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ORIGINAL ARTICLE

CD10 expression in stromal cells of oral cavity squamous cell carcinoma: a clinic and pathologic correlation

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OBJECTIVE: CD10 is expressed on the majority of follicle-center lymphomas and Burkitt lymphomas. CD10 has also been shown to be present in a variety of other neoplasms.
DESIGN: The aim of this study was a correlation of CD10 and several parameters: age, tumor size, presence of lymph node metastases, clinic stage, histologic grading, presence of local recurrences.
MATERIALS AND METHODS: The tissues of 77 consecutive patients with oral cavity squamous cell carcinoma were evaluated using immunostaining with monoclonal antibody for CD10.
MAIN OUTCOME MEASURES: Highly significant correlations were found with the lymph node status, the presence of local recurrences and the histologic grading. The presence of CD10-positive cells was not correlated with the age of patients, tumor size and clinic stage.
RESULTS: The results of the present study show that in oral squamous cell carcinoma CD10 positivity is an indicator of worse prognosis. Another strong correlation was found with the presence of local recurrences. Also the histologic grade was significantly correlated with the CD10 positivity.
CONCLUSION: Our results point to the fact that CD10 expression can, perhaps, have an important role in tumor invasion, probably facilitating the occurrence of metastases.
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Keywords: CD10; lymph node metastases; local recurrence; oral squamous cell carcinoma

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Introduction

CD10 is a zinc-dependent metallopeptidase with a molecular weight of 90 000–110 000 which participates in the postsecretory processing of neuropeptides and peptide hormones (Borscheri *et al*, 2001). It is, from a biochemical point of view, a type II integral membrane protein known as neutral endopeptidase 24.11 (NEP 24.11) and cleaves small biologically active peptides at the amino terminus to hydrophobic residues within the peptide sequences (Xiao *et al*, 2001). CD10 is expressed on the surface of a variety of normal and neoplastic hematopoietic and lymphoid cells (Reyes-Botella *et al*, 1999; Xiao *et al*, 2001; Iwaya *et al*, 2002; Xu *et al*, 2002), including lymphoid precursor cells, germinal center B lymphocytes and some epithelial cells (Ogawa *et al*, 2002; Xu *et al*, 2002). CD10 is expressed on the majority of follicle-center lymphomas and Burkitt lymphomas and in a subset of diffuse large B-cell lymphomas (DLBCL) (Xu *et al*, 2002). CD10 has played an important role in the classification of B-lineage lymphomas and in the characterization of acute leukemias (Xu *et al*, 2002). Other terms for CD10 have been neutral endopeptidase, enkephalinase, neprilysin and common acute lymphoblastic leukemia antigen (Moritani *et al*, 2002; Ogawa *et al*, 2002). CD10 is widely distributed and has been found in the renal tubules, glomeruli, brush border of the intestine, hepatic canaliculi, fetal liver cells, normal liver cells, myoepithelial cells of the normal breast, syncytiotrophoblasts, prostatic stromal and epithelial cells, choroid plexus, brain, gonads, adrenal cortex, leukocytes (Borscheri *et al*, 2001; Xiao *et al*, 2001; Xu *et al*, 2001, 2002; Albrecht *et al*, 2002); it has also been demonstrated in the stromal cells of normal bone marrow and endometrium (Ogawa *et al*, 2002). CD10 may play specific roles in the control of cell growth and differentiation of both hematopoietic and epithelial systems (Groisman *et al*, 2002). CD10 has also been shown to be present in a variety of neoplasms such as renal cell carcinoma, prostatic adenocarcinoma, transitional cell carcinoma, endometrial stromal sarcoma, pancreatic adenocarcinoma,

rhabdomyosarcoma, malignant melanoma, schwannoma, endometrial stromal tumors, intraductal breast papilloma, ductal breast hyperplasia, breast fibroadenoma and phyllodes tumor (Xu *et al*, 2001, 2002; Moritani *et al*, 2002; Oliva *et al*, 2002). A possible role of CD10 in the identification and isolation of apoptosing T cells *in vitro* and *ex vivo* (Cutrona *et al*, 1999) and in the growth of androgen-independent prostate cancer has been suggested (Albrecht *et al*, 2002). CD10 has also been shown to play an important role in the progression of malignant melanoma (Bilalovic *et al*, 2004).

