weinberg 2011). Since the pioneering studies by Folkman established the initial foundations of tumor angiogenesis nearly 40 years ago, research in the field has reached a significant level of maturity, allowing detailed description of the intricate processes of pathological vessel proliferation (Folkman et al. 1971). In this chapter, we compiled the basic mechanisms of blood vessel formation and the biological roles of the main molecular regulators involved in tumor angiogenesis. Furthermore, we describe in detail the "angiogenic switch" occurring in malignant neoplasms, focusing on the role of the complex tumor microenvironment, the differences between physiological and pathological angiogenesis, and the abnormalities found in tumor vasculature.

Molecular Mechanisms of Angiogenesis

Mechanisms Involved in Blood Vessel Formation

Vessels can be described as highly branched and ordered tubular networks that allow transport of gases, nutrients, signaling molecules, and cells. Beyond their nutritive function, blood vessels provide instructive trophic signals essential for organ morphogenesis and the development of every complex organism (Carmeliet and Jain 2011). While the luminal side of all types of blood vessels, including arteries, veins, and capillaries, is formed by a lined monolayer of endothelial cells, vessels are covered in the outside by a basement membrane followed by a layer of mural accessory cells (pericytes and vascular smooth muscle cells (SMCs)).

Prototypically, vasculogenesis and sprouting angiogenesis are the two main mechanisms responsible for neovascularization (Fig. 1a, b). Sprouting angiogenesis is defined as the formation of new vascular structures from a preexisting vessel, while vasculogenesis refers to de novo blood vessel formation due to vascular progenitor cell differentiation. Both mechanisms contribute to the formation and remodeling of the vessel network during development, remain nearly inactive in the adult body, and are only reactivated to allow tissue repair or in the event of a disease.

Beyond vasculogenesis and sprouting angiogenesis, other less frequent mechanisms have recently been reported in neoplasms, including vessel co-option, intussusception, and vasculogenic mimicry (Fig. 1c, d). In most cases, mutual exclusivity between different mechanisms does not exist; indeed, they simultaneously participate both in physiological and pathological angiogenesis.

Vasculogenesis

Vasculogenesis (Fig. 1a) has been extensively described in the early stages of vascular development. It was not until 1997 that the growth of new blood vessels in postembryonic tissues was considered to occur also through vasculogenesis (Asahara et al. 1997). Moreover, compelling evidence suggests that bone marrow-derived circulating endothelial progenitor cells (EPCs) contribute to the induction and progression of postnatal neovascularization (Kopp et al. 2006). EPCs, also known as angioblasts, are circulating cells that express several endothelial lineage-specific markers such as CD34, CD31, VEGFR-2, and Tie-2.

Together with EPCs, mature circulating endothelial cells derived from blood vessel renewal

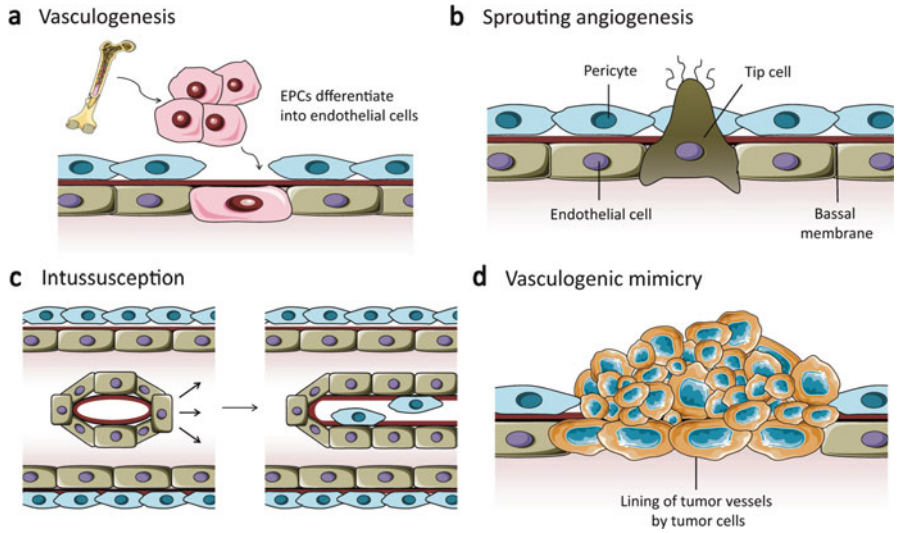

Fig. 1 Mechanisms involved in blood vessel formation. In normal tissues vessels can grow by the recruitment of bone marrow-derived endothelial progenitor cells (EPCs) that differentiate into endothelial cells (**a**), by sprouting angiogenesis (**b**), or by intussusceptive microvascular growth, a process that involves vessel splitting (**c**). Additionally, tumor cells use other mechanisms such as vasculogenic mimicry, during which vessel-like structures are lined by tumor cells (**d**)

also take part in adult vasculogenesis. Moreover, in order to facilitate incorporation of those circulating endothelial adult and progenitor cells and to sustain the stability of the nascent vasculature, hematopoietic stem and progenitor cells are recruited (Kopp et al. 2005). Several chemokines, cytokines, and growth factors that are produced in response to tissue ischemia and tumor growth promote mobilization and recruitment of EPCs. For instance, tumor cells produce proangiogenic factors, such as VEGF, and cytokines (i.e., stromal-derived factor-1) that recruit bone marrow-derived dendritic cells and promote their proliferation and differentiation. The precise mechanisms governing mobilization of precursor cells from the bone marrow and their posterior homing to neo-angiogenic spots are yet not fully understood.

Mobilization of EPCs starts following the activation of the matrix metalloprotease 9 (MMP9) in the osteoblastic zone by the tumor-derived proangiogenic factors. The activation of MMP9 triggers the proteolytic processing of membrane-bound Kit ligand to its soluble active form. The soluble Kit ligand is a stem cell-active cytokine that promotes migration of hematopoietic and endothelial progenitor cells to the vascular zone of the bone marrow and their posterior release in the circulation (Heissig et al. 2002).

When homed, endothelial progenitor cells can be incorporated into the endothelial monolayer of a vessel or recruited to angiogenic sprouts. At this step, P-selectin, E-selectin, and integrins are critical for the correct adhesion of EPCs to the vessel walls (Deb et al. 2004). Differentiation into mature endothelial cells is mainly mediated by VEGF and physically contributes to vessel size since it increases the diameter of the vessel. In addition to the physical contribution of the EPCs to the newly formed vessels, EPCs support angiogenesis by a paracrine mechanism that includes the release of proangiogenic factors in neovascularization sites of the tumor stroma or ischemic tissues (Urbich and Dimmeler 2004).

Formal demonstration of the contribution of vasculogenesis to tumor angiogenesis has been achieved through the use of knockout mice for inhibitors of differentiation factors. These factors initiate the mobilization of bone marrow-derived dendritic cells to angiogenic sites in the tumor. Genetic ablation of inhibitors of differentiation factors disrupted tumor vascularization and

blocked tumor growing as a consequence of an impaired angiogenesis (Benezra et al. 2001). The restoration of the mobilization capability of bone marrow-derived cells by transplantation of wild-type bone marrow rescued tumor neovascularization and growth in these knockout mice.

The contribution of vasculogenesis to tumor vessel formation ranges from 0.1 to 50% depending on the experimental cancer model and tumor type. Lymphomas and hematological tumors are more dependent on bone marrow-derived dendritic cells in comparison to other tumors. The knowledge obtained from the study of tumor vasculogenesis has sparkled the development of new applications at the clinical setting. The potential existing correlation between the levels of endothelial progenitor and circulating cells in blood and the outcome in patients undergoing an anti-angiogenic treatment could be used as a cellular biomarker for monitoring the response to antitumor therapy (Bertolini et al. 2003).

Recently, the study of tumor vasculogenesis has shifted from its role in primary tumor growth toward the study of its implication in dissemination and metastasis. In addition to angiogenesis activation, EPCs are able to promote metastatic growth by homing into metastatic sites prior to tumor cell arrival (Kopp et al. 2005). The spontaneous secretion of SDF-1 by EPCs generates a gradient that could promote the extravasation and development of the pre-metastatic niche (Jin et al. 2012).

Sprouting Angiogenesis

Sprouting angiogenesis is the best described mechanism used by tumors to promote their own vascularization by inducing new capillary sprouts from preexisting host capillaries (Fig. 1b). The mechanism involves several well-defined sequential steps and an extensive interplay between soluble factors, extracellular matrix (ECM) components, and cells (Paku and Paweletz 1991). At the onset of sprouting angiogenesis, there is a destabilization of the endothelial-pericyte contacts which are essential for vessel integrity and quiescence maintenance. Endothelial and mural cells share a complex basement membrane that forms a protective coat around endothelial tubules, preventing resident endothelial cells from leaving their location. Once destabilized, endothelial cells undergo an endothelial-mesenchymal transition that enhances their migratory, invasive, and proliferative properties. These activated cells are then able to degrade the surrounding ECM and the basement membrane by activated proteases (such as MMPs), opening the path for guided migration and proliferation. Vessel lumen is then formed by polarization of the migrating endothelial cells (Ferrara et al. 2003). At this step, an immature blood vessel is formed, and the opposite mesenchymal-endothelial transition directs the reversal of the proliferative state of endothelial cells to the previous resting state. In detail, the return to quiescence is retrieved by synthesis of new basement membrane and pericyte and mural cell recruitment (Jain 2003). This latter step is known as vessel maturation and is characterized by a lack of tumor angiogenesis.

Recent studies have remarked the specialization that endothelial cells undergo in order to enroll the angiogenic process. To achieve locally needed vascular patterns, the multistep process of sprouting angiogenesis requires functional specialization of endothelial cells in the angiogenic sprout together with vascular guidance cues that allow regulation of the topological extension of the forming vessel. The main group of signaling pathways essential for the initial morphogenetic events includes VEGF and Notch (Iruela-Arispe and Dvorak 1997). Attracted by proangiogenic signals, the phenotype of the sprouting endothelial cells gains an invasive and motile behavior, protease activation, cell-cell contact remodeling, and apical-basal polarity reversal. The endothelial cells that are selected to guide the sprouting are located at the tip of the angiogenic sprout and are, therefore, commonly known as “tip cells.” These leading cells respond to VEGF signaling by dynamically extending large filopodia in order to sense and guide the forming vessel along the forthcoming vascular bed. Recent studies show similarities between the molecular regulation of guidance cues of neural and endothelial cells.

These specialized nonproliferative endothelial cells also release different molecular signals that promote the recruitment of mural cells like pericytes, SMCs, and fibroblasts, guaranteeing the stabilization of emerging vessels.

