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# Angiogenesis

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# Definition

Blood vessels develop and grow by three different basic mechanisms: vasculogenesis in which vessels form by concatenation of vascular precursor cells into solid cords that then lumenize; angiogenesis that is the growth of new blood vessels from pre-existing ones; and intussusception in which new blood vessels form by the proliferation of endothelial cells that form a pre-existing vessel into the vessel lumen, originating two blood vessels that split into two opposite sides[(1)].

Angiogenesis, the growth of new vessels from pre-existing ones by sprouting of endothelial cells into a previously avascular tissue, is an essential process both in embryonic development and in adulthood[(1,2)]. It is a complex multistep process involving extracellular matrix degradation and proliferation, survival, migration and anastomosis of endothelial cells[(2)].

The release of extracellular matrix proteases leads to the degradation of the basal membrane of blood vessels. Endothelial cells change shape, proliferate, invade stroma and form tubular structures that coalesce. This requires the coordinated action of a variety of anti and pro-angiogenic factors and cell-adhesion molecules in endothelial cells. Angiogenesis is of paramount importance as it promotes tissue repair; however, in certain conditions it may cause tissue damage. If not tightly regulated, the angiogenic process is frequently imbalanced, and associated with several pathological situations[(1,3)]
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# Angiogenic mediators and modulation of their expression

The angiogenic process requires the activation of a series of receptors by numerous ligands including Placental Growth Factor (PlGF), Fibroblast Growth Factors (FGFs), Angiopoietin-1 and -2 (Ang-1 and -2), Platelet-derived Growth Factor (PDGF), Hepatocyte Growth Factor (HGF), Connective Tissue Growth Factor (CTGF) and Transforming Growth Factors (TGF-α e TGF-β), among many others[1, 3-9].

However, there is a consensus that Vascular Endothelial Growth Factor (VEGF) is the most important angiogenic factor and represents the crucial rate-limiting step during angiogenesis[3,10,11].

VEGF-A is the prototype member of a gene family that also includes placental growth factor (PlGF), VEGF-B, VEGF-C, VEGF-D, and the orf-virus-encoded VEGF-E[11]. Alternative exon splicing results in the generation of four main VEGF isoforms, which have respectively 121, 165, 189, and 206 amino acids (VEGF121, VEGF165, VEGF189, VEGF206). Less frequent splice variants have also been reported, including VEGF145, VEGF183, VEGF162, and VEGF165b[8,11].

VEGF mediates its biological functions at the endothelial level by binding two highly related tyrosine kinase receptors (RTKs), VEGFR-1 and VEGFR-2. It is generally agreed that VEGFR-2 is the major mediator of the mitogenic, angiogenic and permeability-enhancing effects of VEGF-A[3]. VEGFR-1 binds to both VEGF-A and Placenta Growth Factor and fails to mediate a strong mitogenic signal in endothelial cells. It is now generally agreed that VEGFR-1 plays a role in the modulation of VEGF activity[10]. VEGFR-3 is mainly a VEGF-C receptor but it plays important roles in lymphangiogenesis and angiogenesis[12].

The mediators of the angiogenic process can be modulated by some molecules and microenvironmental conditions. VEGF is upregulated by cyclo.oxygenase (COX-2)[13]. Inflammatory cells within a hypoxic environment release huge amounts of factors that exert effects on endothelial cells and degrade the extracellular matrix[2].

Angiogenesis can also be suppressed by inhibitory molecules, such as interferon-α, thrombospondin-1, angiostatin, endostatin or pigment epithelial-derived factor (PEDF)[14-18].

It is the balance of stimulators and inhibitors that tightly controls the normally quiescent capillary vasculature. When this balance is upset angiogenesis develops[19].

Cell-cell and cell-matrix interactions may also play an important role in angiogenesis. Special focus has been recently given the Rac1 GTPase[20]. Recently, microRNA regulation of gene expression has been implicated in the control of pathologic ocular angiogenesis[21].

The angiopoietin (ANG)–TIE signalling pathway has been identified as the second vascular tissue-specific receptor Tyr kinase system. The ANG–TIE pathway is required for lymphatic and blood vessel development and is important for the development of mature blood vessels that originate from the VEGF induced endothelial sprouting. This pathway controls vascular permeability, inflammation and pathological angiogenic responses in adult tissues[(22)]. Maturing of blood vessels include pericyte and coating of endothelial cell walls.

