Author's response to reviews

Title: Clinical and experimental studies regarding the expression and diagnostic value of carcinoembryonic antigen-related cell adhesion molecule 1 in non-small-cell lung cancer

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Version: 3 Date: 3 June 2013

Author's response to reviews: see over
Reviewer’s report
Title: Experimental and clinical experimental studies on carcinoembryonic antigen-related cell adhesion molecule 1 in non-small-cell lung cancer: how does CEACAM1 manifest
Version: 2 Date: 25 March 2013
Reviewer: Robert Kammerer
Reviewer’s report:
The paper by Zhou et al. is dealing with a very interesting issue. The authors have analyzed the amount of CEACAM1 in the serum of NSCLC patients and healthy controls, in order to estimate the impact of serum CEACAM1 levels as a tumor marker. This is of particular importance since CEA and NSE, serum markers currently used, are of limited value. The authors demonstrate that serum CEACAM1 levels can differentiate between cancer patients and healthy controls. Surprisingly the CEACAM1 levels were more pronounced during early disease than at tumor stage III and IV, respectively. Remarkably, mRNA levels in tumor tissues were not significantly higher in tumor tissues than in adjacent tumor free lung tissues. However, there was a negative correlation between CEACAM1 mRNA level and extension of tumor invasion. Finally the authors describe the expression of CEACAM1 short and long isoforms in tumor and normal tissues. Interestingly, they found that in tumor tissues the short isoform is predominantly expressed while in normal lung tissues the long isoform dominates. This is an interesting paper, however the mechanism underlying their observations were not addressed. This limits the impact of the paper. In addition, there are some result that are not sufficiently discussed in the present form of the paper.

Major Compulsory Revisions

Reviewer1 main point
1. The comparison between CEACAM1 as a serum tumor marker with CEA and NSE is problematic, since sensitivity and specificity strongly dependent on the cut off level and also on each other, therefore sensitivity should be compared at similar specificity levels and vice versa. This has to be corrected. (Supp. Table 2 and page 4 “CEACAM1 serum levels” last paragraph.)

Main point response:
1. First, we would like to thank professor Kammerer for his positive and constructive comments. We made Supplementary Table 2 according to Cioffi, M [1]. It is true, as reviewer 1 suggested that the sensitivity and specificity are strongly dependent on the cut off level and each other. Considering the reviewer’s suggestion, we amended the Figure 1B and added the receiver operating characteristic (ROC) curves for CEA and NSE to account for the sensitivity and specificity at the same time. As the ROC curve is a graph of the sensitivity (y-axis) versus 1 – specificity (x-axis), it is often used to determine a cut off value for clinical tests [2-3]. Our ROC curve may provide another perspective. Moreover, the “CEACAM1 serum levels” section in the Results was rewritten, and relevant changes were made in the related parts of our revised manuscript (mainly on Page 7-8, marked in yellow).
2. Another problem of the study is that there is no information of the tissue included into the study. Is the CEACAM1 expression of the sample relevant to the complete tumor? Why are the S-L ratios so different within the normal tumor samples? Inclusion of immunohistological data would improve the paper.

Another problem of the study is that there is no information of the tissue included into the study.

Main point response:
1. We are thankful for professor Kammerer's comments. We apologise for our carelessness in not mentioning Supplementary Table 1, which summarises the clinical and pathological details of the patients described in the Methods. Relevant changes have been made according to the reviewer's suggestions (Page 5-6, Methods, marked in yellow).

Is the CEACAM1 expression of the sample relevant to the complete tumor?

Main point response:
1. Yes, CEACAM1 expression of the sample is relevant to the complete tumour and could reflect tumour burden. The serum levels of CEACAM1 in NSCLC patients are significantly higher than that for healthy donors. Furthermore, previous works have demonstrated that CEACAM1 expression in lung cancer was associated with tumour metastasis and progression [4-6]. In addition, our immunohistological data verified the association between lung cancer and CEACAM1 expression.

Why are the S-L ratios so different within the normal tumor samples?

Main point response:
1. We would like to thank professor Kammerer for his attention and consideration. However, after carefully analysing the data, we found that: 1, the variation of S-L ratios within the normal tissues adjacent to tumour is not much high (Table 1 below, marked in bold and italic, not included in the manuscript); 2, it is true that the variation of CEACAM1 S-L ratios in tumour tissue samples is much wider than that for all the other groups. As we mentioned in our manuscript, the high variation may be due to the limited sample size. Large prospective studies may diminish the variation in our future studies.

Table 1 Descriptive information for the IOD values for all groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Median</th>
<th>95% CI</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour S-form (IOD)</td>
<td>15.89</td>
<td>11.95~60.44</td>
<td>40.13</td>
</tr>
<tr>
<td>Normal S-form (IOD)</td>
<td>9.16</td>
<td>7.23~16.45</td>
<td>7.63</td>
</tr>
<tr>
<td>No.</td>
<td>Sex</td>
<td>Age</td>
<td>T</td>
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</tr>
<tr>
<td>1</td>
<td>M</td>
<td>46</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>77</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>53</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>65</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>72</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>56</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>52</td>
<td>3</td>
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<tr>
<td>8</td>
<td>F</td>
<td>65</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>61</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>50</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>69</td>
<td>2</td>
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<td>M</td>
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<td>2</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>68</td>
<td>1</td>
</tr>
</tbody>
</table>
The staging was made according to the 7th edition for the TNM classification of lung cancer by the International Association for the Study of Lung Cancer (IASLC).

