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ORIGINAL ARTICLE

Glycosaminoglycans and glycolipids as potential biomarkers in lung cancer

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Abstract In this report, we used liquid chromatography-mass spectrometry and Western blotting to analyze the content and structure of glycosaminoglycans, glycolipids and selected proteins to compare differences between patient-matched normal and cancerous lung tissues obtained from lung cancer patients. The cancer tissue samples contained over twice as much chondroitin sulfate (CS)/dermatan sulfate (DS) as did the normal tissue samples, while the amount of heparan sulfate (HS) and hyaluronan (HA) in normal and cancer tissues were not significantly different. In HS, several minor disaccharide components, including NS6S, NS2S and 2S were significantly lower in cancer tissues, while the levels of major disaccharides, TriS, NS and 0S disaccharides were not significantly different in normal and cancer tissues. In regards to CS/DS, the level of 4S disaccharide (the major component of CS-type A and DS) decreased and the level of 6S disaccharide (the major component of CS- type C) increased in cancer tissues. We also compared the content and structure of GAGs in lung tissues from smoking and non-smoking patients. Analysis of the glycolipids showed all lipids present in these lung tissues, with the exception of sphingomyelin were higher in cancer tissues than in normal tissues. Western analysis showed that syndecan 1 and 2 proteoglycans displayed much higher expression in cancer tissue/biopsy samples. This investigation begins to provide an understanding of patho-physiological roles on glycosaminoglycans and glycolipids and might be useful in identifying potential biomarkers in lung cancer.

Keywords Glycosaminoglycans · Glycolipids · Glycoprotein · Compositional analysis · Biomarker · Lung cancer

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Introduction

Lung cancer is one of the leading causes of mortality and the most common cause of cancer death worldwide [1]. Only 15% of lung cancer cases are diagnosed at an early stage and over half of the patients diagnosed with lung cancer die within one year [2]. A recent study reported that more than 50% of non-small cell lung carcinoma (NSCLC) are positive for one of 10 known molecular biomarkers (i.e. Kirsten rat sarcoma virus 2, Epidermal growth factor receptor, etc.) [3]. While the development of various '-omics' techniques, including genomics, proteomics [4, 5] has made it possible to discover identity of promising candidates as prognostic biomarkers and therapeutic

targets, the glycomics of cancer is less well established [6–9]. Despite significant effort, information on composition of glycoconjugates, such as proteoglycans (PGs) and glycolipids (GLs), in cancerous lung tissue remains limited.

PGs are a subset of glycoproteins and represent a major component of the extracellular matrix consisting of a core protein to which one or more glycosaminoglycan (GAG) chains are covalently attached. GAGs are ubiquitous in animal cells and regulate cellular behavior and function by interacting with other extracellular matrix components, cell surface receptors, growth factors, and cytokines [10]. As glycosylation is one of the most common types of posttranslational modifications, the presence and structure of glycans can determine protein localization and function.

Abnormal glycosylation, including the altered structure and expression of glycans, is a characteristic feature associated with cancer transformation and progression [11–14]. In lung cancer, numerous alterations in glycosylation have been described, including aberrant expression and glycosylation of mucins, elevated level of *O*-glycans, altered branching of *N*-glycans, and increased presence of sialic acid on glycoproteins and glycolipids [15]. There has been a report of alterations and decreased expression of PGs, such as glypican 3 [16, 17], and the overexpression of lumican [18], the increased expression Sialyl lewis X (SLe) and sialylated glycosphingolipids [19] detected in tissue or serum derived from lung cancer patients as considerable biomarkers.

The aim of this study is to perform an extensive analysis for GAGs, GLs and expression levels of selected proteins in matched samples from both normal human lung and cancerous human lung tissues. An integrated approach for the characterization of potential biomarkers in lung cancer is suggested.

Materials and methods

Tissues preparation

Tumor and corresponding normal lung tissue specimens were obtained from Korean patients with non-small cell lung cancer (NSCLC). Ten patients with squamous cell carcinomas who underwent curative resection at the Konyang University Hospital (Daejeon, South Korea) were analyzed (Table 1). None of the patients had received chemotherapy or radiotherapy prior to surgery and four out of ten patients were smokers. Informed consent was obtained from each patient. The Bioethics Committee of Konyang University Hospital approved this study. All of the tumor and macroscopically normal lung tissue samples were obtained at the time of surgery and were rapidly frozen in liquid nitrogen and stored at −80 °C until analysis. Tissue samples were confirmed histologically by hematoxylin-eosin staining.

Sample preparation for glycolipid and GAG analysis

Lung tissues obtained from Konyang University Hospital were lyophilized, processed by homogenization and separated into portions for protein analysis, Western blot analysis, GAG and GL analysis.

Tissues for lipid and GAG analysis, homogenized lung tissues were serially extracted with chloroform-methanol mixtures of increasing polarity [(2:1 solution A; 1:1 solution B; 1:2 solution C)]. Tissues were submerged twice into solvent mixture for 24 h each time as previously described [6]. Solutions A, B and C were combined and solvent was evaporated and stored in the freezer for GL analysis. All sample solid residues were then allowed to completely dry for 48 h. For GAG disaccharide analysis, dried tissues were proteolyzed by Actinase E at 55 °C for two days then applied to Vivapure Mini Q-H spin columns (Sartoriou Stedim, Bohemia, NY) to isolate negatively charged GAGs. After loading and washing steps with 100 mM NaCl, GAGs were recovered from the spin columns by washing with 16% aq. NaCl. GAGs released with 16% NaCl were precipitated by adding 80 vol% methanol and incubating overnight in an explosion-proof refrigerator at 4 °C. The precipitated GAGs were recovered by centrifugation and dissolved in water for analysis.

