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Aberrant glycosylation and cancer biomarker discovery: a promising and thorny journey

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Abstract

Glycosylation is among the most important post-translational modifications for proteins and is of intrinsic complex character compared with DNAs and naked proteins. Indeed, over 50%–70% of proteins in circulation are glycosylated, and the “sweet attachments” have versatile structural and functional implications. Both the configuration and composition of the attached glycans affect the biological activities of consensus proteins significantly. Glycosylation is generated by complex biosynthetic pathways comprising hundreds of glycosyltransferases, glycosidases, transcriptional factors, transporters and the protein backbone. In addition, lack of direct genetic templates and glyco-specific antibodies such as those commonly used in DNA amplification and protein capture makes research on glycans and glycoproteins even more difficult, thus resulting in sparse knowledge on the pathophysiological implications of glycosylation. Fortunately, cutting-edge technologies have afforded new opportunities and approaches for investigating cancer-related glycosylation. Thus, glycans as well as aberrantly glycosylated protein-based cancer biomarkers have been increasingly recognized. This mini-review highlights the most recent developments in glyco-biomarker studies in an effort to discover clinically relevant cancer biomarkers using advanced analytical methodologies such as mass spectrometry, high-performance liquid chromatographic/ultra-performance liquid chromatography, capillary electrophoresis, and lectin-based technologies. Recent clinical-centered glycobiological studies focused on determining the

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regulatory mechanisms and the relation with diagnostics, prognostics and even therapeutics are also summarized. These studies indicate that glycomics is a treasure waiting to be mined where the growth of cancer-related gly-comics and glycoproteomics is the next great challenge after genomics and proteomics.

Keywords

analytical methodologies; cancer biomarker; glycomics; glycosylation

Introduction

Glycosylation, a fundamental and prominent post-translational modification of proteins, plays crucial roles in a variety of cellular activities such as cell growth, differentiation, transformation, adhesion, and immune surveillance of tumors [1, 2]. Two main types of glycosylation, *N*-glycosylation and mucin-type *O*-glycosylation, are a series of enzymatic reactions occurring in the endoplasmic reticulum and Golgi complex [1]. Apart from the rare congenital disorders of glycosylation (CDG), aberrant glycosylation has reportedly permeated through every aspect of human diseases including autoimmune diseases [3, 4], infection with bacteria [5, 6], viruses [7, 8] and parasites [9, 10], and especially cancer [11, 12].

Aberrant glycosylation has been pinpointed as a hallmark of cancer [13] due to its contributions in carcinogenesis, cancer progression and metastasis, conferring a new perspective for cancer research involving underlying mechanisms investigation and clinical translation and application [14–17]. The majority of tumor biomarkers currently used in the clinic are glycoproteins (e.g. AFP for liver cancer, CA125 for ovarian cancer, CEA for colon cancer, and PSA for prostate cancer) or are glycan-related such as CA19–9, also known as sialyl-Lewis A for gastrointestinal and pancreatic cancer [11]. Currently used glyco-biomarkers and recent (in the past 5 years) findings of potential cancer glyco-biomarkers are summarized in Table 1. Here, “glyco-biomarker discovery” refers in particular to detect the specific glycosylation changes.

Enormous efforts have been devoted to identify specific tumor-associated alterations in glycosylation which could serve as a distinct feature of cancer in cancer diagnostics and prognostics [12, 46]. This review highlights the most recent advances in glycomics-based biomarker studies, including the state-of-the-art analytical methodologies (e.g. mass spectrometry [MS], high-performance liquid chromatographic [HPLC]/ultra-performance liquid chromatography [UPLC], capillary electrophoresis [CE], and lectin-based methods) and their application in biomarker discovery. Additionally, as glycosylation is a complex and non-template driven process, several clinical-centered glycobiological studies which were devoted to unveil regulatory mechanisms and interplay with other pathways are also briefly refined.

Recent advances in glycomics-based biomarker discovery

Mass spectrometry (MS)

MS-based approaches have the advantages of high sensitivity, high mass accuracy, and most importantly, detailed structural information, which provides a major advance in detecting changes, especially subtle changes, in glycan structures that can be used as markers for cancer. Derivatization of glycans by permethylation [47, 48] and reductive amination [49, 50] is often applied prior to MS analysis to improve the ionization efficiency and intensity, and ultimately the sensitivity of detection. Improvements in ion mobility-MS for the structural characterization of glycans have been reviewed recently [51]. This section focuses on discussing two main MS methods, liquid chromatography (LC)-ESI-MS and MALDI-MS, in terms of their recent advances in characterization and quantitation of glycan changes derived from target serum glycoproteins or global serum/plasma/tissue proteins for glyco-biomarker discovery.

LC-MS

LC-MS has been widely employed to determine glycosylation changes in medium-abundance glycoproteins in patient serum, such as α_1 -antitrypsin (A1AT) [52], α_1 -acid glycoprotein [36], haptoglobin (Hp) [53–55], and immunoglobulin G (IgG) [28], whose aberrant glycosylations are highly associated with cancer progression. Due to the immense structural complexity and heterogeneity of glycans, glycan separation is required prior to MS detection, where LC is extensively coupled with MS to achieve a more complete characterization of the glycome, which includes reverse phase (RP)-LC, hydrophilic interaction chromatography (HILIC)-LC, and porous graphitized carbon (PGC)-LC.

The Lubman group monitored fucosylated *N*-glycan structures at low levels in serum Hp by a quantitative HILIC-LC-MS method, which revealed that the fucosylated *N*-glycan structures in serum Hp were significantly up-regulated in hepatocellular carcinoma (HCC) compared to cirrhosis [56]. The Lubman group also detected the site-specific core-fucosylation (CF) level in serum α_2 -macroglobulin (A2MG) [57] and serum ceruloplasmin (CP) [41] by RP-LC-MS/MS, which determined that CF levels at site 138 of CP significantly increased in HCC compared to cirrhosis patients. Similarly, using RP-LC-MS/MS, the Hancock group characterized site-specific *N*-glycan changes of clusterin in the plasma of clear cell renal cell carcinoma patients before and after curative nephrectomy [58].

Using a PGC-LC-MS/MS approach, the Lebrilla group identified the global serum *N*-glycome and determined relative abundances of *N*-glycans released from human serum [59]. Interestingly, the most abundant *N*-glycan, disialylated biantennary species, is not from the most abundant glycoprotein, IgG, but originated from several glycoproteins including A2MG, transferrin, and A1AT [59]. By utilizing this method, the Fanayan group identified a strong representation of high mannose and α -2,6-linked sialylated complex *N*-glycans on the cell membrane in colorectal cancer (CRC) tissues, which was distinctly associated with CRC [60]. The Rudd group profiled the total serum *N*-glycans from advanced breast cancer patients and healthy individuals by HPLC coupled with MS, demonstrating a significant increase in the sialyl-Lewis X epitope, which harbors potential to be a better indicator to

monitor breast cancer progression than the currently used biomarker, CA15–3 [61]. Most recently, the Li group optimized the HILIC-LC-MS parameters of the targeted Multi-Notch MS3 method for quantifying TMT labeled *N*-glycans derived from human serum proteins, which significantly improves the quantification performance of the *N*-glycome compared to traditional quantification methods and has great potential to be widely applied in quantitative glycomics analysis for biomarker discovery [62].

