Dr. Jeremy Berg and Editorial Board
*Science Advances*

29 June 2019

Dear Dr. Jeremy Berg and Editorial Board:

On behalf of all authors, please consider our submitted research article entitled “Circulating cell-free DNA based low-pass genome-wide bisulfite sequencing aids non-invasive surveillance to hepatocellular carcinoma” for publication in *Science Advances*.

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). 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 (HCC) 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 with 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 ($Methyl_{HBV}$) exhibited excellent predictive performance in distinguishing HCC from other liver diseases. Finally, using the same data, we introduced cell-free DNA fragment size distribution effects into our predictive model yielding a powerful HCC discriminating ability. As this study details a novel approach and strategy generating clinically compelling findings, we believe the readers of Science Advances will find this manuscript highly interesting.

This manuscript has not been submitted elsewhere and all authors declare no conflicts of interest. Thank you for your consideration.

Sincerely, for the authors,

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