PERGAMON

European Journal of Cancer 39 (2003) 1296–1301

European Journal of Cancer

www.ejconline.com

Differential expression of inducible nitric oxide synthase and peroxisome proliferator-activated receptor gamma in non-small cell lung carcinoma

Tak W. Lee[a], George G. Chen[a,c,*], Hu Xu[a], Johnson H.Y. Yip[a], Ernest C.W. Chak[a], Tony S.K. Mok[b], Anthony P.C. Yim[a]

[a]*Department of Surgery, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong*
[b]*Department of Clinical Oncology, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong*
[c]*Sir Y.K. Pao Center for Cancer, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong*

Received 19 March 2002; received in revised form 4 September 2002; accepted 1 November 2002

Abstract

Both inducible nitric oxide synthase (iNOS) and peroxisome proliferator-activated receptor gamma (PPARγ) are closely associated with the development of human cancer. Although the expression of iNOS has been studied in non-small cell lung carcinoma (NSCLC), the level of PPARγ has not been examined in tumorous and non-tumorous tissues from NSCLC. The present study analysed the levels of both iNOS and PPARγ in NSCLC tissues and in lung cell lines. The possible role of these two molecules in the carcinogenesis of lung cancer was investigated. The expression of iNOS was significantly higher in the tumorous tissues than in the non-tumorous ones. In contrast to this pattern of iNOS protein expression, the level of PPARγ was much lower in the tumorous tissues than in the non-tumorous samples. A similar result was also obtained *in vitro* using human lung cancer cell lines and normal lung cells. Immunohistochemical examination revealed that PPARγ expression in the non-tumorous tissues was more likely to be located in the nucleus whereas it was present in both the nucleus and cytoplasm of the tumorous tissues. The intensity of iNOS expression was stronger in the nucleus than in the cytoplasm of the tumorous tissues. More than 50% of the cases tested did not express iNOS protein in the non-tumorous tissues. Statistical analysis indicated a negative correlation between iNOS and PPARγ levels in the NSCLC tissues. In conclusion, this study demonstrated differing expressions for iNOS and PPARγ in NSCLC tissues. Since activated PPARγ is able to inhibit the expression of iNOS and the generation of iNOS is particularly associated with the inflammatory and environmental factors of lung cancer risk, this discrepant expression pattern may be associated with the pathogenesis of NSCLC.

Keywords: Lung cancer; Inducible nitric oxide synthase; Peroxisome proliferator-activated receptor gamma

Although both inducible nitric oxide synthase (iNOS) and peroxisome proliferator-activated receptor gamma (PPARγ) have been shown to be involved in cell proliferation, cell growth and cell cycle control [1,2], their roles in the development of malignant tumours remains controversial. iNOS is an enzyme responsible for the production of nitric oxide (NO). NO is an important bioregulatory agent and signalling molecule that mediates a variety of actions such as vasodilatation, neurotransmission, host defence and apoptosis. It is now widely accepted that iNOS is expressed in response to several stresses, including inflammatory cytokines, bacterial endotoxin and cigarette smoking [3–5]. The link between cigarette smoking and the alteration of iNOS is particularly relevant to the pathogenesis of lung cancer, as cigarette smoking is one of primary risk factors that cause lung cancer [6]. There are several publications describing the expression of iNOS in lung cancer [7–10]. Although most of these studies have observed an increase in the expression of iNOS or its activity, the source of iNOS and the level detected were very different. A study by Liu and colleagues suggested that the

* Corresponding author. Tel.: +852-2632-3934; fax: 852-2645-0605.
E-mail address: gchen@cuhk.edu.hk (G.G. Chen).

0959-8049/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0959-8049(02)00733-5

increased NO production mainly resulted from the tumour-associated nonspecific immunological and inflammatory processes of the host rather than the tumour cells [7]. In a report by Fujimoto and colleagues [8], the activity of NOS was found in lung adenocarcinomas, but not in other types of lung cancers and an increase occurred in approxamately 50% of patients with lung adenocarcinoma. Ambs and colleagues failed to detect an increase in NOS in non-small cell lung carcinoma (NSCLC) [9]. Marrogi and colleagues observed a high iNOS level in lung large cell carcinoma and lung adenocarcinoma, but not in squamous cell carcinoma [10].

