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SNAIL EXPRESSION IN ORAL SQUAMOUS CELL CARCINOMA

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ABSTRACT

Epithelial-Mesenchymal Transition [EMT] describes a process in which epithelial cells undergo alterations in cellular architecture, adhesion, migratory and invasive capabilities. Among the mechanisms largely associated with the metastatic conversation of epithelial cells and the EMT, the loss of E-Cadherin-mediated cell adhesion is prominent and is considered to be a fundamental event. SNAIL (SNAI1), the first EMT inducer originally implicated with tumor progression belongs to a family of zinc-finger transcription factors. Binding of SNAI1 to E-box elements in the E-Cadherin promoter region leads to transcriptional repression of the CDH1 gene and resulting in loss of E-cadherin expression, which is considered a "hallmark" of EMT. This study is designed to evaluate and compare SNAIL1 expression in Oral Squamous Cell Carcinoma [OSCC] to normal oral mucosa [NOM]. Formalin fixed paraffin embedded tissues of diagnosed cases of Oral SCC (n=30) and oral mucosa (n=30), were examined for the expression of SNAIL by standard immunohistochemistry protocol using snail1 antibody. All data were tabulated and statistically analyzed. Snail1 expression was observed in the epithelium and in the stromal elements of the sections, and the expression was significantly associated with the lesion's extension. Highly significant difference was reported among the two groups. ($P < 0.001$). Expression of Snail1 at the invasive front in oral squamous cell carcinomas suggests a key role of EMT in the tumorogenesis of this cancer.

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the most prevalent malignancy in oral cavity [1], which is a dis- ease found particularly in low income communities and mainly a problem of older men, 90% being in > 45 year age group who are exposed to the known risk factors of tobacco and/or alcohol [2,3].

The landmark of carcinoma progression during the invasive and metastatic phases is epithelial cell plasticity and dedifferentiation, which is similar to epithelial-mesenchymal transition (EMT) that occurs during embryonic development [4]. Deregulation of epithelial-mesenchymal transition (EMT) signaling, has been associated with aggressive malignancies and tumor progression to metastasis in several cancer types [5]

The epithelial-mesenchymal transition (EMT) is a biological event in which epithelial cells lose many of their phenotypic features and gain extra properties typical of mesenchymal cells, like transformation of cohesive and polarized epithelial cells into cells that exhibit no polarization and high mobility. This migratory phenotype is the hallmark of an important type of EMT that involves neoplastic cells originating from epithelial malignant neoplasms during tumor invasion and metastasis [6-11].

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These cells undergoing EMT exhibit down-regulation of many epithelial markers, and up-regulation of mesenchymal markers [12-14]. Snail is a master gene in regulating E-cadherin during the process of EMT [15]. The various transcription factors which promote EMT and inhibit E-Cadherin production include Snai1 (snail), Snai2 (slug), Twist, EF1/ZEB1, SIP1/ZEB2 [16].

The detection of epithelial dysplasia and early oral squamous cell carcinoma would be of great support to treat the case and improve the prognosis. Snail could be one such marker which could give the overview of EMT in oral squamous cell carcinoma. The present study aims at detecting the expression levels of Snail in NOM and OSCC by immunohistochemistry and statistically analyzed.

MATERIALS AND METHODS

Formalin fixed paraffin-embedded tissue samples of 30 cases each of OSCC and NOM were retrieved from the archives of Department of Oral & Maxillofacial Pathology, Government Dental College & Research Institute, Bangalore. Histopathological diagnosis of H&E stained sections of OSCC were confirmed by experienced oral pathologists.

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For immunostaining, routine formalin fixed and paraffin embedded tissue were cut into 3µm thick sections, deparaffinized in xylene and rehydrated in graded alcohols and deionized water. Antigen retrieval was carried out by microwave treatment of the slides in TRIS buffer (0.01m, Ph 9.0) in 3 cycles of 5 minutes each at a temperature of 80°c. The slides are cooled and washed thrice with phosphate buffer saline (PBS, 0.01M, ph 7.2). Endogeneous peroxidase activity was blocked with 3% hydrogen peroxide for 20 minutes at room temperature and washed three times with PBS.

After binding of the non-specific antigen with power block (super sensitive HRP IHC detection kit) for 30 minutes at 37°C, the sections were stained with Rabbit polyclonal antibody against SNAIL1 (primary antibody, diluted 1:100, NBP2-27293SS, Novus biologicals, US.) The slides were incubated over night at 4°c with primary antibody and then washed 3 times with PBS. Subsequently, the tissue sections were reacted with pre-diluted biotinylated anti-rabbit antibody (secondary antibody, HRP) for 30 minutes at 37°C and washed thrice with PBS. The color was developed with 3-3'diaminobenzidene tetrachloride and counter stained with Harris hematoxylin. Distinct dark brown staining of the nuclei was considered to be positive and calculated.

