Mucoepidermoid Carcinoma of the Salivary Gland

- A clinico-pathologic and immunohistochemical study for c-erbB-2 oncoprotein -

Twenty-five cases of mucoepidermoid carcinoma of the salivary glands were studied with respect to clinico-pathologic features, prognostic factors, and c-erbB-2 oncoprotein expression. Fourteen cases were located in parotid glands, 2 in submandibular glands and 9 in minor salivary glands. Fourteen patients were confirmed to have local recurrences, 6 patients manifested systemic metastases, and 10 patients died of the disease. They were histologically graded as I in 5, II in 12 and III in 8 cases respectively, and the histologic grade was significantly correlated with disease-free interval and overall survival of the patients. c-erbB-2 overexpression was observed in 9 cases (36%), with a trend to be associated with the higher grade, but was not evaluated as a significant survival-related factor in this series. Larger size and major gland location of the tumors were correlated with more frequent regional lymph node metastases. The present study showed that c-erbB-2 overexpression was not uncommonly present in mucoepidermoid carcinoma of the salivary glands, especially of higher grade, and the histologic grade was the most important and handy prognostic indicator. ***(JKMS 1997; 12: 499~504)***

Key Words : *Carcinoma, Mucoepidermoid; Salivary gland neoplasms; Oncogene proteins c-erb-2 B; Immunohistochemistry; Prognosis*

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INTRODUCTION

Mucoepidermoid carcinoma (MEC) is one of the most common malignancies of salivary glands. This neoplasm had once been regarded as a benign lesion and called 'mucoepidermoid tumor' before the revised WHO classification (1990) which settled on the term 'mucoepidermoid carcinoma' (1). As expected, MEC was reported to manifest variable biologic aggressiveness, basically showing some correlation with histology which is best graded by a 3-tiered grading system (2). The c-erbB-2 oncogene was initially identified as a transforming gene (*neu*) in a chemically induced rat neuroblastoma (3). The gene encodes a 185kD transmembrane glycoprotein of the tyrosine kinase family with growth factor receptor function (4). Amplification or overexpression of c-erbB-2 has been documented in various human tumors, mostly adenocarcinomas of stomach, kidney, breast and ovary, and has shown association with a poor prognosis in some tumors (5, 6). Regarding salivary gland tumors, a small number of studies on c-erbB-2 oncogene have been reported. We performed an immunohistochemical study for c-erbB-2 overexpression on 25 cases of mucoepidermoid carcinoma (MEC) of salivary glands and compared the results with various clinico-pathologic factors.

MATERIALS AND METHODS

Twenty-five cases of mucoepidermoid carcinoma of salivary glands were retrieved from the Surgical Pathology files of the Korea Cancer Center Hospital from 1986 through 1995. Pathologic and medical records were reviewed and follow-up data were obtained.

The tumors were histologically graded as described by Batsakis and Luna (2). Five carcinomas were grade I, characterized by macrocysts, microcysts, and an abundance of differentiated mucin-producing cells (Fig. 1). Grade II mucoepidermoid carcinomas were most common, comprising 12 cases. They showed a preponderance of solid growth of intermediate cells with mild pleomorphism (Fig. 2). In 8 cases of grade III MEC, tumor cells showed considerable pleomorphism and grew in a solid and invasive pattern (Fig. 3). Ductal-type adenocarcinomatous areas were often encountered. Recognition of clear cells or mucinous cells was helpful in differential diagnosis from other high-grade salivary carcinomas. One case had undergone transformation from grade II to grade III with loss of mucinous cells and addition of cellular atypism through 2 recurrences.

Immunohistochemical staining was performed on the formalin-fixed, paraffin-embedded tissue sections using

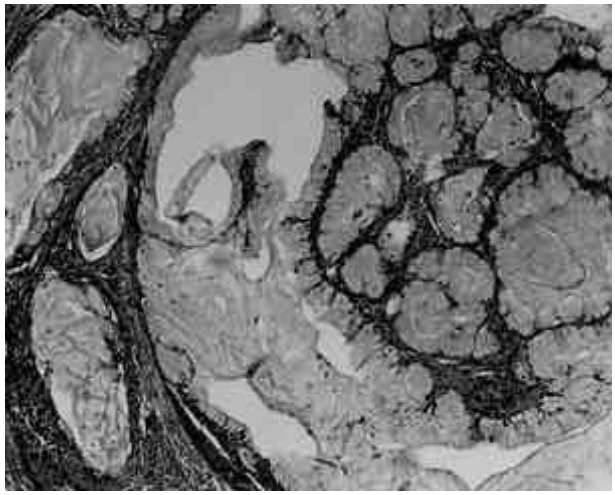

Fig. 1. Photomicrograph of mucoepidermoid carcinoma, grade I, being composed of mucin-producing columnar cells forming macro- and microcysts (H & E).

