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—Full Paper—

Changes in Expression and Localization of X-linked Inhibitor of Apoptosis Protein (XIAP) in Follicular Granulosa Cells During Atresia in Porcine Ovaries

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Abstract. Follicular selection predominantly depends on granulosa cell apoptosis in porcine ovaries, but the molecular mechanisms regulating the induction of apoptosis in granulosa cells during follicular selection remain incompletely understood. To determine the role of X-linked inhibitor of apoptosis protein (XIAP), which suppresses caspase-3, -7 and -9 activities and acts as an endogenous inhibitor of apoptotic cell death, in the regulation of granulosa cell apoptosis during follicular atresia, we examined the changes in the expression level and localization of XIAP mRNA and protein in granulosa cells during follicular atresia using reverse transcription-polymerase chain reaction (RT-PCR), *in situ* hybridization, Western blotting and immunohistochemistry, respectively. High levels of XIAP mRNA and protein were noted in the granulosa cells of healthy follicles, and decreased levels were noted during follicular atresia. *In situ* hybridization and immunohistochemistry demonstrated that XIAP mRNA and protein were strongly expressed in the granulosa cells of healthy follicles, but negative/trace stainings were noted in those of atretic follicles. The present findings strongly indicate that XIAP is a candidate molecule which acts as an anti-apoptotic/pro-survival factor by inhibiting intracellular apoptosis signaling and is involved in the regulation of apoptosis in porcine granulosa cells.
Key words: Apoptosis, Caspase, Granulosa cell, Pig ovary, X-linked inhibitor of apoptotsis protein (XIAP)

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In mammalian ovaries, more than 99% of follicles disappear during follicular growth and development, and this appears to be primarily due to apoptosis of follicular granulosa cells, as the biochemical and morphological characteristics of apoptosis have been observed in the granulosa cells of atretic follicles [1–3]. Apoptotic stimuli and intracellular apoptosis-signal transduction pathways in granulosa cells have not yet been determined, and many researchers have attempted to confirm the primary trigger of apoptosis and how the intracellular apoptotic signal is transmitted in granulosa cells [4–7]. Many apoptosis-related factors are implicated in follicular atresia, including cell death ligands and receptors, intracellular pro- and anti-apoptotic molecules, cytokines and growth factors. In particular, cell death ligand-receptor signaling has been revealed to be the dominant regulatory system for apoptosis in granulosa cells [4–7]. To date, two major intracellular-signal pathways in cell-death ligand and receptor-dependent apoptosis (type I and type II: mitochondrion-independent and -dependent pathways, respectively) have been found [8–12]. In type I and II apoptotic cells, apoptotic signaling is initially triggered by members of the cell death ligand and receptor superfamily [Fas ligand (FasL) and Fas (also called Apo-1, CD95 or TNFRsf6), tumor necrosis factor (TNF) α and its receptors (TNFRs), TNF-related apoptosis-inducing ligand (TRAIL; also called Apo2L) and its receptors (TRAILRs), etc.]. These cell death ligands are grouped in to the TNF family and synthesized as type-II membrane proteins. Cell death receptors are classified as type-I membrane proteins and are part of the TNF receptor superfamily. They have an intracellular death domain (DD), which is essential for the induction of apoptosis. First, the ligands are trimerized and bind to the extracellular domain of trimerized cell death receptors, each of which contains an intracellular DD. The DD of the death receptor binds with the DD of the adaptor proteins (TNF receptor 1-associated death domain protein [TRADD], Fas-associated death domain protein [FADD], etc.) through homophilic interaction. An initiator caspase (procaspase-8; also called FLICE) binds to FADD through homophilic interaction with the death effector domain (DED, the resulting complex is called the death-inducing signaling complex (DISC). Dimerization of procaspase-8 induces auto-proteolytic cleavage and activation. In Type I apoptotic cells, caspase-8 directly activates the effector enzyme, i.e., caspase-3. In Type II apoptotic cells, however, activated caspase-8 cleaves Bid, and then the truncated Bid releases cytochorome c from the mitochondrion, which results in interaction of procaspase-9 with apoptotic protease-activating factor 1 (Apaf1) [13, 14]. The activated caspase-9 then cleaves procaspase-3. Finally, caspase-3 activates endogenous endonuclease (caspase activated DNAse: CAD), resulting in apoptosis.

