Сведения об авторах

Сабырбаев Газиз Болатович – магистр сельскохозяйственных наук, ТОО «Казахский НИИ земледелия и растениеводства», Казахстан, Усть-Каменагорск, e-mail: gaziz_sabyrbaev@mail.ru, https://orcid.org/0000-0001-9349-3147

Information about the authors

Sabyrbayev Gaziz Bolatovich – Master of Agricultural Sciences, Kazakh Research Institute of Agriculture and Crop Production LLP, Kazakhstan, Öskemen, e-mail: gaziz_sabyrbaev@mail.ru, https://orcid.org/0000-0001-9349-3147

DOI:
IRSTI: 68.39.29

Zhongliang Jiang
College of Animal Science and Technology, Key Laboratory of Animal Genetic, Breeding and Reproduction in Shaanxi Province, Northwestern A&F University, Yangling, Shaanxi 712100, China
jiangzhongliang@nwsuaf.edu.cn

INDUCTION OF AUTOPHAGY IN BOVINE OVARIAN GRANULOSA CELLS BY ESTROGEN RECEPTOR B (ERB) EXPRESSION VIA THE AKT/MTOR SIGNALING PATHWAY

Abstract: *The aim of our study was to determine whether silencing or overexpression of estrogen receptor β (ERβ) regulates cell proliferation, steroidogenesis, autophagy and signalling pathways in bovine ovarian granulosa cells in vitro. In this study, bovine ovarian granulosa cells (BGCs) were cultured and transfected with ERβ siRNA (si-ERβ) or a plasmid overexpressing ERβ (oe-ERβ), and CCK-8 kit was used to assess cell proliferation. Real-time PCR was used to measure gene transcription. Western blotting was used to measure protein expression, and a specific kit was used to measure the production of steroid hormones [1]. The results showed the expression level of ERβ af- fects BGC proliferation according to the gene transcription levels of FSHR, CYP19A1, HSD3β1 and STAR and the production of E2 and P4. ERβ was identified as an important nuclear receptor that induced BGC autophagy based on the mRNA and protein ex- pression of autophagy-related genes. Furthermore, the role of ERβ in BGC autophagy was confirmed through treatment with rapamycin (RAPA) or 3-methyladenine (3-MA) in BGCs by cotransfection with si-ERβ or oe-ERβ in BGCs [2, 3]. The results related to AKT/ mTOR signalling and phosphorylation suggested that ERβ induces BGC autophagy through attenuating AKT/mTOR signalling. In summary, this study demonstrates that silencing or overexpression of ERβ regulates BGC proliferation and function and in- duces BGC autophagy by targeting AKT/mTOR signalling. These data reveal a novel regulatory mechanism of autophagy via ERβ and provide insights into the role of au- tophagy in BGCs [4, 5].*

Key words*: cattle, cell death, estrogen receptor β, ovarian development, signalling transduction*

Introduction

Ovarian follicular granulosa cells play a critical role in follicle ovulation or atresia through steroid hormone synthesis and communication with oocytes during follicular development (Adhikari & Liu, 2009), which is affected by many factors, including hormones (Hulas-Stasiak & Gawron, 2011). Numerous studies have indicated that apoptosis of granulosa cells (GC) results in follicular atresia, while cumulative evidence indicates that cell autophagy is involved in follicular atresia (Krysko et al., 2008; Liu et al., 2022; Ma et al., 2019; Zheng et al., 2019). Hence, it is necessary to understand the destiny and regulatory mechanism of granulosa cells to provide a new view of follicular ovulation or atresia.As two members of the nuclear receptor superfamily, estrogen receptors (ERα and ERβ) are ligand-dependent receptors and respond to the presence of oestrogen (Korach, 1994). ERα and ERβ are highly homologous in structure, are distributed in different tissues and have different biological effects with ligand binding (Krege et al., 1998). Both ERα and ERβ are expressed in mammalian ovaries but localize to distinct cell types.

Generally, ERα is expressed in the interstitium and theca cells, while ERβ is only expressed in the granulosa cells of growing follicles (Katherine et al., 2017). It has been confirmed that ERα knockout female mice are infertile because they are anovulatory, have disruption in LH regulation and have uteri that are insensitive to oestrogen. In contrast, ERβ knockout female mice are sub-fertile and primarily lack efficient ovulatory function (Couse et al., 2000; Hewitt & Korach, 2003). Several studies have indicated that autophagy is also triggered by ERβ through lysosome formation (Ruddy et al., 2014) [6]. For instance, in Hodgkin's lymphoma cells, autophagy is induced by ERβ-mediated autophagosome formation and results in high expression of LC3-II even when the lysis function of lysosomes is inhibited (Pierdominici et al., 2017). In addition, as a selective agonist of ERβ, arctigenin has been shown to inhibit autophagy in T-cell lines through the restriction of mTORC1 expression (Yang et al., 2020). In castrated male mice, the expression of autophagosome formation was upregulated by testosterone to activate AMPKα, demonstrating that ERβ induces autophagy (Pierdominici et al., 2017). In bovine, activin A attenuates BGC apoptosis in atretic follicles by ERβ-mediated autophagy signalling (Liu et al., 2022). However, how ERβ activates autophagy and signalling pathways remain unknown.In the ovary, autophagy plays an important role during follicular development and constant expression of ATG7 was observed in oocytogenesis stages as a key autophagy-related gene involved in autophagosome formation (Zhou et al., 2019). Our previous study indicated that bovine ovarian granulosa cells (BGCs) undergo autophagy, and VEGFA and FGF2 are a target of BGC autophagy that is inhibited by miR-21-3p (Ma et al., 2019; Ma et al., 2020) [7, 8]. Nevertheless, it remains unknown whether ERβ is involved in autophagy regulation in ovarian granulosa cells. In this study, we hypothesized that BGC autophagy is regulated by ERβ. To test this hypothesis, ERβ small interference RNA (si-ERβ) and ERβ overexpression (oe-ERβ) were transfected into BGCs, and the effects of ERβ on BGC proliferation and autophagy were determined

