MIB-1, Bcl-2 and p53 in odontogenic myxomas of the jaws

Mib-1, Bcl-2 e p53 nei mixomi odontogeni dei mascellari

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SUMMARY

Odontogenic myxoma is a rare benign neoplasm occurring in the jaws. Microscopically, it is composed of spindle or stellate-shaped cells arranged in a mucinous matrix. In some cases (20%), odontogenic epithelial islands may be found. The Authors evaluated p53, MIB-1, and Bcl-2 expressed by the epithelial and stromal elements in 12 cases of odontogenic myxoma of the jaws. The cells of the odontogenic epithelium were positive for Bcl-2, p53 and MIB-1. The stromal cell showed a high positivity for MIB-1. Proliferation of both the epithelial and stromal components could be related to the growth of this odontogenic tumour.

KEY WORDS: Jaws • Benign neoplasm • Odontogenic myxoma • Apoptosis • Bcl-2 • MIB-1 • p53

INTRODUCTION

Odontogenic myxoma (OM) is a relatively rare, benign neoplasm occurring in the jaws. OM represents 3-20% of all odontogenic tumours (OT) and, in most studies, OM is the third most frequent OT. An annual incidence of 0.07 per million has been reported. It represents from 1.1% to 3.7% of total surgical specimens submitted to an oral pathology department. The incidence of OM is about 1/6 that of ameloblastoma. Slootweg and Wittkamp found that OM constituted 0.1% of all specimens studied in a 40-year period. OM is a locally invasive neoplasm that shows little encapsulation and often extends through the bone. A high recurrence rate has been reported and local infiltration is thought to account for the high recurrence rate. The histogenesis of OM is poorly understood, it is still a matter of debate and the underlying molecular mechanisms of OM remain unknown. According to most investigators, it represents a tumour unique to the jaws arising from the mesenchymal part of the tooth germ. Mutations of the Gs alpha gene are rarely, if ever, associated with sporadic jaw myxoma tumourigenesis, also a mutation in the protein kinase A regulatory subunit type IA (PRKAR1A) has been reported. OM may originate from either mesenchymal elements of the dental papilla, dental follicle, periodontal membrane, or non-dental mesenchyme. Also myxomas located in the soft tissues of the head and neck have been reported. The odontogenic nature of the tumour is suggested by the location within alveolar bone in tooth-bearing regions, the association with missing, incompletely formed or non-erupted teeth, the early age of the patients, the presence of islands and strands of odontogenic epithelium, and clinical behaviour resembling that of ameloblastoma. Several mechanisms may be used by neoplastic cells to provide a growth advantage over normal tissue. Neoplastic cells may present an increased rate of cell division and/or a decreased rate of apoptosis. Apoptosis allows control of the number of cells in a given tissue and tumour cells that inhibit apoptosis show a growth advantage over normal tissue and apoptosis is regulated, to a large extent, by the Bcl-2 gene family. One hypothesis has been recently put forward that OM may present an alteration in the apoptotic mechanisms that helps the growth of these tumours, and, in fact, an increase in cells staining positively for Bcl-2 was found. The production, then, of anti-apoptotic proteins with a decrease in the programmed cell death of the cells constituting OM, may provide a growth advantage in this type of tumour.

RIASSUNTO


PAROLE CHIAVE: Mascellari • Tumori benigni • Mixoma odontogeno • Apoptosi • Bcl-2 • MIB-1 • p53
Ki-67 antigen expression has been observed in the nuclei of proliferating cells and it can be a marker to estimate the state of tissue growth. It has been reported that Ki-67 antigen expression increases in pre-neoplastic and neoplastic lesions of the oral mucosa, and in all states of high cell turnover. MIB-1 is the monoclonal antibody that reacts with the epitope of the Ki-67 nuclear antigen in formalin-fixed, paraffin-embedded sections. Bcl-2 is an anti-apoptotic protein that seems to be important in cancer development and progression. Also p53 seems to be important in oncogenesis. Ki-67, Bcl-2 and p53 have been extensively studied in neck cancerogenesis, in some types of OTs and salivary gland tumours, in odontogenic cysts, and actinic cheilitis. The presence of Bcl-2 seems to be associated with a better prognosis in some tumours, but not in others.

