ORIGINAL ARTICLE

Expression of peroxisome proliferator activated receptor γ and cyclo-oxygenase 2 in primary and recurrent ovarian carcinoma

Sylvia Stadlmann, Uwe Gueth, Edward Wight, Leoni A Kunz-Schughart, Arndt Hartmann, Gad Singer

See end of article for authors' affiliations

J Clin Pathol 2007;**60**:307–310. doi: 10.1136/jcp.2005.035717

Correspondence to:
PD Dr G Singer, Institute of
Pathology, University
Hospital Basel,
Schönbeinstrasse 40, CH-
4031 Basel, Switzerland;
gsinger@uhbs.ch

Accepted 23 February 2006
Published Online First
12 May 2006

Aim: Peroxisome proliferator-activated receptor γ (PPAR γ) has emerged as a potential therapeutic target in several types of cancer. In ovarian carcinomas, limited and conflicting data on PPAR γ protein expression have been reported.

Methods: The immunoexpression of PPAR γ and its putative target cyclo-oxygenase 2 (COX2) was investigated in tumour tissues from 80 patients with primary and corresponding recurrent ovarian serous carcinomas after conventional platinum-based chemotherapy.

Results: PPAR γ expression was observed in 29% of primary and recurrent carcinomas. In the recurrent tumours, PPAR γ expression inversely correlated with COX2 overexpression in both chemosensitive ($p=0.02$) and chemoresistant ($p=0.04$) carcinomas.

Conclusions: The data indicate that PPAR γ may represent a potential target for second-line treatment in ovarian cancers.

Ovarian cancer is the leading cause of death from gynaecological malignancies, in most cases because of the development of resistance to platinum-based chemotherapy in primary and recurrent tumours.¹ Assessment of molecular therapeutic targets that are more strictly related to individual tumour biology and intrinsic chemoresponse could help identify high-risk patients and facilitate management of this disease.

One potential therapeutic target is the peroxisome proliferator-activated receptor γ (PPAR γ), a member of a nuclear hormone receptor superfamily.² Treatment with PPAR γ ligands inhibits the growth of various human cancer cells as well as tumour angiogenesis and invasion.^{3–11} In addition, PPAR γ agonists have been shown to exhibit chemopreventive activities.^{12–14} Preliminary data on the success of treatment in clinical trials, however, are controversial. In patients with chemotherapy-resistant metastatic colorectal cancer, no objective response to the PPAR γ ligand troglitazone was observed.¹⁵ Treatment of patients with metastatic breast cancer also failed to show any clinical benefits.¹⁶ In patients with prostate cancer, however, treatment with troglitazone based on PPAR γ protein expression in tumour tissues led to stabilisation of prostate-specific antigen in 20% of patients.¹⁷

PPAR γ ligands can interact with or have an effect on common pathways with cyclo-oxygenase 2 (COX2), a prostaglandin synthetase. Recent studies suggest that expression of COX2 is regulated by a negative feedback loop mediated through PPAR γ .^{18–21} PPAR γ agonists have been shown to down regulate COX2 and potentiate the apoptotic effects of chemotherapeutic agents.²² COX2 is often overexpressed in ovarian carcinomas, and seems to be associated with chemoresistance and poor outcome.^{23–26} Recently, it has been shown that COX2 is an independent prognostic factor for overall survival in patients with ovarian cancer receiving platinum-based chemotherapy.²⁷

In ovarian carcinomas, two studies have reported very different frequencies of PPAR γ expression. In particular, both studies arrived at different conclusions for overexpression in comparison with benign tumours.^{28–29} On the basis of these

results, we performed a thorough investigation of PPAR γ in a large series of primary ovarian serous carcinomas and their corresponding recurrences after platinum-based chemotherapy. In particular, we aimed to investigate PPAR γ as a therapeutic target for second-line treatment.