Materials and methods

Chemicals and reagents. All chemicals and reagents used in this study were purchased from Sigma Chemical Co. (St. Louis, MO, USA) unless otherwise stated.

Granulosa cell isolation and culture.The isolation and culture of granulosa cells were described by Jiang et al. (2013). Briefly, ovaries were collected from a local abattoir from dairy cattle aged 3-6 years, irrespective of the stage of the oestrous cycle. The ovaries were transported to the laboratory at 30°C in saline with penicillin (100 IU/ml) and streptomycin (100 mg/ml) within 2 h. The 2-to 6-mm follicles filled with clear liquid were selected, and GCs with follicular fluid were aspirated from follicles using a 10-ml syringe. At least 10 ovaries were collected to obtain enough GCs. A 150-mesh steel sieve was used to filter the cell suspension, and the mixture was centrifuged at 1000 g for 5 min to remove the follicular fluid. DMEM/F12 medium was used to resuspend the granulosa cell pellet, and the number of cells was counted using Trypan blue dye exclusion. To culture the cells, a 24-well tissue culture plate was used to seed the cells at a density of 5×105 viable cells in 1 ml of DMEM/ F12 including 10 mM sodium bicarbonate, 4 ng/ml sodium selenite, 0.1%, W/V of bovine serum albumin (BSA), 100 U/ml of penicillin, 100 µg/ml streptomycin, 2.5 µg/ml transferrin, 1.1 mmol/L non- essential amino acid mix, 10 ng/ml bovine insulin, 10-7 M androstenedione and 10 ng/ml bovine FSH (BIONICHE Inc. Ontario, Canada). Cultures were maintained at 37°C in 5% CO2 and 95% air for 2 days. On day 2, the medium was replaced using 70% fresh medium in each well, and then, the treatments were applied [9,10].

Plasmid construction.Bovine specific ERβ siRNA (si-ERβ) and ERβ overexpression (oe-ERβ) plasmids were synthesized by and purchased from RiboBio Company (Guangzhou, China). Sequences were cloned into pcDNA3.1 (+) eukaryotic expression vectors.

Cell transfection and treatments.Lipofectamine™ 3,000 (Life Technologies) was used in this study to transfect si-ERβ or oe-ERβ constructs into BGCs according to the manufacturer's instructions. Negative control (50 nM), ERβ siRNA (si-ERβ, 50 nM), overexpression ERβ (oe-ERβ, 50 nM) plasmids, rapamycin (autophagy inducer, 10 nM) and 3-methyladenine (3-MA, autophagy inhibitor, 10 nM) were applied to the cells on day 2 for 6 h at 37°C in 5% CO2 and 95% air, respectively, after which the medium was replaced with fresh medium, and the cells were cultured for 42 h before they were harvested for the next step of the experiment. The cell culture medium was stored at −20°C for steroid hormone determination.

Assessment of cell proliferation. A MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-2H-tetrazolium bromide) kit was used to assess the proliferation of GCs according to the manufacturer's guidelines. Briefly, after transfection for 6 h and another 42 h of culture, 20 μl MTT solution (5 mg/ml) was added to the medium and mixed gently. The mixture of cells with MTT solution was then incubated for 4 h at 37°C and 5% CO2. A microplate reader (BioTek, Winooski, VT, USA) was used to read the absorbance of samples at 450 and 620 nm. The cell viability was calculated ac- cording to a standard curve, which was prepared by culturing and measuring GC at different plating densities in quintuplicate (from 5×103 to 5×106/ml) for 4 h.

Analysis of steroid hormone production. The secretion of 17β-oestradiol (E2) and progesterone (P4) in cultured granulosa cells was determined using specific ELISA kits (DIAsource, Louvain-laNeuve, Belgium) according to the manufacturer's guidelines. Briefly, based on competition between free hormone and hormone linked to an acetylcholinesterase, the im- munoassay was performed for a limited number of antibody-binding sites. A spectrophotometer was used to determine the intensity of acetylcholinesterase to determine the production of hormones. The inter- and intra-assay CVs averaged 15% and 10.5%, respectively, and the minimum detectable concentration were 5 pg/ml for E2 and 0.2 ng/ml for P4.

RNA extraction and real-time PCR. After transfection, the cell culture medium was removed and TRIzol reagent was used to extract total RNA from BGCs. Total RNA concentration was measured by absorbance at 260 nm. cDNA was synthesized from purified total RNA (1 μg) using 5× All-In-One RT Master Mix (Abm, Richmond, BC, Canada). The ABI real-time PCR system (Applied Biosystems 7900) was used to quantify gene expression using SYBR Green I PCR Master Mix in a 20-μl reaction volume. To amplify each transcript, the thermal cycling parameters were as follows: 3 min at 95°C, 40 cycles of 15 s at 95°C, 30 s at 59°C and 30 s at 72°C. The housekeeping gene GAPDH was used for nor- malization of the mRNA expression levels. The 2−Ct method was used to calculate the relative expression of mRNA. All samples were run in triplicate. The specific primers for cattle quantitative real-time PCR for the target genes are listed in Table 1.