PPARγ plays a key role in the control of adipocyte differentiation and in the regulation of genes involved in lipid and glucose metabolism [2]. It also plays an important role in modulating cell growth and the cell cycle. It has been shown to inhibit the growth of cancer cells and to drive their differentiation processes to the terminal point, that is apoptosis [2,11,12]. The expression of PPARγ has been observed in human lung cancer cell lines and tissues [13,14], although the level of expression has not yet been compared with normal or nontumorous lung cells. Human lung cancer cells positive for PPARγ can be induced by PPARγ ligands to growth arrest or apoptoses [13,14] suggesting PPARγ may be significant in lung cancer therapy. However, the anticancer mechanism of PPARγ is not clear. Recent studies indicate that PPARγ is able to inhibit the expression of iNOS induced by various agents [15–17]. Therefore, the level of PPARγ and its interaction with iNOS may affect the development of lung cancer. Unfortunately, to the best of our knowledge, the examination of $PPAR_{\gamma}$ in both tumorous and nontumorous tissues from lung cancer and the association between PPARγ and iNOS have not yet been investigated. The aim of the present study was to examine the levels of iNOS and PPARγ in lung tumorous and nontumorous tissues and in lung cell lines, and to determine whether there is a correlation between the expression of iNOS and the level of PPARγ.

1. Materials and methods

1.1. Patient population

Thirty-five NSCLC tissue samples and their corresponding non-cancer tissue samples were obtained from patients with NSCLC, who underwent surgical resection at the Department of Surgery, Price of Wales Hospital, Chinese University of Hong Kong. All tumour and nontumour tissue specimens were confirmed by histological examinations. Tissue samples were stored in a liquid nitrogen tank until use. Patients included 25 males and 10 females. 12 of them were cigarette smokers with an average pack/year history of 38. Histologically, 22 cases (63%) had adenocarcinoma, 10 (29%) had squamous cell carcinoma, and 3 (9%) had large cell carcinoma.

1.2. Chemicals

Cell culture medium, Dulbecco's modified Eagle's medium (DMEM), and fetal calf serum were purchased from Life Technologies (Grand Island, NY, USA). Anti-human iNOS and PPARγ antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). ABC reagent was from VECTOR Laboratories (Burlingame, CA, USA). The chemiluminescent detection kit (ECL system) was obtained from Amersham Pharmacia Biotech (Piscataway, NJ, USA) and Total NO Assay kit from R&D Systems (Minneapolis, MN, USA). Unless otherwise indicated, all other chemicals were from the Sigma Chemical Company (St. Louis, MO, USA).

1.3. Cell culture

Three human lung cell lines were used in this study from the American Type Culture Collection (Manassas, VA, USA). HTB-175, an adenocarcinoma cell line, was derived from a patient with NSCLC. Both CCL-75.1 and CCL-202 were lung fibroblast cell lines. All three cell lines were cultured in DMEM.

1.4. Immunohistochemical examination

Tissues used for immunohistochemistry were embedded in paraffin. In order to preserve the antigenic determinants analysed in this study, formalin fixation before embedding was less than 30 h throughout. Tissues were sectioned into 4-μm thick slices. Immunostaining was performed on the paraffin sections according to the standard procedure of ABC kit from Vector Laboratories (Burlingame, CA, USA). In brief, tissue sections were deparaffinised and rehydrated through three changes of xylene and graded alcohol. Tissue sections were then boiled in citrate-based antigen unmasking solution for 1 min and cooled in Milli-Q water, the endogenous peroxidase activity in the tissue sections was quenched with 3% (v/v) hydrogen peroxide solution for 5 min. 1.5% (v/v) normal blocking serum supplemented with avidin solution (Avidin/Biotin blocking kit, VECTOR Laboratories, Burlingame, CA, USA) was used to block the tissue sections for 30 min. Afterwards, preparations were incubated with a primary antibody overnight at 4 °C. The primary antibody was prepared in 1.5% (v/v) normal blocking serum supplemented with biotin solution from Avidin/Biotin blocking kit (VECTOR Laboratories, Burlingame, CA, USA) and used at a working dilution of 1:200. After tissue sections were washed with phosphate-buffered solution (PBS), a biotinylated-labelled secondary antibody, IgG,