A correlation between these different factors has been hypothesized. Studies in human breast cancer and in cancer cell lines have shown that p53 can down-regulate Bcl-2 expression and that apoptosis induced by p53 can be blocked by Bcl-2, in cultured cancer cells. p53 has been shown to down-regulate Bcl-2 via binding to a negative regulatory element outside the Bcl-2 gene promoter. Ravi et al. reported an increased Bcl-2 expression in oral dysplasia and carcinoma. Cruz et al. demonstrated that p53 supra-basal expression was significantly associated with the development of carcinoma. Murti et al. found that the over-expression of p53 protein was significantly more common in severe than in mild epithelial dysplasia, and that p53 expression peaked close to the time of transition from pre-cancer to cancer rather than earlier in the natural history of oral pre-cancer. Apoptosis can be important in tumour growth and prognosis: Langlois et al. found, in colorectal carcinoma, that tumours with higher apoptotic counts seemed to have good prognosis and this may mean that neoplasms with higher levels of apoptosis are slower growing. In renal cell carcinoma, Hindermann et al. found a decrease in cells undergoing apoptosis in less differentiated tumours with an increase in the number of tumour cells and of tumour growth. This decrease in apoptosis was correlated with an increase in the proliferative activity. The presence of Ki-67 closely coincided with p53 protein. Aim of the present study was to analyse MIB-1, p53 and Bcl-2 in stromal and epithelial cells of OM of the jaws.

