Thiazolidinediones Induce Osteocyte Apoptosis by a G Protein-coupled Receptor 40-dependent Mechanism*

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Background: Thiazolidinediones (TZDs) mediate osteocyte apoptosis and sclerostin up-regulation by an unknown mechanism.

Results: Osteocyte apoptosis is mediated through activation of Erk1/2 and p38, whereas sclerostin up-regulation is through peroxisome proliferator-activated receptor-γ (PPARγ) signaling.

Conclusion: TZDs signal not exclusively through PPARγ, as thought, but also via a surface receptor called GPR40.

Significance: Learning how TZDs signal in bone cells is crucial to prevent adverse effects associated with the use of these drugs.

Thiazolidinediones (TZDs),2 also known as glitazones, represent a class of pharmaceutical compounds approved for the treatment of type 2 diabetes mellitus. However, adverse effects such as heart problems and bone fractures have already been reported. Previously, we reported that pioglitazone and rosiglitazone induce osteocyte apoptosis and sclerostin up-regulation; however, the molecular mechanisms leading to such effects are unknown. In this study, we found that TZDs rapidly activated Erk1/2 and p38. These activations were mediated through Ras proteins and GPR40, a receptor expressed on the surface of osteocytes. Activation of this pathway led only to osteocyte apoptosis but not sclerostin up-regulation. On the other hand, TZDs were capable of activating peroxisome proliferator-activated receptor-γ, and activation of this signaling pathway led to sclerostin up-regulation but not osteocyte apoptosis. This study demonstrates two distinct signaling pathways activated in osteocytes in response to TZDs that could participate in the observed increase in fractures in TZD-treated patients.

Also, an unexplained increased risk of bone fracture has been documented mostly in women, but to date, the causes are unknown (2–5). Several cell types coexist in bone, osteoblasts (bone-forming cells), osteoclasts (bone-resorbing cells), osteocytes (which control bone remodeling), and bone marrow cells, including adipocytes. Adipocytes and osteoblasts come from a common progenitor upon activation of specific transcription factors. Activation of Runx-2 drives progenitors to become osteoblasts, whereas activation of PPARγ results in adipocyte differentiation. As TZDs are PPARγ agonists, it has been postulated that TZDs increase adipocyte differentiation at the expense of osteoblasts in vitro (6, 7). In vivo models showed that TZDs decrease bone formation and increase adiposity in bone marrow, although bone resorption is not affected (8–12).

The effects of TZDs on osteocytes are poorly understood. In the adult skeleton, osteocytes make up >90–95% of all bone cells compared with 4–6% osteoblasts and 1–2% osteoclasts. These cells are regularly dispersed throughout the mineralized matrix, connected to each other and to cells on the bone surface through dendritic processes generally radiating toward the bone surface and the blood supply. Osteocytes are a target of drugs affecting bone metabolism such as bisphosphonates because of their connections with blood vessels (14, 15). Osteocytes can conduct and control both bone resorption and bone formation by expressing key mediators such as RANKL and sclerostin (16). Recently, we reported that TZDs induce osteocyte apoptosis in a dose-dependent manner (17). Furthermore, we also demonstrated that TZD-treated osteocytes up-regulate the expression of sclerostin, a bone formation inhibitor, whereas RANKL expression is unchanged compared with untreated cells (17). However, the molecular pathways involved in such effects are totally unknown.

As TZDs are PPARγ agonists, they were thought to signal exclusively through this nuclear receptor. Several recent studies show that TZDs also activate a membrane G protein-coupled receptor called GPR40 (G protein-coupled receptor 40) (18, 19). GPR40 is a fatty acid receptor activated by long-chain fatty acids. Furthermore, GPR40 is involved in glucose- and
fatty acid-induced insulin secretion (20, 21). However, the expression and role of GPR40 in bone are unknown.

The aim of this study was to investigate the signaling pathways involved in osteocyte apoptosis and sclerostin expression. We found that although TZDs induce sclerostin expression through a PPARγ mechanism, osteocyte apoptosis is mediated via GPR40 and activation of Erk1/2 and p38.

**EXPERIMENTAL PROCEDURES**

*Reagents*—Rosiglitazone and troglitazone were purchased from Cayman Chemical (Ann Arbor, MI). Pioglitazone was purchased from Molekula (Shaftesbury, United Kingdom). Signaling inhibitors were purchased from Calbiochem. α-Minimal essential medium, FBS, bovine calf serum, penicillin, and streptomycin were purchased from Lonza (Wokingham, United Kingdom). Antibodies were purchased from Cell Signaling Technology (Danvers, MA) unless indicated otherwise. All other chemicals were purchased from Sigma-Aldrich.

