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ORIGINAL RESEARCH

JMY is required for asymmetric division and cytokinesis in mouse oocytes

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ABSTRACT: JMY is a transcriptional co-factor of p53. Latest work has revealed that JMY is also an actin nucleation factor that regulates new filament assembly and activates Arp2/3 complex in somatic cells; however, roles of JMY in mouse oocyte are unknown. Here we showed the expression and functions of JMY during mouse oocyte meiotic maturation. JMY mRNA is expressed largely from germinal vesicle to metaphase I stage, and gradually decreased during anaphase I, telophase I (TI) and metaphase II (MII) stages. Immunostaining results showed that JMY localized at the spindle and cytoplasm of oocytes. Depletion of JMY by RNAi resulted in symmetric division, failure of spindle migration and cytokinesis during oocyte meiotic maturation, showing a 2-cell-like MII oocyte and TI stage arrest. Actin cap and cortical granules-free domain formation were also disrupted after JMY RNAi, indicating the failure of spindle migration. JMY antibody injection results were consistent with those of JMY RNAi, further confirming the involvement of JMY in oocyte polarity. Our data indicate that JMY is required for spindle migration, asymmetric division and cytokinesis during mouse oocyte maturation.

Key words: JMY / actin / oocyte / asymmetric division / cytokinesis

Introduction

Mammalian oocyte meiotic maturation is characterized by a unique asymmetric division. During this process, the oocyte is transformed into a highly polarized big metaphase II (MII)-arrested oocyte with a small polar body extruded, which is essential for the retention of maternal components for early development (Maro and Verlhac, 2002). Failure of asymmetry usually occurs in the oocytes that are of low quality or have experienced post-ovulatory aging. Asymmetric division depends on oocyte polarization, which includes spindle migration, spindle anchoring and cortical reorganization (Sun and Schatten, 2006; Brunet and Verlhac, 2011). After germinal vesicle breakdown (GVBD), the centrally positioned spindle translocates to the cortex of the oocyte in an actin-dependent way. Furthermore, microfilaments are enriched to form an actin cap, cortical granules (CGs) are redistributed to form a CG-free domain (CGFD) and microvilli are lost in the region overlaying the spindle (Longo and Chen, 1985; Van Blerkom and Bell, 1986; Deng *et al.*, 2003). Together, these changes are called cortical reorganization and polarization. When cortical reorganization is completed, the oocyte extrudes the polar body, leaving a highly polarized oocyte. Different from common ligand-mediated cell polarity, oocyte polarity formation and cortical reorganization is independent of any external ligand, and the signal is intrinsic to the oocyte (Kutsuna *et al.*, 2004). Meanwhile, meiotic spindles in oocytes lack true centrosomes, indicating that specialized mechanisms may be responsible for the off-centre positioning of the spindles. Until now, the molecular details of oocyte polarization have been poorly understood.

Microtubule and microfilament cytoskeletons are widely involved in multiple processes during oocyte polarization (Brunet and Verlhac, 2011). Actin nucleation was mediated by actin nucleation factors, including Formin family proteins (Chesarone *et al.*, 2010), Arp2/3 complex with its activators (Chesarone and Goode, 2009; Campellone and Welch, 2010) and tandem actin monomer-binding proteins (Renault *et al.*, 2008). Arp2/3 complex was activated by nucleation promoting factors including WASP (Bosticardo *et al.*, 2009), N-WASP (Lommel *et al.*, 2001; Snapper *et al.*, 2001), WAVE1 (Dahl *et al.*, 2003; Soderling *et al.*, 2003), WAVE2 (Yamazaki *et al.*, 2003; Yan *et al.*, 2003), WAVE3, WASH (Linardopoulou *et al.*, 2007), WHAMM (Campellone *et al.*, 2008), Abp1 (Goode *et al.*, 2001), Pan1, Cortactin (Weed *et al.*, 2000; Uruno *et al.*, 2001; Weaver *et al.*, 2001) and the newly identified JMY (Zuchero *et al.*, 2009).

JMY was originally identified as a co-factor of p53 (Shikama *et al.*, 1999). An exciting later study has showed that JMY is also an actin nucleation factor for activating Arp2/3 and assembling filaments directly using a Spire-like mechanism (Zuchero *et al.*, 2009). JMY contains a series of WH2 domains that facilitate actin nucleation, and the isolated C-terminus of JMY stimulates Arp2/3-dependent actin assembly as potently as neural-Wiskott-Aldrich syndrome protein-WH2, connector, acidic (N-WASP-WWCA). Perturbation of JMY expression affects cell mobility in HL-60 cells (Zuchero *et al.*, 2009), and the ability of JMY is dependent

on E-cadherin (Coutts *et al.*, 2009). These findings demonstrate a pathway that links the cytoskeleton with the p53 response.

Until now, the knowledge of this novel actin nucleation regulator in somatic cells is limited, and the roles of JMY in oocytes are unknown. The current study has investigated whether JMY is involved in oocyte polarization during oocyte meiotic maturation. JMY was found to show a unique expression pattern and its inhibition by RNAi and antibody injection caused failure of peripheral spindle migration, cytokinesis completion and asymmetric division.

Materials and Methods

Antibodies

Goat polyclonal anti-JMY antibody was purchased from Santa Cruz (CA, USA); Phalloidin-TRITC, Lectin-FITC and mouse monoclonal anti-α-tubulin-FITC antibody was obtained from Sigma (St Louis, MO, USA). Alexa Fluor rabbit-anti-goat 488 and donkey anti-goat 568 antibodies were purchased from Invitrogen (Carlsbad, CA, USA).

Oocyte collection and culture

All animal manipulations were conducted according to the guidelines of the Animal Research Committee of Chungbuk National University, Korea. Germinal vesicle (GV)-intact oocytes were collected from ovaries of 6- to 8-week-old imprinting control region mice and cultured in M16 medium (Sigma Chemical Co., St Louis, MO, USA) under paraffin oil at 37°C, 5%CO_2. Oocytes were collected after various times in culture for immunostaining and microinjection.

