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ORIGINAL PAPER

Changes in estrogen receptor-α variant (ER-α36) expression during mouse ovary development and oocyte meiotic maturation

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Abstract The biological effects of estrogens are largely mediated through estrogen receptors (ERs), which belong to the nuclear receptor gene family of transcription factors. ER-α36 has been recently identified as a new variant of ERα, but its expression and roles in female reproduction system remain unknown. Immunocytochemistry and confocal microscopy were employed to observe ER-α36 distribution in mouse ovary during postnatal development and in oocyte during meiotic maturation. ER-α36 was consistently present in the nuclei of oocytes regardless of follicular growth stage and mouse age until germinal vesicle breakdown (GVBD). Its immunosignal was smeared in granulosa cells. However, the ER-α36 signal is up-regulated and found in cytoplasm with little or no nuclear staining during corpus luteum development. ER-α36 was also found in theca cells. We showed by Western blot that ER-α36 was expressed in mouse oocytes at various maturation stages. When the function of nuclear ER-α36 was blocked by microinjecting anti-ER-α36 specific antibody into the germinal vesicle (GV) of mouse oocytes, the first polar body emission occurred earlier in a higher proportion of oocytes compared to the control. These results suggest that ER-α36 may play critical roles in mouse ovarian folliculogenesis and oocyte development.

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Abbreviations

ER	Estrogen receptor
GV	Germinal vesicle
GVBD	Germinal vesicle breakdown

Introduction

Estrogens are essential components of female reproduction, with vital roles in the uterus, ovary, mammary gland and hypothalamic-pituitary axis (Hewitt and Korach 2003). However, the exact roles of estrogens in early ovarian folliculogenesis and oocyte meiotic maturation have not been fully defined. Estrogen receptor (ER) knockout (i.e. ERKO), aromatase null (ArKO), and ER overexpression mouse models developed recently have thrown new light on the actions of estrogens in mammalian reproduction (Britt et al. 2002, 2004; Couse et al. 1999b, 2003; Dupont et al. 2000; Fisher et al. 1998; Tomic et al. 2007). Impairment of follicular development and postnatal sex reversal of the ovaries have been reported for immature ER *αβ* knockout mice, suggesting that both ERs are required for early ovarian folliculogenesis and the maintenance of germ and somatic cells in the postnatal ovary (Couse et al. 1999b; Dupont et al. 2000). Moreover, ArKO mouse, which possesses functional ERs but does not make endogenous estrogen, can improve the ovarian phenotype, decreased development of Sertoli cells and induced ovulation in some cases after treatment with exogenous estrogen (Britt et al. 2004). In summary, these data show that the biological effects of estrogens are mediated mainly through ERs.

Estrogen receptors (ER) are ligand-dependent transcription factor that regulate transcription of estrogen-responsive genes in the cell nucleus and belong to the steroid/thyroid hormone receptor superfamily (Evans 1988; Kong et al. 2003), all of which show an evolutionarily and functionally conserved structure (Laudet et al. 1992). The classical estrogen receptor (ER) exists as two subtypes, α and β (Gustafsson 2000; Katzenellenbogen et al. 2000; Kuiper et al. 1998). On other hand, there are also a number of splice variants for the isoforms, some of which influence the activity of the wild type receptor (Hopp and Fuqua 1998; Jazaeri et al. 1999; Schreihofer et al. 2000; Shupnik et al. 1998; Wang et al. 2006). An alternatively spliced variant of ER-α66, ER-α36 has been identified recently (Wang et al. 2005). It lacks both transcriptional activation domains of ER-α66, retains portions of DNA-binding domain, partial dimerization and ligand-binding domains, and possesses a unique 27 amino acid domain that replaces the last 138 amino acid of ER-α66 (Wang et al. 2005). Recent reports revealed that ER-α36 localized to plasma membrane and cytoplasm with little or no nuclear staining in breast cancer patients' tissue and breast cancer cell lines (Lee et al. 2008; Wang et al. 2006). Moreover, ER-α36 can inhibit the transactivation of both ER-α66 and ER-β, stimulate MAPK signaling pathway and induce cell growth in breast cancer cell lines (Wang et al. 2006).

The expression and function of classical ER α and β in mammalian ovary were reported in several studies. But the distribution of ER-α36 in mammalian ovary and oocyte, and its role in ovarian folliculogenesis and oocyte meiotic maturation is not known. Therefore, in this study, we examined the expression of ER-α36 in follicular growth and oocyte maturation in mice.

Materials and methods

Chemicals and antibodies

All chemicals used in this study were purchased from Sigma Chemical Company (St Louis, MO) unless otherwise noted. Rabbit polyclonal anti-ER-α36 antibody was a gift from Dr Zhao-Yi Wang (Creighton University, California Plaza, USA).

