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RESEARCH

Expression of Peroxisome Proliferator Activated Receptor-Gamma (PPAR-γ) in Human Non-small Cell Lung Carcinoma: Correlation with Clinicopathological Parameters, Proliferation and Apoptosis Related Molecules and Patients' Survival

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Abstract Peroxisome proliferator-activated receptor-γ (PPAR-γ) has currently been considered as molecular target for the treatment of human metabolic disorders. PPAR-γ has also been implicated in the pathogenesis and progression of several types of cancer, being associated with cell differentiation, growth and apoptosis. The present study aimed to evaluate the clinical significance of PPAR-γ expression in non-small cell lung carcinoma (NSCLC). PPAR-γ protein expression was assessed immunohistochemically in tumoral samples of 67 NSCLC patients and was statistically analyzed in relation to clinicopathological parameters, proliferation and apoptosis related molecules and patients' survival. Positive PPAR-γ expression was prominent in 30 (45 %) out of 67 NSCLC cases. PPAR-γ positivity was more frequently observed in squamous cell lung carcinoma cases compared to lung adenocarcinoma ones (p=0.048). PPAR-γ positivity was significantly associated with bcl-2 positivity (p=0.016) and borderline with c-myc positivity (p=0.052), whereas non associations with grade of differentiation, TNM stage, Ki-67, p53, bax proteins' expression and patients' survival were noted. In the subgroup of squamous cell lung carcinoma cases, PPAR-γ positivity was significantly associated with tumor size (p=0.038), while in lung adenocarcinoma ones with histopathological grade of differentiation (p=0.026). The present study supported evidence for possible participation of PPAR-γ in the biological mechanisms underlying the carcinogenic evolution of the lung. Although the survival prediction using PPAR-γ expression as a marker seems uncertain, the observed correlation with apoptosis related proteins reinforces the potential utility of PPAR-γ ligands as cell cycle modulators in future therapeutic approaches in lung cancer.

Keywords PPAR-γ · Non-small cell lung carcinoma · Immunohistochemistry · Cell cycle · Cell proliferation · Apoptosis

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Introduction

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor superfamily, initially described as molecular targets for compounds, which induce peroxisomal proliferation [1, 2]. To date, three different isotypes have been identified in various species: PPAR-α, PPAR-β/δ and PPAR-γ [1, 2]. PPAR-γ constitutes

the most extensively studied amongst them and functions as a ligand-activated transcription factor by binding to specific DNA sequences, termed to as peroxisome proliferator response elements (PPREs), in the promoter of the target genes as a heterodimer with the Retinoid X Receptors (RXRs) [3, 4]. It can also regulate gene expression independently of PPRE, either by suppressing growth hormone protein-1 (GHP-1) or by interfering with the function of activator protein (AP)-1, signal transducer and activator of transcription (STAT)-1 and nuclear factor-κB (NF-κB) [5]. PPAR-γ has currently been considered as a crucial regulator of adipocyte differentiation and peripheral glucose utilization [6, 7]. Notably, certain PPAR-γ ligands have been introduced in the market as oral antidiabetic agents for the treatment of human metabolic disorders [8, 9]. Pleiotropic functions beyond this limit, such as anti-proliferative and anti-inflammatory effects against several pathological states, including neoplasia, bone malignancies, ischemia/reperfusion injury and gestational diseases are being explored in clinical studies [10–16].

Lung cancer is the leading cause of cancer mortality in the world, causing more deaths than colorectal, breast, and prostate cancers combined [17]. Primary malignant cancers of the lung are classified into small cell lung cancer (SCLC) and nonsmall cell lung cancer (NSCLC). NSCLC is aggressive and highly invasive and accounts for 80 % of all cases [18]. Despite the development of new surgical procedures and chemotherapeutic protocols, the 5-year survival rate remains less than 15 % [17, 18]. Smoking is the most important risk factor in the development of most pulmonary carcinomas [17, 18]. However, even in a smokeless society, it is predicted that lung cancer due to prior exposure to carcinogens will continue to be a major health problem for the future [19, 20]. In this aspect, accumulative evidence has recently suggested that PPAR-γ affects lung tumor cell biology [21, 22]. PPAR-γ was reported to be expressed in several human lung cancer cell lines [23–25], as well as in primary tumors from both SCLC and NSCLC patients [24–29]. Notably, previous studies by our group and others showed increased frequency of positive PPAR-γ expression in well-differentiated adenocarcinoma cases from NSCLC patients compared to moderately- and poorly-differentiated ones [28, 29]. Moreover, decreased PPAR-γ expression was significantly associated with poor prognosis [28].

