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Original Article

$P2Y_6$ purinergic receptor regulates steroid synthesis and proliferation of ovarian luteal cells

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Abstract: Aim: As an important messenger, Uridine 5'-diphosphate (UDP) is involved in series of physiological and pathological processes through activating $P2Y_6$ purinergic receptor. However, the detailed function and possible mechanism of $P2Y_6$ in ovarian luteal cells remain unclear. Methods: Primary murine luteal cells were isolated and cultured. CCK-8 assay was employed to analyze the cell viability after $P2Y_6$ agonist (UDP) and $P2Y_6$ selective antagonist (MRS2578) treatment. Radioimmunoassay was used to assess the progesterone and estradiol production. The expression of three essential steroidogenic enzymes, including the p450 cholesterol side-chain cleavage enzyme (CYP11A), 3β-hydroxysteroid dehydrogenase (3β-HSD) and steroidogenic acute regulatory protein (StAR) were examined by Western blotting. We also examined the ERK1/2 phosphorylation level and the expression of steroidogenic factor 1 (SF1). Results: We found that $P2Y_6$ was highly expressed in murine luteal cells. UDP decreased the progesterone secretion and MRS2578 rescued the effect of UDP. Further studies showed that CYP11A, 3β-HSD and StAR were regulated by UDP. UDP treatment also decreased ERK1/2 phosphorylation, concomitant with decreased expression of SF1, while MRS2578 treatment relieved the effect of UDP. Conclusions: In conclusion, this study demonstrated for the first time that UDP/$P2Y_6$ purinergic signaling regulated progesterone secretion by inhibiting the expression of CYP11A, 3β-HSD and StAR in luteal cells. The ERK1/2 MAPK signaling and downstream SF1 may contribute to the process.

Keywords: $P2Y_6$, UDP, progesterone, luteal cell

Introduction

Nucleotide receptors, also known as P2 receptors, together with P1 receptor subfamily, form the purinergic receptor family [1]. P2 receptors consist of two classes of purinergic receptors. P2X receptors belong to cationic channels and P2Y receptors are G protein-coupled receptors (GPCR) [2]. In mammals, seven different P2X subunits ($P2X_1$-$P2X_7$) and eight P2Y subtypes have been identified [3]. Eight P2Y receptors are subdivided into two distinct clusters based on different coupled G proteins: G_q-coupled receptors ($P2Y_{1, 2, 4, 6, 11}$) and G_i-coupled receptors ($P2Y_{12, 13, 14}$) [4]. Among them, $P2Y_6$ is widely expressed in diverse tissues including placenta, spleen, thymus, intestine, leukocytes, heart, liver, blood vessels, ovary, and microglial and rat aorta, spleen, stomach, intestine, lung, dorsal root ganglia, spinal cord, ovary, etc [5-9]. Extensive expression of $P2Y_6$ indicates it may play numerous critical roles in diverse systems.

$P2Y_6$ is a uridine 5'-diphosphate (UDP) preferring receptor, as UDP derivatives have higher potential in $P2Y_6$ activation than corresponding 5'-triphosphates [10, 11]. Accumulating data showed that UDP/$P2Y_6$ played a vital role in immune response. $P2Y_6$ modulated IL-8 secretion from monocytes [12, 13]. UDP/$P2Y_6$ signaling prevented microglia phagocytosis of viable neurons [14]. As a danger signal, UDP and $P2Y_6$ were found to protect mice from virus infection by increasing IFN-beta production [15]. In osteoclasts, functional $P2Y_6$ receptors initiated NF-κB signaling to enhance osteoclasts survival, as a result of inflammation and mechanical stimulation [16]. What's more, $P2Y_6$ triggered the pressure overloaded-induced cardiac fibrosis and mediated vascular inflammation [17, 18]. These studies indicate that UDP/$P2Y_6$ is

Table 1. Oligonucleotides used for reverse transcription PCR

Gene	Gene bank number	Sense and antisense primer	Product size (bp)
$P2Y_1$	NM008772.5	5'-TGGCGTGGTGTACCCTCTCAAGTC-3' 5'-CGGGACAGTCTCCTTCTGAATGTA-3'	557
$P2Y_2$	NM008773.4	5'-CTGCCAGGCACCCGTGCTCTAACTT-3' 5'-CTGAGGTCAAGTGATCGGAAGGAG-3'	341
$P2Y_4$	NM020621.4	5'-ACTGGAACTAAGATGGTGCTCCT-3' 5'-GCAGATGCCCATGTAGCGGT-3'	558
$P2Y_6$	XM011241739.2	5'-AGCCCACCCATCCTGTCT-3' 5'-GGCCGAGTGCCTTTGTAG-3'	322
$P2Y_{12}$	NM027571.3	5'-CCATTGACCGCTACCTGA-3' 5'-GGAACTTTGGCTGAACCC-3'	330
$P2Y_{13}$	NM028808.3	5'-CTATGAGACGATGTATGTGGGTAT-3' 5'-CTTGTGCCTGCTGTCCTTAC-3'	378
$P2Y_{14}$	NM133200.4	5'-CCTTGCTGTCCCAAACAT-3' 5'-ACCTTCCGTCTGACTCTTT-3'	336

involved in a comprehensive range of physiological and pathological functions in different tissues. In the ovary, various P2 receptors have been found and proved to play indispensable roles. $P2X_7$ receptor was specifically expressed on porcine ovarian thecal cells and murine luteal cells. Its activation induced calcium-dependent cell apoptosis and decreased cell proliferation [19, 20]. Recent study showed that the $P2Y_6$ receptor was expressed in ovarian thecal cells and its activation promoted cell proliferation via triggering ERK1/2 signaling pathway [21].

