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Short communication

Dynamic subcellular localization of estrogen receptor alpha during the first two cleavages of mouse preimplantation embryos

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ABSTRACT

Zygotic gene activation (ZGA) is one of the most important events after mouse fertilization, but the mechanisms underpinning it are still unclear. Estrogen receptor alpha (ERα) is a versatile player in animal development. Our preliminary studies showed that ERα-specific antagonists blocked mouse 2-cell development and inhibited ZGA related gene expression, indicating an indispensable role of maternal ERα in early mouse preimplantation embryo development (PED). Here, we performed immunostaining detection to investigate the cell cycle specific subcellular localization of ERα, and serine 118 phosphorylated ERα (pERα-S118), during the first two cleavages of mouse PED. Our results showed that ERα nuclear localization appeared at 1-cell S-phase, disappeared at metaphase, and reappeared since the G1 phase of 2-cell embryos. Nuclear expression of pERα-S118 started at the 1-cell S-phase, but was absent at the G1 phase of 2-cell embryos, and reappeared since the 2-cell S-phase. Interestingly, pERα-S118 showed a dynamic expression pattern among nuclear, nuclear surface, cytoplasm and cell membrane. These results indicate that maternal derived ERα might still function in the first two cleavages of mouse PED, during which ZGA occurs.

1. Introduction

Mammalian preimplantation embryo development (PED) begins with the combination of mature oocytes and sperms, and ends up with the formation of competent blastocysts that can implant onto the uterus wall. In mice, during the first two cleavages of PED, oocyte-inherited proteins and mRNAs are degraded dramatically. Meanwhile, two waves of zygotic gene activation (ZGA) are produced: the first one (minor ZGA) occurs at the mid-to-end of 1-cells plus the beginning of 2-cells, and the second one (major ZGA) occurs at about G2 phase of 2-cells (Aoki et al., 1997). Molecular events in the first two cleavages of mouse PED are highly timely and spatially organized by "key factors". However, the existence and functions of these putative core regulators are extensively explored but poorly defined.

Our previous studies demonstrated that the mouse blastocyst development ratio was promoted when cultured in the medium supplemented with epigallocatechia gallate (EGCG); EGCG is one kind of green tea extract, which has an estrogen-like effect. The gene expression of Esr1 (also known as estrogen receptor alpha, ERα) in 2-cell embryos was up-regulated after EGCG treatment (Zhang et al., 2014). Besides, ERα-specific antagonists blocked early mouse PED, along with the down-regulation of ZGA related gene MuERV-L (Zhang et al., 2015a). These results suggest a possible role of ERα in early mouse PED, especially during the period of ZGA.

ERα contains two activation function domains: AF-1 and AF-2, the former one mediates ligand-independent activation of ERα; the latter one was activated upon estrogen binding (Lees et al., 1989). Classically, ERα mediates the versatile roles of estrogen in physiological and developmental processes, which also mediates the effects of other hormones and growth factors through "non-classical" pathways, including post-translational modifications of ERα: such as the phosphorylation and dephosphorylation on certain critical sites (Bunone et al., 1996; Denton et al., 1992). Actually, aromatase knockout experiments revealed the dispensable role of

Abbreviations: ERα, estrogen receptor alpha; PED, preimplantation embryo development; pERα-S118, serine 118 phosphorylated estrogen receptor alpha; ZGA, zygotic gene activation.

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estrogen during mouse PED (Huynh et al., 2004). Moreover, ERα-null mice were proved viable, though infertile (Hamilton et al., 2014). Thus, the possible role of ERα during mouse PED, as our previous results indicated, might depend on its non-classical pathways, which can compensate the loss of estrogen binding and on the maternal stored proteins and mRNAs that may mask the effects of gene knockout experiments.

There has been several papers report the developmental stage-specific expression of ERα mRNA in mouse PED. In these earlier studies, extracts of oocytes, 1-cells, 2-cells, 4/8-cells, morulae and blastocysts were examined by means of PCR; and the dynamic expression of ERα mRNA was revealed. It was demonstrated that ERα mRNAs existed in the oocytes with high amount, and reached a lowest level at the 2-cell stage (possibly because of maternal degradation), and re-expressed upon blastocyst formation (Gorski and Hou, 1995; Hiroi et al., 1999; Wu et al., 1992). However, as early mouse PED cell cycles are characterized by the unique long intervals, during which, genes show "wave-like" expression patterns (Hamatani et al., 2004). Thus, samples extracted from early, middle, late periods of one PED stage might differ greatly from each other. So that, it is of need to conduct the "expression pattern" experiments of mouse PED more detailed, for example, cell cycle stage-specifically: as the occurrence of mouse ZGA correlates well with cell cycle.

In the present study, we asked whether the expression of ERα during the first two cleavages of mouse PED is cell cycle stage-specific. Immunofluorescence staining was performed to observe the subcellular localization of ERα. We also detected the expression of serine 118 phosphorylated ERα (pERα-S118), which is phosphorylated on AF-1 and might mediate the actions of second messenger signaling pathways induced by autocrine embryotrophins. The results showed frequent nuclear-cytoplasm shuttling of ERα and the highly dynamic expression of pERα-S118 in mouse early embryogenesis, suggesting that maternal derived ERα might play an important role during this critical period of development.

