



## Immunohistochemical expression of SOX2 in oral normal epithelial dysplasia and squamous cell carcinoma

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### ABSTRACT

**Objective:** Oral squamous cell carcinoma (SCC) is an aggressive neoplasm with serious morbidity and mortality, which typically spreads through local invasive growth. A key approach to this tumor would be to detect potentially malignant lesions at their early stages. The transcription factor, Sex determining region Y-box 2 (SOX2) is an essential regulator of pluripotent stem cells and promotes development and maintenance of squamous epithelia.

**Materials and Methods:** We retrospectively reviewed the charts of 24 samples of SCC, oral epithelial dysplasia and control group, then immunohistochemical staining was performed to detect SOX2 expression.

**Results:** Comparisons between SCC and oral epithelial dysplasia group did not reveal any significant difference ( $p=0.496$ ); also values of control and oral epithelial dysplasia group were statically different ( $p<0.001$ ). Similarly, a significant difference was observed between the values of SCC and controls' ( $p<0.001$ ).

**Conclusion:** In conclusion, SOX2 overexpression is seen in oral dysplasia and SCC and their detection in early stages could be crucial for early tumor identification.

**Keywords:** Oral squamous cell carcinoma, Oral epithelial dysplasia, SOX2.

### Introduction

Oral squamous cell carcinomas (SCC) is a heterogeneous group of head and neck carcinoma with incidence rate of 30,260 in 2015. Despite the overall decline in incidence of oral SCC, due to presentation of these tumors in advance stages, outcomes are sub-optimal in comparison to other head and neck areas

and 5-year survival for advanced stages oral SCC is about 33-42% [1-3]. One of the most important factors which can be attributed in poor survival rate among oral SCC cancer patients, is advance stages in presentation and lack of early detection [4]. These tumors spread mostly through local progressive invasive growth and current studies have

focused mainly on understanding the biological behavior of the invasive oral cancer at their early stages [5,6]. Although molecular progression models exist for oral carcinogenesis, the precise molecular targets and pathways remain unclear [7].

Sex-determining region Y [SRY]-box 2 (SOX2) belongs to a family of embryonic developmental transcription factors. SOX2 plays a crucial role in embryonic development, lineage determination and also cooperate in the induction of pluripotent stem cell [4, 8-9]. SOX2 is usually absent in normal epidermis but starts to express in the majority of pre-neoplastic skin tumors, and continues to overexpress in cancer stem cells compared to the parental cell population in invasive oral SCC [10-11]. Recently, SOX2 was determined as an oncogenic factor in some cancers with epithelial origin. SOX2 locus amplification was found in SCC of esophagus (15%) and lung (23%) in human. In contrast to other SCCs, which SOX2 is frequently genetically amplified, the expression of SOX2 in skin SCC is transcriptionally regulated [11]. In addition, it has been assumed that expression of SOX2 in oral SCC is associated with small tumor size and early tumor stage, and better disease-free survival. Moreover, SOX2 expression in oral SCC has been shown in different pattern and the diffuse staining pattern was significantly associated with lymph node metastasis [12-16].

To the best of our knowledge, studies focused mainly on the expression of SOX2 in oral and esophageal SCC and compared it with the adjacent normal tissue and expression of SOX2 was not evaluated frequently in dysplastic lesions of oral mucosa. In our project, we intended to design a study to evaluate the expression of SOX2 in different level of malignant transformation, so we compared SOX2 expression by using immunohistochemical staining in oral SCC, epithelial dysplasia and normal mucosal tissues.

## Materials and Methods

### Ethic statement

The project was approved by the Ethic Committee of Tehran University of Medical Sciences. All controls who volunteered to take part in this project signed the consent form. The data of SCC and epithelial dysplasia groups were collected retrospectively and no informed consent was requested by the ethic committee.

### Patients

We retrospectively reviewed the charts of 24 SCC

patients who were diagnosed with primary SCC and 24 epithelial dysplasia between July 2013 and March 2014 in our institute. Control group selected among patients who referred for surgery of third molar teeth.