Real-time quantitative PCR analysis

Analysis of JMY gene expression was measured by real-time quantitative PCR and the $\Delta\Delta C_T$ method. Total RNA was extracted from 50 oocytes using a Dynabead mRNA DIRECT kit (Invitrogen Dynal AS, Norway), and first strand cDNA was generated with a cDNA synthesis kit (Takara), using Oligo(dT)12–18 primers (Invitrogen). A cDNA fragment of JMY was amplified using the following primers:

Forward, TTCAAATTACAAGCCGTGCACCCG;
Reverse, AGCTGCCTTCTGGACCTTTACTGA.

We used the DyNAmo HS SYBR Green qPCR kit (FINNZYMES) with a DNA Engine OPTICON 2 Continuous Fluorescence Detector (MJ Research) under the following conditions: 95°C for 10 s, and 38 cycles of 95°C for 5 s and 59°C for 32 s.

JMY siRNA and antibody injection

Approximately 5–10 pl 50 μM JMY siRNA (Sigma, CUA AGC UCU UGG UCC CAC A, UGU GGG ACC AAG AGC UUA G; CCA GUA UGC AGU UUC UAA A, UUU AGA AAC UGC AUA CUG G) was microinjected into the cytoplasm of fully grown GV oocytes using a Eppendorf FemtoJet (Eppendorf AG, Hamburg, Germany) with a Nikon Diaphot ECLIPSE TE300 inverted microscope (Nikon UK Ltd, Kingston upon Thames, Surrey, UK) equipped with a Narishige MM0-202N hydraulic three-dimensional micromanipulator (Narishige Inc., Sea Cliff, NY, USA). After injection, the oocytes were cultured for 24 h in M16 medium containing 5 μM milrinone, and then washed five times in fresh M16 medium, 2 min each time. The oocytes were then transferred to fresh M16 medium and cultured under paraffin oil at 37°C in an atmosphere of 5% CO_2 in air. The control oocytes were microinjected with 5–10 pl of negative control siRNA. The spindle, actin cap phenotypes and chromosome localization were examined using confocal microscopy (Zeiss LSM 710 META, Germany).

For JMY antibody injection, the same method was adopted as above, except that after injection, the oocytes were cultured in M16 medium directly.

Confocal microscopy

The protocol was basically the same as described in our previous work (Sun *et al.*, 2008, 2009, 2010a,b). Oocytes were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) for 30 min at room temperature and then transferred to membrane permeabilization solution (0.5% Triton X-100) for 20 min. After 1 h in blocking buffer (1% bovine serum albumin-supplemented PBS), oocytes were incubated overnight at 4°C, or for 4 h at room temperature with 1:100 goat anti-JMY antibody. After three washes in washing buffer (0.1% Tween 20 and 0.01% Triton X-100 in PBS), the oocytes were labeled with 1:100 Alexa Fluor rabbit-anti-goat 488 IgG or donkey anti-goat 568 IgG for 1 h at room temperature. For Phalloidin-TRITC, Lectin-FITC and α-tubulin-FITC staining, after incubation for 1 h, the oocytes were washed three times in PBS containing 0.1% Tween 20 and 0.01% Triton X-100 for 2 min, then the samples were co-stained with Hoechst 33342 (10 μg/ml in PBS) for 10 min, followed by three washes in washing buffer.

The oocytes were mounted on glass slides and examined with a confocal laser-scanning microscope (Zeiss LSM 710 META, Germany). At least 20 oocytes were examined for each group.

Data analysis

For each treatment, at least three replicates were performed. Statistical analyses were conducted using an analysis of variance. Differences between treated groups were evaluated with Duncan's multiple comparison tests. Data were expressed as mean ± SEM, and $P < 0.05$ was considered significant.

Results

Expression and localization of JMY during mouse oocyte meiotic maturation

Samples were collected after being cultured for 0, 4, 8, 9.5 or 12 h, which represents the time points when most oocytes reach the GV, GVBD, metaphase I (MI), anaphase/telophase I (ATI) and MII stages, respectively. Samples containing 50 oocytes were used for real-time RT–PCR. As shown in Fig. 1A, JMY mRNA expressed largely from the GV stage to MI stage, and then gradually decreased from the ATI stage to MII stage during mouse oocyte meiotic maturation. As evidence of this, the mRNA levels of JMY at the GV, Pro-MI, MI and ATI stages were 256 ± 21, 220 ± 43, 260 ± 19 and 158 ± 64% of that at the MII stage, respectively.

The subcellular localization of JMY at different stages of meiotic maturation was examined by JMY antibody immunofluorescent staining. As shown in Fig. 1B, in GV stage, JMY distributed uniformly in the cytoplasm of oocyte; after GVBD, JMY accumulated around the chromosomes and localized at spindle microtubules from the MI to MII stage. Besides the spindle localization, we also detected the JMY signal in the cytoplasm. Negative control showed no signal of JMY. We also co-stained JMY and α-tubulin, the signals of JMY and α-tubulin were all overlapped, which further confirmed the JMY localization pattern (Fig. 1C).

