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Semaphorin4D expression in inflammatory tissue

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CHAPTER I

Introduction

Inflammation is important in disease progression and healing.

Various exogenous and endogenous stimuli can provoke a complex reaction in the vascularized connective tissue called inflammation. Inflammation is fundamentally a protective response the ultimate goal of which is to rid the organism of both initial cause of cell injury and the consequences of such injury. Without inflammation infections would go unchecked, wound would never heal, and injured organs might remain permanent festering sores. Inflammation is divided to acute and chronic patterns. Acute inflammation is relatively short duration and its main characteristics are the exudation of fluid and plasma proteins and the emigration of leukocytes, predominantly neutrophils. Chronic inflammation is a longer duration and is associated histologically with the presence of lymphocytes and macrophages, the proliferation of blood vessels, fibrosis, and tissue necrosis. Many factors modify the course and histologic appearance of both acute and chronic inflammation.

Angiogenesis is a critical step in chronic inflammation and tumorogenesis.

Angiogenesis is a biological process of endothelial cell (EC) sprouting to form new blood vessels from existing ones. In adults, angiogenesis occurs physiologically in the ovarian cycle and wound healing, and also in pathological processes, including chronic inflammation associated with rheumatoid arthritis and psoriasis, and malignancies such as glioblastoma and osteosarcoma. [12]

Increased formation of new blood vessels is one of the characteristic features in chronic inflammation in which neovascularization is persistent but controllable at the early stages of pathology. Another type of vascularization, namely adaptive arteriogenesis, is also generated in chronic inflammatory tissues. In adaptive arteriogenesis, the preexisting collateral arterioles grow chronically until a supply artery is occluded. This is different from the true angiogenesis that defines the growth of newly formed capillaries. The vessels in adaptive arteriogenesis can provide a

functional and sufficient blood circulation to the damaged site. Angiogenesis in inflammation has its pros and cons. Newly formed blood vessels provide oxygen and nutrients and bring leukocytes to the lesion, which are required for tissue repair. However, activated leukocytes release inflammatory cytokines and proteolytic enzymes that may cause tissue damage, leading to excessive angiogenesis and the persistence of inflammatory responses. Moreover, chronic and persistent inflammation may predispose a lesion to malignant transformation such as in the development of skin and gastric cancer. [12]

In cancer, the physiologic control angiogenesis through a series of "on" and "off" regulatory is broken. Angiogenic growth factors are produced in excess of angiogenesis inhibitors or inhibitors are present in less than stimulators. [12] Commonly know pro-angiogenic factors are vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), placental growth factor (PlGF), and matrix metalloproteinases (MMPs). As the tumor grows into an avascular area, there is an increasing need for nutrients and oxygen supply from the blood, leading to induced transcription of the gene encoding VEGF. Interaction between VEGF and its receptor VEGFR results in proliferation and migration of bone marrow-derived circulating endothelial precursors, which subsequently differentiate into vascular endothelial cells. At the same time, tumor-related monocytes and macrophages, derived from hemopoietic stem cells, secrete angiogenic growth factors as well as proteases to remodel the neovasculature. As the tumor grows larger, increasing need for oxygen causes hypoxia in the tissue, leading to increased production of angiogenic factor, which in turn, stimulate angiogenesis further. [10]

A role of semaphorins and plexins in tumor-induced angiogenesis.

The semaphorins are a large family of phylogenetically conserved membrane bound and secreted molecules originally identified as factors that promoted axon growth cone collapse. Early studies can identify them on red blood cells and lymphocytes. In fact, semaphorins and their receptors, the plexins and neuropilins, now have been implicated in a host of motility responses in different cell types and tissues in both health and disease, including regulation of neural crest cell migration,

loss of cell–cell contacts and branching morphogenesis in epithelium, lymphocyte activation and chemotaxis in the immune response, regulation of angiogenesis, and growth and metastasis of tumors.

