Biochemical and Biophysical Research Communications 507 (2018) 173–177

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Detection of *N*-glycolylneuraminic acid biomarkers in sera from patients with ovarian cancer using an engineered *N*-glycolylneuraminic acid-specific lectin SubB2M

L.K. Shewell [a], J.J. Wang [a], J.C. Paton [b], A.W. Paton [b], C.J. Day [a, *], M.P. Jennings [a, **]

[a] Institute for Glycomics, Griffith University, Gold Coast, Australia
[b] Research Centre for Infectious Diseases, Department of Molecular and Biomedical Science, University of Adelaide, Adelaide, Australia

ARTICLE INFO

Article history:
Received 25 October 2018
Accepted 1 November 2018
Available online 8 November 2018

Keywords:
Ovarian cancer
Biomarker
N-glycolylneuraminic acid
Neu5Gc
Lectin
Diagnostic

ABSTRACT

N-glycolylneuraminic acid (Neu5Gc)-containing glycans are a prominent form of aberrant glycosylation found in human tumor cells and have been proposed as cancer biomarkers. The B subunit of the subtilase cytotoxin (SubB) produced by Shiga toxigenic *Escherichia coli* recognises Neu5Gc containing glycans. We have previously engineered this lectin, SubB2M, for greater specificity and enhanced recognition of Neu5Gc-containing glycans. Here we further explore the utility of SubB2M to detect Neu5Gc tumor biomarkers in sera from patients with ovarian cancer. Using surface plasmon resonance (SPR) we show that SubB2M can detect the established ovarian cancer biomarker, CA125, in a highly sensitive and specific fashion in the context of human serum. These studies established conditions for screening serum samples from patients with ovarian cancer for Neu5Gc glycans. We found that serum from patients with all stages of ovarian cancer had significantly elevated mean levels of Neu5Gc glycans compared to normal controls. Serum from patients with late stage disease (stages IIIC, IV) had uniformly elevated levels of Neu5Gc glycans. Detection of Neu5Gc-glycans using SubB2M has the potential to be used as a diagnostic ovarian cancer biomarker, as well as a tool for monitoring treatment and disease progression in late stage disease.

1. Introduction

Ovarian cancer has the highest rate of mortality among the gynecologic malignancies [1]. The five-year survival rate is less than 20% in women diagnosed with late stage ovarian cancer but increases to over 90% when detected in early stages (stage I-II). However, patients are typically diagnosed in the late stages due to the largely asymptomatic nature of this disease in the early stages [2] and the lack of highly sensitive and specific biomarkers. The best currently available biomarker for ovarian cancer is the human cancer antigen 125 (CA125), also known as MUC16, a heavily glycosylated mucin [3]. Serum CA125 levels are elevated in approximately 80% of ovarian cancer cases at the time of diagnosis [4]. However, CA125 serum levels may also be elevated in nonmalignant conditions such as endometriosis [5], pregnancy [6], ovarian cysts [7], pelvic inflammatory disease [8], hepatitis [9], cirrhosis [10] and in the follicular phase of the menstrual cycle [11]. Due to the limitations of CA125 as a biomarker, it is currently only approved by the FDA to be used to monitor the response to therapy and disease recurrence [12].

Glycans terminating with *N*-glycolylneuraminic acid (Neu5Gc) are not expressed at significant levels on healthy human tissues, as humans express an inactive cytidine monophosphate *N*-acetylneuraminic acid (Neu5Ac) hydroxylase (CMAH) enzyme [13,14]. However, Neu5Gc-containing glycans are found in human tumor tissues and cells [15–19] and have been proposed as a tumor antigen [18,20,21]. While there are analytical methods available [22,23] and a commercially available IgY antibody [24] for the detection of Neu5Gc, we improved upon this by developing a lectin with enhanced sensitivity and specificity for this glycan in complex biological samples. We engineered the B subunit of the Shiga toxigenic *Escherichia coli* (STEC) Subtilase cytotoxin (SubAB) to reduce the recognition of Neu5Ac and broaden the Neu5Gc linkage

* Corresponding author. Institute for Glycomics, Griffith University, Gold Coast campus, QLD 4222, Australia.
** Corresponding author. Institute for Glycomics, Griffith University, Gold Coast campus, QLD 4222, Australia.
E-mail addresses: c.day@griffith.edu.au (C.J. Day), m.jennings@griffith.edu.au (M.P. Jennings).

https://doi.org/10.1016/j.bbrc.2018.11.001

recognition. This improved Neu5Gc-specific lectin was termed SubB2M [25,26]. Here we describe the analysis of serum samples from patients with ovarian cancer compared to normal, cancer-free females using SubB2M via surface plasmon resonance (SPR).

