Overexpression of Interleukin-8 in Salivary Adenoid Cystic Carcinoma Correlates with Distant Metastasis

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Objective: *To evaluate the protein expression of interleukin-8 (IL-8) in salivary adenoid cystic carcinoma (SACC) and to analyse its correlation with the clinicopathologic characteristics and prognosis.*
Methods: *A total of 43 cases of SACC and eight of normal salivary tissues were examined for the expression of IL-8 using immunohistochemistry, and the relationship of IL-8 expression with patients' clinicopathologic features was analysed.*
Results: *While the positive staining rate of IL-8 expression in SACC was 79.1% (34/43), the normal salivary tissues showed negative staining of IL-8 (0/8). IL-8 expression in SACC was positively related to distant metastasis of SACC (*$P < 0.05$*), but not related to the gender, age, tumour location, and pathological types.*
Conclusion: *The overexpression of IL-8 in SACC might be involved in metastasis of SACC. The potential mechanism underlying IL-8 expression related to the prognosis and treatment of SACC needs further research.*
Key words: *salivary adenoid cystic carcinoma, interleukin-8, metastasis, prognosis*

Salivary adenoid cystic carcinoma (SACC) is a highly infiltrative malignant tumour with a tendency for distant metastasis mostly to the lung, and has a high mortality rate despite good loco-regional control of the tumour[1]. Radical surgery, sometimes followed by radiation therapy, represents the main treatment approach for this disease[2]. However, the long-term survival and survival rate are still unsatisfactory. Defined genetic alterations have not been ascribed to the distinct stages of tumour development in SACC. Understanding the mechanism of metastatic behaviour of this carcinoma might help to design new strategies for the diagnosis and treatment of SACC.

Recent investigations have led to an insight into the mechanism of the chemokine network and its influence in the development of primary tumours and metastases[3]. Interleukin-8 (IL-8) was the first multifunctional chemokine found by Yoshimura[4] in 1987 and was believed to be closely related to inflammatory response and several key stages of tumour progression, such as tumour growth, angiogenesis, invasion and metastasis[5-7]. However, there are few studies about the role of IL-8 in SACC development. In this study, the IL-8 protein expression in normal salivary tissues and primary SACC tissues was investigated and its correlation with the clinicopathologic characteristics and prognosis of SACC was analysed.

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Table 1 Tumour location of 43 cases of SACC

	Total	Tumour location	Number of cases
Major salivary glands	26	Parotid gland	8
		Sublingual gland	9
		Submandibular gland	9
Minor salivary glands	17	Palate	8
		Maxilla region	5
		Mandibular posterior region	1
		Tongue	2
		Lip	1
Total	43		

Materials and Methods

Patients and specimens

Archived formalin-fixed paraffin-embedded tissue blocks from 43 patients with SACC during 1999 and 2007 were obtained from the Department of Oral Pathology, Peking University School and Hospital of Stomatology. Among these patients, 20 were males and 23 were females, aged from 17 to 71 years with a median age of 45 years. The distribution of tumour location is shown in Table 1. A total of 17 out of the 43 patients presented with synchronous or developing metachronous metastasis as identified by following up 5 to 10 years after the primary surgery. Eight cases of normal parotid gland tissues were used as the control.

Immunohistochemistry

The antibodies and reagents used for immunohistochemistry (IHC) were obtained from Boster Biological Technology (Wuhan, China). The tumour specimens were fixed in formalin, paraffin embedded, and cut into 4 μm sections for the IHC analysis of IL-8 expression. Sections were deparaffinised in xylene and rehydrated by immersion in a graded series of ethanol dilutions. The sections were dipped in freshly prepared 3% H_2O_2 in distilled water for 10 min to block endogenous peroxidase activity and rinsed with distilled water three times. For antigen retrieval, the sections were boiled in 0.01 mmol/L sodium citrate buffer solution by a microwave oven for 5 min. The sections were blocked in blocking buffer for 20 min and then incubated with anti-IL-8 antibody overnight at 4 °C (1:40 dilution) in a humid chamber. For negative control, the sections were incubated without the primary antibody. The sections were washed with phosphate-buffered saline thoroughly three times and stained using SABC method. The color was developed with diaminobenzidine, followed by counterstaining with hematoxylin. Photographs were taken with a microscope (OLYMPUS BX60 and DP71, Japan).

Assessment of IL-8 immunoreactivity

Immunostaining was assessed in three randomly selected fields in each section. The distribution and intensity of IL-8 immunostaining were assessed semi-quantitatively[8]. Briefly, the scores of each section were summed up from the percentage of positive cells (1: less than 25% positive cells; 2: 25% to 50% positive cells; 3: more than 50% positive cells) and the staining intensity (0: negative; 1: weak; 2: moderate; 3: strong). Scores between 0 and 2 were regarded as negative, 3 and 4 as weak positive, and 5 and 6 as strong positive.

Statistical analysis

Statistical analysis was performed with SPSS 11.5 for Windows. The chi-square test or Fisher's exact probability was performed to examine the correlation of IL-8 expression with clinicopathologic indexes. For all of the tests, the level of significance was set at $P < 0.05$.

