Expression and distribution of the zinc finger protein, SNAI3, in mouse ovaries and pre-implantation embryos

Shujuan GUO1)*, Xingyu YAN2)*, Feifei SHI1)*, Ke MA1), Zi-Jiang CHEN3, 4) and Cong ZHANG1, 3, 4)

1) Key Laboratory of Animal Resistance Research, College of Life Science, Shandong Normal University, Ji’nan, Shandong 250014, China
2) Hebei Medical University Nursing School, Shijiazhuang 050000, China
3) Center for Reproductive Medicine, Ren Ji Hospital, School of Medicine, Shanghai jiao Tong University, Shanghai 200135, China
4) Shanghai Key Laboratory for Assisted Reproduction and Reproductive Genetics, Shanghai 200135, China

Abstract. The Snail gene family includes Snai1, Snai2, and Snai3 that encode zinc-finger-containing transcriptional repressors in mammals. The expression and localization of SNAI1 and SNAI2 have been studied extensively during folliculogenesis, ovulation, luteinization, and embryogenesis in mice. However, the role of SNAI3 is unknown. In this study, we investigated the expression of SNAI3 during these processes. Our immunohistochemistry data showed that SNAI3 first appeared in oocytes by postnatal day (PD) 9. Following this, SNAI3 was found to be expressed consistently in theca and interstitial cells, along with oocytes. In gonadotropin-treated immature mice, the expression of SNAI3 did not change significantly during follicular development. The expression of SNAI3 was reduced during ovulation, after which it increased gradually during luteinization. Similar results were obtained from western blot analyses. Furthermore, real-time polymerase chain reaction (RT-PCR) analyses revealed varying mRNA levels of different Snail factors at a given time in gonadotropin-induced ovaries. During early embryo cleavage, SNAI3 was localized to the nucleus, except the nucleolus at the germinal vesicle and one-cell stages. From two- to eight-cell stages, SNAI3 was localized only to the nucleolus. Thereafter, SNAI3 was detected only in the cytoplasm, except during the blastocyst stage when it was localized to the nucleus of the trophoderm and the inner cell mass. RT-PCR results showed that the expression of Snail superfamily genes was decreased during the blastocyst stage. From the eight-cell to morula stage, when compaction occurs that is a prerequisite for blastocyst formation, Sna13 mRNA was expressed at very low levels and was opposite to the highest expression level of the compaction-related gene, E-cadherin, at the eight-cell stage. Taken together, our results suggest that SNAI3 likely plays some roles during folliculogenesis, luteinization, and early embryonic development.

Key words: E-cadherin-catenin complex, Mouse, Ovaries, Pre-implantation embryos, SNAI3

Received: June 20, 2017
Accepted: January 27, 2018
Published online in J-STAGE: February 15, 2018
©2018 by the Society for Reproduction and Development
Correspondence: C Zhang (e-mail: zhangxinyunlife@163.com)
* S Guo, X Yan, and F Shi contributed equally to this work.
This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (by-nc-nd) License. (CC-BY-NC-ND 4.0: https://creativecommons.org/licenses/by-nc-nd/4.0/)
When oocytes reach full size, transcription ceases and the somatic cell-type nucleus is transformed inside the NPB [13].

The Snail family of proteins are zinc-finger transcription factors with conserved structures across many species, including Drosophila and numerous vertebrates. These proteins are thought to be involved in various processes during cell differentiation [14, 15]. Three members of the Snail family have been described in mammals: Snail1 (also called Snail), Snail2 (Slug), and Snail3 (Smuc). These genes encode DNA binding zinc finger proteins. The C2H2 zinc fingers of the Snail proteins are similar and conserved with almost identical amino acid sequences. Members of the Snail family bind to E-box consensus sites (CAGGTG or CANNTG) to repress transcription [16]. This repressor activity not only depends on the zinc-finger region, but also on a SNAG (Snai/Gfi) domain [17].

