Preliminary Screening of Glycoprotein Profiles in Human Normal and Lung Cancer Sera by Different Staining Methods

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ABSTRACT

In this study, the purposes were to compare the staining methods for detection of glycoprotein profiles in normal and lung cancer serum samples and to preliminary screen the carbohydrate specificity of glycoproteins in both serum samples. Using the sequential staining methods of Pro-Q Emerald 488 glycoprotein gel staining, SYPRO® Ruby staining and silver staining revealed that the Emerald 488 glycoprotein gel staining has the specific detection of glycoproteins compared with SYPRO® Ruby and silver staining and provided the differential glycoprotein profiles between normal and lung cancer serum samples. In addition, seven FITC-labeled lectins; Con A, PNA, ECL, WGA, MAL 1, AAL and UEA, were used to detect the differentially expressed glycoproteins and the carbohydrate specificity of glycoproteins in both samples. The results of lectin staining showed that Con A, WGA and AAL lectins have the strong reactivity to the glycoproteins in both samples compared with other lectins and gave the differentially expressed glycoproteins in lung cancer sera. It indicated that the serum glycoproteins in both samples contain the high contents of mannose, GlcNAc, sialic acid and fucose (α1-3/4, α1-6) residues, in which their expression levels may be correlated to the lung cancer development. Therefore, this preliminary detection method was not only used to screen the altered glycoproteins in human normal and lung cancer sera, but also provided the different carbohydrate specificities of glycoproteins that are very useful for further selective glycoprotein purification and analysis.

Keywords: glycoprotein, lung cancer, human serum, detection, FITC-labeled lectin.

1. INTRODUCTION

Human serum is the most common body fluids and has been widely used in the investigation of altered glycoproteins that frequently observed in many diseases, including cancers [1, 2]. For example, the N-glycan profiles from human blood sera of
healthy individuals and prostate patients revealed significant difference in these two groups and the fucosylation of glycan structures is generally higher in the cancer serum samples [3]. Therefore, the serum is an important source of body fluids and used to find the glycoprotein markers.

In the present, the two most widely used methods for the detection of glycoproteins in gels or on PVDF membranes involve the fluorescent periodate Schiff-base stain and the lectin-based detection system. Indeed, lectins are carbohydrate-binding proteins that present in various sources, such as bacteria, plants and animals, and are highly specific for their mono- and oligosaccharide residues [4]. In a past few years, lectins have been developed as tool to detect aberration of carbohydrate residues in many cancers that involved in malignant transformation, tumor cell differentiation, adhesion and metastasis [5-7]. The immunohistochemical technique was also used the lectin to detect altered glycoproteins in human cancer tissue sections; for example, the Phaseolus vulgaris leukoagglutinating (L-PHA) lectin staining demonstrated the significantly detection of glycoproteins in melanoma, breast and colon neoplasia compared to normal and benign lesions [8-10]. Likewise, the Helix pomatia agglutinin (HPA) lectin was also used to detect the metastasis in breast cancer and cutaneous malignant melanoma [11, 12]. In addition, the lectin staining or blotting is another technique of western blotting and used for detection of glycoproteins on blots. The lectin staining procedure has been used for sequential incubation of the blot with biotin-labeled lectin and peroxidase-labeled avidin or alkaline phosphatase, which have many steps and take a long time. Currently, the use of fluorescein-isothiocyanate (FITC)-labeled lectin is the simplified lectin blotting method that provides the high sensitivity of glycoprotein detection and the useful information of carbohydrate specificity of target glycoproteins. Moreover, lectins has also been developed as the glycoarrays for investigating the increased sialylation and fucosylation in pancreatic cancer and as the enzyme-linked lectin assay (ELLA) for investigating the aberrant glycoproteins or confirming the interested glycoprotein markers between normal and uterine cervix cancer serum samples [13, 14]. Therefore, lectins are useful tools for the detection of glycoproteins in many material sources.

In this study, we compared the different staining methods for the detection of the glycoprotein profiles in normal and lung cancer serum samples. Seven FITC-labeled lectins were also used to detect the different glycoprotein profiles and the carbohydrate specificity of glycoproteins in both serum samples that are the useful information for further glycoprotein purification and analysis.

