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# A REVIEW ON ANGIOGENESIS

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## ABSTRACT

Angiogenesis is the extension of blood vessels from the pre existed vasculature. It is an important process which involves the formation and development of blood vessels, so it is supportive in healing of wound while its inhibition may help to restrict the size of tumor. The angiogenesis activity was involved in many physiological and pathological conditions. When this balance is disturbed, the result is either too much or too little angiogenesis. The body controls angiogenesis by producing a precise balance of growth and inhibitory factors in healthy tissues. Excessive angiogenesis occurs in diseases such as cancer, diabetic blindness, age-related macular degeneration, rheumatoid arthritis, psoriasis and for these Anti angiogenic therapies are aimed to halt new blood vessel growth. Insufficient angiogenesis occurs in diseases such as coronary artery disease, stroke, and chronic wounds. Therapeutic angiogenesis aimed to stimulate new blood vessel growth with growth factors is being developed to treat these conditions. Angiogenic gene therapy is also being developed as a method to deliver angiogenic growth factors to the heart, limbs, and wounds. Currently there is no angiogenic gene therapy and drugs approved by FDA for the treatment of ischemic cardiovascular disease. But FDA approved device is used to stimulate NBV to grow in diseased hearts is a laser used in a technique called Direct Myocardial Revascularization.

**KEYWORDS:** Angiogenesis, physiological and pathological conditions.

## INTRUDUCTION

Angiogenesis is the extension of blood vessels from the preexisted vessels. It is an essential process in the formation and development of new blood vessels, so it is supportive in healing of wound while its inhibition may be helpful to restrict the size of tumor. Angiogenesis is a key process which involves in physiological and pathological functions. The physiological

functions include wound healing, repairing, growth and reproduction. The pathological functions like cancer, diabetic ulcer and other deadly diseases also(Vinoth Prabhu V & Seraj Kuma N, 2010).

Angiogenesis can be mediated via two distinct pathways. They are splitting and sprouting. Splitting may cusses by the excess micro vascular shear stress which leads to the intra luminal splitting of a micro vessel linear into two vessels, but tissue hypoxia triggers sprouting angiogenesis and budding of a new capillary from preexisting vessel.

Angiogenesis play a role in spreading of cancer. To that, blood supply is necessary tumors to grow beyond a few millimeters in size. Tumors can cause this blood supply to form by giving off chemical signals that stimulate angiogenesis. Tumors can also stimulate nearby normal cells to produce angiogenesis signaling molecules. The resulting new blood vessels feed growing tumors with oxygen and nutrients, allowing the cancer cells to invade nearby tissue, to move throughout the body, and to form new colonies of cancer cells, called metastases. This total process is called Tumor –angiogenesis.

**Types of Angiogenesis**

Sprouting angiogenesis and intussusceptive angiogenesis both occur in uterus and in adults. Sprouting angiogenesis was discovered nearly 200 years ago and intussusceptive angiogenesis was discovered by Burri about two decades ago. Sprouting angiogenesis has sprouts composed of endothelial cells which grow towards an angiogenic stimulus such as VEGF-A. Sprouting angiogenesis can therefore add blood vessels to portions of tissues previously devoid of blood vessels. On the other hand, intussusceptive angiogenesis helps in formation of blood vessels by a splitting process in which elements of interstitial tissues invade existing vessels, forming transvascular tissue pillars that expand. Both types of angiogenesis occur in all tissues and organs (Adair & Montani, 2010).

**Sprouting Angiogenesis**

Sprouting angiogenesis was the first identified form of angiogenesis. The process of sprouting angiogenesis involves several sequential steps. Angiogenesis starts with the activation of endothelial cells by specific growth factors like vascular endothelial growth factors (VEGF) and basic fibroblast growth factor (bFGF) that bind to their receptors. As a result, the extracellular matrix (ECM) and basement membrane, surrounding the endothelial cells, are degraded locally by activated proteolytic enzymes. This allows the endothelial cells

to invade into the surrounding matrix and, subsequently, to proliferate and migrate through the matrix. By polarization of the migrating endothelial cells a lumen is created, and an immature blood vessel is formed. The stabilisation of the immature vessels is established by recruitment of mural cells and generation of extracellular matrix. The capillaries get matured by deposition of pericytes and ECM.