The corpus luteum is a temporary but dynamic endocrine gland and originates from remaining granulosa and theca cells of the ovulated follicle. The main function of corpus luteum is secreting large amounts of progesterone, which plays a central role in regulating estrous cycle and the establishment and maintenance of pregnancy [22]. Luteinization of ovulated follicle is the most sophisticated event among mammalian reproduction, including the formation, maintenance and regression. Multiple factors, including hormones, cytokines, immune cells and nerves tightly regulate these processes [23, 24]. In this study, we found that $P2Y_6$ was highly expressed in murine luteal cells. However, the physiological role of $P2Y_6$ receptor in luteal cells is not clear. We will explore the role of UDP/$P2Y_6$ signaling in murine luteal cells and possible mechanisms.

Methods and materials

Steroidogenic acute regulatory protein (StAR; D10H12), phospho-p44/42 MAPK (Erk1/2) and p44/42 MAPK (Erk1/2) were purchased form Cell signaling Technology (Beverly, MA, USA). 3β-Hydroxysteroid dehydrogenase (3β-HSD; sc-30820) was ordered from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Steroidogenic factor 1 (SF1; BA3-823), p450 cholesterol side-chain cleavage enzyme (CYP-11A; BA4136), β-actin, horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG and horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG were obtained from BOSTER (Wuhan, China). Collagenase (Type I), DNase I, UDP, and MRS2578, a $P2Y_6$ receptor-selective antagonist, were ordered from Sigma (St.Louis, MO, USA). GoScript Reverse Transcription System (A5001) was from Promega (Madison, WI, USA). Trizol reagent was acquired from Invitrogen (Carlsbad, CA, USA). DNA polymerase was obtained from NEB (Ipswich, MA, USA). Cell counting kit-8 was purchased from Beyotime (Shanghai, China).

Animals and treatments

Immature Kunming mice (26 days old) were purchased from the Animal Facility of Nanchang University and housed under a temperature and light controlled conditions with free access to water and food. The experimental protocols were approved by the ethical committee of Nanchang University. The mice were injected intraperitoneally with 36 IU of pregnant mare's serum gonadotrophin (PMSG; NingBo Biological Technology, Zhejiang, China) to induce follicular maturation. 72 hours later, they were treated with 36 IU human chorionic gonadotropin (hCG; Ningbo Biological Technology, Zhejiang, China) to induce ovulation and luteinization, as previously described, with a slightly modification [25]. Ovaries were collected 7 days after hCG administration.

Luteal cell culture

The isolation of luteal cells from luteinized ovaries was performed as described in previous studies with slight modification [20, 26]. Briefly, fat and capsule tissue was removed from the luteinized ovaries. After mechanical dissection,

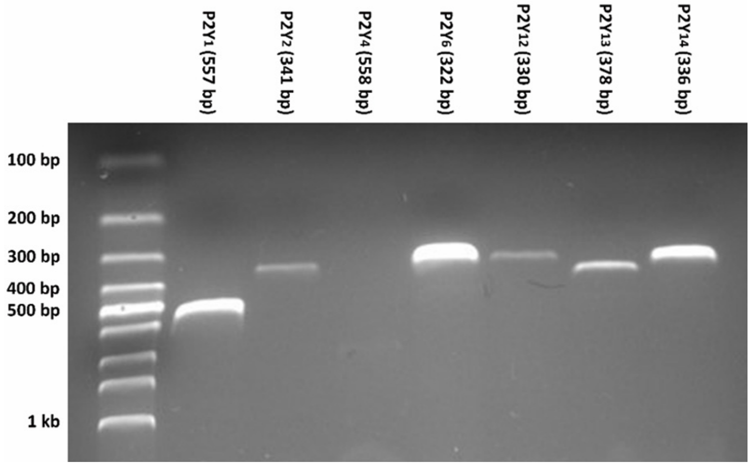

Figure 1. Expression profiles of P2Y purinergic receptors in murine luteal cells. Expression level of $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$ receptors was examined by RT-PCR. Amplification products were electrophoresed on 2% agarose gel and visualized by GoldView staining. Left lane shows DNA markers. Amplicon length is indicated in base pairs (bp) on the top.

ovaries were incubated in medium containing 1 mg/mL collagenase, 0.025% trypsin, and 0.02 mg/mL DNase I for 5 minutes at 37°C. The digested suspension was filtered through 75 μm strainers in order to remove debris and centrifuged at 500 g for 5 minutes. Following two washes, the cells were seeded in Dulbecco's Modified Eagle Media: Nutrient Mixture F-12 (DMEM/F12) culture medium, supplemented with 5% fetal bovine serum (FBS), 100 IU/mL penicillin and 100 μg/mL streptomycin sulfate and cultured overnight for adhesion. After this period the cells were cultured only in fresh medium (Control) or in fresh medium with UDP, MRS2578 or UDP + MRS2578, as described below.

