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ORIGINAL ARTICLE

Glycoprofiling of Early Gastric Cancer Using Lectin Microarray Technology

Taijie Li [1, *], Cuiju Mo [1, *], Xue Qin [1], Shan Li [1], Yinkun Liu [2], Zhiming Liu [3]

[*] *Taijie Li and Cuiju Mo contributed equally to this work and should be considered as co-first authors*
[1] *Department of Clinical Laboratory, First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi, China*
[2] *Liver Cancer Institute, Zhongshan Hospital, Fudan University, Shanghai, China*
[3] *Department of General Surgery, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi, China*

SUMMARY

Background: **Recently, studies have reported that protein glycosylation plays an important role in the occurrence and development of cancer. Gastric cancer is a common cancer with high morbidity and mortality owing to most gastric cancers are discovered only at an advanced stage. Here, we aim to discover novel specific serum glycan-based biomarkers for gastric cancer.**
Methods: **A lectin microarray with 50 kinds of tumor-associated lectin was used to detect the glycan profiles of serum samples between early gastric cancer and healthy controls. Then lectin blot was performed to validate the differences.**
Results: **The result of the lectin microarray showed that the signal intensities of 13 lectins showed significant differences between the healthy controls and early gastric cancer. Compared to the healthy, the normalized fluorescent intensities of the lectins PWA, LEL, and STL were significantly increased, and it implied that their specifically recognized GlcNAc showed an especially elevated expression in early gastric cancer. Moreover, the binding affinity of the lectins EEL, RCA-II, RCA-I, VAL, DSA, PHA-L, UEA, and CAL were higher in the early gastric cancer than in healthy controls. These glycan structures containing GalNAc, terminal Galβ 1-4 GlcNAc, Tri/tetra-antennary N-glycan, β-1, 6GlcNAc branching structure, α-linked fucose residues, and Tn antigen were elevated in gastric cancer. While the two lectins CFL GNL reduced their binding ability. In addition, their specifically recognized N-acetyl-D-galactosamine structure and (α-1,3) mannose residues were decreased in early gastric cancer. Furthermore, lectin blot results of LEL, STL, PHA-L, RCA-I were consistent with the results of the lectin microarray.**
Conclusions: **The findings of our study clarify the specific alterations for glycosylation during the pathogenesis of gastric cancer. The specific high expression of GlcNAc structure may act as a potential early diagnostic marker for gastric cancer.**
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Correspondence:
Zhiming Liu
Department of General Surgery
The First Affiliated Hospital of
Guangxi Medical University
Nanning, Guangxi
China
Phone: +86 07715356701
Email: liuzhiminggy@163.com

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KEY WORDS

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INTRODUCTION

Gastric cancer is a very common cancer with high morbidity and one of the leading causes of cancer deaths worldwide [1]. The World Health Organization (WHO) has reported that there were 951,600 newly diagnosed cases of gastric cancer and 723,000 deaths in 2012 [2].

In China, gastric cancer was the second most common cancer and ranked third in mortality among all malignant tumors with 420,000 new cases and 300,000 deaths annually in 2011 [2]. Although the incidence of gastric cancer has declined, the prognosis and 5-year overall survival of gastric cancer patients still remains unsatisfactory. Surgery and chemotherapy are the primary treatments, while the 5-year overall survival of patients with advanced gastric cancer is only 30% - 50%. Unfortunately, most of the gastric cancer patients are discovered only at the advanced stage because of the lack of markers characterizing early stages, and the diagnostic rate of early gastric cancer is less than 10% [1,3]. Until now, there have been no reliable serologic markers of the early diagnosis, monitoring, and prognosis for gastric cancer. Therefore, it is urgent and necessary to find new markers which can facilitate early diagnosis and increase patient survival.

Protein glycosylation is one of the most common post translational modifications which play an important role in various biological processes, such as cell growth, differentiation, transformation, intercellular signaling, protein-protein interaction, and protein folding [4-6]. Many studies have indicated that aberrant glycosylation was closely associated with the malignant transformation of tumor cells. It was not only involved in carcinogenesis, but also related to tumor development, invasion, and metastasis [7]. Therefore, the glycosylation change of the glycoprotein may be used as a biomarker for tumor diagnosis and therapy. Currently, several serum glycoproteins have been commonly used in the diagnosis and prognosis of gastric cancer including CEA, CA19-9, CA72-4, serum pepsinogen I. CEA is a marker of broad spectrum and it is elevated in all digestive system neoplasms. A previous study reported that the serum CA19-9 level was closely correlated with the invasion, lymph node metastasis, and tumor size of gastric cancer [8]. While the sensitivity and specificity of CEA, CA19-9, and CA72-4 were not very satisfactory for early diagnosis of gastric cancer [8]. Therefore, the discovery of novel glycan-based biomarkers with high sensitivity and specificity for gastric cancer is essential.

