Reviewer's report

Title: Smoking Status Impacts microRNA Mediated Prognosis and Lung Adenocarcinoma Biology

Version: 3 Date: 13 August 2014

Reviewer: Jacek Jassem

Reviewer's report:

The study aims are to identify miRNAs that specifically drive lung carcinogenesis in smokers and never-smokers, and to define prognostic relevance of these alterations.

Comments

1. Analysis of prognostic significance may be seriously biased owing to small number of patients and heterogeneity of the population.

2. The abstract is misleading, as it suggests that 188 pairs of lung adenocarcinoma and normal lung samples were analyzed, whereas in fact there were only 94 pairs.

3. MiRNA expression profiles cluster based on malignancy and smoking histories: malignant and normal lung clustered separately. Within malignant samples and normal lung, 3 clusters were found to correlate with smoking status. As it is impossible to access the supplementary files, it is unclear whether these clusters are based on the same miRNAs in both malignancy and normal lung.

4. There are a few “irreversible” expression changes found in former smokers – those that remain and are found also in cancer. The authors claim that these alterations are important in carcinogenesis. However, they may just be passenger changes that simply persist in tumor formation and through progression. Needs more precise phrasing.

5. The authors found that (83%) of miRNAs are upregulated in tumors vs. normal lung, irrespective of smoking status. This is surprising given the biological function of miRNAs – one might expect to find downregulation of microRNAs in tumors vs. normal lung. Indeed, most studies so far have shown the opposite – this necessitates interpretation. The authors do not describe the normalization procedures used for miRNA expression. They only conclude that it was ensured pathologically for 80% of cancer or lung tissue contents. How much of RNA was added for library construction? The same amount of RNA or the same volume of RNA that was of variable concentrations? Other studies suggest that normal lung tissue yields much smaller concentrations of RNA. This could explain why the authors found 85% of miRNAs overexpressed in cancer vs. normal lung, and put in doubt the study results.

6. Were microRNAs in general underexpressed in smokers vs. non smokers within tumors and within normal lung specimens?
7. One needs to be careful in defining current smoker status. Long-lasting smokers diagnosed with lung cancer tend to quit smoking shortly before surgery. Hence, those who smoked until a few days before surgery would be truly current smokers (and microRNAs related to detoxification processes would be activated in this groups), in contrast to patients who quit more than a month before surgery (who would be expected to exhibit irreversible epigenetic changes in their respiratory epithelium). This is the major concern, as the paper’s main ambition is to trace the effects of smoking on epigenetics of lung cancer.

8. Almost 60% of never smokers were Asians. The race likely influences the molecular pathways of carcinogenesis, therefore this factor adds to the heterogeneity of the population and should be addressed.

9. Stage II, III and IV patients constituted 40% of the population. As the stage is the most powerful prognostic factor, the prognostic value of miRNAs must be analyzed in multivariate analysis including stage. It is possible that most of the miRNAs claimed as prognostic are in fact correlated with stage (nodal involvement). Without such information the prognostic aspects of this paper seems completely spurious. Finally, it is important to analyze additionally metastasis free survival, as this end-point depends on tumor aggressiveness, whereas overall survival in lung cancer patients is strongly affected by random factors and competing risks (COPD, vascular diseases, etc).

10. The miRNAs that might be considered as the most relevant to different paths of carcinogenesis in smokers and non-smokers may be: (i) different between tumors of smokers and non-smokers, and (ii) not different between normal lung of smokers and non-smokers. Such analysis is lacking.

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

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I have no conflict of interest