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Troglitazone enhances tamoxifen-induced growth inhibitory activity of MCF-7 cells

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ABSTRACT

Peroxisome proliferator-activated receptor γ (PPARγ) ligands have been identified as a potential source of therapy for human cancers. However, PPARγ ligands have a limitation for breast cancer therapy, since estrogen receptor α (ER_α) negatively interferes with PPARγ signaling in breast cancer cells. Here we show that ER_α inhihits PPARγ transactivity and ER_α-mediated inhibition of PPARγ transactivity is blocked by tamoxifen, an estrogen receptor blocker. The activation of ER_α with 17-β-estradiol blocked PPRE transactivity induced by troglitazone, a PPARγ ligand, indicating the resistance of ER_α-positive breast cancer cells to troglitazone. Indeed, troglitazone inhibited the growth of ER_α-negative MDA-MB-231 cells more than that of ER_α-positive MCF-7 cells. Combination of troglitazone with tamoxifen led to a marked increase in growth inhibition of ER_α-positive MCF-7 cells compared to either agent alone. Our data indicates that troglitazone enhances the growth inhibitory activity of tamoxifen in ER_α-positive MCF-7 cells.

The peroxisome proliferator-activated receptors (PPARs), nuclear receptors that regulate transcription, are members of the nuclear receptor superfamily that includes steroid, retinoid, and thyroid hormone receptors [1–4]. Three isoforms, PPARα, PPARδ, and PPARγ, have been characterized, and each can form heterodimers with retinoid X receptors (RXRs) [5–11].

PPARγ is expressed by cell lines derived from primary and metastatic breast tumors, and its activation may alter the growth characteristics of breast cancer cells [12,13]. In addition, PPARγ critically mediates cellular events such as growth and differentiation in cancer cells [10,11]. Indeed, PPARγ ligands can induce apoptosis both in vitro and in vivo [13–16]. PPARγ ligands in the form of thiazolidinediones are commercially available as drugs with a well-described and well-tolerated side effect profile. Unfortunately, a phase II trial of troglitazone (TRO) as therapy for advanced, refractory breast cancer failed to yield any clinical benefit [17]. As a result, some have suggested that although the mechanism of action of thiazolidinediones in breast cancer cells is not fully understood, it involves interactions with other nuclear hormone receptors and transcriptional co-activators and repressors, as well as PPARγ-independent effects. A better understanding of these mechanisms is needed before PPARγ ligands can be useful in the treatment of breast cancer patients [12,18].

There is some important crosstalk among nuclear receptors, including PPAR/estrogen receptor (ER) or PPAR/RXR/ER [19–21]. Recent reports indicate that estrogen receptor α (ER_α) binds to PPAR response element (PPRE) sequences and negatively interferes with PPARγ signaling in breast cancer cells [22]. This crosstalk could be partly responsible for the failed application of TRO against breast cancer. Accordingly, we investigated the impact of the anti-estrogen tamoxifen (TAM) on the actions of a PPARγ ligand (TRO) in breast cancer cells, and explored the possibility of a PPARγ ligand as a supplemental agent for TAM therapy in breast cancer patients.

Materials and methods

Reagents. Anti-PARP, Bcl-2, and Bax antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). HBSS (Hanks balanced salt solution), MTT, propidium iodide (PI), 17-β-estradiol (E2), TAM, dimethyl sulfoxide (DMSO), phenol red-free DMEM, and anti-β-actin antibody were obtained from Sigma Chemical Co. (St. Louis, MO, USA). TGO was purchased from ALEXIS Biochemicals

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(Lausen, Switzerland). All stock solutions of drugs were stored at −70 °C and further diluted to the appropriate concentrations with cell culture medium immediately before use.

Cell culture. Human breast cancer cell lines (MCF-7, MDA-MB-231) obtained from the American Type Culture Collection (Rockville, MD, USA) were cultured in DMEM medium containing 10% fetal bovine serum (FBS), 2 mM glutamine, and antibiotics (Penicillin G, 60 mg/L; Streptomycin, 100 mg/L; Amphotericin B, 50 μl/L) in a humidified atmosphere (37 °C, 5% CO_2, 95% air). To elevate the estradiol effect, the FBS in the medium was replaced with 10% charcoal-dextran-treated FBS.

MTT assay. For the mitochondrial tetrazolium assay (MTT), the cells were seeded on 96-well plates at a density of 1×10^4 cells/well. After 24 h, the cell culture medium was changed to serum-free phenol red-free DMEM and the cells were starved for 18 h for synchronization. The cell growth assays were then performed in phenol red-free medium containing 10% charcoal-stripped serum to enhance the treatment with 100 nM E2. Different concentrations of TAM and TGO (alone or combined) were also added to the culture medium. After incubation for 72 h, cultures were washed twice with phosphate-buffered saline (PBS), and MTT (100 μg/0.1 ml PBS) was added to each well. Cells were incubated at 37 °C for 1 h, and 100 μl DMSO was added to dissolve the formazan crystals. The plate was read in a microplate reader (Model 3550, BIO-RAD, Richmond, CA, USA) at 570 nm.

