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Expression of Glucosamine-6-Phosphate Deaminase (GNPDA) in Mouse Ovary

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생쥐 난소에서 Glucosamine-6-Phosphate Deaminase (GNPDA)의 발현

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ABSTRACT: The expression of glucosamine-6-phosphate deaminase (GNPDA) was examined in mouse ovary from neonate to adult. In western blot, band of Mr. 31 kDa antigen sharply increased 2 weeks after birth onward. In immunostaining of the adult ovary, GNPDA expression was constitutive in the theca and interstitial cells. However, expression in the granulosa cells was different according to folliculogenesis. Cytoplasm of the oocyte of some primary follicle showed positive signal but not in the antral follicle. Granulosa cells of antral follicles showed no visible sign of GNPDA expression. In the corpora lutea, the signal intensity in granulosaluteal cells increased according to luteal development and became the highest in the luteolytic phase. In summary, the differential expression of GNPDA was found in follicle cells according to folliculogenesis. It suggests that GNPDA might be involved in tissue remodeling in mouse ovary.

Key words: GNPDA, Ovary, Mouse.

요 약: 생쥐 난소에서 glucosamine-6-phosphate deaminase (GNPDA)의 발현을 조사하였다. Western blot 상에서 Mr. 31 kDa의 항원을 검출하였으며 출생 후 2주에 급격한 발현의 증가가 확인되었다. 난소절편의 면역염색 결과 협막세포와 간충조직에서는 균질한 발현을 보인 반면 난포내 발현양상은 난포의 발달에 따라 다르게 나타났다. 일부 1차 난포의 난자에서 GNPDA의 발현이 관찰되었으나 강소형성 난포의 난자에서는 관찰되지 않았다. 황체화 과립세포에서의 발현은 황체발달에 따라 증가하였으며 황체퇴화 부위에서 강한 신호가 검출되었다. 난포발달에 따른 GNPDA 발현의 차이는 GNPDA가 생쥐 난소 조직 재구성에 관여하고 있음을 암시한다.

INTRODUCTION

Follculogenesis preceeds the series of growing and maturation of follicles, and finally terminates by luteolysis under control of ovarian steroids and peptides acting along the brain-gonadal axis and several locally regulated factors (Spangelo et al., 1995; Knight, 1996; Apter, 1997). These series of events are good model for study of hormonal regulation of cell differentiation in tissue remodeling. Follicular growth, follicular maturation and atresia, ovulation and the nidation of the fertilized oocyte require intense tissue remodeling which can be accomplished only through the action of hydrolytic enzymes whose activities were higher in theca than in granulosa cells (Banos et al., 1996). During this process, there are large need for energy for transport and synthetic activity. Undoubtedly, hexosamine is an important source for energy production. The pathways of glucose utilization for energy production have been studied extensively. Little is known, however, about the reactions by which glucose is converted into complex carbohydrates and expression of a key enzyme of glucosamine catabolism in ovary. Glucosamine-6-phosphate deaminase (isomerase) (GNPDA) (EC 5.3.1.40) which catalyzes the reversible conversion of D-glucosamine 6-phosphate into D-fructose 6-phosphate is important for energy metabolism. In addition, hexosamine is important for construction of complex oligosaccharide chain as well as building of glycosaminoglycans (GAG) which are abundant in extracellular matrix (ECM) of different tissues. In ovary, hexosamine was found in ECM in follicular fluid (Bellin & Ax, 1987; Eriksen et al., 1999), granulosa cells (Yanagishita & Hascall, 1985),

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cumulus cell's matrix (Eppig, 1979; Nakayama et al., 1996), and egg's extracellular coats (Moller et al., 1990; Parillo et al., 1998; Parillo & Verini-Supplizi, 1999). Recently, a mammalian protein displaying similarity to bacterial GNPDA was purified from hamster (Parrington et al., 1996), human (Shevchenko et al., 1998) and beef kidney (Lara-Lemus & Calcagno, 1998). GNPDA was localized selectively to tissues with high energy requirements such as the apical zone of transporting epithelia of the kidney tubules and the small intestine, nerve terminals in the brain, and motile sperm (Wolosker et al., 1998). However, the expression of GNPDA during folliculogenesis was totally uncovered. Herein we examined the GNPDA expression in mouse ovary.

