PAX8-PPARγ Oncogene in Follicular Thyroid Tumors: RT-PCR and Immunohistochemical Analyses

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ABSTRACT

US-guided fine needle aspiration cytology is currently the best diagnostic tool for thyroid nodules. However, it is not sensitive and specific enough for differentiating between benign and malignant follicular tumors. A potentially useful marker for this differentiation is the PAX8-PPARγ rearrangement, identified in follicular thyroid carcinomas, but not in follicular adenomas or other types of thyroid tumors. The aim of this research was to determine the clinical significance of the PAX8-PPARγ oncogene in diagnostics follicular thyroid tumors. The study included 62 patients with follicular or Hürthle cell tumors. Gene expression was determined by reverse transcription-polymerase chain reaction (RT-PCR) from paraffin embedded tissues, and PCR products were checked using the agarose gel electrophoresis. The immunohistochemical analysis was performed on archive paraffin embedded tissues with the monoclonal PPARγ antibody. The statistical analysis has indicated that neither the expression of PAX8-PPARγ mRNA, nor the immunohistochemical analysis with the PPARγ antibody correlate with the pathological diagnosis. The oncogene PAX8-PPARγ has not met the expectations as a reliable tumor marker for differentiation between benign and malignant thyroid tumors, which makes the only reliable histological criteria – capsular and vascular invasion.

Key words: follicular thyroid tumors, PAX8-PPARγ rearrangement, RT-PCR, immunohistochemistry

Introduction

According to the 2004 World Health Organization classification of thyroid tumors, they occur as carcinoma, adenomas and related tumors, and other thyroid tumors1.

Whereas for most thyroid tumors there are clear cytological criteria, the boundaries between adenomatoid nodule, a follicular adenoma and well differentiated follicular carcinoma are not cytologically well defined. It is therefore necessary to find clinically reliable tumor markers which would make this differentiation possible and thus reduce the number of unnecessarily surgeries2–5.

The most common mutations of follicular carcinomas include RAS mutations and PAX8-PPARγ rearrangement, chromosomal translocation between the thyroid transcription factor PAX8 and the nuclear receptor peroxisome proliferator-activated receptor γ (PPARγ). PAX8-PPARγ was, et first, thought to be restricted to FCa (Kroll et al., 2000)6, but other groups have detected the expression of PAX8-PPARγ gene not only in FCa but also in follicular thyroid adenomas7–13. In the present study, we report the analysis of PAX8-PPARγ gene in a series of follicular thyroid tumors, as well as immunohistochemical analysis with PPARγ antibody.

Methods

The research included 62 patients with cytologically diagnosed follicular or Hürthle cell tumors, histologically verified as follicular or Hürthle cell adenomas or carcinomas.
Gene expression was determined by reverse transcription-polymerase chain reaction (RT-PCR). Total mRNA was isolated from paraffin embedded tissues using High Pure RNA Paraffin Kit (Roche Applied Science, EU) and translated into cDNA by reverse transcriptase PCR (SuperScript II RT, Invitrogen, EU). PAX8-PPARγ rearrangements were detected by PCR using one reverse (PAX8-R) and three forward pairs of primers (PAX8-7, PAX8-8 and PAX8-9). The nucleotide sequence of the primers was based on the mRNA sequences deposited in GenBank. Human hypoxanthine phosphorybosyl-transferase 1 (hHPRT1) was used as a referent housekeeping gene. Products of PCR reactions were separated by 1% agarose gel and analyzed. A 362-bp amplification band corresponds to the presence of exons 7, 8 and 9 of the PAX8 gene, a 173-bp band corresponds to PAX8 exons 7 and 9 and a 68-bp band is the result of the fusion between PAX8 exons 7 and exon 1 of PPARγ.

