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Sphingosine 1-phosphate suppresses the viability and estradiol secretion of mouse granulosa cells by activating Gα12/13-YAP signaling

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ABSTRACT: Polycystic ovary syndrome (PCOS) is a disease with abnormal hormones affecting 6–8% of women in reproductive age. Accumulating evidence supports the critical role of sphingosine 1-phosphate (S1P), a biologically active lipid, in PCOS mechanisms. Downstream of S1P, the G protein-coupled receptor (GPCR) family member S1PRs transduce the signal to the Hippo/yes-associated protein 1 (YAP) signal axis which might be involved in the survival, growth, and death of ovarian granulosa cells (GCs), which let us suppose whether S1P contributes to the development of PCOS by regulating Gα12/13-YAP signaling in GCs. This study was designed to examine the action of the S1P/GPCR/YAP signal axis on GCs with a focus on estrogen response of cell viability. The distribution of all S1PRs in mouse ovarian GCs was determined, and it was found that S1PR2 exhibited the strongest expression. In GCs, S1P inhibited YAP phosphorylation at Ser127 and promoted YAP translocation from cytosol to the nucleus. S1P attenuated viability of GCs through activating YAP from Gα12/13 under GPCR activation, which was restored by excessive β-estradiol, indicating the role of S1P/GPCR/YAP signal in estrogen response. Furthermore, S1P also activated YAP to suppress the secretion of estrogen from GCs. The findings presented here provide an inviting therapeutic option for PCOS which involves the dysfunction of GCs.

KEYWORDS: polycystic ovary syndrome (PCOS); sphingosine 1-phosphate (S1P); YAP; granulosa cells

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a disease with abnormal hormones that happens in 6–8% of women of reproductive age [1]. As the major cause of anovulatory infertility, PCOS brings large economic and social burdens to affected women [2]. In PCOS patients, the small sacs of fluid called cysts develop along the outer edge of the ovary, and the small fluid-filled cysts contain follicles that fail to release eggs [3]. As known, the primary oocyte quality plays an utmost important role in the cleavage and implantation of the embryo as well as the maintenance of pregnancy and even fetus growth [4]. The characteristics of PCOS such as hyperandrogenemia and hyperinsulinemia impair folliculogenesis, leading to the premature arrest of immature developing oocytes. As a result, oocytes collected from PCOS patients by *in vitro* fertilization always exhibit low quality [5, 6], and the rare embryos produced by these oocytes were difficult to develop into the next stages [2]. The exact etiology of PCOS which would offer an innovative therapeutic strategy is still unknown.

Ovary is a solid, ovoid structure surrounded by a layer of germinal epithelium. Under this layer, a dense connective tissue capsule is divided into the outer cortex and inner medulla. The cortex stores ovarian follicles in different stages of development, where granulosa cells (GCs) proliferate and develop into multiple cell types. PCOS pathogenesis is associated with the abnormality of GCs, including the growth and programmed cell death of these cells [7].

Estrogen signaling in PCOS pathogenesis has received much recent attention in the field. The GCs serve as the major source of estrogen in ovaries, and as feedback, the estrogen (including β-estradiol) regulates the growth and apoptosis of GCs [8]. Therefore, the estrogen signal in GCs offers a distinctive therapeutic opportunity for PCOS.

With more than 800 members, G-protein-coupled receptors (GPCRs) are considered the largest family of membrane receptors in mammalian cells [9]. Recent reports indicated that multiple ligands of GPCRs, including proton, metabolites, peptide hormones and secreted proteins, regulate Hippo/(YAP) pathway [10], an evolutionarily conserved signaling cascade playing indispensable roles in the development and function of GCs [11–13]. Hence, it would be intriguing to explore if the GPCR signaling exerts its effects on the survival and growth of GCs through YAP and the relevance to PCOS pathogenesis.

Sphingosine 1-phosphate (S1P) is a biological active lipid generated by the ceramidase-catalyzed conversion of ceramide to sphingosine and subsequent

sphingosine kinase (SK)-catalyzed conversion of sphingosine to S1P. GPCRs named S1PR1-S1PR5 function as S1P receptors that coupled to distinct heterotrimeric G proteins and Rac or Rho to control a broad range of downstream effectors [14]. A recent study included 107 women with PCOS and 37 healthy women demonstrated the involvement of S1P in PCOS, which was implicated by the metabolomic approach using liquid chromatography-mass spectrometry that reported a significant elevation of S1P in the serum of PCOS patients [15]. In a mouse model of PCOS where acute hyperandrogenism on ovulation was induced by injecting pregnant mare serum gonadotropins (PMSG)-primed pubertal mice with dihydrotestosterone, the mimics of androgen, YAP1 was necessary for the proliferation of GCs but was down-regulated by luteinizing hormone through the extracellular-regulated kinase-1/2 cascade [16]. However, the action of the S1P/GPCR/YAP signaling axis in PCOS remains unclear.

