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p53 and CyclinX D1 Protein Expression in Carcinomas of the Parotid Gland

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Abstract. *Background: p53 and cyclin D1 genes play a central role in the regulation of the G1 phase of the cell-cycle, and are frequently involved in head and neck tumorigenesis. Materials and Methods: By means of immunohistochemistry, we retrospectively investigated the overexpression of cyclin D1 and p53 genes in a series of 28 parotid gland carcinomas. The imrnunohistochemical analysis was performed using the ABC method and the antibodies DCS6 (for cyclin D1) and CM1 (for p53). Results p53 was overexpressed in 12 (42.9%) and cyclin D1 in 6 cases (21.4%). No significant association was found between p53 or cyclin D1 expression and the evaluated clinicopathological parameters of tumor extension, clinical stage, and lymph node or distant metastases. Nevertheless, it is worth noting that all of the patients with a high expression of p53 died of their disease. Conclusions: The present data confirm the role of p53 abnormalities in the pathogenesis of salivary gland carcinoma and report, for the first time, the involvement of cyclin D1 gene in these tumors.*

Malignant parotid tumors represent about 10-20% of all parotid gland neoplasms (1,2), and are characterized by a variety of histological subtypes that have different clinical behaviours (3).

p53 and cyclin D1 play a key role in the regulation of the G1 phase of the cell cycle (4,5). It is known that the p53 tumor suppressor gene blocks the replication of cells with damaged or faulty DNA, thus causing Gl arrest and allowing either DNA repair or apoptosis (6). The PRAD1/cyclin D1 gene encodes for a protein that exerts its function in the late G1 phase of the cell cycle by complexing with cdk4 and cdk6. The regulatory function of the cyclin Dl/cdk complexes takes place through the phosphorylation of the pRb protein (5) which in turn promotes cell cycle progression. The formation of the cyclin D1/cdk complexes is negatively regulated by wild-type p53 through the activation of the p21 gene (7).

Point mutations of the p53 gene represent the most frequent genetic lesions in human cancer (8). However, they lead to an increase in the half-life of the protein, and thus allow its immunohistochemical detection (9).

The cyclin D1 gene has been shown to be deregulated as a result of various genetic lesions, including chromosomal inversion in parathyroid adenomas (10) and translocation in B-non-Hodgkin lymphomas (11), and gene amplification in breast (12), esophagus (13), head and neck (5,14,15) and liver carcinomas (16). Recent studies have demonstrated a close correlation between the translocation or amplification of the cyclin D1 gene and protein overexpression, thus suggesting that immunohistochemistry may be a reliable method of detecting cyclin D1 gene alterations in human tumors (17).

Although p53 has not yet been extensively studied in salivary gland tumors, and most of the reported studies rely on small case series (18,19,20,21), its overexpression has been found to have an adverse effect on the prognosis of patients with parotid gland cancer (22).

To our knowledge, the role of cyclin D1 has never been investigated in parotid gland neoplasms.

In order to gain a better insight into the role and clinical significance of p53 and cyclin D1, we retrospectively investigated their overexpression in a series of 28 parotid gland carcinomas by comparing immunohistochemical results with the traditional clinicopathological parameters of clinical stage, the presence of lymph node metastasis and patient survival.

Materials and Methods

Pathological samples. Twenty-eight formalin-fixed and paraffin embedded surgical specimens of parotid gland carcinomas collected between 1983 and 1995 (8 NOS adenocarcinomas, and 11 mucoepidermoid, 4 adenoid cystic, 2 squamous cell, 1 acinic cell, 1 salivary duct and 1 epithelial-myoepithelial carcinoma) were selected

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Table I. *p53 and cyclin D1 protein expression in parotid gland carcinomas.*

Histology	No. tumors analysed	p53 positivity No.	%	Cyclin D1 positivity No.	%
Mucoepidermoid carcinoma	11	6	55	4	36.3
Adenocarcinoma	8	4	50	1	12.5
Adenoid cystic carcinoma	4	0	-	1	25
Squamous cell carcinoma	2	1	50	0	-
Salivary duct carcinoma	1	1	100	0	-
Acinic cell carcinoma	1	0	-	0	-
Epithelial-myoepithelial carcinoma	1	0	-	0	-
Total	28	12	42.9	6	21.4

from the files of Clinica Otorinolaringoiatrica I of the University of Milan. The main clinical characteristics of the patients are shown in Table I: nineteen were male and nine female, and their mean age was 62.8 years (range 24-87). All of the patients underwent total parotidectomy, and 13 also underwent neck dissection. The patients with lymph node metastases received post- operative radiotherapy. The median duration of follow-up was 24 months (mean 38 months): 18 patients died as a result of their disease, one patient with an active adenoid cystic carcinoma is still alive; the remaining 9 patients are alive without disease. The identification and clinical staging of the tumors were based on the UICC TNM classification of malignant tumors (23): 6 tumors were in stage I, 9 in stage II, 6 in stage III and 7 in stage IV. Nodal and distant metastases were present in respectively 12 and 3 cases.

