

Correlation of SOX2 expression with clinicopathologic parameters in larynx squamous cell carcinoma

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Abstract

Aim: Laryngeal cancers are the most common tumors in the respiratory system after the lung and bronchial system. It is important to identify new biological markers which may also help us to understand the molecular and biological mechanisms of tumor development. SOX2 is a cancer stem cell, that is an indicator of lymph node metastasis, biomarker or potential therapeutic target in some cancer types. The aim of this study is to investigate whether there is a correlation between SOX2 expression and the clinicopathological features of laryngeal squamous cell carcinoma.

Material and Methods: In this retrospective study, we analyzed surgical tissue samples of 61 patients with laryngeal squamous cell carcinoma who had undergone total laryngectomy and bilateral cervical lymph node dissection without preoperational radiotherapy and/or chemotherapy between 2012 and 2016. SOX2 expression was evaluated in 61 laryngectomy and cervical lymph node dissection materials using immunohistochemistry and statistically analyzed for its correlation with clinicopathological parameters.

Results: Of the 61 patients, only two were female. The average age of the patients at the time of diagnosis was 60,9 ±8,7 years. SOX2 overexpression was detected in 83,6% of cases. There was a statistically significant correlation between SOX2 overexpression and anatomical location, histological differentiation, local cervical lymph node metastasis, perineural invasion and vascular invasion; however, no significant correlation was found between cartilage invasion.

Discussion: SOX2 expression levels in LSCC showed a statistically significant correlation with local cervical lymph node metastasis. Future studies on SOX2 expression levels in preoperational biopsy specimens are warranted to evaluate its potential prognostic parameters, which may guide treatment decisions in patients with LSCC.

Keywords

Laryngeal Cancer; Squamous Carcinoma; SOX2 Expression; Immunohistochemistry

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Introduction

Laryngeal cancers are the most common tumors in the respiratory system after the lung and bronchial system [1]. Almost all laryngeal cancers are squamous cell carcinomas [SCC] [2]. Laryngeal cancers pose a significant risk of morbidity and mortality for patients as the overall 5-year survival rate is 60.3% per SEER Cancer Statistics Review website [accessed on 4/23/2020]. Since tumors in the same location with similar histologic differentiation degree may act differently, new parameters are needed to elucidate the underlying mechanisms of tumor development. New biomarkers, including various immunohistochemical techniques, are needed to determine the prognosis in LSCC and are performed in relation to the correlation of clinicopathology. In studies with cyclin D1 protein [3] and BCL2L12 and BAX protein [4] expression, a significant relationship was found with the clinicopathological parameters in LSCC patients.

SOX2 [SRV-related Hmg-box gene 2] encodes a transcription factor that is crucial for maintaining embryonic stem cell pluripotency. SOX2 has also been shown to play a role in proliferation, migration, invasion, and metastasis of cancer cells, as well as the continuation of tumor cells and stem cells, cell programming, apoptosis and development of chemoresistance. Recent studies showed that SOX2 acts as an anti-apoptotic factor in cancer cells [5,6]. Bass et al. [2009] found that SOX2, a transcription factor at chromosome 3q26.33, at a genomic amplification peak, is effective in esophageal and lung squamous cell carcinomas, and concluded that the lung and esophagus can be identified as a lineage-survival oncogene in SCC. They showed that the expression of pluripotency markers is involved in squamous cell differentiation in SOX2-driven tumors [7]. Recent studies have demonstrated that SOX2 plays a role in the development of the normal esophageal squamous cell structure. Similarly, some others studies have reported the induction of pluripotent stem cells, providing both differentiation and proliferation of basal tracheal cells [8,9]. We assumed that SOX2 expression may be critical in LSCC pathogenesis as larynx, lung and esophagus SCC show significant similarity in histomorphological and clinical features.

In this study, we aimed to investigate the tumor behavior of SOX2 expression in laryngeal carcinoma and the correlation with clinicopathological parameters such as differentiation level of the tumor, cartilage invasion, perineural invasion, lymph node metastasis.