Apart from tip cells, endothelial cells can specialize to highly proliferating cells located in the stalk of the angiogenic sprout. The proliferative potential of the "stalk cells," capable of forming tubes and branches, assures the expansion of the structure. In contrast to tip cells, stalk cells extend fewer filopodia but proliferate to support vessel elongation in response to VEGF-A (Gerhardt et al. 2003). Tip cells are migratory and polarized, whereas stalk cells proliferate during the extension of the sprout and form the new vascular lumen cell population. Stalk cells also produce components of the basement membrane and establish adherens and tight junctions with neighboring cells, thus strengthening the integrity of the new sprout and luminal-abluminal polarity (Dejana et al. 2009).

In order to form a new vascular connection, the tip cell phenotype must be switched off after connecting with the tips of other sprouts or existing vessels. Tip cells build vessel loops by anastomosing with cells from neighboring sprouts. The sprouting process is rehearsed until proangiogenic signals decrease, a new basement membrane is formed, quiescence is reestablished, and VEGF levels dampen (Leslie et al. 2007). In the transition from active sprouting to quiescence, endothelial tip cells adopt a "phalanx"-like phenotype, with features of lumenized, nonproliferative, and immobile cells (Bautch 2009). Eventually, maturity and stabilization are achieved through the generation of a lumen and the migration of pericytes along the basement membrane until vessels are covered, initiating blood flow and allowing perfusion.

The correct extension and morphology of the nascent vessels is regulated by the precarious balance between tip cell navigation and stalk cell proliferation. The phenotypic specialization to tip or stalk cell depends on the balance between proangiogenic factors and endothelial cell proliferation suppressors (Geudens and Gerhardt 2011). The abnormal vascular structures generally found in cancer are a consequence of the imbalance between these two processes. The biological nature of the molecules and signals that initiate the angiogenic cascade from the initial destabilization to the formation of mature and functional vasculature has been profoundly studied and characterized and is further described in the following sections of this chapter.

Alternative Ways of Blood Supply in Tumors

Although sprouting angiogenesis is regarded as the most important contributing mechanism to tumor angiogenesis, there are alternative processes such as intussusceptive microvascular growth (IMG; Fig. 1c) and vasculogenic mimicry (VM; Fig. 1d). These nonconventional pathways introduce an additional level of complexity to the understanding of tumor vascularization mechanisms.

Intussusception

Intussusception (IMG) is a variant of angiogenesis that was first observed in postnatal remodeling of lung capillaries (Caduff et al. 1986; Fig. 1c). This developmental intravascular growth mechanism is based on the splitting of preexisting vessels into two new vessels after the formation of a transvascular connective tissue column, called tissue pillar, into the lumen of the vessel.

In contrast to sprouting angiogenesis, IMG is a fast process that can occur within hours, or even minutes, since it does not require proliferation of endothelial cells. Even though sprouting has the advantage of being invasive and permits joining vascular gaps, it is a slow process that highly relies on endothelial cell proliferation and basal membrane degradation. In IMG, the remodeling of endothelial cells is a consequence of their volume increase and narrowing. It is believed that IMG happens after vasculogenesis or sprouting angiogenesis in order to expand the capillary plexus without a high metabolic demand (Burri et al. 2004).

The onset of IMG is the "touching contact" of endothelial cells from opposite walls. Following the transendothelial cell bridge created from the touching spot, interendothelial junctions are

reorganized, and the endothelial bilayer is performed. Then, the interstitial pillar forms to reinforce the bridges, and mural cells are recruited to cover this new interstitial wall. Due to their contractile features, pericytes are believed to be the main triggers of this phase. Finally, the interstitial pillars widen, endothelial cells retract, and two independent vessels are created (Burri et al. 2004). By IMG, a large vessel can split into smaller vessels.

Even though the precise mechanism of intussusception is poorly understood, there are some key mediators that influence pillar formation. Alterations in blood flow dynamics, changes in shear stress on endothelial cells sensed and transduced by molecules such as CD31, modifications of wall stress on the pericytes, and absences of VEGF are some among the possible factors driving biochemical cascades that result in cytoskeletal rearrangements and intussusception initiation (Djonov and Makanya 2005). For instance, it was observed that human melanomas bare a high number of intraluminal tissue folds together and that a correlation between VEGF and intussusceptive angiogenesis exists in these tumors (Ribatti et al. 2005). In this context, sprouting angiogenesis inhibition might stimulate the process of intussusceptive angiogenesis. Since IMG can only occur on existing vessel networks, its most important contribution is its ability to increase the density and complexity of tumor microvessel networks already established by sprouting angiogenesis. Moreover, IMG also provides additional surface for further sprouting angiogenesis. IMG has been observed in colorectal, melanoma, and mammary tumors (Dome et al. 2007).

Vasculogenic Mimicry

Vasculogenic mimicry (VM; Fig. 1d) describes the ability of some tumor cells to dedifferentiate into multiple cellular phenotypes, obtaining endothelial-like properties (Maniotis et al. 1999). This process leads to the creation of de novo vasculogenic-like matrix embedded networks. The new perfusable vascular-like structures are composed of red blood cells and plasma and contribute to blood circulation (Frenkel et al. 2008). Endothelial cells undergoing VM mimic the pattern of embryonic vascular network, possibly providing tumor cells with a secondary circulation system, independent from angiogenesis.

Molecular analysis comparing highly invasive and noninvasive melanoma cells derived from the same patient suggests a genetic reversion of the aggressive cells to an embryonic-like cell fate and increased cell plasticity. The undifferentiated phenotype includes the expression of endothelium-associated genes such as VE-cadherin and Ephrin-A2, among others (Hendrix et al. 2003). The activation of transmembrane metalloproteinases, release of ECM components, and low levels of oxygen are known to promote VM (Seftor et al. 2005). Although the exact mechanism remains to be unraveled, it involves deregulation of the lineage-specific phenotype and the concomitant transdifferentiation to endothelial-like cells.

VM occurs mainly in aggressive tumors such as melanomas, and even though their occurrence is relatively rare within tumors, the presence of VM-associated patterned networks in tumor tissue correlates with an increased risk of metastasis and poor clinical outcome (Sun et al. 2004). Until now, VM has been described in melanomas, breast carcinoma, prostatic carcinoma, hepatocellular carcinoma, bladder carcinoma, and other aggressive tumors. Increasing evidence demonstrates that tumor cell-dominant VM has a key role in tumor progression and metastasis.

Biological Processes Involved in Angiogenesis

As stated above, in order to build new and fully functional vascular structures, several biological processes must be accurately regulated. Different pro- and anti-angiogenic regulators are needed to perform and control each specific step of the angiogenic cascade. Multifunctionality among those factors is one of their relatively common features, empowering some molecules with the outstanding ability to either activate or inhibit vascularization. On account of simplification, we will describe the molecular regulation of the main biological processes involved in vessel formation:

sprouting and proliferation (Fig. 2a), guidance and navigation (Fig. 2b), stabilization, maturation, and remodeling (Fig. 2c), and regression.

Sprouting of Blood Vessels and Endothelial Cell Proliferation

Endothelial cells in the adult organism remain quiescent and are protected against external insults by autocrine maintenance signals. These cells form a monolayer of phalanx cells and are interconnected by junctional molecules like VE-cadherin and claudins. The surface of the endothelium monolayer is covered by pericytes, which suppress endothelial cell proliferation, and releases pro-survival signals such as VEGF and Ang-1. When quiescent vessels sense an angiogenic signal such as VEGF, fibroblast growth factors (FGFs), or chemokines, released by hypoxic, inflammatory, or tumor cells, sprouting angiogenesis is triggered (Fig. 2a).

VEGF-VEGFR signaling pathway is established as the master regulator of the formation and remodeling of vasculature. VEGF ligands are the prototypical, multifunctional pro-angiogenic factors that control endothelial cell proliferation and migration and regulate cardiovascular system homeostasis (Carmeliet and Jain 2011). Until now, VEGF molecules are allegedly the most potent vascular permeability factors and vasodilatation inductors. Besides, endothelial precursor cell differentiation and vascular guidance of tip cells are also controlled by VEGF family.

The VEGF family is composed of six different members: VEGF-A (referred herein as VEGF), VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placental growth factor (PlGF). Contrary to other angiogenic superfamilies, the VEGF family distinguishes itself by the nonredundant role of its members. At least five different isoforms of VEGF are generated by alternative splicing of a single gene: $VEGF_{121}$, $VEGF_{145}$, $VEGF_{165}$, $VEGF_{189}$, and $VEGF_{206}$. These isoforms differ in their binding affinity for heparin, which might affect their diffusion rates in the extracellular space. The predominant splicing variant of VEGF both in normal and tumor cells is the $VEGF_{165}$ isoform. All the members of the VEGF family and their corresponding receptors usually work as homodimers, although heterodimers between different members have also been reported (DiSalvo et al. 1995).

During tumor progression, VEGF levels are controlled through diverse mechanisms such as hypoxia, oncogene activation, loss of tumor suppressors, cytokines, and growth factors levels. There is also an autocrine production of VEGF by endothelial cells which is critical for vascular homeostasis and early stages of vascular development (Lee et al. 2007). In general terms, paracrine VEGF, released by tumor or stromal cells, increases vessel branching and promotes tumor vessel abnormalities (Stockmann et al. 2008), whereas autocrine VEGF, released by endothelial cells, empowers vascular homeostasis. The deletion of a single allele of VEGF-A causes embryonic lethality, reinforcing the believed key role of this family in developmental vascular physiology.

There are three different tyrosine kinase-type receptors for the VEGF family: VEGFR-1 (or Flt1 in mouse), VEGFR-2 (also known as kinase insert domain-containing receptor in humans or Flk1), and VEGFR-3 (or Flt4). These receptors are principally expressed in endothelial cells (Roskoski 2008). Furthermore, certain VEGF isoforms are also able to bind to non-tyrosine kinase coreceptors such as neuropilins 1 and 2 to enhance VEGFR-2 activity. Neuropilins are best known for their interaction with semaphorin and their angiogenesis-independent function in axonal guidance (Gluzman-Poltorak et al. 2000).

