Abstract of Doctoral Dissertation

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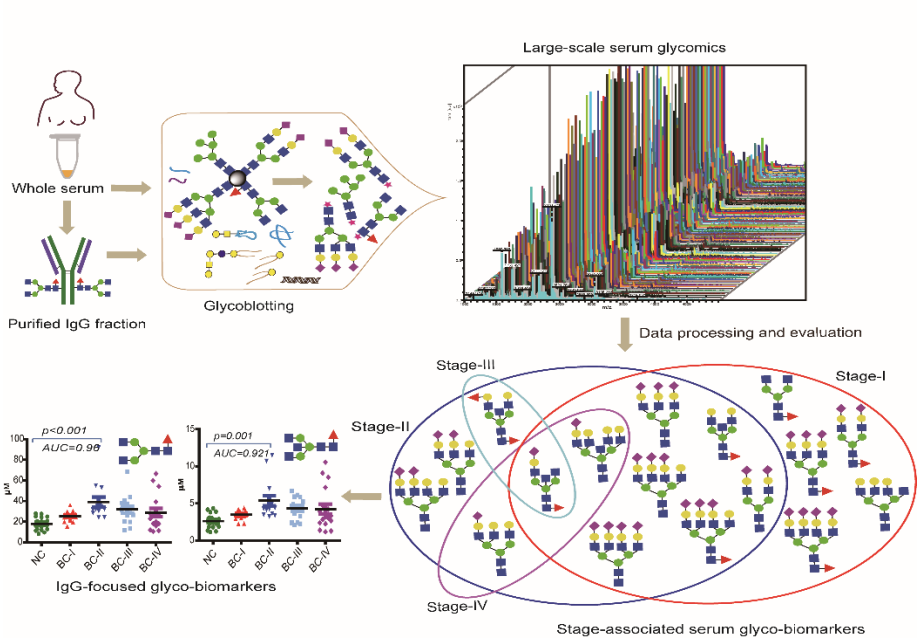
Title of Doctoral Dissertation

Human serum *N*-glycans as highly sensitive cancer biomarkers: Potential benefits and the risks

(高感度がんマーカーとしてのヒト血清*N*-グリカン：その潜在的な恩恵と危険性)

In cancer progression, aberrant serum *N*-glycan alterations have been associated with proliferation, invasion, metastasis, aggressiveness, angiogenesis, oncogenic signaling, and immune regulation of tumor cells. Deciphering cancer-associated alterations in protein glycosylation has been of critical interest not only to understand the oncogenic mechanisms, but also to suggest distinct glycan signatures of cancer that can be considered for diagnostic biomarker and therapeutic opportunities. In this context, more than half of current FDA-approved protein cancer markers are glycoproteins (including CA15-3 and CA27-29 for breast cancer; CA19-9 for pancreatic, gastric, and colonic cancers; CA-125 for ovarian cancer; CEA for Colorectal Cancer; PSA for prostate cancer; and α-fetoprotein for hepatocellular cancer). Breast cancer (BC) has become the most common malignancy and the leading cause of cancer death among women globally. Although there have been improvements in patient diagnosis and survival for breast cancer, there is no clinically validated serum biomarker for its early diagnosis.

This study primarily aimed to explore the potentials of *N*-glycans directly released from patient serum glycoproteins and purified immunoglobulin G (IgG) fraction in distinguishing breast cancer patients from matched normal controls (NC) towards the discovery of non-invasive markers. I used a comprehensive glycomics approach by integrating glycoblotting-based glycan purification with MALDI-TOF/MS based quantitative analysis using sera of BC patients belonging to stages I-IV and normal controls collected from Ethiopian women during 2015-2016. IgG was purified from serum of all subjects by affinity chromatography using protein G spin plate and further subjected to glycoblotting for glycan release. Mass spectral data were further processed and evaluated rigorously, using various bioinformatics and statistical tools. From total of 46 detected serum *N*-glycan structures, 35 were significantly up-regulated in the sera of all BC patients within which 17 *N*-glycans (comprising core-fucosylated, multiply branched and sialylated structures) showed strong diagnostic potential (AUC, 0.8-1) for early stage (I and II). High abundance of these glycans has been strongly associated with greater invasive and metastatic potential of cancer. In a more detail analysis, *N*-glycans from IgG confirmed consistent abundance in BC patients, particularly two core-fucosylated and agalactosylated glycans specifically distinguished (AUC, 0.944 and 0.921, $p \leq 0.001$) stage II patients from NC. Our findings of increased IgG core-fucosylation and agalactosylation were reported to be associated with a decrease in immunosuppressive potential of IgG towards tumor cells, which in part may correlate with the aggressive nature of BC commonly noticed in black population. Altogether, these findings reveal characteristic tumor-associated glycoforms which demonstrate significant distinguishing potential not only between the whole BC patients and the healthy controls but also in a cancer stage dependent manner.



Breast cancer stage specific serum and IgG *N*-glyco-biomarker candidates identified by glycoblotting-assisted MALDI-TOF/MS analysis

Most glycomics studies have previously focused on understanding disease mechanisms and proposing serum markers for various cancers, yet the influence of confounding factors like ethnic variation on the identified glyco-biomarker remains poorly addressed. Hence, my second study aimed to investigate the inter-ethnic variation in serum *N*-glycan and free sialic acid patterns among US origin control, Japanese, Indian, and Ethiopian healthy volunteers in association with the identified glycan biomarkers for hepatocellular (HCC) and other cancers. Ethnic specific detections and differential expression levels, particularly highest abundance ($p < 0.001$) of high mannose, core-fucosylated, hyperbranched/hypersialylated *N*-glycans were demonstrated in Ethiopians. Glycan abundance trend of healthy Ethiopians was nearly close to that of Japanese HCC patients. Surprisingly, some of the glycoforms greatly elevated in the Ethiopian subjects have been identified as sensitive serum biomarkers of various cancers. Fluorescence HPLC-based quantification of free sialic acid demonstrated significant up-regulation primarily in the Ethiopians, compared to the other ethnicities.

In conclusion, the patient based comprehensive study has addressed for the first time both whole serum and IgG *N*-glycosylation signatures of native black women suffering from BC and revealed novel glyco-biomarkers with marked overexpression and distinguishing ability at early stage patients. The informative findings from the inter-ethnic serum *N*-glycomic study emphasize the substantial influence of ethnic difference in human serum *N*-glycome variation, the ignorance of which may provide unclear and imprecise conclusion of the diagnosis by using glycan-related disease biomarkers.

Finally, I have clearly shown the potential of serum glycomics profiling as non-invasive approach to discover early stage specific cancer biomarkers, as well as the need to strictly consider ethnic matching in population based glyco-biomarker discovery research to avoid inaccurate diagnosis.