2. MATERIALS AND METHODS

2.1 Chemical Materials

Human serum samples with a diagnosis of lung cancer patients and healthy donors were provided by Tri-Service General Hospital, Taipei, Taiwan. All fluorescein isothiocyanate (FITC)-labeled lectins; Concanavalin A (ConA), Peanut agglutinin (PNA), Erythrina cristagalli agglutinin (ECL), Wheat germ agglutinin (WGA), Maackia amurensis agglutinin (MAL1), Aleuria aurantia lectin (AAL) and Ulex europaeus agglutinin (UEA), biotinylated labeled AAL lectin and FITC–labeled streptavidin were purchased from Vector Laboratories (Burlingame, CA, USA). SYPRO® Ruby and Pro-Q Emerald 488 glycoprotein gel stain were purchased from Molecular Probes (Eugene, OR, USA). Silver nitrate was purchased from ACROS Organic (New Jersey, USA). The 25% Ammonium solution was purchased from Merck (Darmstadt, Germany). Glutaraldehyde, 2,7-
napthalene-disulfonic acid and formaldehyde were purchased from Sigma (St. Louis, MO, USA).

2.2 Serum Preparation

All of human serum samples were obtained from four healthy donors and four adenocarcinoma lung cancer patients at Tri-Service General Hospital, Taipei, Taiwan. Lung cancer sera were obtained from patients range age 65-76 years with diagnosed lung cancer at various clinical stages (T1N2M0, IIIB; T2N2M0, IIIA; T1N0M0, IA; T1N1M0, IIA). The tumor stage of cancer was determined according to the International System for Staging Lung cancer [15]. The healthy and lung cancer blood samples were allowed to clot at room temperature for 1 h and centrifuged at 2500 \( \times \) g for 15 min. The serum solution samples were stored at -80°C until used.

2.3 Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The serum samples were analyzed by 12.5% polyacrylamide (1.0 mm \( \times \) 10 well) with loading amount 10 \( \mu \)g of protein each well. The proteins were resolved in the SDS running buffer (25 mM Tris-HCl, pH 8.3, 192 mM glycine and 0.1% SDS). The SDS-PAGE was pre-run at constant voltage of 60 V for 10 min then increased to 120 V for 1.30 h. The total proteins separated on SDS-PAGE gel was detected in the same gel by the sequential staining method, as shown in Figure 1A.

2.4 Pro-Q Emerald 488 Glycoprotein Gel Staining

Detection of glycoproteins on SDS-PAGE gel using Pro-Q Emerald 488 glycoprotein gel stain was followed the manufacturer’s instruction (Molecular Probes). Briefly, the gels were fixed overnight in a fixative solution containing 50% methanol and 5% acetic acid. The gels were washed three times with 3% acetic acid for 5–10 min each, and incubated in the oxidizing solution of 1% periodic acid and 3% acetic acid for 20 min. After washed four times with 3% acetic acid for 5-10 min each, the gels were incubated in Pro-Q Emerald 488 dye solution for 2 h with a gentle shaking. After that, the gels were incubated 3 times with 3% acetic acid for 5-10 min each and then rinsed briefly with water. The gels were scanned using a Typhoon 9200 laser scanner (GE Healthcare) with the excitation wave length of 510 nm and 525 nm band pass for emission.

2.5 SYPRO® Ruby Staining

After detection of glycoproteins with Pro-Q Emerald 488 staining, the gel was post-stained with SYPRO® Ruby protein gel stain, according to the manufacturer’s instruction (Molecular Probes). Briefly, the gels were incubated with a fixing solution containing 50% methanol and 7% acetic acid with shaking for 30 min. The gels were soaked in SYPRO® Ruby gel stain solution overnight with gentle agitation. The gels were transferred to a clean container and soaked in a washing solution containing 10% methanol and 7% acetic acid for 1 h. The gels were rinsed with distilled water and scanned using a Typhoon 9200 laser scanner (GE Healthcare) with the excitation wavelength of 532 nm and 565 nm for emission.