Disaccharide compositional analysis of GAGs by liquid chromatography-mass spectrometry (LC-MS)

Briefly, isolated GAGs were digested overnight with a mixture of chondroitinase ABC (10 mU) and ACII (5 mU) at 37 °C and the disaccharide products were recovered in the flow-thru by 10 kDa MWCO centrifugal filtration using the spin column (Millipore, Billerica, MA). The remaining undigested GAGs in the column retentate, were then incubated overnight with a mixture of heparinase I, II, and III (10 mU each) at 35 °C. The resulting disaccharides were similarly isolated by centrifugal filtration. The isolated and freeze-dried samples were labeled by reductive amination by 2-aminoacridone (AMAC) to improve sensitivity and accuracy, and LC-MS analysis was performed as described previously [20]. Unsaturated disaccharides standards of chondroitin sulfate (CS) 0S, ΔUA(1 → 3)-GalNAc; 4S, ΔUA(1 → 3)GalNAc4S; 6S, ΔUA(1 → 3)GalNAc6S; 2S, ΔUA2S(1 → 3)GalNAc; 2S4S or SB, ΔUA2S(1 → 3)GalNAc4S; 2S6S or SD, ΔUA2S(1 → 3)GalNAc6S; 4S6S or SE, ΔUA(1 → 3)GalNAc4S6S; and TriS, ΔUA2S(1 → 3) GalNAc4S6S; unsaturated disaccharides standards of heparan sulfate (HS) 0S, ΔUA(1 → 4)GlcNAc; NS, ΔUA(1 → 4)GlcNS; 6S, ΔUA(1 → 4)GlcNAc6S; 2S, ΔUA2S(1 → 4)GlcNAc; NS2S, ΔUA2S(1 → 4)GlcNS; NS6S, ΔUA(1 → 4)GlcNS6S; 2S6S, ΔUA2S(1 → 4)GlcNAc6S; and TriS, ΔUA2S(1 → 4)GlcNS6S; and unsaturated disaccharides standard of hyaluronan (HA) ΔUA(1 → 3)GlcNAc were obtained from Iduron Co (Manchester, UK).

Table 1 Clinical characteristics of patients providing tissues for studies

Patient number	Sample number	Tissue	T (tumor size)	N (lymph node)	M (metastasis)	Stage	Age/Sex	Smoking
1	1 2	Normal Cancer	2a	0	0	IB	74/M	N
2	3 4	Normal Cancer	1b	0	0	IA	74/M	Y
3	5 6	Normal Cancer	2b	0	0	IIA	69/M	N
4	7 8	Normal Cancer	1a	0	0	IA	55/M	N
5	9 10	Normal Cancer	3	1	0	IIIA	59/M	Y
6	11 12	Normal Cancer	2a	0	0	IB	69/M	N
7	13 14	Normal Cancer	2a	0	0	IB	76/M	N
8	15 16	Normal Cancer	2a	1	0	IIA	70/M	Y
9	17 18	Normal Cancer	2b	1	0	IIB	82/M	Y
10	19 20	Normal Cancer	2a	0	0	IB	73/F	N

Lipid analysis by ultra-high performance hydrophilic interaction liquid chromatography

A UP-HILIC column was used to fractionate the glycolipids extracted in the chloroform methanol washer. Lipid samples were dissolved and diluted 10-fold in 1:1 mixture of mobile phase A (5 mM ammonium acetate in high performance liquid chromatography grade acetonitrile with 2% of HPLC grade acetic acid) and B (5 mM ammonium acetate in HPLC grade methanol with 2% of HPLC grade acetic acid.). The LC column was directly connected to electrospray ionization source of LTQ-Orbitrap XL FTMS. The optimized conditions used for mass spectrometric analysis were determined previously [6]. Quantification analysis of disaccharides was performed using calibration curves established by separation of increasing amounts of unsaturated disaccharide standards. The total amounts of each type GAGs were calculated based on the amount of disaccharides.

Protein detection by Western blotting

Dried lung tissue samples were homogenized and lysed in Nonidet-P40 lysis buffer (10 mM Tris-Cl 10 mM NaCl, 3 mM $MgCl_2$, 0.5% Nonidet P-40 (NP-40), pH 7.4) containing a cocktail of protease and phosphatase inhibitors on ice. Total protein (10 μg) was loaded and separated on 4–15% gradient SDS-PAGE and transferred to nitrocellulose membrane for further incubation with primary antibodies for the proteins of interest. Beta-actin was used as a loading control. Proteins of interest were detected using relevant primary and HRP conjugated secondary antibodies, followed by chemiluminescent (Pierce) exposure on high performance chemiluminescence film (GE Healthcare, Little Chalfont, UK). Primary antibodies used were anti-carcinoembryonic antigen (CEA, Cat # ab183365, Abcam, Cambridge, MA), anti-epidermal growth factor receptor (Cat # ab40815, Abcam), anti-decorin (Cat # ab67449, Abcam), anti-syndecan 1 and 2 (Cat # ab181789 and ab205884, Abcam), antiglypican 1 and 3 (Cat # ab199343 and ab66596, Abcam) and anti-β-actin (Cat # ab8227, Abcam).

