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Heat shock proteins HSP90, HSP70 and GRP78 expression in medullary thyroid carcinoma

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ABSTRACT

Background: Medullary thyroid carcinoma management consists mainly of surgical resection and is largely chemoresistant. There is ongoing effort to discover novel therapies for medullary thyroid carcinoma. Increased levels of heat shock proteins have been associated with multiple cancers and are being studied as potential therapeutic targets. The purpose of this study was to determine the expression levels of heat shock proteins 90 and 70 and of glucose related protein 78 in medullary thyroid carcinoma tissues compared with normal thyroid tissues.
Methods: 20 tissue specimens of medullary thyroid carcinoma and 10 specimens of thyroids without malignancy were analyzed by immunohistochemistry.
Results: Medullary thyroid carcinoma specimens showed 27% higher expression level of heat shock protein 90 immunostaining, and a 43% higher expression level of heat shock protein 70 immunostaining versus normal controls. These differences, however, were not statistically significant. A significantly higher expression level was noted for glucose related protein 78 in the medullary thyroid carcinoma specimens than in the controls.
Conclusion: This study indicates increased expression levels of heat shock proteins 90 and 70 and glucose related protein 78 levels in medullary thyroid carcinoma. These findings, though preliminary imply that these proteins may have a role in medullary thyroid carcinoma's tumor biology and may have and future therapeutic options. Larger cohorts are needed to corroborate these results.

1. Introduction

Medullary thyroid carcinoma (MTC) is an endocrine neoplasm that arises in the parafollicular C cells of the thyroid gland and may secrete calcitonin and carcinoembryonic antigen. It accounts for 2% to 5% of all thyroid carcinomas [1]. Seventy-five percent of cases are sporadic and the rest are inherited. Up to 50% of sporadic cases and 95% of inherited cases are associated with mutations in the RET proto-oncogene [2]. The clinical course is less aggressive than in anaplastic carcinomas but more aggressive than in well-differentiated thyroid cancers. Regional and distant metastases are frequent (up to 50% of cases). Current management is mainly consisted of surgical resection which includes total thyroidectomy and lymph-node dissection. Radioactive iodine treatment is not effective because, owing to their parafollicular C-cell origin, MTCs do not take up iodine. External beam radiation therapy has also not been found effective, and there is no effective chemotherapy regimen [1,3]. The prognosis of patients with MTC is directly related to disease stage. The overall 10-year survival rate is 61% to 75%, but it decreases to 45% if cervical nodes are involved [3,4]. Therefore, researchers are seeking promising new therapeutic modalities for patients with MTC unresponsive to traditional therapy. Potential targets are thyroid molecular signaling pathways and factors associated with cancer cell biology including vascular endothelial growth factor receptors and antibodies, angiogenesis inhibitors, tyrosine kinase inhibitors, and heat shock protein (HSP) inhibitors [1].

In 1963, HSPs were identified as a unique family of proteins that are expressed following exposure to protein-damaging environmental stresses to promote protein renaturation, restore homeostasis and enhance survival. The process is termed the heat shock response [5,6].

The heat shock family of proteins is divided into classes by molecular weight: HSP100, 90, 70, 60 and 27. Each class displays relatively specialized functions, although they often act work in concert to achieve an optimal outcome.

Recently HSPs have been shown to have a role in cancer cell growth and survival and also as potential biomarkers and therapeutic targets (7–28). Our objective was therefore to determine whether MTC is

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associated with elevated expression levels of HSP90, HSP70 and GRP78 compared to normal thyroid. These findings may shed a light on the role of these HSPs on the MTC's tumor biology and may have clinical implications for the prognostic analysis and treatment of patients with MTC.

2. Materials and methods

Following approval of the study by the institutional review board (#4101), primary medullary thyroid carcinoma tissue specimens were obtained from 20 patients treated in our center (study group). Primary non cancerous thyroid tissues were obtained as a control group from 10 subjects with either follicular adenoma , colloid goiter or postmortem normal thyroid. Tissue specimens were verified to by a senior pathologist (S.M) to include MTC tissue for the study group or normal thyroid for the control group prior to proceeding with the immunohistochemical study.

