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- Note -

Opposite Localization of Luteinizing Hormone Receptors and Galectin-3 in Mature Mouse Ovaries

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The present study evaluated the localization of luteinizing hormone receptors (LHR) and galectin-3 (Gal-3), a beta-galactoside-binding animal lectin, in the mature mouse ovaries by immunohistochemical analysis. Intense LHR immunoreactivity was detected in the active corpus luteum (CL), whereas expression of Gal-3 was high in the regressing CL and atretic follicle. In the CL of pregnant mice, LHR immunoreactivity was intense, but Gal-3 expression was low. Thus, LHR and Gal-3 had opposite patterns of expression in mature mouse ovaries, suggesting that both proteins have stage-specific expression patterns and are possibly involved in CL formation and regression.

Key words : Luteinizing hormone receptor, galectin-3, corpus luteum, follicle, mouse ovary

Introduction

Luteinizing hormone receptor (LHR), a member of the G-protein-coupled receptor superfamily, plays a crucial role in corpus luteum (CL) function by interacting with gonadotropin LH [10,11]. Moreover, luteal progesterone production is dependent on LHR [2].

Galectin-3 (Gal-3) is a 26-kDa beta-galactoside-binding animal lectin belonging to the galectin family. To date an estimated 15 family members have been detected in mammals. Gal-3 is expressed in a variety of tissues and cell types, depending on cell cycle stage and proliferative state [1,6,8,9]. In the ovary, Gal-3 is one of the predominant galectin family subtypes, and Gal-3 mRNA expression changes markedly during the estrous cycle and pregnancy [12]. However, little is known about the localization of LHR and Gal-3 proteins in the ovary in adult mice.

In this study, the expression of LHR and Gal-3 proteins in the mature mouse ovary were investigated by immunohistochemical analysis to elucidate their localization patterns in the CL and follicle during the estrous cycle and pregnancy.

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Materials and Methods

Animals and tissue preparation

Five female Institute of Cancer Research (ICR) mice, 8-9-weeks-of-age, and four pregnant ICR mice (16-17 gestational days) were obtained from a specific-pathogen-free colony at Oriental, Inc. (Seoul, Korea). The mice were killed and their ovaries were immediately removed. The ovaries were embedded in paraffin wax after routine fixation in 4% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.4). The Institutional Animal Care and Use Committee at Chonnam National University approved the protocols used in this study, and the animals were cared for in accordance with the Chonnam National University Guidelines for Animal Experiments.

Antibodies

Rat anti-Gal-3 monoclonal antibody (1 mg/ml) was purified from the supernatants of hybridoma cells (clone TIB-166™, M3/38.1.2.8. HL.2; American Type Culture Collection, Manassas, VA, USA). Rabbit anti-LHR polyclonal antibody (H-50) and a goat anti-cathepsin D polyclonal antibody (R-20) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Fluorescein isothiocyanate (FITC)-labeled isolectin B4 (IB4) derived from *Griffonia simplicifolia* (Sigma-Aldrich, St. Louis, MO, USA) was used to label macrophages, as IB4 has a strong affinity

for terminal αD-galactosyl residues, which are abundant in macrophages [7].

Immunohistochemistry

Five micron-thick sections were deparaffinized, hydrated, and incubated with rat anti-Gal-3 (1:1,000 dilution) and rabbit anti-LHR (1:200 dilution) for 2 hr. The sections were then incubated with biotinylated rabbit anti-rat IgG and goat anti-rabbit IgG (Vector ABC Elite Kit; Vector Laboratories, Burlingame, CA, USA) for 45 min. Immunoreactivity was detected through incubation for 45 min with the avidin-biotin peroxidase complex (Vector ABC Elite Kit; Vector Laboratories) prepared according to the manufacturer's instructions. The peroxidase reaction was visualized using a diaminobenzidine substrate kit (SK-4100; Vector Laboratories). As a control, the primary antibodies were omitted for a few test sections in each experiment. The sections were counterstained with Harris hematoxylin before