To date, a large body of evidence has suggested that PPAR-γ ligands can induce cell cycle arrest and apoptosis in several types of cancer cells, including lung cancer ones [12, 21]. PPAR-γ activation by synthetic and natural ligands, such as thiazolidinediones and polyunsaturated fatty acids, respectively, suppressed the growth of lung cancer cells through induction of differentiation and apoptosis [23, 24, 30, 31]. Notably, the growth inhibitory effect of PPAR-γ activation on human lung cancer cells was associated with the reduction of bcl-2 and bcl-w expression and the increase of extracellular signal regulated kinase (ERK) 1/2 and p38 activation [32, 33]. PPAR-γ ligands also induced the expression of death receptor (DR)-5 and increased DR-5 distribution at the cell surface in addition to reducing c-FLIP levels, suggesting that these agents can cooperate with TRAIL to enhance apoptosis in human lung cancer cells [34]. It was also shown that PPAR-γ activation exerted selective effects on differentiation and metastasis of NSCLC [35, 36]. A recent retrospective study further demonstrated a 33 % reduction in lung cancer risk among patients with diabetes using rosiglitazone [37]. However, there is no comprehensive research so far concerning the clinical relevance of PPAR-γ expression in the management and prognosis of NSCLC patients and its correlation with proliferation and apoptosis related molecules.

In view of the above considerations, the present study aimed to assess the immunohistochemical expression of PPAR-γ in tumoral samples of 67 NSCLC patients. The correlation of PPAR-γ expression with clinicopathological parameters and patients' survival was investigated in order to evaluate its clinical significance. The immunohistochemical expression of certain proliferation and apoptosis related molecules, Ki-67, c-myc, p53, bcl-2, bax was also assessed in order to evaluate their potential correlation with PPAR-γ.

Material and Methods

Clinical Material

Sixty-seven NSCLC specimens obtained from equal number of patients who underwent surgical resection due to lung cancer were included in this study. This study was approved by the ethical committee of Laikon General Hospital. None of the patients received any kind of anti-cancer treatment prior to surgery. Fifty-seven (85 %) of the patients were men and 10 (15 %) were women with a mean age of 63 years old (range 43–82 years old). The vast majority of the patients (62/67, 93 %) were smokers. The resected tumors were classified histologically as squamous cell lung carcinoma in 38 (57 %) cases and lung adenocarcinoma in 29 (43 %) cases. The histopahological grading was assessed according to the criteria described in Word Health Organization (WHO) [38]. The histopathological stage of the tumor was assessed according to the TNM-system and the criteria of the International Union Against cancer [39, 40]. The patients were followed-up for a time interval of 60 months (mean 17 months). Study population characteristics are depicted in Tables 1, 2 and 3.

Immunohistochemistry

Formalin-fixed paraffin-embedded tissue sections of 4 μm thickness were dewaxed in xylene and were brought to

Table 1 Correlation of PPAR-γ expression with clinicopathological parameters and proliferation and apoptosis related molecules in NSCLC cases