Table 1 − Specific primer sequences of cattle target genes

Gene	(5′-3′) Forward	(5′-3′) Reverse	ID
GADPH	CACCCTCAAGATTGTCAGCA	GGTCATAAGTCCCTCCACGA	NM_001034034.2
ERβ	CTTCGTGGAGCTCAGCCTGT	TCTCCACATCAGACCCACCA	XM_027554293.1
ATG7	ATTGCTGCATCAAGAGACCCA	CCTTCTGGCGATTATGGTCA	XM_027523157.1
ATG3	GGTTGTTCGGCTATGATGAG	GGGAGATGAGGGTGATTTTC	XM_027519324.1
LC3	TTATCCGAGAGCAGCAGCATCC	AGGCTTGATTAGCATTGAGC	XM_027513856.1
P62	AGGACTGAAGGAAGCTGCAC	GAGAGGGACTCAATCAGCCG	XM_027548457.1
FSHR	GCCCCTTGTCACAACTCTATGTC	GTTCCTCACCGTGAGGTAGATGT	NM_174061.1
BECN1	AGTTGAGAAAGGCGAGACAC	GATGGAATAGGAACCACCAC	NM_001033627.2
CYP19A1	GTGGACGTGTTGACCCTCAT	GGCACTTTCATCCAAGGGGA	NM_174305.1
HSD3B1	ACAATCTGACCGCATCGTCCT	CCACTTGCACCAGTGTCTTG	NM_174343.1
STAR	GCCCAGAAACCTCAGCTCTTA	AGCTTTCCTGCTCCTAAGCAA	NM_174189.1

Western blotting. After treatment, RIPA buffer containing protease inhibitors was used to lyse the cultured granulosa cells. The protein concentrations were determined by BCA assays (Pierce Inc., IL, USA). The protein samples (20 μg) were resolved on 10% SDS-PAGE gels and electrophoretically transferred to polyvinylidene difluoride (PVDF, Millipore) membranes using a Bio-Rad wet Blot Transfer Cell apparatus (transfer buffer containing 20% methanol, 48 mM Tris-base, 39 mM glycine and 1% SDS, pH 8.3). At room temperature, blocking buffer containing 5% BSA, 25 mM Tris, 150 mM NaCl, 2 mM KCl and 0.05% Tween 20, pH 7.4 was used to block the membranes for 2 h, and then, the membranes were incubated with primary antibodies against LC3 (anti-LC3I/II, # L8918, 1:2000; Abcam, UK), P62 (anti-P62, # ab101266, 1:2000; Abcam), AKT (anti-AKT, # ab64148,

1:3000; Abcam), phospho-AKT (anti-pAKT, # ab8932, 1:3000;Abcam), mTOR (anti-mTOR, #ab2372, 1:2000; Abcom), phosphomTOR (anti-pmTOR, 1:2000, #ab84400, phospho S2448; Abcom) and β-actin (anti-β-actin, 1:5000; #4970, Cell Signalling Technology, USA) diluted in BSA blocking buffer

at 4°C. Membranes were then incubated for 2 h at room temperature with 1:4,000 anti-rabbit HRP-conjugated IgG (LK2003, Sungene Biotechnology, Tianjin, China) diluted in QuickBlock™ Secondary Antibody Dilution Buffer (Beyotime Biotechnology, China). Membranes were washed in blocking buffer, and protein bands were revealed by enhanced chemiluminescence (ECL, Millipore, Billerica, USA). Semi-quantitative analysis was per- formed using NIH ImageJ software.

Double staining with MDC and DAPI. MDC (monodansylcadaverine) has been used to stain autophagosomes in vitro, and an autophagosome was labelled as a clear punctum by fluorescence microscopy. In this experiment, BGCs were cultured in 12-well plates and transfected with si-ERβ or oe-ERβ, or treated with rapamycin or 3-MA on day 2 for 6 h. Then, the cells were grown with 0.05 mM MDC and/or 1 μg/ml

DAPI (4′6-diamidino-2-phenylindole) at 37°C for 15 min and fixed immediately with paraformaldehyde (4%) in PBS for 20 min. After washing three times with PBS, a fluorescence microscope (Nikon company, Japan, excitation: 390 nm, emission: 460 nm) was used to visualize the cells immediately. The percentage of cells with puncta in total cells showed the ratio of cell autophagy. A total of 200 cells in each sample were counted.

Statistical analysisIn this study, each experiment was performed in triplicate. GraphPad Prism 6 software (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses. Independent t-tests were used to evaluate the significance of the results between groups. ANOVA was used to test the main effects among treatments, and culture replicates were included as a random variable in the F-test. If the data were not normally distributed (Shapiro–Wilk test), they were transformed to logarithms. Data were presented as the means ± SEM.

