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RESEARCH ARTICLE

Morphological and Hormonal Identification of Porcine Atretic Follicles and Relationship Analysis of Hormone Receptor Levels During Granulosa Cell Apoptosis *In vivo*

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Abstract

Recent reports have demonstrated that follicular atresia is initiated or caused by granulosa cell apoptosis followed by theca cell degeneration in mammalian ovaries, but the mechanism of follicular atresia is still to be elucidated. Therefore, our present study was designed to examine our hypothesis that the changes of follicular microenvironment induce the granulosa cell apoptosis during pocrine follicular atresia *in vivo*. We firstly isolated intact porcine antral follicles and identified them into three groups, healthy follicles (HF), early atretic follicles (EAF) and progressed atretic follicles (PAF) through morphology and histology. To further confirm their status, we detected hormone levels in follicular fluids and the expression level of apoptosis gene *Bax* in granulosa cells. The rate of progesterone (P) and estradiol (E2) was increased with the expression of *Bax*, indicating hormone can be used as a marker of granulosa cell apoptosis or follicular atresia. Finally, we analyzed the expression level of hormone receptor genes in granulosa cells and their relationship with follicular atresia. In PAF, the expression of Progesterone receptor (*PGR*) was increased significantly while estradiol receptor (*ER*) had no notable changes, which suggesting the increased-PGR accelerated the effect of P-stimulated granulosa cell apoptosis. The dramatic increasing of androgen receptor (*AR*) expression in PAF and the obvious increase of tumor necrosis factor-α receptor (*TNFR*) in EAF indicated that there are different pathways regulating granulosa cell apoptosis during follicular atresia. Together, our results suggested that different pathways of granulosa cell apoptosis was induced by changing the follicular microenvironment during follicular atresia.

Key words: granulosa cell apoptosis, follicular atresia, progesterone, estradiol, porcine

INTRODUCTION

Most follicles (99%) undergo atresia at various stages during follicular development, therefore it has great importance to the swine industry by understanding of the mechanism of regulation of follicular growth and regression (Kaipia and Hsueh 1997; Jiang *et al.* 2003). Some studies showed that follicular atresia is caused by granulosa cell apoptosis in mammalian ovaries (Manabe *et al.* 2004; Matsuda-Minehata *et al.* 2006, 2007), and species-specific differences have been noted in apoptotic cell localization of granulosa cells and theca internal cells during follicular atresia (Manabe *et al.* 2000; Tabarowski *et al.* 2005). All these indicated that the regulation mechanism of granulosa cell

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apoptosis is critical for follicular atresia and may be different among mammalian species.

The microenvironment of the antral follicle plays a critical role in the survival of granulosa cells and oocyte. The antral follicle represents a physiological unit of ovarian steroid genesis (Moor *et al*. 1998; Hussein 2005; Kayampilly *et al*. 2010) and can be used as an *in vitro* model to study the mechanism of follicular atresia (Lin and Rui 2010; Lin *et al*. 2010). Therefore, we isolated porcine atretic follicles to examine the follicular microenvironment and analyze the role of different factors especially follicular hormones and their receptors in granulosa cells during follicular atresia because the fate of follicles finally depends on a sophisticated balance between survival and atretogenic molecules.

On the other hand atretogenic, many cell death ligand-receptor systems were reported in granulosa cells of mammalian ovaries (Inoue *et al*. 2007; Lin and Rui 2010; Lin *et al*. 2010). Tumor necrosis factor α (TNFα) and its receptor (TNFR) is an important apoptotic signaling machinery in granulosa cells (Inoue *et al*. 2007; Lin and Rui 2010; Lin *et al*. 2010). To further understand its role in porcine atretic follicles, we examined the expression level of *TNFR* in granulosa cells during follicular atresia *in vivo*.

Some reports were involved in the mechanism of porcine follicular atresia and the role of granulosa cell apoptosis during follicular atresia *in vivo* (Lin *et al*. 2010b; Moosavifar *et al*. 2010; Sugimoto *et al*. 2010; Wang *et al*. 2010; Zhang *et al*. 2010a, b). In this study, we firstly isolated intact porcine antral follicles and their healthy status was identified through morphology and histology, then the concentration of follicular P and E2 and the expression of different hormone receptors and *TNFR* were investigated. Through relationship analysis, we got more important information about gene expression and granulosa cell apoptosis during porcine follicular atresia *in vivo*.

RESULTS

Morphological and histological features of isolated antral follicles

According to morphological and histological features, the health status of follicles was classified into healthy follicles (HF), early atretic follicles (EAF) and progressed atretic follicles (PAF) under a microscope (Jolly *et al*. 1997; Tabarowski *et al*. 2005; Lin *et al*. 2010). HF was characterized by a well-vascularized follicular wall (Fig. 1-A) and an intact, well-organized granulosa cell layer with few pyknotic cells (Fig. 1-D), while PAF with opaque surface of the follicle (Fig. 1-C) and disorganized granulosa cell layer (Fig. 1-F). However EAF were with few or no blood vessels (Fig. 1-B) and the granulosa cell layer became partially detached from the basement membrane (Fig. 1-E).

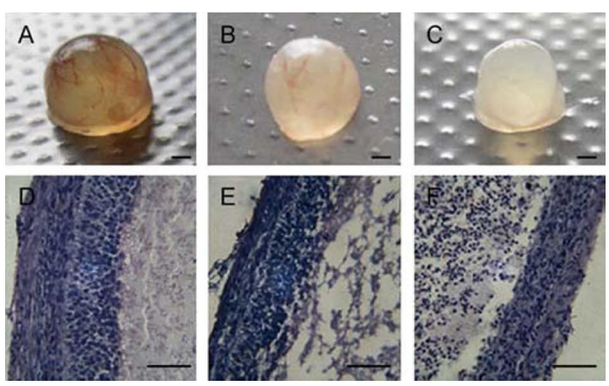

Fig. 1 Comparison of morphological characteristics and histological classification of isolated porcine antral follicles (400×). A and D, healthy follicle. B and E, early atretic follicle. C and F, progressed atretic follicle. Bar=100 μm.

Concentrations of progesterone and estradiol in follicular fluids

The healthy follicles were characterized by a well-vascularized follicular wall and an intact, well-organized granulosa cell layer, while opaque surface of the follicle and disorganized granulosa cell layer in progressed atretic follicles. Our results showed that progesterone (P) concentration increased in EAF and PAF compared with HF (Fig. 2-A), while estradiol (E2) decreased (Fig. 2-B) during follicular atresia. We further found the P/E2 rate in PAF was 20 folds than that in HF (Fig. 2-C), implying it can be used as a marker for the atretic status of antral follicles.

Bax expression in granulosa cells

We used *Bax* as apoptosis gene to indicate the