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Maspin expression in normal and neoplastic salivary gland

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BACKGROUND: Maspin inhibits cell motility, invasion and metastasis. Loss or reduction in maspin expression has been associated with tumoral progression.
METHODS: The presence of maspin was studied immunohistochemically in salivary gland tumours presenting cells with myoepithelial differentiation in their composition, and in normal salivary gland.
RESULTS: Pleomorphic adenoma (PA) presented high expression of maspin, except in the spindle cells and occasional luminal cells. Epithelial–myoepithelial carcinoma and tubular adenoid cystic carcinoma (ACC) showed intense expression in all cells. Cribriform ACC evidenced only few positive cells of the luminal type, while solid subtype showed rare positive cells. Normal salivary gland tissue has shown low levels of maspin positivity.
CONCLUSIONS: Maspin has small participation in normal salivary gland, is increased in PA, and decreases as the histological malignancy raises. Hence, in salivary gland, its expression is not exclusive of myoepithelial cells; thus, it should not be used as a marker for this cell. Nevertheless, we believe it is an important marker of biological behaviour in these tumours.
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Keywords: maspin; myoepithelial cell; salivary gland tumours

Introduction

Maspin (Mammary serine protease inhibitor) is a member of the serpin superfamily of protease inhibitors, and it has a peculiar role as tumour supressor (1). It was first identified by Zou et al. (2), in normal mammary epithelial cells by subtractive hybridization, and its gene is located to 18q21.3 (3). Since its first description, it has been localized in a broad number of human tissues – including placenta, small intestine, uterus, kidney (4) – and lesions, like prostatic cancer, head and neck carcinoma, pancreatic cancer and ovarian carcinoma (5–7).

It has been demonstrated several times that maspin is expressed in normal cells, down-regulated in neoplastic cells and absent in metastatic cells (2, 8, 9). Many evidences demonstrate that maspin suppresses tumour growth and metastasis by inhibiting tumour cell invasion and motility (2, 5, 10, 11).

Other activities have also been described or suggested for maspin: it has an inhibitory effect over angiogenesis through endothelial cell motility inhibition, and causes reduction in the density of microvessels associated with the tumour (12); although it does not directly induces apoptosis, maspin sensitizes breast carcinoma cells to staurosporine (STS)-induced apoptosis *in vitro* (13); it can bind to collagen types I and III and restore cell adhesion (14, 15); it is phosphorylated on tyrosine residues in both normal and neoplastic mammary cell lines, suggesting a role as signal transduction molecule that might be involved in some signalling pathway that does not involve adhesion molecules (16). It has also been found that p53 directly induces maspin transactivation by binding to the gene promoter, and this might be an important pathway in p53 protein function (17).

Because maspin is present in the epithelium of other glands, it is possible that it participates in salivary gland biology as well. The aim of the present work was to detect by immunohistochemistry the protein maspin in normal salivary gland plus benign and malignant tumours of this origin.

Materials and methods

Based on the latest WHO classification of salivary gland tumours (18), eight cases of pleomorphic adenoma (PA), two cases of epithelial–myoepithelial carcinoma (EMC), and seven cases of adenoid cystic carcinoma (ACC) (two of the tubular type, three cribriform, and two solid), plus three cases of normal salivary gland, were retrieved from the archives of the Oral Pathology Department of the School of Dentistry of the University of São Paulo.

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

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Three-micrometer paraffin sections were submitted to streptavidin–biotin method of immunohistochemistry. Dewaxed sections were submitted to a waterbath antigen-recovery step at 95°C immersed in 10 mM citrate solution (pH 6.0), during 30 min. After this, the sections were incubated with a methanol –6% hydrogen peroxide, on a 1:1 solution, to quench endogenous peroxidase activity. The immunohistochemical staining was performed on the Dako Autostainer (Dako, Carpinteria, CA, USA). Briefly, primary antibody antimaspin (BD PharMingen, San Diego, CA, USA) was incubated for 30 min on a 1:250 dilution. Goat anti-mouse immunoglobulin conjugated to peroxidase labelled-polymer was incubated on a one-step technique using EnVision system (Dako), for 30 min. Diaminobenzidine was used as the chromogen, followed by counterstaining with Mayer's haematoxylin. Sections of normal mammary gland were used as positive control for maspin. Negative control was provided by incubation with TRIS buffer instead of primary antibody.

Results

Three patterns of immunostaining were seen: cytoplasmic, nuclear or both cytoplasmic and nuclear positivity. These different patterns were seen in the neoplastic sections and in normal salivary gland as well, and were usually present in all cases studied. For this reason, we will not take this into consideration in the Results description.

