J Oral Pathol Med (2005) 34: 295–7

www.blackwellmunksgaard.com/jopm

Expression of IgSF in salivary adenoid cystic carcinoma and its relationship with invasion and metastasis

Fan Xue[1], Yincheng Zhang[2], Feng Liu[1], Jie Jing[1], Min Ma[1]

[1]*Department of Stomatology, Affiliated Hospital of Ningxia Medical College, Ningxia, 750004, China;* [2]*Department of Maxillofacial Surgery, Stomatological College of Xi'an Jiaotong University, Xi'an, 710004, China*

BACKGROUND: The aim of the present work was to study the potential effect of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and neural cell adhesion molecule (NCAM) of immunoglobulin superfamily on the invasion and metastasis of salivary adenoid cystic carcinoma (SACC).
METHOD: The expressions of ICAM-1, VCAM-1 and NCAM of forty cases with SACC were examined by immunohistochemical methods using respective kits.
RESULTS: A significant relation showed between the expression level and histological differentiation, expression of ICAM-1 and VCAM-1 in solid SACC was greatly increased compared with NCAM; SACC with metastatic lymph node or local recurrence displayed significant relationship to the up-regulation of ICAM-1 and VCAM-1, and to the down-regulation of NCAM.
CONCLUSIONS: It is proposed that ICAM-1 and VCAM-1 may play a role in the invasion and metastasis of SACC. NCAM may be an invasion-resistant adhesion molecule.
J Oral Pathol Med (2005) **34**: 295–7

Keywords: immunoglobulin superfamily; intercellular adhesion molecule-1; neural cell adhesion molecule; salivary gland neoplasms; vascular cell adhesion molecule-1

Introduction

Salivary adenoid cystic carcinoma (SACC) is one of the most common malignant tumors of salivary gland, which accounts for 24% of salivary gland neoplasms. It is characterized by a high rate of recurrence and strong invasion to peripheral nerves or blood vessels at early phase (1, 2). Cellular adhesion molecules (CAMs), a transmembrane glycoprotein on cell membrane, have been proved to mediate the interaction between tumor cells and extracellular matrix, vascular endothelial cell and parenchymal organ cells or other tumor cells, and have an apparent relationship to the invasion and metastasis of tumors (3). Immunoglobulin superfamily (IgSF) is a family of CAMs, including intercellular adhesion molecule-1, 2, and 3 (ICAM- 1, 2, and 3), vascular cell adhesion molecule-1 and 2 (VCAM-1 and 2) and neural cell adhesion molecule (NCAM). The expression of IgSF has been detected in a variety of neoplasms and proved to link the prognosis, metastasis and histological pattern of neoplasms, but similar studies have not been reported in SACC. In this article, the expression of IgSF in 40 cases of SACC was detected by immunohistochemical methods to discuss the potential effect of IgSF on the invasion and metastasis of SACC.

Correspondence: Fan Xue MD, Department of Stomatology, Affiliated Hospital of Ningxia Medical College, Ningxia, 750004, China. Tel.: 086 0951 406 5205. E-mail: xuefan99@yahoo.com.cn
Accepted for publication November 11, 2004

Methods and materials

Archival, formalin fixed tumor specimens from 40 SACC patients (23 men, 17 women, aged 23–79 years) who underwent surgical treatment in the department of surgery, Stomatological Hospital of Xi'an Jiaotong university between 1999 and 2002, were obtained from the department of pathology for immunohistochemical staining. Sixteen tumors originated from major salivary gland and 24 were from minor ones. Diagnoses and pathologic classification were confirmed by using World Health Organization (1991) (4) classification criteria of salivary gland tumors: 20 cribri-tubiform, five mixed and 15 solid.