### Antibodies and Immunohistochemistry

Immunohistochemical staining was performed on 4µm paraffin section. The arrays were deparaffinized in heat oven for 20 minutes at 55 degree Celsius followed by serial xylene washes. They were rehydrated by graded alcohols, and exposed to antigen retrieval by using citrate buffer with pH=6, PT module set for 20 min at 92 C. The endogenous peroxidase activity was omitted by application of 3% hydrogen peroxide in methanol for 20 min. Slides were then incubated with a SOX2 rabbit polyclonal antibody (Glory Science Co; Ltd Add: 2400 Veterans Blvd. Suit 16-101, Del Rio. TX, USA). We used Avidin-biotin model with DAB and Hematoxylin for assessment of colored degree pattern. The evaluation was performed by two independent pathologist without any prior knowledge of each patient's clinical information. If disagreement occurred (intensity score discrepancy >1 or percentage level >10%), the slides were re-evaluated together to obtain a consensus diagnosis.

Each slide was examined under a light microscope (BX43 Olympus, Japan) and based on primary staining (semi quantitatively) scored on the number of stained cells which includes [17]:

0 = No staining

1 = Less than 5% cells were stained

2 = Between 5% to 25% of cells were stained

3 = Between 25% to 50% of cells were stained

4 = More than 50% of cells were stained

Then the staining intensity for each slide reviewed to determine the overall tonality of each slide (qualitative) that includes:

0 = No staining

1 = Weak staining

2 = Moderate staining

3 = Intense staining

### Statistical Analysis

All Statistical analysis were performed using SPSS software, version 18.0. Differences among all tree groups were determined using Kruskal-Wallis and differences between two groups were analyzed by Dunn method. P value <0.05 was considered to indicate a statistically significant difference

### Result

We retrospectively reviewed the charts of 24 SCC and 24 oral epithelial dysplasia and 24 patient as control group. In the SCC group 13 were males and 11 were females with a mean age of 58.04 years (range 26 to 79 years). In the oral epithelial dysplasia group there were 15 males and 9 females with a mean age of 60.58 years (range 49 to 89 years) which among them 7 patients had mild dysplasia, 10 had moderate dysplasia and 7 patients had severe dysplasia. Control group consisted of 11 males and 13 females with a mean age of 22.25 years (range 18 to 32 years).

All patients of SCC and oral epithelial dysplasia samples were positive for SOX2, although in control group 17 patients were positive for SOX2 and 7 patients were negative. In the SCC group 8 patients had mild staining (Fig.1A), 9 patients had moderate staining (Fig.1B) and 7 patients had severe staining for SOX2 (Fig.1C). In the oral epithelial dysplasia group 10 patients showed mild staining, 12 patients showed moderate staining and 3 patients showed severe staining (Table 1). In the SCC group, 10 patients had score 4 (more than 50% of cells were stained), 11 patients had score 3 (25% to 50% of the cells were stained), 2 patients had score 2 (5% to 25% of the cells were stained)

and one patient had score 1 (Less than 5% cells were stained). In the oral epithelial dysplasia group 5 patients had score 4, 11 patients had score 3, 6 patients had score 2 and 2 patients had score 1. In the control group 6 patients have score 2 and 11 patients have score 1 (Table 2).

Comparisons between SCC and oral epithelial dysplasia groups regarding the intensity of staining and percentage of stained cells did not reveal any significant results (P=0.496, P=0.204), although significant difference existed between the values of control and oral epithelial dysplasia group (P<0.001). Similarly a significant difference was observed between the values of SCC and controls' (P<0.001).

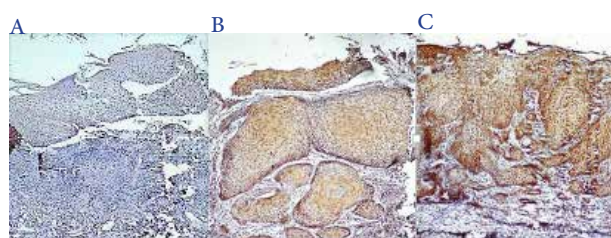


Fig. 1. Immunohistochemical analysis of SOX2 expression in human oral SCC: The intensity was scored as 0 when no positive cells were identified; weak staining as 1; moderate as 2, and strong as 3. A: Weak staining, B: Moderate staining, C: Intense staining.

