

# Immunohistochemical detection of PPAR $\gamma$ receptors in the human pituitary adenomas: correlation with PCNA

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**Abstract:** The occurrence of peroxisome proliferator-activated receptors gamma (PPAR $\gamma$ ) was investigated in 51 human pituitary adenomas and in 6 non-tumoral human pituitary tissue samples. Moreover, the correlation between PPAR $\gamma$  and the proliferating cells nuclear antigen (PCNA) - immunocytochemical proliferation marker was evaluated. The receptors and PCNA were detected by immunohistochemical methods using the polyclonal anti-PPAR $\gamma$  and the monoclonal anti-PCNA antibodies, respectively. PPAR $\gamma$  were found in all examined tissues. The mean percentage of cells with positive nuclear reaction was 3-fold higher in pituitary adenomas in comparison with non-tumoral pituitary tissues. The strongest expression of PPAR $\gamma$  was observed in somatotropinomas. Besides the nuclear reaction, which is typical for PPAR $\gamma$ , positive immunostaining was also observed in the cytoplasm. It was clearly stronger in pituitary adenomas than in non-tumoral pituitary tissues. A slight, statistically insignificant tendency towards negative correlation between PPAR $\gamma$  and PCNA was found in somatotropinomas, prolactinomas, corticotropinomas and gonadotropinomas. On the other hand, in null cell adenomas and "silent" corticotropinomas, a strong positive correlation between the expression of PPAR $\gamma$  and PCNA was observed. The strong expression of PPAR $\gamma$  in human pituitary adenomas and its possible involvement in control of cell proliferation in these tumors give a good reason for the attempts of their treatment with PPAR $\gamma$  ligands.

**Key words:** PPAR $\gamma$  - PCNA - Immunohistochemistry - Pituitary adenomas

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## Introduction

The peroxisome proliferator-activated receptors gamma (PPAR $\gamma$ ) are nuclear receptors involved in many physiological and pathological processes including glucose and lipid metabolism, atherosclerosis, inflammation and carcinogenesis [14]. PPAR $\gamma$  forms a heterodimer with retinoid X receptor (RXR) and regulates expression of target genes by binding to the PPAR $\gamma$  responsive element [16]. Polyunsaturated fatty acids and prostaglandin metabolite 15-deoxy- $\Delta^{12,14}$ -prostaglandin J<sub>2</sub> have been identified as endogenous ligands for PPAR $\gamma$  [9, 17]. It was shown that thiazolidinediones (TZD) used as insulin sensitizers in type 2 diabetes and some nonsteroidal antiinflammatory drugs are high affinity synthetic PPAR $\gamma$  ligands [21, 22]. The highest levels of PPAR $\gamma$  are detected in adipocytes, but these receptors are also widely

expressed in other normal cells and tissues such as monocytes, liver, skeletal muscle and neoplastic cells [8, 19]. It was proven in experimental investigations that activation of PPAR $\gamma$  receptors inhibits growth of many types of tumors [19]. The mechanism by which PPAR $\gamma$  ligands exert their oncostatic effects is very complex and still not clear. Several studies have proved that TZD (troglitazon, pioglitazon and rosiglitazon) can inhibit proliferation and induce apoptosis of cancer cells [19]. Moreover, in liposarcoma and breast, colon and prostate cancers, the synthetic PPAR $\gamma$  ligands cause the terminal differentiation [6, 15, 23, 24]. Recent studies have indicated that PPAR $\gamma$  are expressed in the endothelium of tumoral vessels and that TZD suppress tumor growth also *via* antiangiogenic action [26].

It was also shown that PPAR $\gamma$  agonists inhibited the *in vitro* growth of experimental and spontaneous pituitary adenomas and *in vivo* growth of experimental animal pituitary tumors [11, 12]. The antitumoral effects of these compounds seem to depend on the presence of PPAR $\gamma$  in tumor cells. In pituitary adenomas, the over-

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expression of PPAR $\gamma$  was found as compared with normal tissues [12].

