SET Improved Oocyte Maturation by PP2A and Inhibited Oocyte Apoptosis in Mouse Oocytes

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Abstract

Background: SET is a multifunctional protein involved in a variety of molecular processes such as transcription control, chromatin remodeling, cell apoptosis, and cell-cycle regulation. In ovaries SET is predominantly expressed in theca cells and oocytes. And in PCOS patients the expression of SET was increased than normal people. The current study was designed to determine whether SET play a role in oocyte maturation and apoptosis, which may provide clues for the underlying pathological mechanism of follicular development in PCOS patients.

Methods: Oocytes at GV stage were collected from 6-week-old female ICR mice. 20-25 oocytes per group were placed in M16 medium. Then these oocytes were randomly divided into control group or treatment group for further study. Normal distribution was assessed by the Shapiro-Wilk test, and the student t test was used to compare the mRNA and protein levels. Chi-square analysis was used to compare the ratios of oocytes.

Results: SET overexpression improved oocyte maturation whereas SET knockdown inhibited oocyte maturation. Moreover, SET negatively regulated PP2A activity in oocytes. Treatment with PP2A inhibitor okadaic acid (OA) promoted oocyte maturation. Furthermore, PP2A knockdown confirmed the key role of PP2A in oocyte maturation, and OA was able to block the AdH1-SiRNA/SET-mediated inhibition on oocyte maturation. The central role of PP2A in SET-mediated regulation of oocyte maturation was confirmed by the finding that SET increased the expression of BMP15 and GDF9 and PP2A inhibited their expression. Besides, SET inhibited oocyte apoptosis through decreased the expression of caspase 3 and caspases 8, while PP2A had no effect on oocyte apoptosis.

Conclusions: SET promoted oocyte maturation by inhibiting PP2A activity and inhibited oocyte apoptosis in mouse in-vitro cultured oocytes, which may provide a pathologic pathway leading to oocyte development disorder in PCOS.

Background

SET was initially identified as a translocated gene in a patient with acute undifferentiated leukemia in 1992. It encodes a multifunctional protein involved in a variety of molecular processes such as histone acetylation, transcription control, nucleosome assembly, chromatin remodeling, cell apoptosis, and cell-cycle regulation[1]. It is widely expressed in different cells of various tissues, such as steroidogenic cells within the central nervous system, adrenal glands, and gonads [2–4]. In rat ovaries, SET is expressed in theca cells and oocytes [4], and in human and mouse ovaries SET is also predominantly expressed in theca cells and oocytes[5]. SET regulated cyclin B-CDK1 activity with p21 and participate in G2/M transition of cell cycle in COS and HCT116 cells[1]. Some studies suggested that SET induced neuronal death. Moreover, the cell apoptosis was associated with an increase in the level of cytoplasmic SET[6, 7]. By biding Jcasp domain of the amyloid precursor protein SET specifically activated caspase-3 activity and induced neuronal death[6]. On the contrary, other studies suggested that overexpression of SET in

293T human embryonic kidney cells increased the cells in S phase and downregulated the expression of Bax, thus decreased their susceptibility to induced apoptosis[8]. In SET knockout mice loss of SET resulted in activation of selective p53 responsive genes, which caused growth delay and apoptosis[9].

Polycystic ovary syndrome (PCOS) is one of the most common heterogeneous endocrine disorder diagnosed in 5–10% of women in reproductive age. It is primarily characterized by oligo-ovulation or anovulation, biochemical or clinical hyperandrogenism, and polycystic ovaries on ultrasound. The underlying mechanism for the abnormalities in anovulatory PCOS remains uncertain. It is possible that there are intrinsic differences in folliculogenesis between polycystic ovaries and normal ones which affect preantral and antral follicles[10]. The total number of growing follicles was significantly higher in PCOS ovaries than in normal ovaries. PCOS patients had 2.7-fold more primary follicles, 1.8-fold more secondary follicles, and 2-fold more antral follicles than normal people[11]. But the number of nongrowing primordial follicles in PCOS was normal, suggesting that the increase of growing follicles in PCOS was not due to the increase of primordial follicle recruitment. Overgrowth of preantral follicles and accumulation of small antral follicles arrested in their development, with some atretic features, has been shown in the ovaries of PCOS[12]. Although there are many preantral and antral follicles in PCOS, they can’t develop into dominant follicles and ovulate. The mechanism of follicular dysplasia in PCOS may be related to the dysregulation of follicular selection, development and atresia. Previous studies have confirmed that the growth of follicles was closely related to intrinsic oocyte competence[13–15]. In addition, oocyte development disorders often exist in the ovaries of PCOS patients, suggesting that follicular dysplasia in PCOS patients is related to oocyte development disorder[16–19].

Until now the exact etiology and pathophysiological mechanism of PCOS is still unclear. The family aggregation of PCOS suggests that genetic factors may play an important role in the pathogenesis of PCOS. In recent years, cDNA microarray, proteomics, and genome-wide association studies have been used to find the genes and proteins related to PCOS, and to further understand the pathogenesis of PCOS. The microarray results of Diao et al. showed that the mRNA expression of SET was up-regulated in ovaries of PCOS patients than in normal ones[20]. It was further confirmed that the mRNA and protein expression of SET was increased in PCOS patients[21]. And immunohistochemistry showed that SET protein was highly expressed in thecal cells and oocytes of PCOS ovaries. Combined with previous finding on the role of SET in cell cycle and apoptosis, it is conjectured that overexpression of SET in oocytes may be related to oocyte development disorder in PCOS.

The current study was designed to determine whether SET play a role in oocyte maturation and apoptosis, which may provide clues for the underlying pathological mechanism of follicular development in PCOS patients.

Methods

Animals

The ICR mice were purchased from the Laboratory Animal Center of Yangzhou University (Yangzhou, China) where they were maintained as a breeding colony. Mice were housed under controlled temperature and daily lighting (12h light and 12h dark). Food and water were provided adlibitum. All animal procedures were approved by the Institutional Animal Care and Use Committee of Jiangsu Province and were performed in accordance with the Guide for the Care and Use of Laboratory Animals.

