University of Groningen

The possible FAT1-mediated apoptotic pathways in porcine cumulus cells

Wu, Xinhui; Fu, Yao; Liu, Chang; Chai, Menglong ; Chen, Chengzhen ; Dai, Lisheng; Gao, Yan; Jiang, Hao; Zhang, Jiabao

Published in:
Cell Biology International

DOI:
[10.1002/cbin.10695](https://doi.org/10.1002/cbin.10695)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2017

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Wu, X., Fu, Y., Liu, C., Chai, M., Chen, C., Dai, L., Gao, Y., Jiang, H., & Zhang, J. (2017). The possible FAT1-mediated apoptotic pathways in porcine cumulus cells. *Cell Biology International*, 41(1), 24-32.
<https://doi.org/10.1002/cbin.10695>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

RESEARCH ARTICLE

The possible FAT1-mediated apoptotic pathways in porcine cumulus cells

Xinhui Wu¹, Yao Fu¹, Xulei Sun^{1,2}, Chang Liu¹, Menglong Chai^{1,3}, Chengzhen Chen¹, Lisheng Dai¹, Yan Gao¹, Hao Jiang^{1*} and Jiabao Zhang^{1*}

¹ College of Animal Sciences, Jilin University, Changchun, Jilin, China

² Shenyang Jiuzhou Hospital, Shenyang, Liaoning, China

³ College of Animal Science and Technology, China Agriculture University, Beijing, China

Abstract

Porcine cumulus cells are localized around oocytes and act as a specific type of granulosa that plays essential roles in the development and maturation of oocytes, the development and atresia of follicles, and the development of embryos. Studies of FAT1 have demonstrated its functions in cell–cell contact, actin dynamics, and cell growth suppression. To understand whether the *FAT1* gene affects the apoptosis of porcine cumulus cells and to elucidate the mechanism of this potential action, FAT1 was knocked down using RNA interference. The lack of *FAT1* resulted in stable expression of *CTNNB*, enhanced expression of cleaved CASP3, but decreased the BCL2/BAX ratios at both the mRNA and protein levels. These results indicated that FAT1 inhibited porcine cumulus cell apoptosis via different pathways. Taken together, these data provide new insights into the mechanisms of the association between FAT1 and porcine cumulus cell apoptosis.

Keywords: apoptosis; cumulus cells; FAT1; follicle

Introduction

Cumulus cells are a type of granulosa cell in mammalian ovaries and are located around the oocyte. Cumulus cells communicate with each other and with the oocyte through specialized gap junctions that allow the metabolic exchange and transport of signaling molecules, which indicate that cumulus cells play vital roles in protecting and providing nutrition to the oocyte (Uyar et al., 2013). Additionally, granulosa cell apoptosis is an active, congenital, and alternative process of cell death, and the induction of granulosa cell apoptosis is one potential mechanism of follicle atresia. During the normal process of the ovarian cycle, follicular atresia in each stage is strongly related to the granulosa cell apoptosis, and granulosa apoptosis is widely recognized as the active mechanism of follicle atresia (Feng et al., 2007; Fuko et al., 2012; Sun et al., 2015). In bovine normal estrus cycle, only one or two follicles develop into the

dominant follicle; however, as to the polyovulatory species, such as pig, approximately 10–20 oocytes are ovulated during each estrus cycle. The development of other follicles is slowed down, and these follicles then gradually atrophy and ultimately undergo atresia. Due to the close relationship between the oocyte and the granulosa cells, the morphological function of the granulosa affects the development of oocytes. Thus, understanding the correlations of the proliferation and the apoptosis of cumulus cells with the development of oocytes will aid subsequent studies of the survival potential of embryos.

BMP15 is one of the TGFB family members, which is a well-known oocyte-secreted growth factor that acts on cumulus cells to regulate multiple functions, such as the proliferation, expansion, and apoptosis of cumulus cells (Sudiman et al., 2014). Previous high-throughput sequencing results from our group demonstrated that BMP15 increased *FAT1* mRNA (Zhai et al., 2013). Studies have

*Corresponding authors: e-mail: jhhaojiang@jlu.edu.cn (H.J.); zhangjiabaojlu@163.com (J.Z.)