Groups	Staining severity			
	No	Mild	Moderate	Severe
SCC group	0	8	9	7
Dysplastic group	0	10	12	2
Control group	7	14	3	0
Total	7	34	22	9

Table 1: Comparing staining severity in SCC, dysplastic and control groups.

Groups	Staining amount				
	No	< 5%	5% to 25%	25% to 50%	> 50%
SCC group	0	1	2	11	10
Dysplastic group	0	2	6	11	5
Control group	7	11	6	0	0
Total	7	14	14	22	15

Table 2: Comparing staining amount in SCC, dysplastic and control groups.

### Discussion

The cancer stem cell hypothesis assumes that cancer may be initiated and maintained by a subset of cells that acquire or maintain some stem-cell properties and a small subpopulation of these cells that sustain their ability to differentiate into multiple cell lineages exists in each tumor. This subpopulation may be similar to the stem like cells or stem cells [14]. Understanding genetic pathways that are involved in tumor initiation and progression is crucial for identification of tumors biology leading to the discovery of useful diagnostic, prognostic and predictive markers as well as potential targets for actionable therapies [15]. The SRY-box 2 (SOX2) gene located on chromosome 3q26.33, encodes a member of the SRY related HMG-box family of transcription factors and upregulation of SOX2 induces cell proliferation [17].

In the present study, first of all, we found significant difference between the expression of SOX2 in control and SCC groups ( $p < 0.001$ ). Similar to prior studies, our results confirmed overexpression of SOX2 protein in oral SCC which also have been found in lung, oropharyngeal, esophageal and sinonasal Cancers [4,6,8,14-21].

He et al. [22] studied the expression of SOX2 in oral SCC and showed that it was significantly associated with the pathological grade. In addition, they showed a significant difference in SOX2 staining between oral SCC, epithelial dysplasia and normal oral mucosa. However, in our study comparisons between SCC and oral epithelial dysplasia group did not reveal any significant results ( $P = 0.49$ ), but the difference between normal oral mucosa and oral SCC and dysplasia was statistically significant. In a study by Ting-Ying Fu et al. [17] it has found that SOX2 expression in tumor adjacent normal tissue was significantly higher than that in normal ( $P = 0.021$ ) and tumor tissues ( $P < 0.001$ ) and higher values of SOX2 expression were associated with

better disease specific survival ( $P = 0.002$ ). They have concluded that SOX2 is a biomarker of early stage oral SCC and tumorigenesis. In addition, Cha et al. [23] analyzed the abundance of copy number alterations of dysplastic transitional areas of oral SCC using array-CGH on fresh tissues. Their results appear to support the hypothesis that SOX2 gene amplifications are an early event in tumorigenesis. However in our study, statistically similar levels of SOX2 expression occurred in all stages of the oral dysplasia regardless of mild, moderate or severe dysplasia. Also, because of small patients number in oral dysplasia group, this finding needs to confirm with more comprehensive studies.

Several studies brought out that SOX2 overexpression in head and neck and tongue SCC cancer cells leads to a worse prognosis with lower survival rates. Wang et al. [24] established that SOX2 expressions were significantly associated with higher histological grade ( $P < 0.001$ ), indicating their correlation to dedifferentiation in these tumors and also a significant correlation was observed between increasing levels of immunostaining for SOX2 and decreasing survival for the patients ( $P < 0.001$ ). Ge N et al. [14] revealed that expression of SOX2 was significantly related to the histological grade of patients hypopharyngeal SCCs. However, some articles mentioned that SOX2 protein expression was associated with better overall survival in oral and lung SCC and others showed expression of SOX2 was not associated with prognosis. SOX2 may also act as a promising marker for directing OSCC diagnosis and therapy [12-16].

In conclusion, SOX2 expression is common in oral dysplasia and SCC, their detection in early stages could be crucial for early identification and more accurate prognosis of SCCs. We can offer SOX2 expression analysis as a helpful tool to detect early suspicious oral SCC cancer cases in high risk and dysplastic lesions.

This study was a retrospective study, and immunohistochemical staining was done on available tissue biopsies of oral dysplasia and SCC, so there was not detailed clinic

al information of patients for more accurate data analyses.

### Conflict of Interest

There is no conflict of interest to declare.

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