Collection and culture of mouse oocytes

Germinal vesicle (GV) oocytes were collected from 6-week-old female ICR mice. Mice were intraperitoneally injected with 10 IU of pregnant mare serum gonadotropin (PMSG, Folligon, Intervet, Castle Hill, Australia). After 48 hours, the mice were sacrificed and the ovaries were immediately placed in M2 medium (Sigma, St. Louis, MO). The ovaries were dissected by repeatedly puncturing the surface with fine steel needles, and the oocytes at GV stage were transferred to M16 medium (Sigma, St. Louis, MO) by the glass micropipette under a dissecting microscope. 20-25 oocytes per group were placed in 32μl droplets of M16 medium containing 10% fetal bovine serum (FBS; Invitrogen, Grand island, NY) under a layer of mineral oil in 5% humidity environment at 37°C. Then these oocytes were randomly divided into control group or treatment group for further study. In order to prepare zona pellucida free oocytes, the oocytes were placed in acidic Tyrode's solution (pH 2.5-3.0) within 30s until zona pellucida disappeared. Then the zona-free oocytes were immediately transferred to M2 medium and washed three times before cultured in M16 medium.

Real-time PCR

Total mRNA was extracted using TRIZOL reagent (Invitrogen, Carsbad, CA) and RNeasy Mini kits (Qiagen, Valencia, CA) from the pooled oocytes (20-25 oocytes/tube). cDNA was generated by Sensiscript Reverse Transcription Kit (Qiagen, Valencia, CA) with oligo-dT primer at 37°C for 60 minutes. Primers used for real-time PCR are listed in supp Table. SYBR Green PCR Kits (Takara Shuzo Co Ltd, Kyoto, Japan) and ABI Prism 7300 Sequence Detection System (PerkinElmer Applied Biosystems, Foster City, CA) were used for real-time PCR. Melting curve analysis was performed to confirm the specificity of products. The experiment was repeated at least three times using different sets of oocytes. Data was calculated according to the $2^{-\Delta\Delta CT}$ method and presented as relative fold to GAPDH internal reference gene.

Immunofluorescence

Oocytes were fixed in 4% paraformaldehyde in PBS (PH7.4) for at least 30 min at room temperature and then permeabilized with 0.5% Triton X-100 in PBS for 30 min at 37°C, followed by blocking in 1% BSA for 1h at room temperature. Then they were incubated with SET antibody (1:100, Santa Cruz, CA) at 4°C overnight. After washing, oocytes were incubated with a fluorescein isothiocyanate-conjugated secondary antibody (1:100; Beijing Zhong-Shan Biotechnology Co., Beijing, China) for 1h at 37°C, and DNA was counterstained with propidium iodide (Sigma). Finally, oocytes were mounted on glass slides with DABCO and examined using ZEISS 510 laser confocal microscopy (ZEISS Fluorescent Microsystems, Gottingen, Germany).

Annexin-V staining in oocytes

Annexin-V staining was performed with an Annexin-V kit according to the manufacturer's instructions (KenGentec, Nanjing, China). Annexin-V, a phospholipid-binding protein, detects the translocation of phospholipid phosphatidylserine from the inner to the outer cytoplasmic membrane, which is known to occur during the early stage of apoptosis. At the same time, oocytes were stained with propidium iodide to distinguish viable oocytes from dead ones. Oocytes from different groups were washed twice in PBS and then stained with 500μl of binding buffer, which contained 5μl annexin-V-fluorescein isothiocyanate (FITC) and 5μl PI for 5-15min in the dark. Then the samples were mounted on siliconized slides and observed under a laser confocal scanning microscope (ZEISS Fluorescent Microsystems, Gottingen, Germany).

Western blot analysis

Western blotting was performed as previously described[5] with 1:1000 rabbit anti-SET polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA), 1:1500 mouse anti-PP2A monoclonal antibody (Upstate, Temecula, CA), 1:100 goat anti-GDF9 polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA), 1:500 rabbit anti-caspase 3 polyclonal antibody(Cell Signaling, Danvers, MA), 1:1000 rabbit anti-caspase 8 polyclonal antibody(Abcam, Cambridge, MA). Loading levels were normalized using 1:2000 rabbit anti-tubulin antibody or 1:6000 rabbit anti-GAPDH antibody (Abcam, Cambridge, MA). The results were analyzed by Quantity One version 4.62 (Bio-Rad, Hercules, CA).

PP2A activity assay

PP2A immunoprecipitation phosphatase assay kit (Upstate, Temecula, CA) was used to measure phosphate release as an index of phosphatase activity according to the manufacturer's instructions. Briefly, 100μg protein isolated from oocytes was incubated with 4μg mouse anti-PP2A monoclonal antibody (Upstate, Temecula, CA) overnight. 40μl of protein A agarose beads were added and the mixture was incubated at 4^{o}C for 2 hours. Subsequently, the beads were washed three times with 700μl ice-cold TBS and one time with 500μl Ser/Thr Assay Buffer. The beads were further incubated with 750mM phosphopeptide in assay buffer for 10 minutes at 30^{o}C with constant agitation. 100μl of Malachite Green Phosphate Detection Solution was added and the absorbance at 650nm was measured on a microplate reader.

Statistical analysis

Each experiment was repeated at least three independent times. Normal distribution was assessed by the Shapiro-Wilk test, and the student t test was used to compare the mRNA and protein levels. Chi-square analysis was used to compare the ratios of oocytes. Statistical analysis was performed by Stata 15 software (Stata Corp LP, College Station, Texas). A value of $P < 0.05$ was determined to be statistically significant.

Results

Expression and localization of SET in mouse oocytes

To examine the expression of SET in different phases of mouse oocytes, oocytes were collected for real-time PCR at 0, 3, 8 and 12 h after cultured in vitro, corresponding to the germinal vesicle (GV), germinal vesicle breakdown (GVBD), first metaphase (MI), and second metaphase (MII), respectively. The results showed that there was no significant difference in SET mRNA expression from GV to MII stages (Fig. 1A).

The cellular localization of SET protein in different stages of mouse oocytes was examined by immunofluorescence coupled with confocal microscopy. As shown in Fig. 1B, SET was mainly distributed in nucleus and less in cytoplasm in GV phase. In GVBD phase, the signals of SET reside in the entire oocyte. With the oocyte entering MI phase, SET accumulated strongly in the cytoplasm. In MII phase, set was dispersed in cytoplasm.