Statistical analysis

Data are expressed as mean ± standard error (SE). Statistical analysis was performed using Student's t-test (GraphPad, San Diego, USA). All results were considered statistically significant at $*p < 0.05$ versus the normal tissue.

Results

Total GAG and disaccharide analysis

The differences examined in the composition of GAGs from the normal part and cancerous part of lung tissue disaccharide analysis was performed by LC-MS. In lung tissues the total GAG content was 1–10 mg/g of dry tissue (Table 2). The most prominent GAG present was

Table 2 Summary of GAG analysis in lung tissue samples

	Relative peak area (%)								Total amount (mg/g tissue)		
CS/DS	TriS	2S4S	2S6S	4S6S	2S	4S	6S	0S	CS/DS	HA	
Normal	-	0.02 ± 0.02[1]	0.06 ± 0.04	0.41 ± 0.09	-	79.63 ± 2.06	16.72 ± 2.08	3.18 ± 0.32	1.90 ± 0.44	1.30 ± 0.26	
Cancer	-	-[2]	0.01 ± 0.01	0.72 ± 0.29	-	67.24 ± 3.06**	27.84 ± 2.20**	4.18 ± 1.21	5.18 ± 0.92**	2.24 ± 0.68	
HS	TriS	NS6S	NS2S	2S6S	NS	6S	2S	0S	HS		
Normal	11.15 ± 1.76	5.23 ± 0.91	9.61 ± 1.11	0.24 ± 0.16	21.62 ± 1.85	2.50 ± 0.29	0.74 ± 0.05	48.91 ± 3.49	0.78 ± 0.11		
Cancer	12.25 ± 2.77	2.26 ± 0.32**	7.46 ± 0.90*	-	25.66 ± 1.44	1.77 ± 0.19	0.56 ± 0.09*	50.03 ± 2.89	0.82 ± 0.17		

[1] average ± standard error [2] not detected under these conditions; **$p < 0.001$, *$p < 0.05$ compared with normal Tissue

the CS family which includes DS, followed by HA and finally by HS. The amount of HS and HA in normal and cancer tissues were not significantly different. In contrast, the cancer tissue samples contained over twice as much CD/DS as the normal lung tissue samples (Fig. 1). Next we examined the disaccharide composition of normal and cancer tissues (Table 2, Figs. 2 and 3). In HS, the major disaccharides, TriS and 0S were not significantly different in normal and cancer tissues; minor disaccharides, NS6S, NS2S and 2S in cancer tissues were all lower than in normal tissues. In CS/DS, the major CS/DS disaccharides were 4S and 6S. The level of 4S decreased and the level of 6S increased in cancer tissues.

Comparison of total GAGs and composition in normal lung tissue and lung cancer tissue from smoking and non-smoking patients

This was a retrospective analysis on a limited quantity of ten matched cancer and normal lung tissue samples from 10 patients. The only information available on these patients is presented in Table 1. While some of the patients are listed as smokers we have no detailed history on this behavior.

With the set of GAG analysis data, the total GAGs and compositions were further compared in normal lung tissue and lung cancer tissue from smoking and non-smoking patients (Figs. 4 and 5). The amount of HS and HA in normal and cancer tissues from smoking patients were increased, while the CS amount was not significantly different. In HS, the Tri S and NS6S were in normal and cancer tissues from smoking patients were increased, 0S was decreased. In CS/DS composition, no significant difference was observed in smoking and non-smoking patients.

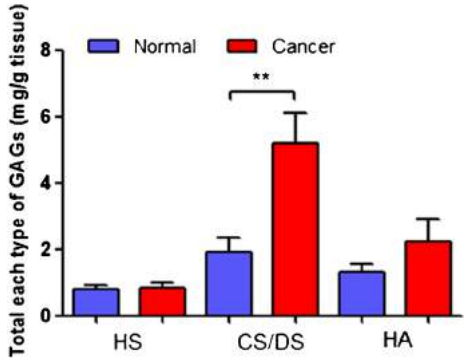

Fig. 1 Comparison of total GAGs in normal lung tissue and lung cancer tissue: N: Normal tissue, C: cancer tissue (**$p < 0.001$)

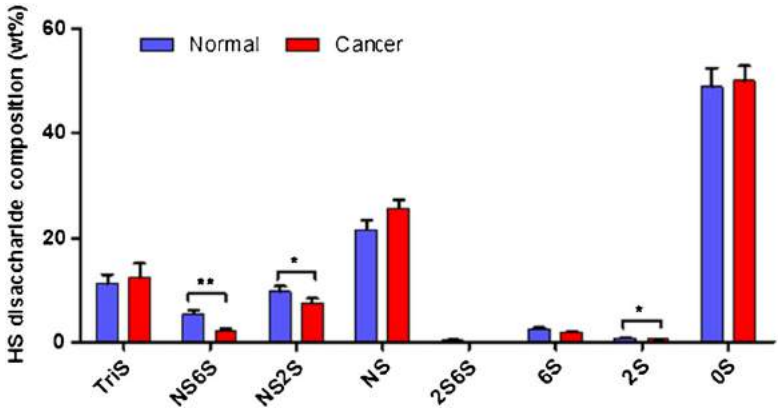

Fig. 2 Comparison of HS composition in normal lung tissue and lung cancer tissue: N: Normal tissue, C: cancer tissue (**$p < 0.001$, *$p < 0.05$)

