Extracellular matrix in invasion and metastasis of oral squamous cell carcinoma

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Abstract

Oral squamous cell carcinoma is a common cancer in developing countries with highly invasive and metastasis credentials. The Lymphnode metastasis in oral squamous cell carcinoma is regarded as the factor that decides on disease survival of patients. Steps have been made towards research in the field of Oral squamous cell carcinoma for better understanding of the molecular events involved in invasion and metastasis. Recently, the role of Extracellular matrix (ECM) of oral squamous cell carcinoma in invasion and metastasis has gained interest, as ECM is known to actively contribute in events that regulate transcriptional controls and cell signalling mechanisms involved in invasion and metastasis. Understanding such contributing role of ECM may pave way for newer methodologies in early detection, prevention and therapeutic strategies for oral squamous cell carcinoma.

Keywords: Cancer nomatrix and Matrikines, extracellular matrix (ECM), Invasion, Metastasis, oral squamous cell carcinoma,

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INTRODUCTION

Oral cancer along with pharyngeal cancer is regarded as the sixth most common cancer in the world with an increased incidence tagged to developing countries.[1] In India, oral cancer ranks as the most prevalent cancer in men[2] and the third most common cancer after cervical and breast cancer among women.[3] The age-standardized incidence rate of oral cancer in India accounts to 12.6/100,000 people. This high incidence of oral cancer in India has been attached to high exposure to sunlight due to farming, smoking, smokeless tobacco habits, alcohol, spicy food, neglected overall oral health and human papilloma virus.[3,4] The incidence of oral cancer appears to be increasing in several parts of the world, particularly in countries such as Australia, Japan and parts of Europe. Thus, oral cancer projects as a significant "Global burden."[3]

Malignancy may be defined as a neoplastic growth with potency to metastasize. The devastating aspects of cancer are attributed to the formation of metastatic foci. Occult metastatic tumor cells may become obscure and remain in a dormant state for longer duration following the resection or elimination of the primary tumor and then be activated by a stimulus leading to the formation of metastatic foci, which may promote mortality among patients.[5,6] Oral cancers metastasize in regional lymph nodes before

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actually metastasizing to distant sites, and this ability of regional lymph node metastasis is regarded as the factor that governs and influences the "Disease Survival Rate" in patients with oral squamous cell carcinoma (OSCC).[7]

The good lymphatic drainage from oral cavity and invasive potential acquired (cell motility factor) by malignant cells of OSCC establishes effective possibilities of regional lymph node metastasis. This establishment occurs through collective contribution of factors responsible for degradation of basement membranes, extracellular matrix (ECM) and action of matrix metalloproteinases (MMPs). The motility of cancer cells in tumor environment is brought by cleavage of ECM and thereby helps the propagation of tumor cells into the adjacent spaces. Therefore, cell motility, a prerequisite for a tumor cell for invasion, is under a coordinated balance between the cell adhesion receptors and the ECM. At present, the role of extracellular matrix or neomatrix in tumor development and progression is well established as ECM plays a vital role in cell adhesion, migration, proliferation, differentiation and gene expression.

Further, there exists also studies which have explored ECM components such as collagen[8] and laminin[9-11] which are known to be abnormally increased and abnormally lost, respectively, in cancer. Therefore, a complete understanding of ECM or neomatrix role in OSCC becomes necessary to aid in the identification of new biomarkers for early prediction of OSCC that relies on components of the ECM, which may facilitate newer strategies for more effective therapy against oral cancers.