VEGFR-1 has been identified as the high affinity receptor, whereas VEGFR-2 is the low-affinity receptor. While placental growth factor and VEGF-B bind to VEGFR-1, VEGF-A can bind both VEGFR-1 and VEGFR-2, VEGF-C and VEGF-D bind to VEGFR-2 and VEGFR-3, and VEGF-E binds to VEGFR-2. Binding of the ligand leads to the dimerization of the receptors; this in turn initiates the autophosphorylation of several intracellularly located tyrosine residues. The activated dimers now expose new docking sites for the recruitment of different types of intermediary signaling molecules by protein-protein interactions through specific SH2 and SH3 domains. These large signaling complexes are known as signalosomes and can be different

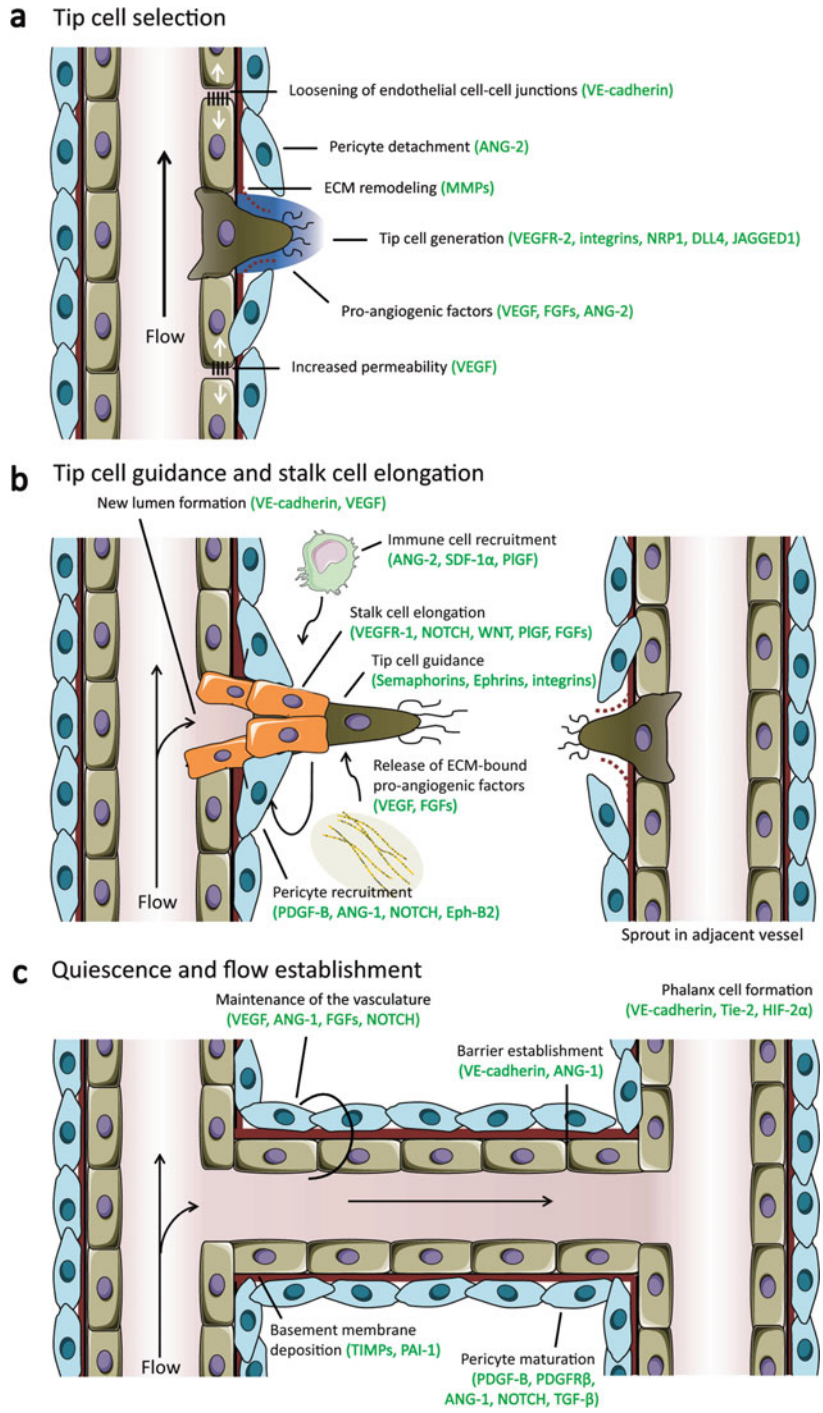

Fig. 2 Molecular basis of angiogenesis. Sequential steps of blood vessel formation and their features are depicted. The most important molecular players involved in each process are denoted in parentheses. (**a**) Upon angiogenic stimulation by proangiogenic factors, the quiescent vessel dilates and an endothelial tip cell is selected. Tip cell generation requires basement membrane degradation, loosening of endothelial cell-cell junctions, and pericyte detachment. A provisional matrix layer is deposited by extravasation of plasma proteins (e.g., fibrinogen) due to increased permeability. Cell migration is favored by protease-mediated matrix remodeling. (**b**) Tip

depending on the combination of VEGF ligand and receptor. In fact, differences between signalosomes allow a broad range of biological effects to VEGF stimulation that include increased endothelial cell proliferation, migration, survival, permeability, and ECM degradation.

Several studies indicate that VEGF stimulates both physiological and tumor angiogenesis by signaling through VEGFR-2 in a dose-dependent manner (Carmeliet and Jain 2011). In fact, VEGFR-2 null mice die during embryonic development by defects in the vascular system, reinforcing its role in proliferation, survival, and migration of vascular endothelial cells. The activation of VEGFR-2 promotes differentiation of progenitors, mitogenesis, chemotaxis, survival, and vascular permeability. It also increases the expression of matrix metalloproteinases and plasminogen activators for ECM degradation and further endothelial cell migration. In detail, VEGF release causes plasma proteins extravasation and the deposition of a provisional ECM scaffold toward which endothelial cells migrate in response to integrin signaling. The activated proteases liberate the angiogenic molecules stored in the ECM such as VEGF and FGF, and the ECM is remodeled into an angio-competent milieu. Once VEGF is released, it binds to the VEGFR-2 receptors of the endothelial cells. Tip cell migration is regulated by VEGF gradient, whereas stalk cell proliferation depends on VEGF concentration (Gerhardt et al. 2003). VEGF/VEGFR-2 signaling axis induces the formation and extension of filopodia and the expression of delta-like ligand 4 (Dll4) protein in tip cells, which activates Notch in stalk cells. Notch, in turn, downregulates VEGFR-2 expression in stalk cells, rendering them less responsive to VEGF and ensuring tip cell leading. The blockade of VEGFR2 signaling is associated with sprouting defects (Bentley et al. 2009).

On the other hand, VEGFR-1 is only slightly activated by proangiogenic factors, and its precise role in angiogenesis is poorly understood (Schwartz et al. 2010). Nevertheless, it has been shown that VEGFR-1 acts as a decoy receptor, since it is able to sequester VEGF, implying a negative role for this receptor in angiogenesis. In addition, mice lacking VEGFR-1 present a higher number of endothelial cells than wild-type mice, whereas the decreased expression of VEGFR-1 increases VEGF availability and VEGFR-2 activity. In fact, a soluble isoform of VEGFR-1 (sVEGFR-1), which encodes the extracellular ligand-binding domain, can be produced by surrounding cells. While soluble VEGF isoforms promote vessel enlargement, matrix-bound isoforms stimulate the branching pattern. sVEGFR-1 inhibits angiogenesis by acting as a molecular trap for VEGF ligand, assisting the guidance of emerging branches or inhibiting the sprouting. The imbalance between the functions of VEGFR-1 and VEGFR-2 causes hemangiomas and benign tumors with increased aberrant angiogenesis (Jinnin et al. 2008).

In pathological conditions, PlGF contributes to the angiogenic switch by affecting multiple cell type directly and indirectly and also activates bone marrow-derived endothelial progenitor cells. PlGF can induce its own signaling and amplify VEGF-driven angiogenesis through direct effects on endothelial cells (Autiero et al. 2003). The synergism between VEGF and PlGF is also stated by the fact that PlGF upregulates the expression of VEGF. PlGF might also indirectly influence SMC proliferation and migration through activated endothelial cell cytokine release (Luttun et al. 2002).

Fig. 2 (continued) cells sense the environment and navigate in response to guidance cues (e.g., semaphorins and ephrins) while adhering to the extracellular matrix (ECM) in order to migrate. Stalk cells behind the leading tip cell proliferate and elongate, attracting pericytes in the process. Stalk cells are further stabilized by the deposition of the new basement membrane. Immune myeloid cell recruitment of tumor-associated macrophages (TAMs) and TIE-2-expressing monocytes (TEMs) produces additional proangiogenic factors and triggers the release of ECM-bound factors. (**c**) Fusion of adjacent branches and posterior lumen formation drive neovessel perfusion, which concludes with quiescence by a phalanx phenotype promotion, deposition of the new basement membrane, maturation of pericytes, reestablishment of cell-cell junctions, and release of vascular maintenance molecules

Other prototypical proangiogenic signaling pathway involves the *fibroblast growth factor* (*FGF*) family, comprised by 23 different ligands and four tyrosine kinase-type receptors (FGFR-1–4) expressed widely in the organism (Presta et al. 2005). FGFR-1 and FGFR-2 are expressed by endothelial cells, and their binding to ligands FGF-1, FGF-2, FGF-4, and FGF-5 leads to the induction of critical stages of angiogenesis in vivo. Among the four pleiotropic proangiogenic ligands, at least FGF-1 and FGF-2 directly stimulate endothelial cell proliferation, detachment, migration, and ultimate differentiation into a functional capillary vessel. For the maintenance of vascular integrity, endothelial quiescent cells require low levels of FGF, since vessel disintegration has been observed as a consequence of FGFR signaling inhibition (Murakami et al. 2008). FGF ligands exert their functions in endothelial cells after paracrine release by stromal or tumor cells, or by endogenous FGF in an autocrine fashion.

Even though VEGF has a pivotal role during angiogenesis, an important cross talk takes place between FGF and VEGF. For instance, VEGF system activation is required for later FGF induction and in vivo angiogenesis promotion. The opposite cross regulation has also been demonstrated, and FGF also seems able to stimulate tumor angiogenesis under certain experimental conditions. The effects of FGF are due to its dual action including a direct effect over endothelial cells and an indirect effect concerning the regulation of the production of other proangiogenic molecules like VEGF, angiopoietin-2 (Ang-2), or interleukin-8 (IL-8) by tumor or stromal cells (Beenken and Mohammadi 2009). Both in mouse and human tumors, the role of FGF in tumor growth and neovascularization has been described (Presta et al. 2005).

The third proangiogenic pathway includes the *epidermal growth factor (EGF)-like family* of growth factors and their receptors (ErbB). The integrants of this family play various functions in different tissues, but they are basically involved in cell proliferation and survival stimulation. Some of the ligands included are EGF and transforming growth factor alpha (TGF-α), which bind to the ErbB family of tyrosine kinase receptors. The ErbB family of receptors is composed of ErbB1/HER1/EGFR, ErbB2/HER2/Neu, ErbB3/HER3, and ErbB4/HER418.

Both in tumor and physiological settings, EGF family members display proangiogenic activity. However, whether the effects of EGF are direct or indirect remains unclear. EGF stimulation produces the release of proangiogenic factor such as VEGF, IL-8, and FGF by tumor and stromal cells. The ErbB family receptors in endothelial cell membranes enable these cells to respond to EGF-like factors by increased proliferation and survival. Furthermore, other angiogenic factors like TGF-β induce the autocrine production of EGF-like molecules such as TGF-α, thus promoting endothelial cell survival through PI3K-Akt signaling (Viñals and Pouyssegur 2001).