It is interesting that studies have shown inverse correlations exist between E-cadherin, SNAI1, and SNAI2 in many different cell systems [8]. E-Cadherin is the main cell-cell adhesion molecule in maintaining the integrity of epithelial tissues, and mediates cell-cell adhesion during preimplantation development [18]. Redistribution of E-cadherin, and initiation of E-cadherin-mediated adhesion, are regulated by the catenins. β-Catenin binds α-catenin and the cytoplasmic domain of E-cadherin, which anchors the adhesion complex to the actin-based cytoskeleton to implement functions together [19, 20]. SNAI1 and SNAI2 are associated with follicular maturation, ovulation, luteinization, embryogenesis, and the epithelial-mesenchymal transition (EMT) in mice [21]. SNAI3 also actively represses transcription [16] and is expressed in skeletal muscle, thymus, and myeloid cells [16, 21]. Human SNAI3 has been identified via in silico analysis, contains the same SNAG and zinc finger domains as the mouse protein [22], and its distribution has been examined [23]. However, Sna3 knockout mice are viable and fertile [23]. Thus, the roles of SNAI3 in female reproduction, especially during embryogenesis, require further study.

To elucidate the location of SNAI3 during the development of follicles and pre-implantation embryos, and to explore whether there are redundant roles among SNAI1, SNAI2, and SNAI3, we examined the temporal and spatial expression of SNAI3 during follicular development, ovulation, luteinization, and embryogenesis. In addition, we examined the transcription of Snail1 and Snail2 during these processes. Our results indicate that SNAI3 might play important roles during follicular development, luteinization, and early embryonic development, and there may exist redundant roles among SNAI1, SNAI2, and SNAI3.

Materials and Methods

Animals

Mice (8 weeks old) were obtained from the Laboratory Animal Center of Shandong University (Ji’nan, Shandong, China). All mice were housed at Shandong Normal University at 23 ± 2°C under a 12/12 h light/dark cycle with access to chow and water ad libitum. All experiments were performed with strict adherence to the Guidelines of Shandong Normal University for the Care and Use of Laboratory Animals.

Ovary, oocyte, and embryo collection

The day of delivery was designated as postnatal day (PD) 0. Ovaries from neonatal and prepubertal mice were collected on PD 1, 4, 9, 18, and 23. To stimulate follicular and luteal development, immature 21-day-old female mice were treated intraperitoneally with 5 IU of pregnant mare serum gonadotropin (PMSG) followed 48 h later by 5 IU human chorionic gonadotropin (hCG) (both from Ningbo Sansheng Pharmaceutical, Zhejiang, China). The ovaries of hormone-treated mice were collected at 24 and 48 h following PMSG injection, and at 16, 24, and 48 h following hCG administration.

For oocyte collection, adult female mice were treated with 10 IU PMSG and sacrificed 46 h later to isolate antral follicles. Fully grown germinal vesicle (GV) oocytes were then selected and collected from the antral follicles using a mouth pipette. For embryo collection, adult females were treated with 10 IU PMSG followed by hCG administration (10 IU) 48 h later, and mated with adult males. Zygotes, two- and four-cell, were retrieved from oviducts at 22, 42, and 55 h post-hCG. Because embryo compaction starts from the eight-cell stage and lasts about 10 h in mice, to study the association between SNAI3 and E-cadherin expression, and compaction, eight-cell embryos and morulae were recovered from oviducts at 66 and 88 h after hCG administration, respectively. Blastocysts were collected from uterus at 90–96 h post-hCG as described earlier [24].

Immunohistochemistry

Immunohistochemistry was carried out as described previously [25, 26]. Briefly, the collected tissues were immediately snap frozen in liquid nitrogen and stored at −80°C. Ovaries were cryosectioned at 7 mm, fixed in acetic acid at −20°C for 10 min, and incubated in 0.3% (v/v) Triton X-100 in phosphate-buffered saline (PBS), pH 7.2, for 20 min. Endogenous peroxidase activity was quenched by incubating the samples in 3% (v/v) H2O2 for 20 min, followed by washing with PBS. Sections were blocked with immunoglobulin G from the same animal species as the primary antibody for 1 h at room temperature (22–26°C), and then incubated at 4°C overnight with the primary antibody to SNAI3 (1:20 dilution, sc-10439; Santa Cruz Biotechnology, Santa Cruz, CA USA). Thereafter, the samples were incubated with a horseradish peroxidase-conjugated secondary antibody for 60 min at room temperature before being developed with a diaminobenzidine peroxidase substrate kit (ZLI-9033; ZSGB-BIO, Beijing, China). Negative controls were incubated with pre-immune serum instead of the primary antibody. Finally, the sections were counterstained with hematoxylin, dehydrated, mounted, and digitally photographed using a microscope (Olympus, Tokyo, Japan).