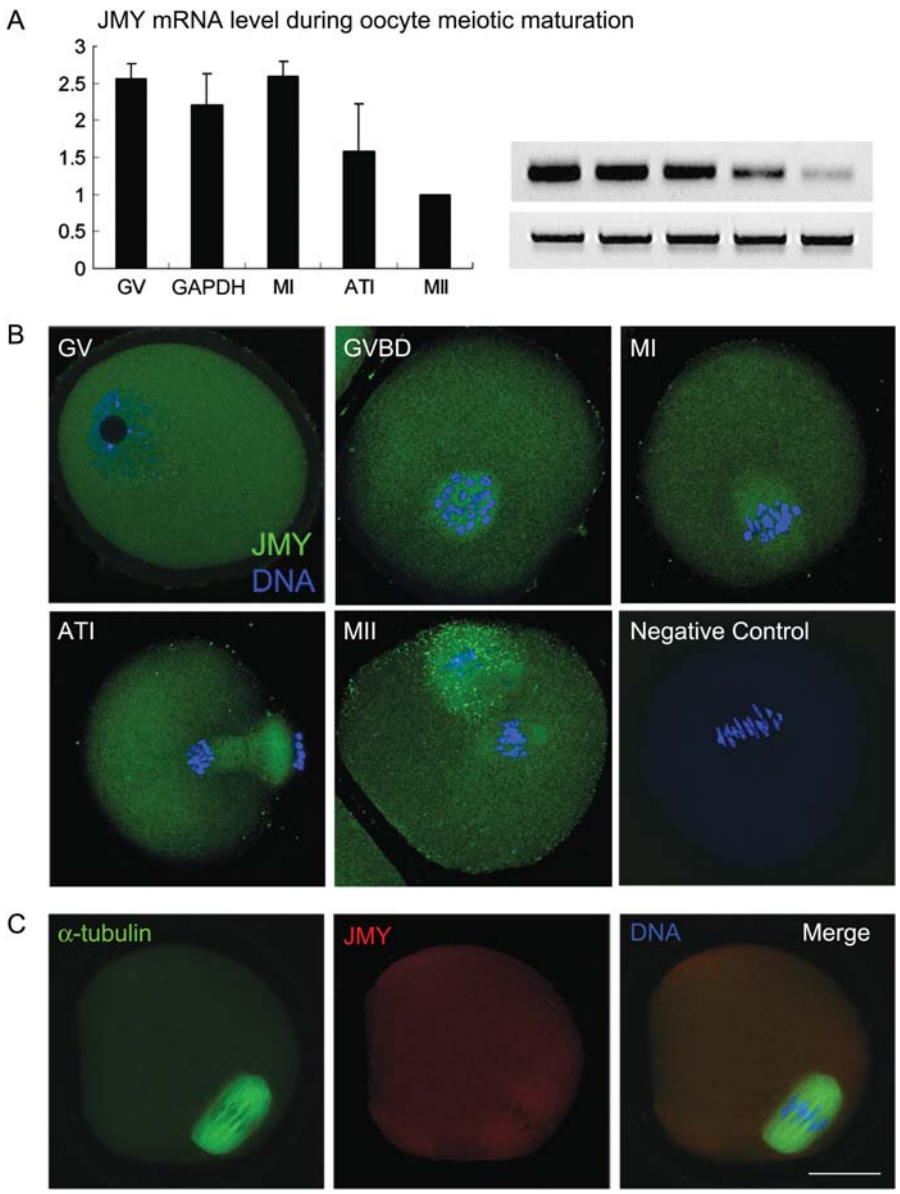

Figure 1 Expression of transcriptional cofactor JMY during mouse oocyte meiotic maturation. (**A**) Levels of JMY mRNA as revealed by real-time RT–PCR analysis. Samples were collected after culturing for 0, 4, 8, 9.5 or 12 h, when most oocytes reached the GV, Pro-MI, MI, ATI and MII stages, respectively. Each sample contained 50 oocytes. JMY mRNA expressed largely from GV stage to MI stage, and then gradually decreased from ATI to MII stage during oocyte meiotic maturation. (**B**) Subcellular localization of JMY during mouse oocyte meiotic maturation as revealed by JMY antibody staining. During mouse oocyte meiotic maturation, JMY accumulated at the spindle of the oocytes and in the cytoplasm. Green, JMY; blue, chromatin. Bar = 20 μm. (**C**) Co-localization of JMY and α-tubulin. JMY co-localized with spindle microtubule. Green, α-tubulin; red, JMY; blue, chromatin. Bar = 20 μm.

JMY RNAi causes failure of asymmetric division and cytokinesis

To further investigate the roles of JMY during mouse oocyte meiotic maturation, JMY siRNA injection was used to down-regulate the expression of JMY, which successfully depressed the mRNA level of JMY (13.2 ± 6.6% versus 100%; Fig. 2A). We employed time-lapse microscopy to examine the dynamic changes occurring in maturing oocytes. As shown in Fig. 2B, in the control group, the oocyte segregated chromosomes at 10 h, extruded polar body and rotated chromosomes at 12 h. While in the JMY RNAi group, one oocyte segregated chromosomes at 10 h, but was arrested at telophase I (TI) stage, showing unrotated chromosomes. The chromosomes of another oocyte did not move to the cortex, but segregated at 9 h 20 min in the central position and underwent symmetric division.

We also used immunofluorescent staining to examine the oocytes after 12 h in culture. As shown in Fig. 2C, depletion of JMY caused TI stage arrest and 2-cell-like MII oocytes, consistent with time-lapse microscopy results. After 12 h in culture, most oocytes (48.6 ± 2.4%,