In renal malignancies the CD10 expression proved to be an additional marker for the differential diagnosis of histologic subtypes (Langner *et al*, 2004; Pan *et al*, 2004); in skin tumors CD10 might be an indicator of tumor invasiveness if overexpressed in stromal cells, while it might be a marker of follicular differentiation of folliculosebaceous apocrine units if expressed in the actual tumor cells of cutaneous epithelial neoplasms (Yada *et al*, 2004).

The aim of this study was a correlation of CD10 and several different parameters such as the age of the patients, tumor size, presence of lymph node metastases, clinic stage, histologic grading, presence of local recurrences.

Materials and methods

The tissues of 77 consecutive patients with squamous cell carcinoma of the oral cavity were evaluated in the present study. In all tissues was present normal epithelium at the periphery of the lesion. The tissues were retrieved from the archives of the Department of Pathologic Anatomy and Histopathology of the University of Ancona, Italy. The clinic parameters that were correlated with CD10 positivity were the age of the patients, tumor size, presence of lymph node metastases, clinic stage, histologic grading, presence of local recurrences. Immunostaining was performed with monoclonal antibody directed against CD10 (Novocastra Laboratories Ltd, Newcastle upon Tyne, UK). For immunostaining, sections were deparaffinized in xylene and dehydrated in an alcohol series. Immunostaining with CD10 required pretreatment with 1 mM ethylenediaminotetraacetic acid (at pH 8.0) for 20 min at 250 W in a microwave oven. CD10 (dilution 1:25) was administered for 1 h at 37°C in a moist chamber, followed by incubation with biotinylated anti-mouse IgG/anti-rabbit IgG (1:200; Vector Laboratories, Wiesbaden, Germany) and avidin biotin complex (ABC) alkaline phosphatase reagent, each for 30 min at room temperature. Between steps the sections were washed in Tris-buffered saline. The immunoreactions were visualized with the ABC method applying a vectastain ABC alkaline phosphatase staining (Camon, Wiesbaden, Germany) or an Ultratech HRP streptavidin-Biotein universal detection system (Immunotech, Marseilles, France). Fast Red and 3,3-diaminobenzidine-tetrahydrochloride respectively served as chromogens. A section of lymph node with lymphoid hyperplasia was used as control tissue. Primary antibodies were omitted for negative controls. Brown staining of the cell membrane was considered positive.

The normal epithelium was CD10 negative. CD10 expression is evident in the stromal cells but not in the tumor cells. When the neoplastic cells were surrounded by the stromal cells expressing CD10, the expression was considered to be positive. In this study, if 10% of the stromal cells in the area of invasive growth were positive for CD10, the case was regarded as having a proliferation of CD+ stromal cells and defined as diffusely CD10+.

Statistical evaluation. Correlation between stromal CD10 expression and clinic–pathologic factors was evaluated using the chi-square test and a *P*-value less than 0.05 was considered significant.

Results

Results are summarized in Table 1.

CD10 stained lymphoid cells, granulocytes, epithelial and stromal cells. Expression of CD10 by the neoplastic cells was not evaluated in the present study. CD10 was considered to be positive in 25 (32.5%) of 77 oral squamous cell carcinoma. The CD10-positive stromal cells were distributed around the neoplastic cells in the intratumoral and peritumoral areas. The intensity of positive CD10 staining was stronger in the areas of stroma close to neoplastic cells (Figure 1). Of the 25 CD10+ cases, 16 (64%) were associated with laterocervical lymph node metastases. CD10+ stromal cells related to recurrences were present in 14 (56%) of the 25 CD10+ cases. CD10+ cells were present in six cases (24%) of well-differentiated tumors (G1), nine (36%) of moderately differentiated tumors (G2) (Figure 2) and 10 (40%) of poorly differentiated tumors (G3).

