Molecular Mediators of Angiogenesis

Areck A. Ucuzian, M.D.[*],[1],[2], **Andrew A. Gassman, M.D.**[*],[1], **Andrea T. East, M.D.**[4], and **Howard P. Greisler, M.D.**[1],[2],[3]

[1]Department of Surgery, Loyola University Medical Center, Maywood, IL

[2]Department of Cell Biology, Neurobiology, & Anatomy, Loyola University Medical Center, Maywood, IL

[3]Research and Surgical Services, Edward J. Hines Jr. V.A. Hospital, Hines, IL

[4]Department of Surgery, University of Michigan, Ann Arbor, MI

Abstract

Angiogenesis, or the formation of new blood vessels from the preexisting vasculature, is a key component in numerous physiologic and pathologic responses and has broad impact in many medical and surgical specialties. In this review, we discuss the key cellular steps which lead to the neovascularization of tissues, and highlight the main molecular mechanisms and mediators in this process. We include discussions on proteolytic enzymes, cell/matrix interactions, pertinent cell signaling pathways, and end with a survey of the mechanisms which lead to the stabilization and maturation of neovasculatures.

Keywords

angiogenesis; proteases; pericytes; growth factors; extracellular matrix

I. Introduction

The study of angiogenesis has clinical applications in numerous fields including peripheral and coronary vascular disease, oncology, hematology, wound healing, dermatology, ophthalmology, and many others. Experimental evidence demonstrates that the promotion of angiogenic processes can have potential therapeutic benefit in ischemic tissue, while the study of anti-angiogenic therapies have yielded several FDA approved additions to chemotherapeutic regimens in the field of oncology.[1] In wound physiology, neovascularization is a key component in the adaptive repair response, promotes prompt coverage of thermal and traumatic wounds, and is required for the survival of grafts and flaps. In addition, inadequately vascularized tissue increases the risk for bacterial and fungal infections which complicate wound management, increase hospital admissions and clinic visits, prolong hospital stays, and add to the cost of health care for patients. For these reasons, a clear and fundamental understanding of the molecular mechanisms which regulate angiogenesis should allow for the development of targeted pro- and anti-angiogenesis therapies which would provide for benefits in the management of complicated wounds, the development of biologic tissue substitutes, and the treatment of pathologies across numerous medical and surgical specialties.

Correspondence: Howard P. Greisler, M.D. Loyola University Medical Center 2160 South First Ave. Maywood, IL 60153, USA OFC: 708-216-8541 Fax: 708-216-6300 hgreisl@lumc.edu.
[*]Contributed equally to this manuscript.

This review focuses on the relevant growth factors, cell-matrix interactions, matrix remodeling enzymes, and pertinent intracellular signaling pathways involved in modulating angiogenesis. We will also discuss the cellular and molecular processes which contribute to the prevention of uncontrolled growth and regression of newly developed vessels.

II. The Process of Angiogenesis

Overview

New blood vessel formation, or neovascularization, occurs either by angiogenesis or by vasculogenesis. Vasculogenesis is the creation of new vessels *de novo* from precursor cells, such as angioblasts, which differentiate into endothelial cells (ECs), form lumens, and create primitive blood vessels. In contrast, angiogenesis is the formation of new capillaries from the preexisting vasculature. Arteriogenesis, or collateralization, results from the hypertrophy and luminal distention of preexisting vessels in response to mechanical stresses caused by redirected blood flow from occluded or stenosed distal vessels, mediated in part by mechanosensitive signaling in vascular wall cells and by macrophage-derived biomolecular signals.[2] While a clinically important process, arteriogenesis is not the formation of new blood vessels and will not be discussed.

The process of angiogenesis involves a complex and dynamic interaction between ECs and the corresponding extracellular environment. *In vivo*, angiogenesis occurs either by the sprouting of vascular ECs from pre-existing capillary endothelia into the surrounding tissues, or by intussusception (aka non-sprouting angiogenesis), which involves the division of capillaries by tissue pillars into two or more daughter vessels.[3, 4] This review will focus primarily on sprouting angiogenesis, to which our usage of the term "angiogenesis" will refer. However, the distinctions made between sprouting and non-sprouting angiogenesis, angiogenesis and vasculogenesis, and the individual processes described below, may be somewhat arbitrary, as they all likely occur in concert and are regulated by related cellular and molecular mechanisms.

Endothelial Cell Sprouting

In a normal blood vessel, a 100-200 ηm thick basement membrane (BM) lies immediately deep to the EC monolayer in the arterial intima. Composed mainly of laminins, type-IV collagen, type-VIII collagen, and proteoglycans, the BM must be degraded prior to EC invasion into the surrounding extracellular matrix (ECM), which itself is comprised of fibrillar collagens, elastins, and various other ECM proteins.[5] ECs, in response to angiogenic stimuli, convert from a quiescent to a synthetically active phenotype characterized by a high mitotic index and increased capacity for migration and matrix proteolysis. These activated ECs are capable of disrupting the tight junctions, adherens junctions, and gap junctions which exist between neighboring intimal ECs and perivascular cells, and invade into the BM and surrounding ECM. [3, 6] Once freed from the capillary intima and in the extravascular space, ECs proliferate and migrate towards chemotactic and angiogenic stimuli in a 3-D extracellular environment and form new angiogenic sprouts (Fig 1). [7]

Lumenogenesis and Tubulogenesis

Lumen formation (and subsequent tube formation) can be considered a hallmark of angiogenesis as it is a relatively specific behavior attributed mainly to cells of the epithelium and endothelium. It is the genetically programmed capacity of ECs to create luminal compartments within multicellular chains which allows for the flow of blood from the pre-existing vasculature to the neovasculature, without which new capillary networks would be unable to perform their central function of oxygen and nutrient transport to normal or pathologic tissues. Indeed, one of the earliest demonstrations of angiogenesis *in vitro* by Folkman and

Haudenschild provided evidence that, in the presence of "direct" angiogens, ECs in culture form capillary-like structures with rudimentary luminal compartments.[8]

While several mechanisms for lumen formation have been suggested, the most widely investigated mechanism is the process of intracellular vacuolization (or intracellular canalization). The earliest observations of angiogenesis described the presence of "seamless" EC lined capillary lumens *in vivo* which were lacking in apparent cross-sectional EC-EC junctions.[9, 10] Since then, several *in vitro* and *in vivo* studies have provided for a mechanistic model of lumen formation consistent with this observation. Mediated by the $\alpha_2\beta_1$ integrin and members of the Rho GTPase family, ECs undergo pinocytosis, leading to the formation of intracellular vacuoles which coalesce and form one larger intracellular lumen.[11-16] This would explain the appearance of "seamless" EC lined capillary lumens lacking apparent junctional contacts among several lumen lining cells. The association of caveolin-1 expression, a protein involved in receptor mediated endocytosis and the formation of caveolae (cellular invaginations which often precede vacuole formation), with EC lumenogenesis is an interesting observation in light of this mechanistic hypothesis.[17] Concurrently, cytoplasmic projections have been suggested to sense and form junctional contacts with neighboring ECs to thereby form more complex multicellular capillary tubes.[15]

While intracellular vacuolization is the most widely studied model of lumen formation in ECs, there are descriptions of other mechanisms. These include intussusception, or the insertion of tissue pillars into the newly forming capillaries,[18] autophagy by lysosomes within individual ECs which leave behind luminal structures,[19] trans-luminal ingrowth of cytoplasmic filopodial projections which create a network of luminal structures,[16] as well as the exocytosis of vacuoles between two (or more) ECs which are sprouting in close apposition to one another to form intercellular lumens (intercellular canalization).[11, 13] Other groups have proposed that apoptosis of centrally located ECs within a cluster of many ECs can lead to tubular neovascular structures.[20, 21] This is consistent with data that demonstrates deficiencies in embryologic lumen formation and microvascular development in transgenic mice which conditionally express the anti-apoptotic protein Bcl-2 in ECs.[22]

It is important to note that these processes are often described or observed using *in vitro* assays of angiogenesis, and that the translation of these finding to the modeling of physiologic lumen formation *in vivo* is challenging.[23] Nonetheless, these models of angiogenesis are invaluable in attempting to understand the basic cellular and molecular mechanisms regulating lumenogenesis and angiogenesis in general. It is also important to note that while the above mechanisms for lumen formation have been proposed as separate entities, they may in fact all be contributing to EC lumen formation concurrently, and the relative importance of any one particular mechanism may be dependent on factors such as the location of the cells undergoing lumenogenesis (i.e. the sprouting tip vs. the trunk/stalk of the sprout).[24] It has also been proposed that heterogeneous populations of ECs with different functional characteristics can contribute to lumen and tube formation simultaneously. Meyer et. al. suggested a model of angiogenic tube formation which relies on three different populations of ECs: 1) those with phagocytic functions which create matrix channels and later undergo apoptosis, 2) those which form vacuoles which coalesce and get exocytosed to the intercellular space leading to a primitive lumen which is subsequently remodeled, and 3) those which are added into the capillary tube at the final stage of angiogenesis and aid in lumen growth and remodeling.[25]

A further consideration is the temporal relationship of lumen formation to sprouting. Whether one precedes the other, or, as suggested above, these processes occur simultaneously, is not entirely clear. There is experimental evidence to suggest both possibilities. Davis and Camarillo have demonstrated that vacuoles can be visible in ECs which are actively sprouting,[14] while

other studies demonstrate that branching and new sprout formation can occur in the absence of lumen formation.[26, 27]

Inosculation

Inosculation is the fusion or anastomosis of two vascular lumens or luminal segments to form one continuous lumen. Although an understudied process in angiogenesis, some literature from the field of skin graft vascularization can be extrapolated to provide some insights into this later step of angiogenesis. In 1980, Tsukada demonstrated that the preservation of the subcutaneous vascular network of a skin graft facilitated free tissue transfer, and that this was associated with both the formation of a neovasculature, as well as the inosculation between the preexisting host and donor vasculature.[28] By analyzing the establishment of donor/host vascular continuity in grafted skin, studies have demonstrated that skin neovascularization with complete inosculation occurs between three to seven post-operative days following skin grafting.[29, 30] Immunohistochemical staining of human skin transplanted onto athymic mice has documented recipient EC invasion into the graft between days 3 and 21 via neovascularization with blood flow commencing on days 3-7. Of note, recipient beds were not seen to have donor EC ingrowth.[31] The molecular mechanisms involved in inosculation, however, are still relatively unknown, and further study of this phase of angiogenesis is required.

III. Molecular Mediators of Angiogenesis

Growth Factors and Cytokines

Among the most commonly described angiogenic growth factors and cytokines include vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), tumor necrosis factor-alpha (TNF-α), transforming growth factor-beta (TGF-β), and the angiopoietins (Ang). Sources of these growth factors include endothelial cells, fibroblasts, smooth muscle cells, platelets, inflammatory cells, and cancer cells. Some growth factors, including fibroblast growth factor-2 (FGF-2, or basic FGF), are sequestered in the ECM, and serve as reservoirs for sustained cell-demanded growth factor release. The local concentrations of specific growth factors in the extracellular milieu may be impacted by physiologic or pathologic responses such as those seen during injury response, chronic inflammatory states or cancer. Platelet derived growth factor (PDGF), also an important mediator of angiogenesis, will be discussed later in the context of neovessel stabilization and maturation.

Vascular endothelial growth factor—VEGF is one of the most well studied growth factors involved in EC migration, mitogenesis, sprouting, and tube formation. Upregulated VEGF and VEGF-receptor (VEGF-R) mRNA has been detected in the tips of invasive angiogenic sprouts, and antibody blockade of VEGF significantly decreases microvessel outgrowth.[32, 33] This heparan sulfate binding molecule exists in several isoforms resulting from alternative splice variants from a single gene product. The soluble or membrane bound form binds most commonly to 2 transmembrane receptor tyrosine kinases, VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk-1).[34, 35] A third transmembrane receptor neuropilin-1 (Nrp-1) binds the VEGF-A splice variant of $VEGF_{165}$, is involved in capillary morphogenesis, and appears to be required for EC filopodial tip directionality during angiogenesis.[36] VEGF also promotes von Willebrand factor release, integrin expression, interstitial collagenase expression, plasminogen activator (PA) and plasminogen activator receptor (PA-R) expression, and increases both vascular permeability and fenestration, which accounts for its original name, vascular permeability factor. Heparin and heparan sulfate proteoglycan binding contributes to VEGF/VEGF-R binding, stabilizes the active conformation of VEGF, prevents its degradation, and has been shown to greatly increase the angiogenic response of ECs to VEGF in a type-I collagen matrix.[37]

Its role in angiogenesis has made VEGF a popular target in both pro- and anti-angiogenesis therapies, and anti-VEGF/VEGF-R antibodies and receptor tyrosine kinase inhibitors have become agents of chemotherapeutic regimens in lung and colon cancer. [1, 38] However, its utility as a targeted drug or gene therapy has also been associated with some potential and observed side effects including gastro-intestinal toxicity, hypothyroidism, proteinuria, coagulation disorders, neurotoxicity, impaired wound healing, and excessive chaotic neovascularization.[39, 40] It is probable that these potential side effects are partially attributable to a potentially narrow therapeutic range, making accurate therapeutic concentrations for both pro- and anti-angiogenesis therapy critical.[41] Thus, the clinical applicability of VEGF (or any angiogen) therapy can likely greatly improve with a better understanding of its absorbance and release kinetics from ECM.[42]

The above concept is especially true for therapeutic neovascularization strategies. For example, the attachment of VEGF to ECM scaffolds, such as fibrin or collagen, has been shown to promote sustained and controlled release of growth factor, and is associated with more organized vascular networks as compared to the chaotic and highly branching structures that commonly result from the local administration of their soluble counterparts.[43, 44] Recently, VEGF-R binding and vascular ingrowth induced by a fibrin binding variant of $VEGF_{121}$ demonstrated low continuous receptor binding and increased subcutaneous vascular invasion with eventual pericyte recruitment compared to wild type controls.[45]

