Differences in CD44s expression in HNSCC tumours of different areas within the oral cavity

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Background and Aims. There is evidence of differences in p63 expression in HNSCC (head and neck squamous cell carcinoma) tumours of different sub-sites within the oral cavity which appear to have an important role in CD44 regulation. The aim of this study was to investigate CD44 expression in HNSCC tumours in different areas of the oral cavity.
Methods. Formalin-fixed and paraffin-embedded oral squamous cell carcinoma specimens from 29 patients were investigated. Expression of CD44s was detected by immunohistochemistry with specific CD44 antibodies and analysed using the H-score. The samples were classified by anatomic location *(*tongue; floor of the mouth; gingiva and other group included hard palate, tonsils, naso- and oropharynx) and histological tumour grade (G1 and G2-G3). Fischer's exact test with a Bonferroni correction was used for the statistical analysis.
Results. Positive immunostaining for CD44s in carcinomas of the tongue was significantly lower (0%) than that of the floor of the mouth (85%; *P*=0.003) or other group (100%; *P*=0.003). No statistically significant differences in CD44s expression were found for different histological tumour grades.
Conclusions. The results indicate that CD44 expression is strongly reduced in tongue tumours compared with other sub-sites within the oral cavity. This should be taken into consideration in assessing the prognostic value of CD44s in this tumour group.

Key words: CD44s, oral cancer, sub-sites, immunohistochemistry

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INTRODUCTION

The families of proteins known collectively as adhesion molecules are cell surface glycoproteins with relatively strong bonding to specific ligands. CD44, one of the main cell surface receptors for hyaluronic acid in the extracellular matrix, is a widely expressed cell surface glycoprotein that serves as an adhesion molecule in cell-substrate and cell-cell interactions[1-3]. This glycoprotein appears to have many varied functions depending on the extracellular structure of the protein which can be produced in many isoforms. The wide range of functional proteins is produced from a single gene by both alternative splicing and post-translational modification[4].

There are considerable differences between sub-sites in structure and epithelial differentiation within normal oral epithelium. Boldrup et al. reported that there were clear differences in p63 expression in normal samples as well as in HNSCC tumours of different sub-sites within the oral cavity[5]. Furthermore, the same authors indicated that p63 seems to have an important role in CD44 regulation[6]. This study was initiated to investigate the CD44 expression in HNSCC tumours of different areas within the oral cavity.

MATERIALS AND METHODS

Routinely formalin-fixed and paraffin embedded specimens of primary oral squamous cell carcinomas from 29 patients were retrieved from the files of the Department of Clinical and Molecular Pathology, Palacky University in Olomouc, Czech Republic. Specimens were taken from tumours of the floor of the mouth (n=13), tongue (n=7), gingiva (n=3), hard palate (n=3), tonsils (n=1), naso-(n=1) and oropharynx (n=1). For clinical details see Table 1.

The samples were classified by the anatomic location (floor of the mouth, tongue, gingiva and other included hard palate, tonsils, naso- and oropharynx) and histological grade of tumour (G1 and G2-G3). Expression of CD44s was detected by immunohistochemistry with specific antibodies (monoclonal mouse antibody NCL-CD44-2, clone DF1485; Visionbiosystems-Novocastra; dilution 1:50) and analysed using the H-score. The H-score is a method of assessing the extent of immunoreactivity taking into account the percentage of cells (0-100%) in each intensity category (0-3) and computes a final score, on a continuous scale between 0-300. The human tonsil- paracortex and germinal centre were used as positive and negative control, respectively. Fischer's exact test with a Bonferroni correction was used for statistical analysis.

RESULTS

The comparative evaluation of CD44s expression in oral squamous cell carcinomas revealed statistically significant decreased expression in tongue tumour group (0%) compared with that of the floor of the mouth (85%; *P*=0.003) and other group (100%; *P*=0.003). The percentage of CD44s positive and negative samples of different areas of the oral cavity are shown in the Table 2. For H-score levels of CD44s expression in tumours of different anatomic locations see Fig.1.

There was, no statistically significant differences in CD44s expression between G1 and G2-3 groups.

DISCUSSION

p63, a member of the p53 family of genes, is vital for normal epithelial development and is also suggested to be involved in epithelial tumour formation[7-9]. Differences in p63 expression have been found in HNSCC tumours of different sub-sites within the oral cavity. Specifically, significantly decreased expression was seen in all tumours of the tongue compared to corresponding normal tongue tissue[5].