Immunohistochemical staining was carried out by means of an avidinbiotin complex (ABC) immunoperoxidase method (5–7). The specimens were sliced at 4 μm thickness, dewaxed in xylene for 15 min, and rehydrated with ethanol. The slides were treated with 3% hydrogen peroxide for 30 min at room temperature. After antigen restoration was performed by microwave in citrate buffer (pH 6) for 5 min, sections were immunostained for ICAM-1, VCAM-1 (polyclonal antibodies 1:100, Dako Corp., Carpinteria, CA, USA) and NCAM (monoclonal antibodies, 1:50, Santa Cruz Biotechnology, CA, USA) and incubation was carried out overnight at 4°C. The sections were then incubated with secondary antibodies for 30 min. Staining was performed using ABC reagents (Dakopatts, Glostrup,

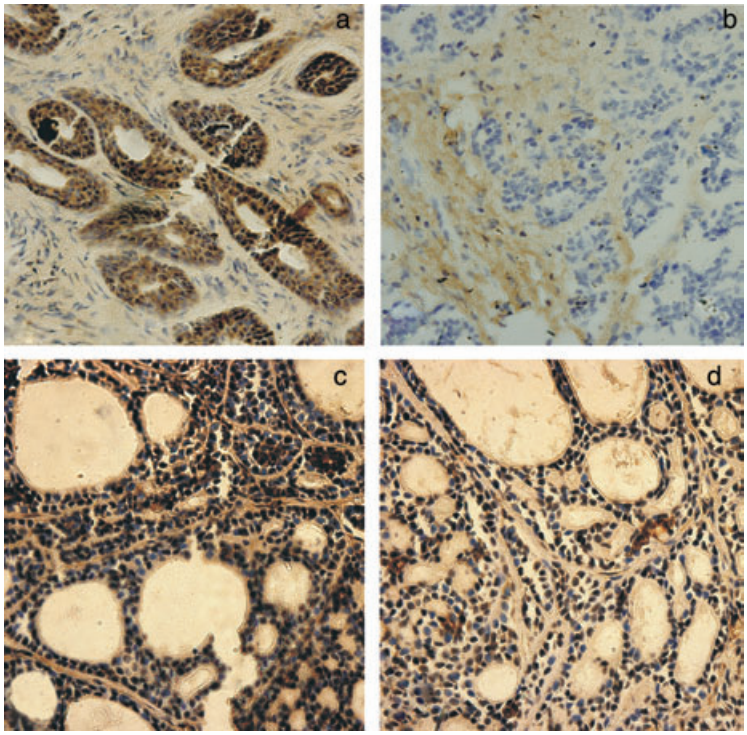

Figure 1 Positive expression of ICAM-1 in tubiform pattern (a), weak expression of NCAM in solid pattern (b); Strongly positive expression of ICAM-1 (c) and positive expression of VCAM-1 in cribiform pattern (d).

Denmark) and diaminobenzidine/hydrogen peroxide as substrate. The sections were counterstained with hematoxylin for 30 s and were washed with phosphate buffered saline (PBS) buffer.

For negative controls, the first antibodies were replaced with PBS solution during the immunohistochemical staining. After the elimination of non-specific staining, the percentage of positive tumor cells in each case was counted using a semiquantitative method reported previously (7). The degree of expression of IgSF was determined as follows: (3+), more than 50% of the cells showed a positive immunoreaction in the cell membrane or cytoplasm; (2+), 1050% of the cells were positive; (1+), <10% of the cells were positive. (−), cells were completely negative throughout the specimens (8). Fisher's exact probability test was used to analyze the statistical significance. $P < 0.05$ was considered significant.

Results

The expression of ICAM-1, VCAM-1 and NCAM were seen at cell membranes or cytoplasm of positive cells, and showed different intensity in different histological patterns of SACC. In most cases, ICAM-1 was strongly expressed than VCAM-1, whereas NCAM was relatively weak expressed (Fig. 1) ICAM-1 and VCAM-1 was expressed in 29 of 40 cases SACC, and NCAM was expressed in 10 cases. All morphologic patterns classically described in SACC, including cribiform, tubular and solid, were presented. Expression of ICAM-1 and VCAM-1 in solid SACC was greatly increased compared with NCAM. There was a significant relation between the expression level of IgSF and histological differentiation degree of SACC (Table 1).