2.6 Silver Staining

After SYPRO® Ruby staining was performed, this gel was post-stained with silver staining. The silver staining method was modified from Heukeshoven and Dernick [16]. Briefly, the SDS-PAGE gels were washed with deionized water for 5 min in a clear container, soaked in ethanol/acetic acid/water
(40/10/50) for 1 h, and subsequently in ethanol/acetic acid/water (5/5/90) for 2 h or overnight. The gels were washed 3 times with deionized water for 5 min each. The gels were soaked in a fixative solution containing 1% glutaraldehyde and 0.5 M sodium acetate for 30 min and washed 3 times with deionized water for 6 min each. After that the gels were soaked twice in a 2,7 naphthalene-disulfonic acid solution (0.05%/w/v) for 20 min and then rinsed 4 times with deionized water for 5 min each. After staining in freshly made amonical silver nitrate solution for 45 min, the gels were washed 4 times with deionized water for 3 min and then developed in the developing solution, containing 0.005% w/v citric acid.
and 0.05% v/v formaldehyde, for 5-10 min. When a slight background stain appeared, the development was stopped by addition of stopping solution, containing 5% Tris and 2% acetic acid. The gels were washed 3 times with deionized water for 20 min and scanned using a general scanner (EPSON).

2.7 Fluorescent Lectin Staining

After SDS-PAGE, the separated proteins on gel were transferred to the PVDF membranes with a constant current 250 mA for 1 h at room temperature. After that PVDF membranes were washed three times with PBS containing 0.05% Tween-20 (PBST) and incubated in a blocking solution containing PBST and 5% non-fat milk for 2 h. PVDF membranes were washed three times with PBST and stained with 10 μg/ml FITC-labeled lectins (ConA, PNA, ECL, WGA, MAL1 and UEA) for 1 h. For double labeling (biotin-avidin FITC) of AAL lectin, PVDF membranes were incubated with biotin-conjugated AAL lectin for 1 h and washed 3 times with PBST. Then, PVDF membrane were incubated with avidin-FITC for 30 min and washed 3 times with PBST. The membranes were scanned using a Typhoon 9200 scanner (GE Healthcare) with a wavelength at 526 nm. The lectin staining procedure is shown in Figure 1B.

2.8 Image Analysis

All electrophoresis and staining experiments were performed in triplicate. The number of protein/glycoprotein bands and the intensity of each band were quantified using the Image Master VDS analysis software version 3.00 (GE Healthcare). The data was presented as mean and standard derivation.

3. RESULTS AND DISCUSSION

3.1 Detection of Proteins by Three Staining Methods

The SDS-PAGE gel of serum samples from normal healthy volunteers and adenocarcinoma lung cancer patients was stained with the sequential staining method of Pro-Q Emerald 488 glycoprotein gel stain, SYPRO® Ruby gel stain and silver stain, respectively (Figure 1A). The SDS-PAGE gel image of each staining method is shown in Figure 2. Based on three staining methods, there are different staining-based theories. Pro-Q Emerald 488 glycoprotein gel stain is generally used for fluorescence detection of glycoprotein in gel and involved reacting carbohydrate groups by a periodate/Schiff’s base (PAS) mechanism, in which the glycols presented in glycoproteins are initially oxidized to aldehydes using periodic acid and then the dye reacts with the aldehydes on the glycoproteins to generate the fluorescent conjugate [17]. SYPRO® Ruby protein gel stain is a ruthenium based fluorescent dye for the detection of proteins in gels and widely used serially to detect glycosylated and unglycosylated proteins in the same gel [18, 19]. Silver stain is a metal-based stain for the detection of proteins, but it depends on the binding of silver ions to the amino acid side chains followed by reduction of free metallic silver. The proteins are visualized as bands where the reduction occurs [20]. Thus, the Pro-Q Emerald 488 glycoprotein gel stain was used to detect the glycoproteins in normal and lung cancer serum samples, while the sequential stains with SYPRO® Ruby protein gel stain and silver stain were used to compare the whole proteins and glycoproteins in both samples. In addition, the sequential staining method was carried out in the same gel in order to avoid the problem of different gel quality and to compare the protein bands within the same gel with different staining methods. Using the Image Master VDS analysis software, the detected protein bands on SDS-PAGE gel stained with Pro-Q
Emerald 488 glycoprotein gel stain, SYPRO® Ruby gel stain and silver stain were 20, 27 and 22 bands, respectively. The comparison of total serum proteins stained with different staining methods revealed that most of serum proteins were glycoproteins. Likewise, it has been reported that the majority of human serum proteins at least 50% of the proteins are glycosylated [21]. The glycoprotein expression in lung cancer serum samples was slightly more than that in normal serum samples. Our results were agreed with the previous study that N-linked oligosaccharides were significantly increased in non-small cell lung cancer patient serum [22]. However, the expression of glycoproteins in normal serum samples at the molecular weight (MW) lower than 45 kDa was higher than that expression in lung cancer serum samples. SYPRO® Ruby gel staining gave the total number of protein bands was higher than silver staining. Some serum protein bands with the MW around 30-20 kDa and upper than 66 kDa could not detect by silver staining. It was indicated that SYPRO® Ruby gel stain gave a higher sensitivity of protein detection than silver stain. It could be explained that the silver stain preferentially interacted with lysine residues, while SYPRO® Ruby stain detected lysine, arginine, and histidine residues [23].