PATIENTS AND METHODS

Patients

Primary tumours from 80 patients with advanced ovarian serous carcinomas from 1985 and 2003 and their recurrences were included in this study. Paired tissue samples from the primary carcinomas and their corresponding recurrences in 74 patients were available for analysis. Tissues were examined at the Institute for Pathology, University of Basel, Basel, Switzerland, and the Cantonal Hospitals of Baden, Liestal and St Gallen, Switzerland. All patients underwent debulking surgery before systemic chemotherapy. Patients, after surgery, received at least three cycles of platinum-based chemotherapy. In all, 73 (91.3%) patients completed first-line chemotherapy (six cycles). Patients were aged 20–77 years (median age 59 years). Only high-grade ovarian serous carcinomas (3.7% Federation of Gynaecology and Obstetrics stage II and 96.3% stage III) were included in this study.^{30–31} The clinical data, including surgical and systemic treatment characteristics, were collected from the medical records. Recurrence was defined as an elevation of CA-125 levels, with confirmation of tumour in the radiological examination or during secondary surgical procedures. Time to recurrence of ≤ 6 months after completion of platinum-based chemotherapy was defined as chemoresistance and time to recurrence of >6 months as chemosensitivity.³²

Tissue microarray

Tissue samples were fixed in 4% buffered formalin, embedded in paraffin wax and used to construct a tissue microarray

Abbreviations: COX2, cyclo-oxygenase 2; IHC, immunohistochemistry; PPAR γ , peroxisome proliferator-activated receptor γ ; TMA, tissue microarray

Figure 1 Immunohistochemical staining for peroxisome proliferator-activated receptor γ (PPAR γ) and cyclo-oxygenase 2 (COX2) in ovarian serous carcinomas. (A) Strong cytoplasmic expression of PPAR γ . (B) Strong cytoplasmic COX2 expression.

(TMA).³³ Haematoxylin and eosin-stained sections were made from each selected primary block (donor blocks) to define representative tissue regions. Tissue cylinders (0.6 mm in diameter) were then punched from the region of the donor block with the use of a custom-made precision instrument (Beecher Instruments, Silver Spring, Maryland, USA). Tissue cylinders were transferred to a 25 mm $\times$ 35 mm paraffin-wax block to produce the TMA block used for the study. The resulting TMA block was cut into 3- μ m sections that were transferred to glass slides by use of the Paraffin Sectioning Aid System (Instrumedics, Hackensack, New Jersey, USA). Sections from the TMA block were used for the different analyses.

Immunohistochemistry

Standard indirect immunoperoxidase procedures were used for immunohistochemistry (IHC; ABC-Elite, Vectra Laboratories, Burlingame, California, USA) on TMAs. Immunohistochemistry was performed with a goat polyclonal antibody to PPAR γ (dilution 1:200, final concentration 1 μ g/ml Santa Cruz Biotechnology, Santa Cruz, California, USA) and a mouse monoclonal antibody to COX2 (dilution 1:100; final concentration 5 μ g/ml, Cayman Chemical, Ann Arbor, Minnesota, USA). TMA was analysed by light microscopy by use of a 10 $\times$ objective. Immunostaining was evaluated semiquantitatively by

Table 1 Patients' characteristics in relation to peroxisome proliferator-activated receptor γ and cyclo-oxygenase 2 protein expression in advanced high-grade ovarian serous carcinomas

	PPAR γ expression, n (%)	COX2 expression, n (%)
Age (years)		
<60	8/29 (27.6)	29/30 (96.7)
$\geq$ 60	8/26 (30.8)	26/26 (100)
p Values	0.436	1
Residual tumour (cm)		
<2	3/15	15/16
20		93.8
$\geq$ 2	3/15	15/15
20		100
p Values	0.378	1
Time to recurrence (months)		
$\leq$ 6	2/14	14/14
14.3		100
>6	14/41	41/42
34.1		97.6
p Values	0.134	1

COX2, cyclo-oxygenase 2; PPAR γ , peroxisome proliferator-activated receptor γ .

two pathologists (GS and SS). PPAR γ or COX2 expression in <10% of tumour cells was considered negative. Appropriate positive and negative controls were used.