Variables	PPAR-γ expression		p-value
Patients	Negative	Positive	
N=67	37 (55 %)	30 (45 %)	
Age	64.32+8.19	62.37+10.18	0.386
Gender			0.308
Male	30 (45)	27 (40)	
Female	7 (10)	3 (5)	
Histopathological type			0.048
Squamous cell carcinoma	17 (25)	21 (31)	
Adenocarcinoma	20 (30)	9 (14)	
Histopathological grade			0.283
Well	2 (3)	5 (7)	well vs mod+poor
			0.134
Moderately	13 (19)	11 (16)	well+mod vs poor
Poorly	22 (33)	14 (22)	0.296
T			0.126
T1	2 (3)	6 (9)	
T2	18 (25)	10 (15)	
T3	14 (22)	12 (18)	
T4	3 (5)	2 (3)	
N			0.487
N0	15 (22)	16 (24)	
N1	10 (15)	6 (9)	
N2	10 (15)	6 (9)	
N3	2 (3)	2 (3)	
M			0.868
M0	32 (48)	27 (40)	
M1	5 (7)	3 (5)	
Ki-67 expression			0.502
Negative	14 (22)	9 (14)	
Positive	23 (33)	21 (31)	
c-myc expression			0.052
Negative	9 (14)	2 (3)	
Positive	28 (41)	28 (42)	
p53 expression			0.789
Negative	21 (31)	18 (25)	
Positive	16 (24)	12 (20)	
bcl-2 expression			0.016
Negative	22 (33)	9 (14)	
Positive	15 (22)	21 (31)	
bax expression			0.485
Negative	4 (6)	5 (7)	
Positive	33 (49)	25 (38)	

Table 2 Correlation of PPAR-γ expression with clinicopathological parameters and proliferation and apoptosis related molecules in squamous cell lung carcinoma cases (*n*=38)

Variable	PPAR-γ expression		p-value
Patients	Negative (%)	Positive (%)	
N=38	17 (45)	21 (55)	
Age	64.48+8.25	61.96+10.01	0.342
Gender			0.878
Male	16 (42)	20 (52)	
Female	1 (3)	1 (3)	
Histopathological grade			0.686
Well	1 (3)	3 (8)	well vs mod+poor
			0.401
Moderately	5 (13)	5 (13)	well+mod vs poor
			0.859
Poorly	11 (29)	13 (34)	
T			0.038
T1	0 (0)	6 (16)	
T2	9 (24)	6 (16)	
T3	6 (16)	8 (20)	
T4	2 (5)	1 (3)	
N			0.340
N0	5 (13)	11 (29)	
N1	5 (13)	5 (13)	
N2	6 (16)	4 (10)	
N3	1 (3)	1 (3)	
M			0.451
M0	14 (37)	19 (50)	
M1	3 (8)	2 (5)	
Ki-67 expression			0.438
Negative	6 (16)	5 (13)	
Positive	11 (29)	16 (42)	
c-myc expression			0.089
Negative	4 (10)	1 (3)	
Positive	13 (35)	20 (52)	
p53 expression			0.492
Negative	10 (26)	10 (26)	
Positive	7 (19)	11 (29)	
bcl-2 expression			0.135
Negative	7 (19)	4 (10)	
Positive	10 (26)	17 (45)	
bax expression			0.132
Negative	1 (3)	5 (13)	
Positive	16 (42)	16 (42)	

water through graded alcohols. To remove the endogenous peroxidase activity, sections were treated with freshly prepared 0.3 % hydrogen peroxide in methanol in the dark, for

Table 3 Correlation of PPAR-γ expression with clinicopathological parameters and proliferation and apoptosis related molecules in lung adenocarcinoma cases (n=29)

Variable	PPAR-γ expression		p-value
Patients	Negative (%)	Positive (%)	
N=29	20 (69)	9 (31)	
Age	64.12+8.03	62.85+10.29	0.412
Gender			0.665
Male	14 (48)	7 (24)	
Female	6 (21)	2 (7)	
Histopathological grade			0.061
Well	1 (3)	2 (7)	well vs mod+poor
			0.159
Moderately	8 (28)	6 (21)	well+mod vs poor
			0.026
Poorly	11 (38)	1 (3)	
T			0.732
T1	2 (7)	0 (0)	
T2	9 (31)	4 (14)	
T3	8 (28)	4 (14)	
T4	1 (3)	1 (3)	
N			0.992
N0	10 (35)	5 (17)	
N1	5 (17)	1 (3)	
N2	4 (14)	2 (8)	
N3	1 (3)	1 (3)	
M			0.161
M0	18 (62)	8 (28)	
M1	2 (7)	1 (3)	
Ki-67 expression			0.822
Negative	8 (28)	4 (14)	
Positive	12 (41)	5 (17)	
c-myc expression			0.393
Negative	5 (17)	1 (3)	
Positive	15 (52)	8 (28)	
p53 expression			0.076
Negative	11 (38)	8 (28)	
Positive	9 (31)	1 (3)	
bcl-2 expression			0.295
Negative	15 (52)	5 (17)	
Positive	5 (17)	4 (14)	
bax expression			0.220
Negative	3 (10)	0 (0)	
Positive	17 (59)	9 (31)	