Material and Methods

Patient Selection

In this study, we included 61 patients, consisting of 59 male and 2 female patients, who were operated due to laryngeal squamous cell carcinoma. In order to compare SOX2 equally with all parameters and to predict the prevalence and correlation of prognostic parameters in general larynx squamous cell cancer, all cases who underwent total laryngectomy and neck dissection operation without preoperational radiotherapy and/or chemotherapy within 4 years were included in this study.

Formalin-fixed, paraffin-embedded tissues [FFPE] of pathological specimens that were stained with Hematoxylin-Eosin were retrieved. Paraffin blocks that contain viable tumor

cells were chosen for analysis. The study was approved by the Research Ethics Committee.

Immunohistochemical Analysis

After identifying suitable FFPE, an unstained section from each block was retrieved. Three-micron thick sections were taken from paraffin- embedded blocks on positively charged microscope slides for immunohistochemical assays.

Immunohistochemical staining of SOX2 [rabbit mab, #SP001, klon: EP103, cell signaling technologies, USA] was performed using the DAP peroxidase method. This procedure was carried out in Leica Bond III device. The immunostaining procedure was performed on a [Leica Bond III] device after slides were incubated at 80°C for 3 hours. Briefly, Bond-Dewak solution was applied for 10 minutes at 60°C, slides were then deparaffinized and rehydrated through graded ethanol solutions. Antibody retrieval was carried out by applying ER1 at 96°C for 20 minutes, followed by H2O2 blocking for 13 minutes at room temperature. The primary antibody [SOX2, rabbit monoclonal antibody, #SP001, klon: EP103, cell signaling technologies, USA, 1:100] was applied for 30 minutes, then it was washed and secondary antibody was applied for 8 minutes at room temperature. DAB was used as a chromogen and hematoxylin was used for counterstaining. Coverslipping followed graded alcohols and xylene.

Evaluation of staining

Immunohistochemical stainings were analyzed under a 20X light microscope. The diencephalon part of fetus human brain was used as a control group. Only cells with nuclear SOX2 staining were accepted as positive staining. Cases were graded according to the intensity of the staining as follows: no staining, weak/yellowish staining, strong/brownish staining (Figure 1). According to the proportion of positively stained tumour cells in 5 high power field: 0 [0-5% stained group], 1+ [6-33% stained group], 2+ [34-66% stained group] and 3+ [67% and over stained group] (Figure 2).

Statistical Analysis

SPSS 22.0 program was used for statistical analysis. Descriptive statistics were given as numbers and percentages for categorical variables; mean, standard deviation, minimum, and maximum for numerical variables. Chi-square analysis was used to compare the ratios between independent groups. Monte Carlo Simulation was used when conditions are not met. $P < 0.05$ was considered statistically significant.

Results

We identified 61 patients who were diagnosed with laryngeal squamous cell carcinoma and underwent total laryngectomy with bilateral neck dissection. Among these patients, 59 [96.7%] were male and 2[3.3%] were female. The mean age of the patients was 60.2 [±]. Histopathologic examination revealed that 26 [42.6%] of tumors were poorly differentiated, 22 [36.1%] were slightly differentiated, and 13 [21.3%] were well differentiated. According to the anatomical location of the tumor, cases were grouped into 6 different groups. The tumor was located in supraglottic region in 25 patients [41.0%], glottic and subglottic in 18 [29.5%], confined to glottis in 11 [18.0%], transglottic in 4 [6.6%], supraglottic and glottic in 2 [3.3%], and confined to subglottis in 1 [1.6%]. In 34 [55.7%] of the patients,

Table 1. Metastasis and invasion characteristics of tumors based on tumor differentiation

	Tumor Differentiation			p
	Well differentiated	Moderately differentiated	Poorly differentiated	
	n[%]	n[%]	n[%]	
Thyroid Cartilage Invasion	6[46,2]	12[54,5]	16[61,5]	0,653
Perineural Invasion	1[7,7]	8[36,4]	15[57,7]	0,010
Vascular Invasion	1[7,7]	7[31,8]	17[65,4]	0,001
Lymph Node Metastasis	2[15,4]	3[13,6]	16[61,5]	0,001

