OPEN ACCESS
Saudi Journal of Pathology and Microbiology
Abbreviated Key Title: Saudi J Pathol Microbiol
ISSN 2518-3362 (Print) |ISSN 2518-3370 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Review Article

Mechanism of Action and Clinical Significance of Angiogenesis: A Review

Dr. Deepa K.K[1*], Dr. Anubhav Jannu[2], Dr. Mithun Kulambi[3]

[1]Assistant Professor, Department of Oral Pathology Subbaiah Institute of Medical and Dental Sciences, Shimoga-577004, India
[2]Associate Professor, Department of Oral and Maxillofacial Surgery Subbaiah Institute of Medical and Dental Sciences, Shimoga-577004, India
[3]Associate Professor, Department of Oral and Maxillofacial Pathology Subbaiah Institute of Medical and Dental Sciences, Shimoga-577004, India

DOI: 10.36348/sjpm.2021.v06i06.004 | **Received:** 16.04.2021 | **Accepted:** 03.06.2021 | **Published:** 08.06.2021

***Corresponding author:** Dr. Deepa K.K

Abstract

Angiogenesis is a complex, sophisticated process, subjected to many different conditions. A number of basic mechanisms of angiogenesis have been deciphered and several targets for therapeutic intervention have been identified. In recent decades, numerous pro and antiangiogenic molecules, as well as their ligands and intracellular signaling pathways, have been identified for treating numerous diseases. For the past decades angiogenesis has been a field under extensive investigation. Tumours depend on the growth of a vascular network, which is stimulated by a variety of angiogenic mediators, providing them with blood and oxygen. Inhibition of these factors and its pathways, there by reducing the growth of blood vessels was major breakthrough in treatment of cancer. Even though anti-angiogenic therapy has gained a lot of progression in the past decade, combination of conventional methods like chemotherapy and radiotherapy along with the antiangiogenic therapy would be more beneficial to the patient. This is a review article to understand the mechanism of action and clinical significance of angiogenesis.

Keywords: Vasculogenesis, Angiogenesis, Neovascularization, bEGF, VEGF, Angiogenic Switch, Sprouting, Intussusceptive, Lymphangiogenesis, Phalanx, Chemokines.

INTRODUCTION

Our cells need oxygen and nutrients to survive. The cells of the small organisms fullfil their needs from the environment. Nutrients and gases pass from areas of high concentration to low concentration through cell membrance of the cells. But in larger animals including human beings need extensive vascular system for nutrition supply and for the excretion of the waste products where simple diffusion doesn't work. The vascular system can adapt to the body's changing needs by changing the amount of blood it delivers to the tissues. Blood vessels constitute the first organ in the embryo and form the largest network in our body. The formation of new blood vessels is a fundamental process that occurs during embryonic and post-natal development and also in a number of pathologies ranging from chronic inflammatory disease to cancer. Blood vessels can be generated by either the process of angiogenesis or vasculogenesis. Vasculogenesis involves the de novo differentiation of endothelial cells from mesoderm-derived precursor cells, called angioblasts, which assemble into a primary capillary plexus. Whereas, Angiogenesis is a process of "formation of blood vessels from the preexisting blood vessels". Vasculogenesis is differentiated by angiogenesis, where new blood vessels arise from sprouting, branching and intussusceptive growth from pre-existing capillaries. The other terms used for formation of blood vessels include arteriogenesis (which is the formation of larger diameter arteries from preexisting capillaries or arterio-arteriolar anastomoses), neovascularization (a general term used to suggest any new vessel formation, of any size, in the adult), capillogenesis (universal formation of new capillaries, during development and in the adult, without any implications regarding possible underlying mechanisms, processes). In adults new blood vessel formation (angiogenesis) is involved in many physiological and pathological situations, such as wound healing, tissue remodeling and regeneration, the female reproductive cycle and ischemia, rheumatoid arthritis and tumor neovascularization. The pioneering work by Folkman and his colleagues through series of experiments observed and confirmed that, under normal conditions, a tissue or tumor cannot grow beyond 1 to 2

mm in diameter without neovascularization. The growth and metastatic spread of tumor cells require oxygen and nutrients; this demand can be fulfilled by angiogenesis. Identification of angiogenesis as a major player in tumor growth and metastasis has made the field of angiogenesis a major focus of research. Angiogenesis is a complex, sophisticated process, subjected to many different conditions. A number of basic mechanisms of angiogenesis have been deciphered and several targets for therapeutic intervention have been identified. In recent decades, numerous pro- and antiangiogenic molecules, as well as their ligands and intracellular signaling pathways, have been identified for treating numerous diseases. This is a review article to understand the mechanism of action and the clinical significance of angiogenesis.

Mechanism of angiogenesis

In the embryo, angiogenesis establishes the primary vascular tree as well as an adequate vasculature for growing and developing organs. In adults transient blood vessels are formed by angiogenesis in ovarian cycle and in physiological repair processes such as wound healing [8]. Process of angiogenesis is regulated by balance between certain molecules called as angiogenic activators or pro-angiogenic factors and angiogenic inhibitors or anti-angiogenic factors. Pro-angiogenic factors are the ones which encourage the growth of new blood vessels, example: vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bEGF), epidermal growth factor (EGF). And anti angiogenic factors are the ones which prevents the growth of blood vessels, example: angiostatin, endostatin, thrombospodins. In physiological conditions this regulation is well balanced.

Physiological process of angiogenesis involves following steps.

