Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide, with increasing incidence, and is the third most common cause of cancer death. The clinical management of patients suffering from HCC is not satisfactory, mainly because of the expense and lack of availability of current diagnostics to predict and monitor the disease. OHSU and Ymir Genomics have teamed up to discover urinary biomarkers that will detect HCC in at-risk patient populations. Ymir has developed a new method for the rapid and effective isolation of urine extracellular vesicles (EVs) and integrated it into Mass Spectrometry (MS) and miRNA array protocols. We have applied these protocols to 3 groups of 20 urine samples from 1) healthy controls, 2) patients suffering from cirrhosis and 3) patients suffering from cirrhosis and untreated HCC. MS data shows that this protocol is capable of identifying over 2400 proteins from only 4 mls of urine; far less sample than previously reported for urine EV proteomics. Furthermore, we have identified 10 liver-selective proteins in the urine of all three groups; proof-of-concept for the isolation of liver-derived EVs in urine. We can also produce high-quality miRNA array data with even less volume of sample. Preliminary experiments on pooled samples from each group have identified several candidate protein and miRNA biomarkers that can distinguish HCC from healthy controls. Candidate biomarkers developed in these experiments will be suitable for a larger longitudinal study, as the method is already compatible with standard clinical chemistry laboratory assay requirements. Thus, this study provides proof-of-concept for the use of a rapid, simple, and inexpensive protocol for the isolation of urine EV-associated cancer biomarkers.

The Use of a Novel Extracellular Vesicle Isolation Method to Discover Urine Hepatocellular Carcinoma Protein Candidate Biomarkers

Ymir Genomics and OHSU have performed a pilot discovery study for urine EV-associated protein and miRNA liver disease biomarkers. Several interesting candidate biomarkers have been identified. Furthermore, the study has perfected methods for sample procurement, sample preparation of EV-associated biomolecules, and high-throughput biomolecule identification with MS and miRNA array. Follow-up larger scale studies are now being planned.