



PARENCHYMAL DOWN REGULATION AND ENHANCED STROMAL EXPRESSION OF SYNDECAN-1 IN ORAL SQUAMOUS CELL CARCINOMA

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ABSTRACT

Background and aim: In normal stratified squamous epithelia, syndecan-1 works as cell adhesion molecule, and exhibits an important role in the regulation of cell growth and differentiation during the development. It is recognized as tumor marker with diagnostic and prognostic role in different cancer types. This study was planned to explore the role of syndecan-1 in oral squamous cell carcinoma.

Materials and Methods: Twenty two cases were histologically diagnosed as Oral Squamous Cell Carcinoma (OSCC) that simultaneously contained dysplastic epithelium and carcinoma in situ (CIS) foci. Immunohistochemical staining for syndecan-1, alpha smooth muscle actin and Ki-67 were done.

Results: Decreased expression of syndecan-1 from normal epithelia to CIS. In OSCC parenchyma, transition from cell membrane localization into cytoplasm was detected. In addition, strong expression of syndecan-1 in stromal fibroblasts was observed.

Conclusion: Decreased expression of syndecan-1 in parenchymal cells together with its appearance in stromal fibroblasts might be used as a reliable tool in oral squamous cell carcinoma early diagnosis.

KEYWORDS: Syndecan-1, parenchymal, stromal, oral squamous cell carcinoma.

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INTRODUCTION

Oral cancer is considered as one of the most common cancers and the incidence of oral squamous cell carcinoma (OSCC) has continued to be increased. OSCC is characterized by a high degree of local invasiveness and a high rate of metastasis to regional cervical lymph nodes ^[1,2].

Syndecans are a heparan sulfate proteoglycan cell surface adhesion molecules; they are involved in cell–cell adhesion and interactions with the extracellular matrix and are capable of binding several ligands, including growth factors. They play a critical role in cell growth, differentiation and cell migration. Four members of the syndecan family have been identified including syndecan-1, -2, -3, and -4 ^[3].

Among the syndecans, syndecan-1 is expressed mainly in stratified squamous epithelial cells as well as plasma cells. Its interaction with the extracellular matrix includes binding to collagens, fibronectin, tenascin, thrombospondin and basic fibroblast growth factors (bFGF) modulates several key processes associated with tumorigenesis, including tumor cell proliferation, apoptosis, angiogenesis, and metastasis ^[4,5].

Decreased expression of syndecan-1 in tumor cells correlates with tumor invasiveness, metastasis, and poor prognosis in different cancer types including lung cancer, and cervical cancer ^[6,7]. The level of syndecan-1 expression in tumor cells inversely correlates with tumor invasiveness, metastatic potential and overall prognosis ^[8].

The expression of syndecan-1 in the parenchyma and stroma of oral squamous cell carcinoma has not been fully studied in detail. Thus this study was planned to elucidate the expression of syndecan-1 in OSCC.

MATERIALS AND METHODS

Selection of cases

For the present study, 22 archival Oral Squamous Cell Carcinoma (OSCC) from the tongue were

collected from Oral Pathology Department, Faculty of Dentistry, Tanta University during the last four years from January 2014 to January 2018. These cases were histologically diagnosed as OSCC that simultaneously contained dysplastic epithelium and carcinoma in situ (CIS) foci. Normal oral epithelium is supplied from 5 gingivectomy cases after obtaining a written consent. The experimental protocol for analyzing surgical materials was reviewed and approved by the Ethical Board of Faculty of Dentistry, Tanta University.

Conventional hematoxylin and eosin staining

The surgical samples were fixed in 10% formalin and routinely processed and embedded in paraffin. Serial sections were cut at 5 μ m from paraffin blocks were used for hematoxylin and eosin (HE) and immunohistochemical staining.

Antibodies

Mouse monoclonal antibodies against human CD138 (clone MI 15), Ki-67 (MIB 1) and alpha smooth muscle actin (1A4, IgG2a) was purchased from Dako (Glostrup, Denmark).

Immunohistochemistry

Immunohistochemistry was performed using the ChemMate EnvisionTM system (Dako). For Syndecan-1 (CD138) and Ki-67 sections were autoclaved in citrate buffer (pH 6.0) for 10 min at 121 °C. For α SMA sections were autoclaved in Tris-EDTA (pH 9.0) for 10 min at 121°C. Then sections were treated with 0.3% hydrogen peroxide in methanol for 30 min at room temperature to block endogenous peroxidase activity and incubated with 5% milk protein in 0.01 M phosphate-buffered saline (PBS, pH 7.4) containing 0.05% Triton X-100 (T-PBS) for 1 hour at room temperature to block non-specific protein binding sites. They were then incubated overnight at 4°C with the primary antibodies diluted at 1:100 (Syndecan-1, Ki-67 and α SMA) in PBS. After overnight incubation, the sections were incubated with the Envision reagents for 1 hour at room temperature. Reaction products

were visualized with 0.02% 3, 30-diaminobenzidine in 0.05 M Tris-HCl buffer (pH 7.6) containing 0.005% hydrogen peroxide. Finally, the sections were counterstained with hematoxylin. For control studies, the primary antibodies were replaced with preimmune IgGs [9].