Data analysis

Statistical tests included the Wilcoxon's signed rank test, the Mann-Whitney U test and the Pearson's test, and were two sided. A p value of $\leq$ 0.05 was considered to be significant. Statistical analyses were performed with SPSS V.12.0 software.

RESULTS

PPAR γ protein expression in ovarian serous carcinoma

PPAR γ immunostaining could be evaluated in 99 primary and recurrent tumours. Failures in analysis were due to either lack of tissue on the MA section or lack of vital tumour cells in the array samples. The staining pattern in the tumour cells was cytoplasmic in all cases, with single tumour cells also exhibiting nuclear staining (fig 1A). The overall expression of PPAR γ in primary and recurrent neoplasms was nearly identical. It was also nearly identical in the cases of tumours with matched recurrences. In all, 16 of 55 (29.1%) primary and 13 of 44 (29.5%) recurrent tumours showed positive staining for PPAR γ , with no significant differences between chemosensitive and chemoresistant tumours. Table 1 summarises the overall PPAR γ expression in primary carcinomas in relation to patients' characteristics.

COX2 protein expression in ovarian serous carcinoma

COX2 immunostaining could be evaluated in 103 primary and recurrent ovarian tumours. COX2 staining pattern in the tumour cells was cytoplasmic in all cases (fig 1B). Overall, primary ovarian carcinomas showed a significantly higher frequency of COX2 expression than did recurrent tumours; $p=0.046$; this was also true for the cases of tumours with matched recurrences. Regarding the frequency of COX2 expression, there was no significant difference between chemosensitive and chemoresistant tumours. Table 1 summarises the patients' characteristics in relation to the overall COX2 expression in primary ovarian carcinomas.

Associations of PPAR γ and COX2 expression in ovarian serous carcinoma

We found no significant differences in the PPAR γ and COX2 coexpression patterns of primary and recurrent ovarian tumours. Ovarian carcinomas were positive for both PPAR γ and COX2 in 16 of 55 (29.1%) primary and in 9 of 42 (21.4%) recurrent tumours. The vast majority of ovarian tumours proved to be negative for PPAR γ but positive for COX2 in 39 of 54 (72.2%) primary and in 28 of 42 (66.7%) recurrent tumours. None of the 55 primary but 3 of 42 (7.1%) recurrent neoplasms were positive for PPAR γ and negative for COX2. One of 54 (1.9%) primary and 2 of 42 (4.8%) recurrent tumours were negative by both PPAR γ and COX2. PPAR γ immunostaining was not associated with COX2 expression in primary ovarian carcinomas ($p = 0.527$). In the recurrent tumours, however, decreased PPAR γ expression significantly correlated with COX2 overexpression in both the chemosensitive ($p = 0.020$) and the chemoresistant ($p = 0.040$) groups.

DISCUSSION

PPAR γ ligands have been reported to induce cell differentiation and apoptosis in several carcinomas, suggesting potential use as anticancer agents.^{3 5 7 8 10 11 34 35} For example, treatment of patients with prostate cancer with a PPAR γ agonist such as troglitazone led to stabilisation of the disease in 20% of the cases.¹⁷ The patients were selected on the basis of PPAR γ expression in their tumour tissues.

In ovarian carcinomas, limiting and conflicting data on PPAR γ protein expression have been reported. Zhang *et al*²⁸ investigated several histotypes of ovarian carcinomas. They showed PPAR γ expression in 11 of 12 (92%) grade 3 tumours. No data were provided on COX2 expression. By contrast, Sakamoto *et al*²⁹ reported 10 of a total of 30 (33%) ovarian serous carcinomas with PPAR γ immunopositivity. This is in line with the results from our study, showing PPAR γ expression in approximately 30% of both primary carcinomas and, in particular, in tissues from their recurrences. This fraction might therefore reflect the expected response rate with second-line PPAR γ targeting agents.

