Maspin Protein Expression: A Special Feature of Papillary Thyroid Carcinoma

TAHANY M. SHAMS, M.D.*; REHAB M. SAMAKA, M.D.**, and MOHAMED E. SHAMS, M.D. ***
The Departments of Pathology, Faculty of Medicine, Suez Canal University and General Surgery, Faculty of Medicine, Suez Canal University.

ABSTRACT

Background and Aim: Mammary serine protease inhibitor (Maspin) is down regulated in breast and prostate cancers and is considered as a tumor suppressor gene. On the contrary, it is over expressed in pancreatic and ovarian carcinomas and is reported to be an oncogene rather than a tumor suppressor gene. The studies of maspin expression in thyroid neoplasia, the focus of this study, are limited. We, therefore, carried out this work in order to detect the frequency and pattern of maspin expression in thyroid neoplasia.

Material & Methods: An immunohistochemical approach was performed on 63 thyroid specimens showing different benign and malignant thyroid lesions. Also, five specimens of the surrounding normal thyroid tissue were included as control. A monoclonal anti-human antibody has been used to detect maspin.

Results: Maspin was only detected in papillary thyroid carcinoma (PTC) and it was negative in all other studied thyroid tissues. In PTC 18/25 (72%) cases were maspin positive. Most of them 11/18 (61.1%) showed both cytoplasmic and nuclear maspin expression, two cases 2/18 (11.1%) were nuclear and the rest of the specimens, 5/18, (27.8%) were cytoplasmic only. There was no statistically significant relation between maspin positive cases and the studied clinicopathological parameters including patient's age, sex and tumor stage. On the other hand, it was statistically significant as regards tumor multicentricity, vascular and lymphatic invasion, as well as lymph node metastasis.

Conclusions: Maspin expression is a special feature of papillary thyroid carcinoma (PTC) which can be used as a therapeutic target. It may be suggested that the genesis of PTC may be different from other types of thyroid carcinoma. Further studies regarding its prognostic role in patients with PTC are recommended.

Key Words: Thyroid carcinoma – Maspin – Immunostaining – Clinicopathological features.

INTRODUCTION

Proteases that remodel the extracellular matrix (ECM) play an important role in the progression of neoplasia [1]. Excessive protease activity can lead to major changes within the microenvironment of tumor tissue to promote cell migration, and it thereby contributes to metastasis. Moreover, subtle changes in the levels and activities of proteases can expose cryptic sites in ECM molecules that alter integrin usage, and release matrix-bound growth factors, which both potentiate proliferation and survival of tumor cells, and induce angiogenesis [2]. Thus, several of the hallmarks of tumor progression occur as a result of alteration in protease activity within the extracellular environment of a growing tumor [3]. The two main classes of ECM-degrading proteases are matrix metalloproteinases (MMPs) and serine proteases. Serpins are a class of serine protease inhibitors; one such inhibitor of the serpin family, namely maspin, encodes a 375-amino-acid protein (Mr of 42,000) with sequence homology to other inhibitory serpins including plasminogen activator 1 and 2 (PAI-1 and PAI-2) and non-inhibitory serpins such as ovalbumin [4,5]. The maspin gene is part of a serpin locus cluster at chromosome 18q21.3-q23 [6].

Maspin has been shown to have a tumor-suppressive activity attributed to inhibition of breast cancer cell motility, invasion, and metastasis [7,8]. Loss of maspin protein expression has been observed frequently and is associated with poor prognosis in breast, prostatic, and oral cancer [9,10]. Although, at present, the molecular and biological mechanisms of the function(s) of maspin remains largely unknown,
studies have suggested that maspin interacts with the p53 tumor suppressor pathway [11], and may function as an inhibitor of angiogenesis [12] in vitro and in vivo. These observations suggest that maspin acts as a tumor suppressor gene.