30 min, at room temperature. Non-specific antibody binding was then blocked using a specific blocking reagent (Snipper, Biocare Medical, Walnut Creek, CA, USA) for 5 min. A mouse (IgG_1) monoclonal antibody (E-8) that recognizes the carboxy terminus of human and rat PPAR-γ (Santa Cruz Biochemicals, Santa Cruz, CA, USA), reacting with PPAR-γ1 and -γ2 was applied. A panel of different primary mouse monoclonal antibodies that recognize proliferation and apoptosis related molecules was also applied according to the manufacturer's instructions; anti-Ki-67 (clone MIB-1, IgG_{1k}, Dakopatts, Glostrup, Denmark), anti-p53 (clone D07, IgG_{2b}, Zymed), anti-c-myc (clone 9E10, IgG_1, Zymed), anti-bcl-2 (clone Bcl-2-100, IgG_1, Zymed), anti-bax (clone 2DZ, IgG_1, Zymed). Antigen retrieval was performed only for PPAR-γ and Ki-67 antigen detection by microwaving slides in 10 mM citrate buffer (pH 6.0) for 15 min according to the manufacturer's instructions [41].

The sections were incubated for 1 h, at room temperature, with the primary antibodies diluted 1:100 in phosphate buffered saline (PBS) according to the manufacturer's instructions. After washing three times with PBS, sections were incubated at room temperature with biotinylated linking reagent (4 plus Universal Goat link, Biocare Medical, Walnut Creek, CA, USA) for 10 min, followed by incubation with peroxidase-conjugated streptavidin label (4 plus HRP label, Biocare Medical) for 10 min. The resultant immune peroxidase activity was developed in 0.5 % 3,3'-diaminobenzidine hydrochloride (DAB; Sigma, Saint Louis, MO, USA) in PBS containing 0.03 % hydrogen peroxide for 3 min. Sections were counterstained with Harris' hematoxylin and mounted in Entellan (Merck, Darmstadt, Germany). Appropriate negative controls were performed by omitting the primary antibodies and/or substituting them with an irrelevant antiserum. As controls for PPAR-γ positive staining, pancreatic and colon cancer positive cases were used [41, 42].

Immunohistochemical Evaluation

Stained sections were independently assessed by S.T. and J.S. blinded to the clinical data with very good inter-observer agreement (κ=0.973, SE: 0.019). Specimens were considered "positive" for PPAR-γ, Ki-67, c-myc, p53, bcl-2 or bax proteins when more than 5 % of tumor cells within the section were positively stained [29, 41, 42]. The pattern of cellular distribution of PPAR-γ immunostaining was also examined, being characterized as: cytoplasmic and nuclear, cytoplasmic only and nuclear only. The intensity of PPAR-γ immunostaining was graded as mild, moderate and intense. The extent of PPAR-γ immunostaining was calculated by the percentage of the PPAR-γ positive cells in the total number of tumor cells in each specimen [29, 41, 42].

Statistical Analysis

Statistical analysis was performed using the chi-square test for categorical parameters and t-test for quantitative ones.

Survival curves were constructed using the Kaplan-Meier method and compared using the log rank test. The influence of each prognostic factor on patients' survival was evaluated using Cox regression analysis. A p-value less than 0.05 was considered as the limit of statistical significance. All analyses were performed by SPSS for Windows Software (SPSS Inc., 2003, Chicago, USA).

Results

Positive PPAR-γ expression was noted in 30 (45 %) out of the 67 examined NSCLC (Table 1). Twenty-one (55 %) out of 38 squamous cell lung carcinoma cases and 9 (31 %) out of 29 lung adenocarcinoma cases showed positive PPAR-γ expression (Table 1). Representative PPAR-γ immunostainings for squamous cell lung carcinoma and lung adenocarcinoma are depicted in Figs. 1 and 2, respectively. The pattern of cellular distribution of PPAR-γ immunostaining was predominately cytoplasmic in 24 (80 %) out of 30 positive NSCLC cases. Three (10 %) NSCLC cases showed nuclear pattern of PPAR-γ immunostaining and another 3 (10 %) NSCLC cases showed both cytoplasmic and nuclear staining. The intensity of PPAR-γ immunostaining was moderate in 6 (20 %) and intense in 24 (80 %) out of 30 positive NSCLC cases.

