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"TUMOR ANGIOGENESIS- A REVIEW"

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ABSTRACT
Angiogenesis is a complex process depending on the coordination of many regulators and thereby activating angiogenic switch. Primary tumor cells, stromal cells and cancer stem cells strongly influence vessel growth. Recent advances in understanding of angiogenic mechanism have lead to the development of several anti-angiogenic and anti-metastatic agents that use the strategy of regulation of angiogenic switch. Targeting angiogenic factors from VEGF (vascular endothelial growth factor) family has become an effective strategy to inhibit tumor growth and so far the most successful results are seen in metastatic squamous cell carcinoma (SCC), renal cell carcinoma (RCC), colorectal cancer (CRC) and non-small cell lung cancer (NSCLC). This review article presents the practicability of several angiogenic parameters and markers that have been employed to conjecture regional metastasis including micro vessel density, positive and negative regulators of angiogenesis and genetic markers for angiogenesis.

KEY WORDS: Angiogenesis, Angiostatin, Endothelial cells, Neovascularisation, Vasculogenesis.

INTRODUCTION
Angiogenesis is a complex process in which there is growth of new blood vessels from the pre-existing ones and it is an essential phenomenon for the growth and survival of solid tumors. The complex network of tumor blood micro vessels guarantees adequate supply of neoplastic cells with nutrients and oxygen and provides efficient drainage of metabolites. Folkman and his colleagues demonstrated that solid neoplasms cannot grow larger than 2-3mm in diameter without inducing their own blood supply.[1] Tumor angiogenesis starts with the release of molecules by tumor cells that send signals to the surrounding normal host tissue, activates certain genes to make protein that encourages the growth of new blood vessels.[1, 2]

A sign of pathologic angiogenesis is the relentless growth of blood vessels (i.e., sustained neo-vascularization). Angiogenesis that continues for months or years supports the progression of many neoplastic and non-neoplastic diseases. However, both physiologic and pathologic angiogenesis are usually focal. An angiogenic focus appears as only a small fraction or a small "hot spot" of proliferating and migrating endothelial cells that arise from a single layer of resting endothelium of approximately 1000 m. According to Folkman, the fundamental objective of all anti-angiogenic therapy is to return a pathologic neovascular focus to its normal resting state or to prevent its initiation.[2, 3, 4]

FACTORS IN TUMOUR ANGIOGENESIS
Most blood vessels in an adult organism remain passive but have the potential to divide in response to stimulus and that leads to angiogenic process. The molecules which have been considered as the positive regulators of angiogenesis are

1. Vascular endothelial growth factor (VEGF)
2. Platelet Derived Growth Factor (PDGF)
3. Fibroblast growth factor (FGF)
4. Epidermal growth factor (EGF)
5. Transforming growth factor (TGF)
6. Matrix metalloproteinases (MMPs)
7. TNF (Tumour necrosis factor)
8. Angiopoietins.[1, 3]

VEGF (Vascular endothelial growth factor)
VEGF is also known as vascular permeability factor (VPF), a heparin binding protein and its level is enhanced in various tumours.

Actions of VEGF
✓Powerful inducer of angiogenesis.
✓Stimulates growth and proliferation of endothelial cells.

✓ Act as survival factor for endothelial cells.
✓ Prevent the apoptosis of endothelial cells.
✓ Regulates the vascular permeability.

VEGF Family and Receptors
Consist of closely related factors that are VEGF A, VEGF B, VEGF C, VEGF D, VEGF E and placental growth factor (PIGF). They signal through three tyrosine kinase receptors VEGF R1, VEGF R2, VEGF R3. VEGF receptors are a group of transmembranous proteins with a single transmembrane domain. Extracellular regions are established by seven immunoglobulins like domain. Intracellular region exhibits tyrosine kinase activity and divided into two fragments (TK1 and TK2) by an inter kinase insert. VEGF R2 is located on the endothelial cells and it is the main receptor for the vasculogenic and angiogenic effects of VEGF.

PDGF (Platelet Derived Growth Factor)
Family of PDGF ligand is made of four structurally related soluble peptides in the form of five various homodimers & heterodimers and they are involved in vessel maturation and also in the recruitment of pericytes.

FGF (Fibroblast Growth Factor): It is composed of 23 different proteins and classified in to six various groups. These ligands are among the untimely angiogenic factors and involved in endorsing cell proliferation, migration and segregation of vascular endothelial cells.

EGF (Epidermal Growth Factor)
It is composed of 11 members and 4 EGF receptors. Activation of EGFR pathway results in up regulation of proangiogenic factors such as VEGF and thus viewed as an indirect regulator of angiogenesis.