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from oocytes and embryos with the E.Z.N.A. MicroElute Total RNA Kit (Omega Bio-tek, Guangzhou, China) or from ovaries using TRIzol Reagent (Tiangen Biotech, Beijing, China) as per the manufacturers’ instructions. At each time point, the numbers of CLs were roughly equal. Total RNA was treated with RNase-free DNase (Tiangen Biotech). Reverse transcription was performed with the Fast Quant RT Kit (Tiangen Biotech). qRT-PCR was carried out using SYBR Green Master Mix on the LightCycler 96 System (Roche Diagnostics, Basel, Switzerland). The primers used are listed in Table 1. PCR was performed under the following conditions: 3 min at 95°C, then 40 cycles of 15 sec at 95°C, 30 sec at 59°C, and 30 sec at 72°C. This was followed by 5 min at 72°C. Melting-curve analyses were performed to confirm product
Table 1. Target transcripts and primer sequences used for the quantification of mRNA levels in the ovaries and embryos

| Primer | Sequence | Size (bp) |
|--------|----------|-----------|
| β-actin | 5′-TGTTACAACTGGGACGACA-3′ | 165 |
| α-catenin | 5′-GGGCTGTGGAAGTCTCAA-3′ | 177 |
| β-catenin | 5′-CCGTCGCTTTATGCT-3′ | 190 |
| E-cadherin | 5′-GAGATGGGAGAAAGGAAAAA-3′ | 171 |
| SNAI1 | 5′-CCATTCTCTGGTCCCTGAT-3′ | 100 |
| SNAI2 | 5′-GCTTCACCTCCACTCTCTT-3′ | 122 |
| SNAI3 | 5′-GCAAGGAGTTGGGAGAAGG-3′ | 129 |
| Cyclin D2 | 5′-CAGGATGATGAGAAGGAGAACA-3′ | 159 |
| P21cip1 | 5′-GGGTATTGGTGATGCTCCT-3′ | 110 |

For immunofluorescence staining, embryos were fixed in 4% paraformaldehyde for 30 min and permeabilized in incubation medium (0.5% Triton X-100 in 3 mM MgCl2, 20 mM HEPES, 50 mM NaCl, 300 mM sucrose, and 0.02% NaN3, pH 7.4) for 30 min (blastocysts for 40 min). This was followed by incubating with 1% bovine serum albumin in PBS for 30 min at room temperature. The embryos were incubated with a goat anti-SNAI3 polyclonal antibody (1:50, sc-10439; Santa Cruz Biotechnology) overnight at 4°C. After washing thrice in PBS for 5 min each, the embryos were incubated with fluorescein-isothiocyanate-conjugated rabbit anti-goat IgG (1:100, sc-2777; Santa Cruz Biotechnology) for 60 min at room temperature. The embryos were then washed thrice in PBS for 5 min each, stained with 10 μg/ml 4’,6-diamidino-2-phenylindole (Sigma-Aldrich), washed, and finally mounted on 1,4-diazabicyclo(2.2.2) octane hydrochloride-containing (Sigma-Aldrich) glass slides. The mounted embryos were observed under a confocal laser scanning microscope (TCS SPE, Leica, Wetzlar, Germany).

Statistical analyses
All statistical analyses were performed using GraphPad Prism (GraphPad Software, La Jolla, CA, USA). For the experiments described in Figs. 3, 4 and 6, one-way ANOVA followed by Dunnett’s test was performed to evaluate statistical significance. Data were considered statistically significant at P < 0.05. Graphs are presented as means ± standard error of the mean. All experiments were repeated independently at least three times.