Collection of ovaries and oocytes

Animal care and use were conducted in accordance with the Animal Research Committee Guidelines of the Institute of Zoology, Chinese Academy of Sciences. The mice with color gene type of *aabbcc* were from an inbred strain of Kunming white mice, a native breed widely used in biological research in China. To explore the developmental expression patterns of ER-α36 in mouse ovaries, ovaries were removed from postnatal mice at 1, 3, 8, 16, 30, 60 days, respectively, placed in the optimal cutting temperature (OCT) compound (Tissue-Tek), snap frozen in liquid nitrogen, and sectioned at 6 μm in a leica cryostat microtome (Leica CM1900, Germany) for immunostaining. Oocytes displaying a germinal vesicle (GV) were collected from ovaries of 6-week-old female mice in M2 medium (Sigma, St Louis, MO). Then, the oocytes were washed thoroughly and cultured in M16 medium (Sigma, St Louis, MO) at 37°C in a humidified 5% CO_2 incubator. At different times after culture, only oocytes that underwent GVBD between 1 and 2 h of culture were used for either immunostaining or Western blot analysis.

Immunofluorescent staining and confocal microscopy

Tissue sections from three mice for each time point were randomly selected and immunostained for ER-α36. Sections were fixed with 4% paraformaldehyde in PBS (pH 7.4) for 5 min, rinsed three times for 5 min each in PBS, permeabilized with 0.5% Triton X-100 for 10 min and then blocked in 1% BSA-supplemented PBS (blocking solution) for 1 h at room temperature. Following the blocking, sections were exposed overnight to anti-ER-α36 antibody diluted 1:100 with blocking solution. After three washes in PBS containing 0.1% Tween 20 and 0.01% Triton X-100 (washing solution) for 5 min each, the sections were labeled with FITC-conjugated goat anti-rabbit IgG antibody diluted 1:100 with washing solution for 1 h at room temperature. After three washes in washing solution, nuclear status of cells were evaluated by staining with PI (propidium iodide, 10 μg/ml in PBS) for 5 min. Following extensive washing, sections were mounted on glass slides.

For staining of ER-α36 in oocytes, oocytes were fixed with 4% paraformaldehyde in PBS (pH 7.4) for at least 30 min at room temperature. After being permeabilized with 0.5% Triton X-100 at room temperature for 20 min, oocytes were processed identically as mentioned above. Each experiment was repeated three times, and at least 30 oocytes were examined each time.

Nonspecific staining was determined by substituting primary antibodies with normal rabbit IgG. Sections and cells were observed under a Confocal Laser-Scanning Microscope (Zeiss LSM 510 META, Germany). The same instrument settings were used for each replicate.

Western blot analysis

For detection of ER-α36 expression, proteins from 300 oocytes at the appropriate stage of maturation were collected in SDS sample buffer and heated for 4 min at 100°C. After cooling on ice and centrifugation at 10,000*g* for

3 min, samples were frozen at −20°C until use. The total proteins were separated by SDS-PAGE with a 4% stacking gel and a 10% separating gel at 90 V, 0.5 h and 120 V, 2.5 h, respectively, and then electrophoretically transferred onto polyvinylidene difluoride membrane for 2.5 h, 200 mA, at 4°C. Membranes were blocked in TBST buffer (10 mM Tris, 150 mM NaCl, 0.1% Tween 20, pH 7.4) containing 5% skimmed milk for 2 h at room temperature and then incubated with polyclonal rabbit anti-ER-α36 antibody, diluted 1:1,000 in TBST containing 0.5% skimmed milk, overnight at 4°C. After three washes of 10 min each in TBST, the membrane was incubated for 1 h at 37°C with HRP-conjugated goat anti-rabbit IgG diluted 1:1,000 in TBST. Then the membrane was processed using the enhanced chemiluminescence (ECL) detection system (Amersham, Piscataway, NJ). The prestained protein molecular weight marker (Fermentas Life Sciences) used for evaluation of molecular weight of ER-α36 was a mixture of six proteins with apparent molecular weight of 117, 85, 48, 34, 26, and 19 kDa, respectively. Equal protein loading was confirmed by detection of β-actin. For reprobing of β-actin, the membrane was washed in stripping buffer (100 mM β-mercatoethanol, 20% SDS, 62.5 mM Tris, pH 6.7) to strip off bound antibody after ECL detection at 50°C for 30 min. The membrane was reprobed with mouse anti-β-actin antibody (Zhongshan Goldenbridge Biotechnology Co.) diluted 1:1,000, using the same procedure as described above. The secondary antibody was HRP-conjugated goat anti-mouse lgG (1:1,000). All experiments were repeated at least three times.

Antibody microinjection

Microinjection was performed using a Nikon Diaphot ECLIPSE TE 300 (Nikon UK Ltd, Kingston upon Thames Surrey, UK) inverted microscope equipped with Narishige MM0-202N hydraulic three-dimensional micromanipulators (Narishige Inc., Sea Cliff, NY, USA). A microinjection volume of 7 pl of anti-ERα36 antibody was injected into the nucleus of a fully-grown GV oocyte in all experiment. The oocytes were kept in M2 medium supplemented with 0.2 mM IBMX to prevent GV breakdown during the injection period. After microinjection, oocytes were washed twice in M16 and then cultured. Control oocytes were microinjected with the same amount of rabbit IgG. Each experiment was repeated three times, and at least 30 oocytes were examined each time.

