

Peroxisome Proliferator Activated Receptor Gamma Immunohistochemical Expression in Human Papillary Thyroid Carcinoma Tissues. Possible Relationship to Lymph Node Metastasis

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Abstract. *Background:* Peroxisome proliferator activated receptor gamma (PPAR γ) involvement in thyroid tumorigenesis has recently been studied, especially in follicular neoplasms. Conflicting results concerning the regulation of this receptor in human papillary carcinoma have been reported. Therefore, we quantitatively assessed PPAR γ immunohistochemical expression in papillary carcinoma in comparison with other types of thyroid tumors and we evaluated its relationship with clinical criteria of aggressiveness. *Materials and Methods:* Immunohistochemistry (IHC) was performed on 56 human thyroid papillary carcinomas (PTC), 9 follicular carcinomas (FTC), 20 follicular adenomas (FA) and 18 Hürthle cells adenomas. PTC were divided into subgroups according to some aggressiveness criteria: tumor size, capsular invasion, lymph node metastasis. Immunostaining was semi-quantitatively analyzed using image analysis software. *Results:* Strong nuclear PPAR γ expression was detected in a large number of PTC (42%), similar to that found in FTC (44%) or FA (63%). Only Hürthle cell adenoma showed a significantly lower proportion of PPAR γ -positive immunoreactivity (11%, $p < 0.05$). Cases of PTC-associated lymph node metastasis showed a higher percentage of PPAR γ -positivity than other case categories (63% vs. 20%), a result which was also noticed when comparing large PTC with infracentimetric tumors (60% vs. 39%, $p < 0.05$). *Conclusion:* These results, combined with recently published data, suggest that the intense PPAR γ immunostaining revealed in PTC could be related to high

wtPPAR γ gene levels. Moreover, they corroborate a strong relationship between PPAR γ expression and tumor progression. PPAR γ IHC evaluation is not a valuable differential diagnostic tool for thyroid tumors but it could be a reliable marker of papillary carcinoma aggressiveness and a potential predictor for an eventual therapy by PPAR γ agonists.

The prognosis of papillary thyroid carcinomas, the most common form of thyroid cancer, remains uncertain and clinical, histopathological or epidemiological features are assessed as predictors of tumor aggressiveness (1-3). In order to improve prognostic evaluation, some immunohistochemical markers have been proposed, namely galectin-3 (4), p27 and cyclin D1 (5).

Peroxisome proliferator activated receptors (PPARs), members of the nuclear hormone receptor superfamily, play an important role in carcinogenesis, particularly in cell cycle control and differentiation. A high expression of PPAR γ was reported in several types of tumor cells (6).

Recently, PAX8/PPAR γ rearrangements were described in follicular thyroid carcinomas and adenomas and a suppressive function on wild-type PPAR γ (wtPPAR γ) was demonstrated (7). No PAX8/PPAR γ fusion oncogene was identified in other types of thyroid tumor (8, 9).

Antiproliferative effects of PPAR γ ligands already noticed in breast, colorectal carcinoma or liposarcoma (10, 11) were reproduced in papillary thyroid carcinoma cell lines expressing PPAR γ mRNA (12, 13). Few data have been published using PPAR γ 's immunohistochemical (IHC) detection on routinely processed thyroid tissue sections (7-9, 14). Initially, strong diffuse PPAR γ immunostaining in follicular thyroid neoplasia was considered relevant for the presence of PAX8/PPAR γ oncogene (7, 8), though a recent study has questioned this (15). The results concerning papillary thyroid carcinoma are controversial (13, 16). The