Immunohistochemistry. One paraffin block corresponding to the most representative slides was selected for immunohistochemical investigation. Cyclin D1 expression was detected using the DCS6 monoclonal antibody (Novocastra Laboratories Ltd., Newcastle upon Tyne, UK) at a working concentration of 1: mg/ml and p53 overexpression using CM1 antiserum (Novocastra Laboratories Ltd., Newcastle upon Tyne, UK) at a working concentration of 1:5000. A standard immunohistochemical ABC technique was used, as previously reported (24,25). Sections from a formalin-fixed, paraffin-embedded breast carcinoma were used as a positive control for DCS6 and CM1 Abs; negative controls were obtained by substituting the primary reagent with non-immune mouse or rabbit sera.

The number of immunoreactive (IR) cells was counted by means of a gridded. eye-piece at 250X magnification in at least 30 tumoral fields. The scoring system was the following: 0: unreactive cases and those expressing less than 10% IR neoplastic cells; 1: 10-30% IR neoplastic cells; 2: 30-50% IR neoplastic cells; 3: more than 50% IR neoplastic cells. The cases classified 2 and 3 were considered high expressors (HE), and those classified 0 and 1 as low expressors (LE).

Statistical methods. The statistical analysis was performed by means of Chi-squared tests, using Yates' correction when necessary (26). The cumulative survival rate was calculated by means of Kaplan-Meier product limit estimates (27). For statistical purposes, the initial step was to compare the positive and negative cases; we then matched the p53 HE with the p53 LE and negative tumors.

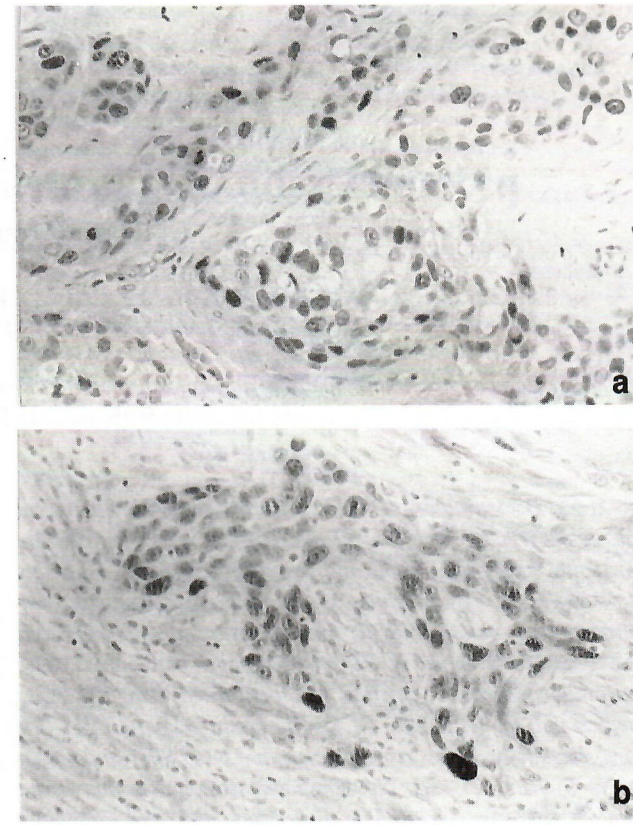

Figure 1. *An example of p53 (a) and cyclin D1 (b) immunoreactivity in two different cases of mucoepidermoid carcinoma: the immunostaining is limited to the nucleus and its intensity ranges from weak to strong.*

Results

The immunohistochemical results are summarized in Table I: p53 proved to be overexpressed in 12 (42 9%) and cyclin D1 in 6 (21 4%) of the 28 parotid gland carcinomas. In particular, p53 protein expression was detected in 6/11 (55%) mucoepidermoid carcinomas, 4/8 (50%) adenocarcinomas, 1/2 (50%) squamous cell carcinomas, and in the only salivary duct carcinoma. Cyclin Dl protein expression was observed in 4/11 (36%) mucoepidermoid carcinomas, 1/4 (25%) adenoid cystic carcinomas, and 1/8 (12%) adenocarcinomas. p53 and cyclin Dl were absent in the cases of epithelial-myoepithelial and acinic cell carcinoma. p53 and cyclin D1 were coexpressed in 3 of the 28 cases (14%); all of which were morphologically classified as mucoepidermoid carcinomas. Whatever the antibody used, immunoreactivity was restricted to the nucleus, and its intensity ranged from weak to strong in a given tumor (Figure 1). The non-neoplastic tissue adjacent to the infiltrative tumors did not show any p53 or cyclin Dl expression.