Abbreviations: BAX, BCL2-associated X protein; BCL2, B-cell CLL/lymphoma 2; BMP15, bone morphogenetic protein; CASP3, caspase 3 apoptosis-related cysteine peptidase; CASP8, caspase 8 apoptosis-related cysteine peptidase; CASP10, caspase 10 apoptosis-related cysteine peptidase; FAT1, FAT atypical cadherin 1; FCM, flow cytometry; TGFB, transforming growth factor beta

demonstrated that the *FAT1* gene encodes a ω -3 fatty acid desaturase enzyme that converts ω -6 to ω -3 polyunsaturated fatty acids (PUFAs) and thus increases ω -6/ ω -3 ratio (Chen et al., 2013a,b). A balanced ω -6/ ω -3 PUFAs ratio has been linked to health benefits and the prevention of many chronic diseases, and increases in this ratio might suppress the growth of cancer cells (Astarita et al., 2014). However, FAT1 is also a member of the transmembrane protein family and is thus localized to the cell membrane (Tanoue and Takeichi, 2005; Morris et al., 2013b). In mammalian animals, the FAT family includes FAT1, FAT2, FAT3, and FAT4, and these proteins play significant roles in various processes of developments (Tanoue and Takeichi, 2005; Katoh and Katoh, 2006; Katoh, 2012; Valletta et al., 2012; Morris et al., 2013a). Thus, based on the structure of FAT1, which consists of 34 cadherin repeats, five EGF-like motifs, a laminin A-G domain in the extracellular region, and a cytoplasmic tail, we believed it would have a significant role in apoptosis (Tanoue and Takeichi, 2005; Sadeqzadeh et al., 2011). Although FAT1 has been demonstrated to be able to modulate the cell–cell contact and actin dynamics (Tepass, 1999; Yagi and Takeichi, 2000; Sopko and McNeill, 2009; Sadeqzadeh et al., 2014), and *FAT1* transgenic animals have been studied to link to its metabolic and modulatory functions of fatty acids contents (Cheng et al., 2015; Fan et al., 2016), there are currently little reports regarding whether the *FAT1* gene affects porcine cumulus cell apoptosis, and the mechanism of FAT1-mediated-apoptosis is still unclear.

The aim of this study is to understand the mechanism of FAT1-mediated porcine cumulus cell apoptosis, and thus porcine cumulus cell were isolated from ovaries and cultivated in vitro previously and RNA interference was used to knockdown the *FAT1* gene. We proposed that various proteins might regulate the FAT1-mediated apoptosis in porcine cumulus cells. To test this hypothesis, molecular technologies were used to examine the expression of target genes in porcine cumulus cell.

Materials and methods

In vitro porcine cumulus cell cultivation

Porcine ovaries were collected from a local abattoir, placed in warm saline (0.9% NaCl containing 100 U/mL penicillin and 100 U/mL streptomycin; the NaCl, penicillin and streptomycin were purchased from Sigma–Aldrich), and transported to the laboratory within 2 h at 37°C. Upon arrival in the laboratory, the ovaries were rinsed three times with warm saline containing 100 U/mL penicillin and streptomycin at approximately 37°C. The follicular fluid was collected from the surfaces of the intact healthy follicles that measured 3–8 mm in diameter using an 18-gauge needle and a 20-mL syringe containing 2 mL of aspiration media (HEPES-buffered DMEM/F-12, Sigma–Aldrich). All the animal experiments were approved by the local ethics committee, Jilin University, China, and the experimental protocol conformed to the Animal Welfare Act Guide for Use and Care of Laboratory Animals.

Only COCs (Figure 1A) with homogeneous granulated cytoplasm and at least three to four layers of compact cumulus cells were selected based on examination under a dissecting microscope. These COCs were washed two to three times in HEPES-buffered DMEM/F-12, and the cumulus cells were dissociated by incubation with hyaluronidase (0.1%) for 3 min and pipette continuously to isolate the cumulus cells from oocytes. The fluid with isolated oocytes and cumulus cells was then centrifuged at 1,000 rpm for 5 min, and pour out the supernatant. Repeat the step mentioned above to wash the precipitation two to three times using DMEM/F-12. The suspension cells were treated in a cell culture plate, DMEM/F-12 (penicillin and streptomycin and 10% fetal calf serum) was added to the plate, and the plate was then incubated at 37.5°C with 5% CO₂ in humidified air (Figure 1B).

siRNA transfection

One pair of FAT1-specific synthetic siRNAs was designed and synthesized by and purchased from Gene Pharma

Figure 1 The separation and cultivation of porcine cumulus cells in vitro. (A) Representative photographs of porcine cumulus–oocyte complex (COCs) showing at least three layers of cumulus around the oocytes were selected. Scale bar is shown in 20 μm. (B) Porcine cumulus cells were separated from the cumulus–oocyte complex (COCs) and the cumulus cells were cultivated in vitro. Scale bar is shown in 50 μm.

