METASTATIC PROCESS OF ORAL SQUAMOUS CELL CARCINOMA

(PROSES METASTASE SEL KARSINOMA ORAL SKUAMOUS DI RONGGA MULUT)

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Abstract

Squamous cell carcinoma (SCC) is the most common malignant tumour in oral cavity. This cancer arises from oral mucosal epithel and also it often causes high rate of morbidity and mortality. Metastasis is one of the major causes of mortality in patient with cancer. This is because metastasis has often occurred before the primary tumor it self is detected. Lymphogenous metastasis is specific for carcinoma. In the late stage, cancer cells from the lymphatic system may enter blood circulation. In brief, steps of the metastatic process may be outlined as follows: genetic alteration, modulation of extracellular matrix and proteolysis, damage cell adhesion, damage tumour cell migration, and also angiogenesis. The involved process factors are genetic factors such as nm23, p16, Lin-7C; proteolitic enzyme such as MMP-1, MMP-2, MMP-3, MMP-8 MMP-9, MMP-14, MMP-15, MMP-17, cathepsin B, D, and O; adhesion between adhesive proteins for example integrin, laminin, cadherin, maspin. In conclusion, the whole process from genetic alterations until the occurrence of independent cell is a complex process that involves interactions among the related factors.

Key words: squamous cell carcinoma, metastasis, extracellular matrix, proteolytic enzyme

Abstrak

Karsinoma sel skuamosa (KSS) merupakan jenis keganasan yang paling sering dijumpai di rongga mulut. Kanker ini berasal dari epitel mukosa mulut serta seringkali menyebabkan tingkat morbiditas dan mortalitas yang tinggi. Metastasis merupakan salah satu penyebab utama kematian pada penderita kanker. Hal ini disebabkan karena metastasis seringkali sudah terjadi sebelum tumor primer itu sendiri terdeteksi. Metastasis secara limfogen spesifik pada karsinoma. Pada stadium akhir, sel kanker dari sistem limfatik dapat pula memasuki sirkulasi darah. Secara garis besar tahap-tahap terjadinya metastasis meliputi perubahan genetik, modulasi matriks ekstraselular dan proteolisis, rusaknya adhesi sel, migrasi sel tumor, serta angiogenesis. Faktor-faktor yang terlibat antara lain faktor genetik seperti nm23, p16, Lin-7C; enzim proteolisis seperti MMP-1, MMP-2, MMP-3, MMP-8 MMP-9, MMP-14, MMP-15, MMP-17, cathepsin B, D, dan O; adhesi antar protein perlekatan seperti integrin, laminin, cadherin, maspin. Sebagai kesimpulan, keseluruhan proses yang terjadi mulai dari perubahan genetik sampai timbulnya kemampuan sel yang terlepas untuk hidup mandiri merupakan suatu peristiwa yang kompleks serta melibatkan serangkaian interaksi antara faktor yang terlibat di dalamnya.

Kata kunci: karsinoma sel skuamosa, metastasis, matriks ekstraselular, enzim proteolisis

INTRODUCTION

The most common oral cancer is squamous cell carcinoma which derives from oral mucosal epithel. High mortality rate due to its ability to invade surrounding tissue, spread throughout the body and metastasize to other areas. Metastasis is one cause of death risk in cancer patients. This is because metastasis has often occurred before the primary tumour it self is detected.

Metastasis process is influenced by several factors, such as matrix metalloproteinases (MMPs), genetic and angiogenesis factors. The purpose of this paper is to provide information about the stages of the process of metastasis in squamous cell carcinoma of oral cavity and the factors that contribute in the process.

Squamous Cell Carcinoma

Oral squamous cell carcinoma (OSCC) is a malignant tumour most often occurs in oral cavity. As

with other carcinomas, OSCC has properties as cancer in general such as; invade the underlying connective tissue and metastasize.[1] Lesions can occur at any place in oral cavity. The lips are most often affected as extraoral lesion, while in oral cavity, tongue is most often affected. OSSC etiology is unknown but the cause is multifactorial.