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In pig ovaries, procaspase-8 protein is expressed in granulosa cells, and cleaved procaspase-8, the active form, is detected in atretic follicles [15–17]. TRADD is not detected in the granulosa cells of healthy follicles, but it is highly expressed in atretic follicles [18]. Another adaptor protein, FADD, is also present in pig granulosa cells [17]. Moreover, the caspase-9 precursor (procaspase-9) decreases, while caspase-9 activity increases as follicular atresia progresses [19]. Apaf1, the activator of caspase-9, is present in the granulosa cells of pig ovaries. These observations confirm that the porcine granulosa cell is a Type II apoptotic cell whose death signal is mediated by mitochondria and suggest that apoptosis and cell proliferation/survival in the granulosa cells of porcine follicles are regulated by TNF and TNFR family proteins [4, 5].

Inhibitor of apoptosis (IAP) gene products which play an evolutionarily conserved role in regulating programmed cell death in diverse species ranging from insects to humans prevent apoptosis by acting as endogenous suppressors of caspase activity [20]. IAPs were first identified in baculoviruses, where they act to keep the host cell alive [21, 22]. The most thoroughly characterized member of the IAP family is X-linked inhibitor of apoptosis (XIAP; also known as MIHA and ILA) [20–25]. XIAP is the most potent endogenous inhibitor of caspases and binds with caspase-9, -3 and -7 with high affinity. Thus, XIAP acts as an inhibitor of apoptosis downstream in the intracellular signaling pathway. In rats, XIAP suppresses granulosa cell apoptosis *in vitro* and *in vivo* [26, 27]. We have reported that protein in granulosa cells, cellular FLICE-like apoptosis inhibiting protein (cFLIP), which has two DEDs and homophilically binds with caspase-8 and/or FADD through their DEDs, acts as an inhibitor of apoptosis upstream in the intracellular signaling pathway [28–32]. To date, there is no information available concerning the roles of the downstream inhibitor, XIAP, in porcine granulosa cell apoptosis. In the present study, to reveal the role of XIAP in the regulation of granulosa cell apoptosis and in follicular selection in porcine ovaries, we examined the changes in the expression levels and localization of XIAP mRNA and protein in granulosa cells during follicular atresia by reverse transcription-polymerase chain reaction (RT-PCR) analysis, *in situ* hybridization, Western blotting analysis and immunohistochemical staining, respectively.

Materials and Methods

Preparation of granulosa cells

As previously reported [31–34], ovaries were obtained from mature sows weighing more than 120 kg at a local slaughterhouse (Kasama, Japan), and each experiment was performed within 1 h after butcher collection. Each follicle, 3–5mm in diameter, was dissected free from extraneous tissue and opened using forceps under a surgical dissecting microscope (SZ40; Olympus, Tokyo, Japan) to obtain the granulosa cells. Granulosa cells were classified as morphologically healthy, early atretic and advanced atretic by the absence or presence of cellular debris in their follicles. To retrospectively confirm the follicle classification, follicular fluid from each follicle was collected and separated by centrifugation at 3,000 rpm (500 g) for 10 min at 4 C. The estradiol-17β (E2) and progesterone (P4) levels were measured using [^{125}I]-RIA kits (Bio-Mesieux, Marcy-1'Etolle, France). Follices with a P4/E2 ratio of less than 15 were classified as healthy according to previous findings [35]. The oocyte-cumulus cell complex in each follicle was removed. The granulosa cells were collected and washed 3 times with phosphate-buffered saline (PBS; pH 7.2) by centrifugation at 3,000 rpm (500 g) for 5 min at room temperature (20–25 C) to separate them from the follicular fluid.

