

Relationship of Angiogenesis and Oral Squamous Cell Carcinoma

Marla V,¹ Hegde V,² Shrestha A¹

¹Department of Oral Histology and Pathology

College of Dental Surgery

BP Koirala Institute of Health Sciences,

Dharan, Sunsari, Nepal.

²Department of Oral Pathology and Microbiology

SDM College of Dental Sciences,

Sattur, Dharwad, Karnataka.

Corresponding Author

Vinay Marla

Department of Oral Histology and Pathology

College of dental surgery

BP Koirala institute of health sciences,

Dharan, Sunsari, Nepal.

Email: vinaymarla85@gmail.com

Citation

Marla V, Hegde V, Shrestha A. Relationship of Angiogenesis and Oral Squamous Cell Carcinoma. *Kathmandu Univ Med J* 2015;50(2):178-85.

INTRODUCTION

The formation of new capillary blood vessel, a process termed 'Angiogenesis' is one of the most pervasive fundamentally essential biological process encountered in mammalian organisms.¹ Angiogenesis is an important event in a variety of physiological settings, such as embryonic development, chronic inflammation wound repair.² Although, angiogenesis is a feature of a limited number of physiological processes; the etiology pathogenesis of a much larger increasingly expanding number of pathological conditions have been shown to be a consequence of an angiogenic response. Ocular disorders, vascular malformations, neoplasm, etc are few of the examples in which angiogenesis plays an important role.³ The aim of this review is to study the significance of angiogenesis in relation to neoplasms.

ABSTRACT

Angiogenesis is an important aspect of a variety of physiological and pathological processes; and depends on the alteration of the balance between pro-angiogenic and anti-angiogenic factors. The role of angiogenesis in the progression and metastasis of neoplasm is a well established phenomenon. With regards to oral squamous cell carcinoma, it is a field of ongoing research and requires validation for it being used as a mode of anti-cancer therapy. This review focuses on the concept of angiogenesis, the factors associated with it, the relationship of angiogenesis with oral epithelial dysplasia and oral squamous cell carcinoma; the methods of studying angiogenesis and anti angiogenic therapy.

KEY WORDS

Angiogenesis, neoplasm, oral squamous cell carcinoma.

LITERATURE

Blood vessels are critical for the maintenance of cellular homeostasis of virtually all cells in the human body therefore all cells must reside within 100µm of a capillary. The development of vascular tree starts early during embryogenesis involves the development of endothelial cells from mesenchymal precursor cells and leading to the formation of the primitive vascular plexus. This process is called vasculogenesis.¹ Recruitment of other mesenchymal cells as smooth muscle cells and fibroblasts subsequently stabilizes the early vascular network, which is also expanded by a process called angiogenesis. Angiogenesis is defined as the generation of new capillaries from pre-existing blood vessels e.g. by sprouting or by intussusception.⁴

Angiogenesis is driven by a cocktail of growth factors and pro-angiogenic cytokines, and is tempered by an

equally diverse group of inhibitors of neo-vascularization. Angiogenesis is tightly regulated in the adult organism. It is only induced during the female reproductive cycle, tissue repair and wound healing; and is organized into the three stages of initiation, proliferation/invasion and differentiation/maturation. Through the pioneering work of Folkman, it was recognized that angiogenesis plays an important role in tumour development, progression and metastasis.⁵ Research into the molecular and cellular components of angiogenesis has also identified an immense heterogeneity between physiologic and tumour angiogenesis as well as between angiogenesis in different tumour entities, different stages of tumour progression and tumour development in different organs.⁶

Tumours begin to grow as small aggregates of neoplastic cells outgrowing the basal layer, without any significant input of blood. Therefore, these aggregates are avascular. This pre-vascular form of the tumour is denominated in-situ carcinoma and is highly dependent on the proximal blood vessels for oxygen and nutrients supply. The size of the carcinoma remains steady, without significant increase over time and limited by the balance of cellular proliferation and apoptosis/cell death.⁷ Without vasculature, the tumour cannot grow beyond 1-2 mm long, because it only takes the oxygen and nutrients by diffusion. The oxygen only diffuses up to 100 μ m between the capillary and the cell, only covering 3 to 5 lines of cells around the capillary.⁸ Once the tumour is connected to circulation by new blood vessels, it can grow and tumour cells disseminated throughout the body, metastasizing. This demonstration generated the novel concept that cancer can be therapeutically treated by antagonizing the angiogenic process.⁹

Research in the field of tumour angiogenesis follows three main directions:

- 1) Identification of positive and negative regulators.
- 2) Characterization of the mechanisms of action and the identification of either natural or synthetic inhibitors;
- 3) Quantification of the neo-vascularization in tumour biopsies as predictive tool for diagnosis.¹⁰