The aim of our study was to estimate the occurrence of PPAR $\gamma$  in the human pituitary adenomas and in the non-tumoral human pituitary gland. Since the proliferating cells nuclear antigen (PCNA) is a good marker for immunohistochemical assessment of cell proliferation in pituitary adenomas [18], we examined the correlation between PPAR $\gamma$  and PCNA.

## Materials and methods

**Human pituitary adenomas.** Fifty one surgically removed pituitary adenomas were investigated. The tumors were fixed in Bouin-Hollande fixative and embedded in paraffin. Histological and immunohistochemical diagnoses were performed using the Herlant's tetrachrome staining and immunostaining with antisera against the pituitary hormones and their subunits, respectively. The following pituitary adenomas were included into present study: 11 somatotropinomas, 8 prolactinomas, 6 corticotropinomas (Cushing disease), 5 "silent" corticotropinomas (lack of clinical symptoms of hypercorticism), 14 gonadotropinomas and 7 null cell adenomas (tumors immunonegative for all the investigated pituitary hormones and their subunits). Moreover, 6 non-tumoral pituitary tissue samples (adjacent to removed microadenomas) were studied.

**PPAR $\gamma$  and PCNA immunostaining.** The paraffin sections (4  $\mu$ m) were immunostained with polyclonal anti-PPAR $\gamma$  antibody (Calbiochem, La Jolla, California, USA) and monoclonal anti-PCNA antibody (Dako Cytomation, Denmark). Both antibodies were used at 1:1000 dilution. The binding of anti-PPAR $\gamma$  antibody was detected using anti-rabbit IgG biotinylated goat antibody, streptavidin complex (StreptABC Complex/HRP, Dako) and 3,3'-diaminobenzidine. Detection of anti-PCNA antibody was performed using DakoCytomation Envision System, AP (Fast Red). The kit includes levamisole as an inhibitor of endogenous alkaline phosphatase and a Fast Red chromogenic substrate system. Finally, the sections were counterstained with hematoxylin. A negative control was obtained by omitting the incubation with primary antibodies. Under  $\times$  1000 magnification, the number of cell with immunopositive nuclear reaction was counted among 1000 randomly chosen tumor or pituitary cells.

**Statistical analysis.** The correlation between PPAR $\gamma$  and PCNA was analysed by the Pearson's correlation coefficient. Statistical significance was set at  $P < 0.05$ .

## Results

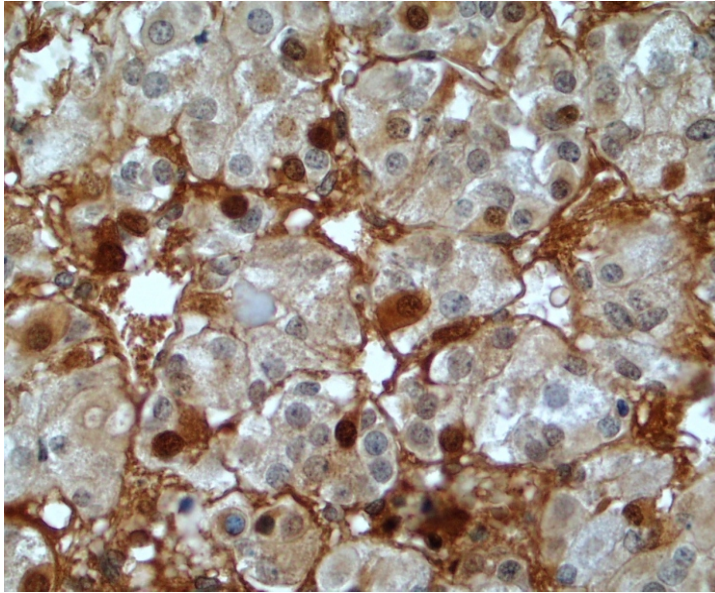
The occurrence of PPAR $\gamma$  was demonstrated in all the examined tissues (Figs. 1, 2). Besides the nuclear reaction, which is typical for PPAR $\gamma$ , positive immunostaining was also observed in the cytoplasm (Fig. 3) and in case of non-tumoral pituitary tissues also in the stroma (Fig. 1). The latter staining seems to be unspecific. Three types of PPAR $\gamma$  localization were noticed: in the nuclear region, in the cytoplasm and in both nucleus and cytoplasm. The mean percentage of the PPAR $\gamma$ -immunopositive cell nuclei in human pituitary adenomas and in non-tumoral pituitary tissues are shown in Figure 4. The strongest expression of PPAR $\gamma$  was observed in somatotropinomas. A relatively high number of immunoposi-