Statistical analysis

All percentages from three repeated experiments were expressed as mean ± SEM and the number of oocytes observed was labeled in brackets as (*n*=). Data were analyzed by χ^2 test. Differences at $p < 0.05$ were considered to be significant.

Results

ER-α36 protein expression in developing mouse ovary

The distribution of ER-α36 in mouse ovaries collected from mice at different ages was shown in Fig. 1. Strong ER-α36 immunoreactivity was detected in surface epithelium cells and oocytes at all post-natal age groups examined. In the ovaries of 1-day post-natal (P1) mouse, ER-α36 immunoreactivity was evident in both oocytes and somatic cells. However, oocyte clusters showed more intense immunosignal compared to other clusters (Fig. 1 P1). When morphologically distinct follicles can be identified at P3, strong ER-α36 staining was primarily associated with granulosa cells and oocytes, whereas interstitial cells exhibited low staining. In oocytes, ER-α36 immunosignal was more intense in nucleus than in cytoplasm, while ER-α36 immunostaining was not clearly delineated to a specific subcellular compartment in granulosa cells. However, the nuclear staining was somewhat more stronger than cytoplasm compartment (Fig. 1 P3). With further development of the follicles by P8, a distinct theca cell layer is formed in secondary follicles. Beside oocytes and granulosa cells, these long-stretched fiber-like theca cells showed evident ER-α36 immunostaining (Fig. 1 P8). The patterns of ER-α36 expression had no obvious changes in subsequent development before ovulation (Fig. 1 P16 and P30). After administering the ovulatory stimulus, granulosa cells of mature follicle were luteinized and transformed into corpus luteum cells, which presented stronger ER-α36 immunostaining than that of granulosa cells. In corpus luteum cells, ER-α36 localized to cytoplasm with little or no nuclear staining (Fig. 1 P60). No ER-α36-specific immunoreactivity could be detected in sections incubated with normal rabbit IgG, which is a substitute for the primary antibody (Fig. 1 P1nc).

Expression of ER-α36 in mouse oocytes during meiotic maturation

The ER-α36 distribution during oocyte meiotic maturation was examined by immunofluorescence. As shown in Fig. 2, in oocytes at germinal vesicle (GV) stage, ER-α36 was mainly localized to the nucleus. From germinal vesicle breakdown (GVBD) to metaphase II stage during mouse oocyte maturation, there was no concentrated signal of ER-α36 immunoactivity in oocytes. ER-α36 could distribute homogenously in the cytoplasm, since Western blot analysis showed that ER-α36, which has an approximate molecular weight of 36 kDa, was expressed in mouse oocytes at all

