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Histological features and prognosis of patients with mucoepidermoid carcinoma of the parotid gland

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Abstract

Histological features and prognosis of patients with mucoepidermoid carcinoma of the parotid gland were analysed. Tumours from 13 patients were classified according to histological grades and immunoreactivity for HER-2/*neu*. Surgical resection of the tumour was performed for all patients, and the overall five-year survival rate was 69 per cent. The patients whose histological grades were 1 or 2 showed a 100 per cent five-year survival rate, but no patient with grade 3 survived five years. Also, patients who had tumours that overexpressed HER-2/*neu* had a lower survival rate (25 per cent) than patients with tumours that had weaker immunostaining (89 per cent). We considered tumours classified as grade 3 plus strong HER-2/*neu* expression to be 'high malignancy', and compared them with 'low malignancy' tumours that were grade 1 or 2 and had weaker HER-2/*neu* staining. Patients with high malignancy tumours had shorter recurrence-free intervals and shorter overall survival than patients with low malignancy tumours. The overall survival period of the low malignancy cases was much longer than the recurrence-free interval; unlike that in the high malignancy tumour patients. These results suggest that the combination of histological grades and expression of HER-2/*neu* may be a useful predictor of the prognosis for mucoepidermoid carcinomata.

Key words: Parotid neoplasms; Immunohistochemistry; Neoplasm staging; Survival rate

Introduction

Mucoepidermoid carcinoma of the parotid gland, which used to be called mucoepidermoid tumour, is classified as carcinoma because all varieties are considered capable of metastasis regardless of their macroscopic or histological appearance. However, there are relatively good and poor prognosis with respect to local recurrence and metastatic ability.

To identify a marker predicting the clinical course of mucoepidermoid carcinomata, evaluation by histopathological grade has been studied (Healey *et al.*, 1970; Batsakis and Luna, 1990; Clode *et al.*, 1991), although the gradings are not always absolute in individual cases. Recently it was proposed that the HER-2/*neu* oncogene could be a useful marker of poor prognosis, independent of the histopathological grade (Press *et al.*, 1994a); but some studies of expression of this oncogene show contradictory results (Kernohan *et al.*, 1991; Sugano *et al.*, 1992).

Since there appears to be no single reliable marker to predict the prognosis for this type of tumour, we investigated whether the combination of these histological parameters was a more accurate predictor. From this point of view, we analysed our mucoepidermoid carcinoma cases histopathologically and compared these results with the clinical outcome.

Patients and methods

Patient information

Between 1981 and 1997, 13 patients were treated at Oita Medical University Hospital for mucoepidermoid carcinoma of the parotid gland. The tumours were staged according to the TNM classification (International Union Against Cancer, 1987). The number of patients who were classified as stages I, II, III, and IV were five, one, five, and two, respectively.

All patients had local tumour resection, comprising four partial parotidectomies and nine total parotidectomies. For six patients, extended resection of the neighbouring tissue was performed with total parotidectomy. Neck dissection was performed on nine patients. Six patients received irradiation as post surgical therapy. Chemotherapy was given to six cases. Clinical data for the individual patients are shown in Table I.

Histological grading

All tumours were graded histopathologically according to the criteria described by Batsakis and Luna (1990). Briefly, a low-grade (grade 1) tumour is characterized by macro- and micro-cysts, differen-

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TABLE I
CLINICAL DATA OF THE PATIENTS

Number	Age/sex	Stage		Treatment	Recurrence	Follow-up	At month	Histology grade	Histology HER
1	40/F	$T_{2a}N_0M_0$	I	PP	—	A	87	2	++
2	41/M	$T_{2a}N_0M_0$	I	PP, Rad	—	A	36	1	+
3	44/M	$T_{2a}N_0M_0$	I	TP+ND	—	A	98	1	+
4	66/M	$T_{2a}N_0M_0$	I	PP, Rad	—	A	174	2	−
5	77/M	$T_{2a}N_0M_0$	I	PP+ND	—	A	45	2	±
6	78/M	$T_{3a}N_0M_0$	II	TP+ND, Rad	+ Local	DOD	30	3	−
7	16/F	$T_{3b}N_0M_0$	III	TP+Ex+ND, CAP	+ Local	A	57	1	±
8	42/M	$T_{2b}N_1M_0$	III	TP+Ex+ND, Rad	+ Local	DOD	110	2	±
9	62/M	$T_{4a}N_0M_0$	III	TP+Ex+ND, Rad	—	A	34	3	−
10	69/M	$T_{2a}N_1M_0$	III	TP+Ex+ND, Rad	+ Local	DOD	20	3	++
11	71/M	$T_{4a}N_0M_0$	III	TP, CDDP	+ Local	DOD	91	1	±
12	72/M	$T_{3b}N_{2b}M_0$	IV	TP+Ex+ND	+ Lung	DOD	12	3	++
13	55/M	$T_{3b}N_{2b}M_0$	IV	TP+Ex+ND, CDDP	+ Neck	DOD	9	3	++