Results

Effects of expression of ERβ on proliferation and steroidogenesis in BGCs.To investigate the role of ERβ in BGC function, small interfering RNAs for ERβ (si-ERβ) and an ERβ overexpression construct (oe-ERβ) were transfected into BGCs to determine the effects of level of ERβ on cell proliferation and steroid hormone production in BGCs. The effects of level of ERβ on BGC proliferation were assessed using an MTT kit in this experiment, and the results indicated that transfection of si-ERβ promoted, while transfection of oe-ERβ inhibited BGC proliferation (Figure 1a). We then measured the mRNA abundance levels of *FSHR*, *CYP19A1*, *HSD3β1* and *STAR* in cultured granulosa cells, which are involved in BGC survival and function (Figure 1b). The results showed that si-ERβ transfection upregulated *FSHR*, *CYP19A1*, *HSD3β1* and *STAR* mRNA abundance, and oe-ERβ trans- fection downregulated *CYP19A1*, *HSD3β1* and *STAR* mRNA abun- dance; however, oe-ERβ transfection did not change the *FSHR* mRNA level. To study the effects of ERβ on BGC function, E2 and P4 production levels in BGCs transfected with si-ERβ or oe-ERβ were detected (Figure 1c). The results showed that si-ERβ transfection significantly increased E2 production but did not change P4 production. The oe-ERβ transfection significantly decreased E2 production but increased P4 production. These data indicated that expression of ERβ regulates BGC proliferation and steroid hormone production.

Expression of ERβ induces BGC autophagy.To study the role of ERβ in BGC autophagy, si-ERβ or oe-ERβ was transfected into BGCs, MDC staining was used to label the autophagic vacuole, and gene mRNA abundance and protein expression were evaluated in this experiment. BGC autophagy was detected using a fluorescence microscope (Figure 2a), and the statistical results showed that the percentage of autophagy was significantly lower in BGCs transfected with si-ERβ and higher in BGCs transfected with oe-ERβ (Figure 2b). Quantitative real-time PCR was used to determine gene mRNA abundance in BGCs following transfection with si-ERβ or oe-ERβ, and *LC3*, *BECN-1*, *ATG3*, *ATG7* and *P62* mRNA abundance levels are shown in Figure 2c. The *LC3*, *BECN-1*, *ATG3* and *ATG7* mRNA levels were significantly downregulated in the cells transfected with si-ERβ, while *P62* mRNA levels were upregulated; however, the mRNA levels of *LC3*, *BECN-1*, *ATG3* and *ATG7* were upregulated in the cells transfected with oe-ERβ, while *P62* mRNA level was downregulated. Furthermore, Western blotting was used to determine the LC3 and P62 protein levels (Figure 2d), and the results indicated that the expression patterns were similar to the mRNA levels. LC3 protein level was decreased in BGCs transfected with si-ERβ, while it was increased in BGCs transfected with the oe-ERβ plasmid. (Figure 2e). that silencing or ERβ knockdown rescues BGC autophagy induced by rapamycin.

It has been confirmed that autophagy is induced by rapamycin (RAPA). To investigate the role of ERβ knockdown in BGC autophagy, a rescue experiment was designed in which 10 nM RAPA and/or 10 nM si-ERβ was used to challenge cultured BGCs. Gene expression analysis revealed that the expression levels of *LC3*, *BECN*, *ATG3* and *ATG7* mRNAs were significantly upregulated in BGCs by RAPA challenge, while those genes were downregulated by transfection with si-ERβ. Meanwhile, in BGCs treated with both RAPA and si-ERβ, the mRNA abundance levels of *LC3*, *BECN*, *ATG3* and *ATG7* were lower than those of cells challenged with RAPA alone but higher than those of cells transfected with si-ERβ alone, in addition to the *ATG3* gene (Figure 3a). The *P62* mRNA abundance was significantly downregulated in BGCs in the single treatment and in both the RAPA and si-ERβ treatments (Figure 3a). It is likely that the protein levels of LC3 and P62 in BGCs treated with RAPA and/or transfected with si-ERβ had similar patterns as the mRNAs (Figure 3b). These results indicate that ERβ knockdown rescues BGC autophagy induced by rapamycin. ERβ overexpression accelerates BGC autophagy inhibited by 3-methyladenine. The 3-Methyladenine (3-MA) has been confirmed as an inhibitor of autophagy. To study the role of ERβ overexpression in BGC autophagy, rescue experiments were designed in which 10 nM 3-MA and/or 10 nM oe-ERβ were used to challenge cultured BGCs. The gene expression results showed that the mRNA abundance levels of *LC3*, *BECN*, *ATG3* and *ATG7* were significantly upregulated in BGCs transfected with oe-ERβ, while those genes were downregulated by challenge with 3-MA. In addition, the mRNA abundance levels of *LC3*, *BECN*, *ATG3* and *ATG7* were lower after treatment with both 3-MA and oe-ERβ than after single oe-ERβ transfection, but higher than after 3-MA challenge (Figure 4a). *P62* mRNA abundance was significantly downregulated in BGCs transfected with oe-ERβ but upregulated by 3-MA challenge. However, the *P62* mRNA level was higher after treatment with both 3-MA and oe-ERβ than after single oe-ERβ transfection but lower than that after 3-MA treatment (Figure 4a). In the same way, the LC3 and P62 mRNAs and proteins had similar expression patterns (Figure 4b). These results suggest that oe-ERβ accelerates BGC autophagy inhibited by 3-MA.

