Author's response to reviews

Title: Stomatin-like protein 2 is overexpressed in epithelial ovarian cancer and predicts poor patient survival

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Version: 5 Date: 9 April 2015

Author's response to reviews: see over
Dear Editor,

We appreciate much the comments of reviewers on our manuscript and have revised the manuscript in accordance with the comments. We have had the manuscript’s language edited by sending it to a professional English editing Company (http://webshop.elsevier.com/languageservices/languageediting/). The following was the comments of reviewers and the point to point responses (please see next page).

Best wishes,

Yanfang Li, Professor,
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Reviewer #1

Major Compulsory Revisions

Comment #1: Is there any available information of the histological subtype of the evaluated EOC samples? Recent studies show that the different subtypes (e.g. endometrioid, mucinosus, serous) are originated from different tissue types, and even low-grade and high-grade serous tumors are genetically highly different. (Vaughan, S., et al., Rethinking ovarian cancer: recommendations for improving outcomes. Nat Rev Cancer, 2011)

Response #1: We have shown the information of the histological subtype of the evaluated EOC samples in Table 1(Page 24 Line 487-493). Analyses suggested that there are no significantly differences among the expression of SLP-2 protein in different histological subtype and grade of the EOC respectively.

(Part of Table 1)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number of cases (%)</th>
<th>SLP-2 expression (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathological type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serous</td>
<td>80(57.1)</td>
<td>20(25.0)</td>
<td>60(75.0)</td>
</tr>
<tr>
<td>Mucinous</td>
<td>14(10.0)</td>
<td>8(57.1)</td>
<td>6(42.9)</td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>36(25.7)</td>
<td>8(22.2)</td>
<td>28(77.8)</td>
</tr>
<tr>
<td>others①</td>
<td>10(7.1)</td>
<td>2(20.0)</td>
<td>8(80.0)</td>
</tr>
<tr>
<td>Grade of differentiation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>59(42.1)</td>
<td>11(18.6)</td>
<td>48(81.4)</td>
</tr>
<tr>
<td>G2</td>
<td>46(32.9)</td>
<td>13(28.3)</td>
<td>33(71.7)</td>
</tr>
<tr>
<td>G3</td>
<td>18(12.9)</td>
<td>9(50.0)</td>
<td>9(50.0)</td>
</tr>
<tr>
<td>Unknown</td>
<td>17(12.1)</td>
<td>5(29.4)</td>
<td>12(70.6)</td>
</tr>
</tbody>
</table>

① Endometrioid adenocarcinoma 2 cases, clear cell carcinoma 3 cases, mixed epithelial carcinoma 5 cases.

Comment #2: Connected to the previous question, epithelial ovarian carcinoma is known to originate from different tissues, for example high grade serous ovarian tumors originate from the
fallopian tube tissue (Lee, Y., et al., A candidate precursor to serous carcinoma that originates in the distal fallopian tube. J Pathol, 2007.). This suggests that normal ovarian epithelial tissue is probably not the sufficient control material. How do the authors explain this? If they have available fallopian tissue samples I would recommend measuring the SLP2 expression in these too.

Response #2: Ovarian epithelial cancer belongs to a heterogeneous group of tumors, of which tissue type of origin is uncertain. The current researches on the origin of ovarian epithelial malignancy mainly contain three theories including ovarian surface epithelium doctrine(1-4), the secondary müllerian system doctrine(5-7), extraovarian tissue of origin doctrine and stem cell doctrine(8). And extraovarian tissue of origin doctrine ranged over fallopian tube tissue of origin doctrine (9-15), urothelial nest theory (16) and endometriosis doctrine (17, 18). Therefore, the origin of ovarian cancer remains still uncertain and needs further researches. And normal ovarian epithelial tissue may be one option for the control material.

Reference:


**Comment #3:** Can the authors explain that no previous studies (including some genome wide expression array based studies) has found correlation between SLP2 expression and ovarian cancer tissue compared to normal, or SLP2 expression and patient survival? Did the authors evaluate the TCGA ovarian cancer data related to their results about SLP2 expression?
**Response #3:** No previous study has investigated the correlation between SLP2 expression and ovarian cancer tissue compared to normal and the correlation between SLP2 expression and the survival of EOC patients. And we can not find any data from the TCGA about SLP2 expression.

**Minor Essential Revisions**

**Comment #1:** Please upload a high resolution image of figure 6, since the axis labels are unreadable.

**Response #1:** We have uploaded a new figure 6.

**Comment #2:** In the methods sections the authors list the providers of the used reagents, however, in some cases they give full details (company, country, city or state), sometimes just the providers, and sometimes none. Please provide that information systematically through the methods section.