Evaluation of Immunostaining results:

Evaluation of immunohistochemistry was performed by 2 independent investigators. Expression of Snail1 was compared between OSCC cells and normal epithelial cells from oral mucosa. Snail positive expression was defined as detectable immuno reaction in the nuclear and sometimes other cytoplasmic regions of cancer cells. SNAIL positive tumor cells were clearly identified by their brown nuclear staining (predominantly). 10 areas (within the tumor and at the invasive front) with highest expression (hotspots) were selected under a magnification 100x. SNAIL positive cells were counted in 10 selected fields per section at 200x magnification. The cells in the entire selected field were counted and the Snail positive cells (predominantly nuclear positive) were also counted. Percentages of the Snail positive cells were calculated.

$$\text{Percentage (\%)} = \frac{\text{Snail positive cells}}{\text{Total number of cells}}$$

An average percentage of all the cases of each group were tabulated.

Statistical analysis:

Statistical analysis of group differences was performed using the Mann Whitney U-test. The P value of <0.05 was considered to indicate statistical significance. All statistical analyses were performed using statistical package for social sciences (SPSS 14) software. .

Results:

In this study, A highly significant difference was detected among the two groups for epithelial scores (Mann-Whitney U-test, <0.001). By post hoc analysis, it was found that the mean rank scores of OSCC group were significantly higher than the NOM group. Snail expression was seen in more than 80% of OSCC patients. The expression was in both cytoplasm and nucleus, but predominantly nucleus. The invasive front of the tumor exhibited positive cells losing its epithelial morphology and gaining fibroblastoid morphology.

Table 1. Differences between OSCC and NOM using Mann-Whitney U-test

Group variable	N	Mean Rank	Sum of Ranks	*P* value
OSCC	30	23.73	712.00	**0.001**
NOM	30	21.53	323.00	

*Significant at P <0.05

Figure 1. normal oral mucosa exhibiting rare snail positive cells. Figure 2, 3 & 4. showing snail positive epithelium at the tumor invasive front and islands /cords present in the connective tissue. Figure 5: showing fibroblastoid appearing snail 1+ tumor cells