Cell viability assay

Luteal cells were digested with trypsin and seeded in 96-well plates at 5×10^3 cells per well and incubated in fresh medium with 1 μM UDP, 10 μM UDP and 100 μM UDP or 1 nM MRS2578, 10 nM MRS2578, 100 nM MRS2578, 1 μM MRS257 and 10 μM MRS2578 or 100 μM UDP, 1 μM MRS2578 and 100 μM UDP + 1 μM MRS2578. 48 hours later, cell viability assays were performed by using cell counting kit-8 (CCK-8). CCK-8 reagent (10 μl) was added to each well of 96-well plate and incubated for 2 hours. Then the absorbance at 450 nm of reduced WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium), was recorded using an enzyme-linked immunosorbent assay plate reader.

Hormone measurements

Luteal cells were cultured in fresh medium and treated with 100 μM UDP, 1 μM MRS2578 or 100 μM UDP + 1 μM MRS2578. After 48 hours, the supernatant of media was harvested and stored at -80°C. The progesterone and estradiol values were measured with a commercial radioimmunoassay kit at a commercial laboratory (Beijing Sino-uk institute of Biological Technology). The intra- and inter-assay coefficients of variation were less than 10%. The cross-reactivity with other peptides and steroid hormones in these kits did not exceed 4%. The sensitivity of the progesterone and estradiol were 0.25 ng/mL and 2 pg/mL, respectively.

Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated from luteal cells using Trizol Reagent. 2 μg total RNA was used to synthesize cDNA by reverse transcriptase. The cDNA was used to amplify $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$ fragments. PCR was performed in 25 μl reaction volume which contained template cDNA, 0.5 U Phusion High-Fidelity DNA Polymerase, 200 μM dNTPs, forward and reverse primers 1 μM, Phusion High-Fidelity buffer and nuclease-free water. Following denaturation at 98°C for 30 sec, amplification was carried out using 32 cycles of 98°C for 10 sec, 60°C for 20 sec and 72°C for 20 sec, finished at 72°C for 10 min. The sequences for specific primers were listed in **Table 1**. After amplification reactions, samples were run on 2% agarose gel, stained with GoldView and visualized by ChemiDoc XRS + Molecular Imager (Bio-Rad).

Western blotting

Luteal cells were lysed in radioimmune precipitation assay (RIPA) lysis buffer containing com-