Abbreviations: F: female; M: male; PP: partial parotidectomy; TP: total parotidectomy; Ex: extended resection of the neighbouring tissue; ND: neck dissection; Rad: radiation; CDDP: cisplatin; CAP: cyclophosphamide + doxorubicin + CDDP; A: alive; DOD: died of disease; HER: HER-2/*neu*.

tiated mucus and epidermoid cells, minimal to absent pleomorphism, and rare mitoses. An intermediate-grade (grade 2) tumour is characterized by solid nests of cells, a preponderance of intermediate cells, and slight to moderate pleomorphism. A high-grade (grade 3) tumour shows invasive growth patterns with nuclear and cytoplasmic pleomorphism and easily identified mitoses.

*Immunohistochemistry for HER-2/*neu

Formalin-fixed, paraffin-embedded tumour tissues were used for immunostaining. The specimens were sectioned at 6 μm thickness and mounted on glass slides. Deparaffinized sections were rinsed in 0.01 M phosphate-buffered saline (PBS, pH 7.2), exposed to five per cent normal goat serum and incubated with a rabbit anti-HER-2/*neu* polyclonal antiserum (A8010; 1:20 dilution; Oncor, Gaithersburg, MD, USA). The sections were then rinsed in PBS, flooded with a 1:200 dilution of biotin-conjugated goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA, USA), rinsed in PBS and incubated in Vectastain ABC reagent (Vector Laboratories). The sections were rinsed in PBS, and the reaction product was visualized by development in 0.5 per cent 3,3′–diaminobenzidine – 0.01 per cent H_2O_2 substrate medium in 0.1 M phosphate buffer. After counter-staining with veronal acetate-buffered 1 per cent methyl green solution, a coverslip was placed over the section for observation. Membrane staining was interpreted as HER-2/*neu* expression. The amount of staining was scored in a blinded fashion as described by Press *et al.* (1994a), i.e., negative (no immunostaining), trace positive (few, detectable immunostained cells scattered through the tumour or located along one edge of the specimen), moderate (distinct membrane staining in the majority of cells), or strong (intense membrane staining in the majority of cells).

TABLE II
SURVIVAL RATE FOR THE PATIENTS ACCORDING TO TNM CLASSIFICATION

TNM classification	One-year	Five-year	10-year
Stage I	100%	100	100
Stage II and III	100	67	0
Stage IV	0	0	0
Total	85	69	26

Analysis of the data

Kaplan-Meier product-limit estimates of time to recurrence and survival time were plotted to evaluate if these outcome measures were correlated with the TNM stages or histopathological findings. As there was only one patient who was classified as TNM stage II, stages II and III were combined for the analysis.

Results

Correlation between TNM stages and clinical outcome

Table II shows the survival rate for the mucoepidermoid tumour patients. The overall five-year survival rate was 69 per cent. Six (46 per cent) patients died of their tumours, six (46 per cent) had no evidence of disease at their last clinic visit, and one (eight per cent) was a cancer-bearing survivor. None of the patients with TNM stage I disease had recurrence of the tumour. However, most of the patients with stages II and III showed local recurrence of the tumour. Patients with stage IV developed pulmonary or neck lymph node metastases after the intial treatment. The five-year survival rate was 100 per cent for the patients with TNM stage I, 67 per cent for those with stages II and III, and 0 per cent for those with stage IV disease.