Table II statistically compares the immunohistochemical results and the evaluated clinico-pathological parameters: no significant association was found between p53 or cyclin D1

Table II. *Correlation between clinico-pathological features and p53 and cyclin D1 protein expression in parotid gland carcinomas.*

	p53 positive tumors			Cyclin D1 positive tumors		
	No.	%	p value	No.	%	p value
Clinical stage						
I-II	5			3		
III-IV	7		.4	3		1
Tumor extension						
T1-T2	9			4		
T3-T4	3		.4	2		1
Grading						
G1-G2	4			3		
G3	8		.1	3		1
Lymph node metastases						
Negative	5			3		
Positive	7		.2	3		.9
Distant metastases						
Negative	6			4		
Positive	6		1	2		.3

immunoreactivity and histological grade (G1-G2 *vs* G3; p= .1 and p=1 respectively), tumor extension (T1-T2 *vs* T3-T4; p= .4 and p=1, respectively), clinical stage (I-II *vs* III-IV; p= .4 and p=1, respectively), the presence of lymph nodes (N0 *vs* N+; p= .2 and p=.9, respectively), or the presence of distant metastases (M0 *vs* M+; p=1 and p=.3, respectively).

Finally, no difference in overall survival was found between the p53 or cyclin D1 positive and negative cases (data not shown). Nevertheless, all of the patients who were HE of p53 died of their disease. The median survival time of the LE and HE patients was respectively 24 months (mean 41 months) and 15 months (mean 23 months).

Discussion

This study immunohistochemically analyzed the expression of p53 and cyclin D1 in a series of malignant tumors of the parotid gland. p53 overexpression was found to be a common event: 12 of the 28 (43%) tumors proved to be immunoreactive to the CM1 antiserum. These data are in line with those of others (22), and confirm the role of p53 abnormalities in the pathogenesis of salivary gland tumors.

Gallo *et al* (22) found that parotid gland carcinomas showing moderate and high p53 oncoprotein immunoreactivity were more frequently associated with regional and distant metastases, and had lower disease free and overall actuarial survival rates than those with little or no p53 expression. In our series, p53 expression was not related to the clinicopathological parameters of histological grade, tumor extension, clinical stage, or the presence of lymph nodes or distant metastases (see Table II). Likewise, no significant difference in overall survival was found between the groups of p53 positive and negative cases, although it is worth noting that all of the patients who were high expressors of p53 died of their disease. Moreover, the median survival time of the low expressors was higher than that of the high expressors of p53 (see Results). Taken together, these data suggest that further studies, based on larger series of patients, are needed to confirm the putative prognostic role of p53 in these tumors.

This is the first report in which the overexpression of cyclin D1 has been investigated in salivary gland tumors. Immunoreactivity to DCS6 was found in 6 of the 28 cases (21.4%). It has been demonstrated that cyclin D1 protein expression is strictly associated with the presence of cyclin D1 gene abnormalities, such as amplification, translocation and chromosomal inversion (10,11,24). As frozen tissue was not available, we did not perform molecular analysis of the cyclin D1 gene. Therefore, further studies are needed in order to establish the molecular abnormalities which lead to cyclin Dl protein expression in salivary gland tumors. It has been shown that cyclin D1 overexpression adversely affects the clinical outcomes of patients with head and neck cancer (24,28,29); nevertheless, Michalides *et al* have reported that it had little or no clinical value in patients with breast cancer (30). In the present series, cyclin D1 overexpression was not associated with histological grade, clinical stage, the presence of lymph node or the presence of distant metastases. Although obtained in a relatively small series, these data suggest that cyclin D1 overexpression might not be a useful prognostic marker in salivary gland cancer.

We found p53 and cyclin D1 coexpression in three of the 11 cases of mucoepidermoid carcinoma. It is well known that the accumulation of multiple genetic lesions (chromosomal deletions and oncoprotein deregulation) critically influences the progression of human cancer (31). Likewise, Kaimo *et al* have recently reported the frequent coexpression of p53 and c-erbB-2 proteins in a cohort of malignant salivary gland tumours (32). It is therefore tempting to speculate that the coexpression of p53 and cyclin D1 might influence the progression of some hystotypes of salivary gland carcinoma.

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