The ECM itself provides a link between vascular cells and their surrounding environment. Proteolytic degradation of the basement membrane and the surrounding ECM is an integral part of angiogenesis. In this step, several *proteinase families* are involved, including matrix metalloproteinases (MMP and their tissue-type inhibitors or TIMP), plasminogen activators (uPA and its inhibitor PAI-1), heparanases, tryptases, chymases, cathepsins, etc. Besides breaking down ECM components and clearing a path for endothelial cell migration, proteinases are able to switch on angiogenesis by the liberation of matrix-bound angiogenic activators (bFGF, VEGF, TGF-β, HGH, etc.) and proteolytically activating angiogenic chemokines such as IL-1β. Whereas VEGF isoforms cleaved by MMPs preferentially enlarge vessels, MMP-resistant matrix-bound VEGF is involved in vessel branching (Iruela-Arispe and Davis 2009). Moreover, proteases such as MMP9 participate in the mobilization of bone marrow progenitors by the liberation of cytokines such as the Kit ligand (Heissig et al. 2002) and by the establishment of a pre-metastatic niche (Kaplan et al. 2005). The proteolytic remodeling of the ECM occurs in a sharply controlled mode, and the pleiotropic activities of proteinases are context and concentration dependent. In fact, excessive breakdown removes guidance cues for endothelial cell migration, thus inhibiting

angiogenesis, while insufficient degradation prevents vascular cell mobility (Carmeliet and Jain 2011).

Moreover, proteinases can switch off angiogenesis, as they liberate matrix-bound angiogenic inhibitors such as arrestin, angiostatin, TSP-1, and inactivate angiogenic cytokines like SDF-1. The basement membrane of quiescent vessels is composed mainly of collagen IV and laminin, whereas the interstitial matrix of collagen I and elastin between vascular cells provides further viscoelasticity and strength to the endothelial cell wall. Proteinases expose novel epitopes of these ECM proteins, which ultimately induce endothelial cell and perivascular cell migration and generate the angiogenic scaffold for neovascularization.

Specific ECM molecules have cell surface receptors such as the heterodimeric *integrins*, which transmit bidirectional information between vascular cell cytoplasm and their surrounding environment. Integrin signaling assists vascular cells at new vessel building by favoring association of endothelial cells with ECM proteins such as vitronectin, fibrinogen, and fibronectin. In addition to their contribution to the ligation of ECM components, integrins interact with several extracellular molecules, functioning as "hubs" that modulate endothelial cell and perivascular cell behavior during angiogenesis. Hence, the binding of integrins to VEGF, FGFs, and Ang-1 or their receptors stimulates vessel growth. Among the integrin family, αvβ3 and αvβ5 integrins are reported to positively regulate the angiogenic switch (Desgrosellier and Cheresh 2009).

In this same context, *VE-cadherin* is an endothelium-specific protein that mediates in homotypic cell-cell interactions (Cavallaro et al. 2006). This protein is coexpressed with VEGFR-2 in developing blood vessels, suggesting a potential interaction between both receptors. VE-cadherin is critical for the final steps of capillary development and lumen formation and is associated with the inhibition of endothelial cell migration. During neovessel sprouting, the adhesive function of VE-cadherin is reduced by endocytosis in response to VEGF and angiogenic factors (Dejana et al. 2009). Meantime, the localization of VE-cadherin at filopodia allows the establishment of new contacts by tip cells with cells on the outreaching vessel sprouts.

Chemokines are a large group of molecules secreted by stromal cells that promote inflammation and pathological angiogenesis by the recruitment of immune cells and EPCs to malignant, inflammatory, and ischemic tissues. Besides, chemokines directly activate the G protein-coupled chemokine receptors (GPCRs) signaling in endothelial cells. Endothelial cells express chemokine CXCR receptor such as the angiogenic CXCR2 and CXCR4 receptors, which are bound by GROa, IL-8 and SDF-1, and angiostatic CXCR3, whose ligands are PF-4, MIG, etc. Hence, SDF-1 binds to the CXCR4 receptor on tip cells (Duda et al. 2011) and is upregulated in hypoxia, supporting mobilization and retention of proangiogenic bone marrow-derived cells to promote revascularization. Depending on the temporospatial balance of these modulators, chemokine release has an overall role in initiating or terminating angiogenesis.

Cues that Guide Vessel Navigation

There are many similarities between tip cell environment sensing and axonal cone growing, and therefore it is not surprising that many molecular, mechanistic, and morphological aspects of vascular guidance are shared with the axon guidance process during nervous system development (Adams and Eichmann 2010; Fig. 2b). In the last decade, a bundle of attractive (VEGF, Slit) and repulsive (semaphorin, ephrin, netrin) vascular guidance cues have been described. However, some cues such as semaphorins, netrins, and Slits are able to function as either attractive or repulsive depending on the molecular context. Specific receptors for each vascular signal are expressed by endothelial cells. Some attractive cues such as *VEGF* are displayed as gradients of soluble factors, whereas others establish communication networks through transmembrane proteins like ephrins and their EPH receptors.

One of the main families governing vessel guidance comprises *Ephrin/EPH*. This family is involved in the regulation of arterial-venous plexus formation, remodeling and maturation,

axonal outgrowth, fasciculation and pathfinding, and lymphatic plexus formation (Mosch et al. 2010). Ephrin and EPH are membrane-bound proteins that function as a bidirectional receptor-ligand pairs whose signaling regulates cell-cell contact-dependent patterning. Whereas EPH becomes autophosphorylated and activates intracellular signalosomes (known as forward signaling), the phosphorylation of ephrin cytoplasmic tail prompts the binding of PDZ domain-containing proteins (known as reverse signaling).

Among the different family members, ephrin-B2 and its receptor EPHB4 have been found to play a key role in angiogenesis by vessel morphogenesis regulation. During vasculogenesis, the vascular plexus is characterized by ephrin-B2-positive arteries and EPHB4-positive venous regions. These two populations of cells prevent intermingling and segregate from each other by avoiding repulsive actions, suggesting an "artery-to-vein push and pull" model of angiogenesis. Besides, ephrin-B2 reverse signaling in tip cells induces VEGFR-2 internalization, which is necessary for downstream signaling of the receptor to cause tip-cell filopodial extension (Sawamiphak et al. 2010). Ephrin-B2 also drives mural cell and EPC recruitment. Moreover, EPHB4 upregulation stimulates tumor angiogenesis and induces malignant transformation, classifying the receptor as a proangiogenic and tumorigenic molecule. Other ephrin ligands and EPH receptors, like ephrin-A1 and EPHA2, have also a role in vessel growth and maturation. Since the discovery of the ephrin/EPH family, several members have been described to be deregulated in different tumor types (Mosch et al. 2010).

Semaphorins belong to a large family of membrane-bound and secreted proteins involved in both attractive and repulsive activities in the vascular and nervous system formation. Semaphorin family ligands are characterized by the presence of a highly conserved extracellular sema domain that mediates the binding to multimeric receptor complexes, mainly formed by plexins and neuropilins (NRPs) (Suchting et al. 2006). Regarding the dual role in promoting or inhibiting the angiogenic response, the signaling cascade initiated after plexin and neuropilin coreceptors (NRPs) activation remains to be completely elucidated.

SEMA3A, SEMA3B, SEMA3D, SEMA3F, and SEMA4A are regarded as negative regulators of tumor angiogenesis, whereas SEMA3C and SEMA4D promote tumor angiogenesis. For instance, SEMA3A expression by endothelial cells of developing vessels inhibits endothelial cell migration as it interferes with integrin function (Serini et al. 2003). The loss of Plexin D1 receptor produces aberrant sprouting into SEMA3E expressing tissues, as seen in zebrafish embryos (Adams and Eichmann 2010). In mice, Plexin D1 removal induces erroneous navigation, since endothelial cells are not able to recognize the repulsive SEMA3E signals in the surrounding environment. Moreover, SEMA3E signaling regulates the balance of tip and stalk cells necessary for growing sprouts by coordinating the activity of VEGF in a negative feedback loop (Kim et al. 2011). SEMA3F has recently been described as a metastasis suppressor in different animal models due to its ability to block peritumoral vessel sprouting, tumor cell adhesion, and migration (Bielenberg et al. 2006). Indeed, tumors have been reported to produce a soluble form of neuropilin 1 (sNpn-1), which might function as a VEGF trap, and therefore inhibit tumor angiogenesis and growth (Guttmann-Raviv et al. 2006). On the contrary, SEMA4D induces endothelial cell migration and tubulogenesis apart from stimulating blood vessel formation in vivo through interaction with its Plexin B1 receptor in endothelial cells (Basile et al. 2004).

Similar dual attractive and repulsive roles are found in two other families of secreted factors: *Slits* and their roundabouts (Robo) receptors and by *netrins* and their receptors uncoordinated 5 (UNC5) and deleted in colorectal cancer (DCC). Slits and Robo are proteins with multiple binding domains that are involved in physiological vasculogenesis (Park et al. 2003). In detail, Robo-4 receptor for Slits is reported to be expressed in sites of active angiogenesis in the adult, which include tumor vessels. Robo-4 expression in endothelial cells maintains vessel integrity, and its deficiency induces leakiness

and hypervascularization (London et al. 2009). The permeability-promoting actions of VEGF are counteracted by Robo-4 activity, which impedes VEGFR-2 activation of Src. In addition, in vitro studies showed that the exposure of endothelial cells expressing Robo-1 to a Slit-2 source promoted their chemotaxis (Wang et al. 2003). Indeed, Slit-2 has been found to be expressed in many tumor cell lines and biopsies.

Netrins are a protein family that contains a laminin VI domain and a carboxy-terminal domain that binds heparin, several proteoglycans, and membrane glucolipids, thus allowing interaction with the cell surface or with ECM components (Barallobre et al. 2005). UNC5B is a netrin receptor whose expression is enriched in tip cells. Netrin-1 binding to UNC5B receptor has shown to act as a repellent in blood vessel guidance (Lu et al. 2004). Other studies have reported stimulation of endothelial cell and perivascular cell proliferation and migration in vitro by Netrin-1 and Netrin-4 (Wilson et al. 2006). In fact, UNC5B inactivation results in enhanced sprouting, whereas netrin-1 promotes filopodia retraction of endothelial cells, consistent with a suppressive function of UNC5B and netrins on vessel growth (Adams and Eichmann 2010).

Vessel Remodeling, Stabilization, and Maturation

In order to achieve functionality, vessels must mature both at the level of the endothelium and vessel wall and as a vascular network. This maturation involves the remodeling of the network into a hierarchically branched structure, which implies the formation of large and small vessels, the association with perivascular cells, the establishment of directional flow, and the adjustment of density to meet the nutritional requirements of the surrounding tissue (Fig. 2c). Preceding the phenomenon of vascular remodeling, capillary retraction takes place. This occurs during the rapid growth phase of capillaries and is generally associated with the elongation of other capillaries in the vicinity (Clark and Clark 1939).