Motivated by accumulating evidence supporting the critical roles of S1P and GPCR/YAP in GCs, the overarching goal of this study is to examine the action of S1P/GPCR/YAP signal axis on GCs with emphasis on estrogen response and the relevance to PCOS mechanisms. The distribution of all S1PRs in mouse ovarian GCs was determined which found that S1PR2 exhibited the strongest expression. In these cells, S1P inhibited YAP phosphorylation at Ser127 and promoted YAP translocation from cytosol to the nucleus. GPCR, coupled by its downstream effectors, Gα12/13, mediated S1P signaling for YAP in mouse GCs. S1P attenuated the growth of GCs through activating YAP from Gα12/13, which was restored by excessive β-estradiol, indicating the role of S1P/GPCR/YAP signal in estrogen response. Furthermore, S1P also activated YAP to suppress the synthesis and secretion of estrogen from GCs. The findings are vital for delineating signal transduction mechanisms involved in GCs in PCOS, where beneficial therapeutic outcomes can be achieved.

MATERIALS AND METHODS

Preparation of primary GCs

Three female C57BL/6j mice (6–8 weeks, 22–25 g) purchased from Provincial Laboratory Animal Public Service Center (Wuhan, China) were injected with PMSG (Sigma-Aldrich, MO, USA) (10 IU) and human chorionic gonadotrophin (HCG; Sigma-Aldrich) (10 IU) and then killed 24 h later. Ovaries were dissected, and the GCs were extracted. The GCs were collected and then centrifuged in Trypsin at 37 °C for 3 min, and DMED Medium with 10% FBS and 1% Penicillin-streptomycin solution was added. The GCs were maintained at 37 °C containing 5% CO_2. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This study was approved by the Hubei Provincial Hospital of Integrated Chinese and Western Medicine (Approval no. KY-2022-10-02).

Treatment and transfections of GCs

The GCs were treated with 0.3 μM S1P (Sigma-Aldrich) for 12, 24, and 48 h, and the treatment time of 0 h served as the control group.

For G12/13 knockdown, 2 short hairpin (sh) RNAs targeting G12 (sh-G12-1 and sh-G12-2) or G13 (sh-G13-1 and sh-G13-2) were inserted into the pLKO.1 vector (Addgene, Cambridge, MA, USA). The GCs were transfected with the above-mentioned plasmid using Lipofectamine® 2000 reagent in accordance with the manufacturer's manuals for 48 h.

Immunofluorescence staining

GCs growing on the glass slide were washed in PBS, fixed in Immunol Staining Fix Solution (Beyotime Biotechnology, Beijing, China) for 10 min, permeabilized with 0.5% Triton-X100 (Beyotime Biotechnology) for 10 min, blocked for 1 h at RT with 5% BSA (Sigma-Aldrich), and then incubated with primary YAP antibodies (Cell Signaling Technology, MA, USA; #14074, 1:200 dilution) at 4 °C for overnight. Cells were then rinsed 3 times in PBS and sequentially incubated with Alexa Fluor 555 donkey anti-rabbit secondary antibody (Beyotime Biotechnology, 1:1000) for 1 h at RT. Cells were finally washed in PBS to omit the antibody mixture. An anti-fade Mounting Medium with DAPI (Solarbio, Beijing, China) was used for samples. Fluorescence images were acquired with an Olympus BX61 fluorescence microscope (at 200 × magnification).

Quantitative real-time PCR (RT-qPCR) and reverse transcription PCR (RT-PCR)

Total messenger RNA was extracted from cells with TRIzol reagent (Thermo Fisher Scientific, USA). The mRNA was then reverse-transcribed to produce cDNA using Revert-AidTM cDNA Synthesis Kit (Thermo Fisher Scientific). The RT-qPCR reactions were conducted with cDNA (2 μg) employing SYBR Mix (Thermo Fisher Scientific), taking GAPDH as the internal reference. The primers for PCR reactions described in this manuscript were designed by Invitrogen software, and their sequences were listed in Table 1. The RT-qPCR was conducted on the Real-time PCR 7300 System (Applied Biosystems, USA).