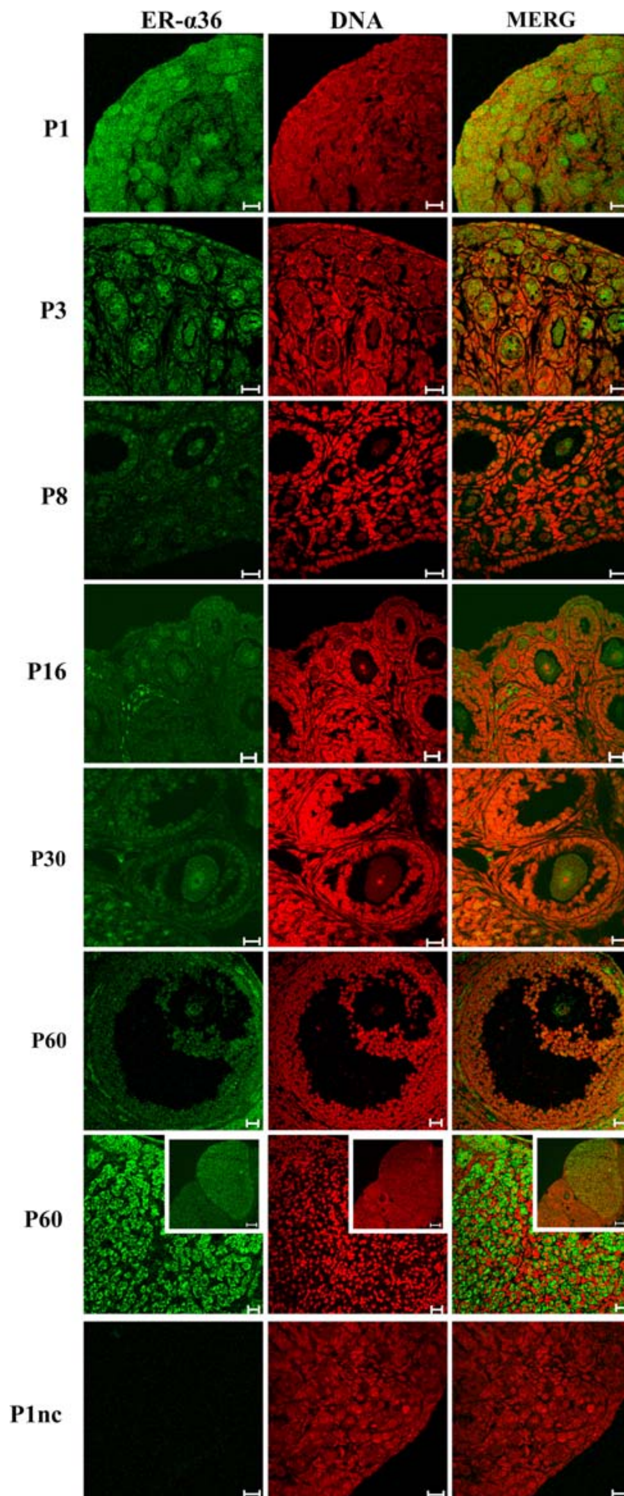

Fig. 1 Immunofluorescent localization of ER-α36 in mouse ovary during the postnatal development. *Green* ER-α36, *red* chromatin, *yellow* overlapping of green and red. Sections were from ovaries collected at 1-day-old (P1), 3-days-old (P3), 8-days-old (P8), 16-days-old (P16), 30-days-old (P30) and 60-days-old (P60) post birth. Sections of 1-day-old (P1nc) postnatal mice ovaries stained when the primary antibody was substituted with normal rabbit lgG were used as control. *Bar* 20 μm. The upper inserts in P60 showed ER-α36 immunosignal was more intense in corpus luteum cells than in granulosa cell. *Bar* 100 μm

stages during meiosis with no evident changes (Fig. 2b). To investigate the potential role of ER-α36 in regulating meiotic maturation of mouse oocyte, we depleted the protein function by microinjecting anti-ER-α36 specific antibody into the nucleus. After 9.5 h of culture, 32.3% (61/195) of anti-ER-α36 antibody injected oocytes displayed first polar body (PB1), which was significantly higher than 17.0% (18/106) in the control group ($p < 0.05$) (Fig. 3).

Discussion

This study for the first time revealed the ER-α36 distribution in postnatal mouse ovary development and in oocyte at different maturation stages. It is found that ER-α36 was constitutively present in the nuclei of oocytes in spite of follicular growth stage and mouse age until GVBD occurrence. Its distribution changes in granulosa cells, theca cells, and corpus luteum cells were also observed. These data may lay ground for the future research on the critical roles of ER-α36 in mouse follicle/oocyte growth and maturation.

As described above, ER-α36 is devoid of both the AF-1 and AF-2 transactivation domains of ER-α66 but retains the DNA-binding domain, partial dimerization and ligand-binding domains, which implies that ER-α36 may effectively compete with ER-α66 for the DNA-binding elements (EREs) in estrogen-responsive genes and suppress the transactivation of ER-α66. It has been demonstrated that ER-α36 inhibits estrogen-dependent and estrogen-independent transactivation activity of ER-α66 and ER*β* in vitro (Wang et al. 2006). However, the correlation between ER-α36 and ER-α66 or ER*β* in vivo as well as the roles of ER-α36 in early ovarian folliculogenesis and oocyte meiotic maturation is not known. In the present study, our data showed that ER-α36 immunosignal is evident in theca and granulosa cells. Several lines of evidence show that ERα is highly expressed in the interstitial/theca compartment, whereas ER*β* expression is limited to granulosa cells of growing follicles in the mouse ovary (Couse et al. 1997; Tremblay et al. 1997). Our results imply the possibility of the actions between ER-α36 and ERα or ER*β* within the same cell type. Additionally, examination of the ovaries of ERα*β*KO mice indicated a phenotype distinct from those observed in ovaries from ERαKO or ER*β*KO mice (Couse et al. 1999a, b; Dupont et al. 2000). Therefore, it is reasonable to speculate that a delicate balance of ERs interactions may ultimately determine the nature of estrogen signaling, which is critical for the differentiation of ovarian somatic cells.