(Shanghai, China). The siRNA sequences were as follows: sense FAT1: 5'-CCUGGUGACUGAGGUAAAATT-3'; antisense FAT1: 5'-UUUAACCUCAGUCACCAGGTT-3'; sense negative control (NC): 5'-UUCUCCGAACGUGU-CACGUTT-3'; and antisense negative control (NC): 5'-ACGUGACACGUUCGGAGAATT-3'. Porcine cumulus cells were transfected with siRNA-FAT1 and NC-siRNA using LipofectamineTM RNAi MAX Reagent (Shanghai Invitrogen Biotechnology, China). Briefly, RNAi duplexes, LipofectamineTM RNAi MAX, and DMEM/F-12 medium were combined and incubated for 20 min at room temperature. The transfection complex was added to the cells, which were subsequently incubated for 24 h at 37°C. The cells were then collected for use in other experiments.

Apoptosis assays

The cells that were transfected with the si-FAT1 were collected after 24 h, stained with FITC annexin V and propidium iodide (FITC Annexin V Apoptosis Detection kit, BD PharmingenTM), and analyzed by flow cytometry (FACSCanto II; BD Biosciences). All experiments were performed in biological triplicates, and the data are representative of at least three independent experiments.

cDNA quantitative real-time PCR

Total RNAs were isolated from the cells at the appropriate time points using Tripure Isolation Reagent (Roche, Germany) and quantified using a NanoDrop 2000 spectrophotometer. DNase treatment was applied, and 1 µg of total RNA was used for cDNA synthesis, which was performed with a FastQuant RT Kit (Tiangen Biotech, China). The quantitative real-time PCR (RT-qPCR) reactions were performed in triplicate in 20-µL reaction volumes (10 µL of 2X Super Real Premix Plus, 1 µL of 1:10 diluted cDNA, and 0.5 µL each of the forward and reverse primers) on a Mastercycler[®] ep realplex (Eppendorf, Germany) with SYBR-Green detection (SuperReal Premix Plus, Tiangen Biotech, China) according to the manufacturer's instruction. The average threshold cycle (Ct) was calculated for each sample, and the relative expression levels were calculated via the $2^{-\Delta\Delta C_t}$ method.

Immunofluorescence

The si-FAT1-transfected porcine cumulus cells were washed three times with PBS after 24 h. The cells were then fixed with 4% formaldehyde solution in PBS for 15 min at room temperature and washed with PBS twice. The fixed cells were permeabilized with 0.1% Triton X-100 in PBS for 15 min on ice and then washed three times with PBS. The cells were incubated with blocking buffer (1% BSA in PBS) at 37°C for 1 h. Thereafter, the cells were incubated with the appropriate

antibodies in 1% BSA in PBS overnight in a dark humidified chamber. Next, the cells were washed five times for 5 min with PBS and incubated with fluorescein-conjugated secondary antibodies (Boster, Wuhan, China) containing 1% BSA within PBS in a dark humidified chamber at 4°C for 1 h. After five washes with PBS, DAPI was used to counterstain the cell nuclei. Immunofluorescence was examined and captured using a confocal ultra-spectral microscope system A1[®], (Nikon Instruments, Tokyo, Japan). The post-capture image analyses were performed using the Nikon Confocal software package NIS-Elements C[®] (Nikon Instruments). The images for presentation were directly obtained from the NIS-Elements C software program.

Western blot

The cumulus cells were lysed in lysis buffer for Western blotting (Beyotime Biotechnology, China) on ice for 15 min, and the resultant cell suspension was then centrifuged at 13,000 rpm for 30 min. The supernatant was removed, and the protein concentration was measured with a BCA protein assay kit (Beyotime Biotechnology, China). Then, 10- to 20-µg samples were supplemented with 2× protein loading buffer (Tiangen Biotech, China) and boiled for 5 min at 95°C. The cell lysates were separated on 4–12% (w/v) sodium dodecyl sulfate–polyacrylamide gels and then transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA). The following procedures were performed using the SNAP i.d.[®] 2.0 Protein Detection System (Millipore, Billerica, MA) according to the instructions of the SNAP i.d.[®] 2.0 Quick Start Guide. The signals were detected as chemical luminescence with X-ray films using an Immobilon Western Chemiluminescent HRP Substrate kit (Millipore, Billerica).