Table 2. Correlation between SOX2 protein expression percentage rate and clinicopathological characteristics

	SOX2 Expression Percentage Rate				p
	%0-5	%6-33	%34-66	≥%67	
	n[%]	n[%]	n[%]	n[%]	
Age					
<60	4[40,0]	6[50,0]	7[63,6]	13[46,4]	0,718
>60	6[60,0]	6[50,0]	4[36,4]	15[53,6]	
Localization					
Supraglottic	0[0,0]	4[33,3]	3[27,3]	18[64,3]	0,002
Glottic	2[20,0]	1[8,3]	3[27,3]	5[17,9]	
Subglottic	0[0,0]	0[0,0]	0[0,0]	1[3,6]	
Transglottic	2[20,0]	0[0,0]	1[9,1]	1[3,6]	
Supraglottic+Glottic	0[0,0]	1[8,3]	0[0,0]	1[3,6]	
Glottic+Subglottic	6[60,0]	6[50,0]	4[36,4]	2[7,1]	
Tumor Differentiation					
Well Differentiated	6[60,0]	3[25,0]	3[27,3]	1[3,6]	<0,001
Moderate Differentiated	3[30,0]	6[50,0]	6[54,5]	7[25,0]	
Poorly Differentiated	1[10,0]	3[25,0]	2[18,2]	20[71,4]	
Thyroid Cartilage Invasion	6[60,0]	3[25,0]	8[72,7]	17[60,7]	0,113
Perineural Invasion	1[10,0]	2[16,7]	6[54,5]	15[53,6]	0,023
Vascular Invasion	1[10,0]	3[25,0]	4[36,4]	17[60,7]	0,021
Lymph Node Metastasis	1[10,0]	3[25,0]	2[18,2]	15[53,6]	0,037

Table 3. Correlation between SOX2 protein expression intensity and clinicopathological characteristics

	SOX2 Expression Intensity			p
	No staining	Weakly staining	Strong staining	
	n[%]	n[%]	n[%]	
Age				
<60	4[40,0]	7[43,8]	19[54,3]	0,641
>60	6[60,0]	9[56,3]	16[45,7]	
Localization				
Supraglottic	0[0,0]	6[37,5]	19[54,3]	0,010
Glottic	2[20,0]	3[18,8]	6[17,1]	
Subglottic	0[0,0]	0[0,0]	1[2,9]	
Transglottic	2[20,0]	0[0,0]	2[5,7]	
Supraglottic+Glottic	0[0,0]	2[12,5]	0[0,0]	
Glottic+Subglottic	6[60,0]	5[31,3]	7[20,0]	
Differentiation grades				
Well Differentiated	6[60,0]	3[18,8]	4[11,4]	0,026
Moderate Differentiated	3[30,0]	6[37,5]	13[37,1]	
Poorly Differentiated	1[10,0]	7[43,8]	18[51,4]	
Thyroid Cartilage Invasion	6[60,0]	7[43,8]	21[60,0]	0,532
Perineural Invasion	1[10,0]	5[31,3]	18[51,4]	0,045
Vascular Invasion	1[10,0]	5[31,3]	19[54,3]	0,028
Lymph Node Metastasis	1[10,0]	4[25,0]	16[45,7]	0,073