Semaphorins play a critical role in blood vessel guidance and endothelial precursor cell homing during physiological and pathological blood vessel development. Among the vertebrate semaphorins, the class 3, which are secreted semaphorins, is the most thoroughly investigated in this regard. Many semaphorins from this class have been shown to be involved in vascular development and exhibit mostly inhibitory or anti-angiogenic effects on endothelial cells for instance Semaphorins3A, 3C, 3E, 3F, 4A, 4D, 6A and 6D. [9]

Semaphorin4D (Sema4D, also called CD100) appears to bind to its receptor, Plexin-B1, directly. These receptors promote branching morphogenesis and axonal guidance in neuronal tissues and proliferation, enhanced cell motility and metastasis in tumor cells when bound by the ligand scatter factor-1 (also called hepatocyte growth factor or HGF). Plexin-B1 is phosphorylated on tyrosine residues upon binding to Sema4D, suggesting that activation of a protein tyrosine kinase phosphorylation cascade may take place as part of the Plexin-B1-initiated signal. Work done by Conrotto and colleagues showed that the Plexin-B1/c-Met interaction may be responsible for a pro-angiogenic response observed in Sema4D treated endothelial cells. [11] Basile and et al. have observed phosphorylation and activation of Plexin-B1 in Sema4D treated endothelial cells and were the first to show a pro-angiogenic response in these cells to Sema4D. [9]

From gene array and immunoblot analyses, Basile and colleagues detected high levels of both Sema4D protein and mRNA in a panel of cell lines derived from primary and metastatic HNSCC, but not in non-tumorigenic epithelial cell line controls. Immunohistochemical analysis of Sema4D in a large collection of tumor samples revealed robust Sema4D expression in the cytoplasm and in particular on the cell surface of invading islands of SCC of the oral cavity and head and neck, as well as in other epithelial derived tumors, such as prostate, colon, breast and lung cancers, but not in normal or dysplastic but non-invasive epithelial cells. Knockdown of Sema4D via expression of shRNAs in HNSCC lines resulted in attenuation of the ability of

HNSCC to induce endothelial cell migration and significantly reduced growth and vascularity of tumor xenografts in nude mice, collectively providing the first in vivo observations supporting a role for Sema4D in the control of tumor growth and angiogenesis. [14]

It has long been known that chronic wounds are at risk for neoplastic progression. The risk of squamous cell carcinoma (SCC) is markedly increased, suggesting that keratinocytes are especially vulnerable to malignant transformation. Very little is known about the molecular inducers of tumorogenesis in chronic wounds. The tumor cell microenvironment, in particular the tumor stroma, appears to play an important role in the control of neoplastic progression through dynamic reciprocity between tumor cells and their surrounding tissue. The inflammatory response within the tumor stroma, which is closely linked to fibroplasia and angiogenesis, has been considered to play a critical role in carcinogenesis. Inflammation, fibroplasia, and angiogenesis are cardinal events that are intimately linked to wound repair. Thus the chronic inflammatory microenvironment of the non-healing wound could be a risk factor for malignant transformation. [5]

Research questions

Does Semaphorin4D express by epithelial cells in an inflammatory tissue?

CHAPTER II

Literature Review

Inflammation

Inflammation is the response of living tissue to injury, characterized by heat, redness, pain, swelling and loss of function. The inflammation stimuli include physical agents, chemical agents, microbiological agents such as bacteria, viruses and immunologically mediated damage through antigen-antibody complexes. The functions of the inflammatory responses are to provide an exudate which brings proteins, fluids and cells to an area of damage to act as a local defense mechanism, to destroy and/or eliminate the injurious agent, and to break down the damaged tissue and remove the debris. The inflammatory process is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue. In the absence of inflammation, wounds and infections would never heal and progressive destruction of the tissue would compromise the survival of the organism. The body's ability to replace injured or dead cells and to repair tissues after inflammation is critical to survival.

Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells which are present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process. The exudates is derived from local vessels in the area of the tissue damage and contains fluid including salts; proteins including immunoglobulins; the end-product of the coagulation system-fibrin; and cells including polymorphs in the early stages and lymphocytes, plasma cells and mononuclear cells at a later stage.