RNA isolation and reverse transcription (RT)-polymerase chain reaction (PCR)

As previously reported [32], total RNA of granulosa cells was extracted using a RNeasy mini kit (Qiagen, Chatsworth, CA, USA) and was then reverse-transcribed using a T-primed first-strand kit (Amersham Pharmacia, Piscataway, NJ, USA) to synthesize cDNA. PCR was performed to determine the expression levels of *XIAP* mRNA in the granulosa cells. Briefly, 1 μl (100 ng) of each cDNA mixture was mixed with 45 μl of Platinum PCR SuperMix (Invitrogen, Carlsbad, CA, USA) and 2 μl (0.2 μM) of each primer (forward primer of 5'-TGT CCG ATG TGC AAC ACA GT -3', reverse primer of 5'-CCA TGT CAG TAC ATG TAG GC -3' and expected PCR product size of 654 bp). *Glyceraldehyde-3phosphate dehydrogenase* (*G3PDH*; used as an intrinsic control) mRNA was amplified using the following primers (forward primer of 5'-GGA CTC ATG ACC ACG GTC CA T-3' and reverse primer of 5'-TCA GAT CCA CAA CCG ACA CG T-3' and expected PCR product size of 220 bp). The mixture was subjected to PCR in a thermal cycler (GeneAmp PCR Systems 9700; PE Applied Biosystems, Foster City, CA, USA), and then a hot-start PCR cycle was performed as follows: 5 min at 94 C; 35 cycles of 30 sec at 94 C, 30 sec at 55 C and 30 sec at 72 C; and then a final extension period of 7 min at 72 C. Each PCR product was electrophoresed in 2% (w/v) agarose gels (Sigma-Aldrich Chemicals, St. Louis, MO, USA) and stained with ethidium bromide (Wako Pure Chemicals, Osaka, Japan). A ready-load 100-bp DNA ladder marker (Gibco BRL, Grand Island, NY, USA) was used as a molecular weight marker for electrophoresis. After electrophoresis, the stained gels were recorded with a digital fluorescencerecorder (LAS-1000; Fuji Film, Tokyo, Japan), and then the intensity of each mRNA band was quantified using the ImageGauge software (Fuji Film) on a Macintosh computer. The relative abundance of specific mRNA was normalized to the relative abundance of *G3PDH* mRNA. To confirm the expression of porcine *XIAP* mRNA, the DNA sequence of the PCR product was determined using an automatic DNA sequencer (ABI prism 310; PE Applied Biosystems).

In situ hybridization

As previously reported [32], to visualize the localization of *XIAP* mRNA by *in situ* hybridization, digoxigenin (DIG)-labeled antisense and sense cRNA probes for *XIAP* mRNA were synthesized using a Lig'n scribe (Ambion, Austin, TX, USA) and DIG RNA labeling kits (Roche Diagnostics, Rotkreuz, Switzerland) according to the manufacturers' instructions. Briefly, the PCR products described above were added to the T7 phage RNA polymerase promoter by ligation to generate antisense and sense RNA

probe templates. This was followed by a PCR using promoter-specific primers and a target gene-specific 5'-primer and 3'-primer, respectively. The antisense and sense probe templates were transcribed, and then DIG was attached.

The ovaries obtained from mature sows as described above were fixed with 20% (v/v) buffered (pH 7.4) formalin (Wako), dehydrated and embedded in paraffin (Histosec; Merck, Darmstadt, Germany). Serial sections 3-μm thick were mounted on glass slides precoated with 3-aminopropyltriethoxysilane (Sigma), deparaffined, rehydrated and washed well in diethyl pyrocarbonate (DEPC; Sigma)-treated water. They were then treated with 0.2 N HCl for 10 min at room temperature, washed with PBS and digested with proteinase K (10 μg/ml; Roche) in PBS for 25 min at 45 C. Subsequently, they were post-fixed with 10% (v/v) buffered (pH 7.4) formalin, immersed in 2 mg/ml of glycine -PBS (Wako) for 10 min twice, washed well with PBS and prehybridized with hybridization cocktails (Amresco, Solon, OH, USA) for 1 h at 25 C. The slides were hybridized with the sense- or antisense-DIG-XIAP cRNA probe for 18 h at 45 C. Each probe (1 μg/ml) was diluted with hybridization cocktail. They were then washed well with 2 × SSC (Invitrogen) containing 50% (v/v) formamide (Invitrogen) for 1 h, 0.5 × SSC containing 0.075% (v/v) Brij 35 (Sigma) for 1 h and 0.2 × SSC containing 0.075% (v/v) Brij 35 for 30 min at 45 C and equilibrated with 100 mM Tris-HCl (pH 7.5) containing 150 mM NaCl (THS). Next, they were treated with blocking solution (Roche) for 1 h at room temperature, incubated with alkaline phosphatase (AP)-conjugated sheep anti-DIG antibody (diluted 1:500 with blocking solution; Roche) for 18 h at 4 C, washed with THS and rinsed with 100 mM Tris-HCl (pH 9.5), 100 mM NaCl and 100 mM $MgCl_2$ (THSM). Subsequently, they were incubated with THSM containing 0.4 mM nitroblue tetrazolium chloride (Sigma), 0.4 mM 5-bromo-4-chloro-3-indolyl-phosphate-4-toluidine salt (Sigma) and 1 mM levamisole (Sigma) for 90 min at 25 C. The sections were immersed in PBS, mounted with Histofine (Nichirei, Tokyo, Japan) and examined with a light microscope (BX51; Olympus). DEPC-treated water was used throughout the *in situ* hybridization staining process. The specificity of the hybridization signal obtained was confirmed by parallel incubation with antisense and sense cRNA probes. As further negative controls, serial sections were hybridized without any probes or were incubated without anti-DIG antibody. In the present examination, all controls yielded completely negative results.