2. Materials and methods

2.1. Expression and purification of SubB2M

The recombinant SubB2M protein was expressed and purified as previously described [25,27]. Briefly, SubB2M was expressed in *E. coli* BL21 (DE3) cells transformed with the SubB2M expression construct as a His_6-tagged fusion protein, which was purified by Ni-NTA affinity chromatography.

2.2. SPR analysis of CA125 spiked into normal human serum and detection of Neu5Gc glycans in human serum samples

SPR was conducted using the Biacore S200 system (GE) with immobilisation of SubB2M performed as described previously [28]. SubB2M (capture level: 4974–5483 Response Units (RU)) and anti-CA125 mAb X325 (Bio-Rad) (capture level: 6538–7531 RU) were immobilized onto flow cell 2 and 3 of a series S sensor chip CM5 (GE) using the NHS capture kit and flow cell 1 was run as a blank immobilisation. Human cancer antigen 125 (CA125) was purchased from MyBioSource (San Diego, USA) and was purified from a human ovarian carcinoma cell line and provided at a concentration of 84,040 units/ml. Human CA125 was spiked into 1% normal human serum diluted in PBS (the zero CA125 concentration control). Human CA125 was serially diluted 1:2 from 75 units/ml to 0.1465 units/ml in 1% normal human serum with the range from 18.75 to 0.1465 units/ml (equivalent to 1875 units/ml – 14.65 units/ml in 100% serum) shown in Fig. 1. Normal female serum is reported to contain <35 units/ml of CA125 [4,29] or similar levels (63.2, 39.8) [28], while serum from patients with advanced stage ovarian cancer have been reported to contain 215.8–1977 CA125 units/ml [30], a mean of 645 CA125 units/ml [31] and up to 8100 CA125 units/ml [4]. SPR analysis was performed using multi-cycle analysis and double reference (values from flow cell 1 and 1% normal human serum only) subtraction using the Biacore S200 evaluation software, as previously described [26].

SPR analysis of human serum samples was performed as described above. The serum samples were diluted 1:100 in PBS and analysed in duplicate individually. SPR analysis was performed using multi-cycle analysis and double reference (values from flow cell 1 and PBS buffer only) subtraction using the Biacore S200 evaluation software.

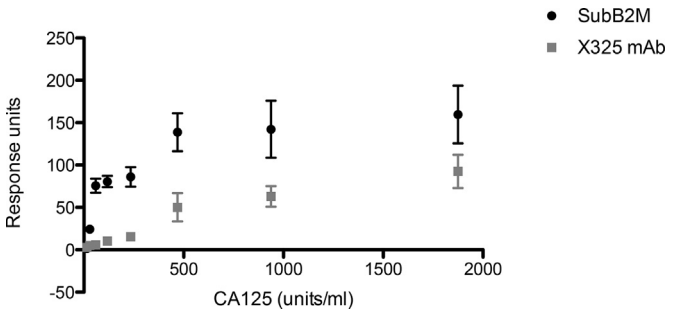


Fig. 1. SPR analysis of SubB2M and anti-CA125 mAb X325 detecting human CA125 spiked into normal human serum. 1% normal human serum was spiked with a concentration range of human CA125 (18.75–0.1465 CA125 units/ml) and detected with SubB2M and the X325 mAb. 1% normal human serum served as the zero concentration control.

