Dear editors of Journal of Cancer:

We are submitting the revised manuscript titled Aberrant methylation of CDH13 can be a diagnostic biomarker for lung adenocarcinoma for your consideration for publication as an article and giving a point-by-point respond to the concerns. The authors wish to acknowledge the anonymous reviewers for their detailed and helpful comments to the manuscript. In the following, a detailed reply to the general and specific comments by the anonymous reviewers are provided in which the reviewer’s comments are reported in normal style while the corresponding authors’ reply is reported in red color.

Response to Reviewer 1:

The diagnostic role of CDH13 methylation in lung cancer has already been studied and reported by other people: Zhong YH, Peng H, Cheng HZ, Wang P, Quantitative assessment of the diagnostic role of CDH13 promoter methylation in lung cancer, Asian Pac J Cancer Prev. 2015;16(3):1139-43. The work described in this manuscript is quite similar to the one published, where both of them performed meta-analysis with searching PubMed and Web of Science, got odds ratio around 6, and came to the same conclusion as the major found of the work. The only difference is that the work in this manuscript performed TCGA and GEO analysis to validate their conclusion. But this is not enough to make this work to be a different and novel research. So I recommend the authors to do more new studies in this filed and publish something is totally different from what is already reported.

Response

Thanks for your comments. We have carefully examined the paper titled as “Quantitative assessment of the diagnostic role of CDH13 promoter methylation in lung cancer”, and we found that though the main objective of this paper is similar with ours, there is a great distinction between the two papers.

Firstly, in our study, we combined the microarray data from GEO and TCGA database for validation. Obviously, nearly all the studies included in our research concerning the relationship between CDH13 promoter methylation and lung cancer are based on the MSP/qMSP method, while the high-throughput microarray technology implemented in GEO and TCGA data validate the conclusions with a different method, thus our conclusions are based on different methods, which makes the conclusions much more robust. And as shown from Figure 3 and Supplementary Figure4 and Supplementary Figure5, the methylation data from different micro-array datasets showed strong consistency and suggested that the high-throughput microarray data is reliable and essential for a robust conclusion. In addition, due to the strong correlation between gene methylation and gene expression, we also conducted the expression analysis with TCGA RNA-Seq analysis and found the expression profile is in accordance with the methylation data, which also strengthened our conclusions.

Secondly, in terms of the subgroup analysis, the published paper only conducted the subtypes of the race, histological type and sample source, while we have conducted the subtypes of age, stage, gender, method, object, histological type, race as well as the subtypes of lung cancer, which made our study much more comprehensive than the published one.

Most importantly, we found that the conclusions from the published paper is suspicious. They draw the conclusion that “the CDH13 methylation test could be a promising diagnostic biomarker which could be applied in the clinical diagnosis of lung adenocarcinoma with remote non-invasive media detection”. In fact, no subtype analysis has conducted concerning the difference between lung adenocarcinoma and lung squamous cell carcinoma which makes the conclusion unconvincing. However, we have conducted the subtype analysis as well as combining the dataset from TCGA and GEO and found the difference between lung adenocarcinoma and lung squamous cell carcinoma.

Due to the previous differences and advantages in our study, we think that our study is totally different from the published paper and our work and conclusions are based on a more solid basis and more reliable at the same time.

Response to Reviewer 2:  
The authors conducted a meta-analysis to comprehensively assess the association between the diagnostic ability of CDH13 methylation in NSCLC. Thirteen studies were identified by literature search. The major comparison is CDH13 promoter methylation between lung cancer and normal tissues. The major conclusion is that the methylation status of the CDH13 promoter is strongly associated with lung adenocarcinoma and could be a potential diagnostic biomarker. Meta-analysis is a well-established method. However, several concerns need to be addressed. Specific comments are listed below.

1. Meta-analysis should be conducted for independent studies. However, it is apparent that some of the studies collected and analyzed by the authors are not independent. For example, results from the study conducted by Hsu et al were used twice (tumor vs control plasma, tumor vs adjacent normal) in the meta-analysis. The two parts from the same study should not be simply treated as two independent studies.

