Expression of the Pituitary Tumor Transforming Gene (*PTTG1*) in Pheochromocytoma as a Potential Marker for Distinguishing Benign Versus Malignant Tumors

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Abstract- The Distinction between malignant and benign pheochromocytoma has always been a diagnostic challenge over the last decades. To date, the only reliable criterion is metastasis. The aim of the present study was to investigate the possible expression of pituitary-tumor transforming gene (*PTTG1*) and retinoblastoma (Rb) in benign and malignant pheochromocytoma. Paraffin blocks of 44 and 11 patients diagnosed with benign and malignant pheochromocytoma were collected. Parameters such as sex, age, tumor size, necrosis, and histological features were compared between the benign and malignant groups as well as immunohistochemical labeling using specific antibodies. *PTTG1* showed negative expression in all (44) benign and 9 out of 11 (81.8%) malignant tumors with only 2 out of 11 (18.2%) malignant tumors showed positive reactivity for *PTTG1* (*P*: 0.037) with spindle cell histological pattern in both of them (*P*: 0.013). Although Rb expression in malignant tumors (81.8%) was slightly more than the benign ones (52.3%), no statistically significant correlation was observed (*P*: 0.087). These results suggest that *PTTG1* immunostaining may play a key role in distinguishing between benign and malignant phaeochromocytoma. However, larger studies are necessary to confirm the outcomes of the present study.

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Introduction

Predicting the behavior of pheochromocytoma has been a challenge for many years. While total remission of most of the malignant cases is achieved by early detection and treatment, the survival rate of those patients with malignant pheochromocytoma is very poor; due to the fact that the only reliable criteria for diagnosis of a malignancy remains the occurrence of metastases (1). Controversial results have been obtained for determining different predictive parameters of malignant pheochromocytoma. High preoperative 24-h urinary dopamine, weighty tumors, extra-adrenal tumors, MIB-1-positive cell rate, depletion of S100-positive sustentacular cells, necrosis, higher age at resection, sporadic occurrence, and increased CgA serum levels are among the features that were associated with malignancy (2-5). However, routine immunohistochemical evaluation for estimation of proliferative activity can be applied for distinguishing benign versus malignant pheochromocytoma (6).

Pituitary-tumor transforming gene (*PTTG1*) is on chromosome 5q33 (7), implicates as an inhibitor of chromatid separation, regulates secretion of basic fibroblast growth factor (8-9), and consists of at least three homologous genes in the human. *PTTG1* regulates chromatid separation and disrupts cell division, increases cell susceptibility and induces mutations during consequent division (10). It has been also found that *PTTG1* that is involved in tumorigenesis acts as a

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transcription factor by activating c-myc oncogene (11) and affects cell signaling via the MAP kinase cascade (12). Moreover, angiogenesis is induced by *PTTG1*; terminating in metastatic spread and tumor progression (8). Both p53-dependent and independent manners are regulated by *PTTG1* (13).

The retinoblastoma gene (RB) encodes a nuclear phosphoprotein that acts as a tumor suppressor (14) and its mutation terminates in a wide range of malignancies, as well as retinoblastomas (15-17). The probable pathway that is disrupted as a consequence of loss of RB is p16INK4a-CDK4/6-RB (18). G1 to S-phase transition of the cell cycle is regulated by phosphorylation of RB and its separation from E2F family members that inhibits cell transcription (14).

Despite all these findings, the role of these genes in neuroendocrine tumors is not clear yet. In the present study, we investigated the immunohistochemical expression of human *PTTG1* and Rb in 55 patients with benign and malignant pheochromocytoma. We aimed to determine any possible correlation between *PTTG1* overexpression and behavior of pheochromocytoma as well as its utility as a predictive marker in early stages when metastasis has not occurred.

Materials and Methods

Study design

The paraffin blocks of formalin-fixed samples representing 55 patients with pheochromocytoma referred to the pathology center from March 1986 to March 2006 were collected. Complete clinical data and information about the patients' follow-up were accessible for all patients.

All specimens were categorized as either malignant or benign tumors. According to 7- 23 years follow-ups 44 and 11 patients were diagnosed with benign and malignant pheochromocytoma, respectively. Distant and lymph node metastasis or extensive invasion of surrounding organs, were considered as a criterion of malignancy.