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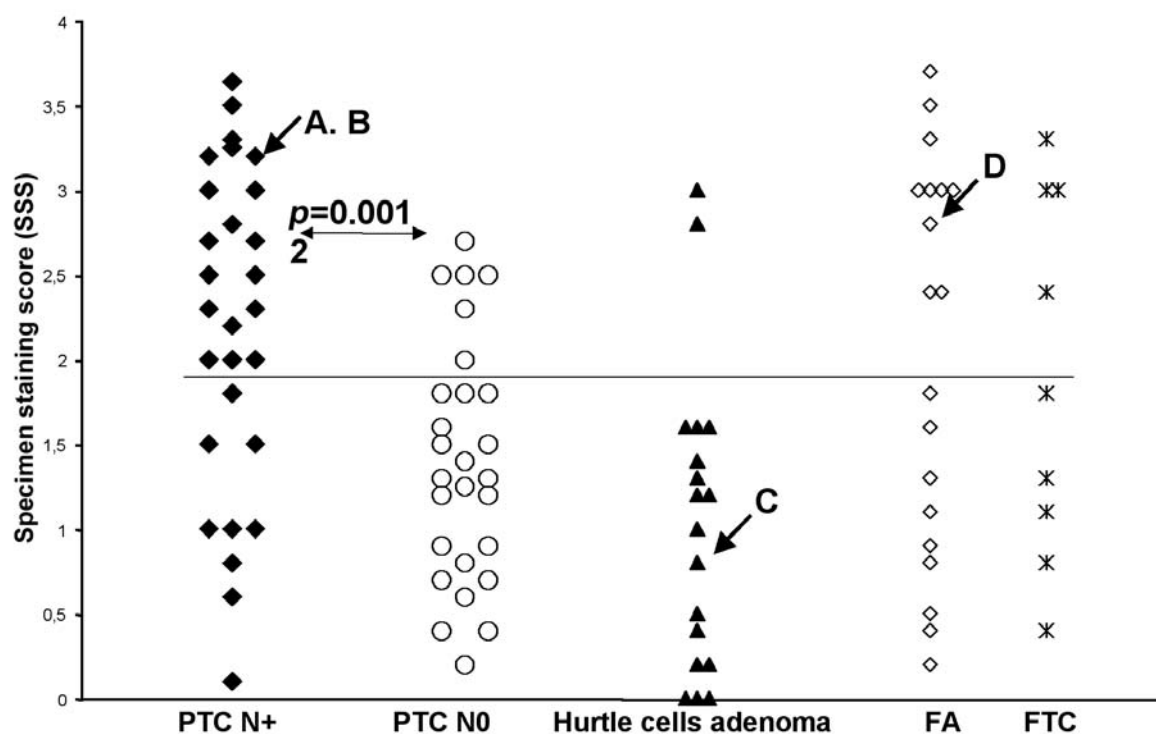


Figure 1. *PPAR* γ specimen staining score (SSS) in studied thyroid tumor groups: similar distribution in PTC and follicular neoplasia; lower values in Hürthe cell adenoma. The SSS cut-off value ≥ 2 revealed a higher percentage of positive immunoreactivity among PTC N0 than PTC N+ (χ -2 test $p=0.0012$)

PTC, papillary thyroid carcinoma with (N+) or without lymph node metastasis (N0); FTC, follicular thyroid carcinoma; FA, follicular adenoma. Flashed cases (A,B,C,D) correspond to photos from Figure 2.

present study was performed to semi-quantitatively assess the immunohistochemical *PPAR* γ expression in papillary tumors comparatively with other types of thyroid neoplasia and to evaluate its relationship with clinical criteria of aggressiveness.

Materials and Methods

Tissue samples. We evaluated thyroid neoplasms, including 56 papillary thyroid carcinomas (PTC), 9 follicular thyroid carcinomas (FTC), 20 follicular adenomas (FA) and 18 Hürthle cells adenomas. The tumors were classified according to the widely accepted histopathological criteria (17). Papillary carcinomas were diagnosed based on characteristic cytological features and architectural (papillary or follicular) patterns. Subgroups of this category were based on some aggressiveness criteria: tumor size (> 1 cm), capsular invasion, lymph node metastasis, revealed before or immediately after the surgery (histological or isotopic confirmation). Follicular carcinomas were distinguished from adenomas on the basis of cytological patterns (18) and invasion of the edges of the tumor and/or invasion of blood vessels in or beyond the capsule. Tumors were classified as Hürthle cell neoplasm when the predominant cell subtype (almost 75 percent of cells) had oncocyctic features (19).

