Liu *et al. Clinical Proteomics* (2024) 21:64
https://doi.org/10.1186/s12014-024-09516-2

Clinical Proteomics

RESEARCH Open Access

Identification of serum *N*-glycans signatures in three major gastrointestinal cancers by high-throughput *N*-glycome profiling

Si Liu[1,2†], Jianmin Huang[3†], Yuanyuan Liu[2], Jiajing Lin[2], Haobo Zhang[2], Liming Cheng[4], Weimin Ye[1*] and Xin Liu[2*]

Abstract

Background Alternative N-glycosylation of serum proteins has been observed in colorectal cancer (CRC), esophageal squamous cell carcinoma (ESCC) and gastric cancer (GC), while comparative study among those three cancers has not been reported before. We aimed to identify serum *N*-glycans signatures and introduce a discriminative model across the gastrointestinal cancers.

Methods The study population was initially screened according to the exclusion criteria process. Serum *N*-glycans profiling was characterized by a high-throughput assay based on matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). Diagnostic model was built by random forest, and unsupervised machine learning was performed to illustrate the differentiation between the three major gastrointestinal (GI) cancers.

Results We have found that three major gastrointestinal cancers strongly associated with significantly decreased mannosylation and mono-galactosylation, as well as increased sialylation of serum glycoproteins. A highly accurate discriminative power (> 0.90) for those gastrointestinal cancers was obtained with serum *N*-glycome based predictive model. Additionally, serum *N*-glycome profile exhibited distinct distributions across GI cancers, and several altered *N*-glycans were hyper-regulated in each specific disease.

Conclusions Serum *N*-glycome profile was differentially expressed in three major gastrointestinal cancers, providing a new clinical tool for cancer diagnosis and throwing a light upon the disease-specific molecular signatures.

†Si Liu and Jianmin Huang contributed equally to this work.

*Correspondence:
Weimin Ye
ywm@fjmu.edu.cn
Xin Liu
xliu@mail.hust.edu.cn
[1]Department of Epidemiology and Health Statistics, School of Public Health, Fujian Medical University, Fuzhou 350122, China
[2]The Key Laboratory for Biomedical Photonics of MOE at Wuhan National Laboratory for Optoelectronics-Hubei Bioinformatics & Molecular Imaging Key Laboratory, Systems Biology Theme, Department of Biomedical Engineering, College of Life Science and Technology, Huazhong University of Science and Technology, No. 1037 Luoyudong Road, Hongshan District, Wuhan 430074, China
[3]Digestive Endoscopy Center, South Branch of Fujian Provincial Hospital, Fuzhou 350000, China
[4]Department of Laboratory Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430074, China

Introduction

The gastrointestinal cancers including colorectal cancer (CRC), gastric cancer (GC) and esophageal cancer (EC), are three of the most common cancers worldwide [1]. In China, esophageal squamous cell carcinoma (ESCC) accounts for nearly 90% of all EC cases [2]. Those three major gastrointestinal cancers have considerable social, psychological and financial impacts on the patients' life and pose heavy global burden to public health [3], necessitating the efficient biomarker discovery and increasing the uptake rate of cancer screening. Several tumor markers for these cancers have been established with clinical usefulness, such as carcinoembryonic antigen (CEA) and carbohydrate antigen 19−9 (CA 19−9), but their diagnostic accuracy are limited [4]. Besides, the absence of non-invasive and reliable molecular indicator significantly impedes the generalization of early screening to a wider population. Although particular molecular subtypes could stratify the gastrointestinal cancer in clinical settings, such molecular panels still require refinement [5]. Therefore, it is crucial to find novel non-invasive biomarker for diagnosis and stratification of those three gastrointestinal cancers.

As one of the most common post-translational modifications (PTM) in mammalian, glycosylation could contribute to disease initiation and progression due to its involvement in many key physiological and pathological processes, such as cell adhesion, molecular trafficking and clearance [6]. Glycans regulate protein and cell functions, and alterations in glycan structure cause pathologic events, suggesting the potential of specific *N*-glycans biomarkers for diagnosis and prediction of disease progression [7]. Uncovering the glycome would not only contribute to reveal the underlying molecular mechanism of disease, but also to spur development of a new generation of therapeutics targeting glycans [8, 9]. Emerging evidences have demonstrated the profound effect of intestinal epithelial glycosylation on host genetics and gut microbiota that interface with the gastrointestinal pathogenesis [10]. Hence, characterization of glycome profile in gastrointestinal cancers promotes to explore the hallmark of cancer progression.

Aberrant *N*-glycosylation plays an important role in cancer development, and understanding the tumor-associated *N*-glycosylation signatures is essential for discovering anti-cancer targets and indicators of therapy monitoring and diagnosis [11]. Previously, it was reported that fucosylated *N*-glycans on haptoglobin in the sera of five types of gastroenterological cancer, including esophageal, gastric, colon, gallbladder and pancreatic cancer [12]. Additionally, serological IgG *N*-glycosylation was regarded as a potential candidate for noninvasive diagnosis of GI cancers [13], particularly for IgG galactosylation [14]. For colorectal cancer, Gao et al. constructed two diagnostic models (CRCglycoA and CRCglycoB) based on the serum *N*-glycan markers [15]. Ren et al. reported that specific serum *N*-glycans signatures have valuable potential in the clinical application of early detection and early relapse prediction of CRC [16, 17]. Comparative glycomic profiling of esophageal cancer revealed the capability of a subset of serum *N*-glycans to distinguish disease-free from different stages of EC patients [18]. Furthermore, serum IgG *N*-glycans featured with fucosylation and mannosylation may contribute to the early prevention of EC [19]. Gastric cancer cases could be differentiated from nonatrophic gastritis with *N*-glycans profiles of serum or tissue [20, 21]. Besides, a nomogram based on glycomic biomarkers in serum and clinicopathological characteristics has valuable potential to assist clinical decision-making before surgery [22]. However, most of these findings were derived from a single cancer group or based on the altered *N*-glycans in one specific glycoprotein. Hence, a full picture of the changes in *N*-glycans profile and differential distribution of glycome between those three gastrointestinal cancers have not been investigated.

Liquid biopsy is promising for precision medicine in cancer care, which has been expanded to profile the products of proteins modified by glycosylation [23]. Of note, blood test is one of the most accessible approach to evaluate the abnormal indicators for diseases, such as CRC screening [24]. Glycans on secretory proteins are influenced by genetic, cellular and environmental factors, the signature of which probably indicates the transformation from normal to carcinoma [25]. Serum *N*-glycome profile could be used for cancer diagnosis, patients' outcomes prediction or therapeutic response [26–29], which is regarded as an attractive source for biomarker discovery. In addition, particular glycan-derived traits enabled the stratification of patients with different subtypes of inflammatory bowel disease (IBD), showing disease course-specific *N*-glycans signatures [30]. However, little is known about the distribution of serum *N*-glycans between CRC, GC and ESCC. Furthermore, altered glycosylation is a universal feature in cancer progression [31], such as higher sialylation of serum proteins, and glycomics studies depicting the *N*-glycans profile across the three gastrointestinal cancers have not been reported.

In this study, we exploited the mass spectrometry based high-throughput assay to characterize serum *N*-glycome profile in the three major gastrointestinal cancers, including CRC, ESCC and GC. *N*-glycomic profile was analyzed through serum collection, *N*-glycans release, methylamidation of carboxyl group followed by matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and data processing. Biomarker panels were established for each cancer type by random

forest analysis. The distribution of glycome profile among the three cancers was evaluated by multivariate analysis.

