Maspink expression in carcinoitma ex pleomorphic adenoma

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Aims: To investigate the presence and distribution of the protein maspin in carcinoitma ex pleomorphic adenoma (CXPA).

Methods: Maspink expression was studied by means of immunohistochemistry in 16 cases of CXPA, using the labelled polymer method.

Results: According to the extent of invasion, the tumours were subdivided into: intracapsular (five cases), minimally invasive (four cases), and invasive (seven cases). Twelve patients had carcinoitma with only epithelial differentiation, whereas four had a malignant myoepithelial component. Non-luminal cells in the duct-like structures of the remnant pleomorphic adenoma were strongly positive for maspin, whereas only a few luminal cells were immunopositive. A few positive cells were seen in the frequent hypocellular and hyalinated areas. Maspink was abundantly expressed, mainly in non-luminal cells, in transitional areas of CXPA with only epithelial differentiation. In frankly carcinomnous areas there was a gradual decrease in maspin expression. Almost all cells were maspin positive in CXPA with a myoepithelial component. When present, luminal cells were in general negative for maspin.

Conclusions: When only epithelial cells undergo malignant transformation, maspin expression is gradually lost. In cases with a myoepithelial component, maspin expression is high, and this might be related to the tumour suppressor activity attributed to this cell.

Pleomorphic adenoma (PA), the most frequent tumour in salivary glands, can undergo malignant transformation. Although rare, this condition has been reported to be responsible for 4.5–15% of salivary gland malignancies. Carcinoitma ex pleomorphic adenoma (CXPA) has no distinctive sex predilection, and is more frequent in the 3rd–6th decades of life. These tumours are highly aggressive, with high rates of metastasis, and carry a poor overall prognosis. It has been suggested that a carcinoitma may develop within a recurrent PA or when it remains untreated over an extended period of time. Despite the recognised clinical importance of CXPA, little is known about its biology. The detection of early transformation in PA is a diagnostic challenge in pathology. Using the interpretation of cellular atypia, increased mitotic index, and lack of encapsulation as signs of malignant transformation is controversial, because these signs can also be seen in ordinary PA.

"The detection of early transformation in pleomorphic adenoma is a diagnostic challenge in pathology."

Maspin (mammary serine protease inhibitor) is a member of the serpin superfamily of protease inhibitors, which has a peculiar role as a tumour suppressor. Its expression has been inversely correlated with malignant behaviour. It is expressed in normal cells, downregulated in neoplastic cells, and absent in metastatic cells. Evidence suggests that maspin suppresses tumour growth and metastasis by inhibiting tumour cell invasion and motility.

We recently detected maspin in a series of salivary gland tumours. Maspin expression correlated with tumour malignancy—more aggressive histological types showed lower immunostaining for maspin. Hence, maspin was abundant in PA.

MATERIALS AND METHODS

For our study, 16 cases of CXPA were retrieved from the files of the department of pathology of the University of Campinas and department of oral pathology, School of Dentistry, University of São Paulo, Brazil.

For morphological analysis, 5 µm sections were obtained from formalin fixed, paraffin wax embedded samples and routinely stained with haematoxylin and eosin.

In each selected case, a malignancy was seen in association with PA. However, for the purposes of our study the histological diagnosis of the malignant component was not important; it was the nature of the neoplastic cells that had undergone malignant transformation—epithelial cells only or epithelial and myoepithelial cells—confirmed morphologically and by a panel of antibodies that was of interest. The tumours were also classified according to the extent of invasion related to the capsule of the previous intracapsular (five cases), minimally invasive (four cases), and invasive (seven cases). Twelve patients had carcinoitma with only epithelial differentiation, whereas four had a malignant myoepithelial component. Non-luminal cells in the duct-like structures of the remnant pleomorphic adenoma were strongly positive for maspin, whereas only a few luminal cells were immunopositive. A few positive cells were seen in the frequent hypocellular and hyalinated areas. Maspink was abundantly expressed, mainly in non-luminal cells, in transitional areas of CXPA with only epithelial differentiation. In frankly carcinomnous areas there was a gradual decrease in maspin expression. Almost all cells were maspin positive in CXPA with a myoepithelial component. When present, luminal cells were in general negative for maspin.

