Thiazolidinediones induce proliferation of human bronchial epithelial cells through the GPR40 receptor

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Gras D, Chanez P, Urbach V, Vachier I, Godard P, Bonnans C. Thiazolidinediones induce proliferation of human bronchial epithelial cells through the GPR40 receptor. Am J Physiol Lung Cell Mol Physiol 296: L970–L978, 2009. First published April 3, 2009; doi:10.1152/ajplung.90219.2008.—Thiazolidinediones (TZDs) are synthetic peroxisome proliferator-activated receptor-γ (PPARγ) ligands that are widely used in type II diabetes treatment. In addition to their ability to improve glucosyl homeostasis, TZDs possess anti-inflammatory properties and inhibit growth of many cells, particularly cancerous airway epithelial cells. However, the functional effects of PPARγ ligands on nonmalignant human bronchial epithelial cells have never been investigated. In the present study, we questioned whether PPARγ ligands may regulate proliferation of human bronchial epithelial cells, and we studied their potential molecular mechanisms. We found that synthetic PPARγ agonists, rosiglitazone (RGZ) and troglitazone (TGZ), induced proliferation of human bronchial epithelial cells, whereas the endogenous PPARγ ligand, 15-deoxyΔ12,14-prostaglandin J2 (15d-PGJ2), inhibited cell growth. RGZ and TGZ (10 μM) induced a rapid and transient intracellular Ca2+ mobilization from thapsigargin-sensitive intracellular stores, whereas 15d-PGJ2 (5 μM) did not induce any Ca2+ signal. The PPARγ antagonist GW-9662 did not inhibit any biological responses, but it reversed the effect of 15d-PGJ2 on cell growth. Using RT-PCR, we detected mRNA expression of the GPR40 receptor, a G protein-coupled receptor recently identified as a receptor for free fatty acids and TZDs, in human bronchial epithelial cells. Downregulation of GPR40 by small-interfering RNA led to a significant inhibition of TZD-induced Ca2+ mobilization and proliferation. This study provides evidence for the proliferative effect of anti-diabetic drug TZDs in nonmalignant human bronchial epithelial cells through GPR40 receptor activation, involving an intracellular Ca2+ signaling pathway.

airway epithelium; glitazone; calcium; peroxisome proliferator-activated receptor-γ; G protein receptor 40

THIAZOLIDINEDIONES (TZDs), such as troglitazone (TGZ), rosiglitazone (RGZ), pioglitazone (PGZ), and ciglitazone (CGZ), are synthetic compounds used in the treatment of type II diabetes to improve insulin sensitivity and reduce blood glucose and triglyceride levels (28, 40). TZDs are selective ligands for a nuclear hormone receptor known as peroxisome proliferator-activated receptor-γ (PPARγ), a member of the PPAR family. The four PPAR isotypes that have been identified to date (α, β, δ, and γ) display different tissue distribution and function (21). In addition to the synthetic anti-diabetic TZDs, some endogenous mediators, including the cyclopentenone prostaglandin 15-deoxyΔ12,14-prostaglandin J2 (15d-PGJ2), some polyunsaturated fatty acids, and eicosanoids, bind and activate PPARγ (17, 23). Activation of PPARγ results in heterodimerization of the receptor with the retinoid X receptor and binding to specific peroxisome proliferator-responsive elements located within the promoter region of target genes involved in lipid biosynthesis and glucose homeostasis. PPARγ is highly expressed in the liver, kidney, intestinal mucosa, and in adipose tissue where it plays a critical role in adipocyte differentiation (15). PPARγ is also expressed in numerous cell types, including epithelial cells of the breast, colon, ovary, and lung (26, 39). Anti-inflammatory properties have been described for PPARγ ligands (34). In addition, anti-tumorigenic actions for TZDs have been reported. A number of in vitro and in vivo studies showed that PPARγ ligands inhibited growth of many cancer cell types, including colon (2, 32), breast (14), prostate (25), and lung (7, 10, 22).