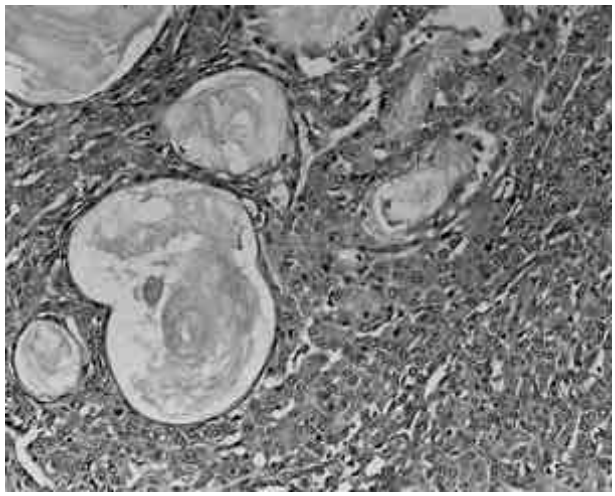

Fig. 2. Grade II mucoepidermoid carcinoma, consisting of predominant solid growth of intermediate cells and microcysts with mucinous epithelium (H & E).

conventional avidin-biotin-peroxidase complex method. After deparaffinization and rehydration, endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol. In order to enhance the immunostaining, sections were treated with Target Unmasking Fluid (KREATECH, Netherlands) for 5 minutes at 100 ℃. After incubation with normal serum (Research Genetics, AL, U.S.A.) to minimize background staining, sections were incubated for 30 minutes at 45℃ with rabbit anti-c-erbB-2 antibody (Zymed, CA, U.S.A.). Treatment with streptavidin-HRP complex (Research Genetics, AL, U.S.A.) and visualization with diaminobenzidine followed.

Associations between variables were measured by Fisher's Exact test and chi-square test. Time to recurrence and time to death were plotted using Kaplan-Meier method, and compared by log rank test.

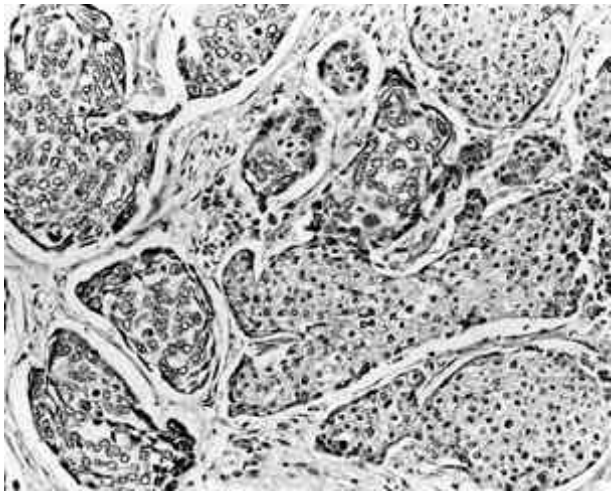

Fig. 3. Mucoepidermoid carcinoma of grade III, showing invasive growth, stromal reaction, and solid nests with cellular pleomorphism. Clear cell component is still focally recognizable (H & E).

RESULTS

Clinical findings and outcome

The patients consisted of 14 males and 11 females, with an age range from 17 to 75 (mean 47.6 years). Sites of the tumors were the parotid gland in 14, submandibular gland (SMG) in 2, and minor salivary gland in 9 cases. Durations of their manifestations, such as mostly swelling or mass, varied from 2 months to 14 years. Size of the tumors ranged from 1 cm to 10 cm (mean 3.7 cm). All patients underwent surgical excision of the tumors. Six patients had regional lymph node metastases at the time of operation. Sixteen patients received adjuvant radiotherapy, and 3 received both radiotherapy and chemotherapy. Recent follow-up results were available in 20 cases. Fourteen patients developed local recurrences from 2 months to 5 years after the first operation. Six of them later developed systemic metastases to various sites including the lung, pleura, pericardium, bone, brain, and liver. Ten patients died of metastatic and/or recurrent tumors, with the survival period ranging from 7 months to 14 years. Three patients are alive with recurrent disease, and 7 are alive with no evidence of disease. Clinicopathologic data are summarized in Table 1.

