Application of SpatialOMx for the discovery of biomarkers of hepatocellular carcinoma

Anand Mehta

Cell and Molecular Pharmacology and Experimental Therapeutics, College of Medicine, Medical University of South Carolina

Goal: Identify glycoprotein biomarkers of hepatocellular carcinoma (HCC) that originate directly from cancer tissue.

Methods: Formalin-fixed human liver tissue from patients with healthy livers, cirrhotic livers, or HCC were evaluated using N-glycan MALDI imaging mass spectrometry on three machines, a SolariXTM 7T Hybrid FTMS System, a rapifleXTM MALDI Tissuetyper and a timsTOF flex mass spectrometer. In all cases, N-linked glycan attached to tissue was removed through the application of a thin molecular coating of PNGase F Prime™ using a TMSprayer (HTX Technologies LLC). Data was analyzed using FlexImaging and SCiLS Lab software. In total, 238 HCC tissue samples and 235 control tissue samples were analyzed. Subsequently, serum glycoproteomics was performed using a recombinant lectin with greater affinity toward branched and fucosylated glycan and over 35 glycoproteins were identified as containing the same glycans observed in HCC tissue. To examine these glycoproteins in serum, we developed a novel multiplexed antibody based platform that allowed us to leverage the power of SpatialOMx to perform glycan analysis simultaneously on hundreds of glycoproteins at once.

Results: Through tissue-based glycan imaging, increased levels of fucosylation were observed in 96% of the HCC tissues examined. The oligosaccharide most often increased in HCC tissue was a tetra-antennary glycan with one to three fucose residues. Importantly, one hundred percent of the cancer tissues had one or more changes in glycosylation. Using a recombinant sugar binding protein with affinity towards fucosylated and branched glycan, over 35 serum glycoproteins that contained altered glycans were identified. Subsequently a novel assay was developed to exploit these findings. This assay combined antibody microarray technology with imaging mass spectrometry to the identify altered glycans on proteins and highlights the role of SpatialOMx in both biomarker discovery and as a biomarker platform.

Conclusions: Using a novel tissue-based glycan imaging mass spectrometry platform, we were able to identify glycan changes that occur directly in cancer tissue and used this information to identify potential biomarkers of HCC that are far superior to those currently used.