Transcription activity assay. MCF-7 cells cultured in six well plates were transfected with 5 μg of PPRE-luciferase (tk-PPRE ×3-luciferase, Addgene) using the Lipofectamine reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's specifications. PPRE-luciferase construct contains three copies of the PPRE (peroxisomal proliferators response element) found in the thymidine kinase (tk) promoter upstream of the luciferase reporter gene. After a 24-h transfection, cells were washed with PBS, 25 μM troglitazone or 100 nM E2 was added in the medium, and cells were incubated further for 24 h. The cells were harvested and lysed in Reporter Lysis Buffer (Promega, Madison, WI, USA), and cell lysates were used for firefly and *Renilla* luciferase activities using the Dual-Luciferase Reporter Assay System (Promega). Luciferase activity was reported as relative light units and is normalized to luciferase activity for each condition. Transfection efficiency was determined by cotransfection of pRL null vector using same protocol.

Cell cycle analysis. For cell cycle analysis, 5×10^5 cells were seeded onto 6-well plates and treated as described above. At the indicated times, cells were harvested by trypsinization, centrifuged at 1500 rpm for 3 min, washed with PBS, and fixed for 1 h in 70% ethanol at 4 °C. Next, the cells were collected by centrifugation, resuspended in PBS containing 5 μg/ml RNase and 50 μg/ml PI, and incubated at 4 °C for 1 h, protected from light. DNA content was analyzed using a Becton–Dickinson FACScan and Cell Quest software. Subsequent data analysis was performed using ModFit software (Becton–Dickinson United Kingdom Ltd., Cowley, United Kingdom).

Western blot analysis. After washing with PBS and harvesting, cell pellets were lysed with lysis buffer (50 mM Tris–HCl (pH 7.6), 1% Triton X-100, 2 mM EDTA, 0.5% sodium dodecyl sulfate (SDS), 150 mM NaCl, 1 mM sodium orthovanadate, 2 mM EGTA, 4 mM *p*-nitro-phenyl phosphate, and 100 mM sodium fluoride) supplemented with protease inhibitors (0.5% leupeptin, 0.5% aprotinin, and 0.02% phenylmethylsulfonyl fluoride). After incubation for 30 min at 4 °C, cellular debris was removed by centrifugation at 10,000*g* for 30 min, and supernatants were analyzed by 12% SDS–PAGE. Electrophoretic transfer from the slab gel to nitrocellulose paper and subsequent immunoblotting was performed by incubation with primary antibodies and followed by further incubation with HRP-conjugated secondary antibody. Reactive proteins were detected using enhanced chemiluminescence (ECL, Amersham Life Sciences, Arlington Heights, IL).

Measurement of apoptosis with enzyme-linked immunosorbent assays (ELISA). Fragmented DNA was assessed as a specific measurement of apoptotic cell damage. The cells were plated on 96-well plates and allowed to attach for 24 h. Cellular DNA fragmentation was measured with a commercially available cellular DNA fragmentation ELISA kit. Proliferating cells in 96-well microtiter plates were labeled with 10 μm bromo-deoxyuridine (BrdU) overnight, washed with sterile PBS, and treated with 0.1 μM E2 for 72 h with TAM and TGO alone or in combination. After treatment, the cells were washed with PBS and incubated with the kit lysis buffer [bovine serum albumin (BSA), EDTA, and Tween 20] for 30 min at room temperature, and soluble BrdU-labelled DNA fragments present in the buffer were quantified using the ELISA kit following the manufacturer's instructions (Roche Diagnostics, Heidelberg, Germany). DNA fragmentation was expressed by the fold increase over the control values.

Data analysis. All experimental data are presented as mean ± standard error (SE). Statistical analysis was performed using Student's *t-test*, and $p < 0.005$ was considered to be significant.

Results and discussion

Effect of ER_α on cell viability and cell cycle in TGO-treated MCF-7 and MDA-MB 231 breast cancer cells

In a previous study, we demonstrated that TGO induces PPARγ-dependent G_1 arrest in breast cancer cells [16]. Although there remains some controversy regarding PPARγ dependency [23–25], PPARγ signaling is still an important factor in TGO-induced anti-tumor actions in breast cancer cells. In contrast, ER_α inhibits PPARγ signaling in breast cancer cells [22]. In this study, we first investigated whether ER_α inhibits TGO- induced cell cycle arrest as well as cell death in breast cancer cells. To evaluate the effects of ER_α on cell viability and cell cycle in TGO-treated breast cancer cells, ER_α-positive MCF-7 cells and ER_α-negative MDA-MB-231 cells were cultured with various concentrations of TGO (0–75 μM) in E2-free culture medium. Exposure to TGO for 48 h produced a dose-dependent decrease in cell viability for both cell lines, as measured by MTT assay. However, ER_α-negative MDA-MB-231 cells were more sensitive to TGO than ER_α-positive MCF-7 cells (Fig. 1A). ER_α-negative MDA-MB-231 cells were also more sensitive to TGO-induced G_1 arrest (Fig. 1B). These results indicate that ER_α inhibited the TGO-induced decrease of cell viability and G_1 arrest in breast cancer cells. In addition, using luciferase assay of a reporter gene containing PPAR response element (PPRE), we found that PPRE transactivity was induced by troglitazone, but this response was completely blocked by 17-β-estradiol treatment (Fig. 1C). In agreement with a previous report [22], we also found that ER_α inhibited PPARγ ligand signaling in breast cancer cells. From these results, we speculated that TGO (a PPARγ ligand) would enhance the growth inhibitory and apoptotic activity of TAM (an anti-ER agent) in breast cancer cells.

Effect of TGO on the growth inhibitory activity of TAM in 17β-E2-treated MCF-7 cells

In order to investigate the effect of TGO on the growth inhibitory activity of TAM in E2-treated ER_α-positive breast cancer cells, MCF-7 cells were seeded into 96-well culture plates at a density of 1×10^4 cells/well in culture medium containing 100 nM E2. MCF-7 cells were treated with various concentrations of TAM (0–5.0 μM) in the presence or absence of 25 μM TGO. Cell proliferation was

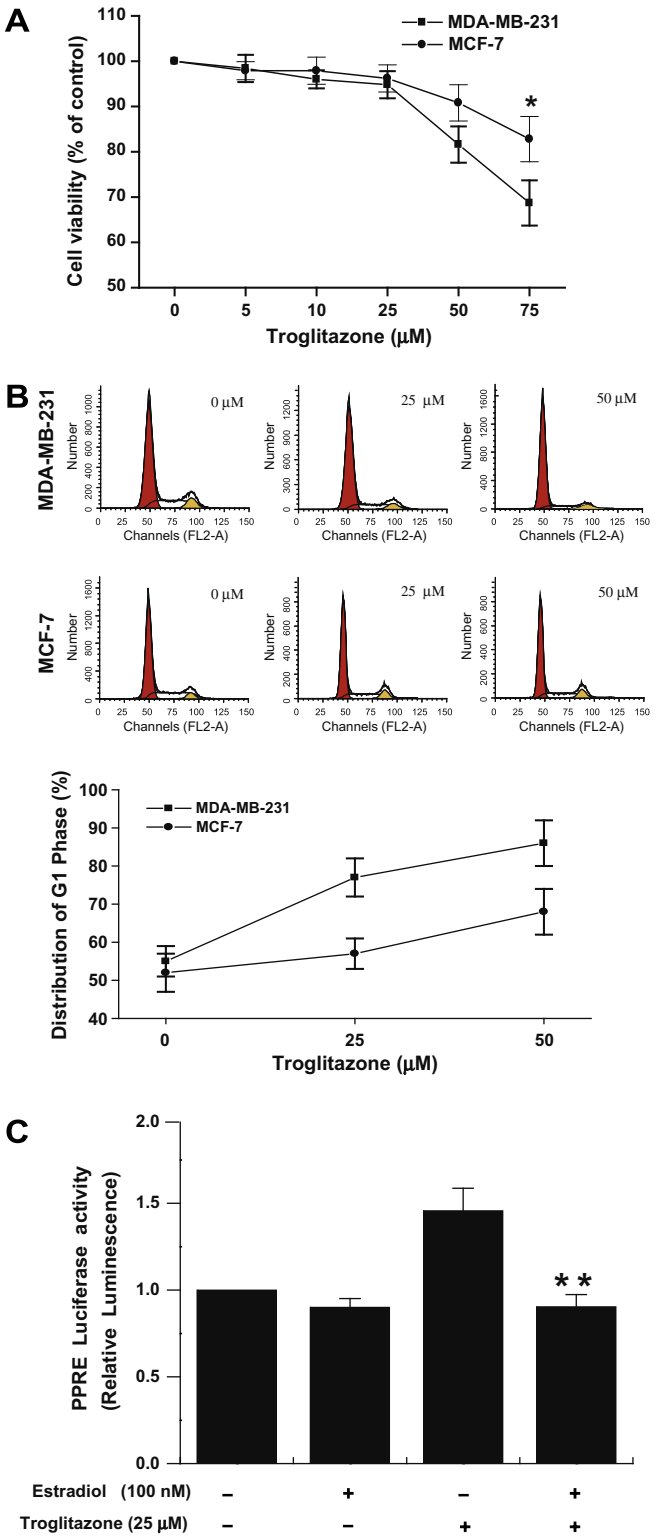

Fig. 1. ER_α inhibits troglitazone activity in breast cancer cells. (A) Cells were treated with the indicated concentrations of troglitazone for 48 h. Cell growth of breast cancer cells was determined by MTT assay. (B) Flow cytometry analysis demonstrating the effect of troglitazone on the cell cycle of breast cancer cells. Cells were treated with troglitazone for 48 h at various concentrations. The cells were harvested, stained with propidium iodide (PI) solution, and analyzed using flow cytometry for cell cycle distribution. The means of four separate experiments are shown (bars, SE). (C) Luciferase assay of a reporter gene demonstrating the effect of troglitazone on PPARγ activation of breast cancer cells. *P* values were determined using Student's *t*-test ($^{*}p < 0.005$ versus MCF-7; $^{**}p < 0.002$ versus troglitazone).

determined by BrdU-labelled ELISA kit. 100 nM E2 increased growth of MCF-7 cells by 63% compared to no E2 treatment. TAM inhibited E2-dependent growth of MCF-7 cells in a dose-dependent manner, with complete inhibiting E2-dependent growth at a concentration of 5 μM (Fig. 2). Interestingly, combination of 5 μM TAM with 25 μM TGO reduced cell proliferation to approximately 50% of control cells (Fig. 2). These results indicate that TGO significantly enhanced the growth inhibitory activity of TAM in E2-treated MCF-7 cells. As expected, the combination of TGO and TAM synergistically increased growth inhibition of breast cancer cells.

Effect of TGO on G_1 arrest activity of TAM in 17β-E2-treated MCF-7 cells

To further investigate the combined effects of TGO and TAM on cell cycle, MCF-7 cells were treated with 25 μM TGO and 0-5 μM TAM alone or in combination for 48 h. The DNA content of the cellular nuclei was analyzed by flow cytometry. TAM caused an increase in G_1-phase and a reduction in S-phase in a dose-dependent manner (Fig. 3A). In TGO-treated cells, the percentage of cells in G_1 or S-phase was markedly different than in cells treated with TAM alone. These results suggest that the combination of TGO and TAM synergistically affected MCF-7 cell cycle.

The cell cycle is tightly regulated through a complex network of positive and negative regulatory molecules such as cyclins [25–27]. In particular, cyclin D1 is an important regulator of cell

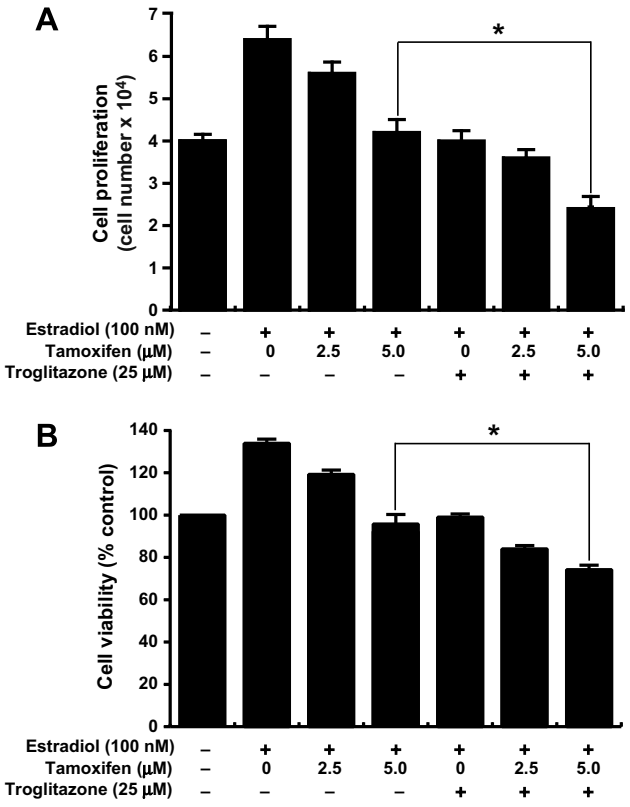

Fig. 2. Troglitazone enhances the growth inhibitory activity of tamoxifen in 17β-estradiol–treated MCF-7 cells. To investigate the combined effect of TGO and TAM on proliferation (A) and cell viability (B) in E2-treated ER_α-positive breast cancer cells, MCF-7 cells were seeded into 96-well culture plates at a density of 1×10^4 cells/well in culture medium containing 100 nM E2. MCF-7 cells were treated with various concentrations of tamoxifen (0–5.0 μM) in the presence or absence of 25 μM troglitazone. Cell proliferation was measured with a BrdU-labelled ELISA kit and cell viability was determined by MTT assay. The means of four separate experiments are shown (bars, SE). P values were determined using Student's *t*-test ($^{*}p < 0.005$ versus troglitazone alone).

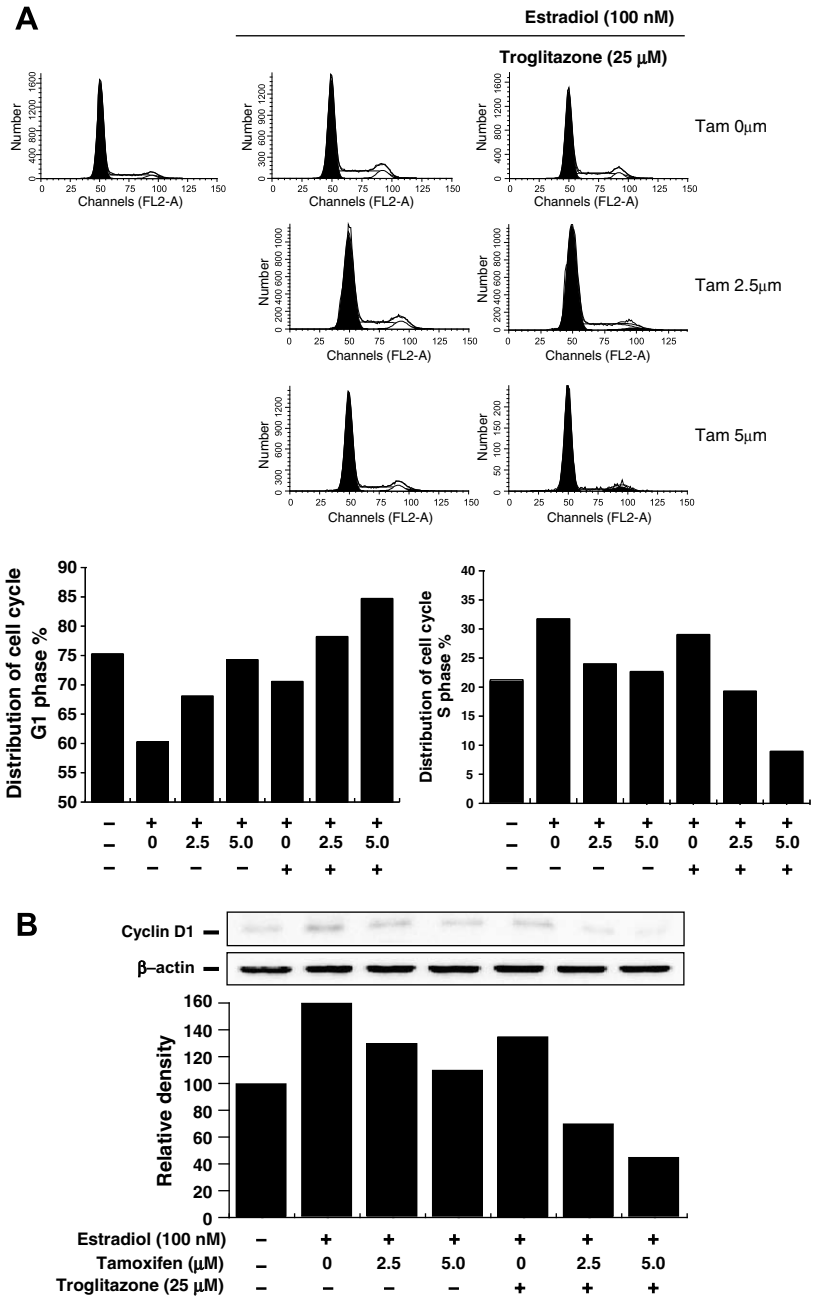

Fig. 3. Troglitazone enhances the G_1 arrest activity of tamoxifen in 17β-estradiol–treated MCF-7 cells. To investigate the effects of combining troglitazone and tamoxifen on cell cycle (A) and cyclin D1 expression (B) in MCF-7 cells, cells were treated with 25 μM TGO and 0-5 μM TAM alone or in combination for 48 h. The cells were then harvested, stained with propidium iodide (PI) solution, and analyzed using flow cytometry for cell cycle distribution. For cyclin D1 expression, cell extracts were separated by SDS/PAGE, followed by Western blotting. β-Actin was used as a loading control. Electrophoretic band analysis was performed with an LAS-1000 (Fujifilm, Japan).

proliferation [27–29]. PPAR ligands have been shown to down-regulate cyclin D1 expression in breast cancer cells [13,30,31]. Accordingly, we investigated the role of cyclin D1 in the change of cell cycle induced by TGO and TAM in breast cancer cells. Protein extract was prepared from cells treated with 25 μM TGO and 0-5 μM TAM alone or in combination for 48 h. Western blotting was performed using antibody against cyclin D_1. As shown in Fig. 3B, TAM treatment dose-dependently caused a decrease in cyclin D1 expression. In TGO-treated cells, cyclin D1 expression was markedly lower than with TAM alone. These results suggest that the combination of TGO and TAM synergistically down-regulated cyclin D1 in MCF-7 cells.

Effect of TGO on apoptotic activity of TAM in 17β-E2-treated MCF-7 cells

The underlying mechanisms of growth inhibition are cell cycle arrest and/or apoptosis. Apoptosis is a distinctive form of cell death that can result in the deletion of specific cell populations. Cell apoptosis can be one of the consequences of cell cycle arrest. We investigated whether the combination of TGO and TAM synergistically affected apoptosis of MCF-7 cells. To determine the effect of combined TGO and TAM on apoptosis of MCF-7 cells, cells were treated with 25 μM TGO and 0–5 μM TAM alone or in combination for 48 h. The induction of apoptosis in MCF-7 cells was measured

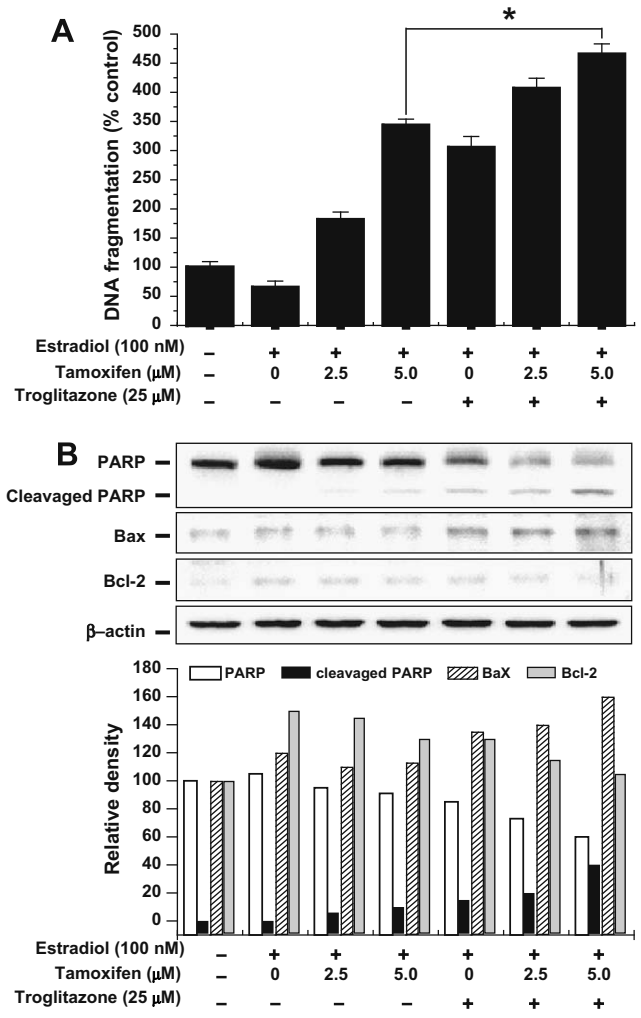

Fig. 4. Troglitazone increases the apoptotic activity of tamoxifen in 17β-estradiol–treated MCF-7 cells. The effect of troglitazone on the apoptotic activity of tamoxifen in E2-treated ER_{α}-positive breast cancer cells was investigated by measuring DNA fragmentation (A) and the expression of apoptosis-related proteins PARP, Bax, and Bcl-2 (B). Cells were treated with 25 μM troglitazone and 0–5 μM tamoxifen alone or in combination for 48 h. The induction of apoptosis in MCF-7 cells was measured with a DNA fragmentation ELISA kit. For apoptosis-related proteins, cell extracts were separated by SDS/PAGE, followed by Western blotting. β-Actin was used as a loading control. Electrophoretic band analysis was performed with an LAS-1000 (Fujifilm, Japan). The means of four separate experiments are shown (bars, SE). *P* values were determined using Student's *t*-test ($^{*}p < 0.005$ versus troglitazone alone).

by a DNA fragmentation ELISA kit. As shown in Fig. 4A, TAM treatment dose-dependently caused an increase in DNA fragmentation. In TGO-treated cells, DNA fragmentation was significantly greater than with TAM alone. These results suggest that the combination of TGO and TAM synergistically enhanced apoptosis in MCF-7 cells. To determine whether these synergistic effects involved apoptotic signaling, we used Western blotting to show that MCF-7 cells exposed to TGO exhibited the typical PARP cleavage or Bax expression of apoptosis compared to cells exposed to TAM alone (Fig. 4B).

In conclusion, we have demonstrated for the first time that the combination of TGO and TAM resulted in a synergistic effect on both growth inhibition and apoptosis induction. Our results suggest that TGO could play a potent role in hormone therapy in breast cancer patients, including hormone resistant or adjuvant therapy. Further research is necessary to establish clinical approaches using TGO or modified analogs of the thiazolidinedione class of drugs and TAM or other anti-estrogens.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

[1] P. Tontonoz, E. Hu, B.M. Spiegelman, Stimulation of adipogenesis in fibroblasts by PPARγ 2, a lipid-activated transcription factor, Cell 79 (1994) 1147–1156.
[2] E. Mueller, P. Sarraf, P. Tontonoz, R.M. Evans, K.J. Martin, M. Zhang, C. Fletcher, S. Singer, B.M. Spiegelman, Terminal differentiation of human breast cancer through PPARγ, Mol. Cell 1 (1998) 465–470.
[3] S. Kersten, B. Desvergne, W. Wahli, Roles of PPARs in health and disease, Nature 405 (2000) 421–424.
[4] E. Mueller, M. Smith, P. Sarraf, T. Kroll, A. Aiyer, D.S. Kaufman, W. Oh, G. Demetri, W.D. Figg, X.P. Zhou, C. Eng, B.M. Spiegelman, P.W. Kantoff, Effects of ligand activation of peroxisome proliferator-activated receptor γ in human prostate cancer, Proc. Natl. Acad. Sci. USA 97 (2000) 10990–10995.
[5] I. Issemann, S. Green, Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators, Nature 347 (1990) 645–650.
[6] S.A. Kliewer, K. Umesono, D.J. Noonan, R.A. Heyman, R.M. Evans, Convergence of 9-cis retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors, Nature 358 (1992) 771–774.
[7] C. Dreyer, H. Keller, A. Mahfoudi, V. Laudet, G. Krey, W. Wahli, Positive regulation of the peroxisomal beta-oxidation pathway by fatty acids through activation of peroxisome proliferator-activated receptors (PPAR), Biol. Cell 77 (1993) 67–76.
[8] S.A. Kliewer, B.M. Forman, B. Blumberg, E.S. Ong, U. Borgmeyer, D.J. Mangelsdorf, K. Umesono, R.M. Evans, Differential expression and activation of a family of murine peroxisome proliferator-activated receptors, Proc. Natl. Acad. Sci. USA 91 (1994) 7355–7359.
[9] P. Sertznig, M. Seifert, W. Tilgen, J. Reichrath, Present concepts and future outlook: function of peroxisome proliferator-activated receptors (PPARs) for pathogenesis, progression, and therapy of cancer, J. Cell Physiol. 212 (2007) 1–12.
[10] D. Panigrahy, S. Huang, M.W. Kieran, A. Kaipainen, PPARγ as a therapeutic target for tumor angiogenesis and metastasis, Cancer Biol. Ther. 4 (2005) 687–693.
[11] C.D. Allred, M.W. Kilgore, Selective activation of PPARγ in breast, colon, and lung cancer cell lines, Mol. Cell. Endocrinol. 235 (2005) 21–29.
[12] M.H. Fenner, E. Elstner, Peroxisome proliferator-activated receptor-γ ligands for the treatment of breast cancer, Expert Opin. Investig. Drugs 14 (2005) 557–568.
[13] F. Yin, S. Wakino, Z. Liu, S. Kim, W.A. Hsueh, A.R. Collins, A.J. Van Herle, R.E. Law, Troglitazone inhibits growth of MCF-7 breast carcinoma cells by targeting G1 cell cycle regulators, Biochem. Biophys. Res. Commun. 286 (2001) 916–922.
[14] E. Elstner, C. Muller, K. Koshizuka, E.A. Williamson, D. Park, H. Asou, P. Shintaku, J.W. Said, D. Heber, H.P. Koeffler, 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 95 (1998) 8806–8811.
[15] N.P. Nunez, H. Liu, G.G. Meadows, PPAR-γ ligands and amino acid deprivation promote apoptosis of melanoma, prostate, and breast cancer cells, Cancer Lett. 236 (2006) 133–141.
[16] H.N. Yu, Y.R. Lee, E.M. Noh, K.S. Lee, J.S. Kim, E.K. Song, M.K. Han, Y.C. Lee, K.B. Kwon, S.J. Lee, H.J. Youn, S.H. Jung, Induction of G(1) phase arrest and apoptosis in MDA-MB-231 breast cancer cells by troglitazone, a synthetic peroxisome proliferator-activated receptor γ (PPARγ) ligand, Cell Biol. Int. 32 (2008) 906–912.
[17] H.J. Burstein, G.D. Demetri, E. Mueller, P. Sarraf, B.M. Spiegelman, E.P. Winer, Use of the peroxisome proliferator-activated receptor (PPAR) γ ligand troglitazone as treatment for refractory breast cancer: a phase II study, Breast Cancer Res. Treat. 79 (2003) 391–397.
[18] K.L. Cheung, Endocrine therapy for breast cancer: an overview, Breast 16 (2007) 327–343.
[19] C.K. Glass, J.M. Holloway, O.V. Devary, M.G. Rosenfeld, The thyroid hormone receptor binds with opposite transcriptional effects to a common sequence motif in thyroid hormone and estrogen response elements, Cell 54 (1988) 313–323.
[20] H. Keller, F. Givel, M. Perroud, W. Wahli, Signaling cross-talk between peroxisome proliferator-activated receptor/retinoid X receptor and estrogen