Results
Localization of SNAI3 during postnatal ovarian follicular development
The distribution of SNAI3 during ovarian development was detected by immunohistochemistry. The expression patterns of SNAI3 are shown in Figure 1. SNAI3 was not detected in the ovary at PDs 1 and 4 (Fig. 1a, b). It first appeared in oocytes on PD 9. At this time, SNAI3 was also found to be expressed in theca and interstitial cells. Following this, SNAI3 expression was detected consistently in these sites at all stages studied (PDs 18 and 23) (Fig. 1c–e).

Localization of SNAI3 during follicular maturation, ovulation, and luteinization
To stimulate synchronized follicular development, ovulation, and luteinization, immature 21-day-old female mice were injected with gonadotropins. The results demonstrated that in pre-antral, antral, and pre-ovulatory follicles, immunoreactivity of SNAI3 was evident in interstitial cells, theca cells, ovarian surface epithelial cells, and oocytes, which was similar to SNAI1 and SNAI2 (Fig. 2a–c). Following ovulation, GCs initiate a program of terminal differentiation and transform into luteal cells within a few hours. During this time, SNAI3 was evident in CL at 16 and 48 h following hCG treatment (Fig. 2d, e). As the CL regressed, the expression level of SNAI3 increased (Fig. 2f).

Next, we examined the transcription of Snai1, Snai2, Snai3, cyclin D2, and p21 in ovaries from gonadotropin-treated mice and compared the mRNA levels by qRT-PCR. The results demonstrated that PMSG increased the transcription of Snai1 dramatically during follicular development and ovulation when compared with 0 h (P 0). However,
Fig. 1. Immunohistochemical localization of SNAI3 during postnatal ovarian follicular development. Ovaries were collected from mice at (a) postnatal day (PD) 1, (b) PD 4, (c) PD 9, (d) PD 18, and (e) PD 23. SNAI3 was visualized with diaminobenzidine staining (brown). (a, b) SNAI3 immunoreactivity is not evident in the ovary (O, oocytes). (c, d) Positive staining for SNAI3 is evident in O, theca cells (TC), and interstitial cells (IT). (GC, granulosa cells.) (e) During ovarian development, immunostaining of SNAI3 is localized to IT, TC, and O. (f) Negative control. Bars = 50 μm.

Fig. 2. Ovarian localization of SNAI3 during gonadotropin-induced follicular maturation, ovulation, and luteinization in immature mice. (a) Postnatal day (PD) 21. (b) Treatment with pregnant mare serum gonadotropin (PMSG) for 24 h. (c) Treatment with PMSG for 48 h, followed by treatment with human chorionic gonadotropin (hCG) for (d) 16 h and (e) 48 h. (f) Negative control treated with nonimmune serum instead of the primary antibody. CL, corpus luteum. Bars = 50 μm.

Fig. 3. Quantitative real-time polymerase chain reaction analyses of Snai1, Snai2, and Snai3 mRNA expression in ovaries from mice treated with pregnant mare serum gonadotropin (P) for 0 h (P0), 24 h (P24), or 48 h (P48) followed by treatment with human chorionic gonadotropin (H) for 16 h (H16), 24 h (H24), or 48 h (H48). Relative expression of (a) Snai1, (b) Snai2, and (c) Snai3 mRNA to β-actin. (d) Comparison of Snai1, Snai2, and Snai3 expression. Relative expression of (e) cyclin D2 and (f) p21 mRNA to β-actin. Data are expressed as means ± SEM. n = 3. * P < 0.05, ** P < 0.01.
following luteinization, the transcription of Snai1 decreased 48 h after hCG treatment (Fig. 3a). On the contrary, the expression of Snai2 did not change significantly during follicular development and decreased significantly 16 h after hCG treatment (Fig. 3b). The expression of Snai3 increased dramatically at 48 h following PMSG treatment and then increased steadily during oovulation and luteinization (Fig. 3c). When comparing the mRNA levels of Snai1, Snai2, and Snai3, we found that the expression of Snail superfamily members were different at a given time (Fig. 3d). We also determined the transcription of cyclin D2 and p21 and discovered that their expression was opposite to the levels of Snai3 after hCG treatment (Fig. 3e, f).

