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Immunohistochemical detection of PPARγ receptors in the human pituitary adenomas: correlation with PCNA

Katarzyna Winczyk and Marek Pawlikowski

Department of Experimental Endocrinology and Hormone Diagnostics, Institute of Endocrinology, Medical University, Łódź, Poland

Abstract: The occurrence of peroxisome proliferator-activated receptors gamma (PPARγ) was investigated in 51 human pituitary adenomas and in 6 non-tumoral human pituitary tissue samples. Moreover, the correlation between PPARγ 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-PPARγ and the monoclonal anti-PCNA antibodies, respectively. PPARγ 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 PPARγ was observed in somatotropinomas. Besides the nuclear reaction, which is typical for PPARγ, 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 PPARγ and PCNA was found in somatotropinomas, prolactinomas, corticotropinomas and gonadotropinomas. On the other hand, in null cell adenomas and "silent" corticotropinomas, a strong positve correlation between the expression of PPARγ and PCNA was observed. The strong expression of PPARγ 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 PPARγ ligands.

Key words: PPARγ - PCNA - Immunohistochemistry - Pituitary adenomas

Introduction

The peroxisome proliferator-activated receptors gamma (PPARγ) are nuclear receptors involved in many physiological and pathological processes including glucose and lipid metabolism, atherosclerosis, inflammation and carcinogenesis [14]. PPARγ forms a heterodimer with retinoid X receptor (RXR) and regulates expression of target genes by binding to the PPARγ responsive element [16]. Polyunsaturated fatty acids and prostaglandin metabolite 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 have been identified as endogenous ligands for PPARγ [9, 17]. It was shown that thiazolidinediones (TZD) used as insulin sensitizers in type 2 diabetes and some nonsteroidal antiinflamatory drugs are high affinity synthetic PPARγ ligands [21, 22]. The highest levels of PPARγ are detected in adipocytes, but these receptors are also widely expressed in other normal cells and tissues such as monocytes, liver, skeletal muscle and neoplasmatic cells [8, 19]. It was proven in experimental investigations that activation of PPARγ receptors inhibits growth of many types of tumors [19]. The mechanism by which PPARγ 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 PPARγ ligands cause the terminal differentiation [6, 15, 23, 24]. Recent studies have indicated that PPARγ are expressed in the endothelium of tumoral vessels and that TZD suppress tumor growth also *via* antiangiogenic action [26].

It was also shown that PPARγ 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 PPARγ in tumor cells. In pituitary adenomas, the over-

Correspondence: K. Winczyk, Institute of Endocrinology, Medical University, Sterlinga 3, 91-425 Łódź, Poland;
e-mail: kwinczyk@os1.kappa.pl

expression of PPARγ was found as compared with normal tissues [12].

The aim of our study was to estimate the occurrence of PPARγ 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 PPARγ 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 tetrachome 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.

PPARγ and PCNA immunostaining. The paraffin sections (4 μm) were immunostained with polyclonal anti-PPARγ 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-PPARγ 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 × 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 PPARγ and PCNA was analysed by the Pearsons correlation coefficient. Statistical significance was set at $P<0.05$.

Results

The occurrence of PPARγ was demonstrated in all the examined tissues (Figs. 1, 2). Besides the nuclear reaction, which is typical for PPARγ, 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 PPARγ localization were noticed: in the nuclear region, in the cytoplasm and in both nucleus and cytoplasm. The mean percentage of the PPARγ-immunopositive cell nuclei in human pituitary adenomas and in non-tumoral pituitary tissues are shown in Figure 4. The strongest expression of PPARγ was observed in somatotropinomas. A relatively high number of immunopositive 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 PPARγ was also stronger in pituitary adenomas than in non-tumoral tissues (data not shown). Estimating the relationship between PPARγ and PCNA, a slight, statistically insignificant tendency towards negative correlation between PPARγ and PCNA was found in somatotropinomas, prolactinomas, corticotropinomas and gonado- tropinomas (Fig. 5). On the other hand, in null cell adenomas and "silent" corticotropinomas, a strong positve correlation (statistically significant) was observed between the expression of PPARγ and PCNA (Fig. 6).