Immunohistochemistry was performed on formalin-fixed paraffin-embedded sections attached to silanized slides. Microwave antigen retrieval was performed at 95°C for 30 minutes in 10-mM citrate buffer (pH 6.0). Slides were incubated with PPARγ monoclonal antibody E8 (SC-7273P, 1:100, Santa Cruz, CA, USA) for 60 minutes on a DAKO Autostainer. The Universal DAKO LSAB-Plus Kit detection system (avidin-biotin complex) and DAB chromogen (DAKO) were used according to manufacturer’s protocol for immune complex detection. Sections were counterstained with Mayer’s haematoxylin, dehydrated and mounted. A tumor with PAX8-PPARγ rearrangement detected by RT-PCR was used as a positive control. Nuclear staining was scored as none (0), weak (1+), moderate (2+), or strong (3+).

The statistical analysis was conducted using the SPSS 9.0, and included the analysis of categorical variables (2x2 and RxC contingency tables), as well as the analysis of correlation and variance.14-16.

Results
Detection of the PAX8-PPARγ rearrangement

RT-PCR method has proven the expression of PAX8-PPARγ mRNA in 33% of follicular carcinomas, 14% of Hürthle cell carcinoma, but also in 33% of follicular and 19% of Hürthle cell adenoma (Figure 1). Therefore, neither individually nor globally (steam comparison of individual categories) there is no differences in expression of PAX8-PPARγ mRNA among the analyzed histologic categories (p=0.631, Freeman-Halton exact test, Table 1). Furthermore, there is no difference in expression between different cytological categories (p=0.281, Fisher exact test), or between adenomas and carcinomas (p=0.177, Fisher exact test).

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p=0.631, Freeman-Halton exact test; FA – follicular adenoma, FC – follicular carcinoma, HA – Hürthle adenoma, HC – Hürthle carcinoma

Immunohistochemistry

Immunohistochemical staining with PPARγ antibodies revealed medium and strong positive results in 3/16 follicular adenomas (18%), 3/18 follicular carcinomas (17%), 1/15 Hürthle cell adenomas (7%), 2/13 Hürthle cell carcinomas (15%) (Figure 2). Contingency table analysis has shown that there are no differences in PAX8-PPARγ expression, neither globally nor in individually-paired comparisons of individual categories, between the analyzed histological (global p=0.911, Freeman-Halton exact test, Table 2) and cytological categories (global p=0.926, Freeman-Halton exact test).

Fig. 1. RT-PCR analysis of thyroid tumors. 362 bp: presence of exons 7, 8 and 9 of the PAX8 gene; 68 bp: fusion between PAX8 exon 7 and exon 1 of PPARγ.

Table 1

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p=0.631, Freeman-Halton exact test; FA – follicular adenoma, FC – follicular carcinoma, HA – Hürthle adenoma, HC – Hürthle carcinoma

Fig. 2. Immunohistochemical staining with PPARγ antibody; strong nuclear staining (3+).
found the PAX8-PPAR$_g$ oncogene in 35% follicular carcinomas, and in as many as 55% of follicular adenomas.$^{10}$

Hibi et al. have analysed the expression of PAX8-PPAR$_g$ gene in Japanese with nodular thyroid changes. They explain the negative findings in follicular adenomas and carcinomas, papillary carcinomas, nodular hyperplasia, and in normal thyroid tissue by a likely ethnic-based tendency for translocation.$^{17}$

The expression of PAX8-PPAR$_g$ gene results in the increased synthesis of PPAR$_g$ protein. In most studies cited above, immunohistochemical staining using PPAR$_g$ antibody was also conducted. In PAX8-PPAR$_g$ positive tumors was found strong positivity of the nucleus to PPAR$_g$ antibodies.$^{6,18}$ Light and medium positivity of the nucleus and cytoplasm is found in adenomas, non-tumour changes, and in normal thyroid tissue. In 2005 Sahin et al. analysed a group of 108 patients with histologically determined follicular tumors and performed the immunohistochemical staining using PPAR$_g$ antibody in order to determine the value of immunohistochemistry as an alternative RT-PCR method to distinguish between adenomas and carcinomas. They found the immunopositivity in 57% follicular carcinomas, 4% Hurthle cell carcinomas, and in 13% follicular adenomas, and concluded that this method is useful in distinguishing between these diseases. Moreover, according to the clinical findings they collected, they state that there is a better prognosis for patients with immunopositivity to PPAR$_g$ antibody.$^{19}$ Most authors state the opposite, stressing a more frequent vascular invasion and worse long-term prognosis for patients with PAX8-PPAR$_g$.$^{7,14,22}$