2.3. Human serum samples

22 serum samples from normal human females, 12 serum samples from patients with stage I ovarian cancer, 11 with stage II ovarian cancer, 10 with stage IIIC ovarian cancer and 14 with stage IV ovarian cancer were obtained from the Victorian Cancer Biobank under application 17020. 'Normal' controls are defined as patients with an apparent non-malignancy diagnosis at the time the sample is taken. The patient data and serum samples used in this project were provided by the Victorian Cancer Biobank with appropriate ethical approval. The Victorian Cancer Biobank, through the Cancer Council Victoria as Lead Agency, is supported by the Victorian Government through the Victorian Cancer Agency, a business unit of the Department of Health and Human Services. Information regarding each of the serum samples used in this study is summarized in Supplementary Table 1.

2.4. Statistical analysis

Statistical analysis was performed using GraphPad Prism 5.0. The differences between normal serum samples compared to cancer patient serum samples were analysed by two-tailed *t*-tests, with a P value of <0.05 considered significant.

3. Results

3.1. SubB2M can detect CA125 spiked into normal human serum

In our previous study we showed that SubB2M can detect bovine glycoproteins, known to be decorated with Neu5Gc, when spiked into normal human serum [26]. In the current study, we investigated whether SubB2M could detect the human tumor antigen CA125 when present in normal human serum. CA125 is heavily glycosylated, with 249 potential N-glycosylation and over 3700 potential O-glycosylation sites [3,30,32]. It has been shown that CA125 from the serum of patients with ovarian cancer has increased levels of sialylated glycans compared to normal controls [30], therefore we used CA125 in our SPR analysis as an example of the type of Neu5Gc-containing tumor antigens that would be expected to be overexpressed in human ovarian cancer.

Purified CA125 from a human ovarian cancer cell line was spiked into normal human serum at concentrations found in normal female serum (<35 CA125 units/ml) [4] up to concentrations previously reported for serum from patients with advanced stage ovarian cancer (215.8–1977 CA125 units/ml) [30]. These spiked samples were prepared in 1% normal human serum, as our previous study showed that this dilution of human serum gave the best signal-to-noise ratio [26].

To verify that our method of detection using SubB2M was reliable, we also analysed the CA125-spiked serum samples with an anti-CA125 monoclonal antibody, clone X325. This mAb recognises epitope group B of CA125, similar to the M11 antibody.

SubB2M showed concentration dependent recognition of CA125 (Fig. 1) and was able to detect down to 0.1465 CA125 units/ml in 1% serum. This is equivalent to a sensitivity of 14.65 CA125 units/ml in 100% serum. These data confirm that SubB2M can detect Neu5Gc-containing human tumor antigens in the context of a complex sample, such as human serum, in a concentration-dependent fashion. The anti-CA125 antibody, X325, also showed concentration-dependent detection of CA125 via SPR, verifying our method for detection of this human tumor antigen. We note that SubB2M had superior sensitivity in detecting CA125 (14.65 CA125 units/ml) compared to the anti-CA125 mAb X325, which could only detect down to 58.6 CA125 units/ml.

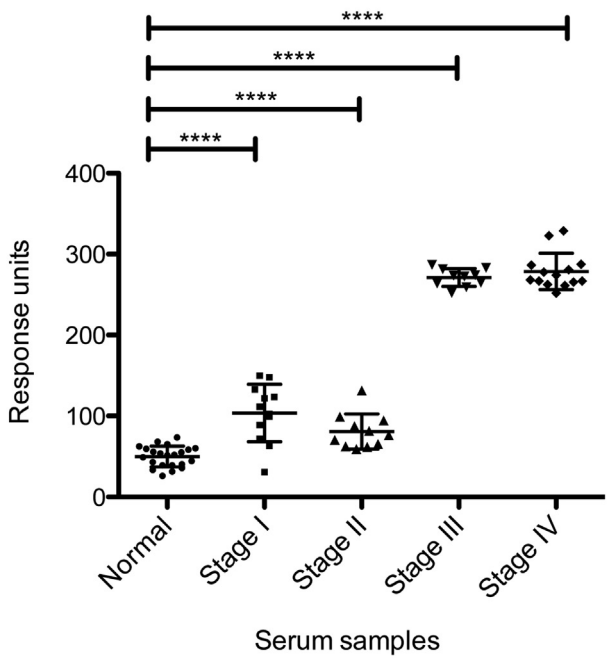
3.2. SubB2M can detect elevated levels of Neu5Gc in serum samples in early and late stage ovarian cancer