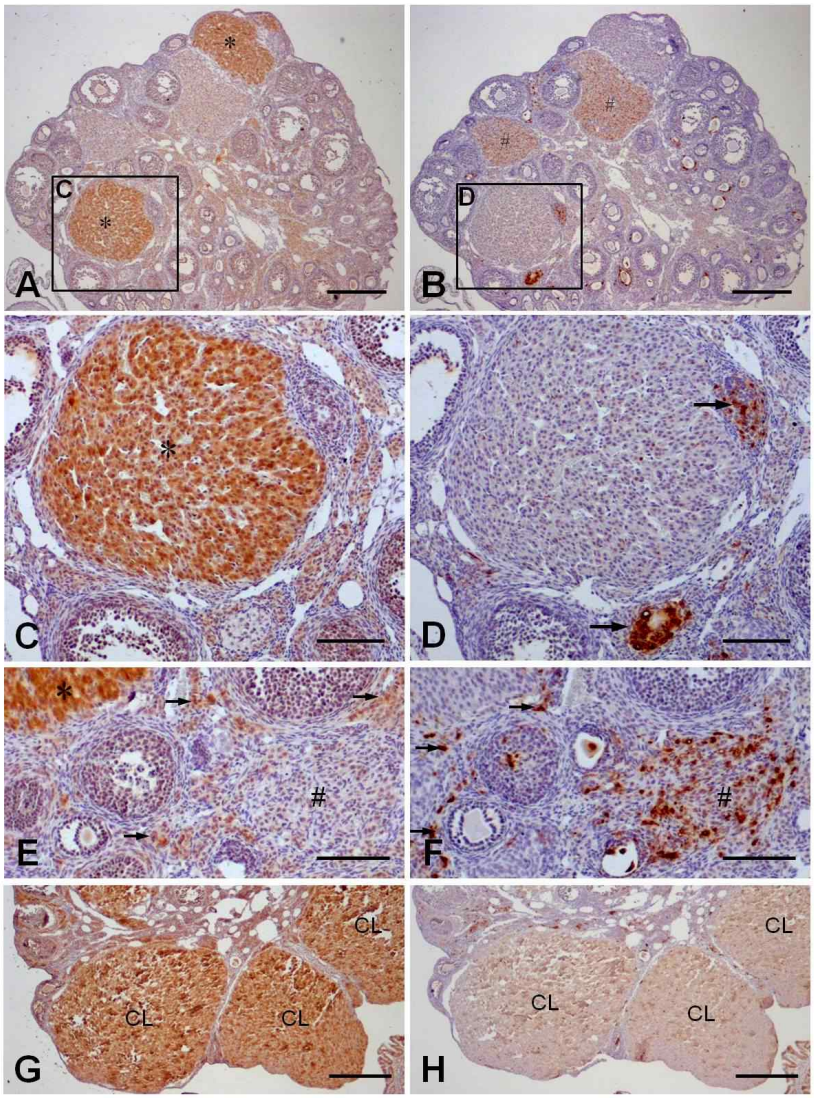

Fig. 1. Immunohistochemical analysis of Gal-3 and LHR in the mouse ovary. Representative images of the expression patterns for LHR (A, C, E and G) and Gal-3 (B, D, F and H) in the non-pregnant mouse ovary (A-F) and the pregnant mouse ovary (G and H). Intense LHR immunoreactivity was detected in the active CL (asterisks), whereas strong Gal-3 immunoreactivity was detected in the regressing CL (pound signs) as well as atretic follicles (D, arrows). Arrows in E and F identify LHR- and Gal-3-positive cells in the interstitium, respectively. The expression patterns for LHR (G) and Gal-3 (H) in the pregnant mouse ovary, showing marked LHR immunoreactivity in the CL, whereas Gal-3 immunoreactivity was very weak. Panels A and B, C and D, E and F, and G and H show pairs of adjacent serial sections. Sections were counterstained with Harris' hematoxylin. Scale bars = 100 μm (C-F), and 300 μm (A, B, G and H).

being mounted.

Double-immunofluorescence

Colocalization of Gal-3 with either cathepsin D or IB4 was examined by double-immunofluorescence labeling in the same sections. In brief, sections were incubated overnight at 4°C with rat anti-Gal-3 antibody (1:500 dilution) and were then treated with tetramethylrhodamine isothiocyanate-labeled anti-rat IgG (Sigma-Aldrich). Next, the sections were incubated for 2 hr at room temperature with either goat anti-cathepsin D (1:200 dilution) followed by FITC-labeled anti-goat IgG (Vector Laboratories) or FITC-labeled IB4 (20 μg/ml). The sections were counterstained during incubation for 30 min at room temperature with 5 μg/ml 4′,6-diamidino-2-phenylindole 2HCl (DAPI; Thermo Fisher Scientific, Rockford, IL, USA) in PBS. The double-immunofluorescence-stained sections were examined using a BX-40 fluorescence microscope (Olympus, Tokyo, Japan) equipped with a ProgRes® CFscan digital camera (Jenoptik, Jena, Germany).