Immunohistochemistry. All thyroid specimens were fixed in 10% formalin for 24 hours. In all cases immunohistochemical analysis was performed on routinely paraffin-embedded sections. The tissues were sectioned onto positively-charged slides (SuperFrost Plus, Menzler-Glaser, Freiburg, Germany) and dewaxed with xylene and alcohol. Water bath antigen retrieval was performed at 97°C for 40 minutes in TRS (Target Retrieval Solution, DAKO Corporation, Carpinteria, CA, USA) pH 9.9. Thereafter, slides were incubated with the primary antibody (*PPAR* γ , SC-7273, 1:75, Santa Cruz Biotechnology, Inc. Santa Cruz, CA, USA) for 40 minutes at room temperature on a DAKO TechMate 500 Plus. The EnVision detection system and DAB (diamino-benzidine) chromogen (K4006, DAKO Corporation) were used according to the manufacturer's protocol for immune complex detection.

Cell immunostaining evaluation. Two semiquantitative methods, visual and a computerised image analysis, were employed. **Visual analysis.** Immunostaining was evaluated by two independent observers (BG, JMD) blinded to clinical data previously established. Nuclear staining of *PPAR* γ was semiquantitatively scored by a method previously described (4, 20). We simultaneously evaluated both the distribution heterogeneity and the intensity of the brown-stained cells. Counts were performed in 10 random high-power fields. At least 1000 cells were counted. Each of the following degrees of staining intensity: absent or weak (-), moderate (1+), strong (2+, 3+, 4+), were adjusted by