TGF β (Transforming Growth Factor-β)
TGF β is produced by nearly every cell type and takes part in angiogenesis, embryonic development & wound healing and has potent growth hindrance properties. It has both pro and anti angiogenic properties, depending on its levels. Low levels result in angiogenesis by up regulating angiogenic factors & proteases and high levels inhibit endothelial cell growth and proliferation by inhibiting phosphorylation of pRB and thereby arresting endothelial cells at late G1 phase of the cell cycle.

MMPs (Matrix Metalloproteinases)
Matrix metalloproteinases induce tumour angiogenesis by degrading the extracellular matrix and releasing angiogenic mitogens that are accumulated in the matrix. MMP-9 and MMP-2 proteolytically cleave and activate dormant TGF-β and encourages the process of tumor angiogenesis.

TNF (Tumour necrosis factor): Tumor necrosis factor is a cytokine released from macrophages, mast cells and T-lymphocytes. It functions as a macrophage activating factor and activates these cells to release angiogenic factors.

Angiopoietins
Angiopoietin 1 and 2 can function both as proangiogenic and anti angiogenic because of their respective agonist and antagonist signal through Tie receptors. Angiopoietin 1 stimulates Tie 2 while Angiopoietin 2 does not activate receptor and act as competitive inhibitor of Angiopoietin 1.[1, 3]

MECHANISM OF ANGIOGENESIS IN CANCER
According to the experimental and clinical data, most human tumors do not induce angiogenesis and exist in situ, without blood supply for months to years, when some cells within small tumor, change to an angiogenic phenotype by a phenomenon known as angiogenic switch. The molecular basis of this mechanism may be an enhanced secretion of angiogenic factors or loss of angiogenesis inhibitors. Thus the switch to an angiogenic phenotype is regulated by a change in the equilibrium between positive and negative regulators of angiogenesis.

In 1945, Algire and Chalkley were the first to conclude that the growth of a solid tumor is intimately connected to the development of an intrinsic vascular network. In addition to primary tumor growth, metastatic tumor growth depends upon neovascularization in two steps: Firstly, neoplastic cells must exit from a primary tumor into the blood circulation after the tumor becomes neovascularized. Secondly, after arrival at distant organs, metastatic cells should again promote angiogenesis for a tumor to expand to a noticeable size. In 1970, the surgeon Folkman was the first to hypothecate that targeting the blood supply by preventing blood vessel formation will lead to the arrest of tumor growth or even tumor shrinkage.[1, 2]

Once a neoplastic mutation has occurred, an avascular episode of tumor growth follows. Tumor cells are supplied by diffusion, and tumor growth is arrested at a size of 1– 2 mm3. The following stadium of 'tumor latency' can last up to years. Currently, the theory of an angiogenic switch, controlled by the balance between pro- and antiangiogenic molecules in the solid tumor microenvironment, is accepted. The switch clearly involves more than simple up regulation of angiogenic activity and is thought to be the result of a net balance of positive and negative regulators.

When proangiogenic factors overcome the effect of angiostatic molecules, the tumor acquires an angiogenic phenotype that results in neoangiogenesis. Endothelial progenitor cells, the cross talk between angiogenic factors and their receptors and the interaction between vasculogenesis and lymphangiogenesis are all factors that may contribute to the switch.[3]

The acquisition of the angiogenic phenotype is considered to be a key step in early tumor progression, which allows the tumor to transform from a microscopic lesion to a rapidly expanding mass with metastatic thread. Oncogene-derived protein expressions as well as a number of cellular stress factors, such as hypoxia, low pH, nutrient deprivation, or inducers of reactive oxygen species, are the important stimuli of angiogenic signalling. An important extension of the angiogenic switch model is that the switch may be tripped in the angiostatic direction at the site of a primary tumor but in the opposite angiogenic direction at the site of distant metastases. In the surgical treatment of certain solid tumors, a growth of metastatic tumors following removal of a large primary tumor has been observed.

Several sequential steps can be highlighted during tumor angiogenesis. In mature, non-growing capillaries, the vessel wall is composed of an endothelial cell lining, a basement membrane, and pericyte coverage. Angiogenic factors produced by tumor cells bind to endothelial cell receptors and initiate the sequence of angiogenesis. Angiogenesis is the result of a highly organized series of molecular and cellular events, resulting in the migration, proliferation, and differentiation of endothelial cells into newly formed capillaries that can subsequently develop into more mature vessels. The angiogenic cascade includes both an activation and resolution phase.