Statistical analyses

All data collected in this study were obtained from three or more different repetitions for each condition. The SPSS 19.0 analysis software was used for the statistical analyses, and the statistical evaluations of the data were performed with one-way ANOVA or *t*-tests for evaluations of the differences between two groups. *P* values <0.05 was considered significantly difference, and *P* < 0.01 was considered highly significant. The data are presented as the means ± the standard errors of the mean (SEM).

Results

Expression of *FAT1* in porcine cumulus cells

To detect whether *FAT1* was silenced in the porcine cumulus cells following transfection with si-FAT1, the expression

levels of *FAT1* in the cumulus cells were measured with RT-qPCR. The results revealed that *FAT1* was significantly downregulated in the porcine cumulus cells that had undergone the *si-FAT1* treatment (Figure 2A). Moreover, examinations of the immunofluorescences of the cultured cumulus cells revealed similar results; that is, stronger signals were observed on the membranes of the porcine cumulus cells of the negative control group (Figure 2B) compared with the *si-FAT1* group (Figure 2C). The immunofluorescence results revealed that *FAT1* localized to the cell membrane; however, the knockdown of *FAT1* resulted in decreased expression of *FAT1* on the cell membrane, which confirmed that *FAT1* is a membrane protein in porcine cumulus cells. Taken together, these results indicated that *FAT1* was successfully knocked down in the porcine cumulus cells, and these cells could thus be used for further research.

Knockdown of *FAT1* increases porcine cumulus cell apoptosis

To study the role of *FAT1* in porcine cumulus cell apoptosis, flow cytometry analyses were used to measure the cells that were dyed with annexin V and propidium iodide. The

average apoptosis rate in the *si-FAT1* group was 18.51%, whereas the average apoptosis rate in the negative control group was 14.55% (Figure 3). Thus, the knockdown of *FAT1* significantly increased the porcine cumulus cell apoptosis rate, which indicates that the cumulus cells lacking *FAT1* died via apoptosis more rapidly than the control cells.

FAT1 sensitizes CTNNB during the apoptosis

The Western blot results (Figure 4A) indicated that the silencing of the *FAT1* gene in the porcine cumulus cells did not alter the expression of *CTNNB*. This finding might suggest that *CTNNB* induces cumulus cell apoptosis by moving from the cell membrane to the nucleus rather than via alterations in its expression.

The *BCL2*/*BAX* ratio in *FAT1*-mediated apoptosis

BAX and *BCL2* are well-known apoptosis marker genes. *BAX* is an inducer of apoptosis, and *BCL2* acts as an inhibitor of apoptosis. Hence, we detected the expressions of *BAX* and *BCL2* in porcine cumulus cells after the transfection of *si-FAT1* by RT-qPCR. The knockdown of *FAT1* significantly increased the *BAX* mRNA level and decreased the *BCL2*

Figure 2 RNA interference effects of *si-FAT1* in porcine cumulus cells. (A) Quantitative real-time PCR detection of *FAT1* expression in porcine cumulus cells after *si-FAT1* was transfected into the porcine cumulus cells for 24 h. The column of *si-FAT1* shows *FAT1* was knocked down over 60%. All assays were performed in triplicate. (B–C) Representative images of porcine cumulus cells in immunofluorescences assays. Cells were treated with the indicated siRNAs. Twenty-four hours after transfection, cells were stained with DAPI (blue) and antibody against *FAT1* (green). Yellow arrows denote plasma membrane localization. Scale bars, 20 μm. The porcine cumulus cells in NC group (B) exhibited positive *FAT1* staining at the lamellipodial edges or on the cell membrane, whereas the *FAT1* knockdown cells exhibited vague and diffuse *FAT1* signal on the cell membrane (C). NC, negative control; *si-FAT1*, knockdown of *FAT1* by *si-FAT1*

Figure 3 Apoptosis of the porcine cumulus cells. (A and B) Representative images of flow cytometry analysis of the apoptotic fraction of porcine cumulus cells treated with si-FAT1. (C) Twenty-four hours after transfection, the average rates of apoptosis in the control porcine cumulus cells and FAT1 knockdown cells were 14.55% and 18.51%, respectively. NC, negative control; si-FAT1, knockdown of FAT1 by si-FAT1.