1. Endothelial cell activation: This occurs in response to angiogenic factors like, VEGF, bFGF- which are induced by hypoxia/ischemia which in turn is mediated by hypoxia inducing factor (HIF).
2. Degradation of the capillary wall by extracellular proteinases: this is mediated by MMPs (matrix metalloproteinases) and urokinases which causes degradation of capillary wall.
3. Formation of branch point in the vessel walls: degradation of extracellular matrix increases the concentration of growth factors. Beginning of proliferation and migration of endothelial cells which is mediated by intrgrins.
4. Migration and proliferation of endothelial cells: towards the angiogenic stimulus, this occurs with elongation of sprouts, which is mediated by integrins.
5. Re-organization of endothelial cells: to form tubules with lumen formation, mediated by angiopointins
6. Maturation and stabilization: to complete the maturation, a vascular basement membrane is deposited and periendothelial cells are recruited to stabilize the vessels by inhibiting endothelial cell proliferation and migration (figure 1).

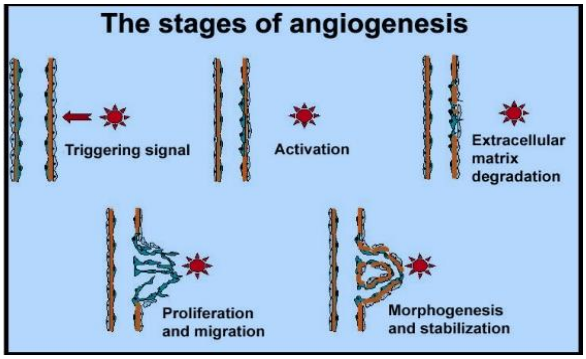

Fig-1: Stages of Angiogenesis

In normal or physiological angiogenesis once the process of maturation and stabilization of blood vessels is over, the concentration of angiogenic factors will be reduced and concentration of angiogenic inhibitors like thrombospondin 1, prolactin, angiostatin, platelet factor 4 are increased. The intact pericyte-endothelial associations prevent vessels from regressing and aberrant remodeling. Additionally, activated transforming growth factor-β (TGF-β) produced in co-cultures with endothelial cells and pericytes inhibits further proliferation of endothelial cells and stabilizes the nascent vessels. The above factors maintain the vasculature in equilibrium [14].

Any disturbance in the balance between pro-angiogenic and antiangiogenic factors may result in too little or too much of angiogenesis which in turn leads to either excessive or insufficient blood supply resulting in numerous disease conditions (FIGURE 2).

Diseases related to insufficient angiogenesis	Diseases related to excessive angiogenesis
• Alzheimer's disease • Amyotrophic lateral sclerosis (ALS or Lou Gehrig's disease) • Diabetes • Atherosclerosis • Hypertension • Crohn's disease • Lupus • Preeclampsia • Nephropathy • Chronic wounds • Coronary artery disease • Diabetic ulcers	• Cancer • Multiple sclerosis • Vascular malformations • Obesity • Psoriasis • Warts • Allergic dermatitis • Kaposi's sarcoma in AIDS • Diabetic retinopathy • Primary pulmonary hypertension • Asthma • Cystic fibrosis • Inflammatory bowel disease • Periodontal disease • Liver cirrhosis • Endometriosis • Ovarian cysts • Uterine bleeding • Arthritis • Osteomyelitis • Diabetic nephropathy

Fig-2: Diseases of insufficient and excessive angiogenesis

Alteration in this balance leads to tumor neovascularization - referred to as **"angiogenic switch"**. Coined in the late eighties, the term "angiogenic switch" refers to a time-restricted event during tumor progression where the balance between pro- and anti-angiogenic factors tilts towards a pro-angiogenic outcome, resulting in the transition from dormant avascularized hyperplasia to outgrowing vascularized tumor and eventually to malignant tumor progression [12]. Induction of the angiogenic switch depends on how heavily that balance tips is in favour of pro- angiogenesis. Angiogenic switch is controlled by changes in the fine-tuned balance between pro- and anti-angiogenic factors secreted either by tumor cells or by cells of the tumor microenvironment. Pro-angiogenic gene expression is increased by physiological stimuli, such as hypoxia, which results from increased tissue mass, and also by oncogene activation or tumour-suppressor mutation. The angiogenic switch can occur at different stages of the tumour- progression pathway, depending on the tumour type and the environment. It is the major step in tumor growth and development [13] (figure 3).

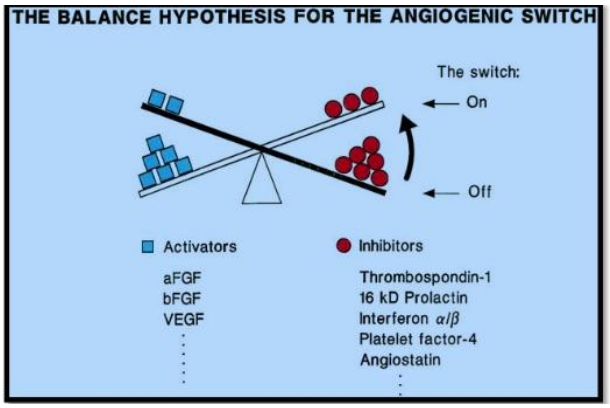

Fig-3: Angiogenic Switch Angiogenesis has been enormously studied in the field of cancer - its development, growth and metastasis

In vertebrates, angiogenesis is accomplished by

1. Sprouting angiogenesis or endothelial sprouting
2. Non sprouting (intussusceptive) microvascular growth [15]. Several other mechanisms of neovascularization have been identified in tumours, including intussusceptive and sprouting which are: The recruitment of endothelial progenitor cells, Vessel cooption and Vasculogenic mimicry [16].