MALDI-MS

For analysis of clinical samples in limited quantities, MALDI-TOF MS is a premier approach for glycan profiling [63] except that it has difficulties in determining the structural isomers with different branching patterns and linkage positions [64]. With a quantitative MALDI-QITMS/MS approach using only 10 μ L of serum in individual patients, the Lubman group identified that fucosylated *N*-glycans in Hp were significantly elevated in pancreatic cancer compared to chronic pancreatitis [65] and a unique pattern of bi-fucosylated tetra-antennary *N*-glycan was identified in HCC patients, which can outperform the clinically used AFP in discriminating early stage HCC from cirrhosis [32]. Nakagawa et al. utilized MALDI-TOFMS to determine *N*-glycan structures of AFP from HCC patients and demonstrated these glycans were affected by specific glycosyltransferases in HCC [66]. Palmigiano et al. utilized MALDI-MS for a high-throughput screening of differential serum *O*-glycans and *N*-glycans in five patients with genetic defects of COG5-CDG, showing a significant increase of apoC-III_{0a} (aglycosylated glycoform) and decrease of apoC-III₁ (mono-sialylated glycoform) in support of clinical diagnosis of COG5-CDG patients [67].

HPLC/UPLC

UPLC has been extensively used for *N*-glycan analysis, and it has advantages of high-throughput, speed and capability to provide branch specific information, which holds great promise for biomarkers related to immune responses [68, 69].

The Rudd group combined HILIC-UPLC with CE-LIF for characterization of *N*-glycans released from IgG in human serum samples, demonstrating a complete structural annotation of IgG *N*-glycans within 20 min, which provides a powerful platform for rapid IgG *N*-glycan structural elucidation of therapeutic antibodies with great potential for clinical use [70]. The group further profiled human serum *N*-glycome using HILIC-UPLC, with more than 140 *N*-glycans assigned, where an increase in sialylation, branching, and outer-arm fucosylation and a decrease in high-mannosylated and bi-antennary core-fucosylated glycans are the most significant alterations between breast cancer patients and healthy controls [27]. Knezevic et al. developed a rapid UPLC method to profile *N*-glycans from plasma samples where *N*-glycans were labeled with aniline, 2-aminobenzamide, and 2-aminoacridone individually and then co-injected for UPLC-fluorescence detection (UPLC-FLD), permitting a superior throughput analysis of 864 plasma samples per day that can greatly facilitate serum-based glyco-biomarker discovery [71].

Capillary electrophoresis (CE)

Over the past years, CE has been used more as a means for oligosaccharide separation, usually coupling with LC and MS for glycomic analysis due to the high properties of

separation, resolving and sample processing [72, 73]. However, combined with laser-induced FLD (LIF), CE-based methods have become a powerful analytic technique, not merely a separation method, for carbohydrate characterization particularly in the field of glycan-based biomarker identification [74–77]. Furthermore, an integrated multiplexing CE method has been developed on the basis of different separation mechanisms and evaluated for analyzing complicated samples [78].

When utilizing CE-LIF for glycan profiling, the derivatization of glycans is needed to facilitate the separation and enhance the sensitivity. Among different kinds of tags, the 8-aminopyrene-1,3,6-trisulfonic acid (APTS) is the most commonly used. An *N*-glycomic profiling method using a DNA sequencer was established by Callewaert et al. [79, 80] which has been widely applied to characterize APTS labeled *N*-glycome in bio-fluids [81–83] and purified proteins [84]. The Gao group has adopted and modified this method to investigate the *N*-glycan-based biomarkers and thus constructed the diagnostic models for various types of cancer including HCC, CRC, multiple myeloma, gastric cancer and extrahepatic cholangiocarcinoma [23, 85–88]. Recently, several high-throughput variants using a fragment analysis module with clinical application potential have been reported [89, 90]. Using an artificial intelligence (AI) algorithm, the predictive diagnostic model might be developed based on the CE pattern.

Lectin-based technologies

Lectins are a group of proteins or glycoproteins derived from plants, animals (including human beings) or microorganisms, which can recognize and reversibly bind to specific free glycans or oligosaccharide moieties on various glycoconjugates [91, 92].

Lack of glyco-specific antibodies has become the major obstacle for the detection of specifically glycosylated proteins. Fortunately, lectins have been considered as an extremely useful tool for glycomic and glycoproteomic study that can be used alone or in conjunction with other methods such as MS and CE because of the wide availability, affinity and relative specificity [93]. Several approaches based on lectins including lectin blot [94, 95], lectin histochemistry/cytochemistry [29, 96], lectin-antibody sandwich enzyme-linked immunosorbent assay (ELISA) [33, 44, 97], lectin affinity chromatography [98, 99], lectin microarray [100–102] and lectin microfluidics [103] have been applied to capture the specific glycan of the glycoproteins [102, 104–106], enhancing the understanding of aberrant glycosylation and carcinogenesis, accelerating biomarkers recognition and quantitative detection. The Gao group has investigated the diagnostic and prognostic value of fucosylated fetuin A in HCC patients by AAL-based ELISA [107]. The Lubman group identified and validated the abnormal sialylation levels of clusterin, complement factor H, hemopexin in the serum of ovarian cancer patients compared to benign diseases using an SNA-based ELISA [108]. With the development of a reverse AAL-based ELISA, the Lubman group also evaluated fucosylated Hp in ovarian cancers, which, in combination with CA125, showed improved performance to distinguish early-stage ovarian cancer from benign diseases compared to CA125 alone [33].

More typical examples of cancer glyco-biomarker discovery using the aforementioned technologies are summarized in Table 1.

Bioinformatics

The databases, softwares, and bioinformatic tools developed to interpret glycomics data as glycan structural information in different glyco-analysis methods including MS (GlycoWorkbench, UniCarb-DB, GlycoDigest) and HPLC/UPLC (GlycoBase) have been recently reviewed [109, 110]. The information on how to utilize glycan structural information to query databases that associate glycans with proteins (UniCarbKB) and with interactions with pathogens (SugarBind) has also been included [110].

Basic glycobiological studies contribute to development of clinical glycomics

Cancer-specific glycosylation changes are mainly based on the aberrant expression and activity of relevant glycosyltransferases and glycosidases. Sialyltransferases (STs) [111], fucosyltransferases (FUTs) [112, 113] and N-acetylglucosaminyltransferases (GnTs) [114, 115] represent a part of glycosyltransferases responsible for the formation of a manifold of glyco-phenotypes, and are currently the most studied molecules in glycobiology [116, 117]. Below, we briefly discuss relevant background and recent progress of cancer glycobiology.

The sugar chains of glycoconjugates are often terminated by diverse sialic acids, which play critical roles in cellular interaction, cancer cell dissociation and metastasis, and also affords another view of biomarker research [118]. Generally, total serum sialylation levels appear to be increased in various malignancies and present application potentials for cancer diagnosis, monitoring and prognosis [119]. Given aberrant sialylation favors cancer initiation and progression via multilevel effects, mediated by more than 20 distinct STs [120–123], inhibitors of STs are of pharmaceutical interest, in particular, for cancer therapy [124–126].

As for FUTs, a systematic, multidisciplinary strategy has been recently employed to confirm FUT8 as a pro-metastatic regulator of melanoma and potential therapeutic target [127]. In addition, FUT8 also has tight connections with lung cancer [112], liver cancer [128, 129], prostate cancer [130], etc. For instance, Tu et al. found that FUT8 could stimulate breast cancer cell invasion and metastasis by remodeling TGF- β receptor CF [94]. A new study has reported that inhibition of FUT8 and blockage of CF enhances T cell anti-tumor immune responses [131]. Additionally, use of FUTs (FUT2 and FUT3) genotype-dependent cut-off values for CA19–9 could improve sensitivity and reduce false-positive results [132], shedding light on the personalized application for glycomics, especially when combined with genetic and epigenetic analysis [133, 134].