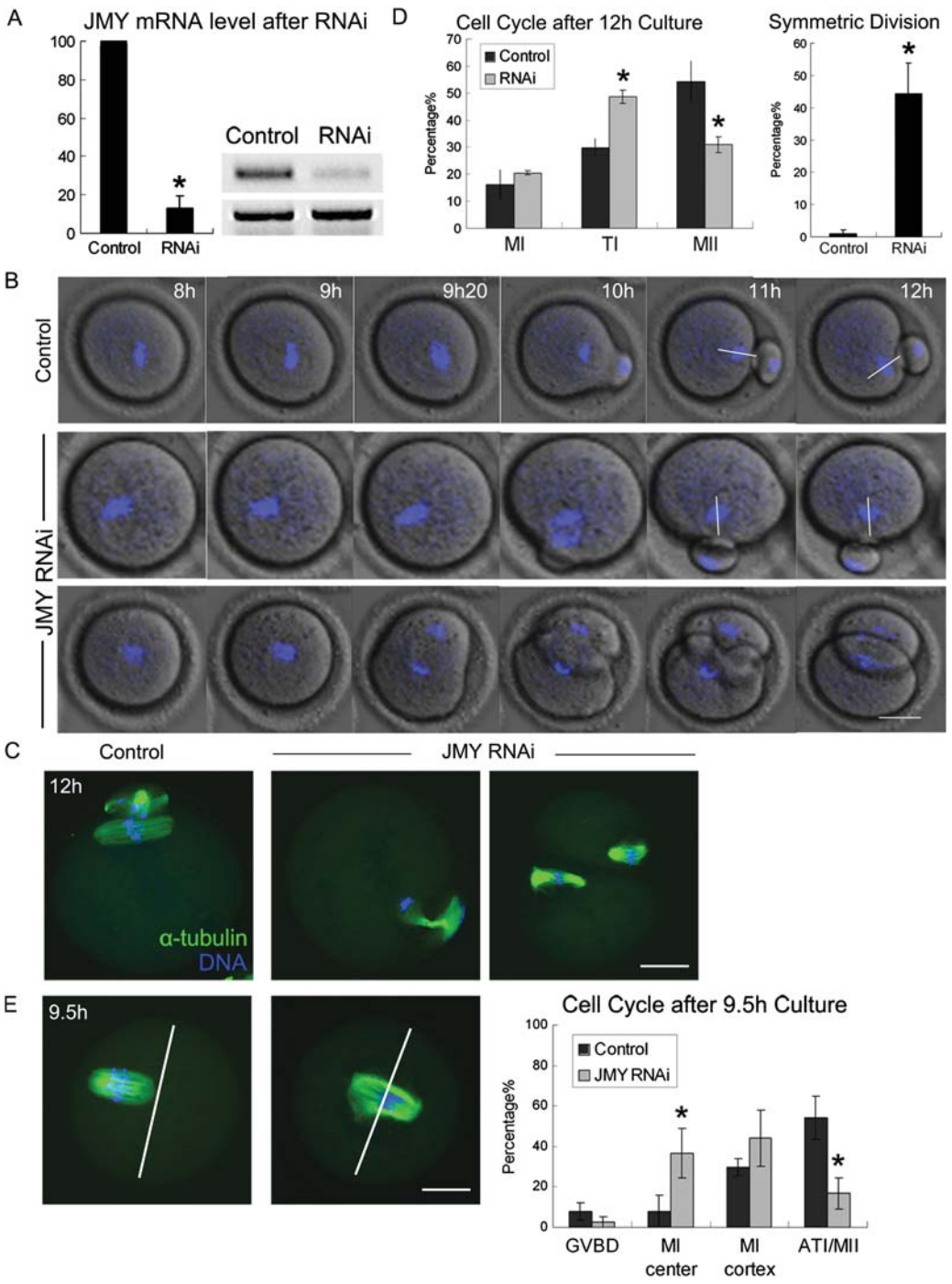

Figure 2 Effects of JMY RNAi on asymmetric division and cytokinesis in mouse oocytes. (**A**) JMY mRNA level after JMY siRNA injection. JMY mRNA level was significantly decreased after JMY siRNA injection. *, significantly different ($P < 0.05$). (**B**) Time-lapse microscopy of chromosome behavior in maturing oocytes after JMY RNAi. In the control group, the oocyte extruded polar body normally. In JMY RNAi group, one oocyte was arrested at the TI stage, another oocyte underwent symmetric division. Bar in the control group showed the chromosomes rotated after polar body extrusion. Bar in the RNAi group showed that the oocytes were arrested at the TI stage and the chromosomes did not undergo roration. (**C**) Immunofluorescent staining of the oocytes after JMY RNAi by 12 h culture. The oocytes showed TI stage arrest with condensed chromosomes and 2-cell-like MII oocytes. Green, α-tubulin; blue, chromatin. Bar = 20 μm. (**D**) Rates of different stages and symmetric division when the oocytes were cultured for 12 h in M16 medium after JMY RNAi. (**E**) Rates of different stages when the oocytes were cultured 9.5 h after JMY RNAi. Bar showed the central position of oocyte. In the control group, the spindle of most oocytes migrated to the cortex, while in the RNAi group, the spindle of most oocytes remained at the center after 9.5 h culture.

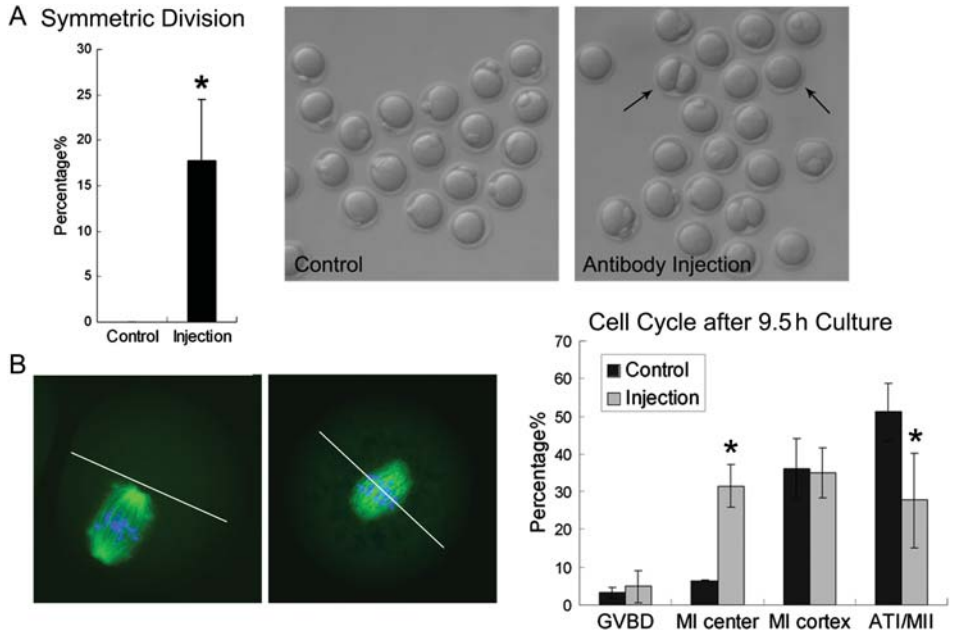

Figure 3 Effects of JMY antibody injection on asymmetric division and spindle migration in mouse oocytes. (**A**) Rate of symmetric division after JMY antibody injection. Arrows show symmetric division. (**B**) Rate of different stages after 9.5 h of culture in oocytes after JMY antibody injection. Bar showed the central position of oocyte. In the control group, the spindle of most oocytes migrated to the cortex, while in the antibody injection group, the spindle of most oocytes remained at the center after 9.5 h culture. *, significantly different ($P < 0.05$).