Late events in the angiogenic process include the stabilization of the newly formed vessels and the maintenance of the vasculature. Vessel integrity in different vascular beds is maintained by a coordinated regulation between several cellular (including endothelial cells, pericytes, fibroblasts, SMC, inflammatory cells, etc.) and noncellular components such as the ECM (Stratman and Davis 2011). In short, pericyte recruitment, adhesion, and wrapping around endothelial cells are fundamental events during blood vessel stabilization and maturation. Whereas pericytes establish direct cell-cell contacts with endothelial cells in capillaries and immature vessels, vascular SMC are separated from endothelial cells by a matrix and function as covers of veins and arteries (Gaengel et al. 2009).

Vascular remodeling is a complex process that requires an extensive array of molecular signaling. During vessel maturation, while endothelial proliferation is detained, endothelial cells express survival signals in order to maintain integrity of the vessel lining. One such survival factor is the canonical *VEGF*, produced by endothelial cells themselves. The "intracrine" VEGF activates the PI3K/AKT survival pathway, thus preventing apoptosis in nonpathological conditions (Warren and Iruela-Arispe 2010). This pro-survival activity of VEGF differs from its paracrine function described in the previous section, where the loss of endothelial VEGF does not cause developmental vascular defects. Moreover, *FGFs* have also been involved in the maintenance of vascular integrity for their ability to strengthen adherens junctions (Beenken and Mohammadi 2009). The inhibition of FGF causes dissociation of tight junctions and further endothelial cell loss and vessel disintegration (Murakami et al. 2008). Another important survival cue is *blood flow*, as shear stress inhibits endothelial cell apoptosis by KLF2 activation. Active KLF2 evokes quiescence by downregulating VEGFR-2 and upregulating nitric oxide synthase and thrombomodulin, favoring vessel dilation, perfusion, and absence of clots.

Nevertheless, the prototypical vascular remodeling factor family par excellence is the *angiopoietin/Tie* family, formed by angiopoietins (Ang) and their receptors, known as Tie (Augustin et al. 2009). The angiopoietin family comprises

the ligands Ang-1, Ang-2, and Ang-4 and the Tie tyrosine kinase receptors Tie-1 and Tie-2. Since all known angiopoietins bind only to Tie-2 receptor, Tie-1 persists as an orphan receptor that might act as a negative regulator of Tie-2. The main function of angiopoietins is the control of the switch between endothelial cell quiescence and activation. At the molecular level, the activation of the angiopoietin/Tie signaling cascade modulates, in a positive or negative fashion depending on the molecular context, pro-survival pathways (such as PI3K-Akt) and endothelial cell permeability (by Src kinase regulation).

The binding of Ang-1 produced by cells in the vicinity of developing vessels (mural cells, fibroblasts, and tumor cells) to the Tie-2 receptor expressed in endothelial cells promotes vessel maturation through endothelial cell quiescence and pericyte recruitment. Ang-1 or Tie-2 deficiency causes premature death in mice due to severe defects in the vascular system characterized by a poorly organized and immature capillary network. As a result, the Ang-1 functions to induce vasculature stabilization by a mature and nonproliferative state maintenance of endothelial cells. Moreover, Ang-1 tightens vessels via effects on PECAM, VE-cadherin and occludin and favors endothelial cell-pericyte interactions by serving as a sticky ECM-associated and α5-binding protein (Saharinen et al. 2008). Roles for Ang-1 in endothelial cell growth and capillary tube formation by its synergistic activity with VEGF and in circulating EPC mobilization have also been described (Hattori et al. 2001).

Opposed to Ang-1, Ang-2 is produced by endothelial tip cells in angiogenic and vascular remodeling sites and acts as an Ang-1 antagonist, contributing to the detachment of perivascular cells. Ang-2 binds specifically to Tie-2, hence competing with Ang-1 for the binding to the same receptor, and its action depends on the endothelial cell state. Intriguingly, whereas Ang-2 inhibits Tie-2 signaling in the resting vasculature, it stimulates Tie-2 signaling on stressed endothelium (Augustin et al. 2009). Even though deficiency of Ang-2 does not impair normal development, adult mice lacking Ang-2 present vascular defects in angiogenically active organs. This suggests that the dual role of Ang-2 is related to vascular remodeling activation, being its final effect dependent on the presence or absence of other proangiogenic factors (Gale et al. 2004). For instance, in the absence of VEGF, Ang-2 promotes vascular regression by endothelial cell apoptosis. On the contrary, VEGF presence stimulates Ang-2 activation of pericyte detachment, enabling endothelial cell exit, proliferation, and migration, thus contributing to new vessel formation.

The overall effects of angiopoietin/Tie signaling on tumors depend on the context (Augustin et al. 2009). Moderate Ang-1 or strong Ang-2 overexpression have been observed in tumor cells. Induced overexpression of Ang-1 in tumor cells stabilizes the vasculature and diminishes angiogenesis, thus promoting antitumor effects. Contrarily, Ang-2 overexpression activates angiogenesis and enhances tumor growth, while its systemic delivery results in tumor vessel regression. Tumor-derived Ang-2 also stimulates angiogenesis by recruiting proangiogenic monocytes (de Palma et al. 2005). On the whole, these studies highlight the significance of maintaining an accurate balance of angiopoietin/Tie signaling for normal vascular homeostasis. Ang-1/Ang-2 balance shifting in favor of Ang-2 makes the vasculature more plastic and susceptible to sprouting.

Other of the main molecular families involved in vessel maturation contains *platelet-derived growth factor* (*PDGF*) and its receptors. The PDGF family comprises four different isoforms (PDGF-A, PDGF-B, PDGF-C, PDGF-D) closely related to the VEGF family and are expressed and impact different types of cells including fibroblasts, SMC, neurons, and endothelium (Andrae et al. 2008). Although PDGF ligands act as homodimers, functional heterodimers (such as PDGF-AB) are also found. Two tyrosine kinase receptors have been described: PDGFR-α and PDGFR-β. Consequent to ligand binding, the receptor dimerizes forming homo- or heterodimeric receptor complexes. Some of the typical interactions are PDGF-AA and PDGF-CC interacting with PDGFR-α and PDGF-BB and PDGF-DD binding to PDGFR-β. In contrast to isoforms, PDGF-A

and PDGF-B, PDGF-C and PDGF-D are secreted as zymogens and require a previous activation by proteolytic cleavage. Similar to VEGF, PDGFs also contain "retention matrix" structural motifs that allow their interaction with ECM proteins and the regulation of their biological availability (LaRochelle et al. 1991).

The proangiogenic PDGF/PDGFR family works in a paracrine fashion. Endothelial and stromal cells produce PDGF factors that bind to their receptors in mural cells (pericytes and SMC). In order to stabilize endothelial cell channel, angiogenic cells release PDGF-B to chemoattract PDGFR-β expressing pericytes (Gaengel et al. 2009). Hence, PDGF-B functions as an attractant, stimulating cell migration, proliferation, and cell fate. Consequently, genetic ablation of either ligands or receptors of the PDGF family in mice provokes pericyte deficiency, which in turn causes vessel leakage, microaneurysm formation, tortuosity, and bleeding, leading to defects in the blood-brain barrier and premature death (Quaegebeur et al. 2010). Among the signaling pathways stimulated upon ligand binding and receptor dimerization are the Ras-ERK, PI3K-Akt, and phopholipase C-γ that induce proliferation, migration, and survival of mural cells.

During tumor development, paracrine PDGF-B produced by tumor cells recruits pericytes and elicits angiogenesis. In addition, PDGF stimulates also tumor cells directly in an autocrine manner, as reported in gliomas. For instance, PDGF-D has been described as a potent stimulator of tumor neovascularization (Li et al. 2003). Besides, tumor-derived PDGF-B also recruits pericytes in an indirect manner by upregulating SDF-1α. In metastasis, PDGFR-β expressing pericytes have a dual role. In primary tumors, pericytes are a physical barrier for tumor cell intravasation, so the absence of pericyte correlates with metastasis (Gerhardt and Semb 2008). Nevertheless, other studies have reported that pericytes at micrometastatic sites support tumor colonization by proangiogenic factor release.

The third major group of remodeling factors involves the *transforming growth factor-beta* (*TGF-β*) family of cytokines which includes, among others, TGF-β, bone morphogenetic proteins (BMPs), and activins. The TGF-β family is involved in the control of many biological responses and cellular functions such as proliferation, apoptosis, or differentiation and is produced by nearly every cell type. Three members of the TGF-β family with partially overlapping expression patterns but distinct functions have been identified (TGF-β1–3). These growth factors are secreted as latent forms and later activated proteolytic processing or binding to thrombospondin-1 (TSP-1). The different effects of TGF-β signaling depend on the molecular, temporal, and spatial context. Genetically modified mice lacking various TGF-β signaling components harbor extensive vascular defects, remarking their key role in angiogenesis (Pardali et al. 2010). In the adult organisms, TGF-β exerts a proangiogenic role on activated sprouting endothelial cells by the stimulation of their proliferation and migration, whereas it induces quiescence and maturation in the resting endothelium.

Signal transduction by TGF-β requires a series of receptors that have a serine/threonine kinase intracellular domain, accessory receptors, Smad proteins and Smad transcription factors (Akhurst 2006). There are two different TGF-β receptor families: type I receptors (TGFβRI, also known as activin-like kinase (ALK)) and type II receptors (TGFβRII), which are constitutively active serine/threonine kinases. Ligand binding of TGF-β to its TGFβRII receptor induces heterodimerization with TGFβRI, which is then phosphorylated at the serine and threonine residues by TGFβRII kinase. Once activated, TGFβRII phosphorylates intracellular proteins such as the Smad family of transcription factors. In turn, the active Smad proteins are translocated into the nucleus where they activate the transcription of target genes.

Two different TGFβRI receptors are expressed in endothelial cells: ALK1 and ALK5. ALK1 stimulates endothelial cell proliferation and migration, whereas ALK5 inhibits these processes, maintains endothelium quiescence, and induces ECM deposition. Furthermore, endoglin (CD105) is a TGF-β coreceptor (or type III TGF-β receptor), which is highly expressed in

proliferative endothelial cells, and is required for ALK1 signaling. The ratio of ALK5/ALK1 expression explains the dual role exerted by TGβ in angiogenesis, since their net balance dictates the outcome of TGF-β response (Lamouille et al. 2002). While proliferation is stimulated through ALK1 signaling in endoglin-positive sprouting endothelial cells, resting endothelium-lacking endoglin is subjected to TGF-β/ALK5-induced quiescence and inhibition of cell proliferation and migration. Besides, differential TGF-β concentration also triggers different responses. At low doses, it contributes to the angiogenic switch, through the upregulation of angiogenic factors such as VEGF and several proteinases. At high doses, TGF-β inhibits endothelial cell proliferation and migration by stimulating the reformation of the basement membrane and the recruitment and differentiation of mesenchymal cells via PDGF-B and SM22a upregulation (Taylor and Khachigian 2000). Endothelial cell proliferation inhibition occurs as a result of the impeded pRb phosphorylation that provokes endothelial cell cycle arrest at G1 phase (Gupta and Qin 2003).