Chronic inflammation is the one of the possible sequelae of acute inflammation where there is continuing inflammation at the same time as attempts at healing. Tissue destruction, with damage to both parenchymal cells and stromal framework, occurs in necrotizing inflammation and is a hallmark of chronic inflammation. As a consequence, repair cannot be accomplished solely by regeneration of parenchymal cells, even in organs whose cells are able to regenerate. Attempts at repairing tissue damage then occur by replacement of nonregenerated parenchymal cells by connective tissue, which in time produces fibrosis and scarring. There are four components to this process: formation of new blood vessels (angiogenesis), migration and proliferation of fibroblasts, deposition of ECM, maturation and organization of the fibrous tissue, also known as remodeling.

Repair begins early in inflammation. Sometimes as early as 24 hours after injury, if resolution has not occurred, fibroblasts and vascular endothelial cells begin proliferating to form (by 3 to 5 days) a specialized type of tissue that is the hallmark of healing, called granulation tissue. The term derives from its pink, soft, granular appearance on the surface of wounds, but it is the histologic features that are characteristic: the formation of new small blood vessels (angiogenesis) and the proliferation of fibroblasts. These new vessels are leaky, allowing the passage of proteins and red cells into the extravascular space. Thus, new granulation tissue is often edematous.

Successful repair after tissue injury requires resolution of the inflammatory response. However, whereas the knowledge about mechanisms and molecules inducing and perpetuating the inflammatory response is constantly increasing, mechanisms, that limit and down regulate this activity are less appreciated. Such mechanisms might include: downregulation of chemokine expression by anti-inflammatory cytokines such as IL-10 or TGF-b1 or upregulation of anti-inflammatory molecules like IL-1 receptor antagonist or soluble TNF receptor; resolution of the inflammatory response mediated by the cell surface receptor for hyaluronan CD44 receptor unresponsiveness or downregulation by high concentrations of ligands. Interestingly, recent in vitro data suggested that matrix metalloproteinases (MMPs)

can downregulate inflammation via cleavage of chemokines, which then act as antagonists.

The major cytokines that mediate inflammation are IL-1 and TNF (α and β). IL-1 and TNF share many biologic properties. IL-1 and TNF-α are produced by activated macrophages, TNF- β by activated T cells, and IL-1 by many other cell types as well. Their secretion can be stimulated by endotoxin, immmune complexes, toxins, physical injury, and a variety of inflammatory processes. Similar to growth factors, they can act on the same cell that produces them (an autocrine effect); on cells in the immediate vicinity, as in lymph nodes and joint spaces (a paracrine effect); or systematically, as with any other hormone (an endocrine effect). Their most important actions in inflammation are their effect on endothelium, leukocytes, and fibroblasts and induction of the systemic acute –phase reactions. In endothelium, they induce a spectrum of changes mostly regulated at the level of gene transcription refereed to as endothelial activation. In particular, they induce the synthesis of endothelial adhesion molecules and chemical mediators including other cytokines, chemokines, growth factors, eicosanoids, and nitric oxide (NO); production of enzymes associated with matrix remodelin; and increases in the surface thrombogenicity of the endothelium. TNF also causes aggregation and priming of neutrophils, leading to augmented responses of these cells to other mediators and the release of proteolytic enzymes from mesenchymal cells, thus contributing to the tissue damage.

Two receptors, TNF-R1 (TNF receptor type 1) and TNF-R2 (TNF receptor type 2), bind to TNF. TNF-R1 is constitutively expressed in most tissues, and can be fully activated by both the membrane-bound and soluble trimeric forms of TNF, while TNF-R2 is only found in cells of the immune system and respond to the membrane-bound form of the TNF homotrimer. As most information regarding TNF signaling is derived from TNF-R1, the role of TNF-R2 is likely underestimated. Upon contact with their ligand, TNF receptors also form trimers, their tips fitting into the grooves formed between TNF monomers. This binding causes a conformational change to occur in the receptor, leading to the dissociation of the inhibitory protein SODD from the intracellular death domain. This dissociation enables the adaptor protein TRADD to bind to the death domain, serving as a platform for subsequent protein binding.

Following TRADD binding, three pathways can be initiated; activation of NF-kB, activation of MAPK pathways and induction of death signaling.

