Lectin-binding sites in epithelial cells of the mouse prostate gland

By

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Summary: The prostate is an exocrine gland in the male reproductive tract that secretes seminal fluids. To gain insight into the cytochemical properties of prostatic epithelial cells, the characteristics of glycoconjugates in mouse prostate sections were examined by lectin histochemistry and immunohistochemistry. Characteristic staining patterns were observed, depending on the type of lectins present in the epithelia. Luminal cells reacted specifically with mannose-binding lectins (Galanthus nivalis lectin, Hippeastrum hybrid lectin, Narcissus pseudonarcissus lectin) and Maclura pomifera lectin in all lobes of the prostate. Luminal cells also expressed galactose, N-acetyl-D-galactosamine (GalNAc), N-acetyl-D-glucosamine (GlcNAc), and fucose residues in the lateral and ventral lobes. Basal cells expressed GlcNAc and fucose, and reacted with Datura stramonium lectin and Aleuria aurantia lectin in all lobes. These results indicate that in the mouse prostate, the selectivity of lectin-binding sites for distinct cell types and lobe-dependent staining may relate to cellular and regional differences in function. Furthermore, some lectins selectively bound to prostatic epithelial cells, indicating their potential use as markers for the histopathological evaluation of prostatic diseases, cancer diagnosis, or male infertility.

Introduction

The prostate is an exocrine gland in the male reproductive tract that secretes seminal fluids and plays important roles in the reproductive process. Although the prostate can be affected by several disorders, including benign prostate hyperplasia and cancer, the cytological characteristics and functions of normal prostatic epithelial cells have not yet been determined.

The mouse prostate gland is comprised 4 lobes, namely the anterior, dorsal, lateral, and ventral lobes\(^1,2\). These lobes differ in terms of their histological examinations, ductal patterns, and secretory functions\(^3,4\). Epithelial cells lining the prostatic lumen are primarily composed of luminal cells and basal cells. The function of luminal cells is to generate various secretory products that are characteristically rich in glycoconjugates\(^5-8\), while the function of basal cells remains to be determined.

Lectins are proteins that bind to specific carbohydrate residues of glycoconjugates due to their individual affinities for particular sugars. Lectins bind not only to sugar chains, but also to proteins, lipids, and other small molecules and support various physiological processes in a variety of cell types. Thus, they have been widely used for detecting sugar chains in histochemical studies and for characterizing prostatic cells in several species, including humans\(^9-12\), rats\(^13-16\), mice\(^5,17\), goats\(^7,8\), rhesus monkeys\(^18\), and water buffalos\(^19\).

The purpose of this study was to obtain new information regarding the distribution of various lectin-binding sites in the prostate and to characterize glycoconjugates present in epithelial cells of the mouse prostate, using lectin histochemistry combined with immunohistochemistry.

Materials and Methods

Tissue Collection and Processing

Nine-week-old adult male C57BL/6 mice were purchased from Japan SLC (Shizuoka, Japan). Experiments using laboratory mice were approved by the Committee on Animal Research at Kumamoto University.
Mice were anesthetized with ether and sacrificed by intracardiac perfusion with 4% paraformaldehyde (PFA) fixative. After perfusion, prostates were removed and further immersed in PFA for at least 4 hr at 4°C. After dehydration, specimens were embedded in paraffin, and 3–4-μm-thick sections were prepared.

**Lectin histochemistry**

Lectin histochemical staining was performed as described in our previous paper. Briefly, deparaffinized sections were incubated with citrate buffer solution (pH 6.0), and their antigenicities were enhanced in an autoclave (121°C, 1 min). Sections were treated with 0.3% H₂O₂ in methanol to block endogenous peroxidase activity. Non-specific binding was blocked by treating sections with 1% bovine serum albumin (BSA) in 10 mM phosphate-buffered saline (PBS; pH 7.4). Sections were incubated with 16 biotinylated lectins diluted in 1% BSA in PBS for 40 min. After washing with PBS, sections were incubated with fluorescein isothiocyanate (FITC)-labeled streptavidin (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 30 min. Subsequently, the sections were 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). 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 then washed with PBS and counterstained with Hoechst 33258 (Sigma-Aldrich, St Louis, MO, USA), mounted with mounting medium (Vector Laboratories), and observed with a BX-51 fluorescence microscope (Olympus, Japan). Control specimens were prepared by omitting the primary antibody-staining step.

**Histochemical controls**

As controls for lectin-binding specificity, sections were pre-incubated with a 0.1–0.5 M concentration of appropriate inhibitory sugars (Wako, Japan), listed in Table 1, for each lectin. Sections were then incubated with a solution containing biotinylated lectins and inhibitory sugars. Nonspecific staining was controlled for by omitting the lectin incubation step.

