Author's response to reviews

Title: Hepatocyte and Keratinocyte growth factors and their receptors in human lung emphysema

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Version: 2 Date: 12 August 2005

Author's response to reviews: see over
Reviewer's report 1

Title: Hepatocyte and Keratinocyte growth factors and their receptors are not decreased in human lung emphysema

Version: 1 Date: 22 July 2005

Reviewer's report:

General

In this carefully done human study, the authors have measured levels of KGF and HGF mRNA and protein in lung samples from patients with emphysema compared to controls without emphysema. Although the study is highly descriptive, it does provide new information on levels of tissue expression of KGF and HGF protein and mRNA in emphysematous lungs compared to non-emphysematous controls. Contrary to the authors' hypothesis, levels of HGF and KGF were not different between controls and emphysema patients although levels of HGF protein and mRNA did have some correlation with the degree of emphysema as measured by pulmonary function tests. Although this study is of limited interest, it is methodologically sound. The major limitation of the study is the use of lung homogenates for the entire study. Future studies should address the expression of HGF and KGF in a more cell specific fashion.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. It would be helpful to include a more thorough discussion of the pathophysiologic significance of the correlation of high levels of HGF with more severe emphysema. The authors suggest that preserved expression of HGF-R suggests a possible role for HGF or other growth factor therapy.
2. Do the authors postulate that the higher HGF levels observed in more severe emphysema are helpful or harmful?
3. A more thorough discussion of the limitations would also be useful including the limitation of the analysis only of lung homogenates.

Discretionary Revisions (which the author can choose to ignore)

What next?: Accept after minor essential revisions

Level of interest: An article of limited interest

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:
I declare that I have no competing interests
Reviewer's report 2
Title: Hepatocyte and Keratinocyte growth factors and their receptors are not decreased in human lung emphysema
Version: 1 Date: 31 July 2005
Reviewer's report:
General
This paper investigates if the expression of HGF and/or KGF (and their receptors) in the lungs of patients with emphysema is different from that seen in smokers with normal lung function or never-smokers. Results show that, on average, there are not significant differences between groups. However, the authors found a significant negative relationship between the expression of HGF and the degree of airflow obstruction (FEV1) in patients with emphysema. Overall, I found the paper well written and interesting. However, I think that the authors should improve the following aspects:

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. Authors conclude that lung emphysema in humans is NOT associated with a global decrease in the expression of HGF or KGF in the lungs. However, they also report an inverse relationship between FEV1 and the expression of HGF, which suggest the contrary. I think that they need to reconcile these two observations. To this end, I would suggest that: (1) they include in the correlation analysis data from smokers without emphysema; (2) they clarify the “definition” of emphysema. It is unclear if this diagnosis was done on the basis of lung function test, CT scan results or both, or other criteria. Also, it would be very interesting to try to quantify the degree of emphysema present (and to include in the analysis other lung function measurements, such as DLCO) to see if there is any relationship between it and the expression of HGF and KGF. In summary, I think that the authors have to re-think very carefully the interpretation of their results.

2. Authors claim that patients with emphysema had severe airflow obstruction. However, it is obvious from the individual data points shown in Figure 3 that this was not the case in all of them. Actually, it is surprising that some patients with emphysema had normal or only minimally reduced FEV1 values. This should be explained and, probably, has something to do with the point I raised above (interpretation of data).

3. Page 6. Two patients without emphysema were receiving inhaled steroids. Were they asthmatics? Can this bias the results? What if they are excluded from analysis?

4. I found the discussion section too short. For instance, potential limitations of the study are not discussed and I believe they should (small sample size, presence of cancer and, among all, criteria used to classify patients). Above it, however, I think that the authors should resolve the apparent internal contradiction indicated above in point 1, this is, whether HGF expression is abnormal or not in patients with emphysema (mean values being not different but authors showing very convincing negative relationship with FEV1). The conclusion of the paper would then be completely opposite to the current one.

5. Figure 3 needs substantial work-up. First, I would suggest to exchange X-Y axis. I believe that, for a respiratory audience, it is more intuitive to have FEV1 values on the X axis. Second, if the correlation test used is, as indicated, the Spearman’s rank order test, “r” should be substituted by “Rho” and the line in the figure omitted (it is not a “linear” regression test). Third, and more importantly, I suggest to include in the analysis data on smokers without emphysema (using a different symbol). I think that this may help to resolve the item indicated on points 1 and 4.
Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. Ref 18 on page 3 relates to pulmonary fibrosis, not to emphysema
2. Page 5. Last sentence “Increased cumulative …” Unclear meaning. Do you mean that there were significant differences between groups. Please, clarify.
3. Table 1. The expression “non relevant” should not be included here. Why is it “non relevant” to know, for instance, the period since smoking cessation in non-emphysema smokers (while it is in patients with emphysema)?

Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable
Statistical review: No
Declaration of competing interests: I declare that I have no competing interests
Answers to Reviewers‘ comments

We thank the Reviewers for their comments and have modified the manuscript accordingly.

Reviewer 1:

General comment

We thank the Reviewer for his positive comments and agree that future studies should address the expression of HGF and KGF in a more cell specific fashion.

Minor essential revisions

1. As suggested by the Reviewer, we modified the discussion section of the revised manuscript.

2. Our results suggest that lung emphysema in humans is not associated with a global decrease in the expression of HGF, KGF and their receptors. As HGF is a key factor in the process of alveolar repair (Ware LB, Matthay MA. Am J Physiol Lung Cell Mol Physiol 2002, 282), we hypothesized that HGF production could be helpful in human lung emphysema. In our hypothesis, HGF production might be not adapted to the degree of alveolar injury. Moreover, in animal models of lung emphysema HGF has been shown to stimulate pulmonary regeneration and to improve pulmonary function (Ishizawa K, Kubo H, et al. Biochem Biophys Res Commun 2004, 324; Shigemura N, Sawa Y, et al. Circulation 2005, 111).

3. We agree with the Reviewer that this study has some methodological limitations and added a paragraph in the discussion section: “This study has some methodological limitations. A limited number of patients was studied in each group, especially in non emphysema groups which were mostly composed of lung biopsies obtained at a site distant from localized carcinoma. Furthermore, the patients could be only evaluated at one time point of the course of their disease.
Inclusion of smokers without emphysema allowed to differentiate emphysema-related and tobacco-related events. Because only one tissue sample from surgically resected material was available for examination, the expression of HGF, KGF and their receptors reflects regional disease activity and may be unrepresentative of the entire lung. Indeed, it is well admitted that emphysema affects different lung regions to a varying extent. Moreover, we evaluated HGF and KGF only in lung homogenates. Future studies should address the expression of HGF and KGF in a more cell specific fashion.”

**Reviewer 2:**

We thank the Reviewer for his very contributing comments. Particularly, the analysis of correlations in smokers allowed us to suggest that HGF expression was correlated with airflow obstruction, and to reconcile the apparent internal contradiction of our results.

**Major Compulsory Revisions**

1. (1) As suggested by the Reviewer, we included in the correlation analysis, data from smokers without emphysema. Again, significant correlations between HGF mRNA and FEV1 (Rho= -0.53, p=0.009, n=25), and FEV1/FVC (Rho= -0.49, p=0.017, n=25) were found. However, the correlation between HGF mRNA and TLC was no more significant (Rho= 0.28, p=0.18, n=24).

(2) - We clarify emphysema definition and description of emphysema patients in the revised manuscript. In all patients, lung emphysema was suspected on CT-scan and confirmed by the pathological examination of lung resection samples. The methods section was modified accordingly.

- We agree that it would be very interesting to quantify the degree of emphysema present and to assess the relationship between it and the expression of HGF and KGF. However,
emphysema quantification was not possible retrospectively on CT-scan, since these exams were not performed with standardized procedures. Moreover, pathological quantification was not possible on frozen samples. Therefore, the severity of emphysema was approached through pulmonary function abnormalities. The methods section was modified accordingly.

As suggested by the Reviewer, we searched for a correlation with DLCO. However, DLCO was available for 10 patients only (7 emphysema patients had no DLCO measure and no smoker patient was evaluated). We did not find a statistical correlation between HGF mRNA and DLCO (Rho=0.055, p=0.87, n=10).

2. We apologize for the typographical mistake. Indeed, in the methods section, it should be read “mild to severe” obstruction. This was corrected in the revised manuscript.

When analysing individual data points from figure 3, we agree that 2 patients evidenced FEV1 > 80% predicted value. Careful examination confirmed that these 2 patients had mild and heterogeneous lung emphysema as assessed by CT-scan and confirmed by histological examination. Therefore, these patients were included in the study as mild emphysema.