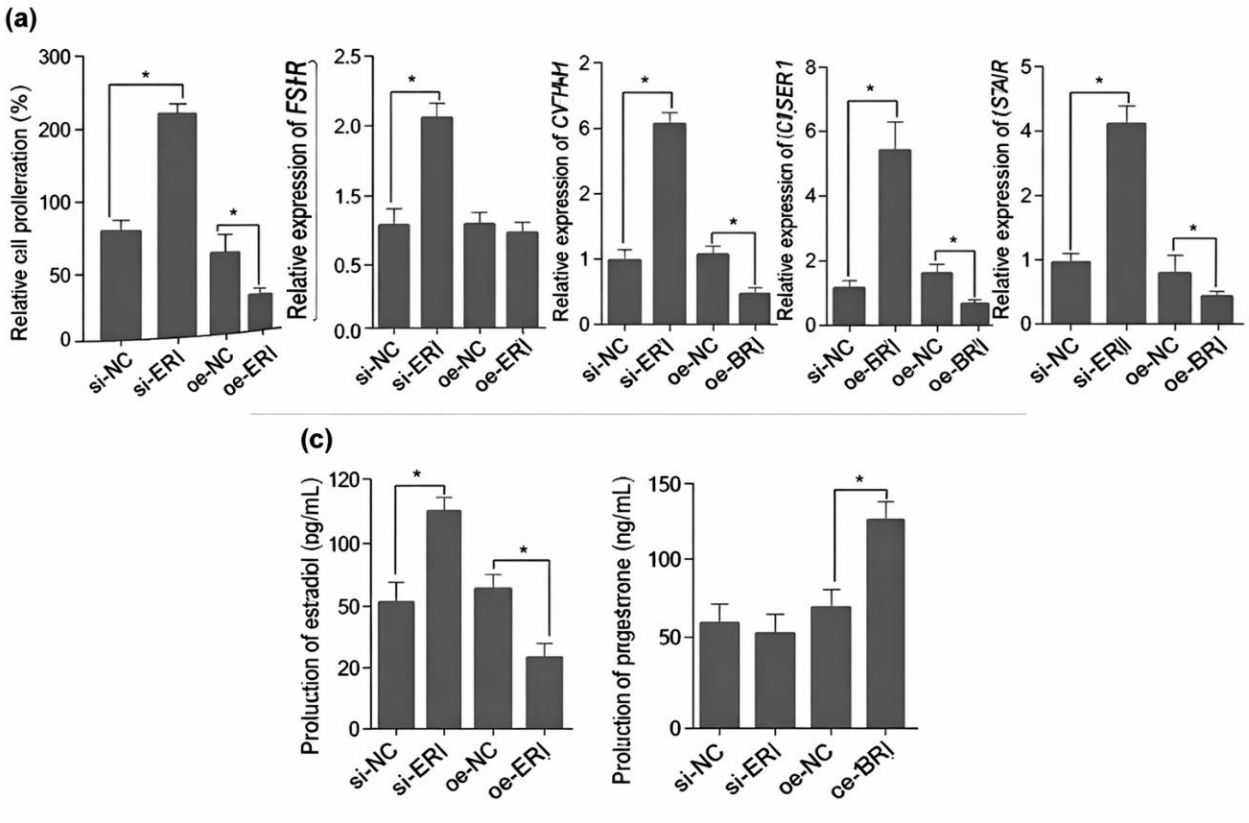

Figure 1 − Effects of ERβ on BGC proliferation and steroidogenesis

BGC proliferation was measured by MTT kit (a), qRT-PCR was used to measure mRNA abundance of *FSHR*, *CYP19A1*, *HSD3B1* and *STAR* genes (b), and E2 and P4 concentration levels in BGCs transfected with si-ERβ or oe-ERβ (c). NC means the negative control. Data are means ± SEM of three independent replicates (*p < .05). ERβ overexpression inhibits AKT/mTOR signalling in BGCs. The AKT/mTOR pathway has been confirmed as one of the impor- tant signalling pathways for autophagy (Krege et al., 1998).

To determine whether expression of ERβ regulates the AKT/mTOR pathway, phosphorylated AKT (p-AKT) and mTOR (p-mTOR) were detected by western blotting in BGCs following transfection with si-ERβ or oe-ERβ. As shown in the results, p-AKT expression was significantly higher in BGCs transfected with si-ERβ (Figure 5a), while it was significantly lower in BGCs transfected with oe-ERβ than in negative control.

The p-mTOR in the BGCs transfected with si-ERβ and oe-ERβ had a similar expression pattern as p-AKT in the BGCs transfected with si-ERβ and oe-ERβ (Figure 5c, d). These data suggest that overexpression of ERβ inhibits the phosphorylation of AKT and mTOR in BGCs.

Discussion

It is well understood that follicular atresia is closely related to the apoptosis of granulosa cells; however, granulosa cell autophagy has been confirmed in a previous study during follicular atresia Ma et al., 2019. In rat granulosa cells, serum starvation induces the accumulation of autophagosomes, leading to apoptosis activation by reducing Bcl-2 expression and resulting in caspase activation (Choi et al., 2011).

