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ANGIOGENESIS IN CARDIOVASCULAR DISEASE: A REVIEW

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ABSTRACT

Angiogenesis is process of new blood vessel formation that occurs under both normal and pathological conditions. In the normal state, two distinct processes can be seen. One utilizes endothelial progenitor cells (EPC) and the second utilizes existing vasculature to generate new vessels. The healthy body controls angiogenesis through a series of "on" (angiogenesis-stimulating growth factors) and "off" (angiogenesis inhibitors) switches. However, the structures formed are often functionally abnormal; possibly due to an imbalance in the angiogenic process Angiogenesis represents an excellent therapeutic target for the treatment of cardiovascular disease. The healing of damaged tissues urgently required the rapid formation of new vessels which is brought about by various growth factors: VEGF, FGF, PDGF, Angiopoitins and Ephrins. Therapeutic angiogenesis is the clinical use of methods to enhance or promote the development of collateral blood vessels in ischemic, thrombotic, atherosclerotic tissue. This new form of treatment is an alternative to high risk percutaneous coronary intervention or coronary artery bypass surgery. There are three major ways to promote angiogenesis: protein therapy (by growth factor proteins including VEGF, bFGF), gene therapy (sustained production of angiogenic factors, the ability for local delivery and so less systemic exposure) and cellular therapy (monocytes, EPC and marrow stromal cells).

INTRODUCTION: Angiogenesis is the process of new blood vessel formation as a natural event that occurs under both normal and pathological conditions. In the normal state, two distinct processes can be seen One that utilizes endothelial progenitor cells (EPC), these are usually derived from bone marrow and initiate endothelial growth and vascular tube formation & other one to utilizes existing vasculature to generate new vessels, and is highly dependent on endothelial cell activation and protease secretion. Under pathological conditions, many of the same steps involved in normal vessel formation are repeated. However, the structures formed are often functionally abnormal, possibly due to an imbalance in the angiogenic process.

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Multiple factors contribute to angiogenesis, including soluble growth and differentiative factors, extracellular matrix components, membrane-bound receptors, and intracellular signaling molecules.

Control of Angiogenesis: Angiogenesis occurs in the healthy body for healing wounds and for restoring blood flow to tissues after injury or insult. In females, angiogenesis also occurs during the monthly reproductive cycle (to rebuild the uterus lining, to mature the egg during ovulation) and during pregnancy (to build the placenta, the circulation between mother and fetus).

The healthy body controls angiogenesis through a series of "on" and "off" switches:

- The main "on" switches are known as angiogenesis-stimulating growth factors
- The main "off switches" are known as angiogenesis inhibitors

Excessive Angiogenesis:

- Occurs in diseases such as cancer, diabetic blindness, age-related macular degeneration, rheumatoid arthritis, psoriasis, and more than 70 other conditions.
- In these conditions, new blood vessels feed diseased tissues destroy normal tissues, and in the case of cancer, the new vessels allow tumor cells to escape into the circulation and lodge in other organs (tumor metastases).
- Excessive angiogenesis occurs when diseased cells produce abnormal amounts of angiogenic growth factors, overwhelming the effects of natural angiogenesis inhibitors.
- Antiangiogenic therapies are being developed to treat these conditions by halting new blood vessel growth.

Insufficient Angiogenesis:

- Occurs in diseases such as coronary artery disease, stroke, and delayed wound healing.
- In these conditions, inadequate blood vessels grow, and circulation is not properly restored, leading to the risk of tissue death.
- Insufficient angiogenesis occurs when the tissue cannot produce adequate amounts of angiogenic growth factors.
- Therapeutic angiogenesis, aimed at stimulating new blood vessel growth with growth factors, is being developed to treat these conditions.

Pathophysiology of Angiogenesis: Vasculogenesis involves the migration and expansion of endothelial cells precursor angioblasts into sites of blood vessel formation in the embryo and yolk sac, in situ differentiation of angioblasts into endothelial cells and that connect to the extra embryonic vasculature. Development of new blood vessels from preexisting blood vessels called angiogenesis.

There are three main types of Secondary Angiogenesis, they are:

1. **Sprouting:** This is the most frequently used mechanism whereby new blood vessels form by 'sprouting'. Blood vessels sprout from the side or the ends of pre-existing blood vessels. During this process, a large sinusoidal capillary is divided into two smaller capillaries, which then grow separately by variety of mechanisms includes vasodilation of parent blood vessels by production of NO. Vasodilation also promoted by VEGF & its downstream signaling targets as well as by VE-cadherin which all promotes the extravasations of plasma proteins to form provisional matrix for cell migration.

   EC in the parent vessel alter their attachments to basement membrane & pericytes. Change in the flow, mechanical force transmitted through the endothelium activates proteases that remodel the vascular basement membrane causing it to very thin. Matrix remodeling has several sequences for regulating angiogenesis. Mechanical force converges on thinned regions of the basement membrane to facillate sprouting remodel ECM. Altered EC responsiveness to growth factors & release growth factors or inhibitors sequestered in the matrix to proliferate or limit angiogenesis.

Angiogenic growth factors such as VEGF or FGF bound to specific receptors such as VEGF-R1 (Flt1) protein on the endothelial cells, thereby activating them. The activated endothelial cells produce matrix metalloproteinases (MMPs), a special class of degradative Enzymes. The MMPs will break down the extracellular matrix, hence permitting the migration of endothelial cells. Proliferation & Migration of EC contributing increase the length of developing vessels; but migration EC forms the tip of capillary sprout. Angiogenesis factors: VEGF, FGF, modulate both proliferation & migration of EC. Complex of metaloprotease & integrins on EC coordinate migration with exposure to matrix molecules to facillate adhesions. As endothelial cells migrate into the surrounding tissues, they will begin to proliferate and migrate out of the existing blood vessel.

