Adhesion molecule CD44 expression in non-tumour epithelium adjacent to tongue cancer

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Summary An immunohistochemical study was performed of changes in the expression of adhesion molecule CD44 in 32 adjacent non-tumour epithelia (ANTE) to lingual carcinomas and in the tumour tissue, using anti-CD44 monoclonal antibody DF1485. The aim was to evaluate the importance of these changes as an early event in lingual carcinogenesis. The ANTE was histologically normal in 22 cases (68.8%) and presented epithelial dysplasia in 10 (32.2%). Three cases of normal and dysplastic ANTE, respectively, were CD44+/− (9.4%). Negative CD44 expression was significantly more frequent in tumours with lower percentages of CD44+ cancer cells, both in normal (P=0.014) and dysplastic (P=0.033) ANTE. The expression in normal ANTE was also significantly associated with clinical stage (P=0.040) and presence of extracapsular nodal spread (P=0.013). Therefore, loss of CD44 expression in ANTE can be considered an early event in lingual carcinogenesis and a marker of major alterations of CD44 expression in the derived tumour tissue.

KEYWORDS
CD44; Tongue carcinoma; Oral carcinoma; Adjacent non-tumour epithelium

Introduction

It is now accepted that many solid tumours, including oral squamous cell carcinomas (OSCCs), are the result of a multi-step process of accumulated genetic alterations that begin before the development of a malignant histopathological phenotype.

This observation underlies the genetic progression model for head and neck carcinoma proposed by Califano et al.,1 in which some molecular events appear very early, even when the mucosa is histologically benign. Study of these early oncogenic molecular disorders is limited by the difficulty of gathering an adequate sample of precancerous lesions with objective documentation of their progression towards cancer. Many authors have circumvented this problem by analysing non-tumour epithelium adjacent to the invasive carcinoma.
Histopathological (epithelial dysplasia) and molecular abnormalities in this epithelium are not induced by the tumour but rather precede the development of the tumour, representing earlier oncogenic alterations in the progression to malignancy. Hence, the study of oncogenic molecular alterations in adjacent non-tumour epithelium (ANTE) may be of value to establish molecular criteria for the early detection of the progression of precancerous lesions.

Oncogenic molecular events in OSCC include alterations in adhesion molecules, producing tissue infiltration and destruction. Adhesion molecule CD44 has proven to be a major factor in cell–cell interactions and cell adhesion. It seems reasonable to hypothesise that the loss of CD44 expression releases cells from their attachment to neighbouring cells, favouring their invasiveness. Studies of laryngeal ANTE have presented contradictory results on CD44 expression and its role as an early event and risk marker for progression to oral cancer. However, few studies have specifically focused on the importance of alterations in CD44 expression as an early event and risk marker for progression to oral cancer. Among oral cancers, lingual squamous cell carcinoma carries an especially poor prognosis, prompting the present study. We analysed the loss of CD44 expression in non-tumour epithelia adjacent to lingual SCCs in order to evaluate its role as an early event in lingual carcinogenesis and as a risk marker for progression to cancer.

Patients and methods

We studied a series of 32 patients, corresponding to a part of a series of patients with lingual SCC previously reported by our group. These patients had been treated for their cancer at the University Hospital of Granada before 1996. The inclusion criterion was the presentation of a lingual SCC in which optical microscopy of hematoxylin–eosin-stained tumour tissue sections had demonstrated the presence of non-tumour epithelium adjacent to the carcinoma. The mean age of the patients was 60.0 years (range 41–84 years) and 28 were male.

Hospital medical records for each patient were searched for the clinical T value, increase in cervical lymph node involvement as determined by clinical methods, presence of distance metastasis according to IUAC and AJCC criteria, and pathologic T value from the pathology report.

The histopathologic data and measurement of tumour thickness were obtained by hematoxylin–eosin staining of formalin-fixed and paraffin-embedded operative tissue sections. Tumour involvement of cervical lymph nodes (pathologic N) was assessed according to IUAC and AJCC criteria. The extracapsular nodal spread of the tumour, the degree of differentiation and the status of the surgical margin were also evaluated. The tumour thickness was measured using a method reported elsewhere. The histopathologic studies were all carried out by a single pathologist (IRA), who classified the ANTE samples as normal or dysplastic.

For the immunohistochemical staining, 4 μm sections were cut from the paraffin blocks. After blocking endogenous peroxidase with H2O2 in methanol for 30 min, sections were immersed in citrate buffer (pH 6.0) in a microwave-resistant container. The anti-CD44 antibody used was Clone DF1485 from Dako (Dako Corporation, Carpinteria, CA). Sections were incubated overnight. Immunoperoxidase detection was employed using the ABC method (Dako) and diaminobenzidine substrate. Counterstaining was performed with hematoxylin. Antigen retrieval methods were used in the study. Staining of infiltrating lymphocytes was considered as positive internal control. A carcinoma section that had not received the primary antibody was used as negative internal control. The membrane expression was considered for the immunohistochemical evaluation of the CD44 molecule. CD44 expression in the tumour tissue associated with the ANTE was determined by calculating the percentage of positive malignant cells with respect to the total number of cells encountered in 20 representative high power fields. The intensity of the staining was disregarded. Groups were formed according to the percentage of positive cells (0–24, 25–49, 50–74 and 75%) (Fig. 1). Following criteria used in previous studies, CD44 expression in basal and parabasal layers with absence of expression in superficial layers of the ANTE was considered normal (Fig. 2).

