A PPARγ agonist inhibits aldosterone-induced mesangial cell proliferation by blocking ROS-dependent EGFR intracellular signaling

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Yuan Y, Zhang A, Huang S, Ding G, Chen R. A PPARγ agonist inhibits aldosterone-induced mesangial cell proliferation by blocking ROS-dependent EGFR intracellular signaling. Am J Physiol Renal Physiol 300: F393–F402, 2011. First published December 1, 2010; doi:10.1152/ajprenal.00418.2010.—Mesangial cell (MC) proliferation is a key feature in the pathogenesis of a number of renal diseases. Peroxisome proliferator-activated receptor-γ (PPARγ) has attracted considerable attention for its effects on stimulating cell differentiation and on inducing cell cycle arrest. We previously showed that aldosterone (Aldo) stimulates MC proliferation via the phosphoinositide 3-kinase (PI3K)/Akt signaling pathway, which was dependent on reactive oxygen species (ROS)-mediated epithelial growth factor receptor (EGFR) transactivation (Huang S, Zhang A, Ding G, and Chen R. Am J Physiol Renal Physiol 296: F1323–F1333, 2009). In this study, we examined whether the PPARγ agonist rosiglitazone inhibited Aldo-induced MC proliferation by modulating ROS-dependent EGFR intracellular signaling. Rosiglitazone at 1–10 μM dose dependently inhibited Aldo-induced MC proliferation of cultured mouse MCs. The inhibitory effect was blocked by the PPARγ antagonist PD-68235, indicating that the rosiglitazone effect acted through PPARγ activation. Rosiglitazone also arrested Aldo-induced cell cycle progression and suppressed expression of cyclins D1 and A. Moreover, rosiglitazone dose dependently blocked Aldo-induced ROS production, EGFR phosphorylation, and PI3K/Akt activation. These results suggest that the PPARγ agonist rosiglitazone may inhibit Aldo-induced MC proliferation directly, by affecting ROS/EGFR/PI3K/Akt signaling pathways and cell cycle-regulatory proteins. PPARγ might be a novel therapeutic target against glomerular diseases.

peroxisome proliferator-activated receptor-γ; glomerular disease

MESANGIAL CELLS (MCs) are resident cells of the glomerulus that are important for maintaining structure and function. Abnormal growth of glomerular MCs is a predominant histological feature of several glomerular diseases, and growth regulation may influence the outcome of glomerulonephritis (13, 30, 37). Elucidating the mechanisms of MC proliferation may contribute to the development of effective treatment strategies for glomerular diseases. However, the stimulation of MC proliferation in response to glomerular injury has been attributed to multiple factors, hindering our understanding of the molecular basis of this process (23, 24, 28, 43).

Recently, attention has focused on the role of aldosterone (Aldo) in the development and progression of cardiovascular disease and chronic kidney disease (1, 14, 16, 38). As an important mediator of the renin-angiotensin-aldosterone system, plasma and tissue Aldo are elevated in diabetic and other progressive nephropathies and may cause various physiological effects through both genomic and nongenomic mechanisms (4). Increasing evidence suggests that Aldo modulates the growth of renal cells, both in culture and in vivo (53, 54). In particular, Aldo stimulates the proliferation of MCs (40, 49). Our previous study demonstrated that Aldo stimulates MC proliferation via the phosphoinositide 3-kinase (PI3K)/Akt signaling pathway, and this was dependent on reactive oxygen species (ROS)-mediated epithelial growth factor receptor (EGFR) transactivation (23).

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors in the nuclear hormone receptor superfamily and are related to retinoid, glucocorticoid, and thyroid hormone receptors (29). Of the three PPAR subtypes that have been identified, PPARα, PPARγ, and PPARδ (also known as PPARβ), PPARγ is the most widely studied. It regulates a diverse spectrum of physiological processes, including adipogenesis, lipid metabolism, insulin sensitivity, and inflammation, and is involved in diseases such as diabetes, obesity, and atherosclerosis (3). After binding to a ligand, PPARγ heterodimerizes with the retinoid X receptor, forming a complex that recognizes PPAR response elements in the promoters of target genes, leading to transcription regulation (15). PPARγ plays a critical role in adipocyte differentiation and glucose homeostasis and is a regulator of cellular proliferation and inflammatory responses in the kidney (5). PPARγ is widely distributed in human and animal tissue, including MCs and proximal tubular cells (2).