tive cell nuclei was found in prolactinomas. The mean percentage of cells with positive nuclear reaction was 3-fold higher in pituitary adenomas (mean 126%) in comparison with non-tumoral pituitary tissues (mean 41%). The cytoplasmic expression of PPAR $\gamma$  was also stronger in pituitary adenomas than in non-tumoral tissues (data not shown). Estimating the relationship between PPAR $\gamma$  and PCNA, a slight, statistically insignificant tendency towards negative correlation between PPAR $\gamma$  and PCNA was found in somatotropinomas, prolactinomas, corticotropinomas and gonadotropinomas (Fig. 5). On the other hand, in null cell adenomas and "silent" corticotropinomas, a strong positive correlation (statistically significant) was observed between the expression of PPAR $\gamma$  and PCNA (Fig. 6).

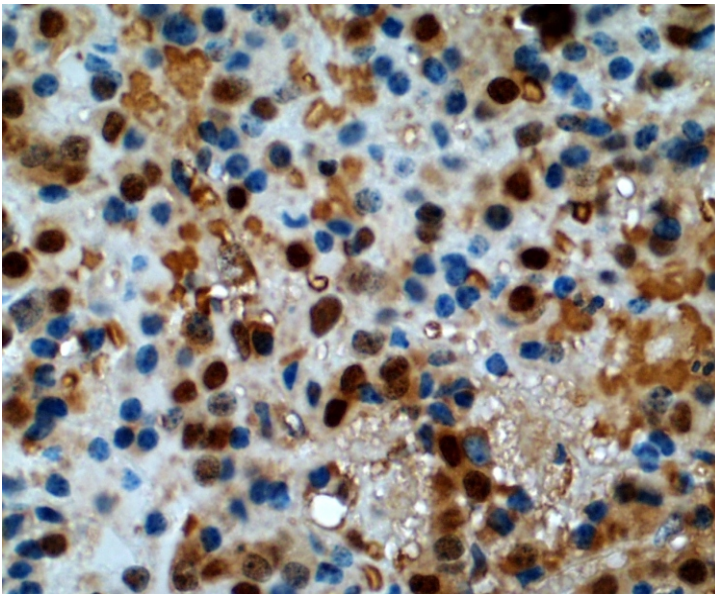
## Discussion

Our finding of increased expression of PPAR $\gamma$  in pituitary adenomas as compared to non-tumoral pituitary tissue is an agreement with the earlier observation of Haeney *et al.* [12]. This finding is also compatible with data showing the enhanced expression of PPAR $\gamma$  in several cancers such as: breast cancer [7], colon cancer [5], testicular cancer [13], glioblastoma [30], urinary bladder cancer [29] and differentiated thyroid cancers [10]. However, in some other cancers the expression of PPAR $\gamma$  is lower than in the corresponding normal tissues, *e.g.* in esophageal cancer [28] and choriocarcinoma [4]. Since PPAR $\gamma$  are nuclear receptors, their immunodetection within the nuclei was expected. The immunostaining in the cytoplasmic region observed in some cells is more difficult to explain. Such immunostaining was also observed in some other tumors like esophageal cancer and non-small lung cancer [28, 20]. Moreover, in salivary duct cancer only cytoplasmic PPAR $\gamma$  immunopositivity was detected [25]. Although the significance of the cytoplasmic PPAR $\gamma$  overexpression remains unknown, it might be speculated that it results from the retention of receptor protein in the cytoplasm. Such a retention could diminish or even make impossible the proper biological action of the receptors within the cell nucleus. Summing up, these findings indicate a role of PPAR $\gamma$  in oncogenesis including pituitary tumorigenesis.