Hematoxylin and eosin (H&E) stained slides were reviewed and scored according to Pheochromocytoma of the Adrenal gland Scaled Score (PASS) (1). Several parameters such as sex, age, tumor size, necrosis, and histologic features were compared between the benign and malignant groups. The histological pattern of tumor, i.e., solid, nest, trabecular, apindle, and small cell were also studied. Blocks with minimum hemorrhage and necrosis were selected for immunohistochemistry staining. After being sectioned at 3 μm, deparaffinized with xylene and alcohol, rehydrated, and immersed in 3% hydrogen peroxide for 15 minutes, immunostaining with anti-*PTTG1* (Sigma Prestige, HPA008890) and anti-Rb antibodies (Novocastra, NCL – Rb358) was performed according to the manufacturer's protocol.

The slides were then evaluated by two separate pathologists who were totally blind to the study. They would discuss the case in cases of the discrepant diagnosis. If they did not reach consensus that case was reviewed by a third pathologist. Each slide was scored according to a semiquantitative scoring method (score 1-4) based on the extent of nuclear and cytoplasmic staining intensity for *PTTG1* and nuclear staining intensity for Rb (Table 1).

Table 1. Immunohistochemical scoring

Immunohistochemical Scoring	Extent of Nuclear/cytoplasmic staining (%)
1	<10
2	10-40
3	41-70
4	>71

Data analysis

Statistical analysis was performed using SPSS®, version 19. Parametric quantitative variables were compared using the independent sample t-test. Comparison of non-parametric quantitative variables was performed by Man Whitney U test while. Chi-square and Fisher tests were utilized for analyzing qualitative data. Differences were considered significant at P <0 .05.

Results

The complete results were previously reported in our recent article in which the expression of Galectin-3, nm-23, and Cyclooxygenase-2 were studied to discriminate between benign and malignant pheochromocytoma (19). In summary, of 55 cases studied, 44 and 11 cases were categorized as benign and malignant, respectively. Although the range of patients' ages was wide (approximately from 10- 70), half of the patients were 30-50 years old. Although the mean age of patients with a malignant tumor was slightly higher than those with benign pheochromocytoma, no significant correlation was detected between the age and malignancy according to statistical analysis. The prevalence of pheochromocytoma was a little more in the female population compared to the male population. However, no significant correlation was observed between sex and

possibility of malignancy as well. The mean tumor size was 6.92 ± 2.67 cm and 9.18 ± 5.02 cm in benign and malignant pheochromocytoma, respectively. Results showed that the risk of malignancy intensifies with increase of tumor size (*P*=0.044). The prevalence of extensive necrosis was 64% and 32% of malignant and benign tumors with no statically significant difference, respectively (*P*=0.05) (19).

Table 2 shows different histological patterns in malignant and benign tumors; indicating that there is a significant correlation between uncommon histological patterns such as spindle or small cell and malignancy (*P*=0.04). Additionally, there was a significant correlation between PASS score and malignancy (*P*=0.002) (19).

Immunohistochemical staining for *PTTG1* was negative for most of both malignant and benign tumors. However, few positive specimens were detected in malignant tumors with spindle cell pattern (*P*<0.013). As showed in Table 3; although positive staining for Rb marker was observed more commonly in malignant tumors, no significant difference was observed between malignant and benign tumors (*P*=0.087). Table 4 shows the results of *PTTG1* staining regarding tumor histological pattern. Specificity and positive predictive value (PPV) of *PTTG1* staining for distinction between malignant and benign cases were 100% (Table 5).

Table 2. The prevalence of different Histologic patterns of the tumors regarding behavior (19)

Histologic feature	Tumor type		
	Benign	Malignant	Total
Solid	7	1	8
Nest	22	1	23
Trabecular	13	1	14
Spindle	1	4	5
Small cell	1	4	5
Total	44 (100%)	11 (100%)	55 (100%)

P. Value =0.04

Table 3. Expression of Rb and PTTG in 44 Benign and 11 Malignant Pheochromocytomas

Immunohistochemical scoring	Rb			PTTG		
	Benign	Malignant	Total	Benign	Malignant	Total
1	21 (47.7%)	2 (18.2%)	23 (41.8%)	44 (100%)	9 (81.8%)	53 (96.4%)
2	4 (9.1%)	1 (9.1%)	5 (9.1%)	0 (0%)	1 (9.1%)	1 (1.8%)
3	8 (18.2%)	1 (9.1%)	9 (16.4%)	0 (0%)	0 (0%)	0 (0%)
4	11 (25.0%)	7 (36.6%)	18 (32.7%)	0 (0%)	1 (9.1%)	1 (1.8%)
Total	44 (100%)	11 (100%)	55 (100%)	44 (100%)	11 (100%)	55 (100%)
	P-Value =0.087			*P*- Value= 0.037		

Table 4. The prevalence of tumors with a certain histologic pattern regarding *PTTG1* score