Maspin is expressed in normal human mammary and prostate epithelial cells but is down-regulated during their cancer progression [13,15]. The loss of maspin gene expression with increasing malignancy is regulated at the transcriptional level [6]. Recent studies have reported on the role of cytosine methylation and chromatin condensation in the down-regulation of maspin expression during neoplastic progression [16]. However, other findings by Maass et al. [17] and Sood et al. [18] showed that maspin was overexpressed in pancreatic and ovarian cancers. Specifically, 5 of 9 pancreatic cancer cell lines had maspin expression along with 23 of 24 tumor specimens. Three of four ovarian cancer cell lines expressed maspin and 57 of 80 invasive ovarian cancers were maspin positive, whereas maspin expression was not detected in normal pancreatic or ovarian tissues [17,18].

Thyroid malignancy is the most common endocrine malignancy as it makes up to 95% of endocrine gland cancer, its estimated cancer death rate is 1500 cases annually, 630 were males and 870 were females [19]. Differentiated tumors (papillary or follicular) are highly treatable and usually curable. Poorly-differentiated tumors (medullary or anaplastic) are much less common, aggressive, metastasize early, and have a much poorer prognosis [20]. The incidence of this malignancy has been increasing. One important risk factor is radiation exposure to the head and neck as radiation therapy is used to treat acne and to reduce swelling and infection in organs such as the thymus, tonsil and lymph nodes. Radiation exposure as a consequence of nuclear fall-out has also been associated with a high risk of thyroid cancer, especially in children [21]. Other risk factors for the development of thyroid cancer include a history of goiter, family history of thyroid disease, female gender and Asian race [22]. The regulation mechanisms of maspin function are not fully understood and their studies on thyroid cancer are very limited so the aim of our study is to examine the maspin expression in thyroid tissue regarding normal tissue, thyroid adenomas and carcinomas. We also aim to investigate whether the maspin positive thyroid cancer is correlated with clinicopathological parameters as patient's age, sex, tumor stage, tumor multicentricity, vascular and lymphatic invasion, lymph node and distant metastasis.

MATERIAL AND METHODS

Tissue samples:

The cases were retrieved from the surgical pathology files of the Suez Canal and Minoufiya University hospitals during the period from January 2001 to December 2004. The study included sixty-three thyroidectomy specimens divided into 15 multinodular goiters (MNG), 10 follicular thyroid adenomas (FTA), 25 papillary thyroid carcinomas (PTC), 8 follicular thyroid carcinomas (FTC), 3 medullary thyroid carcinomas (MTC) and 2 anaplastic thyroid carcinomas (ATC). Additional five normal surrounding thyroid tissue specimens were included. Tumor stage was based on the TNM tumor classification system including tumor stage (T), regional lymph node metastasis (N) and distant metastasis (M).

Formalin-fixed, paraffin-embedded samples for maspin immunohistochemical staining:

The Hematoxylin & Eosin stained sections of the selected tumors were reviewed, then formalin-fixed, paraffin embedded blocks were sectioned at 5 µm for immunohistochemical staining. Maspin immunohistochemical staining was performed using the universal ABC, peroxidase kit (ultra-vision detection system, Anti-polyvalent, ready to use, LAB VISION, USA). The monoclonal antibody used in this study was ready to use mouse monoclonal anti-human maspin (LAB VISION, USA, cat. 1767). All of the slides were deparaffinized using xylene and then rehydrated in decreasing concentrations of ethanol. Antigen retrieval using microwave heating (three times of 10min; 10mM citrate buffer, pH 6.0) after inhibition of endogenous peroxidase activity (0.3 hydrogen peroxidase for 15min). The slides were incubated overnight with the primary antibody at room temperature, then washed using phosphate buffered solution (PBS) and then incubated with secondary antibody for 15min followed by PBS wash. Finally the detection of bound antibody was accomplished using the ABC reagent for 20min then PBS wash. A 0.1% solution of diaminobenzidine
(DAB) was used for 5min as a chromogen. Slides were counterstained with Mayer’s hematoxylin for 5-10min. Negative controls were obtained by omitting the primary antibody. Prostate adenocarcinoma was used as a positive control.