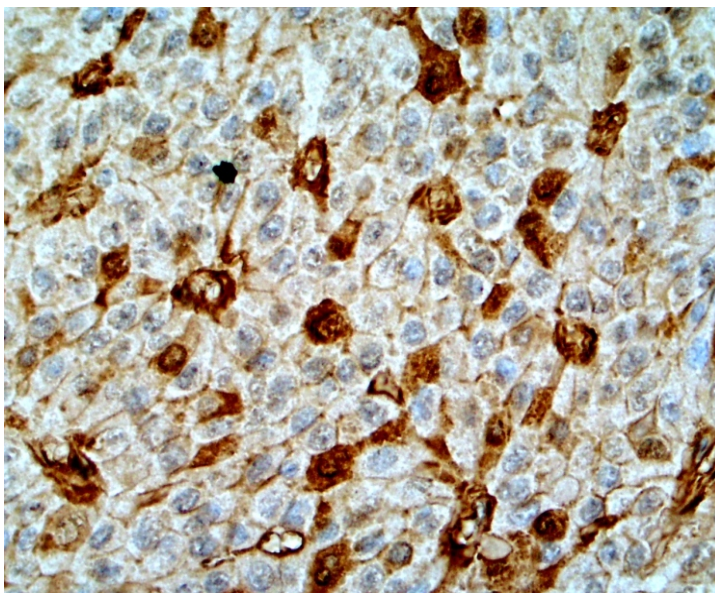
Since PCNA immunoreactivity was shown as a marker of proliferative activity and aggressiveness of pituitary adenomas [2, 27], we estimated the relationship of PPAR $\gamma$  and PCNA in the investigated tumors. The observed tendency towards the negative correlation in pituitary adenomas with exception of null cell adenomas and "silent" corticotropinomas suggests the involvement of PPAR $\gamma$  in the control of cell proliferation in these tumors. On the other hand, a strong positive correlation between PPAR $\gamma$  and PCNA in null cell adenomas and "silent" corticotropinomas indicate that this involvement



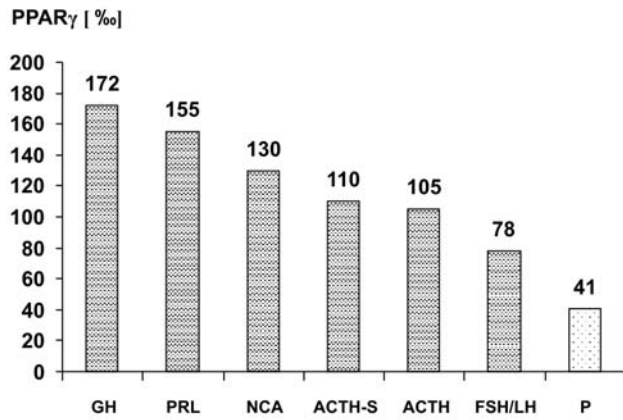
**Fig. 1.** The human non-tumoral pituitary tissue immunostained with anti-PPAR $\gamma$  antibody, showing a positive reaction (brown) in a few nuclei of glandular cells. The unspecific brown coloration can be seen also in the stroma.  $\times 400$ .