Effect of SET on oocyte maturation

AdCMV-SET and AdH1-SiRNA/SET adenoviruses had been constructed in our previous work to overexpress or knockdown SET expression and the efficacy had been validated on both mRNA and protein levels[22]. Mouse zona-free GV oocytes cultured with M16 medium containing IBMX(50μM) were infected with AdCMV-SET or AdCMV-GFP for 24h. Then the media was washed to remove IBMX which inhibiting GVBD. After 3h of in vitro culture the ratio of GVBD was significantly increased in AdCMV-SET infected oocytes when compared with AdCMV-GFP group (Table 1). After another 11h of in vitro culture the ratio of MII was also significantly increased in SET overexpressed oocytes, higher than the control group (Table 1).

Table 1
Effect of SET protein on oocyte maturation

Treatment	Total	n of GVBD	Ratio of GVBD	n of MII	Ratio of MII
AdCMV-GFP	350	250	71.4%	224	64.0%
AdCMV-SET	368	300	81.5%**	270	73.4%**
AdH1-SiRNA/NS	345	247	71.6%	221	64.1%
AdH1-SiRNA/SET	349	198	56.7%**	165	47.3%**
*P < 0.05, **P < 0.01 vs. control					

As compared with the AdH1-SiRNA/NS group, knockdown of SET with AdH1-SiRNA/SET remarkably decreased the ratio of GVBD and MII(Table 1).

Effect of SET on oocyte apoptosis

Mouse zona-free GV oocytes cultured with M16 medium containing IBMX were infected with AdCMV-SET or AdCMV-GFP for 24h. Then the oocytes was transferred to fresh M16 medium and cultured for another 3h or 8h. After that oocytes were collected and classified into three groups by Annexin-V staining as Anguita et al described[23]. (A) necrotic oocytes with PI-positive red nuclei indicative of membrane damage and discontinuous green signal originating from the remnant portions of membrane. (B) viable oocytes which were negative for Annexin-V staining in the cytoplasmic membrane. (C) early apoptotic oocytes with a clear green signal from Annexin-V in the membrane. The results indicated that the ratio of early apoptotic oocytes was significantly decreased in SET overexpressed oocytes after 3 and 8h of in vitro culture (Table 2).

Table 2
Effect of SET protein on oocyte apoptosis

Treatment	Total	n of early apoptosis oocytes at 3h(%)	Total	n of early apoptosis oocytes at 8h(%)
AdCMV-GFP	150	45(30.0%)	148	50(33.8%)
AdCMV-SET	149	30(20.1%)*	155	36(28.4%)*
AdH1-SiRNA/NS	152	47(30.9%)	154	53(34.4%)
AdH1-SiRNA/SET	142	68(47.9%)**	153	78(51.0%)**
*P < 0.05, **P < 0.01 vs. control				

In contrary, knockdown of SET with AdH1-SiRNA/SET resulted in increased ratio of early apoptotic oocytes both at 3h and 8h (Table 2). These results suggest that SET inhibit the apoptosis of oocytes.

Effect of SET on the maturation-related factors of oocyte

AdCMV-SET or AdCMV-GFP adenoviruses were used to infect zona-free oocytes in GV phase. After 16 hours of in vitro culture, the oocytes of each treatment group were collected. Real-time PCR and Western blot were used to detect the changes of bone morphogenetic protein 15(BMP15) and growth differentiation factor 9(GDF9) at mRNA and protein levels. The results showed that overexpression of SET increased the expression of BMP15 and GDF9 at both mRNA (Fig. 2A) and protein levels(Fig. 2B), which were related to oocyte maturation. To confirm the observation from SET overexpression experiments, we performed SET knockdown experiments by infecting zona-free oocytes with AdH1-SiRNA/SET or AdH1-SiRNA/NS. Similarly, the results showed that knockdown of SET decreased the mRNA and protein levels of BMP15 and GDF9 (Fig. 2C-D).

Effect of SET protein on the apoptosis related factors of oocyte

In order to elucidate the SET-related apoptotic pathways in oocytes, the expressions of four apoptosis related factors (caspase 3, caspases 8, caspase 9, and cytochrome c) were measured by real-time PCR and western blot after different treatment. The results showed that up-regulation of SET decreased the expression of caspase 3 and caspases 8 at both mRNA and protein levels, while no changes in caspase 9 and cytochrome c mRNA expression were detected(Fig. 3A-B). In the same way, if SET was down-regulated, the expression of caspase 3 and caspases 8 was increased at mRNA and protein levels, and there was no significant difference in caspase 9 and cytochrome c expression (Fig. 3C-D).

The effect of SET on PP2A phosphatase activity in mouse oocytes

SET was validated to inhibit PP2A phosphatase activity in mouse theca-interstitial cells and some other cells in previous study [5, 24, 25]. To study whether SET affected PP2A phosphatase activity in oocytes, zona-free oocytes in GV phase were infected with AdCMV-SET or AdCMV-GFP. Then the cells were lysated and subjected to immunoprecipitation with anti-PP2A, followed by PP2A activity assay. The results indicated that when SET was overexpressed the activity of PP2A was significantly decreased (Fig. 4A). To confirm the observation from SET overexpression experiments, SET knockdown experiments were also performed. The results showed that PP2A activity was significantly increased after SET knockdown (Fig. 4B).Taken together, these data strongly suggested that SET inhibited PP2A activity in oocytes.

Effect of PP2A on oocyte maturation and maturation-related factors

We subsequently studied whether PP2A could affect oocyte maturation by PP2A inhibitor, okadaic acid (OA). Mouse GV oocytes were cultured with M16 medium containing 10nM OA or DMSO, respectively. After 3h of in vitro culture the ratio of GVBD was significantly increased in OA treated group compared with DMSO group (Table 3). After another 11h of in vitro culture the ratio of MII was calculated and the oocytes were collected for real-time PCR to detect the changes of BMP15 and GDF9. The results showed that OA increased the ratio of MII and the mRNA expression of BMP15 and GDF9 (Fig. 5A), which was consistent with the result obtained from SET overexpression.