In 40 cases SACC, 15 with metastatic lymph node or local recurrence, one total body metastasis and 24 without metastatic lymph node or local recurrence were examined with pathologic and clinical diagnose. SACC with metastatic lymph node or local recurrence displayed significant relationship to the up-regulation of ICAM-1 and VCAM-1, and to the down-regulation of NCAM (Table 2).

Table 1 Expression of IgSF in 40 cases SACC with different histological patterns

		ICAM-1		*VCAM-1*		*NCAM*	
Patterns	*Cases*	−	+	−	+	−	+
Cribri-tubiform	20	9	11	8	12	12	8
Mixed	5	1	4	2	3	4	1
Solid	15	1	14	1	14	14	1

$P < 0.05$ (cribri-tubiform compared with solid pattern).

Table 2 Expression of IgSF and its relation with recurrence and metastasis

Recurrence and lymph node metastasis	*Cases*	*ICAM-1*		*VCAM-1*		*NCAM*	
		−	+	−	+	−	+
Yes	16	1	15	2	14	15	1
No	24	10	14	9	15	15	9

$P < 0.05$.

Discussion

IgSF functions as an activity molecule involved in the recognition and adhesiveness, and is associated with the invasion and metastasis of neoplasms by mediating the homotypic and heterotypic intercellular adhesion and binding (9). ICAM-1 and VCAM-1 are two members of IgSF that have been studied most. Liu et al. (10, 11) measured the sera concentration of ICAM-1 and VCAM-1 in patients with head and neck carcinomas and found the high level expression in nasopharyngeal carcinoma, oral carcinoma and laryngeal carcinoma, and also suggested that the elevated levels of ICAM-1 and VCAM-1 at three different head and neck regions are different, and involved in these tumors classification, but may be unrelated to tumor progression.

ICAM-1 and VCAM-1 are extensively expressed in the cell surface including endothelial cell, fibroblast and tumor cell and bound with the ligands leukocyte function-associated antigen (LFA-1) and very late antigen-4 (VLA-4) respectively, and have a significant role in mediating the immune and inflammatory reaction as well as the metastasis of tumors. The overexpression of ICAM-1 and VCAM-1 in tumor cells inhibits the growth of tumors by mediating the killing of cytotoxic T lymphocyte and natural killer (NK) cell (12), while the expression of ICAM-1 and VCAM-1 in tumor cells surface causes an attachment to ligands expressed in leucocyte, and tumor cells are attached to the angioendothelium by the leucocyte acting as a bridge, thereby, increases the potential for hematogenous metastasis (13). The soluble ICAM-1 and VCAM-1 shedding from the surface of tumors cell into circulatory system may help tumor cells escape the immune surveillance of cytotoxic T lymphocyte and NK cell, and enhance the dissemination (14).

The NCAM is a polypeptide first found by Edelman in nerve tissue and involved in the neuritogenesis and neuromuscular interaction, which functions in the cell-cell binding activities to promote the homogeneous attachment between tumor cells and decreases the invasion of tumors cell (15). Edvardsen K et al. found that a rat glioma cell line transfected with the human NCAM-140 cDNA showed a slower growth rate and less invasiveness than the parent cell line, and using the RT-PCR, the expression of NCAM-140mRNA showed negative correlation with the invasion of tumors cell (16–18).

In this study, we detected the expression of ICAM-1, VCAM-1 and NCAM, the three main cell adhesion molecules of IgSF, which showed different expressions in SACC and there was a significant relation between the expression level and histological differentiation degree. SACC with metastatic lymph node or local recurrence displayed significant relationship with the expression of IgSF. It is proposed that ICAM-1 and VCAM-1 may play a role in the invasion and metastasis of SACC and NCAM may be an invasion-resistant adhesion molecule.