METASTATIC CASCADE

The metastatic cascade fallows a sequential step which involves single tumor cell or groups of tumor cells to dissociate from the primary tumor, invade the surrounding ECM, including both basement membranes and interstitial compartments, enter the vascular or lymphatic space, escape immune surveillance and mechanical disruption, arrest at a distant site, escape from the vascular or lymphatic circulation, penetrate the target tissue and proliferate and metastasize subsequently. Fully potent malignant invasive cell phenotypes are fully equipped to negotiate all steps in the sequence. However, failure in any of these steps will result in loss of metastatic behavior and elimination of the tumor cells. Very small percentage of cell which reaches to circulation will survive and form metastases, which may be attributed to the development of competency for every step of the cascade independently, in a random and reversible fashion.[12]

Further, it is now evident by the work of Kerbel *et al.*[13] that the subpopulation of cells capable to metastasize dominate in the primary tumor mass early in its growth. In extension to these findings, the studies of Cornil *et al.*[14] and Kerbel[15] have shown that factors that behaved as growth inhibitors in early stages of benign tumors can transform as mitogens when tumor cells attain metastatic competence. These metastatically competent cells are therefore responsible for clonal dominance even at distant metastatic foci.

The steps in metastasis formation necessitate specific interactions with the ECM like decreased adhesiveness to the tumor or stromal matrix. Tumor-specific patterns of metastasis formation may be guided by specific tumor endothelial interactions and selective binding to specific matrix components.[16,17] The tumor cells also respond differently to various extracellular matrices and stromal cells that are encountered during metastasis formation. The concept of dynamic reciprocity[18] (normal cells that produce ECM are also influenced by that matrix) in normal cells is also valid for tumor cells and the extracellular matrices that they encounter. However, the responses of malignant tumor cells to various matrix components deviate from the normal cells.[15] The behavior of malignant tumor cell with the ECM is characterized by its tendency to cross tissue boundaries, intermix with cells of the various compartments and metastasize to distant sites,[19] and this may be the resultant of loss of control over the expression of the invasive phenotype observed in these normal cells.[20]

The acquisition of invasive phenotype by a cell is better understood by studying tumor cell interaction with the basement membrane, as the breach in basement membrane is defined as the critical event of tumor invasion, that signals the initiation of the metastatic cascade.[12,20] This event is not a simple process, as basement membrane puts forward connective tissue barriers at multiple key points in the metastatic cascade. These basement membranes are composed of a dense meshwork of collagen Type IV, laminin and heparan sulfate proteoglycans and do not contain pores that would allow passive tumor cell migration. The ability of cell to traverse basement membrane barriers is defined by acquisition of an invasive phenotype which is governed by three steps, i.e., attachment, local proteolysis and migration. Furthermore, these three steps describe tumor cell interaction with all extracellular matrices and not restricted to basement membranes. The nature of the specific interaction (i.e., tumor cell types and type of matrix) may result in selection of certain steps over others at particular points in the metastatic cascade. Thus, establishment of successful metastatic ability of cell is brought through the repetitive cycling of these three

steps.[12,20] Metastasis is therefore a multistep process, defined by the invasive phenotype that is dominated by the ability of tumor cells to attach to the ECM, to degrade matrix components and then migrate through these matrix defects. However, these functions is not unique to tumor cell behavior but also be found in normal cells.[19] An understanding of the factors that control cellular processes essentially pertaining to the malignant invasive phenotype will open up research area for identification of new biomarkers of ECM for early detection, therapeutic targets for prevention and metastasis.

MEDIATORS OF CELL TO EXTRACELLULAR MATRIX INTERACTIONS

Cell adhesion and invasion in malignancy is through a number of specific cell surface-associated molecules which mediate cell to ECM and intercellular interactions. These include the integrins, 67 kD laminin-binding protein, cadherins, Ig superfamily and CD44. The tumor cells for effective metastatic process should demonstrate decreased cell and matrix adhesive properties at various stages of the metastatic cascade. Hence, apparent contribution of each class of cell adhesion molecules to the net cellular and matrix adhesiveness of tumor cells will be dependent on a variety of factors inducing the metastatic capacity of the tumor cell population under study and the model system used to study these cells.