was applied for 30 min. Tissue sections were then washed with PBS and ABC reagent (Avidin/Biotin kit, VECTOR Laboratories, Burlingame, CA, USA) conjugated with horseradish peroxidase was applied for 30 min. Antigen staining was visualised by diaminobenzidine (DAB) substrate (Vector Laboratories, Burlingame, CA, USA). The reaction was terminated by rinsing the sections in tap water. Unless otherwise indicated, all incubations were done in a humidified environment at room temperature. Finally, sections were counterstained with Vector Gill's haematoxylin. After dehydration through graded alcohol and being cleared with xylene, they were mounted with dibutyl phthalate with xylene (DPX) permanent mountant. Negative controls were prepared by replacing the primary antibody with PBS.

1.5. Western blot analysis

Tissue samples were homogenised with ice-cold PBS and then lysed in a solution containing 8 M urea, 0.1 M Na_2H_2PO4 and 0.01 M Tris–HCl. Supernatants were obtained after centrifugation at 10,000g. After boiling, proteins were separated on 10% (v/v) sodium dodecyl sulphate (SDS)-polyacrylamide gels. Proteins were then electrophoretically transferred from the gel onto nitrocellulose membranes and the membranes were blocked for 1 h in PBS–Tween buffer containing 5% (v/v) dry milk powder (fat-free) at room temperature. The membranes were then incubated with a primary antibody (1:1000) for 1 h. After washing, the membranes were incubated with a secondary antibody, IgG-HRP. Finally, they were treated with the reagents in the chemiluniinescent detection kit according to the manufacturer's instruction. The densities of the protein bands were determined and analysed by Quantity One program (version 4.2, BioRad Laboratories, Hercules, CA, USA).

1.6. NO activity assay

The frozen tissues were homogenised at 4 °C in a buffer containing 0.1 mM ethylene diamine tetra acetic acid (EDTA), 10 μg/ml leupetin, 10 μg/ml aprotinin, 10 μ/ml soybean trypsin inhibitor, 100 μm p-amidino-

