Lectin Cytochemical Analysis of Lacrimal Pleomorphic Adenomas

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Abstract: The activity of seven different types of biotinylated lectin were examined in normal and tumorous lacrimal gland tissue. In the normal lacrimal gland tissue, the glandular cells and the tubular epithelium were labeled by *maclura pomifera* agglutinin (MPA), *soybean* agglutinin (SBA), and *bauhinia purpurea* agglutinin (BPA). The myoepithelial cells were stained by *griffonia simplicifolia* agglutinin 1 (GS1). In the primary pleomorphic adenoma tissue, the epithelial components were labeled by MPA, *ulex europaeus* agglutinin, SBA, peanut agglutinin, and BPA. The mesenchymal components showed negative labeling. In contrast, the recurrent pleomorphic adenoma tissue showed a positive reaction in the mesenchymal components when GS1 and BPA were used. These results revealed differences in the glycoconjugate composition among normal and tumorous lacrimal gland tissues from patients with primary and recurrent pleomorphic adenomas.

Key Words: Human histopathology, lacrimal gland, lectin-binding properties, pleomorphic adenoma.

Introduction

Pleomorphic adenoma (PA) is one of the most common benign neoplasms of the lacrimal gland. Histologically, PA consists of myxomatous stroma and cellular areas. A series of immunohistochemical studies have been employed using antisera against cytoskeletons (keratin, actin, vimentin, glial fibrillary acidic protein) or membranous proteins (epithelial membrane antigen, carcinoembryonic antigen) as a means of further characterization of the tumor components. These studies have also helped to elucidate the origin of each structural component in the PA.

Lectin represents a structurally heterogeneous group of glycoproteins that have the common property of binding to specific carbohydrate residues. It has been shown that a variety of lectins are useful as cytochemical markers of the glycoconjugates in various tissues. However, there have been few reports concerning lectin binding to the normal lacrimal gland and PA.

In the present study, we examined the activity of seven different types of biotinylated lectin in normal lacrimal gland tissue and tumor tissue from three cases of PA, one having recurrent PA. Our purpose was to seek differences in the composition of glycoconjugates among these structures.

Materials and Methods

Reagents

Biotinylated *maclura pomifera* agglutinin (MPA), *concanavalin A* agglutinin (ConA), *ulex europaeus* agglutinin (UEA), *soybean* agglutinin (SBA), *peanut* agglutinin (PNA), *griffonia simplicifolia* agglutinin 1 (GS1), and *bauhinia purpurea* agglutinin (BPA) were obtained commercially (Vector Laboratories, Burlingame, CA, USA). Haptenic sugars, N-acetyl-D-galactosamine (GalNAc), α-methyl-D-mannoside, L-fucose, GalNAc, D-galactose, GalNAc, and GalNAc were
used against MPA, ConA, UEA, SBA, PNA, GS1, and BPA, respectively (Sigma Chemical Co., St. Louis, MO, USA).

**Specimen Preparation**

We obtained the tumor tissues from 3 patients with PA. Case 1 (53-year-old man) and case 2 (55-year-old woman) had primary PA. Case 3 (22-year-old man) had recurrent PA.

The excised tumor tissues and adjacent normal lacrimal gland tissues were fixed immediately in a solution of 4% paraformaldehyde in 0.1 mol/L phosphate buffer (pH 7.4) for 2 hours, trimmed into small blocks, and embedded in paraffin. Some specimens were embedded using OCT compound 4593 (Miles Scientific, Naperville, IL, USA) for lectin cytochemistry. Specimens were sectioned with a cryostat at −20°C. Endogenous peroxidase activity was inhibited by treatment for 30 minutes in methanol containing 0.3% hydrogen peroxide. Thirty minutes before use, the avidin-biotin complex (ABC) was prepared by mixing 32 μL of avidin and 32 μL of biotinylated horseradish peroxide in 50 mmol/L Tris-buffered saline (TBS) for 30 minutes; then incubated with 25 μg/mL biotinylated lectin in 0.1% BSA-TBS at room temperature for 30 minutes. The sections were washed with TBS to remove excess lectins and incubated with ABC at room temperature for 30 minutes. After several rinses with TBS, the sections were developed with 3,3′-diaminobenzidine-4HCl (0.2 mg/mL)-H₂O₂ (0.005%) in TBS, and observed under a light microscope.

Specificity of lectin binding was confirmed by competitive inhibition of haptenic sugar at 200 mmol/L.

**Results**

**Light Microscopic Findings**

**Normal part of lacrimal gland.** The tumor mass was removed with a portion of the adjacent normal-appearing lacrimal tissue in Cases 1 and 3. Under a light microscope, the lacrimal gland was divided into two parts, the acinus and the tubule. The acinus was seen as a round, oval, or irregularly shaped cellular island with central lumen. The epithelial cells of the tubules were smaller than the glandular cells. In some sections, the configuration of the myoepitheliocytes surrounding the inner glandular cells was identified.

**Pleomorphic adenoma. Case 1.** In light microscopic findings, the tumor was surrounded by a connective tissue capsule, and characterized by variously dilated cystic tubules (Figure 1a). Secreted eosinophilic ma-

![Figure 1](image1.png)

**Figure 1.** (a) Light micrograph of hematoxylin-eosin-stained paraffin section of pleomorphic adenoma obtained from Case 1. Note cellular stroma and cystoid space (*) filled with secreted mucus (×65, Bar = 250 μm). (b) High magnification of Case 1 pleomorphic adenoma showing characteristic tubular structures lined by almost two layers of epithelium. s: stromal components among tubular structures (×150, Bar = 100 μm).

terial was present in the lumen of the tubule. Tubular structures were arranged in an irregular anastomosing pattern, and their walls were lined with a double layer of the epithelium. In the vicinity of the tubular structures, an aggregation of peritubular cells was found. The stromal cells were relatively rich in myxomatous matrix (Figure 1b).

**Case 2.** The tumor had a myxomatous matrix with cords of epithelial cells (Figure 2). There were small numbers of stromal cells and peritubular cells in the matrix.

**Case 3.** The tumor seemed to be comprised of multiple nodules surrounded by connective tissue capsules (Figure 3a). Each nodule contained similar structures, myxomatous matrix and tubular structures, as found in primary PA (Figures 3b, 3c). The inner layer of the tubular epithelium occasionally underwent squamous metaplasia (Figure 3b).

**Lectin Cytochemistry**

**Normal part of lacrimal gland.** In the glandular cells of the acinus, the zymogen granules were intensely
stained by MPA (Figure 4a), SBA, and BPA (Figure 4d). ConA seemed to be bound to the cytoplasm of the glandular cells (Figure 4b). The luminal surfaces of all lacrimal glands were stained by GS1 (Figure 4e). *Griffonia simplicifolia* agglutinin 1 was also bound to the basal lining of the lacrimal gland. In the tubules, only the apical surface of the epithelial cells was weakly stained by MPA, ConA, SBA, GS-1, and BPA. The basal parts of the tubules did not show any staining by GS-1. We could not detect any labeling in the interstitial connective tissues.

Observations of lectin binding are summarized for the acinus (glandular cell and myoepithelial cell), tubule, and interstitial cell in Table 1.

**Pleomorphic adenoma. Case 1.** The mucus and the luminal surface of the tubular structures were stained by MPA (Figure 5a), ConA, UEA, SBA (Figure 5c), PNA, GS1 (Figure 6a), and BPA (Figure 6c).

**Case 2.** The results of staining reactions in Case 2 were identical to those in Case 1. MPA, ConA, UEA (Figure 5b), SBA, PNA (Figure 5d), GS1, and BPA were bound to the luminal surface of the tubule.

**Case 3.** *Maclura pomifera* agglutinin and UEA bound to the mucus and the luminal surface of the tubule. *Griffonia simplicifolia* agglutinin 1 (Figure 6b) bound to the mucus, inner and outer layer of the tubule, peritubular cells, and stromal cells. *Bauhinia purpurea* agglutinin was weakly positive in the inner layer of the tubule, and strongly positive in the outer layer of the tubule, peritubular cells, and stromal cells (Figure 6d).

Observations of lectin binding in each type of PA are summarized for the mucus, inner and outer tubules, peritubular cells, and stromal cells in Table 2. In the specimen incubated with a mixture of lectin and a specific haptenic sugar, reaction to lectins was remarkably reduced in all sites.

### Discussion

**Normal Part of Lacrimal Gland**

In the present study, MPA, SBA, and BPA, which have specific affinity to GalNAc, bound to the zymogen granules of some glandular cells, whereas the zymogen granules of other glandular cells constituting the same acinus showed no reaction to these lectins. These results suggest that there must be at least two categories of glandular cells, distinguished by their affinity pattern to GalNAc in the zymogen granules. Based on the results of the ultrastructural and histochemical studies, it has been considered that the glandular cells can be classified into two, three, or four categories. It is also suggested that at least some may represent different stages in the secretory activity of one or two distinct types. The present results might indicate the possibility that the
affinity to GalNAc represents some stage of the secretory activity or the maturity of the zymogen granules.

Maclura pomifera agglutinin, ConA, SBA, and BPA were also bound to the apical surface of the epithelium of the tubules. These results suggest that the composition of glycoconjugates in the apical surface of the epithelial cells of the acinus and tubules might be similar.

It is of interest that GS1 binds specifically to the basal region of the lacrimal gland. As the flattened myoepithelial cells surround the corresponding portion of the glandular cells discontinuously, it is possible that GS1 may bind to the myoepithelial cells.