**Interpretation of immunohistochemical staining:**

The immunoreactivity to maspin was evaluated semiquantitatively using a light microscope. Ten representative fields of each slide were examined, and positivity was indicated by the presence of diffuse distinct brown nuclear or cytoplasmic staining. The amount of positive cells was determined as a percentage of the total number of cells observed in each slide. Thus, the semiquantitative scoring (SQS) was graded as (−) when less than 5% of the cells were stained, (+) when positive cells represented between 5% and <30% of cells observed, (+++) when positive cells represented between 70% and 100% of cells observed, and (++++) when more than 70% of the cells observed were positive. The intensity of positivity was evaluated as weak, moderate and marked in comparison to the staining of the positive control [9].

**Statistical analysis:**

Either the $\chi^2$ test or Fisher’s exact test was used as appropriate to determine differences between variables. $p$ value ≤0.05 was considered statistically significant [23].

**RESULTS**

I- *Maspin expression in normal thyroid tissue, thyroid adenomas and carcinomas:*

Maspin protein expression was detectable in none of the cases of normal thyroid tissue and in MNG, FTA, FTC, ATC, and MTC. In contrast, 18 out of 25 PTC (72%) showed positive staining; their staining pattern is shown in Table (1) and Fig. (1).

II- *Demographic and clinicopathological data of papillary thyroid carcinomas:*

The demographic data of the studied 25 cases of PTC were 4 (16%) males and 21 (84%) females (M/F ratio was 1: 5.3). The ages of the studied group ranged from 21 to 62 yr with a median age of 42.6 yr. There were two tumors (8%) stage T1 (major tumor diameter ≤2cm), fourteen tumors (56%) stage T2 (major tumor diameter >2cm but not >4cm), nine tumors (36%) stage T3 (major tumor diameter >4cm), while no tumors were at stage T4. There were five cases (20%) with nodal metastasis and only two cases (8%) with distant lung metastasis. Seven tumors (28%) were multicentric, while six tumors (24%) and four tumors (16%) showed lymphatic and vascular invasion, respectively.

III- *Relation between maspin expression and clinicopathological characteristics of papillary thyroid carcinomas:*

We then analyzed the relationship between maspin expression and the clinicopathological parameters of papillary thyroid carcinomas. Loss of maspin expression was more frequent in PTC with positive lymph node metastasis (80%) than in negative cases (20%), showing a statistically significant relationship ($p$=0.01). Moreover, in seven multicentric cases, five (71.4%) were maspin negative and the remaining two cases (28.6%) were maspin positive, and this was statistically significant ($p$=0.01). Although cases with distant metastasis (2/25, 8.0%) showed loss of maspin expression, yet it did not reach statistical significance ($p$=0.07). As regards maspin expression in cases with lymphatic and vascular invasion, there were statistically significant differences between maspin-positive and maspin-negative cases ($p$=0.03 and 0.04, respectively). On the other hand, no significant differences were found between maspin immunoreactivity and age, sex as well as tumor stage ($p$=0.1, 0.3 and 0.7 respectively) (Table 2).

**Table (1): The staining pattern of maspin positive papillary thyroid carcinoma.**

<table>
<thead>
<tr>
<th>Staining pattern</th>
<th>Maspin positive (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(No.)</td>
</tr>
<tr>
<td>Staining (SQS):*</td>
<td></td>
</tr>
<tr>
<td>(+)</td>
<td>5</td>
</tr>
<tr>
<td>(+++)</td>
<td>9</td>
</tr>
<tr>
<td>(++++)</td>
<td>4</td>
</tr>
<tr>
<td>Staining intensity:**</td>
<td></td>
</tr>
<tr>
<td>Weak</td>
<td>3</td>
</tr>
<tr>
<td>Moderate</td>
<td>12</td>
</tr>
<tr>
<td>Marked</td>
<td>3</td>
</tr>
<tr>
<td>Staining localization:</td>
<td></td>
</tr>
<tr>
<td>Cytoplasmic</td>
<td>5</td>
</tr>
<tr>
<td>Nuclear</td>
<td>2</td>
</tr>
<tr>
<td>Both</td>
<td>11</td>
</tr>
</tbody>
</table>

Percentages of the row totals are provided in parenthesis

* (SQS) semi quantitative scoring (+) tumor positive cells 5-<30 %, (+++) 30-70%, (++++) >70%

**It was evaluated compared to the positive control staining of prostatic adenocarcinoma.
Table (2): Relation between maspin protein expression and clinicopathological parameters of papillary thyroid carcinomas.