IL-1 has two forms: IL-1α and IL-1β. These proteins are synthesized as 31 kDa precursors in the cytoplasm of cells and lack leader sequences. In the human most pro-IL-1α is transmitted to the plasma membrane or to the nucleus of cells; some IL-1 α is released from cells as a mature 17 kDa form. After appropriate cell stimulation, pro-IL-1α is processed at the plasma membrane by a specific enzyme, IL-1α converting enzyme (ICE or caspase 1), and released as mature 17 kDa molecules. Pro-IL-1β and the mature forms of both cytokines are biologically active whereas pro-IL-1β lacks biological activity.

There are two specific IL-1 receptors. The biologically active IL-1RI is an 80 kDa protein with a long cytoplasmic domain of 215 residues. The biologically inert IL-1RII is a 60 kDa protein with a short cytoplasmic domain of 29 residues. The IL-1RII functions as a decoy receptor, binding IL-1 both on the plasma membrane and as a soluble receptor in the fluid phase, preventing IL-1 from interacting with the functional IL-1RI. After binding of IL-1α or IL-1β to the single chain IL-1RI, a second chain is brought into the complex, called the IL-1 receptor accessory protein (IL-1R AcP). Signal transduction pathways activated by the approximated cytoplasmic domains of the IL-1RI and the IL-1R AcP include the NF-κB, JNK/AP-1, and p38 MAP kinase pathways. Two cytoplasmic proteins, MyD88 and Tollip, are first recruited to the receptor complex, with subsequent addition of IRAK. This serine-threonine kinase is phosphorylated and then dissociates from the complex to interact with TRAF6. This key protein may lead to activation of the NF-κB pathway through a series of kinases and phosphorylation steps involving TRAF6, the MAPKK kinase TAK1, NKK, and IKK. IκB is eventually phosphorylated, dissociating from NF-κB with subsequent movement of this protein complex to the nucleus and binding to regulatory DNA sequences. The JNK/AP-1 pathway also is activated by TRAF6 through a different mechanism not involving phosphorylation. Stimulation of cells by IL-1 initiates the transcription of many pro-inflammatory genes including IL-6 and IL-8. [6]

Angiogenesis

The final, intricate branching pattern of blood vessels observed in large multicellular organisms is established by the coalescence and organization of primitive capillaries from individual endothelial cells in a process known as vasculogenesis. Vasculogenesis is followed by angiogenesis, the creation and remodeling of new blood vessels from this pre-existing vascular network. It is not known if the pattern of peripheral blood vessel branching is controlled by the endothelial cells themselves or to what degree it is influenced by the surrounding structures. [9]

Common processes of angiogenesis The process of angiogenesis in chronic inflammation and cancer includes the following common sequential steps: (1) activation and formation of the angiogenic phenotype of EC; (2) changes in the extracellular matrix and degradation of the basement membrane; (3) proliferation and migration of EC; (4) formation of preliminary tubules; (5) remodeling of newly formed microvessels.

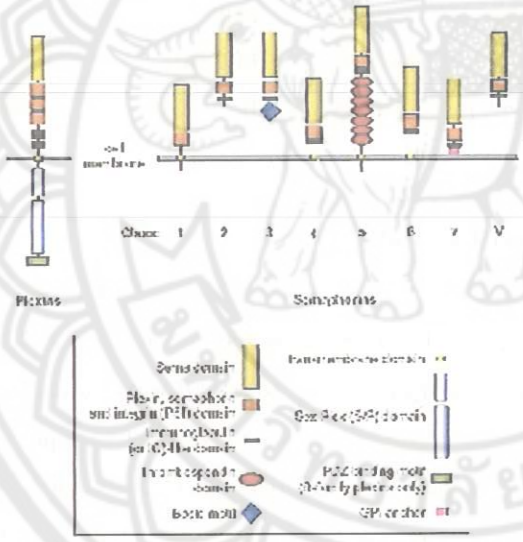
Among these, survival, proliferation and migration of EC from existing vessels are crucial. Imbalanced expression of pro- and antiangiogenic factors and their receptors on EC may determine the generation or regression of new blood vessels. More than 200 proangiogenic factors have been identified. Among those, the most important factors include vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), angiopoietin (Ang) and chemokines. These factors initiate angiogenesis by modulating the migration and/or proliferation of EC and the formation of neovasculature. The main targets of angiogenic stimuli are EC from postcapillary and small terminal venules. On the other hand, antiangiogenic factors including angiostatin, endostatin and vasostatin inhibit various pathways of angiogenesis. [12]