Over the last few years, an increasing number of studies reported that TZDs are able to exert some PPARγ-independent actions (16). The best direct evidence comes from receptor knockout studies where TZDs inhibited tumor growth in both PPARγ+/+ and PPARγ−/− mouse embryonic stem cells (29). Moreover, in several studies, the rapid occurrence of TZD-induced effects (within min to h) suggested that TZDs may have some PPARγ-independent actions that are rather characteristic of cell surface receptor involvement. Recently, a subfamily of deorphanized homologous G protein-coupled receptors (GPCRs) which is activated by saturated and unsaturated long-chain fatty acids, was identified (9). Among them, the GPR40 receptor is described to be specifically activated by free fatty acids and TZDs (9, 20, 24). GPR40 is highly expressed in the brain and pancreatic β-cells and to a lesser extent in other tissues (9). Interestingly, GPR40 is expressed in the lung, but its physiological role in airways is still unknown.

The role of TZDs in lung cancer has been studied in vitro using cancer cell lines and in animal models. However, effect of TZDs on normal human bronchial epithelial cells (HBEC) has not been investigated. In this study, we report for the first time that both TGZ and RGZ induced proliferation of nonmalignant HBEC via Ca2+ mobilization, whereas the non-TZD PPARγ ligand 15d-PGJ2 inhibited cell growth. These unexpected effects of TZDs were independent of PPARγ and were mediated by the G protein-coupled GPR40 receptor. These data provide novel mechanisms for TZD actions in nonmalignant airway epithelial cells.

MATERIALS AND METHODS

Materials. 15d-PGJ2, RGZ, TGZ and GW-9508 were purchased from Cayman Chemical (Ann Arbor, MI). GW-9662 was obtained from Merck Biosciences (Fontenay sous-bois, France). Thapsigargin was purchased from Sigma-Aldrich (St-Quentin-Fallavier, France).
**Cell culture.** The immortalized HBEC line BEAS-2B and human alveolar adenocarcinoma A549 cell line were obtained from ATCC (Rockville, MD). They were cultured in RPMI medium supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% FCS. Primary culture of small airway epithelial cells (SAEC; Clonetics-BioWhittaker, San Diego, CA) were maintained as previously described (5).

**Proliferation assay.** Cell proliferation was determined using the reduction of tetrazolium dye (MTT; Chemicon, Temecula, CA) to cell monolayers (2 days old) in serum-free medium. Cells were then incubated with RGZ (1–30 μM), TZD (0.1–10 μM), 15d-PGJ2 (5 μM), or 10% FCS (used as positive control) or vehicle (medium 2% FCS). When PPARγ antagonist was used, cells were pretreated for 30 min with the antagonist before stimulation with TZDs or 15d-PGJ2. MTT solution (10 μl) was then added to each well and incubated for 4 h (37°C). Dye was extracted from cells with 100 μl of isopropanol-1 N HCl (96:4, vol/vol), and absorbance at 570 nm was determined. A linear relationship was determined for MTT reduction (absorbance at 570 nm) and BEAS-2B cell number [y = 10^{-18s} + 0.0995], r^2 = 0.98.

**Cell cycle analysis.** BEAS-2B cells were plated in six-well plates and were serum starved for 24 h. Cells were then treated with or without RGZ (10 μM) for 24 h in medium containing 0.5% FCS. Medium containing 10% FCS was used as a positive control. Cells were then removed with trypsin-EDTA solution and were fixed by mixing 1 ml PBS with 6 ml cold 100% methanol and kept at −20°C. After removal of methanol, cells were treated with the Coulter DNA-Prep reagents kit (Beckman Coulter, Villepinte, France). Cells were resuspended in 50 μl lysing and permeabilizing reagent and 200 μl propidium iodide solution containing RNase. Flow cytometry analyses were performed using a FACScalibur flow cytometer (Becton-Dickinson, le Pont de Claux, France). The red DNA fluorescence signal was analyzed as pulse width vs. area to eliminate doublets and aggregates. FL2 area histogram has been drawn and formatted to analyze the signal was analyzed as pulse width vs. area to eliminate doublets and aggregates.

**Calcium imaging.** The intracellular Ca^{2+} concentration ([Ca^{2+}]_i) was measured in BEAS-2B cells or A549 cells according to the method previously described (6). Cells were grown on glass bottom dishes (WPI, Stevenage, UK). The emission fluorescence, which is proportional to the level of [Ca^{2+}]_i, was filtered at 510 nm. The transmitted light image was detected using a Photometrics CoolSNAP-fx video camera (Roper Scientific, Evry, France) coupled to the microscope.