Another field called as lymphangiogenesis (development of lymphatic vessels) which has been emerged in recent years and has become a part of angiogenesis [2].

1. Sprouting angiogenesis or endothelial sprouting

Sprouting angiogenesis is the basic mechanism seen in the formation of new blood vessels. It was the first identified form of angiogenesis nearly 200 years ago. The first description of sprouting angiogenesis in tumor growth was reported by Ausprunk and Folkman in 1977 [15].

It is the growth of new capillary vessels out of the preexisting ones.It involves the formation of sprouts composed by endothelial cells which grow towards proangiogenic factors [16]. This type of angiogenesis is seen in physiological conditions like embryonic development, wound healing and reproduction. It also plays an important role in much pathology, like diabetes, rheumatoid arthritis, cardiovascular ischemic complications, and cancer. In cancer, it is not only involved in angiogenesis but also in metastasis [16].

Sprouting angiogenesis is initiated in hypoxic conditions which demand the formation of new blood vessels. The cells respond to hypoxic conditions by releasing the pro-angiogenic factors.

Ausprunk and Folkman identified the stages of sprouting angiogenesis as [15].
Stage I - Degradation of basement membrane
Stage II – Migration of endothelial cells
Stage III – Formation of solid cord of endothelial cells
Stage IV – Lumen formation
Stage V – Network formatiom
Stage VI – Remodelling
Stage VII – Pruning
Stage VIII – Maturation and Stabilization

STAGE I: Degradation of the basement membrane

As sprouting angiogenesis is an invasive process, there will be degradation of basement membrane mediated by proteolytic factors like metrixmetallo proteinases and urokinases, which are released in response to stimulations such as hypoxia and proangiogenic factors.

MMPs, in particular MMP-2, MMP-9 and MT1-MMP, have been recognized as crucial regulators for angiogenesis. MMP activities are controlled by a group of endogenous inhibitors known as TIMPs (Tissue Inhibitors of MetalloProteinases) and by RECK (REversion-inducing Cysteine-rich protein with Kazal motifs) [17].

STAGE II: Migration of endothelial cells

These angiogenic factors stimulate the receptors on the endothelial cells, and interendothelial contacts are weakened and endothelial cells migrate into the connective tissue. Migration of endothelial cells into the connective tissue forms two types of cells called as tip and stalk cells.

Formation of tip and stalk cells:

Gerhardt et al. gave the concept of tip and stalk cell phenotypes of endothelial cells. The endothelial cell which is exposed to angiogenic factors forms into tip and stalk cells bearing different morphologies and functional properties.

Endothelial tip cells primarily migrate and minimally proliferate, in contrast endothelial stalk cells, proliferate. Tip cell is migratory and polarized, while stalk cell proliferates.

Tip cells have cytoplasmic processes called filopodia which secrete proteolytic enzymes which degrade ECM and guides the endothelial cells through ECM towards the angiogenic factors. Tip cells expresses high levels of DII-4(delta like 4), PDGF-b, unc-5 homolog, VEGF receptor 2 and 3 and have low levels of notch signaling activity.

Delta-Notch signaling is a key component of sprout formation. It is a cell–cell signaling system in which the ligand, Dll4 mates with its notch receptor on neighboring cells [15].

Both the receptor and ligand is cell bound and thus act only through cell–cell contact. VEGF-A induces Dll4 production by tip cells, which leads to activation of notch receptors in stalk cells. Notch receptor activation suppresses VEGFR2 production in stalk cells, which dampens migratory behavior compared with that of tip cells. Hence, endothelial cells exposed to the highest VEGF-A concentration are most likely to become tip cells. Although tip cells are exposed to the highest VEGF-A concentration, their rate of proliferation is far less compared with that of stalk cells [15] (figure-4).

