Title: **Researchers develop novel blood test for hepatocellular carcinoma diagnosis and monitoring**

Researchers from the Center for Precision Medicine Research at the Marshfield Clinic Research Institute and Key Laboratory of Genomic and Precision Medicine, Beijing Institute of Genomics, Chinese Academy of Sciences, recently developed a novel approach in which low-pass WGBS was applied to provide a low-cost and effective method to monitor genome-wide cell-free DNA methylation levels for hepatocellular carcinoma patients.

The researchers believe low-cost circulating cell-free DNA monitoring could help those with hepatocellular carcinoma to track their disease progress and to evaluate surgery efficiency. These findings were recently reported in the leading journal, xxxx.

Circulating cell-free DNA (cfDNA) are small double-stranded DNA fragments found in plasma, urine, and other body fluids originating from cell apoptosis and necrosis. In many settings, analyses of cfDNA can be regarded as a way to perform a “liquid biopsy”, which have been produced promising results for genetic testing, early cancer detection. The new approach demonstrate long-range DNA methylation, methylation in HBV integration regions and cell-free DNA fragment size serve as promising features for cell-free DNA-based prediction of hepatocellular carcinoma.

“This method provides a new angle to diagnosis and real-time monitoring of hepatocellular carcinoma patients. We are working on using this novel method to other human cancers, including lung cancer, breast cancer, prostate cancer and cholangiocarcinoma” said Shicheng Guo, Ph.D., one of the primary authors of the study and postdoctoral fellow for Marshfield Clinic Research Institute.

The new approach, termed “low-pass whole-genome bisulfite sequencing (low-pass WGBS)”, was first used to study hepatocellular carcinoma by analyzing the circulating cell-free DNA methylation to hepatitis, cirrhosis, early and advanced hepatocellular carcinoma patients. Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer. Hepatocellular carcinoma occurs most often in people with chronic liver diseases, such as hepatitis and cirrhosis. The risk of hepatocellular carcinoma is higher in people with long-term drinking and individuals infected with hepatitis B or hepatitis C.

“Circulating cell-free DNA has been demonstrated to provide a promising opportunity for non-invasive cancer diagnosis. DNA methylation in circulating cell-free DNA has exhibited particularly promising signals for cancer diagnosis and tissue-of-origin mapping. It is now well-recognized that genome-wide DNA hypo-methylation is a hallmark feature of the human cancer genome and therefore may be usefully applied to cell-free DNA-based cancer diagnosis. However, the amount of circulating cell-free DNA is typically too limited for interrogation with conventional high-depth/coverage genome-wide bisulfite sequencing (WGBS)” said Dr. Steven J Schrodi an Associate Research Scientist at the Marshfield Clinic Research Institute and one of the corresponding authors of the study.

“In this original manuscript, we have proposed a novel strategy to apply low-pass WGBS to monitor DNA methylation levels in cell-free DNA fragments. We have developed a new measurement approach for long-region hypo-methylation which shows utility as a biomarker for cancer surveillance in liver diseases ranging from hepatitis, cirrhosis, early stage hepatocellular carcinoma and advanced HCC. Our study shows that low-pass WGBS provides a stable and powerful diagnostic tool for HCC. Furthermore, our approach enables an evaluation of the efficacy of surgical intervention for HCC. Interestingly, we present evidence of over-representation of differentially methylated CpGs in HBV integration regions based on our low-pass WGBS approach, providing additional insights into the mechanisms of HCC molecular pathophysiology and may aid HCC diagnosis and clinical decisions. Implementation of this approach is favored by the low cost compared to conventional techniques. Using machine learning, we show that HBV integration-based DNA methylation in cell-free DNA (MethylHBV) exhibited excellent predictive performance in distinguishing HCC from other liver diseases. Finally, using the same data, we introduced cell-free DNA fragment size (cfDNAsize) distribution effects into our predictive model yielding a powerful HCC discriminating”

Frequently, diagnosis of hepatocellular carcinoma rely on image test such as CT or MRI or liver biopsy. However, these method usually will be effective when the tumor size < 5mm. Novel technique based on DNA sequencing have been developed in the past several years including cell-fee DNA methylation, cell-free chromatin which provided promising non-invasive diagnosis for human cancers. Recently, cell-free DNA based cancer diagnosis and prognosis has become the most competitive field. GRAIL’s cell-free DNA methylation assay for early pan-cancer diagnosis were approved in May 13, 2019. Memorial Sloan Kettering Cancer Center received approval to MSK-ACCESS on June 11 2019, for their new molecular assay called Analysis of Circulating cfDNA to Evaluate Somatic Status (ACCESS). We hope our novel method can be applied in Marshfield Clinic to provide early screening for high risk liver cancer patients with further technique improvement and validation.

This study was completed through a collaboration between the Marshfield Clinic Research Institute (MCRI), Chinese Academy of Sciences (CAS), Beijing You’an Hospital, Stanford University, Icahn School of Medicine at Mount Sinai, Stonybrook University and University of Wisconsin-Madison.

Authors of the study are Haikun Zhang#, Peiling Dong#, Shicheng Guo#, Chengcheng Tao, Wenmin Zhao, Jiakang Wang, Ramsey Cheung, Augusto Villanueva, Huiguo Ding, Steven J. Schrodi\*, Dake Zhang\*, Changqing Zeng\*

#Authors contributed equally to the work, \*Corresponding authors: Dr. Steven J. Schrodi, Dr. Dake Zhang and Dr. Changqing Zeng.