Highly significant correlations were found with the lymph node status, the presence of local recurrences and

Table 1 Association between clinic–pathologic factors and CD10 stromal cells positivity

	No. of cases	*CD10+*	*CD10−*	P-*value*
Age				
<40 years	12	4	8	0.94 ns
>40 years	65	21	44	
Tumor size				
<2 cm	42	15	27	0.50 ns
>2 cm	35	10	25	
Lymph-node status				
Metastasis	28	16	12	0.0005
No metastasis	49	9	40	
Clinic stage				
I	13	2	11	0.39 ns
II	20	7	13	
III	25	9	16	
IV	19	7	12	
Histologic grade				
1	28	6	22	0.0086
2	32	9	23	
3	17	10	7	
Recurrent				
No	53	11	42	0.0011
Yes	24	14	10	

the histologic grading (Figure 3). The presence of CD10-positive cells was not correlated with the age of the patients, tumor size and clinic stage.

Discussion

Contrasting results have been reported on the prognostic value of CD10. For example, in primary intestinal lymphomas, the CD10+ group showed a trend for a longer overall survival when compared with the CD10– group (Go *et al*, 2002). In childhood acute lymphoblastic leukemia, CD10 has been reported to be a favorable prognostic marker (Cutrona *et al*, 2004). CD10 positivity in lung cancer patients correlated with a significantly higher 5-year survival than that of CD10-negative patients (Tokuhara *et al*, 1991). Improved survival was also reported in CD10 positive diffuse large B-cell lymphoma (Ohshima *et al*, 2001). On the contrary, in diffuse large B-cell lymphoma, Xu *et al* (2001, 2002) reported that cases with a CD10+ phenotype may be associated with an unfavorable clinic course and showed a significantly lower rate of complete remission and in Uherova *et al*'s (2001) patients, the CD10+ group displayed a shorter overall survival. Harada *et al* (1999), for their part, found no differences in the overall survival of CD10-positive and negative DLBCL patients. In breast carcinoma, patients with CD10+ stromal cells had a shorter metastasis-free interval and the frequency of positive stromal staining increased in a significant way in the cases with maxillary lymph node metastases (Iwaya *et al*, 2002). CD10 expression was found to be increased with the increase of tumor dysplasia in colorectal carcinoma (Ogawa *et al*, 2002). Ogawa *et al* (2002), in fact, found that there was no expression of CD10 in the stromal cells of normal colorectal tissue, while CD10+ stromal cells were present adjacent to the tumor cells in 16 of 73 adenomas with mild or moderate dysplasia, 12 of 17 adenomas with severe dysplasia, 10 of 16 intramucosal carcinomas and 50 of 63 invasive carcinomas. Moreover, the stromal expression of CD10 was strongly associated with accumulation of p53 and a large tumor size (Ogawa *et al*, 2002).

The results of the present study show that in oral squamous cell carcinoma CD10 positivity is an indicator of worse prognosis; in fact, the presence of CD10-positive cells was significantly correlated with the presence of metastases (16 out of 28 cases of tumors with lymph node metastases were CD10 positive, while only 9 out of 40 cases without lymph node metastases were positive). Another strong correlation was found with the presence of local recurrences; in patients without recurrences, only 11 out of 53 were CD positive, while in patients with recurrences 14 of 24 were positive. Also the histologic grade was significantly correlated with the CD10 positivity: in grade 1, only six of 28 cases were positive; in grade 2, only nine of 23 cases; while in grade 3, 10 of 17 cases resulted to be CD10 positive.

