

Apoptosis

Apoptotic

The Immunologic Expression of Apoptosis Related Proteins and Apoptotic Cells in Human Ovarian Follicles

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Objective: To investigate the expression of apoptosis related proteins and apoptotic cells on the human ovarian follicles.

Materials and Methods: Thirty five Formalin-fixed paraffin-embedded human ovarian tissue blocks were selected from the surgical pathology files of the department of pathology, College of Medicine, Yonsei University, for the period from 1996 to 1998. All specimen were from premenopausal women aged from 32~45. Ovarian tissues were collected from the patients performing hysterectomy for benign uterine diseases. Immunohistochemical staining was performed for the detection of DNA fragmented cell, Bcl-2, Bax, Fas and Fas-ligand.

Results: Bcl-2 and bax were not expressed on the surrounding cells and oocyte of the primary, primordial and preantral follicles. Fas and Fas-ligand (Fas-L) were not expressed on the surrounding cells on the primordial and primary follicles. But expressed on the surrounding granulosa cells and oocyte in the primordial and primary follicles. In the healthy follicles, Bcl-2 was expressed on the granulosa cells, however, Bax was not expressed. DNA fragmented cells were expressed on the inner granulosa cell layer of atretic follicles.

Conclusion: Fas, Fas-ligand, and Bax may be responsible for the follicular atresia and Bcl-2 may be involved in the follicular survival in the human ovary.

Key Words: Apoptosis, Bcl-2, Bax, Fas, Fas-ligand, Human ovary

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Table 1. Immunologic expression of apoptosis related proteins and DNA fragmented cells in human ovarian follicles

	Bcl-2	Bax	Fas	Fas-ligand	DNA fragmented cells
Primordial follicle	-	-	+‡	+‡	-
Preantral follicle	-	-	+¶	+¶	-
Atretic follicle	-	+†	+	+	+**
Healthy follicle	+*	-	-	-	-

-; Negative staining, +; Positive staining, *; Positive stained on oocyte and granulosa cells, †; Positive stained on granulosa cells and theca cells, ‡; Positive stained on oocyte, ¶; Weakly stained on oocyte and granulosa cells, ||; Positive stained on granulosa cells and theca cells, **; Positive stained inner granulosa cells

가 dimer) Bcl-2 (cell death)
apoptosis¹²
Fas tumor necrosis factor receptor family¹³
가 anti-Fas
Fas-ligand
signal Hakuno¹⁴ Fas
Fas-ligand system
Funayama⁴ apoptosis가
Yuan & Giudice⁵ DNA frag-
mented cell antral follicle preovulatory follicle
가 apoptosis cell)
apoptosis¹⁵
apoptosis⁶
Bcl-2, Bax, Bcl-xlong, Bcl-xshort, P53 ligand apoptosis
Fas⁷⁻⁹
Bcl-2
Bcl-2 follicular lymphoma
t (14; 18) chromosomal translocation breakpoint
Ratts¹⁰ Bcl-2 knock- 35 1996
out mouse surgical pathology files
Fukaya¹¹ Bcl-2가 (formalin-fixed, paraffin-embedded tissue blocks)
(granulosa cell) 32~45
Bax 21-kDa Bcl-2
(heterodimer) Bax (homo- hematoxylin eosin

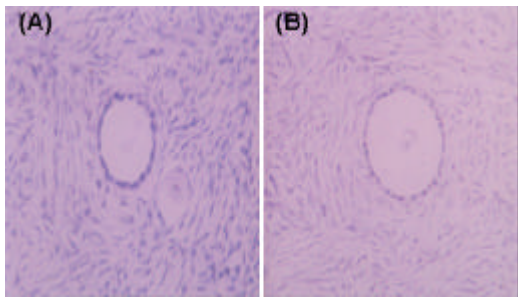


Figure 1. Immunostaining for (A) Bax, showing no reactivity in the primary and the primordial follicle, (B) Bcl-2, showing no reactivity in the primary follicle (original magnification $\times 400$).

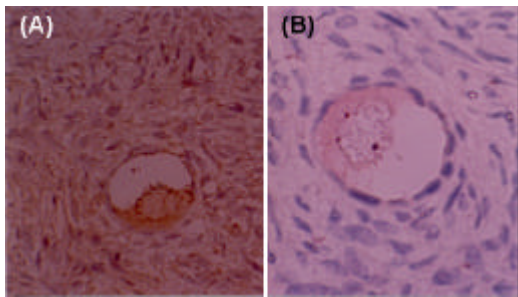


Figure 2. Immunostaining for (A) Fas, (B) Fas-ligand, showing no reactivity except on the oocyte in the primordial follicle (original magnification $\times 400$).

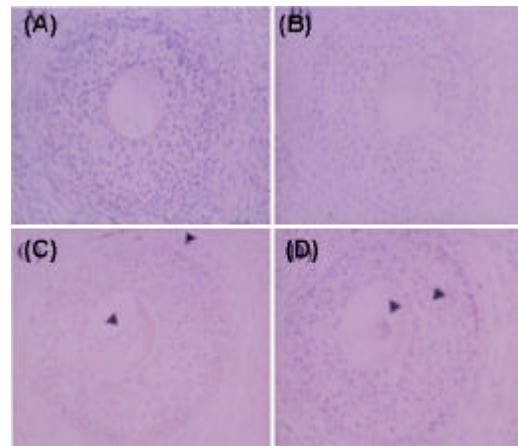


Figure 3. Immunostaining for (A) Bax, showing no reactivity, (B) Bcl-2, showing no reactivity, (C) Fas, weakly stained on the granulosa cells and oocyte, (D) Fas-ligand, faintly stained on the oocyte and some granulosa cells ($\times 400$).