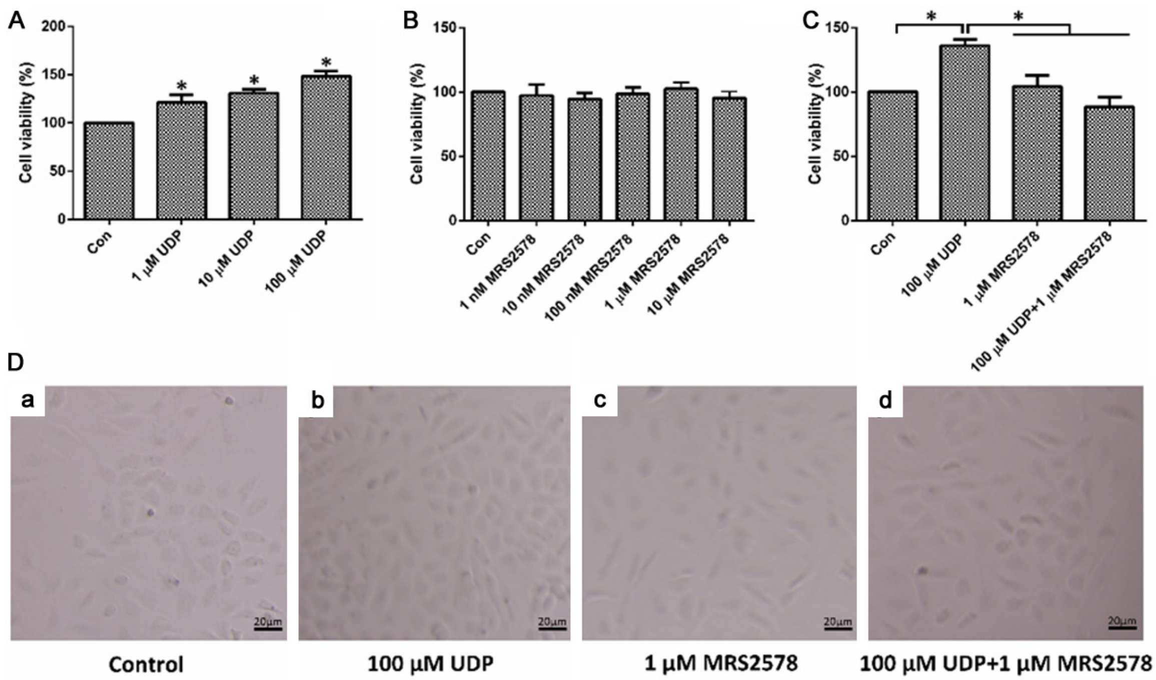

Figure 2. The influence of $P2Y_6$ purinergic signaling on murine luteal cells viability. A: Cell viability assay for cultured luteal cells with fresh medium (control), 1 μM UDP, 10 μM UDP and 100 μM UDP treatment after 48 hours of culture. B: Cell viability assay for cultured luteal cells with fresh medium (control), 1 nM MRS2578, 10 nM MRS2578, 100 nM MRS2578, 1 μM MRS2578 and 10 μM MRS2578 treatment after 48 hours of culture. C: Cell viability assay for cultured luteal cells with fresh medium (control), 100 μM UDP, 1 μM MRS2578 and 100 μM UDP + 1 μM MRS2578 treatment after 48 hours of culture. D: From a to d, primary luteal cells treated with fresh medium (control), 100 μM UDP, 1 μM MRS2578 and 100 μM UDP + 1 μM MRS2578 after 48 hours of culture. Bar graphs shown mean ± standard error of mean (S.E.M.) from three independent experiments. *$P<0.05$.

plete mini protease-inhibitor cocktail tablets (Roche, Mannheim, Germany). The total protein concentration in the supernatants was estimated by using a Bradford assay (Bio-Rad Laboratories, Hercules, CA, USA). Samples were electrophoresed under reducing conditions in 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels and transferred to nitrocellulose membranes. The membrane was blocked with 5% skim milk and incubated overnight with primary antibody at 4°C. Then the membrane was washed with Tris-buffered saline with Tween-20 (TBST, pH 8.0) and incubated with horseradish peroxidase (HRP)-labelled secondary antibody for 1 h at room temperature. Enhanced chemiluminescence (ECL) Western blotting substrate was utilized to visualize the target bands and the intensity of the bands was quantified by densitometry (BIO-RAD Image Lab). The background was subtracted, then semi-quantitative examination of relative protein expression abundance was carried out by normalizing to the amount of β-actin. The following antibody dilutions were used: anti-StAR (1:1000), anti-3β-HSD (1:300), anti-CYP11A (1:400), anti-p-ERK1/2 (1:1000), anti-ERK1/2 (1:1000) anti-SF1 (1:400) and anti-β-actin (1:1500).

Statistical analysis

All statistical analyses were performed using GraphPad Prism v6.01 (GraphPad Software Inc., San Diego, CA, USA). Data are presented as the mean and standard error of the mean (S.E.M). One-way ANOVA followed by a least-significant-difference test was used for statistical comparisons among multiple groups. A *p* value less than 0.05 was deemed as statistically significant.

Results

$P2Y_6$ receptor was highly expressed in murine luteal cells

We examined the expression of seven P2Y receptor transcripts including G_q-coupled P2-

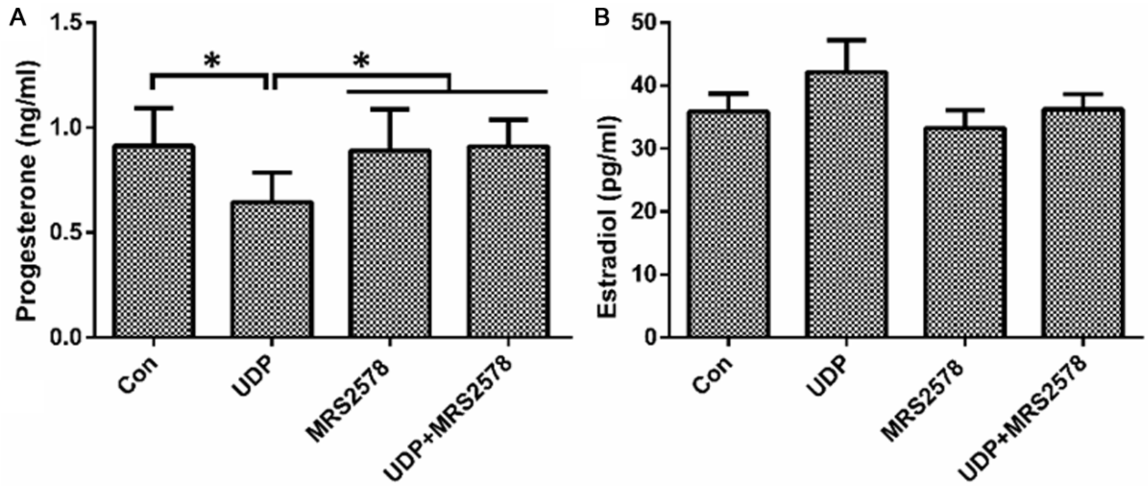

Figure 3. $P2Y_6$ purinergic signalling regulates progesterone secretion in murine luteal cells. The progesterone and estradiol were measured by radioimmunoassay. A and B: Showed the progesterone and estradiol content in cultured media, respectively, after treatment with fresh medium (control), 100 μM UDP, 1 μM MRS2578 and 100 μM UDP + 1 μM MRS2578. Bar graphs are Mean ± S.E.M. of three biological replicates. *P<0.05.