An RT-PCR was performed with the same program as RT-qPCR for GCs. The PCR products were run on a 2% agarose gel electrophoresis along with a PCR marker (Thermo Fisher Scientific).

Western blot analysis

The lysate of whole cells was quickly generated in a pre-cold RIPA buffer. NE-PER™ Extraction Reagents (Thermo Fisher Scientific) were used to prepare the

Table 1 Sequences of PCR primers used in this study.

Gene	Primer		Product size (bp)
S1PR1	Forward (5′–3′) Reverse (5′–3′)	ATGGTGTCCACTAGCATCCC CGATGTTCAACTTGCCTGTGTAG	112
S1PR2	Forward (5′–3′) Reverse (5′–3′)	ACAGCAAGTTCCACTCAGCAA CTGCACGGGAGTTAAGGACAG	131
S1PR3	Forward (5′–3′) Reverse (5′–3′)	ACTCTCCGGGAACATTACGAT CCAAGACGATGAAGCTACAGG	121
S1PR4	Forward (5′–3′) Reverse (5′–3′)	CTGGCTACTGGCAGCTATCC AGACCACCACACAAAAGAGCA	128
S1PR5	Forward (5′–3′) Reverse (5′–3′)	GCTTTGGTTTGCGCGTGAG GGCGTCCTAAGCAGTTCCAG	218
GAPDH	Forward (5′–3′) Reverse (5′–3′)	AATGGATTTGGACGCATTGGT TTTGCACTGGTACGTGTTGAT	213

cytosolic and nuclear fractions according to the protocol provided along with the reagents by the manufacturer. Protein was measured for concentration using a BCA kit (Thermo Fisher Scientific) with the protocol provided by manufacturers. The protein extract was resolved on the 10–15% SDS-PAGE gel, transferred, and electro-blotted onto a nitrocellulose membrane (Millipore). Membrane with transient wash was blocked by incubation with 5% non-fat milk, and then added with primary antibodies against YAP (Cell Signaling Technology; #14074, 1:1000 dilution), phospho-YAP (Ser127) (Cell Signaling Technology; #4911, 1:1000 dilution), or GAPDH (Beyotime Biotechnology; #AF1186, 1:1000 dilution) at 4 °C for 16 h. The secondary antibodies with conjugation of horseradish peroxidase (Cell Signaling Technology) were used for chemiluminescent signal determination. Immunoreactive signals were revealed by the kit of ECL chromogenic substrates (Bio-Rad, Hercules, USA). The expression of GAPDH was used as a normalization control for protein loading.

Hormone analysis

GCs with or without transfection were cultured in the presence and absence of 0.3 μM S1P. After 48 h, cell supernatant was collected by centrifugation (3,000*g*; 5 min) to detect estradiol with the commercial Enzyme-Linked Immunosorbent Assay kit (Sigma-Aldrich; #SE120084) according to the manufacturer's instructions.

Statistical analysis

All data were from 3 independent experiments and reported as the mean ± standard deviation (SD). Statistical analysis was done using GraphPad Prism 8.4.2 software (San Diego, CA, USA). A two-tailed unpaired Student's *t*-test was conducted to compare 2 groups. A one-way ANOVA combined with Tukey's post-multiple test was conducted to compare among multiple groups.

RESULTS

Distribution of S1P receptors in mouse ovarian GCs

To evaluate if S1P enables signal transduction in mouse GCs, the expression of mouse S1PR1- S1PR5 was examined in mouse GCs. RT-PCR was conducted for total RNA extraction from these cells. Though S1PR1-S1PR3 were all expressed, S1PR2 exhibited the strongest expression level (Fig. 1).

Activation of the nuclear translocation of YAP in mouse GCs by S1P

To elucidate the effects of S1P on YAP in mouse GCs, the cells were treated with S1P, and the subcellular distribution of YAP was determined by immunofluorescent staining. S1P increased the translocation of YAP from cytosol to nucleus in a time-dependent manner (Fig. 2A). With the inactivation of Hippo signal, the nuclear translocation of YAP is caused by dephosphorylation of YAP at Ser127 residue. Supporting the inhibitory role of S1P on Hippo, Western blot analysis showed that the Ser127 phosphorylation of YAP was reduced in mouse GCs by treatment of S1P in a time-dependent manner, while the expression level of total YAP displayed no observable difference after the treatment of S1P (Fig. 2B). Thus, YAP function in nucleus was activated in mouse GCs under S1P stimulation.

