PPARγ/PGC-1α Pathway in E-Cadherin Expression and Motility of HepG2 Cells

HUI-JU LEE[1], YEU SU[2], PEN-HUI YIN[3], HSIN-CHEN LEE[1*] and CHIN-WEN CHI[1,3*]

[1]*Department and Institute of Pharmacology, School of Medicine and*
[2]*Institute of Biopharmaceutical Science, School of Life Science, National Yang-Ming University;*
[3]*Department of Medical Research and Education, Taipei Veterans General Hospital, Taipei 112, Taiwan, P.R.C.*

Abstract. *Background: Peroxisome proliferator-activated receptor gamma (PPARγ) activation has been proposed as a potential therapeutic strategy for various types of human cancer. The aim of this study was to activate PPARγ and overexpress PGC-1α in HepG2 cells in order to analyze their effects on cell motility and E-cadherin expression. Materials and Methods: Adenovirus-mediated gene transfer was performed to overexpress PGC-1α in HepG2 human hepatoma cells. Small interference RNA (siRNA) was used to silence the expression of E-cadherin and PPARγ. Cell motility was assessed by transwell cell migration analysis. Measurements of mRNA and protein expression were done by quantitative RT-PCR and Western blotting. Results: Treatment with synthetic PPARγ agonists, thiazolidinediones (rosiglitazone; troglitazone), and adenovirus-delivered overexpression of PPARγ transcriptional coactivator-1α (PGC-1α) up-regulated E-cadherin expression and reduced motility of HepG2 cells. Using PPARγ antagonist GW9662, we demonstrated that both PPARγ-dependent and -independent pathways were involved in PGC-1α-induced up-regulation of E-cadherin. In addition, siRNA-mediated knockdown of E-cadherin expression restored the motility of PGC-1α-overexpressing HepG2 cells, indicating that up-regulated E-cadherin is responsible for the lower migratory ability of these cells. Intriguingly, siRNA-mediated silencing of PPARγ abolished E-cadherin protein expression but also reduced the motility of HepG2 cells. Conclusion: PPARγ/PGC-1α pathway plays a crucial role in modulating E-cadherin expression and motility of HepG2 cells and may be a potential target for the prevention of HCC metastasis.*

Hepatocellular carcinoma (HCC) is a leading cause of cancer-related deaths in Taiwan as well as some other developing countries (1). Surgical resection and liver transplantation are currently treatments of choice for HCC because of limitations in radiotherapy and the resistance to chemotherapy (2). To make matters worse, early diagnosis of HCC is difficult and recurrence or metastasis is a common cause of postoperative deaths. Thus, research in new preventive and therapeutic means targeting HCC is highly warranted (3).

Peroxisome proliferator-activated receptor gamma (PPARγ) is a member of the ligand-activated nuclear hormone receptor super family that plays a central role in adipocyte differentiation and insulin sensitivity (4). In addition to well-defined mechanisms of action for diabetes treatment, PPARγ activation has been shown to inhibit proliferation, induce differentiation and apoptosis, and prevent metastasis of various tumor cells (5, 6). One of the suppressive effects of PPARγ agonists on tumor growth and metastasis has been attributed to an increased expression of E-cadherin, a transmembrane protein of the adherens junction which functions as a metastasis suppressor by regulating cell cell interaction (7-9). Based on their *in vitro* and *in vivo* tumor-suppressive effects and favorable side-effect profiles, the synthetic PPARγ ligands, thiazolidinediones (TZDs), are currently being investigated as potential chemotherapeutic drugs (6, 10).

By docking on transcription factors and/or interacting with multiprotein complexes, coactivators usually increase transcriptional efficiency through affecting chromatin structure and RNA polymerase complex recruitment (11, 12). PPARγ coactivator-1α (PGC-1α), a highly inducible protein, was first identified as a PPARγ-interacting coactivator that regulates adaptive thermogenesis in mice (13). Inspired by the fact that PGC-1α plays a crucial role in regulating metabolism in response to a variety of environmental cues, its aberrant expressions have been vigorously studied and it is now believed to be linked to several metabolism-dysregulated diseases including cancer (14). Although

*Both authors contributed equally to this study.

Correspondence to: Dr. Chin-Wen Chi, Department of Medical Research and Education, Taipei Veterans General Hospital, No. 201, Section 2, Shih-Pai Road, Taipei 112, Taiwan, PRC. Tel: +886 228757627, Fax: +886 228751562, e-mail: cwchi@vghtpe.gov.tw

Key Words: siRNA, migration, metastasis, hepatocellular carcinoma, peroxisome proliferator-activated receptor gamma.

decreased PGC-1α expression has already been linked to the progression of breast and colon cancer clinically, prognosis based on PPARγ expression level in these and other types of cancer remains controversial (15-18). In our previous study, we not only demonstrated a role of PGC-1α in regulating E-cadherin expression in HepG2 human hepatoma cells but also showed a concomitantly reduced expression of these two genes in paired HCC specimens (19). However, the precise role of the PPARγ/PGC-1α pathway in the pathogenesis of liver cancer needs to be further investigated. To achieve this goal, we used TZDs to activate PPARγ and overexpressed PGC-1α *via* adenovirus infection in HepG2 cells to analyze their effects on cell motility and E-cadherin expression.