Pleomorphic adenoma
In duct-like structures non-luminal cells were strongly positive, as were a few luminal cells. Maspin was very abundant in highly cellular areas, although not all cells were positive (Fig. 1a). Cells of different morphology were similarly stained. Spindle-shaped cells of the myxomatous areas, as well as chondroid metaplasia, were both negative to maspin (Fig. 1b). In one case it was possible to note the decrease of maspin expression at the tumour periphery.

Epithelial–myoepithelial carcinoma
Maspin presented strong nuclear and cytoplasmic reactivity in both cell populations. In a few areas, where larger duct-like structures could be observed, luminal cells showed a faint immunostaing or were negative to the antibody (Fig. 1c).

Adenoid cystic carcinoma
Tubular type: both, luminal and myoepithelial cells were strongly positive for the antibody studied. Sometimes, well-defined luminal cells were negative for maspin (Fig. 1d). *Cribriform type*: only a few cells, lining the duct-like structures, were positive for maspin. Cells with myoepithelial differentiation were negative (Fig. 1e). *Solid type*: cells in the core region of the nests were eventually positive, while luminal cells were always negative. In all the three variants of ACC areas of anaplasia were totally negative for maspin (Fig. 1f).

Normal salivary gland
Myoepithelial cells lining acini periphery showed a strong nuclear and cytoplasmic immunoreactivity for maspin (Fig. 2a). In the ducts, the staining was seen mainly in the basal cells, but a few luminal cells were also positive (Fig. 2b). No staining was seen in the salivary secretory cells or any cells in the supporting stroma.

Discussion

It is well-known that proliferation capacity is fundamental in tumoral perpetuation. However, cell–extracellular matrix (ECM) interaction is essential for neoplastic invasion and metastasis. In this context, protease and protease inhibitors play important roles by degrading ECM and preventing this process, respectively (19). Maspin, a recently identified protein, belongs to the serpin family of protease inhibitors – more specifically, it is a ov-serpin, due to structural homology to chicken ovalbumin – and inhibits cell motility, invasion and angiogenesis (2, 5, 10–12).

In the present study, immunohistochemistry was used to detect maspin in the salivary gland neoplasms PA, EMC and ACC, and in normal salivary gland. All specimens studied were, in different degrees, positive for maspin. Three patterns of staining were seen: nuclear, cytoplasmic or both nuclear and cytoplasmic compartment staining. No particular distribution of these patterns could be seen and they were present in all specimens studied, and any of the cells constituent of the lesions could be marked. Early studies described maspin exclusively in cytoplasm and close to cytoplasmic membrane (4). It was only recently that nuclear expression of maspin was noted (20, 21). Reis-Filho et al. (22) pointed for nuclear staining in previous published data from other authors that have not been discussed. Authors were not confident to point out this finding as no function for maspin in the nucleus has been identified yet, and all its known activities would depend on a cytoplasmic situation (22).

In general, the results for the neoplasms studied have revealed concordance between biological behaviour and the expression expected for maspin, i.e. more aggressive histological types showed less positive cells. Maspin was related to prognosis for the first time by Xia et al. (23), studying a group of patients with squamous cell carcinoma of the head and neck. They observed that the high expression of maspin was related to better rates of survival and absence of nodal metastasis. Studies in breast cancer with similar results are also available (24, 25).

The PA revealed a very intense and abundant staining, which agrees with the benign nature of this lesion. Hence, areas mimicking stroma, where ECM is very abundant, showed lack of maspin. This may be connected to ECM digestion, pointing to a possible protease-inhibitor role for maspin. Actually, there is controversy regarding the protease inhibitory activity for this protein. Early studies have found that it probably is a non-inhibitory serpin; however, afterwards, some