Since 2005, lectin microarray has emerged as a new, high-throughput and high-sensitivity technique for the comprehensive analysis of protein glycosylation. Our previous studies used lectin microarray to discovery potential glycan biomarkers for hepatocellular carcinoma (HCC) [9-11]. In the present study, we comprehensively analyzed the glycan profiles of serum samples between gastric cancer and healthy controls using a lectin microarray with 50 kinds of tumor-associated lectins. This study was aimed to obtain a specific serum glycan profiling of gastric cancer which may contribute to tumor early diagnosis and improve patient survival rate.

MATERIALS AND METHODS

Clinical specimens

All the specimens were from the First Affiliated Hospital of Guangxi Medical University (Nanning, China) between April to December 2015. The patients were first diagnosed with gastric cancer [tumor-node-metastasis (TNM) = I,II] by histopathological examination. Patients were excluded if they had undergone any prior radiotherapy and chemotherapy, had any other malignant tumors or other serious primary diseases, or associated acute infection. The control group subjects were also from the Medical Examination Center, First Affiliated Hospital of Guangxi Medical University. Healthy controls have no abnormal findings including routine blood test, the level of serum AST, ALT, CA199, CEA, were negative for *Helicobacter pylori* (*H. pylori*), and had no family history of gastric cancer. Statistical analysis showed no significant difference in gender, age, ethnicity, smoking, alcohol consumption, and body mass index (BMI) between the gastric cancer and control group. This study was approved by Medical Ethics Committee of First Affiliated Hospital of Guangxi Medical University and informed consent was obtained from each participant.

Protein collection and Cy-5-labeling

Fasting blood samples were collected from each participant and centrifuged at 3000 rpm for 10 minutes immediately; the serum samples were isolated and stored at -80°C until use. The high abundance proteins (albumin, immunoglobulins) of pooled serum sample were removed by using ProteoExtract^{TM} Albumin/IgG removal kit (Merk, San Diego, CA, USA) according to the manufacturer's instruction. Then the protein solution was exchanged to 0.1 mol/L Na_2C0_3/$NaHC0_3$ buffer (pH 9.3) through PD-10 desalting column (GE, Bethesda, MD, USA). The concentration of extracted protein was determined by the bicinchonininc acid (BCA) method. The albumin/IgG depleted serum protein was labeled by Lighting-Link Rapid CY5 Conjugation Kit (Innova Bio-Sciences) according to the manufacturer's instructions. First, 1 μL LL-Rapid Modifier Reagent was added to the protein suspension and incubated at room temperature for 15 minutes in dark condition. Then 1 μL LL-Rapid Quencher Reagent was added to the mixture and incubated at room temperature for 4 minutes in dark condition. Cy-5-labeled glycoprotein was stored at 4°C for backup.

Lectin microarray

The protocol of lectin microarray preparation followed the previous publication [12]. A total of 50 kinds of tumor-associated lectins were dissolved in 2% bovine serum albumin (BSA)-TBS solution (pH 7.8) at a concentration of 1 mg/mL and spotted with six repeats. The distance between each spot was 400 μm and the diameter of each spot was 150 μm. After spotting, for the purpose of immobilizing the lectins, the hydrogel slides

(CapitalBio, Beijing, China) were incubated in a vacuum chamber at 25°C overnight. The lectins and their specific binding carbohydrates were listed in Table 1. The lectin microarray was incubated with 2% BSA-TBS at room temperature for 1 hour to block the non-specific binding sites, then washed with 0.1% PBS-Tween20 three times. The Cy-5-labeled protein (11 μg in 80 μL PBS) was added into each well and incubated at room temperature with gentle shaking for 3 hours; then the lectin microarray was washed by 0.1% PBS-Tween20 for 15 minutes three times.

We used a LuxScan 10 K/A scanner system (CapitalBio) to scan the lectin microarray and extracted the data. The fluorescence intensities of each spot were calculated by subtracting background from signal intensity. Repeat data were statistically tested using the Grubbs' outlier method and the replicate data from different microarrays were normalized by median. The fluorescence intensity of each kind of lectin spot binding different samples was analyzed by *t*-test, and $p < 0.05$ was considered as statistically significant.