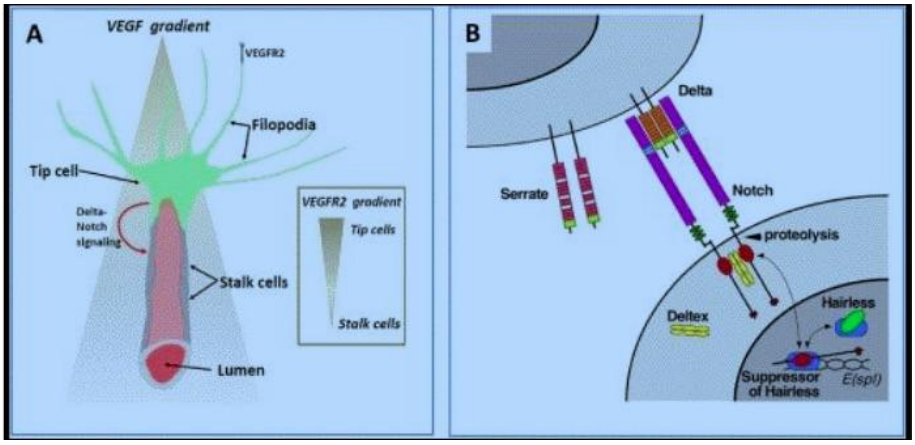

Fig-4: Formation of Tip & Stalk Cells

Recent studies have shown similarities in the molecular regulation in guidance of neural and endothelial cells. Specialized endothelial cells, which resemble axonal growth cones, are located at the tips of growing capillaries. These tip cells extend and retract their filopodia continuously to explore the environment and to define the direction in which a new vascular sprout grows. Both neuronal cells and endothelial cells have Molecular ligand/receptor signalling systems including the family of Ephrins, Semaphorins, Slits, Netrins and Notchs [16]. This specialization of endothelial cells as tip and stalk cells is transient and reversible which depends on proangiogenic factors and the factors which suppresses the endothelial cell proliferation. Regulated balance between both processes establishes adequate shaped nascent sprouts. During the transition from active sprouting to quiescence endothelial cells, tip cell adopts another phenotype called as “phalanx”, these are lumenized, non-proliferating, and immobile cells, which promotes vessel integrity and stabilizes the vasculature through increased cell adhesion and dampened response to VEGF [15].

STAGE III: Formation of solid cord of endothelial cells

After overcoming the ECM and loosening the endothelial cell-cell and cell-matrix adhesions, the endothelial cellsproliferate and migrate to the angiogenic stimulus. Factors such as VEGF, bFGF, Ang(angiopoietins), and chemokines including monocyte chemotactic protein-1 (MCP-1) are involved in this process [17].

STAGE IV: Lumen formation

Endothelial cells that migrate to the ECM subsequently assemble as tubular structures whose diameter is regulated by interplay between VEGF and Ang1. Furthermore, endothelial cells can fuse with other existing vessels to form new ones and develop numerous cell-cell junctions.

Two different ways of lumen formation have been discussed: cord hollowing and cell hollowing. In cord hollowing slit like lumen formation takes place between facing endothelial cells. In the latter there will be a coalescence of intracytoplasmic vescicles resulting in formation of lumen.

During the final stages endothelial cell migration comes to a halt and endothelial cells form a lumen and re-establish functional adherens junctions. This process was shown to be mediated by vascular endothelial cell cadherin (VE-cadherin).

During lumen formation, two functionally different phenotypes of endothelial cells are recognized. The first phenotype, represented by endothelial cells in mature blood vessels, which is characterized by an apico-basal polarity and junction-mediated contact inhibition.

The second phenotype is found in activated tissues and is characterized by the loss of apico-basal polarity and adherents junctions, and have spindle-shaped morphology, and the ability for guided migration.

Formation of apical cell surfaces and electrostatic repulsion of negatively charged apical glycoproteins are sufficient for the initial de-adhesion of adjacent endothelial cells and slit formation. After the lumen formation, lumen diameter increases. Lumen expansion depends on force, hydrodynamic stimuli and F-actin cytoskeleton, fibronectin and/or blood flow [17].

STAGE V: Network formation

Network formation represents a crucial step in the angiogenic process which provides the growing tissue with a newly constituted apparatus of immature and rudimentary vascular channels, upon which acts the fine-tuning process of vascular remodeling.

STAGE VI: Remodelling

The endothelial plexus formed after network formation consists of homogenous web of endothelial cell tubes and sacs. Remodeling involves the growth of new vessels and the regression of others as well as changes in the diameter of vessel lumens and vascular wall thickening, establishment of directional flow [17].

STAGE VII: Pruning.

Pruning means reduction in the extent of (something) by removing superfluous or unwanted parts. Ashton, in 1966 for the first described removal of excess of endothelial cells which form redundant channels. Pruning and remodeling of the vascular network may be stimulated by tissue-derived signaling molecules and blood flow conditions. Vascular pruning accompanying natural remodeling is caused by hyperoxia. Exposure to hyperoxia leads to excessive regression of capillaries, while arteries become refractory to this insult [17].

STAGE VIII: Maturation and stabilization

The stabilization of the newly formed vessel and the maintenance of the existing vasculature are late events in the angiogenic process.

Pericyte adhesion to native capillaries and endothelial cell wrapping by surrounding pericytes are basic events in blood vessel stabilization and maturation.

Several cellular and non-cellular components in the blood vessel, which includes endothelial cells, pericytes, smooth muscle cells, fibroblasts, glial cells, inflammatory cells, and the extracellular matrix, coordinately regulate the maintenance of vessel integrity.

Paku and Paweletz in 1991 integrated the findings of Ausprunk and Folkman as follows

1. Structural alteration in the basement membrane characterized by loss of electron density of the dilated mother vessels, followed by partial degradation of basement membrane.
2. Migration of endothelial cells which gets arranged in parallel, maintaining their basal luminal polarity by forming a slit like lumen and sealed by intact interendothelial junction.
3. Continuous deposition of basement membrane by polarized endothelial cells.
4. Proliferation of pericytes which migrate along the basement membrane of the capillary bud resulting in complete coverage of the new vessels [15].

2. Intussuceptive angiogenesis

Variant of angiogenesis other than sprouting is intussuceptive angiogenesis. It is also called as splitting angiogenesis as it causes the single vessel to split forming two vessels [19].

It is a more recent form of angiogenesis.It was first observed in postnatal remodelling of capillaries in the lung [16]. After studying the microvasculature of rabbit lungs in the light microscope, Short proposed in 1950 that capillary network growth could occur by formation of new meshes. But this study went unnoticed.