Discussion

Our finding of increased expression of PPARγ 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 PPARγ 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 PPARγ is lower than in the corresponding normal tissues, *e.g.* in esophageal cancer [28] and choriocarcinoma [4]. Since PPARγ 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 PPARγ immunopositivity was detected [25]. Although the significance of the cytoplasmic PPARγ 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 PPARγ 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 PPARγ 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 PPARγ in the control of cell proliferation in these tumors. On the other hand, a strong positive correlation between PPARγ and PCNA in null cell adenomas and "silent" corticotropinomas indicate that this involvement

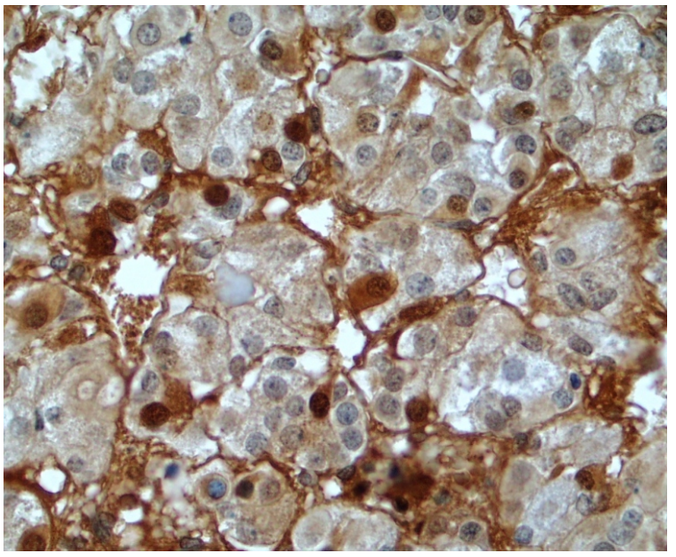

Fig. 1. The human non-tumoral pituitary tissue immunostained with anti-PPARγ antibody, showing a positive reaction (brown) in a few nuclei of glandular cells. The unspecific brown coloration can be seen also in the stroma. × 400.

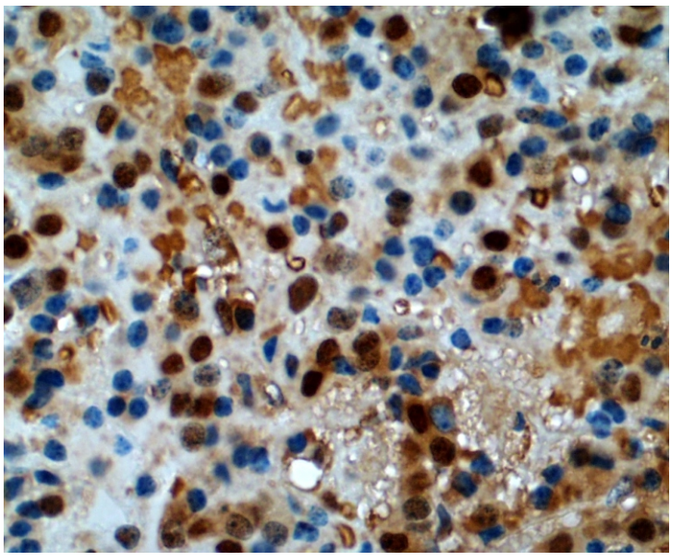

Fig. 2. The human somatotropinoma immunostained with anti-PPARγ antibody, showing a strong nuclear reaction in the tumor cells. × 400.

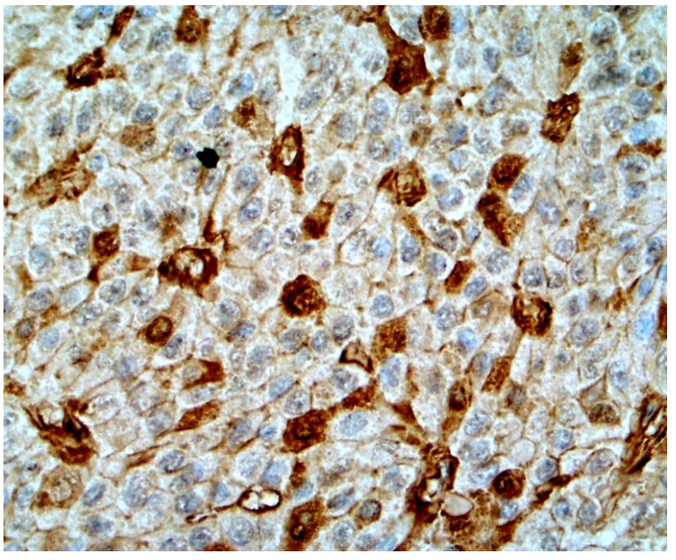

Fig. 3. The human prolactinoma immunostained with anti-PPARγ antibody, showing immunopositive reaction in cytoplasm of tumoral cells besides the nuclear reaction. × 400.