The *Notch* pathway includes another central superfamily of molecules with important roles in vascular biology, controlling not only remodeling but also endothelial cell fate during vascular development and vascular guidance in sprouting angiogenesis (Roca and Adams 2007). There are four transmembrane Notch receptors (Notch-1, Notch-2, Notch-3, Notch-4) which have large extracellular domains named NECD. Unlike the VEGF signaling pathway, their five ligands (Jagged-1, Jagged-2, Delta-like 1, Delta-like 3, and Delta-like 4) are also transmembrane proteins which are exposed by neighboring cells. Thus, interaction requires cell-cell contact. These ligands stimulate Notch-presenting cells in a juxtacrine manner. Upon binding, Notch is subjected to two proteolytic cleavages: extracellular and intracellular (catalyzed by γ-secretase). The intracellular cleavage liberates a portion of Notch known as NICD that translocates to the nucleus and regulates transcription of Notch target genes.

The Notch superfamily has been shown to take part in cell fate decisions, either by initiating differentiation of these cells or by maintaining their undifferentiated state. Notch signaling is critical for the control of endothelial cell fate during arteriovenous differentiation (Gridley 2010). Whereas the inactivation of Notch determines venous identity, its active form determines arterial one. Precisely, Notch-1, Delta-like 1, and Delta-like 4 are expressed in endothelial cell arteries and control arteriogenesis both in the embryo and in the adult.

Besides its roles in vascular development, Notch contributes to sprouting angiogenesis regulation. A deletion of a single copy of Delta-like 4 (Dll4) or Notch-1 provokes vascular defects and embryonic lethality (Gale et al. 2004). Tip or stalk cell specification of endothelial cells is controlled by the Notch pathway (Eilken and Adams 2010). High levels of Notch where noted in stalk cells, whereas Notch signaling was shown to be low in tip cells. During physiological angiogenesis or tumor progression, blockade of Notch or Dll4 augments filopodia and sprouting following excessive tip cell formation (Thurston et al. 2007). Notch-1 expression is critical for tip cell behavior suppression in stalk cells. Both the hypersprouting phenotype and the aberrant number of tip cells after Notch inhibition suggest that tip cell phenotype is the default endothelial response to proangiogenic stimuli. Contrarily to Dll4, the Notch ligand Jagged-1 (JAG1) is primarily expressed by stalk cells. Nevertheless, a modification of Notch by Fringe glycosyltransferase favors the activation of this receptor by Dll4, leaving JAG1 as a poor antagonist that favors phenotype maintenance (Eilken and Adams 2010).

Tip and stalk cell fate are transient phenotypes of endothelial cells. In order to expand the vascular plexus, endothelial cells undergo repetitive cycles of sprouting, branching, and tubulogenesis, requiring active transitions between tip and stalk cells. The coordinated function of VEGFR-2 and Notch pathways controls branching. Indeed, this integrated intercellular feedback works as a "branching pattern generator" and involves the regulation of all VEGFRs by Notch. Dll4 expression is activated through VEGFR-2 signaling in endothelial tip cells. This Dll4 activates Notch signaling in adjacent neighboring endothelial

cells, thus dictating a stalk fate. The inhibition of tip cell behavior occurs as a consequence of Notch-mediated downregulation of VEGFR-2, VEGFR-3, and NRP-1 and VEGFR-1 upregulation (Jakobsson et al. 2010). Through Notch/Dll4 signaling, endothelial cells located at the angiogenic sprout dynamically compete for tip position. Upon VEGF signaling, although all cells upregulate Dll4, the ones that express it quicker or at higher levels have a competitive advantage to become tip cells. Regarding the dynamic shifting of tip-stalk position during sprouting angiogenesis, Dll4 expression is highly regulated at various levels. For example, a TEL/CtBP repressor complex at the Dll4 promoter is transiently disassembled following VEGF stimulation, permitting a restricted pulse of Dll4 transcription (Roukens et al. 2010). Several other pathways such as the Wnt/β-catenin, one converges on the transcriptional control of Dll4 (Corada et al. 2010). Other examples include stalk cell JAG-1 expression, which by antagonizing Dll4 activity, reduces Notch signaling induction in the adjacent tip cells, thus maintaining their responsiveness to VEGF stimulation (Benedito et al. 2009).

Dll4a is highly expressed in tumor blood vessels, implying a role for this protein in the control of tumor angiogenesis. Intriguingly, the inhibition of Notch/Dll4 signaling pathway aberrantly increases tip cell count, leading to augmented vessel density, defective perfusion, and consequently tumor hypoxia and growth inhibition (Thurston et al. 2007). Nevertheless, chronic Dll4 blockade results in vascular neoplasms (Yan et al. 2010).

Regression of Blood Vessels and Endogenous Inhibitors of Angiogenesis

Some endogenous proteins or fragments of proteins formed in the body act as physiological inhibitors of angiogenesis (Ribatti 2009). Apart from inhibiting blood vessel formation, endogenous anti-angiogenic factors block cell cycle progression, migration, and induce apoptosis.

Many of these inhibitors are fragments of larger ECM molecules that are released following proteolysis by enzymes like metalloproteinases, cathepsins, and elastases. For instance, *arresten*, *tumstatin*, and *canstatin* are parts of type IV collagen; *endostatin* is a fragment of type XVIII collagen, and *endorepellin* is part of the proteoglycan perlecan. All fragments bind integrins expressed by endothelial cells. Integrin-dependent signaling pathways are crucial for the anti-angiogenic effects of these molecules. Recombinant tumstatin was reported to specifically induce apoptosis of proliferating endothelial cells and promote a potent anti-angiogenic activity in several in vitro and in vivo angiogenesis models (Maeshima et al. 2002). Similarly, arresten has also been described to inhibit endothelial cell proliferation, migration, tube formation, and growth of primary tumors and metastases in mouse xenograft tumor models (Sudhakar et al. 2005).

Two other molecules that are critical for the negative regulation of angiogenesis are thrombospondin-1 (*TSP-1*) and thrombospondin-2 (*TSP-2*). TSP-1 and TSP-2 are potent anti-angiogenic heparin-binding proteins that although constituents of the ECM can also be secreted and found in blood circulation (Armstrong and Bornstein 2003). Through primary binding to CD36 endothelial cell membrane receptor, TSP-1 is thought to activate anti-proliferative and proapoptotic effects. Furthermore, TSP-1 directly affects the ECM by activation of TGF-β. TSP-2 also inhibits endothelial cell migration and tube formation, as well as specifically increasing apoptosis of these cells (Noh et al. 2003).

Apart from the matrix-derived molecules, the heterogeneous group of other endogenous anti-angiogenic molecules contains several growth factors, cytokines, metabolites of hormones, and clotting factors (Folkman 2004). For example, interferon α (*IFN-α*) and β (*IFN-β*), *interleukin-1β* (*IL-1β*), *IL-4*, *IL-12*, *IL-18*, and *pigment epithelium-derived factor* are all cytokines with the strong capacity of blocking angiogenesis. Both IFNα and IFNβ inhibit angiogenesis in mouse models by modulating the pro-angiogenic signals generated by tumor cells. Moreover, they also modify the activity and expression of several proteases such as MMP-9

(Ma et al. 2001) and downregulate bFGF expression (Dinney et al. 1998). Regarding interleukins, IL-1β inhibits by an autocrine pathway angiogenesis stimulated by FGF. Fragments derived from or related to blood coagulation factors such as *angiostatin* (cleaved from plasminogen produced by tumor cells), anti-angiogenic antithrombin III (derived from antithrombin III) and *platelet factor 4* play a role in angiogenesis inhibition and endothelial cell apoptosis.

The Angiogenic Switch in Tumorigenesis

Without new vessels, tumor outgrowth is usually restricted to no more than 1–2 mm^3. During this phase, known as avascular phase, the tumor is nourished by the diffusion of nutrients and oxygen obtained from nearby blood vessels, and tumor-related new blood vessel formation is not observed. These avascular tumors reach a steady state, where proliferation and apoptosis are balanced, and there is no net increase of tumor volume. In order to sustain unlimited proliferation and to grow beyond the restricted size, tumors demand an extension of the local vessel network, thereby ensuring adequate delivery of oxygen and nutrients to meet their metabolic needs. The transition from the avascular phase to the angiogenic state of tumor development is known as the "angiogenic switch." Since tumor neovascularization is critical for tumor growth, the ability of forming a dense microvascular plexus is a prerequisite acquired early during tumor progression (Folkman 1990). To achieve this end, tumor cells are subjected to numerous genetic and epigenetic changes that endow them with angiogenic potential. The angiogenic phenotype serves the development of malignant neoplasm at multiple stages, since it plays an important role both in the growth and blood supply of the primary tumor and in the tumor metastasis. Several experiments have demonstrated that in the absence of a functional vasculature, tumors become necrotic or apoptotic, reinforcing the dependence of tumors on access to vasculature in order to thrive (Holmgren et al. 1995). The mechanism through which the tumor manages to reactivate the quiescent vasculature from its dormant state to an angiogenic trait and the therapeutic exploitation of its inhibition for cancer treatment has been broadly studied in the past years.

A dynamic balance between positive (pro-angiogenic) and negative (anti-angiogenic) factors controls vascular homeostasis (Hanahan and Folkman 1996). In normal tissues, under physiological conditions, the balance is shifted toward negative regulators of angiogenesis, which maintain the resting state of the vasculature. During tumor progression, several mechanisms contribute to the reversion of this balance. For instance, the loss of tumor suppressor genes and upregulation of oncogenes provoke the loss of the inhibitory phenotype and the gain of inducers that trigger the formation of an excessive and aberrant vascular bed. In early stages of tumorigenesis, tumor cells release high levels of strong angiogenic inducers such as VEGF and FGF. Several studies have appointed VEGF as a key angiogenic player in tumor progression. VEGF is expressed in most types of cancer. Its expression is induced by oncogenes, hypoxia, hypoglycemia, and growth factors and correlates with tumor progression. For example, Myc overexpression leads to a ten-fold increase of VEGF in a B-cell line (Mezquita et al. 2005). Besides, other positive regulators of angiogenesis such as PDGF, FGF, EGF, TGF-β, MMPs, TNF, and angiopoietins (described in the previous sections of this chapter) are also deregulated during the angiogenic switch.

