Reviewer's report

Title: Effects of Retinoic acid on compensatory lung growth

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Reviewer: Matthias Ochs

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In the present paper, Karapolat et al. report on the effects of retinoic acid (RA) in a rat model of post-pneumonectomy compensatory lung growth. Three Groups (A: left posterolateral thoracotomy; B: left posterolateral thoracotomy and left pneumonectomy; C: left thoracotomy, left pneumonectomy, RA treatment) were evaluated 10 days after surgery.

The scientific question is clear, interesting and relevant. However, it is of outmost importance that the data are comprehensive and valid enough to justify any conclusions and suggestions drawn in the present study. This is not the case. There are serious problems concerning the methods used.

Major comments:


1a) Any microscopy-based quantitation of lung structure depends crucially on the fixation and processing protocol. The lungs were fixed in formalin (How? Instillation, perfusion or immersion?) and embedded in paraffin although this is known to result in considerable and variable shrinkage. Controlled fixation with glutaraldehyde and plastic embedding (e.g. in glycol methacrylate) would have been preferable. Thus, in the present study tissue dimensions were not preserved well enough to allow for reliable quantitation.

1b) Any microscopy-based quantitation of lung structure also depends crucially on the sampling protocol. A rat lung contains about 20 million alveoli (see Hyde DM et al. Anat Rec 2004:274:216-226), so the sampling has to be systematic uniform random to ensure that all parts of the lung had the same chance of being analyzed. Otherwise the data are meaningless.

1c) The authors claim that there is a decrease in "mean number of alveoli" in Groups B and C, and an increase in "the mean dimension and average wall thickness" of alveoli in Group C. Unfortunately, these parameters cannot be determined the way the authors did. Their approach violates basic rules of quantitative morphology. For simple geometric reasons, the number of particle
profiles per area (here: alveolar profiles per field of view of a histologic section) is not directly related to particle number per volume (alveoli per lung). Moreover, these data are densities, not totals, and are therefore subject to the so-called "reference trap". Therefore, the data on alveolar number are meaningless. What is meant by "alveolus dimension" with values between 1.7 and 4 µm? Again, for simple geometric reasons, the appearance of a barrier in a thin histologic section cannot be taken directly as barrier thickness.


There are excellent morphometric studies on compensatory lung growth (e.g. by C. Hsia or by H. Fehrenbach). Compared to these studies, the shortcomings of the present manuscript are obvious. I would strongly suggest that the authors consult the relevant literature (see above) and reconsider their approach.

2) The manuscript needs to be edited by a native speaker.

3) The light micrographs are of poor quality and lack scale bars.

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

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

**Statistical review:** No, the manuscript does not need to be seen by a statistician.