PPAR-γ positivity was not significantly associated with patients' age, gender and T/N/M factors of the TNM staging system ($p>0.05$, Table 1). Squamous cell lung carcinoma cases showed significantly increased frequency of positive PPAR-γ expression compared to lung adenocarcinoma cases ($p=0.048$, Table 1). PPAR-γ positivity was more frequently observed in well differentiated NSCLC cases compared to moderately and poorly differentiated ones without reaching statistical significance ($p>0.05$, Table 1). PPAR-γ positivity was significantly associated with bcl-2 positivity ($p=0.016$, Table 1) and borderline with c-myc positivity ($p=0.052$, Table 1), whereas no associations with Ki-67, p53 and bax positivity were noted ($p>0.05$, Table 1). Survival curve stratified by PPAR-γ expression was performed and statistical analysis using the log rank test showed that there was no significant difference in the survival of NSCLC patients presenting PPAR-γ positive tumors and those with PPAR-γ negative tumors ($p=0.529$, data not shown). The intensity, distribution and extent of PPAR-γ immunostaining showed no significant associations with clinicopathological parameters, proliferation and apoptosis related molecules and patients' survival (data not shown).

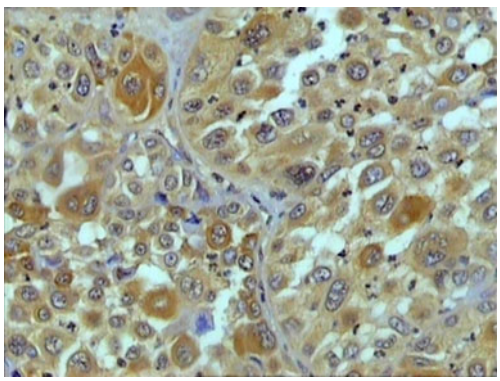

Fig. 1 Intense cytoplasmic immunostaining for PPARγ in tumor cells in one representative case of squamous cell lung carcinoma (magnification x200)

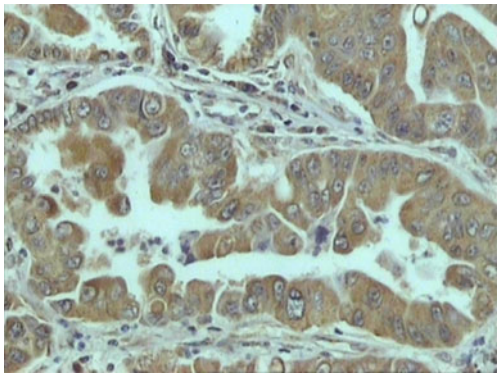

Fig. 2 Intense cytoplasmic immunostaining for PPARγ in tumor cells in one representative case of lung adenocarcinoma (magnification x200)

Statistical analysis was further performed in each histopathological type of NSCLC, separately. In the subgroup of squamous cell lung carcinoma cases, PPAR-γ positivity was not associated with patients' age, gender and histopathological grade of differentiation ($p>0.05$, Table 2). PPAR-γ positivity was significantly associated with the tumor size T- ($p=0.038$, Table 2), whereas it was not correlated with the other components of the TNM staging system ($p>0.05$, Table 2). PPAR-γ positivity showed a trend of correlation with c-myc positivity ($p=0.089$, Table). An increased incidence of PPAR-γ positivity in bcl-2 negative squamous cell lung carcinoma cases was noted without reaching statistical significance ($p=0.135$, Table 2). PPAR-γ positivity was also more frequently observed in bax positive squamous cell lung carcinoma cases without reaching statistical significance ($p=0.132$, Table 2). PPAR-γ positivity did not show any association with Ki-67 and p53 positivity ($p>0.05$, Table 2) and patients' survival ($p=0.313$, data not shown).

In the subgroup of lung adenocarcinoma, PPAR-γ positivity was significantly more frequently observed in well and moderately differentiated cases compared to poorly differentiated ones ($p=0.026$, Table 3). PPAR-γ positivity was not associated with patients' age, gender and TNM stage ($p>0.05$, Table 3). No associations between PPAR-γ positivity and proliferation and apoptosis molecules were

also noted ($p>0.05$, Table 3), except for a trend of correlation with p53 positivity ($p=0.076$, Table 3). PPAR-γ positivity did not affect patients' survival ($p=0.884$, data not shown).

Discussion

It is certainly well established that PPAR-γ is implicated in the biological mechanisms underlying the carcinogenic evolution of the lung. Substantial evidence has currently suggested that synthetic and natural PPAR-γ ligands exerted anti-neoplastic functions by inducing cell cycle arrest and apoptosis in lung cancer cells [21–24, 26, 30, 31]. However, the introduction of PPAR-γ ligands into human advanced cancer clinical trials has generally met with limited success [43]. This seems to be ascribed to the fact that the most comprehensive data so far concerning the implication of PPAR-γ in lung tumorigenesis has been generated in vitro in cell culture conditions, where results are often difficult to be extrapolated to in vivo situations. Moreover, the existing studies on archival clinical material from primary lung tumors, which may reinforce the potential use of PPAR-γ ligands as cancer chemoprevention agents in the clinical setting, remain scarce.

In this aspect, the present study supported clinical evidence for possible participation of PPAR-γ in the biological mechanisms underlying the carcinogenic evolution of NSCLC. In line with previous evidence by our group and others [28, 29], we found significantly increased incidence of PPAR-γ positivity in well and moderately differentiated NSCLC cases compared to poorly differentiated ones. When analyzing all the NSCLC cases, PPAR-γ expression did not show any significant association with TNM stage, which represents the best prognostic index for operable NSCLC [39, 40]. However, separate analysis stratified by histopathological type revealed a significant association between PPAR-γ expression and tumor size (T-factor) in the subgroup of squamous cell lung carcinoma cases. On the other hand, PPAR-γ expression was significantly associated with the histopathological grade of differentiation in the subgroup of lung adenocarcinoma cases. These findings suggested that the clinical relevance of PPAR-γ may be affected by the histopathological type of NSCLC patients.

The present study further showed that PPAR-γ expression was significantly associated with bcl-2 expression, supporting evidence that PPAR-γ ligand activation may affect apoptosis in cancer cells, in vivo. The oncogene bcl-2 has been considered as an inhibitor of apoptosis and bcl-2 overexpression has been shown to suppress apoptotic cell death through a heterodimerization with bax protein [44]. Conversely, bax overexpression led to accelerated apoptotic cell death, supporting evidence that the ratio of bcl-2 to bax may be crucial in determining susceptibility to apoptosis [45]. In this context, it should be noted that beyond the correlation with bcl-2, PPAR-γ positivity was also more frequently observed in bax positive squamous cell lung carcinoma cases, without though reaching statistical significance.

Recent studies have demonstrated that PPAR-γ ligands can induce apoptosis through a decrease of bcl-2 expression and an increase of bax expression in several types of cancer cells, including renal cancer [46] and myeloid leukemia [47], as well as glioblastoma cells [48]. A decrease of bcl-2 expression was also associated with apoptosis induction in breast cancer MCF-7 cells [49], whereas no significant effect of PPAR-γ ligands on bcl-2 and bax expression was noted in both glial brain tumor cells [50] and colon cancer HT-29 ones [51]. Interestingly, the growth inhibitory effect of PPAR-γ activation on human lung cancer cells was associated with reduced expression of bcl-2 and its closest relative bcl-w [32, 33]. PPAR-γ activation by troglitazone resulted in increased extracellular signal regulated kinase 1/2 (ERK1/2) and p38 activation, supporting evidence that cell growth arrest and apoptosis may occur through an ERK1/2 and p38-related mechanism [32, 33]. 15-Deoxy-PGJ_2, a natural occurring PPAR-γ agonist, together with docetaxel, also stimulated apoptosis in NSCLC through inhibition of bcl-2 and cyclin-D1 and overexpression of caspases and p53 [52].