Glycolipid analysis

The GL content was measured by relative ion abundance using UP-HILIC column [6]. In patient samples, all signals of lipid structures except for sphingomyelin (SM) were higher in cancer tissues than in normal tissues. Ten lipid structures, GL1, ceramides (Cer), phosphoethanolamine (LPE) (**$p < 0.001$) and monosialic ganglioside (GM3), phosphatidylcholine (PC3) (*$p < 0.05$) showed statistically significant differences (Fig. 6). The substructures represented by chain length were also found to higher responses in cancer samples. In particular, 10 of 10 patient samples for GL1, Cer, and LPE showed increases in cancer tissues. Phosphatidylcholines, PC1 and PC2, held the first and second place in a relative ion lipid content from both normal and tissue samples, the amount of substructure pattern detected between normal and cancer was not consistent and each ion response from each individual showed large variations. In the case of SM, average of each subunit collected from each patient was significantly higher in normal biopsy samples except C16 (data not shown.) GL1, one potential biomarker, revealed much higher levels in cancer samples, based on analysis of both an absolute lipid response (38,000 in normal versus 120,000 in cancer) and a relative content (Cer > GL2 > GL1 (0.33%) in normal versus GL1 (0.85%) > Cer > GL2 in cancer).

Proteoglycan protein expression levels

Three sets from the ten sets tissue samples, including normal and cancer samples, were available in sufficient quantities for analysis by Western blotting (Fig. 7). The quantity of β-actin (control) observed in each sample was similar and was used to normalize the amounts of the other proteins observed by Western analyses. The protein expression levels of epidermal growth factor receptor (EGFR) and carcinoembryonic antigen (CEA), well-characterized tumor markers were studied first. Cancer samples from two of the three patients showed elevated levels of EGFR and cancer samples from all three patients showed increased levels of CEA. Decorin as a CS/DSPG and syndecan as a HS/CSPG and glypicans as HSPGs were also tested as proteoglycans known to show their altered expression in lung cancers. The 48 kDa of the CS/DSPG decorin core protein was similar in all the samples, however when glycosylated forms (between 60 and 100 kDa) were taken into account

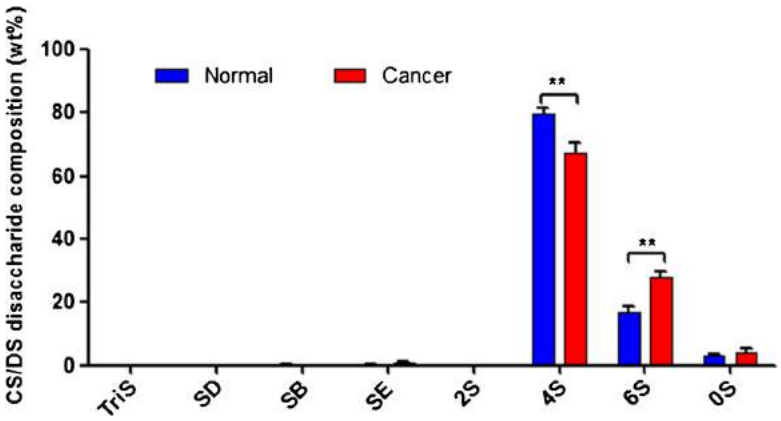

Fig. 3 Comparison of CS composition in normal lung tissue and lung cancer tissue: N: Normal tissue, C: cancer tissue (**$p < 0.001$)

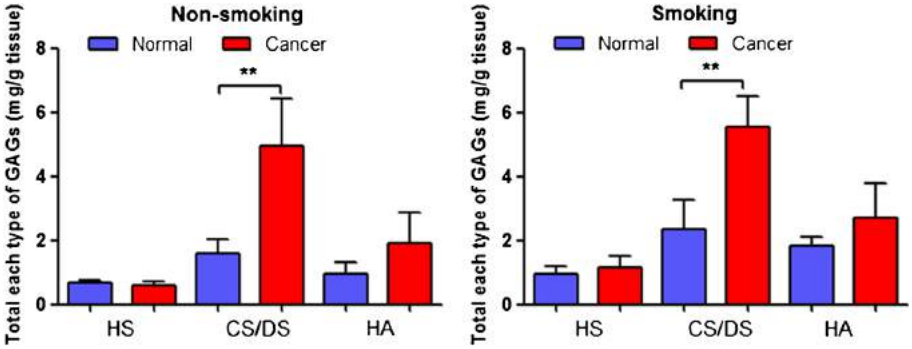

Fig. 4 Comparison of total GAGs in normal lung tissue and lung cancer tissue from smoking and non-smoking patients: N: Normal tissue, C: cancer tissue (**$p < 0.001$)

all three normal tissue were showed slightly higher decorin content than the cancer samples. While no clear trends were observed in HS/CSPGs syndecan 1 and 2, HSPGs glypican 3 and 5 were slightly elevated in all three of the cancer tissues.