2. Materials and methods

2.1. Animal care and ethics statement

Six week-old female ICR mice and ten week-old male ICR mice from SLRC Laboratory Animal Co., Ltd., (Shanghai, China) were housed in Fujian Medical University Laboratory Animal Center with controlled temperature (22 °C ± 1 °C) and light cycle (12 h light/12 h dark), and with ample supply of food and water. The female mice were housed for at least 3–5 days to adapt to the new environment, in order to regulate the menstrual cycle before experimental treatments. All animal experimental procedures were conducted following the guidelines set by the Institutional Animal Care and Use Committee (IACUC) of Fujian Medical University.

2.2. Embryo collection

Female ICR mice were injected with 6 IU PMSG (ProSpec; Rehovot, Isreal), and 46–48 h later followed by 6 IU hCG (Sigma; St. Louis, MO, USA). Female mice were mated with male mice at 1:1 ratio. Embryos were collected from isolated oviducts from 15 to 54 h post hCG, at intervals of about 3 h (see Fig. 1A). A total number of more than 1400 preimplantation embryos from about 140 female mice were used in the present study; around 40 embryos from 4 mice were used in each experimental step. The cells collected at a certain time point, especially around the cleavage period, were consist of blastomeres at different cell cycle stages, and the transient stages around the metaphase were hard to catch, so that a great larger amount of interphase cells were examined than that of metaphase. Nevertheless, at least 6 consistent repeats were observed for each cell cycle stage (see Supplementary Table S1 for detail).

2.3. Antibodies

For the detection of ERα, mouse monoclonal Anti-Estrogen Receptor α Antibody, clone F3-A (04-1564, Merck Millopore), which has been applied in our previous studies (Zhang et al., 2015b), was used at a dilution of 1:3200. The following antibodies were used for the detection of pERα-S118: p-ERα Antibody (Ser 118) (sc-101675, Santa Cruz), which has been applied in the mouse tissue immunoblotting analysis (Xiong et al., 2013), was used at a dilution of 1:400; and Anti-Estrogen Receptor alpha (phosphor S118) antibody (ab31477, Abcam), which has been applied in immunohistochemical analysis (Kim et al., 2015), was used at dilution 1:2000. Phospho-Histone H3 (Ser 10) (6G3) (9706S, Cell signaling) is a mouse monoclonal antibody for detection of Ser 10 phosphorylated Histone H3. The antibody was diluted 1:1600 for use. The following secondary antibodies were used: Alexa Fluor 488 Donkey Anti-Mouse IgG (A-21202, Thermo Fisher), Alexa Fluor 595 Donkey Anti-Rabbit IgG (A-21207, Thermo Fisher), Alexa Fluor 488 Donkey Anti-Rabbit IgG (A-21206, Thermo Fisher).

2.4. Immunostaining detection

Embryos were washed in 0.1% PVA-PBS for three times, and fixed with 4% paraformaldehyde for 30 min at room temperature. After PBST washing, cells were permeabilized in 0.5% Triton X-100 for 30 min, washed thrice in blocking buffer (5% normal donkey serum-0.02% Tween-20 for ERα detection and 0.2% BSA-0.02% Tween-20 for pERα-S118 detection). The cells were blocked for 1 h at room temperature, incubated with primary antibodies (diluted in blocking buffer) 4 °C overnight, and after PBST washing, incubated with secondary antibodies for 1 h at room temperature (protected from light). Negative control was performed by omitting the primary antibodies. Chromatin staining was done with DAPI. After the final washing, embryos were observed under a Ti-E fluorescence microscope (Nikon, Tokyo, Japan).

The staining intensity in different subcellular areas of a cell was measured using the linescan function of NIS-Elements D-4.30.00 software. By setting the average intensity of the subcortical cytoplasmic area as background level, the relative immunofluorescence intensity on the cell membrane was obtained; the staining intensity in the nuclear and on the nuclear surface was calculated similarly by comparing with the average cytoplasmic values (Supplementary Fig. S2). The relative staining levels in various stages were analyzed using GraphPad Prism 5 (graphpad.com). Editing of image files was performed with Adobe Photoshop CS6 software (photoshop.com) by selecting the representative cells of the respective cell cycle stages and arranged them accordingly.

The graphs in Fig. 2 are obtained using Adobe Photoshop CS6 software according to the quantification results (Supplementary Fig. S2). A total number of 140 images obtained with the same conditions and with better clarity (and consistent with the others) were used in the quantification procedure to obtain Fig. 2.

3. Results

Embryos were collected from the oviduct directly from post hCG-15–54 h at about three-hour intervals. The time points of early embryo collection indicated the developmental stages (Fig. 1A). Immunofluorescence staining was applied to observe the expression patterns of ERα and pERα-S118 (Fig. 1B). The nuclear was visualized by counterstaining with DAPI. As it was reported that serine 10 phosphorylated histone H3 (pH3-S10) has a cell cycle