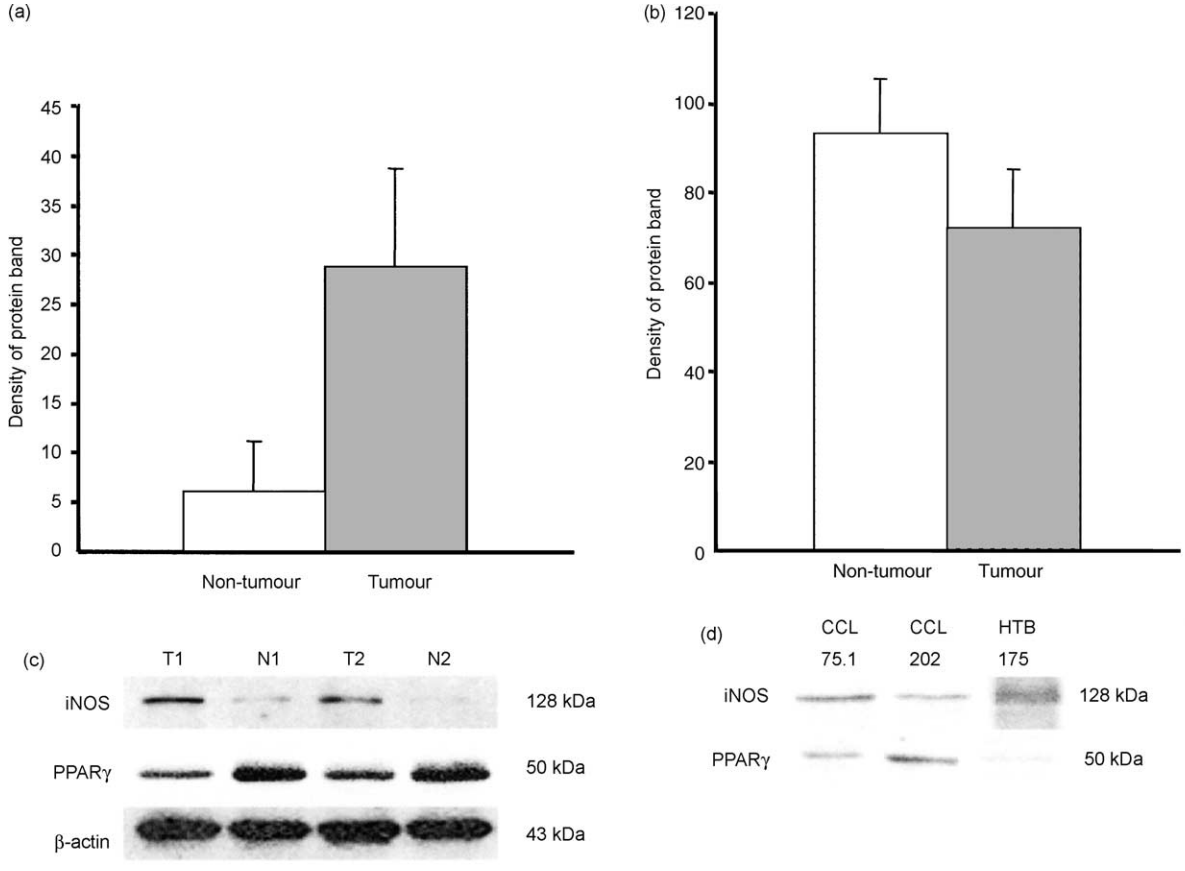

Fig. 1. iNOS and PPARγ protein expression. Proteins were isolated from tumorous and non-tumorous tissues of non-small cell lung carcinoma (NSCLC). Antihuman inducible nitric oxide synthase (iNOS) and peroxisome proliferator-activated receptor gamma (PPARγ) antibodies were used to detect the protein levels by western blotting analysis. The densities of the protein bands for iNOS and PPARγ were quantitated. (a) The average densities of iNOS bands obtained from the tumorous and non-tumorous tissues ($P<0.0001$, $n=35$) are shown. (b) The average densities of the PPARγ bands obtained from the tumorous and non-tumorous tissues ($P<0.05$, $n=35$) are compared. (c) A typical western blot of iNOS and PPARγ for two pairs of tissue samples. T, tumour; N, non-tumour. (d) a western blot obtained from an experiment on the human lung cell lines.

phenymethanesulphonyl fluoride hydrochloride, 1 μM dithiothreitol, 0.32 M sucrose and 15 mM hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES). The homogenates were then centrifuged at 100,000*g* for 1 hour at 4 °C to collect the supernatants. Most of the NO is oxidised to nitrite and nitrate, and the nitrate is later converted to nitrite. The presence of NO in the supernatants was therefore determined by an assay for nitrite, a stable product of NO in solution. The assay was carried out using a Total NO Assay kit from R&D Systems.

1.7. Statistics

All values were expressed as means ± standard errors of the means. Statistical comparisons between the groups were analysed by the Student's *t*-test using InStat software (GraphPad Software, San Diego, CA, USA). Regression and correlation analyses were carried out by the Pearson method. A *P* value of less than 0.05 was considered as statistically significant.

2. Results

The expression of iNOS was significantly increased in the tumorous tissues compared with nontumorous tissues, by both western blotting analysis and immunohistochemical examination (Figs. 1a and 2a and b). Surprisingly, 23 of 35 non-tumorous tissues did not have detectable iNOS protein by western blotting whereas only seven tumorous tissues did not express iNOS. The western blotting results matched the data obtained by immunohistochemical examination of the same set of tissues. In the immunohistochemical examination, iNOS protein was located in both the nucleus and the cytoplasm. However, the signal was much more intense in the former than in the latter (Fig. 2a). There was also an increase in NO activity, reflected by a significant elevation of nitrite, a stable product of NO (Fig. 3).