Materials and methods

Study populations

The samples involved in this study consist of healthy controls ($n=29$), esophageal squamous cell carcinoma ($n=30$), gastric cancer ($n=35$) and colorectal cancer ($n=34$). The age and sex were matched across the four groups involved in this study. Healthy controls were the volunteers identified as disease-free individual. All the samples were collected from Tongji Medical College of Huazhong University of Science and Technology. The study was performed in accordance with the principles of the Declaration of Helsinki criteria, and was approved by the Ethics Committee of Huazhong University of Science and Technology (TJ-IRB20221109). The clinical and histological characteristics of the cohorts are shown in Table S1.

N-glycans release and derivatization

The *N*-glycans were enzymatically released from 10 μL serum as previously described [32]. Briefly, the samples were processed by dissolution and denaturation with Protein Deglycosylation Mix (New England Biolabs), which was subsequently added with 5 mU PNGaseF and incubated overnight at 37 ℃. We removed deglycosylated peptides from the reaction mixture using HyperSep™ Hypercarb™ SPE columns (ThermoFisher). To avoid sialic acid dissociation when directly ionized by MALDI-MS, methylamidation of carboxyl group was conducted according to our previous study [33]. Following the facile derivatization, serum *N*-glycans were purified using self-packed column contained microcrystalline cellulose.

Maldi-Ms analysis

Chemically derivatized *N*-glycans were resuspended in 5 μL acetonitrile: water solution (50:50 ratio), of which 0.5 μL sample aliquots were directly spotted on MALDI plate. After air-dried, an equal volume of 2,5-DHB matrix solution prepared with 10 mg/mL DHB in 50% ACN containing 10 mM sodium acetate was loaded onto the plate in triplicate. The mixture was dried at room temperature prior to MALDI-MS analysis. Mass spectra were acquired in the positive mode by 5800 MALDI-MS equipped with a 355 nm Nd: YAG laser, with the range of *m/z* at 1000–4000 Da. The acquired spectra were the average of 1000 laser shots. MS data were processed by advanced baseline correction, noise filter, and peak deisotoping via Data Explorer 4.0. The *N*-glycans identified herein were depicted and named according to the rules of the Symbol Nomenclature for Glycans (SNFG). All the *N*-glycans structures were visualized using GlycoWorkBench 2.1 software.

Data processing and statistical analysis

The MALDI-MS data for targeted *N*-glycans were exported as text files, which were further directly imported into MATLAB and processed with self-developed code (Mass_Master) [34]. Each dataset for one group can be accomplished within 5 s, resulting in the means of relative abundance of serum *N*-glycans. The distribution of variables was initially tested by Kolmogorov-Smirnov (K-S) test. Data passing the K-S test were further analyzed by parameter test, whereas the group of non-normal distribution was analyzed by nonparametric test. One-way analysis of variance (ANOVA) was performed to examine the difference between three or more groups when the residues conform to Gaussian distribution. On the contrary, Kruskal-Wallis test was performed to test the data with non-Gaussian distribution, and the multiple correction was conducted by Tukey's multiple comparison test. Multivariate partial least squares discriminant analysis (PLS-DA) was conducted to visualize the classification between normal group and three cancers in this study. Random forest (RF) was used to build the three-classification model of ROC curve for distinguishing those three GI cancer groups as described previously [35]. RF was performed by R 4.2.1 with *ranger* package (version 0.14.1), using the default parameter of ntree=500. The dataset was randomly split into a training set and a testing set with an 8:2 ratio. The training set was used for model development while the testing set was employed to assess the performance of the model. The performance of the model was assessed by several commonly used multi-classification evaluation indicators: binary AUC for each category, including CRC_ROC, ESCC_ROC, GC_ROC, MacroROC and MicroROC.

Results

Characterization of n-glycosylation profile by MALDI-MS

The schematic workflow of this study is presented in Fig. 1a, and the MALDI-MS spectra of serum *N*-glycome is displayed in Fig. 1b. Prior to characterizing the profile of healthy controls and gastrointestinal cancer patients, quality control was performed to evaluate the intraday repeatability for the quantitation of serum protein glycosylation. Relative abundance of five representative *N*-glycans was highly repeatable with the relative standard deviation (RSD) of both intraday and interday less than 20% (Table S2), suggesting the good robustness and repeatability of the method. Total 54 *N*-glycans were identified and 16 of *N*-glycans derived traits were also calculated as reported in our previous study [36], such as mannosylation, sialylation, galactosylation and fucosylation (Table S3). Representative MALDI-MS spectra was shown from individuals with healthy controls and patients with CRC, ESCC or GC (Fig S1).

