Editorial

Hepatocellular carcinoma markers in the omics era: the glycomic analysis

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Abstract: Recently, Kamiyama *et al*. performed N-glycan profile in hepatocellular carcinoma (HCC) using the quantitative N-glycomics procedure by way of glycoblotting technology and using an automated glycan purification system. The study showed significant differences of N-glycans profile between healthy volunteers and liver cancer patients. The glycomic approach showed us the usefulness of new tools for identification and application of a new biomarkers for cancer diagnosis and treatment. These findings reveal a new field for future markers discovery.

Keywords: Hepatocellular carcinoma (HCC); glycan; glycomic; tumor markers

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Recent advances in analytical technologies, glycomics and glycoproteomics is gaining momentum in biomarker researches. While metabol-Omics, lipid-Omics, gen-Omics or prote-Omics have been well established and commonly applied to biomarkers discovery, biomarkers research is unexplored through glycomics perspective. Glycomic analysis allows rapid global comparison of glycome within body fluids or tissues of interest, which would allow identification and application of a new type of biomarker for cancer diagnosis and to monitor cancer development and treatment. Unlike genomic or proteomic biomarkers, which directly or indirectly rely on transcriptional or translational information, glycomics allows biomarker researches to focus solely on the posttranslational events within the cells. In the glycomics approach, glycans are harvested and used to determine whether the glycosylation has changed in disease-state samples compared to healthy controls (1-4). Glycosylation plays a critical role in modulating the structure and function of immunoglobulins. Glycosylation alters the biochemical properties of a glycoprotein in a number of ways, including an isoelectric point, conformational stability, thermal and pH stabilities, susceptibility to inorganic solvent and proteolysis and a lectin-binding behavior (5-8). Glycosylation might be considered to be "fine tuning" of the protein and essential for its function (4). Alterations in glycosylation of glycoproteins and glycolipids are present in various cancers, and a considerable amount of them play important roles in carcinogenesis, such as tumor progression, tumor cell differentiation, cell-cell interaction, and tumor cell adhesion and metastasis. Glycosylation changes have been identified in various diseases and in various localized malignancy like ovarian cancer (9), prostate cancer (3). The majority of serum glycoproteins are of hepatic origin. The close relationship between liver and serum glycoproteins suggests that liver abnormalities associated with aberrant glycosylations can be reflected by the changes in serum glycoprotein glycosylation patterns. The changes of glycosylation machinery in the cancer cells can be reflected in the blood circulation by tracing the changes in the glycosylation by the tumor. As tumor cells have different glycosylation machinery, it is hypothesized that identification of tumor-specific glycoforms should improve the specificity of a tumor marker. Glycomics study usually involves a large-scale systemic analysis of glycan pools which usually contain several subtypes such as N-linked and O-linked glycans and glycans from glycolipid. Glycan markers hold considerable

opportunities and challenges for disease diagnosis because the glycosylation machinery is highly sensitive to the biochemical environment. Alterations in glycan structures of immunoglobulins has been observed in a variety of autoimmune disease (10). Glycosylation, whether N-linked glycan or O-linked glycan, is highly sensitive to the biochemical environment and is the most common post-translational modification of proteins on the cell surface and in the extracellular matrix (11-13). Glycans released from serum can be collected and profiled by mass spectrometry. The development of modern mass spectrometers has provided opportunities to obtain systematic information on proteins and their posttranslational protein modifications. Mass spectrometry-based glycomics can provide a single platform to monitor several diseases simultaneously. Methods for releasing the glycans from glycoprotein depend on the attachment of the glycans. N-linked glycans (N-glycans) attach via nitrogen on an asparagine, while O-linked glycans (O-glycans) attach via oxygen on a serine or threonine. The N-glycans are of particular interest since they are more abundant in human sera than O-glycans. Changes in the degree of branching and levels of sialylation and fucosylation in N-glycans have been reported as a consequence of diseases. The extent of glycosylation and the types of O- and N-glycans have been shown to change in cancer. N and O-linked glycans are small molecules and are therefore easy to quantitate like metabolites. The N-linked glycans are of particular interest since are more abundant in human sera than O-linked glycans. Recently, using the quantitative N-glycomics procedure by way of glycoblotting technology and using an automated glycan purification system, Kamiyama *et al*. (14) performed N-glycan profile in hepatocellular carcinoma (HCC). Alterations in the N-glycosylation profiles of glycoproteins have been suggested to play important roles in the growth, differentiation, transformation, invasion, adhesion, immune surveillance and metastasis of tumor. The glycome, which is the glycan analog to the proteome and the genome, is defined as the glycan components of a biological source. Classifying the glycome relative to the genome, the proteome and the metabolome is difficult because it is often bound to proteins directly correlated to the genome. However glycans are produced by proteins making them more like metabolic products. In this regard, glycans are unique in that they link the three major areas of genomic, proteomic and metabolomics, since saccharides are found in all three groups. Using glycomic analysis, Kamiyama *et al*. identified 67 N-glycans that showed differences between HCC and disease-free individuals (normal controls) and investigated the prognostic capabilities. Kamiyama *et al*., using a glycoblotting method, purified N-glycans from preoperative blood samples from a cohort of 369 HCC patients that underwent primary curative hepatectomy. N-glycans were then identified and quantified using mass spectrometry (MS). After evaluation of the relative areas of all the sugar peaks, 67 N-glycans revealed that a proportion had higher relative areas in the HCC cases compared with the normal controls. Fourteen of these molecules had an area under the curve (AUC) greater than 0.80 and provide new information on recurrence of tumor after hepatectomy on patient survival (G2890, G1780, G3195, G3560, G2114, G1809, G3341, G1362, and G3865), on disease free survival (G2890, G1708, G3195, G3560, G3341, G1362 and G3865). Glycans with an AUC value greater than 0.80 were selected for analysis. G2890 was elevated more than a cutoff value in 82.7% and G3560 in 70.7% of HCC patients. In glycan structure both G2890 and G3560 are multiply branched (G2890 is tri-antennary and G3560 is tetra-antennary) glycans with a core fucose. Both glycans have one nonsialylated branch, i.e., G2890 and G3560, are triantennary disialylated glycan, and tetra-antennary tri-sialylated glycan, respectively. G2890 and G3560 glycans were identified as highly promising clinical markers of HCC. In fact both markers were strongly correlated with tumor number, size, stage, microscopic portal vein invasion, microscopic hepatic vein invasion. G2890 and G3560 can predict preoperative HCC tumor malignancy. The study compared the total N-glycans from sera of healthy volunteers and liver cancer patients, and reported dramatic differences of N-glycans profile between these two groups. However it is well established that liver cancer occurs through the process of chronic liver inflammation followed by hepatic cirrhosis (15-19). Liver cancer appears near the end-stage of hepatic cirrhosis, at which time many patients are suffering from loss of liver function and malnutrition. The significance of new information and recognition of clinical patterns in management of HCC should be deeply considered as we strive to improve patient outcomes. Although substantial progress has been made, significant challenges remain on large scale. Quantitative glycomics is a promising approach because the difference in glycans expression between hepatic disease and healthy status is expected to be a useful tool for the diagnosis or prognosis of diseases. The study by Kamyiama *et al*., using glycomic approach, showed us the usefulness of new tools for diagnosis and prognosis of cancer. These findings reveal a new field for future markers discovery.

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