**Response #2:** We have provided the details of all the used reagents in the methods sections. (Page 6 Line 103-104, Page 7 Line 147, Page 8 Line 150,161-163,166,169, Page 9 Line 171, 174-175, 185, 188-189)

**Comment #3:** In legend of figure 2 the authors mention “cancer tissues (T) and adjacent noncancerous tissues (N)” notations, meanwhile they use T and ANT in the figure. Please clarify this.

**Response #3:** We have revised the legend of figure 2, and changed “cancer tissues (T) and adjacent noncancerous tissues (N)” to “cancer tissues (T) and adjacent noncancerous tissues (ANT)”. (Page 25 Line 511-512, Page 26 Line 517)

**Discretionary Revisions**

**Comment #1:** I recommend mentioning the investigated gene’s HUGO approved conventional name (STOML2) in the introduction and the abstract, to make it easier for the readers to look it up in online databases.

**Response #1:** Yes, we did. (Page 2 Line 25, Page 4 Line 72)
Reviewer #2

Discretionary Revisions (which are recommendations for improvement but which the author can choose to ignore)

Comments

- Please read the MS, there are a few typos to correct.
- The title should be present time.

Response: We did as required. (Page 1 Line 1-2)

Minor Essential Revisions

Comment #1: What is the specificity of the primers? I was unable to exactly identify SLP-2 as the targets of the PCR.

Response #1: The primer of SLP-2 was designed using Primer Express v 2.0 software (Applied Biosystems). The sequences of the primers were as follows: forward primer 5'-GTGACTCTCGA CAATGTAAC-3' and reverse primer 5'-TGATCTCATAACGGAGGCAG-3'. And PCR products had tested by the 1.5% AGE (agarose gel electrophoresis). And we tested again through software. The result showed that the primers were specificity.

<table>
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</tr>
</thead>
<tbody>
<tr>
<td>Sense</td>
<td>70</td>
<td>274</td>
<td>26</td>
<td>46.6</td>
<td>-52.0</td>
<td>31.7</td>
<td>1</td>
<td>--</td>
</tr>
<tr>
<td>Anti-sense</td>
<td>68</td>
<td>532</td>
<td>26</td>
<td>58.9</td>
<td>-37.0</td>
<td>31.2</td>
<td>1</td>
<td>--</td>
</tr>
<tr>
<td>Product</td>
<td>52</td>
<td>389</td>
<td>90.0</td>
<td>53.3</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>52.0</td>
</tr>
</tbody>
</table>

Comment #2: Add ethical approval number.

Response #2: We added “(Approval No: B2014-2-26)” on Page 7 Line 144.

Comment #3: Figure 5 looks blurred. Add scale to all IHC figures

Response #3: We have added scale to all IHC figures.

Comment #4: There is no need for three IHC figures; these should be combined into one figure.

Response #4: We used three IHC figures to show different aspects. Figure 3 emphasized SLP-2
protein expression in eight fresh pairs of matched tissues; Figure 4 emphasized SLP-2 protein expression in different kinds of epithelial ovarian tumor; Figure 5 emphasized the different level of SLP-2 staining.

**Comment #5:** Figure 6 has to be replaced by a high quality plot

**Response #5:** We have uploaded a new better Figure 6.

**Major Compulsory Revisions:**

**Comment #1:** There is no chemotherapy treatment data included in the manuscript. Were the patients uniformly treated?

**Response #1:** we added “All patients received surgery. Most patients (except those who had stage IA and grade 1 tumors) had post-operation adjuvant chemotherapy with platinum-based regimen” on Page 6 Line 138-141.

**Comment #2:** It is now possible to perform an independent validation using gene chip data in serous ovarian cancer samples. Use an online database mining tool like www.kmplot.com/ovar. This is especially interesting, as it confirms the correlation to survival described in the manuscript (HR=1.33, p=0.00038).

**Response #2:** We tested “SLP-2” several times in this web (www.kmplot.com/ovar), but got the following result: HR= 0.84, P=0.0075, which is different to our results and to the results mentioned above (HR=1.33, p=0.00038) and which is also opposite to cancer-related nature of SLP-2 as shown by many studies in other cancers mentioned in the “Discuss” section of our paper.

Likely, we also tried a famous drug-resistant gene MDR1 of ovarian cancer on the same website and the results were: HR=0.91, P=0.17, which is also not consistent with the drug resistant and survival negatively impacted nature of the gene.

This validation tool may be an important reference, but it could be affected by many kinds of factors.

**Comment #3:** SLP-2 regulates the biogenesis and the activity of mitochondria. It should be discussed whether it could contribute to the altered energy metabolism typical for cancer cells?