Mediation of S1P activation to YAP in mouse GCs by Gα12/13

Under stimulation, the downstream effectors of GPCRs such as Gα12/13, Gaq/11 or Gαi/o mediate the signal to activate YAP/TAZ. To test whether S1P transduces signal through S1PR2 and Gα12/13 to activate YAP in mouse GCs, the cells were transfected with a lentiviral vector to express shRNA targeting Gα12/13, respectively (the genes of GNA12/13). The efficiency of

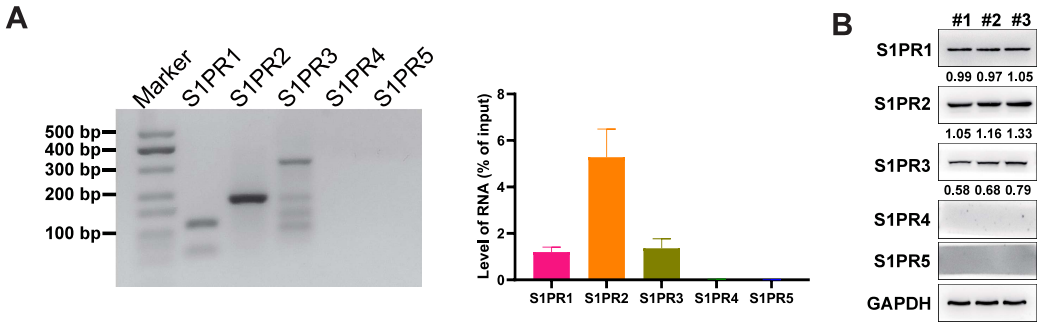

Fig. 1 Distribution of S1PR in mouse GCs. The GCs were prepared freshly, and total RNA was extracted. (A) The RT-PCR analysis showing that only S1PR2 has high expression level, while S1PR1 and S1PR3 also showed mild level expression. (b) Western blot analysis confirming the results of RT-PCR analysis.

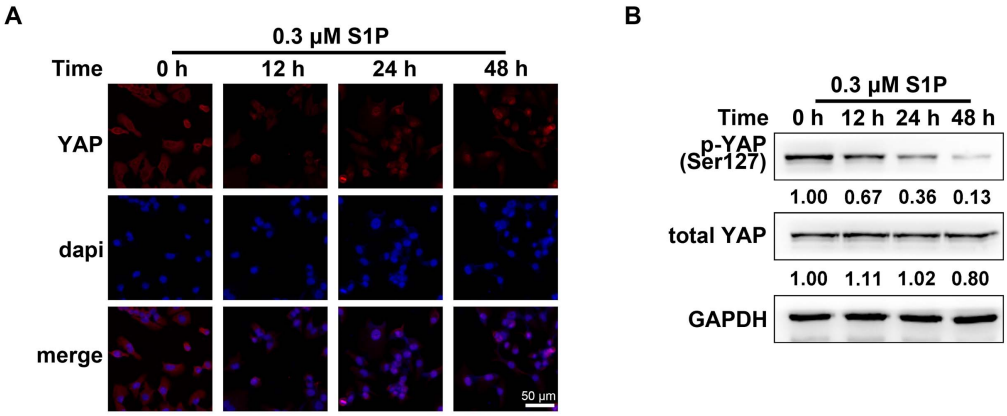

Fig. 2 YAP nuclear translocation and phosphorylation in GCs with S1P treatment. GCs were prepared freshly and treated with S1P for various durations. (A) Immunofluorescent staining of YAP together with DAPI nuclear staining in S1P-treated cells. (B) Western blot analysis for YAP Ser127 phosphorylation, total YAP, with GAPDH as a control.

RNAi knock-down was confirmed. Similar to mouse GCs transfected with RNAi control, the mouse GCs with Gα12 or Gα13 RNAi showed the mainly cytosolic distribution of YAP in immunofluorescent staining. The S1P treatment for mouse GCs transfected with RNAi control promoted nuclear translocation of YAP, but the S1P treatment for mouse GCs with Gα12 or Gα13 RNAi showed unchanged cytosolic YAP distribution (Fig. 3A). Consistently, mouse GCs transfected with RNAi control or with Gα12 or Gα13 RNAi all exhibited a high level of Ser127 phosphorylation of YAP. S1P treatment reduced Ser127 phosphorylation of YAP in mouse GCs transfected with RNAi control, but not in GCs transfected with Gα12 or Gα13 RNAi (Fig. 3B). These findings demonstrated the activity of S1P/S1PR/Gα12/13/YAP signal axis in mouse GCs.

Activation of Gα12/13 and YAP to suppress the estradiol response of mouse GCs by S1P

The survival, growth, and death of mouse GCs affect a plethora of processes involved in PCOS. S1P treatment strongly attenuated the viability of mouse GCs shown by CCK-8 assay (Fig. 4A). Mouse GCs require estrogen signaling for survival and growth, evidenced by the fact that an excessive supply of estradiol in culture medium dramatically increased cell viability. Under such conditions, excessive estradiol also protected mouse GCs from viability reduction by S1P (Fig. 4A), indicating the importance of S1P/Gα12/13/YAP in estrogen response in mouse GCs.

To determine the role of Gα12/13 and YAP in viability weakening in mouse GCs by S1P treatment, the Gα12/13 and YAP were efficiently depleted by RNAi knock-down. The GCs transfected with RNAi control exhibited a reduction of viability by S1P stimulation shown by CCK-8 assay, while the mouse GCs transfected with Gα12 or Gα13 RNAi no longer responded to S1P treatment (Fig. 4B). Drawing a parallel, compared with mouse GCs transfected with non-targeting control RNAi, viability of mouse GCs transfected with YAP RNAi did not show an observable difference between S1P treated group and the control

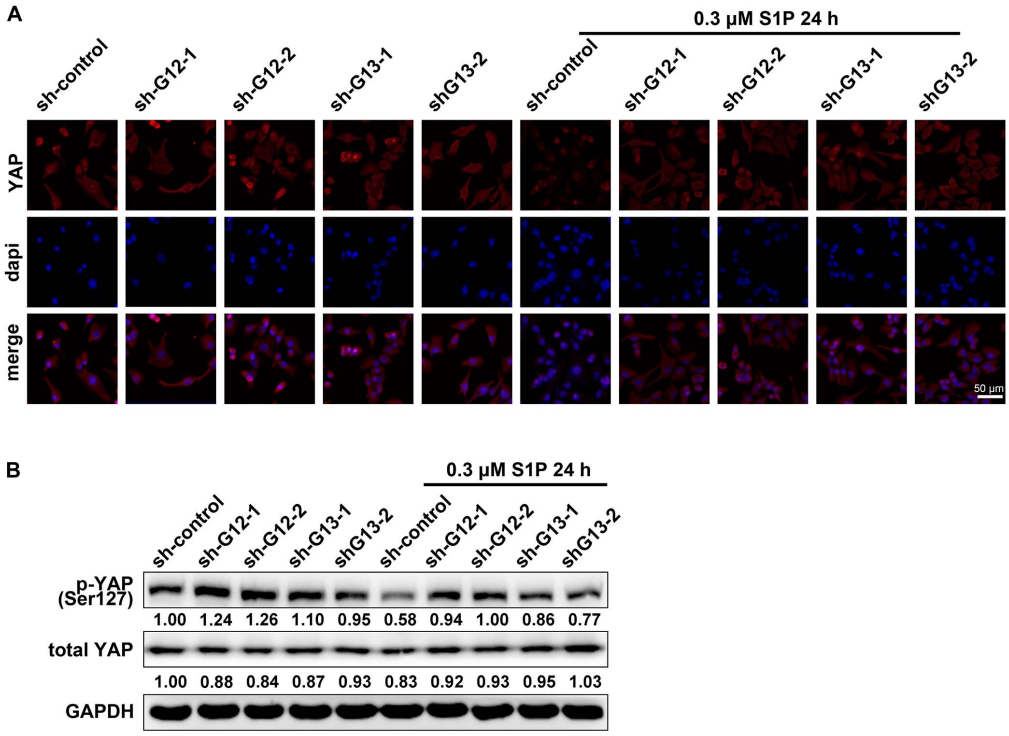

Fig. 3 Knock-down of Gα12 or Gα13 restoring effects of S1P on YAP nuclear translocation and phosphorylation in GCs. GCs were transfected with shRNA-lentivirus to deplete Gα12 or Gα13 and then treated with S1P. (A) Immunofluorescent staining of YAP together with DAPI nuclear staining in S1P-treated control and S1P-treated Gα12- or Gα13-RNAi cells. (B) Western blot analysis for YAP Ser127 phosphorylation, total YAP, with GAPDH as a control.

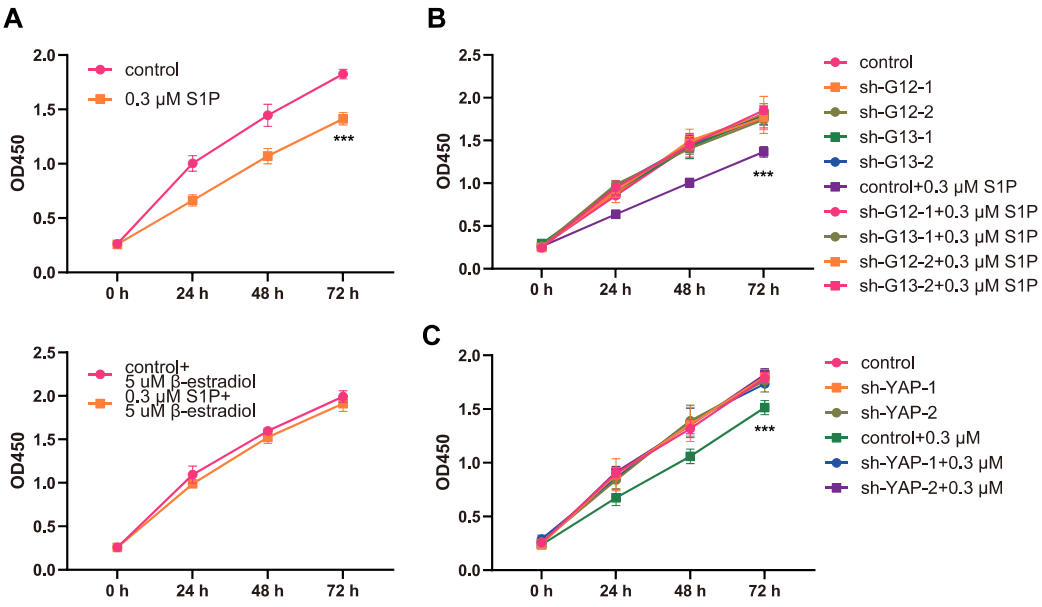

Fig. 4 Activation of Gα12/13 and YAP to suppress estradiol response of cell viability in GCs by S1P. (A) CCK-8 assay conducted to detect cell viability of GCs treated with S1P together with beta-estradiol in multiple time points. GCs transfected with shRNA-lentivirus specific to (B) Gα12 or Gα13 or (C) YAP, treated with S1P. CCK-8 assay was conducted to detect cell viability in multiple time points.

group (Fig. 4C). Therefore, the activation of Gα12/13 and YAP by S1P in mouse GCs mediated the viability reduction.

Activation of Gα12/13 and YAP to suppress the secretion of estradiol in mouse GCs by S1P

It is plausible that GCs serve as the major source of estrogen in ovaries. S1P treatment lowered estradiol released from mouse GCs with transfection of RNAi control into culture medium. The efficient knock-down of Gα12 or Gα13 resulted in an unchanged high level of estradiol in culture medium with or without S1P treatment (Fig. 5). Similarly, S1P did not ameliorate estradiol release from the GCs with efficient knock-down of YAP (Fig. 5). Thus, inactivation of Gα12/13 and YAP is required for estradiol production and secretion in mouse GCs, and S1P activated Gα12/13 and YAP to suppress estradiol supply from mouse GCs.

DISCUSSION

PCOS is one of the most common causes of female infertility and frequently associated with abnormal hormone levels [1]. The studies of mechanisms of PCOS are currently focused on the dysfunction of ovaries and in particular, the GCs within them. Ovarian GCs proliferating in developing follicles are multipotent cells that differentiate into various cell types, like neuronal cells, chondrocytes, and osteoblasts *in vitro* [17]. The proliferation and differentiation of GCs strongly affect the growth and ovulation of follicles and more ovarian functions such as corpus luteum formation and steroid hormone secretion. Together with theca cells, GCs help to synthesize multiple ovarian hormones that support the microenvironment of oocytes [18]. PCOS pathogenesis is associated with abnormality of survival, growth, and death of GCs [7]. Therefore, the present study was designed to evaluate the critical role of GPCR/YAP pathway, under stimulation of S1P, in the growth and hormone synthesis function of GCs.