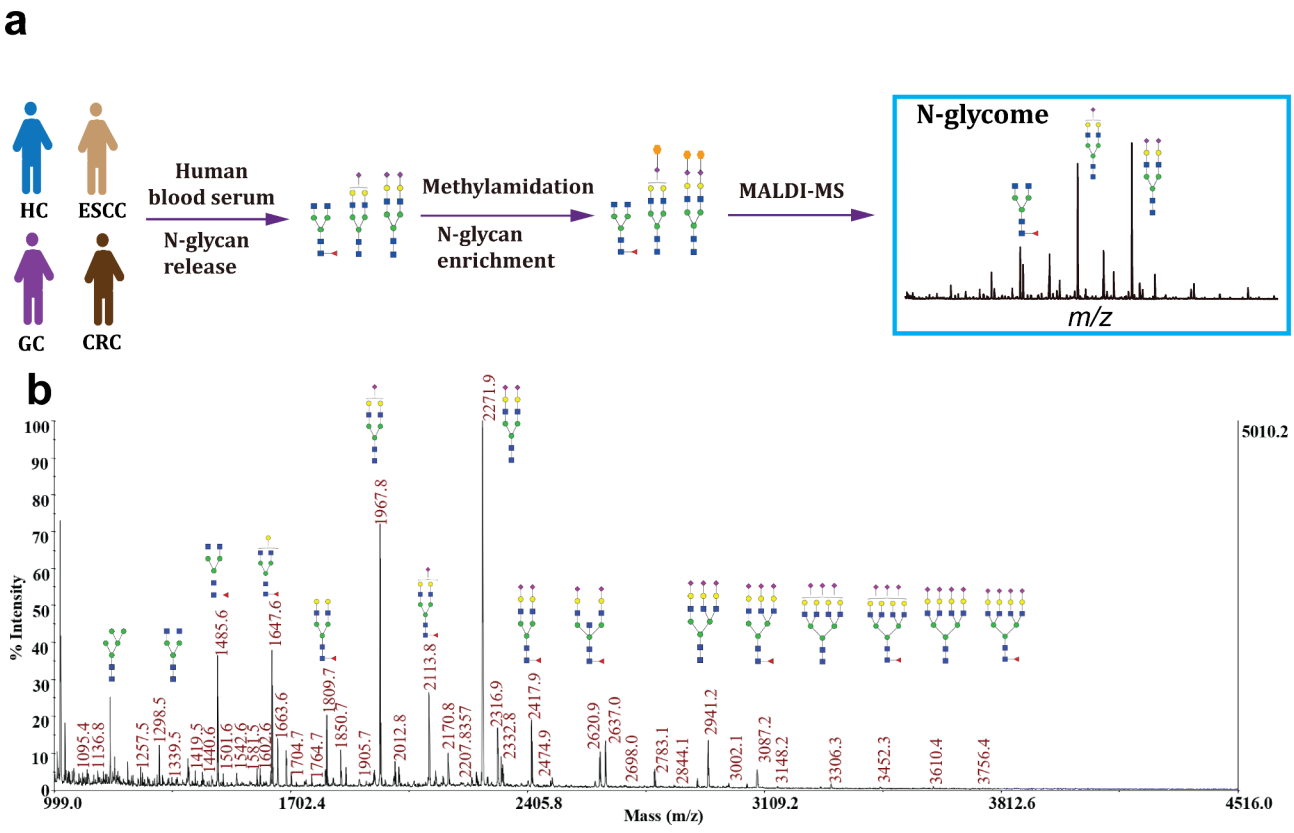

Fig. 1 Experimental workflow of glycomic profiling of serum in healthy controls and patients with three major GI cancer. Blue square denotes N-acetylglucosamine, green circle denotes mannose, yellow circle denotes galactose, purple diamond denotes N-acetylneuraminic acid and red triangle denotes fucose. HC, healthy controls; ESCC, esophageal squamous cell carcinoma; GC, gastric cancer; CRC, colorectal cancer; MALDI-MS, matrix assisted laser desorption ionization mass spectrometry

Changes of serum *N*-glycans in three major gastrointestinal cancers

Significant differences were observed in four types of *N*-glycans features, including mannosylation, galactosylation, bisection and sialylation (Fig. 2, Table S3). Specifically, mannosylation (Man) was lower in all three gastrointestinal cancers compared with controls (Fig. 2.**a**). Further individual *N*-glycan analysis showed that the differential expression of mannosylation in GI cancer patients was primarily reflected by remarkable decrease in *N*-glycan compositions of H5N2, H8N2 and H9N2 (Table S4). Concurrently, mono-galactosylation (G1 total) was substantially decreased in all three cancer groups (Fig. 2c), which were consistent with alterations in *N*-glycans of H4N3F1, H4N4 and H6N3 (Table S4). Additionally, total sialylation was elevated in cancer groups compared with controls (Fig. 2o), especially for ESCC and CRC. Sialylated *N*-glycans of H5N4S1, H5N4S2, H7N6S1, H6N5F1S2, H6N5F1S3 and H7N6F1S2 were likely to contribute to the differential sialylation due to their remarkably increase in gastrointestinal cancers (Table S4).

Identification of dysregulated serum *N*-glycans for three major gastrointestinal cancers

Considering the importance of disease stratification for the therapeutic intervention, we dissected the molecular diversity of three major gastrointestinal cancers at serum *N*-glycome level. It was observed that 8 of serum *N*-glycans (H4N4, H3N5, H8N2, H5N3S1, H9N2, H5N4S1, H5N5S1 and H5N4S2) were significantly changed in the three cancers in the same direction (Fig. 3. **a**), spanning high mannosylation, sialylation and fucosylation and bisection. Further evaluation of diagnostic performance showed that most of those eight *N*-glycans gained moderately accurate AUCs for distinguishing GI cancers from healthy controls (Table S5). Significantly, the other particular serum *N*-glycans were changed distinctively in those three cancers. CRC progression was closely associated with increased neutral mono-antennary *N*-glycan of fucosylation (H3N3F1) and mono-sialylated *N*-glycan of bisection (H4N5S1), concurrently with decreased biantennary mono-sialylated glycan with core-fucose (H4N4F1S1), and tri-antennary bi-sialylated glycan (H6N5S2) (Fig. 3b). Serum *N*-glycans of M6 (H6N2), mono-antennary glycan of mono-sialylation (H4N3S1), neutral biantennary bisected glycan (H5N5), neutral

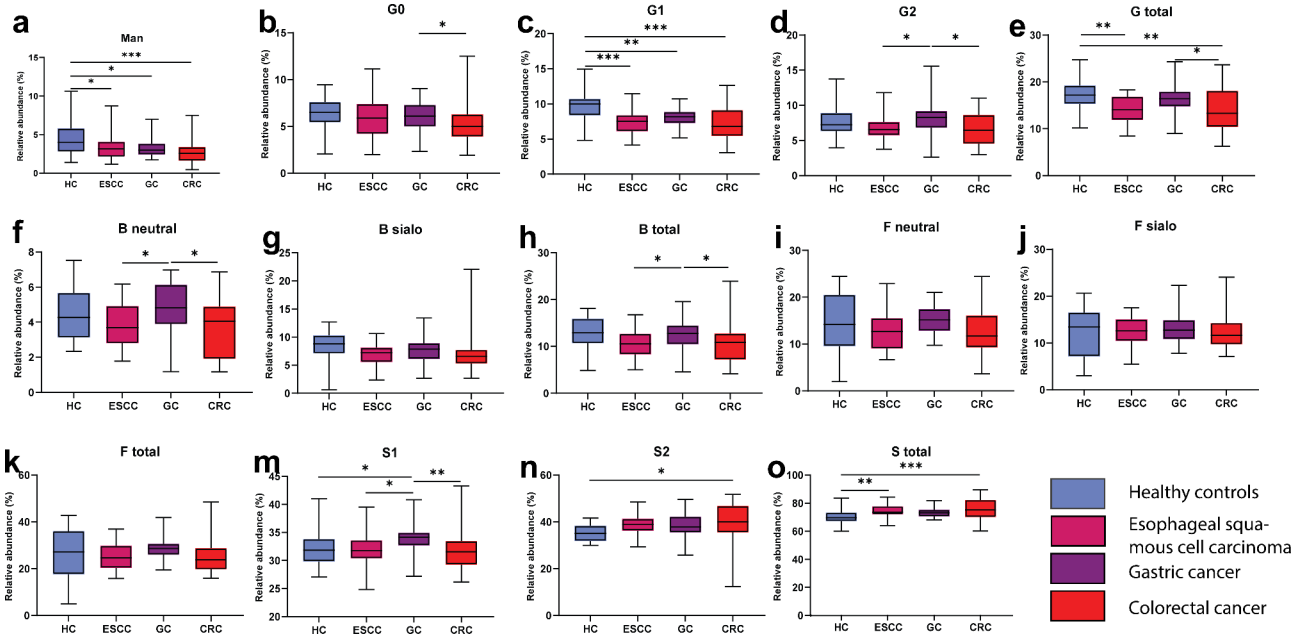

Fig. 2 Box plots of *N*-glycans derived traits from serum *N*-glycome between healthy controls and GI cancer patients. Box plots showing median values and 25% (box) and 75% (line) percentiles for patients and controls was performed to display the main features of total serum *N*-glycome by Tukey's multiple comparisons test. Man, mannosylation; G0, agalactosylation; G1, mono-galactosylation; G2, di-galactosylation; B neutral, neutral bisecting GlcNacylation; B sialo, sialo bisecting GlcNacylation; F neutral, neutral fucosylation; F sialo, sialo fucosylation; F total, total fucosylation; S1, mono-siaylation; S2, di-sialylation; S total, total sialylation

triantennary galactosylated glycan (H6N5) and biantennary bi-sialylated glycan of fucosylation (H5N4F1S1) were uniformly increased in GC (Fig. 3. **c**). For ESCC, two neutral biantennary glycans (H5N4F1 and H5N5F1) were decreased, as opposed to the mono-antennary sialylated glycan (H4N3F1S1) (Fig. 3. **d**). Further ROC analysis showed most of specific *N*-glycans gained an AUC lower than 0.70 or higher than 0.30, while combination of them with biantennary logistic regression significantly improved the diagnostic performance (Table S6).