All tissue samples tested expressed PPARγ. However, in contrast to the results for iNOS expression, the level of PPARγ was significantly lower in the tumorous tissues compared with the non-tumorous ones (Figs. 1b and 2c and d). Although PPARγ is a nuclear protein, we found that this protein was also located in the cytoplasm. A strong signal of PPARγ protein was detected in the nucleus of the respiratory epithelium from non-tumorous tissues (Fig. 2d). PPARγ protein was found infrequently in the nucleus of tumour cells (Fig. 2c), and the intensity was much weaker than that in the nucleus of the non-tumorous cells.

These differing expression patterns for iNOS and PPARγ proteins in tumorous and non-tumorous tissues were confirmed using human lung cell lines. The level of iNOS protein was higher in HTB-175, a human lung cancer cell line, than in normal lung cells (CCL-75.1 and CCL-202) (Fig. 1d). In contrast, PPARγ protein was barely detected in the cancer cells, but was clearly evident in normal lung cells (Fig. 1d).

These results prompted us to examine whether there was a statistical correlation between the levels of both proteins in lung cancer tissues. Although 35 lung tissues from patients with NSCLC were used in this study, only 31 samples qualified for the regression and correlation analyses. This was due to a lack of sample for western blotting analysis in some cases and four other samples were unable to match each other. The regression and

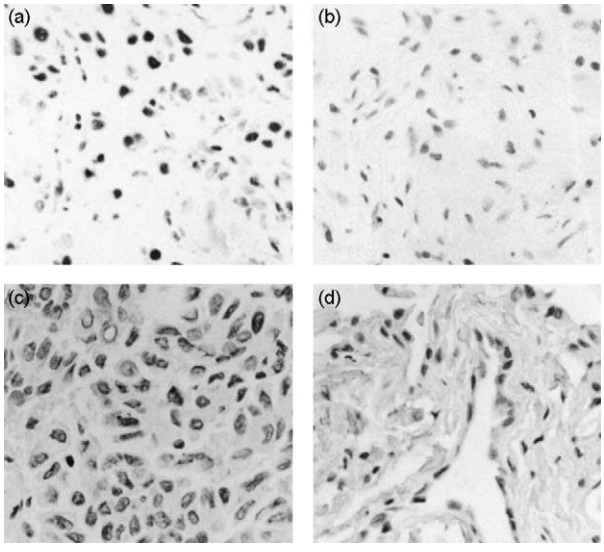

Fig. 2. Immunohistochemical staining of iNOS and PPARγ. The expression of iNOS (a and b) and PPARγ (c and d) proteins in the tumorous (a and c) and non-tumorous (b and d) tissues was studied by immunohistochemical staining (400×).

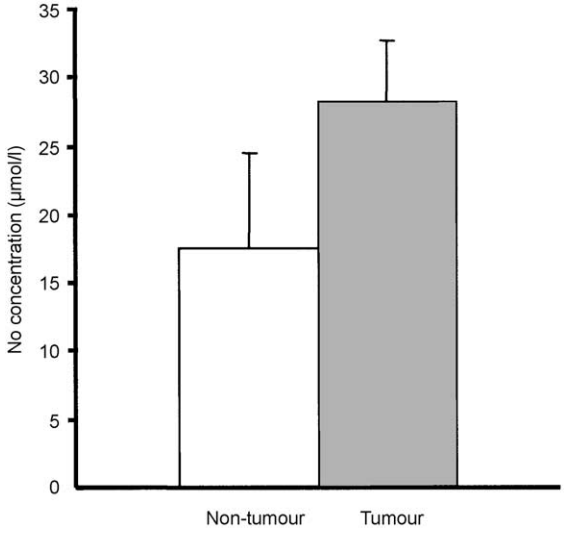

Fig. 3. The level of NO in lung tissues. The frozen tissues were homogenised, the homogenates centrifuged and the supernatants used to assay nitric oxide (NO) activity. The presence of NO in the supernatants was determined by measuring nitrite, a stable product of NO. The NO activity was higher in the tumorous tissues than the non-tumorous ones ($P < 0.05$, $n = 35$).

