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Immunohistochemical localization of matrix metalloproteinase-2 (MMP-2) in medullary thyroid carcinoma

ABSTRACT

Background: *Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes that degrade protein components of the extracellular matrix. These enzymes are believed to play an important role in tumor progression, invasion and metastasis.*

Materials and methods: *We examined the expression of one member of the MMP family, matrix metalloproteinase-2 (MMP-2, collagenase A) in twenty-two paraffin-embedded specimens of medullary thyroid carcinoma (MTC) using an immunohistochemical method and a monoclonal antibody against MMP-2. In addition, we compared MMP-2 expression with clinicopathological data at the time of surgery.*

Results: *Immunohistochemical staining showed moderate to strong positivity in 19/22 cases and weak positivity in 3/22 cases. MMP-2 was immunohistochemically localized mainly in tumor cells, but was also present in surrounding stromal tissue. MMP-2 expression, however, was not associated with histopathological appearance of the tumor tissue, tumor size or the presence of lymph node metastases.*

Conclusion: *Considerble production of MMP-2 is associated with the malignant phenotype of parafollicular thyroid cells, suggesting a potential role of this proteolytic enzyme in tumorigenesis in vivo. However, since MMP-2 expression was not related to clinicopathological data, immunohistochemical staining of MMP-2 does not seem to be helpful in predicting the prognosis of medullary thyroid carcinoma.*

Key words: *Matrix metalloproteinase-2; Medullary thyroid carcinoma; Prognosis; Immunohistochemistry*

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INTRODUCTION

Tumor cells require proteolytic degradation of the extracellular matrix for each step of tumorigenesis, including primary tumor organization, tumor progression and the last stage of tumor progression, metastasis. These events include the ability of malignant cells to invade surrounding stroma by degrading the basement membrane and extracellular matrix components, such as collagen, laminin, fibronectin and proteoglycan (1-4).

Matrix metalloproteinases (MMPs) are a representative endopeptidase family with a broad spectrum of proteolytic activity for a variety of extracellular matrix components. The MMP family of enzymes includes at least 18 members, classified according to substrate specificity. All members share sequence homology, catalytic mechanisms dependent upon a zinc ion, secretion as zymogens (proactive enzymes) and regulation by endogenous inhibitors (5-7).

Under normal conditions these enzymes are responsible for normal tissue remodeling.

During tumorigenesis, levels and localization of these proteases and their endogenous inhibitors may be altered leading to tumor progression.

Among the MMPs, MMP-2 (also called 72 kDa type IV collagenase / gelatinase A), which is able to degrade type IV collagen, is considered to be especially important in the degradation of the extracellular matrix that is associated with the malignant behavior of tumor cells (8).

Overexpression of MMP-2 in tumor tissue compared to adjacent normal tissue has been documented in many types of solid tumors, including breast (9), colorectal (10) and pancreatic carcinomas (11), squamous carcinomas of the head and neck (12) and other carcinomas.

Human thyroid carcinoma tissues have been reported to express MMP-1 and MMP-2 and these MMPs were localized in tumor cells and/or in the fibroblasts adjacent to, or close to the invading tumor cells (13-17).

These studies were mainly focused on thyroid malignancies originating from thyroid follicular epithelial cells, i.e. papillary, follicular and anaplastic carcinomas.

Medullary thyroid carcinoma (MTC) originates from calcitonin producing parafollicular (C) cells of the thyroid and accounts for 5-10% of all thyroid cancers.

MTC shows some unique biologic and genetic features and its prognosis is in general worse than that of well differentiated thyroid carcinomas, but better than that of anaplastic carcinoma (18-21).

The aim of this study was to investigate immunohistochemical expression of MMP-2 in MTC tissue and to correlate its expression with clinicopathological data at the time of surgery.

MATERIALS AND METHODS

Tissue samples

Formalin-fixed and paraffin-embedded tissues of twenty-two cases of sporadic medullary

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thyroid carcinoma were obtained from the archival material of the Institute of Endocrinology, Diabetes and Diseases of Metabolism, Clinical Centre of Serbia, Belgrade. The selection of material was based primarily on the prior diagnosis made by routine histopathological anallysis (22) and positive calcitonin staining.