*Animals*—Calvarias (frontal and parietal bones) from 4-week-old female Swiss mice were removed aseptically. The periosteal layers on both side were carefully stripped off with tweezers under α-minimal essential medium, and calvarias were transferred into a collagen-coated T75 cm² flask prior to culture in α-minimal essential medium supplemented with 5% FBS, 5% bovine calf serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. At confluency, cells were detached with collagenase and plated as described below. This research was conducted in compliance with appropriate guidelines from the Institutional Animal Care and Use Committee.

*MLO-Y4 Cells*—The murine long bone-derived osteocytic cell line MLO-Y4 was kindly provided by L. Bonewald (University of Missouri-Kansas City, Kansas City, MO). These cells present features of osteocytes (16). Cells were cultured in α-minimal essential medium supplemented with 5% FBS, 5% bovine calf serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. Cells were plated at 1 × 10⁴ cells/cm² on type I collagen-coated plates as described previously (22). Growth arrest was investigated in reducing FBS to 0.5% and bovine calf serum to 0.5%.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide Assay—Cell proliferation was investigated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide as described previously (23).

Osteoclast Cultures—Bone marrow macrophages from long bones of 4-week-old female Swiss mice were harvested as described previously (24). Osteoclasts were generated using a mixture of four siRNAs targeting murine PPARγ sequences (CGAAGAACCCAUCCGAAUGA, ACCCAAUGGUCCUAGUA, UCACAAUGCCACAGGUUU, and CGCAUGAAUUCUUAAUG); ON-TARGETplus SMARTpool siRNAs targeting murine PPARγ sequences GAGGAAACCCUGUGAUU, GGCAAAUGUGCCGAAUC, GGUUCAUGUUGAGCCUAU, and GAUAGGAUGAGUUGAGAAAA; and non-specific control siRNA duplexes were purchased from Thermo Scientific. Cells (5 × 10³ cells/cm²) were plated in either 6-wells plates or 25-cm² flasks coated with type I collagen as described above and cultured in the presence of 10⁻⁶ m TZDs. After 24 h, cells were washed twice with Opti-MEM I (Invitrogen) and

Inhibition of Intracellular Signaling—To investigate specific signaling pathways, MLO-Y4 cells were cultured in the presence of the signaling inhibitors bisindolylmaleimide I (50 nM), farnesylthiosalicylic acid (20 μM), FR180204 (10 μM), or SB203580 (10 μM) for 1 h prior to the addition of 10⁻⁶ m TZDs. These concentrations were selected based on a previous pilot study. Sclerostin expression and cell apoptosis were investigated 24 h later as described below.

**Apoptosis Assay**—Apoptosis was determined in TZD-treated cultures as reported previously (17). Briefly, after cell culture, the supernatant containing floating cells was collected and put in previously labeled Eppendorf tubes. Each well was washed with PBS before trypsin was added to detach adherent cells. The mixture containing detached adherent cells was collected and pooled in the Eppendorf tubes containing the cell culture supernatant. Cells were spun at 1500 rpm for 10 min, the supernatant was removed carefully, and cells were incubated with 0.04% trypan blue and transferred into a hemocytometer. Living (clear) and dead (blue) cells were counted under a light microscope, and the percentage of dead cells was determined for each condition as follow: % of dead cells = 100 × (number of dead cells)/(number of dead cells + number of living cells).

**Western Blot Analysis and Immunoblotting**—Cells were cultured in the presence of 10⁻⁶ m TZDs for the indicated time periods. Cells were washed with cold PBS, and lysates were made using lysis buffer containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 50 mM NaF, 3 mM Na₃VO₄, protease inhibitor mixture, and 1% Nonidet P-40. Samples were spun at 13,000 rpm for 30 min at 4°C, the supernatant was collected, and protein concentration was determined with the BCA assay (Thermo Scientific). Samples (20 μg/lane) were run on a 10% acrylamide gel and blotted onto a PVDF membrane. The membranes were washed with TBS and blocked with 5% bovine serum albumin. Samples were incubated overnight with one of the following specific antibodies for Erk1/2: phospho-Erk1/2 (Thr-202/Tyr-204), p38, and phospho-p38 (Thr-180/Tyr-182) (R&D Systems); sclerostin (R&D Systems); PPARγ, phospho-PPARγ (Ser-84), and GPR40 (Santa Cruz Biotechnology); and β-actin (Sigma-Aldrich). Subsequently, membranes were washed with TBS and incubated with the appropriate secondary antibodies coupled to HRP (R&D Systems). Immunoreactive bands were visualized using an ECL kit (Amersham Biosciences). The degree to which the different markers were induced was determined by normalizing the specific signal to that of β-actin using NIH Image) software. Control of loading was assessed by Ponceau red staining of the membrane after transfer.