PDGF is mainly believed to be an important mitogen for connective tissue. PDGF promotes migration and proliferation of endothelial cells as well as an increased recruitment of pericytes. These findings suggest that PDGF is not only important in formation of new blood vessels but it is also very important for their maturation and stabilization[(23)].

Evidence suggests that processes of inflammation and angiogenesis are connected. Newly formed blood vessels enable the recruitment of inflammatory cells, which release a variety of proangiogenic cytokines and growth factors that will perpetuate angiogenesis[(24)].

## Angiogenesis during development of retinal vasculature

During embryogenesis retinal vascularization begins in the most superficial (or inner) retinal layers at the optic nerve head, and radiates outwards from this central point. It reaches the retinal periphery just before birth[(25)].

The migration of large numbers of vascular precursor cells (VPCs) from the optic disc is the first event in human retinal vascularization, and it is apparent before 12 gestational weeks[(26)]. They proliferate and differentiate to form a primordial vascular bed centered on the optic disc. Thus, vasculogenesis is responsible for the formation of the primordial vessels of the inner (superficial) plexus in the central human retina[(27)]. Formation of retinal vessels via vasculogenesis seems to be independent of metabolic demand and hypoxia-induced VEGF expression[(28)].

Angiogenesis is responsible for the formation of the remaining retinal vessels, including increasing vascular density in the central retina, vessel formation in the inner plexus of the peripheral retina, and formation of the outer plexus and the radial peri-papillary capillaries[(28)]. Formation of the outer plexus begins around the incipient fovea between 25 and 26 weeks of gestation, coincident with signals that indicate a functional visual pathway and photoreceptor activity[(27)]. The timing and topography of angiogenesis in the human retina supports the “physiological hypoxia” model of retinal vascular formation, in which angiogenesis is induced by a transient but physiological level of

hypoxia as a result of the increased metabolic activity of retinal neurons as they differentiate and become functional[29].

## Angiogenesis in retina and choroidal pathologies

Retinal anatomy is highly organized and vascular and avascular compartments are strictly segregated in the retina[1]. The blood-retinal barriers, inner and outer, are fundamental for the integrity of structure and optimization of function of the neuro-sensorial retina[30].

Pathological retinal and choroidal angiogenesis generates chaotically orientated and physiologically deficient vessels that do not conform to neuronal histology, which can lead to vision-threatening oedema, exudation and haemorrhage[1].

Angiogenesis is a key aspect in many ocular pathologies that are leading causes of blindness in the world, such as neovascular age-related macular degeneration (AMD), diabetic retinopathy, retinopathy of prematurity, central retinal vein occlusion and other diseases associated with ischemia and neovascularization[31].

Although angiogenesis is a highly complex and coordinated process requiring multiple receptors and ligands in endothelial cells, VEGF is a hypoxia-inducible cytokine that appears to be a pivotal element required for the process in a variety of normal and pathological circumstances[3, 10]. VEGF is a surrogate angiogenic marker, since it acts not only as a mitogen, but also as a survival factor for endothelial cells[2]. Furthermore, it is also involved in the stimulation of the invasive and migration capacity of endothelial cells and in the enhancement of vascular permeability[10]. Bone-marrow derived cells have also been described in choroidal neovascular lesion. Their importance in the pathologic process is still under debate[32].

## Angiogenesis and Age-related Macular Degeneration

The diagnosis of AMD is based on fundoscopic signs observed on the macula, irrespective of visual acuity[33]. The stages of AMD are categorized as early, in which visual symptoms are inconspicuous, (moderate) and late, usually associated with severe loss of vision[34]. Early AMD is characterized by the presence of drusen and/or hyperpigmentations or small hypopigmentations[33]. Late AMD has “dry” and “wet” forms. However, in the same patient we can find the dry form in one eye and the wet in the other eye, or both forms in the same eye. Moreover with time we can see the conversion of wet in dry or dry becoming wet[35].

Age-related changes that predispose to AMD occur in the outer retina, more specifically the region that includes the photoreceptors, the retinal pigment epithelium (RPE), Bruch's membrane and the choriocapillaris.

The aging-dependent alterations in the outer retina have been already discussed in another chapter. AMD-related visual loss is a complex process starting by the deposition of debris in the outer retina[(36)]. The deposition of insoluble material, the calcification and increase in thickness of Bruch's membrane, and a less fenestrated and thinner choriocapillaris leads to photoreceptors/RPE hypoxia resulting in a stimulus for VEGF release[(35, 37-39)].

All the aging changes in outer retina compromise the nutrition of photoreceptors and RPE and create a favourable environment for the development of choroidal neovascularization (CNV). However other factors – genetic and environmental[(40, 41)] – are also important, but its role in the development of CNV is discussed in other chapters of this book.

