Zagazig Veterinary Journal, ©Faculty of Veterinary Medicine,
Zagazig University, 44511, Egypt.
Volume 50, Number 2, p. 126-136, June 2022
DOI: 10.21608/zvjz.2022.128693.1176

RESEARCH ARTICLE

Metabolic Labeling of Malignant Breast Cells Resistance to Chemotherapy with N-Glycolylneuraminic Acid Allows Differential Surface Detection

Dina M.M. AlSadek[1*], Haitham A. Badr[2], Hafiz Ahmed[3]

[1]Department of Histology and Cytology, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44511, Egypt

[2]Department of Biochemistry, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt

[3]GlycoMantra, Inc., Baltimore, MD 21227, USA.

*Corresponding author: dina.alsadek@gmail.com

Article History: Received: 07/05/2022 Received in revised form: 22/05/2022 Accepted: 30/05/2022

Abstract

The ultimate goal of cancer science is to learn how to select a cancer cell surface. Glycans in cancer cells are frequently at varying quantities or have fundamentally various structures than those seen in normal cells. The presence of sialic acid (Neu5Ac), which is prevalent at the end of glycan chains, is significant. N-glycolylneuraminic acid (Neu5Gc) is a “non-human” sugar type that intercept into Neu5Ac natural biosynthetic machinery, presaging as a perspective biomarker. Malignant breast cells resistance to chemotherapy and their non-malignant counterparts, when treated with the Neu5Gc under nutrient deprivation, display cell surface tumor-associated Neu5Gc-rich glycans, which is capture with Neu5Gc linkage specific plant lectins such as *Triticum vulgaris* agglutinin (WGA), *Sambucus nigra* agglutinin (SNA), and *Maackia amurensis* agglutinin I (MAL-I). Nevertheless, MAL-I binding differentiates surface of malignant breast cell line resistance to chemotherapy MCF-7/H compared to normal mammary epithelial cell line MCF-10/A. These findings emphasize the importance of one oxygen atom in Neu5Gc; it’s supplementing of nutrient-depleted cancer cells' resistance to chemotherapy and facilitates the way for the implementation of better diagnostic and prognostic approaches.

Keywords: oxygen atom, neuraminic acids, glycan metabolism, plant lectins, nutrient deprivation.

Introduction

Breast cancer (BC) is still the top second reason for death among women and the increasing death rate is not usually caused by the primary BC, but rather by the spread of win-cancer cells to further tissues/parts of the women-body, resulting in the transformation of more-aggressive metastatic secondary BC-tumors in distant-organs [1]. The lack of easy biomarker(s) testing to detect the BC-disease making females often diagnosed BC too late [2]. This has heightened further research in BC combating, and the identification of novel monitoring biomarker(s) takes place on the front [3]. N-glycosylation generates a large and diversified glycan-molecule repertoire that aids protein folding and cellular secretion [4]. In a process known as "sialylation," glycoproteins typically sprout "sialic acid" sugars (N-Acetyl-Neuraminic-Acid, Neu5Ac) at the termini of their N- and O-glycans [5]. The nomenclature of Neu5Ac was established in early 1957 by Blix research group [6]. Neu5Ac(s) now refers to a group of around 60 found naturally sugar compounds [7]. In addition, there are increasing numbers of chemo-synthetic Neu5Ac-analogues achieved regularly through metabolic-labeling and/or glyco-engineering methods [8]. BC

development is marked by changes in the Neu5Ac-glycosylation machinery regular function, such as enhanced branching of N-linked glycan-sites and sialylation [9]. Elevated glycan-sialylation on the cell surface, in particular, enhances BC proliferation by encouraging cell separation from primary BC via charge repulsion [10]. Cell-surface altered glycan-sialylation has also been shown in BC patients with blood circulating cancer cells [11]. Despite the possibility of enhanced and/or changed glycan-sialylation as a diagnostic biomarker, there is currently no universally applicable early sensing platform that can determine global glycan-sialylation in a variety of BC clinical specimens with high selectivity and sensitivity [12]. Neu5Ac is principally synthesized in the cytosol of mammals' cells from its precursor Uredines-Di-Phosphate-N-Acetyl-Glucosamine (UDP-GlcNAc) by a sequence of chemo-enzymatic bio-reactions [13]. Neu5Ac is produced and used as a nucleotide donor substrate before being transferred to a specific glycan sequence. This substrate is made in the nucleus, where the CMP-Neu5Ac-Synthetase attaches Neu5Ac to Cytidine-5'-Tri-Phosphate to form the donor substrate Cytidine-5'-Mono-Phosphate-Neu5Ac (CMP-Neu5Ac). The CMP-Neu5Ac is transferred to Golgi apparatus by CMP-Neu5Ac transporters and is used there for glycan-sialylation by sialyltransferases which adhere Neu5Ac residues to target glycans via various glycolinkages such α2→3, α2→6, or α2→8 [14]. In parallel, the only known biosynthesis pathway for Neu5Gc (a form of Neu5Ac that vary by a single oxygen atom) occurs by hydroxylation of Neu5Ac via the Cytidine-5′-Mono-Phosphate- Neu5Ac-Hydroxylase (CMPNeu5AcH) which, with the exception of the human, is broadly spread among mammalian species. Human cells cannot express Neu5Gc because the CMPNeu5AcH enzyme is not stimulated owing to exon depletion/frame shift mutation in the encoding-CMPNeu5AcH human gene. Neu5Gc, on the other hand, is metabolically integrated into human cells/tissues through dietary resources (especially red meats and milks), and has been discovered at even higher amounts in some human tumors [15]. The mechanism of Neu5Gc incorporation is not clearly understood. In this context, Varki research group suggested that free Neu5Gc possibly could invade human cells via macropinocytosis to reach the endosome and lysosome regulation followed by transportation into the cytosolic compartment. It could then be converted to CMP-Neu5Gc and transported to the Golgi apparatus, where sialytransferases transfer Sia5Gc molecules to freshly produced sialoglycan structures [16]. Surprisingly, Metabolic Neu5Ac-engineering has noted to be an efficient techniques for modifying glycan structures on cell surfaces, and "Neu5Ac specific plant lectins" are used to investigate various elements of the monitored sialoglycans (a remarkable class of carbohydrate-binding proteins) [17]. In this context, bioavailable Neu5Gc shows the several hierarchical levels by which glycan-sialylation machinery and glycoengineer multi-layered regulation of complicated mammalian systems can be influenced [18]. Zeroing below the cellular level, subtle changes in the Neu5Gc-containing glycans can also enables a variety of biomarkers possibilities [19]. As a result, it stands to reason that activating cancer cell membranes with Neu5Gc in a distinctive metabolic adaptation could allow for sialoglycan metabolism engineering and result in a discrete set of modifications in global glycan sialylation [20]. More recently, it has been shown that Neu5Gc treatment under nutrient deprivation leads to robust glycoconjugate incorporation in BC cells compared with their normal counterparts [21] and particularly, high amount of Neu5Gc in cancer indicates poor prognosis as the cell surface Sia features of BC cancer cells are various fundamentally than those of regular cells [22]. However, to the best of our knowledge, the neo-synthesis of Neu5Gc in BC cancer cells resistance to chemotherapy remains unexplored, mostly because of limitations associated with specific cell surface glyco-sites binding detection. In this study, we demonstrate that Neu5Gc containing differential glycan-sialylation on a BC cancer cell surface allows its preferential detection. Our method offers great promise for the study of BC cancer prognosis and diagnosis.

