

EDITORIAL

Toward Cancer Biomarker Discovery using the Glycomics Approach

Following on from genomics and proteomics, glycomics has become one of the most important research fields in recent years. The techniques for glycomics are rather complicated as compared to those for genomics and proteomics because glycans are very heterogeneous and there are no amplifying or cloning techniques such as those for PCR or molecular cloning, nor are there any synthesizers or sequencers such as those for DNA and proteins. As a result scientists in the other research fields feel it is difficult to become involved in this field and they have not paid attention to the significance of glycans. However, in the post-genomic era, the significance of posttranslational modification of proteins is gaining significance as more than 50% of proteins are glycosylated and it is impossible for us to understand the protein functions without knowing the glycan functions. Owing to the development of mass spectrometry, NMR, and HPLC/CE, although expensive, scientists are finding it easier to analyze glycan structures. Moreover, glycoscience including glycobiology and glycotecnology is expanding enormously these days.

I would like to mention some examples in medical science. Influenza drugs are neuraminidase inhibitors which inhibit growth of the influenza virus, and at the early stage of infectious diseases some bacteria, bacterial toxins and viruses bind firstly to glycans of infected cells. There are more than 30 congenital disorders of glycosylation (CDG), and more than 60 glycosyltransferase gene KO mice have been developed. Interesting phenotypic changes in the mice have been reported in relation to human diseases. Under inflammatory conditions and/or in the process of cancer metastasis, specific glycans of leukocytes, platelets and cancer cells bind specific adhesion molecules, such as selectin with a lectin motif in the endothelial cells which accelerate rolling of cells and cancer metastasis.

Antibody therapies against various cancers, such as breast cancer and non-Hodgkin's lymphoma, are being widely used at present and most of them involve antibodies raised against cell surface receptors such as growth factor receptors. If fucoylation (α 1,6-fucoylation of innermost GlcNAc residues in glycoproteins) is absent in the IgG1 molecules of the above antibodies, the antibodies will facilitate the binding of the Fc γ IIIa receptor of NK cells or mononuclear cells to their Fc portion of IgG1, and activate those cells and destroy the tumor cells. This is called antibody-dependent cellular cytotoxicity (ADCC). The ADCC of antibodies without core fucose is increased up to 50–100-fold as compared to that of ones with core fucose.

In August 2002 we launched the HGPI (Human Glycome/Proteomics Initiative) under HUPO (Taniguchi, N., *Mol Cell Proteomics* 2008, 7, 626–627) and so far two pilot studies involving N-glycan analysis headed by Wada (Wada,



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Y. *et al.* *Glycobiology* 2007, 17, 411–422) and O-glycan analysis headed by A. Delle (manuscript now in preparation) have been performed, and we hope that the technique now being widely used in glycoscience will be easy to use for those who are not familiar with glycan chain analysis. The latest task of HGPI (a new chair, H. Narimatsu) involves facilitating biomarker discovery, especially in cancer prediction, monitoring and therapy. We held a joint meeting at NIH in September, 2006 (Taniguchi, N., Paulson, J., *Proteomics* 2007, 7, 1360–1363) and emphasized the importance of biomarker discovery using the glycomics technique in an NIH white paper (Packer, N. *et al.* *Proteomics* 2008, 8, 8–20).

The cancer biomarkers used today are mostly glycoproteins and glycolipids, but quantification of these markers is usually based on ELISA involving monoclonal antibodies developed a decade ago. Very few markers are useful for the early detection of cancer because even if the values are increased in patients, they are not specific to cancer, and thus we cannot rule out the possibility that increased values are due to benign diseases including inflammation.

There are at least 100 cancer biomarkers available these days, but essentially these cannot be used for the diagnosis of cancer at an early stage. Only a few biomarkers, such as fucosylated α -fetoprotein (L3 fraction), have been recognized as markers for primary hepatocarcinomas which could be used to distinguish between cancer and non-cancer diseases. In 2006 the FDA in the USA approved this marker for the early detection of primary hepatomas. Two years ago the focus group of an NIH workshop entitled "New Frontiers of Glycomics, Biomarker Discovery and Bioinformatics" produced a white paper which emphasized the importance of the glycomics approach for biomarker discovery. NIH/NCI raised funding for the alliance of glycan teams and launched the 5-year biomarker discovery project for cancer.