Fibroblast Growth Factor—FGF is another pro-angiogenic growth factor, which is both stored in the vascular basement membrane to serve as a reservoir supply, and is upregulated during active angiogenesis. The two most commonly studied forms are FGF-2 or basic FGF (bFGF) and FGF-1 or acidic FGF (aFGF) which bind most commonly to the receptor tyrosine kinases FGFR-1 or FGFR-2.[46] *In vitro*, FGF binding of FGFR-1 increases EC migration and promotes capillary morphogenesis when cultured on collagen gels,[47] and activates signaling pathways mediated in part by protein kinase-C (PKC), phospholipase A_2,[48, 49] and numerous others. The observation that FGF-2 enhances endogenous VEGF production, and that VEGF is required for the FGF-2-induced expression of placental growth factor demonstrates the existence of cross talk and synergism between FGF and other growth factor pathways.[50-55] In addition, FGF-2-mediated proteolysis of matrix components (via up-regulation of urokinase receptors), and induction of the synthesis of collagen, fibronectin and proteoglycans by ECs, demonstrate its effects on ECM remodeling during angiogenesis.[47, 56]

The successful maintenance of microvasculatures engineered by the exogenous delivery of FGF, or VEGF, is likely dependent on the local concentration of total growth factor present in the culture environment. [57] Proof of this concept is provided by studies by Uriel, et. al. which demonstrated that persistent low levels of exogenously delivered FGF-1 promoted long-term survival of morphologically healthy appearing microvasculatures *in vitro* compared to higher levels of FGF-1.[58] The observed regression of engineered microvasculatures created by the sustained delivery of angiogens (i.e. VEGF, FGF) in the absence of physiologic demand highlights a potential cautionary paradigm in growth factor therapy of angiogen supply and cellular demand.[45] Unlike $VEGF_{165}$, however, FGF binds with a high degree of affinity to ECM proteins such as fibrin and with a high degree of saturation without a cross-linking molecule (i.e. heparan sulfate proteoglycan), which can potentiate its angiogenic potential.[59, 60] This observation, coupled with the ability of ECs to produce and release FGF during angiogenesis,[61] makes controlling local concentrations of FGF for therapeutic neovascularization purposes challenging. Strategies for sustained or targeted delivery of FGF and designer FGF mutants with biologic properties relatively more beneficial than those of wild type FGF have been investigated to partially address these challenges.[62-65]

Angiopoietin—The angiopoietins are a family of extracellular ligands that bind to the primarily EC-specific Tie receptors. Similar to other growth factor receptors, they represent a group of receptor tyrosine kinases which function primarily during vascular remodeling and angiogenesis.[66, 67] Ang-1 and Ang-2 bind the same receptor, Tie-2, but elicit very different responses. Ang-1 acts as an agonist, causing rapid receptor transphosphorylation, leading to subsequent downstream activation of the protein kinase B/Akt/FKHR (FOX01) pathway to promote EC survival and to inhibit Ang-2 expression.[68]

This latter effect is significant, as Ang-2 is perhaps the most well studied signaling molecule involved in EC activation and the initiation of angiogenesis. The early role of Ang-2 in angiogenesis is evidenced by its dramatic upregulation in ECs after exposure to angiogenic stimuli such as hypoxia and VEGF.[69-75] The release of Ang-2 from Weibel-Palade bodies of vessel ECs induces an autocrine response, which, in the presence of persistent pro-angiogenic signals such as VEGF, acts as a competitive antagonist of Ang-1 to prevent Tie-2 receptor phosphorylation and signaling, is involved in the disruption of the endothelial monolayer, and promotes EC survival, vessel sprouting and angiogenesis.[66, 76-78] Ang-2 has been shown to both promote the formation of neovessels during cerebral ischemia and to act as a critical regulator of postnatal vessel remodeling in the ischemic mouse hind limb.[78-80] Elevated Ang-2 levels in some tumors and direct Ang-2 retinal injections are associated with decreased pericyte coverage of vessel capillaries, which is consistent with a model of Ang-2-mediated dynamic vessel instability which can lead to increased neovascularization in the presence of other angiogenic stimuli.[79, 81-83] A clear understanding of the role of angiopoietins remains elusive and difficult to define however, as their highly context dependent functions vary with the relative levels of Ang-1, Ang-2 , VEGF, and cellular sources of angiogens under specific physiologic and pathologic conditions.

Transforming Growth Factor-β—The TGF-β superfamily is comprised of over 30 structurally similar growth factors and include the three TGF-β isotypes 1-3. They are secreted in an inactive latent form which requires cleavage of the latency associated peptide domain by proteases, often under acidic conditions. *In vivo*, TGF-β promotes ECM deposition and integrin receptor upregulation,[84] is one of the most important cytokines for granulation tissue formation,[85] and modulates wound EC proliferation, migration and capillary tube formation.[86] While *in vitro* results have demonstrated that TGF-β can directly inhibit angiogenesis in a receptor specific manner,[87] its ability to recruit inflammatory cells *in vivo*, which act as a source of pro-angiogenic signals, or its ability to modulate other angiogenesis pathways, may account in part for its pro-angiogenic effects.[86, 88] Its more well established role in mediating EC-pericyte interactions and vessel stabilization will be discussed below.

Tumor Necrosis Factor-alpha—TNF-α is an inflammatory cytokine, secreted primarily by activated macrophages during inflammation and immune response, but has also been associated with mast cells, ECs, fibroblasts, myocytes, adipose tissue, and neuronal tissue. It has a broad array of functions ranging from stimulation of granulocyte-macrophage-colony stimulating factor (GM-CSF) and interleukin-1 (IL-1), and has been suggested to act on ECs both directly, by inducing cell differentiation, and indirectly *in vivo*, by stimulating the production of angiogenic factors from other cells.[89]

Cell-matrix interactions

The proteolysis and remodeling of the ECM is critical in all phases of angiogenesis, affecting EC migration, invasion into the perivascular tissue, and the morphologic formation of luminal structures. The regulation of the activity of these enzymes is highly complex and is impacted by the micro-environmental context within which it occurs. We will discuss the main tools of matrix remodeling, and attempt to highlight the bidirectional nature of cell-matrix interactions.

Matrix metalloproteinases (MMPs) and other proteases—Also call matrixins, MMPs are a family of proteases that degrade ECM proteins and are critical in vascular remodeling, cellular migration, and sprout formation.[90] The observation that there is significant upregulation of MMP activity in ECs during inflammation, wound healing and tumor growth reflect their important role in both physiologic and pathologic angiogenesis.[91] Protein inhibitors of MMP-2 and MMP-9 have been shown to greatly attenuate the migration of both ECs and tumor cells and have been proposed as therapeutic targets in oncology.[92] Knockout mice deficient in MMP-2 demonstrate greatly reduced melanoma tumor angiogenesis and tumor progression.[93] The anti-tumor effects of targeted MMP inhibition is likely due to the dual benefits of inhibiting tumor angiogenesis and metastasis, as these processes both require significant ECM degradation.[94, 95] Disappointing clinical results with MMP inhibition in cancer therapy, however, highlights the complex nature of MMP activity in tumor and angiogenesis progression.[96]

In humans, these zinc-containing endopeptidases may be either secreted or membrane bound (MT-MMP).[91] The secreted forms are typically secreted as inactive pro-peptides which are often activated by a cascade of cell surface protease activity, including that of the MT-MMPs, which cleaves the pro-peptide to the active form. Increased expression of surface urokinase receptors in activated ECs, for example, bind the inactive proenzyme of urokinase plasminogen activator (uPA) (aka urokinase) and convert it to the active form. Active urokinase then promotes the conversion of plasminogen to plasmin.[97] Plasmin then activates MMPs that degrade the extracellular matrix.[98, 99] As such, the inhibition of uPA by plasminogen activator inhibitors (PAI-1 and 2) can affect not only the direct degradation of ECM but also of the activation of latent MMPs,[100] and PAI expression in ECs or pericytes can act to modulate angiogenesis.[101]

In addition to contributing to the proteolytic activation of MMPs, other proteases like uPA, tPA, and plasmin contribute to significant ECM degradation with relative specificity. Plasmin, a serine protease, cleaves both ECM (i.e. fibronectin and laminin), as well as coagulation cascade proteins, and is the enzyme primarily responsible for fibrin degradation, a significant component of the injury response which often is a signal for neovascularization.[102] Plasmin transversely cuts fibrin fibers and after prolonged exposure results in a broad range of progressively smaller fragments, which may themselves modulate the angiogenic response. [103-105] uPA is a secreted enzyme that binds with high affinity to its surface receptor, uPA receptor (uPAR), on a variety of cell types, and upregulated expression of both uPA and uPAR is seen in actively migrating ECs.[56, 99, 106] *In vitro*, TNF-α potentiates VEGF- or FGF-2-induced EC tube formation in fibrin matrices by the mediation of cell surface u-PA expression. [107] Blocking uPA or uPAR, or the presence of anti-plasmin compounds such as ε-aminocaproic acid or aprotinin reduces cellular invasion and matrix degradation,[108] and the upregulation of PAI in an athymic mouse prostate cancer model has been shown to decrease both the density of tumor-associated microvasculatures and the rates of liver and lung metastases.[109]

While the presence of growth factors can modulate the activity and expression of ECM proteolytic pathways, it is clear that the interaction between growth factors and proteases is bidirectional. Proteolytic growth factor activation or release from ECM reservoirs can propagate the angiogenic response by allowing for a sustained cell-demanded local concentration of active pro- (or anti-) angiogenic stimuli within the tissue. In addition, MMP-2 has been shown to directly cleave the extracellular domain of the FGF-1 receptor, which releases a soluble extracellular receptor domain that binds FGF-1 and may modulate angiogenesis *in vivo*.[110] Pathologic dysregulation of this intrinsic positive feedback loop between protease and growth factor activity can have significant clinical consequences as seen during the neovascularization of tumors.[111]

Matrix remodeling proteins also appear to be involved in the formation of luminal and tubular structures during the morphogenic differentiation of ECs in a manner that is specific to the type of MMP involved, with the membrane-bound forms appearing to be more critical in these events than secreted MMPs.[112, 113] This has been supported by experiments utilizing specific tissue inhibitors of metalloproteinases (TIMPs) which demonstrate relative substrate specificity. The inhibition of MT1-MMP, but not MT3-MMP, for example, by TIMP-2 or by siRNA knockdown, inhibits EC tube formation in a 3-D model of angiogenesis.[15, 114] This is consistent with results of an experiment in which aortic rings from MT1-MMP knockout mice displayed attenuated neovessel formation within a 3-D collagen matrix.[115] These results should be viewed cautiously, however, as numerous factors (e.g. EC source), can produce vastly different responses from study to study.[116] The mechanistic explanation for how MMPs contribute to lumenogenesis is unclear, but it has been proposed that MMPs create "vascular guidance tunnels", which serve as molds in the ECM that aid in vascular morphogenesis, pericyte recruitment, and basement membrane assembly.[15]

The extracellular matrix—The ECM is a highly important modulator of angiogenesis during oncogenesis, wound healing, and development. For example, the differentiation of ECs grown as a monolayer *in vitro* into branching tubule structures has been shown to require the coverage of cells with collagen and the establishment of appropriate cell-matrix interactions. [117] Angiostatic molecules like thrombospondin-1 (TSP-1) and interferon-inducible protein-10 have been shown to be less effective in inhibiting VEGF-induced EC proliferation when cultured on type-I collagen, an observation that has intuitive implications in the clinical use of pro- or anti-angiogenesis drugs.[118] Laminin, a non-collagen hetero-trimeric (3 chains: α, β, and γ) glycoprotein found in basement membrane and granulation tissue, has been shown to increase EC-ECM attachment and migration *in vitro*,[86] is produced throughout the length of EC sprouts and has been shown to limit proliferation and lumen size,[119] and is associated with breast and brain cancer neovascularization and membrane invasion (i.e. laminin-8 and laminin-10).[120, 121] Fibronectin, often an injury response protein, is actively unfolded by ECs and serves as an ECM scaffold for cellular matrix deposition and neovascularization,[122] while combinations of other healing proteins like hyaluronic acid, type I collagen and fibrin can have differential angiogenic effects compared to when they are present alone.[123, 124]

While the composition of the ECM is clearly an important factor in the regulation of angiogenesis, the structure of the ECM is also key. Studies have demonstrated in numerous cell types that cell culture in 3-D environments vs. 2-D environments can significantly impact the expression of differentiation markers, diminish cell proliferation, and affect the expression of proteins like MMP-2.[125, 126] Physical characteristics such as fiber thickness, density, orientation, cross-linking, and pore size have all been shown to affect the number, length, and cross-sectional area of angiogenic sprouts.[5, 127, 128] *In vitro*, ECs have been shown to demonstrate decreased cell migration and invasion with increased capillary morphogenesis in fibrin as the rigidity is decreased by altering pH, NaCl concentration, and thrombin content. [108, 129, 130] In chick chorioallantoic membrane (CAM) angiogenesis assays, significantly more sprout invasion is associated with a reduction in fibrin substrate concentration, and the physical characteristics of the ECM were found to be as important as the presence or absence of growth factors such as FGF and VEGF.[123]

Integrins—Among the most important cell surface receptors involved in cell-matrix interactions, the integrins are transmembrane heterodimeric receptors composed of an α and β subunit which engage ECM ligands to transduce environmental signals to cytoskeletal alterations and intracellular signaling pathways which affect phenotypic modulation, motility, mechanotransduction, and cell proliferation,[131-135] and thus impact a broad range of angiogenic processes.[26, 136-145] In addition, downstream integrin signaling events mediate the cellular processes involved in angiogenesis, as exemplified by focal adhesion kinase (FAK)

knockout mice which demonstrate altered EC morphogenesis during blood vessel assembly. [146]

These receptors have the capacity to act in a ligand-specific manner producing specific cellular responses depending on the particular ECM or integrin subunits engaged,[131, 147] with the α_v and β_1 subunits, specifically, proposed to be particularly important in morphologic differentiation.[86] Some evidence suggests that the disruption of cell-matrix interactions by $\alpha_v\beta_3$ and $\alpha_v\beta_1$ integrin receptor blockade actually tends to decrease EC proliferation and to promote the differentiation of ECs to large and multicellular tubules in a matrix specific manner.[148]