Y_1R, $P2Y_2R$, $P2Y_4R$ and $P2Y_6R$, as well as G_i-coupled $P2Y_{12}R$, $P2Y_{13}R$ and $P2Y_{14}R$ [3]. RNA from luteal cells was reverse transcribed and then PCR was carried out with specific oligonucleotides (**Table 1**) for each receptor subtype. As shown in **Figure 1**, $P2Y_1$, $P2Y_2$, $P2Y_6$, $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$ receptors were expressed in murine luteal cells. Compared with other members of P2Y, $P2Y_6$ was highly expressed in murine luteal cells.

UDP increased the viability of murine luteal cells

To investigate the function of $P2Y_6$ receptor in luteal cells, we treated luteal cells with $P2Y_6$ receptor-selective agonist (UDP) and antagonist (MRS2578). CCK8 assay was performed to observe the luteal cell viability. As shown in **Figure 2A**, UDP increased the viability of luteal cell in a dose-dependent manner. However, different concentration of MRS2578 had no obvious effect on the luteal cell viability (**Figure 2B**). MRS2578 significantly blocked the effect of 100 μM UDP on luteal cell viability (**Figure 2C**). In the meanwhile, increased viable primary luteal cell number was observed after treated with 100 μM UDP (**Figure 2D**).

UDP inhibited progesterone secretion

To study the possible effect of $P2Y_6$ purinergic signaling on steroidogenesis, we cultured primary murine luteal cells and harvested the supernatant of media to examine the progesterone and estradiol. As shown in **Figure 3A**, UDP treatment decreased the progesterone secretion. MRS2578 had no obvious effect on progesterone secretion. The decreased progesterone level induced by UDP was rescued by MRS2578. Neither UDP nor MRS2578 treatment altered estradiol secretion (**Figure 3B**).

UDP decreased CYP11A, 3β-HSD and StAR expression

To investigate the possible mechanism of UDP on progesterone secrection, we examined the expression of steroidogenic-related enzymes including CYP11A, 3β-HSD and StAR by Western blot. CYP11A, 3β-HSD and StAR control the rate limiting steps during steroidogenesis [25]. β-actin was used as the internal reference for normalization. The results indicated that the expression of CYP11A, 3β-HSD and StAR was decreased after UDP treatment, as shown in **Figure 4A-C**, respectively. CYP11A, 3β-HSD and StAR had no obvious change after treated with MRS2578. 3β-HSD and StAR returned to normal level in UDP + MRS2578 group.

UDP inhibited the ERK1/2 MAPK signaling pathway

Accumulating evidences showed that UDP/$P2Y_6$ system performed their function mainly through ERK1/2 signaling pathway [21, 27]. We