RESULTS

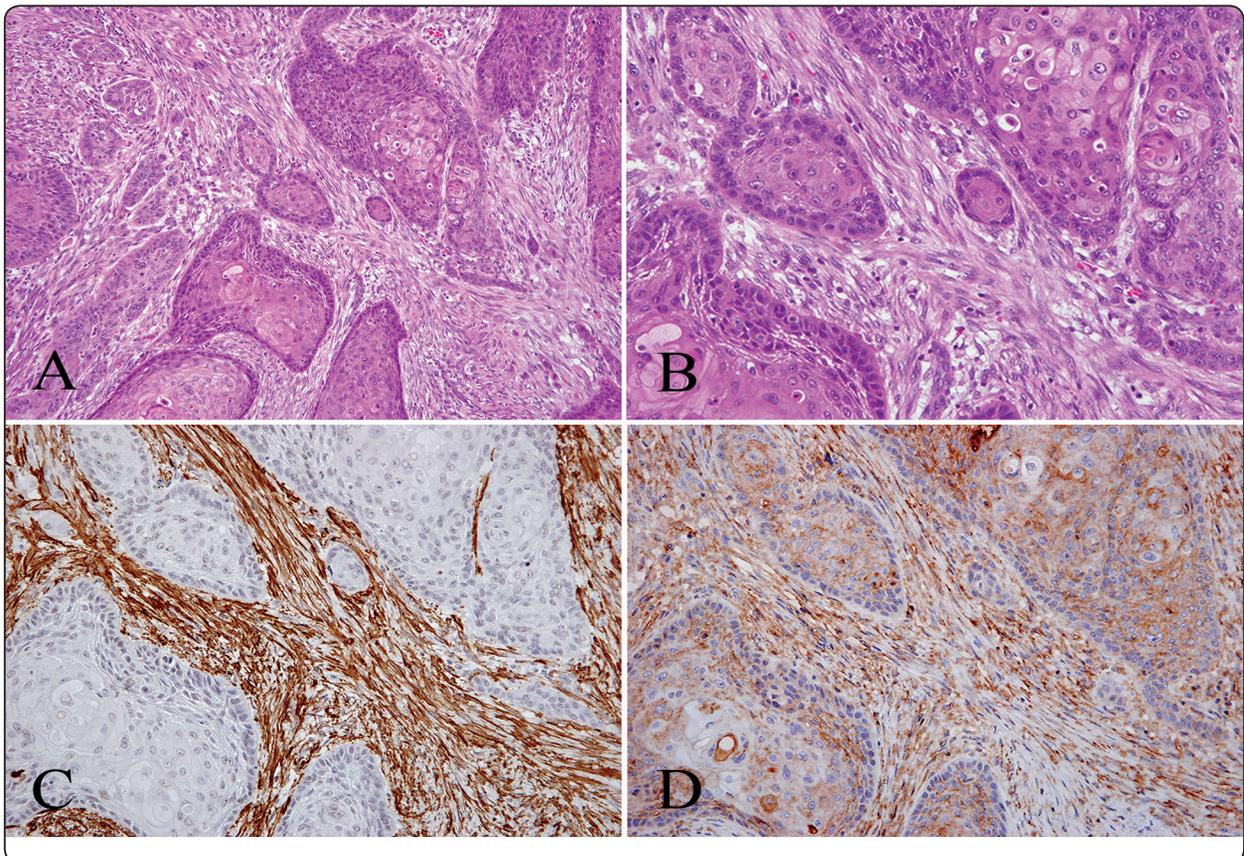
Syndecan-1 expression in normal, dysplastic epithelia and CIS

In normal epithelia (Fig. 1A) obtained from gingivectomy cases, syndecan-1 was expressed in all cell layers of epithelium in a cell membrane pattern except terminally differentiated keratinized layer (Fig. 1B). In dysplastic epithelia (Fig. 1C) where there are dysplastic changes and are characterized by nuclear hyperchromatism, pleomorphism and cellular crowding, syndecan-1 expression was expressed in all prickle cell layer of the epithelium in a cell membrane pattern (Fig. 1D). In CIS (Fig.

1E) where the dysplastic changes extend throughout the entire thickness of epithelium and most cells expressed Ki-67 (Fig. 1E, inset) but the basement membrane still intact, syndecan-1 was expressed in a membranous fashion in the intermediate cell zone between Ki-67 +ve cells and superficial keratinized cells. Stromal cells did not express syndecan-1 except plasma cells (Fig. 1F).

