Author's response to reviews

Title: Overexpression of human sperm protein 17 increases migration and decreases the chemosensitivity of human epithelial ovarian cancer cells

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Author’s response to reviews:

Dear editors:

Thank you very much for informing us the results of the evaluation process of our manuscript submitted to BMC Cancer.

A point-by-point responses to the reviewers’ comments and detail the changes made is as following:

1. Response to reviewer Dimcho Bachvarov’s comments:
   1) The reviewer suggests to perform the inverse experiment, i.e. suppressing the hSp17 gene expression via siRNA in hSp17-expressing ovarian cancer cell lines and performing similar functional analyses.

   Nakazato T group had examined the effect of Sp17 on the chemoresistance of ovarian cancer cells to paclitaxel by suppressing siRNA in hSp17-expressing ovarian cancer cell line ES-2, and found that this treatment decreased the chemoresistance of these cells to paclitaxel. So, we performed the inverse experiment, to confirm similar function of Sp17.

2) According to the review’s suggestion, we have performed an immunocytochemistry analysis using specific anti-hSp17 antibody and proved specific hSp17 overexpression in HO8910/hSp17 cells (seen in revised Fig 2).

3) Page 5, IIrd paragraph. The recombinant plasmid pGEM-T/hSp17 was constructed in our lab. We add a sentence which was constructed in our laboratory using human testicular cDNA and used for generating recombinant HSp17 protein[12]#at page 5, line 17(seen in a detailed list of revisions).

4) Fig.3. The increased migratory capacity of the HO8910/hSp17 cells has been graphically demonstrated (seen in revised Fig 3).

5) The drug concentrations have been changed format in accordance with the paper published in BMC Cancer (seen in revised Fig 4 and in a detailed list of revisions).
6) A retrospective study for all patient specimens was performed, our data is unfavourable for evaluating resistance to chemotherapy because the therapy strategy was not same. However, we think the reviewer’s comment is rational.

7) The complete description of the AKAP3 abbreviation is indicated in the text.

2. Response to reviewer Massimo Broggi’s comments:

1) Although investigators in different lab observed Sp17 present in nucleus, cytoplasm and on cell surface, little knowledge of its function related to its localization has been reported. We found Sp17 could shift from cytoplasm to cell surface during cell cycle and tried to discuss the function of Sp17 present on cell surface on the base of literature (seen in revision).

Although we observe that late stage (#~#) tumors expressed higher amount of Sp17 than early stage (#~#) tumors, the statistical analysis did not show significant difference (data shown as following Tab.)

Table 1. Sp17 expression by IHC and correlation with clinicopathological characteristics in EOC

<table>
<thead>
<tr>
<th>Clinicopathological characteristics</th>
<th>Negative</th>
<th>Positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All tumors, n (%) (n =70)</td>
<td>40</td>
<td>30 (42.9)</td>
</tr>
<tr>
<td>FIGO stage, n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I ~ II</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>III ~ IV</td>
<td>32</td>
<td>17</td>
</tr>
<tr>
<td>Histology (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serous</td>
<td>22</td>
<td>15</td>
</tr>
<tr>
<td>Mucinous</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>Primary peritoneal carcinoma</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Clear cell</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Endometroid</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>
| #, 5%; + , #25%; ++ , 25–50%; +++ , 50–75%; +++#75%.

2) The reviewer suggests to perform in vivo experiments in nude mice using their hSp17-expressing cell line.

We had tried in vivo experiments in nude mice using hSp17-expressing cell line (HO8910/hSp17). Unfortunately, there was no sufficient cancer cells overexpressing HSp17 in transplanted tumor of mice. HO8910/hSp17 cell line might not be a good model for in vivo experiments yet.

3) Response to minor essential revisions

Nakazato T group had examined the effect of Sp17 on the chemoresistance of ovarian cancer cells to paclitaxel using hSp17-expressing ovarian cancer cell line, and found that hSp17 could increase the chemoresistance of these cells to
paclitaxel. So, we tried to determine the response of cells overexpressing Sp17 to platinum containing drugs.