The antiproliferative activity of PPARγ agonists has been observed in a variety of cells (34, 46, 47). PPARγ agonists inhibit MC proliferation in response to VEGF, transforming growth factor-β1, and PDGF (19, 33, 39). The thiazolidinedione (TZD) derivative rosiglitazone is the most potent and selective synthetic PPARγ ligand. It binds to PPARγ with a dissociation constant of ~40 nM and has adiogenic effects on preadipocyte and mesenchymal stem cells in vitro and dramatic antidiabetic effects in vivo (31). Rosiglitazone also inhibits the growth and invasiveness of several kinds of cancer cells (6, 22, 46).

Based on our previous results, we hypothesized that activation of PPARγ might inhibit Aldo-induced MC proliferation. In this report, we tested whether rosiglitazone inhibited MC proliferation by blocking ROS-mediated EGFR activation, leading to blockage of the PI3K/Akt signaling pathway. We also assessed whether rosiglitazone suppressed expression of cyclins D1 and A. The results provide novel insight into the activity and mechanisms of the PPARγ agonist rosiglitazone, in the inhibition of Aldo-induced MC proliferation.
MATERIALS AND METHODS

Reagents and antibodies. Aldo, N-acetyl-L-cysteine (NAC), diphenyleniodonium (DPI), rotenone (Rot), and 2',7'-dichlorofluorescein diacetate (DCFDA) were from Sigma (St. Louis, MO). AG1478 (EGFR antagonist), LY 294002 (PI3K inhibitor), and Akt inhibitor were from Calbiochem (Cambridge, MA). Antibodies against total EGFR, phospho-EGFR, total PI3K, phospho-PI3K, total Akt, and phospho-Akt were from Cell Signaling Technology (Beverly, MA). Secondary horseradish peroxidase-conjugated antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). All other reagents were from Sigma.

MC culture. Primary mouse MCs were established and characterized as previously reported (45). MCs were grown in RPMI 1640 medium supplemented with 10% fetal calf serum, 5 μg/ml insulin and transferrin, 100 U/ml penicillin, and 100 mg/ml streptomycin. For passage, confluent cells were washed with PBS, removed with 0.025% trypsin/0.5 mM EDTA in PBS, and plated in RPMI 1640.

Transient transfection of MCs with EGFR small interfering RNA. For the small interfering (si) RNA assay, MCs grown to 40% confluence in six-well plates were transiently transfected with EGFR-specific siRNA or control siRNA, a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA from Santa Cruz Biotechnology (Santa Cruz, CA). All other reagents were from Sigma.

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Fig. 1. Effects of rosiglitazone on aldosterone (Aldo)-induced mouse mesangial cell (MC) proliferation. Mouse MC proliferation was evaluated by [3H]thymidine incorporation (A and C) and cell counting (B and D). A and B: MCs were pretreated with rosiglitazone (RGZ) at indicated doses for 30 min before stimulation with Aldo at 100 nM for 24 h. C and D: effects of peroxisome proliferator-activated receptor-γ (PPAR-γ) antagonist PD-68235 (PD) on proliferation. MCs were pretreated with PD-68235 (10 μM) and rosiglitazone (10 μM), or rosiglitazone alone for 30 min followed by Aldo (100 nM) for 24 h. Values are means ± SE; n = 6. *P < 0.05, ΔP < 0.01 vs. Aldo-treated group. #P < 0.01 vs. Aldo plus rosiglitazone treatment group by ANOVA.

Fig. 2. Effects of rosiglitazone on Aldo-induced cell cycle progression. Flow cytometry determined the percentage of cells at each cell cycle phase. MCs were pretreated with PD-68235 (10 μM) and rosiglitazone (10 μM) or rosiglitazone alone for 30 min followed by incubation with Aldo (100 nM) for 24 h. A: percentage of cells at G0/G1. B: percentage of cells at S. C: percentage of cells at G2/M. D: percentages of cells at S and G2/M. Values are means ± SE; n = 6. ΔP < 0.01 vs. Aldo-treated group. #P < 0.01 vs. Aldo plus rosiglitazone treatment group by ANOVA.
DNA synthesis and cell count. To estimate DNA synthesis, MCs were stimulated by the indicated agents for 19 h, pulsed with 1 μCi/ml [3H]thymidine for 5 h, washed twice with ice-cold PBS, incubated for 5 min in 5% TCA, washed with methanol, and dissolved in 99% formic acid. [3H]thymidine incorporation into TCA-insoluble material was measured with a liquid scintillation spectrophotometer. To assess cell growth, MCs in six-well plates were stimulated by the indicated agents, and the cell number was counted with a Z1-Coulter Counter (Luton, UK).