S1P is a biological active lipid activating five different GPCRs (named S1PR1–S1PR5) to control a broad range of downstream effectors [14]. S1PR1 selectively couples to members of the Gi family; S1PR2 and S1PR3 couples to members of the Gi, Gq, and G12/13 families [19]. On the contrary, S1PR4 is only detected in the lungs and lymphoid tissues while S1PR5 is only expressed in the brain, skin, and spleen [20]. To test the role of S1P in GCs and the involvement of GPCRs, the present study confirmed the distribution of specific S1P receptors in mouse GCs (Fig. 1) and found the inhibitory effect of S1P on cell growth of GCs (Fig. 4A). Our data showed that S1PR1–S1PR3 were expressed in mouse GCs, while S1PR2 exhibited the strongest expression level. S1PR1 and S1PR3 have been reported to be primarily involved in S1P-induced inflammatory response gene expression [21]. Unlike S1PR1, which couples to Gαi and promotes directed cell migration, S1PR2 inhibits the migration of various cell types [22, 23]. Rather than coupling to Gαi, S1PR2 couples to Gα12/13, 2 closely related G proteins, and possibly also Gαq, thus providing an explanation for the different outcomes of signaling by the 2 receptors [24]. Combined with our data, we wonder whether S1PR2 is involved in the pathological processes of mouse GCs during PCOS by interacting with Gα12/13. The rescue of cell growth attenuation by RNAi knock-down of Gα12/13 under this condition indicated that Gα12/13 served as hub to mediate the action of S1P/S1PR on GCs (Fig. 4B). We also provided compelling evidence that S1P inhibited estrogen release from GCs which was mediated by Gα12/13 (Fig. 5).

One of the pathways downstream of GPCR is Hippo/YAP pathway. It is now universally appreciated that Hippo/YAP is involved in a myriad of core processes governing organ size, including proliferation, apoptosis of differentiated cells, and self-renewal of stem cells [10]. The dysfunction of Hippo/YAP pathway is associated with various types of cancers in humans [9]. When the Hippo signaling is activated, upstream signals promote the phosphorylation of macrophage-stimulating protein (MSP) 1/MST2, large tumor suppressor kinase (LATS) 1/LATS2 kinases, which phosphorylates YAP/Tafazzin family (TAZ) proteins. Phosphorylated YAP/TAZ recruits 14-3-3 proteins that stimulate cytoplasmic retention or proteolytic degradation of YAP/TAZ [25]. Once Hippo signaling is inactivated, YAP/TAZ is phosphorylated at lower level, which promotes their translocation to the cell nucleus, where YAP/TAZ form protein complex with transcription factor TEA domain family member (TEAD) 1-TEAD4 to regulate downstream genes required for a broad range of cellular functions [26].

Accumulating evidence ascertains that Hippo/YAP signaling acts as a critical determinant of the development and function of GCs in ovaries. The exosomes produced and secreted from mesenchymal stem cells contained a large amount of microRNA-21, which was delivered to GCs to inhibit LATS1 kinase. This will promote the production and secretion of estrogen, and LATS phosphorylated YAP and lysyl oxidase like protein 2 to promote StAR expression in GCs [27]. In addition, miR-484 suppressed YAP expression to induce mitochondrial dysfunction in GCs, which reduced cell viability and enhanced cell apoptosis [28]. It is noteworthy that YAP also coordinates with epidermal growth factor (EGF) signaling to regulate the survival and growth of GCs. EGF promoted phosphorylation and reduced expression of YAP [29], while YAP enhanced expression of EGF receptor (EGFR) and its downstream effectors of EGFR such as epiregulin, early growth response 1, and TNF alpha-induced protein 6 [13]. These reports let us suppose that the role of S1P/S1PR2/ Gα12/13 in GCs may be mediated by the

