N-Linked Glycan Branching and Fucosylation are Increased Directly in HCC Tissue as Determined Through in situ Glycan Imaging

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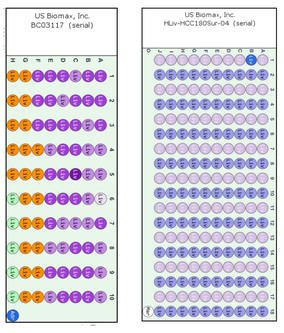
Introduction

Hepatocellular carcinoma (HCC) remains as the 5th most common cancer in the world and accounts for more than 700,000 deaths annually. Changes in serum glycosylation have long been associated with this cancer but the source of that material is unknown and direct glycan analysis of HCC tissues has been limited. With this narrow view, it's difficult to accurately determine if these changes are due to the cancer or due to other confounding factors. To combat these challenges, we used a previously developed method of in situ tissue based N-linked glycan imaging that allows for analysis that bypasses the need for microdissection and solubilization of the tissue prior to analysis.

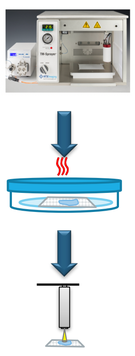
Methods and Materials

A: Tissue Preparation



- Tissue: Liver tissue samples were received from ProSci Inc.
- TMA: TMAs were obtained through US Biomax. Sections were cut at 5 µm.
- Samples were heated prior to dewaxing. Dewaxing utilized washes of xylenes, ethanol, and water.
- Tissues were antigen retrieved in ~4.5 mM citraconic buffer pH 3 using a vegetable steamer. Slides were cooled by exchanging buffer with water after retrieval.

B: N-Glycan Release and MALDI Matrix Application



- Sections were sprayed with 0.1 µg/µL PNGase F using a TM-Sprayer (HTX Technologies).
- Slides were incubated for 2 hours at 37.5°C in a closed cell culture dish with 5 mL of water.
- α-Cyano-4-hydroxycinnamic acid matrix (7 mg/mL in 50% ACN/0.1% TFA) was sprayed onto slides using a TM-Sprayer (HTX imaging).

C: MALDI IMS

- TMA and tissue sections were imaged on a solariX™ FT-ICR in positive ion.
- Images were visualized in FlexImaging 4.0, normalized by total ion current.
- Data was analyzed using SCiLS lab software.

Results

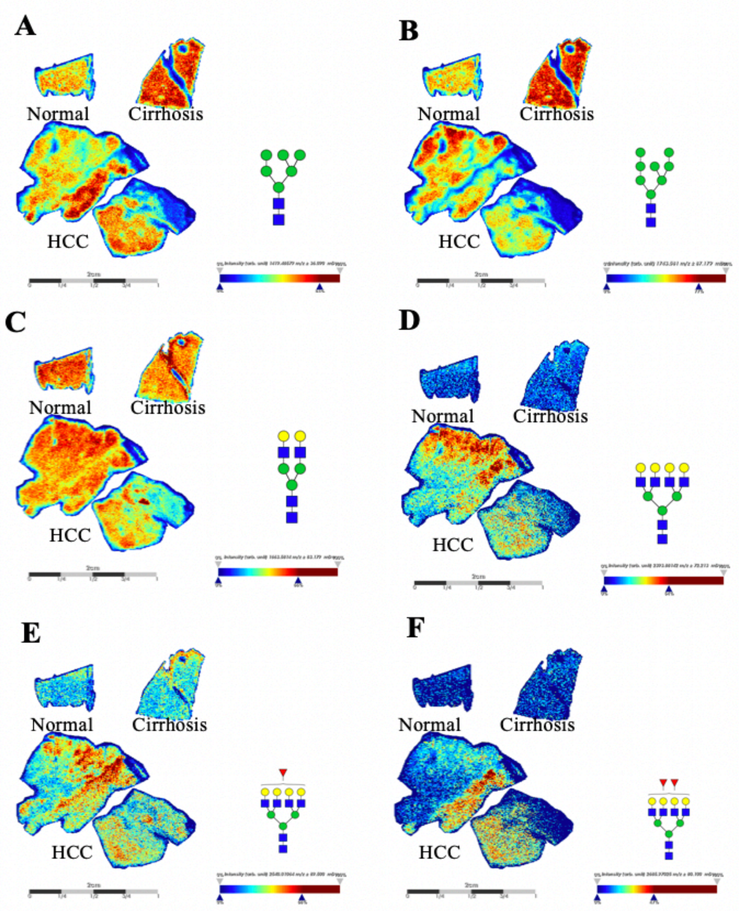


Figure 1. Detection of various N-linked glycans in normal, cirrhotic and HCC tissues. While certain glycans are found in all tissues (A-C), some glycans are found predominantly in the HCC tissue (D-F). Images were acquired with 150 µm raster step size on a Bruker 7T solariX XR ICR FTMS system. Ion intensities are normalized to the TIC of each ion across the tissue. Color scale bars are included and autocorrected for the range of intensities plotted.