c-erbB-2 expression

Nine of 25 (36%) mucoepidermoid carcinomas of the

Table 1. Summary of clinico-pathologic data

Case no	Site	Size (cm)	Histol grade	LN meta	Treatment	Local recur	Meta	Outcome	Survival (months)
1	Parotid	6	I	+	S/R	?	?	Lost	>24
2	Parotid	2	I	−	S/R	−	−	NED	96
3	Parotid	4	I	−	S	?	?	Lost	?
4	Parotid	1.5	I	−	S/R	+	−	AWD	47
5	Parotid	4	I	−	S/R	−	−	NED	57
6	Palate	1.5	II	−	S/R	−	−	NED	108
7	Parotid	5	II	−	S	+	?	Lost	>16
8	Palate	3	II	−	S/R	+	−	DOD	18
9	BOT	3	II	−	C/R/S	−	−	NED	83
10	Parotid	3	II	−	S/R	+	+	DOD	168
11	Palate	1.5	II	−	S/R	?	?	Lost	>7
12	Parotid	3.5	II	−	S	−	−	NED	55
13	FOM	2.5	II	−	S/R	+	−	AWD	42
14	Parotid	8	II	−	S	+	+	DOD	60
15	BOT	3	II	−	S/R	?	?	Lost	?
16	Parotid	10	II	+	R/S	−	−	NED	25
17	Orophx	1	II	−	S	−	−	NED	84
18	Parotid	3.5	III	−	S/R	+	+	DOD	11
19	SMG	3	III	+	S/R	+	+	DOD	16
20	Buccal	3	III	−	S/R	+	+	DOD	72
21	Parotid	3.5	III	−	S/R/C	+	+	DOD	7
22	SMG	7	III	+	S/R	+	−	DOD	19
23	Parotid	6	III	+	S/R	+	−	DOD	13
24	Alv. ridge	1	III	−	S/R/C	+	−	AWD	17
25	Parotid	4	III	+	S	+	−	DOD	15

BOT: base of tongue, FOM: floor of mouth, Orophx: oropharynx, SMG: submandibular gland, S: surgery, R: radiotherapy, C: chemotherapy, NED: alive with no evidence of disease, AWD: alive with disease, DOD: died of disease

Table 2. Comparison of c-erbB-2 expression and clinicopathologic factors

		c-erbB-2 (+)	c-erbB-2 (−)
Age	≤30	2[a]	2[a]
	31-50	5	6
	>50	2	8
Sex	male	5	9
	female	4	7
Site	parotid	5	9
	SMG	1	1
	minor glands	3	6
Size	≤2cm	2	4
	2.1-4cm	5	8
	> 4cm	2	4
Grade	I	0[b]	5
	II	4	8
	III	5	3

[a]: number of cases, [b]: trend test $p<0.05$

salivary glands showed varying degrees of immunostaining for c-erbB-2 oncoprotein along the cell membrane. c-erbB-2 expression was more frequent in high grade tumors than in low grade ones (trend test, $p=0.02$). None of the grade I MECs showed positive membrane staining. The proportion of positive staining was larger in 5 grade III tumors than in 4 grade II ones (Fig. 4). Cytoplasmic staining only without membrane staining was also observed in 1 grade I, 3 grade II, and 2 grade III cases. Comparison of c-erbB-2 expression with other clinicopathologic factors was summarized in Table 2.

Correlation between clinicopathologic factors, c-erbB-2 expression and outcome

Site, size, histologic grade, and c-erbB-2 expression of the tumors were compared with prognosis, represented by regional lymph node (LN) metastasis at the time of presentation, recurrence (disease-free interval) and death (overall survival period) (Table 3).