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

| Lectin group                  | Lectin source                        | Abbreviation | Carbohydrate specificity | Inhibitory sugar |
|-------------------------------|--------------------------------------|--------------|--------------------------|------------------|
| Mannose (Glucose)-binding lectins | Galanthus Nivalis Lectin              | GNL          | α-Mannose                | Mannose          |
|                               | Hippeastrum Hybrid Lectin             | HHL          | α-Mannose                | Mannose          |
|                               | Narcissus Pseudonarcissus Lectin      | NPA          | α-Mannose                | Mannose          |
| Galactose-binding lectins     | Erythrina Cristagalli Lectin          | ECA          | D-GalNAc, D-Gal          | Galactose        |
|                               | Maclura Pomifera Lectin               | MPL          | Galβ1-3GalNAc            | Galactose        |
|                               | Ricina Communis Agglutinin I          | RCA-I        | β-D-Gal                  | Galactose        |
| GalNAc-binding lectins        | Phaseolus Vulgaris Leucoagglutinin    | PHA-L        | Galβ1-4GalNAcβ1-2Man     | GalNAc           |
|                               | Psophocarpus Tetragonolobus Lectin    | PTL-I        | α-GalNAc > α-Gal         | GalNAc           |
|                               | Soybean Agglutinin                    | SBA          | α-D-GalNAc > β-D-GalNAc  | GalNAc           |
| GalNAc-binding lectins        | Datura Stramonium Lectin              | DSA          | β-D-GalNAc               | GlcNAc           |
|                               | Lycopersicon Esculentum Lectin        | LEL          | β-D-GalNAc               | GlcNAc           |
|                               | Wheat Germ Agglutinin                 | WGA          | D-GlcNAc, Sialic acid    | GlcNAc           |
| Fucose-binding lectins        | Aleuria Aurantia Lectin               | AAL          | α-L-Fuc                  | Fucose           |
|                               | Ulex Europaeus Agglutinin 1           | UEA-I        | α-L-Fuc                  | Fucose           |
| Sialic acid-binding lectins   | Limax Flavus Agglutinin               | LFA          | NeuAc > NeuGe            | Sialic acid      |
|                               | Sambucus Sieboldiana Agglutinin       | SSA          | Sia2-6 Gal, GalNAc       | Sialic acid      |

Gal: Galactose, GalNAc: N-acetyl-D-galactosamine, GlcNAc: N-acetyl-D-glucosamine, Fuc: Fucose, Sia: Sialic acid (N-acetylneuraminic acid), NeuAc: N-acetylneuraminic acid, NeuGc: N-glycolylneuraminic acid.
Results

Fourteen of 16 lectins investigated in this study showed variable binding patterns to prostatic epithelial cells (Fig. 1). However, sialic acid-binding lectins (LFA and SSA) were unreactive, which is consistent with a previous study conducted by Akif et al.\(^5\). No non-specific reaction products were seen in any of the control specimens (data not shown).

Mannose-binding lectins

All 3 mannosyl-specific lectins (GNL, HHL, and NPA) showed similar binding patterns with each of the 4 prostatic lobes. These lectins weakly stained the cytoplasm of luminal cells in all lobes. The lectins also stained cytoplasmic granules in the luminal cells of anterior and dorsal prostates (Fig. 1A), as well as basal cytoplasmic granules of lateral and ventral prostates (Fig. 1B). Double-fluorescence staining with an antibody against K5, a marker of basal cells\(^21\), showed that some basal cytoplasmic granules reacting with mannosyl-specific lectins did not colocalize with K5 (Fig. 1C). In the lateral prostate, GNL also stained the luminal surface.

Galactose-binding lectins

In the lateral and ventral prostates, the apical surface of luminal cells showed a positive reaction with all galactosyl-specific lectins. Moreover, ECA weakly stained the Golgi region in luminal cells of the anterior and ventral prostates. Furthermore, MPL weakly stained the Golgi region in luminal cells of all lobes (Fig. 1D). The MPL-positive site was identified as the Golgi complex by colocalization with GM130, which is a known marker for the Golgi complex\(^22\) (Fig. 1E).

GalNAc-binding lectins

In the lateral and ventral prostates, the apical surface and Golgi region of luminal cells reacted positively with all GalNAc-specific lectins (Fig. 1F). In contrast, SBA reacted specifically with basal cells in the anterior and dorsal prostates. Double fluorescence staining of basal cells with an anti-K5 antibody and SBA showed that SBA-positive cells colocalized with K5-positive basal cells.

GlcNAc-binding lectins

In the lateral and ventral prostates, the apical surface of the luminal cells reacted positively with all GlcNAc-specific lectins. In addition, the Golgi region of luminal cells reacted with LEL and WGA. However, basal cells of the anterior and dorsal prostates reacted with all GlcNAc-specific lectins (Fig. 1G). In addition, DSA also reacted with basal cells in the lateral and ventral prostates. All GlcNAc-specific lectin-positive cells were identified as basal cells by colocalization with K5 (Fig. 1H). In these lobes, basal cells often showed a long cytoplasmic extension that infiltrated between adjacent luminal cells toward the lumen (Fig. 1H). Furthermore, WGA weakly stained the Golgi region in luminal cells in the anterior prostate lobe (Fig. 1I). LEL also stained some basal cytoplasmic granules in the lateral prostate lobe, as well as some cytoplasmic granules in the luminal cells of the anterior and dorsal prostates.