GnTs is another family of key enzymes involved in the maturation and alteration of oligosaccharides. The clinical significance of GnTs presents relevance and specificity of cancer type to a certain degree [135–137]. For instance, GnT-V and β 1–6 branching N-linked glycans are associated with good prognosis of bladder cancer [136], whereas GnT-V in gastric cancer correlates with metastasis and poor prognosis [138]. The underlying causes and mechanisms still need to be elucidated. Interestingly, there are interactions not only within different GnTs, but also with other glycosyltransferases [114, 139, 140].

Additionally, it should be mentioned that the altered glycan repertoire of some key regulators such as cadherins [141] in carcinogenesis may lead to dysfunction and abnormal turnover of these molecules, which is associated with the acquisition of cancerous phenotypes. There is growing evidence that glycosylation is not a bystander in the complicated network of malignant transformation, it is indeed a determinant in a series of molecular events during carcinogenesis and can also be a potent immune modulatory or therapeutic target [12, 141, 142]. An intriguing study has demonstrated that the sugar moiety is critical for PD-L1 and PD-1 interaction and targeting glycosylation is a promising immunotherapy strategy for triple-negative breast cancer [143]. An opinion article has attached more importance to deciphering cancer “glyco-code” which modifies immunity and suggested that targeting glycans could offer new therapeutic opportunities [144].

Conclusions and prospects

Glycoproteins are one of the most important glycoconjugates in addition to proteoglycans and glycolipids. Glycans, which covalently attach to the protein backbone, are variant and versatile due to the variant sugar type, number, linkage site, enzyme activity, donor sialonucleotide availability as well as target protein conformation. Glycans greatly enrich the biological information of glycoproteins and thus enhance their interaction in cell behaviors. The complicated features of glycans as well as the limitation of research methodology have made the investigation of glycomics severely lag behind genomics and proteomics. Sialylation, fucosylation and complex branching structures have been revealed to be among the most commonly observed structural patterns in malignancies. The cutting-edge achievements of techniques including MS, CE, UPLC, which focus on glycomics as well as lectin based glyco-capture, microarray and microfluidics which focus on aberrantly glycosylated glycoproteins have greatly accelerated the discovery and application potentialities of glycomics and glycoprotein markers in various cancers.

AFP-L3 for the detection of HCC had long been included in several clinical guidelines by APASL, Japanese and Chinese societies for the treatment of liver cancers. At a cut-off value of 10%, AFP-L3% has a sensitivity of 71% and specificity of 63% for diagnosis of HCC in patients with AFP of 10–200 ng/mL [18]. Serum CA19–9 has a sensitivity of 78.2% and specificity of 82.3% for the diagnosis of pancreatic cancer [20]. Together with the other clinical widely used glycoprotein tumor markers such as PSA, CA125, etc., the extended exploration of glycosylation for tumor diagnostics and even for targeted therapeutics is encouraging.

The circulating proteins at high or medium abundance provide ideal targets for glycosylation detection both individually (like AFP-L3) or as a panel. Panel detection helps to compensate the insufficiency of sensitivity and specificity of individual protein or glycosylated proteins in cancer identification. More well-designed clinical validation needs to be conducted before clinical application. Combined with advanced AI algorithms, the implementation of individual specific as well as panel structure abundance detection is feasible technically and clinically.

The target of pertinent cancer-specific glycosylation as well as lectin based capture is crucial for detection. Although robust lectin based glyco-capture together with immunological detection in microarrays and microfluidics make the determination of specifically glycosylated target proteins easier to standardize and less redundant, the standardization of detection, which should be adapted to clinical utility, is still on the way for glycomic analysis and glycoprotein detection except for AFP-L3. The interference from glycosylation structures of the detection antibody itself often makes the routine protein detection method such as lectin-antibody sandwich ELISA fail. Unfortunately, the antibody against an aberrantly glycosylated peptide site is difficult to obtain due to the low antigenicity of glycans. Very limited types of such antibodies are available. The detection antibodies derived from genetic engineering techniques might be an option in the future.

From the glycan point of view, with the wide application of MS in routine clinical chemistry analysis as well as the advance of MS, the quantitative MS analysis for glycopeptides with site information, which indicates the microheterogeneity information of glycosylation, might be an alternative and promising option in the future. However, it is too early now to expect that the aforementioned analytical methods can remarkably accelerate the anticipated glyco-biomarker revolution. After all, there is a long and thorny journey from the bench to the clinic for the glycobiomarker discovery.

In addition, glycosylation is a template-free process and the expression of relevant genes involved are impacted by various transcription factors, epigenetic changes, macro- or microenvironment, etc. When thinking about glycomics and cancer biomarker discovery, a perspective of molecular pathological epidemiology (MPE) is of great reference value which provides transdisciplinary insights to decipher disease at multilevels integrating bioinformatics, *in vivo* pathology and omics technologies [145–147]. In turn, glycomics has also augmented the scope and connotation of MPE. Recent findings have also highlighted the relationship between glycosylation and immunity, and pinpointed the novel therapeutic strategies based on deciphering cancer “glyco-code” [144].

Thus, although the “sweet” molecules, with complicated physiological and pathological implications, belong to an “old” and “narrow” story, they surely will be uncovered with new promising diagnostic, prognostic, and even therapeutic applications in the future.

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References

1. Ohtsubo K, Marth JD. Glycosylation in cellular mechanisms of health and disease. *Cell* 2006;126:855–67. [PubMed: 16959566]
2. Sperandio M, Gleissner CA, Ley K. Glycosylation in immune cell trafficking. *Immunol Rev* 2009;230:97–113. [PubMed: 19594631]