Lectin blot

Albumin/IgG depleted serum protein was prepared using ProteoExtract™ Albumin/IgG removal kit from 200 μL of 10 gastric cancers and 10 healthy control sera. The bicinchonininc acid (BCA) method was used to detect the concentration of albumin/IgG depleted serum protein. Twenty micrograms of albumin/IgG depleted serum protein were further separated by 10% SDS-PAGE. One gel was stained with Coomassie brilliant blue to contrast the equal amounts of protein and it was used as the internal control. The other gel was transferred onto polyvinylidene fluoride (PVDF) membrane (Millipore, Billerica, MA, USA) using a Bio-Rad SemiDry apparatus. The PVDF membrane was blocked with 3% BSA in TBS-T at room temperature for 1 hour, followed by incubating with biotinylated lectins (LEL 1:400 dilution, PHA-L 1:1,000 dilution, RCA-I 1:4,000 dilution, STL 1:400 dilution) at room temperature for 30 minutes. After washing four times for 15 minutes with TBS-T, the membrane was further incubated with avidin-HRP (1:10,000 dilutions) at room temperature for 30 minutes and then washed with TBS-T four times for 15 minutes. The bands on the membrane were visualized with ECL prime Western Blotting Detection Reagents (GE) and ChemiDoc XRS+ system (Bio-Rad).

Statistical analysis

The statistical analysis was performed using a commercially available statistical software package (SPSS 16.0). Quantitative variables were analyzed by Student's *t*-test. $P < 0.05$ was considered a significant difference.

RESULTS

The glycan profiling of serum glycoprotein in early gastric cancer

Studies have shown that abnormal protein glycosylation was always accompanied by the process of cancer carcinogenesis and development. Therefore, the aim of this study was to find some specific glycan pattern in the development of early gastric cancer using a lectin microarray. All the data were collected from three independent measurements with similar trends. The scan images of serum glycoprotein in healthy controls and early gastric cancer patients for 50 kinds of tumor associated lectins were shown in Figure 1. We discovered that the signal intensity of 13 lectins showed statistically significant differences between the healthy controls and early gastric cancer ($p < 0.05$). The comparison of the binding affinity for each lectin showing with normalized fluorescent intensities and their specific carbohydrates were summarized in Table 2. As shown in Table 2, the normalized fluorescent intensities of PWA and LEL were significantly increased more than three-fold in early gastric cancer compared to the healthy controls. It implied that the specific affinity of N-acetylglucosamine (GlcNAc) showed especially elevated expression in early gastric cancer. Compared to the healthy controls, the binding affinity of eleven lectins (STL, EEL, RCA-I, PHA-L were increased more than two-fold, lectins of UEA, VAL, CAL, RCA-II and DSA were increased one-fold) in the gastric cancer showed significant differences as $p < 0.05$ (Table 2, Figure 2) according to the specific affinity carbohydrates of each lectin. STL can recognize the GlcNAc. EEL and RCA-II prefer to bind to galactosyl (α-1,3) galactose (Gal), N-acetylgalactosamine (GalNAc). PHA-L binds specifically to the glycan with β-1,6-GlcNAc branching structure and tetraantennary complex type oligosaccharides. VAL binds to β-D-Gal residues. UEA and CAL could recognize the α-linked fucose residues and Forssman pentasaccharide (Tn). RCA-I is the binder of terminal Galβ 1-4 GlcNAc, Lac/LacNAc. DSA can specifically recognize the Tri/tetra-antennary N-glycan. This result prompted that those glycan structures mentioned above were elevated in early gastric cancer. On the contrary, the binding ability of CFL and GNL lectins were significantly reduced in early gastric cancer ($p < 0.01$, Figure 2). The N-acetyl-D-galactosamine structure and (α-1,3) mannose residues which were determined by CFL and GNL lectins were decreased in early gastric cancer. The result of lectin microarray hinted at the alteration of protein glycosylation in the development of gastric cancer.

Validation of the differential glycan structures by lectin blot

The result of the lectin microarray showed differences in the protein glycosylation of early gastric cancer compared with the healthy controls. Lectin blot was then performed to validate these differences. The biotin-labeled lectins LEL, PHA-L, RCA-I, and STL in which

Table 1. Lectins and their specific binding carbohydrates.