Intriguingly, it has been shown that cancer cells may escape from ECM-associated endogenous inhibitors by further upregulation of pro-angiogenic factors (Fernando et al. 2008). Nevertheless, an increase of an inducer does not suffice to switch on tumor angiogenesis, since inhibitors like TSP-1 are continuously produced at significantly high levels. The loss of endogenous angiostatic factors by subsequent additional genetic alterations in tumor suppressor genes such as p53 is a necessary step to switch on the angiogenic program (Volpert and Alani 2003). Emerging data also demonstrate that tumor cells play an active role in the vascular stem cell and metastatic niche development in order to ensure

cancer stem and progenitor cell expansion. Additionally, tumor cell metabolism creates an acidic tumor microenvironment that promotes EMT and increased tumor cell stemness.

Hypoxia and Tumor Angiogenesis

Strong evidence supports a role for hypoxia in the activation of tumor angiogenesis. Generally, neoplasms have been described to harbor-extensive regions of hypoxia if compared to the corresponding non-tumoral tissue (Vaupel 2004). Hypoxia occurs as a consequence of the rapid proliferation of the tumor mass and the formation of a distorted and abnormal vasculature, which is inefficient in oxygen transport. Low oxygen levels upregulate inducers and downregulate inhibitors, contribution to switching on angiogenesis. Besides, hypoxia drives upregulation of the expression of endothelial-pericyte destabilizing molecules such as Ang-2, which further contributes to the start of sprouting angiogenesis. The mobilization of multiple types of precursor cells from the bone marrow to the tumor mass, and the recruitment of immune cells are also positively modulated by tumor hypoxia (Blouw et al. 2003). Furthermore, low oxygen concentration downregulates DNA repair mechanisms, promoting genomic instability in cancer cells (Bristow and Hill 2008). Changes in gene expression elicited by hypoxia trigger a switch to anaerobic metabolism, inhibition of apoptosis, increased invasiveness, EMT, and metastasis (Cairns et al. 2011).

Moreover, oxygen-sensing enzymes (prolyl hydroxylases) in endothelial cells have been reported to play a fundamental role in tumor vessel morphology and functionality control (Mazzone et al. 2009). All these findings suggest that a number of key initiating events of tumor angiogenesis are subjected to the control of the hypoxia response program (Fig. 3a). When sufficient oxygen is available, the prolyl hydroxylase domain (PHD) proteins PHD1–3 hydroxylate the hypoxia-inducible factor (HIF) proteins HIF-1α and HIF-2α, regarded as master regulators of the hypoxia driven response. When hydroxylated in a region referred to as oxygen-dependent degradation domain (ODD domain), HIFs are attracted to the von Hippel-Lindau (VHL) protein, member of the E3 multiprotein ubiquitin ligase complex, which marks HIF with a multi-ubiquitin chain that directs it toward proteasomal degradation (Majmundar et al. 2010).

Under hypoxic conditions, PHDs remain inactive, and HIFs initiate their transcriptional activity in order to increase the oxygen supply by angiogenesis, through the upregulation of angiogenic factors (Fraisl et al. 2009). In detail, active HIFs translocate to the nucleus, where they form heterodimers with HIF-1β, which is oxygen independent and constitutively expressed, and bind to hypoxia-response elements (HRE), of the sequence 5′-RCGTG-3′. The binding of HIF-1α/HIF-1β heterodimers to HRE activates the transcription of more than 100 different genes (Semenza 2003; Fig. 3b). Many of these genes, such as VE-cadherin, nodal, VEGF, VEGFR-2, Ephrin-B, CD31, sema4D, plexinB1, integrins, MMPs, and other ECM components, have vasculogenic and prometastatic properties. Other genes include those involved in cell survival, apoptosis, cell motility and cytoskeletal structures, adhesion, transcriptional regulation, and drug resistance.

As a rule, HIF-1α promotes vessel sprouting, while HIF-2α intervenes in vascular maintenance. They have both overlapping and unique target genes and may also trigger specific roles. For instance, whereas HIF-1 has been predominantly regarded as a driver of the initial response to hypoxia (<24 h), HIF-2 seems to be responsible for chronic response (>24 h). Both transcription factors are highly expressed in a wide range of aggressive and metastatic tumors (Yang et al. 2014). Moreover, recent evidence relates HIF-1α overexpression with tumor cell-dominant VM in several types of cancers (Mazzone et al. 2009). HIF-1α depletion in mice impairs embryonic vascular development and revascularization and angiogenesis of injured tissues and tumors. There is also an indirect regulation of tumor angiogenesis by HIF-1α, since it promotes the release of chemoattractants such as SDF-1α to recruit bone marrow-derived progenitors

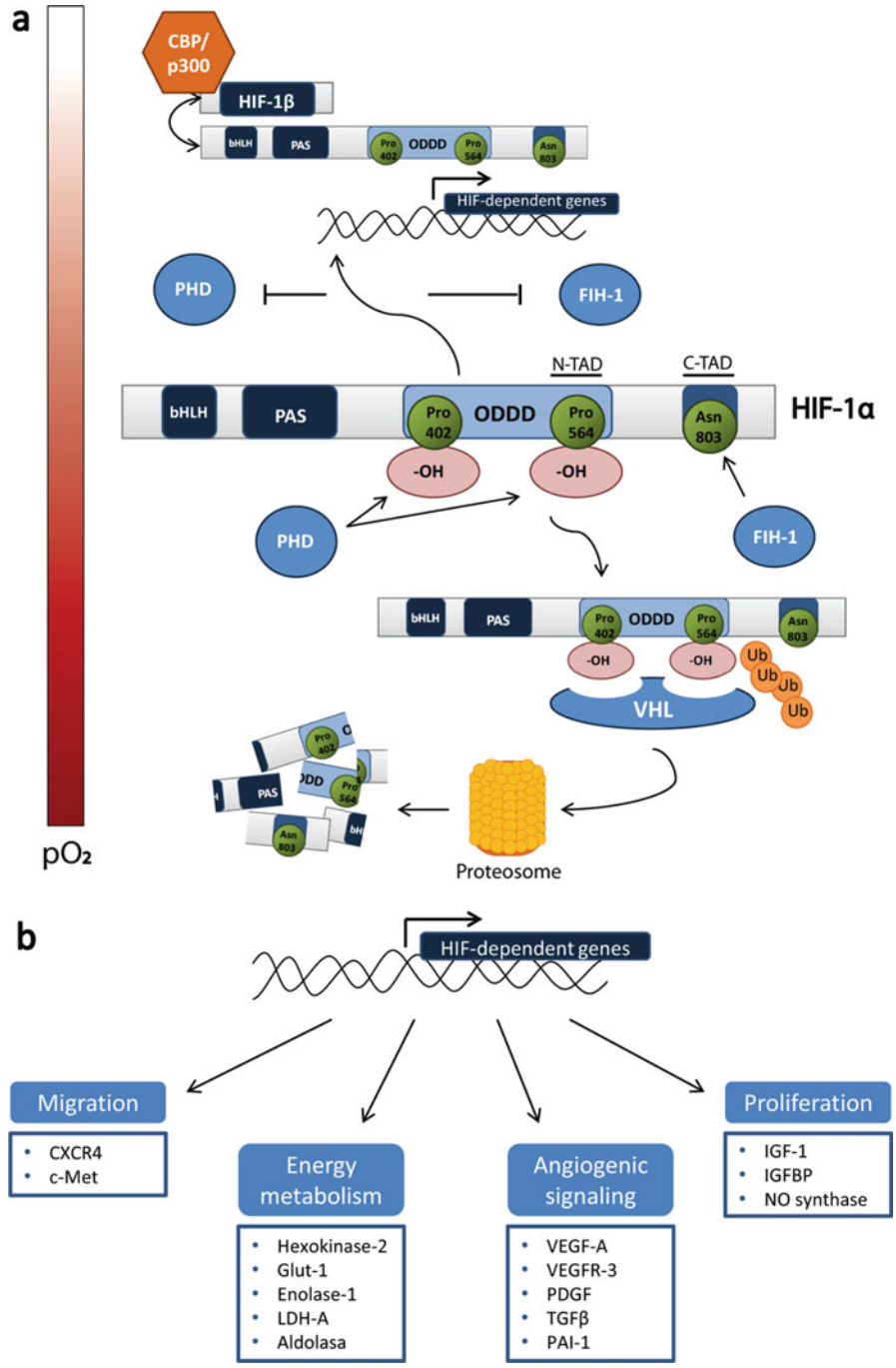

Fig. 3 Stability and transcriptional activity regulation of HIF. (**a**) Under normoxic conditions, prolyl-hydroxylase domain (PHD) proteins hydroxylate two proline residues (402 and 564) located in the oxygen-dependent degradation domain (ODDD) of HIF-1α. Another oxygen sensor, factor-inhibiting HIF-1 (FIH-1), also hydroxylates HIF-1α on an asparagine (803) residue located at its C-terminal transcription activation domain (C-TAF). The hydroxylated protein interacts with the von Hippel-Lindau (VHL) protein that adds a multi-ubiquitin chain to HIF-1α, addressing it for degradation by the proteasome. Under hypoxic conditions, oxygen sensors PHDs and FIH-1 are inhibited, and HIF-1α is no longer degraded. Interaction of HIF-1α with its co-activators such as CBP/p300 and the

(Du et al. 2008). HIF-1α and HIF-2α also regulate TAM polarization and proangiogenic activity with different effects. The relationship between hypoxia and inflammation is illustrated by the interlink between HIF-1α signaling and nuclear factor-kB, which are mutually cross activated. Moreover, HIF activation leads to pro-malignant reprogramming of tumor gene expression and selection of hypoxia-resistant genotypes, like p53 tumor suppressor mutations. Under certain conditions, hypoxic upregulation of VEGF is HIF-1α independent and is mediated by the metabolic regulator PGC-1α, which prepares the ischemic tissue for oxidative metabolism after its revascularization (Arany et al. 2008).

Regarding the regulation of HIFs activation, abundant research shows that it involves reactive oxygen species (ROS) production by mitochondria under limited oxygenation. These ROS have been reported to inhibit PHDs through oxidation to ferric iron or ascorbate depletion (Page et al. 2008). Furthermore, the transcriptional activity of HIFs is regulated at the C-terminal region via two transcriptional activation domains (TAD) known as N-TAD and C-TAD. Precisely, the C-TAD activity is subjected to regulation by the factor inhibition HIF-1 protein (FIH-1) that hydroxylates HIF in an asparagine residue. This hydroxylation abrogates the transcriptional activity by impairing the interaction with other co-activators such as p300 and CBP. In non-hypoxic conditions, HIFs can also be activated by oncogenes and growth factors so as to allow tumor cells to enhance angiogenesis before oxygen deprivation.

