Histochemical evaluation of postnatal lectin-binding sites in the mouse prostate

By

Kentaro SAKUDA, Ryoki MURAGISHI, and Kazuya YOSHINAGA

Department of Anatomy and Cell Biology, Graduate School of Health Sciences, Kumamoto University, 4-24-1 Kuhonji, Kumamoto 862-0975, Japan

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Summary: The prostate is a male accessory genital gland that plays an essential role in reproductive function. To understand the cytological characteristics of differentiating prostatic cells, we used lectin histochemistry combined with immunohistochemistry to examine the distribution of lectin-binding sites on prostatic cells during postnatal development in the mouse. During postnatal development, Hippeastrum Hybrid Lectin (HHL) lectin reacted consistently with the luminal cells of all prostatic lobes (regions), whereas the Ricinus Communis Agglutinin I (RCA-I) and Soybean Agglutinin (SBA) lectins showed remarkable differences with age, region, and cell type. We found that the lectin-binding pattern in differentiating prostatic cells acquired adult characteristics around 3 weeks after birth. The results indicate that prostatic cell differentiation during postnatal development in mice is characterized by the presence of cell- and region-specific lectin-binding sites in the prostate, suggesting that there may also be cellular and regional differences in their function. Furthermore, some lectins (HHL, RCA-I, and SBA) could provide useful markers for research into cell differentiation and for the pathological evaluation of prostatic diseases or in the diagnosis of male infertility.

Materials and Methods

Animals and tissue processing

Male C57BL/6 mice of ages ranging in 1-week intervals, from birth (first day) to 6 weeks, were purchased from Japan SLC (Shizuoka, Japan). All experiments

Corresponding author: Kazuya Yoshinaga, Department of Anatomy and Cell Biology, Graduate School of Health Sciences, Kumamoto University, 4-24-1 Kuhonji, Kumamoto 862-0975, Japan. E-mail: kyoshina@kumamoto-u.ac.jp
using laboratory mice were approved by the Committee on Animal Research at Kumamoto University. Mice were anesthetized with ether, and the prostate was removed and immersed in 4% paraformaldehyde for a minimum of 4 h at 4°C. After dehydration, specimens were embedded in paraffin, and 5-μm-thick sections were prepared.

**Lectin histochemistry**

Three types of biotinylated lectins, purchased from Vector Laboratories (Burlingame, CA, USA), were used. Their full names (natural sources), abbreviations, carbohydrate specificities, and binding inhibitors are listed in Table 1. Lectin histochemical staining was performed as previously described [26, 30]. Briefly, deparaffinized sections were incubated in citrate buffer solution (pH 6.0), and their antigenicities were enhanced in an autoclave (121°C, 1 min). To block endogenous peroxidase activity, sections were treated with 0.3% H2O2 in methanol. Non-specific binding was blocked by treating the sections with 1% bovine serum albumin (BSA) in 10 mM phosphate-buffered saline (PBS; pH 7.4). Sections were incubated with biotinylated lectins diluted in 1% BSA in PBS for 1 h at room temperature. After rinsing the sections with PBS, they were incubated with the VECTASTAIN ABC kit (Vector Laboratories) for 30 min. Lectin binding to the sections was visualized with 0.05% 3,3'-diaminobenzine tetrahydrochloride and 0.01% H2O2 in 50 mM Tris buffer (pH 7.6) for 2–7 min. Sections were counterstained with hematoxylin.

**Double-fluorescence staining using lectin histochemistry and immunohistochemistry**

Double-fluorescence staining was performed as previously described [26, 30]. Briefly, deparaffinized sections were incubated in citrate buffer solution (pH 6.0), and their antigenicities were enhanced in an autoclave (121°C, 1 min). After treatment to block endogenous peroxidase activity and non-specific binding, the sections were incubated with biotinylated lectins diluted in 1% BSA in PBS for 40 min. After rinsing the sections with PBS, they were incubated with fluorescein isothiocyanate (FITC)-labeled streptavidin (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 30 min. The sections were then incubated overnight at 4°C with a primary rabbit anti-GM130 monoclonal antibody (Abcam, Cambridge, UK) or a primary rabbit anti-K5 polyclonal antibody (Covance, Berkeley, CA, USA). After washing with PBS, sections were stained for 30 min with an Alexa Fluor® 568-labeled secondary antibody (Molecular Probes, Eugene, OR, USA). Sections were counterstained with Hoechst 33258 (Sigma-Aldrich, St Louis, MO, USA), and imaged using a BX-51 fluorescence microscope (Olympus, Japan). Control samples were prepared by omitting the primary antibody incubation step.