**RT-PCR.** Total RNA was purified from cell lysates (RNeasy; Qiagen, Valencia, CA), and cDNA were synthesized (Ready-to-Go RT-PCR beads; Amersham, Piscataway, NJ). Semiquantitative human GPR40 gene expression was determined using specific primers (sense primer, 5’-GTG TCA CCT GGG TCT GGT CT-3’ and antisense primer, 5’-GAG CAG GAG AGA GAG GCT GA-3’).

**Western blot.** After specific stimulation, whole cells were lysed in 1% Triton X-100, 1 μM phenylmethylsulfonyl fluoride, 100 μM sodium orthovanadate, and 1:100 protease inhibitor mixtures (Sigma-Aldrich). Total proteins (30 μg) were subjected to SDS-PAGE on 12% Tris–HCl gels and then transferred to nitrocellulose membranes. Blots were probed with an anti-GPR40 rabbit polyclonal antibody (4°C, overnight, 1:200 dilution) (Tebu-bio, le Perray en Yvelines, France). Next, membranes were incubated with horseradish peroxidase-conjugated goat anti-rabbit antibody (37°C, 1 h, 1:10,000 dilution; Sigma-Aldrich). The monoclonal antibody against β-actin (Sigma-Aldrich) was used as an internal control to show equal protein loading. Visualization was performed by chemiluminescence followed by autoradiography. The density of bands was analyzed using a monochrome CCD camera (RS-170; COHU, San Diego, CA) coupled to the NIH Image analysis program (Pierce Biotechnology, Rockford, IL).

**Small-interfering RNA experiments.** A pool of four small-interfering RNA (siRNA) 21-mers matching the human GPR40 sequence was provided pooled from Dharmaco Research (Chicago, IL). To detect off-target effects, a mixture of four siRNA constructs harboring at least four mismatches with known human genes (siControl Non-Targeting siRNA Pool; Dharmaco Research) was used as a negative control siRNA. Transfection of siRNA with a final concentration of 100 nM was accomplished with ExGen 500 (Euromedex, Mundolsheim, France). After transfection (3 days), GPR40 protein expression was determined, and cells were used for Ca^{2+} or proliferation assays.

**Statistical analysis.** Values are expressed as means of three experiments ± SE. Comparison among groups was performed using ANOVA for unpaired (Ca^{2+} images) or paired data followed by a Fisher’s protected least-significant difference test. For all analyses, findings were considered significant at P < 0.05.