### 3.2 Detection of Glycoproteins by FITC-Lectin Staining

In this study, we used seven lectins to detect the glycoproteins and theirs carbohydrate specificity in normal and lung cancer serum samples that have never been reported before. They could be classified to four groups, according to the specific monosaccharide binding: mannose-binding lectin (Con A), galactose/N-acetylgalactosamine (GalNAc)-binding lectin (PNA, ECL), N-acetylglucosamine (GlcNAc)/sialic acid-binding lectin (WGA, MAL1) and fucose-binding lectin (AAL, UEA). Although the lectin staining and the Pro-Q Emerald 488 glycoprotein gel stain-based on colorimetric PAS usually use to detect glycoprotein in gels and on blots, there are different in target binding. The PAS method requires oxidation of glycosyl residues followed by reaction with a chromogenic substrate, a fluorescent substrate or a tag. Then signal is detected directly in the case of the chromogenic or fluorescent conjugates and indirectly using enzyme conjugates of streptavidin or antibodies to the tags [17]. Thus, the glycoprotein pattern using Pro-Q Emerald 488 glyco-
Figure 3. Detection of N-glycoprotein profiles from normal and lung cancer serum samples by using different FITC-labeled lectins. Labels: (A) Total glycoprotein detected by Pro-Q Emerald 488 glycoprotein staining (comparative control), (B) FITC-Con A, (C) FITC-PNA, (D) FITC-ECL, (E) FITC-WGA, (F) FITC- MAL1, (G) biotin-avidin AAL, (H) FITC-UEA. Lanes: 1, protein marker; 2-5, lung cancer serum samples; 6-9, normal serum samples; 10, glycoprotein marker.
Figure 4. Comparative analysis of glycoprotein bands and fluorescence intensities of glycoproteins in normal and lung cancer serum samples by Image Master VDS analysis software program. Labels: (A) Total glycoprotein detected by Pro-Q Emerald 488 glycoprotein gel stain (comparative control; 20 bands), (B) FITC-Con A, (C) FITC-PNA, (D) FITC-ECL, (E) FITC-WGA, (F) FITC- MAL1, (G) biotin-avidin AAL, (H) FITC-UEA.
protein gel stain showed all glycoprotein bands. In contrast, the lectin staining is specific to the oligosaccharide/glycan moieties of glycoproteins and the glycoprotein pattern showed only the glycoproteins containing specific oligosaccharides upon lectin used. The lectin staining procedure for detection of serum glycoproteins has three main steps: (i) the proteins were separated by SDS-PAGE; (ii) the proteins in gels were transferred to PVDF membrane; (iii) the PVDF membrane was stained with FITC-labeled lectin and the fluorescence signal was detected (Figure 1B). The results of lectin staining showed the differential glycoprotein profiles in normal and lung cancer serum samples, depending on the carbohydrate specific binding of FITC-labeled lectins used (Figure 3). The number of glycoprotein bands and theirs intensities of normal and lung cancer serum samples were detected using Image Master VDS analysis software program and used the total glycoprotein number of serum samples visualized by Pro-Q Emerald glycoprotein gel staining as comparative control (Figure 4).

Most of glycoproteins were detected at the MW higher than 37 kDa and a few glycoproteins were detected at the MW about 25-30 kDa. According to Con A recognizes alpha-linked mannose, including high mannose-type and mannose core structures which are common to N-linked glycosylated proteins [24, 25], using Con A lectin staining exhibited 15 glycoprotein bands in normal serum samples and 14 glycoprotein bands in lung cancer serum samples. It indicated that both serum samples contained the mannose residues in the glycoprotein molecules.