Conclusions: When only epithelial cells undergo malignant transformation, maspin expression is gradually lost. In cases with a myoepithelial component, maspin expression is high, and this might be related to the tumour suppressor activity attributed to this cell.

Figure 1 Area of remnant pleomorphic adenoma showing non-luminal cells and occasional luminal cells positive for maspin (original magnification, x100).

Abbreviations: CXPA, carcinoitma ex pleomorphic adenoma; PA, pleomorphic adenoma
PA: intracapsular, minimally invasive (≤ 15 mm of invasion), and frankly invasive.

For immunohistochemistry, 3 μm sections were obtained from formalin fixed, paraffin wax embedded specimens. After dewaxing and rehydration, antigen retrieval was carried out in 10mM citrate solution (pH 6.0) at 95˚C for 30 minutes in a water bath. Sections were then incubated for 30 minutes in a 6% hydrogen peroxide/methanol (1/1) solution to quench endogenous peroxidase activity.

Immunohistochemical staining was performed on a Dako Autostainer (Dako, Carpinteria, California, USA). Briefly, anti-maspin antibody (BD Pharmingen, San Diego, California, USA) was incubated for 30 minutes at a dilution of 1/100. Goat antimouse immunoglobulin conjugated to peroxidase labelled dextran polymer was incubated by a one step technique using the EnVision system (Dako) for 30 minutes. This method avoids non-specific staining as a result of endogenous biotin, which makes it useful for studying salivary gland sections. Diaminobenzidine was used as the chromogen, followed by counterstaining with Mayer’s haematoxylin.

Fragments of lining epithelium and non-neoplastic salivary gland tissue served as internal positive controls. The primary antibody was omitted as a negative control.

RESULTS

According to the extent of invasion, the tumours were subdivided into the following categories: intracapsular (five cases), minimally invasive (four cases), and invasive (seven cases). Twelve cases had carcinoma with only epithelial differentiation (five non-invasive, three minimally invasive, and four frankly invasive), whereas four cases had a malignant myoepithelial component (one minimally invasive, and three of the invasive type).

Although we saw both cytoplasmic and nuclear staining in all specimens, positivity in the cytoplasm disappeared first as malignancy progressed. Areas with faint immunostaining showed nuclear staining only.

Remnant PA

In all cases studied, the area of PA showed the same pattern of staining for maspin. Non-luminal cells in the duct-like structures were strongly positive for maspin, whereas only a few luminal cells were immunostained (fig 1). Maspin was abundant in the rare highly cellular areas, whereas more loosely arranged parts showed decreased expression. Cells in the hyalinised zones were occasionally positive.

CXPA with only epithelial differentiation

In the transitional areas, duct-like structures were still present, with circumjacent benign myoepithelial cells, which were oval or spindle shaped. Strong staining for maspin was seen in these benign myoepithelial cells and rarely in luminal cells, which were large and polygonal. In two cases—both intracapsular—focal areas revealed strong staining of the luminal cells (fig 2). In the frankly carcinomatous areas there was a gradual decrease in maspin expression until it was completely absent in the zones where carcinomatous islets were small. When visible, the periphery of the invasive lesions was negative or weakly positive (fig 3).

CXPA with a myoepithelial component

In these cases, maspin was abundant. In transitional areas, where remnants of duct-like structures were seen, luminal cells were sometimes negative. In frankly malignant areas, where cells with myoepithelial differentiation predominated, all cells were strongly positive for maspin. One case showed areas of squamous metaplasia and these cells were also positive for maspin (fig 4).

Figure 2  Transitional area in a carcinoma ex pleomorphic adenoma with epithelial differentiation only. Strongly positive non-luminal and luminal cells are seen in this specimen (original magnification, ×100).

Figure 3  Frankly invasive area showing only weak staining in occasional cells, restricted to the nuclei (arrowheads) (original magnification, ×200).