Not surprisingly, the expression and activity of the EC proteolytic machinery is mediated by integrin-dependent signaling pathways. Observed colocalization of active cell surface MMP-2 with the $\alpha_V\beta_3$ integrin in angiogenic blood vessels and melanoma cells likely reflects the tight coordination between motility and matrix degradation in invasive cells, once again highlighting the bidirectional nature of cell-matrix interactions.[149] The $\alpha_2\beta_1$ integrin receptor, but not $\alpha_3\beta_1$integrin receptor, has been shown to be required for type I collagen-induced gelatinase A (MMP-2) activation, in a mechanism that also requires MT1-MMP.[105] This, and other studies, demonstrate the highly complex proteolytic cellular responses which are dependent on the nature of the ECM-integrin interaction identified by the specific ECM and corresponding integrin subtype engaged.[150]

In addition to its direct effects on cellular responses, engagement and activation of integrin-dependent pathways mediate responses to growth factors via significant cross-talk with growth factor pathways, allowing for coordination of cell-matrix-growth factor interactions during angiogenesis.[151-153] FGF-2-induced EC migration seems to be mediated in part by the upregulation of the $\alpha_V\beta_3$ integrin. [154] Similarly, VEGF has been shown to upregulate the expression of the collagen binding integrins $\alpha_1\beta_1$ and $\alpha_2\beta_1$, and blockade of these receptors attenuates the angiogenic response to VEGF.[141] Recently, it was shown that VEGF recruits c-src to the EC membrane, which subsequently tyrosine phosphorylates the β_3 integrin and thereby mediates interaction between VEGF-R2 and integrin β_3 after VEGF stimulation.[155]

Intracellular Signaling Pathways

A detailed and extensive review of the intracellular signaling pathways involved in angiogenesis is well beyond the scope of this review. We will attempt to introduce some of the most pertinent pathways here, and have cited recent reviews for further study.[7, 156]

Rho GTPases—The Rho family of guanine nucleotide (GTP) binding proteins (including Rho, Rac, and Cdc42), which are members of the Ras superfamily of GTPases, are intimately involved in growth factor signaling, cytoskeletal dynamics, cell motility, cell shape and spreading, cell phenotype and other processes which significantly impact angiogenesis.[157, 158] VEGF-induced EC chemotaxis has been shown to require activated Rac, and type I collagen-induced migration requires Rac and Cdc42 activity.[159] Interestingly, while both Rac and Rho induce the formation of stress fibers, special actinomyosin filaments arranged to generate the force required for movement, Rho activity may not be involved in either VEGF- or collagen-induced motility, and may even inhibit the migratory response to extracellular matrix.[159] Intracellular signaling molecules phosphatidylinositol 3-kinase (PI3-K), Akt, protein kinase B (PKB), p38 MAPK, FAK, and Rho-associated-kinase (ROCK) all provide molecular linkages among VEGF-R2-mediated Rho GTPase signal transduction pathways in EC migration.[7, 157]

In addition to its effects on EC migration, several studies have demonstrated that Cdc42 plays a crucial role in EC lumenogenesis.[12] Consistent with this, recent evidence demonstrated that

overexpression of Cdc42 GTPase activating protein (Cdc42 GAP), a protein which catalyzes GTP hydrolyzation and consequently decreases the active conformation of Cdc42, attenuates EC lumen formation while siRNA knockdown of Cdc42 GAP increased lumenogenesis.[160] *In vitro*, ECs with lower levels of Cdc42 GAP expression have been shown to be more likely to form tubes and lumens in comparison to ECs with a higher level of expression.[160] Although the exact mechanistic role of Cdc42 in EC lumen formation is still unclear, this and other downstream GTPase pathway proteins demonstrate potentially promising therapeutic targets for pro or anti-angiogenesis therapies.[161]

Protein Kinase C (PKC)—PKC is an intracellular kinase that is an important mediator of endothelial cell migration, proliferation and capillary tube formation. Decreasing PKC activity has been shown to attenuate EC attachment to and migration along ECM coated surfaces, without significantly affecting cell spreading.[162] Its multifaceted role in VEGF signaling is demonstrated by the inhibition of VEGF-induced cell proliferation, MAPK phosphorylation, migration, endothelial tube formation, matrix adhesion, and vessel formation *in vitro* and *in vivo* in cells treated with antisense PKC-α oligonucleotides.[163]

Notch—Signaling through Notch, a cell membrane receptor, is an evolutionarily conserved pathway which impacts cell fate and differentiation determination,[164] and is involved in regulating angiogenesis.[165, 166] Notch receptors and their Delta-like-4 (Dll4) ligand appear to be central components in restricting the formation of tip cells in response to VEGF within angiogenic sprouts, which limits the branching and sprouting patterns of angiogenic networks[167] and limits tube diameter by inhibiting EC proliferation.[168] Blockade of Dll4 in tumor-associated ECs leads to the increased vascularity and branching of tumors, although these microvasculatures demonstrate poor function and are associated with decreased tumor growth.[169, 170]

Cell-Cell Interactions

Perivascular cells such as pericytes (aka mural cells), SMCs, and adventitial fibroblasts, are associated with the endothelium of capillaries and are known to be involved in the later stages of angiogenesis during vessel maturation and stabilization (discussed below). However, these primarily mesenchymally derived cells surrounding the endothelium also appear to have a significant role in mediating the early events of active angiogenesis.[171-174] The co-culture of ECs with pericytes, SMCs and fibroblasts *in vitro* have demonstrated that the secretion of soluble factors such as VEGF or the deposition of ECM proteins such as fibronectin elicit angiogenic activities in ECs such as increased plasminogen activity, proliferation, motility, and cordlike structure formation.[175-180] Evidence that these paracrine interactions may be regulated by the local extracellular environment is provided by the observed endothelin-1-mediated activation of EC mitogenesis by fibroblasts,[176] and the production of Ang-2 and VEGF in mesangial cells (renal pericytes) under hypoxic conditions.[69] *In vivo* observations of pericytes found "leading" angiogenic sprout tips and lining vascular tubes connected to tumor vasculatures which lack ECs *in vivo* suggest a more direct role for pericytes as guiding cells during angiogenesis.[181, 182] These data are consistent with observations that under hypoxic stimulation, VEGF-secreting perivascular cells appear to be the first vascular cells to invade the corpus luteum parenchyma during ovulatory angiogenesis.[183] In our own lab, we have observed that ECs in both direct and indirect co-culture with SMCs form sprouts which extend to greater lengths, form more robust networks, and persist over longer time periods in fibrin hydrogels compared to ECs cultured alone (unpublished results). Thus, while the later stabilization and maturation of the microvasculature by pericytes is well established, the likelihood that they promote angiogenesis given the proper pro-angiogenic environmental context suggests a more broad role for these cells which warrants further study.[184]

Gene Expression

An emerging strategy for understanding the molecular regulators governing many aspects of angiogenic differentiation is the study of genetic expression patterns in activated angiogenic ECs. An analysis of genes expressed in ECs which invade and form tubules in a fibrin rich matrix vs. ECs which remain in a monolayer demonstrated differential regulation of a number of genes.[160] *In vivo*, genetic analyses of tumor-associated ECs, which tend to be more angiogenic than ECs from normal vessels, have identified potential angiogenesis markers, termed tumor endothelial markers (TEMs).[160, 185, 186] The identity and function of many of these genes, are largely still unknown and under study, although their roles in EC survival, integrin signaling, migration, proliferation, and matrix remodeling have been implicated.[160, 187-189] The analysis of TEM expression patterns, and genetic expression patterns in general, offers a unique and exciting methodology which may provide for real translational and therapeutic benefits in a wide variety of patients, and may even provide potential utility in the assessment of cancer prognosis.[190]

IV. Stabilization and Maturation of the Neovasculature

Molecular and cellular feedback is critical in preventing pathologic angiogenesis. This is achieved by the preferential upregulation of anti-angiogenic stimuli and downregulation of the above pro-angiogenic processes. The net result is a final product of stable capillary tubes lined with a quiescent, functional monolayer of ECs demonstrating junctional contacts, low in both mitotic index and apoptotic turnover, supported by surrounding pericytes.

The extracellular matrix

A primary mediator of this final stage of angiogenesis is the composition of the ECM surrounding the endothelial sprouts.[191] Type IV collagen, a major basement membrane protein, has been shown to both promote the lengthening of neovessels and to prevent their regression and continued growth in a dose-dependent manner.[192] It has been shown that the degradation of basement membranes rich in type-IV collagen releases several anti-angiogenic proteins such as arresten (from collagen's α1 chain), and canstatin (α2 chain), which suggests a mechanism for the almost immediate negative regulation of angiogenesis after its induction.[193, 194] Alpha-statin and kinostatin, products of MMP mediated fibrinogen and HMW-kininogen degradation, respectively, possess anti-angiogenic activities and likely regulate the angiogenic response to injury.[91, 96] Thrombospondin is a platelet and inflammatory cell derived protein found within the ECM that is secreted in response to injury[195] which has been shown to inhibit EC proliferation and migration, induce apoptosis, and downregulate MMP activity.[102, 196] For these reasons, thrombospondin and other endogenous inhibitors of angiogenesis, like endostatin, have become attractive targets for cancer treatment, but have unfortunately met with limited clinical success.[197, 198]

Regulation of proteolysis

Given the importance of proteolytic activity and the impact of ECM degradation in modulating angiogenesis, the regulation of MMPs and other proteases is a critical mode of angiogenesis control. Tissue inhibitors of metalloproteinases (TIMPs) are proteins that regulate MMP activity and can act to both activate and inhibit angiogenesis, reflecting the complicated pro- and anti-angiogenic nature of protease activity during angiogenesis.[90, 91, 199] In addition to directly inhibiting MMPs, TIMP-3 prevents VEGF binding to VEGF-R2 and serves to inhibit VEGF induced signaling and angiogenesis.[200]

Pericytes

The recruitment and incorporation of pericytes are key events which mediate the final steps required for the stabilization and maturation of the neovasculature.[6] Pericytes are primarily thought to be derived from cells of mesenchymal origins such as SMCs, fibroblasts or other progenitor cells and possess markers such as α-actin, desmin, PDGF-β receptor, and NG2 which are common to those cells.[6, 173, 201] Several lines of evidence support the role of pericyte incorporation for the stabilization of neovessels and the regulation of uncontrolled angiogenic growth. Early studies showed that mice lacking the ability to recruit pericytes during embryonic vascular development demonstrated increased vessel microaneurysm formation and rupture. [202] Generally speaking, abnormal pericyte coverage and function in the microvasculatures of certain types of tumors is associated with increased EC sensitivity to VEGF withdrawal[203-207] and microvasculatures with disrupted EC/pericyte interactions are associated with more dynamic remodeling.[208]

Direct contact via gap junctions, adhesion plaques, and adherens junctions, as well as indirect paracrine mechanisms between ECs and pericytes, allow for significant interactions which impact cellular differentiation, migration, proliferation, and quiescence.[209-212] Among the paracrine mediators, PDGF is thought to be critical in the recruitment and proliferation of pericytes during angiogenesis. PDGF is a mitogen and chemoattractant for fibroblasts, SMCs, and other mesenchymal cells which is present in four known isoforms A, B, C, and D. Two known PDGF transmembrane receptor tyrosine kinases known as the α and β subunit bind each chain with relative specificity (the α receptor binds the A, B, and C chains primarily while the β receptor binds the B and D chains). Because PDGF exists as a hetero- or homodimer, ligand binding causes receptor hetero- or homodimerization (i.e. PDGF-BB and PDGF-AB can bind α/α, α/β, or β/β, while PDGF AA binds α/α exclusively) and subsequent cell signaling. [213-215] As PDGF-β is the primary receptor present on SMCs and other pericytes, PDGF-B/β signaling is especially critical in the proper recruitment of pericytes to capillaries. Disrupted PDGF-B/β signaling is associated with the attenuated ability to recruit PDGF-β receptor positive pericytes and precursors from the perivascular tissue and the bone marrow and is associated with significant vascular abnormalities in physiologic and pathologic angiogenesis. [202, 205, 216-221]

The source of PDGF during this process is likely from actively angiogenic ECs themselves, which suggests an inherent negative feedback mechanism to prevent uncontrolled neovascularization.[6] Proliferative ECs *in vitro* are potent stimulators of pericyte/SMC growth and migration, in part mediated by PDGF-BB, although this effect is dependent on the time in co-culture, the presence or absence of direct contact, and the deposition of endothelial-specific ECM proteins.[222-230] The importance of EC-specific PDGF-B production in pericyte recruitment *in vivo* was demonstrated in studies which showed that EC-specific knockout of PDGF-B led to significant vascular abnormalities with pericyte loss in mice.[231-233]

Clinically, the simultaneous targeting of pericytes and ECs by pro- or anti-PDGF and VEGF therapy, has been investigated in tissue engineering and oncologic research. Combination therapy has been shown in some studies to be more effective in disrupting tumor vascularity than either agent alone and, as such, combinatorial antitumor therapy is under investigation. [234-237] It is unclear, however, if the apparent improved anti-angiogenic effect observed in combination therapy is specifically attributable to PDGF blockade or to the broad non-specific effects of the receptor tyrosine kinase inhibitors used in most studies of combination therapy. Recently, it was shown that adenoviral expression of soluble anti-PDGF-β receptor provided added inhibition of tumor growth and angiogenesis only when sub-optimal levels of VEGF blockade were present.[238] Clearly, however, PDGF signaling does impact angiogenesis *in vivo*, as the spatiotemporal delivery of PDGF with VEGF has been shown to induce denser and more mature appearing vascular networks within tissue engineered constructs, which is in

part associated with the recruitment of α-actin positive (indicative of pericytes) cells into the neovascular networks.[239, 240]

Although the expansion, spreading, and migration of the SMC/pericyte population on newly formed capillaries may be dependent on PDGF-B/β signaling, it does not appear to be sufficient or necessary for the phenotypic differentiation of undifferentiated mesenchymal cells to pericytes. Instead, this appears to be regulated by TGF-β,[218, 241] as evidenced by observations that ECs direct 10T1/2 (a cell line commonly used to model pericyte precursor cells *in vitro*) to an SMC/pericyte phenotype via a TGF-β-mediated mechanism,[228, 242] and that the phenotypic differentiation of SMCs in co-culture with ECs involves activation of the SMC PI3-K/Akt pathway, which may be mediated by TGF-β.[243, 244]

