October 7, 2019

Greetings Dr. Brian Lacy, Dr. Brennan Spiegel, and the Editorial Board,

On behalf of all authors, please consider our submitted research article entitled, “Genome-Wide Methylation Profiles of Low- and High-Grade Adenoma Reveals Potential Biomarkers for Early Diagnosis of Colorectal Carcinoma” for publication in *The American Journal of Gastroenterology*.

Colorectal cancer (CRC) is the third leading cause of cancer-related death worldwide. Identification and application of early biomarkers of carcinogenesis to enhance cancer screening has become one of the most effective approaches to increase the overall survival probability of patients with cancer. Recent evidence demonstrates that DNA methylation signatures of circulating cell-free DNA (cfDNA) may be a promising approach for early diagnosis of CRC and improved tissue-of-origin mapping ability for patients with metastatic CRC. Over the past decades, a large number of DNA methylation biomarker research has been conducted for CRC; however, this research is based on CRC tissue samples where early aberrations in methylation are assumed to be maintained during progression from the pre-clinical stage to cancer. To identify and evaluate changes in DNA methylation signatures during CRC development, we collected blood samples from two distinct subtypes of colorectal adenoma samples, low-grade adenoma (LGA) and high-grade adenoma (HGA), which could represent early stages of CRC and compared them to normal colorectal tissue and CRC DNA methylation signatures derived from public databases. To the best of our knowledge, this is the first genome-wide DNA methylation research of pre-cancerous colorectal adenomas to identify and compare the methylation patterns between LGA, HGA, and CRC. We identified genome-wide hypo-methylation that occurred at early stages of CRC development (LGA and HGA stages) indicating that LGA may be the best candidate for further development and application of early diagnostic biomarkers for CRC. Further evaluation of differential methylation regions (DMRs) for each group via eedetermined thatfunctions associated compared to normal tissuethe identification and characterization of wherearea under the curve and receiver operatingswas high as , respectivelyWe believe that a comprehensive understanding of genome-wide DNA methylation profiles for early stage pre-cancerous lesions (LGA and HGA) will provide important resources for early diagnosis and treatment of CRC and produce candidate biomarkers for further cfDNA methylation research. Overall, we believe our innovative study provides an opportunity to identify early diagnostic biomarkers and increases our understanding of the pathology and progression of CRC.

This manuscript has not been submitted elsewhere for publication, and all authors declare no conflicts of interest. Thank you for your consideration, and have a great day.

Sincerely,

Shigang Ding, MD, Dake Zhang, PhD, and Changqing Zeng, PhD, on behalf of the author group