Flow cytometry. At the indicated time points, MCs were harvested by trypsinization, fixed in ice-cold 70% ethanol and suspended in 1 ml propidium iodide staining solution (50 μg/ml propidium iodide, 30 U/ml RNase A, 0.1% Triton X-100, 4 mM sodium citrate) and incubated at 37°C for 10 min before addition of NaCl at a final concentration of 138 mM NaCl. Typically, 10,000 gated events were collected on a FACscan machine (Beckton Dickinson, Franklin Lakes, NJ) and analyzed by CELLQUEST software (Beckton Dickinson). Cell cycle analysis used Flowjo Software (Tree Star, San Carlos, CA).

Real-time RT-PCR. Total RNA was isolated from MCs using a TRIzol Total RNA Isolation kit (Invitrogen) according to the manufacturer’s protocol. RNA was eluted with RNase-free water. RT used a Superscript III RT Kit (Invitrogen) according to the manufacturer’s protocol. Briefly, reactions were incubated at 65°C for 5 min, then 50°C for 60 min. Oligonucleotides (cyclin D1: forward 5’-AGT CAG GGC ACC TGG ATT GTT C-3’; reverse 5’-AAC AGA TTA AAT GAT GCA CCG GAG A-3’; cyclin A: forward 5’-GCT CAA GAC TCG ACG GGT TGC-3’; reverse 5’-GCT GCA TTA AAA GCC AGG GCA TC-3’; GAPDH: forward 5’-GTC TTC ACT ACC ATG GAG AAG G-3’; reverse 5’-TCA TGG ATG ACC TTG GCC AG-3’) were designed by Primer3 software (http://frodo.wi.mit.edu/primer3), and synthesized by Invitrogen. Real-time PCR amplification used SYBR Green master mix (Applied Biosystems) and the Prism 7500

Fig. 3. Effects of rosiglitazone on Aldo-induced cyclin D1 and cyclin A expression. MCs were pretreated with rosiglitazone at the indicated doses for 30 min before the addition of Aldo at 100 nM. A and B: real-time RT-PCR analysis for cyclins D1 and A. Cells were treated with Aldo for 12 h, and cyclin D1 (A) and cyclin A (B) mRNA expression was detected. mRNA fold-changes were calculated using GAPDH as a control. C–E: Western blots for cyclins D1 and A. Cells were treated with Aldo for 24 h and cyclin D1 (C) and cyclin A (C and E) protein was detected by immunoblotting using β-actin as the loading control. Values are means ± SE from 3 independent experiments. *P < 0.05, ΔP < 0.01 vs. Aldo-treated group by ANOVA.
Real Time PCR Detection System (Applied Biosystems). Cycling conditions were 95°C for 10 min followed by 40 repeats of 95°C for 15 s and 60°C for 1 min. Relative amounts of mRNA were normalized to GAPDH and calculated using the ΔΔ method from threshold cycle numbers (7).

Western blot analysis. At the indicated time points, MCs were rapidly washed with ice-cold PBS and lysed for 10 min on ice in lysis buffer (50 mM Tris, pH 7.5, 40 mM NaCl, 1% Triton X-100, 2 mM EDTA, 1 μg/ml leupeptin, 2 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride). Lysates were cleared by centrifugation at 14,000 g for 10 min at 4°C. Total protein was quantified by a Bradford assay. Equal amounts of lysates were separated on a 10% polyacrylamide gel, and electrotransferred to Bio-Blot nitrocellulose membranes (Bio-Rad). Membranes were blocked in TBST (20 mM Tris-base, pH 7.6, 150 mM NaCl, 0.1% Tween 20) containing 5% bovine serum albumin for 1 h at room temperature and incubated with primary antibody in blocking solution at 4°C overnight. Membranes were incubated with 1:1,000 diluted horseradish peroxidase-conjugated secondary antibody at room temperature for 1 h and visualized by an enhanced chemiluminescence kit (Amer sham). The chemiluminescent signal was quantified using UVP software (UVP, Upland, CA).