Tumor progression and metastasis in large part depend upon the acquisition of an angiogenic capacity by the cells forming the tumor. Fast growing tumors become hypoxic because the tumor cells overwhelm the ability of the vasculature to meet their high metabolic demands. Paradoxically, it is the hypoxic environment generated in a tumor as it rapidly outgrows its blood supply that eventually leads to the switch from an avascular to a neovascular phenotype. This process turns on the production of

membrane bound pro-angiogenic proteins and soluble survival factors that induce proliferation and migration in surrounding endothelial cells. Many of these factors are components of signaling systems involved in normal angiogenesis that are co-opted by the tumor to promote tumor cell survival and metastasis.

Semaphorin

Semaphorins are secreted and membrane-bound, glycosylphosphatidylinositol (GPI)-anchored proteins that are known to regulate axonal path finding in the developing nervous system [2,15] Nevertheless Semaphorins are expressed in a variety of adult and embryonic tissues outside the nervous system. Recent evidence suggests that semaphorins are involved in the regulation of cell–cell interactions Semaphorins act by binding their cognate receptors, the plexin. [3]



Classification and structure of the semaphorins and plexins. Class 1 and 2 semaphorins are found in invertebrates and 3–7 in vertebrates. Class V represents a class of semaphorins found in some DNA viruses. Class 1, 4, 5 and 6 semaphorins span the cell membrane and class 7 semaphorins are bound directly to the cell membrane via a GPI linkage. Semaphorins belonging to class 2, 3 and V are synthesized in soluble form.

Semaphorin4D

Sema4D, a semaphorin originally identified for its activity in the immune system, where it promotes B cell aggregation and survival, and T-cell activation [1,7,13] This semaphorin exists as a 150-kDa transmembrane molecule and as a soluble form of 120 kDa that originates from cleavage of the extracellular moiety near the transmembrane portion. Experimental evidence shows that the soluble form of the semaphorin can promote many of the biologic effects observed in the immune system.

The activities of Sema4D are mediated by 2 receptors, Plexin B1, the high-affinity receptor widely expressed throughout different tissues and organs,2 and CD72, a low-affinity receptor found in cells of the hemangiopoietic lineage. [8]

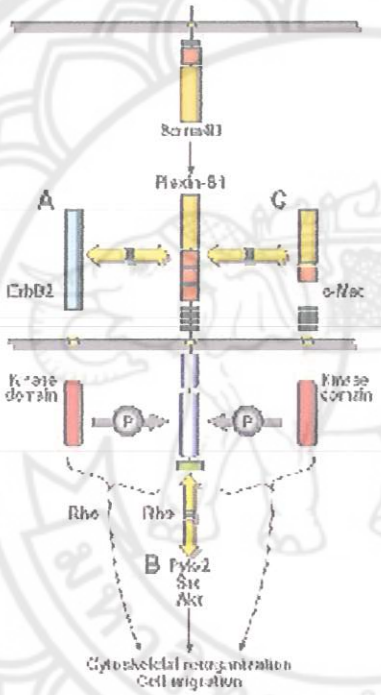
PlexinB1

Plexin, a family of transmembrane molecules sharing structural homology with semaphorins themselves.[3] Plexins have a homologous extracellular domain to the scatter factor receptors and are known to function as semaphorin receptors. Plexins have a large cytoplasmic domain that is conserved among family members and throughout evolution, but lacks homology to other known proteins. Plexin B1 has the highest similarity (28%) to scatter factor receptors and is also present outside the nervous system in tissues and organs where Met is expressed. The ligand for Plexin B1 is Semaphorin4D (Sema4D), a semaphorin that is expressed in both transmembrane and secreted forms (Elhabazi, et al., 2001, pp. 4341-4347). A low-affinity receptor (CD72) for Sema4D is expressed in cells of the immune system, but not in epithelial cells [8]