Establishment of *N*-glycan-biomarker panels for each gastrointestinal cancer

To develop an efficient indicator for cancer screening, the diagnostic performance of serum *N*-glycome for the cancers was evaluated by discriminative model. It was shown that those three major gastrointestinal cancers could be respectively distinguished from controls using serum *N*-glycome (Fig S2. a-c). Interestingly, different *N*-glycan compositions occupied different proportion to discriminate cancer patients from healthy controls, as illustrated in the overall map (Fig. 4a). Furthermore, feature selection through cross-validation showed the best diagnostic performance was obtained with primary 6 *N*-glycans for CRC (Fig S2d), 4 *N*-glycans for ESCC (Fig S2. e), and 22 *N*-glycans for GC (Fig S2. f). Using the primary features as the glycan panels, we found GI patients could be well differentiated from controls with at least accurate AUC scores (Fig. 4 b-d).

Distribution of serum *N*-glycome among three major gastrointestinal cancers

Unsupervised machine learning was applied to explore the diversity of serum *N*-glycome profile among three major gastrointestinal cancers. It was found that those three groups were evidently distinguished from each other by serum *N*-glycome (Fig. 5.**a**), indicating the molecular diversity of those three gastrointestinal cancers at glycome level. Plot of VIP showed the importance of particular *N*-glycans for the contribution to the discriminative model, such as N-glycan composition of peak 33 (H6N5) and peak 48 (H7N6S2) (Fig. 5b). Random forest of serum *N*-glycome identified the primary features for distinguishing those cancers from each other, with acceptable AUC scores (Fig S3). With the main N-glycans used in discriminative model, particular *N*-glycan signatures were shown to be hyper-regulated in each specific cancer (Fig. 5 c).

Discussion

The three cancer groups involved in this study, including CRC, ESCC and GC, dominated the top five fatal cancers in digestive system. Serum based test was mini-invasive approach to cancer screening, while clinically used criteria were lack of sensitivity or specificity. Glycome

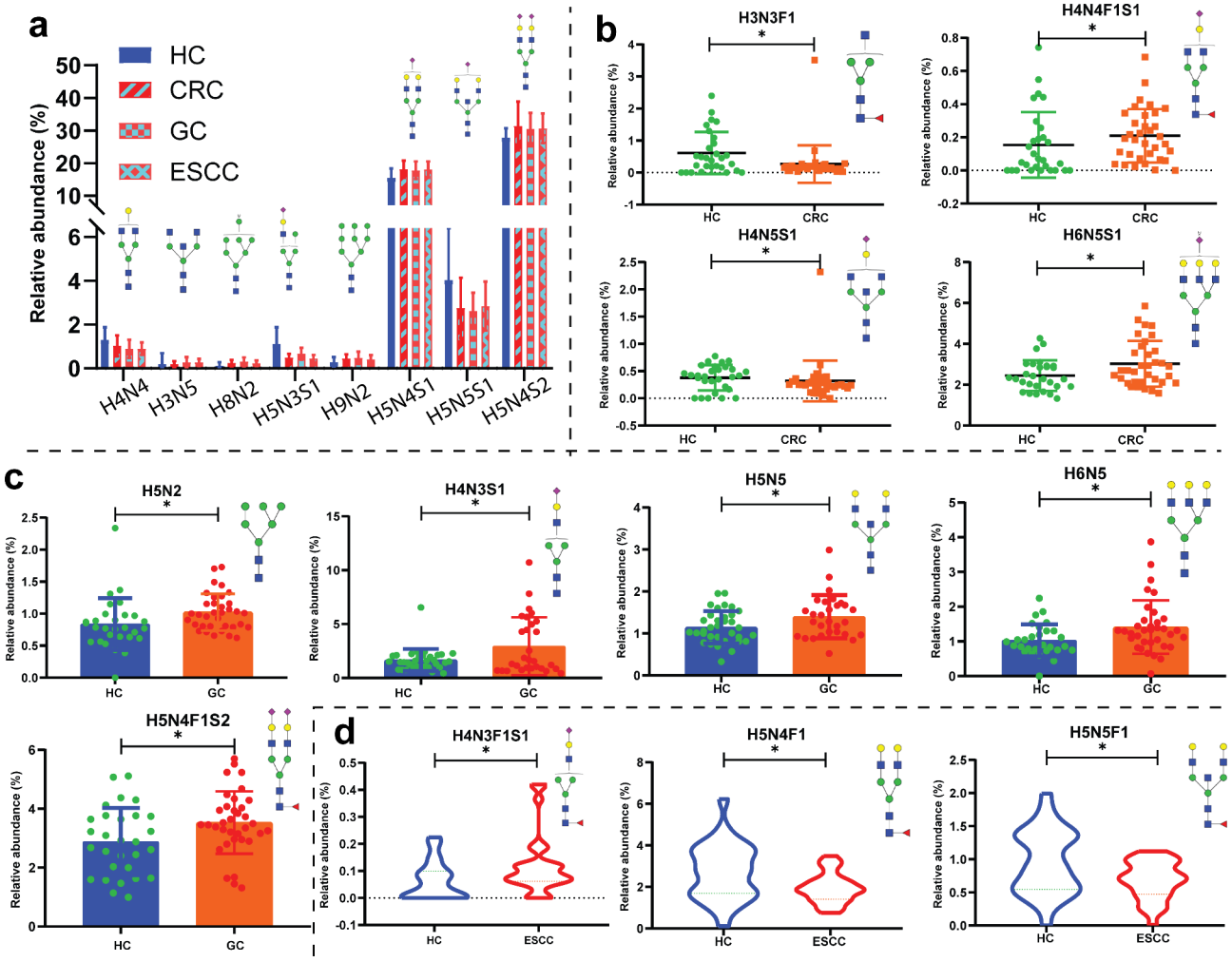

Fig. 3 The similarity and difference of particular *N*-glycans that are significantly changed between three major GI cancers. (**a**) *N*-glycans significantly changed in the comparison between healthy controls and GI cancers with the same trend; (**b**-**d**) Cancer-specific *N*-glycans significantly changed between controls and cancer patients. Mann-Whitney was used for this test, $P > 0.05$, $^{*}P \le 0.05$, $^{**}P \le 0.01$, $^{***}P \le 0.001$

profile was significantly changed in the cancer initiation and tumor metastasis, suggesting the predictive role of particular N-glycan signatures for cancer progression. Importantly, some *N*-glycan patterns have been applied to the clinical trial for disease screening [37]. However, the efficient *N*-glycan-based biomarker panels for CRC, ESCC or GC have not been established. Additionally, the classification of those three cancers poses a great challenge due to the close intertwine even though they showed distinct etiology.

Currently, a high-throughput analytical assay was applied to dissect serum *N*-glycome profile among the three major gastrointestinal cancers. It was observed that those three gastrointestinal cancers share the similar alterations in significant decreased mannosylation and galactosylation, as well as increase in sialylation. Higher abundance of high-mannose-type *N*-glycans were observed in dysplatic region than in colorectal carcinoma [38, 39], implicating its role in cell proliferation. Decreased level of high-mannose type *N*-glycans of serum in GC was also found in previous study [20]. High mannose-binding lectin induces autophagy in GC cells via the downregulation of tumor cell surface integrin/EGFR [40], suggesting the inhibited function of mannosylation in GC. Notably, to our knowledge, this is the first study revealing the altered mannosylation in ESCC, and altered galactosylation in all three gastrointestinal cancers at serum *N*-glycome level. It has been established that sialylated *N*-glycans are fundamental in tumor growth and metastasis [41], suggesting the hallmark of upregulated hypersialylation among cancers. For the three gastrointestinal cancers, increased sialylation in CRC was consistent with the report form previous studies at both serum level [16] and cellular level [42]. Altered level of sialylation modulates CRC malignancy through the mediation of JAK2/STAT3 pathway [43].

