Reviewer's report

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

Version: 2 Date: 28 November 2012

Reviewer: Qunfeng Cai

Reviewer's report:

General

The authors have applied suppression subtractive hybridization to the study of lung cancer. Two subtractive libraries were generated: a forward-subtracted library (FSL) and a reverse-subtracted library (RSL). Two cDNA libraries of differentially expressed genes were constructed using lung adenocarcinoma tissue and adjacent nonmalignant lung tissue. The authors found 177 and 59 genes in each library after subtraction. Sixteen genes were selected for further screening, two novel lung cancer-related genes (ERGIC3 and LPCAT1) were found. ERGIC3 is related to lung cancer proliferation and migration and may be a potential biomarker for lung cancer. The study aims were clearly defined; the methods were appropriate. There are a number of points that require clarification or further work.

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.

Minor Essential Revisions

1. Abstract, methods: period should be put at the end of the 1st sentence
2. Background, 1st paragraph: the global 5-survival rate should be the global five-year survival rate
3. Methods, Construction of the subtractive cDNA library: The information of the tissue for SSH library may be given to understand the differentiation state
4. Results Fig 3 and Fig 2: Figures should be put in a logical order
5. How far away from the cancer tissue was the adjacent nonmalignant lung
tissue? 5cm (Methods: Patients and tissue samples) or 10cm (Methods: Construction of the subtractive cDNA library)

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 line?

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

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

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

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

Declaration of competing interests:

I declare that I have no competing interests'