Results

Protein expression of IL-8 in normal salivary tissues and primary SACC tissues

There was no specific IL-8 staining observed in the eight normal salivary tissues (Fig 1A). Immunoreactivity for IL-8 in SACC tissues is summarised in Table 2. The positive staining was brown–yellow particles predominantly in cytoplasm in SACC tumour cells (Fig 1C and D). Among the 43 cases of SACC specimens, the staining of IL-8 was strong positive in 18 cases (41.9%), weakly

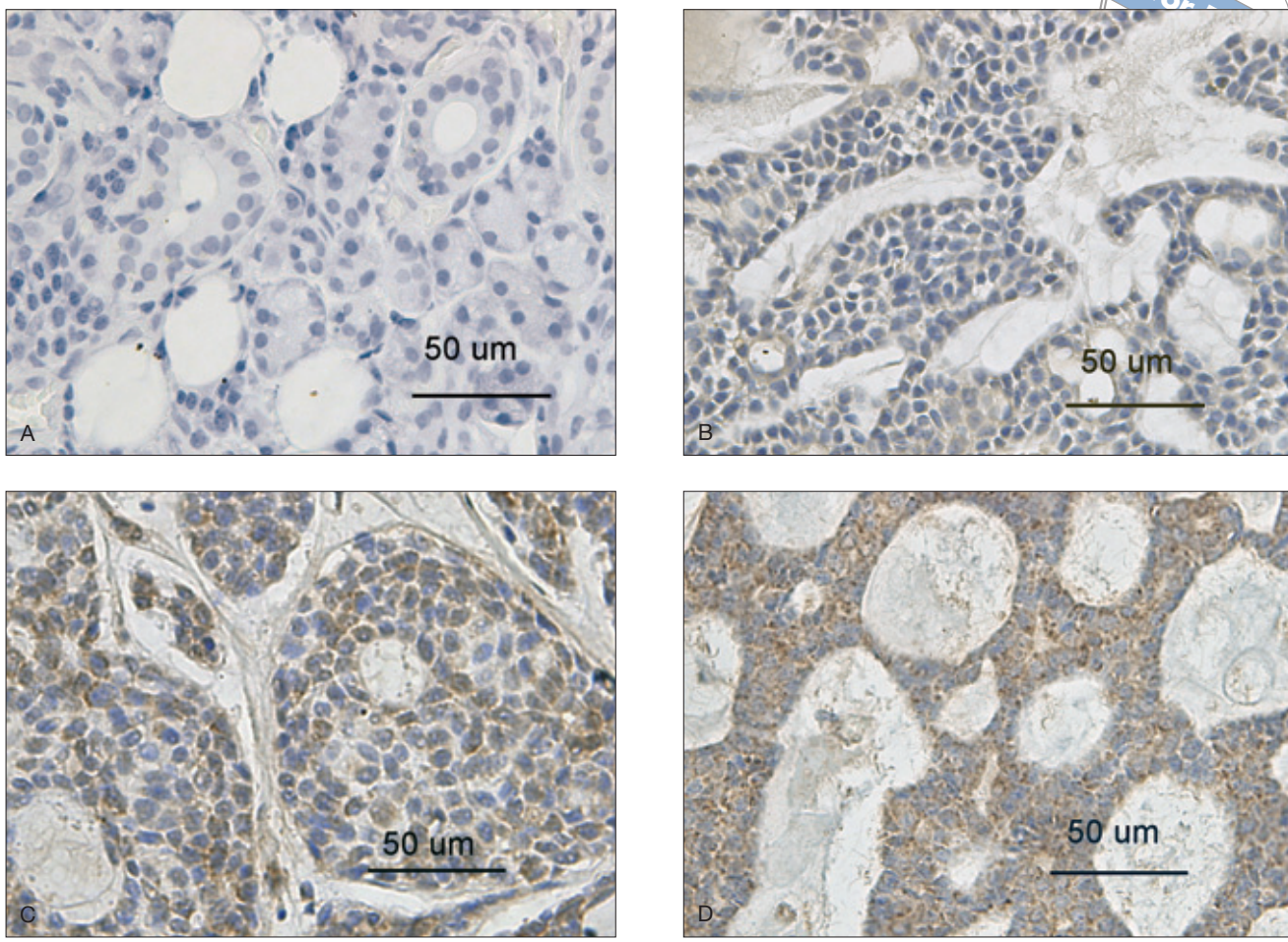

Fig 1 Immunohistochemical detection of IL-8. (A) negative expression in normal salivary tissues; (B) negative control of SACC tissue (substituted the primary antibody with phosphate-buffered saline); (C) weak positive in SACC tissue; (D) strong positive in SACC tissue.

Table 2 IL-8 expression in normal salivary tissues and primary SACC tissues

	Total	IL-8 expression (%)			Positive rate	*P*
		Negative	Weak positive	Strong positive		
SACC tissues	43	9 (20.9%)	16 (37.2%)	18 (41.9%)	79.1%	
Normal salivary tissues	8	8	0	0	0	<0.01

positive in 16 cases (37.2%) and negative in 9 cases (20.9%) (Table 2).