With these above observation taken together, it has been proposed that two modes of pericyte incorporation occur simultaneously: 1) the differentiation of undifferentiated mesenchymal and progenitor cells into pericytes; and 2) the recruitment and co-migration of differentiated mesenchymal cells such as SMCs from a pool of available cells in the perivascular tissue into neovessels.[218]

It is believed that once incorporated, a major function of pericytes is to mediate the quiescence of ECs. *In vitro*, ECs in direct contact with pericytes have lower levels mitosis compared to ECs cultured alone, a process which may require Rho GTPase activity in pericytes.[229, 245, 246] Similarly, ECs in close contact with SMCs in a spherical aggregate of SMCs in collagen gels demonstrated decreased responsiveness to VEGF (in the absence of Ang-2), increased inter-endothelial junctions, decreased PDGF-B growth factor chain expression, and reduced apoptosis.[247] ECs in co-culture with 10T1/2 cells also displayed a significantly higher resistance to the permeability of a tracer molecule biotin-dextran which was associated with tightening of EC-EC tight junctions and with the localization of tight junction proteins plakoglobin, ZO-1, ZO-2, and occludin.[248]

It has also been suggested that pericytes induce vessel stabilization by the production of survival factors. This is supported by several lines of evidence: 1) Neonatal vessels which are resistant to oxygen-induced degeneration are associated with pericytes which express TGF-β_1, which induces VEGFR-1 expression in ECs;[249] 2) 10T1/2 precursor cells produce VEGF upon TGF-β-mediated conversion to pericytes when in direct contact with ECs;[250] 3) Pericytes express Ang-1 *in vitro* and *in vivo*, a growth factor which is associated with basement membrane formation, EC quiescence and endothelial leak resistance, and which can mature vessels lacking pericytes;[67, 251-253] and 4) Pericyte loss caused by PDGF inhibition in a rat model of retinopathy results in overexpression of VEGF/VEGFR2 in ganglion cells and other supporting cells.[254]

A final mechanistic hypothesis for the pericyte-mediated regulation of angiogenesis is that pericytes significantly modulate the ECM remodeling capacity of ECs by directly influencing the local ECM composition. Pericyte-conditioned media has been shown to limit both EC migration and branching *in vitro* by inducing the upregulation of PAI-1 in ECs.[255] In addition, pericytes have been shown to produce TIMP-3 and induce TIMP-2 production in ECs, which specifically targets MT1-MMP, MT2-MMP and ADAM proteinases, with the net effect of decreasing angiogenic potential and tube regression.[112, 256, 257] In addition, pericyte-mediated ECM deposition, specifically the deposition and synthesis of basement membrane, has also been proposed to promote EC quiescence and vessel stability.[191, 258, 259] The importance of the basement membrane in vascular stability is demonstrated in laminin-8 knockout animals which demonstrate poorly developed microvasculature with defective basement membranes, noticeable deformities, and increased responsiveness to pro-angiogenic stimuli.[260]

While the mechanisms discussed above in the maturation and stabilization of existing and newly formed vessels are still topics of current study, it is becoming clear that this final stage of angiogenesis is a significant contributor to the persistence and function of pathologic and physiologic neovasculatures, and will likely provide for future therapeutic strategies.

V. Summary

We have attempted to describe the major cellular processes involved in the angiogenic development of new blood vessels and the molecular mediators involved in regulating these events. It should be noted that while we do not cover the biomechanical environment within which these events occur, the effects of shear stresses and cyclic distention resulting from pulsatile flow is an important consideration.[261, 262]

In summary, the relationships among phenotypically variable vascular (and progenitor and stem) cells, ECM and ECM receptors, growth factors and growth factor receptors, proteases, and intracellular signaling pathways during blood vessel formation represent an infinitely complex set of orchestrated events which are tightly regulated by a myriad of macro and micro-environmental factors in an apparently perpetual state of dynamic interdependent flux. It is a challenge for investigators of such complex biologic systems to develop more intricate and sophisticated models in order to paradoxically apply a more precise and reductionistic investigatory approach. The ultimate benefit of this work will likely be to facilitate the discovery of new anti- or pro-angiogenic therapies that can specifically target pathologic tissues to impact the health of patients in numerous medical and surgical specialties.

Acknowledgments

References

1. Carmeliet P. Angiogenesis in life, disease and medicine. Nature Dec 15;2005 438(7070):932. [PubMed: 16355210]

2. Scholz D, Cai WJ, Schaper W. Arteriogenesis, a new concept of vascular adaptation in occlusive disease. Angiogenesis 2001;4(4):247–257. [PubMed: 12197469]

3. Risau W. Mechanisms of angiogenesis. Nature Apr 17;1997 386(6626):671. [PubMed: 9109485]

4. Burri PH, Djonov V. Intussusceptive angiogenesis--the alternative to capillary sprouting. Mol Aspects Med Dec;2002 23(6S):S1–27. [PubMed: 12537983]

5. Francis ME, Uriel S, Brey EM. Endothelial cell-matrix interactions in neovascularization. Tissue Eng Part B Rev Mar;2008 14(1):19–32. [PubMed: 18454632]

6. Armulik A, Abramsson A, Betsholtz C. Endothelial/Pericyte Interactions. Circ Res September 16;2005 97(6):512–523. 2005. [PubMed: 16166562]

7. Lamalice L, Le Boeuf F, Huot J. Endothelial cell migration during angiogenesis. Circ Res Mar 30;2007 100(6):782–794. [PubMed: 17395884]

8. Folkman J, Haudenschild C. Angiogenesis in vitro. Nature Dec 11;1980 288(5791):551. [PubMed: 6160403]

9. Wolff JR, Bar T. 'Seamless' endothelia in brain capillaries during development of the rat's cerebral cortex. Brain Res Jun 8;1972 41(1):17. [PubMed: 5036034]

10. Guldner FH, Wolff JR. Seamless endothelia as indicators of capillaries developed from sprouts. Bibl.Anat 1973;12:120. [PubMed: 4790339]

11. Davis GE, Bayless KJ. An integrin and Rho GTPase-dependent pinocytic vacuole mechanism controls capillary lumen formation in collagen and fibrin matrices. Microcirculation Jan;2003 10(1):27. [PubMed: 12610662]

12. Bayless KJ, Davis GE. The Cdc42 and Rac1 GTPases are required for capillary lumen formation in three-dimensional extracellular matrices. J.Cell.Sci Mar 15;2002 115(Pt 6):1123. [PubMed: 11884513]

13. Kamei M, Saunders WB, Bayless KJ, Dye L, Davis GE, Weinstein BM. Endothelial tubes assemble from intracellular vacuoles in vivo. Nature Jul 27;2006 442(7101):453. [PubMed: 16799567]

14. Davis GE, Camarillo CW. An alpha 2 beta 1 integrin-dependent pinocytic mechanism involving intracellular vacuole formation and coalescence regulates capillary lumen and tube formation in three-dimensional collagen matrix. Exp.Cell Res Apr 10;1996 224(1):39. [PubMed: 8612690]

15. Davis GE, Koh W, Stratman AN. Mechanisms controlling human endothelial lumen formation and tube assembly in three-dimensional extracellular matrices. Birth Defects Res.C.Embryo.Today Dec; 2007 81(4):270. [PubMed: 18228260]

16. Egginton S, Gerritsen M. Lumen formation: in vivo versus in vitro observations. Microcirculation Jan;2003 10(1):45. [PubMed: 12610663]

17. Liu J, Wang XB, Park DS, Lisanti MP. Caveolin-1 expression enhances endothelial capillary tubule formation. J.Biol.Chem Mar 22;2002 277(12):10661. [PubMed: 11748236]

18. Djonov VG, Galli AB, Burri PH. Intussusceptive arborization contributes to vascular tree formation in the chick chorio-allantoic membrane. Anat.Embryol.(Berl) Nov;2000 202(5):347. [PubMed: 11089926]

19. Stromhaug PE, Klionsky DJ. Approaching the molecular mechanism of autophagy. Traffic Aug;2001 2(8):524. [PubMed: 11489210]

20. Peters K, Troyer D, Kummer S, Kirkpatrick CJ, Rauterberg J. Apoptosis causes lumen formation during angiogenesis in vitro. Microvasc.Res Sep;2002 64(2):334. [PubMed: 12204657]

21. Duval H, Harris M, Li J, Johnson N, Print C. New insights into the function and regulation of endothelial cell apoptosis. Angiogenesis 2003;6(3):171. [PubMed: 15041793]

22. Duval H, Johnson N, Li J, et al. Vascular development is disrupted by endothelial cell-specific expression of the anti-apoptotic protein Bcl-2. Angiogenesis 2007;10(1):55. [PubMed: 17149535]

23. Ucuzian AA, Greisler HP. In vitro models of angiogenesis. World J.Surg Apr;2007 31(4):654. [PubMed: 17372665]

24. Egginton S, Zhou AL, Brown MD, Hudlicka O. Unorthodox angiogenesis in skeletal muscle. Cardiovasc.Res Feb 16;2001 49(3):634. [PubMed: 11166277]

25. Meyer GT, Matthias LJ, Noack L, Vadas MA, Gamble JR. Lumen formation during angiogenesis in vitro involves phagocytic activity, formation and secretion of vacuoles, cell death, and capillary tube remodelling by different populations of endothelial cells. Anat.Rec Nov;1997 249(3):327. [PubMed: 9372166]

26. Davis GE, Camarillo CW. Regulation of endothelial cell morphogenesis by integrins, mechanical forces, and matrix guidance pathways. Exp.Cell Res Jan;1995 216(1):113. [PubMed: 7813611]

27. Vernon RB, Angello JC, Iruela-Arispe ML, Lane TF, Sage EH. Reorganization of basement membrane matrices by cellular traction promotes the formation of cellular networks in vitro. Lab.Invest May;1992 66(5):536. [PubMed: 1374138]

28. Tsukada S. Transfer of free skin grafts with a preserved subcutaneous vascular network. Ann.Plast.Surg Jun;1980 4(6):500. [PubMed: 7002007]

29. O'Ceallaigh S, Herrick SE, Bluff JE, McGrouther DA, Ferguson MW. Quantification of total and perfused blood vessels in murine skin autografts using a fluorescent double-labeling technique. Plast.Reconstr.Surg Jan;2006 117(1):140. [PubMed: 16404261]

30. Okada T. Revascularisation of free full thickness skin grafts in rabbits: a scanning electron microscope study of microvascular casts. Br.J.Plast.Surg Apr;1986 39(2):183. [PubMed: 2421814]

31. Plenat F, Vignaud JM, Guerret-Stocker S, Hartmann D, Duprez K, Duprez A. Host-donor interactions in healing of human split-thickness skin grafts onto nude mice: in situ hybridization, immunohistochemical, and histochemical studies. Transplantation May;1992 53(5):1002. [PubMed: 1585462]

32. Brown KJ, Maynes SF, Bezos A, Maguire DJ, Ford MD, Parish CR. A novel in vitro assay for human angiogenesis. Lab.Invest Oct;1996 75(4):539. [PubMed: 8874385]

33. Gerhardt H, Golding M, Fruttiger M, et al. VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. J.Cell Biol Jun 23;2003 161(6):1163. [PubMed: 12810700]

34. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nat Med Jun;2003 9(6): 669–676. [PubMed: 12778165]

35. Neufeld G, Tessler S, Gitay-Goren H, Cohen T, Levi BZ. Vascular endothelial growth factor and its receptors. Prog Growth Factor Res 1994;5(1):89–97. [PubMed: 7515293]

36. Gerhardt H, Ruhrberg C, Abramsson A, Fujisawa H, Shima D, Betsholtz C. Neuropilin-1 is required for endothelial tip cell guidance in the developing central nervous system. Dev Dyn Nov;2004 231 (3):503–509. [PubMed: 15376331]

37. Steffens GC, Yao C, Prevel P, et al. Modulation of angiogenic potential of collagen matrices by covalent incorporation of heparin and loading with vascular endothelial growth factor. Tissue Eng Sep-Oct;2004 10(910):1502–1509. [PubMed: 15588409]

38. Hurwitz H. Integrating the anti-VEGF-A humanized monoclonal antibody bevacizumab with chemotherapy in advanced colorectal cancer. Clin Colorectal Cancer Oct;2004 4(Suppl 2):S62–68. [PubMed: 15479481]

39. Roodhart JM, Langenberg MH, Witteveen E, Voest EE. The molecular basis of class side effects due to treatment with inhibitors of the VEGF/VEGFR pathway. Curr Clin Pharmacol May;2008 3(2): 132–143. [PubMed: 18690886]

40. Drake CJ, Little CD. Exogenous vascular endothelial growth factor induces malformed and hyperfused vessels during embryonic neovascularization. Proc Natl Acad Sci U S A Aug 15;1995 92(17):7657–7661. [PubMed: 7543999]

41. Ozawa CR, Banfi A, Glazer NL, et al. Microenvironmental VEGF concentration, not total dose, determines a threshold between normal and aberrant angiogenesis. J Clin Invest Feb;2004 113(4): 516–527. [PubMed: 14966561]

42. Sahni A, Francis CW. Vascular endothelial growth factor binds to fibrinogen and fibrin and stimulates endothelial cell proliferation. Blood Dec 1;2000 96(12):3772. [PubMed: 11090059]

43. Ehrbar M, Djonov VG, Schnell C, et al. Cell-demanded liberation of VEGF121 from fibrin implants induces local and controlled blood vessel growth. Circ Res Apr 30;2004 94(8):1124–1132. [PubMed: 15044320]

44. Ishikawa T, Eguchi M, Wada M, et al. Establishment of a functionally active collagen-binding vascular endothelial growth factor fusion protein in situ. Arterioscler.Thromb.Vasc.Biol Sep;2006 26(9):1998. [PubMed: 16794220]