Material and Methods

Materials

Santa Cruz Biotechnology provided N-glycolylneuraminic acid (Neu5Gc; sc-202234-10MG) (USA). Sigma-Aldrich provided the ribonuclease-A (RNase A; R5125-100MG) (USA). Life Technologies Corporation provided the Roswell Park Memorial Institute 1640 Medium (RPMI1640 culture medium; 11875093-500mL), fetal bovine serum (FBS; 10082147-500mL), N-2-Hydroxyethylpiperazine- N-2-Ethane Sulfonic Acid (HEPES; 15630049-20mL), and carbocyanine monomer nucleic acid stain with far-red fluorescence (TO-PRO™-3 stain; T3605-1mL) (USA). Invitrogen provided phosphate-buffered saline (PBS, pH 7.4; 10010002-500mL) (USA). Vector Laboratories provided fluorescein lectins conjugates of Maackia amurensis agglutinin I (MAL-I; FL-1311-10MG particular to Neu5Gc2,3Gal), Sambucus nigra agglutinin (SNA; FL-1301-10MG particular to Neu5Gc2,6Gal), and Triticum vulgaris agglutinin (WGA; FL-1021-10MG particular to Neu5Gc and GlcNAc) [23], (USA). All additional compounds were bought in analytical grade quality from Sigma-Aldrich.

Cell lines and culture conditions

MCF-10/A, a human normal mammary epithelial cell line, and MCF-7/H, a chemotherapy-resistant breast cancer cell line, were a generous gift from the lab of Prof. Chen-Zhong Li (Miami, USA) and cultivated in 175 cm^2 flasks in RPMI1640 culture medium (no antibiotics given to prevent sialyltransferase inhibition), enriched with 1% FBS (to reduce BSA glycan-sialylation interference), at 37 °C under 5% CO_2 [24, 25]. The media for MCF-10/A cells also included 10 g/ml insulin and 5 g/ml hydrocortisone. In all studies, MCF-10/A and MCF-7/H cells were used within the first three passages, cultured for 73 hours to reach mid-exponential growth phase, and gathered by incubating for 5 minutes at 37 °C in a solution containing 0.54 mM EDTA, 154 mM NaCl, and 10 mM N-2-Hydroxy-Ethyl-Piperazine-N-2-Ethane Sulfonic Acid (HEPES), pH 7.4 for < 5 min at 37 °C.

Nutrient deprivation and Neu5Gc treatment

Cells were split and cultivated in RPMI1640 culture media enriched with 1 percent heat-inactivated human-serum (RPMI/HUS) rather than FBS prior to nutrient deprivation tests, culminating with the existing chasing-out Sia5Gc [26]. Following that, cells were either fed 10 mM Neu5Gc in RPMI/HUS medium or starved as described previously [27], briefly: Protocol for nutrient deprivation of cells in suspension: Cells were cultivated in Step 1 according to Section 2. 2. Stage 2: cells were resuspended in RPMI/HUS medium and pelleted for 5 minutes by centrifugation at 1000 x g; cells were deprived of nutrients for 15 minutes in serum-free media lacking Neu5Gc during this step. Stage 3: After centrifuging the cells two times in 37 °C PBS for 5-min, 1 mL aliquots of 10^5 cells mL1 were pipetted slowly into 20 mL BD-Falcon tubes; the cells' metabolism was further nutrient-deprived for an extra 30-min throughout this step. Step 4: Equilibrate 10^5 cell mL1 cell suspension aliquots in Neu5Gc-PBS buffer tubes in a humidified incubator at 37°C and 5% CO_2 with constant shaking at 30 rpms for 45 minutes; negative controls were kept in non-enriched PBS during this Neu5Gc-supplementation step. Step 5: In the absence of supplemental Neu5Gc, the Neu5Gc-PBS solution was decanted, and the BD-Falcon tubes were gently tapped to loosen the gravity-pelleted cells before being washed twice in warm (37 °C) PBS and pelleted by 5 minutes centrifugation each time; this process added an adequate 30 minutes of nutrient-deprivation metabolism. With the exception of lectin staining, which used nutrient-deprived cells under adherent circumstances, the preceding 5-step technique resulted in 2 hours of nutrition deprivation, after which the cells were investigated using the techniques listed below. Step 1: Cells were cultivated for two days on sterilized glass microscope cover slips to create food shortage in adherent cells. Step 2: After 30 minutes on a sterile plastic rack in warm (37 °C) PBS buffer, the cover-slips were replaced with Neu5Gc-PBS solution for 60 minutes of Neu5Gc supplementation (controls were retained in non-supplemented PBS), and then returned to PBS buffer for 30 minutes. At 37 °C and 5% CO_2, all incubations were

