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 28-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.

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-166TM, M3/38.12.8. 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

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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 LIIR (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 LIIR 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 LIIR- and Gal-3-positive cells in the interstitium, respectively. The expression patterns for LIIR (G) and Gal-3 (H) in the pregnant mouse ovary, showing marked LIIR 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).
Double-immunofluorescence

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

<table>
<thead>
<tr>
<th>Site</th>
<th>LHR</th>
<th>Gal-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growing follicle</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Atretic follicle</td>
<td>-</td>
<td>+++*</td>
</tr>
<tr>
<td>Corpus luteum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active corpus luteum</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Regressing corpus luteum</td>
<td>-</td>
<td>+++*</td>
</tr>
<tr>
<td>Pregnant corpus luteum</td>
<td>+++</td>
<td>*</td>
</tr>
<tr>
<td>Interstitium</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Stained sections were scored as follows for each positive reaction per field: -, negative; +, weak; ++, moderate; and ++++, intense.

*Most Gal-3-positive cells were identified as macrophages based on morphological criteria and colabeling with IB4.

*Some Gal-3-positive cells were identified as macrophages based on morphological criteria and colabeling with IB4.

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 mouse 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-
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.

Acknowledgement

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References


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