Immunohistochemistry

Immunostaining was performed on 4-6 mm thick sections using the avidin-biotin peroxidase complex (ABC) techique (23) with reagents supplied by Vector laboratories (Burlingame, CA). Following deparaffination and rehydration, endogenous peroxidase activity was blocked with 0.3% H_2O_2/ methanol followed by non-immune horse serum for 20 min to block the non-specific binding. The sections were then incubated with the primary monoclonal antibody against MMP-2 (clone 42-5D11, purchased from Oncogene Research Products, Calbiochem, USA) at a dilution of 1/100 at 4°C overnight. This was followed by incubation with horse anti-mouse biotinylated IgG 30 min and thereafter with streptavidin-biotin-peroxidase complex (ABC reagent) for 30 min. Among each of the step the sections were washed three times in phosphate buffered saline (PBS). The reaction was visualized using diaminobenzidine tetrahydrochloride (DAB) solution.

After counterstaining with hematoxylin, the slides were dehydrated, coverslipped and examined usnig a Reichert-jung microscope supplied with a Photostar automatic camera system.

For each case, as a negative control, the primary antibody was replaced with PBS, and no positive staining was observed.

RESULTS

The results of the immunohistochemical analysis of MMP-2 expression in medullary thyroid carcinoma tissue in relation to clinical data available at the time of surgery are presented in Table 1.

Light microscopy examination revealed that thirteen out of the twenty-two cases of MTC investigated, had polygonal cells with round or oval nuclei. Small areas with spindle-shaped cells were seen in nine cases. All, but four, cases contained amyloid in variable amounts, from minimal to abundant. In eleven cases regional lymph node metastases (LNM) were present at the time of surgery (pN_1).

Immunohistochemical staining using monoclonal antibody against MMP-2 showed positivity in all of twenty-two cases of MTC examined. In general, staining was diffuse in the cytoplasm of tumor cells, ranging from moderate (+) to strong (++) and was widespread throughout the tumor tissue. Only three cases showed very weak positivity.

Table 1. Clinicopathological for twenty-two cases of sporadic MTC in relation to immunohistochemical staining for MMP-2

Case No.	Age (Year)	Sex	Histological pattern (Type of cells)	Tumor stage* (pTNM)	Immunostaining results** for MMP-2
1.	55	F	polygonal	$T_2N_0M_0$	+
2.	51	F	polygonal	$T_3N_0M_0$	++
3.	40	F	polygonal/spindle	$T_3N_0M_0$	++
4.	74	F	polygonal/spindle	$T_3N_0M_0$	++
5.	66	F	polygonal	$T_3N_0M_0$	++
6.	74	F	polygonal	$T_3N_0M_0$	+
7.	53	F	polygonal	$T_2N_0M_0$	+
8.	70	F	polygonal	$T_2N_0M_0$	++
9.	61	F	polygonal	$T_3N_0M_0$	++
10.	68	F	polygonal/spindle	$T_4N_0M_0$	++
11.	55	F	polygonal/spindle	$T_3N_0M_0$	+/-
12.	21	F	polygonal/spindle	$T_3N_1M_0$	++
13.	67	F	polygonal	$T_4N_1M_0$	++
14.	30	M	polygonal	$T_4N_1M_0$	++
15.	34	F	polygonal/spindle	$T_4N_1M_0$	++
16.	63	F	polygonal	$T_3M_1M_0$	++
17.	47	F	polygonal/spindle	$T_4N_1M_0$	++
18.	41	F	polygonal/spindle	$T_4N_1M_0$	+/-
19.	68	F	polygonal/spindle	$T_2N_1M_0$	+/-
20.	60	M	polygonal	$T_4N_1M_0$	+
21.	65	M	polygonal	$T_4N_1M_0$	++
22.	46	F	polygonal	$T_3N_1M_0$	+

*According to Hermanek and Sobin (24)

** +/- very weak staining
+ moderate staining in the majority of cells
++ strong staining in the majority of cells

Besides MMP-2 expression in the carcinoma cells themselves, positive staining was also found in stromal tissue within the tumor and in the capsule between cancer cell nests and normal surrounding tissue. Positive cells in stroma were spindle shaped, probably fibroblasts and/or macrophages. In addition, endothelial cells of blood vessels within the tumor tissue were also immunoreactive. The mid portion of the tumor showed the same intensity of staining as peripheral margins.

No staining was observed in epithelial cells of normal follicles adjacent to tumor tissue.