Correlation among TNM stages and histological features

Histopathological grading identified four (31 per cent), four (31 per cent), and five (38 per cent) tumours as grade 1, grade 2, and grade 3, respectively. HER-2/*neu* was negative for three (23 per cent) of the mucoepidermoid tumours. Four (31 per

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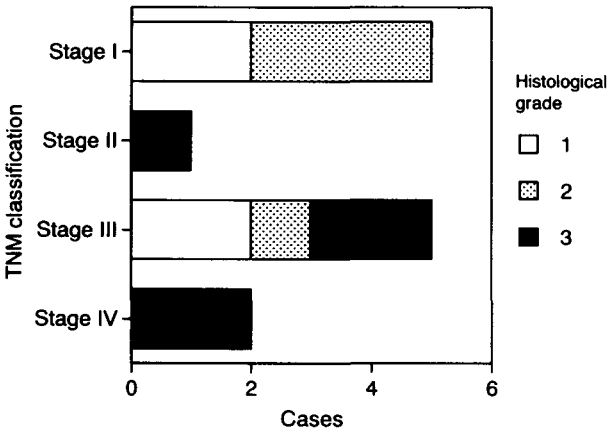

FIG. 1

TNM stages and histological grades of the tumours. Histological grade is not correlated with TNM stage, except for stage IV tumours, which are grade 3.

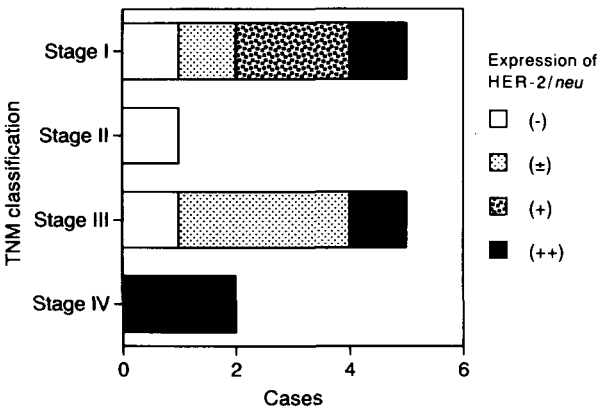

FIG. 2

TNM stages and HER-2/*neu* expression of the tumours. HER-2/*neu* expression is not correlated with TNM stage, except for the stage IV tumours, which are strongly positive for HER-2/*neu*.

cent) tumours were trace positive, two (15 per cent) showed moderate immunostatining, and four (31 per cent) were strongly immunopositive. Neither the histological grades nor the HER-2/*neu* expression levels were well-correlated with the TNM stages, except for the stage IV tumours, which were grade 3 and stongly positive for HER-2/*neu* (Figures 1 and 2).

Although strong HER-2/*neu* immunostaining was found more frequently in tumours with higher histological grades, the correlation between grade and HER-2/*neu* expression was not distinct. For example, two grade 3 tumours lacked expression of HER-2/*neu*, but three other tumours of the same grade showed intense immunostaining.

Correlation between histological features and clinical outcome

There appears to be a correlation between histological grade and patient survival rate. The five-year survival rate was 100 per cent for the tumours graded 1 or 2, and 0 per cent for the patients with grade 3 tumours (Table III). Patients who had tumours with strong immunostaining for HER-2/*neu* had a lower five-year survival rate (25 per cent) than patients with tumours that had weaker immunostaining (89 per cent).

The two histological parameters above were taken together, and the clinical outcome was analyzed. Grade 3 tumours with strong HER-2/*neu* expression were considered 'high malignancy', and compared with 'low malignancy' tumours of grade 1 or 2 and low (no, trace positive, or moderate) HER-2/*neu* expression. The patients with high malignancy tumours had shorter recurrence-free intervals (Figure 3A) and shorter overall survival (Figure 3 A^1) than the patients with low malignancy tumours (Figure 3B, B^1). We also noted that the overall survival periods of the low malignancy cases were much longer than the recurrence-free intervals, unlike that in the high malignancy tumour patients.