Table 3
Effect of PP2A on oocyte maturation

Treatment	Total	n of GVBD	Ratio of GVBD	n of MII	Ratio of MII
DMSO	300	234	78.0%	228	76.0%
OA	318	283	89.0%**	278	86.9%**
AdH1-SiRNA/NS	310	200	71.2%	180	64.1%
AdH1-SiRNA/PP2A	309	250	80.9%**	216	70.0%**
*P < 0.05, **P < 0.01 vs. control					

We further used AdH1-SiRNA/PP2A adenovirus to knockdown PP2A expression in oocytes. When PP2A was knocked down, the ratio of GVBD and MII was all increased (Table 3). Besides, the mRNA expression of BMP15 and GDF9 were also increased(Fig. 5B). Thus, results from both expression manipulation and chemical inhibition experiments point to a negative regulation of PP2A on oocyte maturation.

PP2A-mediated SET regulation of oocyte maturation

To further determine the functional relationship between SET expression/PP2A activity and oocyte maturation, we treated the AdH1-SiRNA/SET or AdH1-SiRNA/NS infected oocytes with PP2A inhibitor, OA. Despite SET knockdown in AdH1-SiRNA/SET infected oocytes, the maturation was rescued by the treatment with OA (Table 4). This result, together with the previous one showing SET inhibition on PP2A activity strongly suggest that the ability of SET to improve the oocyte maturation may be specifically attributed to its inhibition of PP2A activity.

Table 4
PP2A-mediated SET regulation of oocyte maturation

Treatment	Total	n of GVBD	Ratio of GVBD	n of MII	Ratio of MII
AdH1-SiRNA/NS + DMSO	310	204	65.8%	192	61.9%
AdH1-SiRNA/SET + DMSO	320	181	56.6%*	170	53.1%*
AdH1-SiRNA/SET + OA	315	213	67.6%	197	62.5%
*P < 0.05, **P < 0.01 vs. control					

Effect of PP2A on oocyte apoptosis

We subsequently examined whether PP2A could affect oocyte apoptosis. Mouse GV oocytes were cultured with M16 medium containing 10nM OA or DMSO. The results showed there was no significant difference in the ratio of early apoptotic oocytes during these two groups after 3h and 8h in vitro culture (Table 5). Besides, knockdown of PP2A with AdH1-SiRNA/PP2A had no effect on the ratio of early apoptotic oocytes both at 3h and 8h (Table 5). These results suggest that PP2A had no effect on the apoptosis of ovarian oocytes.

Table 5
Effect of PP2A on oocyte apoptosis

Treatment	Total	n of early apoptosis oocytes at 3h(%)	Total	n of early apoptosis oocytes at 8h(%)
DMSO	149	33(22.1%)	145	35(24.1%)
OA	145	34(23.45%)	143	35(24.4%)
AdH1-SiRNA/NS	157	49(31.2%)	149	55(36.9%)
AdH1-SiRNA/PP2A	160	52(32.5%)	150	57(38.0%)
*P < 0.05, **P < 0.01 vs. control				

Discussion

In this study, the in vitro cultured mouse oocyte was used to investigate the effect of SET on oocyte maturation and apoptosis. According to previous reports, SET is a nuclear protein[26], but it also localizes in the cytoplasm[27]. In our study, by immunofluorescence coupled with confocal microscopy we found that SET was expressed predominantly in the nucleus and less in the cytoplasm in GV phase, in the entire oocyte at GVBD stage, in the cytoplasm at MI stage, and uniformly in the cytoplasm at MII stage, which may suggest SET function in regulating oocyte maturation. Expression of SET was upregulated or downregulated in oocytes using adenovirus infection. Interestingly, our results showed that SET improved oocyte maturation by inhibiting PP2A activity and SET also inhibited oocyte apoptosis, which was firstly found by our laboratory. This observation suggested that SET could be a stringent factor in regulating oocyte development, which may promote our understanding in the molecular mechanism of abnormal oocyte competence in PCOS.

In Qi's study overexpression of SET in mouse oocytes caused precocious separation of sister chromatids, but depletion of SET by RNAi showed little effects on the meiotic maturation process[28]. In our study AdCMV-SET and AdH1-SiRNA/SET adenoviruses was constructed to overexpress or knockdown SET expression. After 11h of in vitro culture the ratio of GVBD and MII was significantly increased in AdCMV-SET infected group when compared with control oocytes. Similarly, knockdown of SET with AdH1-SiRNA/SET remarkably decreased the percentage of GVBD and MII. So by in-vitro study it was validated that SET promoted oocyte maturation. This result was further supported by detecting the expression of two important oocyte-derived growth factors-BMP15 and GDF9, which were known to be responsible for controlling fundamental physiological processes in oocyte development and follicular growth[29]. It had been validated that immunization against GDF9 and BMP15 alone or together altered folliculogenesis and ovulation rate in cattle, which indicated both GDF9 and BMP15 appear to be key regulators on oocyte development and follicular growth[30]. In this study, we found overexpression of SET increased the

expression of BMP15 and GDF9 at both mRNA and protein levels in mouse oocytes, suggesting that SET promoted oocyte development through upregulation of BMP15 and GDF9.

It was found that overexpression of SET reduced the detoxification of cells and accumulated cytotoxic substances and carcinogens in cells, leading to cell death or tumor occurrence[31]. The accumulation of SET in the cytoplasm of neurons made cells more sensitive to DNA damage and induced cell death[32]. While in the absence of oxidative stress, the aggregated SET was evenly distributed in the nucleus and cytoplasm of HEK293T cells, which inhibited cell apoptosis. When cells were in mild oxidative stress state, SET mainly gathered in the cytoplasm and promoted cell apoptosis[33]. Under oxidative stress, SET positively regulated cell apoptosis by changing the acetylation of forkhead box protein (FoxO1)[34]. Whether SET is involved in oocyte apoptosis has not been studied. In our study mouse zona-free GV oocytes was infected with AdCMV-SET or AdCMV-GFP for 24h, and after that oocytes were collected and classified into three groups by Annexin-V staining. It was found that the ratio of early apoptotic oocytes was significantly decreased in SET overexpressed group after 3 and 8h in vitro culture. In contrary, knockdown of SET resulted in increased the ratio of early apoptotic oocytes both at 3h and 8h. Some studies have found that SET regulates cell apoptosis through the expression of caspase independent and caspase dependent pathways[35, 36]. Overexpression of SET in HEK293T cells inhibited the activity of caspase 9 and caspase 3, and then inhibited the endogenous apoptotic pathway[37]. In our study the expression of major apoptotic factors caspase 3 and caspase 8 was also dramatically decreased after SET overexpression. Cristobal found that SET inhibited the caspase apoptosis pathway activated by PP2A[38]. We subsequently examined whether PP2A could affect oocyte apoptosis, and the results showed that PP2A had no effect on oocyte apoptosis. These results suggested that SET might inhibit early stage of apoptosis by decreasing the activation of the extrinsic, caspase 8-mediated apoptotic pathway in mouse oocytes.