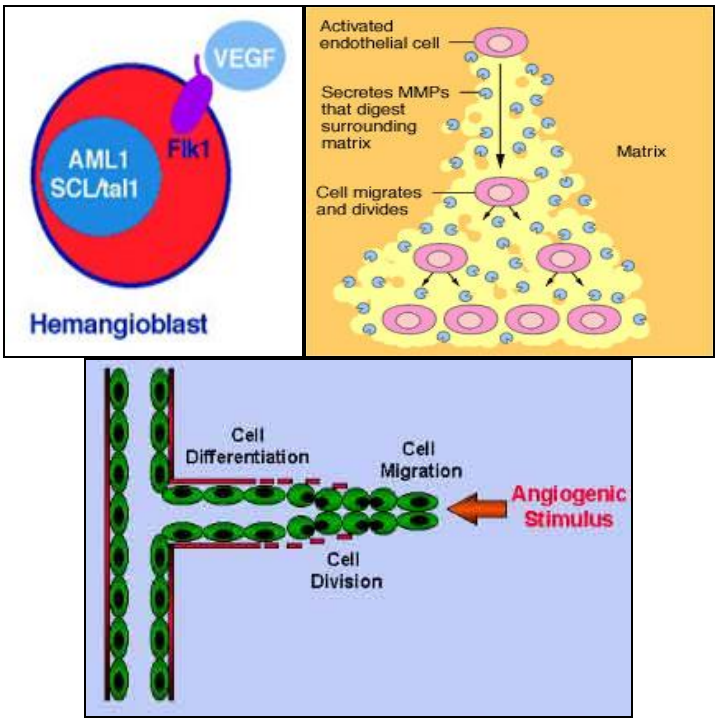
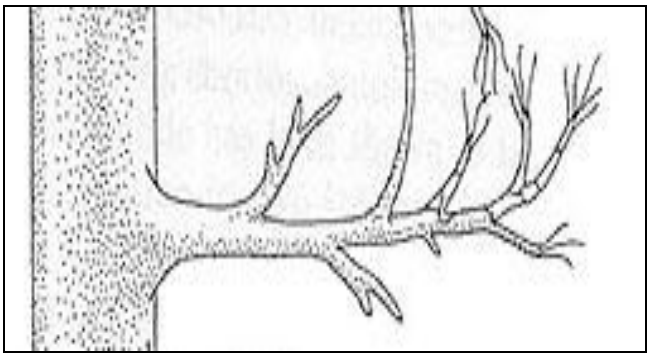


FIGURE 1: ANGIOGENESIS SPROUTING PHENOMENON

2. **Bridging or intussusceptions:** It is connection between EC develop across the lumen to subdivide blood vessel along its length into small parallel vessels (capillaries). As the EC align in the developing capillary, they attach to each other & to the ECM other & to the ECM in manner that create lumen. The lumen develops proximally in the proximally sprout as continuation of lumen from the parent vessels. Lumen formation requires the production of fibronectin & laminin matrix depends on adhesion molecules & cell surface glycoprotein. Specialized molecules called adhesion molecules, or integrins (avb3, avb5) serve as grappling hooks to help pull the sprouting new blood vessel sprout forward. Hence, Sprouting endothelial cells wrap around to form a blood vessel tube.



Parallel capillaries fuse & coacelance along their length or at their tips to form a loop capable of handling blood flow. Blood flow causes further vascular remodeling. Blood cells (like platelets) carry several endothelial factors that facilitate maturation of newly perfused vessels. At the same time, the surrounding mesenchymal cells produce a signal molecule, which binds to the receptor, Tie-2, on the surface of the endothelial cell. This stimulates the migration of the mesenchymal cells toward the vascular endothelium. These mesenchymal cells will form the vascular smooth muscle, known as pericytes, which provide structural support around the blood vessel tubes.

3. **Intercalation of Endothelial cells:** This incorporates additional EC to increase the length & caliber of blood vessel in growing tissues through maturation of basement membrane, stabilization/ incorporation of pericytes & EC survive/apoptosis. The new capillary continuous to remodel the basement membrane by enhance its content in collagen type IV, XV, XVIII thus cause maturation of basement membrane. Maturation of new vessel requires the recruitment of pericyte that regulate blood flow & permeability & produce paracrine signals including VEGF, FGF, PDGF, TGF and ANGIOPOITIN (ANG-1). Pericyte stabilize the newly formed blood vessels & inhibit vascular regression after angiogenic factors. EC apoptosis is enhancing by disruption of cell attachments to the matrix or to other EC.

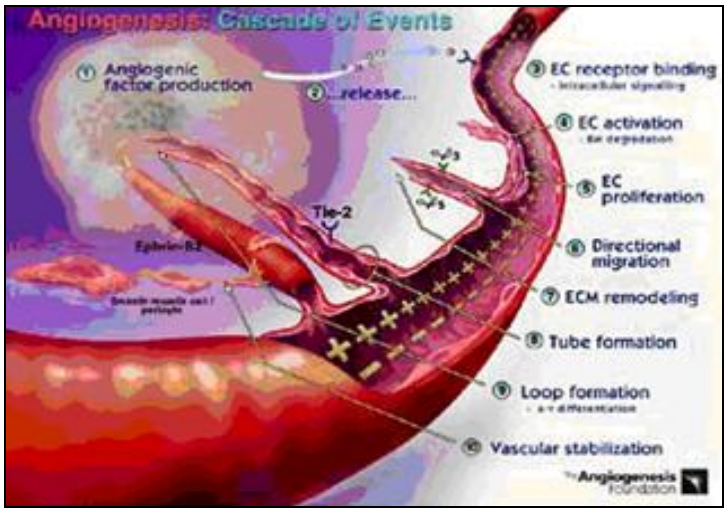


FIGURE 2: PROCESS OF ANGIOGENESIS

In physiologic & pathologic conditions most common involve sprouting & adhere to series of morphologic & biologic events.

Angiogenic Growth Factors:

1. **Vascular Endothelial Growth Factor (VEGF):** Vascular endothelial growth factor (VEGF) is a specific mitogen for vascular endothelial cells (EC). VEGF family consists of seven members – VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F and placental growth factor (PlGF). VEGF is expressed in a wide variety of tissues, including brain, kidney, liver, and spleen, and by numerous cell types. VEGF influences many steps in the angiogenic response. It stimulates degradation of the extracellular matrix surrounding endothelial cells, and it promotes endothelial cell proliferation, migration, and organization into tubular structures.

The biological functions of VEGF are mediated upon binding to receptor tyrosine kinases; VEGFR-1, (Flt-1), VEGFR-2 (KDR/Flk-1), and VEGFR-3 (Flt-4). Neuropilin-1 (Nrp-1) and Nrp-2 are receptors for semaphorins. But they also bind to some members of the VEGF family. VEGF/VEGF-receptor system is a key component in the complex process of angiogenesis that also includes many other stimulators, inhibitors and angiogenic modulators [5]. *In vivo*, VEGF has been shown to regulate vascular permeability, which is important for the initiation of angiogenesis by extravasating plasma proteins that form provisional matrix favoring cell migration [3].