In 1986 Caduff et al. put forward the same idea by investigating the postnatal maturation of rat lung microvasculature in cats by scanning electron microscopy; they observed the appearance of tiny pillars in capillary network. They proposed that the lung capillary network expanded by insertion of slender transcapillary tissue pillars and coined the term "intussusceptional [20]". Burri et al. named this concept as intussusceptive microvascular growth (IMG).

In 1993, the first in vivo intussusceptive microvascular growth was demonstrated by video microscopy in a chick chorioallantoic membrane [16]. Intussuceptive angiogenesis takes place within hours or even minutes as it does not involve proliferation of endothelial cells. Endothelial cells get remodeled by increasing in volume and becoming thinner. This type of angiogenesis occurs throughout life [16].

This type of angiogenesis has been detected in wound healing, in various organs and in tumor. At the growing edge of the tumor both sprouting and intusscetive angiogenesis have been identified.

In addition to forming new capillary structures, intussusceptive growth plays a major role in the formation of artery and vein bifurcations as well as pruning of larger microvessels [19].

Caduff et al. in their study explained the 4 consecutive steps in intussusceptive angiogenesis, depending on the outcomes or phenotypes accomplished at the end of the processes as follows:
Phase I: Creation of a zone of contact between opposite capillary walls
Phase II: Reorganization of the intercellular junctions of the endothelium, with central perforation of the endothelial bilayer.
Phase III: Formation of an interstitial pillar core.
Phase IV: Growth of the slender pillar to a capillary mess.
Phase V: Creation of a zone of contact between opposite capillary walls (formation of a transcapillary interendothelial bridge).

This contact is achieved by the walls protruding into the vessel lumen until contact is made. The contact zone marks the formation of an interendothelial transluminal bridge of approximately 1µm in diameter. There will be formation of dense spots on the cell membrane which is interendothelial adhesive spot. Burrie et al consider this as a initial step in formation of pillar.

PHASE II: Reorganization of the intercellular junctions of the endothelium, with central perforation of the endothelial bilayer

The perforation of the endothelial bilayer points to the beginning of the next steps of pillar formation. They are characterized by the creation of a cylindrical tissue bridge extending across the lumen covered by endothelial cells. In the core of the cylinder, the presence of elements of the interstitial tissue, mainly of cytoplasmic extensions of myofibroblasts with their microfilaments is a classic feature in phase II [20].

PHASE III: Formation of an interstitial pillar core

Cytoplasmic processes of pericytes and myofibroblasts,interstitial fibers frames the pillar alongside the lateral portions of the capillary walls. These pericytes are often seen to cover the interendothelial junctions, which are running around the pillar girdle.

PHASE IV: Growth of the slender pillar to a capillary mesh.

The pillars of this phase show the typical structure of normal intercapillary meshes except that their diameter is smaller than 2.5µm. The core contains all the elements present in phase 3 plus collagen fibrils. The pillar undergoes a series of transformation to end up in a mature mesh. But the reason behind the initiation of this event still remains in dark. Djonov et al. 2002 proposed that hydrodynamic forces may play role in initiating the process (figure-5).

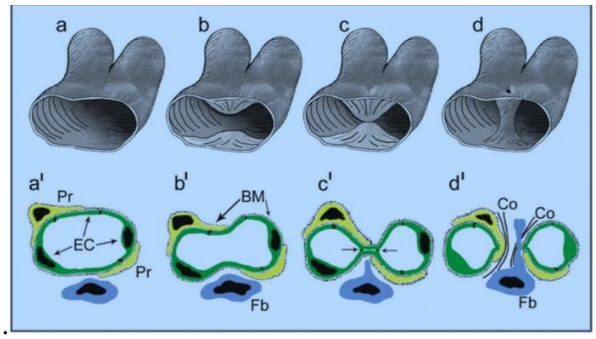

Fig-5: Process of intussuceptive angiogenesis

Patan et al. hypothesized that regulation of pillar formation may be due to changes in intravascular blood flow or changes in shear stress. Experiments on chick chorioallontoic membrane have shown that alteration in blood flow dynamics in arterial branches can induce intussussceptive angiogenesis. This shows that there are several key players that influence pillar formation [21]. Endothelial cells, pericytes, macrophages and blood cells plays role in intussusceptive angiogenesis but interaction between endothelial cells and endothelial cells, endothelial cells and pericytes intercations plays major role. There are several factors like angiopointein, Tie receptors, PDGF B, monocyte chemotactic protein I, ephrins and ephBreceptors which mediates intussusscetive angiogenesis [21].

OTHER MODES OF ANGIOGENESIS

1. Endothelial progenitor cells
2. Vessels co-option
3. Vascular mimcry
4. Lymphangiogenesis

Table-1: Summary of vessel formation in normal and tumor tissue

Modes of vessel formation in normal and tumor tissues			
		Normal tissue	**Tumor tissue**
Vasculogenesis	In developing mammalian embryo angioblasts differentiate into endothelial cells assembling into vascular labyrinth	✓	✓
Angiogenesis	Endothelial sprouting ,development of new blood vessels following the proliferation of the endothelial cells of preexisting vessels	✓	✓
Arteriogenesis	Endothelial cell channels become covered by pericytes or vascular smooth muscle cells	✓	✓
Intussuception	Preexisting vessels split into daughter vessels.	✓	✓
Vessel co option	Tumor cells hijack the existing vasculature. Tumor cell migration along the vessels of the host organ	-	✓
Vascular mimicry	Tumor cells form tubular structures themselves	-	✓
Cancer stem like cells diffrentiate into ecs	Endothelial cells derived from putative cancer stem cells	-	✓