Metastasis

Metastasis is the spreading process of neoplastic cells from the primary to a more distant area. McMahon and Sloan[2] stated that there are various types of metastasis such as lymphogenous, hematogenous, transcoelomic, and implantation metastasis. However, two major pathways in metastasis are through lymphatic vessels (lymphogenous) and through the blood vessels (hematogenous). Sudiono [3] stated that when cancer cells enter the lymphatic system, several possibilities may occur such as defeated the cancer cells; cancer cells survived without causing a specific reaction; cancer cells through the lymph nodes without causing a response; cancer cells proliferate, and finally reach the lymph nodes by following or contrary to regional lymph flow.

The primary spread of carcinoma is through the lymph vessels. First, tumour cells must have the ability to invade the basement membrane as a transition from carcinoma in situ to invasive carcinoma. Therefore, cells must have the ability to synthesize the important enzymes to penetrate the lower membrane and interstitial substance. After penetrating the basal membrane, in the same way, adhesion between cells and cell-matrix damaged, finally cells are ready to metastasize. In general, metastatic OSCC occurs to ipsilateral cervical lymph gland. Carcinoma of the lower lip and floor of mouth tends to metastasize to the submental nodes, posterior oral cavity tumours tend to metastasize to the superior jugular and digastric nodes, while deposits into jugulo digastric retrofaringeal lymph nodes are often found in carcinoma of the oropharynx.[4] At the last stage, the lymphogenous spread can reach the blood vessels through the left thoraxical and right lymph duct.

Extracellular Matrix

Human body tissues are composed of various cells surrounded by extracellular matrix. Extracellular matrix is series of proteins and proteoglycans that supports the structure and function of the regulator on the network and plays a role in cellular migration and proliferation. Extracellular matrix contains of three major groups of biomolecules, which are structural and specialized proteins that have specific functions in the extracellular matrix; and proteoglycans which is a composition of long chains of repeating disaccharides called glycolsaminoglycans. [5]

Matrix Metalloproteinases (MMPs) and Tissue Inhibitors of Metalloproteinases (TIMPs)

Excessive and uncontrolled destruction of extracellular matrix components is signaling abnormallities from a network. MMPs are endopeptidase containing zinc that can degrade various extracellular structures, cell surface receptors, and cytokines.[6] MMPs are classified in different groups. Nagase et al.[7] stated that MMPs are divided in classes of collagenase, gelatinase, stromelisin, matrilisin, membrane type MMPs and other MMPs.

TIMPs are a group of secretory protein that inhibits the activity of MMPs. Currently there are four groups of TIMPs as follows: TIMP-1, TIMP-2, TIMP-3, and TIMP-4.

E. Laminin 5 (Ln-5)

Ln-5 is the main component of the complex hemidesmosome which has an important function in the basal membrane as cell adhesion, migration, proliferation, wound healing, skin homeosatasis, and development. Once secreted, Ln-5 in humans is found in the form of 440 kDa heterometric and 400 kDa.[1] Laminin influences the genetic information inside a cell's nucleus. Laminin has been previously shown to participate in tumor invasion and metastasis. Destruction of laminin can play a detrimental role in the early stages of tumor development.

E-Cadherin

Cadherin connecting epithelial and muscle cells with other cadherin are mostly through *adheren junctions* and desmosomes. Terminal cytoplasmic molecules E-cadherin associated with the actin cytoskeleton via α-catenin and β-catenin, has a role in adhesion of epithelial cells. It is important to establish and maintain intercellular relationships and morphogenesis. E-cadherin expression increased in the early developmental stages of OSCC and decreased in the invasive phase and increased again when the secondary tumour has been successfully established.[8] Detection of E-cadherin expression is very useful in determining metastasis to cervical lymph nodes.[9]

Integrin

Integrins are the major cellular receptor on the

extracellular matrix. Integrins consist of alpha and beta subunits that have the extracellular, trans-membrane and cytoplasmic domain. Integrin expression can be modulated by factors that influence the growth and differentiation, such as TGF-β (Transforming Growth Factor-β). Integrin expression decreased in the early stages of OSCC which then underwent reactivation, and the number increased in the invasive phase. Integrin expression eventually decreased again when the secondary tumour has been successfully established and is able to live independently.[8]

Maspin

Maspin is a cytoplasmic protein in various tissues of epithelial cells and function as the inhibitor protease by inhibited activator plasminogen tissues. Tumour cells produce activator plasminogen for cellular migration and invasion. Decreased expression of maspin associated with a poor prognosis.[10]