TABLE 1: VEGF FAMILY & TYPES

VEGF Types	Location	Function
VEGF-A	Activated ECs, macrophages and differentiated smooth muscle cells (SMCs) in atherosclerotic lesions.	• Induction of angiogenesis and vasculogenesis it causes proliferation, sprouting, migration and tube formation of ECs. • It is vascular permeability Factor.
VEGF-B	Expressed in the most tissues including skeletal muscles, myocardium and brown fat and accounts for more than 80% of the total VEGF-B transcripts.	• Lacks hypoxia-inducible factor-1 and AP-1 sites found in the VEGF-A promoter. Consequently, stimuli such as hypoxia which can induce VEGF-A expression do not appear to regulate levels of VEGF-B.
VEGF-C	Expressed in the heart, small intestine, placenta, ovary and the thyroid gland in adults.	• VEGF-C induces mitogenesis, migration and survival of ECs. • Increase in blood vascular permeability induced by VEGF-C is mediated by VEGFR-2
VEGF-D	Expressed in many adult tissues including the vascular endothelium, heart, skeletal muscle, lung, and bowel. It is secreted glycoprotein.	• The mature form binds to and activates VEGFR-2 and VEGFR-3. • VEGF-D binds only to VEGFR-3. VEGF-D has been shown to be responsible for proliferation of ECs, and angiogenic properties in vitro and in vivo. • It also shows lymphangiogenic potential.
VEGF-E	VEGF-E was discovered in the genome of the parapoxvirus (Orf virus) that infects sheep, goats, and occasionally humans	• Bind specifically to VEGFR-2 and Nrp-1 and are able to stimulate EC mitogenesis and vascular permeability.
VEGF-F	VEGF-F, was identified from snake (viper) venom.	

VEGF-A mediates its responses primarily by activating VEGFR-1 and VEGFR-2 but it also binds to Nrp-1 and Nrp-2. VEGF-A increases permeability by binding to VEGFR-2 and thereafter activating guanylyl cyclase (GC) and cGMP via a nitric oxide dependent pathway. Increased cGMP levels probably enhance endothelial permeability by increasing the vesico-vascular organelles, and transcellular gaps. VEGF-A also causes vasodilatation by induction of endothelial nitric oxide synthase (eNOS) and increasing nitric oxide production. VEGF-A mediated extravasation of fluid and plasma proteins, including fibrin might contribute to enhanced migration of ECs in extracellular matrix Hypoxia induces binding of HIF-1a to the Hypoxia responsive element (HRE) in the VEGF-A gene promoter region, which in turn increases VEGF-A transcription [5].

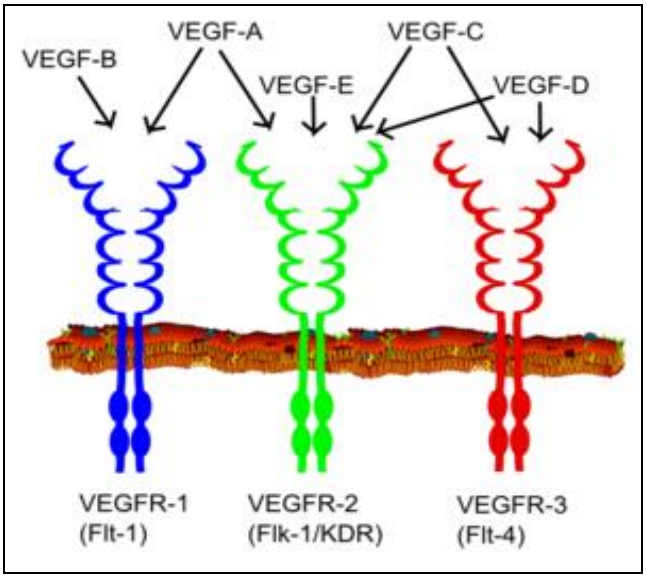


FIGURE 3: VEGF RECEPTOR

Each receptor subtype assembles a different set of signaling molecules, giving rise to the formation of specific signal transduction at the plasma membrane. VEGFR-2 regulates endothelial cell migration, proliferation, differentiation, and survival as well as vessel permeability and dilation. VEGFR-2 is the predominant receptor in angiogenic signaling. Some signaling pathways activated by VEGFR-2 are the PI 3-kinase/Akt pathway and the classical Ras-dependent signaling cascade impinging on MAP kinases such as ERK1 and ERK2 [7].

Fibroblast Growth Factors: Fibroblast growth factors (FGFs) comprise of related polypeptides a family of 20 molecules with a wide range of biological effects; among their actions is the ability strongly to stimulate angiogenesis. The two most extensively studied FGFs in relation to angiogenesis are FGF-1 (also known as acidic fibroblast growth factor; aFGF) and FGF-2 (basic fibroblast growth factor; bFGF). FGFs mediate their effects via four tyrosine kinase receptors, FGFR1 to 4. [3] Human atherosclerosis tissues express increased FGF-1 compared with normal vessels, which may promote plaque revascularization and restenosis. FGF-1 & FGF - 2 do not contain secretion signals, suggesting that cell injury is one mechanism for their release. FGF-2 is expressed at low levels in almost all organs and tissues examined, with high concentrations reached in the brain and pituitary [8].

Two types of receptors have been identified for FGF-2:

- High affinity tyrosine-kinase FGF receptors (FGFRs)
- Low affinity heparin sulfate proteoglycans (HSPGs).

These HSPGs are found in the ECM, the basement membrane and the cell surface. It has been suggested that binding of FGF-2 to HSPGs result in protection of FGF-2 from inactivation in the extracellular environment and in storage of FGF-2 in the ECM and basement membrane. Stored FGF-2 can be released by heparitinase and soluble heparin or after ECM breakdown.

Platelet-derived Growth Factors (PDGF): The PDGFs are a family of peptide growth factors that signal through cell surface tyrosine kinase receptors and stimulate various cellular functions including growth,proliferation, and differentiation.[7] Five isoforms of the PDGF family exist, which include PDGF-AA, PDGF-AB, PDGF-BB, PDGF-C and PDGF-D.

