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ORAL CANCER SHOWING EPITHELIAL MESENCHYMAL TRANSITIONS: NEED FOR A NOVEL THERAPEUTIC APPROACH
Category: Original research

Shree Goyal

Department: Department of Oral Pathology Institute; ITS CDSR Muradnagar, Ghaziabad University, CCS University

Abstract—*Introduction: With the increasing mortality and drug resistance for cancer cases despite advances in anti-cancer treatment strategies, it is imperative to understand the changes occurring at the molecular level at the tumour invasive front. E- Cadherin is a molecular marker which is marker for cell adhesion and vimentin is marker for mesenchymal properties within a cell. Recent development of drug resistant variants of oral squamous cell carcinoma has urged a need for evaluation of epithelial mesenchymal transitions taking place at the tumor invasive front in order to understand pathophysiological reasoning behind this aberrant behavior with therapeutic insights.*

Aim*: To evaluate and characterize epithelial and mesenchymal properties of tumour cells at the invasive front in Oral squamous cell carcinoma and to formulate pathophysiological basis for development of newer treatment strategies in oral cancer cases.*

Materials And Method: *Histopathologically confirmed tissue samples of Oral squamous cell carcinoma (n=5) were selected based on WHO criteria(2005). Immunohistoichemical analysis of E cadherin and Vimentin (Biogenex Inc.) was done using standard streptavidin biotin and heat induced epitope retrieval method. All the slides were evaluated for immunoexpression at the tumour invasive front to assess the epithelial mesenchymal transition.*

Result*: The cells which were proliferating at invasive front showed loss of E-cadherin and were positive for vimentin. These results were indicative of changes occurring at the molecular level in the tumour cells suggestive of attainment of mesenchymal properties in the proliferating epithelial cells.*

Conclusion: *Aberrant expression of E cadherin and vimentin shows that loss of cohesion and acquisition of migratory potential are independent prognostic indicators of Oral squamous cell carcinoma. Those cases showing evidence of EMT are prone to develop recurrence and drug resistance. Therefore, there is a need for development of a novel targeted therapy with molecular considerations in treatment planning.*

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer globally with oral squamous cell carcinoma (OSCC) being the most common malignancy occurring within the oral cavity.[1,2] It is a global health burden with oral squamous cell carcinoma accounting for majority of the cases in the oral cavity.[1] The pathogenesis of oral squamous cell carcinoma is multifactorial with the emphasis now shifting towards epithelial mesenchymal transitions(EMT) taking place within the tumour cells, especially at the invasive tumour front.[1,3] One of the hallmarks of EMT is that the tumour cells acquire plasticity with loss of cell to cell adhesion and increased cell motility with increased ability to invade and metastasize.[4]

EMT is characterized by loss of cell – cell adhesion properties and gain in mesenchymal properties. E-cadherin and β-catenin are molecules which are down regulated and N-cadherin and vimentin are over expressed in cells showing EMT and associated with higher invasive potential.[5] Along with the gain in cellular plasticity with increased ability to invade and metastasize, the cells also acquire possible drug resistance through these mechanisms. Malek et al. have described usefulness of targeted therapy against EMT markers to overcome therapeutic resistance and may have some prognostic significance in future.[4]

E Cadherin is a transmembrane adhesion molecule responsible for cell to cell adhesion in oral keratinocytes.[3] Vimentin is an intermediate filament found predominantly in the cytoplasm of the mesenchymal cells and absent in epithelial cells.[6] Hence, the present pilot study was designed to study the expression of

E-cadherin and Vimentin at the invasive tumour front in Oral squamous cell carcinoma cases.

Material and methods:

A total of 5 cases of Oral Squamous cell carcinoma were included in the study. The tissue were fixed in 10% neutral buffered formalin, processed and embedded in paraffin wax following standard protocol. 2 sections of 4µ thickness were taken, one for haematoxylin and eosin staining for the confirmation of the neoplasm and other for immunohistochemistry procedure.

Study Settings

Tissue specimens were retrieved from the archives of histopathologically confirmed cases of Oral Squamous cell Carcinoma based on WHO criteria (2005). The obtained tissues were fixed in 10% neutral buffered formalin. From each case of formalin fixed paraffin embedded tissue blocks, 3-4µ were obtained. One set of slides was stained by haematoxylin and eosin for reconfirmation of histological diagnosis while other two sets were stained for E- Cadherin and Vimentin using standard immunohistochemical method.

Immunities to chemistry Procedure

Immunohisto chemical analysis for E-cadherin and Vimentin was performed on 3 µm thin paraffin sections which were obtained using a rotary microtome. The sections were immunostained with E-cadherin and Vimentin following a standard protocol of streptavidin-biotin method using heat induced epitope retrieval procedure. After dewaxing, washing and rehydration of the slides through xylene and graded alcohol concentrations, tris buffered saline (TBS) and (PBS) at pH is 7.2-7.4 was used for antigen retrieval. Slides were subsequently treated with 3% hydrogen peroxide to block endogenous peroxidase. Following incubation with the primary antibodies, E-cadherin (Biogenex) and Vimentin (Biogenex), the secondary conjugate antibody was applied and followed by chromogen 3-Diamino benzidine (DAB) and counterstaining with Mayer's hematoxylin.

Immounohistochemical Analysis

All the immune-stained slides were viewed under the light microscope at high power (X400 magnification). Positive immunoexpression of E cadherin was visualised as crisp brown coloured staining membranous staining wheras that of vimentin was visualized as a intracytoplasmic expression within the mesenchymal and tumor cells at the invasive front.