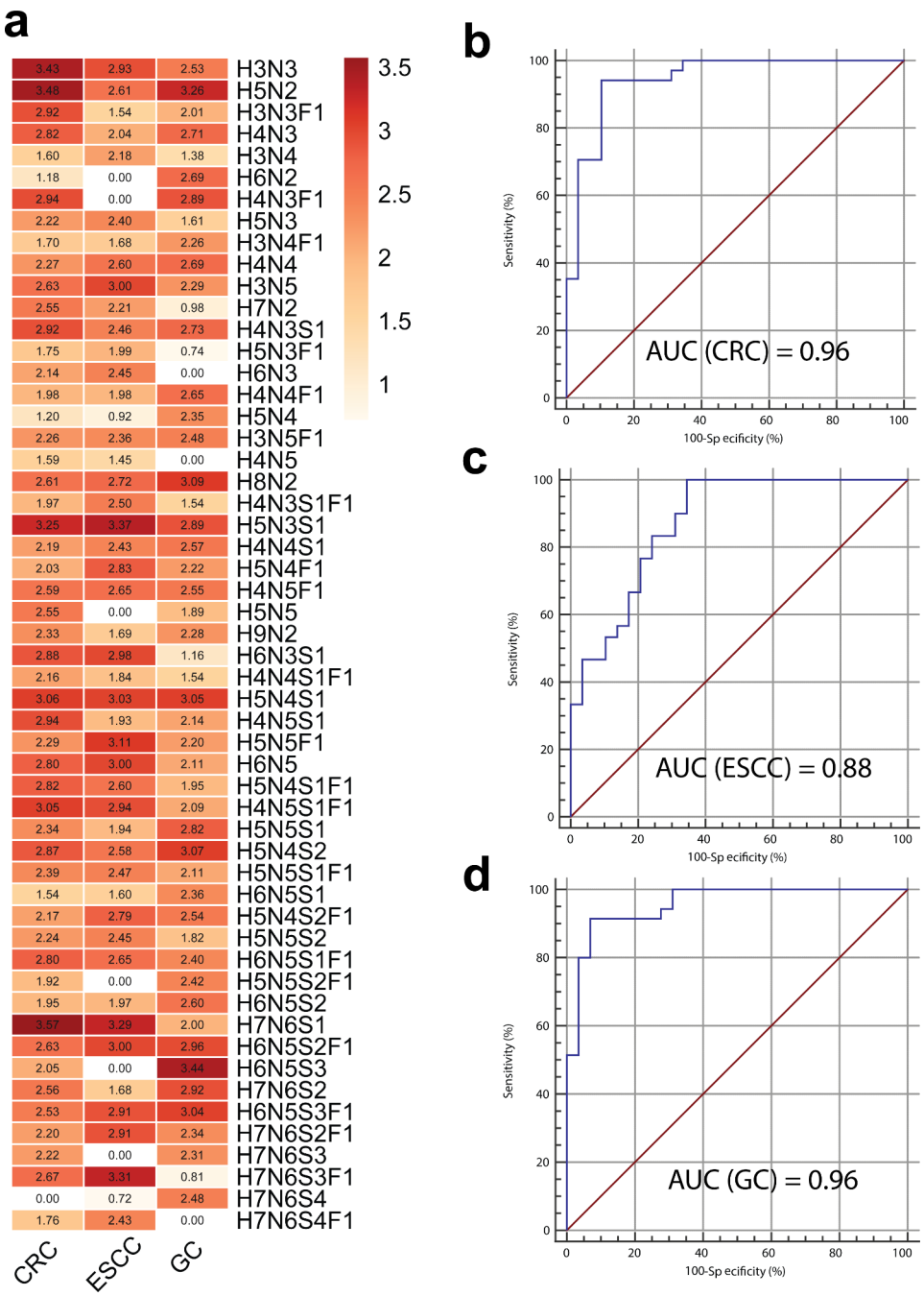

Fig. 4 Evaluation of distribution and discrimination of serum *N*-glycome for three major GI cancers. (**a**) Heatmap shows the permutation importance of N-glycome features in different GI cancer groups. The redder, the more important. The score displayed was calculated by the formula: 5 + log10 (importance). Features with negative importance were set a score of 0. H, hexose; N, N-acetylglucosamine; F, fucose; S, sialic acid; (**b**-**d**) Diagnostic performance of serum *N*-glycome for CRC, ESCC and GC, respectively

Several sialylated *N*-glycans were significantly increased in esophageal adenocarcinoma [18]. Increased sialylation interfering with key signaling pathways and integrin glycosylation is one of the key mechanisms regulating GC malignant behavior [44]. Those findings show the contribution of sialylation to gastrointestinal cancers progression, indicating the value of altered sialylation for digestive diseases screening.

Additionally, we found bisection was slightly increased in GC, as opposed to the alteration in both CRC and ESCC. This finding is consistent to the previous report that bisecting GlcNAc *N*-glycans is involved in the suppression of cancer metastasis [45]. Overexpression of