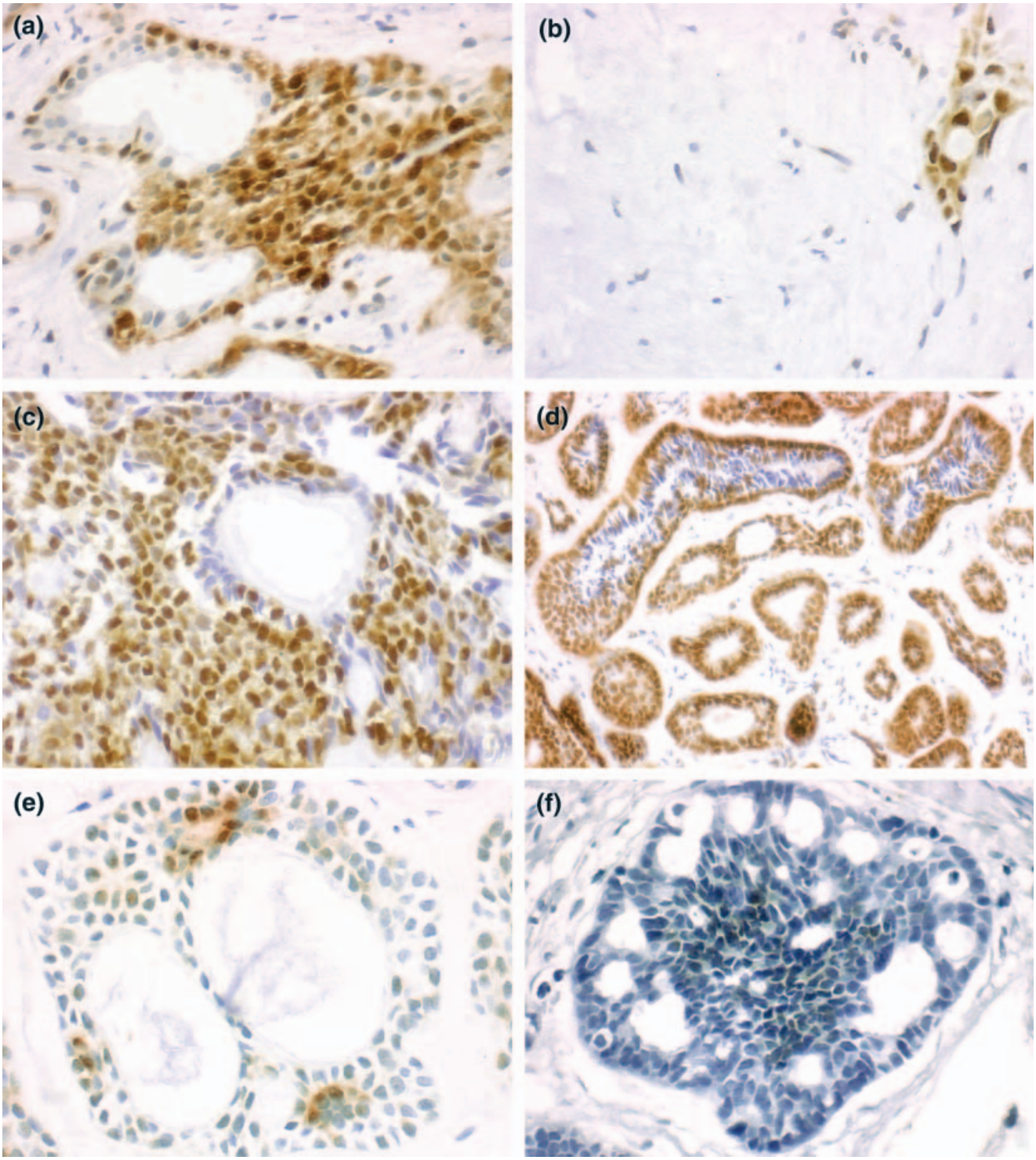

Figure 1 Immunohistochemical staining of maspin in salivary gland tumours. Note that both, nucleus and cytoplasm, are stained. (a) Pleomorphic adenoma: luminal cells of the duct-like structures are negative for maspin, surrounded by block of strongly positive cells. (b) Pleomorphic adenoma: small group of cells positive for maspin close to a myxoid area with spindle-shaped cells negative for the marker. (c) Epithelial–myoepithelial carcinoma: duct-like structure exhibiting luminal cells negative for maspin, surrounded by cells with myoepithelial differentiation positive for the marker. (d) Adenoid cystic carcinoma, tubular subtype: all cells are positive for maspin with the exception of luminal cells in more differentiated duct-like structures. (e) Adenoid cystic carcinoma, cribriform subtype: occasional groups of positive cells. (f) Adenoid cystic carcinoma, solid subtype: a few cells are weakly positive for maspin in the central core of a tumoral nest.

authors have reported that it inhibits plasminogen activators (26–28). Recently, Bass et al. (29) compared maspin and PAI-1 effects in a wide variety of situations in which plasminogen activation is magnified, and concluded that it does not have efficient inhibitory effects over plasminogen.