We next assessed whether the SubB2M lectin could detect elevated levels of Neu5Gc in serum samples from ovarian cancer patients with both early and late stage cancer. We obtained a collection of ovarian cancer serum samples, along with normal female serum samples from a similar age range, from the Victorian Cancer Biobank. 'Normal' controls are defined as patients with an apparent non-malignancy diagnosis at the time the sample is taken. The serum samples were collected immediately pre-operatively and were processed and stored within 2 h of collection. Fig. 2A shows the analysis of 12 stage I, 11 stage II, 10 stage IIIC and 14 stage IV ovarian cancer serum samples, as well as serum from 22 normal females via SPR. We detected significantly elevated levels of Neu5Gc in serum samples from ovarian cancer patients in stage I, II, IIIC and IV compared to normal females, with consistently high levels observed in stages IIIC and IV. Detailed information for each of the serum samples can be found in Supplementary Table 1.

3.3. Elevated Neu5Gc levels observed are not entirely due to elevated CA125 levels

CA125 has been shown to be elevated in the serum of up to 80% of patients with ovarian cancer [4], therefore we were interested to know if the elevated levels of Neu5Gc being detected by SubB2M were due to elevated levels of CA125. The CA125 levels in 14 of the ovarian cancer serum samples were provided by the Victorian Cancer Biobank and were determined within 7 days before or after collection of the serum sample used in our study. There was no correlation between levels of Neu5Gc and CA125 levels for these 14 samples, suggesting that there are other Neu5Gc-containing biomarkers elevated in the serum of these ovarian cancer patients that remain to be identified.

A



B

	Normal	Stage I	Stage II	Stage III	Stage IV
No. of samples	22	12	11	10	14
Age range	38-93	23-84	48-75	38-75	36-83
Mean Neu5Gc levels RU	49.95 (26.32-73.48)	103.7 (30.85-149.9)	80.9 (59.3-131.7)	271.2 (252.2-286.7)	278.7 (251.9-328.9)

Fig. 2. SPR analysis of SubB2M with normal and stage I – IV ovarian cancer serum samples. A) Average response units from duplicates for each serum sample are shown. Error bars = ± 1 SD from the mean for each group. Statistical analysis was performed using two-tailed *t*-tests. **** = *P*-value <0.0001. **B)** Table showing the number of serum samples, age range and mean Neu5Gc levels in SPR RUs for each group. The values in parenthesis equal minimum – maximum RU values for each group of samples determined by SPR.

4. Discussion

Ovarian cancer has the highest mortality rate of the female reproductive cancers, mainly due to late diagnosis resulting from the lack of specific symptoms for this disease [1,2]. Methods for the detection of ovarian cancer in the early stages would therefore improve survival rates. Using our engineered Neu5Gc specific lectin, SubB2M, we showed via SPR that Neu5Gc levels are significantly elevated in serum samples from ovarian cancer patients at stages I, II, IIIC and IV. The range of Neu5Gc levels obtained for the normal serum samples was from 26.32 to 73.48 SPR response units (RUs). Only 3 of the 12 samples with stage I ovarian cancer fell within this range while 5 out of 11 of the stage II ovarian cancer samples were within this range. These data indicate that Neu5Gc levels are elevated in the majority of stage I/II ovarian cancer serum samples analysed in this study and that Neu5Gc-containing tumor antigens have the potential to serve as diagnostic markers for detection of early stage ovarian cancer. None of the serum samples from the stage IIIC and IV cancer patients fell within the normal ranges for Neu5Gc levels demonstrating that Neu5Gc is uniformly elevated in late stage ovarian cancer. Moreover, Neu5Gc levels in all of the stage IIIC and IV samples exceeded the mean level plus 3 standard deviations for the age-matched normal sera, indicating high specificity and sensitivity.