SNAI1 and SNAI2 are associated with follicular maturation, ovulation, and luteinization [24]. Therefore, we measured SNAI3 levels during these processes. We collected ovaries at 24 and 48 h post PMSG, and at 16, 24, and 48 h following hCG administration. The results showed that, in our model, the expression of SNAI3 did not change significantly during follicular development. After ovulation, SNAI3 expression decreased markedly at 16 h, after which the levels increased steadily at 24 and 48 h post hCG treatment (Fig. 4).

Subcellular localization of SNAI3 in oocytes and during early embryonic cleavage

Our results indicated that SNAI3 was localized to the nucleus, except the nucleus at the GV and one-cell stages. At these stages, GV oocytes and zygotes contained many NPBs (Fig. 5a, b’). SNAI3 was localized to the nucleus from the two-cell stage to eight-cell embryos (Fig. 5c–e’), after which SNAI3 was detected only in the cytoplasm (Fig. 5f–f’). During the blastocyst stage, it was localized to the nucleus of the trophectoderm and in the inner cell mass (Fig. 5g–g’).

qRT-PCR was performed to investigate the mRNA levels of α-catenin, β-catenin, E-cadherin, Snai1, Snai2, and Snai3 (Fig. 6). α-Catenin mRNA levels fluctuated during embryo development, and the expression of β-catenin was unstable. Their expression levels were lowest at the blastocyst stage. From eight-cell to morula stages, compaction occurs, which is a prerequisite for blastocyst formation. During this time, E-cadherin was expressed at its highest level at the eight-cell stage. Thereafter, it decreased and was maintained at low levels. The level of Snai1 mRNA changed slightly and reached its lowest level at the blastocyst stage, while Snai2 and Snai3 were expressed at very low levels from two-cell to the blastocyst stage.

Discussion

In this study, we determined the expression of SNAI3 in the oocytes of neonatal and gonadotropin-treated immature mice, as well as in GV oocytes and pre-implantation embryos. Our data showed that SNAI3 appeared in oocytes by PD 9, when it was also expressed in theca and interstitial cells, where it remained at later stages. In gonadotropin-induced immature mice, SNAI3 was expressed in CLs. Western blot analysis showed that SNAI3 did not change significantly during follicular development. SNAI3 was localized to the nucleus but not the nucleolus during the GV and one-cell stages. From two- to eight-cell stages, it was localized to the nucleolus, following which it was found in the cytoplasm. During the blastocyst stage, SNAI3 was located in the nucleus of the trophectoderm and inner cell mass. qRT-PCR results showed that the expression of Snail superfamily genes was decreased during the blastocyst stage. At the same time, the expression of compaction related genes (i.e., E-cadherin and catenins) changed greatly during early embryo development. Taken together, these results suggest that SNAI3 may play roles in the regulation of follicle development and early embryo cleavage.

SNAI3 in follicular development

Our results indicated that the localization of SNAI3 was distinct from the positive staining of oocytes for SNAI2 within primordial follicles, and was similar to Snai1, as we have shown previously [24]. SNAI3 could not be detected in oocytes until PD 9 when oocyte growth begins [28]. Therefore, we speculated that SNAI3 might regulate primary oocyte development, but does not take part in establishment of the primordial follicle pool.