In this research we used the method of polymerase chain reaction with reverse transcription to determine the expression of PAX8-PPAR$_g$ mRNA from paraffin embedded tissues in 62 patients with cytologically and histologically verified follicular or Hurthle cell carcinomas. RT-PCR method has proven the expression of PAX8-PPAR$_g$ mRNA in 33% of follicular carcinoma, 14% of Hurthle cell carcinoma, but also in 33% of follicular and 19% of Hurthle cell adenoma. Therefore, neither individually nor globally (steam comparison of individual categories) there is no differences in expression PAX8-PPAR$_g$ mRNA among the analyzed histological categories. Similarly, adenomas and carcinomas do not differ regarding the expression of mRNA. These findings support the results reported in the studies discussed above.$^{9,10,23,24}$

Immunohistochemical staining with PPAR$_g$ antibody in archive paraffin embedded tissues revealed medium and strong positivity in 18% follicular adenomas, 17% follicular carcinomas, 7% Hurthle cell adenomas, and in 15% Hurthle cell carcinomas. Contingency table analysis has shown that there are no differences in PAX8-PPAR$_g$ expression, neither globally nor in individually-paired comparisons of individual categories, between the analyzed histological and cytological categories. Moreover, adenomas and carcinomas do not differ concerning the PAX8-PPAR$_g$ immunopositivity.

The comparison of the PAX-PPAR$_g$ gene expression and immunohistochemical positivity showed no statistical significance.

This investigation has shown that the expression of PAX8-PPAR mRNA, as well as the immunohistochemical positivity do not correlate with pathohistological diagnosis.

The aim of these study was to determine the value of the PAX8-PPAR oncogene in differentiating between follicular adenoma and carcinoma. Its relation to follicular thyroid tumors is undoubtedly proven, but our results do not confirm the value of this oncogene as a reliable marker for distinguishing these tumors. Therefore, it is questionable whether all patients with cytologically diagnosed follicular tumors may avoid surgery.

Preliminary studies on the significance of PAX8-PPAR gene for differentiating between follicular tumors were conducted on a small number of patients. To confirm the significance of this oncogene as a prognostic factor for thyroid tumors further studies and larger samples are required.

REFERENCES


ONKOGEN PAX8-PPAR U FOLIKULARNIM TUMORIMA ŠTITNJAČE – RT-PCR I IMUNOHISTOKEMIJSKA ANALIZA

SAŽETAK

Ciljana citološka punjka pod kontrolom ultrazvuka metoda je izbora u dijagnostici čvorastih promjena, no nedovoljno objašnjena i specifična za razlikovanje benignih i malignih folikularnih tumora. Potencijalno koristan marker za njihovu diferencijaciju je onkogen PAX8-PPAR, identificiran kod folikularnog karcinoma, a ne kod folikularnog adenoma ni drugih tumora štitnjače. Cilj ovog istraživanja je određivanje kliničkog značaja PAX8-PPAR gena u dijagnostici folikularnih tumora štitnjače. U istraživanju je uključeno 62 ispitanika sa folikularnim ili Hürthleovim tumorom. Ekspresija gena je određena metodom lančane reakcije polimeraze s obrnutom transkripcijom (RT-PCR) iz parafinskih rezova, a PCR produkti provjereni elektroforezom na agaroznom gelu. Na arhivskim parafinskih rezovima je izvršena immunohistokemijska analiza PPAR产物 protutijelom ne korelira s patohistološkom dijagnozom. Onkogen PAX8-PPAR nije opravdava očekivanja u smislu pouzdanog tumorskog markera koji bi omogućio diferencijaciju benignih i malignih tumora štitnjače, te je i dalje jedino pouzdan histološki kriterij – proboj kapsule i vaskularna.