The mechanism by which granulose cells start to differentiate and undergo luteinization has not been well defined. Evidence has accumulated to indicate that ERα is involved

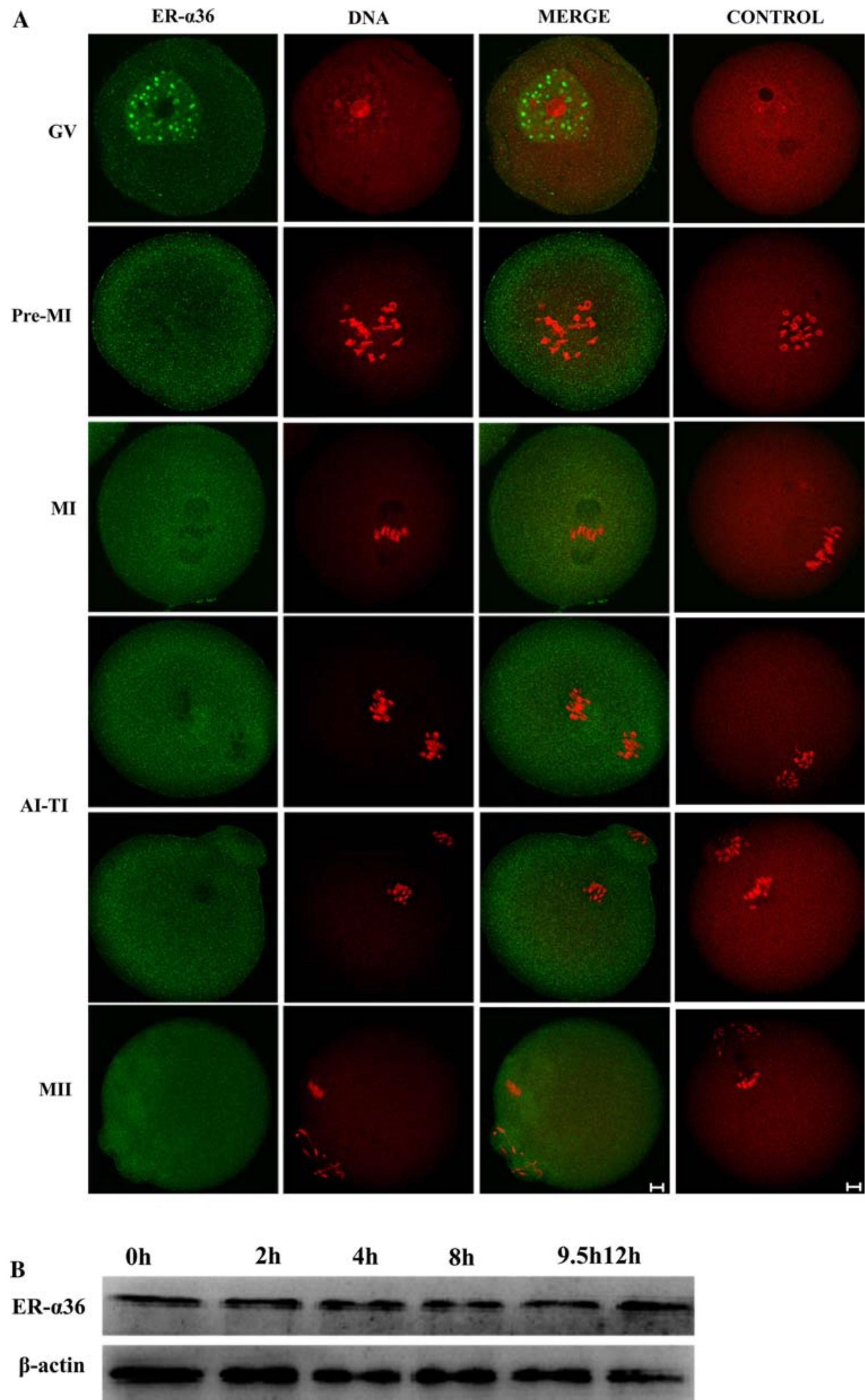

Fig. 2 Localization and expression of ER-α36 during mouse oocyte maturation. **a**. Immunofluorescent localization of ER-α36 in mouse oocytes. *Green* ER-α36, *red* chromatin, *yellow* overlapping of green and red. *GV* oocytes at germinal vesicle, *Pro-MI* oocytes at first prometaphase, *MI* oocytes at first metaphase, *AI-TI* oocytes at first anaphase and telophase, *MII* oocytes at second metaphase. Pictures stained when normal rabbit lgG substituted for primary antibody were used as control. *Bar* 10 µm. **b** ER-α36 immunoblotting of cellular extracts from 300 oocytes cultured for 0, 2, 4, 8, 9.5, and 12 h, respectively. A control blot shows equivalent levels of protein loading between various samples

in this process. Female mice null for ERα (ERαKO) are anovulatory, infertile, and possess hyperemic ovaries devoid of corpus luteum in the presence of elevated LH levels (Couse et al. 1999a, 2003). Although immature ERαKO females do successfully ovulate when treated with exogenous gonadotropins if administered before onset of the overt hypergonadotropic phenotypes in the ovary, but the number of produced corpus luteum is lesser than controls

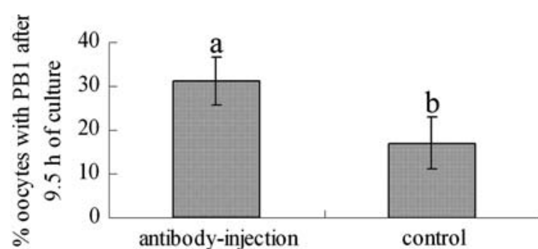

Fig. 3 Microinjection of anti-ER-α36 antibody into the nucleus accelerates the emission of first polar body in mouse oocytes. Data are presented as mean percentage of PB1 ± SEM of three independent experiments after oocytes were cultured for 9.5 h. *PB1* oocytes with first polar body. Different superscripts denote statistical difference at a $p < 0.05$ level of significance