Integrins are a family of cell surface receptors that mediate cell adhesion. The integrins were originally identified as receptors for ECM proteins such as collagens, fibronectin, laminin and vitronectin. Some integrins may also function as cell-cell adhesion molecules. Integrins adhere to more than one ligand, and ligands can be recognized by more than one integrin. Integrins bind through recognition of the RGD sequence common to a number of adhesive molecules including fibronectin, vitronectin and other adhesion proteins.[21-23] Integrin-mediated signaling pathways control cell growth, differentiation, apoptosis, cytoskeletal changes, cell migration and invasion.[24] The cell migration and invasion depends not only on integrin expression levels but also on ligand-binding affinity.[24,25] Studies as that of Albelda *et al*.[26] and Gehlsen *et al*.[27] have demonstrated increased vitronectin receptor in malignant melanoma cells. The major integrin receptors are also been described in OSCC which include $\alpha 2\ \beta 1$, $\alpha 3\ \beta 1$, $\alpha 5\ \beta 1$ and $\alpha 6\ \beta 4$.[24,25] Therefore, changes in ECM composition and integrin profiles can have profound effects on OSCC development and progression.

Laminin is the most important noncollagenic protein matrix in the basal membrane identified by Chung *et al*.[28] However, Timpl *et al*.[29] isolated the first isoform of laminin from murine Engelbreth-Holm-Swarm sarcoma and characterized it as a major noncollagenous basement membrane glycoprotein of molecular weight 850,000–1,000,000 consisting of two major types of polypeptide chains in a ratio 1:2 held together by disulfide bonds.[29] Laminin, a basement membrane-associated glycoprotein, distributed exclusively on the epithelial portion of basement membrane in the lamina lucida, is chemically and immunologically distinct and functions as an adhesive glycoprotein-binding epithelial cell to Type IV collagen and basement membrane.[30,31]

The study conducted by Firth and Reade[32] showed that laminin and collagen IV distribution were continuous in epithelial hyperplasia while dysplastic lesions showed small focal breaks whose number increased in severe dysplasias. Kannan *et al*.[33] reported a gradual increase in the frequency of laminin and collagen IV discontinuity from normal epithelium to hyperplasic, dysplastic and squamous cell carcinomas. Harada *et al*.[34] found that the staining pattern of laminin and collagen IV in primary OSCC s was similar to that of metastatic nodules and observed that cellular population of the deep areas expressed the invasive and metastatic potential of oral carcinoma. These findings of Harada *et al*.[34] may be attributed to development of tumors from the cells which are normally associated with basal lamina production. The basement membrane component like laminin and Type IV collagen produced around the tumor cells may be resultant of differentiated phenotype of the tumor cells.[35]

Laminin isoform – Ln-5 is extensively explored in OSCC. Ln-5 has dual function which can promote adhesion and migration. This dual function of Ln-5 is determined by proteolytic processing which determines whether Ln-5 is an adhesive factor or a migratory factor.[36-40] Loss of Ln-5 expression has been demonstrated in OSCC,[41] which favors the binding of other ligands such as collagen or fibronectin that may be more conducive to tumor growth. The unoccupied Ln-5 receptors can also to bind laminin isoforms that stimulate tumor growth, like Ln-10/11 which binds to the same integrin receptors as Ln-5, has been shown to stimulate keratinocyte proliferation.[42,43] However, several studies have indicated that Ln-5 is also overexpressed in OSCC[44-48] contributing for tumor development and progression. Ln-5 expression has also been described along the invasive edge of OSCC to correlate a poor prognosis in patients with OSCC.[49-52] Recent studies, have now indicated that Ln-5 expression at the invasive front of OSCC is primarily, a means to retard tumor invasion in OSCC.[53-55]

The cadherins are a family of homophilic cell adhesion proteins expressed in a variety of tissues which require Ca^{+2} binding for adhesiveness, rigidity and stability.[56] Three subtypes (E-, N- and P-cadherins) have been identified in mammals and are primarily distinguished on the basis of tissue distribution. Epithelial cadherin also termed as E cadherin or cadherin 1 is a transmembrane glycoprotein, functioning as a cell adhesion molecule.[57] Calcium-dependent adhesion proteins (cadherins) play a critical role in cell adhesion and tissue differentiation.[58] Dysfunctional E cadherin is associated with loss of differentiation and acquisition of invasive phenotype.[56]