<table>
<thead>
<tr>
<th>Clinicopathological parameters</th>
<th>Cases</th>
<th>Maspin expression (n=25)</th>
<th>(*)Positive (n=18)</th>
<th>(**)Negative (n=7)</th>
<th>***p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(No.) (%)</td>
<td>(No.) (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (range), years</td>
<td>25</td>
<td>41.7 (21-62)</td>
<td>43.1 (23-59)</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Gender:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>4</td>
<td>2 (50)</td>
<td>2 (50)</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>21</td>
<td>16 (76.2)</td>
<td>5 (23.89)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pTNM:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pT1</td>
<td>2</td>
<td>1 (50)</td>
<td>1 (50)</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>pT2</td>
<td>14</td>
<td>10 (71.4)</td>
<td>4 (28.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pT3</td>
<td>9</td>
<td>7 (77.8)</td>
<td>2 (22.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph node metastasis (N+)</td>
<td>5</td>
<td>1 (20)</td>
<td>4 (80)</td>
<td>0.01***</td>
<td></td>
</tr>
<tr>
<td>Distant metastasis (M+)</td>
<td>2</td>
<td>0 (0)</td>
<td>2 (100)</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Multicentricity</td>
<td>7</td>
<td>2 (28.6)</td>
<td>5 (71.4)</td>
<td>0.04***</td>
<td></td>
</tr>
<tr>
<td>Lymphatic invasion</td>
<td>6</td>
<td>2 (33.3)</td>
<td>4 (66.6)</td>
<td>0.03***</td>
<td></td>
</tr>
<tr>
<td>Vascular invasion</td>
<td>4</td>
<td>1 (25)</td>
<td>3 (75)</td>
<td>0.04***</td>
<td></td>
</tr>
</tbody>
</table>

Percentages of the row totals are provided in parenthesis.  
*Maspin positive ≥5% of tumor cells were immunoreactive.  
**Maspin Negative <5% of tumor cells were immunoreactive.  
***p-values ≤0.05 were statistically significant.

Fig. (1): Maspin protein expression in papillary thyroid carcinoma. Maspin immunohistochemical staining: (A), weak cytoplasmic staining in the central portion of the tumor; (B), marked cytoplasmic and nuclear staining in the invading tumor tissue; (C), moderate cytoplasmic and nuclear expression in the malignant cells; (D), papillary structures with immunoreactivity in their nuclei. (DAB: A, C x 400; B, D x 250).
DISCUSSION