3. Smoker patients without evidence of emphysema had mild to moderate alterations of pulmonary function test (table 2). Two smoker patients without emphysema received inhaled corticosteroids for COPD at the time of inclusion. None had asthma. The exclusion of the data from these 2 patients did not modify statistical analysis.

4. First point: Limitations of the study

We agree with the Reviewer that this study has some methodological limitations and added a paragraph in the discussion section: “This study has some methodological limitations. A
limited number of patients was studied in each group, especially in non emphysema groups which were mostly composed of lung biopsies obtained at a site distant from localized carcinoma. Furthermore, the patients could be only evaluated at one time point of the course of their disease. Inclusion of smokers without emphysema allowed to differentiate emphysema-related and tobacco-related events. Because only one tissue sample from surgically resected material was available for examination, the expression of HGF, KGF and their receptors reflects regional disease activity and may be unrepresentative of the entire lung. Indeed, it is well admitted that emphysema affects different lung regions to a varying extent. Moreover, we evaluated HGF and KGF only in lung homogenates. Future studies should address the expression of HGF and KGF in a more cell specific fashion.”

We agree with the Reviewer that the small number of patients is a limitation of our study. To our knowledge, lung HGF and KGF expression has never been studied in human emphysema. We consider that our work is a pilot study. Other pathophysiological studies concerning mRNA expression in the emphysema lung have included a number of patients very similar to our study (for example, 22 patients in the study of Kasahara Y, Tuder RM et al. Am J Respir Crit Care Med 2001, 163).

Second point: Apparent internal contradiction

We agree with the Reviewer that the absence of difference of lung HGF expression between emphysema and non emphysema patients and the correlation of HGF with airflow obstruction and lung distension in emphysema patients have to be reconciled. We added in the discussion section: “The correlation between airflow obstruction and HGF mRNA level was similarly observed when all smokers with or without emphysema were studied, suggesting that emphysema was not a main determinant of HGF mRNA level in the lung. This strong correlation between airflow obstruction and HGF mRNA in smokers suggests that the increase of HGF
mRNA was not related to the presence of emphysema but rather to the degree of airflow obstruction. This observation is supported by the correlation between HGF protein in lung homogenates and the FEV1/FVC ratio in our population. These results are in agreement with the observations of Sauleda et al., who have reported that HGF protein concentrations were increased in broncho-alveolar lavage of patients with chronic obstructive pulmonary disease as compared with smokers and non-smokers controls (Sauleda J, Noguera A et al. Eur Respir J 2004, 24 (suppl):320s). Interestingly, the increased lung expression of other growth factors (fibroblast growth factors 1 and 2 and their receptors) has already been reported in chronic obstructive pulmonary disease (Kranenburg AR, De Boer WI et al. Am J Respir Cell Mol Biol 2002, 27)."

At this time, we have no data to explain the correlation between airflow obstruction and HGF mRNA in smokers. However, one may speculate different explanations. We added in the discussion section: “The mechanisms underlying the correlation between airflow obstruction and HGF mRNA in smokers are unclear. Although speculative, we can propose that the mechanical constraints applied to alveolar tissue secondary to airflow obstruction may stimulate HGF production by alveolar epithelial cells, since Yamamoto et al showed that mechanical stretch induced HGF in alveolar type II cells in vitro (Yamamoto H, Teramoto H et al. Respir Physiol 2001, 127). Furthermore, airway inflammation could contribute to increase local HGF expression by neutrophils (Grenier A, Chollet-Martin S et al. Blood 2002, 99) and macrophages (Morimoto K, Amano H et al. Am J Respir Cell Mol Biol. 2001,24). Interestingly, Aharinejad et al. have shown that serum HGF concentrations increased at the time of lung graft rejection, a situation associated with airflow obstruction (Aharinejad S, Taghavi S, et al. Lancet 2004, 363”).

5. Figure 3 has been modified as suggested by the Reviewer.
Minor essential revisions

1. We have deleted the reference 18 which was not related to emphysema.

2. We have clarified this sentence (page 6 of the revised manuscript): “Increased cumulative tobacco exposure was observed in E group as compared with S group”.

3. Table 1 has been modified in agreement with Reviewer’s comment.

Other changes:

1. In view of the modifications of the manuscript, we suggest to slightly modify the title.

2. Moreover, we detected a dramatic omission in the list of authors. Michel Fournier also contributed to this study.