ANGIOGENIC FACTORS

Although most blood vessels in an adult organism remain quiescent, endothelial cells retain the capability of rapid division in response to physiological stimuli, which may result in activation of angiogenesis. Quite a number of molecules are known that can serve as positive regulators of angiogenesis [vascular endothelial growth factors (VEGF), fibroblast growth factors (aFGF and bFGF), transforming growth factors- β (TGF- β), tumour necrosis factor- α (TNF- α), interleukin-8, and angiopoietins]. However, not all of these factors are specific for endothelial cells; only some of them are able to directly influence the endothelial cells.¹¹

VASCULAR ENDOTHELIAL GROWTH FACTORS (VEGF)

Vascular endothelial cell growth factor is one of the most important angiogenic factors. VEGF is a multifunctional cytokine whose biological activity is primarily associated with endothelial cells. There are 5 members of the VEGF family. These include VEGF-A, placental growth factor, VEGF-B, VEGF-C and VEGF-D. These factors are produced by several normal cell types such as macrophages, mesenchymal cells, lung epithelial cells, kidney epithelial cells, mast cells, platelets and also by tumour cells.¹² The functions of various types of VEGF molecules have been summarized in table-1.¹³

Table 1. Functions of VEGF

TYPE	FUNCTION
	ANGIOGENESIS:
VEGF A	Increases migration of endothelial cells
	Increases mitosis of endothelial cells
	Increases $\alpha v \beta 3$ activity
	Creation of blood vessel lumen
	Creates fenestrations
	Chemotactic for macrophages and granulocytes
	Vasodilation
VEGF B	Embryonic angiogenesis (myocardial tissue specifically)
VEGF C	Lymphangiogenesis
VEGF D	Needed for the development of lymphatic vasculature surrounding lung bronchioles.
PLGF	Important for vasculogenesis.

RECEPTORS

There are three receptor protein-tyrosine kinases for the VEGF family of ligands (VEGFR1, VEGFR2, and VEGFR3) and two non-enzymatic receptors (neuropilin-1 and -2). These receptors are located on the surface of endothelial cells. Moreover, several of the VEGF family ligands bind to heparan sulfate proteoglycans that are found on the plasma membrane and in the extracellular matrix. These VEGF binding sites were identified on vascular endothelial cells corresponding to VEGFR1 (Flt-1) and VEGFR2 (Flk-1/KDR). This distribution on endothelial cells accounts for the selectivity and specificity of action of VEGF. VEGFR3 (Flt-4), which is in the same receptor family, binds VEGF-C and VEGF-D.¹²

FIBROBLAST GROWTH FACTORS (FGF)

Fibroblast growth factors are ubiquitous molecules involved in the transmission of signals between epithelium and connective tissues which influence epidermal growth and differentiation, vascular homeostasis, tissue repair and embryonic development. Fibroblast growth factors are derived from mesenchymal cells like fibroblasts,

endothelial cells etc.¹⁴

FGF represents a large family of growth and differentiation factors with at least 18 members. The two most important are FGF-1 (acidic FGF) and FGF-2 (basic FGF), which share more than 50% structural homology. The basic structure of the fibroblast growth factor (FGF)–FGF receptor (FGFR) complex comprises two receptor molecules, two FGFs and one heparan sulphate proteoglycan (HSPG) chain.¹²

Acidic fibroblast growth factor (aFGF) is a member of the fibroblast (heparin-binding) growth factor family of polypeptides and is a potent mitogen for a variety of mesenchymal neuroectodermal cells, including those of vascular origin. Heparin is a critical co-factor for aFGF, although it has no mitogenic potential of its own. Because aFGF is produced by mesenchymal cells and appears to be sequestered in the extracellular matrix, heparin administration might promote an angiogenic response through an interaction with and enhancement of the mitogenic effects of endogenous FGF.¹⁴

Basic fibroblast growth factor (bFGF) is a potent angiogenic factor in normal and diseased tissues. In normal tissues, bFGF is membrane-bound; present in basement membranes and in the sub-endothelial extracellular matrix of blood vessels. In particular, during both wound healing of normal tissues and tumour development, the action of heparan sulphate degrading enzymes activates bFGF, thus mediating the formation of new blood vessels as well as being mutagen for fibroblast cells.¹⁴

FAMILY OF FGF RECEPTORS

Four related FGF receptor tyrosine kinases exist. These receptors are expressed on the surface of endothelial cells. It appears that several FGFs bind to more than one FGF receptor type with high affinity. There are both high and low affinity receptors for FGF. The high-affinity receptor family is represented by FGFR-1-4, which are tyrosine kinase receptors and are responsible for signalling functions. Low-affinity receptors are involved in the sequestration and stabilization of the ligand; and most of them are heparan sulfate proteoglycans (HSPG). They are also present in soluble form in biological fluids and extracellular matrix (ECM). For potent activation of receptors by FGF, the ligand has to be presented via heparan sulphate or heparin; this presentation may involve the creation of a ternary complex involving FGF, heparin: heparin sulfate and the receptor.¹³

ANGIOPOIETINS

Angiopoietins are another family of endothelial cell-specific molecules that play an important role in vessel maintenance, growth and stabilization. There are four types of angiopoietins known: Ang-1, -2, -3 and -4.¹¹