These factors exert their cellular effects through PDGF-α and PDGF-β protein tyrosine kinase receptors. PDGFs activate their receptors by forming receptor dimers: PDGFR-αα, PDGFR-αβ, and PDGFR-ββ. The activated receptors phosphorylate a large number of substrates (more than 20), including themselves, initiating a complex network of signaling cascades. PDGFR-α and PDGFR-β distinctly activate downstream effectors such as Grb2/SOS, PI3K, GAP, Erk, JNK, Src, and Stat.

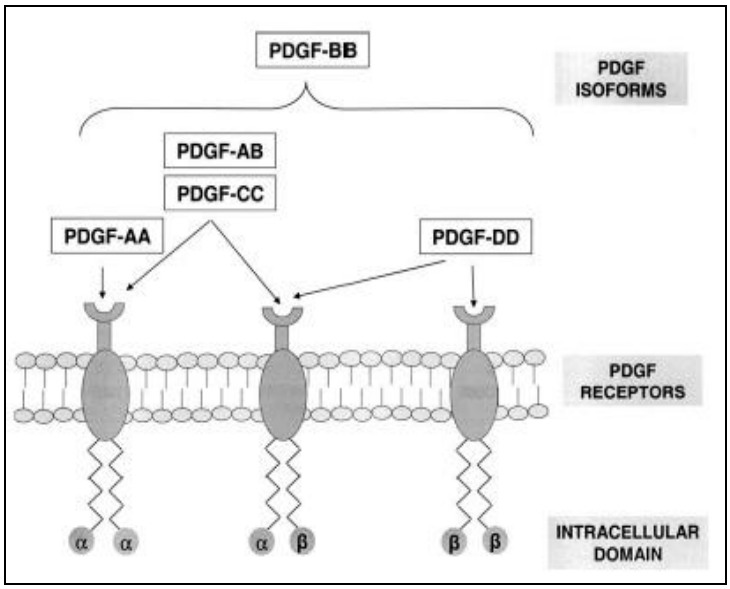


FIGURE 4: FGF RECEPTOR

Uncontrolled PDGFR activation promotes cell migration, proliferation and survival [7, 11]. Recent studies have demonstrated that PDGFs and their receptors are involved in human cancers through autocrine stimulation of tumor cell growth [7].

Hypoxia Inducible Factor (HIF): Tissue oxygenation represents a steady state based upon O2 consumption, primarily by mitochondrial oxidative phosphorylation, and O2 delivery via erythrocytes traveling through tissue capillary networks. This steady state represents a balance between the requirement for O2 as an energy substrate and the inherent risk of oxidative damage to cellular macromolecules.

Hypoxia-inducible factor-1 (HIF-1) is a heterodimeric α, β-transcriptional complex that mediates the cellular response to oxygen availability in multicellular organisms. Dimerisation of the HIF-α and HIF-β subunits under hypoxic conditions activates the transcription of an array of genes involved in the adaptation of cells to the lowered oxygen tension. Medicinal stimulation of the natural HIF mediated response might be used to treat ischemic illnesses such as heart disease; in contrast, inhibition of the HIF mediated hypoxic response might be used for the treatment of tumors via inhibition of angiogenesis.

Short-term hypoxia can also elevate platelet numbers, while prolonged exposure may cause some degree of thrombocytopenia in response to increased levels of erythropoetin (EPO).

Hypoxia-inducible factor-1 (HIF-1) plays a critical part in oxygen homeostasis. The HIF-1 protein consists of two subunits, HIF-1a and HIF-1b. HIF-1α acts as a master transcription switch for regulation of oxygen homeostasis [14].

Non-hypoxic conditions, the von Hippel-Lindau tumor suppressor protein (VHL) targets HIF-1α for rapid ubiquitination. Under hypoxic conditions, the HIF-1 heterodimer recognizes and binds to *cis* elements in the promoter of genes that mediate angiogenesis, such as the gene encoding VEGF.

Angiopoietins: The angiopoietins (Ang1 and Ang2), and their high-affinity receptors Tie1 and Tie2 (for "tyrosine kinase with immunoglobulin and EGF-like domains") are essential for vascular development and potentiate VEGF expression and function [6].

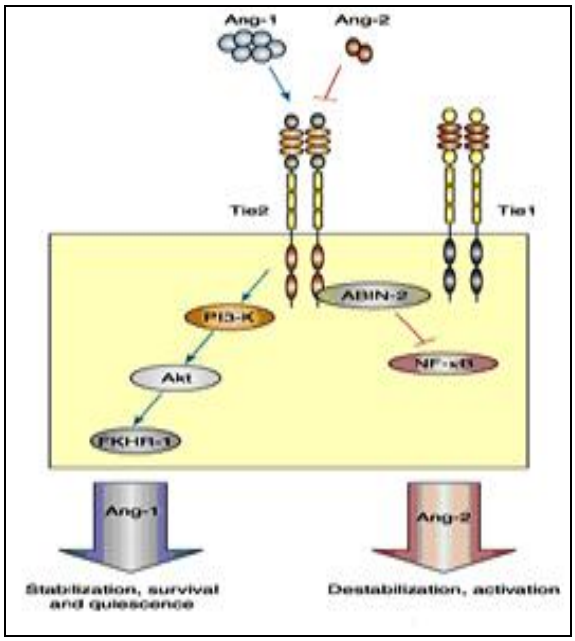


FIGURE 5: ANGIOPOITIN &B TIE RECEPTOR

The ligands for Tie-2 have only recently been discovered: angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2) both bind Tie-2, but only the binding of Ang-1 results in signal transduction and regulation of blood vessel maturation. Therefore, Ang-2 is a natural antagonist of Ang-1 [10]. In a normal adult vessel, Ang-1 is associated with Tie-2 to keep the vessels in a stable state. Upregulation of Ang-2, by hypoxia or VEGF e.g. in the ovary during corpus luteum formation or by tumor cells, disrupts the interaction between Ang-1 and Tie-2 resulting in destabilization of the vessels [8].

Endothelial cells that are no longer surrounded by pericytes and the extracellular matrix acquire a heightened responsiveness to angiogenic signals, and in the presence of VEGF, angiogenesis is promoted [12, 6].

Ang-1-mediated phosphorylation of Tie-2 promotes:

- Cell survival through phosphorylation of Akt threonine kinase p42/44 MAPK
- Reorganization of endothelial cells;
- Recruitment and association of pericytes/vascular smooth muscle cells to mature and stabilize newly formed blood vessels

Ang-1 is constitutively expressed by many different cell types: Ang-1 expression is found in pericytes, smooth muscle cells, fibroblasts and some tumor cells This is in contrast to the expression of Ang-2, which is almost exclusively expressed by endothelial cells themselves, and is also detectable in Kaposi's sarcoma cells, Ang-2 expression is induced by various cytokines, including vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF)-2, and by micro environmental factors (e.g. hypoxia).