(Couse et al. 1999a; Rosenfeld et al. 2000). Furthermore, in ERαKO ovaries, there is an almost total lack of detectable progesterone receptor expression after PMSG stimulation (Couse et al. 2005). Our results demonstrated that ER-α36 expression rapidly increased and its prominent staining was observed in cytoplasm with little or no nucleus staining following ovulation in luteinizing granulosa cells that were undergoing terminal differentiation to form the corpus luteum. During this process, ER-α36 immunoreactivity displayed a shift from nuclear to cytoplasmic distribution. What might be the actual cause of these changes in ER-α36 expression is currently unknown. Several groups have shown that expression and subcellular localization of ERα are related to follicular developmental stage and/or hormonal environment in vitro (Lenie and Smitz 2008; Sharma et al. 1999). There is accumulating evidence showing that ERs continuously shuttle between targets located within various cellular components, that is, membrane, cytoplasm, nucleus, etc., and their steady state localization is a consequence of a fine balance between operational strengths of 'nuclear localization signal' (NLS) and 'nuclear export signal' (NES) (Kumar et al. 2006; Leclercq et al. 2006). This process is regulated by ligand and/or protein-induced ER conformational changes (Leclercq et al. 2006). Another group has found that a pure antiestrogen RU 58668 profoundly modified the subcellular distribution of ER in a specific time-, dose- and protein synthesis-dependent manner. Furthermore, this effect was reversed by RU 58668 withdrawal, together with estradiol treatment (Devin-Leclerc et al. 1998). When granulosa cells proceed to luteinization and shift their main products from estrogen to progesterone, significant changes in the expression of many genes occur. Therefore, we could not exclude the possibility that the NLS of ER-α36 is sequestered by some protein(s), which results in ER-α36 staying in cytoplasm, the site of protein synthesis or that NES of ER-α36 is activated by some protein(s), which leads to ER-α36 translocation from nucleus to cytoplasm. Report has revealed that prolactin-mediated pathway is highly important in the induction of ERα in rodent corpus luteum (Frasor and Gibori 2003; Telleria et al. 1998). A similar intense cytoplasmic ERα staining has been reported in luteinized cells of ovine and mouse (Lenie and Smitz 2008; Zieba et al. 2000). It is now accepted that ERs exert their roles in cells by a number of distinct mechanisms. The classical mechanism of ER action involves estrogen binding to receptors in the nucleus, after which the receptors dimerize and bind to specific response elements known as estrogen response elements (EREs) located in the promoters of target genes to activate or suppress the transcriptional activity of target gene (Bjornstrom and Sjoberg 2005). On other hand, the nongenomic actions of membrane-associated ERs result mainly from rapid activation of cellular signaling systems that eventually act on target transcription factors (Bjornstrom and Sjoberg 2005). Activation of the MAPK signaling pathway by ER-α36 has been established (Wang et al. 2006). Based upon those findings we could speculate that ER-α36 plays an important role in ovulation and luteinization through nongenomic effects, rapidly responding to LH surge. However, all these above speculations need support by solid evidence from ER-α36 knockout mice.

Previous study reported that in response to estrogen, an ERa/p300 complex is recruited to an AP-1 domain located in the proximal BRCA-1 promoter and activates BRCA-1 transcription (Jeffy et al. 2005), whereas BRCA-1 could be a candidate controlling spindle checkpoint component in mitotic and meiotic cells (Joukov et al. 2006; Pan et al. 2008). During GVBD, mammalian oocytes undergo a dramatic change in nuclear organization in which chromatin becomes progressively condensed and forms heterochromatin/chromosomes (De La Fuente 2006). When chromatin is diffused, transcription and translation can take place, but when chromatin becomes condensed, transcription is practically inactive (Curtis et al. 1995; Tomek et al. 2002). In the present study, ER-α36 was mainly concentrated to GV of oocytes regardless of follicular growth stage and mouse age until GVBD occurrence. After GVBD, the signal of ER-α36 was distributed evenly in cytoplasm. Moreover, we blocked the function of nuclear ER-α36 by microinjecting anti-ER-α36 specific antibody into GV of mouse oocytes, and found that the depletion of ER-α36 induced a significantly higher percentage of oocytes with first polar body earlier compared to control. Thus, we speculate that ER-α36 may be involved in regulating transcription or translation of some protein related to cell cycle checkpoint proteins to control cytoplasmic maturation in mammalian oocyte maturation. Of course, we can not exclude the possibility that the new element of antibody–antigen complex may stimulate polar body emission after injection of ER-α36 antibody into the cell. To elucidate and confirm how ER-α36 regulates the progress of meiotic cell cycle, additional biochemical and molecular biological studies are needed.

