

# Maspin immunoreactivity in salivary pleomorphic adenomas

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## Abstract

**Aim:** Maspin is a serine protease inhibitor with possible suppressive effects over tumor progression. The aim of this study was to describe maspin expression pattern in pleomorphic adenomas of the salivary glands, in order to investigate the potential role of this molecule in the benign nature of this neoplasm.

**Materials and Methods:** Immunohistochemistry staining was applied in a sample of 120 pleomorphic adenomas and also in 10 high grade adenoid cystic carcinomas, which served as a control group of malignant biphasic tumors.

**Results:** All pleomorphic adenomas exhibited a strong selective positivity of their myoepithelial component, while luminal cells were invariably not immunostained. On the contrary, all adenoid cystic carcinomas presented a negative profile for maspin, predominantly in the highly malignant areas with a solid pattern of development.

**Conclusion:** It is suggested that maspin expressed selectively by myoepithelial cells of pleomorphic adenomas, may function as a tumor-suppressor factor in salivary neoplasia.

## Introduction

Maspin (mammary serine protease inhibitor) is a member of the serpin (serine protease inhibitor) super-family with tumor-suppressor properties under investigation. Its function is arguably characterized by the ability to hinder tumor

growth and metastasis via an increase in cell adhesion, inhibiting migration and angiogenesis. It is localized in the nucleus, the cytoplasm or it is secreted, while the amount of its expression and its subcellular localization exerts significant prognostic influence in a variety of neoplasms [1].

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The aim of this study is to describe the distribution of maspin expression in pleomorphic adenoma of the salivary glands and to speculate the role of this molecule in the nature of this tumor.

## Materials and Methods

The sample was derived from the records of the First Department of Pathology, Medical School, National and Kapodistrian University of Athens, Greece with the approval of the Ethics Committee of this institution. It consisted of 120 pleomorphic adenomas of the parotid gland; 10 high grade adenoid cystic carcinomas of minor salivary gland origin were also evaluated as a group of malignant tumors with a biphasic nature, similar to that of pleomorphic adenomas. Four-micrometer thick sections were obtained from formalin fixed and paraffin embedded specimens. Antigen retrieval was performed (DAKO, EDTA, pH=8) and anti-maspin antibody were applied (EAW24, dilution 1:10). Diaminobenzidine was used as a chromogen and Gill's hematoxylin as a counterstain. Sections from prostate tissue served as positive controls. Maspin staining intensity was generally quite strong probably due to uniform conditions of fixation and non-oldness of all specimens. In the vast majority of benign tumors, the myoepithelial component was positive at percentages >80% (Table 1). Anti-muscle actin antibody (HHF-35) was utilized as an immunomarker of the myoepithelium at specific sites, where identification of these cells was not conspicuous morphologically.

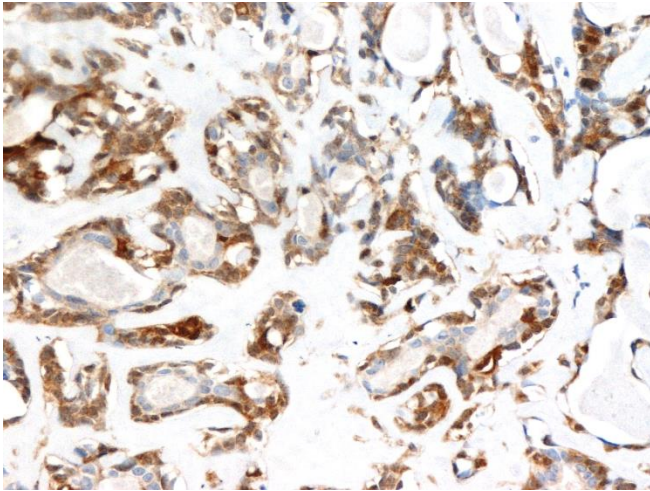
*Table 1. Overview of maspin cytoplasmic immunostaining findings.*

Maspin immunopositivity	Type of sample	
	Pleomorphic adenomas (n=120)	High-grade Adenoid cystic carcinomas (n=10)

≥ 80% of myoepithelial cells	103 (85.8%)	-
60-80% of myoepithelial cells	17 (14.2%)	-
Up to 1% of myoepithelial cells	-	8 (80%)
Totally negative	-	2 (20%)

## Results

All cases of pleomorphic adenoma exhibited selective maspin positivity at their myoepithelial component, the latter often morphologically detectable as a basal layer in glandular structures; immunostaining presented a mainly cytoplasmic staining pattern; combined cytoplasmic and nuclear staining was clearly observed in a minority of pleomorphic adenomas (17 out of 120 cases). On the contrary, identifiable luminal cells were not immunostained, as shown in figure 1. Adenoid cystic carcinomas (ADCCs) with predominantly solid architecture served as a malignant group of salivary tumors also of a biphasic nature (i.e. epithelial-myoepithelial, like pleomorphic adenomas) and presented a diffuse, almost totally negative immunohistochemical profile (except for few immunopositive cells in two cases), always with prominent complete loss of maspin expression at their highly malignant areas with a solid pattern. Thus, as shown in Table 1, the difference in maspin expression between pleomorphic adenomas and ADCCs is evident, as the former presented cytoplasmic immunopositivity in at least 60% of myoepithelial cells, while the latter were totally negative or positive in ≤1% of myoepithelial cells.



*Figure 1. Evident selective maspin immuno-expression in the myoepithelial component of a pleomorphic adenoma (Immunoperoxidase stain,  $\times 250$ )*

## Discussion

In the present study, immunohistochemistry has revealed a specific myoepithelial expression of maspin in benign biphasic salivary tumors (i.e., pleomorphic adenomas) and not in one of their malignant counterparts. It is of interest that all ADCCs examined in the present study were practically maspin- immunonegative, despite the presence of separate myoepithelial cells among the neoplastic cell solid population, as verified by their HHF-35 immunoreactivity. Therefore, it is suggested that maspin- positive myoepithelial cells play a pivotal role to the benign nature of pleomorphic adenomas.

It has been shown in the literature that carcinomas with myoepithelial differentiation, regardless of the amount of myoepithelial cells, are associated with a significantly lower vascular density [2]. As shown in mammary ductal carcinoma in situ (DCIS), myoepithelial maspin has the ability to inhibit invasion by separating ductal cells from the stromal angiogenesis [3]. However, despite their natural tumor-suppressor function,

myoepithelial cells in pleomorphic adenoma have been reported to exhibit an increased expression of Vascular Endothelial Growth Factor (VEGF) and of its receptors, a fact that is probably attributed to the diversity of characteristics of this particular tumor's stroma [4]. In agreement with the above finding, a strong positivity of pleomorphic adenomas for the panendothelial CD34 marker has been reported [5], reflecting high vascularity, especially in their cellular-rich areas. Nevertheless, these benign tumors have been reported to show minimal CD105 staining, demonstrating low relevant density of newly formed microvessels and, consequently, no malignant potential [6]. On the other hand, carcinoma ex pleomorphic adenoma, which is characterized by intense maspin expression in its early stages but a complete loss of maspin in invasive carcinomatous areas [6], is known to be triggered by an angiogenic switch with a considerable increase of neovascularization evidenced by CD105 staining [7]. Consequently, combining our finding with those of previous studies, it can be proposed that myoepithelial maspin expression in pleomorphic adenoma potentially constitutes a tumor-suppressor factor, which may exert remarkable negative control over the tumor's density of newly formed microvessels; further comparative investigation of angiogenesis markers is of course needed to support this suggestion.

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