**RESULTS**

**TZDs induced bronchial epithelial cell proliferation.** To determine the effect of TZDs on cell proliferation, the nonmalignant HBEC line BEAS-2B was treated with RGZ (1, 5, 10, 20, and 30 μM) or TGZ (0.1, 1, 5, and 10 μM) for 24 h, and proliferation was measured by MTT assay (Fig. 1). Both RGZ (Fig. 1A) and TGZ (Fig. 1B) induced a concentration-dependent increase in cell number. The stimulation of cell proliferation by RGZ became significant at a concentration of 5 μM and then reached a plateau at 10 μM that was sustained for up to 30 μM compared with vehicle (n = 5, P < 0.01) (Fig. 1A). At the same concentration (10 μM), a more pronounced effect on proliferation was observed for TGZ compared with RGZ (3.8 ± 0.1 fold induction vs. 1.9 ± 0.2 fold induction, proliferation fold induction calculated compared with vehicle, n = 5, P < 0.01). To confirm the proliferative effect of TZDs, we investigated the consequence of 10 μM RGZ treatment for 24 h on the different phases of the cell cycle (Fig. 1, C and D). Quiescent BEAS-2B cells entered S-phase 6 h after RGZ or TGZ stimulation, whereas they entered S-phase 8 h in the presence of 10% FCS (data not shown). At 24 h, the percentage of cells in G1/G0 phase was lower with RGZ stimulation than vehicle (65.6 ± 2.3 vs. 73.7 ± 3.8%, n = 4, P < 0.05; Fig. 1C). The percentage of cells in G2/M phases after RGZ was significantly higher than vehicle (19.0 ± 0.9 vs. 12.1 ± 3.3%, n = 4, P < 0.05). Moreover, the proliferative index, defined as the total number of cells being in S and G2/M phases, was significantly higher in cells stimulated with RGZ than incubated with the vehicle alone (32.4 ± 0.4 vs. 22.5 ± 2.6%, P < 0.01; Fig. 1D). All data showed that RGZ and TGZ induced proliferation of nonmalignant HBEC via stimulation of the cell cycle.
RGZ-induced proliferation is independent of PPARγ. We next investigated whether the induction of proliferation by TZDs was mediated by the PPARγ receptor. First, we tested the effect of the natural PPARγ ligand 15d-PGJ2 on BEAS-2B cell proliferation. Interestingly, 15d-PGJ2 displayed 45.8 ± 9.5% inhibition of cell proliferation at 24 h compared with vehicle (n = 4, P < 0.05; Fig. 2A). In addition, exposure of cells to the selective PPARγ antagonist GW-9662 (10 µM, 30 min) did not inhibit RGZ-induced proliferation (2.1 ± 0.2 vs. 1.8 ± 0.1, GW-9662 + RGZ compared with RGZ, n = 4; Fig. 2B). Also, TGZ-induced proliferation was not affected by GW-9662 treatment (data not shown). In contrast, GW-9662 treatment reversed the inhibition of proliferation induced by 15d-PGJ2 treatment (0.88 ± 0.1 vs. 0.54 ± 0.09, GW-9662 + 15d-PGJ2 compared with 15d-PGJ2, n = 4, P < 0.05; Fig. 2C). Taken together, these results demonstrate that RGZ-induced proliferation was independent of PPARγ, whereas the inhibition of proliferation by 15d-PGJ2 was mediated via a PPARγ-dependent pathway in HBEC.

**RGZ and TGZ induced [Ca2+]i mobilization.** It has been reported that TGZ can deplete intracellular Ca2+ stores (29). We investigated whether RGZ and TGZ were able to induce a Ca2+ signal in BEAS-2B cells. As shown in Fig. 3A, RGZ caused a rapid and transient increase in [Ca2+]i. The larger response was obtained with 10 µM RGZ [0.36 ± 0.008, mean Δ (ratio 340/380 nm) ± SE, n = 93 cells; Fig. 3B]. Dimethyl sulfoxide (vehicle) used as TZD solvent did not affect [Ca2+]i. At 10 µM, TGZ produced a higher [Ca2+]i response [0.84 ± 0.045, mean Δ (ratio 340/380 nm) ± SE, n = 90 cells, P < 0.05; Fig. 3C] than RGZ. Exposure of cells to 15d-PGJ2 did not produce any change in [Ca2+]i, whereas ATP still produced Ca2+ mobilization, indicating that the Ca2+ signaling was intact [0.38 ± 0.01, mean Δ (ratio 340/380 nm) ± SE, n = 90 cells, P < 0.05; Fig. 3, A and C].

**Involvement of RGZ-induced Ca2+ release in BEAS-2B proliferation.** To identify the source of the [Ca2+]i increase induced by RGZ, we used thapsigargin, a Ca2+-ATPase pump inhibitor. As shown in Fig. 4, A and B, thapsigargin (100 nM) produced a [Ca2+]i increase [0.34 ± 0.02, mean Δ (ratio 340/380 nm) ± SE, n = 61 cells] followed by a return to a plateau value. After 15 min of thapsigargin incubation, exposure of BEAS-2B cells to RGZ (10 µM) did not produce any significant change in [Ca2+]i. The effect of RGZ on [Ca2+]i was completely abolished after thapsigargin treatment [−0.075 ± 0.015, mean Δ (ratio 340/380 nm) ± SE, n = 61 cells, P < 0.05 compared with the RGZ response; Fig. 4B]. Also, thapsigargin treatment had a similar effect on TGZ-induced Ca2+ release (data not shown). These results indicated that the response of HBECs to RGZ mainly involves the Ca2+ release from the thapsigargin-sensitive intracellular stores.