Silencing—siGENOME SMARTpool siRNAs (containing a mixture of four siRNAs) targeting murine PPARγ sequences (CGAAGAACCCAUCCGAAUGA, ACCCAAUGGUCCUAGUA, UCACAAUGCCACAGGUUU, and CGCAUGAAUUCUUAAUG); ON-TARGETplus SMARTpool siRNAs targeting murine PPARγ sequences GAGGAAACCCUGUGAUU, GGCAAAUGUGCCGAAUC, GGUUCAUGUUGAGCCUAU, and GAUAGGAUGAGUUGAGAAA; and non-specific control siRNA duplexes were purchased from Thermo Scientific. Cells (5 × 10³ cells/cm²) were plated in either 6-wells plates or 25-cm² flasks coated with type I collagen as described above and cultured in the presence of 10⁻⁶ m TZDs. After 24 h, cells were washed twice with Opti-MEM I (Invitrogen) and

3 A. Mieczkowska and G. Mabilleau, unpublished data.
incubated with a mixture of 100 nM siRNA, Oligofectamine (Invitrogen), and Opti-MEM I. Cells were exposed to this transfection mixture for 16 h before being returned to normal culture medium. Forty-eight hours after transfection, TZDs (10^{-6} M) were added to the cultures. Knockdown efficiency was assessed by Western blotting.

Generation of Reactive Oxygen Species—MLO-Y4 cells were plated at a density of 1 \times 10^4 cells/cm^2 and cultured for up to 60 min in the presence of TZDs. At the end of the incubation period, intracellular levels of reactive oxygen species were determined using dichlorofluorescein diacetate as described previously (26), and fluorescence was read with a M2 microplate reader (Molecular Devices) with an excitation wavelength of 488 nm and an emission wavelength of 530 nm. As a positive control, MLO-Y4 cells were incubated in the presence of 100 nM phorbol 12-myristate 13-acetate.

Transmission Electron Microscopy—MLO-Y4 cells were fixed in 3.7% paraformaldehyde in Sorensen’s buffer. The cells were then dehydrated in a graded series of ethanol and embedded in Lowicryl K4M. Ultrathin sections were cut, and immunodetection of GPR40 and Bax (R&D Systems) were done using a secondary antibody complexed with 10-nm gold beads.

Statistical Analysis—Statistical analysis was performed with Systat® statistical software release 11.0 (Systat Software, Inc., San Jose, CA). Results are expressed as means ± S.E. The non-parametric Kruskall-Wallis test was used to compare the differences between the groups. When significant differences were observed, data were subjected to the Mann-Whitney test.

**RESULTS**

Rapid Activation of Erk1/2 and p38 Is PPARγ-independent—As represented Fig. 1, within 15 min of incubation with TZDs, a rapid and massive phosphorylation of Erk1/2 and p38 could be noted in osteocytes. This activation lasted for up to 60 min. On the other hand, no phosphorylation of Akt or JNK was recorded in the presence of TZDs (data not shown). As these activations were rapid, we hypothesized that they did not require PPARγ. To test this hypothesis, we performed silencing of this nuclear receptor. Interestingly, after a 15-min treatment with pioglitazone or rosiglitazone, although expression of PPARγ was reduced by 90% after 72 h compared with cells treated with scrambled siRNA, activation of Erk1/2 and p38 was unchanged. This seems to indicate that these two signaling pathways were activated independently of PPARγ. Several previous studies reported a role for TZDs in inducing oxidative stress in cells, leading to activation of MAPK independently of PPARγ. However, no increase in reactive oxygen species generation was recorded in the presence of TZDs (Fig. 1C).