mRNA level and thus decreased the *BCL2/BAX* ratio (Figure 5). The Western blot results were similar (Figure 4); that is, the expressions of BAX and BCL2 proteins increased and decreased, respectively, and the *BCL2/BAX* protein ratio decreased. The increased BAX might lead to BAX/BAX homodimers increase, and cells are more sensitive to the death signal and ultimately activate apoptosis (Hengartner, 2000; Evan and Vousden, 2001; Adams and Cooper, 2007; Chen *et al.*, 2015). Taken together, the mechanism of porcine cumulus cell apoptosis was due to the alteration of the *BCL2/BAX* ratio.

FAT1 affects cleaved CASP3 activation-induced apoptosis

In addition to the BCL2 family, the caspase family also plays a pivotal role in apoptosis. During the progress of apoptosis, CASP8 is the key prototypal initiator caspase, which propagates an apoptotic signal and CASP3, acting as the prototypal executioner caspase that will cleave a panel of proteins that contribute to the structural and bioenergetic integrity of the cell. To examine the direct effect of the caspase family on porcine cumulus cells, we examined cleaved CASP3, which acts as an executor in apoptosis. The

Figure 4 Protein expressions of CTNNB, CASP3, BAX, and BCL2 in porcine cumulus cells. (A) Protein bolts show stable expression of CTNNB after si-FAT1 transfection. (B) Protein bolts show increasing expression of cleaved-CASP3 protein after si-FAT1 transfection. (C) Protein bolts show increasing expression of BAX protein expression after si-FAT1 transfection. (D) Protein bolts show decreasing expression of BCL2 protein expression after si-FAT1 transfection. (E) The column shows decreasing ratio of BCL2/BAX after si-FAT1 transfection. NC, negative control; si-FAT1, knockdown of FAT1 by si-FAT1.

knockdown of FAT1 increased cleaved CASP3 at both the protein and mRNA levels (Figures 4B and 5C). Additionally, the expressions of *CASP8* and *CASP10* increased in the cumulus cells lacking FAT1 (Figures 5A and 5B). These results suggest that the knockdown of FAT1 results in the activations of CASP8 and cleaved CASP3, which indicate that porcine cumulus cell apoptosis may result from the activation of the CASP8-CASP3 pathway.

Discussion

Few researchers have recognized the role of the structure of the *FAT1* gene in reproduction. The majority of researchers who have examined on FAT1 have focused on the physiological and nutritional functions of the *FAT1* gene, which involve the conversion of ω -6 PUFAs to ω -3 PUFAs to reduce the ω -6/ ω -3 PUFA ratio and the effects of FAT1 on apoptosis (Chen et al., 2013a,b; Astarita et al., 2014). In this study, FAT1 was revealed to inhibit porcine cumulus cells apoptosis rather than only play the necessary role in metabolic function by altering the ratio of ω -6/ ω -3 PUFA. Due to the product of FAT1 encodes a transmembrane protein of approximately 500 kDa (Morris et al., 2013a), this large size has been a problematic for protein assays. However, the immunofluorescence results apparently

elucidated that FAT1 was not only expressed on cells membrane, but also confirmed the si-FAT1 used in this study was efficient.

Morris et al. (2013a) connected FAT1 to the WNT/beta-catenin signaling pathway and believed that FAT1 binds CTNNB and fixes it to the cell membrane to impede its translocation from the membrane to the nucleus, which would inhibit the growth of tumor cells and thus indicated that FAT1 may suppress tumorigenesis (Miyazaki, 2011; Morris et al., 2013a). Compared with the results observed by Morris, in the present study, the expression of CTNNB was stable; however, in contrast to the results of Morris, the FCM results indicated that FAT1 inhibited porcine cumulus cell apoptosis rather than inhibiting the proliferation of cumulus cells. Although these two results seemed paradoxical, Morris et al. (2013b) also mentioned that the function of FAT1 is multifaceted and that many opposed functions could be elicited by different mechanisms. Although in this study, we have not confirmed the FAT1-mediated apoptosis in porcine cumulus cells resulted from the alteration of CTNNB; however, we still believe that CTNNB via the WNT/beta-catenin signaling pathway may play its possible role in the apoptosis of porcine cumulus cells, and additional studies should be conducted to study this signaling pathway.

