N-glycosylation of hepatocellular carcinoma correlates with genetic subtypes

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Overview

Introduction:
- Hepatocellular Carcinoma (HCC) is the second leading cause of cancer deaths globally.
- Recent work has identified significant, cancer-linked changes in N-linked glycosylation directly in HCC tissue by MALDI glycan imaging.
- There is significant glycan heterogeneity between HCC tissues, suggesting a correlation between glycan expression and specific molecular subtypes of HCC.

Methods:
- Sample set of consisting of 37 HCC tissues classified using the Hoshida classification system.
- Prepared tissues through antigen retrieval, spraying of PNGase F Prime™, and spraying of CHCA matrix onto the tissue.
- Data was collected using a Bruker MALDI FT-ICR (solariX™ Legacy 7.0 T) and rapifleX TissueTyper™, and analyzed using flexImaging and SCiLS software.

Results:
- Glycan expression trends can be observed, including regarding overall glycan expression and specifically fucosylation expression. These trends can serve to distinguish between tumor subtypes.
- Within Hoshida tumor subtypes, some heterogeneity in glycan expression remains.

Novel Aspect:
- The analysis of glycan information in conjunction with genetic tumor information, which has not previously been done for any cancer type.

Methods

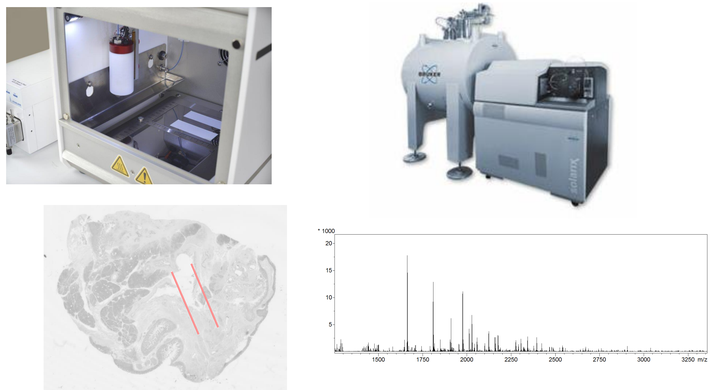

Figure 1. MALDI-IMS Data Collection and Analysis.
MALDI-IMS data was collected using a Bruker MALDI solariX™ Legacy 7.0 T in positive ion, reflector mode. Images were collected at a 125 µm raster on the solariX and 50 µm on the rapifleX, spanning m/z range 600-4500. Tissues were prepared by spraying PNGase F Prime™, and CHCA matrix using a HTX TM-Sprayer M5. Images were visualized in FlexImaging v4.1 (Bruker), normalized by total ion count, and analyzed using SCiLS software (Bruker).

Acknowledgements:
1. Dr. David N. Lewin, Medical University of South Carolina
This work was supported by funding from the South Carolina Centers of Economic Excellence and U01 CA226052.

References:
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2. Hoshida, Y., S.M. Nijman, M. Kobayashi, J.A. Chan, J.P. Brunet, D.Y. Chiang, A. Villanueva, P. Newell, K. Ikeda, M. Hashimoto, G. Watanabe, S. Gabriel, S.L. Friedman, H. Kumada, J.M. Llovet, and T.R. Golub, *Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular carcinoma.* Cancer Res, 2009. **69**(18): p. 7385-92.

MALDI-IMS of HCC Tissues

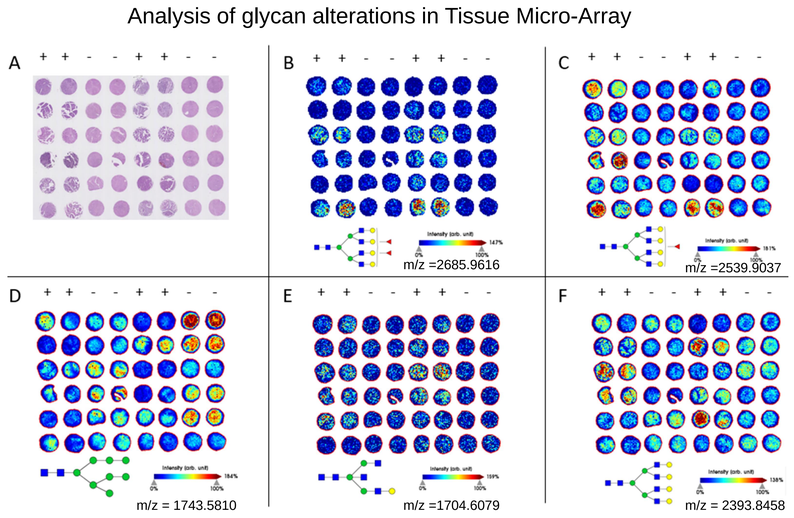

Figure 2. Representative imaging data from a TMA dataset. Representative image data collected for a TMA which included matched TMA cores for 12 HCC cases (marked by +) and 12 non-HCC cases (marked by -). In total, 23 N-glycan structures were significantly increased in HCC cores over non-HCC cores, primarily consisting of branched and/or fucosylated structures (*, P < 0.05). However, there were no structures consistently overexpressed in all HCC cases, suggesting heterogeneity between tumors. The proposed glycan is presented at the bottom of each panel.