carried out in a humidified incubator with a consistent shaking rate of 30 rotations per minute.

Cell viability assay

Without fixation, cells were collected as stated above. Cell pellets were resuspended in PBS consisting of 1 mg/ml propidium iodide and incubated for 5 minutes at room temperature. Flow cytometry was used to examine the cells using a CyAnTM ADP flow cytometer (Beckman Coulter Inc., USA). The proportion of propidium iodide stained cells was used to calculate the number of dead cells.

Flow cytometric analysis

Ethanol-fixed cells were cultured on 96-well plates ($1*10^4$ cells per well) after being rinsed twice with cold PBS. The fluorescein isothiocyanate (FITC)-labeled lectins were subsequently used to stain the plated cells (MAL-I, SNA, and WGA). The equipment parameters for fluorescence and compensation were the same for all studies when comparing mean fluorescence peaks intensity. For each specimen, data was obtained from at minimum 10,000 cells.

Lectin staining and cell imaging

Cells were cultured on a cover-slip surface, and attached cells were fixed in 70% ice-cold ethanol for 15 minutes. Cells were stained with FITC-labeled lectins (MAL-I, SNA, and WGA, 5-g/mL) for 1 hour after being washed with PBS. After staining, cells were medicated with Ribonuclease-A (10 g/mL) and TO-PRO-3 was used to counterstain the nuclei.

Statistical analysis

The independent t-test (SPSS version 19) was used to compare the means of the investigated parameters between the experimental groups. The differences were considered significant when $p<0.05$.

Results and Discussion

Neu5Gc treatment does not affect the cell viability

The propidium iodide (PI) flow cytometric test has been widely used to evaluate apoptosis in several experimental approaches since its introduction. It is predicated on the notion that apoptotic cells are differentiated by DNA fragmentation and, as a consequence, reduction of nuclear DNA content, among other things. The use of a fluorochrome has the ability to bind and label DNA, such as PI, enables for a rapid and exact measurement of cell viability using flow cytometric analysis [28]. The proportion of viable cells for untreated MCF-10/A and MCF-7/H cells (PBS control) were 92.45 percent ±1.9 and 95.27 percent ±2.8, respectively, utilizing a constant count of cells ($3*10^5$ cells mL^{-1}). The cell viability of Neu5Gc-treated MCF-10/A and MCF-7/H cells was 94.16 percent ±1.7 and 96.05 percent ±2.1, consecutively (Fig.1 A and B), indicating that exposure to Neu5Gc at the given conditions had no effect on cell viability.

Sia5Gc treatment promotes differential expression of cell surface sialo-glycoconjugates

To investigate if Neu5Gc medication could influence display of cell surface glycans, particularly sialoglycans, flow cytometry using Neu5Ac linkage specific lectins (MAL-I, SNA, and WGA) was performed. As shown in Figure 2, the shared profile of untreated cells indicated an increase in the binding of SNA and WGA lectins. In contrast, the MAL-I lectin showed weak binding to the surface of untreated cells. Conspicuously, the Neu5Ac treated cells responded similarly but with a marked increase in the binding of SNA and WGA lectins whereas, to our surprise, the MAL-I force elevated binding to MCF-7/H cells but not for MCF-10/A cells (Fig. 2 A and B). We next conducted lectin-mediated staining accompanied by fluorescence microscopy evaluation. As shown in Figure 3, SNA and WGA lectins showed consistently strong fluorescence signals that demonstrated the more Neu5Gc-mediated sialoglycans' accumulation in the metabolic state of treated MCF-10/A and MCF-7/H cells contrasted with those of untreated MCF-10/A and MCF-7/H cells. Lower staining patterns were noted with MAL-I lectin on the surface of untreated MCF-10/A and MCF-7/H cells whereas, the treated MCF-10/A and MCF-7/H cells indicated conversely elevated levels of fluorescence (Fig. 3). Although the examined lectins are distributed in the Golgi region of normal cells, the malignant cells show diffuse

cytoplasm or diffuse cytoplasm/membrane distribution (Fig. 3). The high molecular mass of the MAL-I and SNA lectins renders a passive entrance unlikely, and fluorescence microscopy accepted a membrane-associated fluorescence. As a result, an increase in Neu5Gc-mediated sialoglycans on the outer leaflet of cellular membranes can be ascribed to an increase in SNA and MAL-I staining [29]. Because sialyltransferases associated with the biosynthesis of Neu5Ac (s) have been proven to be effective in the creation of Neu5Gc in mammals, involving humans, Neu5Gc may theoretically exist in any sialoglycans-producing mammalian cell if Neu5Gc is present. However, glycan-bound Neu5Gc is rarely noted in normal mammalian cells due to the extremely low effect of CMP-Neu5Ac-Synthetase catalyzed formation of CMP-Neu5Gc, although a minute amount of Neu5Ac-rich glycan was detected during bioactive synthesis of some types of sialoglycoproteins in normal cells when the free Neu5Gc level in the cell.