Chemokine receptor

In many malignant tumours, cells produce chemokine receptor and use the chemokine to metastasize to the target organs. Chemokine and its receptor are critical in the maturation of dendritic cells, B and T cell development, T-helper (Th1 and Th2) cell responses, infection, angiogenesis, and cell migration.[11]

Tumour suppressor genes

Products of tumour suppressor genes affect the control of proliferation and differentiation of cells. There are many tumour suppressor genes associated with squamous cell carcinoma. This paper will discuss those of which related only to metastases as follows: p16, nm-23, and Lin-7C/VELI3/MALS-3.

p16

P16 gene located in chromosome 9p21-22 binds to CDK4 (cyclin-dependent kinases) and CDK6 for inhibiting the complex catalytic activity of CDK-cyclin D-1 and preventing it to enter S phase cell cycle.[12] CDK4 and CDK6 are normally required for the phosphorylation of PRB (Retinoblastoma Protein) and mediate the transition of G1 to S phase. Loss of p16 gene in oral cancer indicates a poor prognosis.

nm-23

nm-23 provides the code to NDP (Nucleoside Diphosphate) kinase which involved in the transfer of phosphate terminal from a nucleoside triphospate into NDP. Loss of nm-23 gene is closely related to the high incidence of metastasis in some cancers.[12]

Lin-7C/ VELI3/ MALS-3

Lin-7C gene located in chromosome 11p14, it plays a role in metastasis of OSCC. Those genes mediate metastasis of OSCC with a signal from the Lin-7C-Cask (Lin-2)-CTNNB1 or β-catenin and clinically associated with the development of metastases to cervical lymph nodes if Lin-7C expression in primary tumours is decreased.[13]

Angiogenesis

Colonies of cancer cells require an adequate vascular system in order to live independently. The process of new blood vessel formation is called angiogenesis. Tumour cells produce factors that can trigger the formation of new capillaries. Cortesina and Martone[14] stated that there are several key factors that play a role in this process include VEGF, CD105 (endoglin) and interleukin-8.

DISCUSSION

In general way, the stages of metastasis include genetic changes; modulation of extracellular matrix and proteolysis in the form of the destruction of adhesion between cells and between cells with matrix; tumour cell migration; and angiogenesis.[14] A genetic alteration in the early stages of SCC is shown by loss of chromosome 9p21 which is contained of p16 gene. The gene functioned as an inhibitor of CDK4, CDK6 and set the regulation of cell proliferation.[12] CDK4 and CDK6 are normally required for phosphorylation of PRB and mediate the transition of G1 to S phase on the cell cycle. NM23 mechanisms in suppressing the metastatic process have been reported in some studies that in OSCC, there was decreased expression of NM23 in the form of wt-α both NM23-H1 and NM23-H2 that can enhance metastasis. Both NM23-H1 and -H2 repressed transcriptional activity driven by the PDGF-A (platelet-derived growth factor-A) basal promoter (−82 to +8). Activity of the negative regulatory region (−1853 to −883), which contains the 5′-SHS (S1 nuclease-hypersensitive site), was also inhibited modestly by NM23-H1 and NM23-H2. NM23-H1 interacts both structurally and functionally with DNA. NM23 proteins may repressing transcription of a growth factor oncogene, providing a possible molecular mechanism to explain the

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metastasis-suppressing effects therefore their decreasing can enhance metastasis process.

In the metastatic of OSCC has also decreased the expression of tumour suppressor genes Lin-7C. Lin-7C expression is required to increase the expression of β-catenin and to produce a non-invasive phenoltype.[13] Lin-7C is connected to β-catenin through a bond of Lin-7C-Cask (Lin-2)-CTNNB1 (β-catenin). Decreasing in Wnt-1 proto-oncogene also happened in SCC. Normal role of this gene is to stabilize cell adhesion which is mediated by cadherin.

Cortesina and Martone[14] revealed that overexpression of MET proto-oncogene (hepatocyte growth factor receptor) is oftenly found in the OSCC. MET encodes tyrosine kinase for hepatocyte growth factor (HGF). MET is rarely found in normal oral mucosa or in limited amounts in the basal layer. MET is a receptor for HGF. HGF alone can destroy the bonds between the cells by releasing β-catenin at the adheren junction. This can induce cell motility.