$n = 62$) were arrested at the TI stage, only 30.9 $\pm$ 2.9% of oocytes extruded the first polar body in the JMY RNAi group, but a large proportion of oocytes in the control group extruded polar body (54.1 $\pm$ 7.6%, $n = 74$), while only 29.7 $\pm$ 3.5% of oocytes were at the TI stage (Fig. 2D). Symmetric division rate was also examined in the MII oocytes. A total of 44.3 $\pm$ 9.6% ($n = 52$) of the MII oocytes underwent symmetric division in the JMY RNAi group, significantly higher than that of the control group (1.1 $\pm$ 1.1%, $n = 62$; Fig. 2D).

Since the oocytes described above underwent symmetric division, the effect of JMY on spindle migration was analyzed. The spindle of most oocytes in the control group formed and moved to the cortex or entered anaphase I after 9.5 h of culture (MI center 8.1 $\pm$ 8.1%, MI cortex 29.7 $\pm$ 4.4%, ATI/MII 54.1 $\pm$ 10.7%, $n = 37$; Fig. 2E). However, a large proportion of oocytes injected with JMY siRNA possessed centrally located spindles (MI center 36.7 $\pm$ 12.2%, MI cortex 44 $\pm$ 13.9%, ATI/MII 16.8 $\pm$ 7.6%, $n = 55$). Thus, the disruption of JMY by RNAi caused the failure of peripheral spindle migration.

JMY antibody injection causes failure of asymmetric division and cytokinesis

To further confirm the functions of JMY during mouse oocyte meiotic maturation, JMY antibody injection was employed to disrupt the JMY protein activity. As shown in Fig. 3A, similar results were observed with JMY RNAi group. Oocytes underwent symmetric division after JMY antibody injection, showing 2-cell-like MII oocytes. The rate of symmetric division was significantly higher than that of the control group (17.8 $\pm$ 6.7%, $n = 59$ versus 0, $n = 48$).

The effect of JMY on spindle migration was also analyzed. The spindle of most oocytes in the control group formed and moved to the cortex or entered anaphase I after 9.5 h of culture (MI center 6.4 $\pm$ 0.1%, MI cortex 36.1 $\pm$ 8%, ATI/MII 51.2 $\pm$ 7.6%, $n = 47$) (Fig. 3B). However, a large proportion of those injected with JMY antibody possessed centrally located spindles (MI center 31.5 $\pm$ 5.7%, MI cortex 34.9 $\pm$ 6.6%, ATI/MII 27.7 $\pm$ 12.6%, $n = 119$). Thus, the disruption of JMY by antibody injection also caused the failure of peripheral spindle migration.

JMY RNAi and antibody injection cause failure of cortical reorganization

To further confirm the involvement of JMY in spindle migration, we first examined actin cap formation, a feature of oocyte polarization. As shown in Fig. 4, the chromosomes of the control group had already moved to the cortex and formed an actin cap by the latter MI stage, but in the JMY RNAi and antibody injection group, no actin cap was observed. At the ATI stage, the chromosomes segregated at the region of the cortex with an actin cap in the control oocytes, but in the JMY RNAi and antibody injection groups, they segregated in central cytoplasm and cytokinesis was initiated. In the MII stage of the control group, a small polar body and a large MII oocyte formed, and the chromosomes were located under the region of the cortex where the actin cap formed. Conversely, in the JMY RNAi and antibody injection groups, the oocytes formed a 2-cell-like structure with no actin cap. Thus, no actin cap formed after disruption of JMY activity in oocytes.

Formation of the CGFD was examined as a further feature of oocyte polarization. The CGs were absent near the region of the cortex close to the chromosomes at the MI stage in the control group, whilst JMY RNAi and antibody injection resulted in the CGs