45. Ehrbar M, Zeisberger SM, Raeber GP, Hubbell JA, Schnell C, Zisch AH. The role of actively released fibrin-conjugated VEGF for VEGF receptor 2 gene activation and the enhancement of angiogenesis. Biomaterials Apr;2008 29(11):1720–1729. [PubMed: 18155761]

46. Gerwins P, Skoldenberg E, Claesson-Welsh L. Function of fibroblast growth factors and vascular endothelial growth factors and their receptors in angiogenesis. Crit.Rev.Oncol.Hematol Jun;2000 34 (3):185. [PubMed: 10838264]

47. Kanda S, Landgren E, Ljungstrom M, Claesson-Welsh L. Fibroblast growth factor receptor 1-induced differentiation of endothelial cell line established from tsA58 large T transgenic mice. Cell Growth Differ Mar;1996 7(3):383–395. [PubMed: 8838868]

48. Sa G, Murugesan G, Jaye M, Ivashchenko Y, Fox PL. Activation of cytosolic phospholipase A2 by basic fibroblast growth factor via a p42 mitogen-activated protein kinase-dependent phosphorylation pathway in endothelial cells. J Biol Chem Feb 3;1995 270(5):2360–2366. [PubMed: 7836470]

49. Kent KC, Mii S, Harrington EO, Chang JD, Mallette S, Ware JA. Requirement for protein kinase C activation in basic fibroblast growth factor-induced human endothelial cell proliferation. Circ Res Aug;1995 77(2):231–238. [PubMed: 7542179]

50. Landgren E, Eriksson A, Wennstrom S, Kanda S, Claesson-Welsh L. Induction of fibroblast growth factor receptor-1 mRNA and protein by platelet-derived growth factor BB. Exp.Cell Res Mar 15;1996 223(2):405. [PubMed: 8601417]

51. Couper LL, Bryant SR, Eldrup-Jorgensen J, Bredenberg CE, Lindner V. Vascular endothelial growth factor increases the mitogenic response to fibroblast growth factor-2 in vascular smooth muscle cells in vivo via expression of fms-like tyrosine kinase-1. Circ.Res Dec;1997 81(6):932. [PubMed: 9400373]

52. Vernon RB, Sage EH. A novel, quantitative model for study of endothelial cell migration and sprout formation within three-dimensional collagen matrices. Microvasc.Res Mar;1999 57(2):118. [PubMed: 10049660]

53. Xue L, Greisler HP. Angiogenic effect of fibroblast growth factor-1 and vascular endothelial growth factor and their synergism in a novel in vitro quantitative fibrin-based 3-dimensional angiogenesis system. Surgery Aug;2002 132(2):259–267. [PubMed: 12219021]

54. Bonello MR, Khachigian LM. Fibroblast growth factor-2 represses platelet-derived growth factor receptor-alpha (PDGFR-alpha) transcription via ERK1/2-dependent Sp1 phosphorylation and an atypical cis-acting element in the proximal PDGFR-alpha promoter. J.Biol.Chem Jan 23;2004 279 (4):2377. [PubMed: 14593115]

55. Faraone D, Aguzzi MS, Ragone G, Russo K, Capogrossi MC, Facchiano A. Heterodimerization of FGF-receptor 1 and PDGF-receptor-alpha: a novel mechanism underlying the inhibitory effect of PDGF-BB on FGF-2 in human cells. Blood Mar 1;2006 107(5):1896. [PubMed: 16322476]

56. Pepper MS, Sappino AP, Stocklin R, Montesano R, Orci L, Vassalli JD. Upregulation of urokinase receptor expression on migrating endothelial cells. J.Cell Biol Aug;1993 122(3):673. [PubMed: 8393013]

57. Satake S, Kuzuya M, Ramos MA, Kanda S, Iguchi A. Angiogenic stimuli are essential for survival of vascular endothelial cells in three-dimensional collagen lattice. Biochem.Biophys.Res.Commun Mar 27;1998 244(3):642. [PubMed: 9535718]

58. Uriel S, Brey EM, Greisler HP. Sustained low levels of fibroblast growth factor-1 promote persistent microvascular network formation. Am.J.Surg Nov;2006 192(5):604. [PubMed: 17071192]

59. Sahni A, Odrljin T, Francis CW. Binding of basic fibroblast growth factor to fibrinogen and fibrin. J Biol Chem Mar 27;1998 273(13):7554–7559. [PubMed: 9516457]

60. Sahni A, Khorana AA, Baggs RB, Peng H, Francis CW. FGF-2 binding to fibrin(ogen) is required for augmented angiogenesis. Blood Jan 1;2006 107(1):126–131. [PubMed: 16160009]

61. Schweigerer L, Neufeld G, Friedman J, Abraham JA, Fiddes JC, Gospodarowicz D. Capillary endothelial cells express basic fibroblast growth factor, a mitogen that promotes their own growth. Nature Jan 15-21;1987 325(6101):257. [PubMed: 2433585]

62. Zhao W, Chen B, Li X, et al. Vascularization and cellularization of collagen scaffolds incorporated with two different collagen-targeting human basic fibroblast growth factors. J Biomed Mater Res A Sep 1;2007 82(3):630–636. [PubMed: 17315233]

63. Shireman PK, Xue L, Maddox E, Burgess WH, Greisler HP. The S130K fibroblast growth factor-1 mutant induces heparin-independent proliferation and is resistant to thrombin degradation in fibrin glue. J.Vasc.Surg Feb;2000 31(2):382. [PubMed: 10664506]

64. Erzurum VZ, Bian JF, Husak VA, et al. R136K fibroblast growth factor-1 mutant induces heparin-independent migration of endothelial cells through fibrin glue. J.Vasc.Surg May;2003 37(5):1075. [PubMed: 12756357]

65. Brewster LP, Washington C, Brey EM, et al. Construction and characterization of a thrombin-resistant designer FGF-based collagen binding domain angiogen. Biomaterials Jan;2008 29(3):327. [PubMed: 17950455]

66. Maisonpierre PC, Suri C, Jones PF, et al. Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. Science Jul 4;1997 277(5322):55. [PubMed: 9204896]

67. Suri C, Jones PF, Patan S, et al. Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. Cell Dec 27;1996 87(7):1171. [PubMed: 8980224]

68. Daly C, Wong V, Burova E, et al. Angiopoietin-1 modulates endothelial cell function and gene expression via the transcription factor FKHR (FOXO1). Genes Dev May 1;2004 18(9):1060. [PubMed: 15132996]

69. Yuan HT, Yang SP, Woolf AS. Hypoxia up-regulates angiopoietin-2, a Tie-2 ligand, in mouse mesangial cells. Kidney Int Nov;2000 58(5):1912. [PubMed: 11044211]

70. Pichiule P, LaManna JC. Angiopoietin-2 and rat brain capillary remodeling during adaptation and deadaptation to prolonged mild hypoxia. J.Appl.Physiol Sep;2002 93(3):1131. [PubMed: 12183511]

71. Mandriota SJ, Pepper MS. Regulation of angiopoietin-2 mRNA levels in bovine microvascular endothelial cells by cytokines and hypoxia. Circ.Res Oct 19;1998 83(8):852. [PubMed: 9776732]

72. Krikun G, Schatz F, Finlay T, et al. Expression of angiopoietin-2 by human endometrial endothelial cells: regulation by hypoxia and inflammation. Biochem.Biophys.Res.Commun Aug 18;2000 275 (1):159. [PubMed: 10944458]

73. Mandriota SJ, Pyke C, Di Sanza C, Quinodoz P, Pittet B, Pepper MS. Hypoxia-inducible angiopoietin-2 expression is mimicked by iodonium compounds and occurs in the rat brain and skin in response to systemic hypoxia and tissue ischemia. Am.J.Pathol Jun;2000 156(6):2077. [PubMed: 10854229]

74. Oh H, Takagi H, Suzuma K, Otani A, Matsumura M, Honda Y. Hypoxia and vascular endothelial growth factor selectively up-regulate angiopoietin-2 in bovine microvascular endothelial cells. J.Biol.Chem May 28;1999 274(22):15732. [PubMed: 10336473]

75. Ray PS, Estrada-Hernandez T, Sasaki H, Zhu L, Maulik N. Early effects of hypoxia/reoxygenation on VEGF, ang-1, ang-2 and their receptors in the rat myocardium: implications for myocardial angiogenesis. Mol.Cell.Biochem Oct;2000 213(12):145. [PubMed: 11129953]

76. Holash J, Maisonpierre PC, Compton D, et al. Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF. Science Jun 18;1999 284(5422):1994. [PubMed: 10373119]

77. Scharpfenecker M, Fiedler U, Reiss Y, Augustin HG. The Tie-2 ligand angiopoietin-2 destabilizes quiescent endothelium through an internal autocrine loop mechanism. J.Cell.Sci Feb 15;2005 118(Pt 4):771. [PubMed: 15687104]

78. Post S, Peeters W, Busser E, et al. Balance between angiopoietin-1 and angiopoietin-2 is in favor of angiopoietin-2 in atherosclerotic plaques with high microvessel density. J.Vasc.Res 2008;45(3):244. [PubMed: 18182823]

79. Beck H, Acker T, Wiessner C, Allegrini PR, Plate KH. Expression of angiopoietin-1, angiopoietin-2, and tie receptors after middle cerebral artery occlusion in the rat. Am.J.Pathol Nov;2000 157(5): 1473. [PubMed: 11073808]

80. Reiss Y, Droste J, Heil M, et al. Angiopoietin-2 impairs revascularization after limb ischemia. Circ.Res Jul 6;2007 101(1):88. [PubMed: 17540977]

81. Lobov IB, Brooks PC, Lang RA. Angiopoietin-2 displays VEGF-dependent modulation of capillary structure and endothelial cell survival in vivo. Proc.Natl.Acad.Sci.U.S.A Aug 20;2002 99(17):11205. [PubMed: 12163646]

82. Hammes HP, Lin J, Wagner P, et al. Angiopoietin-2 causes pericyte dropout in the normal retina: evidence for involvement in diabetic retinopathy. Diabetes Apr;2004 53(4):1104–1110. [PubMed: 15047628]

83. Zhang L, Yang N, Park JW, et al. Tumor-derived vascular endothelial growth factor up-regulates angiopoietin-2 in host endothelium and destabilizes host vasculature, supporting angiogenesis in ovarian cancer. Cancer Res Jun 15;2003 63(12):3403–3412. [PubMed: 12810677]

84. Collo G, Pepper MS. Endothelial cell integrin alpha5beta1 expression is modulated by cytokines and during migration in vitro. J Cell Sci Feb;1999 112(Pt 4):569–578. [PubMed: 9914168]

85. Roberts AB, Sporn MB, Assoian RK, et al. Transforming growth factor type beta: rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. Proc Natl Acad Sci U S A Jun;1986 83(12):4167–4171. [PubMed: 2424019]

86. Li J, Zhang YP, Kirsner RS. Angiogenesis in wound repair: angiogenic growth factors and the extracellular matrix. Microsc Res Tech Jan 1;2003 60(1):107–114. [PubMed: 12500267]

87. Sankar S, Mahooti-Brooks N, Bensen L, McCarthy TL, Centrella M, Madri JA. Modulation of transforming growth factor beta receptor levels on microvascular endothelial cells during in vitro angiogenesis. J Clin Invest Mar 15;1996 97(6):1436–1446. [PubMed: 8617876]

88. Kim KY, Jeong SY, Won J, Ryu PD, Nam MJ. Induction of angiogenesis by expression of soluble type II transforming growth factor-beta receptor in mouse hepatoma. J Biol Chem Oct 19;2001 276 (42):38781–38786. [PubMed: 11457844]

89. Klagsbrun M, D'Amore PA. Regulators of angiogenesis. Annu Rev Physiol 1991;53:217–239. [PubMed: 1710435]

90. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ Res May 2;2003 92(8):827–839. [PubMed: 12730128]

91. van Hinsbergh VW, Koolwijk P. Endothelial sprouting and angiogenesis: matrix metalloproteinases in the lead. Cardiovasc Res May 1;2008 78(2):203–212. [PubMed: 18079100]

92. Koivunen E, Arap W, Valtanen H, et al. Tumor targeting with a selective gelatinase inhibitor. Nat.Biotechnol Aug;1999 17(8):768. [PubMed: 10429241]

93. Itoh T, Tanioka M, Yoshida H, Yoshioka T, Nishimoto H, Itohara S. Reduced angiogenesis and tumor progression in gelatinase A-deficient mice. Cancer Res Mar 1;1998 58(5):1048. [PubMed: 9500469]

94. Wilson CL, Heppner KJ, Labosky PA, Hogan BL, Matrisian LM. Intestinal tumorigenesis is suppressed in mice lacking the metalloproteinase matrilysin. Proc.Natl.Acad.Sci.U.S.A Feb 18;1997 94(4):1402. [PubMed: 9037065]

95. Montesano R, Pepper MS, Vassalli JD, Orci L. Phorbol ester induces cultured endothelial cells to invade a fibrin matrix in the presence of fibrinolytic inhibitors. J.Cell.Physiol Sep;1987 132(3):509. [PubMed: 2443514]

96. Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. Nat.Rev.Cancer Mar;2002 2(3):161. [PubMed: 11990853]

97. Mignatti P, Rifkin DB. Biology and biochemistry of proteinases in tumor invasion. Physiol.Rev Jan; 1993 73(1):161. [PubMed: 8419965]

98. Murphy G, Stanton H, Cowell S, et al. Mechanisms for pro matrix metalloproteinase activation. Apmis Jan;1999 107(1):38. [PubMed: 10190278]

99. Mazar AP, Henkin J, Goldfarb RH. The urokinase plasminogen activator system in cancer: implications for tumor angiogenesis and metastasis. Angiogenesis 1999;3(1):15. [PubMed: 14517441]

100. Collen D. The plasminogen (fibrinolytic) system. Thromb.Haemost Aug;1999 82(2):259. [PubMed: 10605712]

101. Pepper MS, Montesano R, Mandriota SJ, Orci L, Vassalli JD. Angiogenesis: a paradigm for balanced extracellular proteolysis during cell migration and morphogenesis. Enzyme Protein 1996;49(13): 138. [PubMed: 8797003]