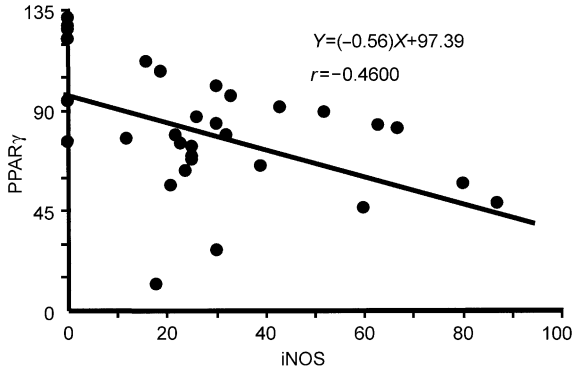

Fig. 4. Correlation between iNOS level and PPARγ expression. The densities of protein bands obtained by western blot were quantitated. Data for iNOS were plotted against those for PPARγ. Regression and correlation analyses were carried out by the Pearson method and revealed a P value of 0.0092.

correlation analyses revealed a negative relationship between the increased level of iNOS protein and the decreased level of PPARγ protein in the lung cancer tissues (Fig. 4).

There was no significant correlation between the expression of iNOS and gender, smoking exposure or histological type of the tumours. A similar non-significant result was also obtained for the expression of PPARγ and gender, smoking exposure or histological type of the tumours.

3. Discussion

Lung cancer, like other epithelial cancers, is believed to arise as a result of a multistep carcinogenic process. The possibility that this process can be blocked or reversed, thereby preventing the development of an invasive cancer, has generated great enthusiasm in the pathogenesis of this disease. The present study demonstrated that the expression of PPARγ was decreased in lung cancer tissues compared with their corresponding normal lung tissues. In agreement with previous studies, we confirmed that the level of iNOS was much higher in tumorous tissues than in non-tumorous tissues. This pattern of an increased iNOS level and a decreased PPARγ expression in NSCLC tissues was also found in the human lung cancer cell lines we examined. Therefore, the above finding suggests that the expression of iNOS was inversely associated with the level of PPARγ.

The involvement of PPARγ in the development of human cancer has recently received much attention [11–14]. To the best of our knowledge, a comparison study on the expression of PPARγ in tumorous and non-tumorous tissues from NSCLC has not been performed before. The low level of PPARγ in the lung cancer tissues as found in this study may be pathogenic. As a transcription factor essential for the differentiation of certain types of cells, activation of PPARγ was able to inhibit the proliferation of cancer cells and thus arrest the growth of cancers [2]. Therefore, the growth control of lung epithelial cells can be affected by PPARγ expression levels. Increasing the level of PPARγ in lung cancer cells may be of therapeutic significance. In fact, a recent *in vitro* study has demonstrated that the activation of PPARγ inhibited human lung cancer cell growth through the induction of apoptosis [13]. It was observed in the present study that the PPARγ protein was mainly accumulated in the nucleus of the lung epithelial cells. This is in line with the fact that PPARγtranslocates into the nucleus after its activation by the binding of specific ligands [2]. Lung cancer cells were more likely to retain PPARγ protein in their cytoplasms. The cytoplasmic location of PPARγ may suggest a failure of PPARγ activation probably due to either an alteration of PPARγ function domains or a lack of PPARγ ligands.

It is now well known that the activation of PPARγ downregulates a number of genes related to the activation of cells and induces a resting phenotype in a cell [2]. iNOS is one of the genes inhibited by PPARγ [15–17]. Therefore, a decrease in PPARγ level is expected to favour iNOS expression. This is true in human NSCLC, as a significant increase in iNOS protein was observed in the NSCLC tissues in which a decrease in the PPARγ level was found. This inverse expression pattern of a decreased PPARγ and an increased iNOS level was also demonstrated in the human lung cancer cell lines in our study and was further confirmed by the regression and correlation analyses. It should be noted that a significant correlation might not necessarily indicate a cause–result relationship between the two parties involved, although it does reveal a link in nature. Our present study did not investigate how a low PPARγ level contributes to the high expression of iNOS seen in NSCLC. It is known that the regulation of iNOS expression by PPARγ involves a transrepression process, which suppresses nuclear factor-kappa B (NF-κB) activity [18]. NF-κB is an important transcription factor for the control of iNOS gene expression [19]. The activity of NF-κB is much higher in human NSCLC [20], which is consistent with the increase in the iNOS level found in the present study. Therefore, it is possible that the lack of a PPARγ signal may result in a loss of transrepression of NF-κB and, subsequently, promote the expression of iNOS in NSCLC.