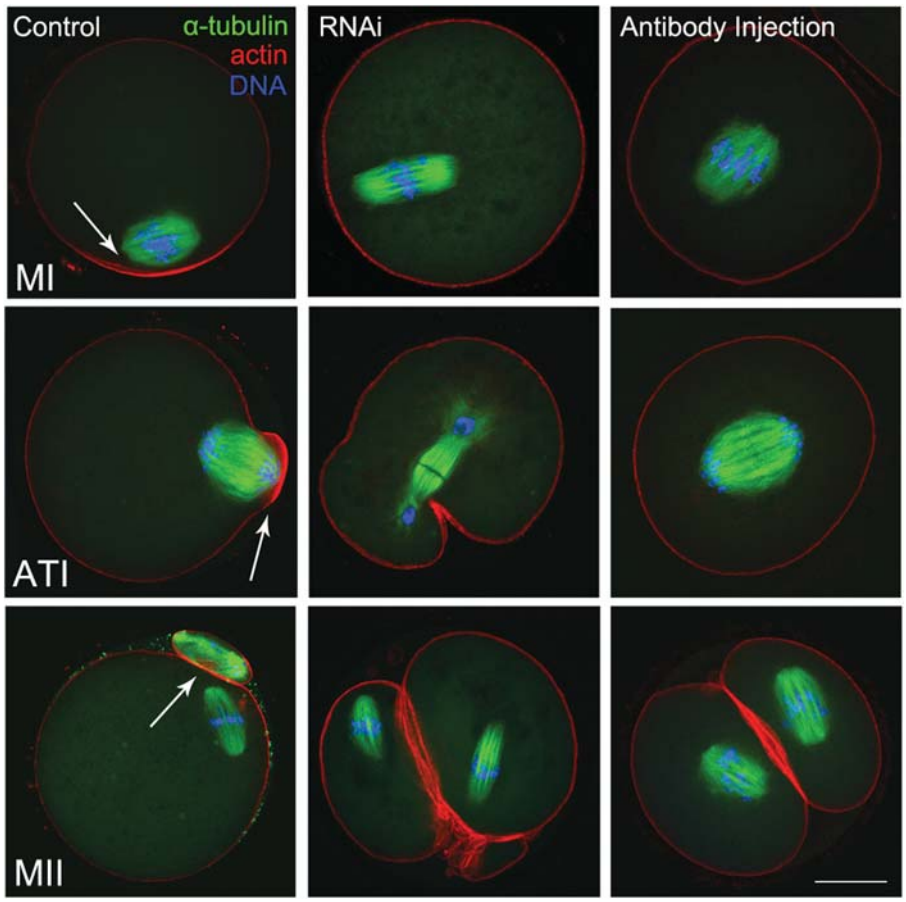

Figure 4 Effects of JMY RNAi and antibody injection on actin cap formation in mouse oocytes. At the MI, ATI and MII stage, an actin cap formed in the control group, whilst no actin cap formed in JMY RNAi and antibody injection oocytes. An arrowhead illustrates the actin cap. Green, α-tubulin; red, actin; blue, chromatin. Bar = 20 μm.

being distributed uniformly across the entire cortex (Fig. 5). The results showed that the formation of the CGFD was disrupted after JMY RNAi and antibody injection. Taken together, the results show that inhibition of JMY disrupted formation of the actin cap and CGFD, indicating a failure of oocyte polarization.

Discussion

In the present study, we investigated the expression, localization and potential functions of JMY during mouse oocyte meiotic maturation. The results demonstrated that JMY was expressed in mouse oocytes. In particular, the inhibition of JMY activity by RNAi and antibody injection affected the formation of actin cap and CGFD, and disrupted peripheral spindle migration, asymmetric division and cytokinesis completion during oocyte meiotic maturation. The study therefore provides direct evidence of the involvement of JMY in oocyte polarization and cytokinesis.

At the MI stage, spindle formed and migrated to the cortex, and JMY mRNA expressed largely in this stage, indicating the possible function of JMY in this process. During oocyte meiotic maturation, JMY was found at the spindle, co-localized with microtubules and it was also observed in the cytoplasm. This localization pattern was similar to a recent finding of Formin (Kwon *et al.*, 2010), which was also reported to be an actin nucleation actor and a critical regulator for spindle migration and cytokinesis in mouse oocyte (Leader *et al.*, 2002; Dumont *et al.*, 2007). The confirmation of spindle localization pattern of Formin linked spindle microtubules to the actin network involved in spindle migration (Azoury *et al.*, 2009; Kwon *et al.*, 2010). The similar localization of JMY with Formin indicates the roles of JMY in spindle migration, which needs the driving force of actin. The cytoplasm localization of JMY may be necessary for the roles of JMY in cytokinesis, which needs actin enrichment in the cortex.

To confirm the assumption, JMY RNAi and antibody injection approaches were employed to investigate the roles of JMY during mouse oocyte meiotic maturation. The results demonstrated that inhibition of JMY activity resulted in symmetric cell division and the disruption of oocyte polarity. Multiple processes appear to be involved in the regulation of asymmetric division, the first of which is spindle positioning, including spindle migration and anchoring. The second process involves cortical reorganization, including the loss of microvilli and CGFD as well as failure of actin cap formation (Azoury *et al.*, 2009; Brunet and Verlhac, 2011). Because the development of the actin cap and CGFD are the key steps in oocyte polarization, these events were examined in this study. Formation of both of these regions was found to be lacking after JMY

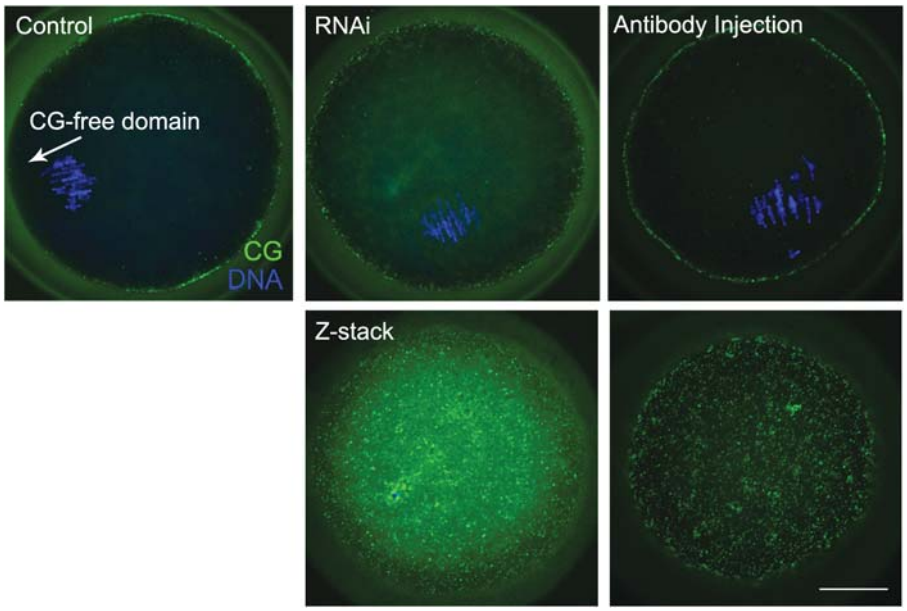

Figure 5 Effects of JMY RNAi and antibody injection on CGFD formation in mouse oocytes. The CGs were absent in the cortex close to where the chromosomes were located at the MI stage in the control group. Conversely, in the oocytes of JMY RNAi and antibody injection, the CGs were distributed throughout the entire cortex. Z-stack showed the presence of different scanned layers. An arrowhead shows the CGFD. Green, cortical granules; blue, chromatin. Bar = 20 μm.