Sakamoto *et al*²⁹ assumed an inverse association between PPAR γ and COX2, based on eight cases that were negative for PPAR γ and simultaneously positive for COX2. In our study, PPAR γ expression was negatively correlated with COX2 expression in the recurrent tumours, providing further evidence that COX2 is regulated by a negative feedback through PPAR γ . Documentation of PPAR γ expression may therefore give valuable information for the selection of a subset of patients who might respond to PPAR γ ligands, thus making PPAR γ a potential target for treatment.

COX2 overexpression has been implicated in platinum-based chemoresistance in ovarian cancer.^{23 24 26} Two studies have investigated different carcinoma histotypes, and one study focused on serous carcinomas. In this present study, a high frequency of COX2 expression was observed, although of weak intensity in most cases. Earlier studies with large tumour groups have reported COX2 expression in 42–79% of ovarian carcinomas and found a positive correlation of COX2 expression with high grade and disease progression.^{26 27 29 36 37} Our selection of only advanced high-grade ovarian serous carcinomas could explain the high frequency of COX2 positivity in this study. In the present study, there was no significant difference between chemosensitive and chemoresistant tumours with regard to the frequency of both PPAR γ and COX2 expression. This suggests that other factors, more important than PPAR γ and COX2, are involved in the development of platinum-based chemoresistance in ovarian serous carcinomas.

Take-home messages

- PPAR γ is an important drug target in many cancer types.
- Up to 29% of ovarian serous carcinomas show an expression for PPAR γ .
- PPAR γ expression is inversely correlated to COX2 overexpression in ovarian serous carcinoma.
- The expression of PPAR γ is not related to chemoresponse in ovarian serous carcinoma.

In conclusion, our data indicate that PPAR γ expression is observed in a considerable fraction of recurrent ovarian serous carcinomas after conventional platinum-based chemotherapy. PPAR γ may therefore represent a potential target for second-line treatment.

ACKNOWLEDGEMENT

We thank Dr PD Beat Thürlimann (Kantonsspital St Gallen), Dr Regula Weber (Kantonsspital Baden) and Dr Ronja Müller (Kantonsspital Liestal) for support in obtaining the clinical data. We also thank Frank van Rey for excellent technical assistance.

Authors' affiliations

S Stadlmann, G Singer*, Institute of Pathology, University Hospital Basel, Basel, Switzerland

U Gueth, * E Wight, Department of Gynecology and Obstetrics, University Hospital Basel, Basel, Switzerland

L A Kunz-Schughart, A Hartmann, Institute of Pathology, University of Regensburg, Regensburg, Germany

*These authors have contributed equally to the work.

Funding: This study was supported by the Swiss Cancer League (Oncosuisse), grant number OCS 01506-02-2004.

Competing interests: None declared.