The angiopoietins bind to Tie (Tyrosine kinase with immunoglobulin-like and EGF-like domains) receptors expressed on endothelial cells. The Tie receptors are tyrosine kinases, so named because they mediate cell

signals by inducing the phosphorylation of key tyrosines, thus initiating the binding and activation of downstream, intracellular enzymes.¹⁵ Tie1 mRNA is highly expressed in embryonic vascular endothelium, angioblasts and endocardium suggesting that the Tie1 receptors are present only on these structures. The Tie2 receptor takes part in vessel maturation by mediating survival signals for endothelial cells. Ang-1 acts as an agonist promoting vessel stabilization in a paracrine manner, whereas Ang-2 is an autocrine antagonist inducing vascular destabilization at high concentrations. Ang-2 has been found to be dramatically increased during vascular remodelling and is implicated in tumour associated angiogenesis and tumour progression. It has been found that VEGF also activates the Tie2 receptor.¹⁵

PLATELET DERIVED GROWTH FACTOR (PDGF)

The PDGF family consists of four different PDGF strands (A-D) establishing functional homodimers (PDGF-AA, PDGF-BB, PDGF-CC, and PDGF-DD) or a heterodimer PDGF-AB. PDGF and VEGF families have much in common in their structure. Analysis of genomic sequences encoding VEGF and PDGF shows that these families originated from a common progenitor.¹⁶

PDGF are expressed by a large variety of normal human tissues and organs. The highest expression of PDGF-A is found in the heart, skeletal muscle and pancreas. PDGF-B is expressed with the highest amounts in the heart and placenta, and moderate levels in other organs. PDGF-C is expressed with higher levels in the heart, kidney, adrenal gland, and pancreas, and with low levels in liver and ovary. The highest expression of PDGF-D is found in the heart, pancreas, ovary and no detectable expression found in the brain, lung and skeletal muscle.¹⁷

PDGF signals through two cell-surface tyrosine kinase receptors, PDGFR α and PDGFR β (present on endothelial cells), and induces angiogenesis by up-regulating VEGF production and modulating the proliferation and recruitment of perivascular cells.¹⁸ The angiogenic activity of PDGF might not only be based on the increased VEGF-A production, because PDGF-B stimulation induces an increased endothelial cells lineage commitment and restricted differentiation of hematopoietic precursors. PDGF-B and PDGFR β signalling have a role in the establishment of functional blood vessels by recruiting and stabilization of perivascular cells.¹⁶

PDGF are major mitogens for many cell types of mesenchymal origin and for some cells that are neuroectodermal in origin, like oligodendrocytes. PDGF have chemoattractant properties and have been involved in bone formation, erythropoiesis, wound healing, angiogenesis; and in the normal development of the kidney, brain, cardiovascular and respiratory systems. A lot of evidences support the implication of PDGF in tumour growth and development of specific lesions from

inflammatory diseases and atherosclerosis. During normal development, cell proliferation significantly increases as a consequence of PDGF overexpression and decreases in PDGF null mutants.¹³

TRANSFORMING GROWTH FACTOR- β (TGF- β)

Transforming growth factor is a multifunctional protein that initiates its diverse cellular responses by binding to and activating specific type I and type II serine/threonine kinase receptors. TGF- β can act as a regulator of proliferation, migration, survival, differentiation, and extracellular matrix synthesis in endothelial cells and vascular smooth muscle cells, as well as in the maintenance of vascular homeostasis.¹⁹ TGF- β is released by a variety of cell types like platelets, T cells, macrophages, monocytes, neutrophils and fibroblasts.¹⁹

The TGF- β proteins affect cell fate by regulating proliferation, differentiation, motility, adhesion, and apoptosis, but are most notable for inhibiting proliferation in a variety of systems.¹⁷ TGF- β 's pleiotropic effects include roles in angiogenesis, but the exact nature of its participation in this process is unclear. TGF- β has been described as angiogenic or anti-angiogenic, depending on the nature of the assay. In vivo angiogenesis studies suggest a role in angiogenesis, but in vitro studies on endothelial cells have demonstrated an inhibitory effect on endothelial cell proliferation, as well as decreased expression of molecules necessary for endothelial cell migration such as plasminogen activators and a correspondent increase in Plasminogen Activator Inhibitors (PAIs).¹⁹ TGF- β has also been shown to increase synthesis and secretion of specific extracellular matrix proteins including fibronectin and collagen. These effects, coupled with its induction of endothelial cell quiescence, have led some to speculate that TGF- β takes part in the resolution phase of angiogenesis in which endothelial cells cease proliferating and functional basement membranes and extracellular matrix complexes are laid down.¹³