**Results**

All the three lectins used in this study, HHL, RCA-I, and SBA, showed a variety of expression patterns in prostatic epithelial cells at each stage, in each lobe, and to each cell type. The lectin-binding patterns are summarized in Table 2. No reaction products were seen in any of the control specimens (data not shown).

HHL weakly reacted with cytoplasmic granules in cells from all prostatic lobes in mice aged 1 week (Fig. 1A). In mice aged 2 weeks or more, HHL showed the same expression pattern as seen in the anterior and dorsal lobes or 1-week old mice, whereas in the lateral and ventral lobes, the positive cytoplasmic granules were observed only at the basal side of the cells. As development progressed, the positive signal gradually increased in both size and intensity (Fig. 1B, C). Double-fluorescence staining with an antibody against K5, a marker of basal cells [31], showed that the HHL-positive cells did not colocalize with K5 (Fig. 1B, C). Accordingly, the HHL-positive cells were identified as luminal cells.

RCA-I was detected in basal cells and at the apical surface of luminal cells in all four lobes of mice aged 1 week (Fig. 1D). The positive signal in the luminal cells of the lateral and ventral lobes was observed until 6 weeks of age (Fig. 1E). The expression was not observed in the anterior lobe in mice aged 3 weeks or more and in the dorsal lobe in mice aged 4 weeks or more. As shown in Table 2, the positive signal in the basal cells was not observed in the lateral lobe in mice aged 3 weeks or more, in the anterior and ventral lobes in mice aged 4 weeks or more, and in the dorsal lobe in mice aged 6 weeks or more. Double-fluorescence staining with an anti-K5 antibody and RCA-I showed that the RCA-I-positive cells colocalized with K5-positive basal cells (Fig. 1D, F).

SBA was detected in the basal cells and at the apical

### Table 1. List of biotinylated lectins used in this study and their inhibitory sugars

| Lectin group       | Lectin source                                                      | Abbreviation | Carbohydrate specificity\(^a\) | Inhibitory sugar |
|--------------------|--------------------------------------------------------------------|--------------|-------------------------------|-----------------|
| Mannose-binding    | *Hippeastrum Hybrida* Lectin                                       | HHL          | α-Mannose                     | Mannose         |
| Galactose-binding  | *Ricinus Communis Agglutinin I*                                    | RCA-I        | β-D-Gal                       | Galactose       |
| GalNAc-binding     | *Soybean Agglutinin*                                               | SBA          | α-D-GalNAc >> β-D-GalNAc       | GalNAc          |

\(^a\)Gal: Galactose, GalNAc: N-acetyl-D-galactosamine
Postnatal lectin-binding sites on prostatic cells

surface of luminal cells in all four lobes of mice aged 1 week (Fig. 1G). No positive signal was observed in luminal cells of the anterior lobe of mice aged 2 weeks or more or in the dorsal lobe of mice aged 4 weeks or more. In contrast, a positive reaction was observed in the lateral and ventral lobes, as well as in the apical region of luminal cells, in mice aged 2 weeks or more (Fig. 1H). The positive reaction in the basal cells of the anterior and dorsal lobes was observed until 6 weeks, but was not observed in the ventral lobe in mice aged 2 weeks or more or in the lateral lobe in mice aged 3 weeks or more (Table 2).

### Discussion

The present study shows that lectin-binding sites are abundant during postnatal development in the mouse prostatic epithelial cells, and that the expression patterns change according to the developmental stage. To our knowledge, this is the first report describing the cytological characteristics of prostatic cells during postnatal development in mice.

In general agreement with previous reports from the studies of rat prostate during postnatal development [23, 25], the lectin-binding patterns in this study suggest that sugar chains expressed in the prostatic epithelium vary with age and among lobes (regions) and cell types. However, some differences in the histochemical findings were noted between the current and previous findings. For example, in this study, PHA-L reacted with the Golgi region of the luminal cells in the ventral lobe, which is not described in the previous report [13]. This discrepancy is likely due to differences in the fixation method or in the sensitivity of the detection techniques.

During postnatal development, HHL reacted consistently with luminal cells in all the prostatic lobes (regions). This suggests that HHL lectin and α-mannose, which is a recognized terminal sugar of HHL, could serve as useful markers for the luminal cells. In addition, in all the prostatic lobes, the HHL-positive cytoplasmic granules in the luminal cells gradually increased in size as the development progressed. Immuno-electron microscopy will be required to clarify the dynamics of these cytoplasmic granules.