E cadherin acts a tumor suppressor and found to be lost during carcinogenesis. This loss of expression of E-cadherin may be attributed to impairment in intercellular association, demarcating the possible initiation of the procarcinogenic process in this epithelial layer.[59] Loss of E-cadherin expression has a negative impact on cellular adhesiveness, the process of cellular differentiation and cellular polarity in the epithelium[60] that induces the cells to attain a migratory phenotype, a significant feature associated with malignant transformation.[60,61] There are several mechanisms that have been proposed regarding the loss of E-cadherin. First, Palacios *et al*.[62] who suggested that Src-dependent endocytosis of E-cadherin followed by its degradation which becomes upregulated during cancer invasion leads to loss of membranous E-cadherin and accumulation of the same in cytoplasm of dysplastic epithelial cells. This cytoplasmic accumulation of the E-cadherin is known to be associated with deregulated transport of cytoplasmic E-cadherin – β catenin complex to the cell membrane as well as enhanced recycling of E-cadherin by endocytosis which eventually undergoes ubiquitination[63] which may thus lead to irreversible alteration in epithelial structural integrity through the loss of E-cadherin.[64] Second, Alvarado *et al*.[65] has suggested two plausible reasons of E-cadherin loss from proliferative layers: (1) the proteolytic cleavage of its ectodomain by MMP-7 which may cause enhanced cytoplasmic accumulation and (2) loss or reduction of E-cadherin expression from the proliferative layer can be caused by somatic mutations, chromosomal deletions, proteolytic cleavage and silencing of the CDH1 promoter which can occur either by DNA hypermethylation or through the action of transcription factors such as Slug, Snail and Twist.[65,66]

CD44, a transmembrane glycoprotein, known to act as a receptor for hyaluronan[67,68] can bind to ECM ligands such as chondroitin sulfate, heparan sulfate, fibronectin, serglycin and osteopontin with lower affinity. Numerous variant isoforms of CD44 are derived as a result of alternate splicing leading to combinations of exons which are inserted into the extracellular domain of proliferating epithelial cells and activated lymphocytes. CD44 plays a vital role in lymphocyte homing. Alternative splicing and glycosylation influence receptor function of the CD44 molecule and thereby reduces the affinity toward hyaluronan. The cytoplasmic domain of CD44 networks itself cytoskeletally through ankyrin and proteins belonging to the ezrin-moesin-radixin family. Studies on CD44 have correlated pattern of CD44 variants produced by neoplastic cells and clinicopathological parameters of tumors, such as grade, stage, presence of metastases and survival in carcinomas of the digestive tract, non-Hodgkin's lymphomas and thyroid carcinomas. The different ligand recognition by CD44 can influence cell motility, invasive properties and metastatic potential of tumors.[69]

Collagens are also found in association with oral epithelium.[70] Type I collagen shows an active role in malignant transformation of epithelial cells and often expressed with well-differentiated OSCC,[71] However, such an active role of collagen IV in malignancy remains obscure, as collagen IV reduced or loss of expression can be correlated to decrease tumor cell differentiation and at other times collagen IV increased expression may lead to nodal metastasis.[72] A study using microarray expression profiling has demonstrated increased expression of collagen IV in OSCC.[73] Mutations in collagen Type VII is known to cause epidermal squamous cell carcinoma in association with dystrophic epidermolysis bullosa.[74] These evidentiary findings although indicate changes in collagen expression promote adhesion, migration and differentiation. The precise mechanism should be further explored before confirming whether loss or overexpression of collagen isoform is a common prerequisite for OSCC tumor invasion.[73]