INFLAMMATORY CYTOKINES

Many inflammatory cytokines are involved in angiogenesis during the wound healing process. These cytokines are released by a variety of inflammatory cells like macrophages and mast cells. Vascular endothelial cell tube and network formation demonstrates the involvement of IL-6, IL-8 and TNF- α . IL-8 is a growth-stimulating cytokine to vascular endothelial cells and induced angiogenesis. TNF- α is also one of the major cytokines involved in mast cell and macrophage-induced angiogenesis.²⁰ Tumour necrosis factor- α (TNF- α) is a growth-inhibitory factor (secreted by inflammatory cells and tumour cells) and even cytotoxic protein when it is overexpressed in endothelial cells. However, exogenous application of TNF- α promotes formation of new blood vessels in several in vivo models. The ability of anti-TNF- α antibodies to completely neutralize the angiogenic activity in the culture medium of activated macrophages confirmed the role of TNF- α as an

angiogenic molecule secreted by macrophages. In addition, macrophages are an important source of IL-6. Studies have reported that IL-6 had no effect or even growth-inhibitory effect on endothelial cells. IL-6 can be synergistic with other cytokines like IL-8 to promote angiogenesis, or it can contribute to the vascular formation by modulating the tissue matrix via induction of target proteins like matrix metalloproteins (MMPs) and VEGF.²⁰

Certain prostaglandins, such as Prostaglandin E1 (PGE1) and Prostaglandin E2 (PGE2), are angiogenic whereas prostaglandins of the A or F series are not. PGE1 stimulates angiogenesis in the cornea and PGE2 stimulates angiogenesis in the chick embryo chorioallantoic membrane. It is not clear how prostaglandins induce capillary growth. However, prostaglandin levels are elevated in tumours, activated macrophages, wounds, and inflammatory exudates. Prostaglandins could act by mobilizing macrophages or by some as yet unknown mechanism.²¹

MATRIX-METALLOPROTEINASES

Matrix-metalloproteinases (MMPs) are a group of zinc-dependent extracellular proteinases, also called matrixins or collagenases, which remodel the extracellular matrix (ECM). The ECM gives tissue its structural integrity and pre-dominantly comprises of the fibrillar collagens, basement membrane, and elastin fibers composed of elastin and fibrillin.²² There are three predominant groups of MMPs: collagenases, gelatinases, and stromelysins. The collagenases (MMP-1, -8, -13, and -18) cleave interstitial (structural) collagens, with MMP-1 as the pre-dominant one. Gelatinases; primarily MMP-2 and MMP-9, degrade basement membrane collagens and denatured structural collagens. The stromelysins (MMP -3, -10, -11, and -19) degrade basement membrane collagens as well as proteoglycans and matrix glycoproteins.²³

Quiescent endothelial cells produce little or no active MMPs, but these proteases are strongly induced and subsequently activated in capillary sprouts during wound healing, inflammation and tumour growth; and in activated endothelial cells in vitro. Tumour cells also produce proteolytic enzymes that can destroy the matrix barriers ambient to the tumour, permitting invasion into surrounding connective tissues.²⁴ MMPs are able to degrade virtually all components of the ECM and connective tissue surrounding the tumour cells and the basement membrane. It was initially believed that MMPs are being produced and secreted by tumour cells, degrading basement membrane and ECM components.^{22,23}

Now, it is known that MMPs are also produced by surrounding stromal cells, including fibroblasts and infiltrating inflammatory cells. It was initially believed that MMPs, via breakdown of the physical barrier, were primarily involved in tumour invasion. There is growing evidence, however, that MMPs have an expanded role, as they are important for the creation and maintenance of a

microenvironment that facilitates growth and angiogenesis of tumours at primary and metastatic sites. In cancer, MMPs are involved in angiogenesis by regulating the bioavailability of vascular endothelial growth factor (VEGF) (e.g., MMP-9) and the cleavage of matrix-bound VEGF.²⁵

NATURALLY OCCURRING ANGIOGENIC INHIBITORS

THROMBOSPONDIN

Thrombospondins are secreted proteins with anti-angiogenic properties and are produced by endothelial cells, platelets and astrocytes in the brain. A considerable amount of research has been conducted on the anti-angiogenic properties of the extracellular matrix molecule thrombospondin. The original work on this field was conducted by Bouck and colleagues, who correlated the anti-angiogenic activity of thrombospondin with the expression of a tumour suppressor gene. Thrombospondin is secreted by endothelial cells and was soon confirmed to be an inhibitor of endothelial cell proliferation, motility, and morphogenesis. The initial correlation of thrombospondin production with tumour suppression was clarified when it was reported that the well-characterized tumour suppressor p53 could repress angiogenesis by up-regulating the production of thrombospondin or other inhibitors in certain tumour cells. In a compelling recent study, the expression of thrombospondin was shown to be inversely related to p53 expression and to angiogenesis in human bladder cancer specimens.²⁶

INTERFERON

The anti-endothelial activity of interferon has been known for some time. It has been demonstrated that interferon could inhibit the migration of capillary endothelial cells, a critical step in angiogenesis. Interferon secreted by leukocytes has subsequently been shown to have some *in vivo* anti-angiogenic activity, but it is not sufficiently potent to be able to cause regression of many tumours when used alone. One potential mechanism of interferon action may be to block the production or efficacy of angiogenic factors produced by tumour cells.²⁷ Few vascular tumours are more sensitive to the inhibitory activity of interferon. Hemangiomas, large benign tumours comprised predominantly of endothelial cells, are particularly sensitive to treatment with interferon. Treatment with α -interferon is one of the first clinically successful treatment protocols for patients with proliferating hemangiomas. Treatment is chronic and of long duration, often lasting a year or longer, but α -interferon is relatively non-toxic and provides the only documented treatment for this potentially disfiguring, sometimes fatal disease.²⁶