Number	Lectins	Company	Specific binding carbohydrates
1	Aleuria aurantia lectin (AAL)	Vector	Terminal αFuc and ± Sia-Le
2	Agaricus bisporus lectin (ABL)	Sigma	Galβ1-3GalNAcα1-Ser/Thr (T-Antigen), Siaα2-3(6) Galβ-1-3GalNAcα1-Ser/Thr
3	Amaranthus caudatus lectin (ACL)	Vector	galactosyl (β-1,3) N-acetylgalactosamine structure
4	Bauhinia purpurea lectin (BPL)	Vector	galactosyl (β-1,3) N-acetylgalactosamine structure
5	Caragana arborescens lectin (CAL)	Sigma	Forssman pentasaccharide Tn
6	Codium fragile lectin (CFL)	Sigma	N-acetyl-D-galatosamine
7	Concanavalin A (Con A)	Vector	α-man biantennary complex-type oligosaccharides
8	Cytisus scoparius Lectin (CSL)	Sigma	D-galactose and by N-acetyl-D-galactosamine
9	Dolichos biflorus agglutinin (DBA)	Vector	α-linked N-acetylgalactosamine
10	Datura stramonium agglutinin (DSA)	Vector	Tri/tetra-antennary, (GlcNAc)n, polyLacNAc and LacNAc(1NA3, NA4)
11	Erythrina cristagalli lectin (ECL)	Vector	galactosyl (β-1,4) N-acetylglucosamine
12	Euonymus europaeus lectin (EEL)	Vector	galactosyl (α-1,3) galactose
13	Galanthus nivalis lectin (GNL)	Vector	(α-1,3) mannose residues
14	Griffonia simplicifolia lectin I (GSL I)	Vector	α-N-acetylgalactosamine residues, α-galactose residues
15	GSL I - isolectin B4 (GSL1b4)	Vector	α-N-acetylgalactosamine residues, α-galactose
16	Griffonia simplicifolia lectin II (GSL II)	Vector	α- or β-linked N-acetylglucosamine residues
17	Helix aspersa lectin (HAL)	Sigma	terminal N-acetyl-α-D-galactosaminyl residues
18	Hippeastrum hybrid lectin (HHL)	Vector	(α-1,3) and (α-1,6) linked mannose structures
19	Helix pomatia lectin (HPL)	Sigma	α-N-acetyl-D-galactosamine
20	Jacalin (JAC)	Vector	galactosyl (β-1,3) N-acetylgalactosamine
21	Lens culinaris agglutinin (LCA)	Vector	Fucα1-6GlcNAc and α-Man, α-Glc, GlcNAc-Asp of the trimannosyl core
22	Lycopersicon esculentum lectin (LEL)	Vector	N-acetylglucosamine
23	Limulus polyphemus lectin (LPL)	Sigma	N-acetylated D-hexosamines
24	Lotus tetragonolobus lectin (LTL)	Vector	α-linked L-fucose
25	Maackia amurensis lectin I (MAL I)	Vector	Siaα2-3Gal
26	Maackia amurensis lectin II (MAL II)	Vector	Siaα2-3Gal
27	Maclura pomifera lectin (MPL)	Vector	Galβ1-3GalNAcα-Ser(T)and GalNAcα-Thr/Ser(Tn)
28	Naja mossambica lectin (NML)	Sigma	exopolysaccharide
29	Narcissus pseudonarcissus lectin (NPL)	Vector	polymannose structures containing (α-1,6) linkages
30	Peanut agglutinin (PNA)	Vector	galactosyl (β-1,3) N-acetylgalactosamine
31	Phytolacca americana lectin (PAL)	Sigma	β-GlcNac
32	Phytolacca americana (PWA)	Sigma	(GlcNacβ4) n
33	Phaseolus coccineus lectin (PCL)	Sigma	sialic acid
34	Phaseolus vulgaris Erythroagglutinin (PHA-E)	Vector	NA2 and bisecting GIcNac
35	Phaseolus vulgaris Leucoagglutinin (PHA-L)	Vector	β-1,6 branching structure, Tetraantennary complex oligosaccharides
36	Pisum sativum agglutinin (PSA)	Vector	α-linked mannose-containing oligosaccharides
37	Psophocarpus tetragonolobus lectin I (PTL I)	Vector	α-linked galactosamine
38	Psophocarpus tetragonolobus lectin II (PTL II)	Vector	β anomeric configuration
39	Ricinus communis agglutinin I (RCA-I)	Vector	Lac/LacNAc, Terminal Galβ 1-4 GlcNAcβl

Table 1. Lectins and their specific binding carbohydrates (continued).