Clinical significance of angiogenesis

Cancer, today is one of the leading severe human disease that claim many lives across the globe. Different countries around the world are in the grip of different types of cancer. Modern methods of treating cancer revolve around cytotoxicity and cell induced necrosis of the cancer cells [22]. For most solid tumours, surgery remains the most effective primary treatment. Despite apparently curative resection, significant numbers of patients develop secondary disease due to growth of undetected micrometastases [23]. These methods have without any doubt have provided effective results when viewed with respect to the cytotoxicity effect but when the effect of the drug on overall systemic regulation of human body is considered these drug tend to have side effect upon the non-targeted system and different organs, hence the targeted drug delivery system has become a leading base of research and cancer therapeutics in recent years.Treatment for the angiogenesis targeted therapy involve many potential methods such as mesenchymal stem cells, Hybrid Liposomes (HL), angio-inhibitors and hematopoietic and endothelial progenitor cells cell-based therapies[22].

Too little angiogenesis or excessive angiogenesis both leads to several diseases. Proangiogenic therapy with angiogenic agents has been recommended in ischemic heart failure, cerebral attacks with thrombosis and some degenerative diseases, such as neuronal degeneration in order to improve vascularity. The pivotal role of angiogenesis in primary tumour growth and metastasis has been recognized for many years, although the mechanisms which control it are incompletely understood. Growth of a tumour beyond 2–3 mm requires development of a microvessel network to facilitate delivery of nutrients and oxygen, and removal of catabolites [22, 23].

Studies on mechanisms regulating angiogenesis in metastatic disease is increasing. Acquisition of a blood supply by micrometastases at the site of implantation is crucial to tumour growth. Tumour cells detaches from the primary tumour to initiate the "metastatic cascade". Angiogenesis is a necessary precursor of metastasis as new proliferating capillaries have incomplete basement membranes and are "leaky", facilitating penetrance by tumour cells. These tumour cells evade immune surveillance in the circulation and also to the site of secondary development, where they cross endothelium, degrade basement membrane, implant, proliferate and establish their own capillary network. Angiogenesis is necessary at both the beginning and the end of the metastatic cascade.

The proliferating capillary endothelial cell offers a unique target for antiangiogenic therapy as antiangiogenic strategies may reduce both the recurrence rate and the metastatic potential of solid tumours [1, 5, 2].

As discussed in this review neovascularization is promoted by a number of proangiogenic factors of tumour and stromal origin. VEGF has been considered a key factor because of its potency and specificity for angiogenic effect, VEGF is the most widely studied angiogenic factor for its clinical significance. Many studies have been done related to clinical significance of VEGF. Recent studies have assessed the prognostic significance of newer members of the VEGF family such as VEGF-C and VEGF-D, both of which are involved in lymphangiogenesis in addition to angiogenesis. Other angiogenic factors which have been studied are bFGF, interferons, cytokines, platelet derived growth factor. All the these factors works by either of the methods (1) interfere with receptor binding or activation of a particular angiogenic factor, (2) inhibit the release of a particular angiogenic factor by tumour cells, (3) enhance the production or action of an

angiogenic inhibitor (4), be similar or identical to a particular angiogenic inhibitor (5), interfere with signal transduction processes or autocrine intracrine activation of capillary endothelial cells, or (6) inhibit the matrix degradation by protease or matrix degrading enzymes[24].

The clinical impression that resection of a primary tumour heralds a phase of increased metastatic growth is of particular interest, but until recently no explanation of this phenomenon exists. Recent studies have shown that the intact primary tumour can regulate growth of associated metastases, either directly or indirectly, through the production of certain antiangiogenic factors, notably angiostatin. Surgical excision of a primary tumour removes the source of the inhibitory angiostatin and other factors, allowing angiogenesis and subsequent growth of previously dormant micrometastases.

Long-term suppression of angiogenesis may become a therapeutic option for induction of long term remission by maintaining micrometastases in a state of dormancy, a dynamic equilibrium during which there is no net tumour growth.

The aim of antiangiogenic treatment will be to reduce and maintain tumours as small relatively dormant clusters of cells which have low metastatic potential, are more susceptible to cell- mediated immunological attack, and which may be more vulnerable to chemotherapy and radiotherapy. Inhibition of angiogenesis results predominantly in a cytostatic, not a cytotoxic, effect, although a recent study suggests that antiangiogenic therapy may lead to tumour regression [25].

Successful antiangiogenic strategies have incorporated a wide range of antiangiogenic agents. Prolonged courses of interferons which induce regression of human haemangiomatous disease, systemic administration of angiostatin has shown to induce regression of primary murine tumours of breast, colon and prostate. Recombinant endostatin inhibits angiogenesis, the growth of metastases and the growth of primary tumours. Angiostatin and endostatin are the direct inhibitors of angiogenesis identified to date, and both have been shown to reduce tumour growth. Systemic administration of endostatin, which directly inhibits endothelial cell mitogenesis, has been suggested as an ideal "dormancy therapy". More recently, it has been reported that repeated cycles of endostatin can prevent tumour recurrence. Indirectly acting antiangiogenic therapies influence the tumor microenvironment which regulates tumour angiogenesis and so indirectly influence endothelial cell behaviour. The majority of antiangiogenic agents currently identified belong to this group.