Pleomorphic Adenoma

By electron microscopy and immunohistochemical study, it has been suggested that the inner layer of the tubule may secrete mucus. On the other hand, the outer layer of the tubular structure may undergo metaplasia and be transformed to a myxoid, fibrous, or cartilaginous stroma. The PA seemed to be comprised of two major cell groups; the epithelial component (inner layer of the tubule) and the mesenchymal component (outer layer of the tubule, peritubular cell, and stromal cell). In the present study, it is of interest that there may be a marked difference in the glycoconjugates, especially in mesenchymal components, between the primary and the recurrent forms of PA. In primary PA, there seems to be no lectin binding in the stromal cells. By contrast, the stromal cells were stained by GS1 and BPA in recurrent PA. It has been known that PA may recur in the

Table 1. Lectin Binding to Normal Lacrimal Glands

<table>
<thead>
<tr>
<th>Lectin</th>
<th>Glandular Cell</th>
<th>Myoepithelial Cell</th>
<th>Tubular Cell</th>
<th>Interstitial Cell</th>
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<tbody>
<tr>
<td>MPA</td>
<td>+++</td>
<td>-</td>
<td>+</td>
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<tr>
<td>ConA</td>
<td>+</td>
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<td>UEA</td>
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<td>SBA</td>
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</tr>
<tr>
<td>PNA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GS1</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>BPA</td>
<td>+++</td>
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Intensity of lectin labeling was qualitatively evaluated by visual judgment as negative (−), weak (+), moderate (+ +), or intense reaction (+ + +).
adjacent soft tissues or the bony wall following incomplete removal. Malignant transformation of recurrent PA has been also reported. Based on these findings, it is suggested that primary PA may change its biological nature after incomplete resection. In the hematoxylin–eosin–stained section of the present case of recurrent tumor, there are no signs of malignancy, such as nuclear atypism or mitosis, in the stromal cells. If this case of recurrent PA is in the process of malignant transformation, the changes in lectin-binding properties seem to precede histological findings. It has been known that the application of some lectins is useful for the diagnosis of specific tumors; UEA for hemangioendocytoma, and PNA for histiocytosis X. Although we need more investigation of the constitutional changes of the glycoconjugates in the other cases of recurrent PA and malignant PA, the present study may indicate that GS1 and BPA are useful markers for the malignant transformation of PA.

Figure 5. Light micrographs of frozen sections from Case 1 and Case 2. Pleomorphic adenomas stained by maclura pomifera agglutinin (MPA) (a), ulex europaeus agglutinin (UEA) (b), soybean agglutinin (SBA) (c), and peanut agglutinin (PNA) (d). Positive reaction was seen in mucus and luminal surfaces of tubular structures in each case. There seemed to be no positive reaction in mesenchymal compartment (×125, Bar = 100 μm).

Figure 6. (a,b) Light micrographs of griffonia simplicifolia agglutinin-1 (GS1)-stained frozen sections of pleomorphic adenomas obtained from Case 1 (a) and Case 3 (b). In Case 1, only luminal surfaces of tubular structures were specifically stained by GS1. In addition to tubular structures, epithelial cell cords and mesenchymal cells were also stained by GS1 in Case 3. (c,d). Light micrographs of bauhinia purpurea agglutinin (BPA) stained frozen sections of pleomorphic adenomas obtained from Case 1 (c) and Case 3 (d). In Case 1, mucus and luminal surfaces of tubular structures were specifically stained by BPA. In addition to these structures, prickle cells surrounding tubular structures (arrowheads) and stromal cells (arrows) were also stained by BPA in Case 3 (×125, Bar = 100 μm).
The myoepithelium-specific markers, such as antikeratin or antimuscle-specific actin antibody, are consistently bound to the myoepithelial cell in the normal lacrimal gland and mesenchymal component in PA. Based on these findings, it has been suggested that myoepithelial cells develop into the mesenchymal component. However, the present results showed that myoepithelial cells develop into the mesenchymal component.

Table 2. Lectin Binding to Lacrimal Pleomorphic Adenomas

<table>
<thead>
<tr>
<th></th>
<th>Mucus</th>
<th>Tubule Inner Layer</th>
<th>Outer Layer</th>
<th>Peritubular Cell</th>
<th>Stromal Cell</th>
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<tbody>
<tr>
<td>MPA</td>
<td>p</td>
<td>+++</td>
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<td></td>
<td>r</td>
<td>+++</td>
<td>++</td>
<td>–</td>
<td>–</td>
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<tr>
<td>ConA</td>
<td>p</td>
<td>–</td>
<td>+++</td>
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<td></td>
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<td>r</td>
<td>+++</td>
<td>+++</td>
<td>–</td>
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<tr>
<td>SBA</td>
<td>p</td>
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<td></td>
<td>r</td>
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<tr>
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<td>p</td>
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<td>+</td>
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Although the present study reveals marked differences in glycoconjugate composition between normal and tumorous lacrimal gland tissue from patients with PA, it seems difficult to discuss the origin of each component of these tumors by their lectin-binding properties.

References