Figure 1

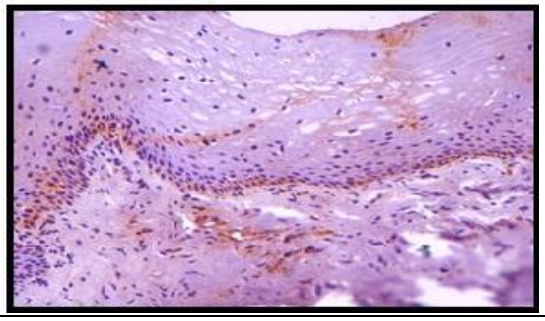


Figure 2

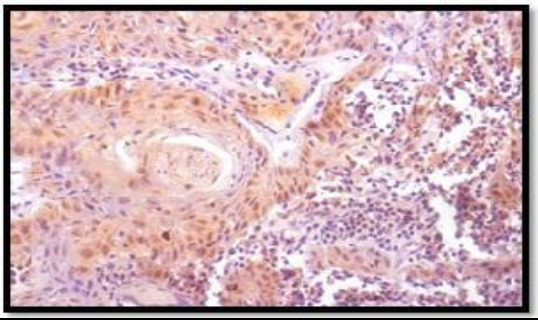


Figure 3

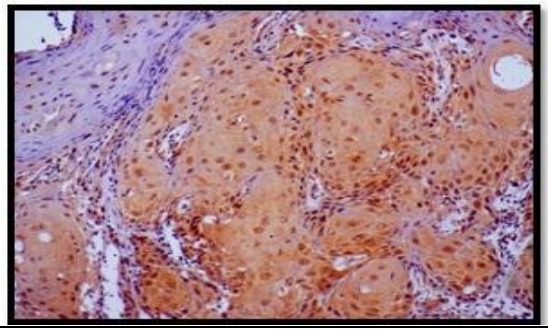


Figure 4

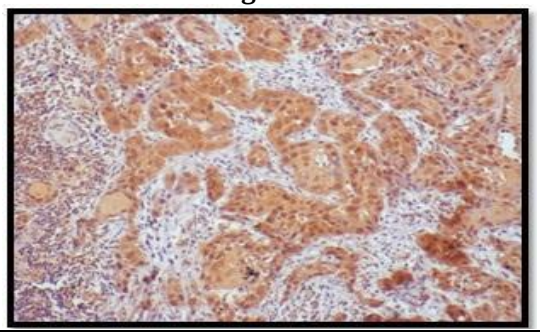
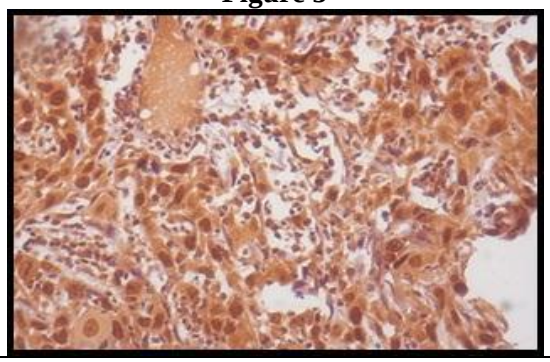
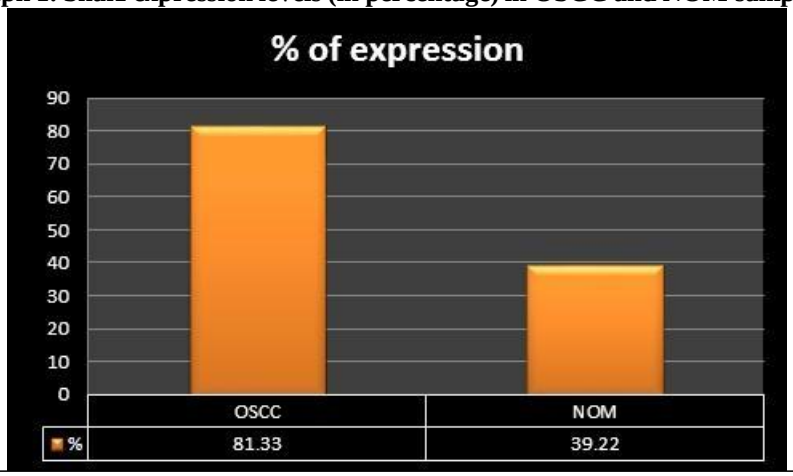


Figure 5



Graph 1. Snai1 expression levels (in percentage) in OSCC and NOM samples.



DISCUSSION

Many researchers have been focusing on the snail expression and its role in EMT in several types of cancers. This study was conducted to assess the EMT marker Snail1 in oral squamous cell carcinoma. Snail1 overexpression usually correlates with increased migration, invasion, and metastasis [17].

Morphologically, cancer cells undergoing EMT switch their epithelial characteristics like cobblestone-like, nonmotile and noninvasive to their mesenchymal elongated, motile, and invasive characteristics [15,18] Oral squamous carcinoma is a case of E-cadherin/Snail1 expression inversion, and the higher the Snail1 expression, the more invasive the cancer [19].

In this study, the tumor cells showed both cytoplasmic and nuclear positivity for the Snail1, but were predominantly nuclear. The expression was found to be very marked in the invasive front and in the infiltrating cords and nests. The cells in the deeper invasive area seemed to show some change in the morphology with lose of cell adhesion. The cells were more of fibroblastoid variant. The statistical analysis revealed a very highly significant difference in the expression of Snail in the normal mucosa and in the oral squamous cell carcinoma cases [P<0.001]. Snail expression was seen in more than 80% of OSCC patients.The tumor stroma in our study exhibited snail positive cells with plump fibroblastoid appearance, which represents the epithelial cells losing its properties and gaining the mesenchymal properties. Carcinoma-associated fibroblasts in tumor stroma have been suggested to derive directly from epithelial cells by EMT [20,21].

CONCLUSION

The increased snail1 expression in oral squamous cell carcinoma specimen and in invasive front showing morphological changes suggests that Snail1 is a usefull marker of EMT and these finding reinforces that the EMT is occurring in the invasive front of the epithelial carcinoma. In conclusion expression of Snail1 has a high contribution to assess whether EMT occurs in the OSCC and its relation to invasiveness and metastasis. There are some limitations like need for follow up of patients for assessing the prognosis and its association with expression of snail1. This study will contribute highly for advances in understanding the process of carcinogenesis, leading to development of new biomarkers which would help in diagnosis, prognosis and targeted therapy in OSCC patients.

Abbreviations;

NOM: Normal oral mucosa
OSCC: Oral squamous cell carcinoma
EMT: Epithelial mesenchymal transition

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