In summary, our results indicated that ER-α36 was mainly present in oocytes, granulose cells, and theca cells in mouse ovary at different developmental stages. Notably, its immunoreactivity increased during luteinzation. When mouse oocytes were cultured in vitro, the signal of ER-α36 was converted from concentrated GV distribution to homogenous cytoplasmic distribution after GVBD. Moreover, ER-α36 depletion from the GV could cause earlier emission of the first polar body. These data imply that ER-α36 may play critical roles in female mammalian reproduction, which needs further evidence in vivo.

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References

Bjornstrom L, Sjoberg M (2005) Mechanisms of estrogen receptor signaling: convergence of genomic and nongenomic actions on target genes. Mol Endocrinol 19:833–842

Britt KL, Kerr J, O'Donnell L, Jones ME, Drummond AE, Davis SR, Simpson ER, Findlay JK (2002) Estrogen regulates development of the somatic cell phenotype in the eutherian ovary. Faseb J 16:1389–1397

Britt KL, Stanton PG, Misso M, Simpson ER, Findlay JK (2004) The effects of estrogen on the expression of genes underlying the differentiation of somatic cells in the murine gonad. Endocrinology 145:3950–3960

Couse JF, Lindzey J, Grandien K, Gustafsson JA, Korach KS (1997) Tissue distribution and quantitative analysis of estrogen receptor-alpha (ERalpha) and estrogen receptor-beta (ERbeta) messenger ribonucleic acid in the wild-type and ERalpha-knockout mouse. Endocrinology 138:4613–4621

Couse JF, Bunch DO, Lindzey J, Schomberg DW, Korach KS (1999a) Prevention of the polycystic ovarian phenotype and characterization of ovulatory capacity in the estrogen receptor-alpha knockout mouse. Endocrinology 140:5855–5865

Couse JF, Hewitt SC, Bunch DO, Sar M, Walker VR, Davis BJ, Korach KS (1999b) Postnatal sex reversal of the ovaries in mice lacking estrogen receptors alpha and beta. Science 286:2328–2331

Couse JF, Yates MM, Walker VR, Korach KS (2003) Characterization of the hypothalamic-pituitary-gonadal axis in estrogen receptor (ER) Null mice reveals hypergonadism and endocrine sex reversal in females lacking ERalpha but not ERbeta. Mol Endocrinol 17:1039–1053

Couse JF, Yates MM, Deroo BJ, Korach KS (2005) Estrogen receptor-beta is critical to granulosa cell differentiation and the ovulatory response to gonadotropins. Endocrinology 146:3247–3262

Curtis D, Lehmann R, Zamore PD (1995) Translational regulation in development. Cell 81:171–178

De La Fuente R (2006) Chromatin modifications in the germinal vesicle (GV) of mammalian oocytes. Dev Biol 292:1–12

Devin-Leclerc J, Meng X, Delahaye F, Leclerc P, Baulieu EE, Catelli MG (1998) Interaction and dissociation by ligands of estrogen receptor and Hsp90: the antiestrogen RU 58668 induces a protein synthesis-dependent clustering of the receptor in the cytoplasm. Mol Endocrinol 12:842–854

Dupont S, Krust A, Gansmuller A, Dierich A, Chambon P, Mark M (2000) Effect of single and compound knockouts of estrogen receptors alpha (ERalpha) and beta (ERbeta) on mouse reproductive phenotypes. Development 127:4277–4291

Evans RM (1988) The steroid and thyroid hormone receptor superfamily. Science 240:889–895

Fisher CR, Graves KH, Parlow AF, Simpson ER (1998) Characterization of mice deficient in aromatase (ArKO) because of targeted disruption of the cyp19 gene. Proc Natl Acad Sci USA 95:6965–6970

Frasor J, Gibori G (2003) Prolactin regulation of estrogen receptor expression. Trends Endocrinol Metab 14:118–123

Gustafsson JA (2000) An update on estrogen receptors. Semin Perinatol 24:66–69

Hewitt SC, Korach KS (2003) Oestrogen receptor knockout mice: roles for oestrogen receptors alpha and beta in reproductive tissues. Reproduction 125:143–149

Hopp TA, Fuqua SA (1998) Estrogen receptor variants. J Mammary Gland Biol Neoplasia 3:73–83

Jazaeri O, Shupnik MA, Jazaeri AA, Rice LW (1999) Expression of estrogen receptor alpha mRNA and protein variants in human endometrial carcinoma. Gynecol Oncol 74:38–47

Jeffy BD, Hockings JK, Kemp MQ, Morgan SS, Hager JA, Beliakoff J, Whitesell LJ, Bowden GT, Romagnolo DF (2005) An estrogen receptor-alpha/p300 complex activates the BRCA-1 promoter at an AP-1 site that binds Jun/Fos transcription factors: repressive effects of p53 on BRCA-1 transcription. Neoplasia 7:873–882