DCFDA fluorescence measurement of ROS. The fluorogenic substrate DCFDA is a cell-permeable dye that is oxidized to highly fluorescent 2',7'-dichlorofluorescein (DCF) by H2O2 and is used to monitor intracellular ROS generation. To quantitate ROS levels, MCs were seeded in 96-well plates. At confluence, cells were washed twice with PBS, incubated for 30 min with 10 μM DCFDA and then treated as indicated. After incubation, cells were washed twice with PBS and relative fluorescence was measured using a fluorescence plate reader (FLUOstar OPTIMA) at 485-nm excitation and 528-nm emission, three times at 90-s intervals.

PI3K assay. Cell lysates were immunoprecipitated with the anti-p85 antibody. A PI3K assay was performed on immunoprecipitates as described previously (56). Briefly, the immunoprecipitates were incubated for 10 min at room temperature with phosphatidylinositol (PI; 0.2 mg/ml) and 50 μmol/l γ-[32P]ATP (10 μCi/point). The kinase assay was stopped by the addition of 80 μl of HCl (1 mol/l) and 160 μl of methanol:chloroform (1:1 mixture). After centrifugation, the upper phase containing the lipids was analyzed. The phospholipids were separated by thin layer chromatography and analyzed by autoradiography. The cell lysates were immunoblotted with anti-p85 antibodies as a loading control.

Statistical analysis. Data are presented as means ± SE. Statistical analysis was by one-way ANOVA and Bonferroni tests, with P < 0.05 considered significant.

RESULTS

Rosiglitazone inhibits Aldo-induced mouse MC proliferation. MC proliferation was evaluated using [3H]thymidine incorporation and by cell counting (Fig. 1). Growth was induced with 100 nM Aldo for 24 h and was significantly, dose dependently suppressed by the PPARγ agonist rosiglitazone (Fig. 1, A and B). To verify the involvement of PPARγ in rosiglitazone inhibition, MCs were pretreated with the specific PPARγ antagonist PD-68235 (10 μM) for 30 min. As shown in Fig. 1, C and D, pretreatment with the PPARγ antagonist abrogated the inhibitory effect of rosiglitazone on Aldo-induced cell growth.

Rosiglitazone arrests Aldo-induced cell cycle progression. Flow cytometry analysis was used to investigate the effect of rosiglitazone on the cell cycle progression stimulated by Aldo (Fig. 2). Aldo treatment induced a 27% increase in S- and G2/M-phase cells compared with the controls, in which >90%
of cells were in G0/G1 phase. Rosiglitazone pretreatment increased the G0/G1-phase cells, and decreased the S- and G2/M-phase cells, suggesting that rosiglitazone suppressed Aldo-induced cell cycle progression by inhibiting the G1-to-S phase transition and arresting cells at G0/G1. In addition, the PPARγ antagonist PD-68235 blocked the rosiglitazone effects on cell cycle progression.

Rosiglitazone suppresses Aldo-induced expressions of cyclin D1 and cyclin A. We tested the expression of cyclins D1 and A in MCs using real-time RT-PCR (Fig. 3, A and B) and Western blot analysis (Fig. 3, C–E). The results indicated that the cyclin D1 and cyclin A expression that was induced by Aldo was significantly and dose dependently inhibited at both the transcriptional and translational levels by rosiglitazone.

Rosiglitazone prevents Aldo-induced activation of PI3K/Akt/mammalian target of rapamycin/p70S6K1 signaling pathway. We previously reported that the PI3K/Akt/mammalian target of the rapamycin (mTOR)/p70S6K1 signaling pathway is activated during Aldo-induced MC proliferation (23). Therefore, we tested the activation of the PI3K/Akt pathway with three different doses of Aldo. Using a PI3K assay and Western blotting to detect PI3K activity and PI3K and Akt phosphorylation, we found that 1 nM Aldo significantly increased the relative levels of phosphor-PI3K and PI3K activity and phosphor-Akt, and the effect became more notable at higher doses (Fig. 4). We examined whether rosiglitazone blocked these phosphorylations. As shown in Fig. 5, rosiglitazone dose dependently reduced PI3K, Akt, mTOR, and p70S6K1 phosphorylation, and 10 μM rosiglitazone almost completely blocked the activation of the Aldo-induced PI3K/Akt/mTOR/p70S6K1 signal pathway.