Results and Discussion

Although LHR expression was weak in growing follicles (Figs. 1A, C, and E), Gal-3 immunoreactivity was extremely weak (Figs. 1B, D, and F). LHR immunoreactivity was very intense in the active CL (Figs. 1A, C, and E), where there were very few Gal-3-positive cells (Figs. 1B, D, and F). Intense Gal-3 immunoreactivity was predominantly detected in the regressing CL and atretic follicles (Figs. 1B, D, and F). LHR and Gal-3 were also detected in the interstitium of mouse ovary (Figs. 1E and F). In the CL of pregnant mice, LHR was abundantly expressed (Fig. 1G), whereas Gal-3-positive cells were rare (Fig. 1H).

The immunohistochemical findings are summarized in Table 1.

Double-immunofluorescence staining showed that cathepsin D and IB4 colocalized with Gal-3 in the CL in the adult mouse ovary (Fig. 2).

A broad consensus holds that the ovary is regulated by diverse hormones and neural signals, and shows dynamic changes in several signals. Among these factors for regulation of ovary, LHR has a crucial role in CL function and also in luteal progesterone production [2]. Recently, Nio-Kobayashi and Iwanaga [13] reported that that LHR mRNA levels were high in the active CL of immature mice in which ovulation was artificially induced. However, there is no evidence about the expression of LHR protein in the follicle and CL of ovary in mature mice during estrous cycle and pregnancy. In the present study, LHR immunoreactivity was strong in the active CL of mature mice and in the CL of pregnant mice, whereas its expression was very low in the regressing CL. Therefore, LHR may play a pivotal role in CL formation and luteal progesterone production during the estrous cycle and in the maintenance of the CL during pregnancy.

Luteolysis is characterized by atrophy, fragmentation, and increased proportion of luteal cells of extracellular matrix and collagen, which is fragmented or engulfed by phagocytes [5]. Luteolysis is induced by a rapid decrease in progesterone production, the release of prostaglandin F2 alpha, and the concomitant expression of 20α-hydroxysteroid dehydrogenase and Gal-3 [4,12,15]. While previous studies revealed strong Gal-3 mRNA and protein expression at 4 weeks postpartum in the CL of mice that were artificially induced to ovulate [12,13], there is little information about the expression of Gal-3 protein compared to the LHR expression in the mature mice ovary during estrous cycle and pregnancy. In the present study, Gal-3 protein was predominantly found in the regressing CL and atretic follicle of ma-

Table 1. Immunohistochemical localization of Gal-3 and LHR in the mature mouse ovary

Site		LHR	Gal-3
Follicle	Growing follicle	+	-
	Atretic follicle	-	+++[a]
Corpus luteum	Active corpus luteum	+++	-
	Regressing corpus luteum	-	+++[b]
	Pregnant corpus luteum	+++	+[a]
Interstitium		++	+[a]

Stained sections were scored as follows for each positive reaction per field: -, negative; +, weak; ++, moderate; and +++, intense.
[a]Most Gal-3-positive cells were identified as macrophages based on morphological criteria and colabeling with IB4.
[b]Some Gal-3-positive cells were identified as macrophages based on morphological criteria and colabeling with IB4.

