Immunoeexpression of c-erbB-2 and p53 in benign and malignant salivary neoplasms with myoepithelial differentiation

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Abstract

**Aim**—To evaluate whether the immunoeexpression of c-erbB-2 and p53 is involved in the pathogenesis and progression of salivary tumours with myoepithelial differentiation.

**Methods**—233 tumours from 211 patients were studied. These included 76 primary and 24 recurrent adenocarcinomas (polymorphous low grade adenocarcinoma, 13; epithelial-like myoepithelial carcinoma, 19; adenoid cystic carcinoma, 56; and basal cell adenocarcinoma, 12) and 133 pleomorphic adenomas and myoepitheliomas, 96 being primary and the remaining recurrent tumours. All cases were formalin fixed and paraffin wax embedded. A StrepABC peroxidase method and polyclonal c-erbB-2 and p53 specific antisera were used.

**Results**—Cell membrane staining of c-erbB-2 was not found in any benign or malignant tumour. There was p53 protein accumulation in one primary and one recurrent pleomorphic adenoma and in 10 adenocarcinomas (polymorphous low grade adenocarcinoma, one; epithelial-like myoepithelial carcinoma, one; adenoid cystic carcinoma, five; and basal cell adenocarcinoma, three), three of them being recurrences.

**Conclusions**—The c-erbB-2 and p53 proteins are not involved in the pathogenesis of pleomorphic adenoma and myoepithelioma and do not constitute biomarkers in assessing the risk of recurrence. c-erbB-2 is not involved in the genesis of low grade salivary neoplasia with myoepithelial differentiation. The percentage of this type of neoplasia with p53 accumulation is low (10%) and does not appear to be related to tumour recurrence.

(J Clin Pathol 1997;50:661-663)

Keywords: p53; c-erbB-2; salivary gland neoplasms

p53 is a nuclear phosphoprotein encoded by a gene located on the short arm of chromosome 17 (17p13.1) and the c-erbB-2 protein is a growth factor receptor encoded at a different region of the same chromosome (17q11.2-12). Both the overexpression of c-erbB-2 and the accumulation of the p53 protein have been reported in a wide variety of human tumours, including those originating in salivary glands. However, there is some controversy about this issue, and the literature is occasionally conflicting, which justifies further investigations to clarify the involvement of both genes and their products in the tumorigenesis of salivary glands.

It is well known that the majority of benign and malignant salivary gland neoplasms show dual differentiation, with a composite epithelial and myoepithelial phenotype. Our previous studies of malignant mixed tumours demonstrated p53 and c-erbB-2 immunoeexpression in high grade carcinomatous areas but not in the benign components, or in the low grade malignant areas, which usually exhibit a modified myoepithelial cell phenotype. These observations suggested that alterations of these protein products are associated with the acquisition of a certain malignant phenotype, supporting the view that low and high grade adenocarcinomatous components of carcinoma ex-pleomorphic adenomas (with and without myoepithelial differentiation, respectively) are related to distinct histogenetic pathways.

The aim of this study was to confirm these conclusions in salivary gland tumours with similar histology but outside the context of malignant transformation of pleomorphic adenoma. We evaluated p53 and c-erbB-2 immunoeexpression in a large series of myoepithelial cell related salivary gland neoplasms, including pleomorphic adenomas, myoepitheliomas, and low grade adenocarcinomas, in an attempt to substantiate our previous results and to evaluate the potential role of the aforementioned proteins in tumour recurrence and progression.

**Materials and methods**

This study was performed on 233 salivary gland tumours: 133 pleomorphic adenomas and myoepitheliomas from 111 patients, and 100 low grade adenocarcinomas from an equal number of patients treated at the Instituto Português de Oncologia de Francisco Gentil, Lisboa, Portugal.

The age of the patients ranged between 10 and 92 years, 67 patients were male and 144 female. In one case the patient's age was not recorded.