Results

The results of the present study showed that there was a decrease in the immunoexpression of E-cadherin which remain confined to cell membrane of the overlying mucosa and in tumor islands present in connective tissue stroma at invasive front(Figure 1). Positive immunoexpression of vimentin was seen within the cytoplasm of mesenchymal cells and epithelial cells at the invasive front(Figure 2). Overall, an increase in vimentin and loss of E cadherin expression was observed in the tumour cells at the invasive front in all the cases.

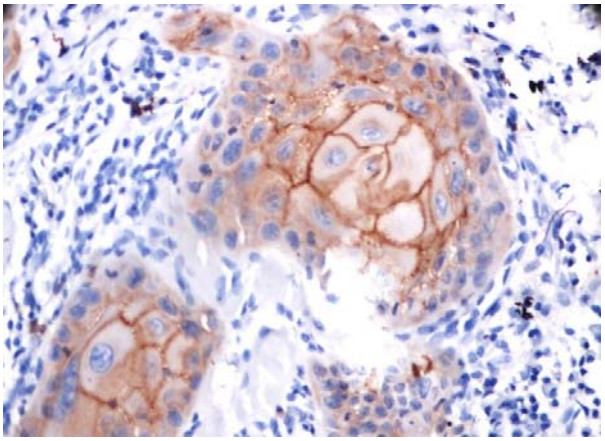

Figure 1: Photomicropgraph showing immunoexpression of E cadherin in the tumour islands (X400 magnification).

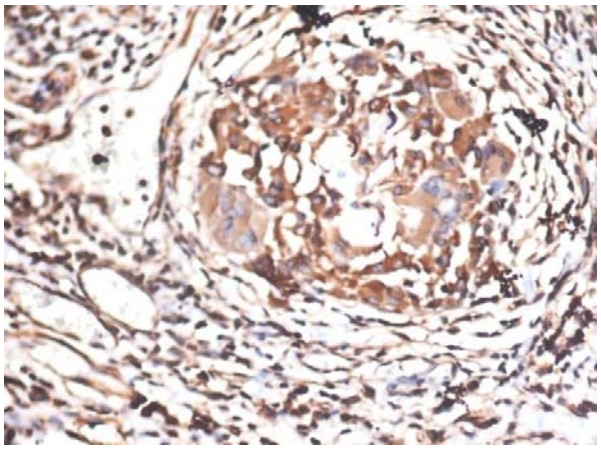

Figure 2: Photomicropgraph showing immunoexpression of vimentin in the tumour islands(X400 magnification).

Discussion:

Development and progression of oral cancer is multifactorial and factors affecting their behavior and outcome are still being studied. One of the processes which has been associated with tumour cells is the acquisition of Epithelial-mesenchymal transitional changes during the process of carcinogenesis. Loss of cell- cell adhesion and gain of mesenchymal properties by the tumour cells is seen in cells undergoing EMT.[6] These changes could be in response to the multiple signals arising from the tumour microenvironment. These signals include

growth factors and cytokines, hypoxia, and extracellular matrix (ECM) components such as TGF beta and BMP in the form of paracrine- autocrine mediators.[4]

We observed a decreased expression in cell adhesion molecule (E cadherin) and increase in expression of mesenchymal marker (Vimentin) in the tumour cells in oral squamous cell carcinoma cases. Similarly, Zhou et al. also observed decrease in E cadherin and increase in vimentin expression within the tumour cells with significant association with lymph node metastases. They also stated that co-expression of these two molecules could be studied for possible EMT taking place within the tunour cells associated with poor outcome.[1]

Balasundram et al. also observed weaker expression of Beta catenin and E cadehrin and weak to moderate expression of vimentin within the tumour cells. However they did not observe any significant correlatiuon between the expression of these molecules and clinicopathologic parameters in their study cases.[6]

Kaur et al. observed that E-cadherin immunoreactivity was found to inversely correlate with the loss of cell differentiation. The expression of E-cadherin decreased significantly in advanced cases of OSCC. They concluded that Loss of the cell adhesion and E-cadherin plays an important role in progression of OSCC, that is, down regulation of its expression is associated with de-differentiation and metastasis.3 Costa et al. observed that E cadherin expression was reduced in the high invasive tumours but there was no difference in vimentin expression in low and high invasive tumours.[2]

Liu et al. correlated E -cadherin and vimentin levels with pathologic parameters and disease specfic survival and observed that low cadherin and higher vimentin expression were associated with poor disease specific survival in tongue squanous cellc arcinoma cases and stated that vimentin could be the most significant prognostic indicator in such cases showing EMT.[7]

Du et al. have stated important role of EMT in cancer progression, metastases and drug resistance. They have also indicated a significant potential role of targeted therapies against EMTs as a future prospect to overcome cancer drug resistance and improved clinical outcomes in cancer patients.[8]

Conclusion:

The present pilot study gives an observational insight into the possible mechanisms involving EMT taking place within the tumour cells in oral squamous cell carcinoma. Within the limitations of the study which include a include a smaller sample size and lack of follow up of the patients for clinical evaluation, it can be stated that study of EMT could be an area of research which needs to be undertaken at a higher level with more sample size and follow up of cases to establish firmly the correlation of EMT and biological behavior of oral cancer cases. The possible role of EMT and its analysis with disease behavior and outcome could be a new avenue in studying molecular changes taking place at the invasive front in OSCC. Also, previously EMT has also been shown to mediate resistance to traditional therapeutic agents. In future, specific targeted therapies could be developed which could help in overcoming drug resistance and increased sensitivity of the tumour cells to anti cancer agents and designing newer treatment strategies for such cases.

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