**Response #3:** we added one paragraph to discussed this (i.e. the 3rd paragraph of “Discuss” on Page 15 Line 322-324 ,Page 16 Line 325-333).
Reviewer #3

Comment #1: Thank you very much for submitting your manuscript to the BMC cancer Journal. I went through the manuscript and had an impression that the paper is well-written, the tables and figures are of high quality, and the authors have clearly worked to produce a comprehensive dataset and detailed description of their methods. However, this paper still needs a considerable revision to be acceptable for the BMC cancer Journal. There are several reports with functional analyses including cell growth, cell adhesion, and tumorigenesis in the antisense approaches. They could perform these functional analyses to gain a deeper understanding of the role of SLP-2 in ovarian cancer.

Response #1: This is a very nice suggestion. We plan to study the function of SLP-2 including cell growth, cell adhesion, and tumorigenesis in the antisense approaches in epithelial ovarian cancer cells in the future work.

Comment #2: I would have also preferred more discussion, since writing often lacks clarity and sharpness and several sections are poorly organized.

Response #2: We made the following revises on “Discuss” section: re-write the 2nd paragraph, added the 3rd paragraph, modified the 5th paragraph, and added the 6th paragraph.

Comment #3: The manuscript would be improved through English editorial review.

Response #3: We have sent our manuscript to a professional English editing Company (http://webshop.elsevier.com/languageservices/languageediting/) to have it edited.
Reviewer #4

Major Compulsory Revisions

Comment #1: 1. Studies over the past several years have definitively shown that the different histological types of ovarian cancer are distinct diseases (including but not limited to high-grade serous, low-grade serous, endometrioid, clear cell), with unique sites of origin, molecular aberrations and pathogenesis. One must therefore study these diseases independently in order to make meaningful conclusions. It has also been shown that the prognostic value of a given biomarker can differ when looking at “ovarian cancers” as a whole, or within a given type. Two notable examples of this are proliferation marker Ki-67 and WT-1, as shown by Kobele et al (PLOS Medicine, 5(12): e232, 2008). Whereas Ki67 was an unfavorable prognostic marker within a cohort of ovarian cancers of all histologies, it did not show a prognostic impact within any specific type. Furthermore, WT-1 was found to be an unfavorable prognostic marker in the overall cohort, but was a favorable prognostic marker within high-grade serous carcinomas. In light of these important findings, the authors are strongly recommended to repeat their survival analysis within each histologic type.

Response #1: We performed further survival analysis in two subgroup (serous cancer and poorly differentiated) which had a bigger size of sample. We added the following results in section “Relationship between SLP-2 expression and patient survival”: “We further performed survival analysis in two subgroup (serous cancer and poorly differentiated) which had a bigger size of sample. In 80 patients with serous cancer, univariate analysis revealed that SLP-2 overexpressions were associated with PFS (P=0.022) and OS (P=0.044) (Figure 6B); Cox regression analysis showed that tumor stage (P=0.05), peritoneal cytology (P=0.001), and SLP-2 overexpression (P=0.003) were independent prognostic factors for poor PFS and also for poor OS (P=0.004, 0.004, and 0.01, respectively). In 36 patients with poorly differentiated cancer, univariate analysis revealed that SLP-2 overexpressions were associated with PFS (P=0.046) and OS (P=0.049) (Figure 6C); Cox regression analysis showed that SLP-2 overexpression was associated with OS (P=0.023), but was not associated with PFS (P=0.058). The above mentioned other factors were not associated with either PFS or OS (P>0.05)”.

We also add Figure 6B and 6C which showed that SLP-2 overexpression affected the survival
of patients with serous cancer or poorly differentiated.

**Comment #2:** In line with the previous comment, the authors should provide more details regarding which types of borderline and benign ovarian tumor specimens were included in their analysis of RNA and protein levels, as it is not meaningful to compare benign/borderline and malignant tumors of different histologic types (e.g. comparing a mucinous borderline tumor to a high-grade serous carcinoma).

**Response #2:** The details regarding types of benign and borderline tumor were added on page 7 Line 135-138.

**Comment #3:** The authors should provide more information on the eight matched pairs of epithelial ovarian cancer specimens (histology, stage, grade) and adjacent noncancerous tissue samples (was this ovarian stroma?).

**Response #3:** The details of eight matched pairs of epithelial ovarian cancer specimens and adjacent noncancerous tissue samples were added on page 7 Line 128-135.

**Comment #4:** Needs some language corrections before being published

**Response #4:** We have sent our manuscript to a professional English editing Company (http://webshop.elsevier.com/languageservices/languageediting/) to have it edited.