Thus, Ang-1-mediated PKB–Akt signaling directly inhibits endothelial-cell apoptosis and prevents activation of the endothelium by inhibiting Ang-2 expression and secretion. This protects endothelial cells from apoptosis and inhibits inflammatory responses.Thus, constitutive Tie2 phosphorylation and signaling involves several signaling pathways that are collectively anti-apoptotic and maintain the quiescent state of the resting endothelium [19].

Ephrins: The family of Eph receptor tyrosine kinases and their ephrin ligands comprise the largest family of growth factor receptors. The family consists of fourteen receptors and ligands divided into two subclasses, A and B. They also play a more general role in development by maintaining functional boundaries in developing embryos. Recent studies have shown that the ephrins play a similar role in compartmentalizing the vascular system, where they are responsible for the general apportionment of the arterial and venous circulation.

Similar to the angiopoietins, interference with ephrin/Eph interactions destabilizes the developing capillary network [6].

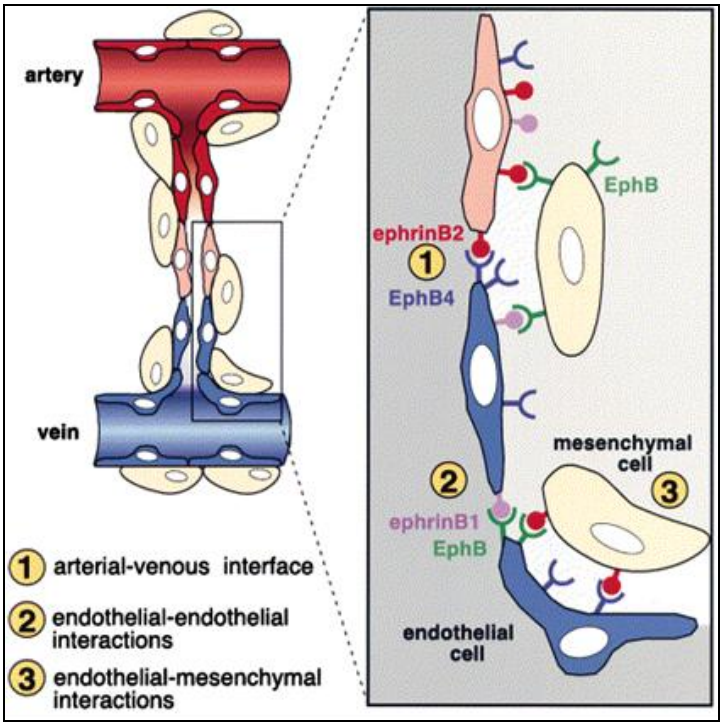


FIGURE 6: EPHRINS RECEPTOR

Possible sites and mechanisms of Eph/ephrin function in the developing vasculature.

(1) Interaction between ephrinB2 and EphB4 at the arterial–venous interface might restrict intermingling of endothelial cells or stimulate formation of new capillary sprouts by angiogenesis.

(2) Co-expression of ligands and receptors by endothelial cells might be required for contact-dependent communication between endothelial cells via bidirectional signaling.

(3) Eph/ephrin molecules expressed by adjacent mesenchymal cells may be involved in the patterning of the vasculature. Interactions could stimulate angiogenesis by the presentation of clustered ephrinB ligands, whereas unclustered monomeric ligands might interfere with bidirectional signaling and thereby inhibit formation of endothelial sprouts.

Angiogenesis in various Cardiovascular Disorders:

Angiogenesis in Atherosclerosis: Angiogenesis is central to many physiological and pathological phenomena. In physiological angiogenesis, new vessels are well shaped and their growth is finely tuned to match the metabolic needs of tributary tissues. Angiogenesis represents an excellent therapeutic target for the treatment of cardiovascular disease. It is a potent, physiological process that underlies the natural manner in which our bodies respond to a diminution of blood supply to vital organs, namely the production of new collateral vessels to overcome the ischemic insult.

Accordingly, neovascularization is activated by physical exercise and destabilized by non-use. A great deal of attention is focused on new approaches for medical manipulation of vascular growth. These methods are aimed at facilitating the reperfusion of ischemic tissues or eradicating pathological vasculature. Therapeutic angiogenesis aims at combating the insufficiency of, or insensitivity to angiogenic factors in the setting of atherosclerotic-induced arterial occlusion. However, clinical evidence indicates that such a defect is not common among patients with ischemic disease, as a whole. Genetic and environmental factors could account for the great heterogeneity in the expression of the master angiogenic factors [23].

Collateral vessel development is an increase in size and caliber of pre-existing arteriolar collateral connections by remodeling, termed arteriogenesis. The presence and number of these native collaterals varies widely within and among species. When an occlusion occurs, such pre-existing collaterals are subject to an increase in the velocity of blood flow and a consequent increase in shear stress within the lumen, ultimately contributing to maturation of collateral conduits.

Arteriogenesis has been suggested to represent a particularly prominent mechanism for development of those medium-sized collaterals that are large enough to be recognized. Bone marrow-derived EPCs circulate in peripheral blood, and incorporate into foci of neo-vascularization in adult animals, are increased in number in response to tissue ischemia, and can augment collateral development following *ex vivo* expansion.

Factors affecting Myocardial Angiogenesis:

- Cardiac growth and the cardiac vasculature are influenced by hormonal and certain physiologic conditions. Elevated or reduced thyroxin levels increase myocardial size and capillary density without inducing significant endothelial cell proliferation, suggest that endothelial cell elongate or increase in size.

- Mechanical forces exerted by contraction and blood flow influence vessel development. Forces are transmitted from the apical endothelial cell surface, across the actin cytoskeleton, to the vascular basement membrane and adjacent cardiomyocytes and back again.

- Molecular mechanism activated by mechanical stimuli involve integrins and their signaling pathways, cell-cell junction molecules, structural molecules in the cytoskeleton, activation of flow sensitive ion channels and up regulation of cell matrix interactions.

- Shear stress and stretch activation of endothelial cells alter patterns of gene expression, cell shape and release of prostacyclins, nitric oxide and other vasoactive molecules. Chronic vasodilators have been employed to increase coronary blood flow and increase capillary density in the myocardium [27].