SET regulated cell proliferation mainly through phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) and extracellular regulated protein kinase (ERK) signaling pathways. In non-small cell lung cancer, knockout of SET up-regulated the activity of PP2A, thereby inhibited Akt and ERK signaling pathways and inhibited cell proliferation[39]. By immunofluorescence microscopy it was found that PP2A was also expressed in ovarian oocytes[5]. It had been validated that SET inhibited PP2A activity in many cells such as theca cells[5], NK cells, and NCI-H295A cells[40]. One of our key observations was that in oocytes SET also inhibited PP2A activity, which was consisted with others' results. Arnaud et al found that the I2NTF and I2CTF fragments of SET interacted with PP2Ac and inhibited PP2A activity[37]. I2CTF interacted with PP2Ac through its carboxy terminal acidic region and in I2NTF fragment valine 92 was essential for its interaction with PP2Ac[37]. Another report showed that SET bound to the B56 of the β-isoform of PP2A in mouse liver[41]. But in this study we could not find evidence for the interaction between SET and PP2A by immunoprecipitation assay. This may be partially due to the inefficiency of immunoprecipitation for detection of transient protein-protein interaction. Recombinant GST-SET protein or cross-linking technology will be applied to enhance the sensitivity of the assay in our future study.

OA has an inhibitory effect on PP1, PP2A, PP2B, and PP2C. At low concentration it is a relative specific inhibitor of PP2A[42]. In our study it was found that inhibition of PP2A activity by OA increased the ratio of MII and the mRNA expression of BMP15 and GDF9. The specificity of PP2A regulation on oocyte maturation was also corroborated by the results from parallel PP2A knockdown[24]. Moreover, when SET was down-regulated by AdH1-SiRNA/SET, oocyte maturation was effectively rescued by OA treatment. Thus, the ability of SET to regulate oocyte maturation appears to be mediated via a direct effect of SET on PP2A.

The current finding on SET-mediated activation of oocyte maturation by inhibition of PP2A activity and inhibition of oocyte apoptosis provides a pathologic pathway leading to oocyte development disorder in PCOS. Given the multifunctional characteristic of SET, it is possible that SET may be involved in other pathologic changes observed in PCOS. The role of SET on the development of PCOS, a factor known to be overexpressed in PCOS ovaries, deserve further investigation.

Conclusions

SET promoted oocyte maturation by inhibiting PP2A activity and inhibited oocyte apoptosis in mouse in-vitro cultured oocytes, which may provide a pathologic pathway leading to oocyte development disorder in PCOS.

List Of Abbreviations

polycystic ovary syndrome(PCOS)

germinal vesicle (GV)

germinal vesicle breakdown (GVBD)

first metaphase (MI)

second metaphase (MII)

okadaic acid (OA)

bone morphogenetic protein 15(BMP15)

growth differentiation factor 9(GDF9)

Declarations

Ethical Approval and Consent to participate

All animal procedures were approved by the Institutional Animal Care and Use Committee of Jiangsu Province and were performed in accordance with the Guide for the Care and Use of Laboratory Animals.

Consent for publication

All authors have consented to the publication of this article.

Availability of supporting data

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

GLL drafted the manuscript, completed the experiments and performed statistical analysis. LD and CYG revised the article. WSY and XJB helped with oocyte culture, acquisition of data and interpretation of data. All authors read and approved the final manuscript.

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Disclosure information

None of the authors have anything to declare.