3. Ohmi Y, Ise W, Harazono A, Takakura D, Fukuyama H, Baba Y, et al. Sialylation converts arthritogenic IgG into inhibitors of collagen-induced arthritis. *Nat Commun* 2016;7:11205. [PubMed: 27046227]
4. Kemna MJ, Plomp R, van Paassen P, Koeleman CA, Jansen BC, Damoiseaux J, et al. Galactosylation and sialylation levels of IgG predict relapse in patients with PR3-ANCA associated vasculitis. *EBioMedicine* 2017;17:108–18. [PubMed: 28169190]
5. Cao Y, Rocha ER, Smith CJ. Efficient utilization of complex N-linked glycans is a selective advantage for *Bacteroides fragilis* in extraintestinal infections. *Proc Natl Acad Sci USA* 2014;111:12901–6. [PubMed: 25139987]
6. Lu LL, Chung AW, Rosebrock TR, Ghebremichael M, Yu WH, Grace PS, et al. A functional role for antibodies in tuberculosis. *Cell* 2016;167:433.e14–43. [PubMed: 27667685]
7. Byrd-Leotis L, Liu R, Bradley KC, Lasanajak Y, Cummings SF, Song X, et al. Shotgun glycomics of pig lung identifies natural endogenous receptors for influenza viruses. *Proc Natl Acad Sci USA* 2014;111:E2241–50. [PubMed: 24843157]
8. van Montfort T, Eggink D, Boot M, Tuen M, Hioe CE, Berkhout B, et al. HIV-1 N-glycan composition governs a balance between dendritic cell-mediated viral transmission and antigen presentation. *J Immunol* 2011;187:4676–85. [PubMed: 21957147]
9. Mickum ML, Prasanphanich NS, Song X, Dorabawila N, Mandalasi M, Lasanajak Y, et al. Identification of antigenic glycans from *schistosoma mansoni* by using a shotgun egg glycan microarray. *Infect Immun* 2016;84:1371–86. [PubMed: 26883596]
10. Tolia NH, Enemark EJ, Sim BK, Joshua-Tor L. Structural basis for the EBA-175 erythrocyte invasion pathway of the malaria parasite *Plasmodium falciparum*. *Cell* 2005;122:183–93. [PubMed: 16051144]
11. Pinho SS, Reis CA. Glycosylation in cancer: mechanisms and clinical implications. *Nat Rev Cancer* 2015;15:540–55. [PubMed: 26289314]
12. Stowell SR, Ju T, Cummings RD. Protein glycosylation in cancer. *Annu Rev Pathol* 2015;10:473–510. [PubMed: 25621663]
13. Munkley J, Elliott DJ. Hallmarks of glycosylation in cancer. *Oncotarget* 2016;7:35478–89. [PubMed: 27007155]
14. Kailemia MJ, Park D, Lebrilla CB. Glycans and glycoproteins as specific biomarkers for cancer. *Anal Bioanal Chem* 2017;409:395–410. [PubMed: 27590322]
15. Vajaria BN, Patel PS. Glycosylation: a hallmark of cancer? *Glycoconj J* 2017;34:147–56. [PubMed: 27975160]
16. Mereiter S, Balmana M, Gomes J, Magalhaes A, Reis CA. Glycomic approaches for the discovery of targets in gastrointestinal cancer. *Front Oncol* 2016;6:55. [PubMed: 27014630]
17. Meany DL, Chan DW. Aberrant glycosylation associated with enzymes as cancer biomarkers. *Clin Proteomics* 2011;8:7. [PubMed: 21906357]
18. Leerapun A, Suravarapu SV, Bida JP, Clark RJ, Sanders EL, Mettler TA, et al. The utility of Lens culinaris agglutinin-reactive alpha-fetoprotein in the diagnosis of hepatocellular carcinoma: evaluation in a United States referral population. *Clin Gastroenterol Hepatol* 2007;5:394–402; quiz 267. [PubMed: 17368240]
19. Cheng J, Wang W, Zhang Y, Liu X, Li M, Wu Z, et al. Prognostic role of pre-treatment serum AFP-L3% in hepatocellular carcinoma: systematic review and meta-analysis. *PLoS One* 2014;9:e87011. [PubMed: 24498011]
20. Poruk KE, Gay DZ, Brown K, Mulvihill JD, Boucher KM, Scaife CL, et al. The clinical utility of CA 19–9 in pancreatic adenocarcinoma: diagnostic and prognostic updates. *Curr Mol Med* 2013;13:340–51. [PubMed: 23331006]
21. O'Brien DP, Sandanayake NS, Jenkinson C, Gentry-Maharaj A, Apostolidou S, Fourkala EO, et al. Serum CA19–9 is significantly upregulated up to 2 years before diagnosis with pancreatic cancer: implications for early disease detection. *Clin Cancer Res* 2015;21:622–31. [PubMed: 24938522]
22. Miyahara K, Nouse K, Dohi C, Morimoto Y, Kinugasa H, Wada N, et al. Alteration of N-glycan profiles in patients with chronic hepatitis and hepatocellular carcinoma. *Hepatol Res* 2015;45:986–93. [PubMed: 25495282]

23. Zhao YP, Xu XY, Fang M, Wang H, You Q, Yi CH, et al. Decreased core-fucosylation contributes to malignancy in gastric cancer. *PLoS One* 2014;9:e94536. [PubMed: 24732908]
24. Liu L, Yan B, Huang J, Gu Q, Wang L, Fang M, et al. The identification and characterization of novel N-glycan-based biomarkers in gastric cancer. *PLoS One* 2013;8:e77821. [PubMed: 24147084]
25. Qin R, Zhao J, Qin W, Zhang Z, Zhao R, Han J, et al. Discovery of non-invasive glycan biomarkers for detection and surveillance of gastric cancer. *J Cancer* 2017;8:1908–16. [PubMed: 28819389]
26. Zhao YP, Zhou PT, Ji WP, Wang H, Fang M, Wang MM, et al. Validation of N-glycan markers that improve the performance of CA19–9 in pancreatic cancer. *Clin Exp Med* 2017;17:9–18. [PubMed: 26714469]
27. Saldova R, Asadi Shehni A, Haakensen VD, Steinfeld I, Hilliard M, Kifer I, et al. Association of N-glycosylation with breast carcinoma and systemic features using high-resolution quantitative UPLC. *J Proteome Res* 2014;13:2314–27. [PubMed: 24669823]
28. Ren S, Zhang Z, Xu C, Guo L, Lu R, Sun Y, et al. Distribution of IgG galactosylation as a promising biomarker for cancer screening in multiple cancer types. *Cell Res* 2016;26:963–6. [PubMed: 27364686]
29. Yi CH, Weng HL, Zhou FG, Fang M, Ji J, Cheng C, et al. Elevated core-fucosylated IgG is a new marker for hepatitis B virus-related hepatocellular carcinoma. *Oncoimmunology* 2015;4:e1011503. [PubMed: 26587313]
30. Vuckovic F, Theodoratou E, Thaci K, Timofeeva M, Vojta A, Stambuk J, et al. IgG glycome in colorectal cancer. *Clin Cancer Res* 2016;22:3078–86. [PubMed: 26831718]
31. Qian Y, Wang Y, Zhang X, Zhou L, Zhang Z, Xu J, et al. Quantitative analysis of serum IgG galactosylation assists differential diagnosis of ovarian cancer. *J Proteome Res* 2013;12:4046–55. [PubMed: 23855414]
32. Zhu J, Lin Z, Wu J, Yin H, Dai J, Feng Z, et al. Analysis of serum haptoglobin fucosylation in hepatocellular carcinoma and liver cirrhosis of different etiologies. *J Proteome Res* 2014;13:2986–97. [PubMed: 24807840]
33. Wu J, Zhu J, Yin H, Buckanovich RJ, Lubman DM. Analysis of glycan variation on glycoproteins from serum by the reverse lectin-based ELISA assay. *J Proteome Res* 2014;13:2197–204. [PubMed: 24575722]
34. Liang Y, Ma T, Thakur A, Yu H, Gao L, Shi P, et al. Differentially expressed glycosylated patterns of alpha-1-antitrypsin as serum biomarkers for the diagnosis of lung cancer. *Glycobiology* 2015;25:331–40. [PubMed: 25347993]
35. Comunale MA, Wang M, Anbarasan N, Betesh L, Karabudak A, Moritz E, et al. Total serum glycan analysis is superior to lectin-FLISA for the early detection of hepatocellular carcinoma. *Proteomics Clin Appl* 2013;7:690–700. [PubMed: 23857719]
36. Tanabe K, Kitagawa K, Kojima N, Iijima S. Multifucosylated alpha-1-acid glycoprotein as a novel marker for hepatocellular carcinoma. *J Proteome Res* 2016;15:2935–44. [PubMed: 27354006]
37. Astrom E, Stal P, Zenlander R, Edenvik P, Alexandersson C, Haglund M, et al. Reverse lectin ELISA for detecting fucosylated forms of alpha1-acid glycoprotein associated with hepatocellular carcinoma. *PLoS One* 2017;12:e0173897. [PubMed: 28296934]
38. Zhang D, Huang J, Luo D, Feng X, Liu Y, Liu Y. Glycosylation change of alpha-1-acid glycoprotein as a serum biomarker for hepatocellular carcinoma and cirrhosis. *Biomark Med* 2017;11:423–30. [PubMed: 28621608]
39. Balmana M, Gimenez E, Puerta A, Llop E, Figueras J, Fort E, et al. Increased alpha1–3 fucosylation of alpha-1-acid glycoprotein (AGP) in pancreatic cancer. *J Proteomics* 2016;132:144–54. [PubMed: 26563517]
40. Weiz S, Wiczorek M, Schwedler C, Kaup M, Braicu EI, Schouli J, et al. Acute-phase glycoprotein N-glycome of ovarian cancer patients analyzed by CE-LIF. *Electrophoresis* 2016;37:1461–7. [PubMed: 26763099]
41. Yin H, Lin Z, Nie S, Wu J, Tan Z, Zhu J, et al. Mass-selected site-specific core-fucosylation of ceruloplasmin in alcohol-related hepatocellular carcinoma. *J Proteome Res* 2014;13:2887–96. [PubMed: 24799124]