Discussion

We investigated the glycosaminoglycans (GAGs), glycolipids, and proteoglycans (PGs) in lung cancer using human biopsy samples for potential carbohydrate-based biomarkers. This involves a retrospective analysis of a limited quantity of ten matched cancer and normal lung tissue samples from 10 patients who were smokers or non-smokers (Table 1). Little is known with respect to smoking and PG/GAG composition. Airway inflammation in smoking is CD44-dependent in mice [21]. Moreover, the lung ECM composition (including PGs) in smokers with chronic obstructive pulmonary disease was different than from non-smokers [22]. Smoking is a well-known addictive behavior and there is some correlation between addiction and PGs [23, 24]. Unfortunately no additional details of the smoking history of the patients providing tissues in this study are available. Mass spectrometric analysis was carried out for the quantitative detection of GAGs and glycolipids and Western blotting used to detect proteoglycans and proteins. While we had sufficient quantities of samples to conduct glycomics analysis on all 10 matched samples, only three sets of these matched samples were available in sufficient quantities to also conduct Western analyses.

The GAG analysis showed: i) the cancer tissue samples contained over twice as much chondroitin sulfate (CS)/dermatan sulfate (DS) as the normal tissue samples; ii) the

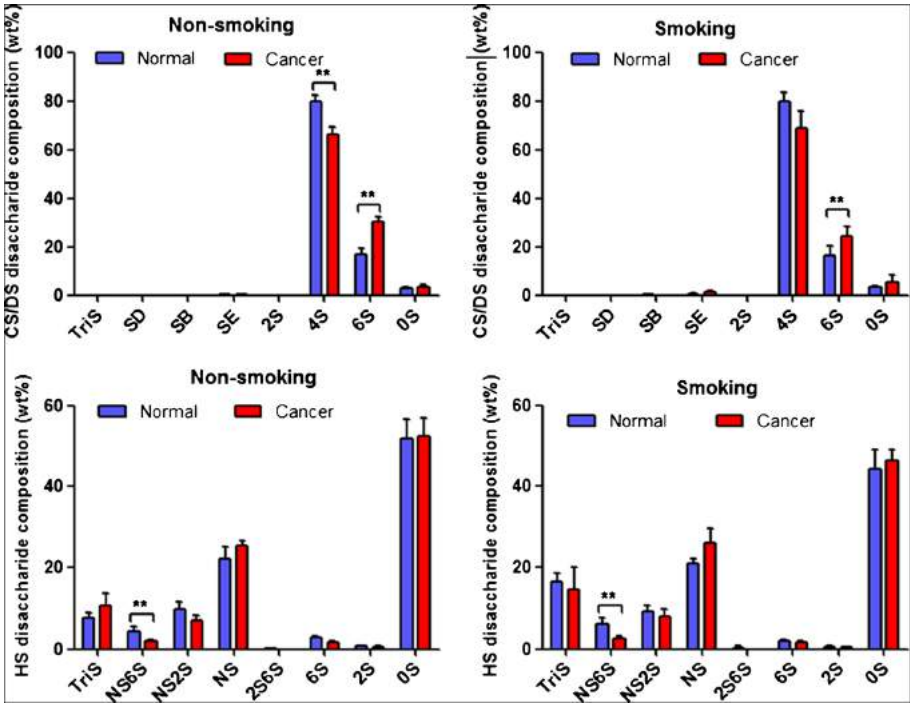

Fig. 5 Comparison of HS/CS composition in normal lung tissue and lung cancer tissue from smoking and non-smoking patients: N: Normal tissue, C: cancer tissue (**$p < 0.001$)

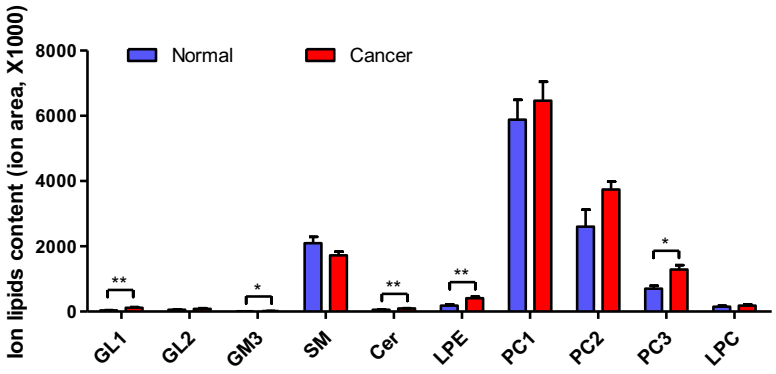

Fig. 6 Glycolipids as total ion lipid content, the data were normalized by the tissue dry weight (cps/mg tissue) in normal lung tissue and lung cancer tissue (**$p < 0.001$, *$p < 0.05$)

total amount of heparan sulfate (HS) and hyaluronan (HA) in normal and cancer tissues were not significantly different; iii) in fine structures of GAGs, there were several minor disaccharide components of HS, including NS6S, NS2S and 2S were significantly lower in cancer tissue, and in CS, the level of 4S disaccharide decreased, but the level of 6S disaccharide increased in cancer tissues. In our previous studies, we did similar study using GAG analysis in colon cancer, breast cancer and stomach cancer [6–8]. All the cases showed different level of changes in both total GAGs and disaccharide components. For example, in breast cancer, cancerous tissue showed an increase in sulfation at the 6S position of CS chains and an increase in the levels of the HS disaccharide NS2S [7]. These differences in the amount and fine structures of GAGs from carcinoma tissues compared with the corresponding normal tissues indicated that GAGs might be potentially used as