Comparing a group of sialic and GalNAc-binding lectins, WGA binds to the Galβ1-3GalNAc residues [30], while ECL binds to the Galβ1-4GlcNAc residues and other Galβ1-related oligosaccharides [31]. It was found that PNA lectin staining exhibited 10 glycoprotein bands, while ECL lectin staining exhibited only 7-8 glycoprotein bands. However, ECL lectin staining showed the fluorescence intensity higher than PNA lectin staining. It was indicated that the serum glycoproteins contained Galβ1-3GalNAc residues more than Galβ1-4GlcNAc and other Galβ1-related oligosaccharides residues.

Comparing a group of galactose and GlcNAc-binding lectins, PNA strongly binds to the Galβ1-3GalNAc residues [30], while ECL binds to the Galβ1-4GlcNAc residues and other Galβ1-related oligosaccharides [31]. It was found that PNA lectin staining exhibited 10 glycoprotein bands, while ECL lectin staining exhibited only 7-8 glycoprotein bands. However, ECL lectin staining showed the fluorescence intensity higher than PNA lectin staining. It was indicated that the serum glycoproteins contained Galβ1-3GalNAc residues more than Galβ1-4GlcNAc and other Galβ1-related oligosaccharides residues.

Comparing a group of fucose-binding lectins, UEA preferentially binds to the Fucα1-2Gal residues [32], while AAL binds to the α1,6-fucosyl residues and broadly specific to α1,2-, α1,3- and α1,4-fucose-containing oligosaccharides [33, 34]. Our results showed that AAL lectin staining could detect 9 bands of serum glycoproteins, while UEA lectin staining could detect only 3 bands of glycoproteins. It was indicated that the serum samples have α1,6-fucosyl residues (core fucosylation) on glycoproteins more than Fucα1-2Gal.

In addition, the lectin used in this study, the carbohydrate specificity of lectin and the glycoprotein detection data are summarized in Table 1. The total numbers of glycoprotein bands stained with various lectins were different, depending on the affinity and specificity of lectins used and the content of
Table 1. Summary of lectins used, carbohydrate specificity and glycoprotein detection.

<table>
<thead>
<tr>
<th>Lectin</th>
<th>Name</th>
<th>Carbohydrate specificity</th>
<th>No. of band</th>
<th>Avg. intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>N</td>
<td>LC</td>
</tr>
<tr>
<td>1. Mannose binding lectin</td>
<td></td>
<td></td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>ConA</td>
<td>Concanavalin A</td>
<td>Mannose(α-mannosidic structures)</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>2. Galactose / N-acetylgalactosamine binding lectin</td>
<td></td>
<td></td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>PNA</td>
<td>Peanut Agglutinin</td>
<td>Gal-Gal-NAc (Galβ1-3GalNAcα1-Ser/Thr )</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>ECL</td>
<td><em>Erythrina cristagalli</em> agglutinin</td>
<td>Galβ1-4 GlcNAc</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>3. Sialic acid / N-acetylgulosamine binding lectin</td>
<td></td>
<td></td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>WGA</td>
<td>Wheat Germ Agglutinin</td>
<td>N-Acetylglucosamine (GlcNAcβ1-4GlcNAcβ1-4GlcNAc), Sialic acid (Neu5Ac)</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>MAL1</td>
<td><em>Maackia amurensis</em> agglutinin</td>
<td>Sialic acid (Neu5Ac/Geα2-3Galβ1-4GlcNAcβ1-R)</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>4. Fucose binding lectin</td>
<td></td>
<td></td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>AAL</td>
<td>Aleuria aurantia lectin</td>
<td>Fucα1-2Galβ1-4(Fucα1-3/4)Galβ1-4GlcNAc; R2-GlcNAcβ1-4(Fucα1-6)GlcNAc-R1</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>UEA</td>
<td>Ulex europaeus agglutinin</td>
<td>Fucose (Fucα1-2Gal-R)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>5. No lectin staining - Whole glycoprotein*</td>
<td></td>
<td></td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