Angiogenesis has been identified in numerous oral lesions extending fron pryogenic granuloma to cancer, as discussed in this review. Antiangiogenic therapy plays an major role in treating these lesions. Antiangiogenic therapy along with curettage has been recommended as novel method for treatment of giant cell lesions of the jaws [26]. The same authors have recommended enucleation of aggressive giant cell lesions and adjuvant treatment with interferon α placed subcutaneously as an excellent strategy for treatment of these lesions [27].

Many studies have the importance of angiogenesis in OLP pathogenesis it has been strongly linked to angiogenesis. Anti-angiogenic drugs, such as bevacizumab, might be introduced as an alternative treatment for contraindicated, non-responsive patients. A marked decrease in vascular endothelial growth factor (VEGF) and interleukin-8 immunoexpression have been noticed in tissue biopsies from bevacizumab-treated lesions when compared with control lesions. Intralesional injection of bevacizumab has been shown too effectively and safely resolved the atrophic/erosive OLP lesions [27].

Micro-RNA (miRNA) and related therapeutic approaches hold great promise in the field of cancer management. Anti-miRNA is miRNA-221 and miRNA-222 which block the angiogenesis process by their regulatory effect on stem cell factor receptor c-kit.

Human papilloma virus (HPV) 16 has been detected in 70% of oropharyngeal cancers. It has been reported that HPV-16 E6 positive cells express high levels of VEGF. E6 oncoprotein upregulates the promoter activity of VEGF in a P53 independent manner, thus suggesting direct stimulation of VEGF gene. These results show that HPV-associated OSCC could respond differently to antiangiogenic therapy. Hence, in future it is very important to consider "HPV-associated OSCC" as a separate entity while studying antiangiogenic therapy. A phenomenon called cellular cannibalism has been identified in OSCC, which is related to nutritional supply to the cancer cells. The nutrition is believed to be mainly received from the blood vessels that grow surrounding the tumour. Hence, OSCC showing increased cannibalistic activity responding to the antiangiogenic therapy would be of great interest to study in future [28].

Multimodality therapy, in which antiangiogenic strategies are combined with chemotherapeutic agents or radiotherapy, is known to be more effective than monotherapy, probably because of the combined effects on both the tumour and vascular components. But optimal methods and timing of administration of antiangiogenic therapies have yet to be determined. Developments in the field of gene therapy may be harnessed to produce long-term low-

grade antiangiogenic substances which maintain micrometastases in a state of dormancy.

Each tumour has a unique complex microenvironment dictated both by tumour histology and site of implantation. Additionally, within any tumour, heterogeneity arises because of variations in stromal density, tumour differentiation, hypoxia and local cytokines, among other factors. As a result, each tumour is exposed to different proangiogenic and antiangiogenic stimuli which ultimately influence net angiogenesis. The dosing schedule for biological antiangiogenic agents is likely to differ significantly from that for pharmaceutical agents.

Inhibition of angiogenesis is the most promising new therapy in the treatment of malignancy. Complete understanding of the factors regulating angiogenesis and the interactions between these factors should lead to valuable new interventions. These may be combined with surgery and other modalities to improve the treatment of many solid tumours. The advantages of anti- angiogenic therapy over standard chemotherapy are its low cytoxicity and drug resistance. The combination of an anti-angiogenic agent, with classical cytotoxic therapy can enhance the effect of either drug alone.

Eventhough antiangiogenic therapy has been recommended as a gold standard method for treating now a day has shown many side effects. Eventhough antiangiogenic therapies are well tolerated compared to traditional cytotoxic chemotherapy agents, asa they are more selective in their cellular effects. The most frequently reported toxicities with sunitinib were diarrhea, hypertension, fatigue, and nausea, while for sorafenib they were diarrhea, skin toxicities, and alopecia1. BV (Bevacizumab) is most often associated with hypertension and proteinuria (excess protein in the urine), followed by mild thrombotic and bleeding events. Other notable but infrequent side effects with BV therapy include serious bleeding, arterial thromboembolism, wound healing complications, GI perforation, and nephrotic syndrome [29].

Careful monitoring and management of patients on antiangiogenic therapy, utilizing a multi-modality approach and closer collaboration between oncologists and specialists, will help mitigate many of these adverse effects and improve quality of care [30].

CONCLUSION

For the past decades angiogenesis has been a field under extensive investigation. Tumours depend on the growth of a vascular network, which is stimulated by a variety of angiogenic mediators, providing them with blood and oxygen. Inhibition of these factors and its pathways, thereby reducing the growth of blood vessels was major breakthrough in treatment of cancer. Even though anti-angiogenic therapy has gained a lot of progression in the past decade, combination of conventional methods like chemotherapy and radiotherapy along with the antiangiogenic therapy would be more beneficial to the patient.

It is now clear that tumour vasculature is not necessarily dependent of endothelial cell proliferation and sprouting of new capillaries. Several additional mechanisms like vascular mimicry, vessel co-option can also provide the tumour with oxygen and nutrients. The current knowledge that antiangiogenesis therapy work best in combination with chemotherapy should be extended to other types of vascularization as well. There is still a long way to fully understand the different mechanisms of tumor vascularization. But the combination of a multimodal anti-vascular approach, representing anti-angiogenesis, anti-lymphangiogenesis and vasculogenic mimicry targeting, together with chemotherapy may become the best possible strategy in the fight against cancer.