Analysis of the relationship between IL-8 expression and clinicopathologic features

The relationship between clinicopathologic features and IL-8 expression in SACC is summarised in Table 3. A total of 17 out of 43 SACC patients showed distant metastasis and 26 cases without metastasis. The positive rates of IL-8 immunostaining in the metastatic group and non-metastatic group were 88.2% and 73.1% respectively ($P < 0.05$), and the strong positive rates were 64.7% and 26.9% respectively. IL-8 expression in SACC was positively correlated with distant metastasis of SACC ($P < 0.05$), but not related to gender, age, tumour location or pathological types ($P > 0.05$).

Table 3 The relationship between IL-8 protein expression and clinicopathologic features in SACC

Characteristics	Total	IL-8 expression (%)			Positive rate	χ^2	*P*
		Negative	Weak positive	Strong positive			
Distant metastasis						6.048	0.049
no	26	7 (26.9%)	12 (46.2%)	7 (26.9%)	73.1%		
yes	17	2 (11.8%)	4 (23.5%)	11 (64.7%)	88.2%		
Pathological type						3.958	0.412
cribriform	22	4 (18.2%)	11 (50.0%)	7 (31.8%)	81.8%		
trabecular	15	4 (26.7%)	4 (26.7%)	7 (46.7%)	73.4%		
solid	6	1 (16.7%)	1 (16.7%)	4 (66.7%)	83.4%		
Tumour location						10.82	0.094
parotid	8	2 (25.0%)	4 (50.0%)	2 (25.0%)	75.0%		
sublingual gland	9	2 (22.2%)	4 (44.4%)	3 (33.3%)	77.7%		
submandibular gland	9	0(0%)	1 (11.1%)	8 (88.9%)	100%		
minor salivary glands	17	5 (29.4%)	7 (41.2%)	5 (29.4%)	70.6%		
Gender						0.125	0.940
male	20	4 (20.0%)	8 (40.0%)	8 (40.0%)	80.0%		
female	23	5 (21.7%)	8 (34.8%)	10 (43.5%)	78.3%		
Age						4.984	0.083
≤50	27	6 (22.2%)	13 (48.1%)	8 (29.6%)	77.8%		
>50	16	3 (18.8%)	3 (18.8%)	10 (62.5%)	81.3%		

Discussion

IL-8 is a multi-origin proinflammatory cytokine with many physiologic functions[9-11] and belongs to the CXC chemokine family. IL-8 can be synthesised and secreted by many malignant tumour cells[12-14], while the corresponding original normal cells or benign tumour cells cannot secrete IL-8. Akiba et al[12] reported that IL-8 was overexpressed in 23 surgically resected hepatocellular carcinoma (HCC) specimens and seven HCC cell lines, and also correlates with venous invasion and bile duct invasion. Gene silencing of IL-8 significantly inhibits the growth of human tumour xenografts in nude mice. Hoffmann et al[15] observed that IL-8 serum levels are elevated in adenoid cystic carcinoma patients compared with healthy individuals. In the present study, there is evidence that IL-8 was overexpressed in primary SACC tissue compared with the normal salivary tissues. IHC showed that SACC cells were the major producer of IL-8 in the tissues. The data suggest that IL-8 may act as an autocrine/paracrine growth factor in the genesis of salivary adenoid cystic carcinoma.

It is known that the majority of SACC deaths result from tumour metastasis rather than from primary tumours. Yet, the biochemical mechanisms regulating invasion and metastasis of SACC remain poorly understood. Despite its physiologic roles in development and immunity, increasing evidence suggests that IL-8 plays an important role during the invasion and metastasis of carcinoma cells[16-19]. IL-8 is elevated in some cancer cells with high metastatic potential[16,20,21]. Rubie et al[22] revealed the upregulation of IL-8 in colorectal liver metastatic tissues relative to the corresponding primary colorectal cancer tissues. In the present study, it was found that both the positive rate and the strong positive rate of IL-8 expression were higher in the metastatic group than in the non-metastatic group. These results suggest that IL-8 may play a role in SACC progression and metastasis.

The exact mechanism of IL-8 involved in tumour genesis and progression differs between various malignancies. Despite the increased expression of IL-8 in cancer cells, polymorphisms of IL-8 have also been characterised in certain cancers[23,24]. The induction of IL-8 signaling activates multiple signaling pathways at transcription or post-translation level. IL-8 increases proliferation and survival of endothelial and cancer cells. It modulates the organisation of the cell cytoskeleton to potentiate the migration of cancer cells, endothelial cells and infiltrating neutrophils at the tumour site. Furthermore, IL-8 was also demonstrated to activate collagenase[19,25] and other metastasis-related genes[26-28] to facilitate cancer metastasis. Further studies, especially with larger sam-

ples, are needed to elucidate the mechanisms and significance of IL-8 and its related molecules in SACC.

In conclusion, this study has shown that IL-8 was overexpressed in SACC and correlated with distant metastasis of SACC. IL-8 may be an important factor facilitating oncogenesis and metastasis in SACC. These results might give an insight into the initiation and progression of the tumour and aid in the design of new strategies for diagnosis and treatment of SACC.

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