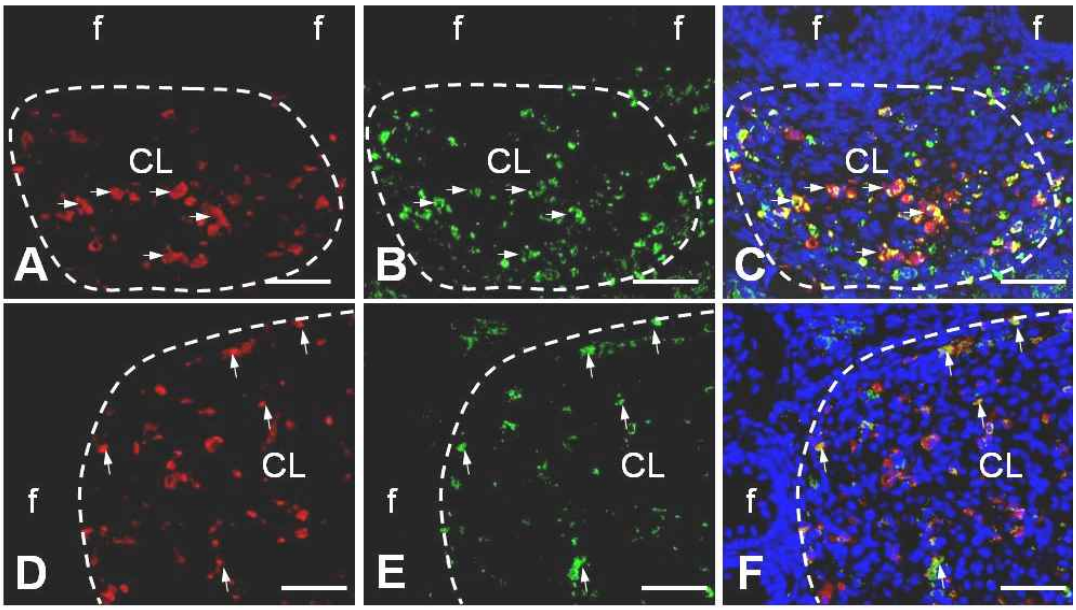

Fig. 2. Double-immunofluorescence images for Gal-3 and either cathepsin D (A-C) or IB4 (D-F) in the non-pregnant mouse ovary. (A) Immunolabeling of Gal-3 (red). (B) Immunolabeling of cathepsin D (green). (C) Merged image. (D) Immunolabeling of Gal-3 (red). (E) Immunolabeling of IB-4 (green). (F) Merged image. Arrows indicate double-stained cells. Slides were counterstained with DAPI (blue). CL, corpus luteum; f, ovarian follicle. Scale bars denote 50 μm.

ture mouse ovary, but was rarely expressed in the active CL and the CL of pregnant mice. Additionally, the present study showed that a lysosomal protease cathepsin D, which cleaves prolactin that plays a pivotal role in CL formation and maintenance [14,15], colocalized with Gal-3. Furthermore, some Gal-3-positive cells in the regressing CL and atretic follicle were identified as macrophages based on morphological criteria and colabeling with IB4. Therefore, on the contrary to LHR, Gal-3 may play a crucial role to eliminate the luteal and follicular cells during CL regression and follicle atresia in the mouse ovary.

In conclusion, LHR is abundantly expressed in the active CL of mature mice and in the CL of pregnant mice. Conversely, Gal-3 immunoreactivity is intense during the luteolytic and atretic stages in the mature mouse ovary. The opposite localizations of LHR and Gal-3 suggest that these proteins have stage-specific expression patterns and its opposite roles in CL formation and regression. Additionally, it is suggested that both Gal-3 and LHR proteins can be useful to determine the stage of the CL as immunohistochemical markers.

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초록 : 성숙마우스의 난소에서 황체형성호르몬수용체와 galectin-3의 상반된 면역조직화학적 발현분포

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본 연구는 성숙마우스의 난소에서 황체형성호르몬수용체(luteinizing hormone receptor, LHR)와 beta-galctoside-binding animal lectin인 galetin-3 (Gal-3)의 발현분포를 면역조직화학방법으로 평가하였다. LHR은 활동(성숙) 황체에서 강하게 발현되는 반면에, Gal-3의 발현은 퇴행중인 황체와 폐쇄난포에서 높게 나타났다. 또한, 임신중인 마우스의 황체에서는 LHR의 면역반응이 강하게 나타났으나, Gal-3의 면역반응은 현저히 낮았다. 위의 결과를 종합해 볼 때, 성숙마우스의 난소에서 LHR과 Gal-3는 난소의 특정한 시기에 따라 다른 분포양상을 나타냄을 알 수 있었으며, 이들 각각의 단백질의 발현으로 보아 황체의 형성과 퇴행에서 상반된 역할을 할 것으로 여겨진다.