Mechanism of signaling of Sema4D-PlexinB1

Activation of the plexins initiates a signaling cascade that in many cases involves G-protein-mediated pathways. The G-proteins act as molecular switches that cycle between an active GTP-bound and inactive GDP-bound form to regulate actin and microtubule dynamics, gene expression, the cell cycle, and cell polarity and mobility trough their numerous downstream effectors. Their endogenous GTPase activity is controlled positively by guanine nucleotide exchange factors (GEFs), and negatively by the GAPs, among others. In addition to inhibiting R-Ras by functioning as a RasGAP, several groups have found that the class B plexins bind the Rho specific GEFs LARG (leukemia-associated RhoGEF) and PRG (PDZ–RhoGEF) at their C-terminal PDZ binding motifs and mediate activation of the small GTPase RhoA in response to ligation with semaphorins. There also is evidence to suggest that the plexins impinge upon pathways mediated by other GTPases such as Rac. There is data showing that Plexin-B1 competes with p21-activated kinase (PAK) for Rac binding, thus sequestering the active form of Rac and inhibiting Rac-dependent processes.

Since the cytoskeleton is required for cells to move and change shape, these results are consistent with a model where the plexins regulate actin and microtubule dynamics upon activation by the semaphorins to exert control over cell motility. Such a mechanism of action can be expanded to encompass moving cells of many different lineages, including endothelial cells responding to stimuli during normal and pathological angiogenesis.[9]



Three possible mechanisms for Sema4D-mediated Plexin-B1 phosphorylation and activation and promotion of angiogenesis in endothelial cells: (A) Binding of Sema4D to Plexin-B1 stimulates the tyrosine kinase activity of HER2/neu (ErbB-2), resulting in the phosphorylation of both Plexin-B1 and HER2/neu, which then induces RhoA activation. (B) Sema4D activates an intracellular tyrosine kinase cascade initiated by the non-receptor tyrosine kinases PYK2 and Src, which results in the tyrosine phosphorylation of Plexin-B1 and activation of Akt and Src in a Rho-dependent manner. (C) Binding of Sema4D to Plexin B1 stimulates the tyrosine kinase activity of Met, resulting in tyrosine phosphorylation of both receptors and a pro-angiogenic phenotype in endothelial cells.

Platelets express Sema4D, Plexin-B1, and CD72, a low-affinity receptor for Sema4D also found on lymphocytes. Sema4D is necessary for promotion of proper thrombus formation and is gradually shed from the surface following platelet activation in a process that requires ADAM17 (also called tumor-necrosis factor-alpha (TNF-α) converting enzyme, or TACE). ADAM17 is a member of a family of proteases that catalyze the turnover of the extracellular matrix and are involved in processing of some cell surface proteins, ligands and receptors (in particular, as the name implies, TNF--α). These findings suggest a dual role for Sema4D in vascular responses to injury; promotion of thrombus formation, and then, as the thrombus

matures, Sema4D is shed from the platelet surface and becomes available to interact with receptors on endothelial cells to initiate vascular repair and recovery of the endothelial monolayer and promotion of new vessel growth. [4]

CHAPTER III

Methodology

1. Tissue collection

All tissues were collected from Dental Hospital, Faculty of Dentistry, Naresuan University, Phitsanulok, Thailand and kept in 10% formalin until use. Granulation tissues, either chronic apical granuloma and other type of inflammatory intraoral tissue were obtained from patients who had conventional dental treatments. All patients were listened and read all the details of this experiment and signed in consent forms before we retrieved the tissue. Ethic approval was obtained from Ethics Committee for Human Research, Naresuan Universtiy, Phitsanulok, Thailand. All tissue had diagnosed by an oral pathologist before use in immunohistochemical analysis.

