

# Clinicopathologic and Immunohistochemical Analysis of Salivary Duct Carcinoma in Major and Minor Salivary Glands

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**Objective:** To investigate the clinicopathologic characteristics of salivary duct carcinoma (SDC) in major and minor salivary glands and the possible role of MMP (matrix metalloproteinase)-2 and MMP-9 in SDC.

**Methods:** Clinicopathologic features of 10 SDCs were described. Immunohistochemical expressions of MMP-2 and MMP-9 proteins were examined in the paraffin samples of the 10 SDCs and 10 basal cell adenomas (BCA). The scoring of the immunoreactivity was estimated by integrated optical density (IOD) through a digital image analyser.

**Results:** The patients of SDC presented clinically with a local mass or swelling, frequently painful (6 of the 10 cases). The symptoms of facial paralysis or lip numbness (3/10) and lymph node metastasis (6/10) were usually detected. SDCs showed increased expression in both MMP-2 and MMP-9 proteins as compared with BCAs. Moreover, MMP-2 and MMP-9 proteins were markedly increased in metastatic compared with non-metastatic SDCs.

**Conclusion:** MMP-2 and MMP-9 may play an important role in invasion and metastasis of SDC. Toothache may be an important clinical characteristic of SDC originated in maxillary sinus. SDC histologically showed various proportions of papillary, comedo, cribriform and solid patterns.

**Key words:** immunohistochemistry, MMP-2, MMP-9, salivary duct carcinoma

Salivary duct carcinoma (SDC) is a rare but highly aggressive salivary gland malignancy with a high rate of rapid regional and distant metastases (generally more than 50%) and dismal prognosis. The tumour is typically found in the parotid gland of elderly men and only 26 SDC cases involving in minor salivary gland have been reported (one originated in maxillary sinus)<sup>1-5</sup>. One of the essential features of carcinomas is the ability to pen-

etrate basement membrane barriers, a prerequisite for tumour cell invasion and metastasis. Matrix metalloproteinases (MMPs) are known to play a central role in the process. Type IV collagen is the main scaffold protein of the basement membrane. MMP-2 (gelatinases A) and MMP-9 (gelatinases B), members of the MMPs proteinase family, are type IV collagenases that degrade type IV collagen of basement membrane. Both MMP-2 and MMP-9 have been reported to play important roles in the extracellular matrix remodelling during the process of tumour invasion and metastasis<sup>6</sup>. Their increased expression has been reported in carcinomas of various organs including the breast, lung, thymus, and cervix, and to correlate with the invasive and metastatic capacities of these tumours and/or their prognoses<sup>7-10</sup>. A few of recent reports have shown that the expression of MMPs was higher in malignant salivary tumours than

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**TABLE 1** The clinicopathologic findings in ten cases of salivary duct carcinoma

Case No.	Sex	Age	Location	Histological classification (main pattern)	Clinical presentation	Size (cm)	Lymph node metastasis	Follow-up
1	F	48	Maxilla (maxillary sinus)	Comedonecrosis	Painful swelling; toothache	4.2	No	Alive without tumour 2 years
2	M	72	Parotid	Comedonecrosis	Asymptomatic mass with facial paralysis	6.9	Level II	Local recurrence at 1.5 years; died of disease 2 years, lost to follow-up
3	M	41	Parotid	Cribriform	Painful mass	6	Level IV	Alive without tumour 8 years
4	M	66	Parotid	Comedonecrosis	Asymptomatic mass	2.5	No	Alive without tumour 5 years
5	M	65	Parotid	Cribriform	Asymptomatic mass	3	Level I B	Alive without tumour 1 year, lost in follow-up
6	M	60	Submandibular gland	Comedonecrosis	Painful mass	6	No	6 months, lost in follow-up
7	M	76	Retromolar area	Comedonecrosis	Painful mass with lower lip numbness	4	Level I B	Local recurrence at 2 months
8	F	40	Parotid	Solid	Asymptomatic mass	3	Tumour cell invasion parotid	Metastasis in lung; died of disease at 1 year
9	M	66	Parotid	Comedonecrosis	Painful mass with facial paralysis	4	Level V	Without treatment
10	M	54	Maxilla (maxillary sinus)	Comedonecrosis	Painful swelling	1	No	

in benign tumours<sup>11,12</sup>. However, there are no data available on the expression of enzymes/proteins that are responsible for tumour invasion and metastasis of SDC.