Protein extraction and Western blotting

As previously reported [32], granulosa cells were prepared from healthy, early atreric and progressed atertic follicles and washed twice with cold PBS. They were then added to 50 μl of lysis buffer [10 mM Tris-HCl (pH 7.8), 1% (v/v) NP40 (Sigma), 150 mM NaCl, 1 mM EDTA and 10 μg/ml aprotinin (Roche)]. After mixing by vortex for 15 min at 4 C and centrifuging at 15,000 g for 15 min at 4 C, the supernatant was collected. The protein concentration in the supernatant was determined using a Bradford assay kit (Bio-Rad Laboratories, Melville, NY, USA) according to the manufacturer's protocol. For Western blotting, the protein fraction (20 μg/lane) prepared from each sample was separated by 10–20% (w/v) gradient sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and then transferred to a PVDF membrane (Millipore, Bedford, MA, USA). The membrane was immersed in blocking solution [600 μl of 1 M Tris-HCl (pH 8, 2), 2 ml of 3 M NaCl, 60 μl of Tween 20 (Sigma) and 1.5 g of BSA (Sigma) for 60 ml] and incubated with goat anti-XIAP polyclonal antibody (0.5 μg/ml; R&D systems, Oxon, UK) for 18 h at 4 C. After washing with wash buffer [10 ml of 1 M Tris-HCl (pH 7.5), 33.3 ml of 3 M NaCl and 1 ml of Tween 20 for 1,000 ml], the membrane was incubated with horseradish peroxidase (HRP)-conjugated anti-goat IgG antibody (1:2,000; Dako, Glostrup, Denmark) for 1 h at room temperature and washed with wash buffer. Chemiluminescence was visualized using an ECL system (Amersham Pharmacia Biotech) according to the manufacturer's protocol and was recorded with a digital recorder. Protein expression levels were quantified using ImageGauge on a Macintosh computer.

Histochemistry

As previously reported [32], to detect apoptotic cells, ovarian sections were stained by the terminal deoxynucleotidyl transferase-mediated biotinylated deoxyuridine triphosphate nick end-labeling (TUNEL) method using a commercial kit (Apop-Tag; InterGen, Manhattanville, NY, USA) according to the manufacturer's directions. Briefly, the sections were pretreated with 20 μg/ml proteinase-K (Sigma) for 15 min at room temperature and immersed in 3% (v/v) H_2O_2 in methanol for 5 min to inhibit endogenous peroxidase activity. They were then incubated with TdT solution containing 45 μM ddATP and 5 μM digoxigenin (DIG)-ddUTP for 1 h at 37 C. After being incubated with peroxidase-labeled anti-DIG antibody solution for 30 min at room temperature, the sections were incubated with dimethylaminoazobenzene (DAB) solution (Dako) for 1 min at room temperature, counterstained with methylgreen, dehydrated, mounted with Entellan (Merck) and examined by light microscope.