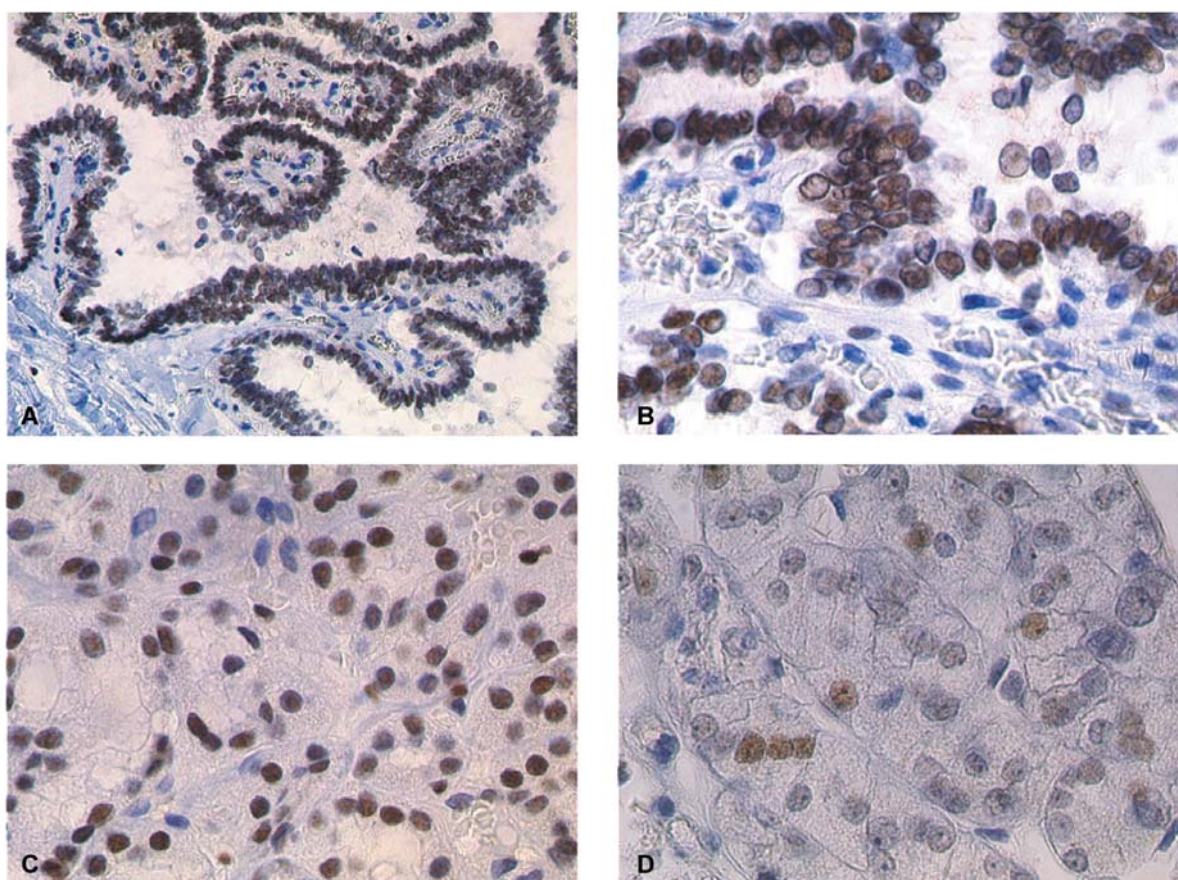


Figure 2. Strong, diffuse PPAR γ nuclear immunostaining (SSS=3.3) in papillary thyroid carcinoma (PTC) N+, original magnification (A) $\times 40$, (B) $\times 100$; similar nuclear immunoreactivity and diffuse distribution (SSS=2.8) in a follicular adenoma, original magnification $\times 100$ (C); scattered faint staining (SSS=0.8) in a Hürthle cell adenoma, original magnification $\times 100$ (D).

the corresponding percentage of cells. The results were added to yield a specimen staining score (SSS). According to others (8), a sample was considered positive when a diffuse staining of strong intensity (≥ 2) was noticed. Thus, a corresponding SSS cut-off value was set at 2. *Computerised image analysis.* All smears stained with PPAR γ antibody were also analyzed by a computerised system (SAMBA Technologies, Grenoble, France) which allowed quantification of the percentage of stained cells (%SC), the average staining intensity (ASI) in these neoplastic cells (at least 1000) and calculation of a Computerised Lecture Index (CLI = %SC \times ASI).

Statistical analysis. Staining characteristics of each category of thyroid tumors were compared by unpaired *t*-test, significant for a $p < 0.05$. When comparing the subgroups of papillary carcinoma a χ^2 test was also employed ($p < 0.05$).

Results

In thyroid tissue samples, PPAR γ immunostaining was nuclear and was heterogeneous in distribution and intensity. Representative images of immunohistochemical staining with anti-PPAR γ antibody are shown in Figure 1.

Table I. IHC expression to anti-PPAR γ antibody in human thyroid tumor tissues; SSS (specimen staining score); CLI (computerized lecture index).

Tumor type	PPAR γ	
	Visual quantitation % of cases PPAR γ - positive (SSS ≥ 2)	Computerized analysis Mean SSS Mean CLI
Follicular adenoma (n=20)	63%	2.03 14.7
Hürthle cell adenoma (n=18)	11%	1.04 6.5
Papillary carcinoma (n=56)	41%	1.78 14.3
Papillary carcinoma N+ (n=27)	63%	2.12 14.5
Papillary carcinoma N0 (n=29)	20%	1.40 11.7
Follicular carcinoma (n=29)	44%	1.90 14.4

The two semiquantitative analysis methods, classical and automatic, were compared in the entire group of specimens and a correlation index of 0.76 was found between SSS and CLI. The means of PPAR γ expression scores (SSS, CLI) in each tumor type are summarised in Table I.

All subtypes of thyroid tumors expressed PPAR γ at a different level. When comparing staining scores of every category of tumors by unpaired *t*-test, only Hürthle cell adenoma showed significantly lower values (1.04 ± 0.05) than FA (2.03 ± 0.26), FTC (1.9 ± 0.3) or PTC (1.78 ± 0.12) ($p < 0.05$). Considering the SSS cut-off value of 2, only 2 out of 18 Hürthle cell lesions were PPAR γ -positive.

PPAR γ immunoreactivity on papillary carcinoma tissue samples was found to be positive in 42% of cases (specimen staining score > 2). Among the 56 cases of papillary carcinomas, lymph node metastasis was identified in 27 cases. The mean SSS was significantly higher in primary lesions of PTC with metastasis (PTC N+) than those without (PTC N0) (2.12 ± 0.19 vs. 1.40 ± 0.13 , $p = 0.0017$) (Figure 2).