Rosiglitazone blocks Aldo-induced phosphorylation of EGFR. Our previous study indicated that signaling downstream of EGFR transactivation is also involved in proliferation in response to Aldo (23). Therefore, we evaluated the effect of rosiglitazone on EGFR tyrosine phosphorylation. EGFR phosphorylation was blocked by rosiglitazone in a dose-dependent manner, and 10 μM rosiglitazone almost entirely blocked generation of phosphor-EGFR (Fig. 6A). The rosiglitazone effect on EGFR was abrogated by the PPARγ antagonist PD-68235 (Fig. 6B).

Rosiglitazone blocks Aldo-induced production of ROS. EGFR phosphorylation is mediated by ROS production originated from NAD(P)H oxidase and mitochondrial respiratory...
chain (23, 36, 41). We first tested the effect of NAD(P)H oxidase and mitochondrial respiratory chain inhibitors on Aldo-induced EGFR activation. As shown in Fig. 6C, DPI, inhibitor of NAD(P)H oxidase, and Rot, inhibitor of mitochondrial respiratory chain complex I, significantly inhibited Aldo-induced EGFR transactivation. We next examined whether rosiglitazone would block Aldo-induced ROS production. To test this hypothesis, MCs were stained with DCFDA, and relative fluorescence was determined (Fig. 7). ROS generation occurred after 60 min of Aldo treatment and was inhibited by rosiglitazone in a dose-dependent manner. Figure 7B shows that the ROS production that was blocked by rosiglitazone was restored by pretreatment with a PPARγ antagonist.

**Inhibitors of ROS-dependent EGFR signaling block Aldo induction of cyclins D1 and A.** Mouse MCs were pretreated with the antioxidant NAC, the EGFR inhibitor AG1478, the PI3K inhibitor LY 294002, or an Akt inhibitor. Aldo induction of cyclins D1 and A was reduced by the inhibitors (Fig. 8). To conform the specificity of the inhibitors, we transfected MCs with vehicle or EGFR siRNA and detected cyclins D1 and A expression and cell proliferation. As shown in Fig. 9, the expression of EGFR protein was inhibited by 80% after exposure to 500 nM EGFR siRNA. When MCs were treated with Aldo in the presence of vehicle siRNA, expression of cyclins D1 and A was increased significantly, and this effect was inhibited significantly in EGFR siRNA-transfected cells. Next, under the same conditions, we measured cell proliferation in response to Aldo. The effects of Aldo on MC proliferation were significantly inhibited, indicating that the ROS-dependent EGFR intracellular signaling pathway was involved in the regulation of cell proliferation. This also demonstrated that rosiglitazone might have suppressed MC proliferation by targeting this pathway.

**DISCUSSION**

In this study, we demonstrated that the antiproliferative effect of rosiglitazone was mediated by PPARγ and inhibition of MC growth occurred through blocking the PI3K/Akt pathway at a point downstream of ROS-dependent EGFR intracellular signaling. Rosiglitazone inhibited Aldo-induced MC proliferation in a dose-dependent manner.
Cell proliferation is ultimately regulated at the level of cell cycle progression through the four stages of G1, S, G2, and M, with important checkpoints in G1 and G2. The G1 checkpoint allows confirmation of a favorable environment for cell proliferation and intact DNA before committing to the S phase. The G2 checkpoint ensures that cells do not enter mitosis until damaged DNA is repaired and DNA replication is complete (25). Our flow cytometry data showed that rosiglitazone increased the proportion of G1 cells, and reduced the proportion of S and G2/M cells, suggesting that rosiglitazone inhibited MC growth by inducing of G1 arrest. This supports findings that rosiglitazone induces growth inhibition in other types of growing cells by G1 arrest (32, 55).