We next investigated the role of RGZ-induced Ca2+ mobilization on BEAS-2B proliferation. For that purpose, cells were pretreated with thapsigargin (100 nM, 30 min) and then exposed to 10 µM RGZ. After 24 h, the proliferation was measured by MTT assay. As shown in Fig. 4C, thapsigargin treatment had no effect on basal BEAS-2B proliferation (1.05 ± 0.28-fold induction, compared with vehicle) but completely attenuated the RGZ proliferative effect (1.1 ± 0.2 vs. 1.8 ± 0.1, thapsigargin + RGZ vs. RGZ, n = 4, P < 0.05). Also, thapsigargin treatment inhibited TGZ-induced proliferation (data not shown). These last results demonstrate that RGZ-induced Ca2+ increase was involved in the proliferative effect of RGZ on HBEC.

**RGZ induced Ca2+ mobilization and proliferation via GPR40 receptor.** Recent reports showing the involvement of the human cell surface G protein-coupled receptor GPR40 in rapid responses to TZDs (24) prompted us to investigate if this receptor could be associated with TZD effects observed in our model. Using semiquantitative PCR, we showed that HBEC (SAEC and BEAS-2B) expressed GPR40 mRNA, whereas the adenocarcinoma epithelial cell line A549 did not (Fig. 5A). We first investigated whether TZDs induce similar biological responses in A549 cells to those observed in HBEC. As shown in Fig. 5B, TGZs did not induce any Ca2+ signal in A549 cells. However, in contrast to HBEC cells, TZDs decreased A549 cell proliferation (0.59 ± 0.06-fold induction, n = 3, P < 0.01 compared with vehicle; Fig. 5C). Also, as shown in BEAS-2B
cells, 15d-PGJ2 did not affect Ca\(^{2+}\) in A549 cells (Fig. 5B) and decreased proliferation in these same cells (Fig. 5C). To determine whether GPR40 is involved in RGZ- and TGZ-induced proliferation in bronchial epithelial cells, we first tested a selective GPR40 agonist (GW-9508) on cell proliferation. As shown in Fig. 6A, GW-9508 induced BEAS-2B proliferation (1.5 ± 0.06-fold induction, \(n = 3\), \(P < 0.05\)), which was abolished by thapsigargin treatment (0.87 ± 0.14-fold induction, \(n = 3\), \(P < 0.01\)). In addition, we performed RNA silencing to reduce endogenous GPR40 mRNA. Transfection of BEAS-2B cells with human GPR40-specific siRNA resulted in a significant decrease of GPR40 protein expression (60% inhibition) compared with control siRNA transfected cells (Fig. 6B). To know whether RGZ induced a Ca\(^{2+}\) signal in cells via GPR40 receptor in BEAS-2B cells, we performed Ca\(^{2+}\) experiments in cells transfected with GPR40 siRNA. We showed that only 50% of cells transfected with GPR40 siRNA responded to RGZ, probably because of the rate of transfection. Also, in the population of responding cells transfected with GPR40 siRNA, the Ca\(^{2+}\) signal was 50% inhibited compared with cells transfected with control siRNA (\(P < 0.01\); Fig. 6C). As shown in Fig. 6C, cell transfection with siRNA affected Ca\(^{2+}\) responses since we showed a 20%
decrease in cells transfected with control siRNA compared with nontransfected cells. In addition, the proliferative effect observed with both RGZ and TGZ was almost abrogated in cells transfected with GPR40 siRNA compared with cells transfected with control siRNA (Fig. 6, D and E). Indeed, RGZ caused a 1.63 ± 0.12-fold induction of proliferation in cells transfected with siRNA control cells and 1.21 ± 0.12-fold induction in cells transfected with GPR40 siRNA (n = 4, P < 0.05; Fig. 6D). TGZ induced 2.64 ± 0.21-fold cell proliferation in siRNA control transfected cells and 1.67 ± 0.14-fold in GPR40 siRNA transfected cells (n = 4, P < 0.01). Moreover, cell cycle experiments showed that the proliferative index, defined as the total number of cells being in S and G2/M phases, is decreased significantly after RGZ stimulation in cells transfected by GPR40 siRNA compared with cells transfected with control siRNA (34.86 ± 0.34 vs. 41.67 ± 0.34%, P < 0.01; Fig. 6E). These results demonstrated that RGZ- and TGZ-induced Ca2+ release and proliferation were mediated via the recently deorphanized G protein-coupled GPR40 receptor in HBEC.