B. The picture should be increased since the bands are almost invisible. And the result for GAPDH should be added.

Main point response:

As suggested by professor Kammerer, we have amended Fig. 3B and added the GAPDH result in our revised manuscript.

The authors discussed in the paper that the level of CEACAM1 in the serum may depend on the amount of soluble splice forms of CEACAM1 expressed in the tumor. Unfortunately, the authors did not analyze the expression of them in the tumor, which could be easily done by RT-PCR.

Main point response:

Special thanks to the reviewer for his good comments. In fact, we have tried to detect the expression of the soluble isoforms of CEACAM1 at the RNA level before. However, although CEACAM1 has been a subject of extensive research since its identification in 1976[7], its role as a new prognostic marker, particularly in serum, is only beginning to be investigated. Much work must be performed to fully understand CEACAM1. In humans, 11 different CEACAM1 splice variants have been detected. The functional difference between individual CEACAM1 isoforms remains unclear. Three isoforms are considered to be expressed in a secreted form because they lack transmembrane domains (Fig. 1 below, marked in red; not included in our manuscript). Normally, the soluble CEACAM1 found in serum must be composed of these 3 isoforms. However, previous reports [8] have found that a soluble CEACAM1 containing A2 domains present in the serum (Fig. 1 below, marked in blue, not included in our manuscript), corresponding to the membrane-bound isoforms of CEACAM1-4L, CEACAM1-4S and the secreted isoform of CEACAM1-4C1. Moreover, it was further demonstrated that apoptosis could induce the cleavage of the intracellular and extracellular domains of CEACAM1, resulting in an increased level of soluble CEACAM1[9]. Still, other reports have provided evidence of a mixed source of soluble CEACAM1 in addition to the naturally occurring secreted isoforms [9-10]. Thus, as CEACAM1 is widely distributed on epithelial and endothelial cells, soluble CEACAM1 may originate either through shedding or from dead cells in addition to active secretion. The exact source of the soluble CEACAM1 protein in serum is unknown. For the reasons mentioned above, the RT-PCR analysis of CEACAM1 in tumour tissues may not sufficiently explain the question of the origin of soluble CEACAM1.
Figure 1 The 11 naturally occurring isoforms of human CEACAM1[11]

Minor Essential Revisions

Table 2
The median (8.60) for Grading G1-G2 seem to be wrong because it is out of range 3.20x10³ – 0.172

Minor point response:
1. We have added the missing “×10^{-3}” into Table 2 (Table 3 in our revised manuscript), and we apologise for our carelessness (Page 20, marked in yellow).

“Squamous cell carcinoma” has a wrong font size.

Minor point response:
1. We have made corrections according to the reviewer’s comments (Page 20, Table 3 in our revised manuscript, marked in yellow).
Reviewer's report
Title: Experimental and clinical experimental studies on carcinoembryonic antigen-related cell adhesion molecule 1 in non-small-cell lung cancer: how does CEACAM1 manifest
Version: 2 Date: 28 March 2013
Reviewer: John Shively

Reviewer's report:
This manuscript demonstrates that serum levels of CEACAM1 can significantly separate normal from NSCLC patients, especially those with early disease. In addition, tissue samples have a discordant ratio of isoforms when compared to tumor vs normal adjacent tissue. These results will be of interest to those looking for potential tumor markers in this disease and since the assay appears to perform better than conventional NSE and CEA assays (with a limited data set), the observation is worth pursuing with larger numbers. Overall the problem is significant and the study well performed with high quality data and data analysis.

There are two issues that should be addressed:

Reviewer2 main point

1. There are many grammatical mistakes- too many to document for this reviewer. The authors should have a native English speaker edit the manuscript for grammar.

Main point response:
1. We apologise for the poorly written manuscript. The manuscript has now been edited by a native English speaker. We hope that the revised manuscript may meet the journal's criteria now.

2. The PCR results that demonstrate the changes in isoform levels needs to better validated. This can be done by performing the analysis with several different numbers of cycles of amplification (only 30 cycles run) and by including an internal standard. In the table documenting this analysis, the legend should explain how the analysis was performed and what is meant by IOD.

Main point response:
1. CEACAM1 PCR was conducted according to the protocols found in Gaur et al. [12] and Wang et al. [13], who used the same primer sequences in the present study. In addition, the experimental procedure was also based on several preliminary experiments that confirmed that 30 cycles were sufficient and feasible (Fig. 2 below not included in our manuscript).
Figure 2  The raw data of PCR with different cycles of amplification in our preliminary experiments. (A) 30 cycles; (B) 35 cycles

1  The GAPDH as internal standard was added as recommended by the reviewer (see Fig. 3B in our revised manuscript).

1  We apologise for our negligence in providing an appropriate introduction for how we analysed the PCR results. The relevant portions of our manuscript have been revised (mainly on Page 7, Statistical analysis, marked in yellow). Briefly, after intensity rectification, IOD was obtained by the ratio of the sum optical density (OD) to the sum area, which is proportional to the quantity of the RNA level. This analysis was performed with Image Pro Plus V6.0 for Windows (Media Cybernetics, Inc., Rockville, MD, USA).

In all, we found the reviewer’s comments to be very helpful, and we have revised our manuscript point-by-point. We thank the editor and reviewers again for their help and hope that the revised manuscript is suitable for publication in BMC cancer.
References:


