

Peroxisome Proliferator-activated Receptor- γ 1 Is Dephosphorylated and Degraded during BAY 11-7085-induced Synovial Fibroblast Apoptosis*

Received for publication, November 30, 2005, and in revised form, June 6, 2006. Published, JBC Papers in Press, June 9, 2006, DOI 10.1074/jbc.M512807200

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Peroxisome proliferator-activated receptor- γ (PPAR- γ) plays a central role in whole body metabolism by regulating adipocyte differentiation and energy storage. Recently, however, PPAR- γ has also been demonstrated to affect proliferation, differentiation, and apoptosis of different cell types. As we have previously shown that BAY 11-7085-induced synovial fibroblast apoptosis is prevented by PPAR- γ agonist 15d-PGJ2; the expression of PPAR- γ in these cells was studied. Both PPAR- γ 1 and PPAR- γ 2 isoforms were cloned from synovial fibroblast RNA, but only PPAR- γ 1 was detected by Western blot, showing constitutive nuclear expression. Within minutes of BAY 11-7085 treatment, a PPAR- γ 1-specific band was shifted into a form of higher mobility, suggesting dephosphorylation, as confirmed by phosphatase treatment of cell extracts. Of interest, BAY 11-7085-induced PPAR- γ 1 dephosphorylation was followed by PARP and caspase-8 cleavage as well as by PPAR- γ 1 protein degradation. PPAR- γ 1 dephosphorylation was followed by the loss of PPAR-DNA binding activity ubiquitously present in synovial fibroblast nuclear extracts. Unlike the phosphorylated form, dephosphorylated PPAR- γ 1 was found in insoluble membrane cell fraction and was not ubiquitinated before degradation. PPAR- γ 1 dephosphorylation coincided with ERK1/2 phosphorylation that accompanies BAY 11-7085-induced synovial fibroblasts apoptosis. 15d-PGJ2, PGD2, and partially UO126, down-regulated ERK1/2 phosphorylation, protected cells from BAY 11-7085-induced apoptosis, and reversed both PPAR- γ dephosphorylation and degradation. Furthermore, PPAR- γ antagonist BADGE induced PPAR- γ 1 degradation, ERK1/2 phosphorylation, and synovial fibroblasts apoptosis. The results presented suggest an anti-apoptotic role for PPAR- γ 1 in synovial fibroblasts. Since apoptotic marker PARP is cleaved after PPAR- γ 1 dephosphorylation but before PPAR- γ 1 degradation, dephosphorylation event might be enough to mediate BAY 11-7085-induced apoptosis in synovial fibroblasts.

Fat cell formation is tightly dependent on ligand-inducible transcriptional factor peroxisome proliferator-activated receptor- γ (PPAR- γ)² (1). PPAR- γ -regulated genes promote storage of fatty acids and repress lipolysis. Although obesity is a risk factor for metabolic diseases such as type 2 diabetes, PPAR- γ -activating drugs lower glucose and lipid levels in such patients (2). These observations illustrate the complexity of PPAR- γ functions, which are not restricted to adipocyte differentiation but also involve proliferation, differentiation, and apoptosis of different cell types (3). The cell- and tissue-specific PPAR- γ effects on apoptosis have been shown. PPAR- γ activators induce apoptosis in multiple myeloma (4), human esophageal squamous cell carcinoma (5), and PPAR- γ transfection suppresses lung tumorigenesis and metastasis (6). However, elevated expression of PPAR- γ was found in epithelial ovarian carcinoma, and PPAR- γ -activating drugs can protect T-cells from apoptosis in a PPAR- γ -dependent fashion (7). Moreover, SV40-transformed human lung fibroblasts express increased amount of PPAR- γ (8). These results suggest the important role of PPAR- γ in neoplastic cell transformation and survival.

The existence of two PPAR- γ isoforms, PPAR- γ 1 and PPAR- γ 2, originated from alternative splicing, has been shown in humans (9). Isoform PPAR- γ 1 lacks the first 30 N-terminal amino acids of PPAR- γ 2. In the presence of PPAR- γ ligands, both PPAR- γ 1 and PPAR- γ 2 can induce adipogenesis, but PPAR- γ 2 more efficiently responds to low ligand concentrations (10). Despite functional similarities (11), PPAR- γ 1 isoform is widely expressed, while PPAR- γ 2 is adipose tissue-restricted (12).

Synovial proliferation and hyperplasia are two of the most detrimental events during persistent inflammatory joint diseases such as rheumatoid arthritis (RA) and osteoarthritis (OA) (13). Nevertheless, spontaneous regression, that includes induction of apoptosis, could be occasionally observed. Mechanisms that prevent synovial fibroblast apoptosis are thus extensively studied (14, 15).

We recently showed that PPAR- γ agonist 15d-PGJ2 protects human synovial fibroblasts from BAY 11-7085-induced apo-

* This work was supported by the Fond d'Investissement de Recherche Scientifique (FIRS) credit 4774 (CHU Sart-Tilman). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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² The abbreviations used are: PPAR- γ , peroxisome proliferator-activated receptor- γ ; 15d-PGJ2, 15-deoxy- $\Delta^{12,14}$ -prostaglandin J2; ERK, extracellular signal-regulated kinase; MAP, mitogen-activated protein; MKP-1, MAP kinase phosphatase-1; RA, rheumatoid-arthritis; OA, osteo-arthritis; EMSA, electrophoresis mobility shift assay; PARP, poly(ADP-ribose) polymerase; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase.

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ptosis (16). Analysis of PPAR- γ expression, as described here, showed that synovial fibroblasts constitutively express PPAR- γ 1, which is dephosphorylated and degraded during BAY 11-7085-induced apoptosis.

EXPERIMENTAL PROCEDURES

Synovial Fibroblasts Isolation—Synovial fibroblasts from OA patients were isolated as explained previously (16). Synovial explants (3–5 mm) from RA patients were obtained by biopsy. Explants were cut and cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum until the attached cells became confluent. Cells were then cultured and used for experiments as synovial fibroblasts from OA patients.

Cell Treatment—Isolated synovial fibroblasts were treated for 24 h with PGE₂, PGF₂ α , PGD₂, 15d-PGJ₂, ciglitazone (BioMol, Plymouth Meeting, PA), and mitogen-activated protein kinase-kinase (MEK1/2)-specific inhibitor UO126 (Cell Signaling, Beverly, MA). Apoptosis was induced by NF- κ B inhibitor BAY 11-7085 (Alexis Corp., San Diego, CA). In some experiments the proteasome inhibitor MG-132 (Alexis Corp.), phosphatase inhibitor okadaic acid (Calbiochem) and PPAR- γ antagonist BADGE (Cayman Chemical, Ann Arbor, MI) were used.

Western Blotting—Cells were collected, lysed, and total proteins separated by SDS-PAGE as explained previously (16). Lysis buffer contained 25 mM Hepes, 150 mM NaCl, 0.5% Triton X-100, 10% glycerol, 1 mM dithiothreitol, phosphatase inhibitors (25 mM β -glycerophosphate, 1 mM Na₃VO₄, 1 mM NaF) and COMPLETE protease inhibitor mixture (Roche Applied Science). Phosphorylated ERK1/2 and caspase-8 were detected with mouse monoclonal antibody that recognize phosphorylated Tyr-204 (E4; catalog number sc-7383) (Santa Cruz Biotechnology, Santa Cruz, CA), and rabbit polyclonal antibody (Pharmingen), respectively, diluted 1:1000, in TBS-T (20 mM Tris (pH 7.6), 500 mM NaCl, 0.2% Tween) supplemented with 10% milk powder. PPAR- γ was detected with rabbit polyclonal antibody (H-100; catalog number sc-7196) (Santa Cruz Biotechnology) that recognize the N terminus of PPAR- γ 1 and mouse monoclonal antibody (E-8; catalog number sc-7273) (Santa Cruz Biotechnology) that recognizes the C terminus of PPAR- γ 1, respectively, diluted 1:1000 in TBS-T. Ubiquitin was revealed by mouse monoclonal antibody (P4D1; catalog number sc-8017) diluted 1:1000 in TBS-T supplemented with 10% milk powder. PARP was detected with mouse monoclonal antibody (Pharmingen), diluted 1:1000 in TBS-T. β -Actin was detected with mouse monoclonal antibody (Sigma), diluted 1:1000 in TBS-T. Incubation of membranes with primary antibodies was done at room temperature for 1–3 h. Western blots were revealed with 1:2000 diluted anti-mouse and anti-rabbit (DAKO A/S, Glostrup, Denmark) and ECL chemiluminescent reagents (Amersham Biosciences, Little Chalfont Buckinghamshire, UK).

Cloning of PPAR- γ 1 and PPAR- γ 2 from Synovial Fibroblasts—Total RNA was extracted from synovial fibroblasts using RNeasy columns (Qiagen, Valencia, CA). 1 μ g of recovered RNA was subjected to reverse transcription using the "First-Strand cDNA synthesis kit for reverse transcription-PCR" (Roche Applied Science). The newly transcribed cDNA was amplified by PCR using Taq Gold (Roche Applied Science) with the primers that correspond to the 5' (forward primer) and 3' (reverse primer) of the human PPAR- γ 1 and PPAR- γ 2,

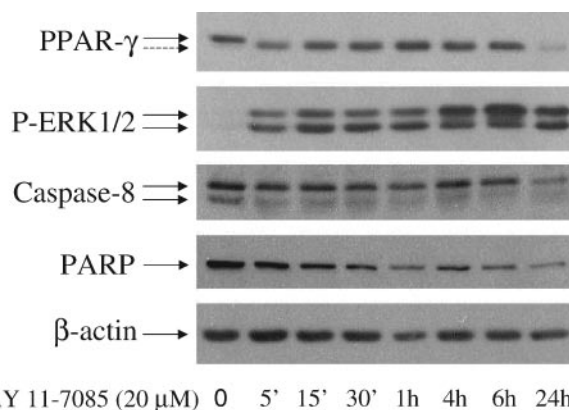


FIGURE 1. PPAR- γ -specific band shift and degradation during BAY 11-7085-induced synovial fibroblast apoptosis. Human synovial fibroblasts were treated with BAY 11-7085 (20 μ M) for the indicated time. The arrows indicate the expression of PPAR- γ , phospho-ERK1/2 (P-ERK1/2), two isoforms of caspase 8, PARP and β -actin, as determined by Western blot in total cell extracts. The hatched arrow indicates PPAR- γ -specific band shift.

respectively (9). DNA products were separated on agarose gel, isolated by gel extraction kit (Qiagen), and cloned in pcDNA3 expression vector (Invitrogen).

Transfection Experiments—HEK293 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. Transfection with the PPAR- γ 1 or PPAR- γ 2 gene cloned in pcDNA3 vector was done by FuGENE reagent (Roche Applied Science). Cell lines expressing PPAR- γ 1 were obtained from single colonies selected for G418 (BioWhittaker, Walkersville, MD) resistance.

Nuclear Protein Extraction and Electrophoresis Mobility Shift Assay (EMSA)—Nuclear extracts and EMSA were performed as described (17). Gel shift oligonucleotides representing DNA binding site for PPAR transcription factors (catalog number sc-2587, Santa Cruz Biotechnology) were labeled with [γ -³²P]ATP using polynucleotide kinase (United States Biochemical, Cleveland, OH).

Protein Phosphatase Treatment of Cell Extracts—Nuclear or total cell extracts were incubated with λ -protein phosphatase (catalog number P0753) (New England Biolabs, Beverly, MA) at 30 °C for 30 min.

Survival Assay—Cell survival was measured by cellular conversion of methyltetrazolium salt (Promega, Madison, WI) into a soluble formazan dye (18). An electron coupling agent phenazine methosulfate was obtained from Sigma. Colorimetric measurement of formazan dye was performed at 490 nm.

Immunoprecipitation—HEK293 cells were simultaneously transfected, as described above, with human PPAR- γ 1 (this work) and c-Myc-tagged human ubiquitin kindly donated by Ron Kopito (19). Sixteen hours after transfection the medium was changed to Dulbecco's modified Eagle's medium supplemented with 1% fetal calf serum. Cells were then treated with MG132 and BAY 11-8075, collected, and total cell proteins extracted. Protein extraction was done by boiling of the cell pellet in 10% glycerol, 2% SDS, and 50 mM Tris-HCl (pH 7.5) for 10 min (20). The lysate was diluted 1:10 with radioimmune precipitation assay-like buffer containing 50 mM Tris-HCl (pH 7.5), 1% Triton X-100, 0.5% sodium deoxycholate, 150 mM

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NaCl, 5 mM EDTA, 25 mM β -glycerophosphate, 1 mM NaF, 1 mM Na_3VO_4 , 100 μM phenylmethylsulfonyl fluoride, supplemented with COMPLETE protease inhibitor mixture (Roche Applied Science). SDS was then added to achieve final concen-

tration of 1% and immunoprecipitation performed with PPAR- γ rabbit polyclonal antibody (H-100; catalog number sc-7196) (Santa Cruz Biotechnology) or c-Myc rabbit polyclonal antibody (A-14; catalog number sc-789) at 4 °C, for 1–3 h. Immunocomplexes were recovered by incubation with protein A-agarose (sc 2001; Santa Cruz Biotechnology) for additional 16 h. The beads were washed four times in radioimmune precipitation assay-like buffer.

Statistics—*p* values were obtained using the Mann-Whitney test and considered significant when lower than 0.05.

RESULTS

PPAR- γ Degradation during BAY 11-7085-induced Synovial Fibroblast Apoptosis—Isolated primary synovial fibroblasts were treated for increasing times with the pro-apoptotic agent BAY 11-7085. Western blot showed that PPAR- γ is constitutively expressed in synovial fibroblasts (Fig. 1). Upon BAY 11-7085 treatment, the PPAR- γ -specific band was shifted into a form of higher mobility, suggesting dephosphorylation. This

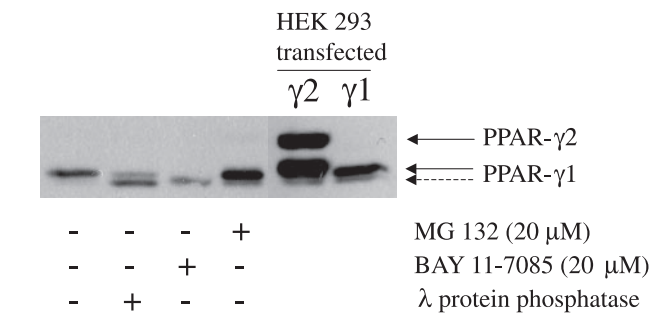


FIGURE 2. Synovial fibroblast PPAR- γ form is phosphorylated PPAR- γ 1. Synovial fibroblasts were treated with BAY 11-7085 for 24 h or with proteasome inhibitor MG-132 for 6 h. Before Western analyses total cell extracts were treated or not with λ -protein phosphatase. As controls, HEK293 cells were transfected with PPAR- γ 1 and PPAR- γ 2 clones (see "Experimental Procedures"). The *hatched arrow* indicates PPAR- γ -specific band shift.

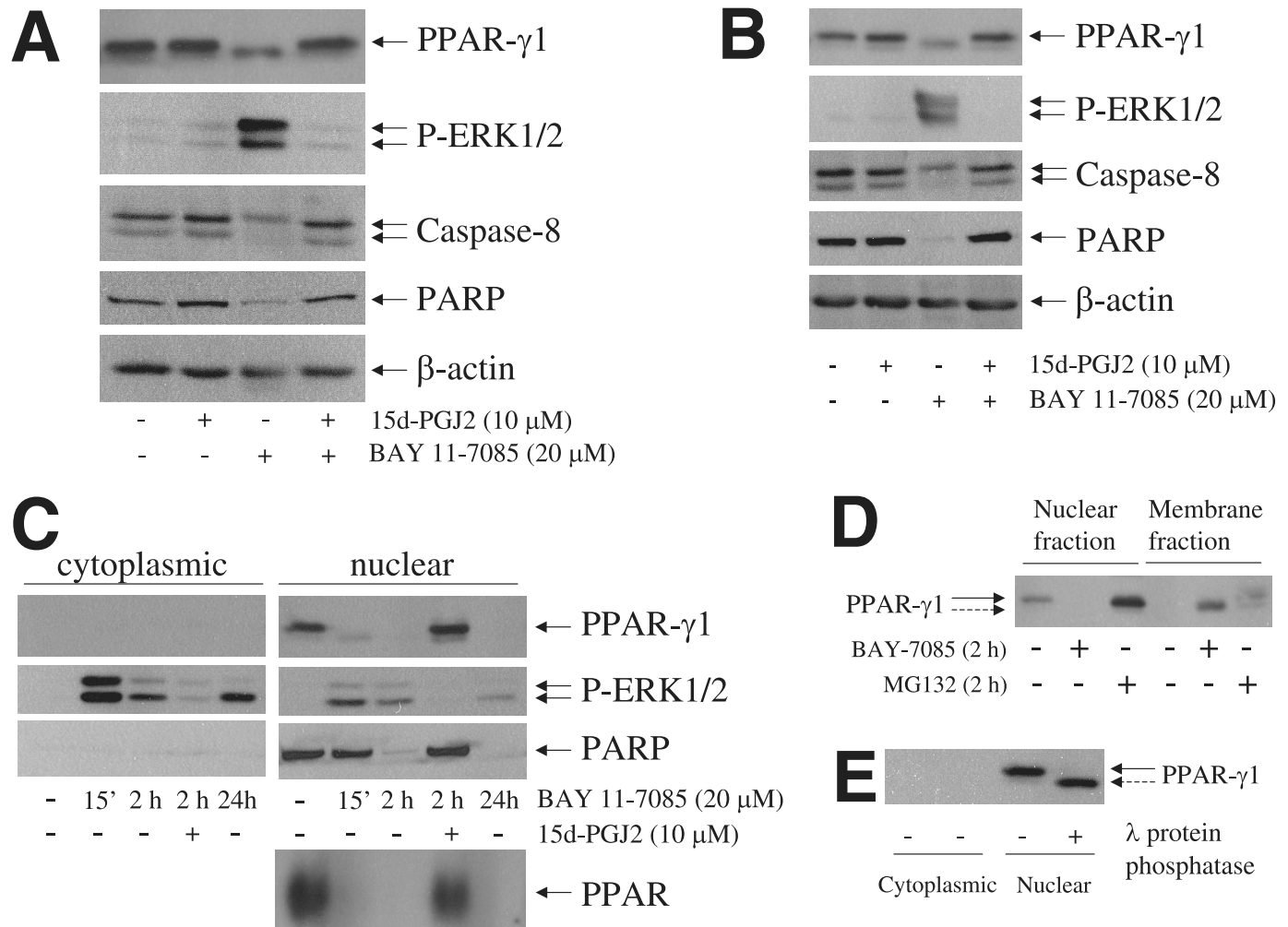


FIGURE 3. 15d-PGJ2 inhibits BAY 11-7085-induced PPAR- γ 1 degradation. Synovial fibroblasts originating from OA (A) or RA patients (B) were pretreated or not with 15d-PGJ2 for 24 h. BAY 11-7085 was then added for an additional 24 h. The *arrows* indicate expression of PPAR- γ , phospho-ERK1/2 (P-ERK1/2), two isoforms of caspase-8, PARP, and β -actin as determined by Western blot in total cells extracts. C, synovial fibroblasts were pretreated with 15d-PGJ2 for 24 h, and BAY 11-7085 was then added for additional 15 min or 2 or 24 h. Western blot shows expression of PPAR- γ , phospho-ERK1/2, and PARP in synovial fibroblast cytoplasmic and nuclear extracts. The *lower panel* shows EMSA performed on the same nuclear extracts with ^{32}P -labeled oligonucleotides representing PPAR-DNA consensus binding site. D, Western blot of nuclear and membrane synovial fibroblasts extracts. E, Western blot of cytoplasmic and nuclear extracts that were treated or not with λ -protein phosphatase. The *hatched arrow* indicates PPAR- γ 1-specific band shift.

(Fig. 3C, lower panel). Protein phosphatase treatment of nuclear extracts, like treatment of total cell extracts (Fig. 2), shifted PPAR- γ 1 into the form of higher mobility (Fig. 3E). These results suggested that the dephosphorylation of PPAR- γ 1 abolished the PPAR-DNA binding activity.

BAY 11-7085-induced PPAR- γ Dephosphorylation and Degradation Involves ERK1/2 Phosphorylation—To test the influence of ERK1/2 phosphorylation on BAY 11-7085-induced PPAR- γ dephosphorylation and degradation, we pretreated synovial fibroblasts with prostaglandins or PPAR- γ activators that suppress (15d-PGJ2, PGD2) or not (PGE2, PGF2 α , and ciglitazone) BAY 11-7085-induced ERK1/2 phosphorylation (Fig. 4A). Only 15d-PGJ2 and PGD2 were able to inhibit BAY 11-7085-triggered PPAR- γ dephosphorylation, suggesting that ERK1/2 activation was involved in BAY 11-7085-induced PPAR- γ dephosphorylation and degradation. Consistently, MEK1/2 inhibitor UO126, which partially inhibits BAY 11-7085-induced ERK1/2 phosphorylation (16), decreased BAY 11-7085-induced PPAR- γ degradation (Fig. 4B).

Phosphorylated but Not Dephosphorylated Form of PPAR- γ 1 Is Ubiquitinated Prior to Degradation—Since PPAR- γ has been shown to be modified by ubiquitin prior to degradation (22), we tested whether this is also the case for dephosphorylated PPAR- γ 1. Of interest, upon BAY 11-7085 treatment, the PPAR- γ 1 dephosphorylated form was found to be translocated to insoluble membrane fraction as well as ubiquitin and P-ERK1 (Fig. 5A). Several ubiquitinated proteins have been found in insoluble membrane fractions, including cystic fibrosis transmembrane conductance regulator (19), and ubiquitinated cellular acute myeloid leukemia protein is associated with the insoluble nuclear matrix (20). However, overexpression of PPAR- γ 1 and Myc-tagged human ubiquitin in HEK293 cells revealed that ubiquitinated PPAR- γ 1 accumulated only in the cells treated with proteasome inhibitor MG132 alone (Fig. 5B, lane 4) but not in the cells treated with both MG132 and BAY 11-7085 (Fig. 5B, lane 5). These results suggest that the dephosphorylated form of PPAR- γ 1 is not ubiquitinated before degradation and that phosphorylation of the protein is necessary for its ubiquitination (23). Nevertheless, the dephosphorylated form of PPAR- γ 1 may still be, at least partially, degraded by proteasome in the absence of ubiquitination, as it was shown for c-Fos (24).

Effects of Phosphatase and Proteasome Inhibitors on PPAR- γ 1 Phosphorylation—Activation of MAP kinase phosphatase MKP-1 (25) and MKP-3 (26) is suggested as the negative feedback loop that regulates deactivation of MAP kinase/ERK. We speculate that BAY11-7085-induced ERK1/2 phosphorylation may trigger the activation of such phosphatases, which could in turn dephosphorylate PPAR- γ 1. To test whether PPAR- γ 1 is sensitive to phosphatases, MKP-1, MKP-2, and protein phosphatase 2A inhibitor okadaic acid was used. Since okadaic acid was highly toxic to synovial fibroblasts and HEK293 cells (results not shown), we have created HEK293 lines that stably express PPAR- γ 1. These lines were less sensitive to toxic effect of okadaic acid than native HEK293 cells (results not shown) and were treated for 24 h with different concentrations of inhibitor. As a positive control the proteasome inhibitor MG132 was used. Western blot showed a marked accumulation of phosphorylated form of PPAR- γ 1 after okadaic acid treat-

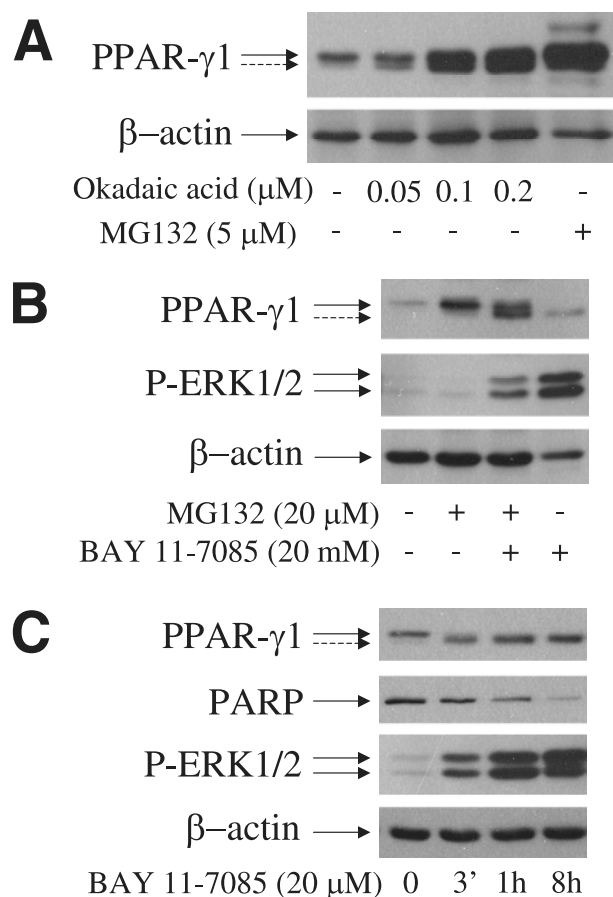


FIGURE 6. Effects of okadaic acid and MG132 on PPAR- γ 1 phosphorylation. A, HEK293 cells, stably transfected with PPAR- γ 1, were treated for 24 h with phosphatase inhibitor okadaic acid or proteasome inhibitor MG132, at the indicated concentrations. The arrows indicate expression of PPAR- γ and β -actin as determined in total cell extracts by Western blot. The hatched arrow indicates PPAR- γ 1-specific band shift. B, synovial fibroblasts were pretreated with MG132 for 24 h, and BAY 11-7085 was then added for additional 1 h. The arrows indicate expression of PPAR- γ 1, phospho-ERK1/2 (P-ERK1/2), and β -actin as determined in total cell extracts by Western blot. The hatched arrow indicates PPAR- γ 1-specific band shift. C, synovial fibroblasts were treated with BAY 11-7085 (20 μ M) for the indicated times. The arrows indicate expression of PPAR- γ , phospho-ERK1/2 (P-ERK1/2), PARP, and β -actin as determined in total cell extracts by Western blot. The hatched arrow indicates PPAR- γ 1-specific band shift.

ment (Fig. 6A). The accumulation of the dephosphorylated form of PPAR- γ 1 was probably due to the inhibitor toxicity. These results suggest that PPAR- γ 1 dephosphorylation involves okadaic acid-sensitive phosphatase.

To test the effect of PPAR- γ 1 phosphorylation and degradation on BAY11-7085-induced apoptosis we have pretreated synovial fibroblasts with proteasome inhibitor MG132, which leads to accumulation of phosphorylated PPAR- γ 1 (Fig. 6B). After 1 h of BAY11-7085 treatment we have observed still more PPAR- γ 1-phosphorylated forms and less BAY11-7085-induced ERK1/2 phosphorylation in the cells pretreated with MG132. However, after a longer BAY11-7085 treatment MG132 did not protect synovial fibroblasts from apoptosis (results not shown). Nevertheless, we have observed that in the synovial fibroblasts PARP is cut after PPAR- γ 1 dephosphorylation but before PPAR- γ 1 degradation (Fig. 6C). These results suggest that dephosphoryl-

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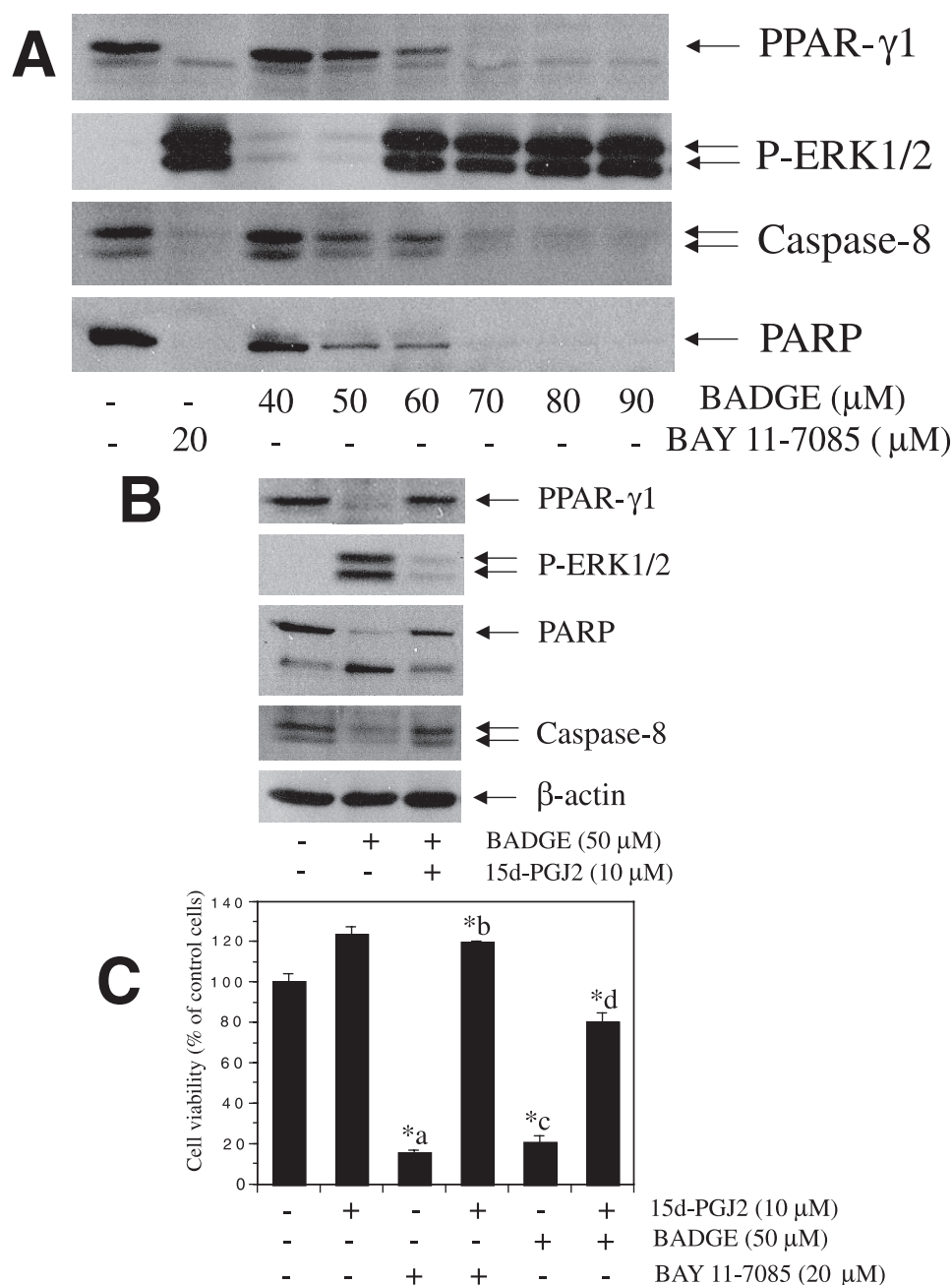


FIGURE 7. PPAR- γ antagonist induces PPAR- γ 1 degradation and synovial fibroblast apoptosis. *A*, synovial fibroblasts were treated with PPAR- γ antagonist BADGE for 24 h at the indicated concentrations. The arrows indicate expression of PPAR- γ , phospho-ERK1/2 (P-ERK1/2), two isoforms of caspase-8, and PARP as determined in total cell extracts by Western blot. *B*, synovial fibroblasts were pretreated or not with 15d-PGJ2 for 24 h. BADGE was then added for an additional 24 h. The arrows indicate the expression of PPAR- γ , phospho-ERK1/2 (P-ERK1/2), and β -actin as determined in total cell extracts by Western blot. *C*, synovial fibroblasts were pretreated with 15d-PGJ2 for 24 h. BADGE or BAY was then added for an additional 24 h. Cell survival was estimated by the methyltetrazolium salt test, and results were expressed as a percentage of surviving cells compared with control cells. **a* and **c*, statistically different from the non-treated control ($p < 0.05$). **b*, statistically different from BAY 11-7085-treated cells ($p < 0.05$). **d*, statistically different from BADGE-treated cells ($p < 0.05$).

ation of PPAR- γ 1 could mediate BAY11-7085-induced apoptosis even without PPAR- γ 1 degradation.

PPAR- γ Antagonist Induces PPAR- γ 1 Degradation—PPAR- γ antagonist BADGE (27) induced synovial fibroblasts apoptosis and PPAR- γ 1 degradation (Fig. 7*A*). BADGE is a ligand for PPAR that, in a radioligand assay, displaces 50% of rosiglitazone

at a concentration of $\sim 100 \mu$ M (27). In our experimental conditions synovial fibroblast apoptosis was induced with 50–70 μ M BADGE (Fig. 7, *A* and *B*). Interestingly, BADGE-induced apoptosis was also accompanied with ERK1/2 phosphorylation, degradation of PPAR- γ , and synovial fibroblast apoptosis (Fig. 7, *B* and *C*). These results suggested an anti-apoptotic role of PPAR- γ 1 in synovial fibroblasts.

DISCUSSION

Beside its essential role in adipocyte differentiation and energy storage, PPAR- γ is involved in cell proliferation, differentiation, and apoptosis, and its role in inflammation and cancer has been recognized (6, 7). PPAR- γ activity has been observed in synovial fibroblasts (28), and PPAR- γ activators troglitazone (28) and 15d-PGJ2 (29) were found to lower interleukin-1- and tumor necrosis factor- α -induced NF- κ B activity. Furthermore, PPAR- γ activators were shown to possess an anti-inflammatory activity against adjuvant-induced arthritis in mice (30). We showed here that synovial fibroblasts express the PPAR- γ 1 form that is being dephosphorylated and degraded during BAY 11-7085-induced synovial fibroblast apoptosis. 15d-PGJ2 and PGD2 that protect synovial fibroblasts from BAY 11-7085-induced apoptosis and inhibit ERK1/2 activation (16) suppressed PPAR- γ 1 dephosphorylation. Furthermore, the PPAR- γ antagonist BADGE (27) was able to induce ERK1/2 phosphorylation, PPAR- γ degradation, and synovial fibroblast apoptosis. In addition, BAY 11-7085-induced PPAR- γ 1 dephosphorylation was partially prevented by MEK1/2 inhibitor UO126. These results suggest that PPAR- γ 1 dephosphorylation

involves ERK1/2 activation. Activation of the ERK1/2 pathway, as a result of mechanical stimuli, has been shown very recently to reduce the expression of PPAR- γ in 3T3-L1 cells (31). Accordingly, our results presented here suggest that ERK1/2 activation leads to reduced PPAR- γ 1 expression in synovial fibroblasts. PPAR- γ 1 harbors the mitogen-activated

protein kinase consensus site and can be phosphorylated on serine 84 (serine 112 in PPAR- γ 2) by ERK1 (32) or ERK2 (33), *in vitro*. Such phosphorylation inhibits both ligand-dependent and -independent PPAR- γ transactivation function. Furthermore, a mutant form of PPAR- γ that cannot be phosphorylated on serine 112 is more adipogenic (32, 33). Curiously, our results showed that upon ERK1/2 activation, PPAR- γ 1, that is ubiquitously phosphorylated, underwent dephosphorylation before being degraded. These results suggest that phosphorylation of endogenous PPAR- γ 1 in synovial fibroblasts depends on another signaling pathway, distinct from ERK1/2. Several recent publications suggested that serine 84 (serine 112 in PPAR- γ 2) is not the unique PPAR- γ 1 phosphorylation site. For example, PPAR- γ phosphorylation that depends on epidermal growth factor receptor signaling has been shown in urothelial cells (34), while in opossum kidney cells PPAR- γ 1 phosphorylation and transcriptional activity depend on phosphatidylinositol 3-kinase (35). The authors showed that insulin triggered PPAR- γ phosphorylation and increased its transcriptional activity, as well as the expression of PPAR- γ -regulated genes. Of interest, insulin effect was not abolished with ERK1/2 phosphorylation. These results suggested the existence of additional, non-described, yet phosphorylation, sites in PPAR- γ .

Mainly, PPAR- γ agonists have been described as anti-inflammatory (36, 37). However, their pro-inflammatory properties have been documented too (38). We showed recently that 15d-PGJ2 protects chondrocytes and synoviocytes from apoptosis, which suggested both anti-inflammatory and pro-inflammatory effects, respectively (16). In this work we showed that the pro-inflammatory effect of 15d-PGJ2 on synovial fibroblasts involves its ability to prevent PPAR- γ 1 dephosphorylation and degradation during apoptosis. Interestingly, synthetic PPAR- γ agonist ciglitazone that protects synovial fibroblasts from nitric oxide donor sodium nitroprusside-induced apoptosis (results not shown) does not protect synovial fibroblasts from BAY 11-7085-induced apoptosis. Activation of PPAR- γ by its agonists has been shown to down-regulate NF- κ B and MAP kinase pathways (39). 15d-PGJ2 but not ciglitazone can suppress BAY11-7085-induced ERK1/2 activation in synovial fibroblasts (16). In rat liver epithelial cells ciglitazone is found to be an efficient ERK1/2 inducer (40). The discrepancy between 15d-PGJ2 and ciglitazone effects on BAY 11-7085-induced apoptosis is probably due to the inability of ciglitazone to suppress BAY11-7085-induced ERK1/2 activation in synovial fibroblasts.

Our data suggest that dephosphorylation of PPAR- γ 1 may be an early event involved in PPAR- γ 1 degradation and synovial fibroblast apoptosis. Furthermore, since apoptosis markers such as PARP are cleaved after PPAR- γ 1 dephosphorylation but before PPAR- γ 1 degradation, our results suggest that the dephosphorylation event might be enough to mediate BAY 11-7085-induced synovial fibroblast apoptosis. Interestingly, both PPAR- γ 1 underphosphorylation and decreased expression were observed very recently to be involved in diseases such as xeroderma pigmentosum (41) and multiple sclerosis (42), respectively.

We also showed that while the phosphorylated PPAR- γ 1 was nuclear and soluble in buffers containing non-ionic detergents,

the dephosphorylated PPAR- γ 1 was found in the insoluble membrane fraction. Furthermore, ubiquitination studies suggested that two forms might be degraded by distinct mechanism. These results further suggest that the two PPAR- γ 1 forms have different cellular localization and function.

Induction of synovial fibroblast apoptosis with the PPAR- γ antagonist further suggests that PPAR- γ might have a prominent role in synovial fibroblast survival as shown for hematopoietic cell line (7) and suggested recently for human lung fibroblasts (8).

Acknowledgments—We thank Aline Desoroux for expert technical assistance, Alain Chariot for helpful discussion, and Ron Kopito for donating the Myc-tagged human ubiquitin plasmid.

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