Association of ANTE CD44 expression with ordered parameters was estimated by Mann–Whitney’s test and with dichotomous variables by Fisher’s exact test.

Figure 1 Positive CD44 expression in lingual carcinoma: ≥75% of CD44+ cancer cells and CD44+ ANTE.
test, using SPSS for Windows, version 11.0 (SPSS Inc. Chicago, Illinois).

Results

Among the 32 tongue OSCCs with ANTE studied, the ANTE was histologically normal in 22 cases (68.8%) and presented epithelial dysplasia in 10 (31.2%). Table 1 exhibits CD44 expression results in both tumour tissue and ANTE. Negative CD44 expression in ANTE was significantly more frequent in tumours with lower percentages of positive cancer cells, in both histologically normal and dysplastic ANTE (Table 2). Tables 3 and 4 display the relationship between the CD44 expression in ANTE and the clinical and pathologic values studied, respectively. Loss of CD44 expression in ANTE was significantly more frequent in tumours with more advanced clinical stage and in tumours with extra-capsular nodal spread.

Discussion

An important issue for the present study was the definition of normal CD44 expression in stratified squamous non-tumour epithelia. We elected to follow authors8,9,16–18 who considered normal CD44 expression to be that in basal and parabasal layers, with no expression in more superficial layers of the epithelium. This is the pattern of a normal maturing epithelium that is differentiating correctly,19 and we speculate that loss of expression at superficial layers may even favour the physiological desquamation of cells of the external epithelium.
Some published studies on CD 44 expression in cancer used ANTE as positive internal control but in most cases did not report pathologic alterations, such as epithelial dysplasia, which may be powerful markers of progression to cancer. The remaining study considered CD44 expression in adjacent dysplastic epithelia but excluded tumours with CD44-negative ANTE on the grounds that this negativity must have been due to technical errors. In our view, the above study designs were misguided, because numerous studies, including the present one, have demonstrated that oncogenic events can occur early in the non-tumour epithelium that is precursor of the cancer. Therefore, the loss of expression in ANTE adjacent to tongue SCC has an undoubted biological significance that is worth careful interpretation.

We observed a loss of expression in 18.6% (six cases) of the ANTE samples studied, in both histologically normal (three cases) and dysplastic (three cases) epithelia. Loss of CD44 expression in ANTE was significantly associated with greater losses of expression in the tumour tissue. Furthermore, CD44-negative ANTE was significantly more frequently related to more advanced clinical stage and extracapsular nodal spread, both recognised features of more aggressive tumours. Our results indicate that alterations in the expression of the CD44 adhesion molecule are an early event in lingual carcinogenesis. These alterations are early markers of a loss of CD44 expression in tumour tissue derived from the epithelium and of the development of aggressive cancers. Thus, some lingual carcinomas develop very early molecular disorders that increase their invasiveness and aggressiveness, even before the epithelium develops the histologic phenotypic features that indicate progression to cancer. There are very few published studies on this issue. Hirvikoski et al. analysed laryngeal dysplasias adjacent to laryngeal SCCs and found loss of CD44 expression in 22% of cases (5/23), interpreted by the authors as an early event in laryngeal carcinogenesis. They also associated loss of CD44 expression with nodal spread of the tumour and a poor prognosis. Similar results were reported by Soukka et al., who considered the absence of CD44 variant exon 6 (v6) in ANTE of laryngeal SCC to be an indicator of early carcinomatous change. There has been scant study of this issue, and the results are sometimes contradictory. Ioachim et al. found a gradual and statistically significant increase in CD44 expression in the progression from keratoses to invasive laryngeal SCC. Studies on oral dysplasias have shown reduction or constitutive CD44 expression. Rautava et al. recently reported that CD44 v6 expression showed no prognostic value as a risk marker of progression to cancer in oral dysplasias; out of 29 cases of oral dysplasia cases, they documented four that progressed to cancer. They described CD44 v6 expression as dysregulated during the malignant process, although

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<th>Variable</th>
<th>Normal (n=22)</th>
<th>Dysplastic (n=10)</th>
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<tr>
<td></td>
<td>CD44+ (%)</td>
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<tr>
<td>N2B</td>
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<td>8 62.5</td>
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a Mann—Whitney test.

b Fisher’s exact test.
the sample size was probably inadequate for statistically meaningful conclusions to be drawn. As mentioned earlier, research on markers of progression to cancer is hampered by the lack of adequate samples of cases in which progression to cancer has been objectively documented. ANTE appears to offer an excellent research model, because it almost certainly constitutes the epithelial origin of the accompanying tumour. Therefore, as previously published, the histopathological and molecular alterations that take place in this epithelium can be considered the earliest events in oral carcinogenesis.2

In conclusion, the loss of CD44 expression in ANTE can be regarded as an early event in lingual carcinogenesis and a marker of major alterations of CD44 expression in the derived tumour tissue.

References

2. Warnakulasuriya KA, Johnson NW. Expression of p53 mutant nuclear phosphoprotein in oral carcinoma and potentially