42. Sogabe M, Nozaki H, Tanaka N, Kubota T, Kaji H, Kuno A, et al. Novel glycan biomarker for ovarian cancer that detects clear cell carcinoma. *J Proteome Res* 2014;13:1624–35. [PubMed: 24498956]
43. Balmana M, Sarrats A, Llop E, Barrabes S, Saldova R, Ferri MJ, et al. Identification of potential pancreatic cancer serum markers: increased sialyl-Lewis X on ceruloplasmin. *Clin Chim Acta* 2015;442:56–62. [PubMed: 25595436]
44. Li L, Gu X, Fang M, Ji J, Yi C, Gao C. The diagnostic value of serum fucosylated fetuin A in hepatitis B virus-related liver diseases. *Clin Chem Lab Med* 2016;54:693–701. [PubMed: 26035113]
45. Betesh L, Comunale MA, Wang M, Liang H, Hafner J, Karabudak A, et al. Identification of fucosylated Fetuin-A as a potential biomarker for cholangiocarcinoma. *Proteomics Clin Appl* 2017;11. doi: 10.1002/prca.201600141.
46. Drake PM, Cho W, Li B, Prakobphol A, Johansen E, Anderson NL, et al. Sweetening the pot: adding glycosylation to the biomarker discovery equation. *Clin Chem* 2010;56:223–36. [PubMed: 19959616]
47. Ciucanu I, Costello CE. Elimination of oxidative degradation during the per-O-methylation of carbohydrates. *J Am Chem Soc* 2003;125:16213–9. [PubMed: 14692762]
48. Kang P, Mechref Y, Klouckova I, Novotny MV. Solid-phase permethylation of glycans for mass spectrometric analysis. *Rapid Commun Mass Spectrom* 2005;19:3421–8. [PubMed: 16252310]
49. Harvey DJ. Electrospray mass spectrometry and fragmentation of N-linked carbohydrates derivatized at the reducing terminus. *J Am Soc Mass Spectrom* 2000;11:900–15. [PubMed: 11014452]
50. Anumula KR. Advances in fluorescence derivatization methods for high-performance liquid chromatographic analysis of glyco-protein carbohydrates. *Anal Biochem* 2006;350:1–23. [PubMed: 16271261]
51. Chen Z, Glover MS, Li L. Recent advances in ion mobility-mass spectrometry for improved structural characterization of glycans and glycoconjugates. *Curr Opin Chem Biol* 2017;42:1–8. [PubMed: 29080446]
52. Krishnan S, Whitwell HJ, Cuenco J, Gentry-Maharaj A, Menon U, Pereira SP, et al. Evidence of altered glycosylation of serum proteins prior to pancreatic cancer diagnosis. *Int J Mol Sci* 2017;18:2670.
53. Chandler KB, Pompach P, Goldman R, Edwards N. Exploring site-specific N-glycosylation microheterogeneity of haptoglobin using glycopeptide CID tandem mass spectra and glycan database search. *J Proteome Res* 2013;12:3652–66. [PubMed: 23829323]
54. Sanda M, Pompach P, Brnakova Z, Wu J, Makambi K, Goldman R. Quantitative liquid chromatography-mass spectrometry-multiple reaction monitoring (LC-MS-MRM) analysis of site-specific glycoforms of haptoglobin in liver disease. *Mol Cell Proteomics* 2013;12:1294–305. [PubMed: 23389048]
55. Pompach P, Brnakova Z, Sanda M, Wu J, Edwards N, Goldman R. Site-specific glycoforms of haptoglobin in liver cirrhosis and hepatocellular carcinoma. *Mol Cell Proteomics* 2013;12:1281–93. [PubMed: 23389049]
56. Zhang Y, Zhu J, Yin H, Marrero J, Zhang XX, Lubman DM. ESI-LC-MS method for haptoglobin fucosylation analysis in hepatocellular carcinoma and liver cirrhosis. *J Proteome Res* 2015;14:5388–95. [PubMed: 26503433]
57. Lin Z, Yin H, Lo A, Ruffin MT, Anderson MA, Simeone DM, et al. Label-free relative quantification of alpha-2-macroglobulin site-specific core-fucosylation in pancreatic cancer by LC-MS/MS. *Electrophoresis* 2014;35:2108–15. [PubMed: 24285556]
58. Gbormittah FO, Bones J, Hincapie M, Tousi F, Hancock WS, Iliopoulos O. Clusterin glycopeptide variant characterization reveals significant site-specific glycan changes in the plasma of clear cell renal cell carcinoma. *J Proteome Res* 2015;14:2425–36. [PubMed: 25855029]
59. Song T, Aldredge D, Lebrilla CB. A method for in-depth structural annotation of human serum glycans that yields biological variations. *Anal Chem* 2015;87:7754–62. [PubMed: 26086522]
60. Sethi MK, Hancock WS, Fanayan S. Identifying N-glycan biomarkers in colorectal cancer by mass spectrometry. *Acc Chem Res* 2016;49:2099–106. [PubMed: 27653471]