102. Pepper MS. Role of the matrix metalloproteinase and plasminogen activator-plasmin systems in angiogenesis. Arterioscler.Thromb.Vasc.Biol Jul;2001 21(7):1104. [PubMed: 11451738]

103. Veklich Y, Francis CW, White J, Weisel JW. Structural studies of fibrinolysis by electron microscopy. Blood Dec 15;1998 92(12):4721. [PubMed: 9845538]

104. Pilcher BK, Dumin JA, Sudbeck BD, Krane SM, Welgus HG, Parks WC. The activity of collagenase-1 is required for keratinocyte migration on a type I collagen matrix. J.Cell Biol Jun 16;1997 137(6):1445. [PubMed: 9182674]

105. Nguyen M, Arkell J, Jackson CJ. Three-dimensional collagen matrices induce delayed but sustained activation of gelatinase A in human endothelial cells via MT1-MMP. Int.J.Biochem.Cell Biol Jun; 2000 32(6):621. [PubMed: 10785359]

106. Yebra M, Parry GC, Stromblad S, et al. Requirement of receptor-bound urokinase-type plasminogen activator for integrin alphavbeta5-directed cell migration. J Biol Chem Nov 15;1996 271(46): 29393–29399. [PubMed: 8910604]

107. Koolwijk P, van Erck MG, de Vree WJ, et al. Cooperative effect of TNFalpha, bFGF, and VEGF on the formation of tubular structures of human microvascular endothelial cells in a fibrin matrix. Role of urokinase activity. J.Cell Biol Mar;1996 132(6):1177. [PubMed: 8601593]

108. Collen A, Koolwijk P, Kroon M, van Hinsbergh VW. Influence of fibrin structure on the formation and maintenance of capillary-like tubules by human microvascular endothelial cells. Angiogenesis 1998;2(2):153. [PubMed: 14517471]

109. Soff GA, Sanderowitz J, Gately S, et al. Expression of plasminogen activator inhibitor type 1 by human prostate carcinoma cells inhibits primary tumor growth, tumor-associated angiogenesis, and metastasis to lung and liver in an athymic mouse model. J.Clin.Invest Dec;1995 96(6):2593. [PubMed: 8675623]

110. Levi E, Fridman R, Miao HQ, Ma YS, Yayon A, Vlodavsky I. Matrix metalloproteinase 2 releases active soluble ectodomain of fibroblast growth factor receptor 1. Proc.Natl.Acad.Sci.U.S.A Jul 9;1996 93(14):7069. [PubMed: 8692946]

111. Chang C, Werb Z. The many faces of metalloproteases: cell growth, invasion, angiogenesis and metastasis. Trends Cell Biol Nov;2001 11(11):S37. [PubMed: 11684441]

112. Saunders WB, Bohnsack BL, Faske JB, et al. Coregulation of vascular tube stabilization by endothelial cell TIMP-2 and pericyte TIMP-3. J.Cell Biol Oct 9;2006 175(1):179. [PubMed: 17030988]

113. Bayless KJ, Davis GE. Sphingosine-1-phosphate markedly induces matrix metalloproteinase and integrin-dependent human endothelial cell invasion and lumen formation in three-dimensional collagen and fibrin matrices. Biochem.Biophys.Res.Commun Dec 26;2003 312(4):903. [PubMed: 14651957]

114. Davis GE, Saunders WB. Molecular balance of capillary tube formation versus regression in wound repair: role of matrix metalloproteinases and their inhibitors. J.Investig.Dermatol.Symp.Proc Sep; 2006 11(1):44.

115. Chun TH, Sabeh F, Ota I, et al. MT1-MMP-dependent neovessel formation within the confines of the three-dimensional extracellular matrix. J.Cell Biol Nov 22;2004 167(4):757. [PubMed: 15545316]

116. Plaisier M, Kapiteijn K, Koolwijk P, et al. Involvement of membrane-type matrix metalloproteinases (MT-MMPs) in capillary tube formation by human endometrial microvascular endothelial cells: role of MT3-MMP. J.Clin.Endocrinol.Metab Nov;2004 89(11):5828. [PubMed: 15531549]

117. Montesano R. Cell-extracellular matrix interactions in morphogenesis: an in vitro approach. Experientia Sep 15;1986 42(9):977–985. [PubMed: 3530800]

118. Addison CL, Nor JE, Zhao H, Linn SA, Polverini PJ, Delaney CE. The response of VEGF-stimulated endothelial cells to angiostatic molecules is substrate-dependent. BMC Cell Biol 2005;6:38. [PubMed: 16262896]

119. Jakobsson L, Domogatskaya A, Tryggvason K, Edgar D, Claesson-Welsh L. Laminin deposition is dispensable for vasculogenesis but regulates blood vessel diameter independent of flow. Faseb J May;2008 22(5):1530. [PubMed: 18073332]

120. Fujita M, Khazenzon NM, Bose S, et al. Overexpression of beta1-chain-containing laminins in capillary basement membranes of human breast cancer and its metastases. Breast Cancer Res 2005;7 (4):R411–421. [PubMed: 15987446]

121. Ljubimova JY, Fujita M, Khazenzon NM, Ljubimov AV, Black KL. Changes in laminin isoforms associated with brain tumor invasion and angiogenesis. Front Biosci 2006;11:81–88. [PubMed: 16146715]

122. Zhou X, Rowe RG, Hiraoka N, et al. Fibronectin fibrillogenesis regulates three-dimensional neovessel formation. Genes Dev May 1;2008 22(9):1231. [PubMed: 18451110]

123. Smith JD, Melhem ME, Magge KT, Waggoner AS, Campbell PG. Improved growth factor directed vascularization into fibrin constructs through inclusion of additional extracellular molecules. Microvasc.Res Mar;2007 73(2):84. [PubMed: 17223139]

124. Kroon ME, van Schie ML, van der Vecht B, van Hinsbergh VW, Koolwijk P. Collagen type 1 retards tube formation by human microvascular endothelial cells in a fibrin matrix. Angiogenesis 2002;5 (4):257–265. [PubMed: 12911012]

125. Haas TL, Davis SJ, Madri JA. Three-dimensional type I collagen lattices induce coordinate expression of matrix metalloproteinases MT1-MMP and MMP-2 in microvascular endothelial cells. J.Biol.Chem Feb 6;1998 273(6):3604. [PubMed: 9452488]

126. Stegemann JP, Nerem RM. Altered response of vascular smooth muscle cells to exogenous biochemical stimulation in two- and three-dimensional culture. Exp.Cell Res Feb 15;2003 283(2): 146. [PubMed: 12581735]

127. Ghajar CM, Blevins KS, Hughes CC, George SC, Putnam AJ. Mesenchymal stem cells enhance angiogenesis in mechanically viable prevascularized tissues via early matrix metalloproteinase upregulation. Tissue Eng Oct;2006 12(10):2875. [PubMed: 17518656]

128. Yao C, Markowicz M, Pallua N, Noah EM, Steffens G. The effect of cross-linking of collagen matrices on their angiogenic capability. Biomaterials Jan;2008 29(1):66–74. [PubMed: 17935778]

129. van Hinsbergh VW, Collen A, Koolwijk P. Role of fibrin matrix in angiogenesis. Ann.N.Y.Acad.Sci 2001;936:426. [PubMed: 11460496]

130. Nehls V, Herrmann R. The configuration of fibrin clots determines capillary morphogenesis and endothelial cell migration. Microvasc.Res May;1996 51(3):347. [PubMed: 8992233]

131. Moiseeva EP. Adhesion receptors of vascular smooth muscle cells and their functions. Cardiovasc.Res Dec;2001 52(3):372. [PubMed: 11738054]

132. Romer LH, Birukov KG, Garcia JG. Focal adhesions: paradigm for a signaling nexus. Circ.Res Mar 17;2006 98(5):606. [PubMed: 16543511]

133. Coppolino MG, Dedhar S. Bi-directional signal transduction by integrin receptors. Int.J.Biochem.Cell Biol Feb;2000 32(2):171. [PubMed: 10687952]

134. Parsons JT. Focal adhesion kinase: the first ten years. J.Cell.Sci Apr 15;2003 116(Pt 8):1409. [PubMed: 12640026]

135. Wiesner S, Legate KR, Fassler R. Integrin-actin interactions. Cell Mol.Life Sci May;2005 62(10): 1081. [PubMed: 15761669]

136. Bayless KJ, Salazar R, Davis GE. RGD-dependent vacuolation and lumen formation observed during endothelial cell morphogenesis in three-dimensional fibrin matrices involves the alpha(v)beta(3) and alpha(5)beta(1) integrins. Am.J.Pathol May;2000 156(5):1673. [PubMed: 10793078]

137. Risau W, Lemmon V. Changes in the vascular extracellular matrix during embryonic vasculogenesis and angiogenesis. Dev.Biol Feb;1988 125(2):441. [PubMed: 3338622]

138. Stupack DG, Cheresh DA. Integrins and angiogenesis. Curr.Top.Dev.Biol 2004;64:207. [PubMed: 15563949]

139. Francis SE, Goh KL, Hodivala-Dilke K, et al. Central roles of alpha5beta1 integrin and fibronectin in vascular development in mouse embryos and embryoid bodies. Arterioscler.Thromb.Vasc.Biol Jun 1;2002 22(6):927. [PubMed: 12067900]

140. Eliceiri BP, Cheresh DA. The role of alphav integrins during angiogenesis: insights into potential mechanisms of action and clinical development. J.Clin.Invest May;1999 103(9):1227. [PubMed: 10225964]

141. Senger DR, Claffey KP, Benes JE, Perruzzi CA, Sergiou AP, Detmar M. Angiogenesis promoted by vascular endothelial growth factor: regulation through alpha1beta1 and alpha2beta1 integrins. Proc Natl Acad Sci U S A Dec 9;1997 94(25):13612–13617. [PubMed: 9391074]

142. Rupp PA, Little CD. Integrins in vascular development. Circ.Res Sep 28;2001 89(7):566. [PubMed: 11577021]

143. Perruzzi CA, de Fougerolles AR, Koteliansky VE, Whelan MC, Westlin WF, Senger DR. Functional overlap and cooperativity among alphav and beta1 integrin subfamilies during skin angiogenesis. J.Invest.Dermatol Jun;2003 120(6):1100. [PubMed: 12787141]

144. Takahashi S, Leiss M, Moser M, et al. The RGD motif in fibronectin is essential for development but dispensable for fibril assembly. J.Cell Biol Jul 2;2007 178(1):167. [PubMed: 17591922]

145. Goh KL, Yang JT, Hynes RO. Mesodermal defects and cranial neural crest apoptosis in alpha5 integrin-null embryos. Development Nov;1997 124(21):4309. [PubMed: 9334279]

146. Ilic D, Kovacic B, McDonagh S, et al. Focal adhesion kinase is required for blood vessel morphogenesis. Circ.Res Feb 21;2003 92(3):300. [PubMed: 12595342]

147. Jalali S, del Pozo MA, Chen K, et al. Integrin-mediated mechanotransduction requires its dynamic interaction with specific extracellular matrix (ECM) ligands. Proc Natl Acad Sci U S A Jan 30;2001 98(3):1042–1046. [PubMed: 11158591]

148. Gamble JR, Matthias LJ, Meyer G, et al. Regulation of in vitro capillary tube formation by anti-integrin antibodies. J.Cell Biol May;1993 121(4):931. [PubMed: 8491784]

149. Brooks PC, Stromblad S, Sanders LC, et al. Localization of matrix metalloproteinase MMP-2 to the surface of invasive cells by interaction with integrin alpha v beta 3. Cell May 31;1996 85(5):683–693. [PubMed: 8646777]

150. Seltzer JL, Lee AY, Akers KT, et al. Activation of 72-kDa type IV collagenase/gelatinase by normal fibroblasts in collagen lattices is mediated by integrin receptors but is not related to lattice contraction. Exp.Cell Res Aug;1994 213(2):365. [PubMed: 7519563]

151. Giancotti FG, Ruoslahti E. Integrin signaling. Science Aug 13;1999 285(5430):1028. [PubMed: 10446041]

152. Senger DR, Ledbetter SR, Claffey KP, Papadopoulos-Sergiou A, Perruzzi CA, Detmar M. Stimulation of endothelial cell migration by vascular permeability factor/vascular endothelial growth factor through cooperative mechanisms involving the alphavbeta3 integrin, osteopontin, and thrombin. Am J Pathol Jul;1996 149(1):293–305. [PubMed: 8686754]

153. Ross RS. Molecular and mechanical synergy: cross-talk between integrins and growth factor receptors. Cardiovasc.Res Aug 15;2004 63(3):381. [PubMed: 15276463]

154. Sepp NT, Li LJ, Lee KH, et al. Basic fibroblast growth factor increases expression of the alpha v beta 3 integrin complex on human microvascular endothelial cells. J Invest Dermatol Sep;1994 103 (3):295–299. [PubMed: 8077694]

155. Mahabeleshwar GH, Feng W, Reddy K, Plow EF, Byzova TV. Mechanisms of integrin-vascular endothelial growth factor receptor cross-activation in angiogenesis. Circ.Res Sep 14;2007 101(6): 570. [PubMed: 17641225]

156. Munoz-Chapuli R, Quesada AR, Medina M Angel. Angiogenesis and signal transduction in endothelial cells. Cell Mol Life Sci Sep;2004 61(17):2224–2243. [PubMed: 15338053]

157. Moon SY, Zheng Y. Rho GTPase-activating proteins in cell regulation. Trends Cell Biol Jan;2003 13(1):13–22. [PubMed: 12480336]

158. Ridley AJ. Rho family proteins and regulation of the actin cytoskeleton. Prog.Mol.Subcell.Biol 1999;22:1. [PubMed: 10081062]

159. Soga N, Namba N, McAllister S, et al. Rho family GTPases regulate VEGF-stimulated endothelial cell motility. Exp.Cell Res Sep 10;2001 269(1):73. [PubMed: 11525641]

160. Engelse MA, Laurens N, Verloop RE, Koolwijk P, van Hinsbergh VW. Differential gene expression analysis of tubule forming and non-tubule forming endothelial cells: CDC42GAP as a counter-regulator in tubule formation. Angiogenesis 2008;11(2):153. [PubMed: 18060510]