The role of iNOS or NO in cancer is multidimensional, and can be either tumour-promoting or tumour-inhibiting [1]. In our study, it seems that an increase in iNOS was associated with the promotion of NSCLC as in more than 50% of non-tumorous tissues tested, iNOS protein was undetectable. It has been reported that NO generated during chronic inflammation and prolonged environmental exposure can initiate and enhance carcinogenesis in humans [1]. Human lung cancer is indeed closely related to chronic inflammation

and environmental factors [21–23]. Furthermore, the lung cancer-associated inflammatory and environmental factors, such as lipopolysaccharide, interleukin 1β and cigarette smoking, have been documented to stimulate the production of iNOS and/or NF-κB [3,24]. However, it is not known whether these factors can attack PPARγ in lung epithelial cells. The carcinogenic effect of iNOS is executed through its enzymatic product, NO, whose level was increased in the NSCLC tissues in our study. NO possesses several properties that might enhance carcinogenesis. For example, NO is able to specifically mediate tumour vascularisation [1,25]. NO is also well known for its role in the DNA damaging pathways and triggers the accumulation of p53 in the nucleus [26,27]. The significance of the nuclear localisation of iNOS in lung cancer cells is not clear at present. However, it may be related to its role in DNA damage.

In conclusion, this study demonstrated differing expression patterns for iNOS and PPARγ in NSCLC tissues. Since activated PPARγ is able to inhibit the expression of iNOS and the generation of iNOS is known to be related to the inflammatory and environmental factors of lung cancer risk in particular, this expression pattern may be associated with the development of NSCLC.

The therapeutic benefit of restoring the PPARγ level will be an interesting question for future analyses.

Acknowledgements

We thank for Isa K.Y. Lee for analysing some of the experimental data.