In general terms there are two basic CNV growth patterns, based on the anatomical position of the abnormal vessels with respect to the RPE monolayer, which are related to Gass's classification of CNV[(42, 43)]. In type 1 CNV, the neovascular complex is located in the plane between the RPE and Bruch's membrane and in type 2 neovascularization the vessels have penetrated the RPE layer to proliferate in the subneurosensory space[(42)].

In the type 1 growth pattern, after breaking through Bruch's membrane, the CNV extend laterally under the RPE in a horizontal fashion that is facilitated by the natural cleavage plane between basal laminar deposits and a lipid rich Bruch's membrane. This growth pattern recapitulates the choriocapillaris and can provide some nutrients and oxygen to an ischemic RPE/outer retina[(43, 44)].

The type 2 growth pattern occurs usually with one or few ingrowth sites with vascular leakage under the RPE/outer retina that usually lead to acute visual simptoms[(43)].

Yannuzzi proposed a type 3 neovascularization, for retinal angiomatous proliferation (RAP), indicating proliferating vessels within or below the retina itself[(45)]. This mixed neovascularization, with a presumed dual origin, may have intraretinal neovascularization driven by angiogenic cytokines from Müller cells, endothelial cells, pericytes, and retinal glial cells, and CNV driven by cytokines from the RPE[(45)]. There is hypothetically neovascularization extending anteriorly from the choroid in conjunction with retinal neovascularization progressing posteriorly, with both circulations eventually anastomosing.

The reason that growth patterns vary according to disease and individuals may be related to genetic predispositions, environmental mechanisms, variations in composition and anatomy of Bruch's membrane, cytokine distribution, or other causes[(43)].

During the dynamic process of development of CNV there is a balance of angiogenesis promoters and inhibitors. In the initiation stage the RPE and photoreceptors produce VEGF[(46)]. There is also production by RPE of Interleukin-8 (IL-8) and Monocyte Chemoattractant Protein-1 (MCP), witch attract monocytes from the choriocapillaris along the outer surface of Bruch's membrane[(47)]. The macrophages tend to concentrate around sites of vascular ingrowth through the Bruch's membrane and express Tumor Necrosis Factor-α (TNF-α) and Interleukin-1 (IL-1), which up-regulate complement factor-B, activates the complement alternative pathway in the subretinal space, and stimulates RPE cells to produce more VEGF[(47,48)].

After initiation, CNV grows to a certain size and progresses through the tissue planes by the action of Matrix Metalloproteinases (MMP) produced by endothelial cells and macrophages[(49)]. During this stage of active growth, Angiopoietins (Ang-1 and 2) are expressed, FGFs are produced by RPE and endothelial cells, and TGF-β is produced by the RPE[(50-52)].

CNV stabilizes during the active stage due to a steady state established between MMP and tissue inhibitors of metalloproteinases, Ang-1 and 2, PEDF and VEGF, PDGF and VEGF, plasminogen and fibrin, and others[(43, 53)].

At some point the balance shifts toward antiangiogenic, antiproteolytic and antimigratory activity resulting in the involutional stage of CNV. When this occurs the angiogenic/proteolitic/migratory cytokine production decreases with a shift toward TGF-β and tissue inhibitors of metalloproteinases production by the RPE[(43)]. In this involutional stage the CNV may become collagenized and form a disciform scar. Subretinal fibrosis is the hallmark of this stage of the disease and is the result of the activity of inflammatory and endothelial cells and fibroblasts[(54)].

## Key points

- Angiogenesis, the growth of new vessels from pre-existing ones, is a complex multistep process involving the activation of series of receptors by numerous ligands.
- Vascular Endothelial Growth Factor (VEGF) is the most important angiogenic factor and represents the crucial rate-limiting step during angiogenesis.
- There are three basic CNV growth patterns, based on the anatomical position of the abnormal vessels with respect to the RPE monolayer and to the proliferation beginning within or below the retina itself: occult (type 1), classic (type 2) and RAP (type 2) lesions.
- Interleukin-8 (IL-8), Monocyte Chemoattractant Protein-1 (MCP), Tumor Necrosis Factor-α (TNF-α), Interleukin-1 (IL-1), Matrix Metalloproteinases (MMP),

Angiopoietins (Ang-1 and 2), Fibroblast Growth Factors (FGFs) and Transforming Growth Factor β (TGF-β), among others, play an active role during the stage of active new vessel growth.

>> References

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