Materials and Methods

Cell culture. HepG2 human hepatoma cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 10 μg/ml gentamycin, 2 mM L-glutamine, 0.1 mM non-essential amino acid (complete DMEM) at 37˚C in a humidified atmosphere containing 5% CO_2. All culture reagents were purchased from Invitrogen (Carlsbad, CA, USA); rosiglitazone and the specific PPARγ antagonist GW9662 were purchased from Cayman Chemical (Ann Arbor, MI, USA); troglitazone was purchased from Sigma-Aldrich (Saint Louis, MO, USA).

siRNA transfection. On-TARGETplus smart pools of small-interference (si)RNAs for non-targeting control, PPARγ (NM_005037) and E-cadherin (NM_004360) were synthesized by Dharmacon Research (Lafayette, CO, USA). Transient transfection was carried out using INTERFERin™ siRNA transfection reagent (Polyplus Transfection, New York, NY, USA) according to the manufacturer's protocol. Briefly, 0.1 nmol siRNAs and 12.5 μl INTERFERin™ were mixed thoroughly with 200 μl serum-free DMEM by vortexing for 10 s. After 10 min incubation at room temperature, this mixture was added to 2×10^5 cells plated in 6-well plates containing 2 ml of complete DMEM. Two days post-transfection, cells were either collected or further infected with adenoviruses.

Ad-PGC-1α virus production and infection. Mass production and storage of Ad-PGC-1α and Ad-GFP viruses were carried out as described previously (19). For infection, HepG2 cells were incubated with the viruses in serum-free DMEM for two hours before virus-containing medium was replaced by fresh complete DMEM containing vehicle (0.1% dimethylformamide (DMF)) or PPARγ ligands (5 μM rosiglitazone, 20 μM troglitazone and 10 μM GW9662). After incubation for 2 days, cells were collected for RT-PCR or Western blot analysis of E-cadherin expression or for transwell migration assay.

Western blot analysis. Whole cell lysates were prepared by M-PER® (mammalian protein extraction reagent) and nuclear extracts were prepared by NE-PER® (nuclear and cytoplasmic extraction reagent) (Pierce, Rockford, IL, USA) supplemented with protease inhibitor cocktail (Calbiochem, La Jolla, CA, USA) according to the manufacturer's protocol. Protein lysates were separated in 10% SDS-polyacrylamide gels and blotted onto nitrocellulose membranes. Primary antibodies, including those for E-cadherin (BD Biosciences, San Diego, CA, USA), β-actin (Sigma), PGC-1α, PPARγ and histone H1 (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), were then used to probe these blots. After incubation at 4˚C overnight, the membranes were washed and then incubated with horseradish peroxidase-conjugated secondary antibodies. Protein visualization was carried out using an enhanced chemiluminescence kit (ECL, Pierce). The signal intensity was obtained by densitometric scanning and relative protein expression was normalized against protein loading control (β-actin).

Sulforhodamine B (SRB) colorimetric analysis. HepG2 cells were seeded at a density of 3,000 cells/well in 96-well plates and were treated with different doses (0, 1, 5, 10, 20, 50 and 100 μM) of PPARγ agonists (rosiglitazone and troglitazone) for 2 days. The total biomass of cells was determined by SRB colorimetric assay (20). Briefly, cells were fixed by cold 10% trichloroacetic acid (TCA; Sigma) at 4˚C for 1 hour. After washing with tap water and air drying, fixed cells were incubated with 0.1% SRB (Sigma) dissolved in 1% acetic acid for 30 min and rinsed five times with 1% acetic acid to remove unincorporated dye. The protein-bound dye was then extracted with 10 mM Tris and the absorbance at 510 nm of this extract was measured.

Transwell cell migration analysis. Cells (1×10^5) of the drug-treated or vehicle control groups were seeded onto the top chambers of 24-well transwell plates (8 μm pore size; Corning Costar Biosciences, Acton, MA, USA). Hepatocyte growth factor (HGF, 20 ng/ml) (Biosource, Camarillo, CA, USA) was added to the bottom chambers as the chemoattractant. After incubation for 16 hours, cells in the top chamber (non-migrated) were removed by PBS rinsed cotton-swab; cells in the bottom chamber (migrated) were fixed by 4% paraformaldehyde and stained with 0.1% Giemsa solution (Sigma). The numbers of migrated cells were counted under a light microscope in 5 randomly-selected fields for each chamber.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis. Total RNA was extracted from cells using Trizol® reagent (Invitrogen, Carlsbad, CA, USA) according to manufacturer's recommendation. The first strand cDNA was synthesized with a random primer (N_6) using RT Beads (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA). Real-time quantitative PCR (qPCR) was performed using the ABI PRISM 7700. The DNA-intercalating SyBr green reagent (PE Applied Biosystems, Foster City, CA, USA) was used for detection of the PCR product. PCR amplification was subsequently performed with primers for *E-cadherin*, sense: 5'-TGA AGG TGA CAG AGC CTC TGG AT-3' and antisense: 5'-TGG GTG AAT TCG GGC TTG TT-3'; for *PPARγ*, sense: 5'-TGC AGG TGA TCA AGA AGA CG-3' and antisense: 5'-AGT GCA ACT GGA AGA AGG GA-3'; for *β-actin,* sense: 5'-TGG CAT TGC CGA CAG GAT-3'and antisense: 5'-GCT CAG GAG GAG CAA TGA TCT-3'. The expression level was estimated using the ΔΔCt method in which all data were normalized against the level of *β-actin* in each sample.