Maspin is a unique serine protease inhibitor with a molecular weight of 42kDa. It has been shown to inhibit tumor cell motility, invasion in cell culture, tumor growth and metastasis. This member of serpin family has significant homology to serpin and contains a carboxyl terminal reactive serpin loop domain, which is essential for its antiprotease activity [24]. Maspin protein made from E-coli, yeast and insects inhibits breast tumor cell migration and invasion [4]. In animal models, maspin has been found to inhibit angiogenesis in rat cornea and xenograft models [12]. It has been demonstrated that maspin is present in the normal epithelium of many organs as breast, prostate, thymus, testis, small intestine and colon [25] but it was negative in normal tissues of pancreas [17] and ovary [18]. In our study, maspin was not detected in normal thyroid tissue and this result is in agreement with previously published data [25, 27]. Futschker et al. [28] demonstrated that maspin expression in normal cells is regulated by epigenetic modification in a cell-type-specific manner. Maspin-positive cells showed no methylation at the CpG islands of the promoter region. In contrast, maspin-negative cells showed extensive methylation. Jaenisch and colleagues and Gaudet et al. [29, 30] have demonstrated that DNA hypomethylation plays a causal role in tumor formation, possibly by promoting chromosomal instability. High maspin expression has been noted in normal mammary and prostatic epithelial cells but is strongly down-regulated in the cancer cells from these respective organs, and is lost in metastatic cells [14, 15]. This indicates that maspin gene has been considered to be a tumor suppressor gene. Interestingly, findings in pancreatic cancer and ovarian cancer are in sharp contrast to those reported in breast and prostate tumors. Specifically, Maass et al. [17] and Sood et al. [18] reported that maspin was overexpressed in pancreatic and ovarian cancer, respectively. However, maspin seems to behave as an oncogene rather than as a tumor suppressor gene in these cancers, and this difference in maspin expression can be explained by epigenic regulation through cytosine methylation of the maspin promoter which has been shown to silence expression of maspin in breast cancer [31]. Paradoxically, the demethylation of the maspin promoter has been found to activate expression in human pancreatic, lung, gastric and ovarian cancers [32, 35].

In our study, 72% of papillary thyroid carcinomas were Maspin positive and was negative in other studied types of thyroid cancers (FTC, ATC, MTC); these results are consistent with those of Boltze et al. [27]. This may be due to DNA methylation or p53 control, as reported by Boltze and colleagues [36, 37] that there was a low methylation rate of 28% in papillary thyroid carcinoma in contrast to the other thyroid carcinomas (89-100%). Also p53 was positive in 2% of maspin positive cells and in 80% of maspin negative cases. These findings suggest that promoter methylation caused maspin repression which plays a major role in gene balance and in the process of tumor determination and dedifferentiation in thyroids. Similarly, the inherent rate of p53 dysfunction within specific cancer cell types may play a significant role in the epigenetic regulation of maspin expression. Finally, maspin may have different functions in different cell types, depending upon protein interactions in each given cell type. In our study, there is significant positive correlation in lymph node metastasis, tumor multicentricity, vascular and lymphatic invasion and maspin loss, and these results are consistent with other studies [14, 15]. This indicates that maspin is lost in metastatic cells and cancer aggression; it has thus been suggested that wild type maspin acts as a metastasis suppressor rather than as a classic tumor suppressor [18]. In contrast, regarding patient age, sex and tumor stage, no significant correlation was found as reported in a previous study [38].

Regarding distant metastasis, there was a trend towards increased distant metastasis to the lung in patients with maspin loss but this finding was statistically insignificant, which may be due to the small number of the studied cases.

It is possible that the localization of maspin may play a critical role in its biological function. However, it is clear that the nuclear localization of maspin is associated with increased survival, whereas the cytoplasmic localization is associated with poor outcome in ovarian carcinoma, [18, 39]. Nuclear maspin may be the biologically active form that plays a critical role in tumor suppression whereas cytoplasmic maspin may be inactive [40]. In our study, most of the tumor samples express maspin with both cytoplasmic and nuclear localization which was proven by
others [10,25]. Few cases showed nuclear or cytoplasmic reaction only. We suggest the possibility of differences in the tumor behavior related to maspin localization and epigenetic regulation and we recommend further study related to these issues, which may render maspin an attractive therapeutic target. Maspin gene therapy was evaluated in prostate and breast cancer which caused suppression of tumor growth through apoptosis induction and angiogenesis inhibition [41,42].

In conclusion: Since maspin positivity was detected in 72% of studied papillary thyroid carcinoma specimens, therefore maspin could serve as a therapeutic target in PTC. Also, its expression in PTC rather than other thyroid carcinomas predict the role of maspin in the genesis of PTC. Regarding the relation between maspin expression and clinicopathological parameters, these data need to be confirmed on a larger scale with follow up of patients for further evaluation of its relation with clinical outcome including overall survival, disease free survival as well as tumor recurrence.

REFERENCES

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