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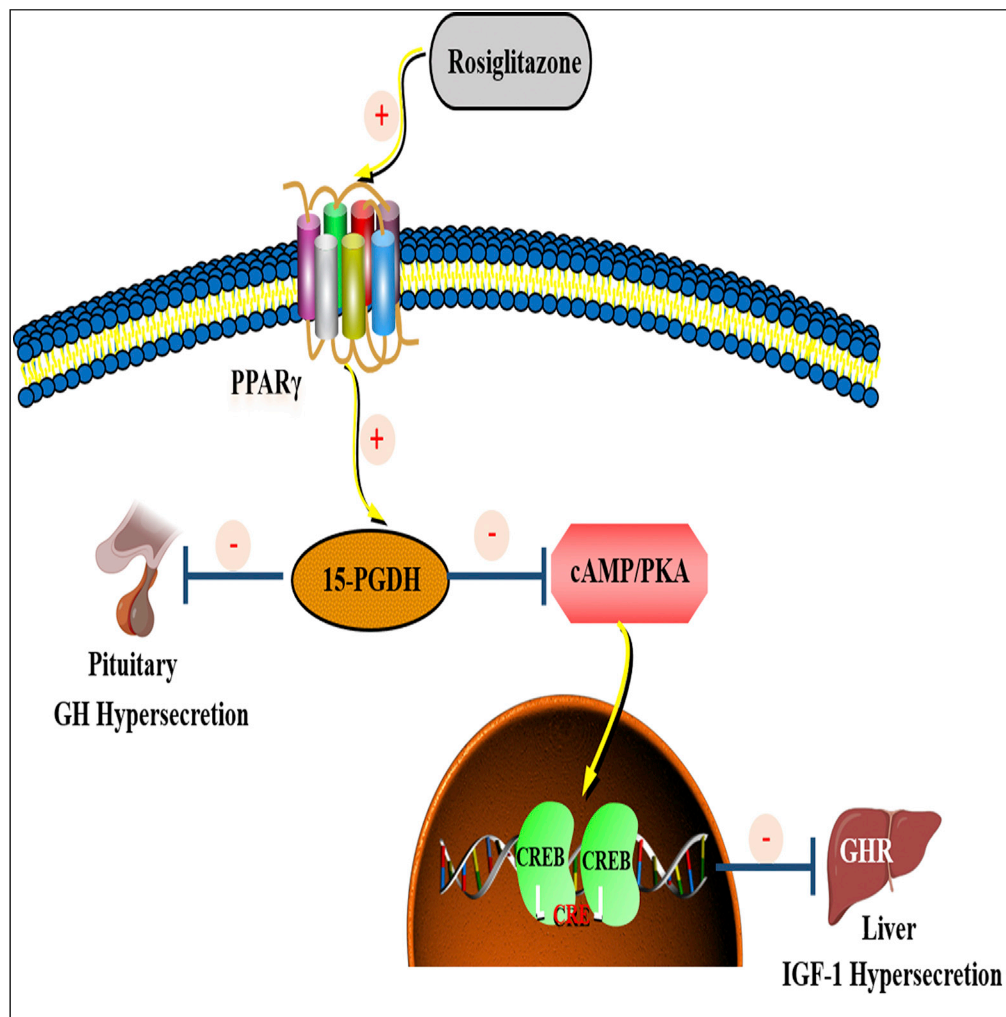

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Highlights

Rosiglitazone reduces GH secretion in GH3 cells via upregulating 15-PGDH expression

Rosiglitazone suppresses IGF-1 secretion in HepG2 cells relied on 15-PGDH expression

Rosiglitazone inhibits tumor growth in mice inoculated subcutaneously with GH3 cells

Rosiglitazone reduces GH and IGF-1 levels in all 19 patients with active acromegaly

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Treatment of acromegaly by rosiglitazone via upregulating 15-PGDH in both pituitary adenoma and liver

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SUMMARY

Rosiglitazone, a synthetic peroxisome proliferator-activated receptor γ (PPARγ) ligand, has been reported to reduce growth hormone (GH) and insulin-like growth factor-1 (IGF-1) in 10 patients with acromegaly. However, the mechanisms remain unknown. Here, we reveal that PPARγ directly enhances 15-hydroxyprostaglandin dehydrogenase (15-PGDH) expression, whose expression is decreased and negatively correlates with tumor size in acromegaly. Rosiglitazone decreases GH production and promotes apoptosis and autophagy in GH3 and primary somatotroph adenoma cells and suppresses hepatic GH receptor (GHR) expression and IGF-1 secretion in HepG2 cells. Activating the PGE2/cAMP/PKA pathway directly increases GHR expression. Rosiglitazone suppresses tumor growth and decreases GH and IGF-1 levels in mice inoculated subcutaneously with GH3 cells. The above effects are all dependent on 15-PGDH expression. Rosiglitazone as monotherapy effectively decreases GH and IGF-1 levels in all nineteen patients with active acromegaly. Evidence suggests that rosiglitazone may be an alternative pharmacological approach for acromegaly by targeting both pituitary adenomas and liver.

INTRODUCTION

Peroxisome proliferator-activated receptor γ (PPARγ), a nuclear receptor involved in adipogenesis and adipose tissue development, governs the expression of genes involved in inflammation, redox equilibrium, trophic factor production, insulin sensitivity, and the metabolism of lipids and glucose (Augimeri et al., 2020; Yousefnia et al., 2018). Synthetic PPARγ agonists (e.g. rosiglitazone) are used to treat type II diabetes. Over the last few years, PPARγ has received much attention for its ability to exert tumor suppressive effects in different types of cancer, such as breast carcinoma, lung cancer, bladder cancer, and leukemia by triggering the proliferation, apoptosis, metastasis, and angiogenesis of cancer cells (Ching et al., 2015; Yousefnia et al., 2018). PPARγ agonists including thiazolidinedione (TZD) are mainly used as therapeutic components such as anti-diabetic drugs and anticancer reagents.

Acromegaly is a rare endocrinopathy, which is prominently caused by growth hormone (GH)-secreting pituitary adenomas with excess levels of GH and insulin-like growth factor-1 (IGF-1) (Dekkers et al., 2008; Katznelson et al., 2014). Without treatment, the mortality rate of patients with acromegaly is twice that of the normal population, and life expectancy is, on average, shortened by 10 years (Sherlock et al., 2011). Current treatment options for acromegaly include surgery, medical therapy, and radiation. As the first-line treatment, surgery can completely remove 90% of microadenomas but only 50% of macroadenomas (Csaba and Dournaud, 2001; Duran-Prado et al., 2009). Somatostatin analogs (SSAs) are the first-line medications, but they normalize both GH and IGF-1 levels in only about 25%–70% patients, depending on different case series (Carmichael et al., 2014; Colao et al., 2016). Dopamine agonists are effective at controlling IGF-1 levels in only a minority of mildly affected patients (Howlett et al., 2013). Pegvisomant (PEG), a GH receptor antagonist, is mostly used as an add-on or second-line therapy due to the need for frequent injections, lack of availability at many centers, high cost, and concern about the potential for tumor growth, though reports are reassuring in this regard. Radiation therapy is considered as the

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third-line option because of the long period of time to achieve biochemical control and concern about adverse effects (Colao et al., 2019).

Noteworthy, GH is produced by the pituitary gland while circulating IGF-1 is mainly secreted from the liver in response to GH stimulation. Currently, most drugs target the pituitary adenomas themselves, except for PEG. As mentioned above, patients partially responsive to SSAs are sometimes prescribed PEG as adjuvant therapy to normalize both GH and IGF-1, while patients resistant to SSAs may switch to PEG monotherapy. Therefore, a medication which targets the pituitary adenoma and the liver would be ideal to treat those patients.

Strikingly, three case reports have shown that rosiglitazone belonging to TZD family decreases both GH and IGF-1 levels in 10 patients with acromegaly, while pioglitazone has no effect, indicating the potential therapeutical effect of rosiglitazone on acromegaly (Bogazzi et al., 2011; Gradiser et al., 2007; Tamez-Perez et al., 2011). Additionally, *in vitro* experiments confirmed that rosiglitazone decreased the secretion of GH by GH3 cells (Bogazzi et al., 2004). Given the limited sample size in previous research, additional longitudinal studies will need to be done to determine the clinical effects of rosiglitazone on acromegaly. Significantly, to date, the mechanisms responsible for the inhibiting effects on GH and IGF-1 have not been illuminated.

In the present study, we showed that 15-hydroxyprostaglandin dehydrogenase (15-PGDH) was the common molecular target for rosiglitazone in both pituitary adenomas and liver, and further, rosiglitazone significantly decreased GH and IGF-1 in patients with active acromegaly.

RESULTS

Rosiglitazone promotes 15-PGDH transcription via PPARγ

To define the mechanism underlying rosiglitazone suppression of GH and IGF-1 production, we first explore the potential target of rosiglitazone. Previous study showing that rosiglitazone treatment upregulated 15-PGDH protein expression in lung cancer cell lines (Hazra et al., 2007). 15-PGDH has been implicated as a tumor suppressor gene with the property that inhibits the growth of several tumor types (Basudhar et al., 2017; Huang et al., 2008; Kim et al., 2017; Pham et al., 2010; Tseng-Rogenski et al., 2010). However, the potential role of 15-PGDH in pituitary somatotroph tumors has never been revealed. Accordingly, we suspected that the suppressive role of rosiglitazone on acromegaly may be associated with 15-PGDH.

First, we investigate the interaction between rosiglitazone and 15-PGDH. We screened the human 15-PGDH promoter and obtained several potential PPARγ-binding sites (Figure 1A). As shown in Figure 1A, three primers, including primer 1 (-3001/-2709), primer 2 (-1966/-1692), and primer 3 (-270/-10), were constructed to verify the major PPARγ binding sites on the promoter of 15-PGDH. Chromatin immunoprecipitation was performed to examine whether PPARγ directly bound to the 15-PGDH promoter. As shown in Figure 1B, anti-PPARγ-immunoprecipitated DNA with an enriched 15-PGDH locus was strongly amplified by all the three primers.

Next, dual luciferase reporter assays were employed to measure the 15-PGDH promoter activity at the presence of PPARγ. HEK293t cells (a commonly used cell line for transfection experiments) were transfected with human 15-PGDH promoter -3001/-10, -3001/-2709 mutation (mut), -1966/-1692 mut, or -270/-10 mut with or without rosiglitazone. The plasmid pGL4.10 was used as the control. As shown in Figure 1C, rosiglitazone significantly increased the luciferase activity in cells transfected with promoter -3001/-10. However, the luciferase activity was much lower in cells transfected with either promoter -3001/-2709 mut or -270/-10 mut compared to the cells which were transfected with promoter -3001/-10. The luciferase activity was not significantly different between the cells transfected with promoter -1966/-1692 mut and -3001/-10. This indicated that the promoters -3001/-2709 and -270/-10 were the major PPARγ binding sites.

A specific PPARγ inhibitor, GW9662, was used to suppress endogenous PPARγ expression. HEK293t cells were transfected with 15-PGDH promoter -3001/-10 and treated with increasing doses of GW9662 (0, 1, 10, and 50 μM) (Figure 1D). GW9662 significantly suppressed the luciferase activity. Additionally, HEK293t cells were transfected with promoter -3001/-10, -3001/-2709 mut, -1966/-1692 mut, or -270/-10 mut in the presence of GW9662 (50 μM). Notably, compared to cells transfected with promoter -3001/-10, those transfected with -270/-10 mut showed higher luciferase activity, indicating that promoter -270/-10 was the most important PPARγ binding site (Figure 1E).

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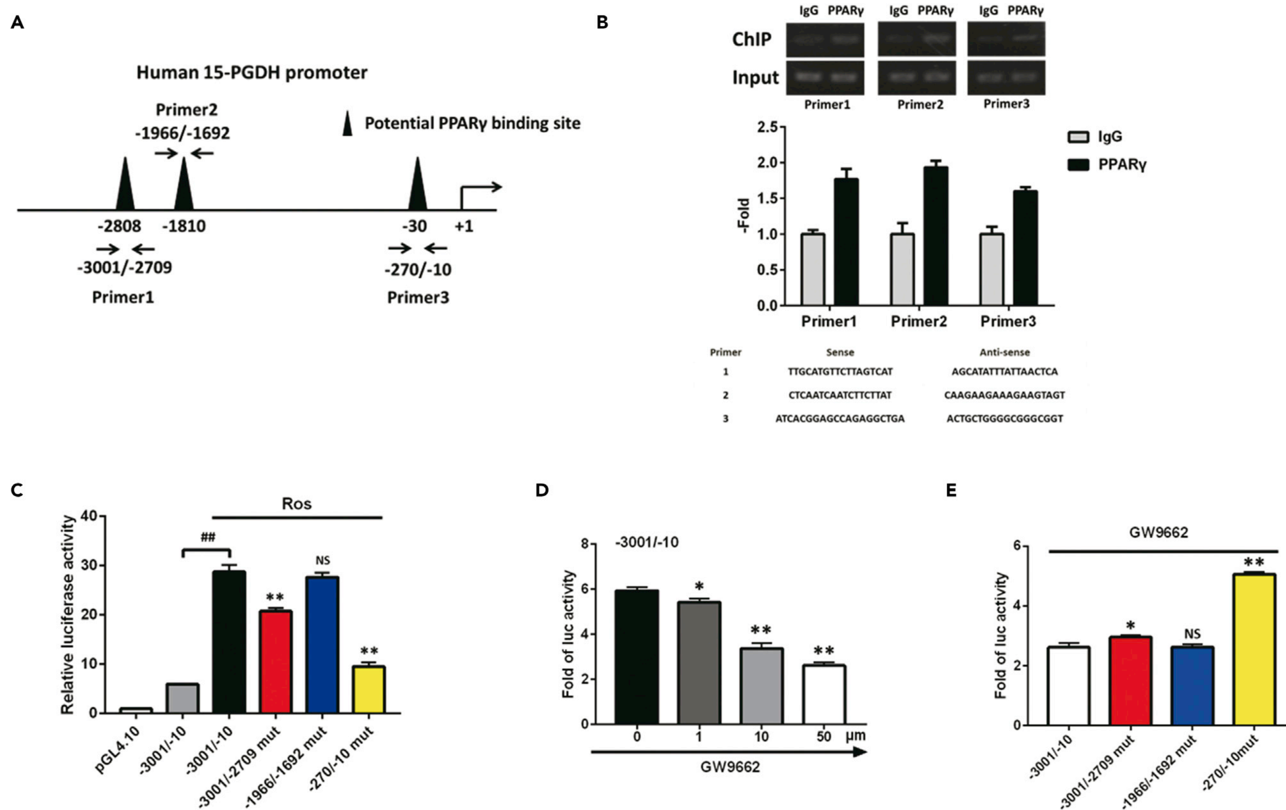

Figure 1. Rosiglitazone promotes 15-PGDH transcription via PPARγ

(A) Three PPARγ-binding motifs on the promoter of human 15-PGDH were predicted, and the primers were designed targeting 3 motifs (-3001/-2709, -1966/-1692, -270/-10), including primer 1, primer 2, and primer 3, to verify the major PPARγ binding sites on the promoter of 15-PGDH.

(B) PPARγ binds to 15-PGDH promoter. HEK293t cells were transfected with the promoters of human 15-PGDH, including primer 1, primer 2, and primer 3, respectively. Anti-PPARγ-immunoprecipitated DNA with an enriched 15-PGDH locus was strongly amplified by all the three primers.

(C) The promoters -3001/-2709 and -270/-10 of 15-PGDH were the major PPARγ binding sites. HEK293t cells were transfected with human 15-PGDH promoter -3001/-10, Ros + human 15-PGDH promoter -3001/-10, Ros + human 15-PGDH promoter -3001/-2709 mutation (mut), Ros + human 15-PGDH promoter -1966/-1692 mut, or Ros + human 15-PGDH promoter -270/-10 mut. The plasmid pGL4.10 was used as control. Data were represented as mean ± SD. ## denotes $p < 0.01$, ** denotes $p < 0.01$ versus Ros + -3001/-10 group.

(D) GW9662 significantly suppressed the luciferase activity. HEK293t cells were transfected with 15-PGDH promoter -3001/-10 and treated with increasing doses of GW9662 (0, 1, 10, and 50 μM). Data were represented as mean ± SD. * denotes $p < 0.05$, ** denotes $p < 0.01$ versus GW9662 (0 μM).

(E) The site -270/-10 in 15-PGDH is the most important site for PPARγ binding. HEK293t cells were transfected with promoter -3001/-10, -3001/-2709 mut, -1966/-1692 mut, or -270/-10 mut in the presence of GW9662 (50 μM). Data were represented as mean ± SD. * denotes $p < 0.05$, ** denotes $p < 0.01$ versus GW9662 +3001/-10.

The expression of 15-PGDH is decreased in pituitary somatotroph adenomas and is negatively correlated with adenoma volume in patients with acromegaly

Then, we examined the expression of 15-PGDH in normal pituitary tissue (n = 13) and pituitary somatotroph adenomas (n = 89) using immunohistochemical (IHC) staining. Compared to normal pituitary tissue, the expression of 15-PGDH in the pituitary somatotroph adenomas was significantly lower (Figure 2A). Additionally, mRNA levels of 15-PGDH were also lower in pituitary somatotroph adenomas than those in normal pituitary tissues (Figure 2B). Interestingly, compared to microadenomas, the expression of 15-PGDH in macroadenomas was even lower (Figure 2C). Furthermore, the expression of 15-PGDH evaluated with the IHC score was negatively correlated with the tumor volume ($R = -0.618$, $p < 0.05$, Figure 2D).

Rosiglitazone reduces GH secretion in somatotroph pituitary adenoma by upregulating 15-PGDH expression

To investigate the direct effects of rosiglitazone on GH secretion of the pituitary adenomas, GH3 cells were treated with 1, 10, or 50 μM rosiglitazone for 48 hr and 72 hr. Both GH secretion and GH mRNA levels were

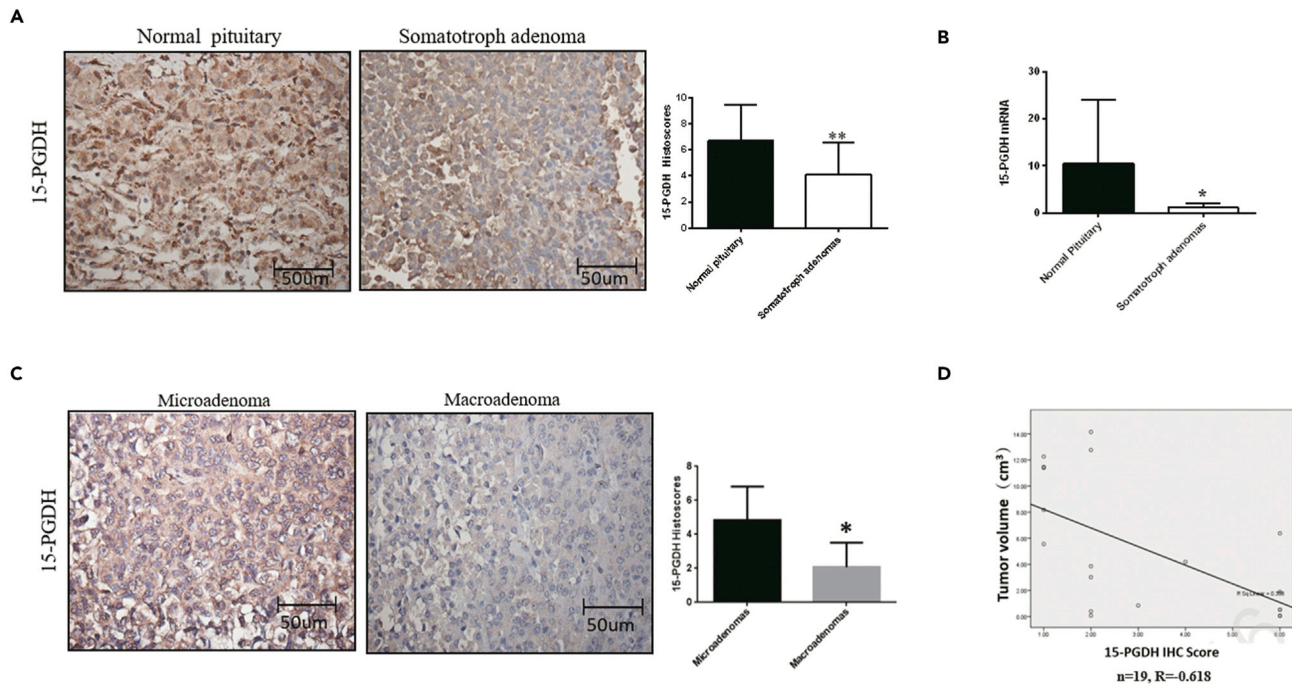

Figure 2. The expression of 15-PGDH is decreased in pituitary somatotroph adenomas and is negatively correlated with adenoma volume in patients with active acromegaly

(A) Compared to normal pituitary tissue, the expression of 15-PGDH in the pituitary somatotroph adenomas was significantly lower. The expression of 15-PGDH was examined by immunohistochemical (IHC) staining in normal pituitary tissue (n = 13) and pituitary somatotroph adenomas (n = 89). Data were represented as mean $\pm$ SD. Scale bar represents 50 μm. ** denotes $p < 0.01$ versus normal pituitary tissue.

(B) mRNA levels of 15-PGDH were lower in pituitary somatotroph adenomas than those in normal pituitary tissues. 15-PGDH mRNA level was evaluated with RT-PCR. Data were represented as mean $\pm$ SD. * denotes $p < 0.05$ versus normal pituitary tissue.

(C) The expression of 15-PGDH was lower in somatotroph macroadenomas compared to somatotroph microadenomas. The expression of 15-PGDH was examined by immunohistochemical (IHC) staining in somatotroph macroadenomas (n = 13) and somatotroph microadenomas (n = 6). Data were represented as mean $\pm$ SD. Scale bar represents 50 μm. * denotes $p < 0.05$ versus somatotroph microadenomas.