Our results revealed decreased CD44s expression in the tongue tumour group which supports the results of a study in which p63 isoforms are considered to have an important role in CD44 regulation[6].

In search for the prognostic value of CD44, several studies with inconsistent results have been reported. According to some authors, loss of CD44 expression is considered to be an adverse prognostic factor in HNSCC tumours[10-12], while others reported that CD44 is part of the signature of head and neck cancer stem cells[13,14] and

Table 1. Clinical data on all 29 patients analysed.

Patient	Sex	Tumour location	Grade
1	M	Floor of the Mouth	3
2	M	Floor of the Mouth	2
3	M	Floor of the Mouth	3
4	M	Floor of the Mouth	2
5	M	Floor of the Mouth	2
6	M	Floor of the Mouth	2
7	M	Floor of the Mouth	3
8	F	Floor of the Mouth	2
9	M	Floor of the Mouth	2
10	M	Floor of the Mouth	2
11	M	Floor of the Mouth	2
12	M	Floor of the Mouth	3
13	M	Floor of the Mouth	2
14	M	Tongue	2
15	M	Tongue	1
16	M	Tongue	2
17	F	Tongue	1
18	M	Tongue	2
19	M	Tongue	2
20	M	Tongue	2
21	M	Gingiva	2
22	M	Gingiva	2
23	M	Gingiva	2
24	M	Other/hard palate	2
25	M	Other/tonsil	2
26	M	Other/oropharynx	1
27	M	Other/oropharynx	1
28	M	Other/nasopharynx	1
29	M	Other/hard palate	1

Table 2. Demonstrates percentage of CD44s positive and negative staining in tumours of different sub-sites within the oral cavity.

Locality		CD44s		Total
		negative	positive	
floor of the mouth	count	2	11	13
	percentage	15.4%	84.6%	100.0%
gingiva	count	2	1	3
	percentage	66.7%	33.3%	100.0%
tongue	count	7	0	7
	percentage	100.0%	0%	100.0%
other*	count	0	6	6
	percentage	0%	100.0%	100.0%
Total	count	11	18	29
	percentage	37.9%	62.1%	100.0%

* included hard palate, tonsils, naso- and oropharynx

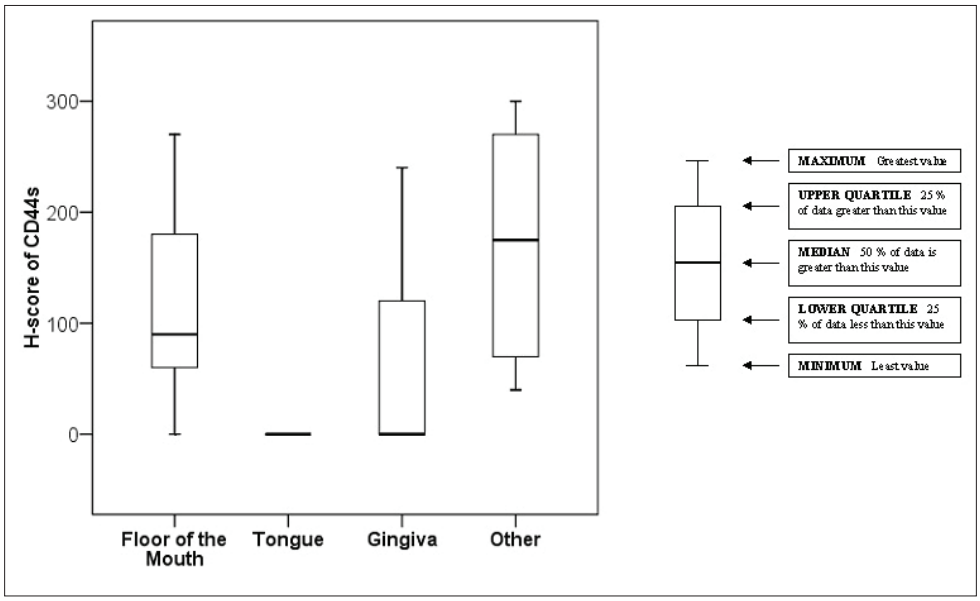

Fig. 1. Box plot summarising H-score for tumours of different anatomic locations.