Number	Lectins	Company	Specific binding carbohydrates
40	Ricinus communis agglutinin II (RCA-II)	Vector	GalNAc, N-acetylgalactosamine
41	Ricin B Chain (RIC)	Vector	galactose/N-acetylgalactosamine
42	Soybean agglutinin (SBA)	Vector	terminal α- or β-linked N-acetylgalactosamine
43	Sophora japonica agglutinin (SJA)	Vector	N-acetylgalactosamine and galactose residues
44	Sambucus nigra lectin (SNA)	Vector	Siaα2-6Gal/GalNAc
45	Solanum tuberosum lectin (STL)	Vector	oligomers of N-acetylglucosamine
46	Ulex europaeus agglutinin (UEA)	Vector	α-linked fucose residues
47	Viscum album lectin (VAL)	Sigma	β-D-galactosyl residues
48	Vicia villosa lectin (VVL)	Vector	α- or β-linked terminal N-acetylgalactosamine
49	Wisteria floribunda lectin (WFL)	Vector	N-acetylgalactosamine linked α or β to the 3 or 6 position of galactose
50	Wheat germ agglutinin (WGA)	Vector	(GlcNAc)n, multivalent Sia and GalNAc

Table 2. Comparison of different lectin affinity glycan profiles of serum glycoprotein in gastric cancer and healthy controls (normalized fluorescent intensities, mean ±SD).

Lectins	Specificity	Healthy controls	Gastric cancer	Gastric cancer/ healthy controls	p-value
PWA	(GlcNAcβ4) n	634.25 ± 175.36	3,040.17 ± 1,038.65	4.79	$p < 0.01$
LEL	N-acetylglucosamine	601.58 ± 121.55	1,865.5 ± 754.55	3.10	$p < 0.01$
RCA-I	Lac/LacNAc, Terminal Galβ 1-4 GlcNAcβl	159.00 ± 14.30	373.75 ± 134.17	2.35	$p < 0.01$
PHA-L	β-1,6 branching structure, Tetraantennary complex oligosaccharides	336.17 ± 21.34	713.08 ± 50.19	2.12	$p < 0.01$
STL	oligomers of N-acetylglucosamine	182.67 ± 43.79	380.67 ± 35.67	2.08	$p < 0.01$
EEL	galactosyl (α-1,3) galactose	967.83 ± 215.50	1,977.5 ± 416.69	2.04	$p < 0.01$
RCA-II	GalNAc, N-acetylgalactosamine	10,113.67 ± 638.73	14,857.83 ± 493.6	1.47	$p < 0.01$
CAL	Forssmanpentasaccharide (T/Tn)	207.75 ± 32.62	264.75 ± 22.02	1.27	$p < 0.01$
DSA	Tri/tetra-antennary, (GlcNAc)n, polyLacNAc and LacNAc (1NA3, NA4)	1,536.5 ± 105.64	1,955.42 ± 153.77	1.27	$p < 0.01$
UEA	α-linked fucose residues	430.83 ± 65.14	528.08 ± 38.14	1.23	$p < 0.05$
VAL	β-D-galactosyl residues	8,628.75 ± 357.21	10,285.25 ± 1,060.16	1.19	$p < 0.01$
CFL	N-acetyl-D-galatosamine	635.08 ± 35.98	433.17 ± 13.97	0.68	$p < 0.01$
GNL	(α-1,3) mannose residues	617.42 ± 62.39	232.17 ± 47.57	0.38	$p < 0.01$

$P < 0.05$ was considered significant difference.

the normalized fluorescent intensities were significantly increased more than two-fold were subsequently selected for further validation (Table 2). Albumin/IgG depleted serum protein was separated by 10% SDS-PAGE and stained by Coomassie brilliant blue. The gray values of the two groups were basically the same (Figure 3). The results of lectin blot showed that the total bands for lectins LEL and STL and their binding GlcNAc were markedly increased in early gastric cancer. Similarly, the results of lectins PHA-L and RCA-I were consistent with lectin microarray. Summing up, there was some differential glycan pattern in the development of

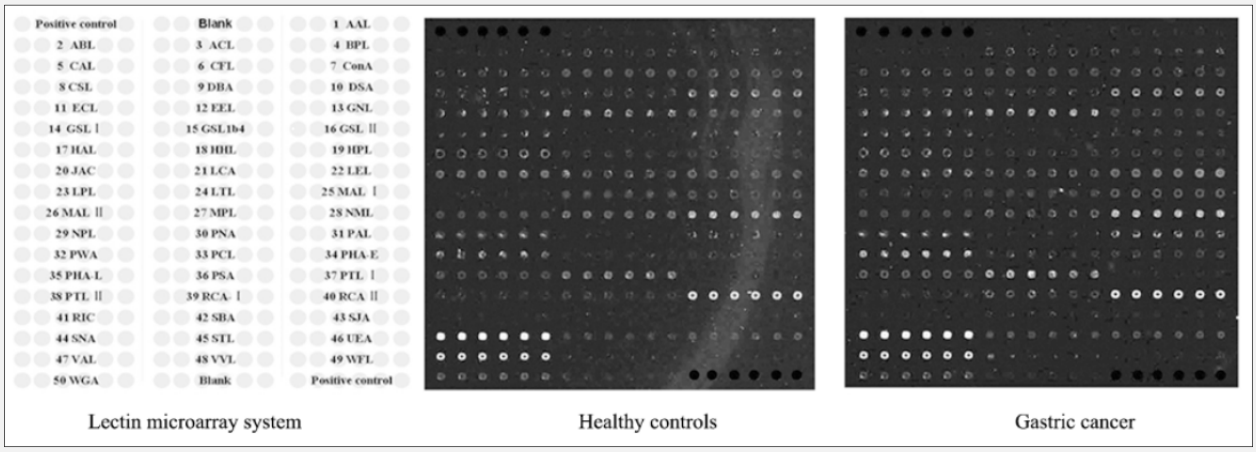

Figure 1. Lectin microarray system; arrangement diagram of 50 tumor associated lectin (left), affinity signal of each lectin for healthy controls and gastric cancer were scanned with fluorescence scanner (middle and right).

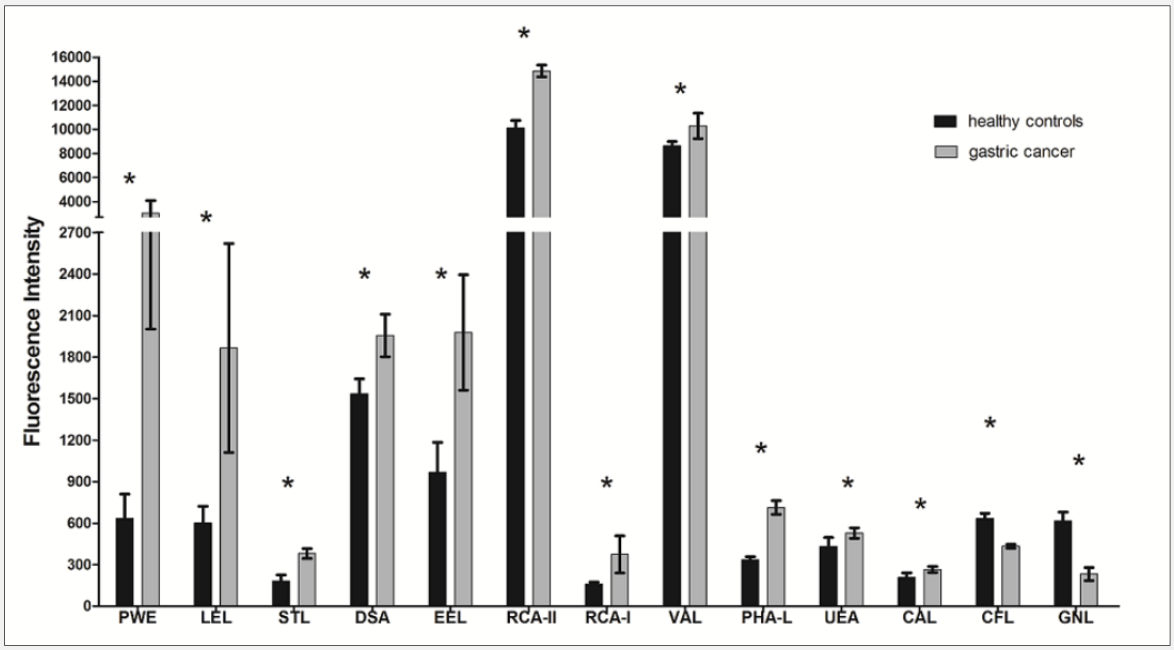

Figure 2. The differential glycan profiling of serum glycoprotein in healthy and gastric cancer cases by bar graph based on lectin microarray data.

Data are the average ± SD of three independent measurements. * represent that $p < 0.05$.

gastric cancer and the specific high expression of GlcNAc structure may act as a potential early diagnostic marker for gastric cancer.