Fucose-binding lectins

AAL reacted with basal cells in all lobes. AAL reacted with the apical surface in luminal cells of the lateral and ventral prostates and the Golgi region of the ventral prostate. Although UEA-I showed a negative reaction with the dorsal prostate, it stained the Golgi region in the anterior and ventral prostates and the apical surface in the lateral and ventral prostates.

Discussion

Using combined lectin histochemistry immunohistochemistry, we characterized the cytochemical properties of sugar chain expression in the mouse prostatic epithelium. To our knowledge, this is the first study demonstrating such a reliable and widely applicable histological method with prostatic tissue. In agreement with previous reports\(^5,17\), we suggest that clear differences in lectin-binding patterns observed among differing cell types or regions (lobes) of the prostatic epithelium reflect differences in their respective functions. It is noteworthy that some of our findings differed from those of previous reports. For example, PHA-L reacted positively with the Golgi complex of luminal cells in ventral prostate in this study, but was unreactive in a previous study\(^5\). This discrepancy may be due to differences in the sensitivities of the detection methods used, or due to differences in the fixation procedures.

Our findings showed that various lectins bound to luminal cells in the lateral and ventral prostates, suggesting that a variety of glycoconjugates are synthesized in these lobes. In contrast, luminal cells in the anterior and dorsal prostates showed negative or weak reactions with the lectins tested. Notably, we found that luminal cells specifically reacted with mannose-specific lectins (GNL, HHL, and NPA) and a galactose-specific lectin (MPL) in all prostatic lobes, indicating that luminal cells contain Galβ1-3GalNAc in the Golgi complex and α-mannose in the cytoplasm. In the lateral and ventral prostates, we found some cytoplasmic granules containing mannose and GlcNAc residues in the basal cytoplasm of luminal cells. These results suggest that cytoplasmic granules may participate in the migration of secretory glycoconjugates from the basal to apical cytoplasm of luminal cells\(^2,23\).

In this study, we showed that basal cells react with DSA and AAL lectins in all prostatic lobes, indicating...
Fig. 1. Histochemical and immunohistochemical lectin staining of epithelial cells from a mouse prostate gland. A: GNL staining of the anterior lobe. The arrows highlight small cytoplasmic granules of luminal cells that stained positively with GNL. B: GNL staining of a ventral lobe section. The arrows show basal cytoplasmic granules of luminal cells that reacted positively with GNL. C: Double-fluorescence imaging of the ventral lobe with an anti-K5 antibody and GNL. Basal cells reacted with K5 (red), and basal cytoplasmic granules of luminal cells reacted with GNL (green). D: MPL staining in the anterior lobe. The Golgi complex (arrow) of luminal cells stained weakly positive with MPL. E: Double-fluorescence imaging of the ventral lobe with a GM130 antibody (red) and MPL (green). Double-positive staining (yellow) with both GM130 and MPL is seen in the Golgi complex (arrow). F: PHA-L staining in the ventral lobe. The apical surface (arrowhead) and Golgi complex (arrow) of luminal cells were positive with PHA-L. G: Positive DSA staining of basal cells in the anterior lobe (arrow). H: Double-fluorescence imaging of the anterior lobe with an anti-K5 antibody (red) and DSA (green). Double-positive staining of basal cells (yellow or white) with both an anti-K5 antibody and DSA (arrow). The arrowhead highlights a DSA-positive basal cell with a cytoplasmic extension projecting to the luminal side. I: Double-fluorescence imaging of an anterior prostate lobe with an anti-GM130 antibody (red) and WGA (green). A weak double-positive reaction (yellow) with both GM130 and WGA was seen in the Golgi complex (arrowheads). Asterisks indicate the lumens of the prostatic ducts. Scale bars = 20 μm.
that β-D-GlcNAc and α-L-fucose are common terminal sugars in basal cells. Basal cells also reacted with all GlcNAc-specific lectins in the anterior and dorsal prostates, suggesting an abundant production of glycoconjugates occurs in these lobes. It should also be noted that some basal cells extend cytoplasmic projections into the luminal side of the anterior and dorsal prostates (Fig. 1H). Further morphological and functional analyses will be helpful for understanding the physiological significance of these flask-shaped basal cells existing in specific lobes.

In conclusion, although the present study does not provide functional information about prostatic epithelial cells, we did observe selectivity of lectin reactivity with distinct cell types and lobe-dependent staining in the prostate, which is suggestive of cellular and regional differences in functions. Furthermore, because some lectins (GNL, HHL, NPA, and MPL) selectively reacted with the luminal cells of all 4 prostatic lobes, these lectins can potentially serve as useful biomarkers for prostate cell differentiation, histopathological evaluation of diseases, cancer diagnosis, or male infertility.

Acknowledgements

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