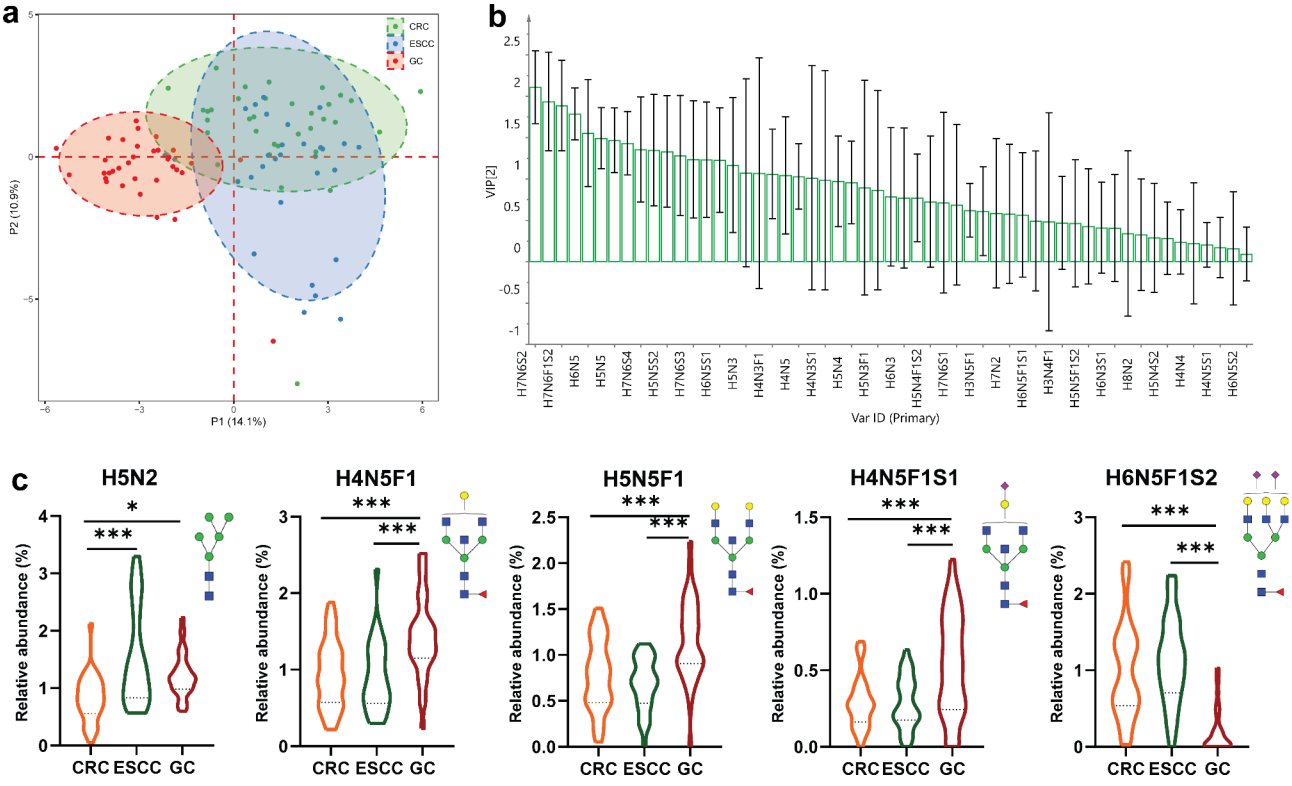

Fig. 5 Assessment of serum *N*-glycome in the comparison between three major GI cancers. (**A**) Partial least-square discriminant analysis (PLS-DA) of serum *N*-glycome for differentiation of three GI cancers; (**B**) Plot of variable importance for projection (VIP) showing the importance of serum N-glycans; (**C**) Violin plot of particular serum N-glycans in the comparison between CRC, ESCC and GC. One-way ANOVA was used for this test, $P > 0.05$, $^{*}P \le 0.05$, $^{**}P \le 0.01$, $^{***}P \le 0.001$

core-fucosylation is an important feature in several cancers [45], while slightly decreased core-fucosylation of total serum glycoproteins was found in those three gastrointestinal cancers. This may be explained by the interference of other factors in the blood system, or the heterogeneity of glycome profile in different organs. Considering the crucial role of glycosylation in digestive system, it is worthy of further investigations about the potential of *N*-glycan signatures.

Further individual *N*-glycans analysis showed the similar alteration in all three cancer groups, such as decreased biantennary mono-galactosylated glycan and increased biantennary di-sialylated glycan. This finding may elucidate the down-regulated galactosylation and up-regulated sialylation in those gastrointestinal cancers (GI) as described above. Indeed, clinical criteria for those three cancers share common features. Interestingly, several significantly changed *N*-glycans were unique to those three cancers, suggesting the differential expression of glycosylation in GI cancer patients. Further evidence showed that those three gastrointestinal cancers could be nearly distinguished by serum *N*-glycome profile, implicating the distinct molecular mechanism in the evolution of carcinogenesis. These data suggest that dysregulated *N*-glycosylation could be an underpinning of digestive cancers pathogenesis, warranting further studies of spatial glycomics.

Different types of glycosylation have been observed in some major diseases [46], while the altered N-glycome profile across various GI cancers has not been investigated. Currently, we found that specific *N*-glycan compositions were significantly changed between GC, CRC and ESCC, particularly for biantennary high-mannosylated *N*-glycan (H5N2), biantennary core-fucosylated *N*-glycan of bisecting GlcNAcylation with mono-galactose (H4N5F1) or bi-galactose (H5N5F1) or mono-galacto sialic acid (H4N5F1S1), and triantennary bi-sialylated *N*-glycan of core-fucosylation (H6N5F1S2). Indeed, clinicopathological characteristics of these three GI cancers are distinct [47]. Our results suggest a new approach to understanding the differential pathological features of the digestive system. Notably, CRC exhibits lower levels of H5N2 compared to GC and ESCC, indicating variations in mannosylation along the digestive tract. This could be due to the fact that gut microbiota utilize different endoglycosidases to modify the same N-glycan substrate [48]. GC is characterized by higher levels of biantennary core-fucosylated N-glycans with bisecting GlcNAcylation and galactose or sialic acid (H4N5F1, H5N5F1, and H4N5F1S1) and lower levels of H6N5F1S2 compared

to both ESCC and CRC. Intriguingly, chronic H. pylori (Hp) infection and gastric inflammation lead to a reconfiguration of the gastric glycophenotype, with increased expression of genes involved in fucosylation, galactosylation, and sialylation [49]. This suggests that the altered N-glycosylation in GC may be linked to Hp infection. Collectively, our findings could serve as additional biomarkers for gastrointestinal cancer through glycomic liquid biopsies. However, despite using the same glycomic methodology for these three diseases, the consistency of glycan measurements under varying clinical conditions remains uncertain. This aspect warrants further systematic investigation to establish disease-specific N-glycome profile.

However, some limitations existed in this study. First of all, it was conducted with relatively small sample size, warranting further investigations with a large-scale sample involvement. Besides, it lacks external validation of the *N*-glycan signatures based predictive model, using data from studies on other populations. The last but not least is that this study was performed at the serum level, further in vitro and in vivo studies will be necessary to advance our understanding of the pathogenetic mechanism of specific *N*-glycan signatures.

In conclusion, our observational study demonstrated the alterations in *N*-glycosylation of total serum glycoproteins were strongly associated with the pathogenesis of GI cancers. Three major gastrointestinal cancers could be differentiated from each other by serum *N*-glycome profile with fairly good diagnostic performance. The serum *N*-glycan profiles displayed a pronounced dysregulation among the three gastrointestinal cancers, thereby highlighting the molecular heterogeneity across the gastrointestinal tract. Notably, the identification of cancer-associated glycan biomarker would significantly enhance the clinical diagnostic potential of liquid biopsy.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12014-024-09516-2.

Supplementary Material 1

Acknowledgements

We thank Professor Liming Cheng at Tongji Hospital of Tongji Medical College, Huazhong University of Science and Technology, for providing the clinical samples.

Author contributions

S. L., study concept and design, drafting of the manuscript, analysis and interpretation of data, statistical analysis; J.-M. H., research performance, administrative support, provision of study material or patients; Y.-Y. L., J.-J. L and H.-B. Z., bioinformatics analysis and data interpretation; L.-M. C., administrative support, provision of study material or patients; W.-M. Y, critical revision of the manuscript for important intellectual content, administrative support; X. L., study concept and design, critical revision of the manuscript for important intellectual content, administrative support. All authors read and approved the final manuscript.

Funding

The work was funded by High-Level Talents Research Start-Up Project of Fujian Medical University (Grant No. XRCZX2023015 and XRCZX2023030), the Key Project of Education and Scientific Research for Young and Middle-aged People of Fujian Province (Grant No. JZ230019), the National Key Research and Development Program of China (Grant No. 2022YFC3400800), and the National Natural Science Foundation of China (Grant No. 81827901 and 82272418).

Data availability

The mass spectrometry glycomic data have been deposited to the GlycoPOST archive with the dataset identifier GPST000435.

Declarations

Competing interests

The authors declare no competing interests.