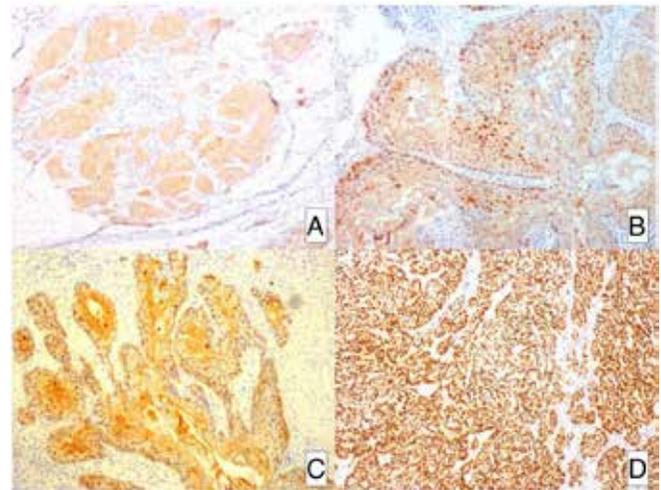


Figure 1. A. SOX2 nuclear staining was not observed, score 0, X100. B. SOX2 nuclear staining percentage rate score 1, X200. C. SOX2 nuclear staining percentage rate score 2, X200. D. SOX2 staining percentage rate score 3, X200.

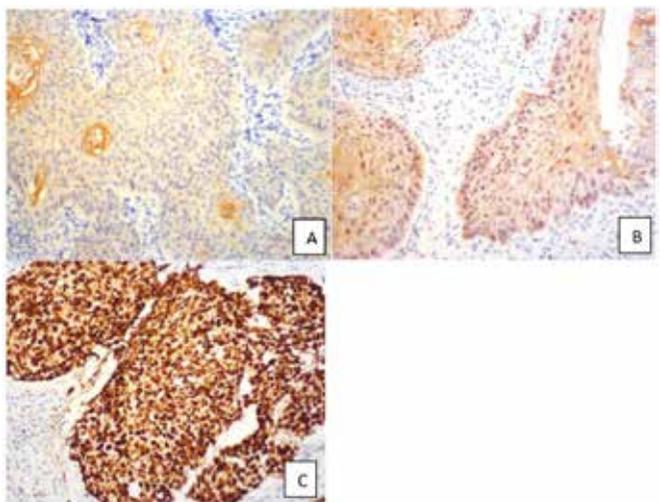


Figure 2. A. SOX2 nuclear staining was not observed, X200. B. SOX2 nuclear staining weakly, X200. C. SOX2 nuclear staining strong, X200.

thyroid cartilage invasion was present, perineural invasion was seen in 24 [39.3%] patients, vascular invasion was detected in 25 [41,0%] patients, and lymph node metastasis was detected in 21 [34,4%] patients. The relation of tumor differentiation degree with invasion [vascular, perineural, cartilage] and cervical lymph node metastasis is shown in Table 1.

In 10 [16.4%] patients, the tumor was not stained with SOX2 [0], in 12 patients % 6-33 of the tumor was stained with SOX2 [1+], in 11 patients %34-66 of the tumor was stained with SOX2[2+] and in 28 patients >67% of the tumor was stained with SOX2 [3+]. In cases showing SOX2 expression, 16 [83.6%] had strong staining in 16 and 35 [57.4%] had weak staining. When these groups were compared, significant differences were found regarding location of the tumor, differentiation level of the tumor, perineural and vascular invasion, lymph node metastasis [p=0,002, p<0,001, p=0,023, p=0.021, p=0.037]. In 3+ stained group, the tumors located in supraglottis were statistically higher than in other groups [p=0,002]. The SOX2 staining percentage rate was found to be slightly differentiated

in the group with score 3 and well differentiated in the group with a score of 0. It was noted that perineural, vascular invasion, and lymph node metastasis rates were higher in groups with SOX2 staining score 2 and score 3. SOX-2 expression percentage rates are shown in Table 2.

When examining the relationship between SOX2 staining intensity and other parameters, location of the tumor, differentiation degree, perineural and vascular invasion rates, a statistically significant difference was observed between them [$p = 0.010$, $p = 0.026$, $p = 0.045$, $p = 0.028$].

As the intensity of staining increases, a higher number of supraglottic locations, vascular and perineural invasion, and poorly differentiated tumor were observed (Table 3).