**Intussusceptive Angiogenesis**

When the blood vessel walls penetrates upto the lumen inside making the vessel split into two, irrespective of the diameter is called Intussusceptive or Splitting angiogenesis. It is less time consuming as it forms from pre-existing endothelial cells. It is very crucial in embryonic vasculation where growth is fast and resources are less. It is also seen in postnatal lungs of rats and humans choroid of the eye, around glands, intestinal mucosa, kidney, ovary, and uterus skeletal muscle, heart, brain, artery and vein (Hillen & Griffioen, 2007).

**Ion Channels**

Ion channel are protein expressed by virtually all living cells that creates a pathway for charged ions from dissolved salts which includes sodium, potassium, calcium, and chloride ions and other. These proteins are typically composed of at least two different domainsa. They are ransmembrane domain which includes the ion pore, and an extracellular domain which includes the ligand binding location. To pass through the lipid cell membrane like cells in the nervous system, contraction of the heart and of skeletal muscle, and secretion in the pancreas. These are examples of physiological processes that require ion channels. In addition, ion channels in the membranes of intracellular organelles are important for regulating cytoplasmic calcium concentration and acidification of specific subcellular compartments. (James Huettner).

**Ion channel effect – mechanism on angiogenesis**

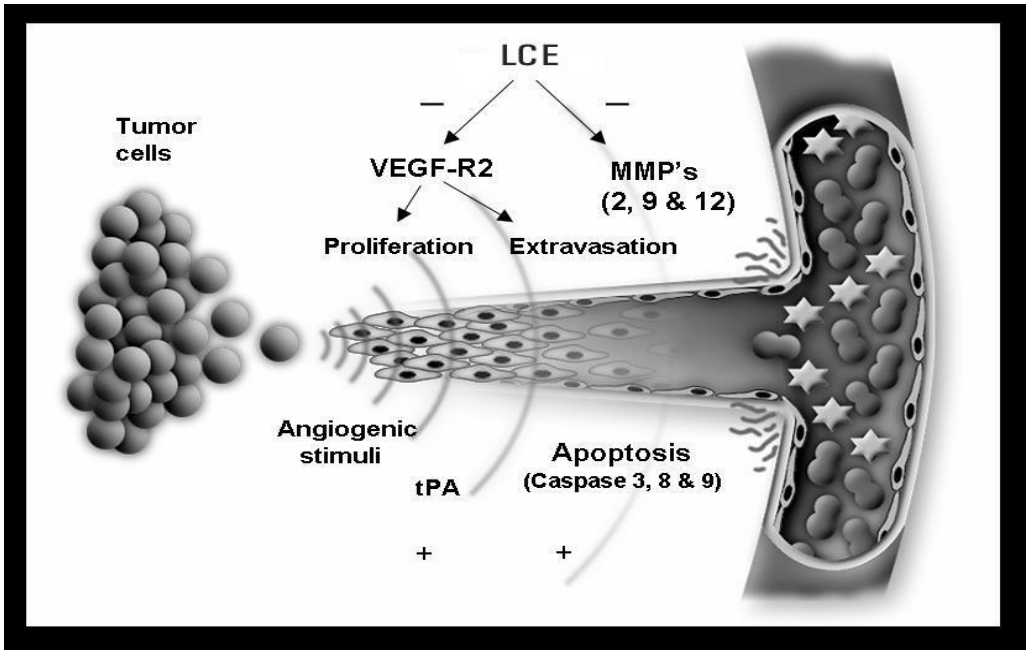
**Mechasim of Angiogenesis**

Angiogenesis is a complex process, which involves between cell soluble factors and extra cellular matrix (ECM) components.

The angiogenesis shows different sequential steps which includes as follows:

- The Release of Proteases From Activatedendothelial Cells.
- Degradation of The Basement Membrane Surrounding The Existing Vessel.
- Migration of The Endothelial Cells Into The Interstitial Space.

- Endothelial Cell Proliferation and Lumen Formation.
- Generation of New Basement Membrane with The Recruitment Of Pericytes.
- Fusion of The Newly Formed Vessels And Initiation of Blood Flow.




Angigenesis is a proliferative type vasculatur e formation with exicted once.For that the adult vasculature is derived from a network of blood vessels that is initially created in the embryo by vasculogenesis. vasculogenesis is a process whereby vessels are formed *de novo* from endothelial cell precursors termed angioblasts.During vasculogenesis, angioblasts proliferate and coalesce into a primitive network of vessels known as the primary capillary plexus.The endothelial cell lattice created by vasculogenesis then serves as a scaffold for angiogenesis.

After the primary capillary plexus is formed, it is remodeled by the sprouting and branching of new vessels from pre-existing ones in the process of angiogenesis. Angiogenesis occurs in the adult during the ovarian cycle and in physiological repair processes such as wound healing.

Endothelial cell basement membrane and extracellular matrix is then degraded and remodeled by specific proteases such as matrix metalloproteinases and new matrix synthesized by stromal cells is then laid down. This new matrix, coupled with soluble growth factors, fosters the migration and proliferation of endothelial cells. After sufficient endothelial cell division has occurred endothelial cells arrest in a monolayer and form a tube-like structure. Blood flow is then established in the new vessel.

Under normal circumstances, angiogenesis is a highly ordered process under tight regulation because it requires inducing quiescent endothelial cells in a monolayer to divide and spread the vascular network only to the extent demanded by the demands of growing tissues. Which can influenced by many positively and negatively acting factors.

**Ion Channel Effect on Cell Invasion**

In glioma cells the secretion of cytoplasm appears to be driven by efflux of Cl− from the cell. The efflux may occur when the Glioma cells may accumulate Cl− above their electrochemical gradient and the opening of Cl− channels which leads to the efflux of Cl− either inhibition of Cl− efflux or replacement of Cl−with impairment anions and impairs the cell invasion.

**Ion Channel Effect on Cell Migration:**

Cell migration is important tumor metastatic. The oscillating activity of Ca sencitive k channel may plays a major role in migration as a part of pre-requisite.

Endo/exo cytotic recycling of plasma membrane with exo cytosis membarane vesicles taking plase at the leading edge of lamellipodium.The intercate interactions of cytoskeleton components exerts force on cytoskeleton-associated adhesion molecules. In this process the lipid flow rate may visuvalize the components of cytoskeleton during migration (Xi Huang & Lily Yeh , 2014).

**Ion Channel Effect on Cell Proliferation**

Cytosolic Ca+2 activity plays a major role in the regulation of cell Proliferation. The growth factors can stimulate Ca+2 release through activated Calcium channel which mediates Calcium entry, thus triggering may causes Ca+2 release from intracellular stores yielding oscillations of cytosolic Ca+2 activity. This type of activity may causes several functions like depolymerization of acting filaments. The depolymerization results in disinhibition of Na/H exchanger and/or Na,K, 2Cl_ co transporter. These type of ions may accumulate and osmotically obliged water and thus increase cell volume which causes the stimulation of calcium. These type of stimulations are pre-requisites for proliferation.

Anion channels may be activated during cell proliferation and also the Cell proliferation may regulated by several potassium channel (k+) by indirect action on apoptosis. Where as the

inhibition of K channels may participates in the stimulation of apoptosis . (T Bose *et al*, 2015)

**Ion Channel Effect on Apoptosis**

Apoptosis is stimulated by activation of K channels and the inhibition of apoptosis may increases by extracellular k concentration or K channel blockage. The level of Cellular loss of K favors apoptosis in a wide variety of cells. The activation of K channels hyperpolarizes the cell membrane thus increases the electrical driving force for Cl exit. Depends upon the Cl_ channel activity the K channel activity leads to cellular loss of KCl with osmotically obliged water which may causes the cell shrinkage and finally undergoes apoptosis.

**Angiogenic factors**

Angiogenesis is subject to a complex control system with proangiogenic and antiangiogenic factors. In adults, angiogenesis is tightly controlled by this "angiogenic balance", i.e., a physiological balance between the stimulatory and inhibitory signals for blood vessel growth. In normal circumstances, the formation of new blood vessels occurs during wound healing, organ regeneration, and in the female reproductive system during ovulation, menstruation, and the formation of the placenta. It is also an important factor in several pathological processes such as tumor growth, rheumatoid arthritis,diabetic retinopathy, and psoriasis. A switch to the angiogenic phenotype depends on a local change in the balance between angiogenic stimulators and inhibitors.

**Vascular Endothelial Growth factors (VEGF)**

VEGF is the most potent angiogenic factors. **VEGF** family including VEGF-A, VEGF-B, VEGF-C, VEGF-D, and VEGF-E. VEGF-A subfamily shows 6 isoforms:

- VEGF-A121
- VEGF-A145
- VEGF-A165
- VEGF-A183
- VEGF-A189
- VEGF-A206.

These are different in their properties and functions(27). VEGF-A has 3 receptors.they are:

- VEGFR-1 (FLT-1)
- VEGRR-2 (FLK-1/KDR)
- VEGFR-3 (FLK-4).

VEGFR-1 has the highest affinity for binding on VEGF and PLGF (placental growth factor), VEGFR-2 binds to VEGF-A.VEGF-C and VEGF-D both bind to VEGRR-2 and VEGFR-3. The effects of VEGF on proliferation and migration are carried out by VEGFR-2. VEGF-A plays a major role in the initiation of vasculogenesis, sprouting angiogenesis and Immature vessels formation. (Ann Hoeben et al, 2004).

VEGF-A is a 34- to 42-kDa, dimeric, disulfide-bound glycoprotein. In normal tissues, the highest levels of VEGF-A mRNA are found in adult lung, kidney, heart,and adrenal gland. Lower, but still readily detectable,quantities of VEGF-A transcript levels occur in liver,spleen, and gastric mucosa. VEGF-A exists in at least seven homodimeric isoforms. The monomers consist of 121, 145, 148, 165, 183, 189, or 206 amino acids. The primary VEGF-A transcript derives from a single VEGF-A gene, coding for eight exons (Poltorak & Neufeld, 1999).

**Angiopoietins**

Angiopoietins are paracrine growth factors which are ligands for tyrosine kinase receptor Tie2.and the Interaction of angiopoietins with Tie-2 on endothelial cells is necessary for maturation and stabilization of the developing vasculature.

Angiopoietin-2 is an antagonist of angiopoitin-1 that can block angiopoietin-1- induced autophosphorylation of Tie-2 in endothelial cells.

**EGF and TGF-α**

EGF (epidermal growth factor) and TGF-α (transforming growth factor-α) are mitogenic for endothelial cells and increase. Both are binds to the EGF receptor (32). EGF receptor signaling demonstrated in cancer cells cause cell proliferation, angiogenesis, Metastasis and decreased apoptosis.

TGF-β promotes VSMC differentiation and deficiency of ENG or ALK-1 impairs mural cell development.

**FGF**

There are 20 distinct FGF (Fibroblast growth factors) and four different types of tyrosine kinase receptors.

FGF-1 (acidic FGF) and FGF-2 (basic FGF) are the primary growth factors for angiogenesis. FGF-1 and FGF-2 have the binding capacity to four FGF receptors and are mitogenic for endothelial cells and fibroblasts.

**Matrix Metalloproteinase (MMP)**

MMP's are plays a major role in degradation of proteins that keep the vessel walls solid. This proteolysis allows the endothelial cells to escape into the interstitial matrix. Inhibition of MMPs prevents the formation of new capillaries. These enzymes are highly regulated during the vessel formation process because destruction of the extracellular matrix would decrease the integrity of the microvasculature. (Verma RP & Corwin H, 2007).

**Anti-angiogenic factors**

**Angiostatin**

Angiostatin is derived from plasminogen from the first 4 kringle domains. Angiostatin can have capacity to suppresses the proliferation, migration, differentiation, and tube formation of endothelial cells and also inhibits angiogenesis. Systemic.

**Endostatin**

Endostatin is derivative of collagen XVIII. It is a enzymatic degradative product which can suppresses migration, proliferation and apoptosis .

**Thrombospondin(TSP)**

The TSPS (thrombospondins) are a family of glycosylated extracellular matrix proteins (42). TSP family includes TSP-1 and TSP-2. TSP-1 can prevent in vivo neovascularization in the cornea pocket assay and inhibits in vitro migration and proliferation of endothelial cells and also blocks tube formation. TSP-2 can have ability to inhibit the tumor growth which significantly stronger than TSP-1.

**Qunatification Of Angiogenesis**

| *In vivo evaluation methods* | *In vitro evaluation methods* | | *In ova evaluation methods* |
|---|---|---|---|
| | *Cell culture assays* | *Organ culture assays* | |
| Sponge implantation assay | Cord formation assay | The aortic ring assay | Chicken chorioallantoic membrane (CAM) assay |
| Matrigel plug assay | Tube formation assay | The chick aortic arch assay | |
| Corneal angiogenesis assay | Cell migration assay | | |
| Dorsal air sack model | Cell proliferation assay | | |
| | Gelatin Zymography | | |

**In Vivo Methods**

**Sponge Implantation Method**

- Sponge implantation method is mainly is used to evaluation of angogenic and anti-angiogenic effects.This method can be done by using forgein substances which are involved in inflamation leads to angiogenesis.
- Rat (wt: > 200g) are anesthetized with an i.p. injection of 5-10mg/kg of xylazine and 80-90mg/kg of ketamine (duration of anastesia - approximately 40 minutes).
- Tramadol (5mg/kg) used in the surgert time as an analgesic.
- The depilatory is washed away with warm running water and betadine/alcohol swabbing is used to disinfect the surgical area.
- In this method sterile sponges should be made and inserted into first layer of skin when the rat become unresponsive to toe-pinch.
- The dosing should be given next day of surgery which is a 13 days study.
- After the dosing compleation 14 day animal groups are sacrificed and the sponges should be dried and weight should be noted.
- Then finally histological examination shoud me made for the evaluation of angogenic and anti-angiogenic agents.

**Corneal Neovascularisation Assay**

- Neovascularization is the growth of the vascular system which plays a major role in both
- health and diseases
- In this corneal Neovascularization model, rats are anasthetised by an intramuscular injection of ketamine (40 mg/kg) and xylazine (8 mg/kg) for cauterization.
- Cauterization. should be done by a stick (end diameter 2.5 mm) which is coated with silver nitrate (75%) and potassium nitrate (25%,) was applied to the central cornea of the right eye of each rat for 10 seconds.
- Theis may causes a chemical burn which is not more than 2 × 2mm.the exes chemical burn should be removed and the measurement of eye burn with calipers.
- The eyes were rinsed immediately with 5 mL of saline solution.
- After the next day of burning,dosing should be done which is a 13days study.
- Then the dosing should be completed the animals are sacrificed and histology should be done.

**Matrigel Plug Angiogenesis Assay**

- The mechanism involved in this midel is injection of forgein substamces leads to stimulation of inflammatory cells.
- Matrigel is a gelatinous material derived from mouse tumor cells like a T175 which contains 80-90% confluent monolayer of tumor cells and washes the cells with phosphate buffered saline (PBS).
- To the matrigel the both pro- angiogenic and anti-angiogenic agents are also added and combined solution is injected into the subcutaneous space of an animal.
- Then the injected sample may causes the formation of the single solid gel plug will allows cell growth and blood vessel formation.
- After inoculation for 7 days, excise the matrigel will be fix with formalin overnight and the samples should be made section onto slides.
- Stain the slides for histological observation.

**In Vitro Methods**

**Rat Aortic Ring Assay method**

- This method is widely used in vitro method for the evaluation of angiogenic and antiangiogenic drugs.

- Healthy male wistar rat was selected and sacrificed, thoracic cavity was opened and the visceral organs were separated. Thoracic aorta was identified and isolated by cutting both the ends. Immediately it was transferred to cold PBS supplied with aeration.
- Fibroadipose tissue was isolated, the proximal and distal 2mm segments of the aorta were cut away. Aorta were cut into 1mm ring sections and washed with PBS.
- These rings were placed in the 24 well plates with 150□l of Matrigel. Rings were overloaded with matrigel. Rings were allowed to polymerize for 1-2 hours at 37$^{o}$ C and then exposed to hypoxic conditions for 2 hours.
- This hypoxic condition stimulates the formation of sprouts from the rings. This was reoxygenated for 7 days and the abundance of blood vessels was quantified.

**Hindlimb Ischemia Model**

- In this Hindlimb Ischemic model, rats were anasthetised by an intramuscular (IM) injection of ketamine (40 mg/kg) and xylazine (8 mg/kg) for surgery.
- Rat was place in the supine position over a draped heated pad on the operating table.Wipe the skin with three alternating Povidine Iodine and alcohol scrubs. Then use a dissection microscope at 10X or 20X magnification to obtain an better view of the hindlimb region
- By using fine forceps and surgical scissors make an incision of the skin approximately 1 cm long from the knee towards the medial thigh.
- phosphate buffered saline (PBS) used to moistened fine pointed cotton swabs and clear the subcutaneous fat tissue surrounding the thigh muscle.
- Apply the cautery transversely to incise and dissect through the subcutaneous fat tissue to reveal the underlying femoral artery and Use a retractor to open the wound and to obtain a better view of the lower extremity vasculature.
- Using fine forceps and a fine pointed cotton swab, gently pierce through the membranous femoral sheath to expose the neurovascular bundle.
- By using set of fine forceps and cotton swab, dissect and separate the femoral artery from the femoral vein and nerve at the proximal location near the groin.
- After the dissection pass a strand of 7-0 silk suture underneath the proximal end of the femoral artery. Occlude the proximal femoral artery using double knots. Place the tie on the vessel as proximal in the wound as possible in order to leave length for the second tie and an intervening segment that will be transected.
- Separate the femoral artery from the femoral vein at the distal location and Occlude the distal femoral artery with a second set of double knots just proximal to the first set of

knots.This second set of sutures will be used for gripping the artery during the transaction procedure.

- Transect the segment of femoral artery between the distal and proximal knots with a fine pointed cotton swab and a pair of spring scissors.The incision using 5-0 Vicryl sutures and allow the animal to resting position.

**In Ovo Method**

**Chick Chorioallantoic Membrane (CAM) Assay**

- CAM assays have been widely used to study angiogenesis which helps to study cell invasion and metastasis. The CAM model greatly promotes the efficiency of tumor cell grafting, high reproducibility and cost effective.
- Fertilized white leghorn chicken eggs were collected from a local hatchery at day'0' and checked for the damage.
- They are grouped randomly into 7 groups each containing 6 eggs. The eggs were cleaned with 70 % ethanol and incubated under condition of constant humidity at $37^0$C. On the 3rd day of incubation a small hole was drilled at the narrow end and 2-3 ml of albumin was withdrawn with 18 gauge hypodermic needle.
- The window was sealed with transparent tape and again incubated. On the 7th day of incubation a small square window was opened in the shell and sterile gel foam (3mm×3mm×1mm) piece was implanted on top of the membrane.
- The vehicle control group was impregnated with sterile normal saline; the tests groups were impregnated with various doses of test drugs.
- The eggs were returned to the incubator and they were incubated undisturbed till day 14. On 14th day of incubation the eggs were removed from the incubator and the CAM tissues directly beneath each sponge was resected from control and treated CAM samples.
- Tissues were placed in 10 % formalin and stained with hematoxylin and eosin and examined under trinocular microscope at 4X magnification.
- The number of vessel branch points contained in the square region equal to the area of each sponge was counted and findings from CAM preparations were analyzed for each treatment group.

**Evaluation Parameters**

**Procedure for determining Hemoglobin content**

The sponges after they were removed from the body of the rats were soaked in double distilled water and homogenized completely over ice platform for 5min and then the liquid obtained was centrifuged at 10,000 rpm in cooling centrifuge for 5 minutes and the supernatant liquid obtained was put in the cell count machine and the hemoglobin content was estimated as g/Dl.(Mahtab Bahramsoltani et al, 2009).

**Procedure for determining Number of blood vessels formed per sponge**

The sponges were bisected and fixed in formal saline at 4°C for 1 h and then immersed in 75% ethanol for 30 min and finally kept in 90% ethanol. Paraffin sections (10 pm) were prepared and stained with Hematoxylin and Eosin. The prepared slides were then observed under trinocular microscope at magnification of 400X and the circular spaces amidst the fibroblast growth regions present were counted as they represent the blood vessels formed in the sponges.

**Hemoglobin Determination in Matrigel Plugs**

In this method, Matrigel should dissected and homozinized after weighing.The homozinization kept for 5-10 min ice at 10,000rpm on centrifuge for 6min.

The obtained supernent is mixed with Drabkin's reagent and the hemoglobin content in sample should be quantified at 540nm in spectrophotometer.

**Histology Examination**

Rats were sacrificed at day 14 after surgery. Eyes were enucleated and immersed in 10% neutral-buffered formalin. Paraffin sections were stained with hematoxylin and eosin.

The corneas were excised and prepared for light microscopy and transmission electron microscopy. For light microscopy, approximately 1µm semithin sections were stained with with hematoxylin and eosin and eyes were examined on a Philips (Eindhoven, Netherlands) electron microscope.

**Procedure For Determine Corneal Neovascularisation Area**

Quantification of CNV Area Rats were examined under microscope on 1, 3, 5, and 7th day after catherisation of cornea. Both the length and clocks of CNV were measured.

Area of CNV On day 1 after operation, the limbal vessels were dilated, with no angiogenesis appeared. On day 3, angiogenesis began to invade pericornea with a brush shape, the area of CNV was low. On day 5, new vessels reached the lower margin, and the area of CNV was Mideum. On day 7, new vessels continued to elongate, parts of them extended as loops and the area of CNV was High. After $7^{th}$ day the CNV area was gradually decreased and finally shows maximum of cure should obtained at13th day (Wei Zhao et al, 2009).

**Procedure For Determine Blood Vessel On Cornea**:

Eyes with corneal Catherisation were examined at 4-day intervals with a stereomicroscope at 25 X and 40x. Maximum vessel length was measured during each observation at 40x with an ocular micrometer. The number of blood vessels and presence of corneal edema were also recorded. Serial diagrams of the vascular reactions were made, and photographs were taken to document major changes.

**Various Antiangiogenic drugs Approved for Clinical use**

Angiogenesis has become an attractive target for drug therapy due to its key role in tumor growth. An extensive array of compounds is currently in pre-clinical development, with many now entering the clinic and/or achieving FDA approval. Several regulatory and signaling molecules governing angiogenesis are of interest, including growth factors (e.g. VEGF, PDGF, FGF, EGF), receptor tyrosine kinases, transcription factors such as HIF, as well as molecules involved in MAPK and PI3K signaling. Pharmacologic agents have been identified that target these pathways, yet for some agents (notably thalidomide), an understanding of the specific mechanisms of antitumoraction has proved elusive. The following review describes key molecular mechanisms and novel therapies that are on the horizon for anti-angiogenic tumor therapy.

| Drug | Target | Company | Indication |
|---|---|---|---|
| Avastin (Bevacizumab) | VEGF | Genentech | mCRC,NSCLC,Advanced breast cancer |
| Erbitux (Cetuximab) | EGFR | Imclone | mCRC & Head and Neck cancer |
| Vectibix (Panitumumab) | EGFR | Amgen | mCRC |
| Lucentis (Ranibizumab) | VEGF | Genentech | Wet Age-related macular regeneration |
| Macugen (Pegaptanib) | VEGF | OSIPharmaceuticals | Wet Age-related macular regeneration |
| Endostar(Endostatin) | Angiogenesis | Shangdong Simcere | Lung cancer |

| | | | |
|---|---|---|---|
| | inhibitor | Medgen | |
| Sorafenib (Nexavar) | VEGFR, PDGFR &c-kit | Bayer AG/Onyx | Advanced RCC |
| Sunitinib (Sutent) | PDGFR & VEGFR | Pfizer | Advanced RCC & GIST |
| Dasatinib (Sprycel) | Bcr-Abl & Src | Bristol-Myers Squibb | Gleevec-resistant CML or Ph+ ALL |

**Clinical Applications**

Pathological angiogenesis is a hallmark of cancer and various ischaemic and inflammatory diseases.Concentrated efforts in this area of research are leading to the discovery of a growing number of pro- and anti-angiogenic molecules, some of which are already in clinical trials. The complex interactions among these molecules and how they affect vascular structure and function in different environments are now beginning to be elucidated. This integrated understanding is leading to the development of a number of exciting and bold approaches to treat cancer and other diseases. But owing to several unanswered questions, caution is needed.

**Angiogenesis and Inflammation**

Inflammatory proteins are closely linked to angiogenesis (Krupinski *et al.*, 2008). Pro-infl ammatory cytokines such as tumor necrosis factor-alpha (TNF-a), interleukin- 1 beta (IL-1b), IL-6, IL-8 and monocyte chemo attractant protein (MCP)-1 strongly promote chronic inflammation of rheumatoid arthritis (RA) and inflammation-related angiogenesis. Specifially, RA is characterized by endothelial cell proliferation and angiogenesis, leukocyte activation (Roobina et al., 2013).

**Peripheral Vascular Disease**

Despite major advances in both surgical and percutaneous revascularization techniques, therapeutic options for patients with peripheral vascular disease of the lower limbs are limited. Conventional drug therapy is of no benefit. When vascular obstruction is extensive, percutaneous

Revascularization may not be feasible. Surgical therapy is complicated by a variable morbidity and mortality and is dependent on long-term graft patency. Therapeutic angiogenesis is another way to affect revascularization but is more difficult in older patients with endothelial dysfunction secondary to diabetes or hyperlipidemia (Rissanen et al., 2001).

**Hypoxia**

Hypoxia is a strong stimulus for angiogenesis in numerous diseases. Cells in tumours, wounds or atherosclerotic plaques become hypoxic when too distant from nearby vessels. The abnormal deposition of extracellular matrix or vascular congestion impairs delivery of oxygen and causes hypoxia in diabetes, Alzheimer's disease and asthma. The supply of oxygen can also become limited by vascular pruning during hypertension or upon exposure of premature babies to high oxygen.