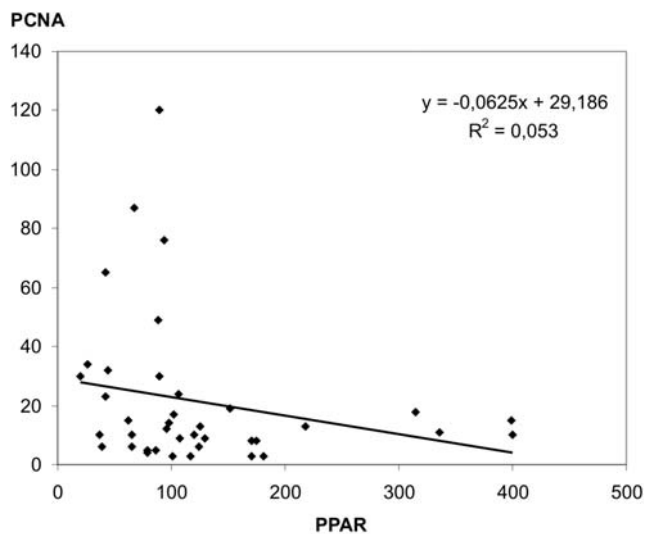
**Fig. 2.** The human somatotropinoma immunostained with anti-PPAR $\gamma$  antibody, showing a strong nuclear reaction in the tumor cells.  $\times 400$ .



**Fig. 3.** The human prolactinoma immunostained with anti-PPAR $\gamma$  antibody, showing immunopositive reaction in cytoplasm of tumoral cells besides the nuclear reaction.  $\times 400$ .

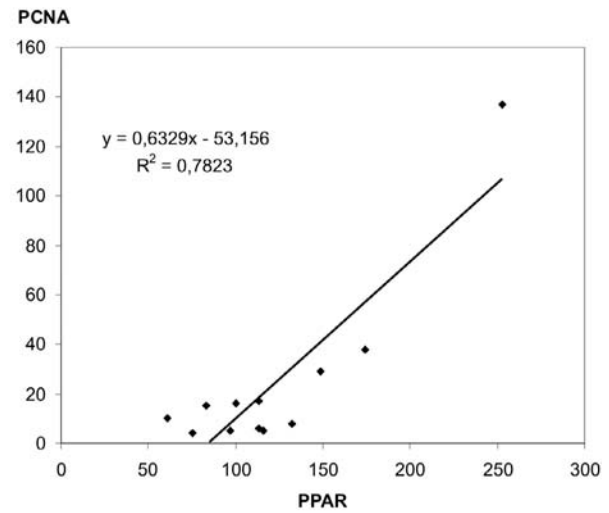


**Fig. 4.** The mean percentage of the PPAR $\gamma$ -immunopositive cell nuclei per 1000 randomly scored cell nuclei in human pituitary adenomas and in non-tumoral pituitary glands. GH - somatotropinomas; PRL - prolactinomas; NCA- null cell adenoma; ACTH-S - "silent" corticotropinomas; ACTH - corticotropinomas; FSH/LH -gonadotropinomas; P - non-tumoral pituitary tissue.



**Fig. 5.** Correlation between PPAR $\gamma$  and PCNA in somatotropinomas, prolactinomas, corticotropinomas and gonadotropinomas ( $r = -0.23$ ;  $p = 0.158$ ).

may be opposite in different pituitary adenoma types. The strong expression of PPAR $\gamma$  in human pituitary adenomas and its possible involvement in control of cell proliferation in these tumors justify the attempts of treatment of pituitary tumors with PPAR $\gamma$  ligands. Such attempts were already made in patients with Cushing disease [3, 1]. It remains to establish in further studies whether the high expression of PPAR $\gamma$  can predict the positive effect of TZD treatment of pituitary tumors.



**Fig. 6.** Correlation between PPAR $\gamma$  and PCNA in null cell adenomas and "silent" corticotropinomas ( $r = 0.88$ ;  $p < 0.001$ ).

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