After initiation process of the tumour, one initial step of metastasis is loss of epithelial integrity involving the attachment of molecules. Based on previous literatures[8,10,11,14] the authors have tried to summarize the factors that involved in the process of invasion and metastasis in these following scheme (Figure 1).

Ln-5 is a major component of basal membrane which is composed by subunits α3, β3, and γ2. Expression ln-5 also found in high-invasive cells and γ2 subunit of laminin breakdown can be caused by bone morphogenetic protein-1 (BMP-1) and besides it there showed overexpression of MMP-2 and MT1-MMP, the protein will induce cell movement. Instead, the solution of the globular domain of laminin α3 would prevent the movement of cells.[8]

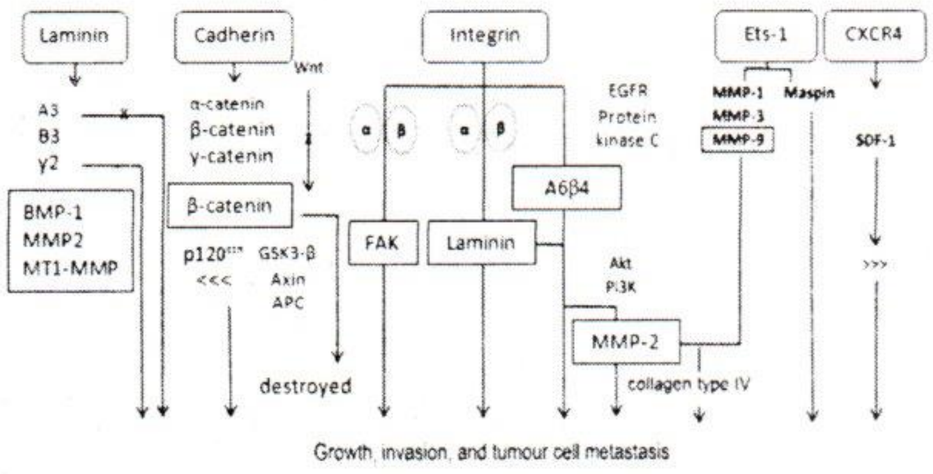

Figure 1. Factors involved in growth, invasion and metastasis process

Elements of cell attachment (adheren junction) consist of α-catenin, β-catenin, and γ-catenin. Catenin mediates transduction of cellular matrix contacts with cadherin. β-catenin can interact with different proteins in cells. Interaction of β-catenin with GSK3 (Glycogen Synthase Kinase 3), APC (adenomatous polyposis coli), and axin can enhance the activity of GSK3 which increased phosphorylation of β-catenin and β-catenin and as consequence is degradation of cellular adhesion matrix among cells. On the contrary, β-catenin can also interact with the lymphoid enhancing factor (LEF). Catenin and LEF complex occured in the nucleus and resulted in Wnt-1 proto-oncogene expression. Wnt-1 binds to the *frizzled* (fz) and activates *dishevelled* (DSH) and DSH activation can inhibit the activity of GSK3 until β-catenin is degraded which allows the presence of β-catenin in the nucleus of cells to interact with transcription factors and regulate gene transcription for both proliferation and apoptosis cells. P120ctn is also an adherent junction component that serves as a tumour suppressor by helping to stabilize E-cadherin.[16] However, in some malignancies decreased expression of p120ctn and the mechanism is not clearly known. Loss of p120ctn can induce the occurrence of metastasis.

Bonds between integrins induce phosphorylation of focal adhesion kinase (FAK). FAK can induce cell migration. High FAK expressions in SCC, increases the chance of tumour cells become invasive and metastasize. According to a study, EGFR (Epidermal Growth Factor Receptor) can trigger the migration of cells so that it induces the release of α6β4 from hemidesmosome.[8] The release of α6β4 can also be induced by the activation of protein kinase-C. α6β4 interacts with laminin and activate several ways of carcinoma cell invasion settings.