Thus, the presence of Neu5Gc in human cells and/or bodily fluid could indicate a problem with Neu5Gc metabolism. Other research studies have been noted that MAL-I, SNA, and WGA diagnose Neu5Gc-sialylated glycans kinds on the terminal branches [30-33]. MAL-I, in particular, identifies glycans composed of Neu5Gc-Gal-GlcNAc with Neu5Ac at the 3 position of galactose, whereas SNA attaches preferentially to Neu5Gc-sialylated glycans bonded to terminal galactose in a (α2→6) and (α2→3), to a lesser extent a linkage. WGA adheres to almost all Neu5Gc-sialylated glycan isomers, in which its signal translates into a general degree of glycan-sialylation. This is in line with the results that both untreated cells have an abundance of α2→6 connected Neu5Gc-sialylated glycans, but terminal α2→3 linked Neu5Gc residues are extremely restricted. More crucially, a contrasting of the treated cell profiles showed that MCF-7/H cells imposed a much wider variety of (α2→3) and (α2→6) Neu5Gc-containing sialylated-glycans than MCF-10/A cells, which demonstrated an extreme level of (α2→6) Neu5Gc-containing sialylated-glycans while retaining restrictions of (α2→3) Neu5Gc-mediated sialylated-glycans. Thus, the current findings imply that Neu5Gc medication reflects differences in new-synthesis of the (α2→3) Neu5Gc-containing sialylated-glycan structures on the surface of cancer cells resistant to chemotherapy rather than normal cells.

Because of their flexibility, glycan structures that differ in their asialotopes have substantial topographic features in common and, as a result of this similarity, will bind to the same lectin molecule [34]. On the other hand, different types of lectins specific for the same asialotopes structure may recognize different binding regions of its surface [35]. To date, there are no antibodies have been identified to bind structural sialylated-glycan, such as α2,3– and α2,6–linked terminal sialic acid residues, whereas lectins have this specificity [36]. To the best of our knowledge, this is the first report demonstrating that Neu5Ac-specific plant lectins, specifically MAL-I, can be utilized in a metabolic-specific manner to distinguish between normal and cancer-bearing Neu5Gc-containing sialylated-glycans, paving the way for rapid translation to clinical surroundings. When compared to Neu5Ac, Neu5Gc biomolecule amplifies the critical influence (by adding a single oxygen atom) in determining the activity of many diverse sialoglycans because all transporters and enzymes that operate on Neu5Ac can also operate on Neu5Gc. Given how naturally Neu5Ac chemical engineering occurs via these enzymatic approaches, Neu5Gc differential cell surface glycan expression may seem to be a promising approach at first, as the approach is focused on controlling the influx rate or modifying the Neu5Gc synthesis enzymes.