- Hypoxia in tissues is a potent inducer of angiogenesis. Hypoxia or hypoglycemia increase HIF-1α, that regulates several gene involved on angiogenesis and erythropoiesis to restore homeostasis in tissue deprived of oxygen or nutrients.

- Increase expression of HIF-1α, in transgenic mice induced blood vessels with increased calibers and more smooth muscles cells compared to capillaries induced by VEGF alone.

- The effect of hypoxia on coronary vessels and heart *in vivo* are very complex, because chronic hypoxia in patients induces other physiologic adaptations. Chronic hypoxia induces pulmonary hypertension, right ventricular hypertrophy and alterations in capillaries and arterioles due to altered pressure load rather than reduced oxygen delivery in the right ventricle.

Angiogenesis Triggering Factors: It is a very complex process. Conceptually the major triggers of this process can be simplified into three broad categories: Mechanical, Chemical and Molecular factors.

Mechanical influences [29]:

Hemodyanmics: Augmentation of blood flow during exercise, the hyperthyroid state (Thyrotoxicosis) and administration of certain drugs have all been shown to stimulate vascular sprouting.

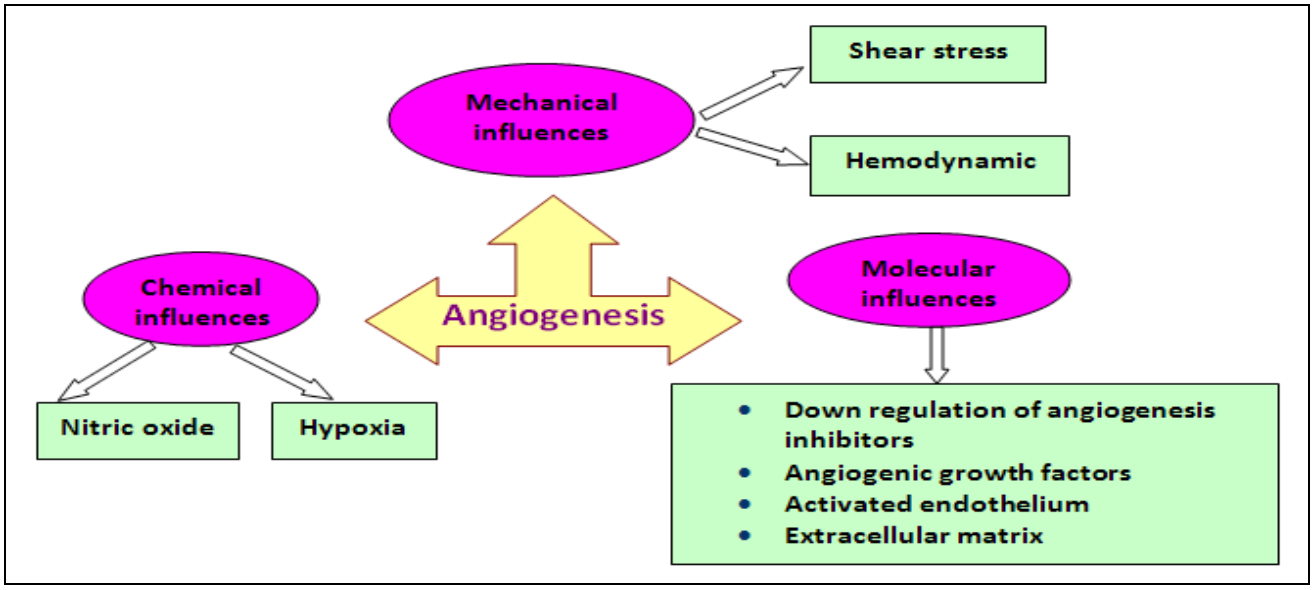
It also has been shown that large vessels with low flow tend to reduce or obliterate their endovascular diameter and the lumens of the small vessels with chronically increased high blood flow tend to enlarge. Augmentation of blood flow therefore may both stimulate vascular sprouting and maintain patency of the newly formed collateral vessels and so providing blood flow to the ischemic area.

Shear Stress: Shear stress has an important influence on the development of collaterals in the ischemic tissues. Shear stress and stretch of the myocardium (i.e. increase left ventricular end diastolic volume) can lead to up-regulation of adhesion molecules in the endothelium, attraction of the inflammatory cells and stimulation of the endothelial cells to produce angiogenic factors. Endothelial cells can be viewed as mechano-receptors that respond to physical forces and therefore play an important role in linking mechanical influences with the molecular signals of angiogenesis.

Chemical influences: This can happen either through hypoxia or through the production of nitric oxide:

Hypoxia and Oxygen Tension Gradient: Hypoxia stimulates macrophages to release various factors including platelets derived growth factor and fibroblast growth factor 1 and 2 Hypoxia has been shown to upregulate vascular endothelial growth factor owing both by increasing transcription mediated by hypoxia inducible factor 1 and by increasing the stability of vascular endothelial growth factor mRNA Hypoxia also increase the expression of vascular endothelial growth factor receptors(FLK and FLT) this renders the endothelium susceptible to either the systematic or the paracrine effect of preexisting vascular endothelial growth factor.

Nitric Oxide: VEGF is known to induce the release of Nitric oxide from endothelial cells, and vascular endothelium inducible NO synthase (iNOS) production is amplified during VEGF- induced angiogenesis The role of Nitric Oxide in VEGF induced angiogenesis have been shown in NOS knocked out mice as well as after NOS inhibition, both result in reduction of angiogenesis. Nitric Oxide has also a regulatory effect on VEGF production.



Angiogenesis in Atherosclerotic Plaques: Normal vessels have microvasculature known as vasa vasorum that is confined to the adventitial and outer medial layers, yet proliferates and extends into the intima in association with atherosclerosis and other types of large-vessel vasculitis including thromboembolic occlusions in arteries and vein.

Anatomically plaque capillaries arise more frequently from the native adventitial vasa vasorum, which are supplied by vessels that originate at branch points of coronary arteries and run lengthwise along arteries.

The causes of coronary lesion progression from an asymptomatic fibroatheromatous plaque to a lesion at high risk for rupture (thin cap fibroatheroma or "vulnerable plaque") are not fully understood. Recently, our laboratory showed that intraplaque hemorrhage is an important process in the progression of asymptomatic plaques into high-risk unstable lesions. Red blood cell (RBC) membranes are rich in phospholipids and free cholesterol, and their accumulation within plaques plays a key role in promoting lesion instability through necrotic core expansion and inflammatory cell infiltration.