Response

Thank you for the suggestion. Yes, you are right. Usually, non-independent studies should not be enrolled in the meta-analysis. However, as you known, we want to evaluate the diagnostic performance for CHD13 in solid tissue and plasma simultaneously. We have already separated them in the subset analysis (tissue and non-tissue) section in our previous version. In the current revision version, we re-analysis the data (Figure 1A) with only independent (only include tissue-based data) to make the conclusion more solid. All the corresponding changes were red labelled in the manuscript.

2. Have you assessed the quality of original publications? Do all publications included in this analysis have a good study design and use proper analytic methods?

Response

Yes, we assessed the quality of original publications with the rule of QUADAS, and we found all the papers have relatively high QUADAS scores with a mean score of 11.23. The detailed score information for each study is listed below. And the QUADAS questions are based on the following published paper:

Whiting P F, Weswood M E, Rutjes A W S, et al. Evaluation of QUADAS, a tool for the quality assessment of diagnostic accuracy studies[J]. BMC medical research methodology, 2006, 6(1): 1. We have added this information in the manuscript at line 292-293 in page 14.

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|  |  | **QUADAS Question** | | | | | | | | | | | | | |  |
| **Study** |  | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **Total Score** |
| Dong et al (2007, Korea) |  | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 11 |
| Feng et al (2008, USA) |  | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 12 |
| Hanabata et al (2004, Japan) |  | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 11 |
| Hsu et al (2007, Taiwan) |  | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 12 |
| Jin et al (2009, Japan) |  | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 12 |
| Nikolaidis et al (2012, UK) |  | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 11 |
| Toyooka et al(2001, USA) |  | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 11 |
| Toyooka et al(2003, USA) |  | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 11 |
| Tsou et al (2007, USA) |  | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 12 |
| Ulivi et al (2006, Italy) |  | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 10 |
| Wang et al (2008, China) |  | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 12 |
| Zhai et al (2014, China) |  | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 10 |
| Zhang et al (2011, China) |  | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 11 |

3. Not clear how the results were extracted from original study. For example, in the same study mentioned above, the association for tumor vs plasma is different between original study and this meta-analysis (i.e. OR=2.759 [0.652–11.669] in the original publication and OR=2.50[0.90-6.94] in the meta-analysis). Please clarify this inconsistency.

Response

Thank you for the carefully checking. Actually, in Hsu’s study, they investigated the diagnostic performance for 6 genes and they applied multiple logistic regression analysis. The OR for each gene was adjusted by other 5 genes. However, we only investigated the diagnostic effect of CDH13. That’s why a little difference is found between these two results. Hsu’s paper is Han‐Shui, et al. Characterization of a multiple epigenetic marker panel for lung cancer detection and risk assessment in plasma." Cancer 110.9 (2007): 2019-2026

4. In the abstract it says “Fifteen” studies were found by literature review but there are actually only 13 independent studies. To avoid confusion, it should be corrected.

Response

Thanks very much for your correction. We have correct them at line 33 in page 2 of the revised manuscript and replace ‘fifteen’ with ‘thirteen’.

5. In Table 1, the footnote says “b with two records since there are Tissue and serum data simultaneously in this article”, but no records were marked by “b”.

Response

Thanks very much for your correction. We have corrected this error in the table 1. We added this record to Hsu et alb ‘s and Zhang et alb ‘s studies.

6. The presentation of Figure 2-E and legends are bad. What does the circle represent? The best AUC cutoff point? Please attach a high quality ROC curve with specific footnotes.

Response

Thanks very much for your suggestion. We updated the Figure 2E with more explicit lines and dots. The dotted circle region represents the estimated 95% confidential regions for sensitivity and specificity based on whole individual studies. The standard deviations underlying confidence intervals for the sensitivities and false positive rates are used to determine the scale of the ellipses on logit-ROC space. These ellipses get back-transformed to ROC space and plotted. The confidence regions of sensitivity and specificity are ellipses on logit-ROC space to show the relationship between sensitivity, specificity and AUC at the same time so that the readers could be more clear about the performance about this biomarker. We have given a more detailed legends for Figure 2E, please check lines 528-530 in page 22

7. If paired samples were analyzed in the TCGA dataset, Wilcoxon signed-rank test should be used instead of Wilcoxon rank-sum test because of paired study design.

Response

Thanks for your correction. We have implemented this method and changed the P-values as well as the annotations in Table 3 and in the main text as well. Please check them in page 25