Statistical analyses. The means and standard deviations (SD) were calculated from at least three independent experiments. Student's *t*-test was used for comparing the means of two treatment groups. Multiple-group comparisons were carried out using one-way analysis of variance (ANOVA) followed by the Tukey *post-hoc* test. Differences

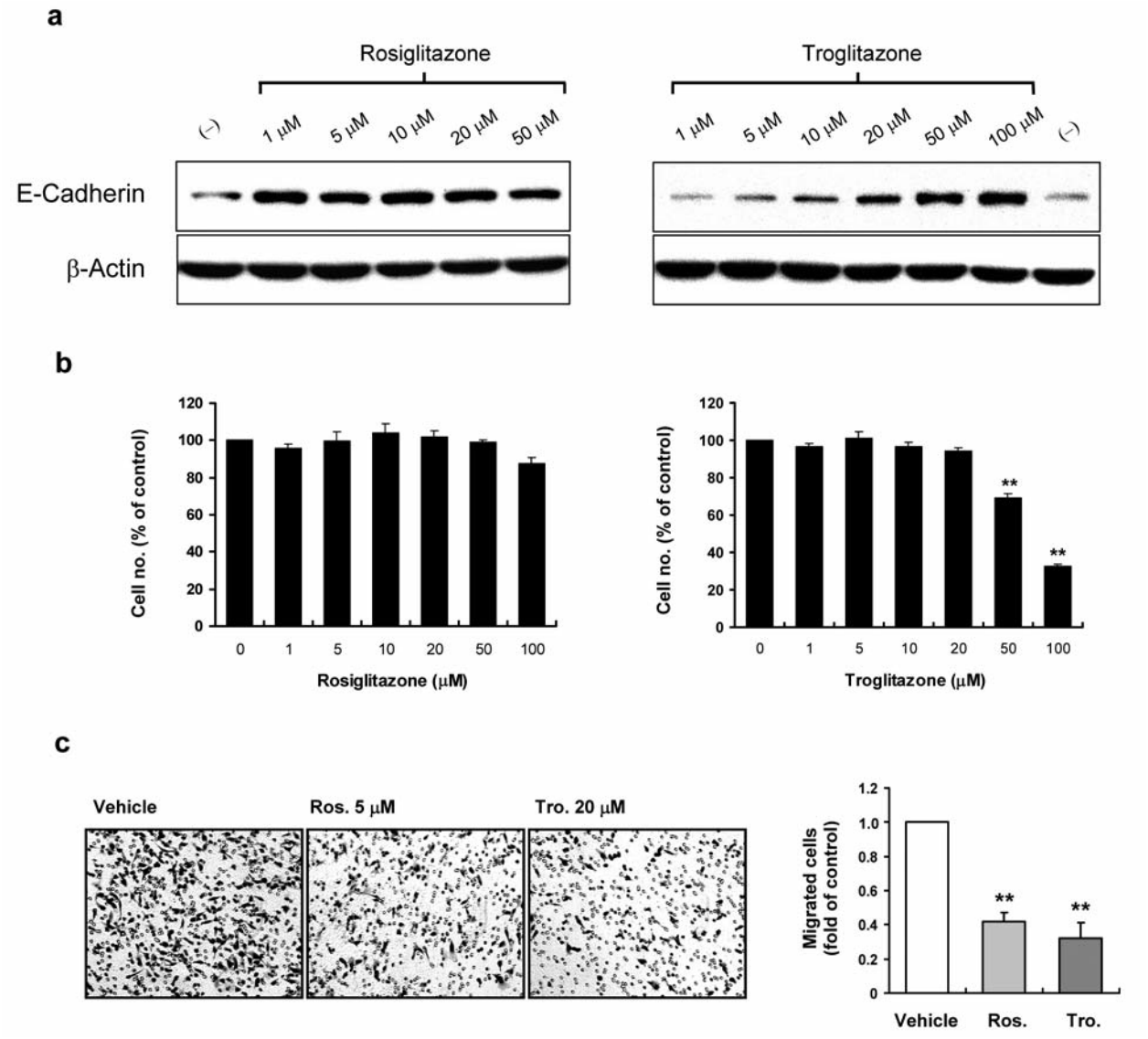

Figure 1. *Effects of thiazolidinediones on E-cadherin expression, cell growth and motility of HepG2 cells. After treatment with different doses of rosiglitazone, troglitazone (from 1 μM to 100 μM), or dimethylformamide (DMF)-vehicle control (–) for 2 days, Western blot analysis was performed to measure the protein level of E-cadherin (a). SRB colorimetric assay showing the effects of different doses of rosiglitazone and troglitazone on the growth of HepG2 cells (b). Microphotographs of the migrated cells were taken at ×200 magnification with an Olympus optical microscope. Cell motility was determined using transwell chamber after cells were treated with 5 μM rosiglitazone (Ros. 5 μM) or 20 μM troglitazone (Tro. 20 μM) for 2 days (c). **p<0.01 when compared with vehicle control cells by Student's t-test.*

were considered statistically significant when $p<0.05$.