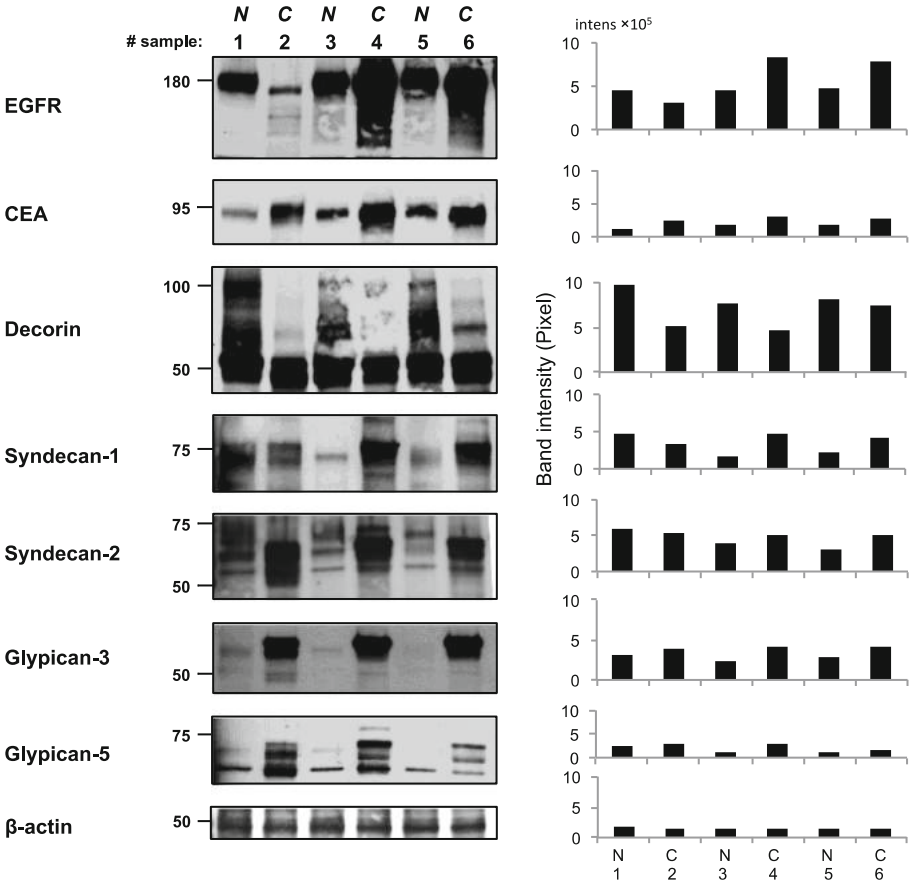

Fig. 7 Proteoglycan protein analysis of normal and cancer tissues. N: normal tissue, C: cancer tissue. Left panel: Western blotting images of PG proteins; Right panel: plots of band intensity based on digitized western blotting images using UN-Scan-IT software

biomarkers to distinguish the different kinds of cancer. Unfortunately, we have no information on the underlying mechanism. We can make no conclusion regarding whether the change in glycome is a cause or effect of cancer and can only conclude that there is an association.

Alteration of PGs in several types of cancers including lung has been studied. At the outset of the current study, we selected eight antibodies to use in these Western analyses, EGFR, CEA (known tumor markers) and decorin, syndecan-1, syndecan-2, glypican-3, and glypican-5 (proteoglycans) and β-actin (control). Decorin, one of the CSPGs, had been shown as a lower expression in NSCLC tissue than in adjacent normal lung tissue [25]. In several studies, different subtypes of glypicans (GPCs) as one of well-studied HSPGs has revealed that, for instance, GPC5 plays a role as a tumor suppressor in lung cancer tissues and lung cancer cell lines by inhibiting Wnt/Beta-catenin signaling, whereas GPC3 was not appeared in lung SCC [26–29]. Some studies indicate a significantly altered expression level of HSPG, including syndecan-1 (SDC1) in human lung cancer is observed with correlation to differentiation status of tumor cells and disease stage [15, 30]. At the outset of our study we selected decorin, syndecans and glypicans to provide a window into changes that we might observe in HS/CS/DS proteoglycans. Unfortunately, the proteins selected for Western analysis, with the exception of CEA, showed no clear trends and offer no real insight into the PGs that carry the increase CS/DS GAG chains. These results serve as an exploratory study that provides guidance for future prospective studies involving a larger number of patients and more extensive exploration of the proteoglycans responsible for the observed significant differences in CS/DS levels. Future, studies will need to examine a wider array of CS/DS PGs including versican and CD44.

There is more GL information than for other glycoconjugates. Ceramides and gangliosides have been known to be associated with survival rate and cancer progression [31]. In the current study we only focused on the GM3 ganglioside because of the limitations of our method. We have no reason to believe that GM3 is the only GSL species altered in lung cancer. Many GSLs (GM1, GM2, GD1, GD3, etc.,) have been detected in cancer cells and tissues and reported in the literature [31].

