Dr. XXX and Editorial Board  *XX* [December](file:///E:\Program%20Files\youdao\Dict\7.5.2.0\resultui\dict\?keyword=December) 2019

*Science Advances*

Dear Dr. XXX and Editorial Board:

On behalf of all authors, please consider our submitted research article entitled “Hypo-methylation in HBV integration regions aids non-invasive surveillance to hepatocellular carcinoma by low-pass genome-wide bisulfite sequencing” 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 in which we utilized DNA methylation around HBV integration regions to apply low-pass WGBS to monitor DNA methylation levels in cell-free DNA fragments. Interestingly, we present evidence of over-representation of cfDNA fragments in intergenic, repeat regions and HBV integration regions compared to functional elements (promoter and gene coding regions), providing additional insights into the mechanisms of HCC molecular pathophysiology and may aid HCC diagnosis and clinical decisions. WGBS of tissue samples demonstrates hypo-methylation around HBV integration sites as a more sensitive indicator to detect HCC compared to genome-wide DNA hypo-methylation. We have developed a new measurement approach for long-range methylation around HBV integration sites which shows utility as biomarkers for cancer surveillance in liver diseases ranging from hepatitis, cirrhosis, early stage hepatocellular carcinoma (HCC) and advanced HCC. Our study shows that hypo-methylation around HBV integration sites by 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. Implementation of this approach is favored by the low cost compared to conventional techniques. Using receiver operating characteristic (ROC) curves, we show that HBV integration-based DNA methylation in cell-free DNA exhibited excellent predictive performance in distinguishing HCC from other liver diseases. Finally, using an independent cohort, we validated HBV integration-based DNA methylation in 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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