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Original article

Evaluation of MMP2 and Caspase-3 expression in 107 cases of papillary thyroid carcinoma and its association with prognostic factors

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ABSTRACT

Papillary thyroid carcinoma (PTC), including its variants and widely varying behavior, constitutes about 80% of all thyroid malignancies. Increased knowledge regarding molecular alterations has led to attempts to identify diagnostic or prognostic factors for a reliable preoperative approach to the classification of patients according to risk of recurrence. In this study, 107 cases of PTC with known histological properties, including vascular or capsular invasion, were assessed for expression of MMP2 and Caspase-3 using immunohistochemistry. Considering 10% as a cutoff to discriminate cases with invasive behavior from the non-invasive group, there was no relationship between expression of MMP2 or Caspase-3 in tumor cells and the presence of capsular invasion ($p = 0.45$ and 0.64, respectively), as well as for the expression of Caspase-3 and vascular invasion ($p = 0.43$). In case of MMP2, a borderline correlation was found between the positive reaction of tumor cells with the presence of vascular invasion ($p = 0.05$). So the evaluation of MMP2 in thyroid PTC appears to be of some benefit to the prediction of tumor behavior while Caspase-3 as a marker of prediction seems to be of no use.

Introduction

Thyroid cancer is the most common thyroid malignancy [31], and its incidence has increased over the last 3 decades [7]. Papillary thyroid carcinoma (PTC), including its large number of variants and variable behavior from indolent micro-carcinoma to invasive tumors with metastatic potential [25], constitutes about 80% of all thyroid malignancies [7].

Evaluation of prognosis at the time of diagnosis leads to a reliable preoperative approach for the classification of patients according to the risk of recurrence and death, so it determines the need for more extensive surgery or adjuvant therapies in those with aggressive potential [7], but avoids unnecessary treatment in cases with an indolent course. Many risk classification systems have been used to estimate disease-free survival in patients with thyroid malignancies [7,8]. For years, factors such as older age, male gender, extra thyroidal invasion, lymph node or distant metastasis, and high tumor stage have been proposed as poor prognostic factors [7,16,18,30,32,36,37,40,42]. Increased knowledge of molecular alterations has led to many attempts to identify more diagnostic and prognostic factors in thyroid carcinoma in order to give a more objective preview of tumor behavior at the time of first surgery.

MMPs are zinc-dependent proteolytic enzymes [1,17] capable of degradation of extracellular matrix during tumor genesis [1]. MMP2, also referred to as Gelatinase A, has been specially considered in this family. It is able to digest type IV collagen, and the overexpression in tumor tissue in different type of malignancies has been evaluated [1,2,5,13,17,21,22,34].

Imbalance between proliferation and apoptosis is another issue that has been proposed as important pathogenic mechanism involved in tumor genesis [10,12,29,33,38]. Caspase, a family of Cystin protease, plays a central role in the initiation phase of apoptosis [27]. Furthermore, it has been introduced as a marker of apoptotic cells [9,12] but its expression in thyroid tissue has not been clarified yet [12].

In this study, we evaluated 107 cases of papillary thyroid carcinoma with known histological properties, including vascular and capsular invasion, for the expression of MMP2 and Caspase-3. Our aim was to determine a probable relationship between the positive or negative expression of these markers by immunohistochemistry (IHC) with the presence of capsular and/or vascular invasion, which are generally regarded as signs of tumor aggressiveness.

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Materials and methods

Paraffin blocks of archival formalin fixed specimens of PTC diagnosed during 16 years (1991–2007) in the Department of Pathology, Shariati Hospital, Tehran, were examined to determine the relationship of MMP2 and Caspase 3 expression with vascular and capsular invasion. In order to eliminate the effect of the histological type of tumors, we selected classic PTC (papillary pattern) cases with available data in which we were interested (the status of vascular and capsular invasion could be confirmed by the authors). At least 3 blocks per case were studied except for the cases with micro papillary carcinomas. (Clinical data, 3 to 5 year follow ups were available. However, unfortunately, we could not achieve information on TNM stage or the presence of distant metastasis in all patients.)

The study was conducted according to the protocol of the Ethics Committee of Endocrinology and Metabolism Research Center. Paraffin blocks with minimal tissue necrosis or hemorrhage and a satisfactory amount of tumoral tissue were selected for IHC staining. Three-micrometer cut sections from representative blocks of every tumor were deparaffinized, rehydrated, placed in 0.5% H_2O_2 for 1 min to block endogenous peroxidase activity, and washed with water. They were processed with 0.01 citrate buffer (pH 6.0) in pressure cooker, placed in a bath of tap water, washed in TBS buffer (pH 7.6) for 5 min, and placed in diluted normal serum for 10 min. After incubation with primary antibody (Novocastra™ Lyophilized Mouse monoclonal antibody Matrix metalloproteinase 2 and Novocastra™ Lyophilized Mouse monoclonal antibody CPP3) (Leica Biosystems Newcastle Ltd., Newcastle, UK) and washing in TBS, sections were consecutively incubated with biotinylated secondary antibody, washed in TBS, incubated in ABC reagent, washed in TBS, incubated in suitable peroxidase substrate, washed in running tap water, counterstained with hematoxylin, dehydrated, and mounted. The process was independently performed on each specimen for both MMP2 and CPP3.