To visualize the localization of XIAP protein, serial sections were incubated with normal rabbit serum (Sigma) to block nonspecific protein binding and were then incubated with goat polyclonal anti-XIAP antibody (5 μg/ml; R&D systems) for 1 h at room temperature. They were subsequently washed with PBS and incubated with biotinylated anti-goat IgG antibody (1:200 in PBS; Vector Laboratories, Burlingame, CA, USA) for 30 min at room temperature. After being incubated with avidin biotin complex (ABC kit; Vector) for 1 h at room temperature, the sections were incubated with DAB solution, washed with distilled water, counterstained with methylgreen, dehydrated, mounted with Entellan and examined by light microscope.

Statistical analysis

All procedures, including the isolation of follicles and preparation of granulosa cells, were repeated three times with separate groups (nine sows/group) for independent observation. Before ANOVA, the homogeneity of variance was assessed using the StatView 4.5 software (Abacus Concepts, Berkely, CA, USA) on a Macintosh computer. ANOVA was performed with Fisher's least significant differences test for biochemical data, and Wilcoxon's signed-rank test was performed for histological estimation using the StatView 4.5 software. Differences with $P<0.05$ were consid-

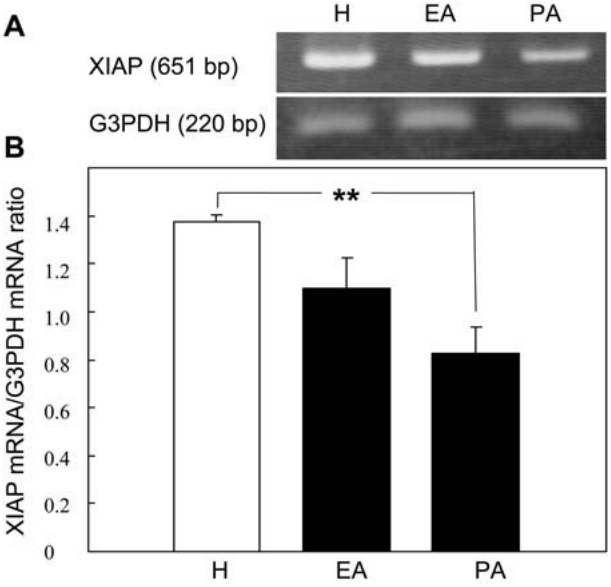

Fig. 1. Changes in the expression of *XIAP* mRNA in granulosa cells during follicular atresia as determined by RT-PCR. A: Representative photographs of RT-PCR products for *XIAP* and *G3PDH* (as an internal control) mRNAs in granulosa cells prepared from healthy (H), early atretic (EA) and progressed atretic (PA) follicles. B: Porcine *XIAP* mRNA level (*XIAP* mRNA/*G3PDH* mRNA ratio) in each follicle. All data are shown as means ± SD. The asterisks indicate a significant difference *vs.* a healthy sample (P<0.01).

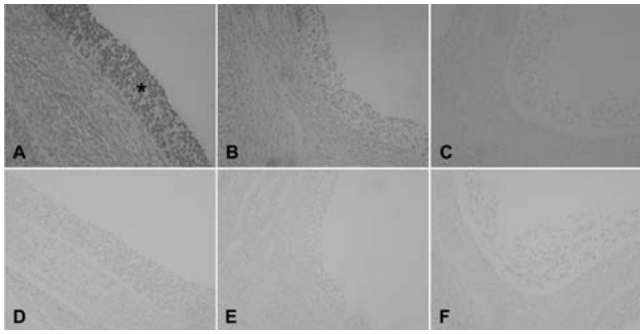

Fig. 2. Representative photographs of *in situ* hybridization for *XIAP* mRNA. Pig ovarian sections from healthy (A and D), early atretic (B and E) and progressed atretic (C and F) follicles were hybridized with an antisense cRNA probe (A, B and C). Strong positive staining for *XIAP* mRNA was detected in granulosa cells in the granulosa layers (asterisk) of healthy follicles. Decreases in *XIAP* mRNA expression were seen in granulosa cells during follicular atresia. No positive staining was detected when sections were hybridized with a sense cRNA probe (D, E and F). Magnification: × 200.