References

1. Sousa J, Sharma RR, Delmendo A, Pawar SJ. Primary orbito-cranial adenoid cystic carcinoma with torcular metastasis: a case report and review of the literature. *J Clin Neurosci* 2001; **8**: 173–6.
2. Wiseman SM, Popat SR, Rigual NR, et al. Adenoid cystic carcinoma of the paranasal sinuses or nasal cavity: a 40-year review of 35 cases. *Ear Nose Throat J* 2002; **81**: 510–4, 516–7.
3. Zetter BR. Adhesion molecules in tumor metastasis. *Semin Cancer Biol* 1993; **4**: 219.
4. Liu F, Liu T. *Neoplasms Pathology*. Beijing: Medical University and China Xiehe Medical University Associated Publishers, 1997; 611.
5. Reed AL, Califano J, Cairns P, et al. High frequency of p16 (CDKN2/MTS-1/INK4A) inactivation in head and neck squamous cell carcinoma. *Cancer Res* 1996; **56**: 3630–3.
6. Zhao Y, Zhang S, Fu B, Xiao C. Abnormalities of tumor suppressor genes P16 and P15 in primary maxillofacial squamous cell carcinomas. *Cancer Genet Cytogenet* 1999; **112**: 26–33.
7. Fujita M, Enomoto T, Haba T, et al. Alteration of p16 and p15 genes in common epithelial ovarian tumors. *Int J Cancer* 1997; **74**: 148–55.
8. Naka T, Oda Y, Iwamoto Y, et al. Immunohistochemical analysis of E-cadherin, α-catenin, β-catenin, γ-catenin, and neural cell adhesion molecule (NCAM) in chordoma. *J Clin Pathol* 2001; **54**: 945–50.
9. Stanners CP, Fuks A. *Cell Adhesion and Communication Mediated by the CEA Family: Basic and Clinical Perspectives*. Amsterdam: Harwood Academic Publishers, 1998; 5771.
10. Liu CM, Sheen TS, Ko JY, Shun CT. Circulating intercellular adhesion molecule-1 ICAM-1) eselectin and vascular cell adhesion molecule-1(VCAM-1) in head and neck cancer. *Br J Cancer* 1999; **79**: 360–2.
11. Liu CM, Ko JJ, Shun CT, Hsiao TY, Sheen TS. Soluble adhesion molecules and cytokines in tumor-associated tissue eosinophilia of nasopharyngeal carcinoma. *Acta Otolaryngol* 2001; **121**: 534–8.
12. Paine R III, Ward PA. Cell adhesion molecules and pulmonary fibrosis. *Am J Med* 1999; **107**: 268–79.
13. Tanabe K, Alexander JP, Steinbach F, Campbell S, Novick AC, Klein EA. Retroviral transduction of intercellular adhesion molecule-1 enhances endothelial attachment of bladder cancer. *Urol Res* 1997; **25**: 401–5.
14. Gho YS, Kim PN, Li HC, Elkin M, Kleinman HK. Stimulation of tumor growth by human soluble intercellular adhesion molecule-1. *Cancer Res* 2001; **61**: 4253–7.
15. Edelman GM. Cell adhesion molecule. *Science* 1983; **219**: 450–7.
16. Edvardsen K, Pedersen PH, Bjerkvig R, et al. Transfection of glioma cells with the neural cell adhesion molecule NCAM: effect on glioma-cell invasion and growth in vivo. *Int J Cancer* 1994; **58**: 116–22.
17. Edvardsen K, Brunner N, Spang-Thomsen M, Walsh FS, Bock E. Migratory, invasive and metastatic capacity of NCAM transfected rat glioma cells. *Int J Dev Neurosci* 1993; **11**: 681–90.
18. Owens GC, Orr EA, Demasters BK, Muschel RJ, Berens ME, Kruse CA. Overexpression of a transmembrane isoform of neural cell adhesion molecule alters the invasiveness of rat CNS-1 glioma. *Cancer Res* 1998; **58**: 2020–8.