Considering other criteria of tumor aggressiveness, infracentimetric PTC ($n = 26$) had a significantly lower SSS score (1.49 ± 0.14) than larger tumors ($n = 30$, 2.04 ± 0.2) ($p = 0.02$). Lymph node metastasis was significantly lower for infracentimetric tumors than for larger ones ($9/26$ vs. $18/30$, χ^2 test $p = 0.05$). SSS values showed no significant difference for extracapsular penetration criteria ($p = 0.14$).

Discussion

In our study we evaluated, by IHC, the expression of PPAR γ on a large cohort of human thyroid tumor tissues. While PPAR γ IHC detection has been described in literature data (7-9, 14), a detailed quantification has rarely been performed (15).

We found PPAR γ -positive cases in each studied category of thyroid neoplasia (11% of Hürthle cell adenoma, 42% of papillary carcinoma, 44% in follicular carcinoma and up to 60% in follicular adenoma). So far PPAR γ IHC is not a suitable differential diagnostic tool for thyroid tumors, in concordance with recent results (15). To our knowledge this study is the first to show that a large proportion of human PTC tumor cells can be strongly stained for PPAR γ .

Recent studies concerning the involvement of PPAR γ in thyroid tumoral pathogenesis or progression have focused on RT-PCR as the main technique of identification. Kroll *et al.* were the first to prove the presence of fusion oncogene PAX8/PPAR γ in follicular thyroid carcinoma (7). Later, two studies detected the same rearrangement in follicular adenoma (9, 14). For papillary carcinoma the absence of this fusion oncogene was demonstrated (8) and Ohta *et al.* detected wtPPAR γ mRNA in several cell lines and in 3 out of 6 cases of human papillary carcinomas (12, 13). Therefore we suggest that the intense immunostaining

revealed in our study for this category could be related to a high wt PPAR γ expression.

We showed a significantly higher percentage of PPAR γ -positive cases in primary PTCs with lymph node metastasis than those without (63% vs. 20%, $p < 0.05$). Similar results were also noticed when comparing larger papillary carcinoma with infracentimetric tumors (60% vs. 39%). Our observations were consistent with the hypothesis that PPAR γ expression might be related to tumor progression. This is in agreement with the Ohta *et al.* study where it was shown that PPAR γ -negative cells, transplanted to nude mice, could not form a tumor (13) and with Martelli *et al.*'s results, suggesting connections of PPAR γ with cell cycle control (12).

Among studied lesions, Hürthle cell adenoma weakly stained for PPAR γ . The absence of PAX8/PPAR γ rearrangement in oncocytomas has already been shown by Nikiforova *et al.* (8). Thus our results suggest a weak expression or the absence of wtPPAR γ in these tumors and give additional strength to the evidence of a different behavior of these tumors compared to other benign thyroid lesions.

Concerning the role of PPAR γ agonists on thyroid tumors progression, two *in vitro* studies showed an antitumoral effect: i) synthetic or natural ligands of PPAR γ inhibited growth of the four cell lines of thyroid papillary carcinoma expressing PPAR γ (13); ii) the effect of ciglitasonone was more pronounced in a highly malignant thyroid cell line consistent with abundant PPAR γ gene expression and absence in a papillary cell line showing no PPAR γ mRNA (12). Unfortunately, Kroll *et al.* showed that the PAX8/PPAR γ fusion oncogene, expressed in a contingent of follicular neoplasia, inhibited thiazolidindione-induced gene trans-activation by wtPPAR γ (7). Hence, for thyroid follicular tumors, a PAX8/PPAR γ detection should be considered before starting a therapeutic trial by PPAR γ agonists.

Our results for PPAR γ IHC expression in papillary thyroid tumors, in association with literature data showing the absence of antitumoral effects of PAX8/PPAR γ and PPAR γ ligands (8), suggest that PTCs, especially those with a high dissemination potential, are putative candidates for treatment with PPAR γ agonists.

In conclusion, PPAR γ IHC evaluation is not a valuable diagnostic tool for the identification of thyroid tumors but it could be a reliable marker of papillary carcinoma lymph node dissemination and a potential predictor for an eventual therapy by PPAR γ agonists.

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