According to recent studies, tumour cell invasion was induced by α6β4 depends on the activity of phosphoinositides-3 OH kinase (PI3K) which produces the formation of specific α6β4. Bonds of α6β4 heterodimer protect cells from apoptosis through a pathway involving PI3K/ Akt (protein kinase B) and increased survival of cells which was lack of p53 gene. In addition, the β4 subunit of α6β4 can stimulate specific signaling role in cells motility, expression of MMP-2, and tumour invasion.[8]

Ets-1 protein is a transcription factor that controls the regulation of several matrix degrading proteinases, including maspin. Ets-1 can also activate expression of MMP-1, -3, and -9. In the invasive SCC, a decline in maspin expression and overexpression of the three MMP-inducing cells forwarded to invasive and metastasize.[10]

OSCC cell also expresses of CXCR4 (chemokines receptors) that interacts with specific ligands stromal cell derived-1 (SDF-1), associated with actin polymerization, pseudopodia formation, and induces cell motility and invasion. CXCR4expression is related with metastasis lymphogenous.[11]

The process of reduction in cell adhesion to matrix as described above, definitely related to matrix metalloproteinases (MMPs) that degraded extracellular macromolecules matrix in the basal membrane. MMPs is also plays a role in the process of angiogenesis. There are strong correlations among MMP-1, MMP-2, MMP-3, MMP-8 MMP-9, MMP-14, MMP-15, MMP-17 and MT-1 MMP in the potential metastatic of OSCC. However, Rosenthal and Matrisian,[14] stated that the prognostic value of MMP regulation is still unclear.

The other endopeptidase is cathepsin. Overexpression of cathepsin D and B on OSCC associated with regional lymph node metastasis.[15] Another study on the tongue carcinoma using *cDNA* microarray analysis stated cathepsin O is a marker in metastatic OSCC.[15]

The ability of cell motility that plays a role in metastasis process is very complex as the consequence of various factors described above. Finally, the detached carcinoma cells can enter lymphatic circulation to form metastatic deposits in the cervical lymph nodes. Cancer cells can also exit from the bloodstream into the lymphatic system in the thoracal or lymph duct. In doing so they may lead to metastasis to other organs.[4]

Finally, the process of angiogenesis is the result of an imbalance between pro and anti angiogenic factor produced by normal and tumour cells. Many angiogenic factors play a role, VEGF, CD105 (endoglin), and IL-8 are pro-angiogenic factors that is most widely studied. During the process of angiogenesis, TGF-β1 (Transforming Growth Factor-β1) and TGF-β3 are expressed. VEGF and TGF-β1 induce angiogenesis but have opposing effects on endothelial cells. VEGF protects endothelial cells from apoptosis; TGF-β1 induces apoptosis. VEGF/VEGF receptor-2 (VEGFR2) signaling mediates TGF-β1 induction of apoptosis. This finding raised an important question: Does this mechanism stimulate or inhibit angiogenesis? TGFB is a multifunctional peptide that controls proliferation, differentiation, and other functions in many cell types. TGFB acts synergistically with TGFA in inducing transformation. It also acts as a negative autocrine growth factor. Dysregulation of TGFB activation and signaling may result in apoptosis. VEGF-mediated apoptosis is required for TGF-β1 induction of angiogenesis. In vitro the apoptotic effect of TGF-β1 on endothelial cells is rapid and followed by a long period in which the cells are refractory to apoptosis induction by TGF-β1. CD105 can bind TGF-β1 and TGF-β3. Schimming and Marme[14] stated that CD105 expression in neoplastic tissue is much higher compared to those of normal mucosa.

There is also an increasing in VEGF-A and C that associates with lymph node metastasis[14] as VEGFs normal function is to create new blood vessels. In addition, poor prognosis is also shown by high expression of interleukin-8 (IL-8) that played a role in the process of angiogenesis. However TGF-β1 can inhibit the secretion and activity of many other cytokines including interferon-γ, tumor necrosis factor-alpha (TNF-α) and various interleukins while macrophages produce cytokines that can increase the production of VEGF and IL-8. Wanatabe et al.[17] suggested that IL-8 can be secreted in the OSCC and facilitated by MMP-7.

As conclusion, the metastatic process is complex series of interaction events among involving factors which are genetic changes, modulation of extracellular matrix and proteolysis, breakdown of adhesion between cells and matrix cells, tumour cell migration, and angiogenesis. Genetic changes that may involved in invasion-metastasis process include increasing or decreasing of gene expression such as decreasing in expression of p16, NM23-H1, Lin-7C, Wnt-1, TGM3, KRT16, and increasing expression of MET and RPS27. Matrix components destruction is influenced by MMPs and cathepsin enzymes as MMP-1, MMP-2, MMP-3, MMP-8 MMP-9, MMP-14, MMP-15, MMP-17, cathepsin B, D, and O. Finally, tumour cells released and migrated somewhere were able to grow and lived independently that required adequate system of vascularization and angiogenesis.