161. Koh W, Mahan RD, Davis GE. Cdc42- and Rac1-mediated endothelial lumen formation requires Pak2, Pak4 and Par3, and PKC-dependent signaling. J.Cell.Sci Apr 1;2008 121(Pt 7):989. [PubMed: 18319301]

162. Yamamura S, Nelson PR, Kent KC. Role of protein kinase C in attachment, spreading, and migration of human endothelial cells. J.Surg.Res Jun;1996 63(1):349. [PubMed: 8661224]

163. Wang A, Nomura M, Patan S, Ware JA. Inhibition of protein kinase Calpha prevents endothelial cell migration and vascular tube formation in vitro and myocardial neovascularization in vivo. Circ.Res Mar 22;2002 90(5):609. [PubMed: 11909826]

164. Schweisguth F. Regulation of notch signaling activity. Curr.Biol Feb 3;2004 14(3):R129. [PubMed: 14986688]

165. Leslie JD, Ariza-McNaughton L, Bermange AL, McAdow R, Johnson SL, Lewis J. Endothelial signalling by the Notch ligand Delta-like 4 restricts angiogenesis. Development Mar;2007 134(5): 839. [PubMed: 17251261]

166. Siekmann AF, Lawson ND. Notch signalling limits angiogenic cell behaviour in developing zebrafish arteries. Nature Feb 15;2007 445(7129):781. [PubMed: 17259972]

167. Hellstrom M, Phng LK, Hofmann JJ, et al. Dll4 signalling through Notch1 regulates formation of tip cells during angiogenesis. Nature Feb 15;2007 445(7129):776. [PubMed: 17259973]

168. Sainson RC, Aoto J, Nakatsu MN, et al. Cell-autonomous notch signaling regulates endothelial cell branching and proliferation during vascular tubulogenesis. Faseb J Jun;2005 19(8):1027. [PubMed: 15774577]

169. Ridgway J, Zhang G, Wu Y, et al. Inhibition of Dll4 signalling inhibits tumour growth by deregulating angiogenesis. Nature Dec 21;2006 444(7122):1083. [PubMed: 17183323]

170. Noguera-Troise I, Daly C, Papadopoulos NJ, et al. Blockade of Dll4 inhibits tumour growth by promoting non-productive angiogenesis. Nature Dec 21;2006 444(7122):1032. [PubMed: 17183313]

171. Kunz-Schughart LA, Schroeder JA, Wondrak M, et al. Potential of fibroblasts to regulate the formation of three-dimensional vessel-like structures from endothelial cells in vitro. Am.J.Physiol.Cell.Physiol May;2006 290(5):C1385. [PubMed: 16601149]

172. Brey EM, McIntire LV, Johnston CM, Reece GP, Patrick CW Jr. Three-dimensional, quantitative analysis of desmin and smooth muscle alpha actin expression during angiogenesis. Ann.Biomed.Eng Aug;2004 32(8):1100. [PubMed: 15446506]

173. Ozerdem U, Grako KA, Dahlin-Huppe K, Monosov E, Stallcup WB. NG2 proteoglycan is expressed exclusively by mural cells during vascular morphogenesis. Dev Dyn Oct;2001 222(2):218–227. [PubMed: 11668599]

174. Ozerdem U, Monosov E, Stallcup WB. NG2 proteoglycan expression by pericytes in pathological microvasculature. Microvasc.Res Jan;2002 63(1):129. [PubMed: 11749079]

175. Kuzuya M, Satake S, Esaki T, et al. Induction of angiogenesis by smooth muscle cell-derived factor: possible role in neovascularization in atherosclerotic plaque. J.Cell.Physiol Sep;1995 164(3):658. [PubMed: 7544360]

176. Davie NJ, Gerasimovskaya EV, Hofmeister SE, et al. Pulmonary artery adventitial fibroblasts cooperate with vasa vasorum endothelial cells to regulate vasa vasorum neovascularization: a process mediated by hypoxia and endothelin-1. Am.J.Pathol Jun;2006 168(6):1793. [PubMed: 16723696]

177. Montesano R, Pepper MS, Orci L. Paracrine induction of angiogenesis in vitro by Swiss 3T3 fibroblasts. J.Cell.Sci Aug;1993 105(Pt 4):1013. (Pt 4). [PubMed: 7693734]

178. Kale S, Hanai J, Chan B, et al. Microarray analysis of in vitro pericyte differentiation reveals an angiogenic program of gene expression. Faseb J Feb;2005 19(2):270. [PubMed: 15579670]

179. Chon JH, Wang HS, Chaikof EL. Role of fibronectin and sulfated proteoglycans in endothelial cell migration on a cultured smooth muscle layer. J.Surg.Res Sep;1997 72(1):53. [PubMed: 9344714]

180. Dietrich F, Lelkes PI. Fine-tuning of a three-dimensional microcarrier-based angiogenesis assay for the analysis of endothelial-mesenchymal cell co-cultures in fibrin and collagen gels. Angiogenesis 2006;9(3):111. [PubMed: 17051343]

181. Ozerdem U, Stallcup WB. Early contribution of pericytes to angiogenic sprouting and tube formation. Angiogenesis 2003;6(3):241. [PubMed: 15041800]

182. Nehls V, Denzer K, Drenckhahn D. Pericyte involvement in capillary sprouting during angiogenesis in situ. Cell Tissue Res Dec;1992 270(3):469–474. [PubMed: 1283113]

183. Reynolds LP, Grazul-Bilska AT, Redmer DA. Angiogenesis in the corpus luteum. Endocrine Feb;2000 12(1):1–9. [PubMed: 10855683]

184. Nehls V, Drenckhahn D. A microcarrier-based cocultivation system for the investigation of factors and cells involved in angiogenesis in three-dimensional fibrin matrices in vitro. Histochem.Cell Biol Dec;1995 104(6):459. [PubMed: 8777732]

185. St Croix B, Rago C, Velculescu V, et al. Genes expressed in human tumor endothelium. Science Aug 18;2000 289(5482):1197. [PubMed: 10947988]

186. Bussolati B, Deambrosis I, Russo S, Deregibus MC, Camussi G. Altered angiogenesis and survival in human tumor-derived endothelial cells. Faseb J Jun;2003 17(9):1159. [PubMed: 12709414]

187. Vallon M, Essler M. Proteolytically processed soluble tumor endothelial marker (TEM) 5 mediates endothelial cell survival during angiogenesis by linking integrin alpha(v)beta3 to glycosaminoglycans. J.Biol.Chem Nov 10;2006 281(45):34179. [PubMed: 16982628]

188. Rmali KA, Puntis MC, Jiang WG. TEM-8 and tubule formation in endothelial cells, its potential role of its vW/TM domains. Biochem.Biophys.Res.Commun Aug 19;2005 334(1):231. [PubMed: 15993844]

189. Wang XQ, Sheibani N, Watson JC. Modulation of tumor endothelial cell marker 7 expression during endothelial cell capillary morphogenesis. Microvasc.Res Nov;2005 70(3):189. [PubMed: 16202431]

190. Rmali KA, Puntis MC, Jiang WG. Prognostic values of tumor endothelial markers in patients with colorectal cancer. World J.Gastroenterol Mar 7;2005 11(9):1283. [PubMed: 15761964]

191. Davis GE, Senger DR. Endothelial extracellular matrix: biosynthesis, remodeling, and functions during vascular morphogenesis and neovessel stabilization. Circ.Res Nov 25;2005 97(11):1093. [PubMed: 16306453]

192. Bonanno E, Iurlaro M, Madri JA, Nicosia RF. Type IV collagen modulates angiogenesis and neovessel survival in the rat aorta model. In Vitro Cell.Dev.Biol.Anim May;2000 36(5):336. [PubMed: 10937837]

193. Colorado PC, Torre A, Kamphaus G, et al. Anti-angiogenic Cues from Vascular Basement Membrane Collagen. Cancer Res May 1;2000 60(9):2520–2526. 2000. [PubMed: 10811134]

194. Kamphaus GD, Colorado PC, Panka DJ, et al. Canstatin, a Novel Matrix-derived Inhibitor of Angiogenesis and Tumor Growth. J. Biol. Chem January 14;2000 275(2):1209–1215. 2000. [PubMed: 10625665]

195. DiPietro LA, Nissen NN, Gamelli RL, Koch AE, Pyle JM, Polverini PJ. Thrombospondin 1 synthesis and function in wound repair. Am J Pathol Jun;1996 148(6):1851–1860. [PubMed: 8669471]

196. Ren B, Yee KO, Lawler J, Khosravi-Far R. Regulation of tumor angiogenesis by thrombospondin-1. Biochimica et Biophysica Acta (BBA) - Reviews on Cancer 2006;1765(2):178–188.

197. Baker LH, Rowinsky EK, Mendelson D, et al. Randomized, phase II study of the thrombospondin-1-mimetic angiogenesis inhibitor ABT-510 in patients with advanced soft tissue sarcoma. J Clin Oncol Dec 1;2008 26(34):5583–5588. [PubMed: 18981463]

198. Kulke MH, Bergsland EK, Ryan DP, et al. Phase II study of recombinant human endostatin in patients with advanced neuroendocrine tumors. J.Clin.Oncol Aug 1;2006 24(22):3555. [PubMed: 16877721]

199. Khasigov PZ, Podobed OV, Gracheva TS, Salbiev KD, Grachev SV, Berezov TT. Role of matrix metalloproteinases and their inhibitors in tumor invasion and metastasis. Biochemistry (Mosc) Jul;2003 68(7):711. [PubMed: 12946252]

200. Qi JH, Ebrahem Q, Moore N, et al. A novel function for tissue inhibitor of metalloproteinases-3 (TIMP3): inhibition of angiogenesis by blockage of VEGF binding to VEGF receptor-2. Nat.Med Apr;2003 9(4):407. [PubMed: 12652295]

201. Bergers G, Song S. The role of pericytes in blood-vessel formation and maintenance. Neuro Oncol Oct;2005 7(4):452–464. [PubMed: 16212810]

202. Lindahl P, Johansson BR, Leveen P, Betsholtz C. Pericyte loss and microaneurysm formation in PDGF-B-deficient mice. Science Jul 11;1997 277(5323):242. [PubMed: 9211853]

203. Morikawa S, Baluk P, Kaidoh T, Haskell A, Jain RK, McDonald DM. Abnormalities in pericytes on blood vessels and endothelial sprouts in tumors. Am J Pathol Mar;2002 160(3):985–1000. [PubMed: 11891196]

204. Benjamin LE, Golijanin D, Itin A, Pode D, Keshet E. Selective ablation of immature blood vessels in established human tumors follows vascular endothelial growth factor withdrawal. J.Clin.Invest Jan;1999 103(2):159. [PubMed: 9916127]

205. Song S, Ewald AJ, Stallcup W, Werb Z, Bergers G. PDGFRbeta+ perivascular progenitor cells in tumours regulate pericyte differentiation and vascular survival. Nat Cell Biol Sep;2005 7(9):870–879. [PubMed: 16113679]

206. Sennino B, Falcon BL, McCauley D, et al. Sequential loss of tumor vessel pericytes and endothelial cells after inhibition of platelet-derived growth factor B by selective aptamer AX102. Cancer Res Aug 1;2007 67(15):7358–7367. [PubMed: 17671206]

207. Sussman MA. Showing up isn't enough for vascularization: persistence is essential. Circ Res Nov 21;2008 103(11):1200–1201. [PubMed: 19028918]

208. Benjamin LE, Hemo I, Keshet E. A plasticity window for blood vessel remodelling is defined by pericyte coverage of the preformed endothelial network and is regulated by PDGF-B and VEGF. Development May;1998 125(9):1591–1598. [PubMed: 9521897]

209. Rucker HK, Wynder HJ, Thomas WE. Cellular mechanisms of CNS pericytes. Brain Res Bull Mar 15;2000 51(5):363–369. [PubMed: 10715555]

210. Gerhardt H, Wolburg H, Redies C. N-cadherin mediates pericytic-endothelial interaction during brain angiogenesis in the chicken. Dev Dyn Jul;2000 218(3):472–479. [PubMed: 10878612]

211. Gerhardt H, Betsholtz C. Endothelial-pericyte interactions in angiogenesis. Cell Tissue Res Oct; 2003 314(1):15–23. [PubMed: 12883993]

212. Tillet E, Vittet D, Feraud O, Moore R, Kemler R, Huber P. N-cadherin deficiency impairs pericyte recruitment, and not endothelial differentiation or sprouting, in embryonic stem cell-derived angiogenesis. Exp Cell Res Nov 1;2005 310(2):392–400. [PubMed: 16202998]

213. Andrae J, Gallini R, Betsholtz C. Role of platelet-derived growth factors in physiology and medicine. Genes Dev May 15;2008 22(10):1276–1312. [PubMed: 18483217]

214. Clowes AW. Regulation of smooth muscle cell proliferation and migration. Transplant.Proc Feb-Mar;1999 31(12):810. [PubMed: 10083350]

215. Fredriksson L, Li H, Eriksson U. The PDGF family: four gene products form five dimeric isoforms. Cytokine Growth Factor Rev Aug;2004 15(4):197. [PubMed: 15207811]

216. Soriano P. Abnormal kidney development and hematological disorders in PDGF beta-receptor mutant mice. Genes Dev Aug 15;1994 8(16):1888–1896. [PubMed: 7958864]

217. Leveen P, Pekny M, Gebre-Medhin S, Swolin B, Larsson E, Betsholtz C. Mice deficient for PDGF B show renal, cardiovascular, and hematological abnormalities. Genes Dev Aug 15;1994 8(16): 1875–1887. [PubMed: 7958863]

218. Hellstrom M, Kalen M, Lindahl P, Abramsson A, Betsholtz C. Role of PDGF-B and PDGFR-beta in recruitment of vascular smooth muscle cells and pericytes during embryonic blood vessel formation in the mouse. Development Jun;1999 126(14):3047–3055. [PubMed: 10375497]

219. Abramsson A, Lindblom P, Betsholtz C. Endothelial and nonendothelial sources of PDGF-B regulate pericyte recruitment and influence vascular pattern formation in tumors. J Clin Invest Oct;2003 112 (8):1142–1151. [PubMed: 14561699]