Differences between Physiological and Tumor Neovascularization

In physiological conditions, most adult blood vessels remain quiescent, with a minimum rate of endothelial cell proliferation for the purpose of maintaining cell turnover and vascular integrity. Angiogenesis is limited to the high metabolic demands of growing tissues or wound healing and tissue repair. During adulthood, three different locations of the female reproductive organs comprise the few adult tissues requiring ongoing angiogenesis: (i) monthly, during the reproductive cycle, so as to rebuild the uterus lining; (ii) in the ovaries during egg maturation in ovulation; and (iii) during pregnancy in order to synthesize the placenta (Jaffe 2000). Particularly, follicular growth and corpus luteum development depend entirely on angiogenesis, which allows initial rapid corpus luteum growth and later the regression of the follicular blood vessels. A coordinated and time-regulated action of inducers and inhibitors of angiogenesis regulates the course of the ovarian cycle (Goede et al. 1998). VEGF is regarded as the master player during vascular growth in ovarian function, with its expression temporally and spatially associated with blood vessel proliferation in the ovary and occurring first in perivascular cells. Besides, nitric oxide, a potent vasodilator and stimulator of VEGF production, is released by endothelial cells of luteal arterioles and capillaries. Therefore, a paracrine signaling loop is established between perivascular cells, which produce VEGF, and endothelial cells, which produce nitric oxide, ensuring a coordinated regulation of angiogenesis and vasodilation (Reynolds et al. 2000).

Even though most of the vascular plexus remains quiescent in the adult tissues, endothelial cells retain the ability of dividing rapidly in response to a physiological stimulus such as inflammation or hypoxia. For instance, during wound healing, angiogenesis is reactivated for the regeneration of damaged tissues. In this context, several proangiogenic factors such as VEGF and Ang-2 are rapidly overexpressed, whereas

Fig. 3 (continued) HIF-1β subunit activates the binding to hypoxia response elements (HRE). (**b**) Main biological processes and genes regulated by HIF mediated transcription. HIF-dependent processes include migration, energy metabolism, angiogenic signaling, and proliferation among others

Ang-1 is downregulated with similar kinetics, allowing the destabilization of preexisting vessels and the formation of new capillaries. Finally, VEGF and Ang-2 decrease to baseline levels to allow immediate maturation and stabilization of the new vessels. The whole process is finely controlled in a specific temporal and spatial sequence that results in a tightly regulated balance of pro- and anti-angiogenic molecules (Bloch et al. 2000). As a result of the tuned balance, these physiological processes lead to the formation of a stable and functional vascular tree.

Contrarily, an alteration of the equilibrium between negative and positive regulators of angiogenesis promotes abnormal vessel growth, as seen in many pathological conditions. The shift in the balance can lead to either excessive or defective angiogenesis that can exacerbate or worsen the pathological symptoms. As explained in the previous sections, the best-known condition in which angiogenesis is switched on is cancer disease. However, other examples of excessive angiogenesis occurring when the diseased cells produce abnormal amounts of growth factors include, among others, ocular and inflammatory disorders, obesity, diabetes, cirrhosis, or asthma. By contrast, insufficient angiogenesis is related to vessel dysfunction and to diseases like osteoporosis, ischemic heart disease, or preeclampsia (Carmeliet and Jain 2011).

Although physiological and pathological angiogenesis share most molecular mechanisms, they differ in many features. Several lines of evidence indicate that molecules like cyclooxigenase-2, proteases, TSP-2, and placental growth factor are specifically involved in pathological neovascularization. Moreover, pathological angiogenesis is normally determined by inflammation or hypoxia and is, therefore, characterized by macrophage and leukocyte infiltration in the diseased tissues. For instance, tumors where described by Dvorak in 1986 as "wounds that never heal."

Differences between Normal and Tumor Vessels

Both at the morphological and functional levels, tumor vessels display unique characteristics that make them different to the normal vasculature (Fig. 4). The tumor microenvironment is characterized by uncontrolled and continuous overproduction of angiogenic factors. Such an extreme stimulation of the endothelium leads to the development of immature and structurally and functionally abnormal vasculature (Goel et al. 2011). In fact, the tumor vascular tree is chaotic, populated by dead-end vascular branches and areas of intermittent and inverted blood flow that impairs the vascular function and punctually leads to regions of lowered perfusion and subsequent hypoxia (Baluk et al. 2005). The resulting irregular perfusion impedes nutrient, oxygen, and drug delivery. Vessel-poor regions are followed by highly dense areas, and, when looked at the microscope, tumor vessels vary from irregular, abnormally wide, dilated, and tortuous serpentine-like shapes, with uneven diameter and excessive branching, to thin capillaries with small lumens. Furthermore, every layer of the tumor vessel wall is also abnormal. Endothelial cells of the tumor vasculature are poorly interconnected, lacking a cobblestone appearance and forming occasional multilayers. Moreover, tumor vessels are characterized by an irregular basement membrane and a lack of functional perivascular cells, which renders them leaky and with numerous openings, widened interendothelial junctions and discontinuous basal membrane. This leads to increased permeability to circulating molecules and even to entire cells, promoting tumor cell intravasation, dissemination, and metastasis. Another frequent outcome of hyperpermeability is an increase in interstitial pressure in the tumors, thereby impeding nutrient and drug distribution, and extravasation of erythrocytes, a process known as microhemorrhaging. At the molecular level, endothelial cells from tumors have been reported to upregulate different genes if compared to normal endothelial cells, termed tumor endothelial markers or TEMs (St Croix et al. 2000).

The structural abnormalities of tumor vessels are consequences of the pathological imbalance of activators and inhibitors of angiogenesis. Molecular studies have shown a marked upregulation of VEGF mRNA in the majority of human tumors (Ferrara et al. 2003). VEGF released by tumor and

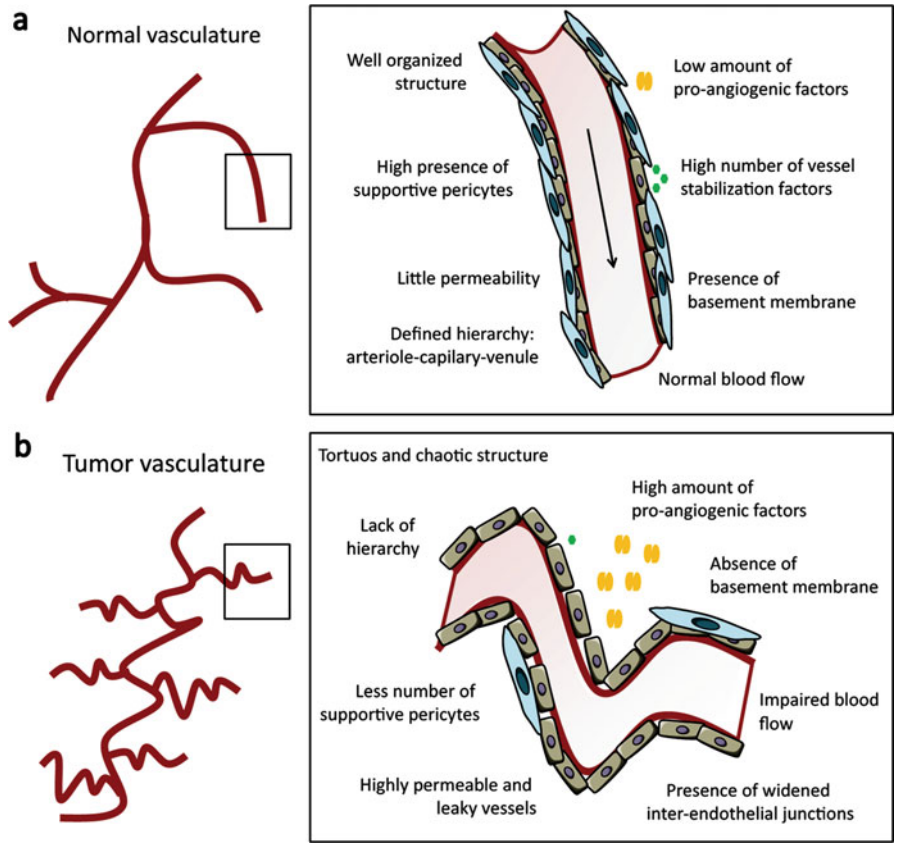

Fig. 4 Schematic differences between (**a**) normal and (**b**) pathological angiogenesis. The uncontrolled overproduction of angiogenic factors in (**b**) pathological conditions leads to an immature and structurally and functionally altered vasculature which is characterized by chaotic and tortuous vascular branches, regions of hypoxia, irregularities in the basement membrane, and lack of coverage of perivascular cells, among others. By contrast, (**a**) the normal vasculature has a well-organized structure, normal blood flow, low amount of proangiogenic factors, and a high presence of supportive pericytes

stromal cells not only triggers proliferation of endothelial cells and sprouting angiogenesis but also promotes increased vascular permeability, which renders tumor vessels highly leaky. Furthermore, tumor-produced Ang-2 mediates in the dissolution of endothelial junctions, while proteases digest the basement membrane and the ECM, thus allowing endothelial cell migration and sprouting. The sustained imbalance in the production of proangiogenic factors and the persistent lack of vessel-stabilizing factors produces an immature and dysfunctional vascular network that resembles a vessel structure that is not able to cope with the rapid growth rate of the expanding tumor mass (Baluk et al. 2005).

Conclusion

Tumor angiogenesis is a well-established hallmark of cancer. Starting with the archetypal sprouting angiogenesis and ending with the less familiar vasculogenic mimicry, the understanding of the different mechanisms that drive the angiogenic process is essential for successful therapeutic targeting. The main biological processes involved in angiogenesis progression are endothelial cell proliferation, vessel guidance, maturation, stabilization and quiescence, and, finally, regression. The interplay between the main molecular families composing each step of the vessel-branching

process is crucial to understand the chemical and physical changes that endothelial cells and their surroundings undergo both in normal and pathological conditions. Moreover, aberrant regulation of some of the molecules involved in normal angiogenesis, such as VEGF, FGF, or MMPs, is critical for the engagement of the angiogenic switch and the subsequent tumor progression. In this context, the role of intratumoral hypoxia as a catalyst of the overproduction of proangiogenic molecules both by tumor and stromal cells is emphasized. However, even though the molecular pathways followed both in normal and pathological angiogenesis are shared, the morphology of the newly formed vascular tree in the tumor stroma is completely different due to an aberrant imbalance of proangiogenic and anti-angiogenic molecules. In conclusion, a profound knowledge of the mechanisms, mediators, and main players of the angiogenic process, together with the focus on the main differences between physiological and tumor angiogenesis, is decisive for an effective development of therapeutic strategies.

Cross-References

- Anti-angiogenic Targets: Angiopoietin and Angiopoietin Receptors
- Mechanisms of Anti-angiogenic Therapy
- Pathology of Tumor Angiogenesis
- The Role of the VEGF Signaling Pathway in Tumor Angiogenesis

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