Materials and methods

The slides of 12 OM of the jaws were retrieved from the archives of the Institute of Pathology, Polytechnic University of the Marche, Ancona, Italy. The patients comprised 7 females and 5 males, mean age 34.4 years (range 18-47). Overall, 11 lesions were located in the posterior mandible, while one was located in the anterior maxilla; the radiographic appearance was of a multi-locular lesion in 8 cases and a uni-locular lesion in 4. All patients were asymptomatic and the lesion had been discovered on X-rays performed for other causes. All lesions had been treated with wide resections. Two recurrences were reported after 4 and 6 years, respectively. All slides were carefully reviewed and the diagnosis of OM of the jaws was confirmed. Immuno-histochemical staining for Bcl-2 protein was performed using the following antigen retrieval system. Sections were deparaffinized in two changes of xylene for 10 minutes each and were then rehydrated through graded alcohols and immersed in 0.3% hydrogen peroxide in methanol for 30 minutes to block endogenous peroxidase activity. Sections were then washed in phosphate-buffered saline (PBS). The tissue sections were placed in a microwave oven (Cookytronic M720, 700 W, Philips, Andover, MA, USA) in a plastic Coplin jar filled with 10 mM sodium citrate buffer (pH 6.0), at 5-minute intervals, for a total of 10 minutes. At each 5-minute interval, the Coplin jar was taken out of the microwave oven and allowed to cool. Slides were incubated overnight with a 1:60 dilution of the primary mouse anti-human Bcl-2 monoclonal antibody (Dako 124, Glostrup, Denmark). A biotin streptavidin detection system was used with diaminobenzidine as the chromogen. Slides were washed twice with PBS and incubated with the linking reagent (biotinylated anti-immunoglobulins) for 15 minutes, at room temperature. After rinsing in PBS, the slides were incubated with the peroxidase-conjugated streptavidin label for 15 minutes, at room temperature. The sections were again rinsed in PBS and incubated with diaminobenzidine for 10 minutes, in the dark. After chromogen development, slides were washed in two changes of water and counterstained with a 1:10 dilution of haematoxylin. The sections were then dehydrated, cleared in xylene, and mounted. A negative control was performed in all cases by omitting the primary antibody, which, in all instances, resulted in negative immuno-reactivity. Sections from a lymph node with follicular lymphoma were used as positive controls. Normal lymphocytes infiltrating the connective tissue of the cyst wall represented an internal positive control for Bcl-2 immuno-staining. In all positive cases, immuno-reactivity was restricted to the cytoplasm. Immuno-histochemical staining for p53 protein was performed using the following antigen retrieval system. Sections were deparaffinized in two changes of xylene for 10 minutes each and then were rehydrated through graded alcohols and immersed in 0.3% hydrogen peroxide in methanol for 30 minutes to block endogenous peroxidase activity. Sections were then washed in PBS. The tissue sections were placed in a microwave oven (Philips, Cookytronic M720, 700 W) in a plastic Coplin jar filled with 10 mM sodium citrate buffer (pH 6.0), at 5-minute intervals, for a total of 10 minutes. At each 5-minute interval, the fluid level in the Coplin jar was removed from the microwave oven and allowed to cool. Slides were incubated overnight with a 1:50 dilution of the primary mouse anti-human p53 monoclonal antibody (Dako DO-7, Glostrup, Denmark). A biotin-streptavidin detection system was used with diaminobenzidine as the chromogen. Slides were washed twice with PBS and incubated with the linking reagent (biotinylated anti-immunoglobulins) for 15 minutes, at room temperature. After rinsing in PBS, the slides were incubated with the peroxidase-conjugated streptavidin label for 15 minutes, at room temperature. The sections were again rinsed in PBS and incubated with diaminobenzidine for 10 minutes, in the dark. After chromogen development, slides were washed in two changes of water and counterstained with a 1:10 dilution of haematoxylin. The sections were then dehydrated, cleared in xylene, and mounted. A negative control was performed in all cases by omitting the primary antibody, which, in all instances, resulted in negative immuno-reactivity. p53 expression and location were evaluated on histological sections using a Leitz Orthoplan...
Microscope equipped with a X 63 objective and with an eyepiece graticule. Only nuclear staining of epithelial cells was observed and the nuclei with a clear brown colour, regardless of staining intensity, were regarded as p53 positive. For MIB-1 immunostaining the slides were pretreated with 3-aminopropyltriethoxysilane (APES-Sigma, St. Louis, MO, USA), which avoided separation of the section from the slide during incubation in the microwave oven. For each case, a 5 μm section was cut and placed on a pretreated slide. The staining protocol of this slide consisted in the applications of a series of reagents in the following manner: overnight drying at 37 °C; dewaxing and rehydration; immersion in a plastic box containing 0.01 M citrate buffer at pH 6.0; incubation for 5 min in microwave oven: initially at 750 watts until boiling began, then at 350 watts for the remaining time; incubation for 5 min in microwave oven at 350 watts; cooling for 20 min, at room temperature; washing in running water and, then, in distilled water for 5 min; washing in tris buffer saline (TBS) for 5 min; removal of any excess TBS; addition of primary monoclonal mouse anti-ki-67 antibody (Immunotech, Praha, Czech Republic) diluted 1:25 in TBS; overnight incubation at 4 °C in a humidified room; washing in TBS for 5 min (3 times); addition of secondary prediluted biotinylated anti-mouse antibody (LSAB-Dako) and incubation for 10 min, at room temperature; washing in TBS for 5 min (3 times); addition of prediluted streptavidin-peroxidase complex (LSAB-Dako) and incubation for 10 min, at room temperature; washing in TBS for 5 min (3 times); immersion in 0.05% DAB and 0.01% H2O2 in TBS for 2-3 min at room temperature; washing in running water, then in distilled water for 5 min; counterstaining with ethyl-green for 30 min; washing in distilled water for 30 sec; washing in buthanol I for 5 sec; washing in buthanol II for 30 sec; dehydration and mounting in Permount (Biomeda, Foster City, CA, USA)

Results

Macroscopically, the lesions had a myxoid consistency, and were partially surrounded by a thin fibrous capsule. Microscopically, the tumours consisted of spindle and stellate cells dispersed in a faintly basophilic ground substance. The degree of cellularity varied in the central and in the peripheral areas. Strands of apparently inactive odontogenic epithelium, surrounded by collagenous fibres, were present in 3 cases. No mitoses were present. In some cells, nuclear atypia was present. The immunoreactivity for p53 protein was present in only 5-10% of the epithelial cells (Fig. 1), while the stromal component was completely negative. The positivity for MIB-1 was overall less than that observed for p53. A higher positivity for MIB-1 of the stromal cells (4%) (Fig. 2) than epithelial cells (1%) (Fig. 3) was observed. With Bcl-2, a strong positivity of the lymphocytes was present: the stroma was completely negative, while only about 2-5% of the epithelial cells were weakly positive (Table I).