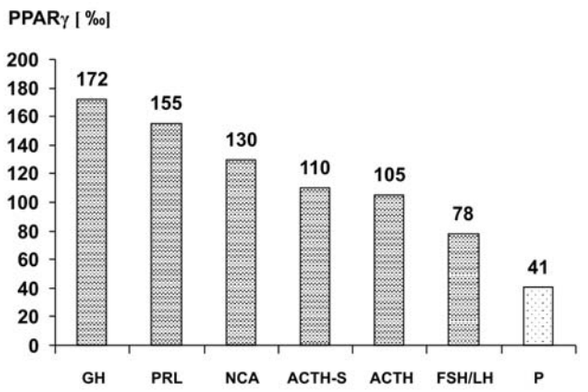

Fig. 4. The mean percentage of the PPARγ-immunopositive cell nuclei per 1000 randomly scored cell nuclei in human pituitary adenomas and in non-tumoral pituitary glands. GH - somatotropinomas; PRL - prolactinomas; NCA- null cell adenoma; ACTH-S - "silent" corticotropinomas; ACTH - corticotropinomas;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

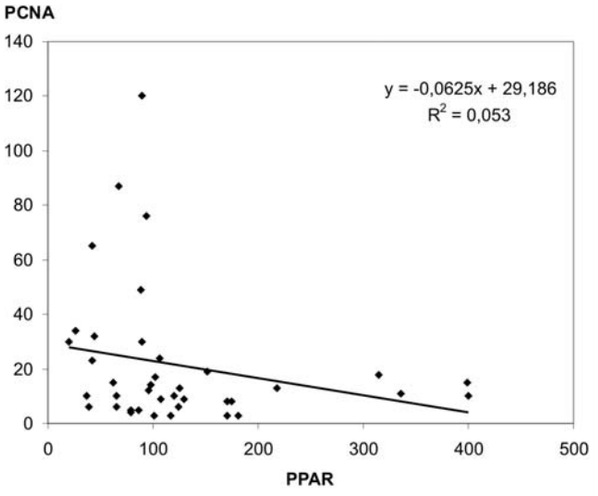

Fig. 5. Correlation between PPARγ and PCNA in somatotropinomas, prolactinomas, corticotropinomas and gonadotropinomas (r = -0 .23; p = 0.158).

may be opposite in different pituitary adenoma types. The strong expression of PPARγ 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 PPARγ 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 PPARγ can predict the positive effect of TZD treatment of pituitary tumors.

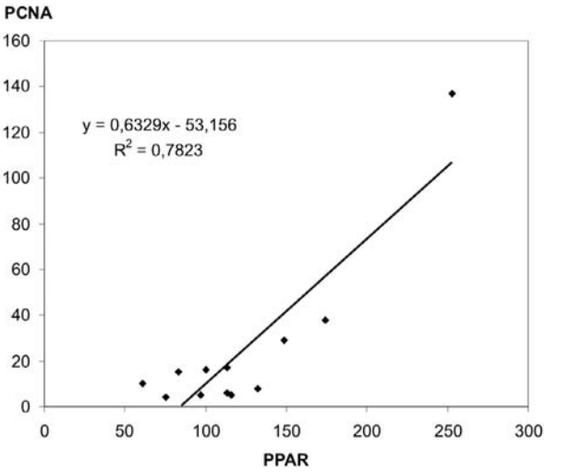

Fig. 6. Correlation between PPARγ and PCNA in null cell adenomas and "silent" corticotropinomas (r = 0.88; p<0.001).