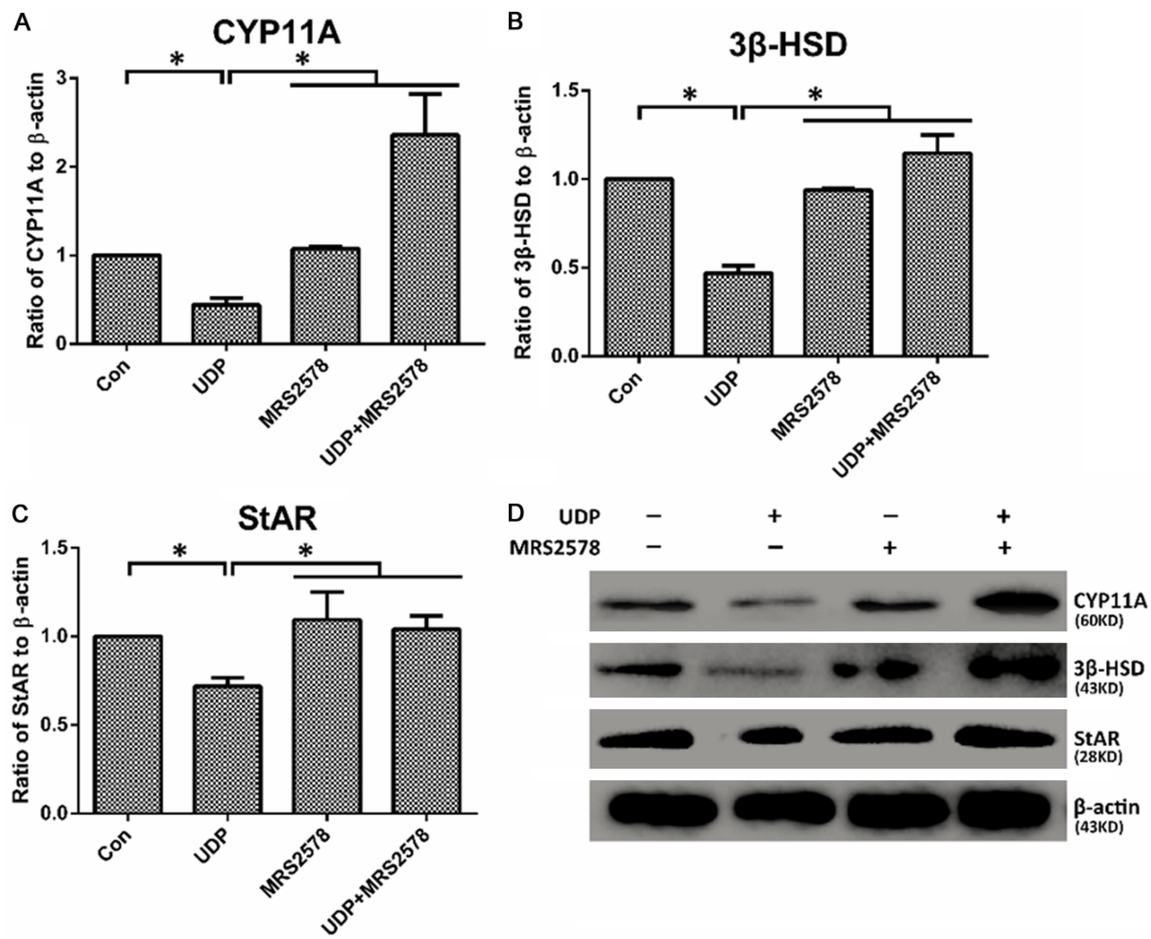

Figure 4. $P2Y_6$ purinergic signaling regulates steroidogenic enzymes in murine luteal cells. (A) CYP11A, (B) 3β-HSD and (C) StAR protein expression level in luteal cells after fresh medium (control), 100 μM UDP, 1 μM MRS2578 and 100 μM UDP + 1 μM MRS2578 treatment. (D) Representative Western blots of CYP11A, 3β-HSD, StAR and β-actin. β-actin serves as a loading control. Values represent the mean ± S.E.M. were from three independent experiments. *$P<0.05$. StAR, steroidogenic acute regulatory protein; 3β-HSD, 3β-hydroxysteroid dehydrogenase.

also examined the change of ERK1/2 signaling pathway by Western blot. As shown in **Figure 5**, compared with control group, UDP inhibited the p-ERK1/2 protein expression level. MRS2578 reserved the effect of UDP on phosphorylated ERK1/2 in murine luteal cells.

UDP inhibited SF1 expression

To explore a potential link between ERK1/2 signaling pathway and CYP11A, 3β-HSD and StAR expression, an imporant mediator of these genes, transcription factor-SF1, was detected by Western blotting [28]. As shown in **Figure 6**, UDP decreased the SF1 expression and MRS2578 partially reserved the influence of UDP on SF1, indicatingthat SF1 was one of the possible mediators.

Discussion

The $P2Y_6$ purinergic receptor is wildly expressed innumerous cell types. It's involved in massive physiological and pathological processes by regulating cell proliferation, survival, cytokines secretion, phagocytosis and etc [13, 16, 29, 30]. In the ovary, $P2Y_6$ signaling was demonstrated to regulate the theca cell proliferation [21]. Whereas, the role of $P2Y_6$ purinergic signaling in murine luteal cells has not yet been elucidated. In this study, we found that $P2Y_6$ receptor was highly expressed in murine luteal cells.

The main function of corpus luteum is to synthesis and secret steroid hormones, mainly progesterone. The progesterone is essential for