61. Abd Hamid UM, Royle L, Saldova R, Radcliffe CM, Harvey DJ, Storr SJ, et al. A strategy to reveal potential glycan markers from serum glycoproteins associated with breast cancer progression. *Glycobiology* 2008;18:1105–18. [PubMed: 18818422]
62. Chen B, Zhong X, Feng Y, Snovida S, Xu M, Rogers J, et al. Targeted MultiNotch MS(3) approach for relative quantification of N-glycans using multiplexed carbonyl-reactive isobaric tags. *Anal Chem* 2017;90:1129–35. [PubMed: 29182308]
63. Rodrigo MA, Zitka O, Krizkova S, Moulick A, Adam V, Kizek R. MALDI-TOF MS as evolving cancer diagnostic tool: a review. *J Pharm Biomed Anal* 2014;95:245–55. [PubMed: 24699369]
64. Yamagaki T, Nakanishi H. Ion intensity analysis of post-source decay fragmentation in curved-field reflectron matrix-assisted laser desorption/ionization time-of-flight mass spectrometry of carbohydrates: for structural characterization of glycosylation in proteome analysis. *Proteomics* 2001;1:329–39. [PubMed: 11680879]
65. Lin Z, Simeone DM, Anderson MA, Brand RE, Xie X, Shedden KA, et al. Mass spectrometric assay for analysis of haptoglobin fucosylation in pancreatic cancer. *J Proteome Res* 2011;10:2602–11. [PubMed: 21417406]
66. Nakagawa T, Miyoshi E, Yakushijin T, Hiramatsu N, Igura T, Hayashi N, et al. Glycomic analysis of alpha-fetoprotein L3 in hepatoma cell lines and hepatocellular carcinoma patients. *J Proteome Res* 2008;7:2222–33. [PubMed: 18479159]
67. Palmigiano A, Bua RO, Barone R, Rymen D, Regal L, Deconinck N, et al. MALDI-MS profiling of serum O-glycosylation and N-glycosylation in COG5-CDG. *J Mass Spectrom* 2017;52:372–7. [PubMed: 28444691]
68. Agakova A, Vuckovic F, Klaric L, Lauc G, Agakov F. Automated integration of a UPLC glycomic profile. *Methods Mol Biol* 2017;1503:217–33. [PubMed: 27743370]
69. Pucic-Bakovic M. High-throughput analysis of the IgG N-glycome by UPLC-FLR. *Methods Mol Biol* 2017;1503:21–9. [PubMed: 27743356]
70. Mittermayr S, Bones J, Doherty M, Guttman A, Rudd PM. Multiplexed analytical glycomics: rapid and confident IgG N-glycan structural elucidation. *J Proteome Res* 2011;10:3820–9. [PubMed: 21699237]
71. Knezevic A, Bones J, Kracun SK, Gornik O, Rudd PM, Lauc G. High throughput plasma N-glycome profiling using multiplexed labelling and UPLC with fluorescence detection. *Analyst* 2011;136:4670–3. [PubMed: 21956149]
72. Khatri K, Klein JA, Haserick JR, Leon DR, Costello CE, McComb ME, et al. Microfluidic capillary electrophoresis-mass spectrometry for analysis of monosaccharides, oligosaccharides, and glycopeptides. *Anal Chem* 2017;89:6645–55. [PubMed: 28530388]
73. Mantovani V, Galeotti F, Maccari F, Volpi N. Recent advances in capillary electrophoresis separation of monosaccharides, oligosaccharides, and polysaccharides. *Electrophoresis* 2017;39:179–89. [PubMed: 28857216]
74. Guttman A, Pritchett T. Capillary gel electrophoresis separation of high-mannose type oligosaccharides derivatized by 1-aminopyrene-3,6,8-trisulfonic acid. *Electrophoresis* 1995;16:1906–11. [PubMed: 8586063]
75. Chen FT, Evangelista RA. Profiling glycoprotein n-linked oligosaccharide by capillary electrophoresis. *Electrophoresis* 1998;19:2639–44. [PubMed: 9848672]
76. Zhuang Z, Starkey JA, Mechref Y, Novotny MV, Jacobson SC. Electrophoretic analysis of N-glycans on microfluidic devices. *Anal Chem* 2007;79:7170–5. [PubMed: 17685584]
77. Kovacs Z, Simon A, Szabo Z, Nagy Z, Varoczy L, Pal I, et al. Capillary electrophoresis analysis of N-glycosylation changes of serum paraproteins in multiple myeloma. *Electrophoresis* 2017;38:2115–23. [PubMed: 28116769]
78. Feng HT, Su M, Rifai FN, Li P, Li SF. Parallel analysis and orthogonal identification of N-glycans with different capillary electrophoresis mechanisms. *Anal Chim Acta* 2017;953:79–86. [PubMed: 28010746]
79. Laroy W, Contreras R, Callewaert N. Glycome mapping on DNA sequencing equipment. *Nat Protoc* 2006;1:397–405. [PubMed: 17406262]

80. Callewaert N, Geysens S, Molemans F, Contreras R. Ultrasensitive profiling and sequencing of N-linked oligosaccharides using standard DNA-sequencing equipment. *Glycobiology* 2001;11:275–81. [PubMed: 11358876]
81. Callewaert N, Van Vlierberghe H, Van Hecke A, Laroy W, Delanghe J, Contreras R. Noninvasive diagnosis of liver cirrhosis using DNA sequencer-based total serum protein glycomics. *Nat Med* 2004;10:429–34. [PubMed: 15152612]
82. Verhelst X, Vanderschaeghe D, Castera L, Raes T, Geerts A, Francoz C, et al. A glycomics-based test predicts the development of hepatocellular carcinoma in cirrhosis. *Clin Cancer Res* 2017;23:2750–8. [PubMed: 27986746]
83. Vermassen T, Van Praet C, Vanderschaeghe D, Maenhout T, Lumen N, Callewaert N, et al. Capillary electrophoresis of urinary prostate glycoproteins assists in the diagnosis of prostate cancer. *Electrophoresis* 2014;35:1017–24. [PubMed: 24254641]
84. Debruyne EN, Vanderschaeghe D, Van Vlierberghe H, Vanhecke A, Callewaert N, Delanghe JR. Diagnostic value of the hemopexin N-glycan profile in hepatocellular carcinoma patients. *Clin Chem* 2010;56:823–31. [PubMed: 20348404]
85. Wang M, Fang M, Zhu J, Feng H, Warner E, Yi C, et al. Serum N-glycans outperform CA19–9 in diagnosis of extrahepatic cholangiocarcinoma. *Electrophoresis* 2017;38:2749–56. [PubMed: 28752594]
86. Chen J, Fang M, Zhao YP, Yi CH, Ji J, Cheng C, et al. Serum N-glycans: a new diagnostic biomarker for light chain multiple myeloma. *PLoS One* 2015;10:e0127022. [PubMed: 26075387]
87. Zhao YP, Ruan CP, Wang H, Hu ZQ, Fang M, Gu X, et al. Identification and assessment of new biomarkers for colorectal cancer with serum N-glycan profiling. *Cancer* 2012;118:639–50. [PubMed: 21853445]
88. Fang M, Zhao YP, Zhou FG, Lu LG, Qi P, Wang H, et al. N-glycan based models improve diagnostic efficacies in hepatitis B virus-related hepatocellular carcinoma. *Int J Cancer* 2010;127:148–59. [PubMed: 19904744]
89. Feng HT, Li P, Rui G, Stray J, Khan S, Chen SM, et al. Multiplexing N-glycan analysis by DNA analyzer. *Electrophoresis* 2017;38:1788–99. [PubMed: 28426178]
90. Feng HT, Lim S, Laserna AK, Li P, Yin X, Simsek E, et al. High throughput human plasma N-glycan analysis using DNA analyzer and multivariate analysis for biomarker discovery. *Anal Chim Acta* 2017;995:106–13. [PubMed: 29126476]
91. Badr HA, Alsaadek DM, Darwish AA, Elsayed AI, Bekmanov BO, Khussainova EM, et al. Lectin approaches for glycoproteomics in FDA-approved cancer biomarkers. *Expert Rev Proteomics* 2014;11:227–36. [PubMed: 24611567]
92. Andre S, Kaltner H, Manning JC, Murphy PV, Gabius HJ. Lectins: getting familiar with translators of the sugar code. *Molecules* 2015;20:1788–823. [PubMed: 25621423]
93. Hashim OH, Jayapalan JJ, Lee CS. Lectins: an effective tool for screening of potential cancer biomarkers. *PeerJ* 2017;5:e3784. [PubMed: 28894650]
94. Tu CF, Wu MY, Lin YC, Kannagi R, Yang RB. FUT8 promotes breast cancer cell invasiveness by remodeling TGF-beta receptor core fucosylation. *Breast Cancer Res* 2017;19:111. [PubMed: 28982386]
95. Yang HF, Yu M, Jin HD, Yao JQ, Lu ZL, Yabasin IB, et al. Fentanyl promotes breast cancer cell stemness and epithelial-mesenchymal transition by upregulating alpha 1,6-fucosylation via Wnt/beta-catenin signaling pathway. *Front Physiol* 2017;8:510. [PubMed: 28798691]
96. Matsuda A, Higashi M, Nakagawa T, Yokoyama S, Kuno A, Yonezawa S, et al. Assessment of tumor characteristics based on glycoform analysis of membrane-tethered MUC1. *Lab Invest* 2017;97:1103–13. [PubMed: 28581490]
97. Zhang S, Jiang K, Zhang Q, Guo K, Liu Y. Serum fucosylated paraoxonase 1 as a potential glycobiomarker for clinical diagnosis of early hepatocellular carcinoma using ELISA Index. *Glycoconj J* 2015;32:119–25. [PubMed: 25702281]
98. Fanayan S, Hincapie M, Hancock WS. Using lectins to harvest the plasma/serum glycoproteome. *Electrophoresis* 2012;33:1746–54. [PubMed: 22740463]