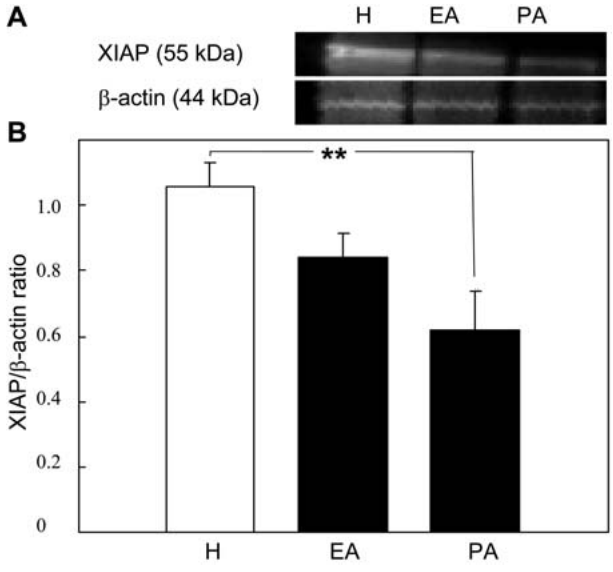

Fig. 3. Changes in the expression of XIAP protein in granulosa cells during follicular atresia as determined by Western blotting. A: Representative photographs of Western blotting for XIAP and β-actin (as an internal control) in granulosa cells prepared from healthy (H), early atretic (EA) and progressed atretic (PA) follicles. B: Porcine XIAP protein level (XIAP protein/ β-actin ratio) in each follicle. All data are shown as means ± SD. The asterisks indicate a significant difference *vs.* a healthy sample (P<0.01).

ered significant.

Results

Changes in the expression levels and localization of XIAP mRNA in follicles

XIAP mRNA was detected in granulosa cells prepared from healthy, early atretic and progressed atretic follicles by RT-PCR (Fig. 1A). The *XIAP* mRNA levels of the granulosa cells as follicular atresia progressed (P<0.01; Fig. 1B).

By *in situ* hybridization, obvious positive reactions for *XIAP* mRNA were detected in the granulosa cell layers of healthy follicles (Fig. 2A). Weaker reactions were observed in those of early atretic follicles (Fig. 2B), and trace reactions were seen in those of progressed atretic follicles (Fig. 2C). When ovarian tissue sections were incubated with the sense cRNA probe for *XIAP* mRNA, no positive staining was seen (Fig. 2D, E and F).

Changes in the expression levels and localization of XIAP protein in follicles

By Western blotting, a strong immunoreaction for XIAP protein at 55 kDa was demonstrated in the granulosa cells of healthy follicles (Fig. 3A). Decreased expression levels were seen in early atretic follicles, and significantly decreased expression levels were noted in progressed atretic follicles (P<0.01; Fig. 3B).

Strong immunohistochemical reactions for XIAP protein were seen in the granulosa layers of healthy follicles (Fig. 4A). Staining intensity decreased with the progression of follicular atresia (Fig. 4B and C). In the granulosa layer, internal theca layer or external theca layer of healthy follicles, no cells with positive TUNEL staining (apoptotic cells) were found (Fig. 4D). At the early stage of

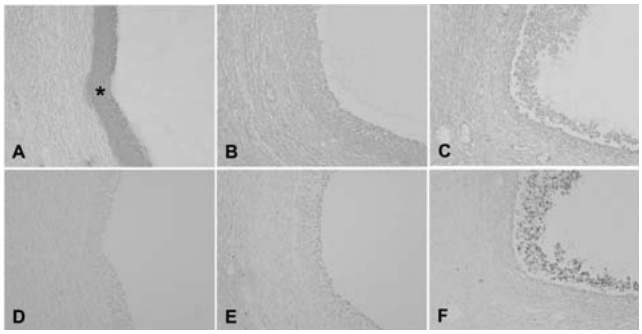

Fig. 4. Representative photographs of immunohistochemistry for XIAP protein (A, B and C) and of TUNEL staining to detect apoptotic cells (D, E and F). Pig ovarian sections from healthy (A and D), early atretic (B and E) and progressed atretic (C and F) follicles. Strong positive staining for XIAP was detected in granulosa cells in the granulosa layer (asterisk) of healthy follicles. Decreases in XIAP expression were seen in granulosa cells during follicular atresia. Magnification: × 200.

follicular atresia, apoptosis occured in the granulosa cells located on the inner surface of the granulosa layers of follicular walls (Fig. 4E), and most granulosa cells showed positive reactions for TUNEL staining in progressed atretic follicles (Fig. 4F).