Although biopsy samples are conventionally tested using histological and immunohistochemical methods, these methods are often insufficient in cancer diagnosis and in guiding therapy. While there are many studies that focus on the clinical proteomics and clinical genomics of cancer, clinical glycomics data of cancer is still very limited. In the current study, the GAGs, PGs and glycolipids in lung cancer tissues were preliminarily profiled in a retrospective study using a small number of clinical samples. Advanced methodology and technology in mass spectrometry area has facilitated glycosaminoglycan analysis from cells to clinic samples and from small to large-scale sets in a rapid and sensitive way [32, 33]. Restrictions associated with sample collection, such as low number of group size and its limited sample amount, make it difficult to arrive at decisive conclusions. In conjunction with our previous studies on colon, stomach and breast cancer, we have discovered that the amount of GAGs and their fine structures might be useful as biomarker to both identify cancers and to guide therapy (i.e., as a theragnostic). By undertaking studies involving a larger number of patients, the GAGs and GLs present in the cancer tissues, might one day be used to distinguish the different types cancer cells (i.e., easily treatable from more aggressive forms). Ultimately, it might even be possible to extend these markers to serum or plasma samples, or to use these markers as theragnostics to guide therapy. However, despite these limitations of the current small retrospective study, we have explored different types of glycoconjugates by advanced analytical approach based on mass spectrometry and suggest GAGs and GL might serve as potential biomarkers for lung cancer.

CEA, carcinoembryonic antigen; CM, ceramide, *N*-acyl-sphing-4-enine; CS, chondroitin sulfate; DS, dermatan sulfate; EGFR, epidermal growth factor receptor; GAG, glycosaminoglycan; GL, glycolipid; GM3, monosialic ganglioside, NeuAcα2-3Galβ1-4Glcβ-ceramide; HILIC, hydrophilic interaction liquid chromatography; HS, heparan sulfate; LPC, lysophosphatidylcholine, 1-acyl-sn-glycero-3-phosphocholine; PC, phosphatidylcholine, 1,2-diacyl-*sn*-glycero-3-phosphocholine; PE, phosphoethanolamine, 1,2-diacyl-*sn*-glycero-3-phosphoethanolamine; PG, proteoglycan; SM, sphingomyelin, *N*-acyl-sphing-4-enine-1-phosphocholine

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. World Health Organization. Cancer. Fact Sheet No. 297 (2013)
2. U.S. National Institutes of Health. National Cancer Institute. SEER Cancer Statistics Review, 1975–2010
3. Sequist, L.V., Heist, R.S., Shaw, A.T., *et al.*: Implementing multiplexed genotyping of non-small-cell lung cancers into routine clinical practice. Ann. Oncol. **22**(12), 2616–2624 (2011)
4. Li, W., Zhao, K., Kirberger, M., Liao, W., Yan, Y.: Next generation sequencing technologies in cancer diagnostics and therapeutics: a mini review. Cell. Mol. Biol. **61**, 91–102 (2015)