Figure 5 The mRNA expressions of *CASP8*, *CASP10*, *cleaved-CASP3*, *BAX*, *BCL2*, and *BCL2/BAX* ratio in porcine cumulus cells. (A) The column shows increasing mRNA expression of *CASP8* after transfection with si-FAT1. (B) The column shows increasing mRNA expression of *CASP10* after transfection with si-FAT1. (C) The column shows increasing mRNA expression of *cleaved-CASP3* after transfection of si-FAT1. (D) The column shows increasing mRNA expression of *BAX* after transfection with si-FAT1. (E) The column shows decreasing mRNA expression of *BCL2* after transfection with si-FAT1. (F) The column shows decreasing ratio of *BCL2/BAX* after transfection with si-FAT1. NC, negative control; si-FAT1, knockdown of FAT1 by si-FAT1.

According to Dominique K., the loss of FAT1 promotes cell apoptosis due to the interaction of FAT1 with CASP8, which is the key initiator caspase in apoptosis (Kranz and Boutros, 2014). In the present study, following the knockdown of FAT1, the expression of CASP8 increased, and the cleavage of CASP3 increased. These results are agreeable to those of the Dominique K. to some extent, which indicates FAT1 may promote porcine cumulus cell apoptosis via a CASP8-CASP3 pathway. Although the cysteine-containing aspartate-specific protease (caspase) family plays a key role in apoptosis via the controls of the activation and execution of programmed apoptosis, other proteins, such as the BCL2 family, also play critical roles in cell apoptosis (Bergmann et al., 1998; Ricky et al., 2002; Fuchs and Steller, 2011; Otterson et al., 2012; Tiwari et al., 2015). According to function, the BCL2 family can be divided into two types, that is, proteins that inhibit apoptosis, such as BCL2, and proteins that promote apoptosis, such as BAX (Bergmann et al., 1998; Ricky et al., 2002; Fuchs and Steller, 2011; Otterson et al., 2012; Albamonte et al., 2013; Tiwari et al., 2015).

In the present study, the BCL2/BAX ratio decreased significantly following the knockdown of FAT1, and this may have resulted from structural variation. Typically, the expressions of both of these proteins are stable, but when BAX is overexpressed, BAX/BAX homodimers increase, and cells are more sensitive to the death signal and ultimately activate apoptosis (Hengartner, 2000; Evan and Vousden, 2001; Adams and Cooper, 2007; Chen et al., 2015). Similarly, when BCL2 is overexpressed, large numbers of BAX/BAX dimers are degenerated, and BCL2/BAX heterodimers increase, which suppress BAX/BAX dimer-induced apoptosis and consequently extend the survival time of the cell. Accordingly, most researchers have indicated that the ratio of these proteins is a key factor in the regulation of death, but whether alterations in this ratio promote or inhibit apoptosis remains controversial (Hanafy et al., 2010; Durdu et al., 2012). Our results indicated that FAT1 may inhibit porcine cumulus cell apoptosis via the BCL2/BAX pathway.

Only several of the representative target genes associated with apoptosis were analyzed in this study; however, apoptosis is a complex physiological process that is controlled

by a variety of cytokines, proteins, and signaling pathways. The potential possible pathways involved in FAT1-induced porcine cumulus cell apoptosis referred to above might be connected to form a single pathway. In the present study, we only separately examined the relationships of FAT1 and CTNNB, FAT1 and caspase, and FAT1 and BCL2/BAX. However, how these pathways could function as a single signaling pathway remains unclear. To systematically explore the role of FAT1 in porcine cumulus cell apoptosis, further studies will focus on WNT/beta-catenin in addition to the caspase signaling pathway, and the CRISPR-CAS9 technique may be used instead of si-RNA.

Conclusions

Our data suggest that FAT1 plays a critical role in inhibiting porcine cumulus cells apoptosis, and this negative role may be mediated via two possible pathways simultaneously: the BCL2/BAX pathway and/or the CASP8-CASP3 pathway. As the proliferation and apoptosis of cumulus cells play important roles not only in oocyte development and maturation but also in the processes of follicle development and atresia, it might be useful to clarify the mechanism of the interaction between cumulus cells and mammalian breeding.

Acknowledgments and funding

This work was supported by the grant from the National Natural Science Foundation of China (31572400).

Conflict of interest

No authors in this manuscript declare any conflict of interest.