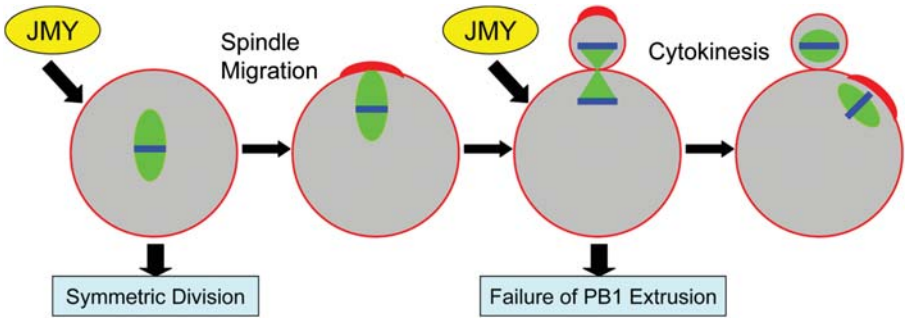

Figure 6 JMY is involved in multiple processes associated with oocyte asymmetric division, including spindle migration, polarization and cytokinesis. Disruption of spindle migration causes central spindle localization and symmetrical division. Disruption of cytokinesis causes cell cycle arrest at telophase I and failure to extrude the polar body.

RNAi or antibody injection; in contrast, actin and CGs were observed to distribute uniformly throughout the cortex. These results are indicative of the disruption of oocyte polarization, which may cause the subsequent symmetric division.

Cortical reorganization is initiated after the spindle migrates to the cortex. We therefore investigated whether JMY was involved in this earlier process of spindle positioning. The results showed that the disruption of JMY activity by JMY RNAi or antibody injection caused the arrest of the spindle in a central location after 9.5 h in culture, a time by which the spindles of most oocytes should have moved to the cortex or entered anaphase I. Spindle migration is known to be dependent on actin; therefore, JMY may regulate spindle migration through its influence on actin nucleation (Fig. 6). Plus, disruption of actin caps and CGFD formation further provided the evidence for the involvement of JMY in spindle migration.

Polar body extrusion is depended upon cytokinesis, which in turn is driven by actin. Since JMY was reported to activate the Arp2/3 complex, which regulates actin nucleation, we speculated whether JMY also regulated cytokinesis. Our results showed that after 12 h of culture, the disruption of JMY activity was largely responsible for the arrested development of oocytes at TI stage, which was characterized by a failure to extrude the polar body. These observations are indicative of a failure of cytokinesis (Fig. 6). The functions of JMY are similar to those described for Formin-2 and cortactin (Dumont *et al.*, 2007; Yu *et al.*, 2010) in mouse oocytes, whose disruption also results in the failure of spindle migration and cytokinesis. This

suggests that there may be a relationship between JMY, Formin-2 and cortactin, which needs further verification.

In conclusion, our results indicate that JMY regulates oocyte asymmetric division through wide-ranging effects on spindle migration, polarization and cytokinesis during meiotic maturation.

Authors' roles

S.C.S. designed and performed the experiments, analyzed the data and drafted the manuscript; Q.Y.S. analyzed the data and revised the paper; N.H.K. designed the experiments, analyzed the data and revised the paper.

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References

Azoury J, Verlhac MH, Dumont J. Actin filaments: key players in the control of asymmetric divisions in mouse oocytes. *Biol Cell* 2009; **101**:69–76.

Bosticardo M, Marangoni F, Aiuti A, Villa A, Grazia Roncarolo M. Recent advances in understanding the pathophysiology of Wiskott-Aldrich syndrome. *Blood* 2009;**113**:6288–6295.

Brunet S, Verlhac MH. Positioning to get out of meiosis: the asymmetry of division. *Hum Reprod Update* 2011;**17**:68–75.

Campellone KG, Welch MD. A nucleator arms race: cellular control of actin assembly. *Nat Rev Mol Cell Biol* 2010;**11**:237–251.

Campellone KG, Webb NJ, Znameroski EA, Welch MD. WHAMM is an Arp2/3 complex activator that binds microtubules and functions in ER to Golgi transport. *Cell* 2008;**134**:148–161.

Chesarone MA, Goode BL. Actin nucleation and elongation factors: mechanisms and interplay. *Curr Opin Cell Biol* 2009;**21**:28–37.

Chesarone MA, DuPage AG, Goode BL. Unleashing formins to remodel the actin and microtubule cytoskeletons. *Nat Rev Mol Cell Biol* 2010; **11**:62–74.

Coutts AS, Weston L, La Thangue NB. A transcription co-factor integrates cell adhesion and motility with the p53 response. *Proc Natl Acad Sci USA* 2009;**106**:19872–19877.

Dahl JP, Wang-Dunlop J, Gonzales C, Goad ME, Mark RJ, Kwak SP. Characterization of the WAVE1 knock-out mouse: implications for CNS development. *J Neurosci* 2003;**23**:3343–3352.

Deng M, Kishikawa H, Yanagimachi R, Kopf GS, Schultz RM, Williams CJ. Chromatin-mediated cortical granule redistribution is responsible for the formation of the cortical granule-free domain in mouse eggs. *Dev Biol* 2003;**257**:166–176.

Dumont J, Million K, Sunderland K, Rassinier P, Lim H, Leader B, Verlhac MH. Formin-2 is required for spindle migration and for the late steps of cytokinesis in mouse oocytes. *Dev Biol* 2007;**301**:254–265.

Goode BL, Rodal AA, Barnes G, Drubin DG. Activation of the Arp2/3 complex by the actin filament binding protein Abp1p. *J Cell Biol* 2001; **153**:627–634.