(D) 15-PGDH expression was negatively ($R = -0.618$, $p < 0.05$) correlated with the tumor volume of pituitary somatotroph adenomas (n = 19).

significantly decreased by rosiglitazone in a dose-dependent manner, and rosiglitazone (50 μM) could significantly reduce GH secretion and GH mRNA levels in GH3 cells (Figure 3A). However, pioglitazone (50 μM) had no such effect on GH secretion and mRNA levels (Figure 3A). Next, we examined the effects of rosiglitazone on 15-PGDH mRNA and protein levels and found that rosiglitazone, especially at dose of 50 μM, prominently upregulated the mRNA and protein expression of 15-PGDH in GH3 cells (Figure 3B). Similar results were obtained in the primary cultured cells of human GH-secreting pituitary adenomas (n = 5): rosiglitazone decreased GH levels and increased mRNA levels of 15-PGDH in a dose-dependent manner, and these effects were more noteworthy at rosiglitazone (50 μM) (Figure 3C). To examine whether 15-PGDH mediated the effect of rosiglitazone on GH3 cells, 15-PGDH was either knocked down or overexpressed in GH3 cells (Figure 3D). We found that compared to cells treated with rosiglitazone alone, knockdown of 15-PGDH significantly increased the GH mRNA levels, while overexpressing 15-PGDH significantly decreased GH mRNA levels in the cells (Figure 3E). Together, these findings suggest that rosiglitazone decreases GH production at least partially by upregulating 15-PGDH expression.

Rosiglitazone increases apoptosis and autophagy in GH3 cells

Next, we investigated whether rosiglitazone had any effects on the proliferation of GH3 cells. We found that proliferation of the cells was significantly decreased by 50 μM rosiglitazone at 24 hr, 48 hr, 72 hr, and 96 hr after treatment (Figure 4A). However, when we added SW033291 (500 nM), a 15-PGDH inhibitor, the growth inhibitory effect of rosiglitazone was partially abolished (Figure 4A). Annexin V-propidium iodide was used to examine apoptosis with flow cytometry. We found that rosiglitazone significantly increased both early and late apoptosis of GH3 cells, which was partially abolished by SW033291 (Figure 4B). Western blot demonstrated that cleaved caspase 3 protein expression was

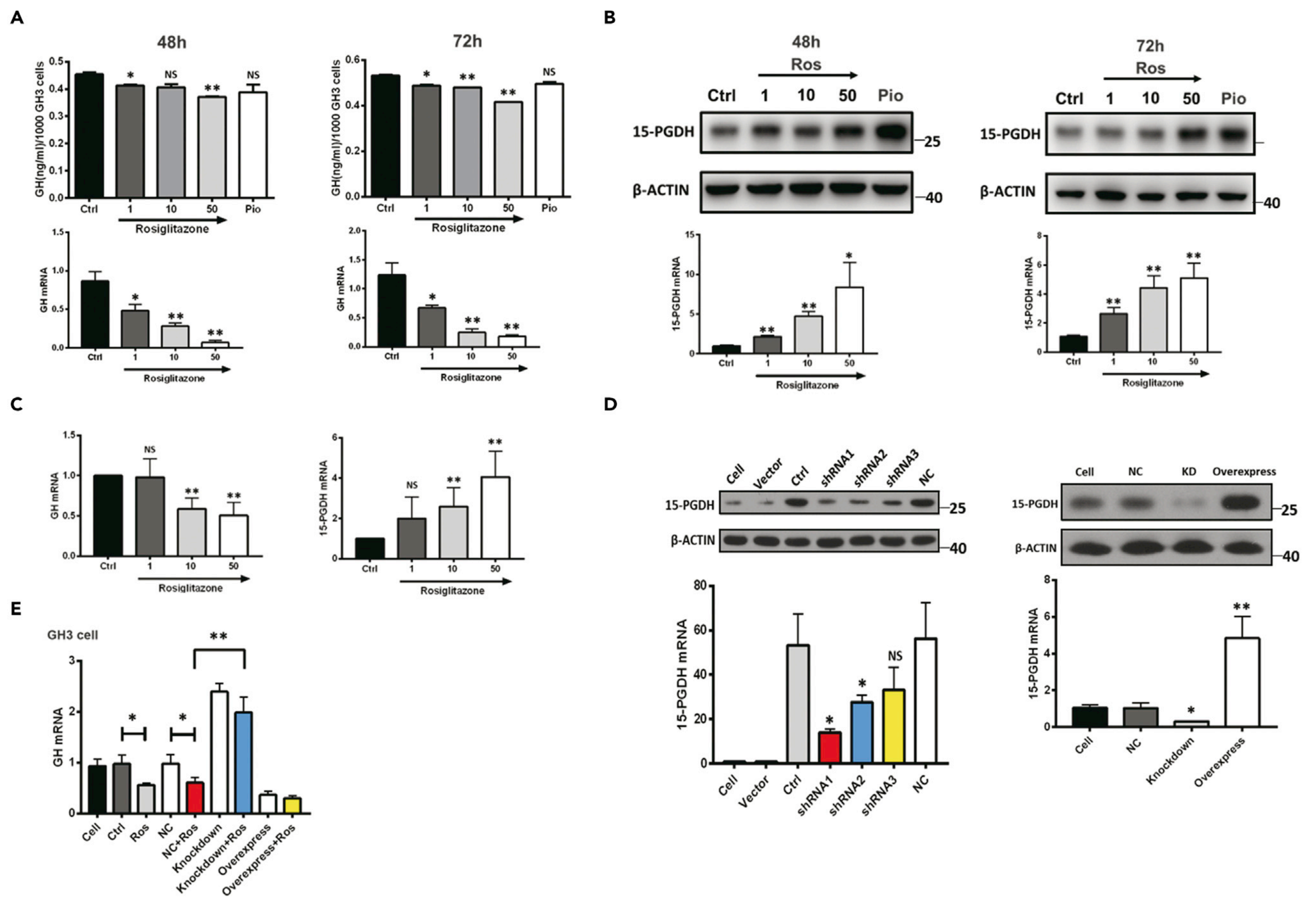

Figure 3. Rosiglitazone reduces GH secretion in somatotroph pituitary adenoma by upregulating 15-PGDH expression

(A) Both GH secretion and GH mRNA levels were significantly decreased by rosiglitazone in a dose-dependent manner. GH3 cells were treated with rosiglitazone (1, 10, or 50 μM) or pioglitazone (Pio, 50 μM) for 48 hr and 72 hr. GH3 cells were added water as control (Ctrl). GH concentration of the medium and GH mRNA level of the cells were both determined. Data were represented as mean ± SD. * denotes $p < 0.05$ and ** denotes $p < 0.01$ versus Ctrl.

(B) Rosiglitazone dose dependently upregulated the mRNA and protein expression of 15-PGDH in GH3 cells. GH3 cells were treated with rosiglitazone (1, 10, or 50 μM) or pioglitazone (Pio, 50 μM) for 48 hr and 72 hr. GH3 cells were added with water as control (Ctrl). 15-PGDH protein and mRNA levels were determined by western blot and RT-PCR, respectively. Data were represented as mean ± SD. *denotes $p < 0.05$ and **denotes $p < 0.01$ versus Ctrl.

(C) Rosiglitazone dose dependently decreased GH mRNA levels and increased 15-PGDH mRNA levels in the primary cultured cells of human GH-secreting pituitary adenomas. Human somatotroph pituitary adenoma cells from 5 patients with GH-secreting pituitary adenomas were treated with rosiglitazone (1, 10, or 50 μM) for 72 hr. Cells were added with water as control (Ctrl). The mRNA levels of GH and 15-PGDH were determined by RT-PCR. Data were represented as mean ± SD. **denotes $p < 0.01$ versus Ctrl.

(D) 15-PGDH was effectively knocked down or overexpressed in GH3 cells. GH3 cells were transfected with 15-PGDH short hairpin RNA (shRNA1, shRNA2, shRNA3) or 15-PGDH lentivirus (overexpression) for 72 hr, and then, 15-PGDH protein expression was determined by western blot. Scrambled shRNA was used as a negative control (NC). Data were represented as mean ± SD. * denotes $p < 0.05$ and ** denotes $p < 0.01$ versus NC.

(E) Knockdown of 15-PGDH significantly increased the GH mRNA levels, while overexpressing 15-PGDH significantly decreased GH mRNA levels in GH3 cells. GH3 cells were treated with rosiglitazone (Ros), scrambled shRNA (NC), scrambled shRNA plus rosiglitazone (NC + Ros), 15-PGDH shRNA1 (knockdown), 15-PGDH shRNA1 plus rosiglitazone (knockdown + Ros), 15-PGDH lentivirus (overexpress), or 15-PGDH lentivirus plus rosiglitazone (overexpress + Ros). GH3 cells were added with water as control (Ctrl). GH mRNA levels were measured by RT-PCR. Data were represented as mean ± SD. * denotes $p < 0.05$ versus Ctrl or NC. ** denotes $p < 0.01$ versus NC + Ros.

significantly increased by rosiglitazone treatment for 24 hr, which was partially reversed by SW033291 (Figure 4C).

Mounting evidence has indicated that rosiglitazone exerts anticancer effects through inducing autophagy (Dang et al., 2018). Thus, we explored whether the effects of rosiglitazone on apoptosis in GH3 cells are associated with autophagy. We found that rosiglitazone significantly changed the levels of several proteins involved in autophagy pathways, including Beclin1 (BECN1), ATG5, ATG7, p62,

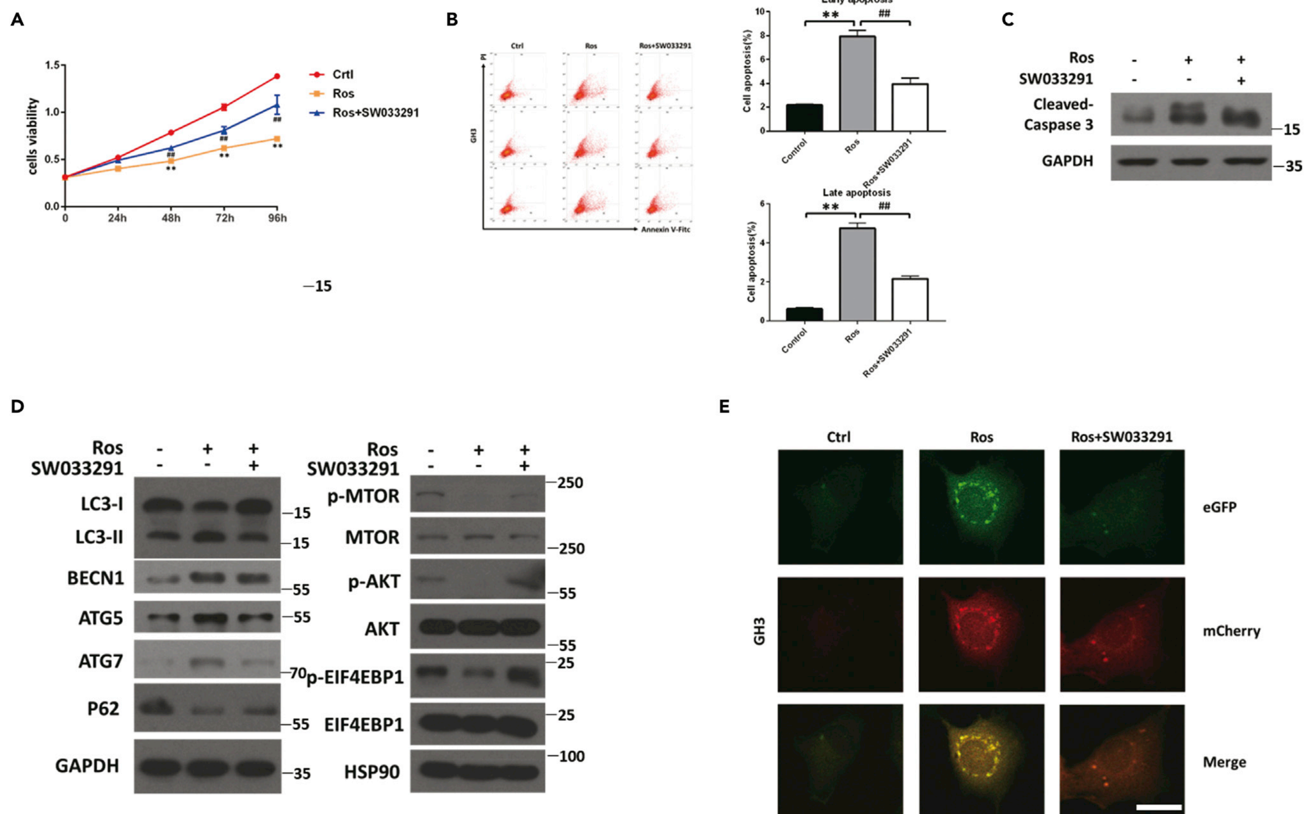

Figure 4. Rosiglitazone increases apoptosis and autophagy in GH3 cells

(A) SW033291 significantly reversed the growth inhibitory effect of rosiglitazone on GH3 cells. GH3 cells were treated with rosiglitazone (Ros, 50 μM) or rosiglitazone (Ros, 50 μM) plus SW033291 (500 nM) (Ros + SW033291) for 24, 48, 72, or 96 hr. Cells were added with DMSO as control (Ctrl). GH3 cell proliferation was assessed using the CCK8 kit. Data were represented as mean ± SD. ** denotes $p < 0.01$ versus Ctrl. ## denotes $p < 0.01$ versus Ros.

(B) SW033291 significantly abolished the increased both early and late apoptosis of GH3 cells induced by rosiglitazone. GH3 cells were treated with rosiglitazone (Ros, 50 μM) or rosiglitazone (Ros, 50 μM) plus SW033291 (500 nM) (Ros + SW033291) for 24 hr. Cells were added with DMSO as control (Ctrl). Cell apoptosis (both early apoptosis and late apoptosis) was assessed by Annexin V/propidium iodide (PI) assay. Data were represented as mean ± SD. ** denotes $p < 0.01$ versus Ctrl. ## denotes $p < 0.01$ versus Ros.

(C) SW033291 impeded the increased cleaved caspase 3 protein expression caused by rosiglitazone in GH3 cells. GH3 cells were treated with rosiglitazone (Ros, 50 μM) or rosiglitazone (Ros, 50 μM) plus SW033291 (500 nM) (Ros + SW033291) for 24 hr. Cells were added with DMSO as control (Ctrl). The protein level of cleaved caspase 3 was assessed by western blot.

(D) SW033291 reversed the rosiglitazone-induced changes of several proteins involved in autophagy pathways. GH3 cells were treated with rosiglitazone (Ros, 50 μM) or rosiglitazone (Ros, 50 μM) plus SW033291 (500 nM) (Ros + SW033291) for 24 hr. Cells were added with DMSO as control (Ctrl). The protein levels of Beclin1 (BECN1), ATG5, ATG7, p62, mammalian target of rapamycin (mTOR), p-mTOR, Akt, p-Akt, p-EIF4EBP1, and EIF4EBP1 were assessed by western blot.

(E) SW033291 reversed the increased autophagy in the rosiglitazone-treated GH3 cells. Confocal analysis of GH3 cells expressing tandem mCherry-GFP-LC3 treated with treated with rosiglitazone (Ros, 50 μM) or rosiglitazone (Ros, 50 μM) plus SW033291 (500 nM) (Ros + SW033291) for 24 hr. Cells were added with DMSO as control (Ctrl). Scale bar represents 10 μm.

mammalian target of rapamycin (mTOR), Akt, p-Akt, p-EIF4EBP1, and EIF4EBP1; and these changes were reversed by SW033291 (Figure 4D). Dual-fluorescence mCherry-eGFP-LC3 also showed that autophagy was increased in the rosiglitazone-treated cells, which was reversed by SW033291 (Figure 4E). These results indicate that rosiglitazone increases apoptosis and autophagy in GH3 cells by upregulating 15-PGDH expression.

Rosiglitazone suppresses GHR expression and reduces IGF-1 secretion in HepG2 cells by upregulating 15-PGDH expression

Rosiglitazone has been used to treat type 2 diabetes mellitus. Characterized as an insulin sensitizer, it improves insulin resistance in the liver (Eliasson et al., 2014). It has been shown that rosiglitazone regulates the

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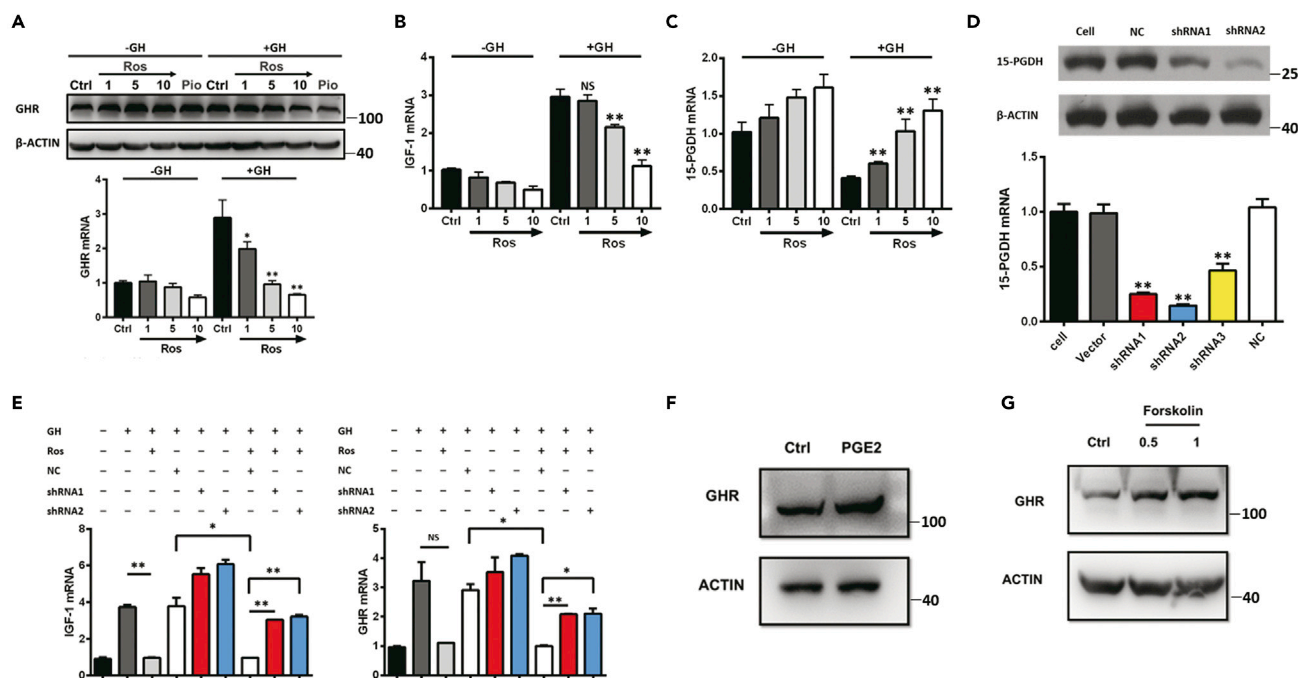

Figure 5. Rosiglitazone suppresses GHR expression and reduces IGF-1 secretion in HepG2 cells by upregulating 15-PGDH expression

(A) Rosiglitazone decreased the expression of GHR in a dose-dependent manner in HepG2 cells treated with recombinant human growth hormone (rhGH). HepG2 cells were treated with rosiglitazone (1, 5 or 10 μM) or pioglitazone (Pio, 50 μM) with or without recombinant human growth hormone (rhGH, 50 nM) for 24 hr. Cells were added with water as control (Ctrl). The protein and mRNA levels of GHR were tested by western blot and RT-PCR, respectively. Data were represented as mean ± SD. * denotes $p < 0.05$ and ** denotes $p < 0.01$ versus Ctrl.

(B) Rosiglitazone decreased IGF-1 mRNA levels in a dose-dependent manner in HepG2 cells treated with rhGH. HepG2 cells were treated with rosiglitazone (1, 5 or 10 μM) with or without recombinant human growth hormone (rhGH, 50 nM) for 24 hr. Cells were added with water as control (Ctrl). The mRNA levels of IGF-1 were tested by RT-PCR. Data were represented as mean ± SD. ** denotes $p < 0.01$ versus Ctrl.

(C) Rosiglitazone increased 15-PGDH mRNA levels in a dose-dependent manner in HepG2 cells treated with rhGH. HepG2 cells were treated with rosiglitazone (1, 5 or 10 μM) with or without recombinant human growth hormone (rhGH, 50 nM) for 24 hr. Cells were added with water as control (Ctrl). The mRNA levels of 15-PGDH were tested by RT-PCR. Data were represented as mean ± SD. ** denotes $p < 0.01$ versus Ctrl.

(D) 15-PGDH expression was successfully knocked down in HepG2 cells. HepG2 cells were transfected with 15-PGDH shRNA (shRNA1, shRNA2) or negative control (NC) for 72 hr. The protein expression of 15-PGDH was tested by western blot. Data were represented as mean ± SD. ** denotes $p < 0.01$ versus NC.

(E) Inhibition of 15-PGDH prevented the suppressing efficacy of rosiglitazone on both IGF-1 and GHR. Under the treatment recombinant human GH (rhGH, 50 nM), HepG2 cells were treated with rosiglitazone (Ros, 10 μM), negative control (NC), 15-PGDH shRNA1 (shRNA1), 15-PGDH shRNA2 (shRNA2), rosiglitazone (10 μM) plus negative control (Ros + NC), rosiglitazone (10 μM) plus 15-PGDH shRNA1 (Ros + shRNA1), or rosiglitazone (10 μM) plus 15-PGDH shRNA2 (Ros + shRNA2) for 24 hr. The mRNA levels of IGF-1 and GHR were tested by RT-PCR. Data were represented as mean ± SD. ## denotes $p < 0.01$ versus Ros. Data were represented as mean ± SD. * denotes $p < 0.05$ and ** denotes $p < 0.01$ versus Ros + NC.

(F) GHR protein expression in HepG2 cells was upregulated by PGE2. HepG2 cells were treated with PGE2 (10 μM, 24 hr). Cells were added with DMSO as control (Ctrl). Western blot is used to measure the level of GHR protein expression.