5. Yeat, N.C., Lin, C., Sager, M., Lin, J.: Cancer proteomics: developments in technology, clinical use and commercialization. Expert Rev Proteomics. **12**, 391–405 (2015)
6. Joo, E.J., Weyers, A., Li, G., Gasimli, L., Li, L., Choi, W.J., Lee, K.B., Linhardt, R.J.: Carbohydrate-containing molecules as potential biomarkers in colon cancer. OMICS. **18**(4), 231–241 (2014)
7. Weyers, A., Yang, B., Yoon, D.S., Park, J.-H., Zhang, F., Lee, K.B., Linhardt, R.J.: A structural analysis of glycosaminoglycans from lethal and non-lethal breast cancer tissues: towards a novel class of theragnostics for personalized medicine in oncology. OMICS. **16**, 79–89 (2012)
8. Weyers, A., Yang, B., Park, J.-H., Kim, Y.-S., Kim, S.-M., Lee, S.-E., Zhang, F., Lee, K.B., Linhardt, R.J.: Microanalysis of stomach cancer glycosaminoglycans. Glycoconj. J. **30**, 701–707 (2013)
9. Ucakturk, E., Akman, O., Sun, X., Baydar, D.E., Dolgun, A., Zhang, F., Linhardt, R.J.: Changes in composition and sulfation patterns of glycoaminoglycans in renal cell carcinoma. Glycoconj. J. **33**, 103–112 (2016)
10. Couchman, J.R., Pataki, C.A.: An introduction to proteoglycans and their localization. J. Histochem. Cytochem. **60**(12), 885–897 (2012)
11. Alper J.: Turning sweet on cancer. Science **201,** 159–160 (2003)
12. Hakomori, S.: Aberrant glycosylation in tumors and tumor-associated carbohydrate antigens. Adv. Cancer Res. **52**, 257–331 (1989)
13. Hakomori, S.I., Cummings, R.D.: Glycosylation effects on cancer development. Glycoconj. J. **29**(8–9), 565–566 (2012)
14. Ohtsubo, K., Marth, J.D.: Glycosylation in cellular mechanisms of health and disease. Cell. **126**(5), 855–867 (2006)
15. Lemjabbar-Alaoui, H., McKinney, A., Yang, Y.W., Tran, V.M., Phillips, J.J.: Glycosylation alterations in lung and brain cancer. Adv. Cancer Res. **126**, 305–344 (2015)
16. Kim, H., Xu, G.L., Borczuk, A.C., Busch, S., Filmus, J., Capurro, M., Brody, J.S., Lange, J., D'Armiento, J.M., Rothman, P.B., Powell, C.A.: The heparan sulfate proteoglycan GPC3 is a potential lung tumor suppressor. Am. J. Respir. Cell Mol. Biol. **29**, 694–701 (2003)
17. Vongchan, P., Linhardt, R.J.: Characterization of a new monocloral anati-glypican antibody specific forhepatocellular carcinoma cell type HepG2. World J. Hepatol. **9**, 368–384 (2017)
18. Matsuda, Y., Yamamoto, T., Kudo, M., Kawahara, K., Kawamoto, M., Nakajima, Y., Koizumi, K., Nakazawa, N., Ishiwata, T., Naito, Z.: Expression and roles of lumican in lung adenocarcinoma and squamous cell carcinoma. Int. J. Oncol. **33**, 1177–1185 (2008)
19. Mujoo, K., Cheresh, D.A., Yang, H.M., Reisfeld, R.A.: Disialoganglioside GD2 on human neuroblastoma cells: target antigen for monoclonal antibody-mediated cytolysis and suppression of tumor growth. Cancer Res. **47**(4), 1098–1104 (1987)
20. Yang, B., Chang, Y., Weyers, A.M., Sterner, E., Linhardt, R.J.: Disaccharide analysis of glycosaminoglycan mixtures by ultra-high-performance liquid chromatography-mass spectrometry. J Chromatogr A. **17**(1225), 91–98 (2012)
21. Kumar, S., Lanckacker, E., Dentener, M., Bracke, K., Provoost, S., De Grove, K., Brusselle, G., Wouters, E., Maes, T., Joos, G.: Aggravation of allergic airway inflammation by cigarette smoke in mice is CD44-dependent. PLoS One. **11**, e0151113 (2016)
22. Annoni, R., Lanças, T., Yukimatsu, T.R., de Medeiros Matsushita, M., de Morais Fernezlian, S., Bruno, A., da Silva Fernando Ferraz, L., Roughley, P.J., Battaglia, S., Dolhnikoff, M., Hiemstra, P.S., Sterk, P.J., Rabe, K.F., Mauad, T.: Extracellular matrix composition in COPD. Eur Respir J. **40**(1362), 1373 (2012)
23. Sanna P.P.: US Patent, 8847003 B2: role of proteoglycans in drug dependence (2014)
24. Chen, J., Repunte-Canonigo, V., Kawamura, T., Lefebvre, C., Shin, W., Howell, L.L., Hemby, S.E., Harvey, B.K., Califano, A., Morales, M., Koob, G.F., Paolo Sanna, P.: Hypothalamic proteoglycan syndecan-3 is a novel cocaine addiction resilience factor. Nat Commun. **4**, 1955–1962 (2013)
25. Shi, X., Liang, W., Yang, W., Xia, R., Song, Y.: Decorin is responsible for progression of non-small-cell-lung cancer by promoting cell proliferation and metastasis. Tumor Biol. **36**, 3345–2254 (2015)
26. Yuan, S., Yu, Z., Liu, Q., Zhang, M., Xiang, Y., Wu, N., Wu, L., Hu, Z., Xu, B., Cai, T., Ma, X., Zhang, Y., Liao, C., Wang, L., Yang, P., Bai, L., Li, Y.: GPC5, a normal epigentically silence tumor suppressor, inhibits tumor growth by suppressing Wnt/Beta-catenin signaling in lung adenocarcinoma. Oncogene. (2016). doi:10.1038/onc.2016.149
27. Zhao, Z., Han, C., Liu, J., Wang, C., Wang, Y., Cheng, L.: GPC5, a tumor suppressor, is regulated by miR-620 in lung adenocarcinoma. Mol. Med. Rep. **9**(6), 2540–2546 (2014)
28. Yang, X., Zhang, Z., Qiu, M., Hu, J., Fan, X., Wang, J., Xu, L., Yin, R.: Glypican-5 is a novel metastasis suppressor gene in non-small-cell-lung cancer. Cancer Lett. **341**(2), 265–273 (2013)
29. Timek, D.T., Shi, J., Liu, H., Lin, F.: Arginase-1, HepPar-1, and Glypican-3 are the most effective panel of markers in distinguishing hepatocellular carcinoma from metastatic tumor on fine-needle aspiration specimens. Am. J. Clin. Pathol. **138**(2), 203–210 (2012)
30. Farnedi, A., Rossi, S., Bertani, N., Gulli, M., Silini, E.M., Mucignat, M.T., Poli, T., Sesenna, E., Lanfranco, D., Montebugnoli, L., Leonardi, E., Marchetti, C., Cocchi, R., Ambrosini-Spaltro, A., Foschini, M.P., Perris, R.: Proteoglycan-based diversification of disease outcome in head and neck cancer patients identifies NG2/CSPG4 and syndecan-2 as unique relapse and overall survival predicting factors. BMC Cancer. **15**, 352 (2015)
31. Hayashi, N., Chiba, H., Kuronuma, K., Go, S., Hasegawa, Y., Takahashi, M., Gasa, S., Watanabe, A., Hasegawa, T., Kuroki, Y., Inokuchi, J., Takahashi, H.: Detection of N-glycolyated gangliosides in non-small-cell lung cancer using GMR8 monoclonal antibody. Cancer Sci. **104**(1), 43–47 (2013)
32. Zhang, Y., Jiao, J., Yang, P., Lu, H.: Mass spectrometry-based N-glycoproteomics for cancer biomarker discovery. Clin. Proteomics. **11**, 18 (2014)
33. Li, G., Li, L., Tian, F., Zhang, L., Xue, C., Linhardt, R.J.: Glycosaminoglycanomics of cultured cells using a rapid and sensitive LC-MS/MS approach. ACS Chem. Biol. **10**, 1303–1310 (2015)