In mice, autophagy of granulosa cells was induced by FSH, which is important to maintain follicular atresia and granulosa cell proliferation (Shen et al., 2016). In porcine, the early induction of autophagic flux contributes to oxidative stressinduced apoptosis in granulosa cells (Zhang et al., 2021). Moreover, autophagy of granulosa cells is suppressed by activation of the mTOR signalling pathway mediated by AKT during follicular atresia (Zhang et al., 2019). In our laboratory, a previous study indicated that BGC autophagy is involved in follicular atresia (Ma et al., 2019; Zheng et al., 2019). Although studies have elucidated the function of GC autophagy in follicular atresia and ovarian primordial follicular reserves (Song et al., 2015), the regulatory mechanism of autophagy in GC is indistinct. In the present study, we demonstrate that (a) ERβ inhibits BGC proliferation and (b) ERβ induces BGC autophagy by decreasing AKT/mTOR phosphorylation. Together, the current results demonstrate that ERβ plays an important role in BGC autophagy.

As a nuclear estrogen receptor, ERβ is highly expressed in mammalian ovaries but is limited to granulosa cells (Katherine et al., 2017). A previous study in ERβ knockout mice treated with pregnant mare serum gonadotropin (PMSG) demonstrated that ERβ plays an important role in FSH-mediated GC differentiation and maturation of the whole follicle (Deroo & Korach, 2006).

In addition, estrogen receptor β-null (βERKO) mice exhibit subfertility, and βERKO female mice treated by super ovulation exhibited a significantly lower percentage of ovulations and trapped oocyte follicles (Hsieh et al., 2015). In this study, BGCs were treated with si-ERβ and oe-ERβ, and the mRNA levels and production of E2 and P4 indicated that ERβ inhibits BGC proliferation and regulates steroid hormone production.

As a form of programmed cell death, autophagy is involved in the regulation of intracellular homeostasis (Doherty&Baehrecke, 2018).

The autophagy was labelled by MDC in BGCs transfected with si-ERβ or oe-ERβ, bars = 10 μm (a), the statistical results of percentage of autophagic BGCs (b), gene expression of *LC3*, *BECN-1, ATG3, ATG7* and *P62* in BGCs transfected with si-ERβ or oe-ERβ (c), protein expression levels of LC3 and P62 in BGCs transfected with si-ERβ or oe-ERβ (d), and the statistical results were shown in (e). NC means the negative control. Data are means ± SEM of three independent replicates (*p < .05, **p < .01).

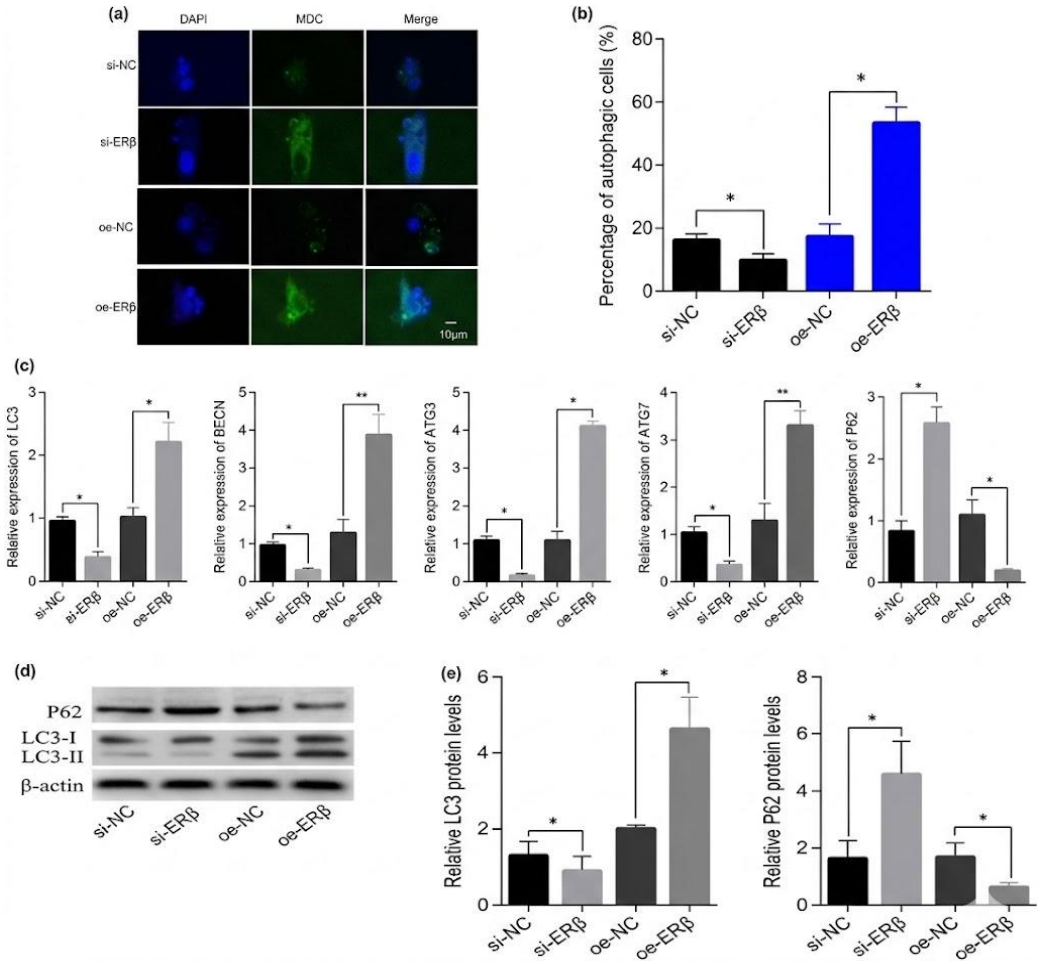

Figure 2 − ERβ induces BGC autophagy

Conclusion

In conclusion, the results above show for the first time that expres- sion of ERβ inhibits BGC proliferation and contributes to BGC autophagy by attenuating PI3K/AKT/mTOR signalling. These findings further our understanding of the mechanisms of follicular develop-ment and atresia in cattle ovaries.