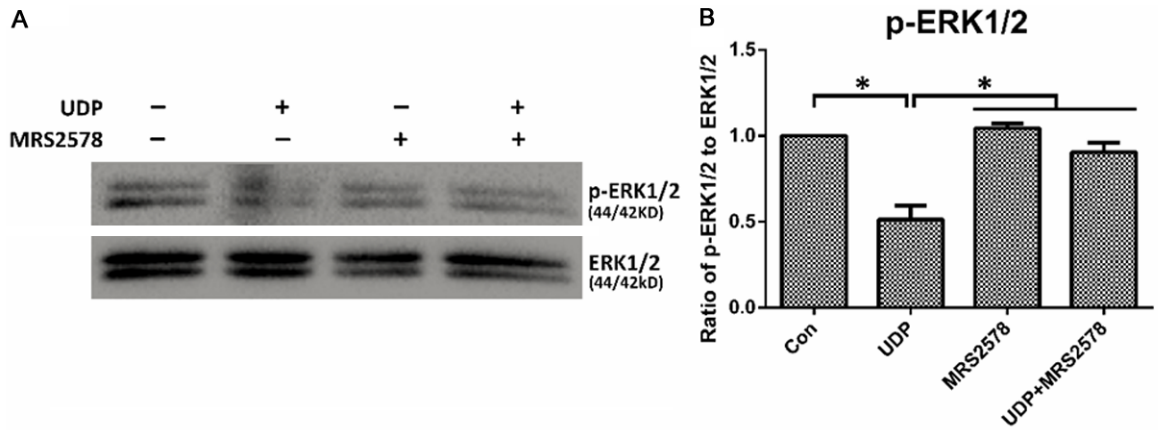

Figure 5. $P2Y_6$ purinergic signaling regulates ERK1/2 phosphorylation in murine luteal cells. (A) Western blotting detection of phosphorylated ERK1/2 after fresh medium (control), 100 μM UDP, 1 μM MRS2578 and 100 μM UDP + 1 μM MRS2578 treatment. B: Densitometric analysis results of phospho-ERK1/2 to total ERK1/2. Data are expressed as means ± S.E.M and bar graph in (B) is representative of three independent experiments. *P<0.05.

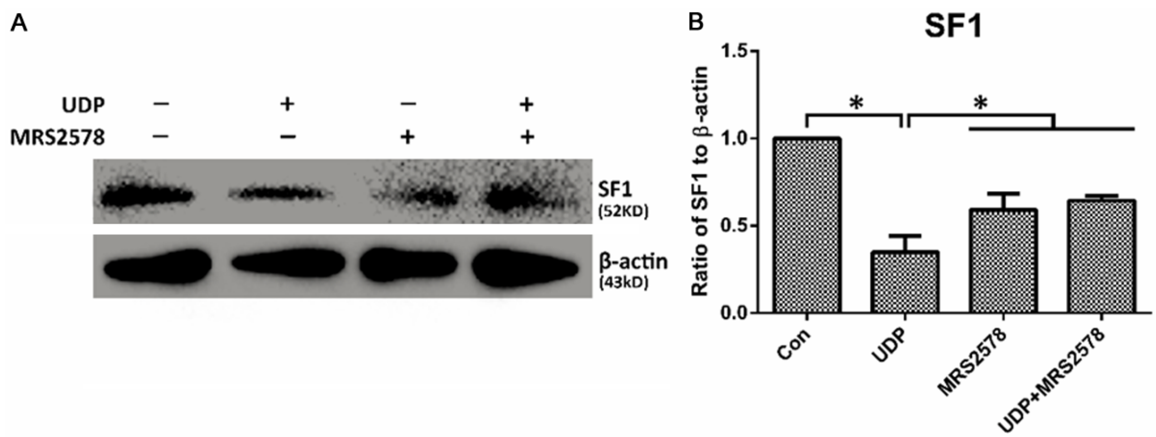

Figure 6. UDP/$P2Y_6$ signaling regulates SF1 in murine luteal cells. (A) Western blotting detection of SF1 after fresh medium (control), 100 μM UDP, 1 μM MRS2578 and 100 μM UDP + 1 μM MRS2578 treatment. (B) Densitometric analysis results of SF1 to β-actin. β-actin serves as a loading control. Total ERK1/2 serves as a loading control. Data are expressed as means ± S.E.M and bar graph in (B) is representative of three independent experiments. *P<0.05.

estrous cycle, embryo implantation and the maintenance of intra-uterine pregnancy [31]. We found that UDP, an agonist of $P2Y_6$, decreased the progesterone secretion and had no obvious effect on estradiol. To confirm the role of $P2Y_6$ purinergic signaling, MRS25678, a specific antagonist, was employed in this work. MRS2578 completely rescued the influence of UDP on progesterone secretion. These data implied that UDP/$P2Y_6$ signaling played a vital role in regulating progesterone synthesis in murine luteal cells.

Progesterone synthesis depends on three key enzymes including CYP11A, 3β-HSD and StAR. StAR mediates the translocation of intracellular cholesterol into inner membrane of mitochondria [32]. CYP11A, an inner membrane bound enzyme, cleaves the side-chain of cholesterol and converts it into pregnenolone [33]. 3β-HSD ultimately catalyses the reaction from pregnenolone to progesterone [34]. We show that the expression of CYP11A, 3β-HSD and StAR protein was inhibited after treatment with UDP. MRS2578 had no effect on these three key enzymes, but reversed the inhibition effect of UDP. These results suggested that UDP/$P2Y_6$ signaling affected progesterone secretion through regulating CYP11A, 3β-HSD and StAR.