2. Tissue preparation

Step	Concentration/Solution	Temperature	Duration
1	10% Formalin	4° C	6 hrs.
2	PBS I	4° C	30 min.
3	PBS II	4° C	30 min.
4	70% EtOH I	4° C	30 min.
5	80% EtOH II	4° C	30 min.
6	95% EtOH I	4° C	30 min.
7	95% EtOH II	4° C	30 min.
8	100% EtOH I	RT	30 min.
9	100% EtOH II	RT	30 min.
10	Xylene I	RT	Until EtOH gone
11	Xylene II	RT	Until EtOH gone

Step	Concentration/Solution	Temp	Duration
12	Xylene III	RT	1 hr.
13	Paraffin I	56° C	1 hr.
14	Paraffin II	56° C	1 hr.
15	Paraffin III	56° C	1 hr.
16	Embedding Pure paraffin	4° C	

3. Immunohistochemistry

Tissue were serially sectioned, and mount on silane coated slides (Superfrost® plus, Thermo Scientific, ESCO) following standard procedures. Slides were hydrated through graded alcohols, incubated in 3% hydrogen peroxide for 30 min and 2% Bovine serum albumin (BSA) in Phosphate buffer saline with 0.1% Tween for 1 h at room temperature, and treated with Sema4D antibody (BD Transduction Laboratories) 1:50 dilution overnight at 4°C. The slides were washed in PBS, incubated with biotinylated secondary antibody (1:400 dilution; Vector Laboratories) for 1 h, and treated with ABC complex (Vector Stain Elite; Vector Laboratories) for 30 minute at room temperature. The slides were developed in 3,3-diaminobenzidine (Serva) and counterstained with methyl green or light green. Images were taken by using a digital camera through an Olympus stereomicroscope.

CHAPTER IV

Results

Figure 1: Chronic apical periodontitis
(A) Hematoxylin and eosin staining of granulation tissue from periapical region with clinical diagnosis of chronic apical periodontitis. (B,C) Immunohistochemistry with Sema4D primary antibody. (D) Granulation tissue with no signal of Sema4D detection in another sample from chronic inflammatory tissue.

Immunohistochemical analysis in granulation tissue of chronic apical periodontitis failed to detect Sema4D. We only found a positive staining from lymphocytes and immune cells which is an internal positive control of Semaphorin4D expression.

In chronic inflammatory tissue, the epithelium is presented as in normal epithelium although some distorted shapes are found because of a boiling method in

antigen retrieval process. (Fig 1B) There is no evidence of positive staining of membrane protein, Semaphorin4D in oral epithelial cells. There are a lot of small capillary vessels in connective tissue layer. Sema4D was detected in the associated chronic inflammatory cells accumulating in the connective tissue layer and the epithelial–connective tissue interface. (Fig. 1B-C) In some chronic inflammatory tissue, most of chronic inflammatory cells are plasma cells which are not express Semaphorin4D. (Fig 1D)

Figure 2 : Lichen planus
(A) Hematoxylin and eosin staining of lichen planus from intraoral region. (B-D) Immunohistochemistry with Sema4D primary antibody.

Oral lichen planus specimen showing hyperkeratosis, interface lymphocytic infiltrate and basilar vacuolization with apoptosis (Fig. 2A) but failed to exhibit Sema4D expression in the epithelial cells expression as same as in chronic periapical periodontitis sections. Sema4D was detected in the associated chronic inflammatory

cells accumulating at the epithelial–connective tissue interface (Fig. 2B-C). In contrast, we detected robust immunohistochemical staining for Sema4D in the cytoplasm and cell surface in invading islands of transformed epithelial cells (Fig. 3C), which is more clearly seen at a higher magnification (Fig. 1*C Insets*). Taken together, these results demonstrate Sema4D expression in HNSCC tumors and their derived cells lines but not in normal and noninvasive oral epithelium.