Syndecan-1 localization in the parenchyma and stroma of oral squamous cell carcinoma

In oral squamous cell carcinoma (Fig. 2A), in which, malignant epithelial cells existed in the form of islands and invaded the connective tissue with intervening plumb stromal fibroblasts (Fig. 2B), that strongly expressed α SMA (Fig. 2C). Syndecan-1 exhibited transition from cell membrane into cytoplasmic localization in some malignant epithelial cells (Fig. 2D). In addition, stromal fibroblasts expressed syndecan-1 in their cytoplasm (Fig. 2D).



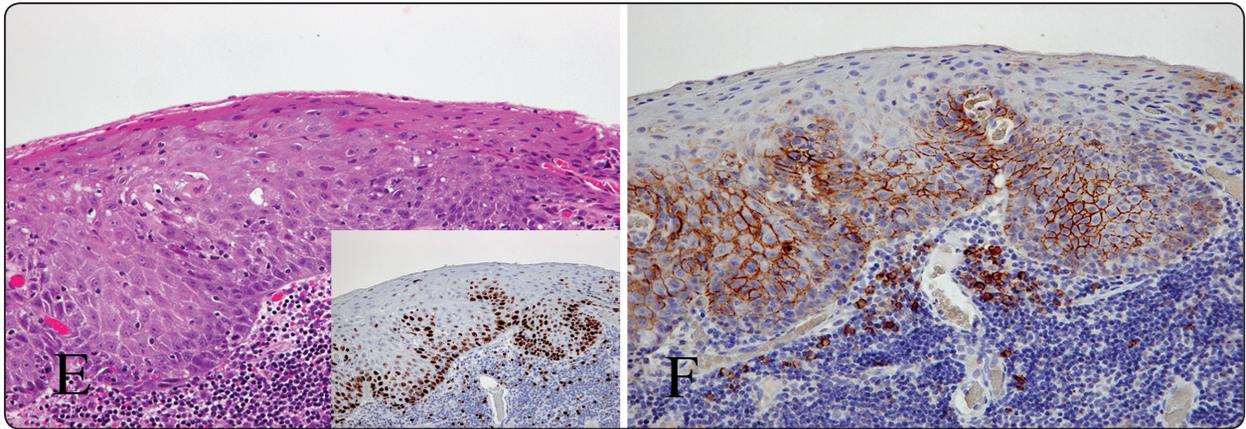


Fig. (1) Immunohistochemical expression of Syndecan-1 in normal, dysplastic epithelia and CIS. HE (A, C, F), Inset (E) immunoperoxidase stains for Ki-67, immunoperoxidase stains for CD138 (B, D, F), hematoxylin counterstain. (A-F) $\times 200$. In normal epithelia (A), syndecan-1 was expressed in cells of all cells layers of epithelium in a cell membrane pattern except outer keratinized layer (B). In dysplastic epithelia (Fig. 1C) where there are dysplastic changes and are characterized by nuclear hyperchromatism, pleomorphism and cellular crowding, syndecan-1 expression was expressed in all prickle cell layer of the epithelium in a cell membrane pattern (D). In CIS (E) where the dysplastic changes extend throughout the entire thickness of epithelium and most cells expressed Ki-67 (E, inset) but the basement membrane still intact, syndecan-1 was expressed in a membranous fashion in the intermediate cell zone between Ki-67 +ve cells and superficial keratinized cells. Plasma cells were positive for syndecan-1 (F).