References

1. Katoh K, Nakanishi Y, Akimoto S, Yoshimura K, Takagi M, Sakamoto M, et al. Correlation between laminin-5 gamma2 chain expression and epidermal growth factor receptor expression and its clinicopathological significance in squamous cell carcinoma of the tounge. Oncology 2002; 62: 318-26.
2. McMahon RFT, Sloan P. Essentials of pathology for dentistry. Churchill Livingstone, China. 2002: 75-85.
3. Sudiono J. Pemeriksaan patologi untuk diagnosis neoplasma mulut. Jakarta: EGC Penerbit Buku Kedokteran. 2008; 34-46.
4. Neville BW, Damm DD, Allen CM, Bouquot JE. Oral and maxillofacial pathology. 3rd ed. India: Saunders. 2009; 409-19.
5. Murray RK, Granner DK, Mayes PA, Rodwell VW. Matriks ekstrasel. Biokimia Harper. Anna PH, Tiara MN. Sikumbang (editor). 25th ed. Jakarta: EGC Penerbit Buku Kedokteran.2003; 662-79.
6. Suojanen J. Studies on matrix metalloproteinase (MMP-2, -9, and -14) and β_2 integrin targeting as potential anticancer therapeutics (dissertation). Helsinki: University of Helsinki; 2009.

https:// oa. doria.fi/ bitstream/ handle/ 10024/ 45529/ studieso.pdf?sequence=1.
7. Nagase H, Visse R, Murphy G. Structure and function of matrix metalloproteinases and TIMPs. Cardiovascular Research 2006; 69: 562-69.
8. Ziober BL, Silverman SS, Kramer RH. Adhesive mechanisms regulating invasion and metastasis in oral cancer. Critical Reviews in Oral Biology and Medicine 2001; 12(6): 499-510.
9. Kaur G, Carnelio S, Rao N, Rao L. Expression of e-cadherin in primary oral squamous cell carcinoma and metastatic lymph nodes: an immunohistochemical study. Indian J Dental Research 2009; 20(1): 71-6.
10. Yoshizawa K, Nozaki S, Okamune A, Kitahara H, Ohara T, Kato K, et al. Loss of maspin is a negative prognostic factor for invasion and metastasis in oral squamous cell carcinoma. J Oral Pathology and Medicine 2009; 38: 535-9.
11. Ishikawa T, Nakashiro Koh-Ichi, Hara S, Sebastian KK, Li C, Shintani S, et al. CXCR4 expression is associated with lymph- node metastasis of oral squamous cell carcinoma. International J Oncology 2006; 28: 61-6.
12. Auerkari EA, Tjahayani A, Djaya W. Tumour suppression in oncogenesis of oral squamous cell carcinoma. Jurnal Kedokteran Gigi Universitas Indonesia 2000; 7 (Edisi Khusus): 72-82.
13. Onda T, Uzawa K, Nakashima D, Saito K, Iwadate Y, Seki N, et al. Lin-7C/VELI3/MALS-3: an essential component in metastasis of human squamous cell carcinoma. Cancer Res 2007; 67(20): 9643-8.
14. Cortesina G, Martone T. Molecular metastases markers in head and neck squamous cell carcinoma: review of the literature. Acta Otorhinolaryngol Ital 2006; 26: 317-25.
15. Shi Z, Stack MS. Review: Molecule of cell adhesion and extracellular matrix proteolysis in oral squamous cell carcinoma. Histol Histopathol 2010; 25: 1-13.
16. Moreno MP, Song W, Pasolli A, Williams SE, Fuchs E. Loss of p120 catenin and links to mitotic alterations, inflamations, dan skin cancer. The National Academy of Sciences of the USA. 2008; 105:15399-15404. http:// www. pnas. org/ content/ 105/40/15399.full.pdf+html.
17. Wanatabe H, Iwase M, Ohashi M, Nagumo M. Role of interleukin-8 secreted from oral squamous cell carcinoma cell lines. Oral Oncology 2002; 38: 670-9.