We are planning a strategy to study if patients with high expression of Sp17 have the low responders to platin-baesd agents.

3. Response to the third reviewer ‘s comments:

First of all, we thank Dr. Taylor for his comments for our manuscript. We learned Dr. Taylor’s wonderful work in gynecology by reading his scientific articles. I admit frankly, to have difficulties in writing manuscript in English and I look forward to any revise comment to consummate our article. Regarding to study design, we referred to the strategy used in recent publications of analogous research#Yang AD et al. Clin Cancer Res 2006; 12: 4147-4153; Nakazato et al. Int J Gynecol Cancer 2007, 17: 426–432 #.

We repeated detection several times, indeed found only 43 % of the tumors of positive for HSp17 in EOC. However, our late research observed 61%-66% positive for Hsp17 in cervical cancer and endometrial cancer. If sample size of EOC is enlarged, the positive rate may be higher. More over, great many factors influence chemoresistance of cancer cell, overexpression of Hsp17 is only one among those.

As we stated in manuscript, our program’s primary focus is to study the clinical relevance of Sp17 aberrant present, to identify a better prognostic marker and new treatment for this deadly disease. Owing to the observation of all metastatic ascitic fluid cancer cells from eight EOC patients were positive for Sp17, we presume Sp17 could promote the migration and metastatic activity of cancer cells. In order to confirm this hypothesis, we generated a cell model with over-expressing Sp17 by transfecting the Sp17 cDNA into an ovarian cancer cell line. Nakazato et al (Int J Gynecol Cancer 2007, 17: 426–432) had showed that expression of the Sp17 gene can be used as a predictor of the chemoresistance of ovarian cancer to paclitaxe. They examined the effect of small interfering RNA targeting the Sp17 gene on the chemoresistance of clear cell adenocarcinoma of the ovary to paclitaxel, found that this treatment decreased the chemoresistance of these cells to paclitaxe. Based on their results, we performed the experiments, using the same cell line over-expressing Sp17, to confirm the similar function of Sp17 to platin-based agent. LD50#the reviewer suggested, here we understand as IC50. Complied suggestion, we have calculated IC50 for cisplatin and carboplatin revised figure 4.

3. Detailed list of revisions:

1) Page 5, IIrd paragraph line 17. a sentence#which was constructed in our laboratory using human testicular cDNA and used for generating recombinant HSp17 protein[12]#was added.

2) Page 5, IIrd paragraph line 28 has been changed to “and confirmed by Western blot and immunohistochemistry analysis.” was added.

3) Page 6, IIrd paragraph line 12, “generated in our laboratory [12] ” was added.

4) Drug (MTT and platin-based agents) concentrations in manuscript have been
changed to mg•ml-1 or µg•ml-1 respectively.

5) Page 6, IIrd paragraph line 12~14, a sentence “Although late stage (#~#) tumors expressed higher amount of Sp17 than early stage tumors, the statistical analysis did not show significant difference (data not shown).” was added. In line 19, “HSp17 was present in cytoplasm and on cell surface in model cells and” was added., and then “Although investigators in different laboratory observed Sp17 present in nucleus, cytoplasm and on cell surface, little knowledge of its function related to its localization has been reported. The role for Sp17 in promoting heparin sulphate-mediated adhesion of lymphoid cells has been proposed by Lacy and Sanderson[22], who showed that Sp17 expressed on the surface of lymphoid-derived cells of a patient with plasma cell leukemia promotes cell–cell adhesion via interaction with the heparin sulphate chain of syndecan 1.” was added.

6) Fig 4, bar graph changed to dosing curve.

7) References list and some spelling mistakes had been corrected.

Thank you for your kind consideration.

Yours sincerely,

Dr. Fang-qiu Li