Plaque Neovascularization: It contributes to atherosclerosis, acute lesion instability and vascular disease by promoting several functions. Plaque neovascularization is comprised of a network of capillaries that arise from the adventitial vasa vasorum and extend into the intimal layer of atherosclerotic lesions and other types of vascular injury. Vasa vasorum-derived microvessels do not extend to the intima of normal arteries, penetrating only the adventitia and outer media .The functions of these plaque capillaries are proposed to be important regulators of plaque growth and lesion instability.

Neovascularization in Atherosclerosis Arteries: In absence of disease, microvessels provide vessel wall nutrients to the tunica media, while the intima is fed by oxygen diffusion from the lumen. As disease evolves and the tunica intima thickens, oxygen diffusion is impaired, and microvessels become the major source for nutrients to the vessel wall. Intimal neo-vascularization appears to be an almost ubiquitous feature of atherosclerotic disease. Majority of microvessels arise from the adventitial vasa vasorum and rarely from the luminal is surface of the parent artery. Microvessel density greatest in lesions with marked macrophage serve as a port of entry for inflammatory cells, from the systemic circulation to the nascent atherosclerotic lesion. As disease progress, microvessels also play a role in intraplaque hemorrhage, lipid core expansion, and plaque rupture.

Mechanisms of Vessel Wall Neovascularization: Initial steps in angiogenesis include an increase in vascular permeability and proteolytic degradation of the surrounding extracellular matrix.

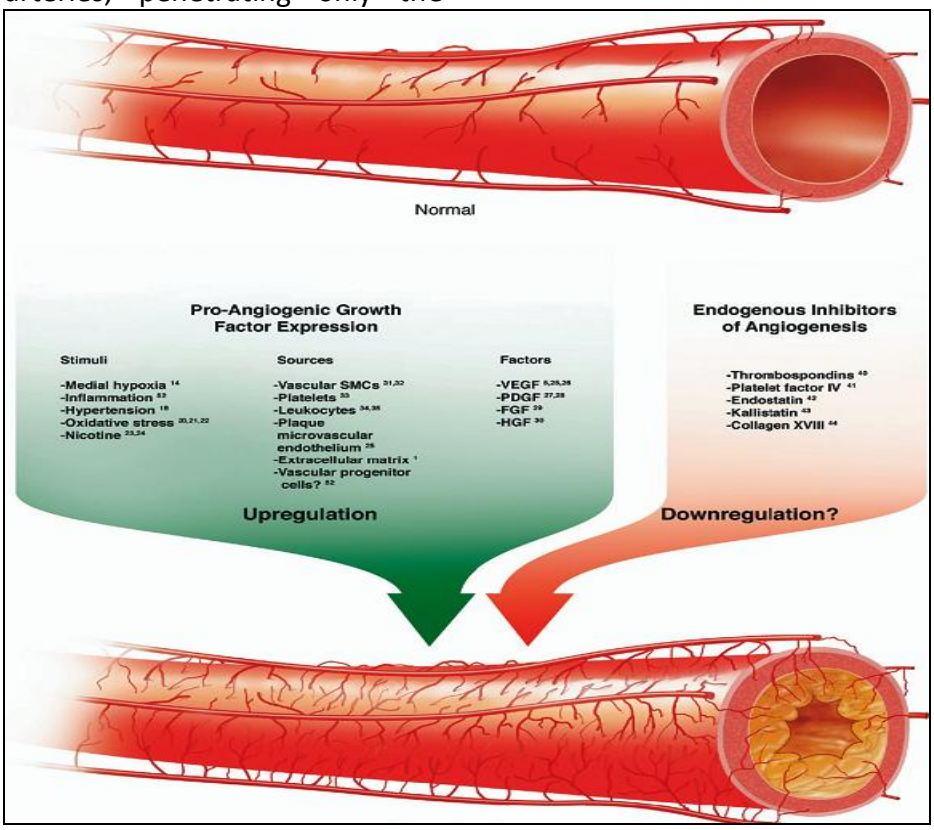


FIG. 7: MECHANISM OF VESSEL WALL NEOVASCULARIZATION IN ATHEROSCLEROSIS

This is followed by chemotactic migration and proliferation of endothelial cells, formation of a lumen, and functional maturation of the newly formed capillary by tightening of interendothelial cell junctions.

Thus, it appears likely that hypoxia within the plaque core is an important trigger of proangiogenic activity in established vascular lesions that exhibit significant neo intimal thickening. Up-regulation of proangiogenic growth factors (including VEGF) within the vessel wall may precede intimal thickening. Several angiogenic factors including VEGF, FGF, chemokines, PIGF, PDGF are abundantly expressed in human atherosclerotic lesions.

Macrophages in atherosclerotic lesions also express IL-8, TGF-β and other cytokines which can further augment the production of some angiogenic factors by other vascular wall cells. The complex regulation of plaque angiogenesis also includes microenvironment factors, such as local hypoxia or oxidative stress, ECM turnover, presence of endogenous factors that antagonize the proliferation of vasa vasorum. TSP-1 and collagen XVIII, the parent molecule of endostatin, is abundant in the vessel wall, which impede the inward growth of vasa vasorum across the media into atheromas.

Angiogenic Therapy in Myocardial Ischemia: Tissues depend on oxygen and nutrients for their metabolic needs. Because there is a threshold limit for the diffusion of these elements, vessels need to grow whenever cells increase in number or size. Accordingly, muscular hypertrophy is associated to physiologic neovascularization, while vessels tend to regress under conditions of non-use [44].

The healing of damaged tissues represents one prototypical situation in which the rapid formation of new vessels is urgently required.

The other way round, in patients with limb ischemia, supply side of angiogenic factors is thought to benefit tissue repair by compensating a putative endogenous deficit in production/release. More recently, trans-plantation of progenitor vascular cells has emerged as a powerful method to provide the substrate for revascularization and regenerate the injured heart [25]. These concepts were initially tested in experimental animal models and are now under scrutiny in the clinic.