REFERENCES

- 1 Fraser MJ, Leung B, Jahani-Asl A, *et al*. Chemoresistance in human ovarian cancer: the role of apoptotic regulators. *Reprod Biol Endocrinol* 2003;**1**:66.
- 2 Sporn MB, Suh N, Mangelsdorf DJ. Prospects for prevention and treatment of cancer with selective PPAR γ modulators (SPARMs). *Trends Mol Med* 2001;**7**:395–400.
- 3 Elstner E, Muller C, Koshizuka K, *et al*. Ligands for peroxisome proliferator-activated receptor γ 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* 1998;**95**:8806–11.
- 4 Sato H, Ishihara S, Kawashima K, *et al*. Expression of peroxisome proliferator-activated receptor (PPAR) γ in gastric cancer and inhibitory effects of PPAR γ agonists. *Br J Cancer* 2000;**83**:1394–400.
- 5 Rumi MA, Sato H, Ishihara S, *et al*. Peroxisome proliferator-activated receptor γ ligand-induced growth inhibition of human hepatocellular carcinoma. *Br J Cancer* 2001;**84**:1640–7.
- 6 Martelli ML, Iuliano R, Le Pera I, *et al*. Inhibitory effects of peroxisome poliferator-activated receptor γ on thyroid carcinoma cell growth. *J Clin Endocrinol Metab* 2002;**87**:4728–35.
- 7 Koeffler HP. Peroxisome proliferator-activated receptor γ and cancers. *Clin Cancer Res* 2003;**9**:1–9.
- 8 Panigrahy D, Shen LQ, Kieran MW, *et al*. Therapeutic potential of thiazolidinediones as anticancer agents. *Expert Opin Invest Drugs* 2003;**12**:1925–37.
- 9 Yoshimura R, Matsuyama M, Segawa Y, *et al*. Expression of peroxisome proliferator-activated receptors (PPARs) in human urinary bladder carcinoma and growth inhibition by its agonists. *Int J Cancer* 2003;**104**:597–602.
- 10 Theocharis S, Margeli A, Vielh P, *et al*. Peroxisome proliferator-activated receptor- γ ligands as cell-cycle modulators. *Cancer Treat Rev* 2004;**30**:545–54.
- 11 Fenner MH, Elstner E. Peroxisome proliferator-activated receptor- γ ligands for the treatment of breast cancer. *Expert Opin Invest Drugs* 2005;**14**:557–68.
- 12 Badawi AF, Badr MZ. Chemoprevention of breast cancer by targeting cyclo-oxygenase-2 and peroxisome proliferator-activated receptor- γ . *Int J Oncol* 2002;**20**:1109–22.

