Feb 7st,2018

Dear Yusuke Nakamura, Editor-in-Chief of *Cancer Science*:

I would like to submit a manuscript titled ‘Identification of hyper-methylated tumor suppressor genes-based diagnostic panel for esophageal squamous cell carcinoma (ESCC) in a Chinese Han population’ for possible publication in Cancer Science.

DNA methylation-based biomarkers have been suggested to be promising for early cancer diagnosis. However, a limited number of DNA methylation-based biomarkers for esophageal squamous cell carcinoma (ESCC), especially in Chinese Han populations have been identified and evaluated quantitatively. To search for the early diagnostic biomarkers for ESCC, candidate tumor suppressor genes (N = 65) were selected through literature searching, and four public high-throughput DNA methylation microarray datasets including 136 samples totally were collected for initial confirmation. Targeted bisulfite sequencing was applied in an independent cohort of 94 pairs of ESCC and normal tissues from a Chinese Han population for eventual validation. In summary, four candidate genes (*ADHFE1, EOMES, SALL1* and *TFPI2*) were identified and validated in the ESCC samples from a Chinese Han population. All four candidate regions were validated to be significantly hyper-methylated in ESCC samples (*ADHFE1*, p = 1.70×10-3; *EOMES*, p = 2.90×10-9; *SALL1*, p = 3.90×10-7; *TFPI2*, p = 3.40×10-6). Logistic regression based prediction model shown a robust ESCC classification performance (Sensitivity = 66%, Specificity = 87%, AUC = 0.81). Moreover, advanced classification method had better performances (random forest and naive Bayes). Interestingly, the diagnostic performance could be improved in non-alcohol use subgroup (AUC = 0.84). In conclusion, we believed that methylation panels of *ADHFE1, EOMES, SALL1* and *TFPI2* could be an effective methylation-based assay for ESCC diagnosis.

I would greatly appreciate could you consider its suitability for publication in Cancer Science.

The authors declare that the paper is being submitted for consideration for publication in Cancer Science, that the content has not been published or submitted for publication elsewhere. The Subject Category that applied to the manuscript: Genetics, Genomics, and Proteomics. All authors have contributed significantly to this work. Minghua Wang, Jiucun Wang, Li Jin, Yinghui Zhou, Shilin Li and Shicheng Guo contributed to the conception and design of the study. Chenji Wang, Dunmei Zhao, Zhenglei He and Xulong Feng contributed to the sample collection and DNA extraction, Ying Wang and Caihua Li conducted the targeted bisulfite sequencing experiments for the validation stage, Weilin Pu, Sidi Chen and Chenji Wang contributed to TCGA and GEO as well as the targeted bisulfite sequencing data analysis. Weilin Pu, Minghua Wang, Jiucun Wang and Shicheng Guo wrote the manuscript. All authors read and approved the final manuscript. All authors are in agreement with the content of the manuscript. Thank you very much for your consideration and help with handling the paper for us.

Sincerely,

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