The pool of primordial follicles at birth represents the total population of germ cells available to female mammals during their entire reproductive life [1]. The zinc finger domains of SNAI3 bind to CAGGGT (CANNNTG) E-box motifs and compete with the basic helix-loop-helix transcription factors [16], such as SOHLH2 and FIGα. In females, Sohlh2 transcripts are confined to oocytes of small follicles in the immature ovary. In adult ovaries, SOHLH2 protein is present in primordial follicles but is not detected in growing oocytes [29]. FIGα plays a key regulatory role in the expression of multiple oocyte-specific genes, including those that initiate folliculogenesis and those that encode the zona pellucida [30]. The persistence of SNAI3 in growing oocytes in our work indicates that SNAI3 may compete with SOHLH2 and FIGα to control follicular development. Because SNAI3 belongs to the Snail superfamily and its expression patterns are similar to Snai1 and Snai2 in primary, secondary, and mature follicles [24], we speculate that it has similar roles in
Fig. 5. Immunofluorescence localization of SNAI3 during the germinal vesicle (GV) stage and early embryonic cleavages. Immunostaining with an anti-SNAI3 antibody (green) and DNA staining with 4′,6-diamidino-2-phenylindole dihydrochloride (blue). (a–a’’) GV. (b–b’’) One-cell stage (white arrow indicates the nucleolus). (c–c’’) Two-cell stage. (d–d’’) Four-cell stage. (e–e’’) Eight-cell stage. (f–f’’) Morula. (g–g’’) Blastocyst (ICM, inner cell mass, TE/white arrow, trophoectoderm). (h–h’’) Negative control for SNAI3. Bars = 25 μm.

Fig. 6. mRNA levels of α-catenin, β-catenin, E-cadherin, Snai1, Snai2, and Snai3 determined by the quantitative real-time polymerase chain reaction in eggs and pre-implantation embryos. UF, unfertilized eggs. ZY, zygote. 2C, two-cell embryos. 8C, eight-cell embryos. M, morulae. BL, blastocysts. At least 40 oocytes/embryos were evaluated in each group. Three independent assays were carried out for each gene. Data are presented as means ± SEM of triplicate simples. * P < 0.05, ** P < 0.01.
promoting follicular development at these stages.

**SNAI3 during formation of CL**

When preantral follicles develop into Graafian follicles, GCs are sensitive to luteinizing hormone. Luteinizing hormone stimulates ovulation and acts as a signal for GCs to differentiate into CLs. The reprogramming of GCs into luteal cells demands they exit from the cell cycle and arrest predominantly in the G0/G1 phase [31]. Termination of cell proliferation during luteinization correlates with the loss of many cell cycle regulators, such as cyclin D2, that promote progression to the G1 phase by activating Cdk4 [31, 32]. The highly conserved promoter region of cyclin D2 contains two consensus E-box sequences that can compete for binding with members of the Snail family [33, 34]. Thus, Snail family proteins directly repress transcription of cyclin D2 to arrest the cell cycle.

A negative correlation was observed between SNAI3 and cyclin D2 in CLs, which led us to propose that SNAI3 participates in luteinization by repressing the promoter activity of cyclin D2 during transformation from GCs to luteal cells. In addition, members of the Snail family might function by cooperating with other E-box containing proteins, such as p21, or by binding to the E-box of proteins such as c-Myc and arrest luteal cells in the G0/G1 phase.

P21 has been long known for inhibiting cyclin/Cdk [35, 36]. However, more recent data have shown that it has positive effects on cyclin/Cdk activation through its “assembly factor” function. Because an inverse correlation was observed between SNAI3 and P21 in CLs, we speculated that SNAI3 had a potential influence on CL formation. The oncogene protein, c-Myc, binds specifically to 5′-CACGTG-3′ E-box sequences to activate transcription. c-Myc is found. A major fraction of the sense-strand nuclear c-Myc transcript is localized to the nucleoli [43]. C-Myc is an oncogenic transcription factor that integrates the cell cycle machinery with cell adhesion, cellular metabolism, and apoptotic pathways [44]. Furthermore, the nucleolus is a key regulator of p53 stability. P53 is stable in a cell unless the nucleolus promotes its degradation through the ubiquitin pathway, with several knock-on effects through pathways controlled by the p53 status [45]. Relatively little is known about the spatial distribution and compartmentalization of RNA within nucleoli, or about the possible significance of subnuclear localization with respect to RNA processing, intranuclear turnover, or transport to the cytoplasm. The function of SNAI3 in the nucleoli is also unclear and further work is needed to delineate its role.