2. DNA fragmented

DNA fragmented cell
DNA fragmented cell
fragmentation detection kit (Tdt Frag, Oncogene)

- 1)
- 2) permeabilization
- 3) Endogenous peroxidase
- 4) Equilibrium and labeling reaction
- 5) Termination of labeling reaction
- 6) DAB (Detection of DAB solution)
- 7) (Counterstaining)

1.

microwave-oven heating

avidin-biotin complex

Xylene dewax

rehydration

Endogenous peroxidase activity 3% hydrogen peroxide 10

incubation

Bcl-2 Bax

citrate buffer (0.01 MM, PH 6.0) immersion

Fas Fas-Ligand 750 W microwave oven 15 EDTA immersion

20 Bcl-2 monoclonal

(Dako) 1 : 80 , Bax polyclonal

(Pharminigen) 1 : 1000 , Fas monoclonal (Dako) 1 : 20, Fas-L polyclonal

(Santacruz) 1 : 100

Bcl-2 Bax 1 (primary follicle)

(primordial follicle)

(oocyte) (Figure 1), Fas

Fas-L (Figure 2).

Fas Fas-L 2 (preantral follicle)

Bcl-2 Bax 2

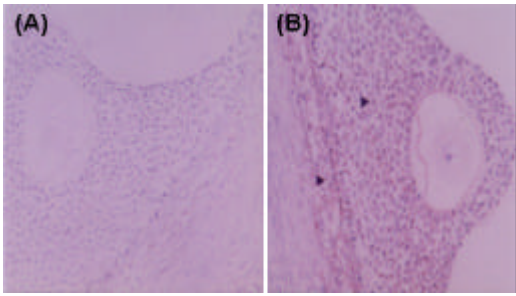


Figure 4. Immunostaining for (A) Bax, showing no reactivity, (B) Bcl-2, showing reactivity on the granulosa cells in the healthy follicle (×400).

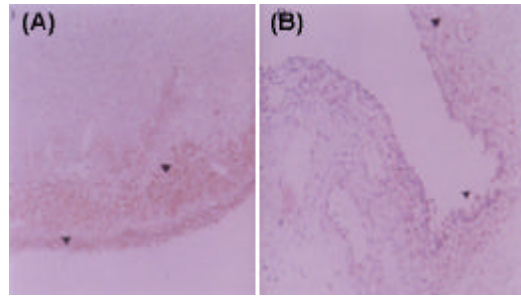


Figure 5. Immunostaining for (A) Fas, showing moderate reactivity on the granulosa cells and theca cells on the surrounding stroma, (B) Fas-ligand, showing weak reactivity on the granulosa cells and theca cells (×200).

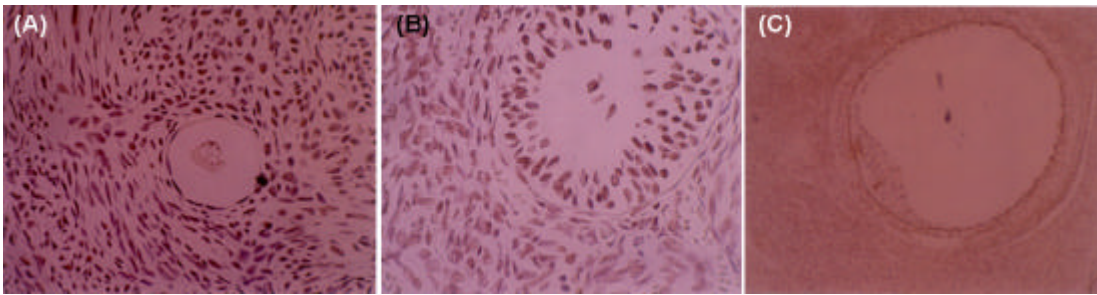


Figure 6. Immunostaining for DNA fragmented cells (A) in primordial follicle, showing no reactivity on the granulosa cells, oocyte and on the surrounding stroma, (B) in preantral follicle, showing no reactivity, (C) inner granulosa cells, showing reactivity (×100).

Fas Fas-L Table 1 .

가

(Figure 4). Bcl-2 apoptosis

(theca cell) Fas Fas-L, Bax

Bcl-2 .

(atretic follicle) Bax가

Bcl-2 가

. Fas , , ,

Fas-L apo-

(Figure 5). DNA fragmented (primordial follicle) 1 (primary follicle)

, 1 2 , Bcl-2 Bax가 . Fas

가 1

(Figure 6). Fas Fas-ligand가

2 (preantral follicle)
 Fas가
 , Fas
 Fas
 가 Bcl-2
 . Sakamaki¹⁶ Fas가
 (granulosa cell) (luteal cell)
 . Kondo⁷ Fas
 , Fas 2 apoptosis
 (preantral follicle) (granulosa cell) Fas apoptosis
 . Fas Fas-ligand, Bax
 Fas가 Fas가 Bcl-2
 Bcl-2 apoptosis
 가
 , Bax (theca cell)
 (granulosa cell) . Bcl-2
 (atretic process)
 가
 DNA fragmented
 가 가
 Bcl-2 가 가
 가 Bcl-2 Bax
 . Fas
 , Fas-ligand Fas
 Fas-ligand
 가 Fas
 Fas-ligand
 apoptosis , ,
 가 .
 , ,
 , I ,
 .¹⁷
 , re-
 active oxygen species,
 -6가 .¹⁷ , ,

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