SPR is a highly sensitive, label-free, and rapid optical detection method and is increasingly being improved for clinical applications (see reference 33 for a recent review). However, the drawbacks of current commercial SPR sensors for clinical settings include their relatively large footprint and high cost [33]. Also, a range of dilutions of the serum samples had to be performed, with the data presented in this study obtained from 1% dilutions. While dilution of the serum samples is advantageous in that very little sample is required from the patient for analysis, the dilution factor of serum samples from some patients may need to be individually optimized. We noticed that with some of the serum samples, at concentrations above 1%, an over-saturation effect was occurring where the RUs would plateau then decrease. The molecular weight of the Neu5Gc-conjugates being detected by SubB2M via SPR may influence the RUs detected, with larger molecular structures potentially causing a greater refractive index shift resulting in higher RUs. For example, we noticed that the patient with the lowest Neu5Gc value recorded (specimen no. 08SH256) had elevated levels of CA19.9 (also known as sialyl Lewis A). This individual may have elevated levels of free Neu5Gc containing glycans, which may result in lower RUs compared to a serum sample with elevated levels of high-molecular weight Neu5Gc-containing glycoproteins or glycolipids. Identifying and characterizing the Neu5Gc-containing glyco-conjugates that are being detected by SubB2M and using these data to improve current ovarian cancer diagnostic methods is currently under investigation.

It has been reported that the origin of Neu5Gc in humans is dietary incorporation of this non-human glycan from animal sources, particularly red meat [34], and it is established that ovarian cancer cells secrete Neu5Gc glycoconjugates [15]. However, it has

recently been proposed that human cancer cells may also produce endogenous Neu5Gc [35]. Our data showing uniformly elevated levels of Neu5Gc in the serum of ovarian cancer patients supports the latter hypothesis.

The capacity to diagnose ovarian cancer in early stages will have a significant impact on patient survival rates. Biomarkers that can monitor response to treatment and disease progression are also needed. SubB2M can detect Neu5Gc-glycans at early stages of ovarian cancer and detects uniformly high levels in late stage disease. The detection of Neu5Gc-glycans using SubB2M has the potential to be used as a diagnostic tool, and as a liquid biopsy for monitoring treatment and disease progression. As a research reagent SubB2M has the potential to help identify novel Neu5Gc biomarkers for ovarian cancer.

Note

Receiver operating characteristic (ROC) analysis of the data in Figure 2 was performed and revealed the ability of Neu5Gc levels to distinguish between two diagnostic groups (diseased/normal). Area under the curve (AUC) values of 1.0 = 100% accurate diagnostic test: Normal vs Stage I: optimal cutoff = >62.94RU (sensitivity = 91.67%, specificity = 86.36%); AUC = 0.9053), Normal vs Stage II: optimal cutoff = >61.02RU (sensitivity = 90.91%, specificity = 81.82%); AUC = 0.9298, Normal vs Stage III: optimal cutoff = >162.9RU (sensitivity = 100%, specificity = 100%); AUC = 1.00, Normal vs Stage IV: optimal cutoff = >162.7RU (sensitivity = 100%, specificity = 100%); AUC = 1.00.

Funding

This work was supported by National Health and Medical Research Council (NHMRC) Program Grant 1071659 (to JCP and MPJ), NHMRC Project Grant 1084050 (to AWP) and NHMRC Principal Research Fellowship 1138466 (to MPJ).

Disclosure

The following authors (JCP, AWP, CJD and MPJ) declare that they are named inventors on a patent on matter contained in this manuscript.

Acknowledgements

We thank the Victorian Cancer Biobank for providing all patient data and serum samples.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2018.11.001.