In the present study, the clinicopathologic characteristics of SDC originating from the major or minor salivary gland are described, and the expression of MMP-2 and MMP-9 proteins in this tumour are examined.

## Materials and Methods

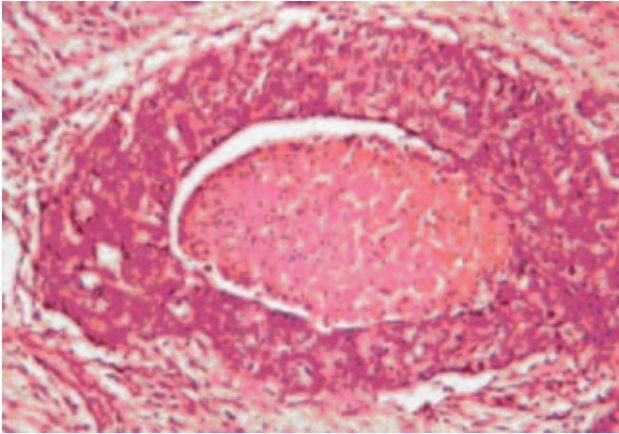
### *Patients and tissue samples*

Ten cases of archival formalin-fixed paraffin samples of primary SDC were obtained from the biopsy or surgical excision specimens of the tumour in the Department of Oral Pathology, West China College of Stomatology,

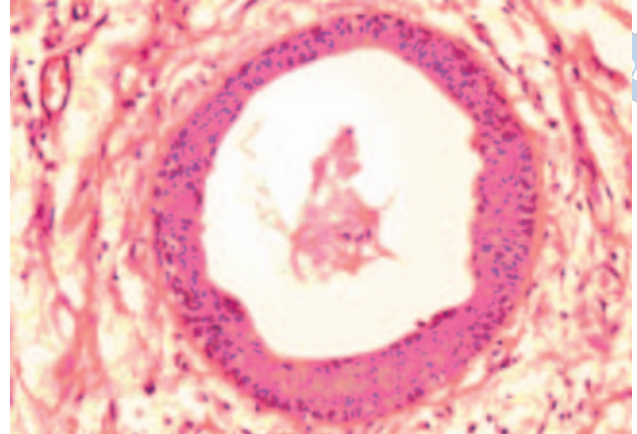
Sichuan University. As a comparison, 10 cases of basal cell adenoma (BCA) of salivary gland from the same department were randomly selected. The histological diagnoses of the samples were re-confirmed by two oral pathologists independently according to the World Health Organization (WHO) criteria<sup>2</sup>. Clinical data of the patients were obtained from the surgical pathological reports and medical records.

### *Immunohistochemistry*

The primary antibodies used for this study were mouse monoclonal antibodies MMP-2 (ZM-0330, Zymed, USA) and MMP-9 (ZM-0335, Zymed, USA). The immunostaining procedure was mainly involved in an anti-



**Fig 1** The comedonecrosis pattern in salivary duct carcinoma (H.E., magnification 200x).



**Fig 2** Salivary duct carcinoma with intraductal tumour proliferation (H.E., magnification 200x).

gen retrieval technique to unmask the MMP-2 and MMP-9 epitopes and a streptavidin/peroxidase technique.

In brief, 5  $\mu$ m sections were placed on silanised slides. The slides were deparaffinised in xylene and rehydrated in a graded ethanol series. Endogenous peroxidase activity was blocked using 3%  $H_2O_2$  for 10 min. For antigen retrieval, the slides were placed in a plastic slide container filled with 10 mmol/l citrate buffer. The immersed sections were placed in a microwave oven at high intensity for 15 min. The sections were rinsed with phosphate-buffered saline (PBS) and the non-specific binding was blocked with 10% normal goat serum (Histostain<sup>TM</sup> – plus Kits, Zhong Shan, Beijing) for 15 min. The slides were subsequently incubated overnight at 4°C with the primary antibodies MMP-2 (1:100) and MMP-9 (1:100), respectively. After washing with PBS, the slides were incubated with secondary antibody goat anti-mouse IgG (Histostain<sup>TM</sup> – plus Kits) for 15 min at room temperature and washed with PBS, followed by incubation with streptavidin/peroxidase (Histostain<sup>TM</sup> – plus Kits) and developed by the diaminobenzidine reaction. All reactions were carried out in an airtight humidified chamber. Slides were briefly counterstained with haematoxylin. Each set of staining reactions included both SDC and BCA as well as known positive controls (breast duct carcinoma) and negative controls (without the primary antibody) to minimise staining intensity variations among different batches.