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Figures

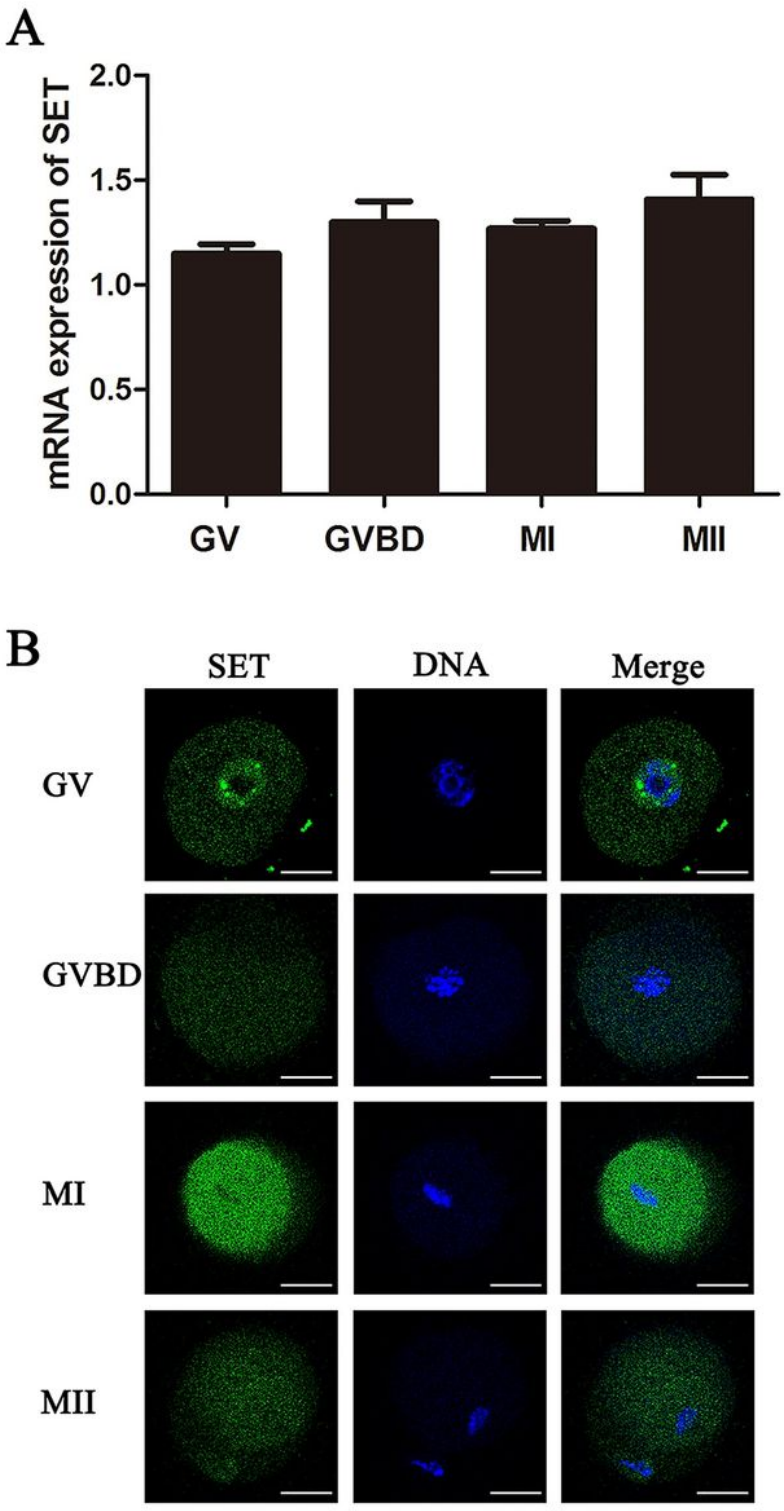

Figure 1

The Expression and localization of SET in mouse oocytes from GV to MII. (A) Real-time PCR analysis of SET mRNA in oocytes. 50 oocytes were cultured in vitro for 0, 3, 8, and 12 hours, respectively. Then the oocytes were collected for real-time PCR . Results were presented as mean ± SD from at least 3 independent experiments. *$p < 0.05$ and **$p < 0.01$. (B) Immunofluorescence staining of SET and

chromosomes in oocytes. Oocytes cultured for 0h (GV), 3h (GVBD), 8h (MI) and 12h (MII) in vitro were stained with specific SET antibody (green) and propidium iodide (blue). Scale bar = 20μm

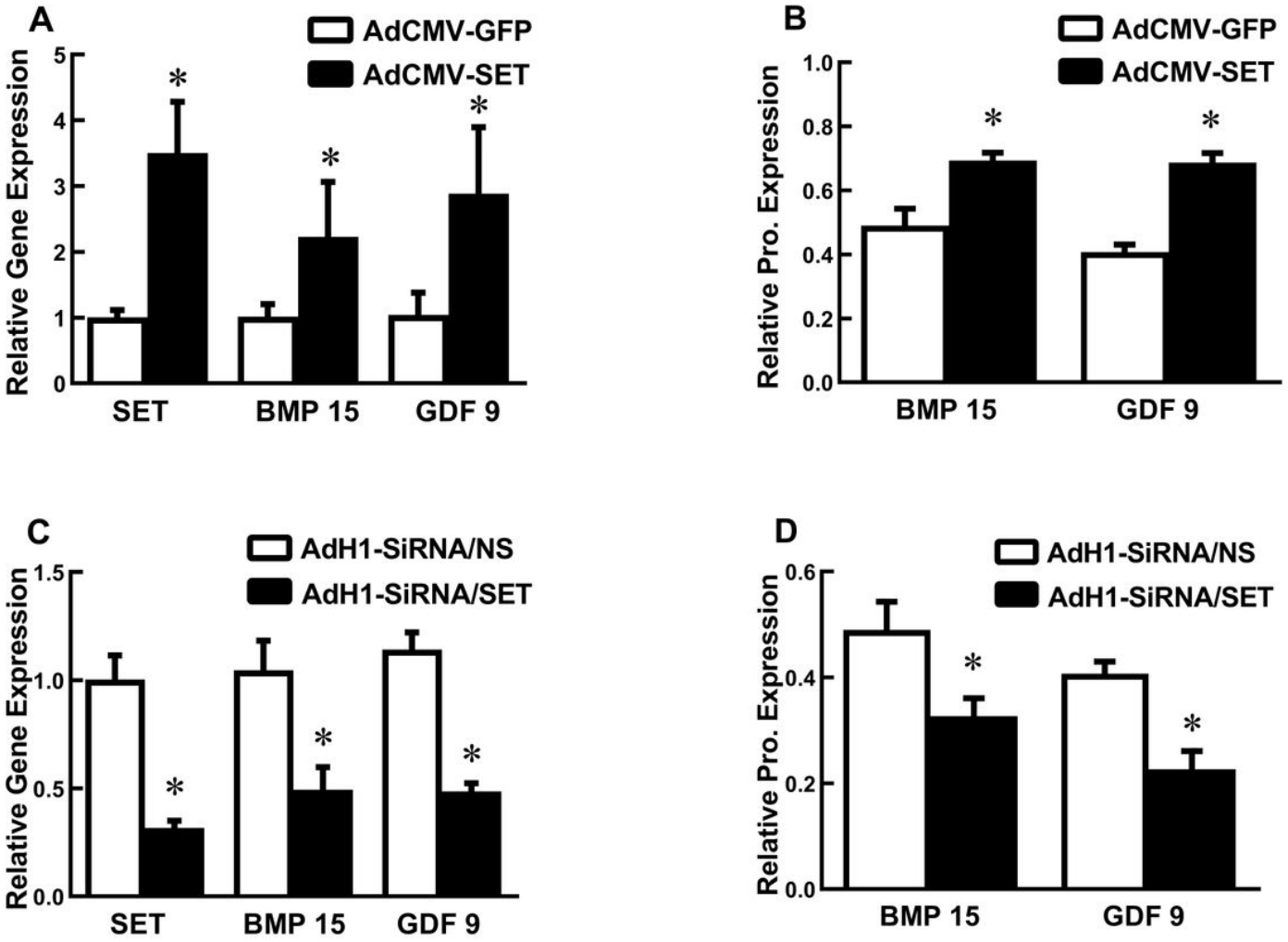

Figure 2

Effect of SET on maturation-related factors of oocyte. (A) Real-time PCR analysis of BMP15 and GDF9 in mouse zona-free oocytes after infected with AdCMV-SET or AdCMV-GFP for 24h. (B) Western blot analysis of BMP15 and GDF9 in mouse zona-free oocytes after infected with AdCMV-SET or AdCMV-GFP for 48h. (C) Real-time PCR analysis of BMP15 and GDF9 in mouse zona-free oocytes after infected with AdH1-SiRNA/SET or AdH1-SiRNA/NS for 24h. (D) Western blot analysis of BMP15 and GDF9 in mouse zona-free oocytes after infected with AdH1-SiRNA/SET or AdH1-SiRNA/NS for 48h. Results were presented as mean ± SD from at least 3 independent experiments. * $p < 0.05$.