In order to ensure the validity of the technical method, positive controls were used which consisted of tonsilar tissue for CCP3 and inflamed large bowel for MMP2, as recommended by the manufacturer. Sections from which the primary antibodies were omitted served as negative reaction controls.

To interpret the results, a cut off of 10% of tumor cells with positive staining was established. The staining pattern was expected to be cytoplasmic for both markers. The tumor was regarded to positively express the antigen if at least 10% of tumor cells showed positive cytoplasmic staining.

The slides were independently reviewed by two pathologists. In case of discordant diagnosis, the two pathologists tried to reach a consensus.

After data collection, the analysis of qualitative and quantitative data was made using Statistical Package for Social Sciences (SPSS) version 16.0 (Chicago, IL, USA). $p < 0.05$ was considered significant.

Results

The 107 cases of PTC in this study ranged in age from 13 to 79 years (mean age 41.3) and comprised 69 females (64.5%) and 38 males (35.5%).

The tumor diameter ranged from 0.5 cm to 7 cm (mean 2.5 cm, SD = 1.25).

Twenty-seven cases (25.2%) showed vascular invasion, whereas 80 (74.8%) cases did not.

Capsular invasion was present in 34 (31.8%) and absent in 73 (68.2%) cases.

Of all 107 PTCs, 16 (15% versus 85%) cases showed extra thyroid extension. Necrosis was present in only 4 (3.7% versus 96.3%) out of 107 tumors.

Using a cut off of 10% (percentage of tumor cells with positive staining) to discriminate between the negative and positive expression of the markers, we obtained the following results:

MMP2 was positively and negatively expressed in 80 (74.8%) and 27 (25.2%) cases, respectively.
Positive CPP3 expression was shown in 92 (86%) cases; 15 (14%) cases were negative for this marker.
Six cases (5.6%) showed dual negativity for both MMP2 and CPP3; 30 cases (28%) were positive for a single marker; and in 71 (66.40%) cases, both markers were positive.

In our study, no statistically significant relationship was found between age or sex of the patients and expression of either CCP3 or MMP2.

We also evaluated the status of MMP2 and CCP3 expression regarding the presence of necrosis or extra thyroid extension (Tables 1 and 2).

Tables 3 and 4 summarize the results of immunoreactivity of papillary thyroid carcinomas for MMP2 and CPP3, categorized according to the status of capsular invasion, as well as to that of vascular invasion (Figs. 1 and 2).

There was no significant relationship between the expression of MMP2 or CCP3 in tumor cells and the presence of capsular invasion ($p = 0.45$ and 0.64, respectively). Also, the same was found for positive expression of CCP3 and tumor vascular invasion ($p = 0.43$), as well as for extra thyroid extension or necrosis (Tables 1 and 2). Just a borderline correlation observed in cases with positive reaction to MMP2 and vascular invasion ($p = 0.05$).

Table 1
Result of immunoreactivity of papillary thyroid carcinoma according to status of necrosis.

PTC	MMP2+	MMP2-	Total	CCP3+	CCP3-	Total
With necrosis	3 (3.8%)	1 (3.7%)	4 (3.7%)	4 (4.3%)	0 (0%)	4 (3.7%)
Without necrosis	77 (96.2%)	26 (96.3%)	103 (96.3%)	88 (95.7%)	15 (100%)	103 (96.2%)
Total	80 (100%)	27 (100%)	107 (100%)	92 (100%)	15 (100%)	107 (100%)
	P=0.736				P=0.541	

Table 2
Results of immunoreactivity of papillary thyroid carcinoma according to status of extra thyroid extension.

PTC	MMP2+	MMP2-	Total	CCP3+	CCP3-	Total
With extra thyroid extension	12 (15%)	4 (14.8%)	16 (15%)	12 (52.17%)	4 (4.76%)	16 (15%)
Without extra thyroid extension	68 (85%)	23 (85.2%)	91 (85%)	11 (47.83%)	80 (95.24%)	91 (85%)
Total	80 (100%)	27 (100%)	107 (100%)	23 (100%)	84 (100%)	107 (100%)
	P=0.626				P=0.234	