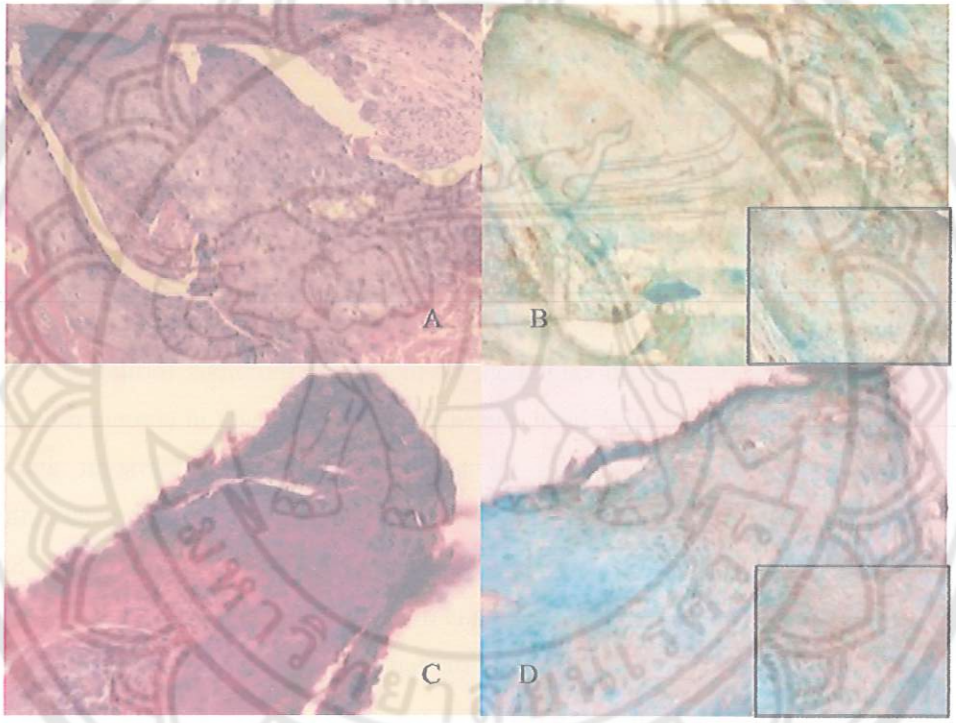


Figure 3: Well-differentiated squamous cell carcinoma
(A, C) Hematoxylin and eosin staining of well-differentiated squamous cell carcinoma from intraoral region. (B, D) Immunohistochemistry with Sema4D primary antibody.

CHAPTER VI

Discussion

Although the study on angiogenesis began almost a century ago, its importance had not been completely appreciated until the recent decades. Numerous studies have focused on the general process of angiogenesis with little attention paid to the differences between angiogenesis in chronic inflammatory diseases and in cancer. While angiogenesis in these two pathological states shares similarities, clear distinction in morphology and ontogeny exists. More careful investigation of the mechanisms underlying the differences should lead to the development of novel and unique therapeutic strategies for diseases involving angiogenesis, in particular, malignant tumors. [12]

Semaphorin4D can express and release by many kinds of cell including cancer cell, lymphocyte and platelet. It functions as a potent proangiogenic molecule results in endothelial cell migration, promotion of thrombus formation and tumor angiogenesis. On the other hand, the role of Semaphorin4D in chronic inflammatory tissue which is characterized by high blood vessel proliferation and active inflammation has not been elucidated. Sema4D might be a key regulator that link between chronic inflammatory process and tumorogenesis. The study of Sema4D molecular mechanism on epithelium and other cells during inflammatory process may be an important basic knowledge that support further advanced studies in treatment of chronic inflammatory disease and cancer.

However, we failed to detect any expression of Semaphorin4D in epithelial cells in chronic inflammatory tissue from intraoral origin. Only internal positive controls, lymphocytes and platelets, were found. This may be because the chronic inflammatory process is a very complicated process. It contains variety of cells and protein which is involve. Semaphorin4D main source is may be from an inflammatory cell which is very abundant in this process. The epithelial cells may not necessary to produce this protein. Commonly know pro-angiogenic factors, VEGF, bFGF, EGF, PDGF, PlGF and MMPs may be sufficient to supply a vascular growth factor for produce new blood supply.

In the other hand, the selected sections may not suitable for looking for Semaphorin4D expression. The transitional process from long term chronic inflammation may be critical. Only chronic inflammatory tissues which are stimulated by external stimuli such as cell stress, UV light or chronic irritation and will become to cancer may be a stage that exhibit Semaphorin4D. However, this is the first study that focuses on Semaphorin4D expression in an inflammatory tissue. In the future we will use a wide variety of chronic inflammatory tissue and try to collect specifically in a transitional stage to cancer. Moreover we will try to create an in vitro mouse model to stimulate chronic inflammation which become a cancer which we can collect and investigate each histopathological process.

BIOGRAPHY

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Professional Societies

1998-present Member, The Dental Association of Thailand

2004-present Academic Subcommittee, Thai Society of Pediatric Dentistry

Research

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