Discussion

SOX2 is a transcription factor located on chromosome 3p26.33, which is responsible for the regeneration of the pluripotent stem cells. This has been reported to take place in embryologic development, organogenesis and several different neoplastic processes. Overexpression of SOX2 in Hep-2 cells is related to activation of the P13K/Akt/mTOR pathway, which causes overexpression of MMP-2 and results in an increased tumor cell proliferation, migration, and invasion in laryngeal squamous cell carcinoma [10].

In the literature, there are only a few studies regarding the association of SOX2 overexpression in laryngeal squamous cell carcinomas. In a recent study, SOX2 staining intensity was not found to be associated with the age and gender of the patient, and tumor location in laryngeal squamous cell carcinoma [11]. Similarly, no significant association with the age and gender of patients was found in our study. We found that the location of the tumor was associated with the intensity of SOX staining, which was prominent in supraglottic tumors. This difference might be due to embryologically, the supraglottic area (3rd and 4th branchial arc) and glottic and subglottic area (5th and 6th branchial arc) developed from different embryological structures.

In our study, differentiation of the tumor was also associated with SOX2 expression, as, for example, Gonzalez et al [12] and Liu et al [13] reported similar findings. The extensiveness of staining was also found to be associated with differentiation of the tumor. However, Rodriguez et al [14] did not find any association between tumor differentiation and SOX2 expression in breast cancer, which might be due to the location and histology of the tumor type.

We also confirmed that the intensity and percentage rates of SOX2 staining were significantly associated with lymph node metastasis, vascular and perineural invasion. Lengerke et al [15] showed that SOX2 overexpression in early-stage breast cancer is an indicator of potential lymph node metastasis. In another study related to tongue SCCs, a significant relationship was shown with lymph node metastasis of SOX-2 expression [13]. Xia-bing et al reported that SOX2 overexpression affects clinical stage, lymph node metastasis and prognosis in laryngeal carcinoma [11].

Since there is significant connection between sox2 prevalence and lymph node metastasis, cases that have been diagnosed as larynx scc, as a result of first biopsy, taken from the larynx,

lymph node metastasis might be anticipated by means of SOX2 immunohistochemical studies. Therefore, larynx dissection supplementation decision may be included in the surgical procedure. Providing identification of cases that are unlikely to cause lymph node metastasis, it will actually prevent lymph node dissection that is not necessary.

In a study done with 94 patients with precancerous lesions, Rocio et al [16] found that SOX2 expression was the only significant independent predictor for laryngeal cancer transformation. These findings support the role of SOX2 in early tumor development and the clinical utility of SOX2 expression in predicting the transformation of precancerous lesions to laryngeal cancer in addition to current WHO histopathological classification.

Identification of genetic alterations in the formation of tumorigenesis. It plays a key role in predicting the behavior of the tumor and discovering prognostic biomarkers, as well as in more effective treatments for potential targets [17]. To develop new strategies in diagnosing and treating laryngeal carcinoma, molecular studies about oncogenic transcription factors like SOX-2 are being conducted. In addition, cancer stem cells being effective in tumor formation and cell motility, improve understanding of the resistance mechanism in radiotherapy resistant head and neck cancers, and it is important to develop new therapeutic agents that can also be used in the treatment of these cancers [18].

It has been found that LSD-1 inhibitors, can stop tumor growth and therefore can be used in targeted therapy in SOX-2 expressing tumors, such as lung SCC [19]. To determine SOX2 expression in larynx biopsies, treatments targeting SOX-2 can also be valuable in the use of larynx SCCs.

In this study, we have shown that SOX2 overexpression has a significant effect on prognostic factors in laryngeal squamous cell carcinoma. These findings will provide insight for future studies about the alternative treatment of laryngeal carcinoma. In this matter, further studies including alternative treatment methods are needed.

Conclusion

SOX2 overexpression shows a statistically significant correlation with cervical lymph node metastasis and clinicopathological parameters. It can be used in the preoperative biopsy specimens with SOX2 expression to guide treatment decisions in laryngeal SCC patients and to evaluate the potential of targeted treatment.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

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