When the endothelial cells are stimulated to grow, they secrete proteases, heparanase, and other digestive enzymes that digest the basement membrane surrounding the vessel. The dissolution of the extracellular matrix allows the release of proangiogenic factors from the matrix. The junctions between endothelial cells become leaky, and newly formed vessel sprouts grow towards the source of the stimulus. Besides further endothelial cell proliferation and migration, hematopoietic endothelial progenitor cells are also considered to contribute to capillary lumen formation. Later resolution results in the maturation and stabilization of the newly formed microvasculature by investment of vessels with pericytes, basement membrane reconstruction, and junctional complex formation.[3, 5]

Within the tumor vascular network, the resolution phase is incomplete, resulting in micro vessels that are irregular and tortuous with partial endothelial linings and fragmentary basement membrane as well as increased micro vascular permeability. Tumor vessels tend to break conventional rules of microcirculation, spreading without organization and changing vessel diameters with missing differentiation in arterioles, capillaries and venules.[2]

ANGIOGENESIS INHIBITORS

Because an adequate vascular response is essential for the initial development and the continued growth of the solid tumours so now a day's anti angiogenic therapy is an attractive modality for preventing the development of malignant neoplasm. The angiogenic inhibitors can be either endogenous (present within the body) or synthetic (drugs). Different mechanisms of antiangiogenesis are based on the step of angiogenic cascade that is inhibited.

a. Increase the secretion of antiangiogenic factors e. g retinoids
b. Prevent the activation of macrophages and other endothelial cells by the tumor cells.
c. Targeting the actions of VEGF.
d. Inhibition of proteases that is essential for penetration of basement membrane and degradation of surrounding ECM to create space in which endothelial cells can proliferate and form new vessels. E.g.- Merimastat, Neovastat
e. Induce the EC apoptosis.
f. Inhibition of EC survival.
g. To make the endothelial cells refractory to angiogenic stimulus. E.g.-Thalidomide, endostatin, squalamine & TNP-470.

The various Endogenous Angiogenesis inhibitors are as follows

A. Interferon
B. Interleukins
C. TIMP
D. Angiostatin
E. Endostatin

INTERFERON

Interferon is the members of secreted glycoproteins which directly or indirectly inhibit the tumor angiogenesis and growth. Administration of optimal dose of IFN α/β decrease the expression of β - FGF m RNA and protein, micro vessel density and also induces the apoptosis of endothelial cells. IFN γ induces its antiangiogenic effects through the secretion of IFN γ – inducible protein 10 (IP-10) and monokine.

INTERLEUKINS

The structure of interleukins (ILs) determines its function to play a role in either promoting or inhibiting angiogenesis.IL1a is a cytokine secreted by activated macrophage induces angiogenesis through the increased expression of angiogenic factors. IL 12 suppresses the expression of VEGF mRNA, promotes the apoptosis and inhibits proliferation rate in human tumors and reduce tumor vessel density. IL-10 down regulate the synthesis of VEGF, IL-1β, TNF-α, IL-6, MMP-9 in tumor associated macrophages.

TIMP

Degradation of basement membrane and remodelling of ECM is required to create a space in which endothelial cells can migrate and proliferate. In the process of remodelling of ECM, matrix metalloproteinases (MMPs) have the central role and tissue inhibitors of matrix metalloproteinase's (TIMP) inhibit the neovascularisation by inhibiting the breakdown of surrounding matrix. The migration of ECs through gelatin is inhibited by TIMP-1.TIMP-2 inhibits the β

FGF induced EC proliferation. Because of the multiple effects of TIMPs, MMPs are the attractive targets for tumor therapy.

ANGIOSTATIN

It is a 38 KDa internal fragment of plasminogen and its antiangiogenic effect is due to down regulation of VEGF expression within tumours. Binding of angiostatin to plasma membrane localized ATP synthatase suppress the endothelial–surface ATP metabolism and thus down regulate the EC (endothelial cell) proliferation and migration. According to many studies angiostatin treatment significantly increases the apoptosis of EC.

ENDOSTATIN

It is a 20 KDa fragment of type XVIII collagen and inhibits ECs proliferation, angiogenesis and tumour growth.

Mode of action of Endostatin

1) Inhibits the binding of VEGF to ECs.
2) Directly binds to receptors but not to VEGF.
3) Blocks the VEGF induced tyrosine phosphorylation.
4) Suppresses the VEGF induced downstream events of KDR/Flk-1 signalling which are involved in the mitogenic activities of VEGF.[1, 3]

GENETIC MARKERS OF ANGIOGENESIS

At the molecular level, the angiogenic switch operates as a shift in the balance of production by tumor cells of molecules that positively and negatively regulate angiogenesis. Over expression of positive factors and down regulation of inhibitors during the early tumor development are triggered by genetic mutations that control angiogenesis, such as

1. Over expression of RAS oncogene increases production of angiogenetic protein VEGF.
2. Deletion of Tp53 down regulates production of angiogenetic inhibitor protein Thrombospondin-1.