Results

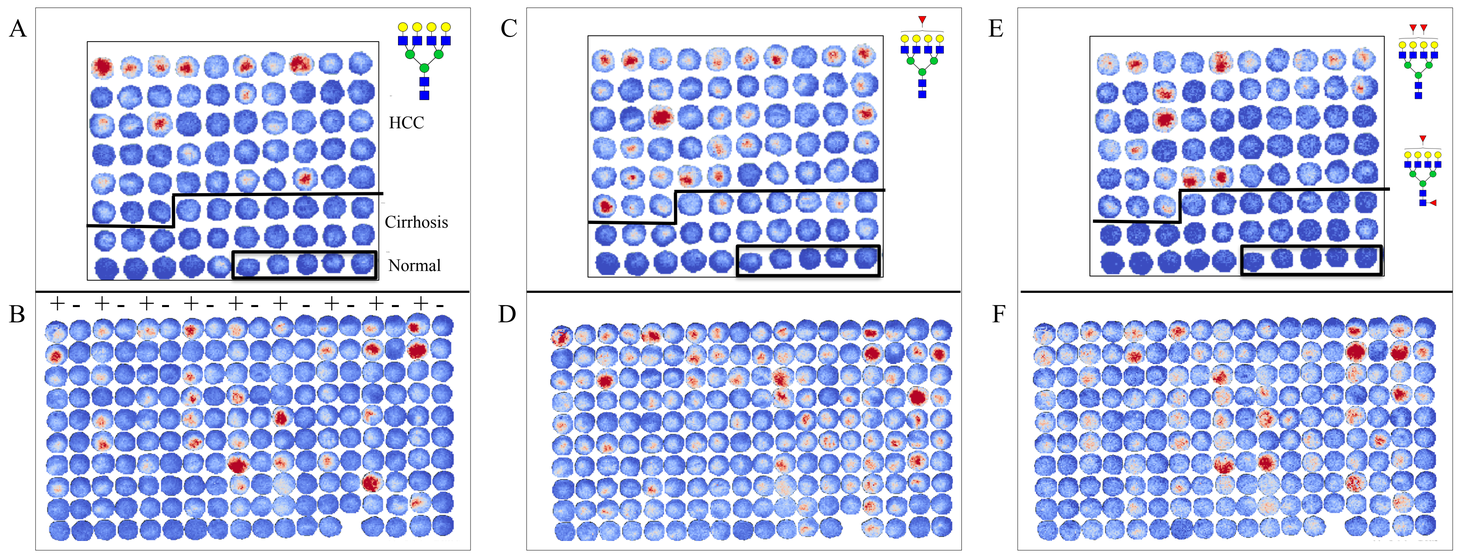


Figure 2. Representative imaging data from both TMA datasets. Representative image data collected for both TMA sets (first TMA with independent samples is on top with the second TMA with HCC and patient-matched untransformed adjacent tissue is on bottom) showing three different glycan structures: A and B) 2393.840 m/z; C and D) 2539.957 m/z; E and F) 2685.969 m/z. For the first TMA, the HCC tissue is indicated at top with the cirrhotic samples in the middle and the healthy tissue samples in the box at the bottom right corner. For the second TMA, the + above each column represents the HCC tissue and the consecutive – column represents the matched normal adjacent tissue section. The proposed glycan is presented at the top of each panel.

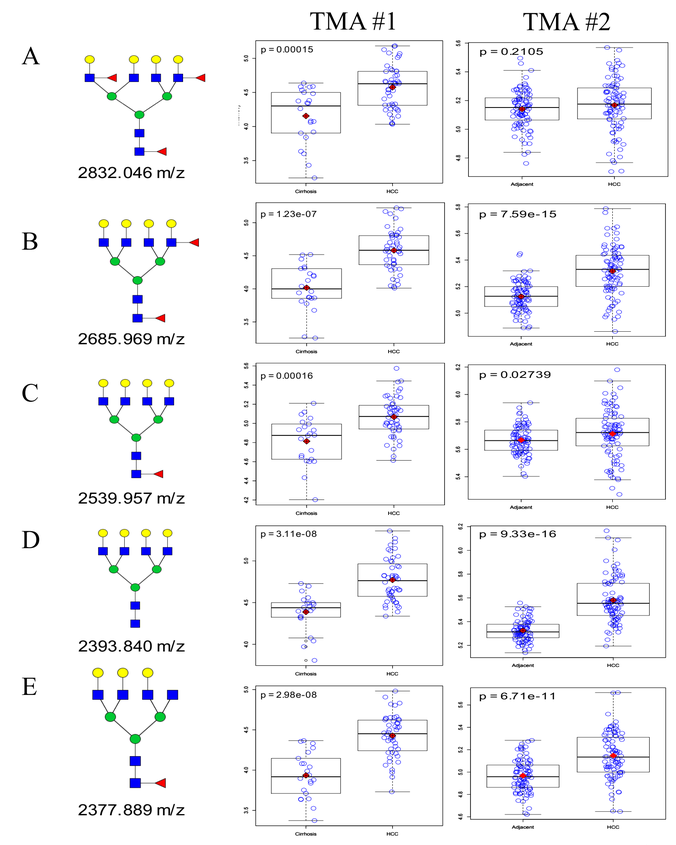


Figure 3. Analysis of human liver TMA datasets. Proposed glycan structure, log transformed intensity scatter plot with the red diamond indicating mean and associated p- value for TMA #1 and TMA #2. In TMA #1, analysis was done comparing HCC versus cirrhotic samples and for TMA #2, analysis was done comparing HCC versus matched untransformed adjacent tissue. A, B, C, and E utilized a student t-test for their p-value while D utilized a Wilcoxon Rank Sum Test.