Results

Thiazolidinedione increases E-cadherin expression and reduces the motility of HepG2 cells. To evaluate the effects of PPARγ activation in HepG2 cells, PPARγ agonists rosglitazone and troglitazone were used to treat these cells. As shown in Figure 1a, E-cadherin protein level increased in agonist-treated cells, with rosiglitazone being more potent of the two. After determining the non-cytotoxic doses of TZDs by SRB cell proliferation assay (Figure 1b), 5 μM rosiglitazone and 20 μM troglitazone were used to treat HepG2 cells for two days, respectively. As can be seen in Figure 1c, the migratory ability of HepG2 cells was reduced 2.5- to 3-fold by treatment with TZDs.

PGC-1α up-regulates E-cadherin expression in both PPARγ-dependent and -independent manners in HepG2 cells. We reported previously that PGC-1α overexpression activated E-cadherin expression at the transcriptional level in HepG2 cells (19). To further examine whether interaction between PPARγ

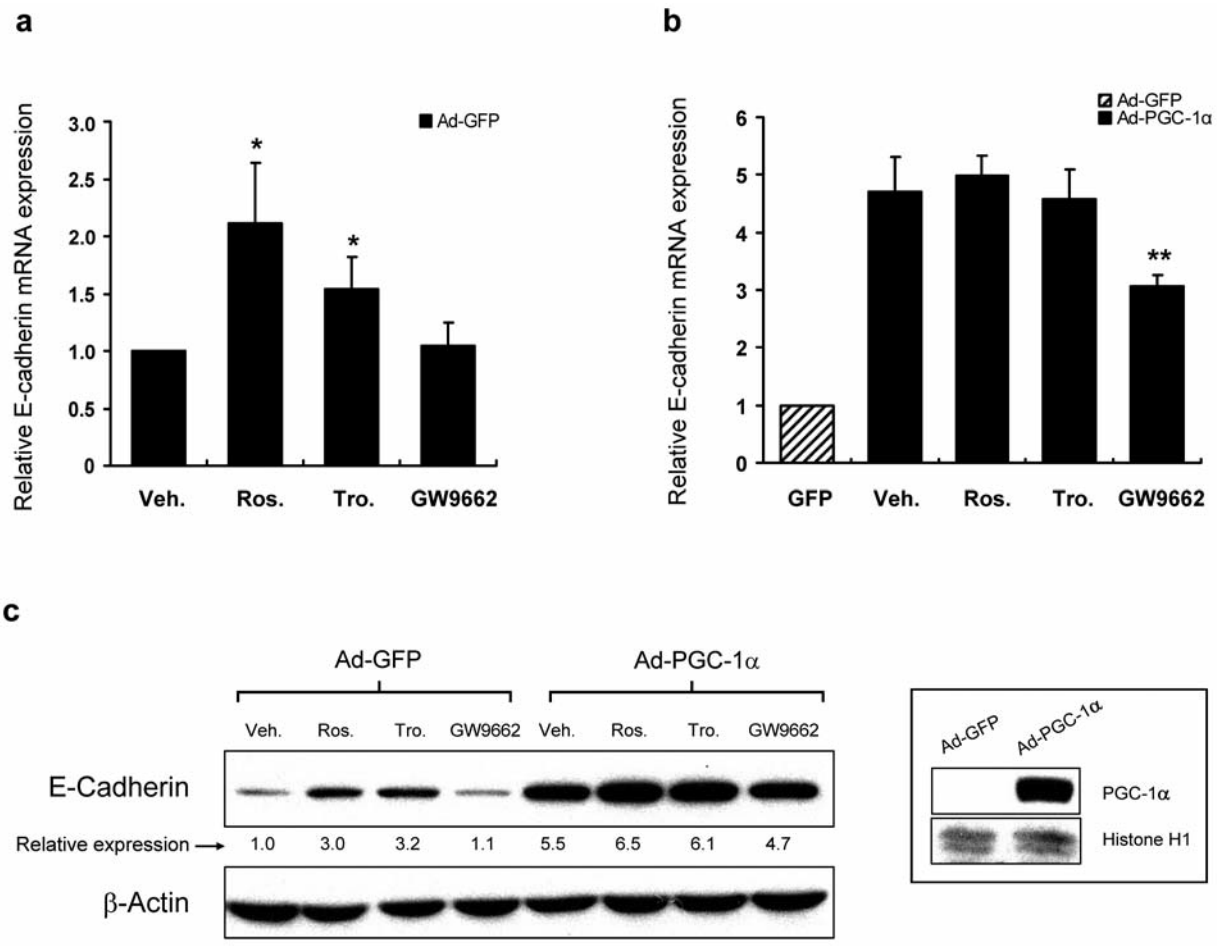

Figure 2. *The role of thiazolidinediones and PGC-1α in regulating E-cadherin expression in HepG2 cells. HepG2 cells were treated with dimethylformamide (DMF, Veh.), 5 μM rosiglitazone (Ros.), 20 μM troglitazone (Tro.), or 10 μM GW9662, respectively, after control Ad-GFP (a) or Ad-PGC-1α (b) infection. Total RNA was extracted 2 days post-thiazolidinedione treatment and RT-qPCR analysis was performed to measure the mRNA experession of E-cadherin. *p<0.05, **p<0.01 when compared with vehicle control cells by Student's t-test. c, Western blot analyses of E-cadherin protein expression in control Ad-GFP- and Ad-PGC-1α-infected HepG2 cells 2 days post-thiazolidinedione treatment. The relative E-cadherin signal intensity, normalized against protein loading control (β-actin) by densitometric scanning, is indicated. The right panel shows the nuclear expression of PGC-1α and histone H1 loading control in infected cells.*

and PGC-1α affects the expression of E-cadherin, we assessed the effects of PPARγ agonists and antagonist on E-cadherin mRNA as well as protein levels in control Ad-GFP and Ad-PGC-1α-infected HepG2 cells. As shown in Figure 2a, rosiglitazone and troglitazone induced 2.2-fold and 1.6-fold increases in E-cadherin mRNA expression respectively in Ad-GFP infected cells, whereas PPARγ antagonist GW9662 had no effect. On the other hand, even though rosiglitazone and troglitazone treatment did not further increase the PGC-1α-mediated E-cadherin transcription, GW9662 inhibited this response by about 40% (Figure 2b). In agreement, E-cadherin protein levels induced by PGC-1α overexpression were slightly increased by TZDs but were reduced moderately (~20%) by GW9662 in PGC-1α-infected HepG2 cells (Figure 2c).

E-Cadherin silencing restores but PPARγ silencing further reduces the motility of PGC-1α-overexpressing HepG2 cells. In order to delineate the role of up-regulated E-cadherin in modulating the motility of PGC-1α-overexpressing HepG2 cells, siRNA was used to knockdown the E-cadherin expression. As shown in Figure 3a, Ad-PGC-1α-infected HepG2 cells showed a significant increase in E-cadherin mRNA expression (3~4-fold) which was effectively abolished in E-cadherin-silenced cells (0.6-fold compared with Ad-GFP-infected control si CTL cells). The efficient reduction of E-cadherin protein expression was also observed (data not shown). As expected, E-cadherin silencing not only increased the motility of Ad-GFP-infected HepG2 cells, but also restored that of PGC-1α-overexpressing ones (Figure 3b). Since

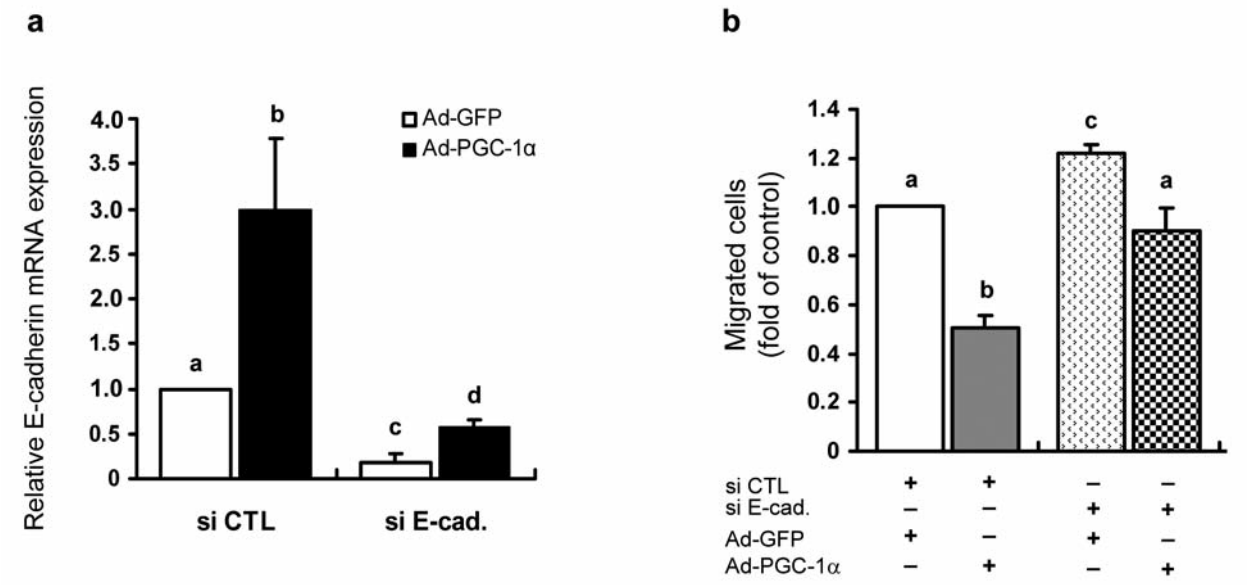

Figure 3. *Up-regulated E-cadherin expression is related to the reduced motility of PGC-1α-overexpressing HepG2 cells. a, RT-qPCR analysis of E-cadherin mRNA expression in 2 days post-Ad-GFP- or Ad-PGC-1α-infected E-cadherin-silenced HepG2 cells. b, Quantification of cell motility by counting migrated Ad-GFP- and Ad-PGC-1α-infected cells under siRNA suppression of their E-cadherin expression (si E-cad.) or non-targeting control (si CTL). Data with different letters are significantly different (ANOVA, $p<0.05$).*

E-cadherin expression was only slightly affected by the PPARγ ligands in PGC-1α-overexpressing HepG2 cells, we further examined whether PPARγ silencing could influence E-cadherin expression and consequently cell motility. As can be seen in Figure 4a, a moderate decrease in PPARγ mRNA level was detected 2 days post-siRNA transfection (~40%). Furthermore, the expression of GFP-PPARγ fusion protein from a transiently transfected plasmid in HepG2 cells was significantly reduced by PPARγ siRNA (Figure 4b). Surprisingly, E-cadherin protein levels dramatically decreased in both GFP- (negative control) and PGC-1α-overexpressing HepG2 cells after PPARγ knockdown (Figure 4c). Contrary to expectation, this treatment resulted in a 2-fold decrease in motility of both Ad-GFP and Ad-PGC-1α-infected cells (Figure 4d).

Discussion

PPARγ activation has been shown to inhibit the proliferation and metastasis of various types of cancer including HCC (6, 21). In this report, we provide clear evidence that PPARγ activation, by either thiazolidinediones (TZDs) treatment or PGC-1α overexpression, not only up-regulates E-cadherin expression in HepG2 human hepatoma cells but also reduces their motility. Furthermore, the role of PGC-1α in PPARγ/PGC-1α-mediated up-regulation of E-cadherin in these cells was clearly demonstrated.

Loss of E-cadherin expression is correlated with the progression to malignant, metastatic carcinoma for a number of tumor types, including HCC (9, 22). Hence, restoration or up-regulation of E-cadherin expression has been proposed to be effective therapy for cancer which might be achieved by treating with PPARγ ligands (7, 23). In this regard, we found that the addition of TZDs not only up-regulated E-cadherin expression but also reduced migration of HepG2 cells, the latter of which has also been observed in other cancer cells treated with TZDs (24, 25). It is thus conceivable that E-cadherin up-regulation may be the main mechanism underlying the suppression of tumor cell migration by PPARγ ligands.

In the absence of PPARγ ligands, PGC-1α interacts with PPARγ physically and their co-expression could activate the PPAR response element-driven reporter gene expression in a ligand-independent manner (13, 26, 27). However, this transcriptional activation is selective, and we found *E-cadherin* to be its target gene in HepG2 cells (19). On the other hand, E-cadherin mRNA expression up-regulated by PGC-1α was markedly (~40%) suppressed by the PPARγ antagonist GW9662, suggesting that both PPARγ-dependent and -independent pathways are involved in PGC-1α-mediated E-cadherin activation in HepG2 cells. Intriguingly, synergism between another PPARγ agonist pioglitazone and valproic acid, a histone deacetylase inhibitor, on E-cadherin up-regulation and growth inhibition in prostate cancer cells has recently been reported (7). Fittingly, an increase of the acetylated histone H3 within the 5'-proximal promoter of *E-cadherin* gene was found by us in PGC-1α-overexpressing HepG2 cells (19). On this basis, we postulate that chromatin