In conclusion, our results strongly support the role of CD10 in the differentiation and growth of neoplastic

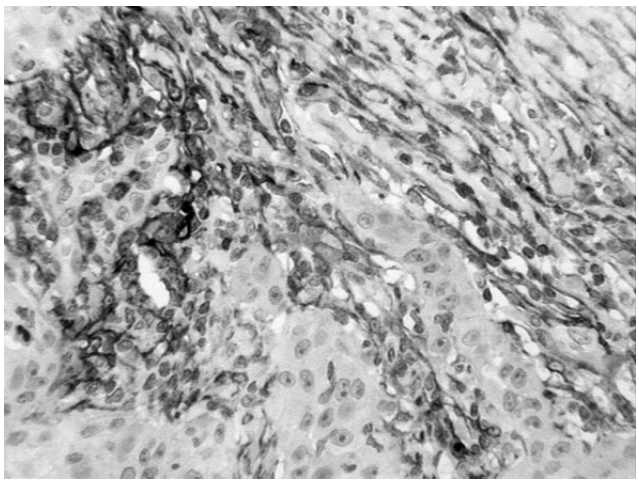

Figure 1 The intensity of positive CD10 staining was stronger in the areas of stroma close to neoplastic cells. Magnification, ×200

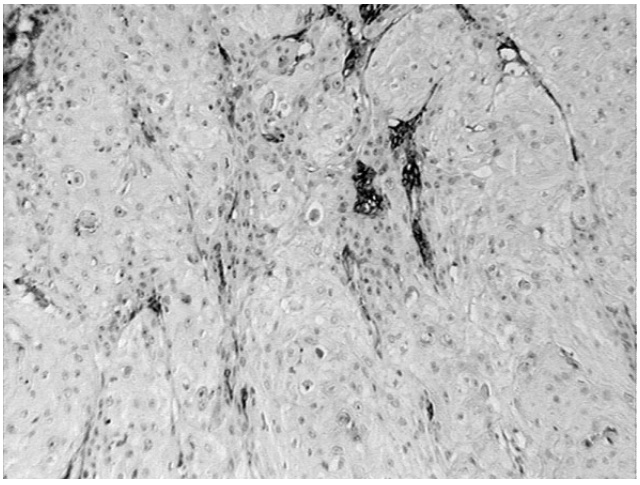

Figure 2 Moderately differentiated squamous cell carcinoma (lfn-): CD10 intratumoral stromal cells positivity. Magnification, ×400

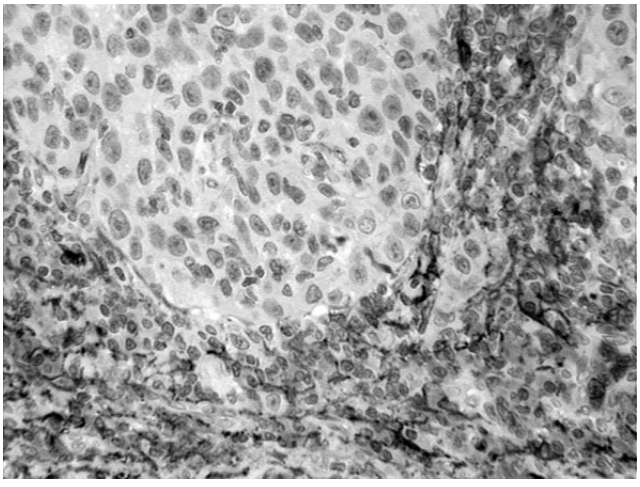

Figure 3 Poorly differentiated squamous cell carcinoma with lymph node metastasis: CD10 lymphoid cells positivity and stromal cells positivity in the peritumoral stromal cells. Magnification (G3), ×200

cells and CD10 expression can, perhaps, have an important role in the tumor invasion, probably facilitating the occurrence of metastases (Ogawa *et al*, 2002).

Further studies are clearly needed to elucidate the role of this surface glycoprotein in cancerogenesis.

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