Author's response to reviews

Title: Prognostic Effect of Matrix Metalloproteinase-9 in Patients with Resected Non Small Cell Lung Cancer

Authors:

Chang Young Lee (cyleecs@yuhs.ac)
Hyo Sup Shim (SHIMHS@yuhs.ac)
Seokkee Lee (cslsk8@naver.com)
Jin Gu Lee (csiglee@yuhs.ac)
Dae Joon Kim (kdjcool@yuhs.ac)
Kyung Young Chung (kychu@yuhs.ac)

Version: 4
Date: 9 January 2015

Author's response to reviews: see over
Dear Editor-in-Chief:

We deeply appreciate your considerate review and comments on our manuscript. We revised our manuscript based on your kind suggestions.

Many researchers are interested in the prognostic value of tumor matrix metalloproteinase-9 (MMP-9) expression for non-small cell lung cancer (NSCLC). But, the prognostic impact of immunohistochemical staining of tumor MMP-9 expression in operable NSCLC is controversial, furthermore there is no common standard for defining positive tumor MMP-9 expression. In the present study, tumor MMP-9 positivity was not determined by a scoring system but by the presence or absence of tumor MMP-9 expression and tumor MMP-9 expression was an independent poor prognostic factor for the relapse of lung adenocarcinoma. We wish this study would be helpful in understanding the association. Your kind consideration of this paper for publication in ‘JOURNAL OF CARDIOTHORACIC SURGERY’ would be greatly appreciated.

Thank you for your time and consideration.

Respectfully,

Kyung Young Chung, M.D.
Department of Thoracic Surgery, Yonsei University College of Medicine
250 Seongsanno Seodaemun-Gu Seoul, Korea (CPO box 8044)
Phone: 82-2-2228-2140
Fax: 82-2-393-6012
E-mail: kychu@yuhs.ac
Reviewer 1 comments:

The only concern that this reviewer raises is that a negative IgG control should be provided. This will validate the expression signals of MMP-9 obtained with MMP-9 antibodies used in the study were specific (Figure 1).

: Thank you for your comments. We added a negative control in Figure 1 and briefly described in methods section (methods page 4, line 13).

We performed immunohistochemistry without the primary antibody as negative control. The result was negative (Supplementary Figure). Because, as shown in Figure 1, the negative or positive for MMP9 was clear in tumor, we thought its expression was specific in positive cases.

![Figure 1 Immunohistochemical analyses of NS CLC representing different expression levels for tumor MMP 9.](image)

(A) no tumor MMP-9 expression
(B) tumor MMP-9 expression
(C) negative control
Reviewer 2 comments:

1) The title of the study is misleading - the study was really an evaluation of MMP-9 expression and prognosis in surgically treated lung cancer. (Major revision)

: Thank you for your comments. According to your suggestion, we corrected the title: "Prognostic Effect of Matrix Metalloproteinase-9 in Patients with Resected Non Small Cell Lung Cancer" (title page 1)

2) In the Methods section the authors state that patients underwent abdominal sonography as part of the preoperative evaluation - this is unusual. Please explain.

: We agree with your comment. In our hospital, abdominal sonography had been performed for preoperative and postoperative evaluation, but since 2007, ¹⁸FDG-PET has been used instead of abdominal sonography (methods page 4, line 19).

3) Also in the methods the authors should state clearly that staining for MM9 was done retrospectively and with IRB approval for the study and review of clinical outcomes. (Major revision)

: We described the IRB approval in methods section (methods page 4, line 8): “This study was approved by the Institutional Review Board of the Yonsei University College of Medicine. The IRB waived the requirement of individual patient consent because the analysis was retrospective in nature.”

4) In the Methods the authors stage that the patient’s characteristics are listed in Table 1 - this should be moved to the Results (Minor Revision)

: Thank you very much for your kind suggestion. According to your comment, this was moved to the Results (results page 6, line 20).
5) In the Results the authors do not state whether patients with stage II or III disease received adjuvant therapy - chemotherapy or radiation (Major Revision)

: Thank you for your comment. According to your comment, we showed the adjuvant therapy in Table 1 and Table 4. We also added a description in results section for whether patients with stage II or III disease received adjuvant therapy (results page 7, line 3).

In this study, the patients of tumor MMP-9 expression had more advanced stage ($p = 0.001$), therefore, they received more adjuvant therapy ($p=0.000$). However, there was no difference in adjuvant therapy for patients with stage II or IIIA (72.4% vs. 71.4%; $p = 0.879$) and there was no statistical difference in adjuvant therapy for histological type.