References

[1] United States Cancer Statistics Working Group, United States Cancer Statistics (USCS): 1999-2014 Cancer Incidence and Mortality Data, 2017 from, https://nccd.cdc.gov/uscs/. (Accessed 17 August 2018).
[2] American CancerSociety, Survival Rates for Ovarian Cancer, by Stage, 2016 from, https://www.cancer.org/cancer/ovarian-cancer/detection-diagnosis-staging/survival-rates.html. (Accessed 16 August 2018).
[3] B.W. Yin, K.O. Lloyd, Molecular cloning of the CA125 ovarian cancer antigen: identification as a new mucin, MUC16, J. Biol. Chem. 276 (2001) 27371–27375.
[4] R.C. Bast Jr., T.L. Klug, E. St John, E. Jenison, J.M. Niloff, H. Lazarus, R.S. Berkowitz, T. Leavitt, C.T. Griffiths, L. Parker, V.R. Zurawski Jr., R.C. Knapp, A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer, N. Engl. J. Med. 309 (1983) 883–887.
[5] L.C. Giudice, A.J. Jacobs, C.E. Bell, L. Lippmann, Serum levels of CA-125 in patients with endometriosis, Gynecol. Oncol. 25 (1986) 256–258.
[6] Y. Haga, K. Sakamoto, H. Egami, R. Yoshimura, M. Akagi, Evaluation of serum CA125 values in healthy individuals and pregnant women, Am. J. Med. Sci. 292 (1986) 25–29.
[7] D.E. Pittaway, J.A. Fayez, J.W. Douglas, Serum CA-125 in the evaluation of benign adnexal cysts, Am. J. Obstet. Gynecol. 157 (1987) 1426–1428.
[8] H. Halila, U.H. Stenman, M. Seppala, Ovarian cancer antigen CA 125 levels in pelvic inflammatory disease and pregnancy, Cancer 57 (1986) 1327–1329.
[9] A. Ruibal, R. Siurana, G. Encabo, A. Cuartero, [Serum CA 1,25 in patients with non-ovarian tumors. Evidence of a relation between the increase of the antigen and hepatic disease], Med. Clin. 84 (1985) 376–377.
[10] J.F. Bergmann, M. Beaugrand, H. Labadie, J.M. Bidart, C. Bohuon, CA 125 (ovarian tumour-associated antigen) in ascitic liver diseases, Clin. Chim. Acta 155 (1986) 163–165.
[11] T. Masahashi, K. Matsuzawa, M. Ohsawa, O. Narita, T. Asai, M. Ishihara, Serum CA 125 levels in patients with endometriosis: changes in CA 125 levels during menstruation, Obstet. Gynecol. 72 (1988) 328–331.
[12] P. Bottoni, R. Scatena, The role of CA 125 as tumor marker: biochemical and clinical aspects, Adv. Exp. Med. Biol. 867 (2015) 229–244.
[13] N.M. Varki, A. Varki, Diversity in cell surface sialic acid presentations: implications for biology and disease, Lab. Invest. 87 (2007) 851–857.
[14] H.H. Chou, T. Hayakawa, S. Diaz, M. Krings, E. Indriati, M. Leakey, S. Paabo, Y. Satta, N. Takahata, A. Varki, Inactivation of CMP-N-acetylneuraminic acid hydroxylase occurred prior to brain expansion during human evolution, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 11736–11741.
[15] S. Inoue, C. Sato, K. Kitajima, Extensive enrichment of N-glycolylneuraminic acid in extracellular sialoglycoproteins abundantly synthesized and secreted by human cancer cells, Glycobiology 20 (2010) 752–762.
[16] A.N. Samraj, H. Laubli, N. Varki, A. Varki, Involvement of a non-human sialic acid in human cancer, Front Oncol 4 (2014) 33.
[17] H. Higashi, Y. Hirabayashi, Y. Fukui, M. Naiki, M. Matsumoto, S. Ueda, S. Kato, Characterization of N-glycolylneuraminic acid-containing gangliosides as tumor-associated Hanganutziu-Deicher antigen in human colon cancer, Cancer Res. 45 (1985) 3796–3802.
[18] Y.N. Malykh, R. Schauer, L. Shaw, N-Glycolylneuraminic acid in human tumours, Biochimie 83 (2001) 623–634.
[19] J. Furukawa, M. Tsuda, K. Okada, T. Kimura, J. Piao, S. Tanaka, Y. Shinohara, Comprehensive glycomics of a multistep human brain tumor model reveals specific glycosylation patterns related to malignancy, PLoS One 10 (2015), e0128300.
[20] G. Marquina, H. Waki, L.E. Fernandez, K. Kon, A. Carr, O. Valiente, R. Perez, S. Ando, Gangliosides expressed in human breast cancer, Cancer Res. 56 (1996) 5165–5171.
[21] H. Higashi, T. Sasabe, Y. Fukui, M. Maru, S. Kato, Detection of gangliosides as N-glycolylneuraminic acid-specific tumor-associated Hanganutziu-Deicher antigen in human retinoblastoma cells, Jpn. J. Canc. Res. 79 (1988) 952–956.
[22] F. Priego-Capote, M.I. Orozco-Solano, M. Calderon-Santiago, M.D. Luque de Castro, Quantitative determination and confirmatory analysis of N-acetylneuraminic and N-glycolylneuraminic acids in serum and urine by solid-phase extraction on-line coupled to liquid chromatography-tandem mass spectrometry, J. Chromatogr. A 1346 (2014) 88–96.
[23] K. Kitajima, N. Varki, C. Sato, Advanced technologies in sialic acid and sialoglycoconjugate analysis, Top. Curr. Chem. 367 (2015) 75–103.
[24] S.L. Diaz, V. Padler-Karavani, D. Ghaderi, N. Hurtado-Ziola, H. Yu, X. Chen, E.C. Brinkman-Van der Linden, A. Varki, N.M. Varki, Sensitive and specific detection of the non-human sialic Acid N-glycolylneuraminic acid in human tissues and biotherapeutic products, PLoS One 4 (2009) e4241.
[25] C.J. Day, A.W. Paton, M.A. Higgins, L.K. Shewell, F.E. Jen, B.L. Schulz, B.P. Herdman, J.C. Paton, M.P. Jennings, Structure aided design of a Neu5Gc specific lectin, Sci. Rep. 7 (2017) 1495.
[26] J. Wang, L.K. Shewell, A.W. Paton, J.C. Paton, C.J. Day, M.P. Jennings, Specificity and utility of SubB2M, a new N-glycolylneuraminic acid lectin, Biochem. Biophys. Res. Commun. 500 (2018) 765–771.
[27] A.W. Paton, P. Srimanote, U.M. Talbot, H. Wang, J.C. Paton, A new family of potent AB(5) cytotoxins produced by Shiga toxigenic Escherichia coli, J. Exp. Med. 200 (2004) 35–46.
[28] C.J. Day, V. Korolik, Identification of specific ligands for sensory receptors by small-molecule ligand arrays and surface plasmon resonance, Methods Mol. Biol. 1729 (2018) 303–317.
[29] T.L. Klug, R.C. Bast Jr., J.M. Niloff, R.C. Knapp, V.R. Zurawski Jr., Monoclonal antibody immunoradiometric assay for an antigenic determinant (CA 125) associated with human epithelial ovarian carcinomas, Cancer Res. 44 (1984) 1048–1053.
[30] R. Saldova, W.B. Struwe, K. Wynne, G. Elia, M.J. Duffy, P.M. Rudd, Exploring the glycosylation of serum CA125, Int. J. Mol. Sci. 14 (2013) 15636–15654.
[31] R.G. Moore, A.K. Brown, M.C. Miller, S. Skates, W.J. Allard, T. Verch, M. Steinhoff, G. Messerlian, P. DiSilvestro, C.O. Granai, R.C. Bast Jr., The use of multiple novel tumor biomarkers for the detection of ovarian carcinoma in patients with a pelvic mass, Gynecol. Oncol. 108 (2008) 402–408.
[32] A. Rump, Y. Morikawa, M. Tanaka, S. Minami, N. Umesaki, M. Takeuchi, A. Miyajima, Binding of ovarian cancer antigen CA125/MUC16 to mesothelin mediates cell adhesion, J. Biol. Chem. 279 (2004) 9190–9198.
[33] J.F. Masson, Surface plasmon resonance clinical biosensors for medical diagnostics, ACS Sens. 2 (2017) 16–30.
[34] P. Tangvoranuntakul, P. Gagneux, S. Diaz, M. Bardor, N. Varki, A. Varki,

E. Muchmore, Human uptake and incorporation of an immunogenic nonhuman dietary sialic acid, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 12045–12050.
[35] P.A. Bousquet, J.A. Sandvik, N.F. Jeppesen Edin, U. Krengel, Hypothesis: hypoxia induces de novo synthesis of NeuGc gangliosides in humans through CMAH domain substitute, Biochem. Biophys. Res. Commun. 495 (2018) 1562–1566.