In Table 2, the lectin-staining patterns of mouse prostatic cells during postnatal development are shown. The patterns change according to the lobes and cell types. For example, HHL lectin showed a positive reaction in the luminal cells of the lateral and ventral lobes and in the apical region of luminal cells in mice aged 2 weeks or more. The positive structures were identified partially as the Golgi complex by colocalization with GM130, which is a known marker for the Golgi complex [32].

Table 2. Lectin-staining patterns of mouse prostatic cells during postnatal development

|       | Anterior prostate | Dorsal prostate | Lateral prostate | Ventral prostate |
|-------|------------------|----------------|-----------------|-----------------|
| HHL   |                  |                |                 |                 |
|       | LC BC            | LC BC          | LC BC           | LC BC           |
| 1w    | + –              | + –            | + –             | + –             |
| 2w    | + –              | + –            | + –             | + –             |
| 3w    | + –              | + –            | + –             | + –             |
| 4w    | + –              | + –            | + –             | + –             |
| 5w    | + –              | + –            | + –             | + –             |
| 6w    | + –              | + –            | + –             | + –             |
|       |                  |                |                 |                 |
| RCA-I |                  |                |                 |                 |
|       | LC BC            | LC BC          | LC BC           | LC BC           |
| 1w    | + +              | + +            | + +             | + +             |
| 2w    | + +              | + +            | + +             | + +             |
| 3w    | + +              | + +            | + +             | + +             |
| 4w    | – –              | – +            | + +             | + +             |
| 5w    | – –              | – +            | + +             | + +             |
| 6w    | – –              | – +            | + +             | + +             |
|       |                  |                |                 |                 |
| SBA   |                  |                |                 |                 |
|       | LC BC            | LC BC          | LC BC           | LC BC           |
| 1w    | + +              | + +            | + +             | + +             |
| 2w    | + +              | + +            | + +             | + +             |
| 3w    | + +              | + +            | + +             | + +             |
| 4w    | – –              | – +            | + +             | + +             |
| 5w    | – –              | – +            | + +             | + +             |
| 6w    | – –              | – +            | + +             | + +             |

LC: luminal cell, BC: basal cell –: negative, +: positive
Fig. 1. Lectin histochemical staining and immunostaining in the developing mouse prostatic epithelia. A: HHL staining in the anterior lobe in mice aged 1 week. A positive signal is seen in cytoplasmic granules (arrows) of epithelial cells. B, C: Double-fluorescence imaging with HHL (green) and a K5 antibody (red) as a basal cell marker in the ventral lobe in mice aged 2 weeks (B) and 5 weeks (C). Arrows indicate HHL-positive cytoplasmic granules in luminal cells. Arrowhead indicates a K5-positive basal cell. D: Double-fluorescence imaging with RCA-I (green) and a K5 antibody (red) in ventral lobe in mice aged 1 week. Positive signals for both RCA-I and K5 are seen in basal cells (arrowheads). RCA-I is also seen on the apical surface (arrow) of luminal cells. E: RCA-I staining in the ventral lobe in mice aged 4 week. The apical surface (arrow) of luminal cells shows a positive reaction. F: Double-fluorescence imaging with RCA-I (green) and a K5 antibody (red) in the dorsal lobe in mice aged 5 weeks. Positive signals for both RCA-I and K5 are present in basal cells (arrowheads). G: Double-fluorescence imaging with SBA (green) and a K5 antibody (red) in the lateral lobe in mice aged 1 week. Positive signals for both SBA and K5 are present in basal cells (arrowhead). SBA is present on the apical surface (arrow) of luminal cells. H: SBA staining in the lateral lobe in mice aged 3 week. A positive reaction is seen on the apical surface and in the Golgi region (arrows) of luminal cells. I: Double-fluorescence imaging with SBA (green) and a GM130 antibody (red) in ventral lobe of mice aged 5 weeks. The apical surface of the luminal cells is positive for SBA. Positive signals for both SBA and GM130 are seen in the Golgi region (arrows). Asterisks indicate the lumen of the prostatic ducts. Scale bars = 20 μm.
may be a reflection of functional differences. Interestingly, we found that lectin-binding patterns in differentiating prostatic cells acquired adult characteristics at around 3 weeks of age. Prostatic cell differentiation is complete and cells become fully mature at 3–4 weeks, indicating prostatic cells acquired adult characteristics at this stage, before androgen production begins.

In conclusion, the present study demonstrated that prostatic cell differentiation during postnatal development in mice is characterized by cell- and region-specific lectin-binding patterns in prostates, and the data suggest that there are cellular and regional differences in function. Furthermore, some lectins (such as HHL, RCA-I, and SBA) could provide markers for research into cell differentiation and for the pathological evaluation of prostatic diseases, or in the diagnosis of male infertility.

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