Received: 12 August 2024 / Accepted: 19 November 2024
Published online: 28 November 2024

References

1. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, Jemal A. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74(3):229–63.
2. Feng R, Su Q, Huang X, Basnet T, Xu X, Ye W. Cancer situation in China: what does the China cancer map indicate from the first national death survey to the latest cancer registration? Cancer Commun (Lond). 2023;43(1):75–86.
3. Huang J, Lucero-Prisno DE 3rd, Zhang L, Xu W, Wong SH, Ng SC, Wong MCS. Updated epidemiology of gastrointestinal cancers in East Asia. Nat Rev Gastroenterol Hepatol. 2023;20(5):271–87.
4. Izquierdo-Sanchez L, Lamarca A, La Casta A, Buettner S, Utpatel K, Klümpen HJ, Adeva J, Vogel A, Lleo A, Fabris L, Ponz-Sarvise M, Brustia R, Cardinale V, Braconi C, Vidili G, Jamieson NB, Macias RI, Jonas JP, Marzioni M, Hołówko W, Folseraas T, Kupčinskas J, Sparchez Z, Krawczyk M, Krupa Ł, Scripcariu V, Grazi GL, Landa-Magdalena A, Ijzermans JN, Evert K, Erdmann JI, López-López F, Saborowski A, Scheiter A, Santos-Laso A, Carpino G, Andersen JB, Marin JJ, Alvaro D, Bujanda L, Forner A, Valle JW, Koerkamp BG, Banales JM. Cholangiocarcinoma landscape in Europe: Diagnostic, prognostic and therapeutic insights from the ENSCCA Registry. J Hepatol. 2022;76(5):1109–21.
5. Fernandes E, Sores J, Cotton S, Peixoto A, Ferreira D, Freitas R, Reis CA, Santos LL, Ferreira JA. Esophageal, gastric and colorectal cancers: looking beyond classical serological biomarkers towards glycoproteomics-assisted precision oncology. Theranostics. 2020;10(11):4903–28.
6. Schjoldager KT, Narimatsu Y, Joshi HJ, Clausen H. Global view of human protein glycosylation pathways and functions. Nat Rev Mol Cell Biol. 2020;21(12):729–49.
7. Verhelst X, Dias AM, Colombel J-F, Vermeire S, Van Vlierberghe H, Callewaert N, Pinho SS. Protein glycosylation as a diagnostic and prognostic marker of Chronic Inflammatory Gastrointestinal and Liver diseases. Gastroenterology. 2020;158(1):95–110.
8. Thomas D, Rathinavel AK, Radhakrishnan P. Altered glycosylation in cancer: a promising target for biomarkers and therapeutics. Biochim Biophys Acta Rev Cancer. 2021;1875(1):188464.
9. Stanley P. Genetics of glycosylation in mammalian development and disease. Nat Rev Genet. 2024;25(10):715–29.
10. Kudelka MR, Stowell SR, Cummings RD, Neish AS. Intestinal epithelial glycosylation in homeostasis and gut microbiota interactions in IBD. Nat Rev Gastroenterol Hepatol. 2020;17(10):597–617.
11. Lin Y, Lubman DM. The role of N-glycosylation in cancer. Acta Pharm Sin B. 2024;14(3):1098–110.
12. Takahashi S, Sugiyama T, Shimomura M, Kamada Y, Fujita K, Nonomura N, Miyoshi E, Nakano M. Site-specific and linkage analyses of fucosylated N-glycans on haptoglobin in sera of patients with various types of cancer: possible implication for the differential diagnosis of cancer. Glycoconj J. 2016;33(3):471–82.
13. Liu P, Wang X, Dun A, Li Y, Li H, Wang L, Zhang Y, Li C, Zhang J, Zhang X, Ma L, Hou H. High-throughput profiling of Serological Immunoglobulin G