References

1. Wink DA, Vodovotz Y, Laval J, Laval F, Dewhirst MW, Mitchell JB. The multifaceted roles of nitric oxide in cancer. *Carcinogenesis* 1998, **19**, 711–721.
2. Debril MB, Renaud JP, Fajas L, Auwerx J. The pleiotropic functions of peroxisome proliferator-activated receptor gamma. *J Mol Med* 2001, **79**, 30–47.
3. Wright JL, Dai J, Zay K, Price K, Gilks CB, Churg A. Effects of cigarette smoke on nitric oxide synthase expression in the rat lung. *Lab Invest* 1999, **79**, 975–983.
4. Lee RP, Wang D, Kao SJ, Chen HI. The lung is the major site that produces nitric oxide to induce acute pulmonary oedema in endotoxin shock. *Clin Exp Pharmaco Physiol* 2001, **28**, 315–320.
5. Jaiswal M, LaRusso NF, Burgart U, Gores GJ. Inflammatory cytokines induce DNA damage and inhibit DNA repair in cholangiocarcinoma cells by a nitric oxide-dependent mechanism. *Cancer Res* 2000, **60**, 184–190.
6. Boyle P, Maisonneuve P. Lung cancer and tobacco smoking. *Lung Cancer* 1995, **12**, 167–181.
7. Liu CY, Wang CH, Chen TC, Lin HC, Yu CT, Kuo HP. Increased level of exhaled nitric oxide and up-regulation of inducible nitric oxide synthase in patients with primary lung cancer. *Br J Cancer* 1998, **78**, 534–541.
8. Fujimoto H, Sasaki J, Matsumoto M, Suga M, *et al*. Significant correlation of nitric oxide synthase activity and p53 gene mutation in stage I lung adenocarcinoma. *Jpn J Cancer Res* 1998, **89**, 696–702.
9. Ambs S, Bennett WP, Merriam WG, *et al*. Vascular endothelial growth factor and nitric oxide synthase expression in human lung cancer and the relation to p53. *Br J Cancer* 1998, **78**, 233–239.
10. Marrogi AJ, Travis WD, Welsh JA, *et al*. Nitric oxide synthase, cyclooxygenase 2, and vascular endothelial growth factor in the angiogenesis of non-small cell lung carcinoma. *Clin Cancer Res* 2000, **6**, 4739–4744.
11. Eibl G, Wente MN, Reber HA, Hines OJ. Peroxisome proliferator-activated receptor gamma induces pancreatic cancer cell apoptosis. *Biochem Biophys Res Commun* 2001, **287**, 522–529.
12. Gupta RA, Brockman JA, Sarraf P, Wilison TM, DuBois RN. Target genes of peroxisome proliferator-activated receptor gamma in colorectal cancer cells. *J Biol Chem* 2001, **276**, 29681–29687.
13. Tsubouchi Y, Sano H, Kawahito Y, *et al*. Inhibition of human lung cancer cell growth by the peroxisome proliferatoractivated receptor-gamma agonists through induction of apoptosis. *Biochem Biophys Res Commun* 2000, **270**, 400–405.
14. Chang TH, Szabo E. Induction of differentiation and apoptosis by ligands of peroxisome proliferator-activated receptor gamma in non-small cell lung cancer. *Cancer Res* 2000, **60**, 1129–1138.
15. Li M, Pascual G, Glass CK. Peroxisome proliferator-activated receptor gamma-dependent repression of the inducible nitric oxide synthase gene. *Mol Cell Biol* 2000, **20**, 4699–4707.
16. Liang YC, Tsai SH, Tsai DC, Lin-Shiau SY, Lin JK. Suppression of inducible cyclooxygenase and nitric oxide synthase through activation of peroxisome proliferatoractivated receptor-gamma by flavonoids in mouse macrophages. *FEBS Lett* 2000, **496**, 12–18.
17. Fahmi H, Di Battista JA, Pelletier JP, Mineau F, Ranger P, Martel-Pelletier J. Peroxisome proliferator-activated receptor gamma activators inhibit interleukin-1beta-induced nitric oxide and matrix metalloproteinase 13 production in human chondrocytes. *Arthritis Rheumatism* 2001, **44**, 595–607.
18. Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK. The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. *Nature* 1998, **391**, 79–82.
19. Chen G, Wang SH, Warner TD. Regulation of iNOS mRNA levels in endothelial cells by glutathione, a double-edged sword. *Free Rad Res* 2000, **32**, 223–234.
20. Mukhopadhyay T, Roth JA, Maxwell SA. Altered expression of the p50 subunit of the NF-kappa B transcription factor complex in non-small cell lung carcinoma. *Oncogene* 1995, **11**, 999–1003.
21. Emmendoerffer A, Hecht M, Boeker T, Mueller M, Heinrich U. Role of inflammation in chemical-induced lung cancer. *Toxicol Lett* 2000, **112–113**, 185–191.
22. Scott FM, Prime W, Walshaw M, Turnbull L, Field JK. Peptide amidating activity in human bronchoalveolar lavage fluid: relationship to lung cancer, inflammation and infection. *International Journal of Oncology* 2000, **16**, 327–332.
23. Haugen A, Ryberg D, Mollerup S, Zienolddiny S, Skaug V, Svendsrud DH. Gene-environment interactions in human lung cancer. *Toxicol Lett* 2000, **112–113**, 233–237.
24. Bogdan C. Nitric oxide and the immune response. *Nat Immunol* 2001, **2**, 907–916.
25. Gallo O, Masini E, Morbidelli L, *et al*. Role of nitric oxide in angiogenesis and tumor progression in head and neck cancer. *J Natl Cancer Inst* 1998, **90**, 587–596.
26. Burney S, Caulfield JL, Niles JC, Wishnok JS, Tannenbaum SR. The chemistry of DNA damage from nitric oxide and peroxynitrite. *Mutation Res* 1999, **424**, 37–49.
27. Huang LC, Clarkin KC, Wahl GM. Sensitivity and selectivity of the DNA damage sensor responsible for activating p53-dependent G1 arrest. *Proc Natl Acad Sci* 1996, **93**, 4827–4832.