References

1. Adhikari D., Liu K. Molecular mechanisms underlying the activation of mammalian primordial follicles // Endocrine Reviews. – 2009. – Vol. 30. – P. 438-464. – DOI: 10.1210/er.2008-0048.
2. Choi J., Jo M., Lee E., Choi D. Induction of apoptotic cell death via accumulation of autophagosomes in rat granulosa cells // Fertility & Sterility. – 2011. – Vol. 95. – P. 1482-1486. – DOI: 10.1016/j.fertnstert.2010.06.006.
3. Choi J., Jo M., Lee E., Choi D. AKT is involved in granulosa cell autophagy regulation via mTOR signaling during rat follicular development and atresia // Reproduction. – 2015. – Vol. 147, № 1. – P. 73-80. – DOI: 10.1530/REP-13-0386.
4. Couse J.F., Hewitt S.C., Korach K.S. Receptor null mice reveal contrasting roles for estrogen receptor α and β in reproductive tissues // Journal of Steroid Biochemistry & Molecular Biology. – 2000. – Vol. 74. – P. 287-296. – DOI: 10.1016/S0960-0760(00)00105-9.
5. Deroo B.J., Korach K.S. Estrogen receptors and human disease // Journal of Clinical Investigation. – 2006. – Vol. 116. – P. 561-570. – DOI: 10.1172/JCI27987.
6. Dikic I., Elazar Z. Mechanism and medical implications of mammalian autophagy // Nature Reviews Molecular Cell Biology. – 2018. – Vol. 19. – P. 349-364. – DOI: 10.1038/s41580-018-0003-4.

7. Doherty J., Baehrecke E.H. Life, death and autophagy // Nature Cell Biology. – 2018. – Vol. 20. – P. 1110-1117. – DOI: 10.1038/s41556-018-0201-5.
8. Dong Z., Jiang H., Liang S., Wang Y., Jiang W., Zhu C. Ribosomal protein L15 is involved in colon carcinogenesis // International Journal of Medical Sciences. – 2019. – Vol. 16. – P. 1132-1141. – DOI: 10.7150/ijms.34386.
9. Hewitt S.C., Korach K.S. Oestrogen receptor knockout mice: roles for oestrogen receptors α and β in reproductive tissues // Reproduction. – 2003. – Vol. 125. – P. 143-149. – DOI: 10.1530/rep.0.1250143.
10. Hsieh D.J., Kuo W.W., Lai Y.P., Shibu M.A., Shen C.Y., Pai P., Yeh Y.L., Lin J.Y., Viswanadha V.P., Huang C.Y. 17β-estradiol and/or estrogen receptor β attenuate the autophagic and apoptotic effects induced by prolonged hypoxia through HIF-1α-mediated BNIP3 and IGFBP-3 signaling blockage // Cellular Physiology and Biochemistry. – 2015. – Vol. 36. – P. 274-284. – DOI: 10.1159/000374070.

Чжунлян Цзян
Колледж животноводства и технологий, Ключевая лаборатория генетики,
селекции и воспроизводства животных провинции Шэньси,
Северо-Западный аграрный университет, Китай, 712100, г. Янглин, провинция Шэньси
jiangzhongliang@nwsuaf.edu.cn

ИНДУКЦИЯ АУТОФАГИИ В ГРАНУЛЁЗНЫХ КЛЕТКАХ ЯИЧНИКА КРУПНОГО РОГАТОГО СКОТА ПРИ ЭКСПРЕССИИ РЕЦЕПТОРА ЭСТРОГЕНА В (ERB) ЧЕРЕЗ СИГНАЛЬНЫЙ ПУТЬ AKT/MTOR

Цель нашего исследования заключалась в изучении того, влияет ли снижение или сверхэкспрессия рецептора эстрогена β (ERβ) на пролиферацию клеток, стероидогенез, аутофагию и сигнальные пути в гранулёзных клетках яичника крупного рогатого скота in vitro. В данном исследовании гранулёзные клетки яичника крупного рогатого скота (ГККРС) культивировали и трансфицировали с использованием siRNA для ERβ (si-ERβ) или плазмиды с сверхэкспрессией ERβ (oe-ERβ). Для оценки пролиферации клеток использовали набор CCK-8. Количественная ПЦР в реальном времени применялась для измерения уровня транскрипции генов. Экспрессию белков определяли методом вестерн-блоттинга, а для оценки продукции стероидных гормонов использовали специализированный набор. Результаты показали, что уровень экспрессии ERβ влияет на пролиферацию ГККРС, что подтверждается изменениями уровней транскрипции генов FSHR, CYP19A1, HSD3β1 и STAR, а также продукцией эстрадиола (E2) и прогестерона (P4). ERβ был идентифицирован как важный ядерный рецептор, индуцирующий аутофагию ГККРС на основании уровней мРНК и белков, связанных с аутофагией. Дополнительно роль ERβ в аутофагии ГККРС была подтверждена обработкой клеток рапамицином (RAPA) или 3-метиладенином (3-MA) при сопутствующей трансфекции с si-ERβ или oe-ERβ. Данные по сигнальному пути AKT/mTOR и уровню его фосфорилирования свидетельствуют, что ERβ индуцирует аутофагию ГККРС путем подавления сигнального пути AKT/mTOR. В заключение, настоящее исследование демонстрирует, что снижение или сверхэкспрессия ERβ регулируют пролиферацию и функцию ГККРС и индуцируют аутофагию за счет воздействия на сигнальный путь AKT/mTOR. Полученные данные раскрывают новый механизм регуляции аутофагии посредством ERβ и предоставляют новые представления о роли аутофагии в гранулёзных клетках яичника.