METALLOPROTEINASE INHIBITORS

Invasive events require both active cell migration and the ability to cause limited degradation of the connective tissue in order to allow passage of the tumour cells through

tissue. This is accomplished, in part, by the activity of metalloproteinases that are sequestered on the tumour cell surface and concentrated at the leading edge of the tumour cell; called matrix metalloproteinases (MMPs) because of their ability to degrade extra-cellular matrix. These enzymes inhibit both angiogenesis and tumour metastasis.²⁴ Naturally occurring MMP inhibitors, known as TIMPs (tissue inhibitors of metalloproteinases), have been found in a variety of cells and tissues; and secreted by fibroblasts and monocytes. All members of the TIMP family inhibit angiogenesis. TIMPs also inhibit tumour growth and metastasis. Although the mechanism whereby TIMPs inhibit angiogenesis and metastasis would appear to be their ability to suppress matrix degradation, other cellular effects of the TIMPs make interpretation more difficult, because these protease inhibitors can also directly block proliferation and migration of both tumour cells and endothelial cells *in vitro*. The combined activities of this class of inhibitor make them potent anti-tumour agents.²⁸

ANGIOGENESIS IN ORAL EPITHELIAL DYSPLASIA

In normal tissues, blood vessels are usually quiescent, and cells usually secrete low levels of inducers and high levels of inhibitors. As normal cells progress toward malignancy, they must develop the ability to induce angiogenesis. To achieve this switch, epithelial cells usually increase the amount of inducers and decrease the amount of inhibitors they secrete. There is considerable interest in determining how cells, progressing from normal to tumourigenic, switch from being anti-angiogenic to angiogenic.⁶ In animal models, a distinct switch to the angiogenic phenotype is seen. In other cases, the cells developing into tumours sequentially become more angiogenic in a stepwise fashion. The exact mechanisms regarding how this occurs in most neoplasm have for the most part remained elusive.⁵

During the premalignant stages, oral epithelial dysplastic cells manifest altered responses to factors present in the stroma, resulting in uncontrolled cell proliferation. However, continued growth of transformed cells requires the induction of angiogenesis, which is thought to occur as a discrete step termed the 'angiogenic switch'. Traditionally, the angiogenic switch occurs when the balance between the levels of activators and inhibitors of angiogenesis at the premalignant lesion tips in favour of pro-angiogenesis.^{5,6} Oncogene activation and/or tumour suppressor gene inactivation can alter the expression of angiogenesis activators (including vascular endothelial growth factors (VEGFs), fibroblast growth factors (FGFs), matrix metalloproteinases (MMPs) and inhibitors (including thrombospondin-1 and statins) in transformed cells.¹⁰

ANGIOGENESIS IN ORAL SQUAMOUS CELL CARCINOMA

A substantial body of work published over the last 40 years has firmly established that solid tumours are "angiogenesis-dependent". This theory, first proposed by Folkman, was based on several key observations.⁸ First, it was shown that

tumours, implanted into isolated perfused organs, where blood vessels did not grow or when introduced into either subcutaneous transparent chambers in mice or the avascular cornea of the rabbit eye, grew ever so slowly, as small 1-2-mm³ spheres or as thin wafers, by absorbing nutrients diffusing from the surrounding tissues. The tumours were able to survive in this dormant state for an extended period of time but were unable to grow progressively.^{8,9} However, as the advancing edge of the tumour approached adjacent microvessels, diffusible “angiogenic factors”, released from the tumour, stimulated endothelial cells to grow and migrate directionally toward the tumour and organize into a capillary network. This switch from the prevascular to vascular phase was accompanied by exponential growth of the tumour.^{8,11,29}

There are numerous examples where these observations have been validated in human tumours. For example, human retinoblastomas that metastasize to the vitreous or the anterior chamber of the eye remain avascular until they settle on the richly vascular iris or retina and become vascularized.⁵ Carcinoma of the ovary metastasizes to the peritoneum as avascular spheres that fail to grow until they become vascularized. The appearance of neo-vascularization at the base of melanomas that enter the vertical growth phase, heralds the onset of rapid growth and increased metastatic potential. An increase in the number of new capillaries in certain types of breast and prostate carcinoma has been shown to correlate with malignant and metastatic potential which is of prognostic significance.³⁰