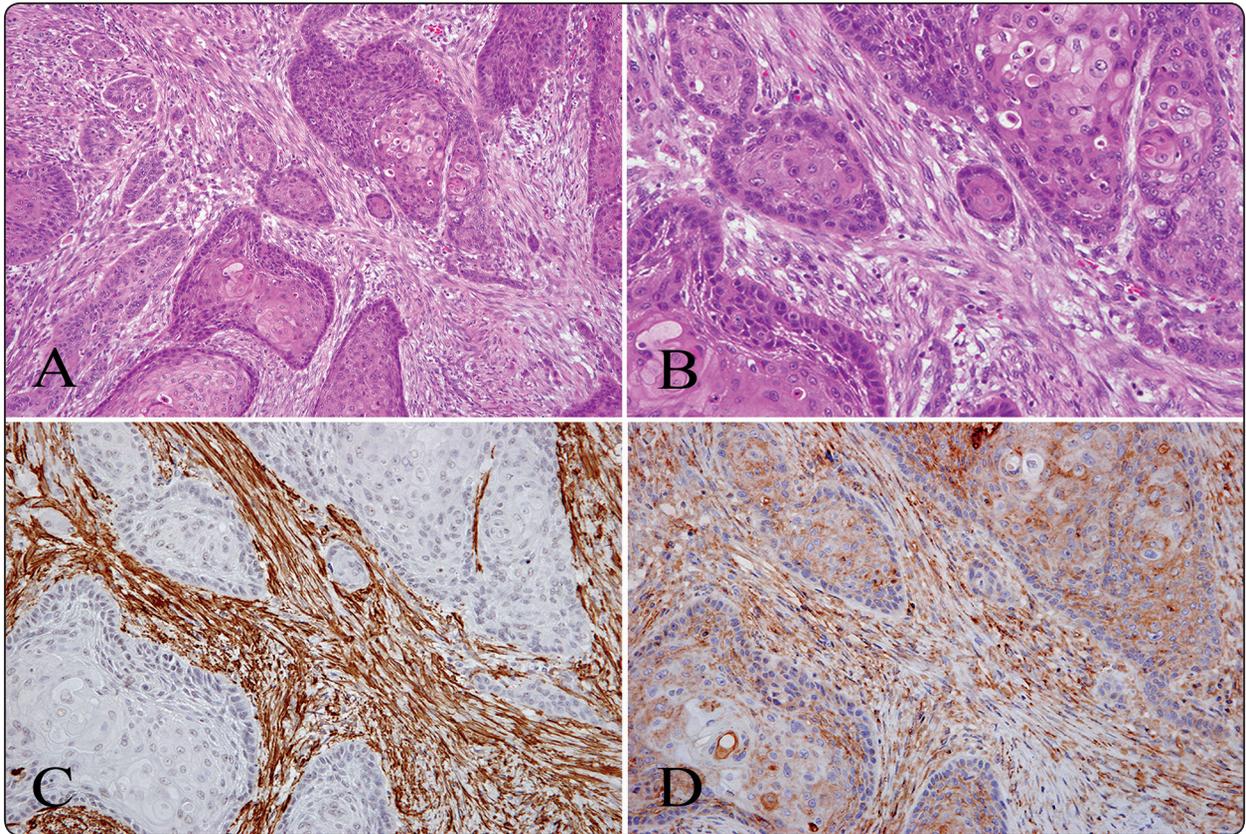


Fig. 2 Immunohistochemical expression of Syndecan-1 in. HE (A, B), immunoperoxidase stains for α SMA and CD138 (D), hematoxylin counterstain. (A) $\times 100$, (C-D) $\times 200$. In oral squamous cell carcinoma (A), in which, malignant epithelial cells in the form of islands were invaded the connective tissue with intervening plumb stromal fibroblasts (B), that strongly expressed α SMA (C). Syndecan-1 exhibited transition from cell membrane into cytoplasmic localization in some malignant epithelial cells (D). In addition, stromal fibroblasts expressed syndecan-1 in their cytoplasm (D).

DISCUSSION

In the normal process of differentiation of stratified epithelia, syndecan-1 is functioned in cell-to-cell and cell-to-matrix interaction, which contribute to maintain the normal epithelia^[10].

Syndecan-1 expression is frequently changed in cancer, but its definite function in tumorigenesis is not clearly understood. In some human cancers, the expression of syndecan is downregulated, while increased in others^[11].

Previous studies revealed that syndecan-1 expression was reduced in head and neck squamous cell carcinoma and correlated it with poor prognosis^[12,13]. However, syndecan-1 expression in the stroma of oral squamous cell carcinoma has not been fully elucidated.

In normal epithelia, syndecan-1 retains its cell membrane localization, however; in the process of transformation to epithelial dysplasia and CIS, its expression was reduced^[14,15] to the level that it disappeared in the lower part of CIS epithelia which also positive for Ki-67. In addition, Deshmane and Khot^[16], indicated that syndecan-1 can be considered as a useful biomarker for assessing dysplastic changes and can be used as a reliable marker in predicting malignant changes. In other words, syndecan-1 keeps the architecture of epithelia but gone when the cells are permanently transformed, proliferating and become ready for invasion.

In preparation for invasion, syndecan-1 shifted from the cell membrane localization into the cytoplasm of transformed malignant cells, as they have reduced intercellular adhesion as well as extracellular matrix interactions, with the ability to move more freely, with invasive behaviour and distant metastasis^[17].

On contrary to reduced expression of syndecan-1 in parenchymal cells, enhanced stromal expression of syndecan-1 was noted; supporting the evidence of the active role played by the stroma in the biologic course of tumors. The stromal induction of

syndecan-1 and its loss from the surfaces of malignant epithelial cells may contribute to the invasiveness and/or metastatic potential of the tumors^[18].

In conclusion, this study revealed that decreased expression of syndecan-1 in parenchymal cells together with its appearance in stromal fibroblasts might be used as a reliable tool in oral squamous cell carcinoma early diagnosis.

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