Ключевые слова: *крупный рогатый скот, гибель клеток, рецептор эстрогена β, развитие яичника, сигнальная трансдукция.*

Чжунлян Цзян
Мал шаруашылығы және технологиялар колледжі, Шэньси провинциясының мал генетикасы,
селекциясы және репродукциясы негізгі зертханасы, Солтүстік-батыс Аграрлық университеті,
712100, Қытай, Янглин қаласы, Шэньси провинциясы
jiangzhongliang@nwsuaf.edu.cn

ЭСТРОГЕН РЕЦЕПТОРЫ В (ERB) ЭКСПРЕССИЯСЫ АРҚЫЛЫ AKT/MTOR СИГНАЛДЫҚ ЖОЛЫ НЕГІЗІНДЕ ІРІ ҚАРА МАЛ АНАЛЫҚ БЕЗІНІҢ ГРАНУЛЕЗА ЖАСУШАЛАРЫНДА АУТОФАГИЯНЫҢ ИНДУКЦИЯСЫ

Зерттеуіміздің мақсаты – эстрогеннің β рецепторының (ERβ) төмендеуі немесе жоғары экспрессиясының сиыр овариінің гранулярлық жасушаларының пролиферациясына, стероидогенезіне, аутофагиясына және сигналдық жолдарына әсерін зерттеу болды. Зерттеуде сиыр овариінің гранулярлық жасушалары (СОГЖ) in vitro жағдайда өсіріліп, ERβ-ге қарсы siRNA (si-ERβ) немесе ERβ-нің жоғары экспрессиясын тудыратын плазмидалармен (oe-ERβ) трансфекцияланды. Жасушалардың пролиферациясын анықтау үшін CCK-8 жинағы қолданылды. Ген транскрипциясының деңгейін анықтау үшін сандық нақты уақыттағы ПТР қолданылды. Белоктардың экспрессиясын вестерн-блот әдісімен бағалады, ал стероид гормондарының өндірісін арнайы жинақпен өлшеді. Нәтижелер ERβ экспрессиясының деңгейі гранулярлық жасушалардың пролиферациясына әсер ететінін көрсетті, бұл FSHR, CYP19A1, HSD3β1 және STAR гендерінің транскрипция деңгейінің өзгерістерімен, сондай-ақ эстрадиол (E2) және прогестерон (P4) өндірісімен расталды. ERβ-гранулярлық жасушалардағы аутофагияны индукциялайтын маңызды ядролық рецептор ретінде анықталды, бұл аутофагияға қатысты мРНҚ және белоктардың деңгейлеріне негізделген. Сонымен қатар, ERβ-ның гранулярлық жасушалардағы аутофагиядағы рөлі рапамицин (RAPA) немесе 3-метиладенин (3-MA) өңдеулері кезінде si-ERβ немесе oe-ERβ трансфекциясымен бірге расталды. AKT/mTOR сигналдық жолы мен оның фосфорлану деңгейі бойынша алынған мәліметтер ERβ гранулярлық жасушалардағы аутофагияны AKT/mTOR жолының тежелуі арқылы индукциялайтынын көрсетті. Қорытындылай келе, бұл зерттеу ERβ төмендеуі немесе жоғары экспрессиясының гранулярлық жасушалардың пролиферациясы мен функциясын реттеп, аутофагияны AKT/mTOR сигналдық жолы арқылы индуцирлейтінін көрсетті. Алынған деректер ERβ арқылы аутофагияны реттеудің жаңа механизмін ашып, овари гранулярлық жасушаларындағы аутофагияның рөлі туралы жаңа түсінік берді.

***Кілт сөздер**: сиыр, жасуша өлімі, эстроген β рецепторы, овари дамуы, сигналдық трансдукция.*

Information about the authors

Zhongliang Jiang – College of Animal Science and Technology, Key Laboratory of Animal Genetic, Breeding and Reproduction in Shaanxi Province, Northwestern A&F University, Yangling, China, jiangzhongliang@nwsuaf.edu.cn, ORCID: https://orcid.org/0000-0001-7822-4520

Сведения об авторах

Чжунлян Цзян – Колледж животноводства и технологий, Ключевая лаборатория генетики, селекции и воспроизводства животных провинции Шэньси, Северо-Западный Аграрный университет, г. Янглин, Китай, jiangzhongliang@nwsuaf.edu.cn, https://orcid.org/0000-0001-7822-4520

Авторлар туралы мәліметтер

Чжунлян Цзян – Мал шаруашылығы және технологиялар колледжі, Шэньси провинциясының мал генетикасы, селекциясы және репродукциясы негізгі зертханасы, Солтүстік-батыс аграрлық университеті, Янглин қаласы, Қытай, jiangzhongliang@nwsuaf.edu.cn, https://orcid.org/0000-0001-7822-4520