99. Song E, Zhu R, Hammoud ZT, Mechref Y. LC-MS/MS quantitation of esophagus disease blood serum glycoproteins by enrichment with hydrazide chemistry and lectin affinity chromatography. *J Proteome Res* 2014;13:4808–20. [PubMed: 25134008]
100. Hirabayashi J, Yamada M, Kuno A, Tateno H. Lectin microarrays: concept, principle and applications. *Chem Soc Rev* 2013;42:4443–58. [PubMed: 23443201]
101. Matsuda A, Kuno A, Nakagawa T, Ikehara Y, Irimura T, Yamamoto M, et al. Lectin microarray-based sero-biomarker verification targeting aberrant O-linked glycosylation on mucin 1. *Anal Chem* 2015;87:7274–81. [PubMed: 26091356]
102. Syed P, Gidwani K, Kekki H, Leivo J, Pettersson K, Lamminmaki U. Role of lectin microarrays in cancer diagnosis. *Proteomics* 2016;16:1257–65. [PubMed: 26841254]
103. Shang Y, Zeng Y, Zeng Y. Integrated microfluidic lectin barcode platform for high-performance focused glycomic profiling. *Sci Rep* 2016;6:20297. [PubMed: 26831207]
104. Patwa T, Li C, Simeone DM, Lubman DM. Glycoprotein analysis using protein microarrays and mass spectrometry. *Mass Spectrom Rev* 2010;29:830–44. [PubMed: 20077480]
105. Kletter D, Curnutte B, Maupin KA, Bern M, Haab BB. Exploring the specificities of glycan-binding proteins using glycan array data and the GlycoSearch software. *Methods Mol Biol* 2015;1273:203–14. [PubMed: 25753713]
106. Haab BB. Antibody-lectin sandwich arrays for biomarker and glycobiology studies. *Expert Rev Proteomics* 2010;7:9–11. [PubMed: 20121469]
107. Agre P, Bertozzi C, Bissell M, Campbell KP, Cummings RD, Desai UR, et al. Training the next generation of biomedical investigators in glycosciences. *J Clin Invest* 2016;126:405–8. [PubMed: 26829621]
108. Wu J, Xie X, Nie S, Buckanovich RJ, Lubman DM. Altered expression of sialylated glycoproteins in ovarian cancer sera using lectin-based ELISA assay and quantitative glycoproteomics analysis. *J Proteome Res* 2013;12:3342–52. [PubMed: 23731285]
109. Walsh I, Zhao S, Campbell M, Taron CH, Rudd PM. Quantitative profiling of glycans and glycopeptides: an informatics' perspective. *Curr Opin Struct Biol* 2016;40:70–80. [PubMed: 27522273]
110. Lisacek F, Mariethoz J, Alocci D, Rudd PM, Abrahams JL, Campbell MP, et al. Databases and associated tools for glycomics and glycoproteomics. *Methods Mol Biol* 2017;1503:235–64. [PubMed: 27743371]
111. Lu J, Gu J. Significance of beta-galactoside alpha 2,6 sialyltransferase 1 in cancers. *Molecules* 2015;20:7509–27. [PubMed: 25919275]
112. Chen CY, Jan YH, Juan YH, Yang CJ, Huang MS, Yu CJ, et al. Fucosyltransferase 8 as a functional regulator of nonsmall cell lung cancer. *Proc Natl Acad Sci USA* 2013;110:630–5. [PubMed: 23267084]
113. Li J, Guillebon AD, Hsu JW, Barthel SR, Dimitroff CJ, Lee YF, et al. Human fucosyltransferase 6 enables prostate cancer metastasis to bone. *Br J Cancer* 2013;109:3014–22. [PubMed: 24178760]
114. Zhao Y, Nakagawa T, Itoh S, Inamori K, Isaji T, Kariya Y, et al. N-acetylglucosaminyltransferase III antagonizes the effect of N-acetylglucosaminyltransferase V on alpha3beta1 integrin-mediated cell migration. *J Biol Chem* 2006;281:32122–30. [PubMed: 16940045]
115. Huang C, Huang M, Chen W, Zhu W, Meng H, Guo L, et al. N-acetylglucosaminyltransferase V modulates radiosensitivity and migration of small cell lung cancer through epithelial-mesenchymal transition. *FEBS J* 2015;282:4295–306. [PubMed: 26293457]
116. Taniguchi N, Kizuka Y. Glycans and cancer: role of N-glycans in cancer biomarker, progression and metastasis, and therapeutics. *Adv Cancer Res* 2015;126:11–51. [PubMed: 25727145]
117. Kizuka Y, Taniguchi N. Enzymes for N-glycan branching and their genetic and nongenetic regulation in cancer. *Biomolecules* 2016;6:e25. [PubMed: 27136596]
118. Bai Q, Liu L, Xia Y, Long Q, Wang J, Xu J, et al. Prognostic significance of ST3GAL-1 expression in patients with clear cell renal cell carcinoma. *BMC Cancer* 2015;15:880. [PubMed: 26552809]
119. Zhang Z, Wuhler M, Holst S. Serum sialylation changes in cancer. *Glycoconj J* 2018;35:139–60. [PubMed: 29680984]