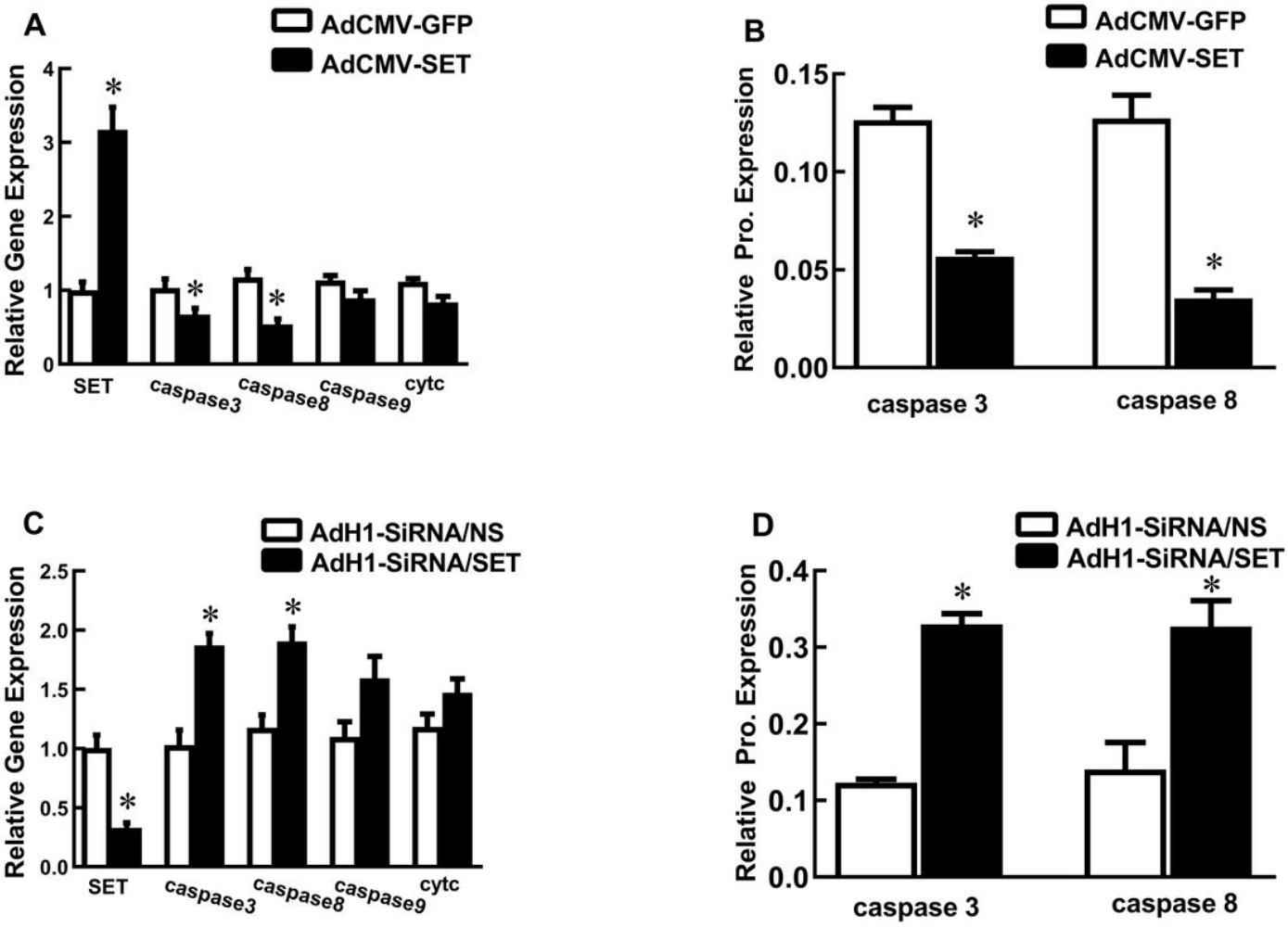

Figure 3

Effect of SET on apoptosis related factors of oocyte. (A) Real-time PCR analysis of caspase 3, caspases 8, caspase 9, and cytochrome c in mouse zona-free oocytes after infected with AdCMV-SET or AdCMV-GFP for 24h. (B) Western blot analysis of caspase 3, caspases 8 in mouse zona-free oocytes after infected with AdCMV-SET or AdCMV-GFP for 48h. (C) Real-time PCR analysis of caspase 3, caspases 8, caspase 9, and cytochrome c in mouse zona-free oocytes after infected with AdH1-SiRNA/SET or AdH1-SiRNA/NS for 24h. (D) Western blot analysis of caspase 3, caspases 8 in mouse zona-free oocytes after infected with AdH1-SiRNA/SET or AdH1-SiRNA/NS for 48h. Results were presented as mean ± SD from at least 3 independent experiments. * $p < 0.05$.