(G) Forskolin upregulated the expression of GHR in HepG2 cells in a dose-dependent manner. HepG2 cells were treated with forskolin (0.5, 1 μM) for 24 hr. Cells were added with DMSO as control (Ctrl). GHR protein was assessed by western blot.

expression of multiple proteins in the liver (Tjokroprawiro, 2006). GH acts by binding to the growth hormone receptor (GHR) in the liver and thus increases the production of IGF-1. Therefore, we hypothesized that rosiglitazone could regulate the expression of GHR. First, rosiglitazone decreased the expression of both GHR and IGF-1 in a dose-dependent manner in hepatocellular carcinoma HepG2 cells treated with recombinant human growth hormone (rhGH) (50 nM, 24hr) (Figures 5A and 5B). In addition, similarly to the effects observed in GH3 cells, rosiglitazone also increased the expression of 15-PGDH in HepG2 cells exposed to rhGH (Figure 5C). Time course analyses showed that the regulation of GHR by rhGH gradually increased until 6 h and then remained stable until at least 36 hr (Figure S1). Next, we examined whether 15-PGDH mediated the regulatory effects of rosiglitazone on IGF-1 and GHR. The expression of 15-PGDH in HepG2 cells was inhibited with lentivirus-mediated shRNA (Figure 5D). Inhibition of 15-PGDH prevented the suppressing efficacy of rosiglitazone on both IGF-1 and GHR (Figure 5E). As 15-PGDH is the key enzyme which oxidizes and inactivates prostaglandin E2 (PGE2), we examined whether the effects of 15-PGDH were

mediated by PGE2. Indeed, GHR protein expression in HepG2 cells was upregulated by PGE2 (Figure 5F). PGE2 exerts multiple effects by binding and activating PGE2 receptors, which are G-protein-coupled receptors. Therefore, we examined whether forskolin, an activator of adenylate cyclase, could regulate the expression of GHR. Consistently, forskolin upregulated the expression of GHR in HepG2 cells in a dose-dependent manner (Figure 5G). Taken together, the above results suggested that inhibition of 15-PGDH led to the accumulation of PGE2 and activation of the G-protein-coupled receptors, which increased the expression of GHR. Rosiglitazone induced the expression of 15-PGDH and thus decreased the expression of GHR and IGF-1 in HepG2 cells.

As shown in Figure 5G, forskolin upregulated GHR expression in HepG2 cells. Adenylate cyclase/cyclic adenosine monophosphate (cAMP) signaling activates protein kinase A (PKA) and thus results in the phosphorylation of cAMP response element-binding protein (CREB), which induces the expression of targeted genes (Quinn, 1993). We explored whether CREB directly promotes the transcription of GHR. HEK293t cells were transfected with human GHR promoter with or without CREB. The plasmid pGL4.10 was used as control. As shown in Figure S2, CREB significantly increased luciferase activity in the cells transfected with the GHR promoter. Therefore, CREB promotes the transcription of GHR.

Rosiglitazone slows the growth of tumors and reduces GH and IGF-1 secretion in nude mice inoculated subcutaneously with GH3 cells

To investigate the effects of rosiglitazone on GH and IGF-1 secretion *in vivo*, male nude mice were inoculated subcutaneously with GH3 cells. The mice were randomly assigned to three groups: the control (treated with saline) group, the Ros (treated with rosiglitazone dissolved in saline) group, and the Ros plus SW033291 (treated with rosiglitazone and SW033291 dissolved in DMSO) group (Figure 6A). As shown in Figures 6A and 6B, compared with the control group, both the tumor volume and weight were significantly decreased by rosiglitazone treatment, which was impeded by SW033291 (Figures 6B and 6C). In the Ros plus SW033291 group, the mean tumor volume and weight were similar to those of the control group (Figures 6B and 6C). The mean serum GH and IGF-1 levels were lower in the Ros group compared to the control group and the Ros plus SW033291 group (Figures 6D and 6E). PGE2 is degraded by 15-PGDH into 13,14-dihydro-15-keto-PGE2 (PGEM) (Molitch, 2017). Thus, we measured urinary PGE2 and PGEM levels to examine whether rosiglitazone affected the metabolism of PGE2 by upregulating 15-PGDH *in vivo*. We found that the urinary PGE2/Creatinine (Cr) was decreased in the Ros group compared to the control group, which was increased by SW033291 (Figure 6F). In contrast, rosiglitazone significantly increased the urinary PGEM/Cr, which was blocked by SW033291 (Figure 6G).

Rosiglitazone decreases GH and IGF-1 levels in patients with active acromegaly

Finally, we evaluated the efficacy of rosiglitazone in controlling GH and IGF-1. Serum random GH and IGF-1 levels are shown in Table 1. As the levels of random GH did not fit a normal distribution, GH data were log-transformed before analysis. The random GH of baseline, 3 months rosiglitazone treatment, and 6 months rosiglitazone treatment were 0.8795 ± 0.4735, 0.8961 ± 0.0880, and 0.7668 ± 0.4447, respectively. Rosiglitazone administration significantly reduces random GH levels (baseline *vs* 3m, $p = 0.8140$; baseline *vs* 6m, $p = 0.0107$; 3m vs 6m, $p = 0.0358$, Figure 7A). The IGF-1 index, defined as IGF-1 level divided by the upper limit of normal (ULN) for age and sex, of baseline, 3 months rosiglitazone treatment, and 6 months rosiglitazone treatment were 2.2026 ± 0.8194, 1.5500 ± 0.5454, and 1.5947 ± 0.7470, respectively. Rosiglitazone significantly decreased IGF-1 (baseline *vs* 3m, $p = 0.0002$; baseline *vs* 6m, $p < 0.0001$; 3m vs 6m, $p = 0.7873$, Figure 7B). After rosiglitazone treatment, all 19 participants had a decrease in IGF-1 levels and 4 out of 19 participants (21.05%) normalized their IGF-1 levels, while 16 out of 19 (84.21%) participants had a decrease in GH levels after treatment.

DISCUSSION

Rosiglitazone is an anti-diabetic drug which has been used to treat type 2 diabetes mellitus for more than two decades. Unexpectedly, several case reports have shown that rosiglitazone reduced GH and/or IGF-1 levels in patients with active acromegaly (Bogazzi et al., 2011; Gradiser et al., 2007; Tamez-Perez et al., 2011). However, the small patient sample size in these case reports and the lack of mechanistic exploration impeded the clinical application of rosiglitazone. Additionally, in all previous case reports, rosiglitazone was used in combination with SSAs or/and PEG (Bogazzi et al., 2011). Thus, it is difficult to evaluate the efficacy of rosiglitazone per se. In the present study, 19 patients with active acromegaly were treated with

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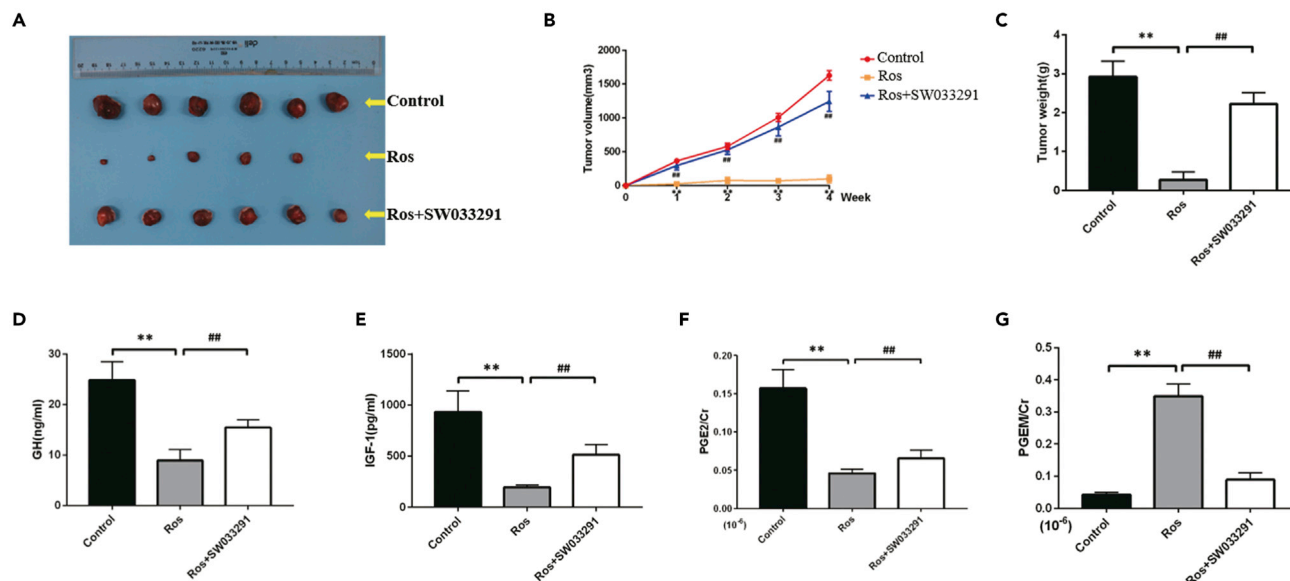

Figure 6. Rosiglitazone slows the growth of tumors and reduces GH and IGF-1 secretion in nude mice inoculated subcutaneously with GH3 cells

(A) Nude mice were subcutaneously injected with 5×10^6 GH3 cells and randomized into three groups, including the rosiglitazone (Ros, 200 mg/kg per day, gavage, n = 6) group, the rosiglitazone plus 15-PGDH inhibitor SW033291 (10 mg/kg per day, intraperitoneal) (Ros + SW033291, n = 6) group, and the normal saline (control) group. After 4 weeks, all the mice were sacrificed, and the tumors were harvested.

(B) SW033291 reversed the decrease of the tumor volume in Ros-treated mice. The tumor volumes of these three groups were measured after 1, 2, 3, or 4 weeks of Ros or Ros plus SW033291 treatment. Data were represented as mean ± SD. ** denotes $p < 0.01$ versus control group, ## denotes $p < 0.01$ versus Ros group.

(C) SW033291 abolished the decrease of the tumor weights in Ros-treated mice. The tumor weights of these three groups were measured after 1, 2, 3, or 4 weeks of Ros or Ros plus SW033291 treatment. Data were represented as mean ± SD. ** denotes $p < 0.01$ versus control group, ## denotes $p < 0.01$ versus Ros group.

(D and E) Mean serum GH and IGF-1 levels were lower in the Ros group compared to the control group and the Ros plus SW033291 group. Mice serum of these three groups was collected and GH and IGF-1 levels were measured. Data were represented as mean ± SD. ** denotes $p < 0.01$ versus control group, ## denotes $p < 0.01$ versus Ros group.

(F) The urinary PGE2/Creatinine (Cr) was increased in the Ros + SW033291 group than that in Ros group. Levels of PGE2 and creatinine (Cre) were measured in urine of mice. Data were represented as mean ± SD. ** denotes $p < 0.01$ versus control group, ## denotes $p < 0.01$ versus Ros group.

(G) Compared to mice in the Ros group, the urinary PGEM/Cr level was decreased in the Ros + SW033291 group. Data were represented as mean ± SD. ** denotes $p < 0.01$ versus control group, ## denotes $p < 0.01$ versus Ros group.

rosiglitazone alone. We found that rosiglitazone decreased GH and IGF-1 levels in all patients treated and normalized IGF-1 levels in nearly 30%, which suggested that rosiglitazone might be effective for biochemical control in patients with active acromegaly.

Currently, drugs used to inhibit GH secretion include SSAs and dopamine agonists (DAs). However, SSAs only normalize GH levels in 30%–50% of patients with acromegaly, whereas DAs may be effective in only 10%–20% of mildly affected patients (Colao et al., 2014; Katznelson et al., 2014; Howlett et al., 2013). The GH receptor antagonist PEG can normalize IGF-1 levels in majority of patients but requires frequent self-injection, is not available at many centers, is expensive, and acts only at the liver to inhibit GH-stimulated IGF-1 production, thereby raising concerns about the potential for tumor growth. Therefore, alternative pharmacological approaches are still needed. Herein, we demonstrate that through upregulating 15-PGDH expression, rosiglitazone inhibits GH secretion. Our mechanistic studies also suggest that 15-PGDH itself may be a potential molecular target for acromegaly treatment.