During embryo compaction, blastomeres acquire polarity and become flat [46, 47]. This compaction is mediated by the cell adhesion molecule, E-cadherin. SNAI1 represses the transcription of E-cadherin, both in vitro and in vivo, by binding to the E-box sequence of its promoter. Clustering of E-cadherin is thought to depend on linkage through cytoplasmic catenin to the actin cytoskeleton. Specifically, β-catenin binds directly to both the E-cadherin cytoplasmic domain [20] and to the actin-binding protein α-catenin [48]. In the current work, SNAI3 was localized to the cytoplasm during the morulae stage, when it may be unable to efficiently repress this activity, thus allowing compaction to proceed.

The EMT, in which polarized epithelial cells are converted into motile cells, is crucial for multicellular organisms to get past the blastula stage of embryonic development (embryogenesis), and is a defining structural change during organ development as well as carcinogenesis [49]. E-Cadherin at the adherens junction is a key molecular target of the EMT [49], and down-regulation of E-cadherin is thought to play a fundamental role during the EMT. SNAI1 is a direct repressor of E-cadherin [49], while SNAI2 represses E-cadherin to promote the EMT in various cancers. For example, it represses E-cadherin via p19Arf in prostate tumorigenesis [49].

Combined with our previous research [24], we found that SNAI3 shows similar tendencies as SNAI2 during early embryo development. Thus, because Snai3 mutant mice are viable and fertile [23], like Slug knockout mice [50], the SNAI1 family might play a similar role during embryogenesis, and there might be redundant roles among SNAI1, SNAI2, and SNAI3. However, whether SNAI3 and other family members repress the same or different sets of target genes, and how such regulation leads to the correct decision in various developmental and physiological processes, remains to be determined.

We noted an inconsistency between Snai3 mRNA levels and the amount of its protein during folliculogenesis and embryogenesis. Because gene expression is a complex process that is controlled at multiple steps, such as transcriptional regulation, post-transcriptional regulation (RNA processing, RNA stability), ribosome occupancy and density, RNA availability, translational regulation, post-translational regulation, and protein stability, RNA and protein levels are not always well-correlated [51–53]. Moreover, the protein-per-mRNA ratio is different for different genes, and may even change for a given gene under different cellular conditions [54]. Therefore, the basis for this discrepancy needs considerable further investigation.

**Conflict of interest:** No financial conflict of interest exists. The authors do not have any potential conflict of interest on the discussed topic.

**Acknowledgements**

This work was supported by grants from the National Natural Science Foundation of China (NSFC: 31471399 and 31671199), National Key R&D Program of China (2017YFC1001403), and Shanghai Municipal Education Commission—Gaofeng Clinical
Medicine Grant Support (20152515) to CZ. Support was also received by a grant from the Major Program of the National Natural Science Foundation of China (81440743) to Z-JC, and by the Shanghai Commission of Science and Technology (funding number: 17DZ2271100).

References

1. Kezeler P, Nilsson E, Skinner MK. Cell-cell interactions in primordial follicle assembly and development. Front Biosci 2002; 7: d1990–d1996. [Medline] [CrossRef]

2. Faddy MJ, Godon RG, Gougeon A, Richardson SJ, Nelson JF. Accelerated disappearance of ovarian follicles in mid-life: implications for forecasting menopause. Hum Reprod 1992; 7: 1342–1346. [Medline] [CrossRef]

3. Sliżynski BM. Meiotic prophase in female mice. Nature 1957; 179: 638. [Medline] [CrossRef]

4. Boras K. Oogenesis in the mouse. A study of the meiotic prophase. Exp Cell Res 1961; 24: 495–507. [Medline] [CrossRef]

5. Pepling ME. Follicular assembly: mechanisms of action. Reproduction 2012; 143: 139–149. [Medline] [CrossRef]

6. McGee EA, Hsieh AJ. Initial and cyclic recruitment of ovarian follicles. Endo Rev 2008; 21: 200–224. [Medline] [CrossRef]

7. Edson MA, Nakamura AK, Matzuk MM. The mammalian ovary from genesis to revelation. Endo Rev 2009; 30: 624–712. [Medline] [CrossRef]

8. Fauser BC, Van Heeumen AM. Manipulation of human ovarian function: physiological concepts and clinical consequences. Endo Rev 1997; 18: 71–106. [Medline]