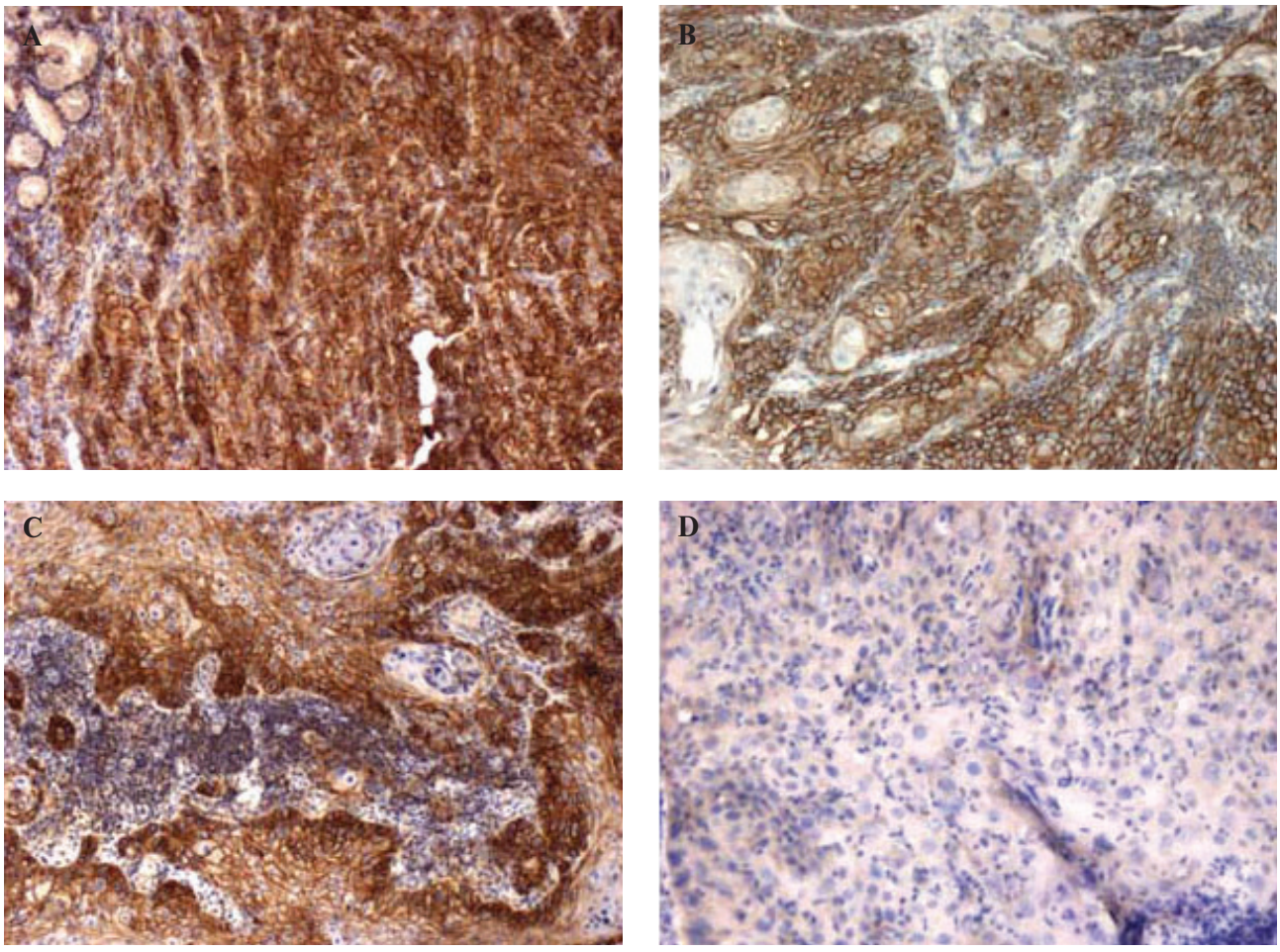

Fig. 2. Immunohistochemical staining for CD44s expression in tumour of the oropharynx (A), hard palate (B), floor of the mouth (C) and tongue (D), respectively.

that only a CD44 positive subset of tumour cells has the capacity to initiate tumour growth[15]. Kokko et al. reported significant association between CD44 overexpression and aggressive behavior in pharyngeal and laryngeal HNSCC, but not in HNSCC of the oral cavity[16]. These contradictory findings complicate the prognostic value of CD44.

The present results indicate that CD44s expression is strongly reduced in squamous cell carcinomas of the tongue compared with other areas of the oral cavity (Fig.2). This should therefore lead us to take different areas into consideration in assessing the prognostic value of CD44s in this tumour group.

CONFLICT OF INTEREST STATEMENT

Author's conflict of interest disclosure: *None declared.*

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