Locations of the tumors were significantly associated with LN metastasis ($p=0.01$), which was absent in all

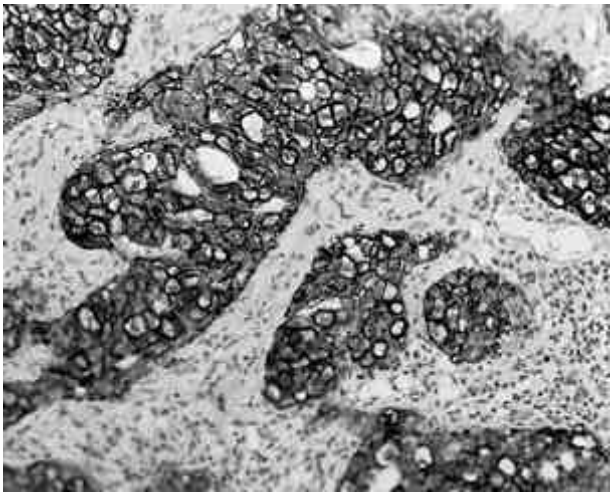

Fig. 4. Immunostaining of grade III mucoepidermoid carcinoma for c-erbB-2 oncoprotein, showing diffuse intense cell membrane positivity (ABC).

minor gland tumors (0/9) and present in all SMG tumors (2/2). Larger size of the tumors was also significantly associated with more frequent LN metastasis ($p=0.01$). Higher grade MECs and c-erbB-2-positive tumors tended to be associated with more frequent LN metastasis, without statistical significance.

Both disease-free intervals and overall survival periods were shorter in cases of major gland location, larger

Table 3. Correlation between clinico-pathologic factors, c-erbB-2 and clinical outcome

			LN meta	Recur	DFI	Death	OS
Site	parotid	(14)a	4[a,b]	8[a]	36	6[a]	47
	SMG	(2)	2	2	10.5	2	17.5
	minor	(9)	0	4	38	2	72
Size	≤2cm	(6)	0[b]	2	84	0	84
	2.1-4cm	(13)	2	9	12	7	42
	> 4cm	(6)	4	4	16.5	3	21.5
Grade	I	(5)	1	1	57[b]	0	57[b]
	II	(12)	1	5	55	3	60
	III	(8)	4	8	8	7	15.5
c-erbB-2	(−)	(16)	3	8	30.5	6	52
	(+)	(9)	3	6	12	3	16.5
LN meta	(−)	(19)		10	38	6	57[b]
	(+)	(6)		4	9	3	16

DFI : median disease-free interval (months),
OS : median overall survival (months),
SMG : submandibular gland,
a : number of cases, b : $p<0.05$

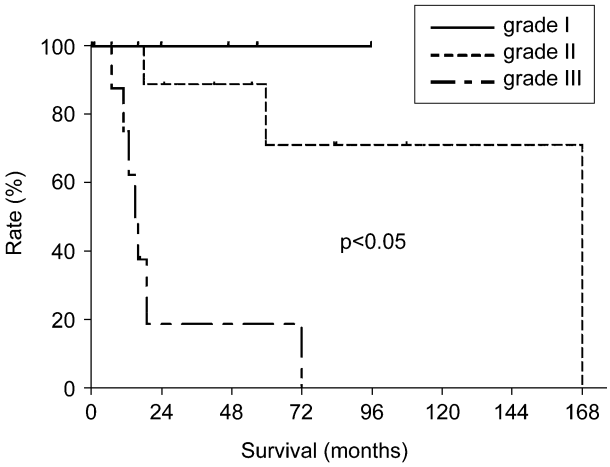

Fig. 5. Overall survivals according to the histologic grade of mucoepidermoid carcinomas.

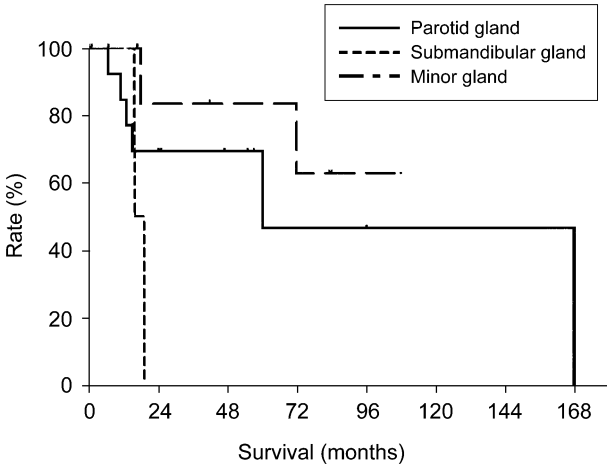

Fig. 6. Overall survivals according to the location of mucoepidermoid carcinomas.

size, higher histologic grade, c-erbB-2 overexpression, and positive regional LNs. Among them, histologic grade was shown to have significant correlation with disease-free interval ($p=0.004$) and overall survival period ($p=0.0005$) (Fig. 5). Regional lymph node metastasis at the time of operation was significantly related ($p=0.048$) to the shorter survival. The two submandibular gland tumors showed definitely shorter survival periods than the parotid or minor gland tumors (Fig. 6), but the number of the cases studied was too small to produce a statistical significance.