A positive staining for MMP-2 or MMP-9 was the appearance of a dark-brown stain in the cytoplasm under a light microscope. The immunostaining of MMP-2 and MMP-9 was measured as the integrated optical density

(IOD) index with MIAS-2000 Image Analysis System (image pro plus 4.10)<sup>13</sup>.

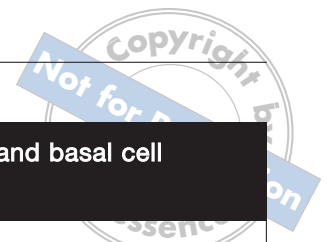
#### Statistical analysis

The statistical analyses were performed using SPSS 10.0 for Windows. The Mann-Whitney test was used to compare the IOD-Index between SDC and BCA.

## Results

#### Clinicopathologic findings

As shown in Table 1, eight of the SDCs patients were male and two were female, with an average age of 59 years (from 40 to 76 years old). Seven of the SDCs originated from the major salivary glands (six in the parotid and one in the submandibular gland), while the rest originated from the minor salivary glands (one in the oral cavity and two in the maxillary sinus). All the patients presented with a local mass (8/10 cases) or swelling (2/10). The mass/swelling was painful in 6/10 patients, including both the patients with SDC in the maxillary sinus. Two of the ten patients had facial paralysis, both presented SDC in parotid gland. The patient with an intra-oral lesion at the retromolar region had numbness of lower lip. The mean dimension of the tumours was  $4 \pm 1.8$  cm (1 to 6.9 cm). Lymph node metastases were confirmed pathologically in six of the operated patients. None of the treated patients had distant metastases. Four patients remain disease-free for 1 to 6 years after surgery, one died of recurrence, two gave up any treatment after biopsy due to personal reasons, and three were lost to follow-up.


**TABLE 2 Immunoreactivity of MMP-2 and MMP-9 in salivary duct carcinomas (SDC) and basal cell adenomas (BCA)**

	SDC	SDC	BCA	BCA
	MMP-2	MMP-9	MMP-2	MMP-9
Case No.	IOD* ± SD**	IOD ± SD	IOD ± SD	IOD ± SD
1	1414.23 ± 160.81	2560.65 ± 53.12	2963.63 ± 21.05	335.69 ± 62.60
2	3129.26 ± 186.20	1803.11 ± 99.67	663.21 ± 58.90	621.97 ± 76.79
3	7635.22 ± 258.23	1411.58 ± 107.87	4469.25 ± 143.97	2650.70 ± 120.09
4	1001.27 ± 58.34	359.26 ± 33.47	411.43 ± 96.28	364.51 ± 22.28
5	8744.45 ± 228.73	758.94 ± 33.69	2237.11 ± 33.49	633.48 ± 40.09
6	1381.21 ± 74.30	968.63 ± 30.90	688.19 ± 53.18	559.11 ± 32.49
7	7691.25 ± 148.97	6520.31 ± 133.52	749.86 ± 52.02	1988.63 ± 130.54
8	9658.22 ± 101.2	741.25 ± 32.22	7445.79 ± 163.45	245.66 ± 33.31
9	5378.89 ± 235.89	403.36 ± 37.41	3599.46 ± 44.26	678.59 ± 49.58
10	425.99 ± 32.99	669.1 ± 43.76	368.55 ± 53.23	329.28 ± 34.44
Mean	4646	1619.62	2359.65	840.75

\*IOD: the integrated optical density, which was a measure of the total amount of MMP-2 or MMP-9 immunostaining intensity.

\*\*SD: the standard deviation of IOD measurements.