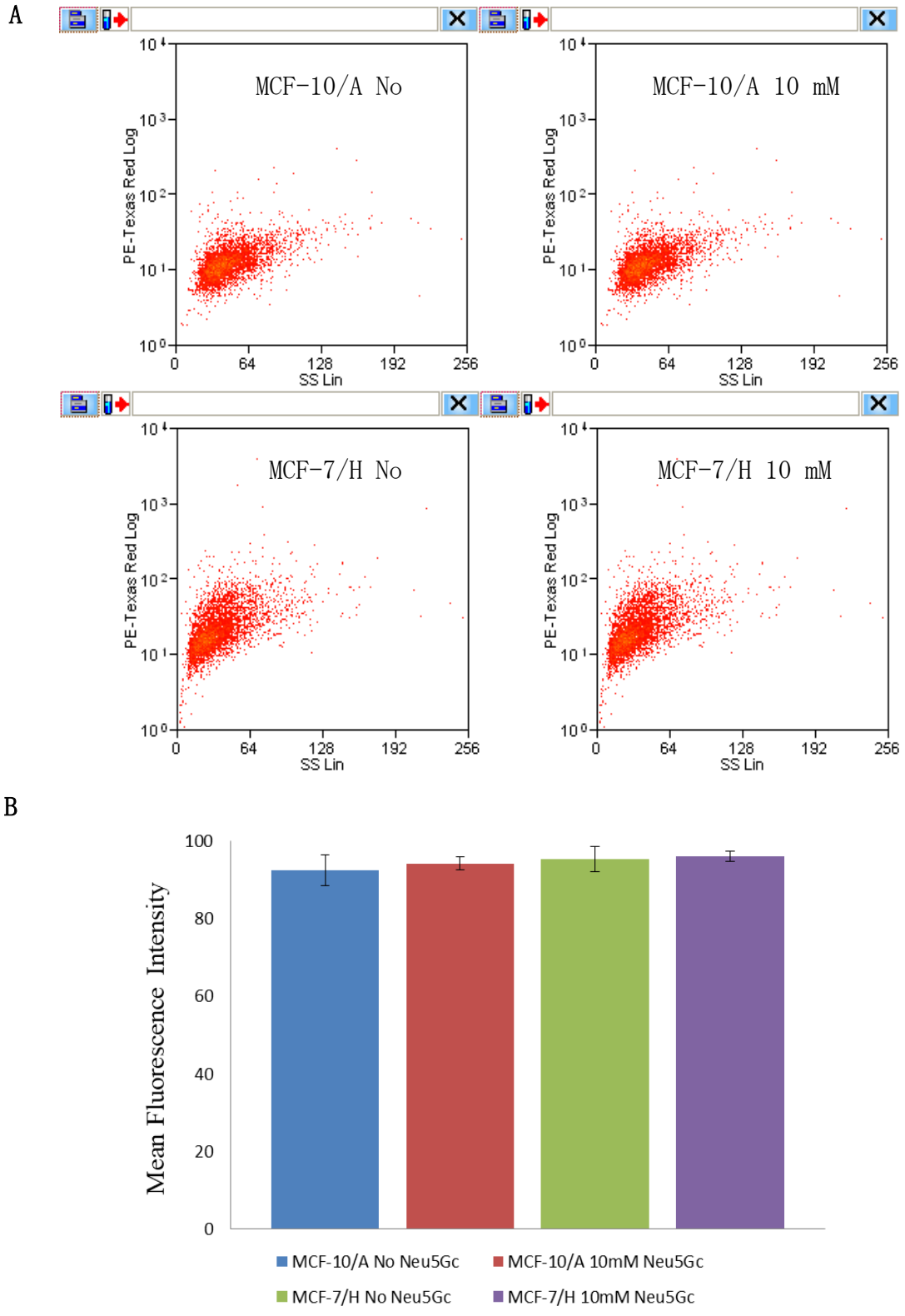

Figure 1: Exclusion of propidium iodide was used to determine MCF-10/A and MCF-7/H cells viability. On ice for 20 minutes, cells were stained with 1 mg/ml propidium iodide (PI) and examined by flow cytometry in the PE-Texas Red setup. The fraction of PI stained cells was utilized to calculate the number of dead cells. The living and dead cells for MCF-10/A and MCF-7/H cells are displayed in cytograms in the exitance or lack of Neu5Gc (A), and the live cells are depicted in a bar diagram (B). The data are from three different tests, with standard deviation indicated by error bars.

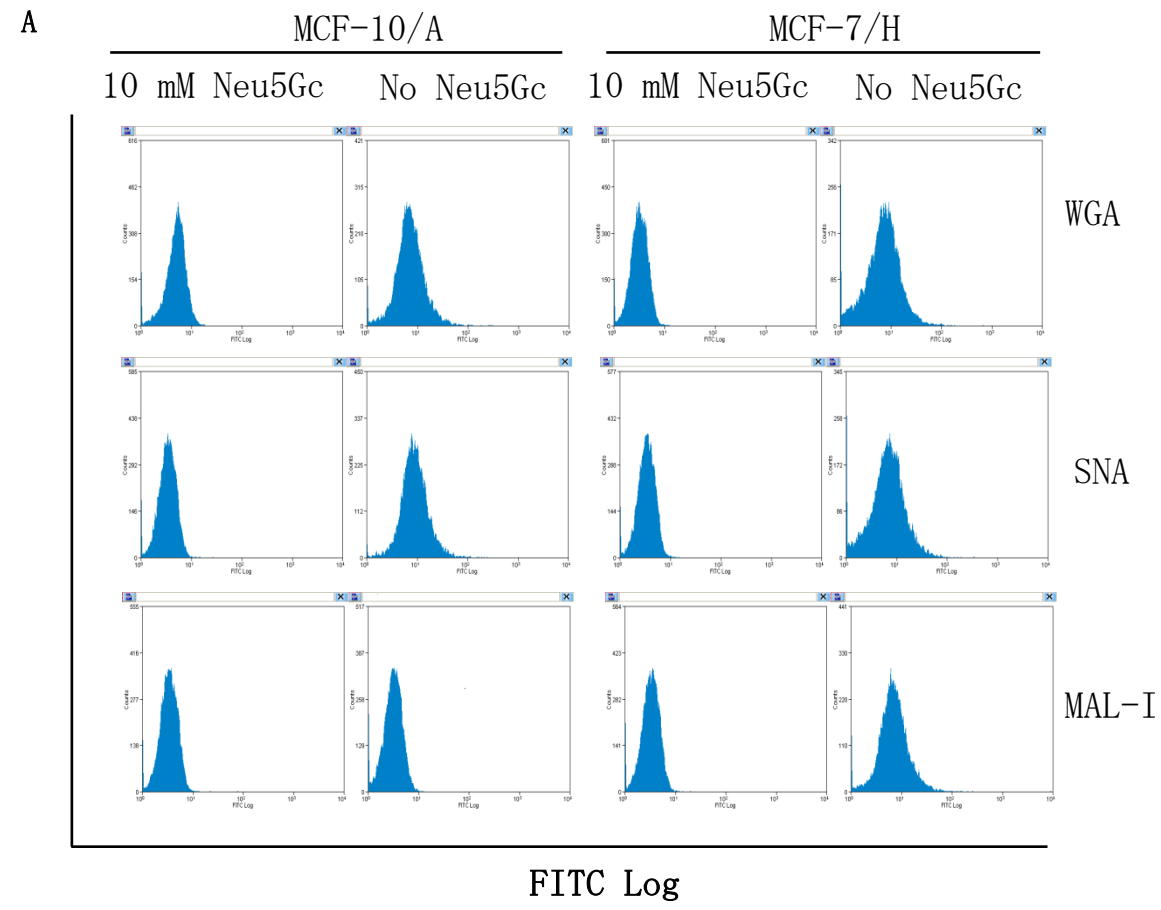

Figure 2: Lectin binding analysis of MCF-10/A and MCF-7/H cells using flow cytometry. Flow cytometry was utilized to record the fluorescence attributed to FITC-lectin binding to -2,6 and -2,3 sialylated surface moieties in the exitance or lack of 10 mM Neu5Gc, and the fluorescence attributed to FITC-lectin binding to α-2,6 and α-2,3 sialylated surface moieties was measured using bivariate sideward scattering (SS Lin) vs. FITC log variables The data comes from three separate experiments, and the error bars express the standard deviation. *p* value <0.001 is indicated by three asterisks.