**DISCUSSION**

In the present study, we have demonstrated for the first time that both TZDs, RGZ and TGZ, stimulate nonmalignant HBEC proliferation. We demonstrated that this effect was not mediated by the nuclear receptor PPARγ but involved the G protein-coupled GPR40 receptor activation, inducing a rapid and transient intracellular Ca2+ mobilization from internal stores. In contrast, the endogenous cyclopentenone prostaglan-
din 15d-PGJ2 did not induce a Ca2+ signal and promoted cell growth arrest via the PPARγ-dependent pathway.

PPARγ ligands such as TZDs and 15d-PGJ2 have been described previously as mediators exhibiting anti-tumoral properties, particularly in lung cancer. Indeed, CGZ and 15d-PGJ2 induced growth arrest of cancer cells through either the induction of apoptosis or the promotion of differentiation (10). In addition, TGZ and PGZ significantly reduced the number of metastasis and restricted nonsmall cell lung cancer tumor progression in vivo (22). These inhibitory effects of TZDs on cancerous cell proliferation are related to PPARγ. In patients with lung cancer, a decrease in PPARγ expression has been associated with a poorer prognosis (33). In contrast, in an animal xenograft model, the overexpression of PPARγ blocked tumor progression (7). Finally, a retrospective study in a male veteran diabetic population showed a 33% reduction in the diagnosis of lung cancer between TZD users compared with nonusers (18). Although antiproliferative effects have been described most frequently, PPARγ ligands mediate a proliferative effect in mesangial cells (31), vascular smooth muscle (35), breast cancer (36), colon cancer (12) and cyclooxygenase-2-depleted cancer cells (13). In accordance with those previous studies, we demonstrate that TZDs induced proliferation of nonmalignant HBEC. In this study, we used a SV40-transformed bronchial epithelial cell line (BEAS-B cells) that is derived from normal lung to investigate the molecular mechanisms underlying TZD-induced proliferation, but the same findings were observed using primary cultures of SAEC (Supplemental Fig. S1) (Supplemental data for this article can be found on the American Journal of...
In addition, performing the cell cycle analysis, we confirmed that RGZ and TGZ induced BEAS-2B proliferation with a significant increase in the number of cells entering in S and G_{2}/M phases. TZDs exhibited opposite actions on the proliferation of cancerous and normal airway epithelial cells. These results provide evidence for a specific cell type-dependent proliferative action of PPAR\(_{\gamma}\) ligands.

Within the past few years, PPAR\(_{\gamma}\)-independent effects for TZDs in different cell types and concerning different functions of PPAR\(_{\gamma}\) ligands have been reported. The evidence for PPAR\(_{\gamma}\)-independent functional effects comes from studies using PPAR\(_{\gamma}\) null cells. TZDs were able to reduce tumor necrosis factor-\(\alpha\) and interleukin-6 release induced by interferon-\(\gamma\) in macrophages derived from PPAR\(_{\gamma}\) null embryonic stem cells (11). In addition, TGZ and CGZ inhibited tumor growth in both PPAR\(_{\gamma}\)^{-/-} and PPAR\(_{\gamma}\)^{+/+} mouse embryonic stem cells (29). Moreover, PPAR\(_{\gamma}\) antagonists were unable to block PPAR\(_{\gamma}\) ligand actions in other studies (1, 30). In the present study, the cellular proliferation induced by TZDs was independent of PPAR\(_{\gamma}\), since a PPAR\(_{\gamma}\) antagonist had no effect. Finally, the rapid occurrence of agonist-induced effects (within min) provides further evidence for a PPAR\(_{\gamma}\)-independent mechanism. Indeed, PPAR\(_{\gamma}\) ligands were able to activate extracellular signal-regulated protein kinase, which occurred rapidly (within 15 min) in smooth muscle (35), human colorectal carcinoma (2), and mesangial (38) cells. It has also been reported that TZDs induced a transient and rapid mobilization of \([Ca^{2+}]_{i}\) (29), which is unusual for a nuclear receptor activation but more compatible for membranous receptor activation, such as a GPCR. Here, TZDs caused an increase in \([Ca^{2+}]_{i}\), by intracellular store emptying that is involved in the cell proliferation induced by these compounds. Elevation of \([Ca^{2+}]_{i}\), as induced by TZDs, may be of particular importance in second messenger signaling pathways and in the regulation of cellular proliferation and gene expression (4).