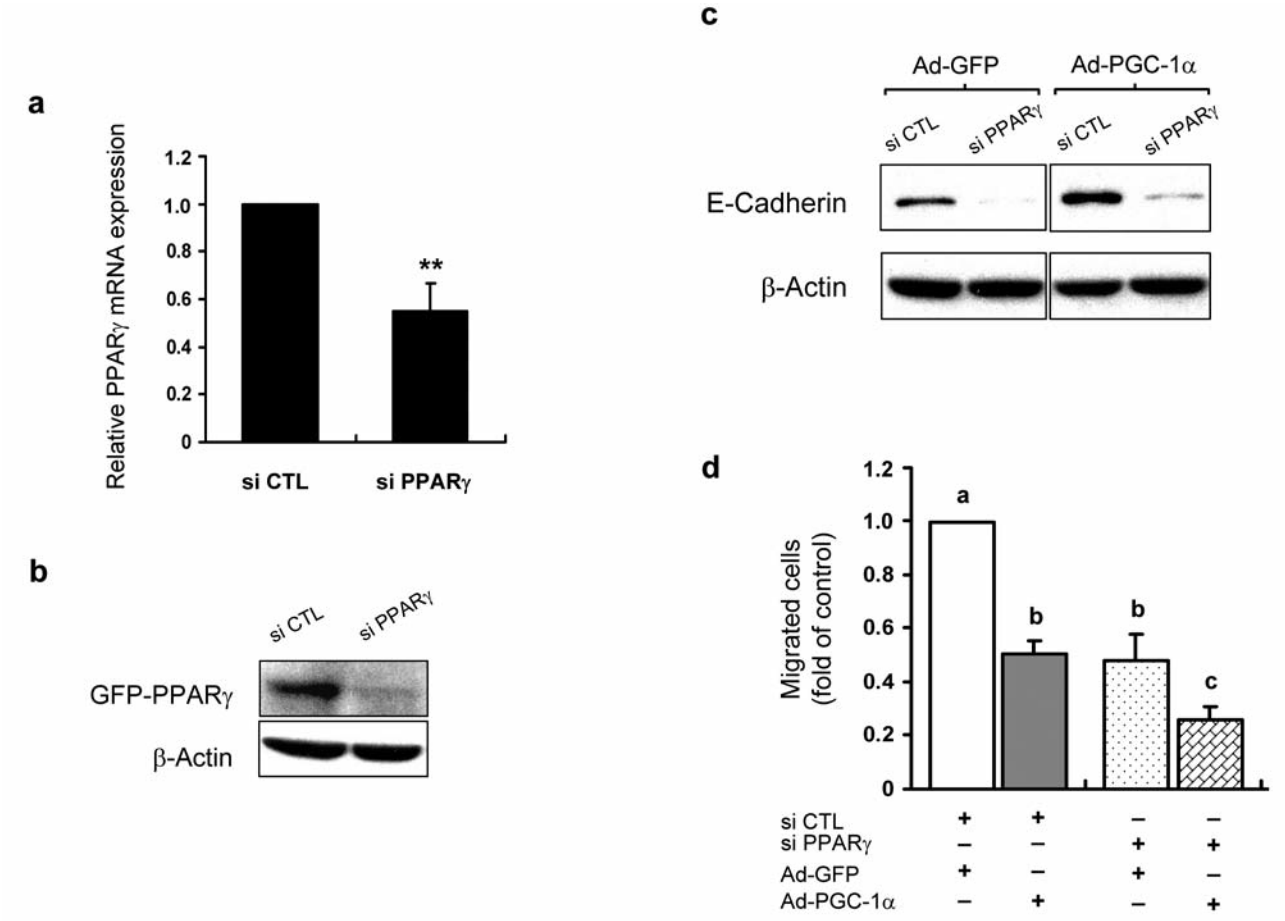

Figure 4. *The role of PPARγ-silencing in modulation of E-cadherin expression and motility of HepG2 cells. Examination of PPARγ siRNA efficiency by (a) RT-qPCR analysis of PPARγ mRNA expression in HepG2 cells 2 days after its siRNA transfection and (b) Western blot analysis of GFP-fused PPARγ protein expression after GFP-PPARγ plasmid and PPARγ siRNA were co-transfected into HepG2 cells for 2 days. Significantly different at **p<0.001. c, E-Cadherin protein expression levels in Ad-GFP- and Ad-PGC-1α-infected, PPARγ- (si PPARγ) or non-targeting (si CTL) silenced HepG2 cells were examined by Western blotting. Total protein was extracted 4 days' post-siRNA transfection (siRNA transfection for 2 days' plus 2 days post-infection incubation). d, Quantification of cell motility by counting migrated Ad-GFP- and Ad-PGC-1α-infected cells under siRNA silencing of their PPARγ expression (si PPARγ). Data with different letters are significantly different (ANOVA, p<0.05).*

modification might be the main mechanism responsible for the up-regulated E-cadherin expression seen in PGC-1α-overexpressing HepG2 cells. Finally, the importance of E-cadherin up-regulation in the PPARγ/PGC-1α-mediated reduction of motility of HepG2 cells was firmly established by the restoration of motility after the knockdown of E-cadherin expression by its siRNA.

Despite E-cadherin up-regulation being previously shown in PPARγ-overexpressing stable clones derived from NSCLC cells (28), our finding that both basal and PGC-1α-stimulated E-cadherin expressions were abolished in PPARγ-silenced HepG2 cells clearly indicates the crucial role of PPARγ in maintaining the cellular E-cadherin level. Intriguingly, suppression of matrix adhesion of hepatoma cells and induction of tumor cell death by PPARγ inhibition have also been reported (17, 29), suggesting the complexity of the roles played by PPARγ in tumorigenesis. In fact, we also detected a reduction in the motility of HepG2 cells after they were treated with PPARγ antagonist GW9662 (unpublished observation). Considering these data, it is our belief that either activation or repression of PPARγ could be an effective strategy for cancer therapy. A discrete modulation rather than complete activation or inhibition of PPARγ may be the most effective strategy for utilizing this pathway to treat HCC or other types of cancer. In conclusion, our findings of the intricate interactions between PGC-1α and PPARγ activation in mediating E-cadherin expression and its associated effects on motility suggest that this axis might provide an important focus for further development of novel anticancer therapies.

Acknowledgements

We would like to thank Dr. Andrew Yau-Chik Shum for his critical reading of the manuscript and editorial help. This work was supported by grants (NSC95-2320-B-075-008-MY2; NSC97-2320-B-075-006-MY3) from the National Science Council, Taiwan.

