Reviewer's report

Title: Expression of CLDN1 and CLDN10 in lung adenocarcinoma in situ and invasive lepidic predominant adenocarcinoma

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Reviewer: Junichi Soh

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Zhang et al investigated the difference of gene expression level between adenocarcinoma in situ (AIS) and invasive lepidic predominant adenocarcinoma (LPA) using cDNA microarray and RT-PCR to find that CLDN1, CLDN10, MMP-2, and c-fos expression levels were associated with histological difference with prognostic significance. In addition, they also examined the impact of expression levels of these genes on prognosis in 81 additional lung adenocarcinomas. Although their findings look like interesting, the strategy of this study and the constitution of this manuscript are poor for publication.

Major Compulsory Revisions

1) How many operations for lung adenocarcinomas were performed in Tian Jin Medical University Hospital during 2000 to 2005? In this study, are all samples constitutively collected? If they are not constitutive, reviewer feels that it is very difficult to evaluate their prognosis correctly and that the authors should reveal how to select 41 and 81 samples for validation study. Reviewer is skeptical for the arbitrariness of this study without the detailed information of selection bias in this study.

2) It seems that the authors performed microarray for all 41 cases in validation cohort to find 550 significant genes relating histological difference, and they narrowed down 13 genes out of 550 genes by investigating the impact of the expression levels of each 550 genes on PFS. If this is correct, reviewer strongly recommends modifying the manuscript because it is very difficult for readers to understand these details, and revealing the meaning of the original cohort (Is the original cohort necessary?). Additionally, it is better to add a figure to show the flowchart of this study to select candidate genes.

3) They should show the list of all significant genes relating with the difference between AIS and LPA for the original (n = 20) and/or validation (n = 41) cohorts (Supplemental tables are suitable).

4) Please show the clinical features of AIS and LPA in all cohorts (Tables 1 and 2).

5) For 81 validated cases, it is very important to confirm the positive relationship between the expression levels of four significant genes and the histological features (AIS and LPA). Authors should show this point. Otherwise, it is inapplicable for further investigation. If there is positive relationship, the authors should investigate the impact of AIS or LPA features on the prognosis in 81 lung
adenocarcinoma patients of validation study.

6) In the “Microarray analysis” section, the authors should show the instrument to examine the OD of RNA.

7) In the “Quantitative real-time reverse transcription-PCR” section, the authors should show the sequences (or manufacture’s IDs) of Taqman probe and primer sets for 13 genes.

8) Table 2 should include the category of AIS or LPA.

9) Table 3A and 3B should be combined into one table. It is very difficult for readers to understand. Please explain more detailed of the P* value (Is this P value calculated after RT-PCR assay?).

Minor Essential Revisions
10) There are a lot of easy and grammatical errors. Intensive careful rereading should be performed by the authors, and the grammatical check by native English speaker is mandatory.

Discretionary Revisions
11) Table 1 is better to be sub-classified into AIS or LPA.

12) Please add a supplemental figure with representative examples of IHC for four genes.

**Level of interest:** An article of limited interest

**Quality of written English:** Not suitable for publication unless extensively edited

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests.