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TUMOR ANGIOGENESIS – AN UPDATED REVIEW

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Abstract:

Angiogenesis is a fundamental process of formation of new blood vessels. This highly synchronized process occurs during human development, reproduction, wound repair and is also a fundamental pathogenic process in cancer and several other diseases. The inhibition of angiogenesis has been a subject of comprehensive research for years. Several studies have found reasonable improvement in considering angiogenesis inhibitors and its success in its use as a traditional form of therapy. This review summarizes several important angiogenic factors, the mechanism of angiogenesis at molecular level and various drugs used for the treatment. The aim of this review was to discuss the various factors involved in angiogenesis so as it can be applied in the battle against cancer and other angiogenic-related diseases.

Keywords : Angiogenesis, anti-angiogenic therapy, cancer.

Introduction

Cancer cell progresses through a series of mutations which is accompanied by activation of certain specific genes and loss of suppressor genes which makes the tumor cells independent in growth signals, insensible to anti growth signals and indifferent to apoptotic signals. This make tumor cell capable of limitless replicative potential and also tumorigenic. But the question arises whether these neoplastic properties are necessary and sufficient enough for a cell to expand into a population which becomes clinically detectable, symptomatic or lethal. The answer to this is that these neoplastic processes may only be necessary but not sufficient enough for the cancer to become metastatic and lethal. The literature review suggests that it is the microvascular endothelial cell which dictates to a cancer cell that it can grow into a tumor and comes to a size which becomes clinically detectable, can kill the host or can metastasize to distant organs. This implies that for any tumor to metastasize or to develop into a lethal phenotype, it must first employ and then sustain its own private blood supply which is called as Tumor angiogenesis. Tumors which cannot induce angiogenesis remain quiescent at a microscopic in situ size and such non-angiogenic cells produce lesions which are usually non-detectable and so are called "No takes".[1-5]

Concepts of Tumor Angiogenesis

The previous concepts of tumor angiogenesis involved simple dilatation of existing blood vessels and an inflammatory reaction which is more precisely a side effect of tumor growth. But it was Sir Judah Folkmann who proposed a visionary hypothesis stating that the most primary solid tumors are those which undergo a prolonged state of avascularity and apparently latent

growth in which the maximum size attainable is only 1-2 mm in diameter. Up to this size, the tumor cells obtain the necessary oxygen and nutrient supplies which they require for growth and survival and this is achieved by simple passive diffusion. These microscopic tumor masses in due course toggle on angiogenesis by conscripting surrounding mature host blood vessels to commence sprouting new blood vessel capillaries which nurture toward and ultimately permeate the tumor mass, thus providing the potential for unrelenting expansion of the tumor mass and also hematogenous metastatic spread. He also proposed that the angiogenic switch was initially hypothesized to set off by the ectopic production and elaboration of a growth factor called "tumor angiogenesis factor" (TAF) by the tumor cells. Lastly it ought to be possible to affect tumor growth by jamming tumor angiogenesis by somehow preventing TAF production. This kind of therapeutic approach can be successful in curative sense also.[4, 5]

Phases of tumor angiogenesis

The process includes two major phases; namely activation phase and formation phase. After the formation of capillaries, maturation and stabilization of blood vessels takes place by pericyte recruitment, vessel sprouting and vessel stabilization. It adapts the same molecular mechanism in both physiological and pathological angiogenesis. The decisive role is of the mesenchymal cells because it releases Angiopoetin -1 which binds to Tie-2 receptors expressed on endothelial cells directing them to recruit pericytes and stabilize. These Tie receptors are tyrosine kinases whose expression follows VEGFR expression. The ligand for Tie receptor is Angiopoetin 1 which upon binding to Tie 2 induces tyrosine phosphorylation which tends to activate Angiopoetin and inducing not only sprout formation but also recruitment of pericytes and stabilizing vessels. These molecules are responsible for supporting angiogenesis. [4, 5]

Characteristics of Tumor blood vessels

The capillaries are characterized by an irregular diameter and are dilated. The endothelial cells are overlapped with each other and are organized in a chaotic way. These vessels present an abnormal branching pattern which lead to abluminal sprouts. They lack smooth muscle coat because the pericytes are absent or detached. These blood vessels have very weak interconnections and focal intercellular openings between the endothelial cells and scanning electron microscopy reveals that the openings are less than 2 micron meters in diameter making them extremely leaky. The leakiness can result in extravasation of plasma proteins and even erythrocytes and it may also lead to traffic of tumor cells into the blood stream and formation of metastases. The tumor vessels have an irregular basement membrane regarding the matrix protein composition, assembly and structure. In addition there is no division between arterioles and venules among tumor vessels; so the blood flow is chaotic leading to a poorly oxygenated tumor tissue.[4, 5]

Different Mechanisms of Tumor Vascularization

Six different mechanisms of tumor vascularization have been hypothesized. They are: 1. Sprouting angiogenesis, 2. Intussusceptive angiogenesis, 3. Recruitment of endothelial progenitor cells, 4. Vessel cooption, 5. Vasculogenic mimicry, 6. Lymphangiogenesis.

1. Sprouting Angiogenesis – Refers to the growth of new blood capillary vessels from pre-existing vessels. These blood vessels endow with oxygen, nutrients to the expanding tissues and also remove the metabolic waste.

2. Intussusceptive microvascular growth - It is a rapid process in which endothelial cells are remodeled by increasing the volume of vessel but decreasing the diameter of vessel. This process takes place usually after vasculogenesis and does not need proliferation of endothelial cells.[4,6]

Process of Intussusception[4,6]

The process of intussusception involves four phases. In the first phase, the endothelial cells from the opposite walls of blood vessels make a contact making a transluminal bridge. Then occurs the reorganization of the inter endothelial junctions followed by perforation of the endothelial bilayer. In the third phase, the interstitial pillar is formed and pericytes, which have contractile characteristics, invade the newly formed interstitial wall. Finally in the fourth phase the pillars grow in diameter and the endothelial cells retract and two separated vessels are formed with recruitment of endothelial cells

In addition to recruiting vascular endothelium from the host, certain tumors may also attract mast cells, macrophages and inflammatory cells. These cells can amplify tumor angiogenesis by releasing proangiogenic molecules such as bFGF, or by releasing metalloproteinases that can mobilize VEGF and other angiogenic proteins. Certain tumor cells may also trigger host stromal cells in the tumor bed to over express the angiogenic protein VEGF.

3. Vessel cooption- Tumor cells exit from microvessels present in the target organ, which begin to grow around these blood vessels which cause the endothelial cells to undergo apoptosis, and finally stimulate neovascular sprouts from neighboring vessels. This process, called "cooption," may represent an intermediate or alternative step in the switch to the angiogenic phenotype.

4. Vasculogenic mimicry- The term "vasculogenic mimicry" was introduced to illustrate the pretense of tumor cells as endothelial cells. This process usually occurs in aggressive tumors wherein the tumor cells dedifferentiate to an endothelial phenotype and make tube-like structures. These mechanisms endow the tumor cells with a secondary circulation system which works independently of angiogenesis.

5. Lymphangiogenesis- Lymphatic vessels are also part of the vascular circulatory system. The lymphatic system is a network of capillaries, collecting vessels and ducts that drain most of the organs. In contrast to the blood vascular network, the lymphatic network is an unrestricted transport scheme, without a driving force which drains extravasated fluid, collects lymphocytes and returns it to circulation. This proves the roleof lymphatic system in tumor progression. [4, 6]

The Angiogenic Switch [7- 9]

After understanding the different mechanisms of angiogenesis the next question arises about when angiogenesis is activated during the development of cancer. Whether angiogenesis is simply an inevitable consequence of limited vascularization in tumor cells or is the angiogenic switch – an important part of the repertoire of qualities that a tumor acquires to be successful. This is an endless debate which is to be answered. The literature suggests that there are 4 different mechanisms of angiogenic switch. They are Prevascular tumors recruit their own blood supply.

1. Circulating endothelial cells in tumor angiogenesis are responsible for activating angiogenic switch.
2. Non endothelial cells may amplify tumor angiogenesis.
3. Process of Vessel cooption

1. Prevascular tumors recruit their own supply

The recruitment of blood supply is the most common mechanism of the angiogenic switch. Majority of carcinomas originate as microscopic lesions in an avascular epithelial layer. These microscopic lesions are separated by a basement membrane from the underlying vasculature in submucosa/dermis. The basement membrane as a temporary physical barrier but as the basement membrane gets breached by the new vessel sprouts, tumor cells from multiple cell layers around each new capillary blood vessel and provides nutrients to the tumors. But these microcylinders are restricted to the oxygen diffusion limit for each specific tumor.[7- 9]

2. Circulating endothelial cells in tumor angiogenesis.

Recent experimental and clinical evidence reveals that circulating endothelial progenitor cells derived from stem cell reservoirs in the postnatal bone marrow can be recruited to the vascular bed of tumors and can thus contribute to tumor growth. VEGF elaborated by a variety of tumor signals through both VEGFR-1 and VEGFR-2 can mobilize progenitor endothelial cells (sine endothelial cells contain VEGFR) into the circulation where they are recruited into the vascular bed of certain tumor types, but not in all tumors.[7- 9]

3. Non endothelial cells may amplify tumor angiogenesis

In addition to recruiting vascular endothelium from the host, certain tumors may also attract mast cells, macrophages and inflammatory cells. These cells can amplify tumor angiogenesis by releasing proangiogenic molecules such as bFGF, or by releasing metalloproteinases that can mobilize VEGF and other angiogenic proteins. The tumor

cells may also trigger host stromal cells in the tumor microenvironment to over express the angiogenic protein VEGF.[7-9]

4. Vessel cooption

Tumor cells sometimes exit from microvessels in the target organ, begin to grow around these vessels thus causing the endothelial cells to undergo apoptosis, and finally induce neovascular sprouts from neighboring vessels. This process, called "cooption," was so considered an intermediate or in some cases as an alternative step in the switch to the angiogenic phenotype.[7-9] (As described previously)

Molecular components of the angiogenic switch

The angiogenic switch is regulated by two components, promoters and inhibitors.

Promoter - VEGF

The most potent angiogenic factor is VEGF. Alternative splicing of human VEGF mRNA gives rise to different isoforms of 121, 145, 165, 189, 206 amino acid residues. It has four types of receptor - Neuropilin, VEGFR1 (Flt1), VEGFR2 (KDR/Flk1), VEGFR3 (Flt4).The majority of effects of VEGF are exerted through activation of VEGFR2 such as proliferation and migration. VEGF stimulâtes microvascular endothelial cell proliferation. It also involves endothelial cell migration and sprouting by inducing tyrosine phosphorylation of VE – cadherins which is a component of adherens type cell to cell junctions responsible for endothelial cell migration. VEGF mediates cell matrix interactions by the expression of 11 and 20 integrin. It inhibits endothelial cell apoptosis and increases endothelial cell permeability. It is possible that certain other positive regulators of angiogenesis may operate through VEGF or be VEGF-dependent. For e.g. the high angiogenic activity of bFGF, which is related because of the following observations: First, bFGF induces the expression of VEGF2; Second, the two endothelial mitogens act synergistically to stimulate capillary tube formation in vitro; Third, systemic administration of a soluble receptor for VEGF (Flk-1) partially blocks cornea angiogenesis induced by implanted bFGF.[10] The molecular mechanism of VEGF as promoter of angiogenesis is depicted in the Table 1.

The other promoters with their respective function are illustrated in the Table 2.

Inhibitors of Angiogenesis

There are two types of inhibitors – Direct and Indirect, the mechanism is explained in the Table 3.

Table 1 - Mechanism of VEGF in tumor angiogenesis

Action	Molecular Mechanism
Endothelial cell migration	VEGF induced tyrosine phosphorylation of VE – cadherins which is a component of adherens type cell to cell junctions are responsible for endothelial cell migration.
Cell – Matrix interactions	VEGF enhances the expression of 11 and 20 integrins which is responsible for cell-matrix interactions.

VEGF – Vascular endothelial growth factor.
VE cadherin – vasculo endothelial cadherin

Table 2 - Promoters of Angiogenesis

Fibroblast Growth factor	• Stimulates EC proliferation. • Promotes microvessel tube formation • Promotes EC migration. • Important promoter of blood vessel remodeling after tissue injury.
Platelet growth factor	• Increases capillary wall stability. • Stimulates the proliferation of cultured pericytes • Increases DNA synthesis on capillary. • Stimulate formation of angiogenic sprouts in vitro.
Angiogenin	• Promotes angiogenesis in vivo • Assists EC adhesion and spreading in vitro
Angiotropin	• Helps activate microvascular ECs during wound healing • Stimulates angiogenesis in vivo • Randomly induces capillary EC migration

Matrix metalloproteinase -9 (MMP-9)	• Thought to help mobilize EPCs by cleaving ECM
Stromal-cell-derived factor-1 (SDF-1)	• Helps guide EPCs to ischemic areas during angiogenesis
Tumor necrosis factor-α (TNF-α)	• Stimulates angiogenesis in vivo. • Stimulates EC tube formation in vitro.
Transforming growth factor-α (TGF-α)	• Promotes EC proliferation • Stimulates angiogenesis in vivo
Angiopoieten-1 (Ang-1)	• Recruits pericytes to recently created blood vessels. • Helps promote EC survival and sprout formation. • Increases the diameter of blood vessels endothelium.
Angiopoieten-2 (Ang-2)	• Antagonist of Tie-2 receptor, reduces levels of pericytes. • Increases plasticity of newly formed blood vessels.

EC– Endothelial cell ECM – Extracellular matrix

Table 3 – Inhibitors of Angiogenesis

Inhibitor	Mechanism	Example
	Inhibits endothelial cells from responding to multiple angiogenic proteins like bFGF and VEGF	Endostatin
	Inhibits synthesis by tumor cells of angiogenic proteins.	Iressa

VEGF– Vascular endothelial growth factor bFGF– Fibro blast growth factor

Balance Hypothesis of Angiogenic switch

The normally quiescent vasculature can be activated to sprout new capillaries (angiogenesis), a morphogenic process controlled by an angiogenic switch mechanism. The prevailing evidence suggests that changes in the relative balance of inducers and inhibitors of angiogenesis can activate the switch. In some tissues, the absence of angiogenesis inducers may keep the switch off, while in others the angiogenesis inducers are present but held in check by higher levels of angiogenesis inhibitors. Thus, either reducing the inhibitor concentration, e.g., for TSP-1, by loss of a tumor suppressor gene; or increasing the activator levels, e.g., for induction of VEGF, by hypoxia, can each change the balance or activate the switch, leading to the growth of new blood vessels.[5,10,11]

Molecular origins of tumor angiogenesis[5,10,11]

After understanding the pathophysiology tumor angiogenesis, the authors worked on the molecular aspects of process of angiogenesis. The 3 basic molecular pathways involved in tumor angiogenesis are

1. The VEGF and VEGF- receptor family in tumor angiogenesis.
2. The Notch Delta-like ligand 4 signaling pathway.
3. Angiogenesis and circulating bone marrow derived cells.

1. The VEGF and VEGF- receptor family in tumor angiogenesis

Induction of or an increase in VEGF expression in tumors can be caused by numerous environmental (epigenetic) factors such as hypoxia, low pH, inflammatory cytokines (e.g., interleukin-6), growth factors (e.g., basic fibroblast growth factor), sex hormones (both androgens and estrogens), and chemokines (e.g., stromal-cell–derived factor 1). Other causes include genetic inductive changes such as activation of numerous different oncogenes or loss or mutational inactivation of a variety of tumor-suppressor genes. This molecule is only one member of a family of proteins that also comprises VEGF-B, VEGF-C, VEGF-D, VEGF-E and PlGF. These proteins interact with three major tyrosine kinases: VEGFR1, VEGFR2 and VEGFR3 and two non-receptor tyrosine kinases; neuropilin 1 and neuropilin 2 that also bind to other ligands. The binding of VEGF to VEGFR 2 leads to a cascade of different signaling pathways, the main being ;

a. Up regulation of genes involved in mediating the proliferation and migration of endothelial cells and migration of endothelial cells.
b. Promoting their survival and vascular permeability.

2. The Notch Delta-like ligand 4 signaling pathway

The interaction of Dll4 and notch receptors through the contact of adjacent endothelial cells leads to a series of

proteolytic events whereby a notch intracellular signaling domain is cleaved and released by a γ-secretase; the domain then translocates to the nucleus. There it interacts with transcription factors and induces the expression of various target genes. The induction of Dll4–notch signaling is thought to act as a damping mechanism to prevent excessive angiogenesis and to promote the orderly development of new blood vessels. Blockade of Dll4–notch signaling interferes with this negative feedback mechanism, resulting in an increased density of vascular sprouts and branches.

3. Circulating bone marrow derived cells in angiogenesis – Promoters and guardians of tumor angiogenesis

a. Myeloid cells in the tumor microenvironment
b. Tumor associated macrophages
c. Mast cells
d. Platelets – Guardians of Tumor vasculature

a. Myeloid cells in tumor microenvironment[12]

Myeloid cells are derived from bone marrow which plays an important role in the growth and metastasis. These cells are easily recruited to the tumor environment and stimulate tumor angiogenesis which is a hallmark of cancer. Besides promoting tumor angiogenesis, myeloid cells suppress the tumor immunity and also promote metastasis to distant and distinct sites.[12] (Table 4)

Pro-tumorigenic myeloid subpopulation

The pro-tumorigenic subpopulation contribute to tumor angiogenesis which are enlisted in Table 5.

Clinical implication of myeloid cells

Several myeloid subpopulations may play roles during neovascularization of tumors, mediating refractiness to anti-angiogenic therapies or the escape from tumor surveillance. Myeloid cells represent novel targets for therapeutic strategies. The mobilization and recruitment of myeloid cells by the tumor defines myeloid cells as a potential delivery system to target the tumor environment.[12]

b. Mast cells and angiogenesis

Mast cells derived components are -

1. Act as effective pro-angiogenic factors such as VEGF, bFGF, TGF-beta, TNF-alpha and IL-8.

2. Proteinases and heparin that are derived from mast cells release heparin-binding pro-angiogenic factors which are blocked on cell surfaces and also in the extracellular matrix (ECM) promoting angiogenesis.

3. Mast cells release histamine, VEGF, and certain lipid-derived chemical mediators of inflammation which induce microvascular hyperpermeability thus showing pro-angiogenic effects.

4. Mast cells also promote chemotactic recruitment of monocytes/macrophages and lymphocytes making them capable of contributing to angiogenesis by releasing angiogenesis modulating molecules. [13]

c. Platelets – the guardians of tumor angiogenesis

Platelets promote angiogenesis by releasing certain soluble factors which may regulate the endothelial stability of the angiogenic tumor vessels. They also support angiogenesis by preventing vascular damage induced by the tumor cells. Moreover these platelet derived soluble factors diminishes the injuries produced by inflammation. Certain factors like angiopoietin-1 (ang-1), serotonin (5-HT), and sphingosin-1-P(S1P) were shown to have vascular permeability too.[13,14]

Clinical importance of platelets

Interference in platelet-tumor vessel cross-talk represents an interesting and challenging approach for manipulating tumor vasculature in order to improve anticancer therapies[13,14]

d. Tumor associated macrophages – TAM's

Macrophages, the eminent cells of wound healing provide aid for tissue growth, remodel the tissue matrix and also promote angiogenesis. These functions of macrophages holds true when they are present in solid tumors. The

literature review suggests that macrophages in tumor microenvironment, called as tumor associated macrophages function in tumor cell proliferation, tumor cell migration and invasion and also tumor angiogenesis.[14]

Mechanism of action of TAM's

Solid tumors show areas of hypoxia which tends to release factors, such as MCP-1 and GM-CSF. These factors are potent chemokines and are chemotactic toward the monocytes in the surrounding blood vessels. So the monocytes are continually recruited from the blood vessels into the tumor get differentiated into TAMs and accumulate themselves in the hypoxic areas. Macrophages, when collected in hypoxic areas respond to hypoxia by up-regulating transcriptional factors HIF1 and HIF2, which are responsible for activating a broad array of genes to support tumor cell invasion and tumor angiogenesis. Ultimately, TAMs function as the contributor of tumorigenic factors and so as regulators for malignant spreading.[14]

Applications of tumor angiogenesis

The tumor cells attract monocytes activating them to secrete angiogenic factors in head and neck squamous cell carcinoma. In addition to them macrophages produce cytokines that act in paracrine fashion on the tumor cells and stimulate them to secrete VEGF. So it is believed that these cells may have an indirect role in the induction of angiogenesis in tumors (includingHNSCC). Beyond its effects on tumor expansion, perhaps the most important way in which angiogenesis can facilitate tumor metastasis is by providing an efficient route of exit for tumor cells. They leave the primary site and enter the blood stream. Angiogenesis enhances entry of tumor cells into the circulation by providing an enhanced density of young, permeable blood vessels which possess little basement membrane and less intercellular junction complexes than normal mature blood vessels. Moreover the number of metastasis formed is proportional to the number of tumor cells shed. A very imperative finding which fascinated the field of interest came out in a study reported by Weidner et al in 1991 who found that in the primary tumor which have a higher degree of angiogenesis have worse prognosis proving that there is a direct relationship between angiogenesis and metastasis. Besides understanding the prognostic implication of tumor angiogenesis, it also served to highlight the extent to which a tumor mass can become contaminated by blood vessels.[5,15,16]

Anti Angiogenic therapy

Anti-angiogenic substances currently under investigation can be divided in agents directly targeting endothelial cell recruitment, endothelial cell proliferation as well as tube formation, whereas indirect inhibitors target tumor cells production of pro-angiogenic growth factors or interfere with their receptors or intracellular signaling pathways. [17, 18]

Advantages of anti-angiogenic therapy over conventional chemotherapy

They are not restricted to a certain histologic tumor entity as all solid tumors depend on angiogenesis. In contrast to chemotherapy, no endothelial barrier has to be crossed by the therapeutic substances. The endothelial cell targeted is genetically stable and therefore subjected to be less prone to development of drug resistance. Moreover, antagonism of angiogenesis is a highly selective therapy promising less serious side effects.[17, 18] (Table 6)

Conclusion

Cancer cells are like teenagers and all they want to do is consume and nurture. To streamline the progress of consumption, these cells send chemical messages that cause blood vessels to fabricate themselves wherever they are. This creation of new blood vessels is called angiogenesis. It is now concluded that without angiogenesis, a tumor cannot grow to a significant size because the growth rate of malignant tissue will surpass the capacity of the normal blood supply in a given region to support malignant growth. In hypothesis, it is believed that if angiogenesis is blocked in the malignant cells , the tumor will be unable to divide quickly and will most likely die off. So after more than three decades of intensive research, there is now proof that antiangiogenic therapy, especially when combined with chemotherapy, results in increased survival in patients suffering from advanced solid tumors.[19-22]

Abbreviations – HNSCC – Head and neck squamous cell carcinoma, VEGF – Vascular Endothelial growth factor, TAM – Tumor Associated Macrophages

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