Subtypes of HCC

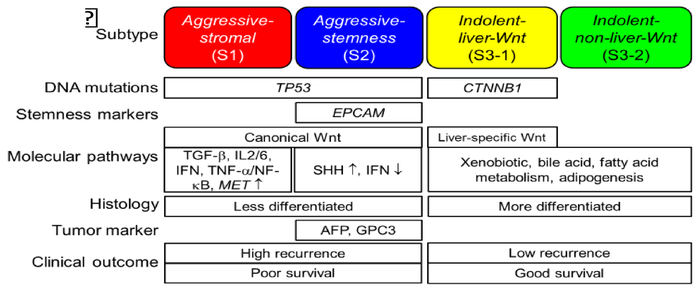

Figure 3. Molecular HCC subtypes. Molecular and clinical characteristics of each subtype are summarized.

Glycan Expression in Subtyped Tissues

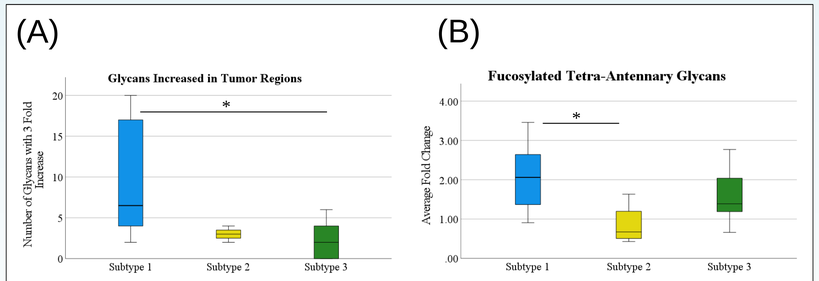

Figure 4. HCC Glycan Expression. (A)**:** N-linked glycan structures were determined to be increased in the tumor region with an area under the peak fold change of >3 from the adjacent tissue to the tumor region. A significant difference between S1 and S2/S3 was determined with a Wilcoxon Rank-Sum Test (* = p<0.05) (B): The average fold change of all measured fucosylated tetra-antennary glycans is significantly higher in S1 than in S2 tumors. Wilcoxon Rank-Sum Test (* = p<0.05)

Analysis of Subtyped HCC Tissues and Results

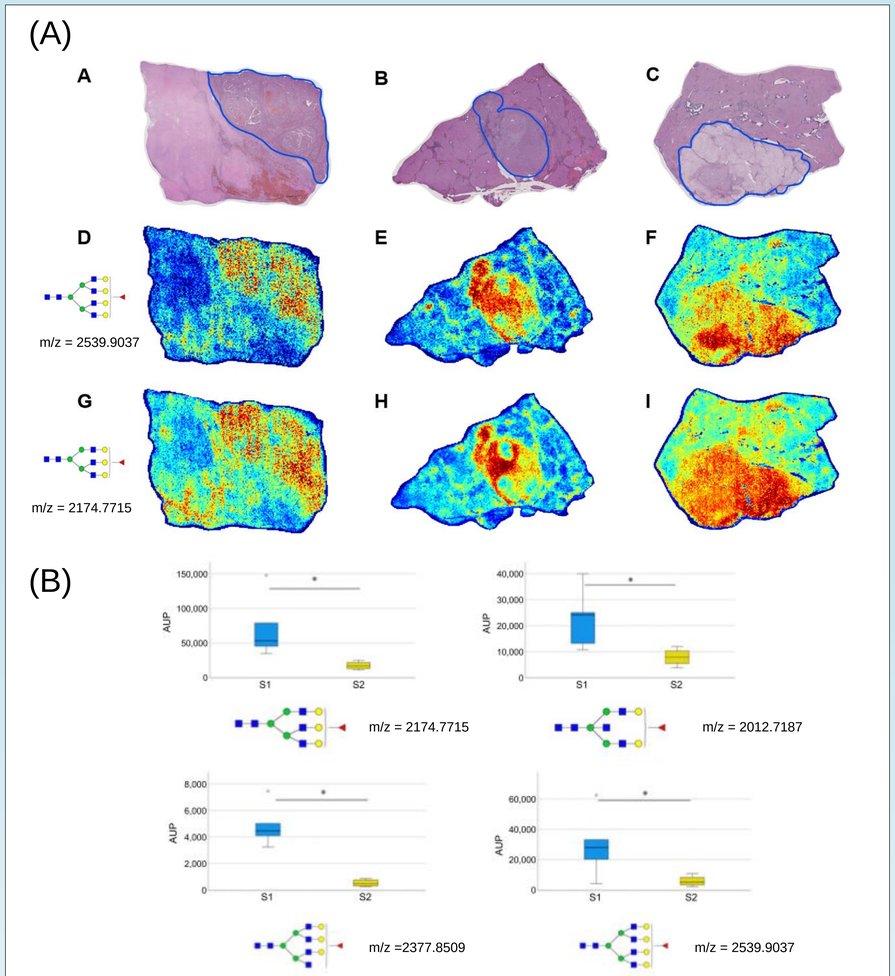

Figure 5. Glycan Analysis of Subtyped HCC Tissues. (A): Representative images of S1 tumors are shown, with corresponding H&E stains that outline the tumors in blue. Fucosylated branched glycans are often tumor associated in S1 tumors, examples of which are shown. (B): Comparison of expression of common branched, fucosylated glycans in S1 and S2 tumors.

Conclusions

- HCC tumors exhibit glycan differences from surrounding normal and cirrhotic tissue that can be identified through MALDI-IMS.
- There is still glycan heterogeneity within each subtype, but differing trends regarding overall glycan expression and fucosylated glycan expression are observed.
- Glycans that exhibited increased branching structures were commonly abundant in tumor tissue of all subtypes.
- Fucosylation of branched glycans is increased in S1 tumors but not in S2 tumors, which is promising considering that S2 tumors have increased AFP expression.
- Understanding how genetic differences of tumors relate to differences in glycan expression allows for the more precise application of glycomic information for HCC detection and prognosis.