Author's response to reviews

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

Authors:

Fang qiu Li (njljfq@jlonline.com)
Yan ling Han (xiaohan_301@163.com)
Qun Liu (xinye0128@163.com)
Bo Wu (wubo0020@sina.com)
Wen bin Huang (wbhuang348912@126.com)
Su yun Zeng (zengsuy@163.com)

Version: 2 Date: 23 January 2009

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’s 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 has 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 Broglini’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>
<th>+ ~ ++</th>
<th>+++ ~ ++++</th>
</tr>
</thead>
<tbody>
<tr>
<td>All tumors, n (%) (n =70)</td>
<td>40</td>
<td>30 (42.9%)</td>
<td>16 (22.9%)</td>
<td>14 (20%)</td>
</tr>
<tr>
<td>FIGO stage, n</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I ~ II</td>
<td>8 13 8 5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III ~IV</td>
<td>32 17 8 9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histology (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serous</td>
<td>22 15 8 7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucinous</td>
<td>11 6 5 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary peritoneal carcinoma</td>
<td>3 4 2 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clear cell</td>
<td>2 2 1 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endometroid</td>
<td>2 2 0 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>0 1 0 1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#, 5%; +, #25%; ++, 25–50%; ++++, 50–75%; +++#75%.
2) The reviewer suggested to perform in vivo experiments in nude mice using their hSp17-expressing cell line.

Thanks for suggestion, we will perform in vivo experiments in nude mice using hSp17-expressing cell line HO8910/hSp17 next study.

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 the low responders to platinum.

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) All of drug concentrations in manuscript have been changed to mg•ml-1 or µg•ml-1.

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) References list and some spelling mistakes had been corrected.

Thank you for your kind consideration.
Yours sincerely,

Dr. Fang-qiu Li