15-PGDH is the key enzyme that degrades and inactivates PGE2 (Seo and Oh, 2017). Recent reports suggest that 15-PGDH is a tumor suppressor gene, and the upregulation of 15-PGDH could inhibit the growth of several tumor cells, including hepatocellular carcinoma, non-small cell lung cancer, and colorectal cancer (Basudhar et al., 2017; Huang et al., 2008; Kim et al., 2017; Pham et al., 2010; Tseng-Rogenski et al., 2010). Here, for the first time, we observe that 15-PGDH expression is decreased in pituitary somatotroph tumors compared to normal pituitary tissue. Further, we found that the expression of 15-PGDH negatively

Table 1. Levels of GH and IGF-1 before and after rosiglitazone treatment

	Random GH (μg/L)			IGF-1			IGF-1 index		
Patient no.	Baseline	3m	6m	Baseline	3m	6m	Baseline	3m	6m
1	2.5	8.8	2.36	391	277	75.54	1.38	0.98	0.27
2	53.3	23.3	33.3	843	773	782	2.35	2.16	2.18
3	15.48	31.3	31.2	801	539	534	2.24	1.51	1.49
4	14.4	10.3	10.6	933	666	604	2.61	1.86	1.69
5	3	3.1	2.3	636	369	319	2.24	1.30	1.12
6	7.7	10.6	6.4	336	278	198	1.41	1.17	0.83
7	7.4	13.5	5.8	612	381	351	1.71	1.06	0.98
8	2.9	2.6	2.7	733	480	381	2.05	1.34	1.06
9	8.72	7.6	4.9	338	290	329	1.19	1.02	1.16
10	25.5	8.8	10.7	1052	662	691	2.94	1.85	1.93
11	14.4	9.2	8.6	770	655	588	2.15	1.83	1.64
12	30.16	7.73	11.1	1126	347.2	979	3.96	1.22	3.45
13	26.1	44.1	35.3	1152	767	781	4.06	2.70	2.75
14	1.38	2.1	1.1	537	436	462	1.50	1.22	1.29
15	1.61	2.35	1.87	421	276	412	1.18	0.77	1.15
16	10.24	4.63	5.25	674	476	417	1.88	1.33	1.16
17	1.9	–	1.75	497	–	485	1.75	–	1.71
18	2.92	4.26	2.74	641	574	581	2.69	2.41	2.44
19	10.18	8.98	6.9	915	776	716	2.56	2.17	2.00

Baseline means before rosiglitazone treatment; 3 m means rosiglitazone treatment for 3 months; 6 m means rosiglitazone treatment for 6 months.

correlates with tumor volume, which suggested that 15-PGDH may exert inhibitory effects on pituitary somatotroph tumors as well. It has been shown that the activation of PPARγ leads to cellular differentiation, apoptosis, and arrest of tumor progression (Giaginis et al., 2012; Shimada et al., 2002). As a synthetic PPAR-γ ligand, rosiglitazone has been shown to inhibit tumor cell proliferation and enhance cell apoptosis (Chang and Szabo, 2000; Wang et al., 2020). Previous studies have shown that rosiglitazone suppresses GH secretion, induces GH3 cell apoptosis, and delays the development of the subcutaneous GH-secreting tumors in nude mice injected with GH3 cells (Heaney et al., 2003; Bogazzi et al., 2004). However, the molecular mechanism remains unknown. Here, our data clearly demonstrated that rosiglitazone increases apoptosis of GH3 cells and reduces the growth and GH secretion in mice inoculated subcutaneously with GH3 cells, and all these effects are mediated by 15-PGDH. These results suggest that 15-PGDH is involved in the suppressive effects of rosiglitazone on pituitary somatotroph adenomas. It has been shown that 15-PGDH expression is regulated by transforming growth factor β1, interleukin-4, histone deacetylase inhibitors, and nonsteroidal anti-inflammatory drugs (Tai, 2011; Tong et al., 2006; Yan et al., 2004; Chi and Tai 2010). In our study, for the first time, we reveal that as a transcription factor, PPARγ directly binds to 15-PGDH promoter and enhances its transcription.

Normalization of IGF-1 levels is a key goal for acromegaly treatment, as it is the best predictor of disease control (Giustina et al., 2010). Presently, only pegvisomant, a GH receptor antagonist, directly acts on the liver to inhibit IGF-1 secretion (Tritos and Biller, 2017), while its application is limited due to those issues mentioned above (Leonart et al., 2019; Elbaum et al., 2019). In this study, we observed that rosiglitazone decreases IGF-1 levels in 19 patients with active acromegaly. Our report is a pilot study with a small patient number and short duration. A randomized trial with a larger patient number is needed to confirm our findings.

It is unclear whether the reduction in IGF-1 levels was caused by a reduction in pituitary GH secretion or direct effects of rosiglitazone on liver. As an anti-diabetic drug, rosiglitazone directly regulates lipid and glucose metabolism in the liver (Sun et al., 2006). Therefore, we speculated whether rosiglitazone inhibited

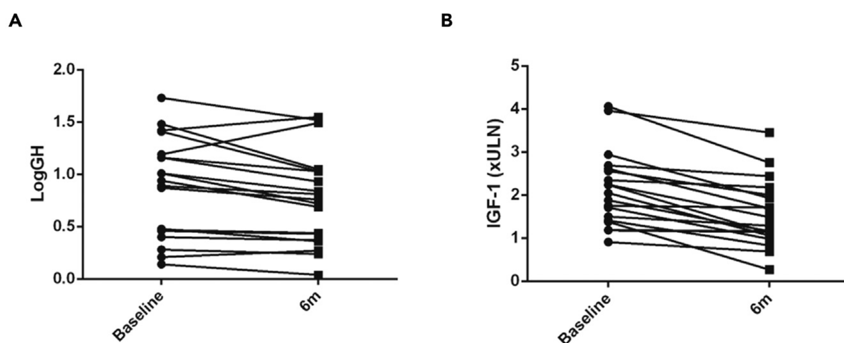

Figure 7. Rosiglitazone decreases GH and IGF-1 levels in patients with active acromegaly

(A) Rosiglitazone (4–8 mg/day, once a day, for 6 months) decreased random GH levels in 19 patients with active acromegaly. Log GH significantly decreased from 0.8795 ± 0.4735 to 0.7668 ± 0.4447 during the study period. Data were analyzed using paired samples *t* tests. $p = 0.011$.

(B) IGF-1 level was significantly decreased from $2.1374 \pm 0.8715 \times$ ULN to $1.5447 \pm 0.7750 \times$ ULN after 6 months of rosiglitazone treatment. Data were analyzed using paired samples *t* tests. $p < 0.0001$.

hepatic IGF-1 production independent of GH reduction. We find that indeed, rosiglitazone directly decreases IGF-1 production in the liver by upregulating 15-PGDH expression. Interestingly, rosiglitazone also inhibits GHR protein expression in HepG2 cells, which is partially by inhibiting the PGE2/cAMP/PKA pathway. However, these effects vanished when 15-PGDH was silenced with shRNA or inhibited by a specific inhibitor. Furthermore, we verify that CREB binds to the GHR promoter and promotes the transcription of GHR. These findings indicate that rosiglitazone reduces GHR expression by elevating 15-PGDH expression and suppressing the cAMP/PKA/CREB pathway, which ultimately decreases IGF-1 production. Therefore, the therapeutic effects of rosiglitazone on acromegaly are attributable not only to inhibiting pituitary GH secretion but also to suppressing hepatic IGF-1 production.

Discordance between GH and IGF-1 status has been reported to be as high as 39.5% of treated patients with acromegaly (Zeinalizadeh et al., 2015). Most discordant patients exhibit normal GH and elevated IGF-1 levels, which is associated with worse metabolic profiles and is implicated as a marker of persistent active disease with a shortened life expectancy (Alexopoulou et al., 2008; Zeinalizadeh et al., 2015). Therefore, drugs that simultaneously target both pituitary adenoma and liver will be ideal for better biochemical control in patients with acromegaly. In the present study, we demonstrated direct effects of rosiglitazone not only on the liver but also on pituitary adenoma by upregulating 15-PGDH, a common molecular target.

Bogazzi et al. (2004) have verified that rosiglitazone at 50 μM prominently increased apoptosis and inhibited proliferation of GH3 cells, as well as reduced the secretion of GH from GH3 cells. Work from Heaney et al. (2003) reports that rosiglitazone at either 50 μM or 75 μM exhibits an inhibitory effect on proliferation of GH3 cell proliferation. Moreover, to investigate the role of rosiglitazone on GH3 cell proliferation, rosiglitazone was used up to 100 μM *in vitro* (Emery et al., 2006). These observations demonstrate that rosiglitazone at 50 μM is safe and practicable. In our study, as shown in Figure 3, we found that GH secretion and GH mRNA levels were both significantly decreased by rosiglitazone in a dose-dependent manner from 1 to 50 μM. Based on previous research and our results, thus we chose the concentration of rosiglitazone at 50 μM.

Work from Bastemir and his colleagues showed that rosiglitazone (8 mg/day for 6 weeks) did not reduce basal and nadir GH levels from the oral glucose tolerance test and the IGF-1 levels in 7 patients with active acromegaly (Bastemir et al., 2007). However, in our study, we exhibited that random GH and the IGF-1 index were both significantly decreased after 6 months of treatment with rosiglitazone (8 mg/day). There are several potential reasons for the difference. First, the duration of rosiglitazone administration (6 weeks *vs* 6 months) might cause the difference. As mentioned in Bastemir's paper, they also suggested that longer duration (other than 6 weeks) should be required to determine the usefulness of rosiglitazone. Second, the patient number might be one of the factors leading to the disparity. In Bastemir's work, 7 patients were enrolled while in our study; 19 patients were included. Third, the tumor volume might be another reason

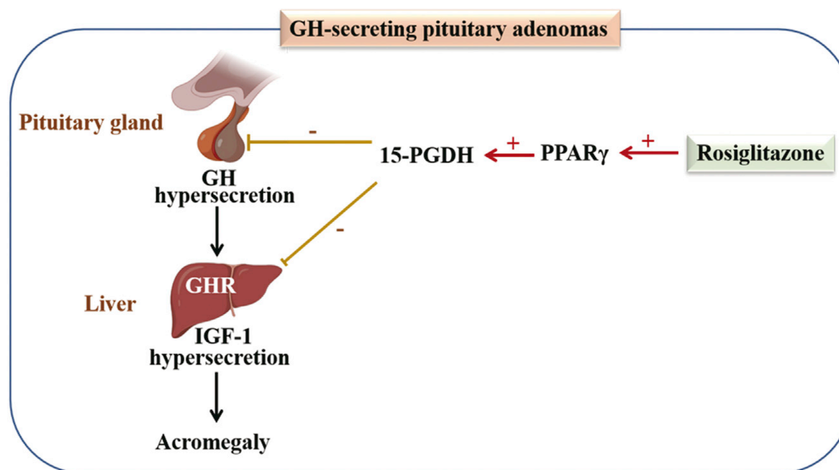

Figure 8. The putative molecular mechanisms of rosiglitazone treatment on acromegaly by targeting the pituitary and liver

Rosiglitazone activates PPARγ, which promotes the transcription and expression of 15-PGDH. Then, 15-PGDH not only acts on the pituitary to inhibit the secretion of GH but also acts on the liver to inhibit IGF-1 secretion, exerting the therapeutic effect on acromegaly.

for the difference. In our study, all patients had received surgeries, and the residual tumor was very small and less than 1 cm in diameter. However, in Bastemir's study, among 7 patients, 6 of them had macroadenomas and only one patient had microadenomas. And 3 of the 6 patients with macroadenomas received rosiglitazone treatment before getting surgeries. We and others have shown that the tumor volume was negatively associated with the sensitivity to SSAs (Colao et al., 2011; Shen et al., 2020; Tortora et al., 2019). The bigger tumors were more resistant to SSA. Thus, although there is no study examining the effects of tumor volume on response to rosiglitazone, we speculate that the efficacy of rosiglitazone might be affected by tumor volume.