Kutsuna H, Suzuki K, Kamata N, Kato T, Hato F, Mizuno K, Kobayashi H, Ishii M, Kitagawa S. Actin reorganization and morphological changes in human neutrophils stimulated by TNF, GM-CSF, and G-CSF: the role of MAP kinases. *Am J Physiol Cell Physiol* 2004;**286**:C55–C64.

Kwon S, Shin H, Lim HJ. Dynamic interaction of formin proteins and cytoskeleton in mouse oocytes during meiotic maturation. *Mol Hum Reprod* 2010 doi 10.1093/molehr/gaq088.

Leader B, Lim H, Carabatsos MJ, Harrington A, Ecsedy J, Pellman D, Maas R, Leder P. Formin-2, polyploidy, hypofertility and positioning of the meiotic spindle in mouse oocytes. *Nat Cell Biol* 2002;**4**:921–928.

Linardopoulou EV, Parghi SS, Friedman C, Osborn GE, Parkhurst SM, Trask BJ. Human subtelomeric WASH genes encode a new subclass of the WASP family. *PLoS Genet* 2007;**3**:e237.

Lommel S, Benesch S, Rottner K, Franz T, Wehland J, Kuhn R. Actin pedestal formation by enteropathogenic Escherichia coli and intracellular motility of Shigella flexneri are abolished in N-WASP-defective cells. *EMBO Rep* 2001;**2**:850–857.

Longo FJ, Chen DY. Development of cortical polarity in mouse eggs: involvement of the meiotic apparatus. *Dev Biol* 1985;**107**:382–394.

Maro B, Verlhac MH. Polar body formation: new rules for asymmetric divisions. *Nat Cell Biol* 2002;**4**:E281–E283.

Renault L, Bugyi B, Carlier MF. Spire and Cordon-bleu: multifunctional regulators of actin dynamics. *Trends Cell Biol* 2008;**18**:494–504.

Shikama N, Lee CW, France S, Delavaine L, Lyon J, Krstic-Demonacos M, La Thangue NB. A novel cofactor for p300 that regulates the p53 response. *Mol Cell* 1999;**4**:365–376.

Snapper SB, Takeshima F, Anton I, Liu CH, Thomas SM, Nguyen D, Dudley D, Fraser H, Purich D, Lopez-Ilasaca M *et al.* N-WASP deficiency reveals distinct pathways for cell surface projections and microbial actin-based motility. *Nat Cell Biol* 2001;**3**:897–904.

Soderling SH, Langeberg LK, Soderling JA, Davee SM, Simerly R, Raber J, Scott JD. Loss of WAVE-1 causes sensorimotor retardation and reduced learning and memory in mice. *Proc Natl Acad Sci USA* 2003; **100**:1723–1728.

Sun QY, Schatten H. Regulation of dynamic events by microfilaments during oocyte maturation and fertilization. *Reproduction* 2006; **131**:193–205.

Sun SC, Xiong B, Lu SS, Sun QY. MEK1/2 is a critical regulator of microtubule assembly and spindle organization during rat oocyte meiotic maturation. *Mol Reprod Dev* 2008;**75**:1542–1548.

Sun SC, Wei L, Li M, Lin SL, Xu BZ, Liang XW, Kim NH, Schatten H, Lu SS, Sun QY. Perturbation of survivin expression affects chromosome alignment and spindle checkpoint in mouse oocyte meiotic maturation. *Cell Cycle* 2009;**8**:3365–3372.

Sun SC, Lee SE, Xu YN, Kim NH. Perturbation of Spc25 expression affects meiotic spindle organization, chromosome alignment and spindle assembly checkpoint in mouse oocytes. *Cell Cycle* 2010a;**9**:4552–4559.

Sun SC, Lin SL, Zhang DX, Lu SS, Sun QY, Kim NH. Borealin regulates bipolar spindle formation but may not act as chromosomal passenger during mouse oocyte meiosis. *Front Biosci (Elite Ed)* 2010b;**2**:991–1000.

Uruno T, Liu J, Zhang P, Fan Y, Egile C, Li R, Mueller SC, Zhan X. Activation of Arp2/3 complex-mediated actin polymerization by cortactin. *Nat Cell Biol* 2001;**3**:259–266.

Van Blerkom J, Bell H. Regulation of development in the fully grown mouse oocyte: chromosome-mediated temporal and spatial differentiation of the cytoplasm and plasma membrane. *J Embryol Exp Morphol* 1986; **93**:213–238.

Weaver AM, Karginov AV, Kinley AW, Weed SA, Li Y, Parsons JT, Cooper JA. Cortactin promotes and stabilizes Arp2/3-induced actin filament network formation. *Curr Biol* 2001;**11**:370–374.

Weed SA, Karginov AV, Schafer DA, Weaver AM, Kinley AW, Cooper JA, Parsons JT. Cortactin localization to sites of actin assembly in

lamellipodia requires interactions with F-actin and the Arp2/3 complex. *J Cell Biol* 2000;**151**:29–40.

Yamazaki D, Suetsugu S, Miki H, Kataoka Y, Nishikawa S, Fujiwara T, Yoshida N, Takenawa T. WAVE2 is required for directed cell migration and cardiovascular development. *Nature* 2003;**424**:452–456.

Yan C, Martinez-Quiles N, Eden S, Shibata T, Takeshima F, Shinkura R, Fujiwara Y, Bronson R, Snapper SB, Kirschner MW *et al*. WAVE2 deficiency reveals distinct roles in embryogenesis and Rac-mediated actin-based motility. *Embo J* 2003;**22**:3602–3612.

Yu D, Zhang H, Blanpied TA, Smith E, Zhan X. Cortactin is implicated in murine zygotic development. *Exp Cell Res* 2010;**316**:848–858.

Zuchero JB, Coutts AS, Quinlan ME, Thangue NB, Mullins RD. p53-cofactor JMY is a multifunctional actin nucleation factor. *Nat Cell Biol* 2009;**11**:451–459.