References

- Adams KW, Cooper KGM (2007) Rapid turnover of Mcl-1 couples translation to cell survival and apoptosis. *J Biol Chem* 282: 6192–200.
- Albamonte MI, Albamonte MS, Stella I, Zuccardi L, Vitullo AD (2013) The infant and pubertal human ovary: Balbiani's body-associated VASA expression, immunohistochemical detection of apoptosis-related BCL2 and BAX proteins, and DNA fragmentation. *Hum Reprod* 28: 698–706.
- Astarita G, Astarita G, McKenzie JH, Wang B, Strassburg K, Doneanu A, Johnson J, Baker A, Hankemeier T, Murphy J, Vreeken RJ, Langridge J, Kang JX (2014) A protective lipidomic biosignature associated with a balanced omega-6/omega-3 ratio in fat-1 transgenic mice. *PLoS ONE* 9: e96221.
- Bergmann A, Agapite J, Steller H (1998) Mechanisms and control of programmed cell death in invertebrates. *Oncogene* 17: 3215–23.
- Chen Y, Mei M, Zhang P, Ma K, Song G, Ma X, Zhao T, Tang B, Ouyang H, Li G, Li Z (2013a) The generation of transgenic mice with fat1 and fad2 genes that have their own polyunsaturated fatty acid biosynthetic pathway. *Cell Physiol Biochem* 32: 523–32.
- Chen TY, Zhang ZM, Zheng XC, Wang L, Huang MJ, Qin S, Chen J, Lai PL, Yang CL, Liu J, Dai YF, Jin DD, Bai XC (2013b) Endogenous n-3 polyunsaturated fatty acids PUFAs mitigate ovariectomy-induced bone loss by attenuating bone marrow adipogenesis in FAT1 transgenic mice. *Drug Des Dev Ther* 7: 545–52.
- Chen C, Zhang X, Zhang S, Zhu S, Xu J, Zheng Y, Han J, Zeng J-Z, Yan X (2015) Quantification of protein copy number in single mitochondria: the Bcl-2 family proteins. *Biosens Bioelectron* 74: 476–82.
- Cheng G, Fu C, Wang H, Adoligbe C, Wei S, Li S, Jiang B, Wang H, Zan L (2015) Production of transgenic beef cattle rich in n-3 PUFAs by somatic cell nuclear transfer. *Biotechnol Lett* 37: 1565–71.
- Durdu S, Deniz GC, Balci D, Zaim C, Dogan A, Can A, Akcali KC, Akar AR (2012) Apoptotic vascular smooth muscle cell depletion via BCL2 family of proteins in human ascending aortic aneurysm and dissection. *Cardiovasc Ther* 30: 308–16.
- Evan GI, Vousden KH (2001) Proliferation, cell cycle and apoptosis in cancer. *Nature* 411: 342–8.
- Fan Y, Ren C, Wang Z, Jia R, Wang D, Zhang Y, Zhang G, Wan Y, Huang M, Wang F (2016) Transgenesis of humanized fat1 promotes n-3 polyunsaturated fatty acid synthesis and expression of genes involved in lipid metabolism in goat cells. *Gene* 576: 249–55.
- Feng WG, Sui HS, Han ZB, Chang ZL, Zhou P, Liu DJ, Bao S, Tan JH (2007) Effects of follicular atresia and size on the developmental competence of bovine oocytes: a study using the well-in-drop culture system. *Theriogenology* 67: 1339–50.
- Fuchs Y, Steller H (2011) Programmed cell death in animal development and disease. *Cell* 147: 742–58.
- Fuko M, Inoue N, Manabe N, Ohkura S (2012) Follicular growth and atresia in mammalian ovaries_ regulation by survival and death of granulosa cells. *J Reprod Dev* 58: 44–50.
- Hanafy SM, Shehata OH, Farahat NM (2010) Expression of apoptotic markers BCL-2 and bax in chronic hepatitis C virus patients. *Clin Biochem* 43: 1112–7.
- Hengartner MO (2000) The biochemistry of apoptosis. *Nature* 407: 770–6.
- Katoh M (2012) Function and cancer genomics of FAT family genes (review). *Int J Oncol* 41: 1913–8.
- Katoh Y, Katoh M (2006) Comparative integromics on FAT1, FAT2, FAT3 and FAT4. *Int J Mol Med* 18: 523–8.
- Kranz D, Boutros M (2014) A synthetic lethal screen identifies FAT1 as an antagonist of caspase-8 in extrinsic apoptosis. *EMBO J* 33: 181–97.
- Miyazaki T (2011) Human FAT1 cadherin controls cell migration and invasion of oral squamous cell carcinoma through the localization of β -catenin. *Oncol Rep* 26(3): 587–92.

