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Loss of Maspin expression is a negative prognostic factor in common salivary gland tumors

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Summary Maspin, a 42 kDa protein, belongs to the serine protease inhibitor (serpin) family and is suggested to have inhibitory effects on tumor-induced angiogenesis, tumor cell motility, invasion and metastasis and influences prognosis of tumor patients. The aim of the study was to analyze Maspin expression in salivary gland cancer as well as its prognostic impact on survival in comparison to clinical parameters. Immunohistochemical staining was carried out in 73 cases of salivary gland malignancies. High proportions of Maspin expression were observed in adenoid cystic carcinomas, mucoepidermoid carcinomas and carcinomas ex pleomorphic adenoma, low proportions were seen in salivary duct carcinomas. Acinic cell carcinomas did not show any Maspin expression. Analysis of the prognostic impact of Maspin expression was restricted to salivary gland carcinoma types of intermediate malignancy grade (adenoid cystic carcinoma, mucoepidermoid carcinoma and carcinoma ex pleomorphic adenoma). For these tumors, univariate analyses revealed that T-stage ($p = 0.025$), age $\geqslant 70$ ($p = 0.0065$), loss of Maspin ($p = 0.0016$) and presence of residual tumor ($p < 0.001$) correlated with poor prognosis. In multivariate analysis age $\geqslant 70$ ($p = 0.005$) and loss of Maspin ($p = 0.036$) were significant prognostic factors. Moreover, negative Maspin staining was associated with lymph node metastasis and residual tumor. According to these findings, Maspin might be useful as a new prognostic marker in adenoid cystic carcinoma and in salivary gland carcinomas with intermediate grade of malignancy where grading systems are still under debate.

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Introduction

Carcinomas of the salivary glands are rare, comprising less than 0.5% of all malignancies and about 5% of head and neck cancers.[1] They are characterized by morphological diversity between different tumor types or even within an individual tumor mass.[2] The assessment of prognostic factors is complex as prognosis depends on clinical factors (gender, age, site, size, TNM-stage, presence of positive resection margins) and histological factors (tumor type, grade).[2–5] Immunohistochemical staining may provide further prognostic criteria as high proliferative activity assessed by the expression of the proliferation marker Ki-67 and mutation of tumor suppressor protein p53 assessed by high expression of wild type p53 have been reported to be of prognostic significance in salivary gland cancer.[6–9]

Maspin (*Ma*mmary *s*erine *p*rotease *in*hibitor) is a 42 kDa protein that belongs to the serpin (serine protease inhibitor) family of protease inhibitors. It is supposed to suppress tumor-induced angiogenesis, tumor cell motility, invasion and metastasis.[10,11] It can be upregulated by pro-apoptotic p53,[12] and seems to sensitize tumor cells to apoptosis through upregulation of Bax.[13] It was first discovered as a gene downregulated in invasive and metastatic breast cancer.[14] Loss of Maspin has been associated with poor prognosis in various malignant tumors like breast cancer, oral squamous cell carcinoma, non-small cell lung cancer and ovarian carcinoma.[15–18] Concerning salivary gland cancer downregulation of Maspin was observed in carcinoma ex pleomorphic adenoma and in the solid subtype of adenoid cystic carcinoma.[19–21] However, to the best of our knowledge there have been no reports to date investigating the prognostic significance with long term follow-up.

Therefore the aim of the following study was to examine the expression of Maspin in the most common entities of salivary gland carcinoma and its relation to clinical factors as well as its prognostic impact on disease-specific survival.

Patients and methods

Patients and tumor samples

Paraffin embedded specimens of 76 patients (35 males, 43 females, mean age 57.3 years, range 24—88 years) with salivary gland cancer, treated at the University of Regensburg, Germany, between 1990 and 2006 were (re-)classified according to the contemporary WHO-classification (Table 1).[2] Clinical data (age, gender, tumor site, tumor size, node involvement, distant metastases and presence of residual tumor) were retrospectively reviewed from the charts. Follow up information was obtained by contact with primary care physicians and affected patients. Survival analyses for clinical and histological factors were investigated separately in adenoid cystic carcinoma, as this was the most frequent entity, and after grouping together with mucoepidermoid carcinoma and carcinoma ex pleomorphic adenoma, as these entities are known to have similar (intermediate) prognosis.

Table 1 Clinical parameters of 76 salivary gland carcinomas according to histological types[a]

	ADCC	MEC	CXPA	SDC	ACCC	Total
Gender						
Male	8	6	9	9	3	35
Female	17	9	4	3	8	41
Age						
<70	20	12	10	5	8	55
⩾70	5	3	3	7	3	21
T-stage						
T1-2	14	12	7	7	7	47
T3-4	11	3	6	5	4	29
N-stage						
N0	19	11	10	3	10	53
N+	6	4	3	9	1	23
M-stage						
M0	23	15	13	12	11	74
M1	2					2
R-stage						
R0	17	15	9	9	11	61
R+	8		4	3		15
Total	25	15	13	12	11	76

[a] ADCC = Adenoid cystic carcinoma, MEC = Mucoepidermoid carcinoma, CXPA = Carcinoma ex pleomorphic adenoma, SDC = Salivary duct carcinoma, ACCC = Acinic cell carcinoma.

Immunohistochemistry

After construction of a tissue microarray block (TMA)[22] immunohistochemical staining with antibodies against Maspin (clone G167-70, Becton Dickinson GmbH, Heidelberg, Germany, dilution 1:1000, microwave antigen retrieval, pH 7.3) and p53 (clone sc-263, Santa Cruz Biotechnology, Inc., Heidelberg, Germany, dilution 1:1000, microwave antigen retrieval, pH 7.3) was performed on a Ventana NexES Autostainer (Ventana Medical Systems S.A., Illkirch, France).

Evaluation of all immunostained slides was carried out twice, and immunoreactivity was assessed semiquantitatively.

For Maspin nuclear and cytoplasmic staining was considered separately and combined and was valued positive if more than 10% of tumor cells were stained. Staining intensity was graded in a semiquantitative way (0 negative staining, 1+ week staining, 2+ moderate staining, 3+ strong staining) (Fig. 1). Tumors with negative or weak staining were considered negative, tumors with 2+ or 3+ staining positive. In combined evaluation staining was positive if either nuclear or cytoplasmic intensity or both were at least moderate (2+).

For interpretation of p53 staining the percentage of stained cells was determined and assessed positive if more than 10% of tumor cells were stained.

Statistical analysis

Relationships between Maspin expression and clinicopathological factors were examined using Fisher's exact probabil-