References

1 Department of Health, Executive Yuan, Health Statistics of the Republic of China, Taipei, Taiwan, ROC: Vital-Statistic, 2007.
2 Bruix J, Boix L, Sala M and Llovet JM: Focus on hepatocellular carcinoma. Cancer Cell *5*: 215-219, 2004.
3 Llovet JM and Bruix J: Molecular targeted therapies in hepatocellular carcinoma. Hepatology *48*: 1312-1327, 2008.
4 Desvergne B and Wahli W: Peroxisome proliferator-activated receptors: nuclear control of metabolism. Endocr Rev *20*: 649-688, 1999.
5 Lehrke M and Lazar MA: The many faces of PPARgamma. Cell *123*: 993-999, 2005.
6 Grommes C, Landreth GE and Heneka MT: Antineoplastic effects of peroxisome proliferator-activated receptor gamma agonists. Lancet Oncol *5*: 419-429, 2004.
7 Annicotte JS, Iankova I, Miard S, Fritz V, Sarruf D, Abella A, Berthe ML, Noel D, Pillon A, Iborra F, Dubus P, Maudelonde T, Culine S and Fajas L: Peroxisome proliferator-activated receptor gamma regulates E-cadherin expression and inhibits growth and invasion of prostate cancer. Mol Cell Biol *26*: 7561-7574, 2006.
8 Nemenoff RA: Peroxisome proliferator-activated receptor-gamma in lung cancer: defining specific *versus* 'off-target' effectors. J Thorac Oncol *2*: 989-992, 2007.
9 Jeanes A, Gottardi CJ and Yap AS: Cadherins and cancer: how does cadherin dysfunction promote tumor progression? Oncogene *27*: 6920-6929, 2008.
10 Ondrey F: Peroxisome proliferator-activated receptor gamma pathway targeting in carcinogenesis: implications for chemoprevention. Clin Cancer Res *15*: 2-8, 2009.
11 Spiegelman BM and Heinrich R: Biological control through regulated transcriptional coactivators. Cell *119*: 157-167, 2004.
12 Puigserver P, Adelmant G, Wu Z, Fan M, Xu J, O'Malley B and Spiegelman BM: Activation of PPARgamma coactivator-1 through transcription factor docking. Science *286*: 1368-1371, 1999.
13 Puigserver P, Wu Z, Park CW, Graves R, Wright M and Spiegelman BM: A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell *92*: 829-839, 1998.
14 Finck BN and Kelly DP: PGC-1 coactivators: inducible regulators of energy metabolism in health and disease. J Clin Invest *116*: 615-622, 2006.
15 Jiang WG, Douglas-Jones A and Mansel RE: Expression of peroxisome-proliferator activated receptor-gamma (PPARγ) and the PPARgamma co-activator, PGC-1, in human breast cancer correlates with clinical outcomes. Int J Cancer *106*: 752-757, 2003.
16 Feilchenfeldt J, Brundler MA, Soravia C, Totsch M and Meier CA: Peroxisome proliferator-activated receptors (PPARs) and associated transcription factors in colon cancer: reduced expression of PPARgamma-coactivator 1 (PGC-1). Cancer Lett *203*: 25-33, 2004.
17 Schaefer KL, Wada K, Takahashi H, Matsuhashi N, Ohnishi S, Wolfe MM, Turner JR, Nakajima A, Borkan SC and Saubermann LJ: Peroxisome proliferator-activated receptor gamma inhibition prevents adhesion to the extracellular matrix and induces anoikis in hepatocellular carcinoma cells. Cancer Res *65*: 2251-2259, 2005.
18 Kristiansen G, Jacob J, Buckendahl AC, Grutzmann R, Alldinger I, Sipos B, Kloppel G, Bahra M, Langrehr JM, Neuhaus P, Dietel M and Pilarsky C: Peroxisome proliferator-activated receptor gamma is highly expressed in pancreatic cancer and is associated with shorter overall survival times. Clin Cancer Res *12*: 6444-6451, 2006.
19 Lee HJ, Su Y, Lui WY, Chau GY, Yin PH, Lee HC and Chi CW: Peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α) up-regulated E-cadherin expression in HepG2 cells. FEBS Lett *582*: 627-634, 2008.
20 Vichai V and Kirtikara K: Sulforhodamine B colorimetric assay for cytotoxicity screening. Nat Protoc *1*: 1112-1116, 2006.
21 Yu J, Qiao L, Zimmermann L, Ebert MP, Zhang H, Lin W, Rocken C, Malfertheiner P and Farrell GC: Troglitazone inhibits tumor growth in hepatocellular carcinoma *in vitro* and *in vivo*. Hepatology *43*: 134-143, 2006.
22 Potter E, Bergwitz C and Brabant G: The cadherin–catenin system: implications for growth and differentiation of endocrine tissues. Endocr Rev *20*: 207-239, 1999.
23 Howard EW, Camm KD, Wong YC and Wang XH: E-Cadherin up-regulation as a therapeutic goal in cancer treatment. Mini Rev Med Chem *8*: 496-518, 2008.
24 Zhang W, Wu N, Li Z, Wang L, Jin J and Zha XL: PPARgamma activator rosiglitazone inhibits cell migration *via* up-regulation of PTEN in human hepatocarcinoma cell line BEL-7404. Cancer Biol Ther *5*: 1008-1014, 2006.
25 Wang PS, Chou FS, Porchia L, Saji M and Pinzone JJ: Troglitazone inhibits cell migration, adhesion, and spreading by modulating cytoskeletal rearrangement in human breast cancer cells. Mol Carcinog *47*: 905-915, 2008.
26 Wu Y, Chin WW, Wang Y and Burris TP: Ligand and coactivator identity determines the requirement of the charge clamp for coactivation of the peroxisome proliferator-activated receptor gamma. J Biol Chem *278*: 8637-8644, 2003.
27 Li Y, Kovach A, Suino-Powell K, Martynowski D and Xu HE: Structural and biochemical basis for the binding selectivity of peroxisome proliferator-activated receptor gamma to PGC-1alpha. J Biol Chem *283*: 19132-19139, 2008.
28 Bren-Mattison Y, Van Putten V, Chan D, Winn R, Geraci MW and Nemenoff RA: Peroxisome proliferator-activated receptor-gamma (PPAR(gamma)) inhibits tumorigenesis by reversing the undifferentiated phenotype of metastatic non-small cell lung cancer cells (NSCLC). Oncogene *24*: 1412-1422, 2005.
29 Weng JR, Chen CY, Pinzone JJ, Ringel MD and Chen CS: Beyond peroxisome proliferator-activated receptor gamma signaling: the multi-facets of the antitumor effect of thiazolidinediones. Endocr Relat Cancer *13*: 401-413, 2006.

Received June 22, 2009
Revised November 3, 2009
Accepted November 10, 2009