Ulrich Mahlknecht, MD, PhD

Editor-in-Chief

Clinical Epigenetics

Jan 5, 2014

Dear Dr. Mahlknecht:

Thank you for your letter of Dec 13, 2013 regarding our manuscript entitled “Quantitative assessment of the diagnostic role of APC promoter methylation in non-small cell lung cancer”. Our appreciation also goes to the reviewers for their helpful comments. We have revised the manuscript following the reviewer’s comments and your instructions.

Enclosed please find the revised version of the manuscript along with a point by point description of our responses to the reviewer’s comments. We hope that the manuscript is now acceptable for publication in Clinical Epigenetics. Thank you again for your letter and for your editorial assistance.

Sincerely yours,

Jiucun Wang, Ph.D.

Professor,

School of Life Sciences

Fudan University

220 Handan Road, Shanghai 200433

People’s Republic of China.

Tel: (+) 86-21-55665499

Fax: (+) 86-21-55664885

E-mail: [jcwang@fudan.edu.cn](mailto:jcwang@fudan.edu.cn)

**Responses to Reviewer 1’s comments**

We first thank the reviewer for the helpful comments. In the revised manuscript, we have incorporated the reviewer’s comments.

**Comments**: “(Discretionary Revisions). This paper has conducted a comprehensive analysis of the APC gene and non-small cell lung cancer. Although the statistical analysis was appropriate, but we just could not understand one aspect. Recently, many studies indicated that the APC gene could be the maker of some cancers. How could we ensured the specificity”

**Response**: Thank you so much. It is really a good question. As you said, many evidences have shown the diagnosis role of DNA methylation in some cancers. Additional, evidences also showed that each type of cancer have its specific aberrant methylation profile, which means we can combine different panels of biomarkers to identify different kinds of cancers.

**Responses to Reviewer 2’s comments**

We first thank the reviewer for the helpful comments. In the revised manuscript, we have incorporated the reviewer’s comments.

(Minor Essential Revisions).This manuscript studied the relation between the APC promoter methylation and non-small cell lung cancer by using meta-analysis of previously published Manuscripts. The study was performed rigorously and the findings are interesting. In general, I'd recommend publication if the authors can address my concerns.

Minor Essential Revisions

**Comments:** The authors need to improve the quality of writing and presentation. It's

Sometimes, not clear what the authors were trying to say, for example,

a. "The proportions of the samples in stage I were counted and the ranges were32.1-100% and 70-100% respectively". What do the two ranges refer to?

b. In Table 1, column headers. What does "M+/M-" mean?

c. In Table 4, "P-values from t-test or Wilcoxon sum-rank test". It should be "rank-sum test" and it's not clear which p-values are from t-test and which are from Wilcoxon test.

**Response:**

1. Thanks for the reviewer’s comments. For each article, it has aproportion for its stage I samples. So for all the articles, the proportion will have a range. For example, as table 1 shows, the stage I proportion of the study of Zhang et al (2011,China) is 32.1%.
2. M+ and M-means methylation positive and methylation negative, respectively. I have noted this under the table 1.
3. You are quite right. We have revised “sum-rank” to “rank-sum” in the main body and Table 4. The choice of t-test or rank-sum test was determined by normal distribution of variable or not? T-test will be chose when the variable is normally distributed, or else rank-sum test will be chose. We have interpreted such problem in the manuscript.

**Comments:** Some spaces were omitted probably due to the document formatconversation. For example,

a. page 4: "17retrieved studies"

b. page 14: "Theestimate"

**Response:** We are sorry about that. It is caused by transfer between different Microsoft Words. Now we have checked all such situations and revise them.

**Comments**: Discretionary Revisions, It's not clear how the analysis of TCGA dataset helps to validate the meta-analysis findings. The authors simply listed numbers from the TCGA analysis without much discussion. A thoroughly discussion and attempts to

"Integrate" this analysis with the meta-analysis, as claimed by the authors in the background section, is desired.

**Response:**

Thanks you for your suggestion, we have added these discussion into the the discussion section. Actually, we have checked all the meta-result with the TCGA dataset. However, these results were scattered all over the result and discussion section, such as

1. TCGA’s T-test, logistic regression results were concordant with the meta-analysis.
2. ORs from logistic regression based on heterogeneous samples were significantly greater than those on the autogenous samples in the condition of Ad2Sc of 4:3, which was concordant with the meta-analysis

Additionally, in the discussion section, we have compared the result based on Meta- analysis and TCGA dataset, such as,

1. Integrated analysis showed that the age at the diagnosis, autogenous or heterogeneous control, the ratio of the adenocarcinoma to squamous cell carcinoma, and primer set of CpG sites were the most important heterogeneity sources, while sample type (tissue or serum), proportion of males, proportion of stage I, and detection methods could not explain the heterogeneity.
2. Age was one of the most important heterogeneity sources from meta-regression analysis (beta = -0.3, P = 2.0×10-5), meanwhile, the OR in the younger subgroup (OR = 4.65) was greater than that in older subgroup (OR = 2.24). However, TCGA NSCLC dataset demonstrated different results. Furthermore, neither Ad nor Sc data supported that age would affect the OR of the APC methylation to the risk of NSCLC in logistic regression model (P > 0.05). Briefly, much more evidence should be collected to make an eventual decision.
3. As to the contribution of Ad2Sc, both subgroup analysis and TCGA analysis showed significantly greater OR in high Ad2Sc than that in low Ad2Sc group, which suggested APC methylation test have better diagnosis performance for adenocarcinoma.