Similarly a role of angiogenesis has been established in oral squamous cell carcinoma. A variety of molecules capable of inducing angiogenesis are directly produced by keratinocytes and inflammatory cells in oral squamous cell carcinoma. Interleukin-8 (IL-8) secreted by the tumour epithelial cells is a major angiogenic factor. In addition, in the closely related bronchogenic carcinomas, IL-8 was the primary mediator of angiogenesis found in fresh tumour homogenates.² Increased levels of VEGF protein expression are seen in many different neoplasm including head and neck squamous cell carcinoma (HNSCC), may play an important role in the induction of angiogenesis in those neoplasm that secrete high levels of this cytokine.^{2,31}

It is also seen that a variety of cell types originating in the bone marrow play crucial roles in pathological angiogenesis. These include the cells of the innate immune system— notably macrophages, neutrophils, mast cells, and myeloid progenitors that infiltrate the premalignant lesions and progressed tumours; and assemble at the margins of such lesions. The peri-tumoural inflammatory cells help to trip the angiogenic switch in previously quiescent tissue and to sustain ongoing angiogenesis associated with tumour growth in addition to facilitating local invasion.³²

EFFECT OF TUMOUR CELLS ON ANGIOGENESIS

The tumour cells recruit blood vessels by several different mechanisms. They produce diffusible angiogenic factors that directly activate endothelial cells stimulating them to sprout and grow towards the developing tumour. They elaborate cytokines, which attract and activate macrophages, mast cells and neutrophils which in turn elaborate angiogenic factors.³³ They are also able to block the production of or become refractory to inhibitors of angiogenesis. They produce enzymes that release angiogenic factors sequestered in the extracellular matrix and stimulate adjacent normal tissues to make enzymes such as stromelysin and collagenase that can be activated to promote angiogenesis.³⁴

EFFECT OF TUMOUR STROMA ON ANGIOGENESIS

Although tumour cells themselves promote the recruitment and expansion of their own blood supply; tumour associated immune/inflammatory cells can modify and contribute to this process by supplying a variety of growth factors, cytokines and proteases comparable to that secreted by tumour cells themselves.¹⁹ The pattern of inflammatory and immune cell infiltration in the tumour microenvironment could contribute to cancer progression and metastasis or to the inhibition of tumour growth. The predominant stromal cells found in cancers are macrophages, lymphocytes, natural killer cells, endothelial cells, fibroblasts, eosinophils and mast cells.³³ The number and type of cells that compose the inflammatory immune cell infiltrates in solid tumours are related to the local production of chemokines and other chemotactic factors by both the tumoural and stromal cells. Mast cells and tumour associated macrophages (TAMs) are prominent inflammatory cell population in many tumour types residing in both perivascular and avascular, hypoxic regions of these tissues and plays a role in angiogenesis.³⁵

ASSESSMENT OF TUMOUR ANGIOGENESIS FOR DETERMINING THE PROGNOSTIC SIGNIFICANCE

A major finding which attracted interest in the field of tumour angiogenesis was reported by Weidner et al. who found that the greater the degree of angiogenesis detected in a primary tumour, the worse is the prognosis. This established a direct relationship between metastasis and angiogenesis. It was first shown in breast cancer subsequently a large diverse array of other tumours, cancer including melanomas, gliomas, lung, bladder prostate cancers, many others.^{9,30} With the development of highly specific immunohistochemical endothelial markers that can be easily assessed in histological archival materials; numerous quantitative studies of assessing intra-tumoural vascularity have been reported in various human solid tumours.³⁶ Tumour vascularity is measured by staining tissue sections with antibodies specific (or highly, specific) for antigens expressed by vascular endothelial cells such as factor VIII (von Willibrand factor), CD-31 or CD-34 and then

counting (under high power) the number of highlighted vessels in so-called vascular 'hotspots' i.e. localized areas where there are unusually high numbers of vessels, as detected first under lower power magnification.³⁷

ANGIOGENIC ASSAYS

An assay is an investigative procedure in laboratory medicine, pharmacology and molecular biology for qualitatively assessing or quantitatively measuring the presence or amount or the functional activity of a target entity which can be a drug or a biochemical substance or a cell in an organism or organic sample. An angiogenic assay is used to study the functional activity of the endothelial cells and various angiogenic molecules, tumour cells and also to measure the efficacy of the various anti-angiogenic drugs that are being developed.³⁹

Assays that quantify the neo-vasculature are critical for studying the mechanism involved in angiogenesis using various angiogenic factors and also for the development of agents to combat angiogenic diseases like cancer and those that stimulate new vessel growth for the treatment of vascular disease. They are a must to validate data from in vitro studies.³⁹ These assays are based on the principle that new vessels will grow in an area that was previously devoid of blood vessels or has a vascular pattern clearly distinguishable from newly formed capillaries. The development of these assays paved the way for understanding the relationship of the angiogenic processes with various pathological conditions.⁴⁰ These assays are now being used for development of newer anti-angiogenic drugs by testing them in vitro and also in various experimental animals.³⁸

Various assays have been developed for studying the angiogenic process. These include:

- In vitro assays: these assays are performed in different types of matrices like matrigel which simulate the extracellular matrix.
- In vivo assays: the test substance (drug) is implanted into the experimental animal and effects are studied by measuring the microvessel density manually or by computer image analysis.
- Organ culture assays: the test substance is studied in a vessel derived from a rat or chick.⁴¹