Recent discoveries have shown that hypoxia activates hypoxiainducible transcription factors (HIFs), which function as master switches to induce expression of several angiogenic factors including VEGF, nitric oxide synthase (NOS), platelet-derived growth factor (PDGF), Ang2 and others. Hypoxia-driven angiogenesis salvages ischaemic myocardium and prolongs survival of stroke patients. However, it can cause blindness in premature newborns and in diabetic patients53, and haemorrhagic rupture of atherosclerotic plaques. Apart from stimulating angiogenesis, hypoxia can also cause vascular remodelling. In chronic obstructive lung disease, hypoxia causes irreversible loss of vessels and thickening of the vascular muscular coat, with resultant life-threatening pulmonary hypertension. This vascular remodelling has been ascribed to an imbalance between vasodilators (nitric oxide) and vasoconstrictors (endothelin-1).

**Angiogenesis and Diabetic Retinopathy**

Proliferative diabetic retinopathy is a vision-related complication of diabetes mellitus caused by excessive angiogenesis (Yadav et al., 2012).

**Arteriosclerosis**

The deficiency of functional blood vessels in cardioand cerebrovascular syndromes contributes to a variety of ischemic symptomswhich includes angina, intermittent claudication and loss of mental function in transient ischemic attacks. Poor perfusion in diabetes is also the leading cause of diabetic limb amputation. A pathological type of arteriogenesis is the 20-fold enlargement of preexisting collateral arterioles after occlusion of a supply artery. As a result of the increased collateral flow, endothelial cells express monokines and monocyte adhesion molecules (such as intracellular adhesion molecule-1) The recruited using proteinases and death factors (TNF-a). Activated endothelial cells then upregulate bFGF, PDGF-B and TGFb-1, thereby inducing the re-growth of smooth muscle cells and vessel enlargement.

It was recognized early on the AIDS epidemic, that the chronic infection with HIV results in unique vascular disorders and later development of vascular tumors i.e. Kaposi sarcoma. However, the pathomechanism of this complication of HIV infection was not known for a long time. HIV-TAT is the transactivating protein of the AIDS virus responsible for the viral transcription. Meanwhile this protein has several biological activites based on the multimodular nature of the protein: RGD, heparin-binding domain and chemokine-like sequences. Therefore, TAT is able to induce proliferation and migration of endothelial as well as transformed endothelial cells (Kaposi), induces uPA expression but only in concert with other inflammatory cytokines such as IL-1, TNFa, IFNg.1 Interestingly, heparin and heparin-like carbohydrates enhance the angiogenic potential of TAT. Accordingly, one can consider TAT as a viral heparin-binding growth factor which is further supported by the observation, that TAT binds the VEGFR, KDR. Meanwhile TAT is not a viral oncogene, since it is unable to transform endothelial cells: this is achieved by transforming effect of HSV8 virus in Kaposi sarcoma.(Agnes JANOVICS 2001).

**Tumor-Induced Angiogenesis**

The genetic background of the "angiogenic switch during tumor progression is not fully understood, but recent discoveries of the main angiogenic factors, VEGF, bFGF, PDGF, suggest that the switch is able to turn on the expression of the genes of these factors in tumors. Later studies identified several anti-angiogenic factors as well, suggesting that the "angiogenic switch" might also control the expression of these factors. Tumor-induced neoangiogenesis therefore means the predominance of pro-angiogenic- over antiangiogenic machineries in cancer. It is now accepted that cancer cells may use physiological pathways to turn on the pro-angiogenic genotype.10,11 In this case hypoxic tumor cells in the growing tumor tissue, which express wt-HIF1 gene, activate the expression of HRE-containing angiogenic factor genes including the main angiogenic mitogen, VEGF. Amplification of several oncogenes in cancers also leads to the overexpression of pro-angiogenic factors. It is a characteristic alteration in a considerable proportion ofhuman cancers that wtP53 expression is lost. An angiogenic consequence of this fact is that such cells would not be able to express thrombospondin, one of the most significant physiological angiogenesis inhibitors.( Jozsef Timar, 2001).

## CONCLUSION

The past decade has led to major advances in the understanding the molecular pathways involved in angiogenesis. This basic research has led to the identification of new targets associated with angiogenesis, leading to the development of an extensive number of pre-clinical anti-angiogenesis agents. Ongoing studies of different approaches are evaluating some of the molecular targets and agents, with some even in clinical trials, and data regarding efficacy and safety is currently emerging.

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