9. Bowman P, McAlister A. Cleavage rate of mouse embryos in vivo and in vitro. J Exp Emb Morph 1970; 24: 203–207. [Medline] [CrossRef]

10. Hardy K, Spanel S. Growth factor expression and function in the human and mouse preimplantation embryo. J Endocrinol 2002; 172: 221–236. [Medline] [CrossRef]

11. Poolekula Minhas, Parfenov V. Nucleolar transformation in mouse antral follicles: distribution of colin and components of RNA-polymerase I complex. Cell Tissue Biol 2008; 2: 522–530. [CrossRef]

12. Biggiero M, Martin TE, Gordon J, Amalfit F, Fakan S. Physiologically inactive nucleoli contain nucleoplasmin ribonucleoprotein: immunoelectron microscopy of mouse spermatids and early embryos. Exp Cell Res 1994; 213: 55–63. [Medline] [CrossRef]

13. Chouinard LA. A light- and electron-microscope study of the nucleolus during growth of the oocyte in the prepubertal mouse. J Cell Sci 1971; 9: 637–663. [Medline]

14. Selfiton M, Sánchez S, Nieto MA. Conserved and divergent roles for members of the Snail family of transcription factors in the chick and mouse embryos. Development 1998; 125: 3111–3121. [Medline] [CrossRef]

15. Boulay JL, Dennefeld C, Alberga A. The Drosophila developmental gene snail encodes a protein with nuclear acid binding fingers. Nature 1987; 330: 395–398. [Medline] [CrossRef]

16. Kataoka H, Murayama T, Yokode M, Sano H, Ozaki H, Yokota Y, Nishikawa S, Iita T. A novel snail-related transcription factor Smuc regulates basic helix-loophelix transcription factor activities via specific E-box motifs. Mol Biol Cell 2004; 15: 626–633. [Medline] [CrossRef]

17. Battle E, Sancho E, Franci C, Dominguez D, Monfar M, Baulida J, Garcia De Herreros A. The transcription factor snail is a regulator of E-cadherin gene expression in epithelial tumour cells. Nat Cell Biol 2008; 2: 84–89. [Medline] [CrossRef]

18. Fleming TP, Sheth R, Fenske I. Cellular adhesion in the preimplantation mammalian embryo and its role in trophoderm differentiation and blastocyst morphogenesis. Front Biosci 2001; 6: D1000–D1007. [Medline] [CrossRef]

19. Hirano S, Nose A, Hatta K, Kawakami A, Takeichi M. Calcium-dependent cell-cell adhesion molecules (cadherins): subclass specificities and possible involvement of actin bundles. J Cell Biol 1987; 105: 2501–2510. [Medline] [CrossRef]

20. Huber AH, Weis WI. The structure of the β-catenin-E-cadherin complex and the molecular basis of diverse ligand recognition by β-catenin. Cell 2001; 105: 391–402. [Medline] [CrossRef]

21. Newkirk KM, Mackenzie DA, Bakaletz AP, Hudson LG, Kusewitt DF. Microarray analysis demonstrates a role for Slag in epiblast differentiation. J Invest Dermatol 2008; 128: 361–369. [Medline] [CrossRef]

22. Katoh M, Katoh M. Identification and characterization of human SNA1L3 (SNA1G) gene in silico. Int J Mol Med 2003; 11: 383–388. [Medline] [CrossRef]

23. Bradley CK, Norton CR, Chen Y, Han X, Booth CJ, Yoon JK, Krebs LT, Gridley T. The snail family gene snai3 is not essential for embryogenesis in mice. PLoS ONE 2013; 8: e65544. [Medline] [CrossRef]

24. Guo C, Meng X, Bai J, Chen C, Liu T, Liu S, Zhang C, Li W-P. Expression and localization of transcription factors SNAI1 and SNAI2 in mouse ovaries and pre-implantation embryos. Cell Tissue Res 2014; 358: 585–595. [Medline] [CrossRef]

25. Cui LL, Yang G, Pan J, Zhang C. Tumor necrosis factor a knobout increases fertility of mice. Theriogenology 2011; 75: 867–876. [Medline] [CrossRef]