MATERIALS AND METHODS

1. Western Blot Analysis

Swiss albino mice (ICR strain) were reared in 12 h light and 12 h dark cycle. Ovaries from 1, 2, 3 and 4-weeks old mice, and day-3 pregnant mice were used. Ovaries were cleared from blood by blotting the filter paper. After washing in the PBS, ovaries were homogenized in PBS containing 1% NP-40 and protease inhibitor cocktail (completeTM, BMS). After centrifugation at 20,000 g for 10 min, supernatant was collected. After quantitation of protein content according to Bradford (1976), protein extarcts were mixed with sample lysis buffer (Laemmli, 1970) and boiled for 5 min at 100C. Proteins were subjected to SDS-PAGE in 10% polyacrylamide gel. After electrophoresis, proteins were electrotransferred to PVDF membrane. Immunodetection of GNPDA was done according to Towbin et al (1979). Membrane was blocked with 5% BSA in TBS. After brief washing twice with TBS, 1:1,000 diluent (0.5 μg/ml in 1% BSA in TBS) of rabbit anti mouse GNPDA peptide antiserum which raised against 12 amino acids of C-terminal peptide of GNPDA was applied to membrane and incubated for 1 hr at RT. After washing (3X each for 10 min) with TBS, anti rabbit IgG antibody conjugate with alkaline phosphatase (Promega) was applied and incubated for 1 hr at RT. After washing (3X each for 10 min) with TBS, blot was equilibrated with alkaline phosphatase buffer (0.1 M Tris, pH 9.5, 0.1 M NaCl, 5 mM $MgCl_2$) for 10 min. Alkaline phosphatase coloring reagent (Promega) was applied to blot and incubated until the band appeared.

2. Immunostaining

Ovary from adult mouse was fixed with 5% formaldehyde in PBS (pH 7.4) for 12 h and processed for paraffin section. After deparaffination with xylene, slide was incubated in PBS for 1 h at RT. Slide was blocked in 5% BSA in PBS. Anti GNPDA antiserum which raised against N-terminal 12 amino acid peptide of GNPDA (Gye et al., 2000) was diluted with 1:50 in 0.5% BSA in PBS and applied to tissue section and incubated for 1 h in humidified chamber at RT. In control experiment, non-immune rabbit serum was used. After washing with PBT (0.1% Triton X-100 in PBS) each for 10 min, goat anti rabbit IgG antibody conjugated with horseradish peroxidase (HRP, Promega) was applied and incubated for 30 min. After washing with PBT for 3 times, coloring reaction was done using diaminobezimidine (DAB) solution (DAKO, USA). After coloring, slides were rinsed in DW and processed for permanent mounting. Photographs were made with color reversal film (ASA 100) under Olympus BX50 microscope with differential interference optics.

RESULTS

In Western blot, band of Mr. 31 kDa antigen corresponding to mouse homologue of GNPDA increased 2 weeks after birth and became the highest in luteal phase ovary (Fig. 1). Immunostaining of GNPDA in ovary was different according to folliculogenesis (Table 1). Granulosa cells of primary follicles showed different staining intensity (arrows in Fig. 2A). Some of the follicles which have degenerative sign showed positive signal in the oocyte (arrowhead in Fig. 2A) and granulosa cells (box

Fig. 1. Western blot of GNPDA in mouse ovaries. NP-40 soluble fractions of ovary homogenates from 1, 2, 3, and 4 weeks-old mice and luteal phase ovary from adult (8 weeks) mice were resolved in SDS-PAGE. A band of Mr. 31 kDa antigen corresponding to mouse homologue of GNPDA increased 2 weeks after birth onward.

Table 1. Expression of GNPDA in mouse ovary.

Follicle stage	Cells				
	Theca	Granulosa	Interstitial	Oocyte	Remarks
Primary	NE	+/−	+++	+/−	Positive sign in the degenerative follicles
Growing	+	+/−	+++	+/−	"
Antral	++	−	+++	−	
Graafian	++	−	+++	−	
Early luteal	++	+/−	+++	NE	
Mid luteal	++	++	+++	NE	
Late luteal	++	+++	+++	NE	
Luteolytic	NE	++++	++++	NE	

Signal intensities are given as −, absent; +, weak; ++, moderate; +++, strong; ++++, very strong. NE, not examined.

in Fig. 2A). However, granulosa cells of antral follicle were free from staining (star in Fig. 2A, and arrowheads in Fig. 2B and C). Interstitial cells showed the strong signals regardless of follicles (asterisks in Fig. 2A). GNPDA was constitutively expressed in theca layers (arrows in Fig. 2B, C, and D). There was the spatially different signals among the luteal follicles (CLs in Fig. 2A, D, and E). The luteal cells in early luteal phase showed weak signal (box in Fig 2D) but signal intensity gradually increased according to the progression of the luteal phase (box in Fig 2E). Eventually the most intensive signal was found in the luteolytic site (box in Fig. 2F).