There was no apparent association between MMP-2 expression and histopathological appearance of the tumor tissue or tumor stage. The advanced stage of MTC (with LNM at the time of surgery, pN_1 eleven cases) showed similar immunostaining results as cases without LNM, pNo, (Table 1).

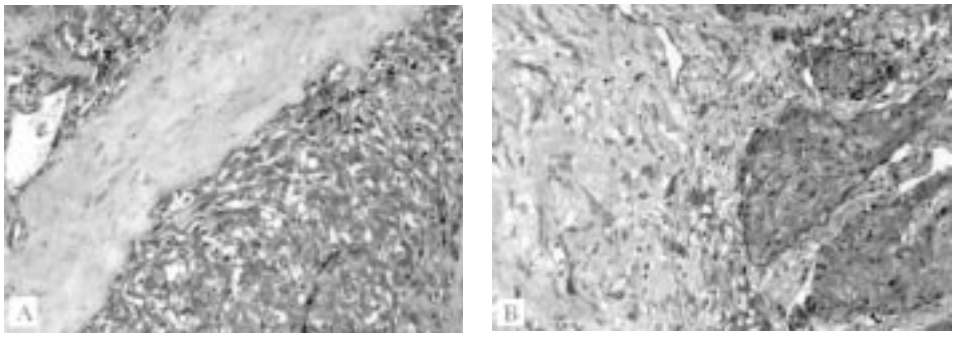

Figure 1. Immunolocalization of matrix metalloproteinase-2 (MMP-2) in medullary thyroid carcinoma. Carcinoma tissues were immunostained with a monoclonal antibody against MMP-2 as described in Material and Methods. MMP-2 was immunohistochemically localized mainly in the cytoplasm of tumor cells but was also present in surrounding stromal tissue. Cases No 5, $pT_3N_0M_0$ (A) and No 16, $pT_3N_1M_0$ (B) are shown. Diaminobenzidine-hematoxylin; original magnification: x20

DISCUSSION

In this immunohistochemical study we analyzed expression of matrix metalloproteinase-2 in a series of twenty-two cases of medullary thyroid carcinoma.

Marked expression of MMP-2 was found in malignant cells of most of carcinomas analyzed, indicating the capacity of these cells to produce high levels of this proteolytic enzyme.

The investigations, concerning the relationship between thyroid tumors and MMPs, are few. involvement of MMP-2 in thyroid cancer has been demonstrated in previous studies by immunohistochemistry of neoplastic thyroid tissue (14), by *in situ* hybridization (15) and by gelatin zymography of follicular thyroid carcinoma cell lines (16) and papillary thyroid carcinoma tissue (17). Since in some of these reports (15,16), MMP-2 was not observed in cancer cells, but only in stroma, there is much dispute whether thyroid cancer cells themselves can produce MMPs or whether cancer cells stimulate surrounding stromal cells to produce MMPs, via a paracrine interaction.

In the present study both the carcinoma and stromal cells were labeled by immunohistochemistry. The major MMP-2 expression was found in the carcinoma cells, perhaps due to the low cellularity of the stromal fibroblasts compared with that of the carcinoma cells.

However, in the MTC cases, examined MMP-2 expression apparently had no relation to clinicopathological data (histopathological appearance of the tumor tissue, tumor size or the presence of lymph node metastases). Thus, MMP-2 immunostaining does not seem to be of prognostic significance for MTC.

There are some possible explanations for that. One of them is that a high rate of production of MMP-2 is a very early event in the history of MTC progression. Also, members of the MMP family of proteolytic enzymes have different substrates and possibly have specific roles in each step of tumorigenesis of various human

tumors.

Thus, MMP-2 is probably just one of several enzymes contributing to tumor progression. In addition, as with other MMPs, latent MMP-2 proenzyme is regulated by proteolytic activation and interaction with tissue inhibitors of matrix metalloproteinases (TIMPs), their specific endogenous inhibitors (25,26). An imbalance between the activity of MMPs and their inhibitors may be one of the mechanisms responsible for tumor progression. Thus, it is possible that the balance between MMPs and their inhibitors will be more informative for predicting tumor progression.

CONCLUSION

Taken together, the results of this study showed that a high rate of production of MMP-2 is associated with the malignant phenotype of parafollicular thyroid cells suggesting a potential role of this proteolytic enzyme in tumorigenesis *in vivo*. However, since MMP-2 expression was not related to clinicopathological data, immunohistochemical staining for MMP-2 itself does not seem to be of prognostic value for MTC.

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