Although the expression levels of p53 and proliferating cell nuclear antigen (PCNA) were also investigated at different cut points, there was no significant correlation between their levels and incidence of occult neck metastasis. A recent study indicates that the expression of cyclin D1 correlates with the presence of occult cervical metastases in head and neck carcinoma patients, thus suggesting that its immunohistochemical evaluation in biopsy samples may be used as an additional tool for identifying patients to be treated with elective neck dissection. However, this study had included advanced lesions in the node positive cases and early lesions in node negative cases. Hence in addition to the retrospective nature of the study, this could have possibly led to a misleading result.[6]

ANGIOGENESIS IN ORAL SQUAMOUS CELL CARCINOMA

Squamous cell carcinoma of the head and neck (HNSCC) is the sixth most common cancer with 5,00,000 diagnosis per year worldwide. In spite of the various advances in the treatment modalities of locally advanced disease, more than 50% of cases will have relapse. Furthermore, combining surgery, radiotherapy, and chemotherapy often results in severe and permanent function deficits with a negative impact on the quality of life of patients'.

Angiogenesis is a hallmark of tumor progression which has been studied in many cancer types including head and neck squamous cell carcinoma. Antiangiogenic agents are to date available and useful for the treatment of many tumors.[7, 8]

Vascular endothelial growth factor A (VEGF-A) is the best known agent that has the potential to induce vasculogenesis. It is a vascular permeability factor that belongs to the platelet-derived growth factor (PDGF) super family, which also includes VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placental growth factor (PlGF). Hypoxia induces VEGF expression through the mediation of hypoxia-inducible factor (HIF-1α). There are many other factors involved in angiogenesis, such as epidermal growth factor (EGF), PDGF, prostaglandins, COX-2, and IL-6. The VEGF family of ligands plays its role through cell surface receptor tyrosine kinases, VGFR-1, VGFR-2, and VGFR-3. VEGFR-2 is the most important one through which VEGF exerts its mitogenic, chemotactic, and vascular permeabilizing effects on endothelial cell. Moreover, VEGF interacts with a family of co receptors -2) that strengthen the link between VEGF and its receptors increasing their biological activity called neuropilins (NRP-1 and NRP).[9, 10]

Over expression of VEGF in HNSCC is associated with more advanced disease, increased resistance to cytotoxic agents, and poor prognosis. In a meta-analysis of 12 studies including 1002 patients affected by cancer of oral cavity (70.8% of patients), pharynx (15.2%), and larynx (14%), VEGF expression was evaluated, and its positivity was associated with a twofold higher risk of death at 2 years.

Hasina et al demonstrated that there are different molecular mechanisms by which each tumor induces angiogenesis. Using sample collected from patients affected by HNSCC and sample of normal and dysplastic mucosa, they conducted an immunohistochemical analysis and gene expression profiling studies. They studied the expression of cytokines (CK) such as VEGF, IL-8/CXCL8, HGF, and FGF-2 in normal, dysplastic, and pathological tissues. These CK are well-known mediators of HNSCC angiogenesis. The authors observed that normal mucosa generally does not express VEGF, IL-8/CXCL8, FGF-2 and HGF and that, where present, the levels of these CKs are very low compared to dysplastic and pathological mucosa. The same CKs are more frequently expressed and at a higher levels in dysplastic oral mucosa.[9, 10, 11]

The incidence and the intensity of expression of VEGF, IL-8/CXCL8, FGF-2 and HGF are highest in HNSCC samples. Moreover, they validated the presence of two different clusters in relation to angiogenesis in HNSCC samples: tumors in Cluster A express high levels of VEGF and FGF-2 and low levels of IL-8/CXCL8 and HGF and are characterized by higher levels of microvessel density than tumors in Cluster B, expressing on the contrary low levels of VEGF and FGF-2 and higher levels of IL-8/CXCL8 and HGF. These data suggest that there are at least two different pathways in inducing angiogenesis in HNSCC. This hypothesis has an important therapeutic implication.[9, 11, 12]

CONCLUSION

The research in vascular signaling has revealed a significant degree of complexity and cross-talk between signaling systems. But, all of them have noted to be active in tumor angiogenesis are reiterations of those operational during developmental angiogenesis. Understanding the mechanism and factors involved in the process improves the quality of treatment. The fundamental requirement of tumor growth is vascular supply, so antiangiogenic therapy for cancer particularly effective in combination with cytotoxic agents. Future experiments and more integrated efforts on the evaluation of the therapy at the molecular level will further aid to refine targeted treatments in tumor biology.

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