Activation of Erk1/2 and p38 Is Ras-dependent—As PKC and Ras are two targets of TZDs in other tissues, we decided to investigate their role in Erk1/2 and p38 activation. The use of bisindolylmaleimide I, a specific inhibitor of PKC, did not reduce the activation of Erk1/2 or p38 in response to pioglitazone or rosiglitazone (Fig. 2A). On the other hand, the use of
farnesylthiosalicylic acid, a specific inhibitor of Ras proteins, hampered activation of Erk1/2 and p38 in pioglitazone- or rosiglitazone-treated cells (Fig. 2B). These results suggest that activation of Erk1/2 and p38 is dependent on proteins from the Ras family.

**Sclerostin Up-regulation Is PPARγ-dependent, whereas MLO-Y4 Apoptosis Is p38-dependent**—As it seems that TZDs have PPARγ-independent mechanisms, we wanted to determine whether osteocyte apoptosis and sclerostin up-regulation are under PPARγ control (Fig. 3). In the presence of TZDs, we observed an increase in osteocyte apoptosis. Silencing of PPARγ did not modify the pattern of osteocyte apoptosis. These results suggest that TZDs induce osteocyte apoptosis through a PPARγ-independent mechanism (Fig. 3B). On the other hand, in the presence of 10 μM SB203580, a specific p38 inhibitor, osteocyte apoptosis was significantly decreased by 69 and 63% in pioglitazone- and rosiglitazone-treated cultures, respectively. As Erk1/2 and p38 are known modulators of cell growth, MLO-Y4 cells were cultured in reduced serum conditions. Arresting cell growth did not affect the pattern of osteocyte death, but the presence of SB203580 significantly lowered the amount of dead osteocytes in low serum conditions (supplemental Fig. S2), suggesting that TZDs induce osteocyte death, independently of cell growth, via a p38-dependent mechanism.

**MLO-Y4 Apoptosis Is Mediated through GPR40**—In other cell systems, it has been reported that TZDs activate a cell surface receptor called GPR40. We investigated whether osteocytes express GPR40. Indeed, by Western blotting, we found that GPR40 was abundantly expressed in osteocytes (Fig. 5A) but also in human osteoblasts and primary human osteoclasts. Furthermore, immunogold labeling revealed that GPR40, in osteocytes, was localized at the cell membrane (Fig. 5B). To
investigate whether GPR40 could be a target for TZDs in osteocytes, we performed silencing experiments. After 72 h, GPR40 expression was significantly reduced by 92% (Fig. 6A). GPR40 silencing resulted in a decreased activation of Erk1/2 and p38 in pioglitazone- and rosiglitazone-treated cultures, although this decrease was more marked for p38 (Fig. 6B). In response to pioglitazone and rosiglitazone, osteocyte apoptosis was significantly decreased by 66 and 70%, respectively, in cells in which GPR40 was silenced (Fig. 6C), confirming that this cell surface receptor is responsible for p38 activation and osteocyte apoptosis. On the other hand, sclerostin expression was unchanged in GPR40-silenced cultures in response to TZDs (Fig. 6D). Moreover, we investigated the intracytoplasmic localization of Bax in TZD-treated cells (Fig. 6E). TZDs triggered a relocalization of Bax from the cytoplasm to the outer membrane of the mitochondria. Silencing of GPR40 or SB203580 treatment reversed the relocalization of Bax.

TZDs Induce Cell Death and Sclerostin Up-regulation in Primary Osteoblasts—To ascertain whether the above findings were restricted to the MLO-Y4 cell line or could be extended to bone-derived osteoblasts, we investigated p38 activation, cell death, and sclerostin expression in response to TZDs in osteoblasts obtained from calvarias of young mice (Fig. 7). After pioglitazone treatment, p38 was rapidly activated in primary osteoblasts (Fig. 7A). Interestingly, TZD induced a significant augmentation of cell death in osteoblast cultures by 60 and 72% in pioglitazone- and rosiglitazone-treated cultures, respectively (Fig. 7B). This increase in cell death was mediated, as for MLO-Y4 cells, through a GPR40/p38-mediated mechanism as evidenced by the reduction in cell death in the presence of siRNA targeting GPR40 or SB203580 (Fig. 7, B and C). On the other hand, PPARγ silencing did not affect cell death in TZD-treated cultures (Fig. 7D). Similar to what was observed with MLO-Y4 cells, TZDs significantly up-regulated sclerostin expression. On the other hand, sclerostin expression was not affected by GPR40 silencing (data not shown), but as for MLO-Y4 cells, sclerostin expression was significantly reduced by 84% in cultures in which PPARγ was silenced.

**FIGURE 3.** Sclerostin expression but not osteocyte apoptosis is mediated through PPARγ. A, MLO-Y4 cells were pretreated with scrambled (white bars) or PPARγ (gray bars) siRNA prior to the addition of TZDs, and osteocyte apoptosis was investigated. Untreated cells (black bars) served as controls. B, sclerostin expression was assessed in untreated cells (and black bars), cells transfected with scrambled siRNA (sc and white bars), and cells transfected with PPARγ siRNA (ppar-γ and gray bars). ***, p < 0.01 versus cells transfected with scrambled siRNA. PIO, pioglitazone; ROSI, rosiglitazone.

**FIGURE 4.** Osteocyte apoptosis but not sclerostin expression is dependent on Erk1/2 and p38 activation. A, MLO-Y4 cells were pretreated with 10 μM FR180204 (white bars), a specific inhibitor of Erk1/2, or 10 μM SB203580 (gray bars), a specific inhibitor of p38, prior to the addition of 10−6 M TZDs to the culture. Untreated cells served as controls (black bars). B, sclerostin expression was assessed in untreated cells (and black bars), FR180204-treated cells (FR and white bars), and SB203580-treated cells (SB and gray bars). ***, p < 0.01 versus untreated cells. PIO, pioglitazone; ROSI, rosiglitazone.
TZDs Decrease Osteoclast Formation through a GPR40/p38-mediated Mechanism—As GPR40 was expressed also in osteoclasts, we investigated the role of TZD in osteoclast physiology. In contrast to what was reported for primary osteoblasts and MLO-Y4 cells, TZDs did not increase the death of osteoclast precursors (Fig. 8). However, although cell death was not affected, treatment of osteoclast precursor cultures with TZDs resulted in a dose-dependent decrease in osteoclast numbers. We then postulated that the same GPR40/p38-mediated mechanism might be responsible for this reduction in osteoclast numbers, and indeed, silencing of GPR40 or use of SB203580 significantly increased the number of osteoclast as evidenced in Fig. 8 (C and D).

DISCUSSION

TZDs represent an interesting class of drugs used in the treatment of type 2 diabetes mellitus. However, several adverse effects, including low bone mass and bone fracture, have been reported in patients treated with these molecules. Previously, we reported that TZDs induce sclerostin expression and osteocyte apoptosis (17). However, little was known about the molecular mechanism leading to these two events and especially whether they were the result of distinct molecular pathways or linked. In this study, our results suggest that although TZDs induce sclerostin expression through activation of PPARγ, osteocyte apoptosis is mediated through a different signaling pathway involving GPR40, proteins from the Ras superfamily, and activation of Erk1/2 and p38.

TZDs have previously been described as agonists of PPARγ. The ligand binding affinity order for PPARγ is rosiglitazone > pioglitazone > troglitazone (27). However, although TZDs mediate osteocyte apoptosis, the ranking order for TZD potency in doing this is pioglitazone > troglitazone > rosiglitazone (27). This finding is in contradiction to the ligand binding affinities. In this study, we have demonstrated that silencing of PPARγ did not affect the pattern of osteocyte apoptosis, whereas silencing of GPR40 significantly decreased osteocyte apoptosis. Furthermore, blockade of p38, which is activated within 15 min in osteocytes, kinetically appears unlikely to reflect actions toward PPARγ. Taken together, these results indicate that although TZDs have been described as strong PPARγ agonists, some effects are mediated through different intracellular signaling pathways. On the other hand, the order of potency regarding sclerostin expression seems to follow the ligand binding affinities and is markedly reduced when PPARγ is silenced, suggesting a PPARγ-dependent mechanism. This idea is further reinforced by the fact that a putative PPARγ-responsive element could be identified in the promoter of the Sost gene. The molecular mechanism leading to PPARγ activation by TZDs is still unclear. PPARγ activity may be modulated by several post-translational modifications, including phosphorylation, sumoylation, ubiquitination, nitrilation, and intracellular compartmentalization (see review in Ref. 28). Some of our results suggest an increase in Ser-84 phosphorylation in response to TZDs; however, this phosphorylation seems to be independent of Erk1/2 and p38 activation, as specific inhibitors such as FR180204 and SB203580 did not modify the pattern of Ser-84 phosphorylation. Further studies are clearly required to fully understand the mechanism behind PPARγ activation and sclerostin up-regulation.

Although TZDs signal undoubtedly through PPARγ, several other signaling pathways have been evidenced (18, 29–34). Among all of these pathways, similarities in intracellular targets have been evidenced with transactivation of the EGF receptor. As such, in liver cells, transactivation of this receptor (30, 32, 33) leads to activation of Erk and p38. This pathway involves the rapid generation of reactive oxygen species to activate Src and then the EGF receptor. However, in our study, we demonstrated that, in osteocytes, TZDs did not generate oxidative stress, and it is unlikely that this pathway is at the origin of osteocyte apoptosis. A strong argument to support the role of GPR40 in osteocyte apoptosis is that MAPK signaling and osteocyte apoptosis are strongly inhibited in the presence of...
GPR40 silencing. Furthermore, several recent studies reported the involvement of GPR40 in PPARγ-independent response to TZDs in other cell types (35, 36). Smith et al. (18) recently reported the mode of binding of TZDs to GPR40 and demonstrated, upon binding, a rapid activation of Gαq/Gα11, resulting in activation of Erk1/2 and p38. Our study suggests similar results, as TZDs induced Erk1/2 and p38 activation upon interaction with GPR40. Our study also seems to suggest that Ras proteins are intermediates between GPR40 and MAPK activation. However, whether this event requires Gαq/Gα11 and augmentation in intracellular calcium remains unknown.

To our knowledge, this is the first report of the expression of GPR40, the free fatty acid receptor 1, on the surface of osteocytes. Cornish et al. (37) previously reported the expression of GPR40 in murine osteoclast precursor cells but not in murine osteoblasts. Cornish et al. demonstrated that the use of GW9508, a GPR40/GPR120 agonist, resulted in decreased osteoclastogenesis. Our results in osteoclast cultures also support a role of GPR40 in reducing osteoclastogenesis. A growing body of evidence suggests that PPARγ is necessary for osteoclastogenesis (38, 39); however, this is intriguing, as TZDs have been shown to decrease osteoclast formation and bone resorption (40–42). One explanation could be that, in osteoclasts, the observed effect associated with the use of TZDs is mediated through GPR40, and as such, further studies on the impact of GPR40 activation in osteoclasts are needed.

FIGURE 6. Osteocyte apoptosis but not sclerostin expression is GPR40-dependent. A, efficiency of GPR40 silencing. B, silencing of GPR40 resulted in decrease activation of Erk1/2 and p38. White bars, cells transfected with scrambled siRNA (sc); gray bars, cells transfected with GPR40 siRNA. **, p < 0.01 versus cells transfected with scrambled siRNA. C, osteocyte apoptosis was determined in response to TZDs in untreated cells (black bars), cells transfected with scrambled siRNA (white bars), and cells transfected with GPR40 siRNA (gray bars). D, GPR40 silencing did not affect the pattern of expression of sclerostin. White bars, cells transfected with scrambled siRNA; gray bars, cells transfected with GPR40 siRNA. E, intracytoplasmic localization of Bax in untreated cells (panel a), pioglitazone-treated cells (panel b), rosiglitazone (ROSI)-treated cells (panel c), pioglitazone (PIO)-treated cells transfected with scrambled siRNA (panel d), and pioglitazone-treated cells transfected with GPR40 siRNA and pioglitazone- and SB203580-treated cells (panel f).
As a conclusion, Fig. 9 summarizes the effects of TZDs on osteocytes. Rapidly after treatment with TZDs, phosphorylation of Erk1/2 and p38 occurs through the involvement of GPR40, expressed on the cytoplasmic membrane, and Ras. This signaling pathway results in recruitment of Bax to the outer membrane of the mitochondria and induction of osteocyte apoptosis.
activate PPARγ leads to recruitment of Bax to the outer membrane of the mitochondria and activates Erk1/2 and p38 through a Ras-dependent mechanism. This pathway

**FIGURE 9.** Schematic representation of TZD action in osteocytes. TZDs activate PPARγ with this result in activation of the sclerostin gene (SOST) and sclerostin up-regulation.

ptosis. In parallel, TZDs cross the cytoplasmic membrane and activate PPARγ. In return, PPARγ induces the expression of sclerostin within 24 h.

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Thiazolidinediones and Osteocytes


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