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References

[1] Ambrosi B, Dall'Asta C, Cannavo S, Libe R, Vigo T, Epaminonda P, Chiodini I, Ferrero S, Trimarchi F, Arosio M, Beck-Peccoz P (2004) Effects of chronic administration of PPAR-gamma ligand rosiglitazone in Cushing's disease. Eur J Endocrinol 15: 173-178

[2] Blevins LS, Verity DK, Allen G (1998) Agressive pituitary tumors. Oncology 12: 1307-1318

[3] Cannavo S, Ambrosi B, Chiodini I, Vigo T, Russo A, Milici C, Barbetta L, Dall'Asta C, Adda G, Arosio M (2004) Baseline and CRH-stimulated ACTH and cortisol levels after administration of the peroxisome proliferator-activated receptor gamma ligand, rosiglitazone, in Cushing's disease. J Endocrinol Invest 27: RC8-RC11

[4] Capparuccia L, Marzioni D, Giordano A, Fazioli F, De Nictolis M, Busso N, Todros T, Castellucci M (2002) PPAR gamma expression in normal human placenta, hydatidiform mole and choriocarcinoma. Mol Hum Reprod 8: 574-579

[5] Chen GG, Lee JF, Wang SH, Chan UP, Ip PC, Lau WY (2002) Apoptosis induced by activation of peroxisome-proliferator activated receptor-gamma is associated with Bcl-2 and NF-kappaB in human colon cancer. Life Sci 70: 2631-2646

[6] Demetri GD, Fletcher CD, Mueller E, Sarraf P, Naujoks R, Campbell N, Spiegelman BM, Singer S (1999) Induction of solid tumor differantiation by the peroxisome proliferator-activated receptor-γ ligand troglitazone in patients with liposarcoma. Proc Natl Acad Sci USA 96: 3951-3952

[7] Elstner E, Muller C, Koshizuki K, Williamson EA, Park D, Asou H, Shintaku P, Said JW, Heber D, Koeffler HP (1998) Ligands for peroxisome proliferator-activated receptor gamma and retinoic acid receptor inhibit growth and induce apoptosis of human breast cancer cells *in vitro* and in BNX mice. Proc Natl Acad Sci USA 95: 8806-88011

[8] Fajas L, Auboeuf D, Raspe E, Schoonlans K, Lefebvre AM, Saladin R, Najib J, Laville M, Fruchart JC, Deeb S, Vidal-Puig A, Flier J, Briggs MR, Steals B, Vidal B, Auwerx J (1997) The organization, promotor analysis and expression of human PPARγ gene. J Biol Chem 272: 18779-18789

[9] Forman BM, Tontonoz P, Chen J, Brun RP, Spiegelman BM, Evans RM (1995) 15-Deoxy-delta12,14-prostoglandin J_2 is a ligand for the adipocyte determination factor PPAR gamma. Cell 83: 803-812

[10] Galusca B, Dumollard JM, Chambonniere ML, Germain N, Prades J, Peoc'h M, Estour B (2004) Peroxisome proliferator activated receptor gamma: immunohistochemical expression in human papillary thyroid carcinoma tissues. Possible relationship to lymph node metastasis. Anticancer Res 24: 1993-1997

[11] Heaney AP, Fernando M, Yong WH, Melmed S (2002) Functional PPAR-gamma receptor is a novel therapeutic target for ACTH-secreting pituitary adenomas. Nat Med 8: 1281-1287

[12] Heaney AP, Fernando M, Melmed S (2003) PPAR-γ receptor ligands: novel therapy for pituitary adenomas. J Clin Invest 111: 1381-1388

[13] Hase T, Yoshimura R, Mitsuhashi M, Segawa Y, Kawahito Y, Wada S, Nakatani T, Sano H (2002) Expression of peroxisome proliferator-activated receptors in human testicular cancer and growth inhibition by its agonists. Urology 60: 542-547

[14] Kersten S, Desvergne B, Wahli W (2000) Roles of PPARs in health and disease. Nature 405: 421-425

[15] Kitamura S, Miyazaki Y, Shinomura Y, Kondo S, Kanayama S, Matsuzawa Y (1999) Peroxisome proliferator-activated receptor γ induces growth arrest and differentiation markers of human colon cancers cells. Jpn J Cancer Res 90: 75-80