2.1. Immunohistochemical study

Tissue specimens were fixed with 10% formalin, embedded in paraffin, cut into 5-μm sections and placed on slides. The sections were deparaffinized, and endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol 100% for 25 min and washed with triethanolamine-buffered saline (TBS) at pH 7.6. For antigen activation, the sections were immersed in citrate buffer (pH 6), boiled, and incubated in a microwave oven at 95 °C for 10 min. After the sections were rinsed with TBS at pH 7.6, they were incubated in 20% swine serum (Wako, Osaka, Japan) for 20 min to block nonspecific reactions. The sections were then incubated overnight with the primary antibodies, HSP90, HSP70 and GRP78, at a dilution of 1:50, 1:40 and 1:500, respectively. The following morning, the sections were rinsed with TBS and incubated with peroxidase-labeled goat anti-mouse/rabbit antibody (Envision™) for 30 min. They were then rinsed again with TBS and incubated with 0.02% 3,3-diaminobezidinetetrahydrochloride (DAB) in 0.05 M Tris buffer for visualization of the peroxide reaction. This was followed by rinsing with tap water for 20 min and counterstaining with Mayer's hematoxylin. Finally, the sections were dehydrated by incubation with alcohol in rinsing dilutions (70%, 95% and 100% respectively) and cleared by incubation with toluene. Sections for negative controls were incubated with peroxidase-labeled goat anti-mouse\rabbit antibody, but without the primary antibody. Sections for positive controls were prepared from breast carcinoma for testing for HSP70 and GRP78 and from endometrial carcinoma for testing for HSP90; the tissues used are known to stain positively for the respective antibodies.

2.2. Immunohistochemical evaluation

Immunohistochemistry evaluation was performed by an experienced head and neck pathologist (SM) . Immunostaining was verified with positive control specimens. Cells were considered positive when immunoreactivity was clearly observed in the cytoplasm. At least 1000 cells in 5 randomly selected fields were carefully monitored and classified into 4 categories: strong positivity 3 (+++), >66% positive cells; diffuse 2 (++), 33% to 66% positive cells; heterogeneous 1 (+), 10% to 33% positive cells; and negative 0 (−), <10% positive cells. Intensity was defined according to the color of staining: deep brown 3 (+++); brown 2 (++); yellowish brown 1 (+); or faint color 0. When the intensity of staining was not uniform across the specimen, an intermediate score was assigned (e.g., 0.5, 1.5, 2.5).

Specimens from the 2 groups were compared for percentage of the area that stained positive and for the intensity of the positive staining. Only the tumor tissue was analyzed in the study group specimens.

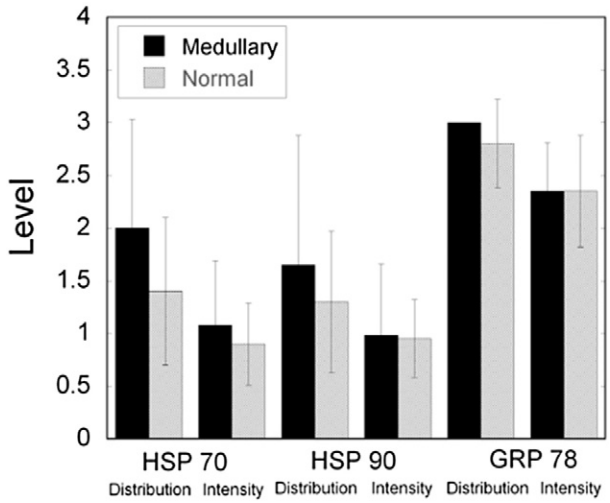

Fig. 1. Histogram of heat shock protein immunohistochemical evaluation.

2.3. Statistical analysis

Statistical analyses were performed using the nonparametric Mann-Whitney test. A p value of <0.05 was considered statistically significant.

3. Results

3.1. Patient characteristics

The study group consisted of 11 women and 9 men of average age 49 years at surgery (range: 9–80 years). Average tumor size was 2.3 cm (range: 0.8–5 cm). The control group consisted of 5 males and 5 females of average age 59 years.

3.2. Immunohistochemistry (Table 1, Fig. 2)

Perinuclear staining was noted in all specimens and membrane staining was not found in any of the specimens (Fig. 1).

3.2.1. HSP90

On immunohistochemistry studies, the average score for percentage positively stained area for HSP90 was 1.65 in the study group and 1.3 in the control group. MTC specimens showed a 27% higher positivity rate than control specimens. The average staining intensity score was 0.98 in the study group and 0.95 in the control group. Neither of these differences was statistically significant (Table 1).

Table 1
Summary of immuno-histocehmical evaluation.

Marker	Diagnosis	No.	Mean ± SD	P value	P value
HSP 70 area of staining	Medullary	20	2 ± 1.03	N.S	**0.08**
	Normal	10	1.4 ± 0.7		
HSP 70 intensity	Medullary	20	1.08 ± 0.61	N.S	0.52
	Normal	10	0.9 ± 0.39		
HSP 90 area of staining	Medullary	20	1.65 ± 1.23	N.S	0.4
	Normal	10	1.3 ± 0.67		
HSP 90 intensity	Medullary	20	0.98 ± 0.68	N.S	0.92
	Normal	10	0.95 ± 0.37		
GRP 78 area of staining	Medullary	20	3 ± 0	0.003	**0.042**
	Normal	10	2.8 ± 0.42		
GRP 78 intensity	Medullary	20	2.35 ± 0.46	N.S	0.63
	Normal	10	2.35 ± 0.53		