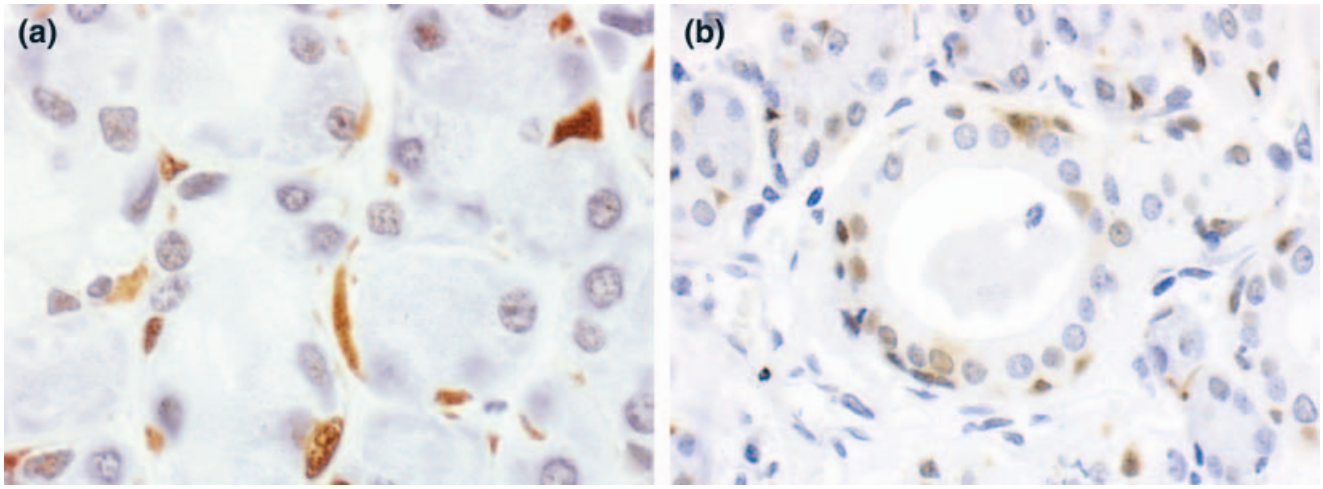

Figure 2 Maspin immunostaining in normal salivary gland. (a) Myoepithelial cells in the acini periphery positive for maspin. (b) Cells in the duct positive for maspin. Note that besides myoepithelial cells, scattered luminal cells are positive for the marker.

It is well-known that PA has a tendency to recur (30, 31). Although it has been closely associated with incomplete surgical excision, other factors might be involved. In this context, maspin down-regulation at the tumoral periphery, as seen in one of our cases, could contribute to this behaviour.

The EMC is an uncommon malignant low-grade neoplasm. Abundant maspin staining seen in almost the totality of cells agrees with this behaviour. Cases of poor prognosis have been described but they present solid histological pattern, and are composed mainly of clear cells (32). None of our cases expressed these features.

Among the three histological patterns of ACC: tubular, cribriform and solid, the tubular subtype showed abundant maspin staining, similar to EMC, while only luminal cells were positive in cribriform ACC, and myoepithelial cells were negative. Solid subtype presented myoepithelial cells of the core areas of nests positive for maspin. Many evidences show that histological ACC subtypes are directly related to prognosis, the tubular pattern presenting the best prognosis, and solid type the worst (33, 34). Therefore, our findings are in agreement with this biological behaviour.

Maspin has been described in different cells of epithelial origin, and is especially abundant in mammary gland myoepithelial cell (20). Myoepithelial cells rest between glandular epithelium and the basement membrane where they contribute to the synthesis of this structure, and form a natural border keeping apart potentially invasive cells from the basement membrane and underlying stroma (35). Neoplasms with origin in the myoepithelial cells usually exhibit a benign or at most, low-grade biological behaviour. When neoplastic, myoepithelial cells could have their ability to produce ECM increased, being responsible for the large amounts of these molecules seen in the lesions (35, 36).

It was also suggested the use of maspin as a reliable myoepithelial cell marker, able to help in the differential diagnosis of breast tumours salivary gland-like (21). Data here presented shows that maspin is not exclusively a myoepithelial marker and even nuclear reactivity is not exclusive of this cell type.

Although all specimens studied showed cells positive for maspin, it was more abundant in neoplasms than in normal salivary tissue, which presented mainly myoepithelial and a few luminal cells that were positive. These findings significantly differ from mammary gland where maspin is very abundant in both populations (2). This might be explained by the more intense renew that mammary gland suffers through life. It was already demonstrated using *in vivo* studies that maspin plays an important role in mammary gland development, regulating cell adhesion and motility. Besides that, it has an increased expression during pregnancy and lactation (37). Maspin distribution in normal tissues suggests that its down-regulation might be a normal event in cell differentiation important to proliferation and migration under an appropriate stimulus (4). Seftor et al. (11) showed that maspin alters integrin profile – including down-regulation of β1-integrin – in mammary gland cells and this could alter their abilities to adhere and interact to ECM molecules, thus preventing invasion by increasing the anchoring of cells to ECM.

Mechanisms underlying maspin expression remain to be elucidated and different explanations for loss of maspin expression have been suggested. No mutations or deletion were found in the maspin gene, but absence of transactivation through the Ets- and AP-1 DNA-biding sites in the promoter region (38), aberrant methylation and chromatin condensation of the promoter (39, 40), hypermethylation and histone de-acetylation (40) are strongly suggested epigenetic mechanisms at the genetic level.

As, in the present study, malignant tumours as EMC and ACC tubular strongly expressed maspin, in salivary gland its use is not indicated in the differential diagnosis of benign and malignant lesions as it has been strongly

suggested for mammary gland and prostate. Nevertheless, we believe it is an important marker of biological behaviour in these tumours.

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