*The whole glycoproteins in human normal and lung cancer serum samples were detected using Pro-Q Emerald glycoprotein staining. Abbreviations: N, normal serum; L, lung cancer serum; Avg, average (± SD).
carbohydrate components. Although the number of glycoprotein bands in the normal and lung cancer serum samples, which stained with the same lectin, were nearly the same, the fluorescence intensity of each band was different in both samples. It was indicated that the carbohydrate content in each glycoprotein were different. The average fluorescence intensities of each glycoprotein band may vary depending on the numbers of sample used and the contents of different carbohydrate components; for example, the high fluorescent intensity of few glycoprotein bands may affect to the standard derivation (SD) of the average intensity of total glycoprotein band, leading to gain a high SD values. The comparative results of lectin staining showed that AAL lectin has higher average fluorescence intensity than other lectins. We suggested that most human serum glycoproteins contain the high content of α1,6-fucosyl residues, but different expression levels between normal and lung cancer serum samples. Alterations in the expression of fucosylated oligosaccharides have been observed in numerous cancers [35]. For example, Przybylo et al. found that fucosylation and α2-6 sialylation on cadherins in human bladder cancer correlated with poor prognosis and Nuck et al. found that Fucα1-2Gal residues were presented in hepatocellular carcinoma, but non-observed in normal liver [36, 37]. In lung cancer, it had also been reported that E-cadherin was core fucosylated in highly metastatic lung cancer cells, but absent in lowly metastatic lung cancer cells [38]. Con A reacted to serum glycoproteins in normal and lung cancer serum samples with the highest number bands, as agreed with the previous study that the total human sera glycoproteins contained N-linked oligosaccharides about 84.1% [22]. According to the lung cancer serum samples had the average fluorescence intensity of Con A binding higher than normal serum samples, it was indicated that the overall N-linked oligosaccharides/glycans in lung cancer serum samples were expressed more than normal serum samples. WGA showed a modulate intensity, but the serum glycoprotein bands were detected more than AAL. The comparative fluorescence intensities between normal and lung cancer serum glycoproteins using WGA showed the significantly difference as comparing to other lectins. We suggested that the glycoproteins in normal and lung cancer serum samples may contain the different content of GlcNAc and sialic acid residues that may correspond to the lung cancer development. It has also been reported that total sialic acid levels in lung cancer sera and other cancers were slightly increased [39, 40]. Although some lectins showed the highest or lowest binding of glycoproteins and the fluorescence intensity in normal and lung cancer serum samples, they are still useful for specific purposes and further studies. For examples, ECL lecin staining was used to detect alterations in glycosylation of cytosolic proteins in malignant tumors [41] and the down-regulation and heavy sialylation of decay-accelerating factor (DAF) was associated with pathology in non-small cell lung carcinoma (NSCLC), in which these alterations make this protein a potential marker for NSCLC [42].

4. CONCLUSION

Due to the glycosylation of glycoproteins in cancers correlated with the cancer development, the detection of altered glycoproteins in cancers is very important. In this study, we described the preliminary screening of glycoprotein profiles in human normal and lung cancer serum samples by different staining methods. The comparison of different staining methods for detection of glycoproteins resulted that Pro-Q Emerald 488 glycoprotein gel staining gave the best detection of total
glycoproteins in both samples, which can be used as a preliminary screening of total glycoprotein detection prior to further analysis of glycoproteins in the samples. In addition, the lectin staining was used to detect the carbohydrate specificity of glycoproteins in the serum samples. The results of lectin staining showed that the high affinity order of lectins on glycoproteins was Con A > WGA > AAL lectins, while the high specific binding order of lectins on glycoproteins was AAL > WGA ≥ ConA. It was indicated that the glycoproteins in serum samples contained the high contents of mannose, GlcNAc, sialic acid and fucose (α1-3/4, α1-6), which exhibited the different expression levels of glycosylation between normal and lung cancer. Therefore, this preliminary detection technique is not only used to screen the serum glycoprotein profiles in normal and lung cancer and the specific altered glycoprotein bands with different carbohydrate specificities and expressions, it can provide the useful information for further selective glycoprotein separation and identification of glycoprotein biomarkers in the next studies and may also used as a screening platform for detection of altered serum glycoproteins in other diseases. However, further investigation of altered glycoproteins in lung cancer sera using the selective lectins for enrichment of glycoprotein and analysis of different glycan structures are required to gain a better understanding of the lung cancer development and may be the basis for further diagnostic and therapeutic applications.

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