**TABLE 3 Relationship between the expressions of MMP-2 and MMP-9 and metastasis of lymph node in SDC**

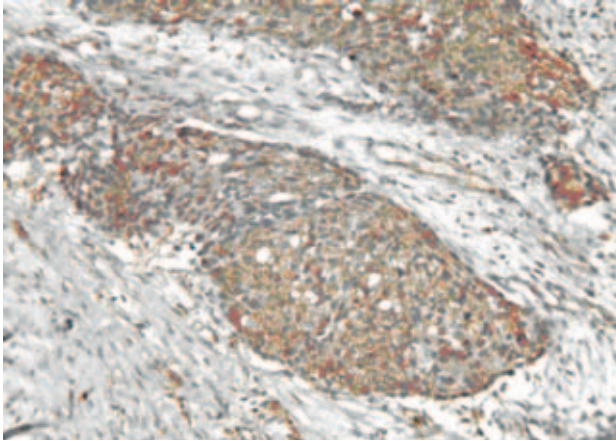
	MMP-2	MMP-9	Lymph node metastasis
Case No.	IOD* ± SD**	IOD ± SD	
2	3129.26 ± 186.20	663.21 ± 58.90	Yes
3	7635.22 ± 258.23	4469.25 ± 143.97	Yes
5	8744.45 ± 228.73	2237.11 ± 33.49	Yes
7	7691.25 ± 148.97	749.86 ± 52.02	Yes
8	9658.22 ± 101.2	7445.79 ± 163.45	Yes
9	5378.89 ± 235.89	3599.46 ± 44.26	Yes
Mean	7039.55	3194.11	
1	1414.23 ± 160.81	2963.63 ± 21.05	No
4	1001.27 ± 58.34	411.43 ± 96.28	No
6	1381.21 ± 74.30	688.19 ± 53.18	No
10	425.99 ± 32.99	368.55 ± 53.23	No
Mean	1055.68	1107.95	

\*IOD: the integrated optical density, which was a measure of the total amount of MMP-2 or MMP-9 immunostaining intensity.

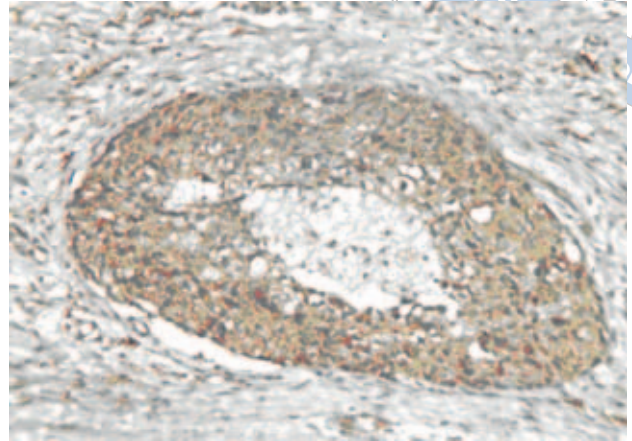
\*\*SD: the standard deviation of IOD measurements

Histologically, the tumours showed various proportions of papillary, comedo, cribriform and solid patterns. The stromal desmoplasia was common in most tumours. Cytologically, the tumour cells were large and polygonal, with eosinophilic cytoplasm and irregular vesicular nu-

clei. There were nuclear pleomorphisms and frequent mitoses. Perineural invasion and infiltration of adjacent structures were frequently observed. The SDC of the minor salivary gland also exhibited a wide range of histological appearances (Figs 1 and 2).



**Fig 3** Immunostaining for MMP-2 in salivary duct carcinoma (solid pattern) is obvious (magnification 200x).



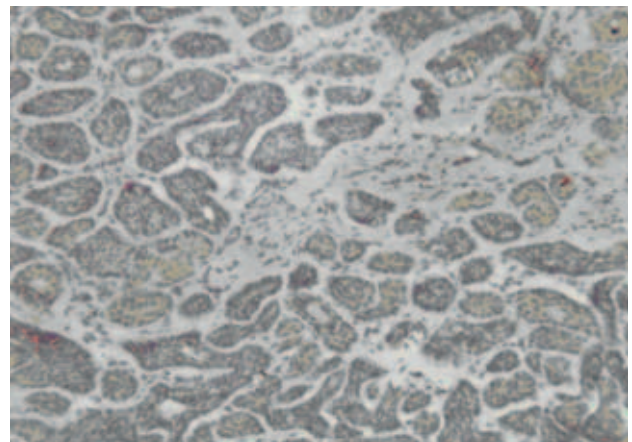
**Fig 4** Salivary duct carcinoma (comedonecrosis pattern) demonstrates strong and homogeneous immunostaining of the tumour cell cytoplasm for MMP-9 (magnification 200x).