receptor through estrogen response elements, Mol. Endocrinol. 9 (1995) 794–804.
[21] J.H. Segars, M.S. Marks, S. Hirschfeld, P.H. Driggers, E. Martinez, J.F. Grippo, M. Brown, W. Wahli, K. Ozato, Inhibition of estrogen-responsive gene activation by the retinoid X receptor β: evidence for multiple inhibitory pathways, Mol. Cell. Biol. 13 (1993) 2258–2268.
[22] D. Bonofiglio, S. Gabriele, S. Aquila, S. Catalano, M. Gentile, E. Middea, F. Giordano, S. Ando, Estrogen receptor α binds to peroxisome proliferator-activated receptor response element and negatively interferes with peroxisome proliferator-activated receptor γ signaling in breast cancer cells, Clin. Cancer Res. 11 (2005) 6139–6147.
[23] F. Turturro, E. Friday, R. Fowler, D. Surie, T. Welbourne, Troglitazone acts on cellular pH and DNA synthesis through a peroxisome proliferator-activated receptor γ-independent mechanism in breast cancer-derived cell lines, Clin. Cancer Res. 10 (2004) 7022–7030.
[24] M.A. Rumi, S. Ishihara, Y. Kadowaki, C.F. Ortega-Cava, H. Kazumori, K. Kawashima, N. Yoshino, T. Yuki, N. Ishimura, Y. Kinoshita, Peroxisome proliferator-activated receptor γ-dependent and -independent growth inhibition of gastrointestinal tumour cells, Genes Cells 9 (2004) 1113–1123.
[25] C. Berthet, P. Kaldis, Cell-specific responses to loss of cyclin-dependent kinases, Oncogene 26 (2007) 4469–4477.
[26] D. Santamaria, C. Barriere, A. Cerqueira, S. Hunt, C. Tardy, K. Newton, J.F. Caceres, P. Dubus, M. Malumbres, M. Barbacid, Cdk1 is sufficient to drive the mammalian cell cycle, Nature 448 (2007) 811–815.
[27] P. Sicinski, J.L. Donaher, Y. Geng, S.B. Parker, H. Gardner, M.Y. Park, R.L. Robker, J.S. Richards, L.K. McGinnis, J.D. Biggers, J.J. Eppig, R.T. Bronson, S.J. Elledge, R.A. Weinberg, Cyclin D2 is an FSH-responsive gene involved in gonadal cell proliferation and oncogenesis, Nature 384 (1996) 470–474.
[28] Z. Li, C. Wang, G.C. Prendergast, R.G. Pestell, Cyclin D1 functions in cell migration, Cell Cycle 5 (2006) 2440–2442.
[29] L.K. Mullany, P. White, E.A. Hanse, C.J. Nelsen, M.M. Goggin, J.E. Mullany, C.K. Anttila, L.E. Greenbaum, K.H. Kaestner, J.H. Albrecht, Distinct proliferative and transcriptional effects of the D-type cyclins in vivo, Cell Cycle 7 (2008) 2215–2224.
[30] C. Wang, M. Fu, M. D'Amico, C. Albanese, J.N. Zhou, M. Brownlee, M.P. Lisanti, V.K. Chatterjee, M.A. Lazar, R.G. Pestell, Inhibition of cellular proliferation through IκB kinase-independent and peroxisome proliferator-activated receptor γ-dependent repression of cyclin D1, Mol. Cell. Biol. 21 (2001) 3057–3070.
[31] H. Lapillonne, M. Konopleva, T. Tsao, D. Gold, T. McQueen, R.L. Sutherland, T. Madden, M. Andreeff, Activation of peroxisome proliferator-activated receptor γ by a novel synthetic triterpenoid 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid induces growth arrest and apoptosis in breast cancer cells, Cancer Res. 63 (2003) 5926–5939.