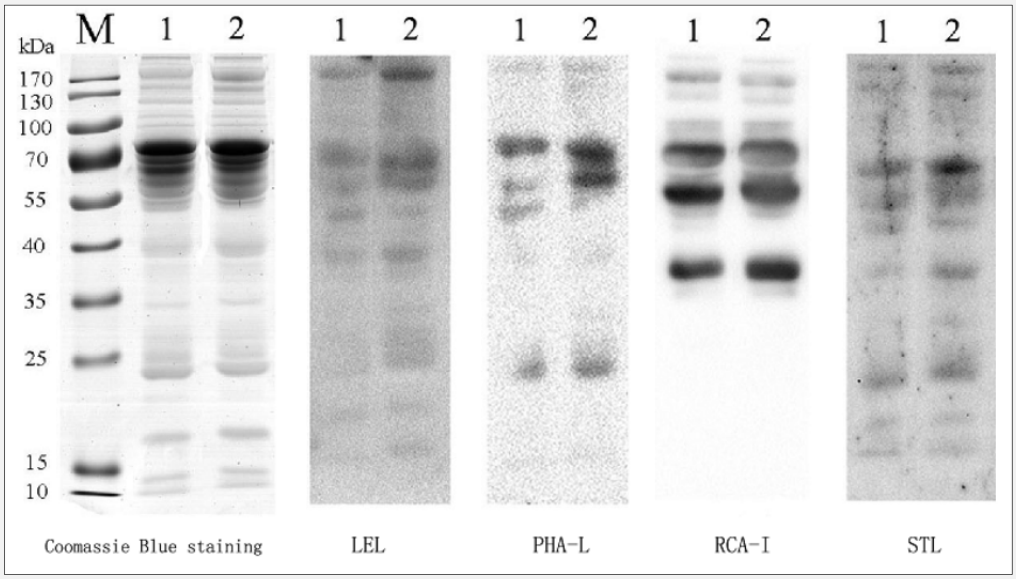

Figure 3. Lectin blot for biotin labeled lectins LEL, PHA-L, RCA-I, and STL (M - marker, 1 - healthy, 2 - gastric cancer).

DISCUSSION

Gastric cancer was the second leading cause of cancer death. The development of gastric cancer is a multi-factorial and multi-step process, including superficial gastritis, atrophic gastritis, intestinal metaplasia, dysplasia, and finally, carcinoma [13]. Abnormal protein glycosylation always accompanied this process. *H. pylori* infection can lead to chronic inflammation by recognizing Leb and H type 1 carbohydrate structures, meanwhile increasing the expression of N-acetyl aminotransferase V (β3GnT5) and Sialyl-Lewis X (Sialyl-LeX) [14, 15]. Previous studies had reported that the A3F1G1 with Sialyl-LeX and N-glycan with fucosylation might be the potential biomarkers for early diagnosis of gastric cancer, the sensitivity and specificity were higher than CA19-9 [16,17]. In this study, we used a lectin microarray to compare the glycan profiles of early gastric cancer and healthy controls. The result of lectin microarray indicated that the glycosylation levels were different between early gastric cancer and healthy controls. Actually, lectin is a kind of glycoprotein which can bind specific glycan structures [18]. As the development of glycomics and glycoproteomics progresses, lectin has been widely used to detect and enrich glycoconjugate. Lectin microarray is a new carbohydrate analysis technology which is rapid and has high-sensitivity and high-throughput and also great potential in cancer biomarker studies. Here, the lectin microarray was established by our laboratory with 50 kinds of tumor-associated lectins. The coefficient of variation of the fluorescence signal was less than 10% when the spotting concentration of lectins was 1 mg/mL. It demonstrated that the lectin microarray system has good reproducibility. Besides, the accuracy of lectin microarray results was verified by detecting the N-glycan structure of standard glycoprotein alpha-fetoprotein and fetuin [12]. The preliminary studies of our research group used lectin microarray to discover potential glycan biomarkers for HCC. Zhang [19] found that the fucosylated Hp-beta subunit might be a potential marker for liver cirrhosis and HCC. Li et al. [10] used this lectin microarray to detect cell surface glycan alterations in the epithelial mesenchymal transition (EMT) process of Huh7 HCC cells. Jing et al. [11] showed that the affinity signals of 14 lectins were increased in HCC patients when this lectin microarray was used to identify specific serum glycan profiles of carcinogenic processes for HCC. To sum up, the results of previous studies confirm that this lectin microarray system was reliable.