The main technical challenge in any study of angiogenesis is the selection of the most appropriate assay. The ideal angiogenesis assay would be robust, rapid, reproducible with reliable readouts, automated computational analysis, multi-parameter assessment, including positive negative controls should relate directly to results seen in the clinic.³⁹ Despite the increasing numbers of both in vitro in vivo assays, a 'gold-standard' angiogenesis assay has yet to be developed; therefore, a combination of assays are required to identify the full range of effects of a test protein or to identify the molecular and or cellular events in angiogenesis.^{38,40}

TARGETING TUMOUR ANGIOGENESIS FOR CANCER PREVENTION

The concept of "anti-angiogenesis" was first proposed in 1971 by Judah Folkman, who hypothesized that inhibition of neovascularization at an early stage of cancer development could prevent tumour growth and metastases; and maintain tumour dormancy.⁸ It has been established based on the vast literature that angiogenesis inhibition is an effective strategy to restrict cancer growth in animal models bearing a wide variety of cancers. To date, more than 300 angiogenesis-inhibitory molecules have been identified as potential drug candidates, including many natural and synthetic chemical entities. Selective targeting of angiogenic blood vessels is possible as a result of differential proliferation rates between normal and tumour-associated endothelium.⁴² The normal vasculature is highly quiescent, with only one in every 10,000 endothelial cells dividing at any given time, and a physiological doubling time ranging from 47 to 20,000 days.⁴³ In contrast, the doubling rate for tumour endothelium is 2-13 days. Thus, anti-angiogenic agents are selective in inhibiting proliferating tumour vasculature, but do not affect normal blood vessels.²⁹

CONCLUSION

The relationship of angiogenesis and neoplasm is a well established factor. However studies related to the role of angiogenesis and oral squamous cell carcinoma is still limited. It is important to understand the potential of anti-angiogenic therapy if we combat this deadly disease from all fronts.

REFERENCES

1. Moehler TM, Ho AD, Goldschmidt H, Barlogie B. Angiogenesis in hematologic malignancies. *Critical reviews in oncology/hematology* 2003; 45: 227-44.
2. Lingen MW. Angiogenesis in the development of head and neck cancer and its inhibition by chemopreventive agents. *Crit Rev Oral Biol Med* 1991; 10(2): 153-64.
3. Pepper MS. Manipulating angiogenesis – From basic science to bedside. *Arteriosclerosis, Thrombosis, and Vascular Biology* 1997; 17: 605-19.
4. Risau W. Mechanisms of angiogenesis. *Nature* 1997; 386: 671-4.
5. Polverini PJ. The pathophysiology of angiogenesis. *Crit Rev Oral Biol Med* 1995; 6: 230-247.
6. Trapero JC, Sanchez JC, Sanchez BP, Gutierrez JS, Gonzalez MA, Martinez AB. Update on molecular pathology in oral cancer and precancer. *Anticancer Res* 2008; 28: 1197-1206.
7. Barrett JC. Mechanisms of multistep carcinogenesis and carcinogen risk assessment. *Environ Health Perspect* 1993; 100: 9 -20.