#### *Immunohistochemical detection of MMP-2 and MMP-9*

Table 2 shows that both MMP-2 and MMP-9 were mostly localised in the cytoplasm of carcinoma cells and few stromal cells also showed a faint staining. The immunoreactivity of MMP-2 was stronger than that of MMP-9 in both SDC and BCA. The expression of MMP-2 was found in 9 out of the 10 SDCs, but only in 4 out of the 10 BCAs, and showed a significantly stronger staining in the SDC compared with the BCA (mean IOD: 4646.00 vs. 1619.62,  $p = 0.029$ ). The expression of MMP-9 was found in 5 out of 10 SDCs and in 2 out of 10 BCAs, and showed a significantly stronger staining also in the SDC than in the BCA (mean IOD: 2359.65 vs. 840.75,  $p = 0.023$ ) (Figs 3 to 5). Table 3 shows that the mean IOD value for MMP-2 and MMP-9 staining was higher in metastatic SDC than in non-metastatic SDC (7039.55 vs 1055.68, 3194.11 vs 1107.95 respectively).

#### **Discussion**

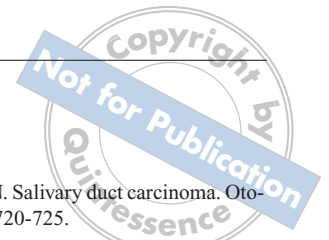
The present study reports ten cases of SDC, a rare but one of the most aggressive salivary gland carcinomas. Of the ten cases, one was an intraoral lesion (extremely rare with only 26 reported cases in the English literature) and two were originated in maxillary sinus (to the best of our knowledge, there is only one reported case in the literature)<sup>3</sup>. The aggressive nature of the tumour was well demonstrated, as six of the ten lesions had facial paralysis or local pain, and six had metastasised to re-



**Fig 5** Basal cell adenoma shows weak cytoplasmic immunostaining for MMP-2. (magnification 200x).

gional lymph nodes. SDC usually appears clinically as a painless nodule in the parotid region<sup>4</sup>. Lewis et al<sup>5</sup> reported 26 patients with SDC, and only six (23%) patients complained of local pain; in comparison, the symptom of local pain seemed more common (six of the ten cases) in the present study. Similar to the reported SDC in the maxillary sinus<sup>3</sup>, one of the two SDCs that originated in maxillary sinus in the present study presented clinically with a chief complaint of toothache, and both manifested as local swelling in the maxilla, a feature not reported in the previous study.

The present study is the first to investigate the expression of enzymes/proteins in SDCs that are responsible



for tumour invasion and metastasis, two features pronounced in SDCs. Both invasion and metastasis are dependent upon the ability of carcinoma cells to degrade extracellular matrix components. MMP-2 and MMP-9 are believed to be particularly involved in this degradation process. The present results showed that the expression of both MMP-2 and MMP-9 were significantly increased in SDC compared with the benign salivary gland tumour BCA, and the increase was more pronounced for MMP-2 than for MMP-9. The results are consistent with the findings of Nagel et al<sup>12</sup>, that MMP-2 expression in particular seems to be related to the invasive properties and the malignant potential of malignant salivary gland tumour, such as mucoepidermoid carcinoma and acinic cell carcinoma.

Recent studies have also suggested a correlation between expression of MMP-2/MMP-9 and tumour lymph node metastasis in various malignant tumours<sup>10,14,15</sup>. Kayano et al<sup>11</sup> reported the activation ratio of the MMP-2 zymogen significantly correlated with histological grade and lymph node metastasis in mucoepidermoid carcinoma. In the present study, there was different expression of MMP-2 and MMP-9 between metastatic and non-metastatic SDC. It would suggest that over-expression of MMP-2 and MMP-9 may be predictive of metastasis for SDC. However, larger multicentre studies are required to improve the impact of this finding and its use as a prognostic tool for metastatic cases of SDC.

## Conclusion

MMP-2 and MMP-9 may play an important role in invasion and metastasis of SDC, and markedly increased MMP-2 and MMP-9 expression in metastatic SDC compared with non-metastatic SDCs may be suggestive of a prognostic role for SDC. Toothache may be an important clinical characteristic of SDC originated in the maxillary sinus. The histological appearance of SDC originated from the minor salivary gland was the same as those originated from major salivary gland.

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