TABLE III

SURVIVAL RATE FOR THE PATIENTS ACCORDING TO HISTOLOGICAL FEATURES

	One-year	Five-year	10-year
Grade 1	100%	100	0
Grade 2	100	100	50
Grade 3	60	0	0
HER-2/*neu* (−) ~ (+)	100	89	33
HER-2/*neu* (++)	50	25	0

Discussion

In the present study, cases of mucoepidermoid carcinoma were classified according to histological grade and HER-2/*neu* expression. We graded the tumours histopathologically according to the criteria of Batsakis and Luna (1990). Their three-level grading system incorporates cyto-differentiation as well as growth patterns, emphasizes the intermediate cell population as an integral histogenetic and histological component, and recognizes poorly differentiated types of carcinomas. Our results, which

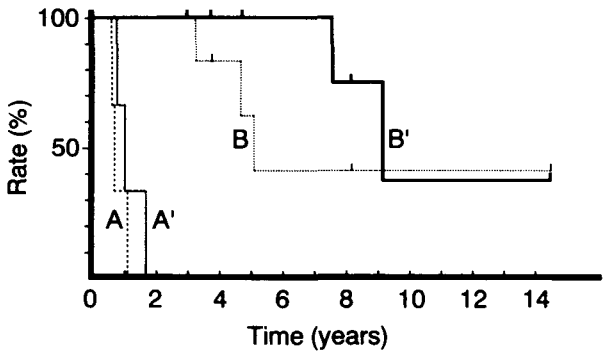

FIG. 3

Actuarial survival curves for patients with mucoepidermoid carcinomas. The patients with 'high malignancy' have a shorter recurrence-free interval (A) and shorter overall survival (A') than the patients with 'low malignancy' (B, B'). It is also noted that the overall survival period of the low malignancy cases (B') is much longer than the recurrence-free intervals (B), unlike that in the high malignancy patients (A, A'). Histological grade 3 tumours with strong HER-2/*neu* expression are considered high malignancy, and tumours whose grade is 1 or 2 and have weaker HER-2/*neu* staining are low malignancy.

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showed that the survival rate was worse in patients with higher histological grades, indicate that this grading system is useful for the evaluation of the prognosis for patients with this parotid tumour.

The HER-2/*neu* oncogene was first identified as a dominant transforming gene in chemically-induced adrenal neuroblastomas in neonatal mice and was referred to as *neu* (Shih *et al.*, 1981; Schechter *et al.*, 1984). Amplification and/or overexpression of HER-2/*neu* in human tumour tissue is associated with a poor prognosis in ovarian (Slamon *et al.*, 1989), endometrial (Hetzel *et al.*, 1992), and breast (Slamon *et al.*, 1987; Press *et al.*, 1993) carcinomata. The association of this oncogene with salivary gland tumours is controversial (Kernohan, *et al.*, 1991: Sugano *et al.*, 1992; Press *et al.*, 1994a), although it was suggested that the number of cases with HER-2/*neu* overexpression may have been underestimated in the study that reported no association (Kernohan *et al.*, 1991), because the antibody used for immunostaining was less sensitive (Press *et al.*, 1994a; Press *et al.*, 1994b). In the present study, we used an anti-HER-2/*neu* polyclonal antiserum that was reported to have fair sensitivity (Press *et al.*, 1994b), and we were able to demonstrate that the overexpression of HER-2/*neu* in mucoepidermoid carcinoma was associated with poor prognosis. The results obtained in this study support the notion that HER-2/*neu* could be a marker of tumour aggressiveness.

Because the expression of HER-2/*neu* appears to be independent of histological grade, it seems reasonable to take both histological parameters into consideration. In the present study, we categorized high malignancy and low malignancy according to histological grades and HER-2/*neu* expression. The patients with high malignancy had shorter recurrence-free intervals and shorter overall survival, which indicates accuracy in categorization.

It is interesting that the survival periods in the low malignancy patients were much longer than the recurrence-free intervals, unlike in the high malignancy patients. Because it is thought that the adequacy of primary surgical excision is related to local recurrence, histological parameters alone cannot predict the recurrence-free interval. The difference between the recurrence-free interval and the survival period, which was observed in the low malignancy cases, suggests that the low malignancy patients have a more favourable outcome than the high malignancy patients, even after a tumour recurs. Such information is useful for follow-up of patients with inoperable tumours, such as our patient number 7 (Table I), who is a cancer-bearing survivor with low malignancy.

As for the TNM stage I cases, the five-year and 10-year survival rates were 100 per cent, regardless of the histological features. Thus, we suggest that the histological classification described above should be a useful predictor for the prognosis of mucoepidermoid carcinomas, especially for patients in the advanced stages.

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