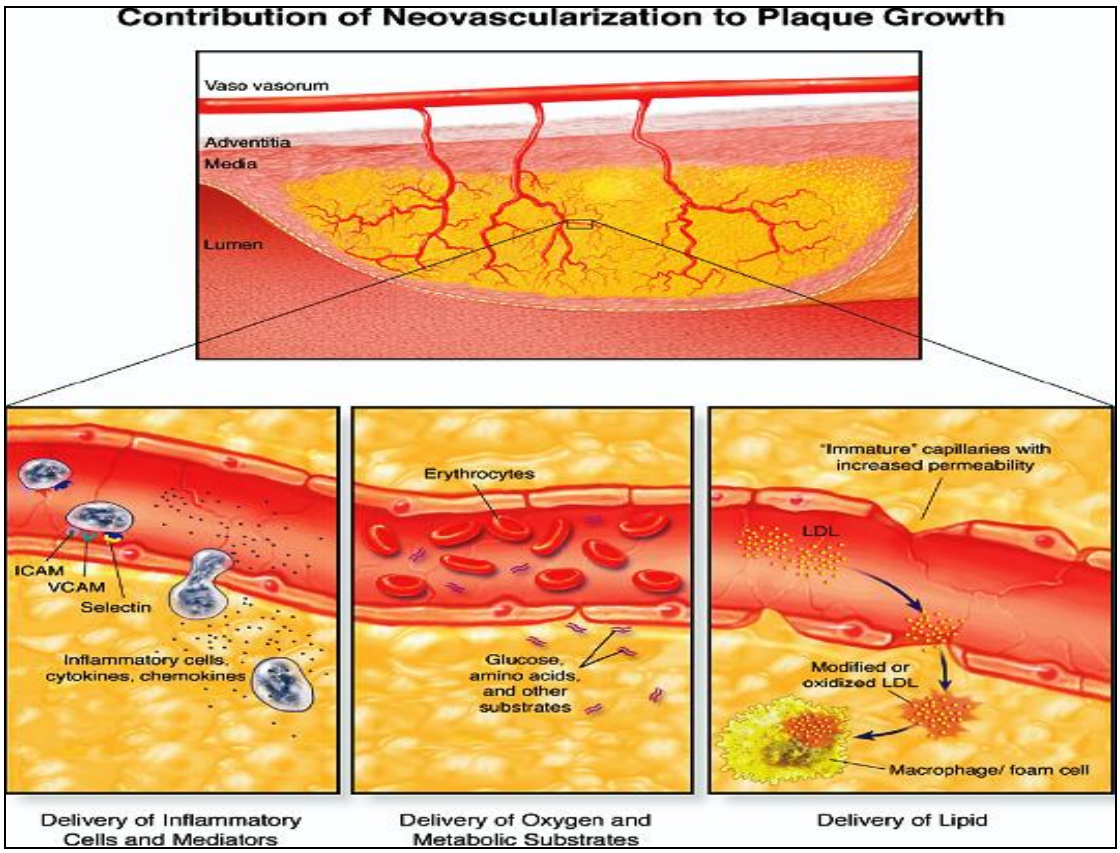


FIGURE 7: NEOVASCULARIZATION IN PLAQUE GROWTH

Clinical applications of angiogenesis research are being pursued along three general lines:

- Prognostic markers in cancer patients
- Antiangiogenic therapy
- Angiogenic therapy

The first angiogenic therapy of ischemic vascular disease was the administration of vascular endothelial growth factor (VEGF)/vascular permeability factor to patients with severe peripheral vascular disease in the lower limbs. Currently available approaches for treating coronary arterial disease aim to relieve symptoms either by reducing myocardial oxygen demand, preventing further disease progression, restoring blood flow a large localized segment of the epicardial coronary arterial tree.

Recent advances in our understanding of the stimuli and control mechanism governing the development of new blood vessels in coronary heart disease have led to an improved picture of compensatory healing process the accompanies myocardial ischemia and infarction. Collaterals appear at the interface between normal and ischemic tissue in response to a gradually developing high grade stenosis or occlusion. Blood flow through coronary collaterals may be sufficient to preserve ischemia and prevent ischemia at rest and reduce or prevent ischemia during stress. It is therefore potentially attractive to emulate and/or enhance this process by delivery of growth factors to promote neovascularization [25].

Therapeutic Angiogenesis: The high-affinity endothelial receptors for various angiogenic growth factors are now well-recognized to mediate proliferation, migration and differentiation of the cellular populations required for the augmented growth of new vascular structures in ischemic tissues [34].

Therapeutic angiogenesis is the clinical use of methods to enhance or promote the development of collateral blood vessels in ischemic tissue. Angiogenic treatment is potentially important as we see more patients that are having ischemia or vascular insufficiency that are not amenable to revascularization.

This new form of treatment is an alternative to high risk percutaneous coronary intervention or coronary artery bypass surgery [29].

Strategies for Drug Delivery in Therapeutic Angiogenesis: Therapeutic angiogenesis can be affected by one of two mechanisms of drug delivery:

- Transfer of the relevant genetic material
- Therapeutic administration of the angiogenic factor itself

The native factor can be administered either via the systemic route or locally. Alternatively, local sustained-release of the native factor or direct gene therapy may provide efficient delivery of angiogenic factors and facilitate more prolonged local exposure to the growth factors and minimize systemic side effects [34].

TABLE 2: VARIOUS MODES OF DRUG DELIVERY

Gene transfer	Native factor
• Plasmid DNA	• Systemic
• Liposomal DNA	• Single vs repeated administration
• Viral vectors	• Local
▪ Recombinant adenovirus	• Polymer-based devices (slow releasing polymers hydrogel, microspheres, etc.)
▪ Retrovirus	• Intravascular delivery (porous balloons, stents iontophoresis, simple injection
▪ Adeno-associated virus	• Cardiac catheterization

Angiogenic Cytokines: Nature's response to the development of profound tissue ischemia includes the up regulation of angiogenic growth factors and mobilization of circulating cellular elements that together enable development of an accessory vasculature. Multiple angiogenic factors and inhibitors have been implicated in the formation and correct patterning of functional blood vessels.

Gene Therapy for Neovascularization: Potential therapeutic genes for improvement of angiogenesis or arteriogenesis include growth factors, which predominantly act on endothelial cells to promote

endothelial cell proliferation, migration. Concomitantly, most of the growth factors render endothelial cells less sensitive for apoptosis induction. Among the first growth factors identified to improve angiogenesis were members of the vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) families. Some of the growth factors may enhance tissue regeneration not solely via the proangiogenic activity but also via promotion of stem/progenitor cell mobilization. VEGF, angiopoietin-1 and erythropoietin mobilized endothelial progenitors from the bone marrow [34].

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