- 13 **Andela VB**, Altuwaijri S, Wood J, *et al*. Inhibition of beta-oxidative respiration is a therapeutic window associated with the cancer chemo-preventive activity of PPARgamma agonists. *FEBS Lett* 2005;**579**:1765–9.
- 14 **Lu J**, Imamura K, Nomura S, *et al*. Chemopreventive effect of peroxisome proliferator-activated receptor gamma on gastric carcinogenesis in mice. *Cancer Res* 2005;**65**:4769–74.
- 15 **Kulke MH**, Demetri GD, Sharpless NE, *et al*. A phase II study of troglitazone, an activator of the PPARgamma receptor, in patients with chemotherapy-resistant metastatic colorectal cancer. *Cancer J* 2002;**8**:395–9.
- 16 **Burstein HJ**, Demetri GD, Mueller E, *et al*. Use of the peroxisome proliferator-activated receptor (PPAR) gamma ligand troglitazone as treatment for refractory breast cancer: a phase II study. *Breast Cancer Res Treat* 2003;**79**:391–7.
- 17 **Mueller E**, Smith M, Sarraf P, *et al*. Effects of ligand activation of peroxisome proliferator-activated receptor gamma in human prostate cancer. *Proc Natl Acad Sci USA* 2000;**97**:10990–5.
- 18 **Inoue H**, Tanabe T, Umesono K. Feedback control of cyclo-oxygenase-2 expression through PPARgamma. *J Biol Chem* 2000;**275**:28028–32.
- 19 **Ikawa H**, Kameda H, Kamitani H, *et al*. Effect of PPAR activators on cytokine-stimulated cyclooxygenase-2 expression in human colorectal carcinoma cells. *Exp Cell Res* 2001;**267**:73–80.
- 20 **Yang WL**, Frucht H. Activation of the PPAR pathway induces apoptosis and COX-2 inhibition in HT-29 human colon cancer cells. *Carcinogenesis* 2001;**22**:1379–83.
- 21 **Badawi AF**, Eldeen MB, Liu Y, *et al*. Inhibition of rat mammary gland carcinogenesis by simultaneous targeting of cyclooxygenase-2 and peroxisome proliferator-activated receptor gamma. *Cancer Res* 2004;**64**:1181–9.
- 22 **Aggarwal BB**, Bhardwaj A, Aggarwal RS, *et al*. Role of resveratrol in prevention and therapy of cancer: preclinical and clinical studies. *Anticancer Res* 2004;**24**:2783–840.
- 23 **Ferrandina G**, Lauriola L, Zannoni GF, *et al*. Increased cyclooxygenase-2 (COX-2) expression is associated with chemotherapy resistance and outcome in ovarian cancer patients. *Ann Oncol* 2002;**13**:1205–11.
- 24 **Lou WZ**, Shen K, Zhang Y, *et al*. Relationship of cyclooxygenase 2 expression and chemotherapy response and prognosis in human ovarian carcinoma. *Zhonghua Fu Chan Ke Za Zhi* 2004;**39**:529–32.
- 25 **Seo SS**, Song YS, Kang DH, *et al*. Expression of cyclooxygenase-2 in association with clinicopathological prognostic factors and molecular markers in epithelial ovarian cancer. *Gynecol Oncol* 2004;**92**:927–35.
- 26 **Raspolini MR**, Amunni G, Villanucci A, *et al*. Increased cyclooxygenase-2 (COX-2) and P-glycoprotein-170 (MDR1) expression is associated with chemotherapy resistance and poor prognosis. Analysis in ovarian carcinoma patients with low and high survival. *Int J Gynecol Cancer* 2005;**15**:255–60.
- 27 **Denkert C**, Kobel M, Pest S, *et al*. Expression of cyclooxygenase 2 is an independent prognostic factor in human ovarian carcinoma. *Am J Pathol* 2002;**160**:893–903.
- 28 **Zhang GY**, Ahmed N, Riley C, *et al*. Enhanced expression of peroxisome proliferator-activated receptor gamma in epithelial ovarian carcinoma. *Br J Cancer* 2005;**92**:113–19.
- 29 **Sakamoto A**, Yokoyama Y, Umemoto M, *et al*. Clinical implication of expression of cyclooxygenase-2 and peroxisome proliferator-activated-receptor gamma in epithelial ovarian tumours. *Br J Cancer* 2004;**91**:633–8.
- 30 **Singer G**, Kurman RJ, Chang HW, *et al*. Diverse tumorigenic pathways in ovarian serous carcinoma. *Am J Pathol* 2002;**160**:1223–8.
- 31 **Singer G**, Oldt R III, Cohen Y, *et al*. Mutations in BRAF and KRAS characterize the development of low-grade ovarian serous carcinoma. *J Natl Cancer Inst* 2003;**95**:484–6.
- 32 **Jazaeri AA**, Awtrey CS, Chandramouli GV, *et al*. Gene expression profiles associated with response to chemotherapy in epithelial ovarian cancers. *Clin Cancer Res* 2005;**11**:6300–10.
- 33 **Simon R**, Mirlacher M, Sauter G. Tissue microarrays. *Methods Mol Med* 2005;**114**:257–68.
- 34 **Kato M**, Kusumi T, Tsuchida S, *et al*. Induction of differentiation and peroxisome proliferator-activated receptor gamma expression in colon cancer cell lines by troglitazone. *Cancer Res Clin Oncol* 2004;**130**:73–9.
- 35 **Nunez NP**, Liu H, Meadows GG. PPAR-gamma ligands and amino acid deprivation promote apoptosis of melanoma, prostate, and breast cancer cells. *Cancer Lett* 2005;**236**:133–41.
- 36 **Matsumoto Y**, Ishiko O, Deguchi M, *et al*. Cyclooxygenase-2 expression in normal ovaries and epithelial ovarian neoplasms. *Int J Mol Med* 2001;**8**:31–6.
- 37 **Erkinheimo TL**, Lassus H, Finne P, *et al*. Elevated cyclooxygenase-2 expression is associated with altered expression of p53 and SMAD4, amplification of HER-2/neu, and poor outcome in serous ovarian carcinoma. *Clin Cancer Res* 2004;**10**:538–45.