REFERENCES

1. Houtman, J. J. Lifes's blood angiogenesis in health and diseases. FASEB J. Welblog: https://www.faseb.org/portals/2/PDFs/Angiogenesis%20 Article.pdf.
2. Bikfalvi, A. (2006). Angiogenesis: health and disease. Annals of oncology, 17(10): 65-70.
3. Costa, C., Soares, R., Schmitt, F. (2004). Angiogenesis: now and then. APMIS, 112(7-8):402-12.
4. Słotwińska, S.M. (2012). Angiogenesis and oral diseases. Centr Eur J Immunol, 37(2):164-166.
5. De Mendonca, R.J. Angiogenesis in wound healing. Department of biological science. Federal university of Triangulo Minciro, Brazil. Weblog. Available from: https://cdn.intechopen.com/pdfs-wm/34634.pdf.
6. Steiner, R. (1992). Angiogenesis--historical perspective. EXS, 61:1-3.
7. Adiar, T.H., Montani, J.P., Rafae, l.S. Overveiw of angiogenesis. Morgan and Claypool Life Sxiences.Weblog.Avialablefrom:https://www.ncbi.nlm.nih.gov/books/NBK13515/.
8. Papetti, M., Herman, I.M. (2002). Mechanisms of normal and tumor-derived angiogenesis. Am J Physiol Cell Physiol, 282(5):C947-70.
9. http://www.mrcophth.com/MRCOphth/mrcophthessay2002/octquestion1.html.
10. http://woundcareresource.com/basic.html
11. https://www.cellworks.co.uk/angiogenesis.php.
12. Baeriswyl, V., Christofori, G. (2009). The angiogenic switch in carcinogenesis. Semin Cancer Biol, 19(5):329-37.
13. Bergers, G., Benjamin, L.E. (2003). Tumorigenesis and the angiogenic switch. Nat Rev Cancer, 3(6):401-10.

14. Hanahan, D., Folkman, J. (1996). Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell, 6(3):353-64.
15. Ribatti, D., Crivellato, E. (2012). 'Sprouting angiogenesis', a reappraisal. Dev Biol. Dec 15; 372(2):157-65.
16. Hillen, F., & Griffioen, A. W. (2007). Tumour vascularization: sprouting angiogenesis and beyond. Cancer and Metastasis Reviews, 26(3), 489-502.
17. Van Hinsbergh, V. W., & Koolwijk, P. (2008). Endothelial sprouting and angiogenesis: matrix metalloproteinases in the lead. Cardiovascular research, 78(2), 203-212.
18. Adiar, T.H., Montani, J.P., Rafae, l. S. Overveiw of angiogenesis. Morgan and Claypool Life Sciences. https://www.ncbi.nlm.nih.gov/books/NBK13515./
19. Burri, P. H., Hlushchuk, R., & Djonov, V. (2004). Intussusceptive angiogenesis: its emergence, its characteristics, and its significance. Developmental dynamics: an official publication of the American Association of Anatomists, 231(3), 474-488.
20. Schlatter, P., König, M. F., Karlsson, L. M., & Burri, P. H. (1997). Quantitative study of intussusceptive capillary growth in the chorioallantoic membrane (CAM) of the chicken embryo. Microvascular research, 54(1), 65-73.
21. Kovacic, J. C., Moore, J., Herbert, A., Ma, D., Boehm, M., & Graham, R. M. (2008). Endothelial progenitor cells, angioblasts, and angiogenesis--old terms reconsidered from a current perspective. Trends in cardiovascular medicine, 18(2), 45-51.
22. Russels, C. Endothelial cells, angiogenesis and vasculogenesis. Stem cell technology. https://www.stemcell.com/media/files/minireview/MR29012.
23. Rahul, J. Significance of angiogenesis in both survival and extermination of the tumor. Journal of Medical and Health Sciences.
24. McNamara, D.A., HArmey, J.H., Walsh, T.N., Redmond, H.P., Bouchier-Hayes, D.J. (1998). Significance of angiogenesis in cancer therapy. Br J Surg, 85(8):1044-55.
25. Craft, P.S., Harris, A.L. (1994). Clinical prognostic significance of tumour angiogenesis. Ann Oncol, 5(4):305-11.
26. Bikfalvi, A. (1995). Significance of angiogenesis in tumour progression and metastasis. Eur J Cancer, 31A:1101-1104.
27. Kaban, L. B., Troulis, M. J., Ebb, D., August, M., Hornicek, F. J., & Dodson, T. B. (2002). Antiangiogenic therapy with interferon alpha for giant cell lesions of the jaws. Journal of Oral and Maxillofacial Surgery, 60(10), 1103-1111.
28. Kaban, L. B., Troulis, M. J., Wilkinson, M. J., Ebb, D., & Dodson, T. B. (2007). Adjuvant antiangiogenic therapy for giant cell tumors of the jaws. Journal of Oral and Maxillofacial Surgery, 65(10), 2018-2024.
29. Mahmoud, M. M., & Afifi, M. M. (2016). Anti-angiogenic therapy (bevacizumab) in the management of oral lichen planus. European journal of oral sciences, 124(2), 119-126.
30. Sarode, G.S., Sarode, S.C., Patil, S. (2016). Antiangiogenic Therapy in Oral Cancer: A Thoughtful Consideration. World J Dent, 7(2):51-53.
31. Lacouture, M.E., Lenihan, D.J., & Quaggin, S.E. (2017). Antiangiogenic Therapy: Tolerability and Management of Side Effects. Angiogenesis foundation. Weblog. available from: http://www.angio.org/pdf/Angio_Poster_Final_6-30.pdf (accessed 10th April 2017).