Discussion

As mentioned above, our recent findings have indicated that the granulosa cells of porcine ovarian follicles are type II apoptotic cells, in which the intracellular apoptotic signal is induced through mitochondria [19], and that the mitochondrial signal pathway, which is predominantly triggered by Apaf-1 and caspase-9, plays a crucial role in determining the fate of granulosa cells during follicular atresia. XIAP, the most potent endogenous inhibitor of caspase-9, -3 and -7 [23–25], has been detected in the granulosa cells of rat ovaries [26]. Based on these results, we hypothesized that XIAP acts as an anti-apoptotic/pro-survival factor by inhibiting caspase-9 and -3 and plays a crucial role in regulating the occurrence of apoptosis in porcine granulosa cells during follicular atresia. In the present study, to confirm the contribution of XIAP to follicular selection in porcine ovaries, we examined the changes in expression and localization of the mRNA and protein of XIAP in granulosa cells during follicular atresia. Both the mRNA and protein of XIAP were detected in granulosa cells prepared from healthy, early atretic and progressed atretic follicles by RT-PCR and Western blotting, respectively, and their levels decreased as atresia progressed. Moreover, the histochemical findings for the mRNA and protein of XIAP demonstrated by in situ hybridization and immunohistochemistry, respectively, affirmed these molecular biochemical findings. High levels of both mRNA and protein were demonstrated in the granulosa layers of healthy follicles, and decreased levels were noted in those of atretic follicles. These findings strongly suggest that follicles with high levels of XIAP in the granulosa layers remain alive, while follicles with low levels of XIAP or without XIAP undergo atresia in the ovaries of pigs which have a complete estrous cycle. These results are consistent with the findings in the ovaries of rats with an incomplete estrus cycle [26]. Thus, XIAP may play an important role in follicular survival by inhibiting the transduction of intracellular signal for apoptosis in type II apoptotic cells in mammals.

To date, six members of the IAP family [XIAP, human IAP-1 (HIAP-1 also called cIAP-2), human IAP-2 (HIAP-2 also called cIAP-1), neuronal apoptosis inhibitor protein (NAIP), survivin and livin (also known as KIAP)] have been identified in mammals [24, 36–40]. The IAP family members contain one to three baculoviral IAP repeat (BIR) domain(s) at the N-terminal. The BIR domain is essential for biological activity. XIAP contains three BIR domains and a really interesting new gene (RING) finger domain at the C-terminal, which is comprised of small protein motifs that bind one or more zinc atoms, contains multiple finger-like protrusions that make tandem contacts with the target molecule and has E3 ubiquitin ligase activity [20, 41]. The 3rd BIR domain (BIR3) of XIAP is responsible for inhibition of caspase-9, and the 2nd BIR domain (BIR2) is the dominant determinant for inhibition of caspase-3 and -7 [42–44]. XIAP inhibits activation of procaspase-9 by binding to it [45–47]. We presume that XIAP binds with procaspase-9 in granulosa cells of healthy follicles, and the granulosa cells continue to survive. When XIAP levels begin to decrease in granulosa cells, procaspase-9 is activated and cells die. Consequently, follicles begin undergoing atresia. Further study of the regulatory mechanism for XIAP expression in granulosa cells, in which apoptosis is mediated by a cell death ligand and receptor system, is needed. Because a recent study [48] showed that, in a type II apoptotic cell (the human T cell lymphoblast-like Jurkat cell) in which the release of cytochrome c and formation of the apoptosome (Apaf-1/caspase-9 complex) occurred when FasL and Fas-mediated apoptosis was induced, XIAP inhibits the activation of procaspase-3 but not procaspase-9. To confirm the interaction of XIAP with procaspase-3 and the procaspase-9 in granulosa cells of porcine ovaries, further studies are needed.

In conclusion, the present study demonstrated high levels of XIAP mRNA and protein in the granulosa cells of healthy follicles in porcine ovaries and decreases during the progression of atresia. XIAP acts as an anti-apoptotic/pro-survival factor by inhibiting procaspase-9's activation in the granulosa cell, a type II apoptotic cell.

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