In the present study, c-myc positivity presented a positive trend of correlation with PPAR-γ positivity when analyzing all NSCLC cases. In this aspect, it was shown that PPAR-γ ligands can force cancer cells to apoptosis through inhibition of c-myc gene expression [51, 53]. In support of this view, PPAR-γ activation reduced c-myc mRNA expression in human peripheral blood mononuclear cells [54]. However, the precise molecular mechanism through which PPAR-γ ligands may interfere with c-myc in order to induce apoptosis has not been elucidated yet. In this context, the observed correlation with c-myc positivity in squamous cell lung carcinoma cases and the absence of a similar correlation in lung adenocarcinoma ones further reinforce the differential clinical impact of PPAR-γ in NSCLC according to the histopathological type.

The present study further revealed that the cellular pattern of PPAR-γ distribution was predominately cytoplasmic in the vast majority of NSCLC cases examined. In line with the present study, similar pattern of cellular distribution was noted in human neuroblastoma [55], epithelial ovarian carcinoma cells [56] and colon carcinoma [42] using immunohistochemistry. Moreover, several previous studies documented that PPAR-γ was present in both the cytoplasm and nucleus of primary human NSCLC tissues [29, 57, 58]. Mechanistically, the intracellular distribution-translocation of nuclear receptors as PPARs and RXR was shown to be achieved by their

phosphorylation [59, 60]. Alterations of the phosphorylation process, which usually occurs in cancer, may result in loss of receptor-mediated effects by blocking PPAR-γ protein in the cytoplasm [59]. This could possibly explain the cytoplasmic pattern of staining observed in our study, since mutation of PPAR-γ gene is a very rare event in human malignancies [61].

We also found a trend of correlation between PPAR-γ and p53 positivity in the subgroup of lung adenocarcinoma cases. The p53 protein has been considered as the product of a tumor suppressor gene and its wild-type was shown to exert a crucial role in the regulation of cell growth. Cells in which DNA was damaged by various genetic alterations were forced to enter cell cycle arrest or programmed cell death by wild-type p53 [62]. It was shown that p53 mutations were implicated in the development of human carcinomas and alterations of p53 precede malignant transformation [63, 64]. Mutations of p53 were identified in more than 50 % of all human carcinoma, including that of lung. The clinical significance of p53 mutations has also been examined with regard to tumor progression and patient prognosis of lung carcinoma [63, 64]. However, in the present study, PPAR-γ expression was not directly associated with clinicopathological factors, which are related with the tumor per se and predict patients' survival, such as TNM, tumor proliferative capacity reflected Ki-67 positivity and the genetic background of tumor, such as p53. In this aspect, the trend of correlation with p53 in lung adenocarcinoma cases needs to be verified by a larger cohort study in order for precise conclusions to be drawn. On the other hand, PPAR-γ expression did not show any significant correlation with Ki-67 expression, which has been considered as an index for tumor proliferation and patients' survival in NSCLC [65]. In the present study, it should be noted that despite the high proportion of patients with advanced disease and undifferentiated tumors, 36 % of tumor samples proved to be Ki-67 negative. This may ascribed to the fact that the growth fraction reflected by Ki-67 expression has been considered to relate only to the number (or the fraction) of proliferative cells but not to the time needed for the completion of the intermitotic cycle; in other words, the estimation of the growth fraction by Ki-67 gives information only about the state but not about the rate of proliferation [65].

In conclusion, the present study showed that PPAR-γ protein was correlated with histopathological type and molecules related to apoptosis, supporting evidence for its possible participation in the biological mechanisms underlying the carcinogenic evolution of the lung. The clinical relevance of PPAR-γ seems to be affected by the histopathological type of NSCLC patients, which needs to be verified by larger cohort studies conducted on each histopathological type, separately. The observed correlation of PPAR-γ with apoptosis related proteins reinforces the clinical utility of its ligands as cell cycle modulators in future therapeutic approaches in lung cancer. As PPAR-γ expression was not correlated with TNM stage, prognostic indicators, such as p53, tumor proliferative capacity and patients' survival, it could not be used as prognostic factor stratifying lung cancer patients. Further in vitro and in vivo studies are required in order for the participation of PPAR-γ and the therapeutic utility of its ligands to be explored. Such studies may delineate the importance of specific PPAR-γ ligands as useful therapeutic agents in the treatment of human lung cancer.

Conflict of interest statement None declared

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