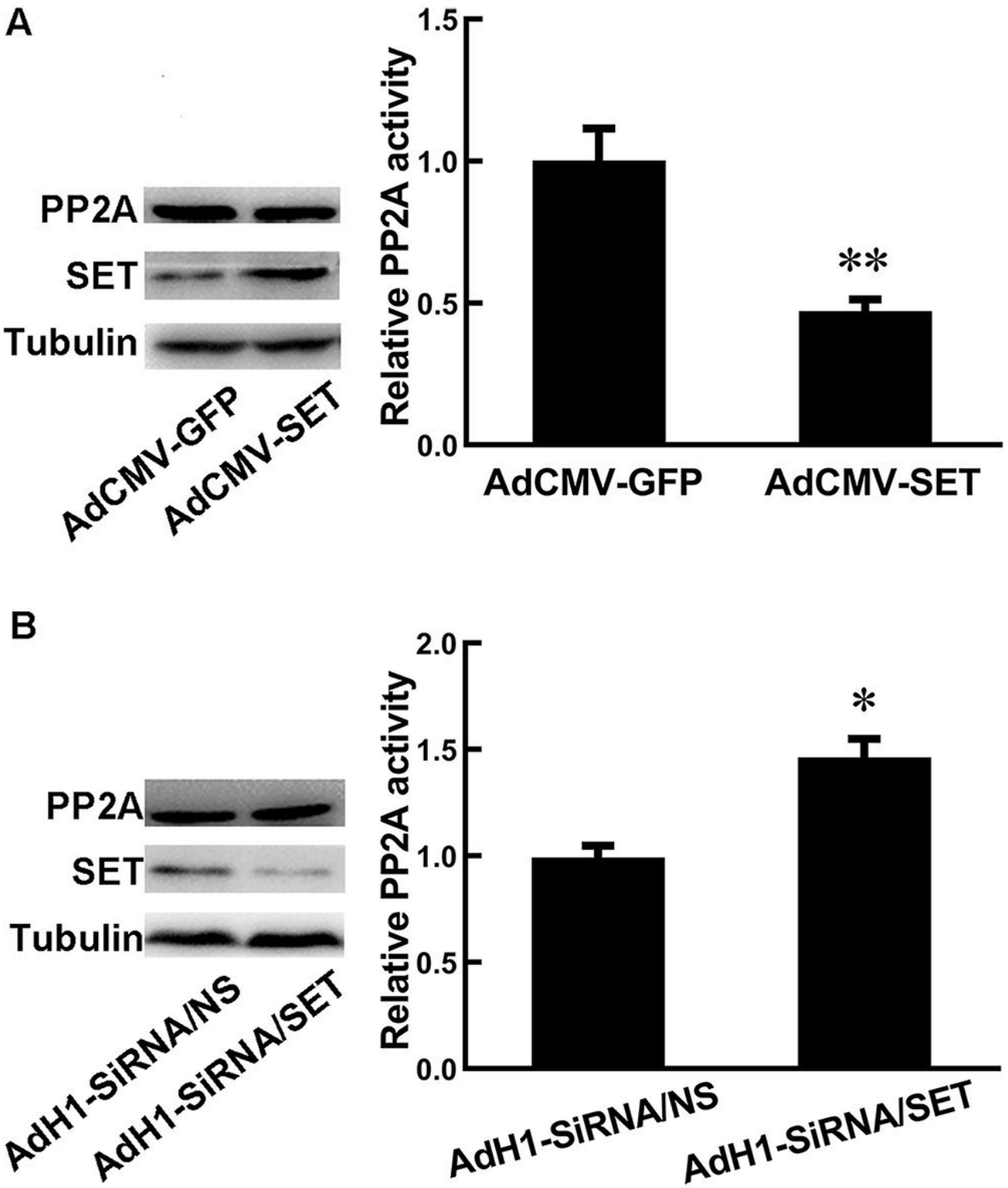

Figure 4

The effect of SET on PP2A phosphatase activity in mouse oocytes. (A) The AdCMV-GFP or AdCMV-SET infected oocytes were collected for PP2A activity assay with PP2A immunoprecipitation phosphatase assay kit. (B) The AdH1-SiRNA/SET or AdH1-SiRNA/NS infected oocytes were collected for PP2A activity by PP2A immunoprecipitation phosphatase assay kit. Results were presented as mean ± SD from 3 independent experiments. *$p < 0.05$ and **$p < 0.01$.

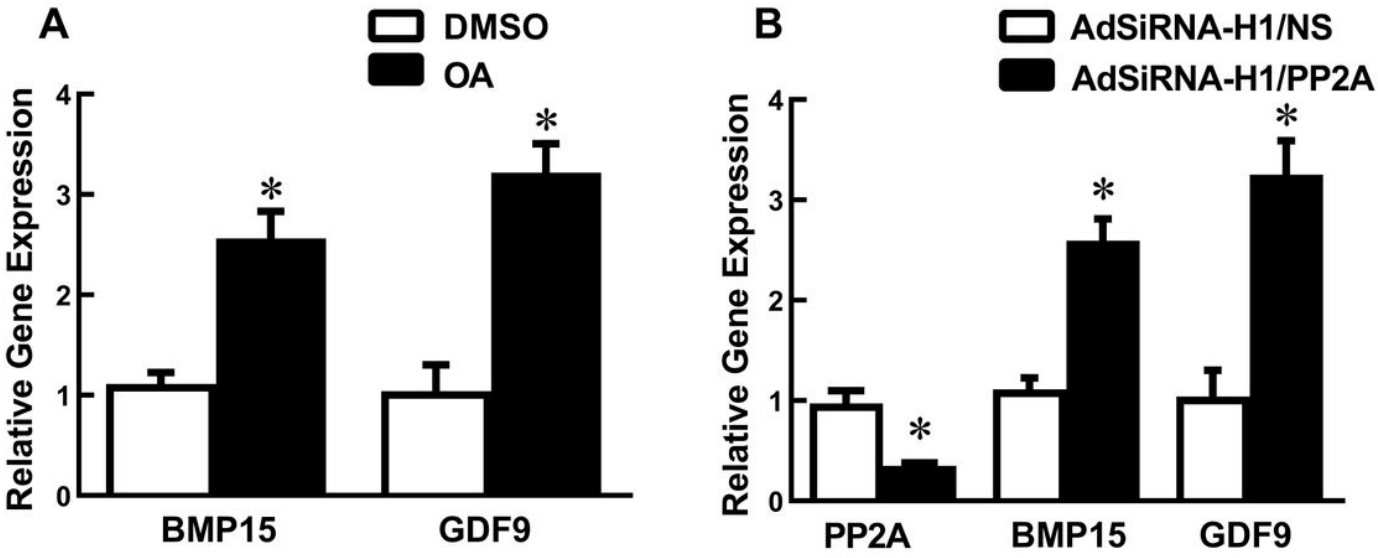

Figure 5

Effect of PP2A on oocyte maturation and maturation-related factors (A) The oocytes were incubated with DMSO or 10nM OA. After 11h post-treatment oocytes were collected for real-time PCR to detect the changes of BMP15 and GDF9. (B) Oocytes were infected with AdH1-SiRNA/PP2A or AdH1-SiRNA/NS adenoviruses. At 48h of post-infection the follicles were collected for real-time PCR to detect the changes of PP2A, BMP15 and GDF9. Results were presented as mean ± SD from at least 3 independent experiments. *$p < 0.05$ and **$p < 0.01$.

Supplementary Files

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