Human Pathology (2014) **45**, 851–857

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Human PATHOLOGY

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Original contribution

SOX2 expression in hypopharyngeal, laryngeal, and sinonasal squamous cell carcinoma☆,☆☆

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Received 11 July 2013; revised 8 December 2013; accepted 11 December 2013

Keywords:
Squamous cell carcinoma;
Sinonasal;
Larynx;
Hypopharynx;
SOX2;
Immunohistochemistry;
Prognosis

Summary Squamous cell carcinoma (SCC) of the head and neck display high frequencies of DNA copy number gains at chromosomal region 3q26-27. Recently *SOX2* has been postulated as a driver oncogene for these amplifications; however, its role as a prognostic marker is still a matter of debate. The aim of this study was to evaluate the involvement of SOX2 protein expression in three different sublocalizations of head and neck SCC and its possible role as prognostic marker. SOX2 expression was analyzed by immunohistochemistry in 102 pharyngeal, 67 laryngeal, and 51 sinonasal SCCs, and the relation to clinicopathological and follow-up data was studied by χ^2 and Kaplan-Meier analysis. SOX2 expression was significantly ($P = .002$) more frequent in hypopharyngeal and laryngeal SCC (38%, 39/101) and (42%, 28/67), respectively, compared to sinonasal cancer SCC (14%, 7/51). SOX2 expression did not correlate to disease stage, T or N classification, lymph node metastasis, recurrence or clinical outcome in any of the three sublocalizations. These results indicate that SOX2 expression is a common event in hypopharynx and larynx, but not in sinonasal SCC. The absence of correlation to clinical outcome, may suggest a role for *SOX2* in tumor initiation, but not in tumor progression.

1. Introduction

Squamous cell carcinoma (SCC) of the head and neck (HNSCC) is the sixth most common neoplasm in the world, representing 2% to 5% of all malignancies [1]. The great majority is located in oral cavity, larynx and pharynx; sinonasal SCC may be considered a rare tumor. Tobacco and alcohol, as well as human papilloma virus infection, are well known etiological factors, the latter especially in oropharyngeal cancer. HNSCC display a characteristic profile of

☆ Disclosure: The authors declare that they are not aware of conflicts of interest, financial or otherwise.

☆☆ Funding sources: This study was supported by grant PI08-1599 and EMER07-048 of Fondos de Investigación Sanitaria (FIS) and RD12/0036/0015 of Red Temática de Investigación Cooperativa en Cáncer (RTICC), Spain, and the FEDER Funding Program from the European Union.

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0046-8177/$ – see front matter
http://dx.doi.org/10.1016/j.humpath.2013.12.004

genetic aberrations, including losses at chromosomal regions 3p, 8p, 9p, 11qter, 17p, 18q, and gains at 3q, 5p, 7p, 7q, 8q, 11q13, 18p, and 20q [2-4]. The most frequent copy number gains and amplifications occur at 3q26-27. This region contains many genes, and this may explain the fact that up to the present time there is still no consensus on the driver gene.

Copy number gains of the 3q26-27 region have been observed in SCC of many localizations [2-7]. In pulmonary and esophagus SCC, the *SOX2* (sex-determining region Y-box 2) transcription factor has been identified as an oncogene and a driver gene for this locus [8-10]. *SOX2* amplification and expression has been implicated in many tumor types, including breast, colon, ovarian, and nasopharyngeal cancer [11-14], but mostly in SCC of various localizations (lung, esophagus, head and neck). *SOX2* has been reported to be amplified and overexpressed in 20% of squamous cell pulmonary cancer, being higher in smokers than in nonsmokers [10,15]. Hussenet et al [9] observed a frequency of *SOX2* amplification similar to *c-MYC*, *EGFR* or *ERRB2*. Freier et al [16] reported copy number gains in 52% and SOX2 expression in 18% of oral SCC.

The role of *SOX2* as a prognostic marker is still a matter of debate. Some studies have shown that expression of SOX2 confers a better prognosis [17,18], but others found an association with worse clinical outcome or adverse clinical parameters, including recurrences, lymph node and distant metastasis, disease-free and overall survival [12-14]. Also in HNSCC the prognostic significance of *SOX2* remains unclear. Therefore, the aim of our study was to analyze SOX2 protein expression in three different sublocalizations of HNSCC: pharynx, larynx and sinonasal SCC, and to evaluate its possible role as prognostic marker using clinicopathological and follow-up data.

2. Materials and methods

2.1. Patients and samples

A total of 220 patients with histologically confirmed SCC were included in our study, after informed consent and approval of the Hospital Universitario Central de Asturias Hospital Ethical Committee and following the Helsinki Declaration guidelines. In detail, there were 3 groups of patients, classified by their primary tumor localization: 102 hypopharyngeal, 67 laryngeal, and 51 sinonasal cancers. All patients had a single primary tumor and received no treatment prior to surgery. No patient had distant metastases at the time of diagnosis. TNM classification was performed according to the 7th Edition of the International Union Against Cancer TNM classification system and histological grade according to World Health Organization (WHO) classification [19]. Sixty-five percent (137/213) of patients received postoperative radiotherapy; for 10 patients this information is missing. Follow-up information was obtained of all patients, until the last occurrence for patients still alive, until the time of death or until the time of lost contact. The median follow-up time was 31 months (range, 0-211). A summary of the relevant clinicopathological features for each tumor localization is shown in Table 1.

2.2. Immunohistochemistry

A total of 10 tissue microarray (TMA) blocks were assembled from formalin-fixed, paraffin-embedded tissues as previously described [20]. Briefly, areas of interest rich in non-necrotic areas were identified on corresponding hematoxylin and eosin–stained sections, and were marked with 2-mm circles on the source paraffin block. The source block was cored, and a 1-mm core was transferred to the

Table 1 Clinical features of the three groups of patients

	Hypopharynx	Larynx	Sinonasal
Total Patients	102	67	51
Gender			
Male	99	67	37
Female	3	0	14
Age			
Average	59	62	66
Range	43-84	36-86	47-91
T classification			
T1-2	29	21	5
T3-4	73	46	46
N classification			
N0	16	33	38
N+	86	34	13
Disease stage			
I-II	7	13	4
III-IV	95	54	47
Histological grade			
Well	23	25	18
Moderate	41	30	10
Poor	38	12	23
Follow-up (months)			
Mean	25	41	31
Median	14	37	18
Range	0-95	1-97	1-211
Radiotherapy			
No	25	39	12
Yes	75	20	39
Lost	2	8	0
Local recurrence			
No	39	36	9
Yes	63	31	42
Distant metastasis			
No	61	49	46
Yes	41	18	5
Patient status			
Alive	23	22	8
Died of disease	62	26	37
Died of other causes	8	9	6
Lost	9	10	0