In the group of patients with benign tumours, 29 had recurrent disease. From these, 14 primaries and 37 recurrent tumours (from 23 patients) were assessed for comparison. The remainder were not studied because the archival tissue was not available, or recurrence had...
Table 1 Results of p53 and c-erbB-2 immunostaining in benign neoplasms (pleomorphic adenoma and myoepithelioma)

<table>
<thead>
<tr>
<th>Tumour type</th>
<th>n</th>
<th>Number examined</th>
<th>p53/c-erbB-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-recurrent primary tumour</td>
<td>82</td>
<td>82</td>
<td>1.0</td>
</tr>
<tr>
<td>Primary tumour with further recurrences*</td>
<td>29</td>
<td>14</td>
<td>0.0</td>
</tr>
<tr>
<td>First recurrence*</td>
<td>28</td>
<td>16</td>
<td>0.0</td>
</tr>
<tr>
<td>Second recurrence</td>
<td>13</td>
<td>13</td>
<td>1.0</td>
</tr>
<tr>
<td>Third recurrence</td>
<td>3</td>
<td>3</td>
<td>0.0</td>
</tr>
<tr>
<td>Fourth recurrence</td>
<td>3</td>
<td>3</td>
<td>0.0</td>
</tr>
<tr>
<td>Total</td>
<td>158</td>
<td>133</td>
<td>2.0</td>
</tr>
</tbody>
</table>

*The case that recurred as a malignant mixed tumour is not included. This was positive for p53 in the adenocarcinomatous component of the tumour.

Table 2 Results of p53 and c-erbB-2 immunostaining in salivary adenocarcinomas with myoepithelial participation

<table>
<thead>
<tr>
<th>Tumour type</th>
<th>Number examined</th>
<th>p53 positive cases (% of +ve cells)</th>
<th>c-erbB-2 +ve cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLGA</td>
<td>13</td>
<td>1 (&gt;10%)</td>
<td>0</td>
</tr>
<tr>
<td>EMC</td>
<td>19</td>
<td>1 (&gt;25%)</td>
<td>0</td>
</tr>
<tr>
<td>ACC</td>
<td>56</td>
<td>3 (&gt;10% in 3; &gt;25% in 2)</td>
<td>0</td>
</tr>
<tr>
<td>BCA</td>
<td>12</td>
<td>3 (&gt;10%; &gt;50% and &gt;75%)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

PLGA, polymorphous low-grade adenocarcinoma; EMC, epithelial-myoeplithelial carcinoma; ACC, adenoid cystic carcinoma; BCC, basal cell adenocarcinoma.

been diagnosed by fine needle aspiration cytology. One tumour recurred as a carcinoma ex-pleomorphic adenoma, and this recurrence was not included in the present study.

In the adenocarcinoma group we analysed 76 primary neoplasms and 24 recurrences. The adenocarcinomas were classified as: polymorphous low grade adenocarcinoma (n = 13), epithelial-myoeplithelial carcinoma (n = 19), adenoid cystic carcinoma (n = 56), and basal cell adenocarcinoma (n = 12). Radiation therapy had been carried out in nine patients, eight with adenoid cystic carcinoma and one with epithelial-myoeplithelial carcinoma.

Haematoxylin and eosin stained sections of each tumour were re-examined and one representative paraffin block was selected for the immunohistochemical study. Sections (4 μm thick) were collected on to gelatine coated slides, dewaxed in xylene, and rehydrated through ethanol. Endogenous peroxidase was blocked with 0.3% H2O2 in methanol, for 10 minutes, at room temperature.

For p53 staining, high temperature antigen unmasking and incubation with rabbit polyclonal p53 specific antibody (CM1; Novocastra, Newcastle upon Tyne, UK) diluted 1:3000, for 30 minutes, was carried out. For c-erbB-2 immunostaining, a polyclonal c-erbB-2 specific antibody (A485; Dako, Glostrup, Denmark) diluted 1:150, for 30 minutes, at room temperature, was used. All slides were then incubated with a secondary biotinylated anti-rabbit antibody (Dako) diluted 1:250, for 30 minutes, at room temperature and StreptABC conjugated with peroxidase (1:1+100) for 30 minutes, at room temperature. Diaminobenzidine tetrahydrochloride solution was used as chromogen. Appropriate control procedures were used.

Tissues were considered to be p53 positive when brown stained nuclei were observed in more than 10% of tumour cells. The percentage of p53 positive nuclei was determined in a semiquantitative way as >10%, ≤25%, 25-50%, 50-75%, and >75%. c-erbB-2 immunostaining was considered positive when the neoplastic cells exhibited a distinct cell membrane decoration.

Results

PLEOMORPHIC ADENOMAS AND MYOEPITHELIOMAS
c-erbB-2 immunoexpression was consistently negative in all 133 cases. p53 positivity was obtained in more than 10% of the neoplastic cells in one primary neoplasm and in one recurrent tumour (second recurrence). In the latter case, both the primary tumour and the first recurrence were negative (table 1).