120. Bull C, Stoel MA, den Brok MH, Adema GJ. Sialic acids sweeten a tumor's life. *Cancer Res* 2014;74:3199–204. [PubMed: 24830719]
121. Bull C, den Brok MH, Adema GJ. Sweet escape: sialic acids in tumor immune evasion. *Biochim Biophys Acta* 2014;1846:238–46. [PubMed: 25026312]
122. Glavey SV, Manier S, Natoni A, Sacco A, Moschetta M, Reagan MR, et al. The sialyltransferase ST3GAL6 influences homing and survival in multiple myeloma. *Blood* 2014;124:1765–76. [PubMed: 25061176]
123. Bos PD, Zhang XH, Nadal C, Shu W, Gomis RR, Nguyen DX, et al. Genes that mediate breast cancer metastasis to the brain. *Nature* 2009;459:1005–9. [PubMed: 19421193]
124. Wang L, Liu Y, Wu L, Sun XL. Sialyltransferase inhibition and recent advances. *Biochim Biophys Acta* 2016;1864:143–53. [PubMed: 26192491]
125. Szabo R, Skropeta D. Advancement of sialyltransferase inhibitors: therapeutic challenges and opportunities. *Med Res Rev* 2017;37:219–70. [PubMed: 27678392]
126. Chen JY, Tang YA, Huang SM, Juan HF, Wu LW, Sun YC, et al. A novel sialyltransferase inhibitor suppresses FAK/paxillin signaling and cancer angiogenesis and metastasis pathways. *Cancer Res* 2011;71:473–83. [PubMed: 21224350]
127. Agrawal P, Fontanals-Cirera B, Sokolova E, Jacob S, Vaiana CA, Argibay D, et al. A systems biology approach identifies FUT8 as a driver of melanoma metastasis. *Cancer Cell* 2017;31:804.e7–19. [PubMed: 28609658]
128. Wang Y, Fukuda T, Isaji T, Lu J, Im S, Hang Q, et al. Loss of alpha1,6-fucosyltransferase inhibits chemical-induced hepatocellular carcinoma and tumorigenesis by down-regulating several cell signaling pathways. *Faseb J* 2015;29:3217–27. [PubMed: 25873065]
129. Ji J, Gu X, Fang M, Zhao Y, Yi C, Wang A, et al. Expression of alpha 1,6-fucosyltransferase 8 in hepatitis B virus-related hepatocellular carcinoma influences tumour progression. *Dig Liver Dis* 2013;45:414–21. [PubMed: 23352314]
130. Wang X, Chen J, Li QK, Peskoe SB, Zhang B, Choi C, et al. Over-expression of alpha (1,6) fucosyltransferase associated with aggressive prostate cancer. *Glycobiology* 2014;24:935–44. [PubMed: 24906821]
131. Okada M, Chikuma S, Kondo T, Hibino S, Machiyama H, Yokosuka T, et al. Blockage of core fucosylation reduces cell- surface expression of PD-1 and promotes anti-tumor immune responses of T cells. *Cell Rep* 2017;20:1017–28. [PubMed: 28768188]
132. Wannho A, Hov JR, Folseraas T, Rupp C, Friedrich K, Anmarkrud JA, et al. FUT2 and FUT3 genotype determines CA19-9 cut-off values for detection of cholangiocarcinoma in patients with primary sclerosing cholangitis. *J Hepatol* 2013;59:1278–84. [PubMed: 23958938]
133. Lauc G, Vojta A, Zoldos V. Epigenetic regulation of glycosylation is the quantum mechanics of biology. *Biochim Biophys Acta* 2014;1840:65–70. [PubMed: 23999089]
134. Lauc G, Essafi A, Human JE, Hayward C, Knezevic A, Kattla JJ, et al. Genomics meets glycomics-the first GWAS study of human N-glycome identifies HNF1alpha as a master regulator of plasma protein fucosylation. *PLoS Genet* 2010;6:e1001256. [PubMed: 21203500]
135. Kim YS, Hwang SY, Kang HY, Sohn H, Oh S, Kim JY, et al. Functional proteomics study reveals that N-acetylglucosaminyltransferase V reinforces the invasive/metastatic potential of colon cancer through aberrant glycosylation on tissue inhibitor of metalloproteinase-1. *Mol Cell Proteomics* 2008;7:1–14. [PubMed: 17878270]
136. Ishimura H, Takahashi T, Nakagawa H, Nishimura S, Arai Y, Horikawa Y, et al. N-acetylglucosaminyltransferase V and beta1–6 branching N-linked oligosaccharides are associated with good prognosis of patients with bladder cancer. *Clin Cancer Res* 2006;12:2506–11. [PubMed: 16638859]
137. Murata K, Miyoshi E, Kameyama M, Ishikawa O, Kabuto T, Sasaki Y, et al. Expression of N-acetylglucosaminyltransferase V in colorectal cancer correlates with metastasis and poor prognosis. *Clin Cancer Res* 2000;6:1772–7. [PubMed: 10815896]
138. Huang B, Wu Q, Ge Y, Zhang J, Sun L, Zhang Y, et al. Expression of N-acetylglucosaminyltransferase V in gastric cancer correlates with metastasis and prognosis. *Int J Oncol* 2014;44:849–57. [PubMed: 24399258]

139. Pinho SS, Reis CA, Paredes J, Magalhaes AM, Ferreira AC, Figueiredo J, et al. The role of N-acetylglucosaminyltransferase III and V in the post-transcriptional modifications of E-cadherin. *Hum Mol Genet* 2009;18:2599–608. [PubMed: 19403558]
140. Lu J, Isaji T, Im S, Fukuda T, Kameyama A, Gu J. Expression of N-acetylglucosaminyltransferase III suppresses alpha2,3-sialylation, and its distinctive functions in cell migration are attributed to alpha2,6-sialylation levels. *J Biol Chem* 2016;291:5708–20. [PubMed: 26801611]
141. Carvalho S, Reis CA, Pinho SS. Cadherins glycans in cancer: sweet players in a bitter process. *Trends Cancer* 2016;2:519–31. [PubMed: 28741480]
142. Dempsey E, Rudd PM. Acute phase glycoproteins: bystanders or participants in carcinogenesis? *Ann N Y Acad Sci* 2012;1253:122–32. [PubMed: 22352780]
143. Li CW, Lim SO, Chung EM, Kim YS, Park AH, Yao J, et al. Eradication of triple-negative breast cancer cells by targeting glycosylated PD-L1. *Cancer Cell* 2018;33:187.e10–201. [PubMed: 29438695]
144. Rodriguez E, Schettters ST, van Kooyk Y. The tumour glyco-code as a novel immune checkpoint for immunotherapy. *Nat Rev Immunol* 2018;18:204–11. [PubMed: 29398707]
145. Ogino S, Nowak JA, Hamada T, Phipps AI, Peters U, Milner DA Jr, et al. Integrative analysis of exogenous, endogenous, tumour and immune factors for precision medicine. *Gut* 2018;67:1168–80. [PubMed: 29437869]
146. Ogino S, Lochhead P, Chan AT, Nishihara R, Cho E, Wolpin BM, et al. Molecular pathological epidemiology of epigenetics: emerging integrative science to analyze environment, host, and disease. *Mod Pathol* 2013;26:465–84. [PubMed: 23307060]
147. Ogino S, Chan AT, Fuchs CS, Giovannucci E. Molecular pathological epidemiology of colorectal neoplasia: an emerging transdisciplinary and interdisciplinary field. *Gut* 2011;60:397–411. [PubMed: 21036793]

Glyco-biomarkers in clinical practice and novel glyco-biomarker discovery in recent 5 years.

Table 1:

Glycomic study target	Cancer	Significant glycosylation alterations	Role
AFP-L3 (used in clinic)	Hepatic	Core-fucosylation [18, 19]	Diagnosis, prognosis
CA19-9 (used in clinic)	Pancreatic	Sialyl-Lewis A structure [20, 21]	Diagnosis, prognosis
Total serum/plasma profiles	Hepatic	Increased multi-antennary glycans with fucose residues [22]	Diagnosis
	Gastric	Decreased core-fucosylated glycans [23, 24]; increased hybrid and multi-branched structures and decreased monoantennary, galactose, bisecting type and core fucose structures [25]	Diagnosis; monitoring progression
Immunoglobulin G (IgG)	Pancreatic	Increased core-fucose residues [26]	Diagnosis
	Breast	Increased sialylation, branching, and outer-arm fucosylation and decreased high-mannosylated and biantennary core-fucosylated glycans [27]	Diagnosis
Colorectal	Hepatic	Decreased galactosylation [28]; increased core-fucosylation [29]	Diagnosis; prognosis
	Colorectal	Decreased galactosylation [28]; decreased galactosylation and sialylation; increased core-fucosylation of neutral glycans and decreased core-fucosylation of sialylated glycans [30]	Diagnosis
Haptogloblin (Hp)	Gastric	Decreased galactosylation [28]	Diagnosis
	Lung	Decreased galactosylation [28]	Diagnosis
Ovarian	Ovarian	Decreased galactosylation [28]; decreased galactosylation [31]	Diagnosis
	Hepatic	Increased bi-fucosylation [32]	Diagnosis
Ovarian	Ovarian	Increased fucosylation [33]	Diagnosis
	Lung	Increased galactosylation, fucosylation and poly-LacNAc structures [34]	Diagnosis
Hepatic	Hepatic	Increased fucosylation [35]	Diagnosis
	Hepatic	Increased multifucosylated tetraantennary structures [36]; increased fucosylation [37]; increased fucosylation and sialylation [38]	Diagnosis
Pancreatic	Pancreatic	Increased α -1,3 fucosylation [39]	Diagnosis
	Ovarian	Increased core-fucosylated bi-antennary structures [40]	Diagnosis
Ceruloplasmin (CP)	Hepatic	Increased core-fucosylation [41]	Diagnosis
	Ovarian	Increased LactiNAc motifs [42]	Diagnosis
Pancreatic	Pancreatic	Increased sialyl-Lewis X levels [43]	Diagnosis
	Hepatic	Increased fucosylation [35, 44]	Diagnosis; prognosis
Fetuin A	Cholangio	Increased fucosylation [45]	Diagnosis