Cell cycle-regulatory proteins guide the transition through cell cycle phases (50), so we analyzed the molecular mechanism by which rosiglitazone causes cell cycle arrest by evaluating changes in levels of these proteins. Cyclin D1 controls cell cycle progression through the G1 phase and the G1-to-S transition (42), and cyclin A-associated kinase activity is required for entry into S, completion of S, and entry into M (20). Levels of cyclins D1 and A were high after Aldo treatment. Rosiglitazone significantly and dose dependently reduced the expression of these proteins, suggesting that rosiglitazone inhibited cell growth and induced cell cycle arrest by inhibiting cyclin D1 and A expression, consistent with previous reports (49).

Our previous study demonstrated that the ROS-EGFR-PI3K-Akt pathway transduces the Aldo-induced proliferation signal in cultured MCs (23). ROS are now known to be signaling molecules in a wide variety of physiological and pathological processes, including proliferation, differentiation, apoptosis, and migration (12). Increasing evidence suggests that many proliferative responses, in various cell types, are dependent on ROS generated by oxidant-generating systems after growth factor stimulation (35). These systems include NADPH oxidase, the mitochondrial respiratory chain, xanthine oxidase, cyclooxygenase, lipooxygenase, cytochrome P-450, and nitric oxide synthase. Glomerular ROS are elevated over the entire course of 5 days in anti-Thy 1.1 mesangioproliferative glomerulonephritis (17). In cultured MCs, ROS production triggered by oxidized LDL promotes cell cycle progression (10). Furthermore, we found that ROS generated by NADPH oxidase mediated angiotensin II-induced human MC proliferation (11). Our findings demonstrated that rosiglitazone dose dependently inhibited ROS production, suggesting that this is how it inhibited MC proliferation. Our results correspond to reports showing that PPARγ agonists suppress agent-induced ROS generation (21, 27).

EGFR appears to be the molecular target of ROS in the Aldo signaling pathway of cultured MCs. Inhibition of EGFR pathways in cancer cells blocks cell cycle progression (Fig. 8).
sion, apoptosis, and angiogenesis (9). Blocking EGFR activation might contribute to rosiglitazone inhibition of MC growth. We found that rosiglitazone blocked EGFR phosphorylation. The PI3K/Akt pathway is implicated in both cell proliferation and survival (48) and is activated by EGFR. PI3K activity is important for G1-to-S transition (44), and Akt regulates a PDGF-induced decrease in p27kip1, a nuclear protein that inhibits both the G1 and S phase (8). In cultured rat glomerular MCs, glial cell-derived neurotrophic factor-induced MC proliferation was associated with dissociation of p27kip1, which was regulated by PI3K (26). Our data showed that PI3K and Akt inhibitors reduced expression of cyclins D1 and A and found that rosiglitazone blocked PI3K/Akt/mTOR/p70S6K1 signaling activation in a dose-dependent manner. These results suggested that rosiglitazone inhibited cell growth via the PI3K/Akt/mTOR/p70S6K1 pathway.

The PPARγ antagonist PD-68235 abolished the effects of rosiglitazone on MC growth and cell cycle arrest, suggesting that PPARγ at least partially mediated the rosiglitazone effects. Ghosh et al. (19) found that TZDs blocked MC proliferation and affected cell cycle regulation by acting on PPARγ. Another study showed that rosiglitazone prevented the high glucose effects on MC signaling and gene expression through PPARγ. These results suggest that the antiproliferative effects of rosiglitazone are mediated via PPARγ (52). However, growing evidence suggests that TZDs may produce pharmacological effects independently of PPARγ.

PPARγ-independent signals that mediate rosiglitazone effects have been demonstrated (18, 22, 51). Han et al. (22) found that rosiglitazone’s effects on phosphorylation of AMPK and p70S6K are not blocked by a PPARγ antagonist. Galli et al. (18) showed that rosiglitazone inhibits the invasiveness of pancreatic cancer cells by PPARγ-independent mechanisms. At present, the PPARγ-independent pathways activated by rosiglitazone and ciglitazone have not been fully defined, and future studies are needed to reveal the PPARγ-independent pathways that mediate the effects of PPARγ agonists.

In conclusion, our results demonstrated that rosiglitazone inhibited Aldo-induced MC proliferation through PPARγ-dependent signals that blocked the ROS-EGFR-PI3K-Akt pathway. Our results provide novel insights into the activity and mechanisms of rosiglitazone inhibition of Aldo-induced MC proliferation and suggest potential therapeutic strategies for treatment of glomeruloproliferative diseases.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.
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