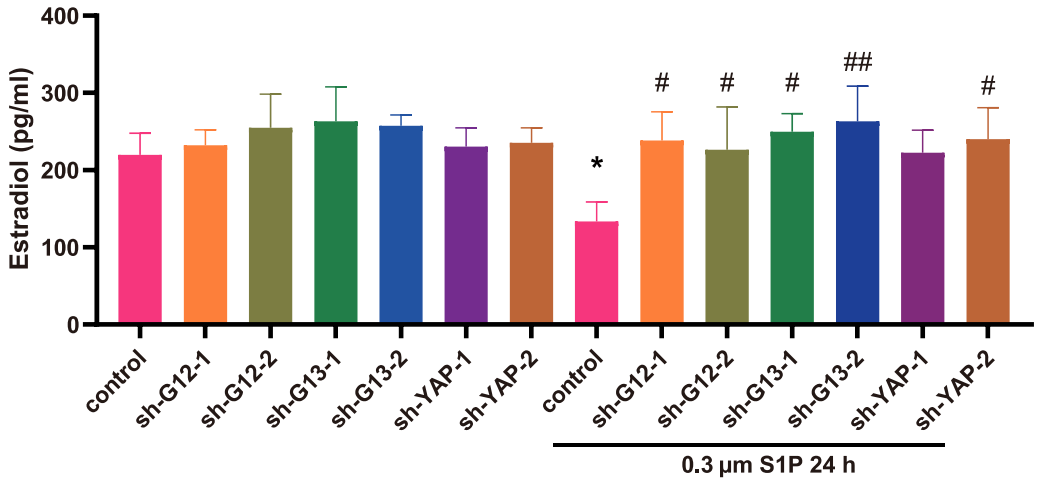

Fig. 5 Activation of Gα12/13 and YAP to suppress estradiol production and release in GCs by S1P. GCs were transfected with shRNA-lentivirus to deplete Gα12, Gα13, or YAP and then treated with S1P. Data represented as mean ± standard deviation (SD). $^{*}p < 0.05$, vs. control; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$, vs. control+S1P group.

YAP signaling. By finding that S1P suppressed YAP phosphorylation and promoted YAP nuclear translocation (Fig. 2), which could be attenuated by RNAi knock-down of Gα12/13 (Fig. 3), the action of signal axis of S1P/GPCR/YAP in mouse GCs was confirmed. Further study demonstrated that YAP knock-down efficiently restored the inhibitory effects of S1P on mouse GC cell growth and estrogen release (Fig. 4D and Fig. 5), which was highly instrumental in revealing the importance of the GPCR/Hippo/YAP signal axis in GCs.

Estrogen signaling in PCOS pathogenesis has received much recent attention. The GCs serve as the major source of estrogen in ovaries, while the estrogen (including estradiol) regulates the growth and apoptosis of GCs. In rat ovaries, the defective 5-HT system resulted in reduced estradiol availability in ovaries which attenuated growth and enhanced apoptosis of GCs [30]. The importance of estradiol was also reported for cultured rabbit ovarian GCs, where the overexpression of Cathepsin S promoted secretion of estradiol to support cell survival and growth [31]. Four types of estrogen receptors (ERs), ERβ1/2/4/5, were highly expressed in human ovarian GCs, and expression of ERβ4 and ERβ5 were controlled by estradiol. ERβ4, compared with other ERβs, played more critical role in apoptosis of GCs [32]. The role of ER-alpha receptor in PCOS was reported in a rat model, where ER-alpha receptor activated the PI3K/Akt/mTOR signal axis to promote survival and growth and inhibit the apoptosis of GCs [33]. The function of estrogen in PCOS was also implicated in a report about Apelin, a cytokine secreted from adipose tissue, that inhibit the activity of estrogen and IGF-1 on GCs to accelerate the PCOS pathogenesis [34]. To address this issue, our findings showed that the role of S1P in mouse GC growth control was strictly dependent on the abundance of estrogen in the environment (Fig. 4A), and the S1P exerted an inhibitory effect on the release of estradiol from GCs, which required the GPCR/YAP signaling axis (Fig. 5). Therefore, the estrogen signal in GCs offers a distinctive therapeutic opportunity for PCOS.

CONCLUSION

In the present study for mouse ovarian GCs, S1P inhibited YAP phosphorylation at Ser127 and promoted YAP translocation from cytosol to the nucleus. GPCR, coupled with its downstream effectors, Gα12/13, mediated S1P signaling for YAP in mouse GCs. S1P attenuated the growth of GCs through activating YAP from Gα12/13, which was restored by excessive β-estradiol, indicating the role of S1P/GPCR/YAP signal in estrogen response. Furthermore, S1P also activated YAP to suppress the synthesis and secretion of estrogen from GCs, indicating the involvement of S1P/YAP signal axis in multiple aspects of the estrogen response of GCs which could be an essential mechanism of PCOS. Overall, the findings reported here provide a faithful model for S1P/GPCR/YAP signal in GCs which enables insights into the clinical therapeutic development of PCOS. The importance of GCs in the physiology and pathology (like PCOS) of ovaries has been universally appreciated in the field, though function and diseases of ovaries require the interplay of various cell types. The signal axis shown here for GCs, the S1P/GPCR/YAP signal, will be reasonably considered a critical mediator and therapeutic target of PCOS pathogenesis, and its roles in other ovary cell types related to PCOS will also be addressed by our group in future studies.

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