Joukov V, Groen AC, Prokhorova T, Gerson R, White E, Rodriguez A, Walter JC, Livingston DM (2006) The BRCA1/BARD1 heterodimer modulates ran-dependent mitotic spindle assembly. Cell 127:539–552

Katzenellenbogen BS, Montano MM, Ediger TR, Sun J, Ekena K, Lazennec G, Martini PG, McInerney EM, Delage-Mourroux R, Weis K, Katzenellenbogen JA (2000) Estrogen receptors: selective ligands, partners, and distinctive pharmacology. Recent Prog Horm Res 55:163–193 discussion 194–165

Kong EH, Pike AC, Hubbard RE (2003) Structure and mechanism of the oestrogen receptor. Biochem Soc Trans 31:56–59

Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, van der Burg B, Gustafsson JA (1998) Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology 139:4252–4263

Kumar S, Saradhi M, Chaturvedi NK, Tyagi RK (2006) Intracellular localization and nucleocytoplasmic trafficking of steroid receptors: an overview. Mol Cell Endocrinol 246:147–156

Laudet V, Hanni C, Coll J, Catzeflis F, Stehelin D (1992) Evolution of the nuclear receptor gene superfamily. EMBO J 11:1003–1013

Leclercq G, Lacroix M, Laios I, Laurent G (2006) Estrogen receptor alpha: impact of ligands on intracellular shuttling and turnover rate in breast cancer cells. Curr Cancer Drug Targets 6:39–64

Lee LM, Cao J, Deng H, Chen P, Gatalica Z, Wang ZY (2008) ER-alpha36, a novel variant of ER-alpha, is expressed in ER-positive and -negative human breast carcinomas. Anticancer Res 28:479–483

Lenie S, Smitz J (2008) Estrogen receptor subtypes localization shifts in cultured mouse ovarian follicles. Histochem Cell Biol 129:827–840

Pan H, Ma P, Zhu W, Schultz RM (2008) Age-associated increase in aneuploidy and changes in gene expression in mouse eggs. Dev Biol 316:397–407

Rosenfeld CS, Murray AA, Simmer G, Hufford MG, Smith MF, Spears N, Lubahn DB (2000) Gonadotropin induction of ovulation and corpus luteum formation in young estrogen receptor-alpha knockout mice. Biol Reprod 62:599–605

Schreihofer DA, Stoler MH, Shupnik MA (2000) Differential expression and regulation of estrogen receptors (ERs) in rat pituitary and

cell lines: estrogen decreases ERalpha protein and estrogen responsiveness. Endocrinology 141:2174–2184

Sharma SC, Clemens JW, Pisarska MD, Richards JS (1999) Expression and function of estrogen receptor subtypes in granulosa cells: regulation by estradiol and forskolin. Endocrinology 140:4320–4334

Shupnik MA, Pitt LK, Soh AY, Anderson A, Lopes MB, Laws ER Jr (1998) Selective expression of estrogen receptor alpha and beta isoforms in human pituitary tumors. J Clin Endocrinol Metab 83:3965–3972

Telleria CM, Zhong L, Deb S, Srivastava RK, Park KS, Sugino N, Park-Sarge OK, Gibori G (1998) Differential expression of the estrogen receptors alpha and beta in the rat corpus luteum of pregnancy: regulation by prolactin and placental lactogens. Endocrinology 139:2432–2442

Tomek W, Torner H, Kanitz W (2002) Comparative analysis of protein synthesis, transcription and cytoplasmic polyadenylation of mRNA during maturation of bovine oocytes in vitro. Reprod Domest Anim 37:86–91

Tomic D, Frech MS, Babus JK, Symonds D, Furth PA, Koos RD, Flaws JA (2007) Effects of ERalpha overexpression on female reproduction in mice. Reprod Toxicol 23:317–325

Tremblay GB, Tremblay A, Copeland NG, Gilbert DJ, Jenkins NA, Labrie F, Giguere V (1997) Cloning, chromosomal localization, and functional analysis of the murine estrogen receptor beta. Mol Endocrinol 11:353–365

Wang Z, Zhang X, Shen P, Loggie BW, Chang Y, Deuel TF (2005) Identification, cloning, and expression of human estrogen receptor-alpha36, a novel variant of human estrogen receptor-alpha66. Biochem Biophys Res Commun 336:1023–1027

Wang Z, Zhang X, Shen P, Loggie BW, Chang Y, Deuel TF (2006) A variant of estrogen receptor-{alpha}, hER-{alpha}36: transduction of estrogen- and antiestrogen-dependent membrane-initiated mitogenic signaling. Proc Natl Acad Sci USA 103:9063–9068

Zieba D, Bilinska B, Schmalz-Fraczek B, Murawski M (2000) Immunohistochemical localization of estrogen receptors in the ovine corpus luteum throughout the estrous cycle. Folia Histochem Cytobiol 38:111–117