In conclusion, we demonstrate that rosiglitazone significantly decreases GH and IGF-1 levels in patients with acromegaly, and we further identify that 15-PGDH is the common molecular target of rosiglitazone in pituitary adenomas as well as the liver. The schematic mechanism by which rosiglitazone exerts its effects on the pituitary gland and liver was collectively shown in Figure 8: through upregulating the expression of 15-PGDH, rosiglitazone inhibits the hypersecretion of GH in pituitary adenoma and the exceeding secretion of IGF-1 in the liver and consequently controls the progression of acromegaly.

These results collectively position 15-PGDH as a potential new therapeutic target for acromegaly and implicate rosiglitazone as a possible alternative pharmacological approach for acromegaly, including cases with normal GH and elevated IGF-1.

Limitations of the study

In this study, we provide a comprehensive analysis of the therapeutic function of rosiglitazone on acromegaly and identify that 15-PGDH is the common molecular target for rosiglitazone in both the pituitary adenoma as well as the liver. However, the main limitation of the current study is the relatively small sample size attributed to the rarity of acromegaly, and these findings need to be further confirmed in larger-scale studies.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2021.102983.

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AUTHOR CONTRIBUTIONS

Z.Z., Y.Z., and M.H. conceived the original idea of this study. Y. Zhang, M.W., C.J., Z.C., M.H., Y. Zhao, and Z.Z. searched literature and designed experiments. H. Yang, L.W., Y.Y., C.J., N.Q., Z.M., Z.Y., X.S., W.L., Y.W., and W.G. performed the statistical and imaging analyses. Y. Zhang, M.W., and Z.Z. wrote the initial draft of the manuscript. V.M., L.H., E.J.L., H. Ye, Y.W., Y.L., M.H., and Y.Z. revised the manuscript critically for important intellectual content. Z.Z. finally approved the version to be submitted.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit polyclonal anti-15-PGDH	Santa Cruz Biotechnology	Cat#sc-98907; RRID:AB_2120417
Rabbit polyclonal anti-cleaved caspase-3	Cell Signaling Technology	Cat#9661; RRID:AB_2341188
Mouse monoclonal anti-β-actin	Santa Cruz Biotechnology	Cat#sc-81178; RRID:AB_2223230
Mouse monoclonal anti-GAPDH	Santa Cruz Biotechnology	Cat#sc-365062; RRID:AB_10847862
Mouse monoclonal anti-LC3-I	Cell Signaling Technology	Cat#4599; RRID:AB_10548192
Mouse monoclonal anti-LC3-II	Cell Signaling Technology	Cat#3868; RRID:AB_2137707
Mouse monoclonal anti-BECN1	Cell Signaling Technology	Cat#3495; RRID:AB_1903911
Mouse monoclonal anti-ATG5	Santa Cruz Biotechnology	Cat#sc-133158; RRID:AB_2243288
Mouse monoclonal anti-ATG7	Santa Cruz Biotechnology	Cat#sc-376212; RRID:AB_10988418
Rabbit polyclonal anti-P62	Cell Signaling Technology	Cat#13121; RRID:AB_2750574
Mouse monoclonal anti-p-MTOR	Santa Cruz Biotechnology	Cat#sc-293133; RRID:AB_2861149
Rabbit polyclonal anti-MTOR	Cell Signaling Technology	Cat#2972; RRID:AB_330978
Rabbit monoclonal anti-p-AKT	Cell Signaling Technology	Cat#4060; RRID:AB_2315049
Mouse monoclonal anti-AKT	Santa Cruz Biotechnology	Cat#sc-81434; RRID:AB_1118808
Mouse monoclonal anti-p-EIF4EBP1	Santa Cruz Biotechnology	Cat#sc-293124
Mouse monoclonal anti-EIF4EBP1	Santa Cruz Biotechnology	Cat#sc-81149; RRID:AB_1118481
Rabbit polyclonal anti-HSP90	Cell Signaling Technology	Cat#4874; RRID:AB_2121214
Mouse monoclonal anti-GHR	Santa Cruz Biotechnology	Cat#sc-137185: RRID:AB_2111405
Rabbit polyclonal anti-PPARγ antibody	Abcom	Cat#ab45036; RRID:AB_1603934
Bacterial and virus strains		
Ad-mCherry-GFP-LC3B	Beyotime	Cat#C3011
Biological samples		
Normal pituitary tissue	Autopsy from cadaver organ donation (Fudan University).	https://www.fudan.edu.cn/
Tumor tissue was obtained from 89 patients diagnosed with acromegaly	Department of Neurosurgery at Huashan Hospital	https://www.huashan.org.cn/
Chemicals, peptides, and recombinant proteins		
Lipofectamine 2000	Invitrogen	Cat#11668019
Bradford reagent	Invitrogen	Cat#23236
TRIzol Reagent	Invitrogen	Cat#15596026
Dulbecco's modified eagle medium	HyClone	Cat#30243
Ham's F-12K Medium	Invitrogen	Cat#21127030
Rosiglitazone	Taiji Group	Cat#188914
SW033291	Sigma-Aldrich	Cat#1485
Critical commercial assays		
Rat GH ELISA kit	FineTest	Cat#ER0993
Cell counting kit 8	Sigma-Aldrich	Cat#96992
FITC Annexin V Apoptosis Detection Kit	BD Biosciences	Cat#666547

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental models: Cell lines		
Rat GH3 cells	American Type Culture Collection	CRL-82.1
HepG2	National Collection of Authenticated Cell Cultures	SCSP-510
Experimental models: Organisms/strains		
BALB/c Nude mice: CAnN.Cg-*Foxn1*^{nu}/Crl	SHANGHAI SLAC LABORATORY ANIMAL CO. LTD	401
Oligonucleotides		
siRNA targeting rat 15-PGDH, see manuscript	This paper	N/A
Primers for 15-PGDH promoter -3001/-2709 mut, see manuscript	This paper	N/A
Primers for 15-PGDH promoter -1966/-1692 mut, see manuscript	This paper	N/A
Primers for 15-PGDH promoter -270/-10 mut, see manuscript	This paper	N/A
Primers for 15-PGDH, see manuscript	This paper	N/A
Primers for GH, see manuscript	This paper	N/A
Primers for IGF-1, see manuscript	This paper	N/A
Primers for GHR, see manuscript	This paper	N/A
Primers for β-actin, see manuscript	This paper	NA
Recombinant DNA		
pGL4.10	Shanghai Siyuan Biotechnology Co., Ltd.	SHB201
Software and algorithms		
GraphPad Software	La Jolla	https://www.docslides.com/mitsue-stanley/prism-5-software-graphpad-software-la-jolla-ca-usa-jmp
SPSS 16.0 statistical software	IBM Corporation	https://www.ibm.com/cn-zh/analytics/spss-statistics-software

RESOURCE AVAILABILITY

Lead contact

Zhaoyun Zhang (zhaoyunzhang@fudan.edu.cn).

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Zhaoyun Zhang (zhaoyunzhang@fudan.edu.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

All data and analytical methods are reported in the main text or in the supplemental information section.

Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animal study

Athymic 5-week-old male BALB/c nude mice, from Shanghai Laboratory Animal Center maintained on a standard chow with free access to water, were used for all *in vivo* experiments. Eighteen nude mice

were subcutaneously injected with rat GH3 cells (5 × 10^6) to form GH3 tumors, and GH3 tumors were formed 2 weeks after injections. Then, mice were randomly divided into three groups, including the rosiglitazone (200 mg/kg per day, gavage, n = 6) group, the rosiglitazone plus 15-PGDH inhibitor SW033291 (10 mg/kg per day, intraperitoneal, n = 6) group, and the normal saline (Control) group. After 4 weeks, the mice were sacrificed. The serum and urine samples were collected, and the tumors were harvested. The serum GH and IGF-1 levels were measured by the GH ELISA kit (Merck Millipore, Germany) and IGF-1 ELISA kit (Cayman, MI, USA) according to the manufacturer's protocol. Urine PGE2, PGEM and creatinine (Cr) were detected by the PGE2 ELISA kit (Cayman), PGEM ELISA kit (Cayman) and creatinine ELISA kit (Cayman). Animal experiments were conducted with the permission of the Institutional Animal Care and Use Committee of Fudan University. Our study kept in accordance with the Guide for the Care and Use of Laboratory Animals, published by the Chinese National Institutes of Health.

Clinical trial

Nineteen patients, (11 females and 8 males, mean age 35 ± 2 years), who had been diagnosed with acromegaly and had received transsphenoidal surgery in Huashan Hospital but had not achieved biochemical remission at least 3 months after surgery, or who had not achieved biochemical remission after at least 3 months treatment with somatostatin analogs were enrolled in our study, and then were treated with rosiglitazone (No.188914, Taiji Group, Chongqing Fuling Pharmaceutical Factory Co., Ltd., Chongqing, China) for 6 months. Patients were treated with rosiglitazone 4 mg/day for 1 week to check if the treatment was tolerable. If there were no significant side effects like edema, the dose would be increased to 8 mg/day for 6 months. In fact, all patients have taken rosiglitazone 8 mg/day after the first week. Patients was followed up 3 months and 6 months after the treatment, including random GH and IGF-1 levels. Clinical characteristics of the 19 patients were presented in Table S1. The study was approved by the ethical review board at Huashan Hospital. This study was approved by the ethics committee at Huashan Hospital (KY2016-360) and was registered on clinicalTrial.gov (NCT03309319). Informed consent was obtained from all subjects.

Human pituitary samples

Normal pituitary tissue (n = 13) were obtained via autopsy from cadaver organ donation (Fudan University). Tumor tissue was obtained from 89 patients diagnosed with acromegaly, based on the presence of classic clinical features, with biochemical confirmation including the lack of serum GH suppression to less than 1 mg/L during an oral glucose tolerance test and/or an elevated serum IGF-1 level for age, who had undergone surgery in the Department of Neurosurgery at Huashan Hospital. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The pilot study was approved by the Huashan Hospital IRB, and written informed consent was obtained from all participants included in the study.

METHOD DETAILS

Human GH and IGF-1 measurements

GH was measured by a two-site chemiluminescent immunometric assay (AutoDELFIA hGH, PerkinElmer Life and Analytical Sciences, Boston, USA), and the intra-assay coefcient of variation (CV) was 5.3–6.5%, inter-assay CV was 5.7–6.2%, and sensitivity was up to 0.01 μg/L (0.026 mU/L). IGF-1 was measured with the Immulite 2000 solid-phase, enzyme-labeled chemiluminescent immunometric assay (Siemens Healthcare Diagnostic Products Limited, Marburg, Germany). The intra-assay CV was 2.3–3.5%; inter-assay CV, 7.0–7.1%; and sensitivity, 20 μg/L.