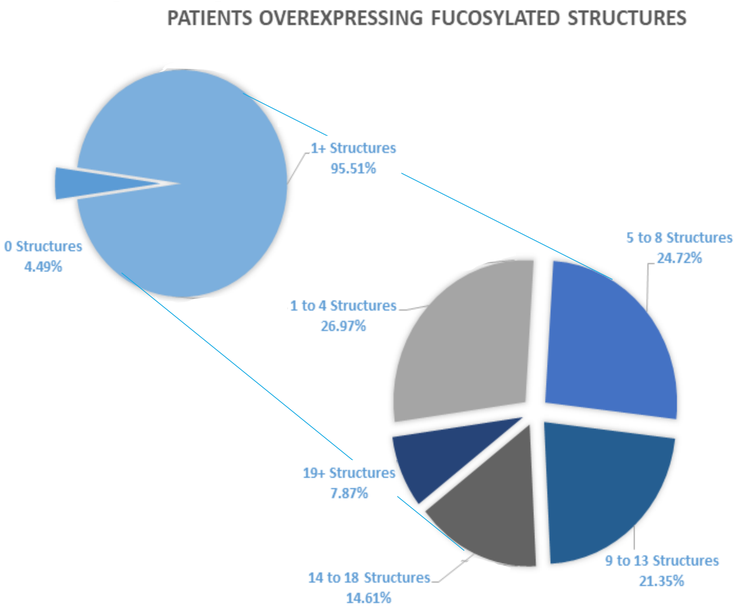


Figure 4. Patients demonstrating elevated levels of fucosylated glycan structures. A total of 33 fucosylated glycans were found elevated in the patient-matched TMA. Comparing HCC to the un-transformed adjacent tissue, a 1.5x relative intensity increase in HCC tissue was used to classify patients as elevated. Of the 89 patients able to be analyzed, 96% (85 patients) demonstrated elevated levels of at least one of these fucosylated structures (left). Of these 85 patients, they were further classified into varying classes based on the number of fucosylated structures they had elevated levels for. 27% (23 patients) had elevated levels of one to four fucosylated structures, 25% (21 patients) had elevated levels off five to eight structures, with 8% of patients showing elevated levels of 19 or more of these fucosylated structures with one patient showing elevated levels all 33 fucosylated structures found.

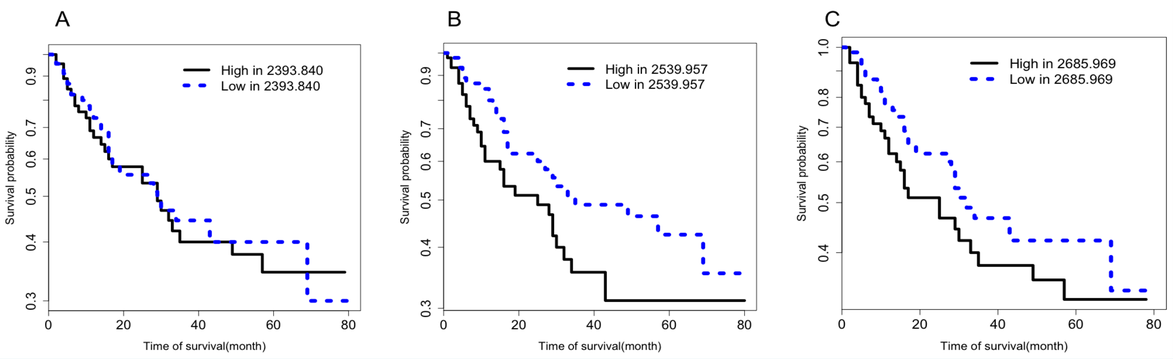


Figure 5. Survival Plots for Branched and Fucosylated Glycans: Kaplan–Meier Survival plots for glycan at m/z 2393.840 (A) 2539.957 (B) and 2685.968 (C) from the 90 patient TMA.

Conclusions

This work supports the hypothesis that the increased levels of fucosylated N-linked glycans in HCC serum are produced directly from the cancer tissue, rather than being a phenomenon that is not cancerous tissue specific. We then validated these findings through comparison of N-glycan imaging compared to HPLC analysis to confirm that these relative glycan levels were also seen through conventional tissue solubilization methods. These findings have implications for the future in which a clinical assay can be used in conjunction with these elevated glycoforms to identify HCC in patients at an earlier stage of progression, particularly if associated protein information can be identified. By targeting these proteins associated with increased branching and fucosylation, we can not only develop a better diagnostic test for patients, but also elucidate the root cause of this behavior and use this as a chemotherapeutic target in the future.

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