- Morris LG, Kaufman AM, Gong Y, Ramaswami D, Walsh LA, Turcan S, Eng S, Kannan K, Zou Y, Peng L, Banuchi VE, Paty P, Zeng Z, Vakiani E, Solit D, Singh B, Ganly I, Liao L, Cloughesy TC, Mischel PS, Mellinghoff IK, Chan TA (2013a) Recurrent somatic mutation of FAT1 in multiple human cancers leads to aberrant Wnt activation. *Nat Genet* 45: 253–61.
- Morris LG, Ramaswami D, Chan TA (2013b) The fat epidemic A gene family frequently mutated across multiple human cancer types. *Cell Cycle* 12: 1011–2.
- Otterson MF, Nie L, Schmidt JL, Link BJ, Jovanovic N, Lyros O, Rafiee P (2012) EUK-207 protects human intestinal microvascular endothelial cells (HIMEC) against irradiation-induced apoptosis through the Bcl2 pathway. *Life Sci* 91: 771–82.
- Ricky W, Johnstone AAR, Lowe SW (2002) Apoptosis a link between cancer genetics and chemotherapy. *Cell* 108: 12.
- Sadeqzadeh E, de Bock CE, O'Donnell MR, Timofeeva A, Burns GF, Thorne RF (2014) FAT1 cadherin is multiply phosphorylated on its ectodomain but phosphorylation is not catalysed by the four-jointed homologue. *FEBS Lett* 588: 3511–7.
- Sadeqzadeh E, de Bock CE, Zhang XD, Shipman KL, Scott NM, Song C, Yeadon T, Oliveira CS, Jin B, Hersey P, Boyd AW, Burns GF, Thorne RF (2011) Dual processing of FAT1 cadherin protein by human melanoma cells generates distinct protein products. *J Biol Chem* 286: 28181–91.
- Sopko R, McNeill H (2009) The skinny on fat: an enormous cadherin that regulates cell adhesion, tissue growth, and planar cell polarity. *Curr Opin Cell Biol* 21: 717–23.
- Sudiman J, Sutton-McDowall ML, Ritter LJ, White MA, Mottershead DG, Thompson JG, Gilchrist RB (2014) Bone morphogenetic protein 15 in the pro-mature complex form enhances bovine oocyte developmental competence. *PLoS ONE* 9: e103563.
- Sun S-C, Liu S, Jiang L, Zhong T, Kong S, Zheng R, Kong F, Zhang C, Zhang L, An L (2015) Effect of acrylamide on oocyte nuclear maturation and cumulus cells apoptosis in mouse in vitro. *PLoS ONE* 10: e0135818.
- Tanoue T, Takeichi M (2005) New insights into fat cadherins. *J Cell Sci* 118: 2347–53.
- Tepass U (1999) Genetic analysis of cadherin function in animal morphogenesis. *Curr Opin Cell Biol* 11: 540–8.
- Tiwari M, Prasad S, Tripathi A, Pandey AN, Ali I, Singh AK, Shrivastav TG, Chaube SK (2015) Apoptosis in mammalian oocytes: a review. *Apoptosis* 20: 1019–25.
- Uyar A, Torrealday S, Seli E (2013) Cumulus and granulosa cell markers of oocyte and embryo quality. *Fertil Steril* 99: 979–97.
- Valletta D, Czech B, Thasler WE, Müller M, Bosserhoff A-K, Hellerbrand C (2012) Expression and function of the atypical cadherin FAT1 in chronic liver disease. *Biochem Biophys Res Commun* 426: 404–8.
- Yagi T, Takeichi M (2000) Cadherin superfamily genes: functions, genomic organization, and neurologic diversity. *Genes Dev* 14: 1169–80.
- Zhai B, Liu H, Li X, Dai L, Gao Y, Li C, Zhang L, Ding Y, Yu X, Zhang J (2013) BMP15 prevents cumulus cell apoptosis through CCL2 and FBN1 in porcine ovaries. *Cell Physiol Biochem* 32: 264–78.

Received 21 June 2016; accepted 15 October 2016.
Final version published online 27 November 2016.