Table I. Proliferative activity and oncoprotein expression in odontogenic myxomas.

<table>
<thead>
<tr>
<th>Epithelial cells</th>
<th>%</th>
<th>Stromal cells</th>
<th>%</th>
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<tbody>
<tr>
<td>p53</td>
<td>5-10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>MIB-1</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Bcl-2</td>
<td>2-5</td>
<td>0</td>
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Discussion

OM is an uncommon, benign neoplasm that is thought to be derived from ectomesenchyme and, histologically, is similar to the dental papilla of the developing tooth [14]. Due to the fact that most OM have been reported in the tooth-bearing areas of the jaws, there is strong support that this is an ectomesenchymal OT [14]. Ultra-structural, biochemical and histochemical studies suggest that the neoplastic spindle cells are fibroblast-like cells called ”myxoblasts” with a capability to synthesize and produce a large quantity of mucopolysaccharides [4]. The neoplastic cells show positivity
for vimentin and, in some areas, for muscle specific actin, but, on the contrary, are negative for keratin, desmin, neurospecific enolase, neurofilament and glial fibrillary acidic protein. The majority of the patients have no clinical signs or symptoms. Pain, dysesthesia, mucosal ulceration, invasion of the soft tissues, tooth mobility may be present. The epithelium that may be found within the lesion shows positivity to cytokeratin and this fact is consistent with an odontogenic origin.

OM is found most frequently in young patients, with a peak incidence in the 2nd and 3rd decades. The mandible is more often involved with the ramus and body being equally affected. It is a slow growing tumour that can cause marked facial asymmetry. The tumour may produce symptoms, depending on the site, from tooth mobility to sinus obstruction if the maxilla is involved. A very rare grossly as mucoid, slimy specimens. The tumour may produce invasion of the soft tissues, tooth mobility may be present. A malignant variant (myxosarcoma) has been reported. Macroscopically, OM appears as a soft, lobulated non-encapsulated mass that, on sectioning, has a yellow-white, glistening, firm, mucoid surface. The cut surface fails to bulge beyond the surrounding tissues upon sectioning. OM may appear also grossly as mucoid, slimy specimens with a semi-gelatinous consistency. In this instance, a capsule is not identified and areas of bone are often present within the cut surface. Microscopically, there are stellate or spindle-shaped cells of mesenchymal origin (vimentin positive), loosely arranged in a soft, mucoid matrix, composed of glycosaminoglycans, primarily hyaluronic acid and chondroitin sulfate. Hyperchromatic nuclei and mitotic figures are rare, and in about 20% of the lesions, scattered islands and strands of inactive-looking odontogenic epithelium may be found. These epithelial rests are not necessary for the diagnosis and are identical by transmission electron microscopy with the rests of Malassez found in the periodontal ligament surrounding teeth.

The odontogenic epithelium may represent nothing more than a foetal remnant, or it may play an important role in the pathogenesis of OM: it has been suggested that myxoma cells develop as a result of an inductive effect of this odontogenic epithelium. The strands of odontogenic epithelium, when present, are generally termed “inactive”. The present results show, on the contrary, that epithelial cells are positive for Bcl-2, p53 and MIB-1: it is possible to document then the presence of proteins involved in the cell replication or the positivity of a proliferation index. From these data, it can be hypothesized that the growth of the tumour could be related also to this epithelial component. Perhaps it is worthwhile pointing out the fact that the cases where the odontogenic epithelium was present had a larger diameter than the others. The stromal cells showed a higher positivity for MIB-1, and this fact could confirm the results of previous studies that showed that OM mainly grows due to the activity of the stromal cells that have been found to be actively proliferating. In fact, fine structural features indicate that the connective tissue cells of myxoma are active cells synthesizing and excreting an extracellular matrix. OM could also present dysregulated apoptotic mechanisms that assist the neoplastic growth. Both proliferation and matrix production could then determine an increase in tumour size.

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