In the present study, the lectin microarray was used to compare the glycan profiles of early gastric cancer and healthy controls. We observed that lectins of PWA, LEL, and STL had significantly increased signals in early gastric cancer, and the GlcNAc which was specifically identificated by these lectins showed especially elevated expression in early gastric cancer. The Tri/tetra-antennary N-glycan which was recognized by DSA was also increased in early gastric cancer. Similarly, we also discovered that the affinity of EEL, RCA-I, RCA-II, and VAL were enhanced in early gastric cancer patients compared to health controls. That is to say, galactose

(Gal) and N-acetylgalactosamine (GalNAc) structures were up-regulated in gastric cancer. Previous studies had reported that GlcNAc and GalNAc were highly expressed in the cell surface of HCC and endometrial cancers and were closely related to cancer development and progression [10,20]. In gastric cancer, Huang et al. suggested that the glycosylation level was higher than in ulcers, and GalNAc bound to MPL and VVA showed a significant increase [21]. Consistent with a previous study, our result showed that GlcNAc and GalNAc structures were highly expressed in early gastric cancer patients. Moreover, we discovered that GlcNAc structure was markedly increased in early gastric cancer. LEL and STL lectin blot confirmed this result. Therefore, the specific high expression of GlcNAc structure might act as a potential early diagnostic marker for gastric cancer.

The synthesis of glycan was catalyzed by a series of glycosyltransferases and glycosides. N-acetylglucosaminyltransferase V (GnT-V) was a key glycosyltransferase involved in cancer metastasis, which could catalyze the GlcNAc β 1-6 branches to N-glycan at the Manα1-6 side of the trimannosyl core. Studies had already confirmed that β-1,6-GlcNAc branching structure was increased in the process of tumor cell malignant transformation and promoted the development of colon cancer by regulating the Wnt pathway [22,23]. Pinho's study found that the β-1,6-GlcNAc branching structure of E-cadherin was increased in the cells of gastric cancer tissue [24]. The up-regulated expression of GnT-V destroyed the stability of the combination for catenin and N-glycan promoting cancer metastasis [25]. In our study, we captured that the serum glycoprotein in gastric cancer has a stronger affinity to PHA-L both by lectin microarray and lectin blot technology. It suggested that β-1,6-GlcNAc branching structure was highly expressed in gastric cancer.

Studies have demonstrated that fucosylation was expressed in many tumors, such as HCC, lung cancer, and breast cancer [26-28]. It is well known that the fucosylated AFP, AFP-L3, is a specific marker for diagnosis of HCC [26]. There is evidence that the core fucose was closely related to cancer proliferation, invasion, and metastasis. However, Liu and Zhao's studies showed that the core fucose and FuT8 were decreased in gastric cancer contrary to previous findings [29-30]. The present study did not find the differential expression of core fucose between gastric cancer and health controls, but the α-linked fucose residues are highly expressed in gastric cancer which might be the potential glycan marker for gastric cancer. The forssman pentasaccharide (Tn) antigen and sialyl-Tn (STn) have high levels in a majority of carcinomas, such as gastric cancer, colorectal cancer, breast cancer [21,31,32]. Tn and STn could be used as specific markers as they appear in the early stage of tumors and not in normal tissue. We discovered that the affinity signal of lectin CAL and its identifying structure Tn antigen were up-regulated in gastric cancer serum samples. It is consistent with previous studies which found T, sialyl-T, and Tn-antigen were elevated in both tissue and serum samples of gastric cancer [21, 33].

The results of present study also showed that the N-acetyl-D-galactosamine structure and (α-1,3) mannose residues were down-regulated in gastric cancer compared to healthy controls. Previous studies indicated that the activity of mannosidase was decreased in many types of cancer, causing decreased trimming of high mannose structures and augmented branching of the high mannose core in tumor secreted glycoproteins [34]. Huang et al. reported that (α-1,3) Man binder GNA had decreased signals in ulcers compared to gastric cancer [21]. Ozcan's study showed that the high mannose oligosaccharides were down-regulated in gastric cancer compare to gastritis [35]. The causes of inconsistent results regarding the mechanism of glycosylation were not clear and many factors could influence this process. Future work needs intensive research into the mechanism of glycan involved in the occurrence and development of gastric cancer.

CONCLUSION

In conclusion, we successfully screened the glycan profiling of serum glycoprotein in early gastric cancer using a lectin microarray. The GlcNAc, GalNAc, Tri/tetra-antennary N-glycan, β-1,6-GlcNAc branching structure, α-linked fucose residues, and Tn antigen were increased, while the N-acetyl-D-galactosamine structure and (α-1,3) mannose residues were decreased in early gastric cancer. The GlcNAc structure was markedly increased in early gastric cancer, both in the result of lectin microarray and lectin blot, which may act as a potential early diagnostic marker for gastric cancer.

Declaration of Interest:
The authors declared that there are no conflicts of interest regarding the publication of this research.

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