Immunohistochemistry

Expression of 15-PGDH was examined on paraffin-embedded pituitary tissue section with 1:100 dilution of 15-PGDH antibody (cat no: sc-98907, Santa Cruz Biotechnology, Boston, USA). Image analysis used Image Pro Plus 6.0 software (Media Cybernetics, Silver Spring, MD, USA).

Cell culture

GH3 cells were grown in Ham's F-12K (Kaighn's) Medium (cat no:21127030, Gibco™, Life Technologies, Carlsbad, USA), supplemented with 15% horse serum, 2.5% fetal bovine serum (FBS), 2 mM glutamine, and antibiotics. Human pituitary tumor cells were obtained by transsphenoidal surgery from 5 patients

with GH-secreting tumors. Tumor tissue from 5 patients with GH-secreting tumors was placed in 0.9% saline immediately after transsphenoidal surgery. Cells were grown in modified essential medium (MEM) containing D-Val instead of L-Val, to prevent fibroblast proliferation (Renner et al., 1994), 10% FBS and insulin 5 mg/L, transferrin 5 mg/L, sodium selenite 20 mg/L and nonessential amino acids. Human HepG2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (cat no: SH30243, HyClone, Logan City, USA) supplemented with 10% FBS and 1% penicillin-streptomycin-glutamine.

Real-time PCR (RT-PCR) analysis

Total RNA was extracted from pituitary tissues with TRIzol Reagent (cat no: 15596-026, Invitrogen, Carlsbad, USA) and was converted to cDNA according to the manufacturer's protocol. The sequences of the primers used were listed below:

human 15-PGDH: 5′-GCGATGGCTGCTAACCTT-3′ and 3′-ATTGCCCCATATTTTCTTCTTTT-5′;

human GH: 5′-AGTCTGTTTGCCAATGCTGTGCTC-3′ and 3′-GTGGGGGCTGGGATGGTCTC-5′;

human IGF-1: 5′-TTCACCAGCTCTGCCACGGC-3′ and 3′-AAGCAGCACTCATCCACGATG-5′;

human GHR: 5′-GCCGTTCACCTGAGCGAGAG-3′ and 3′-TCCCCAGCAGAAACATAATCAGG-5′;

human β-actin: 5′-AGAGGGAAATCCTGCGTGAC-3′ and 3′-CCATACCCAGGAAGGAAGGCT-5′.

Western Blot

Cells were lysed with lysis buffer containing protease inhibitor cocktail (Roche, Basel, Switzerland). Insoluble cell debris was removed by centrifugation and the supernatants saved for analysis. The amount of proteins was quantified using the Bradford reagent (Bio-Rad) (cat no: 23236, Thermo Fisher Scientific, Waltham, USA). For each sample, about 30 μg of total protein was resolved on a pre-cast 4–12% polyacrylamide gel (Invitrogen) and transferred electrically onto a PVDF membrane. The membranes were incubated with antibodies to 15-PGDH (cat no: sc-98907, Santa Cruz Biotechnology, Boston, USA), cleaved caspase-3 (cat no: #9661, Cell Signaling Technology, Boston, USA), β-actin (cat no: sc-81178, Santa Cruz Biotechnology), GAPDH (cat no: sc-365062, Santa Cruz Biotechnology), LC3-I (cat no: #4599, CST), LC3-II (cat no: #3868, CST), BECN1 (cat no: #3495, CST), ATG5 (cat no: sc-133158, Santa Cruz), ATG7 (cat no: sc-376212, Santa Cruz), P62 (cat no: #13121, CST), p-MTOR (cat no: sc-293133, Santa Cruz), MTOR (cat no: #2972, CST), p-AKT (cat no: #4060, CST), AKT (cat no: sc-81434, Santa Cruz), p-EIF4EBP1 (cat no: sc-293124, Santa Cruz), EIF4EBP1 (cat no: sc-81149, Santa Cruz), HSP90 (cat no: #4874, CST), and GHR (cat no: sc-137185, Santa Cruz).

Enzyme linked immunosorbent assay (ELISA)

Enzyme linked immunosorbent assays (ELISA) were performed to measure GH concentrations in the culture supernatant of GH3 cells. GH3 cells were seeded into a 6-well plate with 1×10^5 cells per well and cultured at 37°C for 48 hr and 72 hr with or without rosiglitazone (1, 10 or 50 μM) or pioglitazone (Pio, 50 μM). Then, the cell supernatant of each group was collected and the concentration of GH was measured using rat GH ELISA kit (cat no:ER0993, FineTest, China).

Short hairpin RNA (shRNA) transfection

Cells were seeded into 6-well plates and grown until 60–80% confluent. The shRNA for 15-PGDH was transfected with Lipofectamine 2000 (cat no: 11668019, Invitrogen) following the manufacturer's recommended protocol. The sequences designed for inhibiting 15-PGDH gene expression were GACACTGTTCATC CAGTGT (Rat shRNA1), GCGATGGCTGCTAACCTTA (Rat shRNA2), GCTCTCAATGGTGCCATTA (Rat shRNA3), GCAGGTGTACAGTGTAAAG (Human shRNA1) and GCATGGCATAGTTGGATTC (Human shRNA2). The sequences designed for 15-PGDH gene overexpression were GCGATGGCTGCTAACCTT (Rat). A scrambled siRNA without biological effects was used as negative control (NC). The sequences designed for NC were GAAGCCAGATCCAGCTTCC.

Cell proliferation assay

Cell proliferation assay was performed using cell counting kit 8 (CCK-8, Sigma) according to the manufacturer's instructions. Briefly, GH3 cells were cultured in 96-well plates (5×10^3 cells/well) and then were treated with 50 μM of Ros or 50 μM Ros plus 500 nM SW033291 (cat no: SML1485, Sigma) for 24 hr,

48 hr, 72 hr and 96 hr. After the indicated incubation times, 10 μL of CCK-8 was added to the plates and incubated for an additional 4 hr at 37°C. The absorbance was measured at 450 nm using a microplate reader (Bio-Rad Laboratories, Richmond, CA, USA).

Annexin V/propidium iodide (PI) assay

Apoptotic cell death was detected using the FITC Annexin V Apoptosis Detection Kit (BD Biosciences, San Jose, CA, USA). Briefly, GH3 cells (1×10^5 cells/well) were seeded in six-well tissue culture plates. After exposed to 50 μM Ros or 50 μM Ros plus 500 nM of SW033291 for 24 hr, attached and floating cells were both collected. Cells were washed with PBS and diluted in 100 μL of binding buffer. Then, samples were incubated with Annexin V-FITC and PI at room temperature for 15 min in the dark. After addition of 400 μL of binding buffer to each sample, the stained samples were analyzed using a flow cytometer (FACScan, Becton Dickinson, Franklin Lakes, NJ, USA). Data were analyzed using FlowJo software.

Confocal microscopy assay

Aautophagic flux was detected by transfecting GH3 cells with Ad-mCherry-GFP-LC3B (adenovirus expressing mCherry-GFP-LC3B fusion protein) (cat no: C3011, Beyotime, Shanghai, China). Ad-mCherry-GFP-LC3B expressing cells were grown on coverslips, fixed with 4% paraformaldehyde for 15 min in PBS, 0.2% Trtion X-100 for 15 min in PBS. Coverslips were mounted in antifade (Life Technologies) and examined under a confocal microscope (Zeiss 710, Germany).

Chromatin immunoprecipitation (ChIP) assay

The binding of PPARγ to the promoter of 15-PGDH was examined by ChIP assay. Cells (5.0×10^7) were cross-linked with 1% formaldehyde for 10 min at 37°C, and quenched by glycine (125 mM) for 5 min. Then, cells were resuspended in SDS lysis buffer with protease inhibitors and sonicated to generate chromatin fragments of about 300 bp in length. Samples were diluted with low-salt RIPA buffer and pre-cleared with 50 μL of Protein A and G for 1 hr at 4°C. PPARγ antibody (cat no: ab45036, abcom, Cambridge, UK) were added to pre-cleared supernatants, and the mixtures were incubated overnight at 4°C. 50 μL Protein A and G were then added to the samples, and the mixtures further were incubated for 2 hr at 4°C. The beads were subsequently washed with wash buffer (low-salt RIPA, high-salt RIPA, LiCl and TE). Precipitated chromatin was eluted in 200 μL elution buffer (0.1 M $NaHCO_3$ and 1% SDS). Reverse cross-linking was performed overnight at 65°C, and chromatin was then treated with RNase A and 5M NaCl for 30 min at 37°C and proteinase K overnight at 65°C. Eluted DNA was purified with PCR purification kit (Qiagen) and analyzed by quantitative PCR (qPCR) using the primers as listed in Figure 1B.

Dual luciferase activity assay

Human 15-PGDH promoter reporter construct used was generated by fusing fragments to the firefly luciferase cDNA in pGL4.10. HEK293t cells were transfected with pGL4.10, human 15-PGDH promoter -3001/-10, -3001/-2709 mutation (mut), -1966/-1692 mut, or -270/-10 mut with or without rosiglitazone.

Human 15-PGDH promoter -3001/-2709 mut were constructed with using the following primers:

F: 5′-TAAGGTGAATATGGTATTGTATAATTTAGTGTATAGGTTCTAAAAGC-3′;

R: 3′-ACCATATTCACCTTACATGTG-5′.

Human 15-PGDH promoter -1966/-1692 mut were constructed with using the following primers:

F: 5′-AATATCTATCTTCTCTGTCTACATTTCAGCTGTTCAGTTTTT-3′;

R: 3′-GAGAAGATAGATATTGTCATGGA-5′.

Human 15-PGDH promoter -270/-10 mut were constructed with using the following primers:

F: 5′-GAGACGCGGAGCTCGGGGTGGGCCCGCCCCAGCAGTGGC-3′;

R: 3′-CGAGCTCCGCGTCTCCGCGC-5′.

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Human GHR promoter reporter construct used was generated by fusing fragments to the firefly luciferase cDNA in pGL4.10. HEK293t cells were transfected with the human GHR promoter with or without CREB. After 24 hr, luciferase assays were performed using the Dual-Luciferase™ reporter assay kit (Promega) according to the manufacturer's instructions.

QUANTIFICATION AND STATISTICAL ANALYSIS

All experiments were independently repeated at least three times. All data are expressed as mean $\pm$ SD. Statistical analyses were performed with Prism 7.0a (GraphPad Software, La Jolla, CA) or performed with SPSS 16.0 statistical software (IBM Corporation, Armonk, NY, USA). Means were compared using the Student *t* test when data distribution was normal, or by Wilcoxon rank-sum (Mann-Whitney) test when variables were not normally distributed. Correlations were performed, and Pearson correlation coefficients, or the Spearman Rank correlation coefficients when variables were normally, or not normally distributed, respectively, are reported. A two-sided significance level of 5% was considered statistically significant.

ADDITIONAL RESOURCES

Clinical trial registry numbers is NCT03309319 and Clinical trial registry link is at https://clinicaltrials.gov/ct2/results?cond=&term=NCT03309319&cntry=&state=&city=&dist=.