Normal salivary gland tissue adjacent to neoplasms was consistently negative for both antibodies.

ADENOCARCINOMAS WITH MYOEPITHELIOM DIFFERENTIATION

Immunostaining of c-erbB-2 was not found in any of the 100 cases. In situ ductal carcinoma of the breast, used as a c-erbB-2 positive control, displayed uniformly marked brown staining of the neoplastic cell membrane.

Accumulation of p53 was observed in 10 cases of adenocarcinoma (fig 1): one case of epithelial-myoeplithelial; one polymorphous low grade adenocarcinoma; five adenoid cystic carcinoma; and three basal cell adenocarcinomas (table 2). Three of the positive cases were recurrent tumours. The percentages of positive cases among the groups of primary and recurrent neoplasms were 9.2% (7 of 76) and 12.5% (3 of 24), respectively.

Nuclear immunoexpression of p53 was distributed diffusely throughout the tumour section, without cell type preference, and irrespective of the histological type. Differences in staining intensity were not taken into account. p53 was found to be positive in two of nine cases previously submitted to local radiotherapy.

Discussion

Frequently, recurrence of pleomorphic adenoma is associated with either incomplete excision of the lesion or tumour capsule...
Immunoexpression of c-erbB-2 and p53 in benign and malignant salivary neoplasms

Disruption during surgical removal. Malignant change occurs in 3-4% of all pleomorphic adenomas and is associated with longstanding and recurrent tumours. 

In this study, the immunoexpression of p53 was very low and c-erbB-2 was not immunoexpressed, either in primary or recurrent pleomorphic adenomas, suggesting that these genes are not involved in the pathogenesis of these neoplasms, or in their potential for recurrence. Likewise, they do not seem to constitute biomarkers for the assessment of the risk of their malignant transformation.

These findings support our previous results obtained in the study of a series of carcinoma ex-pleomorphic adenomas in which p53 and c-erbB-2 expression was found to be consistently negative in the benign areas of the tumours. In those studies, p53 and c-erbB-2 immunoexpression was found exclusively in the malignant salivary gland neoplasms with evidence of myoepithelial differentiation which supports the view that this growth factor receptor is not involved in their pathogenesis. This conclusion is in accordance with previous studies performed in cases of salivary gland carcinomas with myoepithelial differentiation but differs from the results obtained by Kärjä et al.

In contrast to our observations in this series of low grade adenocarcinomas, the c-erbB-2 protein is expressed overtly by high grade salivary carcinomas, namely salivary duct carcinoma and high grade mucoepidermoid carcinoma. This gives further support to the existence of distinct molecular mechanisms in salivary gland carcinogenesis.

The p53 protein was detected in a small number of low grade salivary adenocarcinomas, irrespective of their histological subtype. The percentage (10%) of positive cases may be overestimated as, in two of the 10 positive tumours, the patients had received radiotherapy. It is known that cell irradiation can induce p53 wild-type protein accumulation and therefore may be responsible for false positive results that are not mutation associated.

There is some variation in the literature regarding the frequency of p53 alterations in myoepithelial lineage adenocarcinomas of the salivary glands. Distinct methodologies, criteria for positivity, sample types, and subjectivity of the histological classifications are some of the main factors that might explain the disparate results.

To evaluate whether recurrent malignant adenocarcinomas express p53 more frequently than primary neoplasms a large number of lesions was studied by immunochemistry. We verified that the percentage of positivity among primary (9.2%) and recurrent (12.5%) tumours did not differ significantly. In a previous study of a small number of cases, Papadaki et al. suggested that p53 mutation was involved in tumour recurrence. However, the discrepancy of results could be explained by the sample sizes, distinct methodologies, and assessment criteria. In fact, we considered cases containing more than 10% of p53 stained nuclei as positive, assuming that it would be more likely that occasional positive cells (10%) distributed throughout the histological section would be related to stochastic accumulation of the protein, rather than to p53 mutation. Whatever the reasons for the existence of a certain number of p53 positive dispersed cells, in our study, they occurred in 11 primary lesions and only one recurrent lesion, reinforcing the assumption that tumour recurrence is not related to p53 gene alterations.

This work was supported by a grant from the Núcleo Regional de Saúde da Região Sudeste da Universidade Federal de Minas Gerais, Brazil.
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J Clin Pathol 1997 50: 661-663
doi: 10.1136/jcp.50.8.661

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