DISCUSSION

Except for a few categories of high-grade carcinomas such as salivary duct carcinoma and carcinoma ex

pleomorphic adenoma, most salivary gland carcinomas are slowly progressing neoplasms of low-grade malignancy. MEC also presents a difficulty in predicting its prognosis. According to the studies on the prognostic factors of MEC, histologic grade has been regarded as the most important prognostic determinant (7-10). Other than histologic grade, proliferative activity of tumors measured by immunohistochemistry or flow cytometry has been proposed to be a prognostic indicator (10-12).

c-erbB-2 (*neu*) oncogene amplification or overexpression has been associated with poor prognosis in some adenocarcinomas (5, 6). Although amplification of this distinct gene in human cancer was first demonstrated in a human salivary gland adenocarcinoma, studies of c-erbB-2 oncogene in salivary gland tumors have not been extensive. c-erbB-2 overexpression has been described mostly in high-grade salivary carcinomas such as adenocarcinoma, salivary duct carcinoma and carcinoma ex pleomorphic adenoma (13-18). Some studies have demonstrated a correlation between c-erbB-2 expression and the clinical course of high-grade salivary gland carcinomas (14, 16).

Regarding MEC, there had been only individual case descriptions of c-erbB-2 expression (19-21), before a collective study by Press et al. (22). They observed the amplification and overexpression of c-erbB-2 oncogene in 21% and 38% of MECs, respectively. The results were correlated with male sex, older age, positive lymph node, earlier relapse and shorter survival.

Our study showed a similar incidence of c-erbB-2 overexpression in MECs (36%). Cases with c-erbB-2 overexpression showed more common lymph node metastasis and recurrence, and shorter survival, but without statistical significance. There was a trend for an association with histologic grade, which was significantly correlated with clinical outcome.

Among other factors, larger tumors and tumors of major glands showed more frequent regional lymph node metastases. Two cases of SMG tumors, in particular, showed rapid progression and death within 1 year. Submandibular location was known to be associated with frequent lymph node metastasis (23) and shorter survival (24) in adenoid cystic carcinoma patients. However, it was difficult to determine the significance of this location in this study, because only two cases occurred in the SMG and both were histologically grade III.

In a study on adenoid cystic carcinomas, c-erbB-2 overexpression was more intense with tubular and cribriform patterns than with solid pattern (25). This correlation is contradictory to that in MEC. However, the authors of the former study seemed to regard cytoplasmic staining, as well as membrane staining, as positive, since they reported the positivity not only in all tumors but also in normal salivary ductal epithelial cells, which showed no membrane reactivity in any other study. The cytoplasmic c-erbB-2 positivity without membrane staining was observed in 6 of our cases, and its significance is uncertain.

Press et al. reported that male gender was significantly associated with neu oncogene amplification and overexpression in MEC patients (22). In a transgenic mice model, levels of c-neu transgene expression in the salivary glands of male animals were higher than in female carriers, and correlated with parotid gland enlargement (26). However, no gender difference was found with either membrane or cytoplasmic c-erbB-2 expression in our study.

c-erbB-2 oncogene may play an important role in salivary gland tumorigenesis and tumor progression, and may have clinical significance in certain salivary gland carcinomas including MEC. Nonetheless, the results of the present study indicate that histologic grade is the most important and accessible prognostic factor in MEC.

It is often difficult to diagnose a grade III MEC. Differential diagnoses should include salivary duct carcinoma, carcinoma ex pleomorphic adenoma, and adenocarcinoma NOS, especially in major salivary glands. MEC of grade III, often evolved from MEC of lower grades, histologically tended to maintain roundish solid nests of neoplastic cells with abundant eosinophilic cytoplasms, which were occasionally intermixed with clear cells. They lacked predominant glandular differentiation, large comedonecrosis, extensive desmoplastic reaction, or myxochondroid stroma. Thorough examination of the specimens including previous biopsies could provide histologic clues for MEC such as clear or mucinous cells. However, the clinical importance of the differentiation of grade III mucoepidermoid carcinoma from other high carcinomas of the salivary gland is uncertain since the prognosis of the former tumor appears as poor as that of the others.

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