PROTEOLYSIS IN INVASION

Proteolysis and migration through tissue barriers are noted in normal cell functions under specific physiologic circumstances. However, the terms of malignant neoplasia define a shift toward sustained invasive capacity. The invasion is guided by a cyclic attachment to and subsequent release of matrix components in a programmed manner. This implies that enhanced proteolysis in tumor cells is still tightly regulated in a temporal and spatial fashion with respect to cell attachment and migration. The association of proteases with the invasive process is through inappropriate overexpression in the malignant tissue, either by the tumor cells, host cells intermixed or immediately adjacent to the invasion front or both. This overexpression mechanics holds good for all the classes of proteases: thiol-, seryl-aspanyl and metallo-proteases.[75,76] The difference between enzyme

production under physiologic and neoplastic conditions may be that in tumor cells, the enzymes may be regulated by autocrine growth factor stimulation. Tumor cells become unresponsive to signals that would down-regulate MMP expression from host cells and matrix. Activation of proenzyme is an important control point for the development of the invasive phenotype, which occurs in the presence of tissue inhibitors of MMP (TIMPs), the endogenous and ubiquitous TMMPs. The balance between active enzyme and TIMP decides the process local matrix degradation occurs. Overexpression of MMP proenzymes and subsequent activation is the mechanism by which tumor cells achieve a balance in favor of proteolysis. Proteolysis activity is always at a constant check between the local concentration of activated enzymes and their endogenous inhibitors.[77]

Evidence for the role of MMP enzymes in tumor invasion and metastasis comes from *in vitro* studies from murine and human tumor cell lines that transcribe, synthesize and secrete MMP enzymes.[78,79] The ECM components, cell-matrix interactions and the pericellular environment all act as determinants of MMP production. There exists studies that correlate MMP expression with invasive behaviour, and metastatic potential in animal models[80-82] and also studies that demonstrate modulation of these MMP expression by growth factors.[83-85] OSCC cells not only produce many of the ECM proteins but also synthesize and secrete MMPs. ECM components found in the oral epithelium are proteolytically processed. The processing of ECM molecules results in the liberation of peptides, and such peptides are termed as "matrikine." Matrikines affect various cellular activities such as proliferation, migration, apoptosis and angiogenesis.[86] Several matrikines are generated during proteolytic processing of the Ln-5 precursor. Matrikines generated from the α3 and β3 chains of the Ln-5 precursor chains promote migration,[87,88] and N-terminal γ2 chain proteolytic fragments of Ln-5 precursor is detected in patients with pancreatic ductal cell adenocarcinoma.[89] Further, γ2 chain of Ln-5 precursor and its proteolytic fragments have been found at the invasive fronts of OSCC.[88]

Several collagen-derived matrikines also have been reported. The tripeptide glycyl-L-histidyl-L-lysine present in the α2 chain of Type I collagen has demonstrated angiogenesis *in vivo*,[90] stimulate ECM synthesis and increase the expression of MMP-2. Another matrikine produced by C-terminal domain, of the α3 chain of Type IV collagen, decreases tumor cell proliferation and migration.[91] Further, Endostatin (a C-terminal domain of Type XVIII collagen) has demonstrated antiangiogenic properties, and therefore, OSCC which expresses collagen Type XVIII fails to exhibit nodal metastasis..[41] However, the role of matrikines in development and progression of oral cancer requires further exploration.

CONCLUSION

ECM not only acts as a support of epithelial tissues but also acts as a network for regulating transcriptional controls and cell signaling mechanisms that are involved in cell adhesion, migration, tumor development and progression. It is also noted that the proteases and proteases inhibitor balance operates wisely in ECM and thereby influencing cell-matrix interactions which favours proteolytic cleavage of ECM a step a prerequisite for invasion and migration. Understanding the role of ECM in tumor development, invasion and migration may open up new insights in early detection, preventive and treatment strategies for oral cancer.

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Conflicts of interest
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