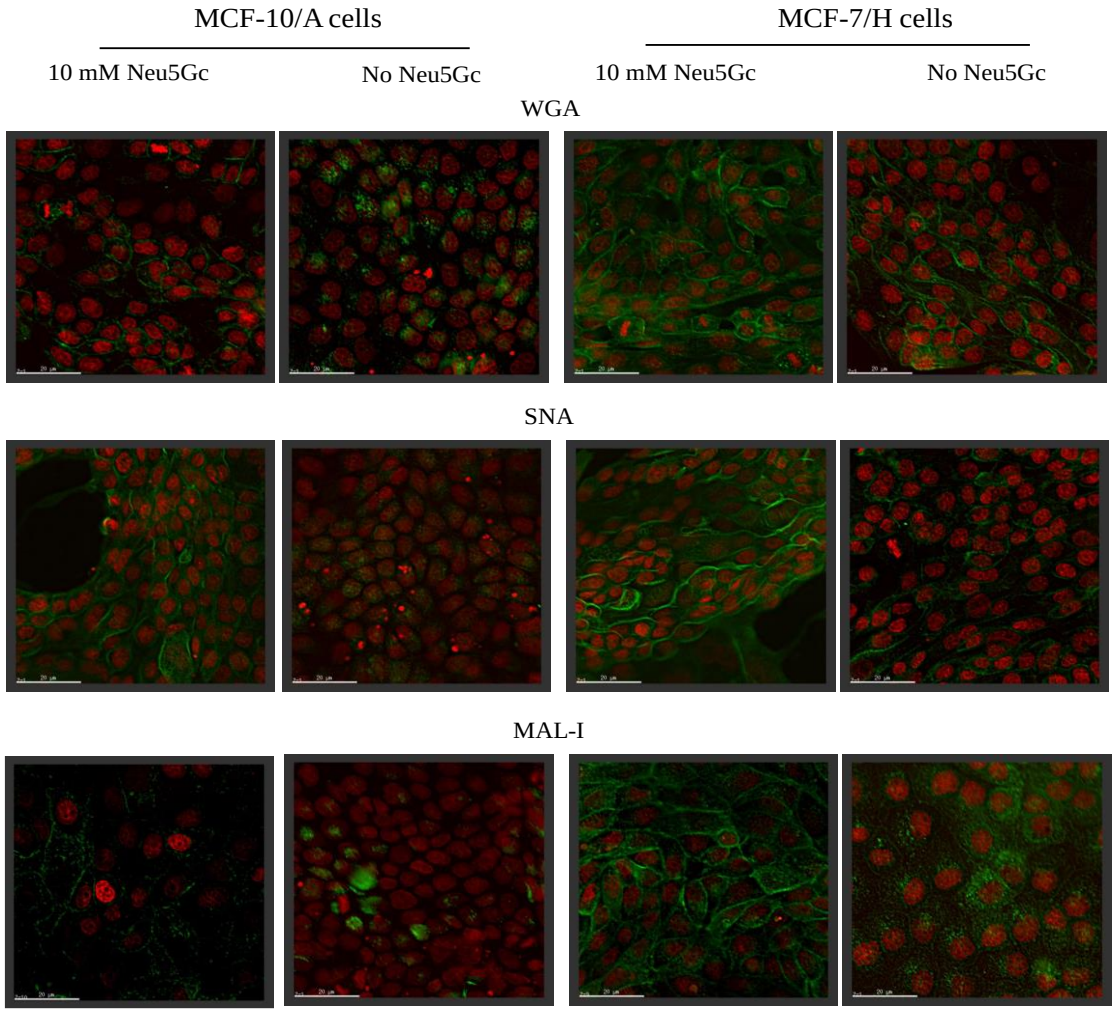

Figure 3: Confocal imaging of MCF-10/A and MCF-7/H cells after lectin-mediated staining. Cells were treated for 2 hours with 10 mM Neu5Gc in the existence or lack of FITC-labeled lectins (WGA, SNA, and MAL-I) at a concentration of 5 µg/ml and stained for 1 hour with FITC-labeled lectins (WGA, SNA, and MAL-I) at a concentration of 5 µg/ml during food deprivation (green fluorescence). The nuclei were counter-stained with TO-PRO-3 after the cells were medicated with 50 µg/ml ribonuclease A. (blue fluorescence). The images have been ten times magnified.

Therefore, insight gained from the study of "nonhuman" Neu5Gc nutrient-deprived environment, utilizing breast normal and BC cells as a model system, can offer a proof-of-concept endeavors to unravel novel glycobiology biomarkers. In future, this biomarker can be used as a tool for both diagnostic and therapeutic, so called 'theranostic'. Ultimately, our results will pave the way for metabolic engineering methods to decorate cell surfaces with specific glycan epitopes, which is crucial for the implementation and optimization of BC diagnostic products.

Conclusion

The major focus of this study is that differential uptake of exogenous Neu5Gc could be used as a diagnostic test to identify BC cells resistance to chemotherapy. The evidence presented shows some differences between BC normal and malignant cells, but the additional studies required to demonstrate that this work could have predictive power for breast cancer prognosis.

Conflict of Interest

GlycoMantra, Inc. of the United States filed a non-provisional complete patent on August 16, 2016, with a priority date of August 17, 2015, entitled "Exploiting nutrient deprivation

for modification of glycosylation in research, diagnostic, therapeutic, and biotechnological applications." One of the three inventors named was Haitham A. Badr. There are no other financial interests that are competing with this one.

References

[1] Hanahan, D. and Weinberg, R.A. (2011): Hallmarks of cancer: the next generation. Cell, 144: 646–674.

[2] DeBerardinis, R.J. and Thompson, C.B. (2012): Cellular metabolism and disease: what do metabolic outliers teach us? Cell, 148: 1132–1144.

[3] Downsett, M. and Dumbier, A.K. (2009): Emerging biomarkers and new understanding of traditional markers in personalized therapy for breast cancer. Cancer Clin. Res. 14: 8019–8026.

[4] Holst, S.; van Pelt, G.W.; Mesker, W.E.; Tollenaar, R.A.; Belo, A.I.; van Die, I.; Rombouts, Y. and Wuhrer, M. (2017): High-throughput and high-sensitivity mass spectrometry-based N-glycomics of mammalian cells. Methods Mol. Biol. 1503: 185–196.

[5] Cohen, M. and Varki, A. (2010): The sialome − far more than the sum of its parts. OMICS 14: 455–464.

[6] Djansugurova L., Badr H., Ahmed H., Khussainova E., Amirgalieva A., Dwek M. Sialic acid treatment makes cancer cells distinguishable from normal cells. FEBS J. 2012; 279: 315.

[7] Prescher, J.A. and Bertozzi, C.R. (2006): Chemical technologies for probing glycans. Cell 126: 851–854.

[8] Varki A. (2009): Multiple changes in sialic acid biology during human evolution. Glycoconj. J. 26: 231–245.

[9] Ohtsubo, K. and Marth, J.D. (2006): Glycosylation in cellular mechanisms of health and disease. Cell 126: 855–867.

[10] Badr, H.A.; ElSayed, A.I.; Ahmed, H.; Dwek, M.V.; Li, C.-Z. and Djansugurova, L.B. (2013): Preferential lectin binding of cancer cells upon sialic acid treatment under nutrient deprivation. Appl. Biochem. Biotechnol. 171: 963–974.

[11] Schultz, M.J.; Swindall, A.F. and Bellis, S.L. (2012): Regulation of the metastatic cell phenotype by sialylated glycans. Cancer Metastasis Rev. 31: 501–518.

[12] Narimatsu, H.; Sawaki, H.; Kuno, A.; Kaji, H.; Ito, H. and Ikehara, Y. A (2010): Strategy for discovery of cancer glyco-biomarkers in serum using newly developed technologies for glycoproteomics. FEBS J. 277: 95–105.

[13] Jacobs, C.L.; Goon, S.; Yarema, K.J.; Hinderlich, S.; Hang, H.C.; Chai, D.H. and Bertozzi, C.R. (2001): Substrate specificity of the sialic acid biosynthetic pathway. Biochemistry 40: 12864–12874.

[14] Varki, A. (2012): Gagneux P. Multifarious roles of sialic acids in immunity. Ann. N. Y. Acad. Sci. 1263: 16–36.

[15] Varki A. (2009): Multiple changes in sialic acid biology during human evolution. Glycoconj. J. 26: 231–245.

[16] Varki A. (2010): Uniquely human evolution of sialic acid genetics and biology. Proc. Natl. Acad. Sci. USA 107: 8939–8946.

[17] Badr, H.A.; AlSadek, D.M.; Darwish, A.A.; ElSayed, A.I.; Bekmanov, B.O.; Khussainova, E.M.; Zhang, X.; Cho, W.C.; Djansugurova, L.B. and Li, C.-Z. (2014): Lectin approaches for glycoproteomics in FDA-approved cancer biomarkers. Expert Rev. Proteom. 11: 227–236.

[18] Bergfeld, A.K.; Pearce, O.M.; Diaz, S.L.; Pham, T. and Varki A. (2012): Metabolism of vertebrate amino sugars with N-glycolyl groups: elucidating the intracellular fate of the non-human sialic acid N-glycolylneuraminic acid. J. Biol. Chem. 287: 28865–28881.

[19] Tangvoranuntakul, P.; Gagneux, P.; Diaz, S.; Bardor, M.; Varki, N.A. and Varki, E. (2003): Much-more, human uptake and incorporation of an immunogenic nonhuman dietary sialic acid. Proc. Natl. Acad. Sci. USA 100: 12045–12050.

[20] Yin, J.; Hashimoto, A.; Izawa, M.; Miyazaki, K.; Chen, G.Y.; Takematsu, H.; Kozutsumi, Y.; Suzuki, A.; Furuhata, K.;

Cheng, F.L.; Cheng, C.H.; Sato, C.; Kitajima, K.; Kannagi, R. (2006): Hypoxic culture induces expression of sialin, a sialic acid transporter, and cancer-associated gangliosides containing non-human sialic acid on human cancer cells, Cancer Res. 66: 2937–2945.

[21] Badr, H.A.; AlSadek, D.M.M.; Mathew, M.P.; Li, C.-Z.; Djansugurova, L.B.; Yarema, K.J. and Ahmed H. (2015): Nutrient-deprived cancer cells preferentially use sialic acid to maintain cell surface glycosylation. Biomaterials 70: 23–36.

[22] Astronomo, R.D. and Burton, D.R. (2010): Carbohydrate vaccines: developing sweet solutions to sticky situations? Nat. Rev. Drug Discov. 9: 308–324.

[23] Geisler, C. and Jarvis, D.L. (2011): Letter to the Glyco-Forum: Effective glycoanalysis with Maackia amurensis lectins requires a clear understanding of their binding specificities. Glycobiology, 21: 988–993.

[24] Badr, H.A.; AlSadek, D.M.M.; Mathew, M.P.; Li, C.−Z.; Djansugurova, L.B.; Yarema, K.J.; Ahmed H. Lectin staining and Western blot data showing differential sialylation of nutrient−deprived cancer cells to sialic acid supplementation. Data in Brief 2015; 5: 481–488.