DISCUSSION

Most of lysosomal antigen mature through the secretion and subsequent endocytosis. N-acetylhexosamines of glycoproteins and glycosaminoglycans (GAGs) are recycled after lysosomal degradation (Rome & Hill, 1986). The lysosomal content increases in the granulosa-luteal cells of developing corpora lutea and there was a maximal increase in the number of lysosomes in the terminal phase of the cycle, resulting in final regression of corpora lutea (Gregoraszczuk & Sadowska, 1997). Regression of the corpus luteum at the late luteal phase has been reported to be associated with both a decrease in the number of proliferating cells and an increase in the number of apoptotic cells (Gaytan et al., 1998) and induction of the formation of primary lysosomes which cause cellular degeneration through autosegregation and enzymatic digestion of cell organelles in secondary lysosomes (Rall et al., 1981). In the interstitial cells and theca cells of ovary, constitutive expression of GNPDA was found and there was no difference in expression of GNPDA in these cells of growing, antral, and Graafian follicles. Undoubtedly, theca cells are active in transport and resorption of fluid during these stages of folliculogenesis. Therefore, it can be suggested the expression of GNPDA in theca cells reflected high energy requirement for transport activity of the theca cells in these follicles. Cytoplasm of the some oocytes from primary follicles showed positive signals but not in the antral follicle. It has been known that apoptotic cell death of the oocyte occurred during the atretic degeneration of follicles during development of primary follicle from primodial follicle (Guraya, 1985). Therefore, it suggested that GNPDA expression possibly involved in the degeneration of oocyte during follicular development.

In Western blot, band of Mr. 31 kDa corresponding to mouse homologue of GNPDA increased during postnatal development of the ovary and became the highest in luteal phase in adult ovary. There was sudden rise in GNPDA expression of 2 weeks-old ovary. After birth, the follicles committed to growth continue cell proliferation and increase in oocyte volume independently of gonadotrophins (primarily FSH) until the late preantral stage when antrum formation is contingent upon FSH (Drummond & Findlay, 1999). The great excess of developing follicles will degenerate rather than ovulate. Follicular formation and development are largely postnatal and accompany the vast changes in tissue composition in the ovary. It resulted in increase in the size of ovary by appearance of growing follicles