N-Glycome as a noninvasive biomarker of gastrointestinal cancers. Engineering. 2023;26:44–53.
14. Ren S, Zhang Z, Xu C, Guo L, Lu R, Sun Y, Guo J, Qin R, Qin W, Gu J. Distribution of IgG galactosylation as a promising biomarker for cancer screening in multiple cancer types. Cell Res. 2016;26(8):963–6.
15. Zhao YP, Ruan CP, Wang H, Hu ZQ, Fang M, Gu X, Ji J, Zhao JY, Gao CF. Identification and assessment of new biomarkers for colorectal cancer with serum N-glycan profiling. Cancer. 2012;118(3):639–50.
16. Pan Y, Zhang L, Zhang R, Han J, Qin W, Gu Y, Sha J, Xu X, Feng Y, Ren Z, Dai J, Huang B, Ren S, Gu J. Screening and diagnosis of colorectal cancer and advanced adenoma by Bionic Glycome method and machine learning. Am J Cancer Res. 2021;11(6):3002–20.
17. Gu Y, Duan B, Sha J, Zhang R, Fan J, Xu X, Zhao H, Niu X, Geng Z, Gu J, Huang B, Ren S. Serum IgG N-glycans enable early detection and early relapse prediction of colorectal cancer. Int J Cancer. 2023;152(3):536–47.
18. Mechref Y, Hussein A, Bekesova S, Pungpapong V, Zhang M, Dobrolecki LE, Hickey RJ, Hammoud ZT, Novotny MV. Quantitative serum glycomics of esophageal adenocarcinoma and other esophageal disease onsets. J Proteome Res. 2009;8(6):2656–66.
19. Pan H, Wu Z, Zhang H, Zhang J, Liu Y, Li Z, Feng W, Wang G, Liu Y, Zhao D, Zhang Z, Liu Y, Zhang Z, Liu X, Tao L, Luo Y, Wang X, Yang X, Zhang F, Li X, Guo X. Identification and validation of IgG N-glycosylation biomarkers of esophageal carcinoma. Front Immunol. 2023;14:981861.
20. Ozcan S, Barkauskas DA, Renee Ruhaak L, Torres J, Cooke CL, An HJ, Hua S, Williams CC, Dimapasoc LM, Han Kim J, Camorlinga-Ponce M, Rocke D, Lebrilla CB, Solnick JV. Serum glycan signatures of gastric cancer. Cancer Prev Res (Phila). 2014;7(2):226–35.
21. Demirhan DB, Yılmaz H, Erol H, Kayili HM, Salih B. Prediction of gastric cancer by machine learning integrated with mass spectrometry-based N-glycomics. Analyst. 2023;148(9):2073–80.
22. Zhao J, Qin R, Chen H, Yang Y, Qin W, Han J, Wang X, Ren S, Sun Y, Gu J. A nomogram based on glycomic biomarkers in serum and clinicopathological characteristics for evaluating the risk of peritoneal metastasis in gastric cancer. Clin Proteom. 2020;17:34.
23. He K, Baniasad M, Kwon H, Caval T, Xu G, Lebrilla C, Hommes DW, Bertozzi C. Decoding the glycoproteome: a new frontier for biomarker discovery in cancer. J Hematol Oncol. 2024;17(1):12.
24. Ladabaum U, Mannalithara A, Weng Y, Schoen RE, Dominitz JA, Desai M, Lieberman D. Comparative effectiveness and cost-effectiveness of Colorectal Cancer Screening with blood-based biomarkers (Liquid Biopsy) vs fecal tests or Colonoscopy. Gastroenterology. 2024;167(2):378–91.
25. Pongracz T, Mayboroda OA, Wuhrer M. The human blood N-Glycome: unraveling Disease glycosylation patterns. JACS Au; 2024.
26. Verhelst X, Geerts A, Callewaert N, Van Vlierberghe H. The potential of glycomics as prognostic biomarkers in liver disease and liver transplantation. Acta Gastroenterol Belg. 2019;82(2):309–13.
27. Wang Y, Liu Y, Liu S, Cheng L, Liu X. Recent advances in N-glycan biomarker discovery among human diseases. Acta Biochim Biophys Sin (Shanghai). 2024;56(8):1156–71.
28. Huang C, Fang M, Feng H, Liu L, Li Y, Xu X, Wang H, Wang Y, Tong L, Zhou L, Gao C. N-glycan fingerprint predicts alpha-fetoprotein negative hepatocellular carcinoma: a large-scale multicenter study. Int J Cancer. 2021;149(3):717–27.
29. Qin R, Zhao J, Qin W, Zhang Z, Zhao R, Han J, Yang Y, Li L, Wang X, Ren S, Sun Y, Gu J. Discovery of non-invasive glycan biomarkers for detection and surveillance of gastric Cancer. J Cancer. 2017;8(10):1908–16.
30. Shubhakar A, Jansen BC, Adams AT, Reiding KR, Ventham NT, Kalla R, Bergemalm D, Urbanowicz PA, Gardner RA, Wuhrer M, Halfvarson J, Satsangi J, Fernandes DL, Spencer DIR. Serum N-Glycomic biomarkers Predict Treatment Escalation in Inflammatory Bowel Disease. J Crohns Colitis. 2023;17(6):919–32.
31. Bellis SL, Reis CA, Varki A, Kannagi R, Stanley P. (2022) Glycosylation Changes in Cancer. In: Varki A, Cummings RD, Esko JD editors Essentials of Glycobiology. Cold Spring Harbor Laboratory Press Copyright © 2022 The Consortium of Glycobiology Editors, La Jolla, California; published by Cold Spring Harbor Laboratory Press; https://doi.org/10.1101/glycobiology.4e.47. All rights reserved., Cold Spring Harbor (NY), pp 631–644.
32. Liu S, Cheng L, Fu Y, Liu B-F, Liu X. Characterization of IgG N-glycome profile in colorectal cancer progression by MALDI-TOF-MS. J Proteom. 2018;181:225–37.
33. Liu X, Qiu H, Lee RK, Chen W, Li J. Methylamidation for sialoglycomics by MALDI-MS: a facile derivatization strategy for both α2, 3-and α2, 6-linked sialic acids. Anal Chem. 2010;82(19):8300–6.
34. Liu S, Yu Y, Liu Y, Lin J, Fu Y, Cheng L, Liu X. Revealing the changes of IgG subclass-specific N-glycosylation in colorectal cancer progression by high-throughput assay. Proteom Clin Appl. 2021;15(2–3):e2000022.
35. Liu S, Tu C, Zhang H, Huang H, Liu Y, Wang Y, Cheng L, Liu BF, Ning K, Liu X. Noninvasive serum N-glycans associated with ovarian cancer diagnosis and precancerous lesion prediction. J Ovarian Res. 2024;17(1):26.
36. Liu S, Liu Y, Lin J, Liu B-F, He Z, Wu X, Liu X. Novel insight into the etiology of Haff Disease by mapping the N-Glycome with Orthogonal Mass Spectrometry. Engineering. 2023;26:63–73.
37. Mereiter S, Balmaña M, Campos D, Gomes J, Reis CA. Glycosylation in the era of Cancer-targeted therapy: where are we heading? Cancer Cell. 2019;36(1):6–16.
38. Ukkola I, Nummela P, Heiskanen A, Holm M, Zafar S, Kero M, Haglund C, Satomaa T, Kytölä S, Ristimäki A. N-Glycomic profiling of microsatellite unstable colorectal Cancer. Cancers (Basel). 2023;15(14):3571.
39. Boyaval F, Dalebout H, Van Zeijl R, Wang W, Fariña-Sarasqueta A, Lageveen-Kammeijer GSM, Boonstra JJ, McDonnell LA, Wuhrer M, Morreau H, Heijs B. High-mannose N-Glycans as malignant progression markers in early-stage Colorectal Cancer. Cancers (Basel). 2022;14(6):1552.
40. Sato Y, Kubo T, Morimoto K, Yanagihara K, Seyama T. High mannose-binding Pseudomonas fluorescens lectin (PFL) downregulates cell surface integrin/EGFR and induces autophagy in gastric cancer cells. BMC Cancer. 2016;16:63.
41. Dobie C, Skropeta D. Insights into the role of sialylation in cancer progression and metastasis. Br J Cancer. 2021;124(1):76–90.
42. Boyaval F, van Zeijl R, Dalebout H, Holst S, van Pelt G, Fariña-Sarasqueta A, Mesker W, Tollenaar R, Morreau H, Wuhrer M, Heijs B. N-Glycomic signature of Stage II Colorectal Cancer and its Association with the Tumor Microenvironment. Mol Cell Proteom. 2021;20:100057.
43. Liu B, Liu Q, Pan S, Huang Y, Qi Y, Li S, Xiao Y, Jia L. The HOTAIR/miR-214/ST6GAL1 crosstalk modulates colorectal cancer procession through mediating sialylated c-Met via JAK2/STAT3 cascade. J Exp Clin Cancer Res. 2019;38(1):455.
44. Pinho SS, Carvalho S, Marcos-Pinto R, Magalhães A, Oliveira C, Gu J, Dinis-Ribeiro M, Carneiro F, Seruca R, Reis CA. Gastric cancer: adding glycosylation to the equation. Trends Mol Med. 2013;19(11):664–76.
45. Pinho SS, Reis CA. Glycosylation in cancer: mechanisms and clinical implications. Nat Rev Cancer. 2015;15(9):540–55.
46. Dotz V, Wuhrer M. N-glycome signatures in human plasma: associations with physiology and major diseases. FEBS Lett. 2019;593(21):2966–76.
47. Zilbauer M, James KR, Kaur M, Pott S, Li Z, Burger A, Thiagarajah JR, Burclaff J, Jahnsen FL, Perrone F, Ross AD, Matteoli G, Stakenborg N, Sujino T, Moor A, Bartolome-Casado R, Bækkevold ES, Zhou R, Xie B, Lau KS, Din S, Magness ST, Yao Q, Beyaz S, Arends M, Denadai-Souza A, Coburn LA, Gaublomme JT, Baldock R, Papatheodorou I, Ordovas-Montanes J, Boeckxstaens G, Hupalowska A, Teichmann SA, Regev A, Xavier RJ, Simmons A, Snyder MP, Wilson KT. A Roadmap for the human gut cell Atlas. Nat Rev Gastroenterol Hepatol. 2023;20(9):597–614.
48. Sastre DE, Sultana N, Huliciak MVASN, Du M, Cifuente J, Flowers JO, Liu M, Lollar X, Trastoy P, Guerin B, Sundberg ME. Human gut microbes express functionally distinct endoglycosidases to metabolize the same N-glycan substrate. Nat Commun. 2024;15(1):5123.
49. Magalhães A, Marcos-Pinto R, Nairn AV, Dela Rosa M, Ferreira RM, Junqueira-Neto S, Freitas D, Gomes J, Oliveira P, Santos MR, Marcos NT, Xiaogang W, Figueiredo C, Oliveira C, Dinis-Ribeiro M, Carneiro F, Moremen KW, David L, Reis CA. Helicobacter pylori chronic infection and mucosal inflammation switches the human gastric glycosylation pathways. Biochim Biophys Acta. 2015;1852(9):1928–39.

Publisher's note