Author's response to reviews

Title: Suppression subtractive hybridization identified differentially expressed genes in lung adenocarcinoma: ERGIC3 as a novel lung cancer-related gene

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Author's response to reviews: see over
Subject: Manuscript “Suppression subtractive hybridization identified differentially expressed genes in lung adenocarcinoma: ERGIC3 as a novel lung cancer-related gene” (MS: 2134587857791898)

Dear Dr Cho,

I thank you for your letter from December 2, 2012. We appreciate the constructive criticism of reviewers, and have revised the text in line with the suggestions as follows:

Reviewer: 1

Specific comments:

1. The authors used two criteria to select the 16 genes for validation by RT-qPCR and the data were shown in Table 1. Two additional columns indicating whether the gene was reported previously and the main function of each gene shall be included in the Table 1. Accepted and revised. Two additional columns, “Main Functions” and “References”, have been added in the Table 1.

2. The figures shall be labeled in the order they appeared in the text. Therefore, figures 2 and 3 shall be re-labeled. Accepted and revised. The order of figure 2 and 3 has been changed.

3. ERGIC3 is an integral membrane protein cycling between the ER and Golgi. Such information and related previous publications shall be given and described in introduction section so the rationale to study subcellular localization ERGIC3 is supported in the Results section. Accepted and revised.

4. The nuclear stain signals in the representative immunohistochemistry figures were strong. The authors shall give explanation to this observation in the Discussion section. Accepted and revised. The antibody of ERGIC3 used in the study was the polyclonal ant-ERGIC3 serum, since at that time the monoclonal antibody to ERGIC3 was unavailable. This may be the reason that the non-specific staining appears. We have given an explanation to this observation in the Results section.
5. The Discussion sections shall be shortened by removing or shortening the descriptions that had given in Introduction and Results sections.

Accepted and revised. The duplicate contents that had been given in Introduction and Results sections, have been eliminated in the Discussion section.

Reviewer: 2

Major Compulsory Revisions

1. In discussion 5th paragraph: “We observed that the distribution of ERGIC3 was associated with the cellular shape. In the round cells, ERGIC3 was located around the nucleus, but it was at the side of the nucleus in the fusiform cells.” From the results Subcellular localization of ERGIC3 protein in cultured cells: “ERGIC3 was distributed at the side of nucleus in EPLC-32M1, 801D, and NCI-H446 cells, but uniformly present around nucleus in SPCA-1, GLC-82 and A549 cells”. The correlation between ERGIC3 distribution and cell shape is interesting, I would like to see a discussion of ERGIC3 distribution with respect to tumor source since these round cells (SPCA-1, GLC-82 and A549) were derived from adenocarcinomas, the fusiform cells (EPLC-32M1) were derived from SCC. Accepted. We also noticed that there was the correlation between the cell shape and tumor source. These round cells (SPCA-1, GLC-82 and A549) were derived from adenocarcinomas, and the fusiform cells, EPLC-32M1, 801D, and NCI-H446, were respectively derived from SCC, large cell carcinoma, and small cell lung carcinoma. Because only one cell line of the each type (SCC, large cell carcinoma, and small cell lung carcinoma) was used in our study, we did not discuss this issue. Of cause, the interesting issue will be further studied in our laboratory.

Minor Essential Revisions:

1. Abstract, methods: period should be put at the end of the 1st sentence. Accepted and revised.

2. Background, 1st paragraph: the global 5-survival rate should be the global five-year survival rate. Accepted and revised.

3. Methods, Construction of the subtractive cDNA library: The information of the tissue for SSH library may be given to understand the differentiation state. Accepted and revised. The phrase “well-differentiated lung adenocarcinoma” has been added in the “Construction of the subtractive cDNA library”.

4. Results Fig 3 and Fig 2: Figures should be put in a logical order. Accepted and revised.

5. How far away from the cancer tissue was the adjacent nonmalignant lung tissue? 5 cm (Methods: Patients and tissue samples) or 10 cm (Methods: Construction of the
The adjacent nonmalignant lung tissue used for construction of the subtractive cDNA library was away from the cancer tissue at least 10 cm, and all the rest were at least 5 cm.

6. Results, Fig 1B: the protein expression of ERGIC3 was increased in three lung cancer cell lines, how about the protein expression in the other three cell lines?

The mRNA levels of ERGIC3 were increased in all 6 lung cancer cell lines (SPCA-1, GLC-82, EPLC-32M1, NCI-H446, A549 and 801D) by q-RT-PC. The protein expression of ERGIC3 was also increased in three lung cancer cell lines (SPCA-1, GLC-82, and EPLC-32M) by western blot. Among the other three cell lines (NCI-H446, A549 and 801D, the protein expression of ERGIC3 was also increased by immunofluorescence stain.

7. Figure legends, Figure 3: culreticulin should be calreticulin.

Accepted and revised.

Discretionary Revisions

1. As a housekeeping gene, #-actin has more variation than GAPDH in tumor tissue, further work may consider it useful to switch to a more stable reference gene.

Accepted. Many thanks! We will use GAPDH as a reference gene in the future.

As a result of the comments of the reviewers, we have included some of the above considerations into the revised manuscript. We thank the reviewers for their comments and hope that our manuscript now will meet the requirements for publication in the BMC Cancer.

Yours sincerely,

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