On July 13–14, 2007, I organized the international symposium of the 27th Sapporo Cancer Seminar entitled "Glycomics: New Perspectives in Cancer Cell Behavior" in Sapporo, Hokkaido, Japan under the auspices of the Sapporo Cancer Seminar Foundation and HGPI, the Core to Core Program and the 21st Center of Excellence Program funded by the Japan Society for Promotion of Science. Many distinguished scientists attended this meeting. The Sapporo cancer seminar has a 27-year history and is held every year, focusing on cancer research, similar to the Gordon Research Conferences.

I was asked to serve as a Editor of this Special Issue, and on this occasion I selected several people who participated in this symposium and also asked other experts in this field to submit original papers or reviews. After careful peer reviews by the editorial board members and outside experts, I am fortunate to have 16 excellent papers which have been accepted for publication.

In this issue, Pierce *et al.* describe that an increase in bisecting GlcNAc and the responsible gene, GnT-III(Mgat3), are the most significant changes in glycan expression in ovarian cancer. Gu *et al.* for the first time reported that Mgat3 expression is regulated *via* the E-cadherin- β -catenin-actin complex. Dennis *et al.*

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report global changes in gene expression in epithelial cells under normal conditions or supplemented with GlcNAc. They also found that high GlcNAc unregulated several genes, such as those of EGF and TGF- β resulting in an increase of β -1,6 GlcNAc branching of N-glycans, and, due to binding to galectin 3 on the cell surface, endocytosis and cell growth were decreased. Downstream of EGFR, MAPK pathway gene expression was decreased, while G1/S checkpoint gene expression was increased. As judged on 2-D gel-based lectin blotting using L-PHA as well as nano-LC-FT-ICFR/LTQ, 26 proteins were identified as target proteins for GnT-V in colorectal cancer (Ko *et al.*). In a human colon epithelial cancer cell line Michalski *et al.* analyzed the difference in α -2,3 and 2,6-sialylation on glycoproteins between proliferating and differentiated HT-29 cells, and also showed the N-glycan profile, observed on lectin-blot analysis as well as MALD-TOF and GC-MS analysis. Miyoshi wrote a review on fucosylated haptoglobin, which had been reported earlier, and suggested a possible pancreatic cancer biomarker. This requires validation for clinical use in the future. The serum levels of CD44v modified with sialyl Lewis x/a glycans may provide information for predicting the risk of developing metastasis and post-operative prognosis in cancer patients (Kannagi *et al.*).

Irimura *et al.* prepared a library of mutated *Maeckia amurensis* hemagglutinin and examined the carbohydrate binding specificity toward various cells, including carcinoma and melanoma. They found that these mutated lectins were useful as tools for high-throughput profiling of various cell-surface glycans. Rudd *et al.* reviewed the identification of serum glycoproteins and its significance in distinguishing between biomarkers for cancer and inflammation.

Four types of sialidase occur in human tissues, and membrane sialidase was found to be useful as a cancer biomarker (Miyagi *et al.*) and may be a potential target for cancer diagnosis and therapy. Furukawa *et al.* describe a possible drug target for malignant melanomas, ganglioside GD3 being a likely candidate.

Kim *et al.* describe forced expression of ganglioside GD3 in K562 cells and increased membrane transglutaminase 2, and proposed the recruitment of TG2 to the membrane by endogenous GD3. This may play a key role in erythroid differentiation in K562 cells.

Mucins play a key role in carcinogenesis and tumor invasion. Yonezawa *et al.* showed the expression profiles of MUC1, MUC2, and MUC among mucins in various human tumor tissues using immunohistochemistry and *in situ* hybridization, and discuss MUC1/4 in terms of tumor aggressiveness in human neoplasms. Siglec-3, a sialic acid-binding Ig-like lectin, binds MUC 2 mucins carrying sialyl Tn antigen and induces apoptosis of monocyte-derived dendritic cells, which is implicated in tumor escape from immune surveillance (Nakata *et al.*).

Muramatsu *et al.* have written a review on glycosaminoglycan-binding cytokines, such as bFGF, IL-8, MCP-1 and VEGF HGF, G-CSF, midkine, and pleiotrophin, which are clinically useful tools for both early detection and prognostic prediction. Matsumoto *et al.* reviewed the roles of HGF and its receptor, met, in tumors and a therapeutic approach using NK-4, an antagonist of HGF.

I sincerely thank all the scientists who kindly submitted these excellent papers in spite of their busy schedules. It is my wish that this Special Issue will be of assistance to the increasing number of scientists working in the field of cancer biomarkers.

I would also like to thank all contributors and reviewers for their efforts in this endeavor. My thanks also go to Dr. Hiroshi Kobayashi, Director of the Sapporo Cancer Seminar Foundation, and Professor Mike Dunn who gave me the opportunity to edit this Special Issue of PROTEOMICS.



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