PTTG1	Histologic features					
	Solid	Nest	Trabecular	Spindle cell	Small cell	Total
1	8(16.3%)	23 (46.9%)	14 (28.6 %)	3 (6.1%)	1 (2.0%)	49(100%)
2	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)
3	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
4	0 (0%)	0 (0%)	0 (0%)	1 (20%)	4 (80%)	5 (100%)
Total	8 (14.5%)	23 (41.8%)	14 (25.5%)	5 (9.1%)	5(9.09%)	55 (100%)

P. Value= 0.013

Table 5. Sensitivity, Specificity, PPV, and NPV

Immunohistochemical marker	Sensitivity	Specificity	PPV	NPV
PTTG1	18.2	100	100	83
Rb	81.8	47.7	28.1	91.3
***PTTG1* and Rb**	18.2	100	100	83
***PTTG1* or Rb**	81.8	47.7	28.1	91.3

Discussion

Pheochromocytoma is an uncommon neuroendocrine tumor of the adrenal medulla or sympathetic ganglia that arises from chromaffin cells that can secrete catecholamines. It can occur sporadically or as part of a genetic syndrome with common symptoms of catecholamine excess such as hypertension and palpitations. Its only potential treatment is complete surgical removal. The prevalence of malignant phaeochromocytoma ranges from 7% (20) to 35% (21) according to the application of different criteria. While the overall survival rate for patients with benign pheochromocytoma is similar to those of aged-matched controls, the 5-year survival rate of patients with metastatic malignant pheochromocytoma is 40% (22).

It should be noted that determining the malignant potential of endocrine tumors based on histological criteria is not reliable, and other clinical and laboratory findings should be also considered. Due to the fact that classical pathologic features of malignancy such as nuclear hyperchromatism and increased presence of mitotic figures are commonly observed in both benign and malignant forms, histological criteria are of limited value in predicting the malignant behavior of pheochromocytoma (1). For this reason, using absolute criteria to distinguish between malignant and benign pheochromocytoma before the incidence of metastases, seems logical. Immunohistochemical markers of cell proliferation can be used to evaluate tumor cell populations; increased expression of which can be associated with a poor prognosis (6,19,23).

Similar reports about predictive factors of malignancy in phaeochromocytoma with conflicting results exist (5-6). As similar to previous studies, significantly larger tumor diameters were found in patients with malignant phaeochromocytoma (4-5,21). However, no significant correlation was detected between age, sex or necrosis with malignant behavior of the pheochromocytoma in current study; this is consistent with some other studies that revealed that no correlation exists between age and behavior of tumor (3,24). Additionally, we observed a significant correlation between rare patterns such as spindle like and malignancy in phaeochromocytoma (19). This confirms the findings of other investigators who suggested that spindle cell components might indicate malignant potentials (25-26).

PTTG1 is over-expressed in multiple tumors and is a novel oncogene with a significant role in a wide range of carcinomas (7,27). The highest expression of *PTTG1* is observed in the testis (7-8). During spermatogenic cycle, *PTTG1* is expressed in germ cell that suggests its potential role in rapidly proliferating cells (28). In most normal human tissues, *PTTG1*1 shows low expression as compared to malignant cells (27,29-30); in which *PTTG1* is expressed at higher than normal levels. Regarding these findings, this gene is possibly involved in tumor angiogenesis. Furthermore, the correlation between higher expression of *PTTG1* and higher pathological stage, lymph node invasion and metastasis has been previously showed; suggesting that *PTTG1* acts as a prognostic marker of invasive cancer (31).

Besides the previously measured parameters, the present study suggests that *PTTG1*-positive immunohistochemical staining in pheochromocytoma may help to discriminate between benign and malignant tumors; considering its high specificity and PPV. As far as the presence of metastatic lesions is the only criteria for diagnosis of malignant pheochromocytoma when the prognosis is very poor, *PTTG1* may prove to be an important prognostic marker to improve patients' survival rate.

Conflicting reports exist about the potential role of Rb for distinguishing benign versus malignant tumors. Gupta *et al.*, reported a significant correlation between Rb-positive staining and malignancy of pheochromocytoma (32) while the findings of other investigations revealed completely opposite results (23,33). According to present findings, although the prevalence of Rb-positive staining was higher in malignant cases, no significant expression was detected in Rb as an indicator of malignancy in pheochromocytoma (P=0.087).

In conclusion, *PTTG1* is differentially expressed by benign and malignant pheochromocytoma with low sensitivity; meaning that positive IHC result for *PTTG1* is of great value to predict malignant behavior of

pheochromocytoma with 100% specificity and 100% PPV; especially when used in a panel with potential sensitive markers.

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