[16] Kliewer SA, Ulmesono K, Noonan DJ, Heyman RA, Evans RM (1992) Convergence of 9-cis retinoic acid and peroxisome proliferator signaling pathways trough heterodimer formation of their receptors. Nature 358: 771-774

[17] Kliewer SA, Sundseth SS, Jones SA, Brown PJ, Wisely GB, Koble CS, Deuchand P, Wahli W, Wilson TM, Lenhard JM, Lehmann JM (1997) Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors α and γ. Proc Natl Acad Sci USA 94: 4318-4323

[18] Klencki M, Kurnatowska I, Slowinska-Klencka D, Lewiński A, Pawlikowski M (2001) Correlation between PCNA expression and AgNOR dots in pituitary adenomas. Endocr Pathol 12: 163-169

[19] Koeffer HP (2003) Peroxisome proliferator-activated receptor γ and cancers. Clin Cancer Res 9: 1-9

[20] Lee TW, Chen GG, Xu H, Yip JH, Chak EC, Mok TS, Yim A (2003) Differential expression of inducible nitric oxide synthase and peroxisome proliferator-activated receptor gamma in non-small cell lung carcinoma. Eur J Cancer 39: 1296-1301

[21] Lehmann JM, Moore LB, Smith-Oliver TA, Wilkinson WO, Wilson TM, Kliewer SA (1995) An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome-activated receptor γ. J Biol Chem 270: 12953-12956

[22] Lehmann JM, Lenhard JM, Oliver BB, Ringold GM, Kliewer SA (1997) Peroxisome proliferator-activated receptors α and γ are activated by indomethacin and other non-steroidal anti-inflammatory drugs. J Biol Chem 272: 3406-3410

[23] Mueller E, Sarraf P, Tontonoz P, Evans RM, Martin KJ, Zhang M, Fletcher C, Singer S, Spiegelman BM (1998) Terminal differentiation of human breast cancer through PPAR-gamma. Mol Cell 1: 465-470

[24] Mueller E, Smith M, Sarraf P, Kroll T, Aiyer A, Kaufman DS, Oh W, Demetri G, Frigg WD, Zhou XP, Eng C, Spiegelman BM, Kantoff PW (2000) Effects of ligand activation of peroxisome proliferator-activated receptor gamma in human prostate cancer. Proc Natl Acad Sci USA 97: 10990-10995

[25] Mukunyadzi P, Ai L, Portilla D, Barnes EL, Fan CY (2003) Expression of peroxisome proliferator-activated receptor gamma in salivary duct carcinoma: immunohistochemical analysis of 15 cases. Mod Pathol 16: 1218-1223

[26] Panigraphy D, Singer S, Shen LQ, Butterfield CE, Freedman DA, Chen EJ, Moses MA, Duensing S, Fletcher C, Fletcher JA, Hlatky L, Hahnfeldt P, Folkman J, Kaipainen A (2002) PPRAγ ligands inhibit primary tumor growth and metastasis by inhibiting angiogenesis. Am Soc Clin Invest 110: 923-932

[27] Pawlikowski M (2001) Immunocytochemical prognostic markers in pituitary tumors. Folia Histochem Cytobiol 39: 105-106

[28] Terashita Y, Sasaki H, Haruki N, Nishiwaki T, Ishiguro H, Shibata Y, Kudo J, Konishi S, Kato J, Koyama H, Kimura M, Sato A, Shinoda N, Kuwabara Y, Fujii Y (2002) Decreased peroxisome proliferator-activated receptor gamma gene expression is correlated with poor prognosis in patients with esophageal cancer. Jpn J Clin Oncol 32: 238-243

[29] Yoshimura R, Matsuyama M, Segawa Y, Hase T, Mitsuhashi M, Tsuchida K, Wada S, Kawahito Y, Sano H, Nakatani T (2003) Expression of peroxisome proliferator-activated receptors (PPARs) in human urinary bladder carcinoma and growth inhibition by its agonists. Int J Cancer 104: 597-602

[30] Zang C, Wachter M, Liu H, Posch MG, Fenner MH, Stadelmann C, von Deimling A, Possinger K, Black KL, Koeffler HP, Elstner E (2003) Ligands for PPARgamma and RAR cause induction of growth inhibition and apoptosis in human glioblastomas. J Neurooncol 65: 107-118

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