220. Guo P, Hu B, Gu W, et al. Platelet-derived growth factor-B enhances glioma angiogenesis by stimulating vascular endothelial growth factor expression in tumor endothelia and by promoting pericyte recruitment. Am J Pathol Apr;2003 162(4):1083–1093. [PubMed: 12651601]

221. Lindblom P, Gerhardt H, Liebner S, et al. Endothelial PDGF-B retention is required for proper investment of pericytes in the microvessel wall. Genes Dev Aug 1;2003 17(15):1835–1840. [PubMed: 12897053]

222. Powell RJ, Carruth JA, Basson MD, Bloodgood R, Sumpio BE. Matrix-specific effect of endothelial control of smooth muscle cell migration. J.Vasc.Surg Jul;1996 24(1):51. [PubMed: 8691527]

223. Yoshida H, Nakamura M, Makita S, Hiramori K. Paracrine effect of human vascular endothelial cells on human vascular smooth muscle cell proliferation: transmembrane co-culture method. Heart Vessels 1996;11(5):229. [PubMed: 9129242]

224. Newcomb PM, Herman IM. Pericyte growth and contractile phenotype: modulation by endothelial-synthesized matrix and comparison with aortic smooth muscle. J.Cell.Physiol May;1993 155(2): 385. [PubMed: 8482730]

225. Axel DI, Riessen R, Athanasiadis A, Runge H, Koveker G, Karsch KR. Growth factor expression of human arterial smooth muscle cells and endothelial cells in a transfilter coculture system. J.Mol.Cell.Cardiol Nov;1997 29(11):2967. [PubMed: 9405172]

226. Wu XJ, Huang L, Song DL, Jin J, Zhao G. Effects of endothelial cell growth states on the proliferation and migration of vascular smooth muscle cells in vitro. Sheng Li Xue Bao Oct 25;2003 55(5):554. [PubMed: 14566403]

227. Fillinger MF, O'Connor SE, Wagner RJ, Cronenwett JL. The effect of endothelial cell coculture on smooth muscle cell proliferation. J.Vasc.Surg Jun;1993 17(6):1058. [PubMed: 8505784]

228. Hirschi KK, Rohovsky SA, D'Amore PA. PDGF, TGF-beta, and heterotypic cell-cell interactions mediate endothelial cell-induced recruitment of 10T1/2 cells and their differentiation to a smooth muscle fate. J.Cell Biol May 4;1998 141(3):805. [PubMed: 9566978]

229. Hirschi KK, Rohovsky SA, Beck LH, Smith SR, D'Amore PA. Endothelial cells modulate the proliferation of mural cell precursors via platelet-derived growth factor-BB and heterotypic cell contact. Circ.Res Feb 19;1999 84(3):298. [PubMed: 10024303]

230. Hirschi KK, Skalak TC, Peirce SM, Little CD. Vascular assembly in natural and engineered tissues. Ann.N.Y.Acad.Sci Jun;2002 961:223. [PubMed: 12081906]

231. Bjarnegard M, Enge M, Norlin J, et al. Endothelium-specific ablation of PDGFB leads to pericyte loss and glomerular, cardiac and placental abnormalities. Development April 15;2004 131(8):1847–1857. 2004. [PubMed: 15084468]

232. Betsholtz C. Insight into the physiological functions of PDGF through genetic studies in mice. Cytokine Growth Factor Rev Aug;2004 15(4):215–228. [PubMed: 15207813]

233. Betsholtz C, Lindblom P, Bjarnegard M, Enge M, Gerhardt H, Lindahl P. Role of platelet-derived growth factor in mesangium development and vasculopathies: lessons from platelet-derived growth factor and platelet-derived growth factor receptor mutations in mice. Curr Opin Nephrol Hypertens Jan;2004 13(1):45–52. [PubMed: 15090859]

234. Bergers G, Song S, Meyer-Morse N, Bergsland E, Hanahan D. Benefits of targeting both pericytes and endothelial cells in the tumor vasculature with kinase inhibitors. J.Clin.Invest May;2003 111 (9):1287. [PubMed: 12727920]

235. Potapova O, Laird AD, Nannini MA, et al. Contribution of individual targets to the antitumor efficacy of the multitargeted receptor tyrosine kinase inhibitor SU11248. Mol Cancer Ther May;2006 5(5): 1280–1289. [PubMed: 16731761]

236. Hasumi Y, Klosowska-Wardega A, Furuhashi M, Ostman A, Heldin CH, Hellberg C. Identification of a subset of pericytes that respond to combination therapy targeting PDGF and VEGF signaling. Int J Cancer Dec 15;2007 121(12):2606–2614. [PubMed: 17691110]

237. Timke C, Zieher H, Roth A, et al. Combination of vascular endothelial growth factor receptor/ platelet-derived growth factor receptor inhibition markedly improves radiation tumor therapy. Clin Cancer Res Apr 1;2008 14(7):2210–2219. [PubMed: 18381963]

238. Kuhnert F, Tam BY, Sennino B, et al. Soluble receptor-mediated selective inhibition of VEGFR and PDGFRbeta signaling during physiologic and tumor angiogenesis. Proc Natl Acad Sci U S A Jul 22;2008 105(29):10185–10190. [PubMed: 18632559]

239. Chen RR, Silva EA, Yuen WW, Mooney DJ. Spatio-temporal VEGF and PDGF delivery patterns blood vessel formation and maturation. Pharm.Res Feb;2007 24(2):258. [PubMed: 17191092]

240. Korpisalo P, Karvinen H, Rissanen TT, et al. Vascular endothelial growth factor-A and platelet-derived growth factor-B combination gene therapy prolongs angiogenic effects via recruitment of interstitial mononuclear cells and paracrine effects rather than improved pericyte coverage of angiogenic vessels. Circ Res Nov 7;2008 103(10):1092–1099. [PubMed: 18832750]

241. Betsholtz C, Karlsson L, Lindahl P. Developmental roles of platelet-derived growth factors. Bioessays Jun;2001 23(6):494–507. [PubMed: 11385629]

242. Darland DC, D'Amore PA. TGF beta is required for the formation of capillary-like structures in three-dimensional cocultures of 10T1/2 and endothelial cells. Angiogenesis 2001;4(1):11. [PubMed: 11824373]

243. Lien S-C, Usami S, Chien S, Chiu J-J. Phosphatidylinositol 3-kinase/Akt pathway is involved in transforming growth factor-[beta]1-induced phenotypic modulation of 10T1/2 cells to smooth muscle cells. Cellular Signalling 2006;18(8):1270–1278. [PubMed: 16310342]

244. Brown DJ, Rzucidlo EM, Merenick BL, Wagner RJ, Martin KA, Powell RJ. Endothelial cell activation of the smooth muscle cell phosphoinositide 3-kinase/Akt pathway promotes differentiation. J.Vasc.Surg Mar;2005 41(3):509. [PubMed: 15838487]

245. Kutcher ME, Kolyada AY, Surks HK, Herman IM. Pericyte Rho GTPase mediates both pericyte contractile phenotype and capillary endothelial growth state. Am.J.Pathol Aug;2007 171(2):693. [PubMed: 17556591]

246. Orlidge A, D'Amore PA. Inhibition of capillary endothelial cell growth by pericytes and smooth muscle cells. J.Cell Biol Sep;1987 105(3):1455. [PubMed: 3654761]

247. Korff T, Kimmina S, Martiny-Baron G, Augustin HG. Blood vessel maturation in a 3-dimensional spheroidal coculture model: direct contact with smooth muscle cells regulates endothelial cell quiescence and abrogates VEGF responsiveness. Faseb J Feb;2001 15(2):447. [PubMed: 11156960]

248. Kurzen H, Manns S, Dandekar G, Schmidt T, Pratzel S, Kraling BM. Tightening of endothelial cell contacts: a physiologic response to cocultures with smooth-muscle-like 10T1/2 cells. J.Invest.Dermatol Jul;2002 119(1):143. [PubMed: 12164937]

249. Shih SC, Ju M, Liu N, Mo JR, Ney JJ, Smith LE. Transforming growth factor beta1 induction of vascular endothelial growth factor receptor 1: mechanism of pericyte-induced vascular survival in vivo. Proc Natl Acad Sci U S A Dec 23;2003 100(26):15859–15864. [PubMed: 14657382]

250. Darland DC, Massingham LJ, Smith SR, Piek E, Saint-Geniez M, D'Amore PA. Pericyte production of cell-associated VEGF is differentiation-dependent and is associated with endothelial survival. Dev Biol Dec 1;2003 264(1):275–288. [PubMed: 14623248]

251. Sundberg C, Kowanetz M, Brown LF, Detmar M, Dvorak HF. Stable expression of angiopoietin-1 and other markers by cultured pericytes: phenotypic similarities to a subpopulation of cells in maturing vessels during later stages of angiogenesis in vivo. Lab Invest Apr;2002 82(4):387–401. [PubMed: 11950897]

252. Thurston G, Suri C, Smith K, et al. Leakage-resistant blood vessels in mice transgenically overexpressing angiopoietin-1. Science Dec 24;1999 286(5449):2511. [PubMed: 10617467]

253. Uemura A, Ogawa M, Hirashima M, et al. Recombinant angiopoietin-1 restores higher-order architecture of growing blood vessels in mice in the absence of mural cells. J Clin Invest Dec;2002 110(11):1619–1628. [PubMed: 12464667]

254. Wilkinson-Berka JL, Babic S, De Gooyer T, et al. Inhibition of platelet-derived growth factor promotes pericyte loss and angiogenesis in ischemic retinopathy. Am J Pathol Apr;2004 164(4): 1263–1273. [PubMed: 15039215]

255. McIlroy M, O'Rourke M, McKeown SR, Hirst DG, Robson T. Pericytes influence endothelial cell growth characteristics: role of plasminogen activator inhibitor type 1 (PAI-1). Cardiovasc Res Jan; 2006 69(1):207–217. [PubMed: 16165110]

256. Baker AH, Edwards DR, Murphy G. Metalloproteinase inhibitors: biological actions and therapeutic opportunities. J.Cell.Sci Oct 1;2002 115(Pt 19):3719. [PubMed: 12235282]

257. Lafleur MA, Forsyth PA, Atkinson SJ, Murphy G, Edwards DR. Perivascular cells regulate endothelial membrane type-1 matrix metalloproteinase activity. Biochem.Biophys.Res.Commun Mar 30;2001 282(2):463. [PubMed: 11401482]

258. Grant DS, Kleinman HK. Regulation of capillary formation by laminin and other components of the extracellular matrix. Exs 1997;79:317. [PubMed: 9002225]

259. Davis GE, Senger DR. Extracellular matrix mediates a molecular balance between vascular morphogenesis and regression. Curr.Opin.Hematol May;2008 15(3):197. [PubMed: 18391785]

260. Zhou Z, Doi M, Wang J, et al. Deletion of laminin-8 results in increased tumor neovascularization and metastasis in mice. Cancer Res Jun 15;2004 64(12):4059. [PubMed: 15205311]

261. Riha GM, Lin PH, Lumsden AB, Yao Q, Chen C. Roles of hemodynamic forces in vascular cell differentiation. Ann.Biomed.Eng Jun;2005 33(6):772. [PubMed: 16078617]

262. Lehoux S, Castier Y, Tedgui A. Molecular mechanisms of the vascular responses to haemodynamic forces. J.Intern.Med Apr;2006 259(4):381. [PubMed: 16594906]
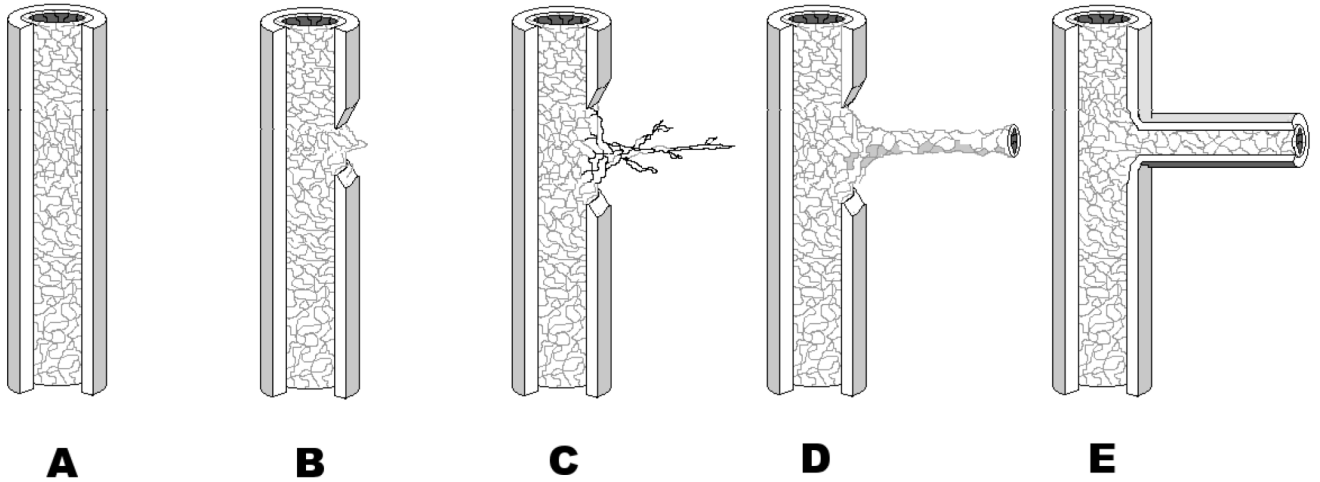
Figure 1.
Diagrammatic description of the steps involved in angiogenesis. **A**) A stable vascular structure comprised of an endothelial monolayer surrounded by mural tissue (i.e. basement membrane, pericytes, and adventitia); **B**) Destabilized vessel demonstrating the disruption of mural tissue by endothelial cells; **C**) Endothelial cell invasion into perivascular tissue and subsequent sprout formation; **D**) Endothelial cell lumenogenesis and tube formation with inosculation (*not shown*) to other pre-existing vascular structures; **E**) Stabilization of the capillary sprout by recruitment of pericytes and deposition of basement membrane.