[25] Li C.-Z. (2015) An insight into the cells' glycans and lectin−glycosensing sites. Sci. Bull. DOI:10.1007/s11434-015-0933-6

[26] Bardor, M.; Nguyen, D.H.; Diaz, S. and Varki, A. (2005): Mechanism of uptake and incorporation of the non-human sialic acid N-glycolylneuraminic acid into human cells. J. Biol. Chem. 280: 4228–4237.

[27] Badr, H.A.; AlSadek, D.M.M.; El-Houseini M.E.; Saeui, C.T.; Mathew, M.P.; Yarema, K.J.; Ahmed, H. Harnessing cancer cell metabolism for theranostic applications using metabolic glycoengineering of sialic acid in breast cancer as a pioneering example. Biomaterials. 2017; 116: 158–173.

[28] Riccardi, C. and Nicoletti, I. (2006): Analysis of apoptosis by propidium iodide staining and flow cytometry. Nat. Protoc. 1: 1458–1461.

[29] Varki, N.M. and Varki, A. (2007): Diversity in cell surface sialic acid presentations: implications for biology and disease. Lab. Invest. 87: 851–857.

[30] Dube, D.H. and Bertozzi, C.R. (2005): Glycans in cancer and inflammation: potential for therapeutics and diagnostics. Nat. Rev. Drug Discov. 4: 477–488.

[31] Fuster, M.M. and Esko, J.D. (2005): The sweet and sour of cancer: glycans as novel therapeutic targets. Nat. Rev. Cancer 5: 526–542.

[32] Schwefel, D.; Maierhofer, C.; Beck, J.G.; Seeberger, S.; Diederichs, K.; Moller, H.M.; Welte, W. and Wittmann, V. (2010): Structural basis of multivalent binding to Wheat Germ Agglutinin. J. Am. Chem. Soc. 25: 8704–8719.

[33] Moremen, K.W.; Tiemeyer, M. and Nairn, A.V. (2012): Vertebrate protein glycosylation: diversity, synthesis and function. Nat. Rev. Mol. Cell Biol. 13: 448–462.

[34] Arnaud J., Audfray A., Imberty A. Binding sugars: from natural lectins to synthetic receptors and engineered neolectins. Chem. Soc. Rev. 2013; 42: 4798−4813.

[35] Gabius H.−J., André S., Jiménez−Barbero J., Romero A., Solís D. From lectin structure to functional glycomics: principles of the sugar code. Trends Biochem. Sci. 2011; 36: 298–313.

[36] Sharon N. Lectins: past, present and future. Biochem. Soc. Trans. 2008; 36(6): 1457−1460.

الملخص العربي

المتلازمة الأيضية لخلايا الأورام السرطانية المقاومة للعلاج الكيماوي عند المعالجه بحمض جليكوسَّيَاليك تشكل زياده في مقترناتٍ سكرية تتبرعم خارجة من أسطح الخلايا بأفضلية كدلالة حيويّة

دينا م.م. الصادق[1] , هيثم ع. بدر[2] , حفيظ أحمد
قسم الأنسجة والخلايا- كلية الطب البيطري- جامعة الزقازيق- الزقازيق-مصر[1]
قسم الكيمياء الحيوية- كلية الزراعة- جامعة الزقازيق - مصر[2]
شركة جليكومانترا- ميرلاند- الولايات المتحدة[3]

الهدف النهائي من البحث في علم السرطان هو تعلم كيف يتم تمييز سطح الخلية السرطانية. تشتمل عمليّة تقدّم السرطان على خطوات عديدة منها ما يقوم علي الإرتباط بأنواع محدده من المقترنات السكرية وهو ما يدير مفتاحا يسمح للسرطان بالنفاذ الي «أبواب» الخلية السليمة. تكتسب وحدات حمض السَّيَاليك للمقترنات السكرية والتي تتبرعم خارجة من أسطح الخلايا أهمية خاصة. وبتعبير أكثر تحديدا انواع السكرات الموجودة في الثدييات «غير شائعة في الخلايا البشرية» من نوع حمض جليكوسَّيَاليك تعد واحده من اهم دلائل الأورام السرطانيه. في هذا البحث تم استخدام نوعين من خلايا الثدي البشريه احدهما خلايا طبيعية سليمه (MCF-10/A) واخري سرطانية مقاومة للعلاج الكيماوي (MCF-7/H) كنموذج لدراسه اختلاف محتوي المقترنات السكرية علي أسطح هذه الخلايا عند المعالجه بحمض جليكوسَّيَاليك تحت ظروف خاصه من الحرمان من التغذيه. وجد زياده في نشاط الإنزيمات التي تتحكم في تخليق الجزيئات الحاملة لسكرات حمض جليكوسَّيَاليك أو تفكيكها عند ظروف الحرمان من التغذيه في كلا النوعين من الخلايا وذلك طبقا لنتائج تجارب الالتصاق باللكتينات النباتية مثل (WGA, SNA, MAL-I لكن لكتين الماكيا (MAL-I) اظهر تحديدا التصاق قوي علي السطح الخارجي لخلايا سرطان الثدي المقاومة للعلاج الكيماوي (MCF-7/H) بينما تقع ضمن الحيز داخل الخلوي في الخلايا السليمة (MCF-10/A) موضحا تخصصيه عاليه لروابط حمض جليكوسَّيَاليك من نوع الفا (2) و (3). يعد هذا اول تقرير ينتفع بأستخدام انواع من اللكتينات وتحديدا لكتين الماكيا (MAL-I) في الأستهداف الانتقائي للخلايا السرطانية. هذه الطريقه اثبتت دقتها في الالتصاق بأنواع محدده من الروابط الخاصه بحمض جليكوسَّيَاليك علي سطح الخليه السرطانيه كدلالة حيويّة في التكهن المبكر للسرطان وتحسين طرق التشخيص.