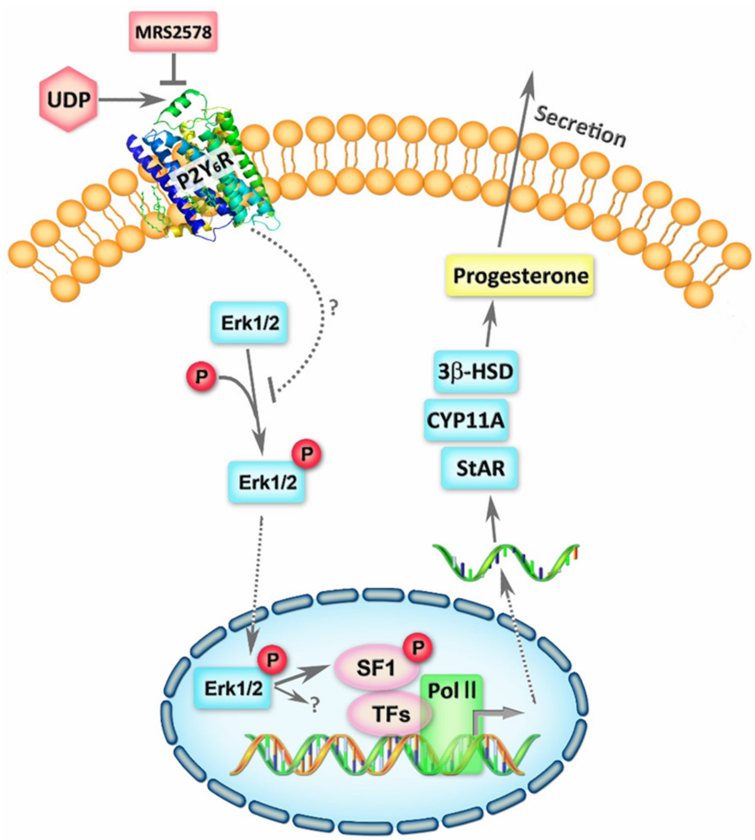

Figure 7. Schematic representation of $P2Y_6$ purinergic signaling regulates progesterone secretion in luteal cells. P, phosphorylation; SF1, steroidogenic factor 1; TFs, transcription factors; Pol II, RNA polymerase II; StAR, steroidogenic acute regulatory protein; CYP11A, p450 cholesterol side-chain cleavage enzyme; 3β-HSD, 3β-hydroxysteroid dehydrogenase.

In ovarian theca cells, neurons, chondrocytes, macrophages, human lung adenoma cells, $P2Y_6$ activation induces subsequent effects predominantly through phosphorylated ERK1/2 [21, 27, 35-37]. Intriguingly, our results showed that UDP inhibited the ERK1/2 phosphorylation in murine luteal cells. MRS2578 reserved the inhibition effect of UDP on p-ERK1/2. ERK1/2 signaling is a vital determinant in regulating diverse cellular processes including proliferation, differentiation, survival, apoptosis and motility [38]. In the ovary, ERK1/2 signaling is fundamental for follicles maturation, ovulation and luteinization [39]. In steroidogenic cells, especially granulosa-lutein cells, the requirement of ERK1/2 activation in progesterone synthesis has been documented [40, 41]. Therefore, we inferred that ERK1/2 signaling pathway mediated the decrease of progesterone and downregulation of CYP11A, 3β-HSD and StAR induced by UDP/$P2Y_6$.

SF1 belongs to nuclear receptor 5A (NR5A) family and plays a pivotal role in the transcriptional regulation of P450scc, 3β-HSD and StAR [28]. Studies showed that ERK signaling influenced the steroid hormone synthesis through regulating SF1 [42, 43]. Our results showed that UDP decreased the SF1 expression. However, MRS2578 partially relieved the effect of UDP/$P2Y_6$ on SF1, indicating that SF1 was one possible mediator between p-ERK1/2 andsteroidgenic enzymes.

Nucleotides are mainly intracellular distribution, however, they can also be released under either physiological or pathological conditions. Extracellular nucleotides mainly act as paracrine factors by binding P2 purinergic receptors on plasma membrane [29]. Massive studies showed that the purinergic signaling regulated the cellular physiology in different ovarian cells types including the oocytes, granulosa cells, theca cells, luteal cells and the ovarian surface epithelium [44]. In previous study, we reported that $P2X_7$ modulated the steroid synthesis and proliferation of murine luteal cells [20]. In this study, we showed that $P2Y_6$ regulated progesterone secretion by inhibiting CYP11A, 3β-HSD and StAR expression in murine luteal cells. The p-ERK1/2 and downstream transcription factor-SF1 were possible links between UDP and steroidogenesis (**Figure 7**). What's more, we also presented that $P2Y_6$ participated in regulating proliferation of luteal cells, but the detailed mechanism needs further exploration.

In summary, this study demonstrated for the first time that UDP/$P2Y_6$ purinergic signaling regulated progesterone secretion by inhibiting the expression of CYP11A, 3β-HSD and StAR in luteal cells. The ERK1/2 signaling and downstream SF1 may contribute to the process. This study improved our understanding on the regulation role of purinergic signaling in the corpus luteum. As luteal phase insufficiency is one of the major causes of female infertility. Nucleotides can be released from the sympathetic nerve ending and influenced the function of corpus luteum. So, exploring the function of purinergic signaling in luteal cells and purinergic abnormality in corpus luteum have implications for diagnosis and treatment for luteal phase insufficiency of luteal phase defect and other diseases related to corpus luteum development.

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Disclosure of conflict of interest

None.

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