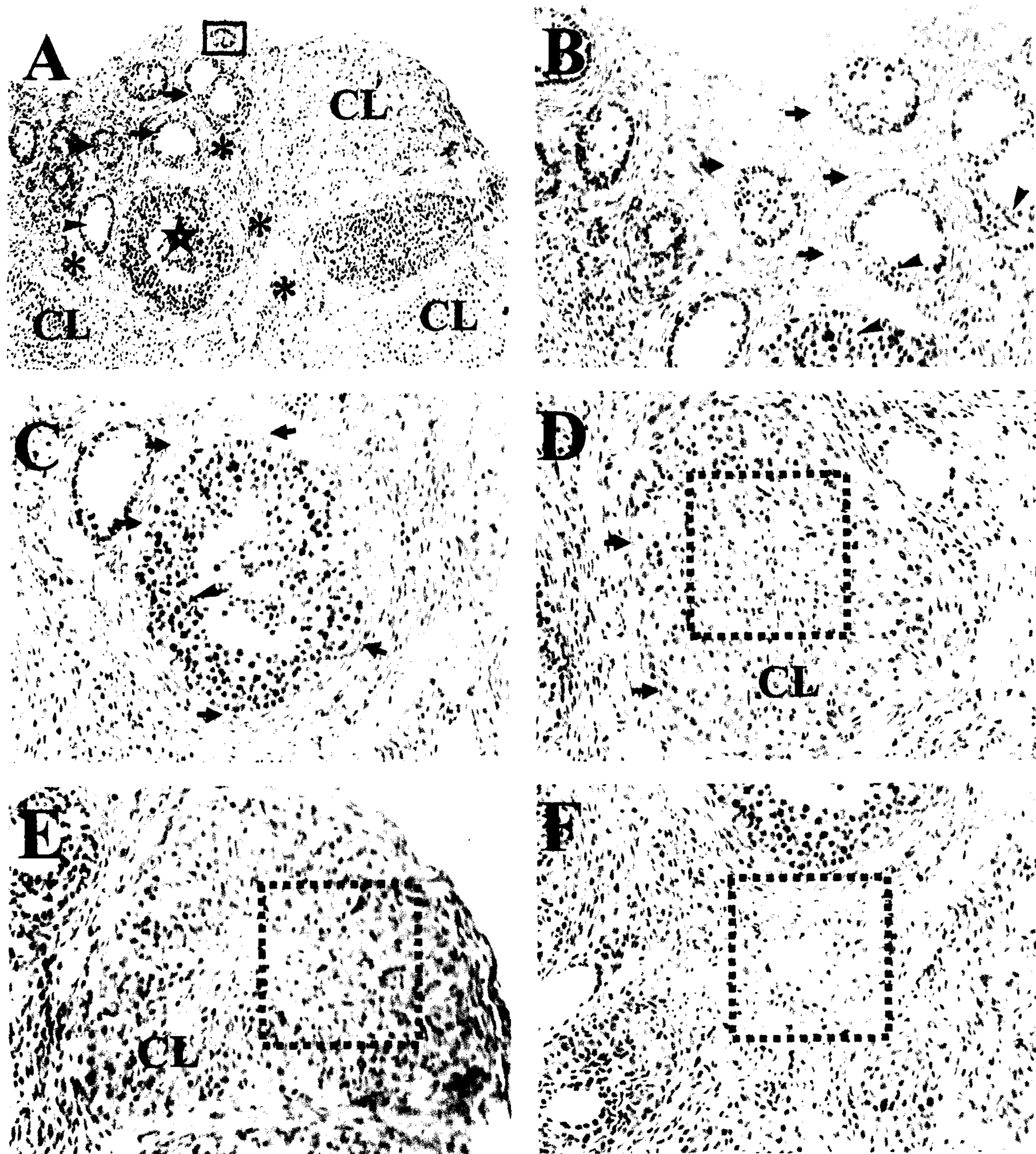

Fig. 2. GNPDA expression in mouse ovary. (A) Low magnification view of mouse ovary. Primary follicles showed different signal intensity (arrows). Some of them which have degenerative sign showed positive signal in the oocyte (arrowhead) and granulosa cells (box). Interstitial cells showed the strong signals regardless of follicles (asterisks). Granulosa cells of antral follicles were clear from staining (star). Different signals among the luteal follicles are seen (CL). Luteal cells in early luteal phase showed no signal (boxed area). (B and C) Enlarged view of (A). Granulosa cells of antral follicles were clear from staining (arrowheads) but theca cells showed positive staining (arrows). (D) Mid luteal phase. Luteal cells showed weak signal (boxed area). Theca cells showed positive staining (arrows). (E) Late luteal phase. Luteal cells showed moderate signal (boxed area). (F) Luteolytic site. Intensive signal was found in the luteolytic site (boxed area).

and increase in interstitial cells. In the immunostaining of ovaries, interstitial expression of GNPDA was constitutive throughout the follculogenesis. Therefore, it can be suggested that increase in GNPDA expression was possibly due to the increase in intestitial cells during postnatal development of the ovary. The luteal cell expression of GNPDA started in

medium-sized corpora lutea and reached the highest in luteolytic one. It suggested that GNPDA might be involved in luteal regression in mouse ovary. The functional luteal regression undergoes structural luteal regression, characterized by cell death and the loss of luteal tissue (Juengel et al., 1993; Young et al., 1997). In immunostaining, intensive signal was detected in the luteolytic site, indicating the involvement of GNPDA in clearing of the corpus luteum. Taken together, it suggested that the expression of GNPDA in ovary was different according to luteal development.

In summary, the differential expression of GNPDA was found in ovarian tissues according to follicular development, suggesting that GNPDA might be involved in ovarian tissue remodeling.

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