<table>
<thead>
<tr>
<th>Tumor MMP-9</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Postop. Tx. (I, II, IIIa)</strong></td>
<td>0.000</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>73 (28.5)</td>
</tr>
<tr>
<td>Radiation therapy</td>
<td>7 (2.7)</td>
</tr>
<tr>
<td>Combination therapy</td>
<td>19 (7.4)</td>
</tr>
<tr>
<td>No treatment</td>
<td>157 (61.3)</td>
</tr>
<tr>
<td><strong>Postop. Tx. (II, IIIa)</strong></td>
<td>0.666</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>49 (50.0)</td>
</tr>
<tr>
<td>Radiation therapy</td>
<td>5 (5.1)</td>
</tr>
<tr>
<td>Combination therapy</td>
<td>17 (17.3)</td>
</tr>
<tr>
<td>No treatment</td>
<td>27 (27.6)</td>
</tr>
<tr>
<td><strong>Postop. Tx. (II, IIIa) in sq</strong></td>
<td>0.879</td>
</tr>
<tr>
<td>Received</td>
<td>71 (72.4)</td>
</tr>
<tr>
<td>No treatment</td>
<td>27 (27.6)</td>
</tr>
<tr>
<td><strong>Postop. Tx. (II, IIIa) in ade</strong></td>
<td>0.826</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>33 (50.8)</td>
</tr>
<tr>
<td>Radiation therapy</td>
<td>5 (7.7)</td>
</tr>
<tr>
<td>Combination therapy</td>
<td>10 (15.4)</td>
</tr>
<tr>
<td>No treatment</td>
<td>17 (26.2)</td>
</tr>
</tbody>
</table>

MMP-9: matrix metalloproteinase-9; Postop. Tx.: postoperative treatment; Combination therapy: Chemotherapy + Radiation therapy; sq: squamous cell carcinoma; ade: adenocarcinoma.
6) In the Discussion the authors need to give more reasons or evidence to support why they chose to use median IHC score to define MMP-9 tumor expression presence or absence.

: Thank you for your comment. As in previous studies, there were many attempts to define positive tumor MMP-9 expression in NSCLC, such as intensity of staining, median value of staining, and scoring system [26]. In this study, we used IHC score (score = intensity * percentage, range 0 ~ 300), and the median IHC score (IHC score=10) was used initially as the cut-off value. By the way, an IHC staining score of 10 was the minimum score for staining of tumor MMP-9 expression, therefore there was no score below of 10. We described “presence or absence of tumor MMP-9 expression”, that literally means the presence or absence of tumor MMP-9 expression. The important point of our study was that tumor MMP-9 positivity was not determined by a scoring system but by the presence or absence of tumor MMP-9 expression.

7) In the Discussion the authors should justify why they performed a subset analysis of just the adenocarcinoma patients - to the reader it appears they noticed post hoc that more adenocarcinoma patients had MMP-9 expression and decided at that point to just look at the adeno patients. Why would adenoca patients have different levels of MMP-9 expression than squamous cell patients of the same stage?

: Thank you for your comments. Unfortunately, we also don’t know what causes more frequent MMP-9 expression in adenocarcinoma patients. As previous studies showed, they had some difference in level of MMP-9 expression by histological type, but there was no statistical difference [12, 14, 27, 30-32]. And in some of the studies, MMP-9 expression was analyzed in adenocarcinoma [24, 28, 29, 33]. In this study, the group with tumor MMP-9 expression had a higher proportion of patients with adenocarcinoma histology (40.2% vs. 61.5%; p = 0.001). As stated previously, the reason was the different in determination of tumor MMP-9 positivity from other studies. So, more studies are needed to assess MMP-9 expression in adenocarcinoma.

8) In the Conclusions the statement that MMP-9 expression is an independent poor
prognostic factor for relapse in lung adenocarcinoma is too strong given the level of evidence.

: Thank you. We corrected this as suggested (conclusions page 11, line 16): “Moreover, in
this study, Cox regression analysis revealed that tumor MMP-9 expression was an
independent poor prognostic factor for the relapse of lung adenocarcinoma. Thus, more
studies will be needed to confirm this, and furthermore, IHC staining to distinguish tumor
MMP-9 expression may be useful to predict clinical outcomes after surgical resection of lung
adenocarcinoma.”