Recently, TZDs have been reported to bind specifically a membranous GPCR named GPR40 (24), which is highly expressed in the brain and pancreatic \(\beta\)-cells. To a lesser extent, GPR40 is also present in other tissues, including lungs (9). The role of GPR40 receptor has been well described with regard to insulin secretion from pancreatic \(\beta\)-cells (20), but its physiological role in airways has never been investigated. Our study provides the first demonstration for a physiological role of TZDs via the GPR40 receptor in airway epithelial cells. Previously, it has been shown that GW-9508, a selective GPR40 agonist, induced \([Ca^{2+}]_{i}\) mobilization in human embryonic kidney 293 cells expressing GPR40 (8). In our study, we showed that GW-9508 induced BEAS-2B proliferation that was abolished by thapsigargin treatment. We also reported that GPR40 receptor was expressed in normal HBEC and, siRNA-targeting GPR40 experiments showed that this receptor is potentially involved in TZD-induced \(Ca^{2+}\) signal and proliferation. In addition, in A549 cells, which do not express the GPR40 receptor, TZDs did not induce a \(Ca^{2+}\) signal and cell proliferation. It has been documented previously that activation of the GPR40 receptor by the unsaturated free fatty acid olate

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Fig. 7. Schematic diagram of TZDs and 15d-PGJ\(_2\) molecular mechanisms in bronchial epithelial cells. TZDs and 15d-PGJ\(_2\) act through different signaling pathways in normal airway epithelial cells. TZDs activate the membranous G protein-coupled GPR40 receptor and induce a rapid and transient \(Ca^{2+}\) release from thapsigargin-sensitive stores. The \(Ca^{2+}\) signal is necessary for the induction of cell proliferation by TZDs independently of PPAR\(_{\gamma}\) receptor. TZDs probably bind PPAR\(_{\gamma}\) in these cells and may be involved in others functions. In contrast to TZDs, the natural prostaglandin 15d-PGJ\(_{2}\) binds PPAR\(_{\gamma}\) and inhibits bronchial epithelial cell proliferation.
led to a rapid and transient rise in cytosolic Ca\(^{2+}\) and induced proliferation in breast cancer cells (19).

Hence, our data demonstrated that synthetic (TZDs) and natural PPAR\(\gamma\) ligands (15d-PGJ\(_2\)) may use different molecular signaling pathways to mediate their opposite action in nonmalignant HBEC. Indeed, the endogenous PPAR\(\gamma\) ligand 15d-PGJ\(_2\) inhibited cell growth in both tumoral (A459) and nontumoral (BEAS-2B) cell lines that was reversed by a PPAR\(\gamma\) antagonist, suggesting a PPAR\(\gamma\)-dependent pathway. Moreover, we did not detect any Ca\(^{2+}\) signal after 15d-PGJ\(_2\) stimulation, emphasizing that TZDs generate other intracellular signals notably via GPR40 activation in nonmalignant HBEC.

In summary, our findings suggest a new potential signaling pathway activated by TZDs in HBEC (Fig. 7). In nonmalignant HBEC, TZDs may preferentially bind to the G protein-coupled GPR40 receptor, resulting in a rise of [Ca\(^{2+}\)], from depletion of internal stores that would promote cell growth. In contrast, the natural prostaglandin 15d-PGJ\(_2\) binds the nuclear receptor PPAR\(\gamma\) and induces cell growth arrest. Taken together, all of these data provide a novel mechanism of action for TZDs in HBEC stimulating cell proliferation via the GPR40 receptor. Thus GPR40 also plays an important role in the control of cell growth in the lung. Of note, TZDs have been described to have potent anti-inflammatory properties in asthma (3, 27, 37). Because proliferation is important in the healing process of the injured bronchial epithelium, the present evidence suggests that TZDs may interfere with epithelium remodeling via GPR40 activation. Structural changes of the epithelium are important features of chronic airway disease, mostly refractory to current standard therapies. Therefore, anti-diabetic TZD actions may be interesting to be investigated in these chronic and persistent conditions and may give alternative therapeutic strategy for these respiratory diseases.

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