Figure 4  Carcinoma ex pleomorphic adenoma with only myoepithelial component showed strong staining for maspin (original magnification, ×25).
DISCUSSION

CXPA is a malignant tumour that develops from the epithelial component of PA. Tumour progression results in frankly carcinomatous islands that gradually invade adjacent tissue. We analysed maspin expression in a series of 16 cases of CXPA presenting intracapsular, minimally invasive, and invasive patterns.

Maspin is a member of the serpin family of proteinase inhibitors. It has been associated with decreased cell motility, invasion capability, and metastasis formation, conferring a tumour suppressor role on this protein.

In the 12 cases where luminal cells had undergone malignant transformation, maspin was abundant in the initial phases (most often in benign myoepithelial cells and rarely in malignant epithelial cells in contact with those cells), and it was gradually lost concomitant with myoepithelial cell disappearance. Cases with myoepithelial differentiation showed high maspin expression in all phases.

"During tumour progression, the disappearance of benign myoepithelial cells results in reduced maspin expression by epithelial cells until it completely disappears."

Maspin has been consistently associated with the myoepithelial cell. Several studies have found it to be present predominantly or even exclusively in these cells, and its use as a myoepithelial cell marker has already been suggested. We reported previously that in normal salivary glands maspin is expressed by myoepithelial cells almost exclusively. Similarly, ordinary PA shows positivity in cells with myoepithelial differentiation, whereas only occasional epithelial (luminal) cells are immunopositive for this protein. In transitional phases of CXPA, when benign myoepithelial cells are still present, this profile is exacerbated, whereas in some areas epithelial cells also highly express maspin. These findings suggest that maspin production is coordinated by myoepithelial cells and that epithelial cells are influenced by them. During tumour progression, the disappearance of benign myoepithelial cells results in reduced maspin expression by epithelial cells until it completely disappears. It has been postulated by Sternlicht and Barsky that myoepithelial cells exert an influence over epithelial cells in a paracrine or contact mediated manner. Our results are in agreement with the theory that the myoepithelial cell is a natural tumour suppressor, and that even when transformed, this cell continues to show low grade behaviour and to have the characteristics of a normal cell (for example, high maspin expression).

Barsky et al have shown that transformed myoepithelial cells retain or even augment the synthesis of basement membrane molecules, an important feature for tumour suppressor activity. Our results suggest that in malignant transformation of luminal cells, remnant myoepithelial cells are stimulated to exhibit their complete phenotype, and exert tumour suppressor activity.

Although its interpretation is subjective, high expression of maspin in the early phases of CXPA might be diagnostically useful. Despite showing the same pattern of maspin expression seen in ordinary PA, areas of PA present in association with CXPA characteristically show conspicuous hypocellularity and hyalinisation, a feature reported in several series. These areas show low maspin expression, contrasting greatly with the strong positivity seen in transitional areas. Unfortunately, this use is restricted to CXPA with a malignant epithelial component only.

Maspin was first described as a cytoplasmic protein, but it was later reported in the cell nucleus. Although all known maspin activities depend on a cytoplasmic distribution, there is probably a biological reason for its presence in the nucleus. Recently, Mohsin et al studied nuclear maspin expression in invasive breast cancer, and found that 96% of samples showed nuclear staining, and this was related to hormone receptor expression. The authors found both cytoplasmic and nuclear staining in myoepithelial cells, but predominantly nuclear staining in luminal cells. In our study, we detected a difference in distribution between both compartments in the frankly invasive areas where staining decreased. Maspin disappeared first in the cytoplasm, whereas faint staining was seen in the nuclei for longer.

In conclusion, when only epithelial cells undergo malignant transformation in PA, maspin expression is downregulated during malignant progression, as would be expected—although expression is higher in the early stages compared with normal salivary glands and benign PA. In contrast, when myoepithelial cells are also transformed, high maspin expression is seen in all phases, and this might be related to the tumour suppressor activity attributed to this cell type.

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