8. Folkman J. Fundamental concepts of the angiogenic process. *Current molecular medicine* 2003; 3: 643-51.
9. Weidner N, Semple JP, Welch WR, Folkman J. Tumour angiogenesis and metastasis – correlation in invasive breast carcinoma. *N Engl J Med* 1991; 324: 1-8.
10. Kerbel RS. Tumour angiogenesis: Past, present and the near future. *Carcinogenesis* 2000; 21(3): 505-15.
11. Folkman J, Klagsbrun. Angiogenic factors. *Science* 1987; 235: 442-7.
12. Gerwins P, Skoldenber E, Welsh LC. Function of fibroblast growth factors and vascular endothelial growth factors and their receptor in angiogenesis. *Crit Rev Oncol Hematol* 2000; 34: 185-194.
13. Roskoski R. VEGF receptor protein-tyrosine kinases: Structure & regulation. *Biochem Biophys Res Commun* 2008; 375: 287-91.
14. Forootan SS, Ke Y, Jones AS, Helliwell TR. Basic fibroblast growth factoring angiogenesis in squamous carcinoma of the tongue. *Oral oncology* 2000; 36: 437-443.
15. Thomas M, Augustin HG. The role of angiopoietins in vascular morphogenesis. *Angiogenesis* 2009; 12: 125-37.
16. Andrae J, Gallini R, Betsholtz C. Role of platelet-derived growth factors in physiology and medicine. *Genes Dev* 2008; 22: 1276-1312.
17. Pardali E, Ten Dijke P. Transforming growth factor-beta signalling and tumor angiogenesis. *Front Biosci* 2009; 14: 4848-61.
18. Siar CH, Oo VPA, Nagatsuka H, Nakano K, Ng KH, Kawakami T. Angiogenic squamous dysplasia-like phenomenon in oral epithelial precursor lesions. *Eur J Med Res* 2009; 14: 315-319.
19. Yu JL, Rak JW. Inflammatory and immune cells in tumour angiogenesis and arteriogenesis. *Breast Cancer Res* 2003; 5: 83-8.
20. Lorusso G, Ruegg C. The tumour microenvironment and its contribution to tumour evolution toward metastasis. *Histochem Cell Biol* 2008; 130: 1091-1103.
21. Chang SH, Liu CH, Conway R, Han DK, Nithipatikom K, Trifan OC. Role of prostaglandin E2-dependent angiogenic switch in cyclooxygenase 2-induced breast cancer progression. *PNAS* 2004; 101(2): 591-6.
22. John A, Tuszynski G. The role of matrix metalloproteinases in tumour angiogenesis and tumour metastasis. *Pathol Oncol Res* 2001; 7(1): 14-23.
23. Deryugina EI, Quigley JP. Matrix metalloproteinases and tumour metastasis. *Cancer Metastasis Rev* 2006; 25: 9-34.
24. Kurahara S, Shinohara M, Ikebe T, Nakamura S, Beppu M, Hiraki A, Takeuchi H, Shirasuna K. Expression of MMPs, MT-MMP, and TIMPs in squamous cell carcinoma of the oral cavity: correlations with tumor invasion and metastasis. *Head Neck* 1999; 21: 627-38.
25. Yoon SO, Park SJ, Yun CH, Chung AS. Roles of matrix metalloproteinases in tumour metastasis and angiogenesis. *J Biochem Mol Biol* 2003; 36(1): 128-137.
26. Bornstein P. Thrombospondins function as regulators of angiogenesis. *J Cell Commun Signal* 2009; 3: 189–200.
27. Indraccolo S. Interferon-alpha as angiogenesis inhibitor: learning from tumor models. *Autoimmunity* 2010; 43(3): 244-7.
28. Baker AH, Edwards DR, Murphy G. Metalloproteinase inhibitors: biological actions and therapeutic opportunities. *J Cell Sci* 2002; 115: 3719-3727.
29. Lopez O, Magarino Y, Delgado R. The angiogenic process and cancer. *Biotechnologia aplicada* 2009; 26: 111-116.
30. Makrilia N, Lappa T, Xyla V, Nikolaidis I, Syrigos K. The role of angiogenesis in solid tumours: An overview. *Eur J Intern Med* 2009; 20: 663-71.
31. Ascani G, Balercia P, Messi M, Lupi L, Goteri G, Filosa A et al. Angiogenesis in oral squamous cell carcinoma. *Acta Otorhinolaryngol Ital* 2005; 25: 13-17.
32. Noonan DM, Ventura A, Bruno A, Pagani A, Albini A. The Angiogenic Switch: Role of Immune Cells. *Immunologic Signatures of Rejection* 2011, 57-75.
33. Alkhabuli J. Significance of Neo-Angiogenesis and Immuno-Surveillance Cells in Squamous Cell Carcinoma of the Tongue. *Libyan J Med* 2007; 2: 30-39.
34. Koontongkaew S. The Tumor Microenvironment Contribution to Development, Growth, Invasion and Metastasis of Head and Neck Squamous Cell Carcinomas. *J Cancer* 2013; 4(1):66-83.
35. Fan F, Schimming A, Jaeger D, Podar K. Targeting the Tumour Microenvironment: Focus on Angiogenesis. *Oncology* 2012; 1-16.
36. Jin Y, Tipoe GL, White FH, Yang L. A quantitative investigation of immunocytochemically stained blood vessels in normal, benign, premalignant and malignant human oral cheek epithelium. *Virchows Arch* 1995; 427: 145-51.
37. Li SH, Hung PH, Chou KC, Hsieh SH, Shieh YS. Tumour Angiogenesis in Oral Squamous Cell Carcinomas: The Significance of Endothelial Markers and Hotspot Selection. *J Med Sci* 2009; 29(2): 67-74.
38. Staton CA, Reed MWR, Brown NJ. A critical analysis of current in vitro and in vivo angiogenesis assays. *Int. J Exp Path* 2009; 90: 195–221.
39. Goodwin AM. In vitro assays of angiogenesis for assessment of angiogenic and anti-angiogenic agents. *Microvasc Res.* 2007; 74(2-3): 172–183.
40. Aeurbach R, Lewis R, Shinnars B, Kubai L, Akhtar N. Angiogenesis Assays: A Critical Overview. *Clin Chem* 2003; 49(1): 32-40.
41. Tahergorabi Z, Khazaei M. A review on angiogenesis and its assays. *Iran J Basic Med Sci* 2012; 15(6): 1110-26.
42. Bertolini F, Mancuso P, Bobbi A, Pruneri G. The thin red line: Angiogenesis in normal and malignant hematopoiesis. *Exp Hematol* 2000; 28: 993–1000.
43. Li WW, Li VW, Hutnik M, Chiou AS. Tumour angiogenesis as a target for dietary cancer prevention. *Oncology* 2011; 1-23.