Author's response to reviews

Title: Expression of tumor-specific antigen MAGE, GAGE and BAGE in ovarian cancer tissues and cell lines

Authors:

Shiqian Zhang (zhangshiqian370112@126.com)
Xiaoliang Zhou (zhou3208@163.com)
Hao Yu (yuhao19@yahoo.cn)
Yunhai Yu (ywhdmlove@yahoo.com.cn)

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

Many thanks for the positive evaluation and for providing us an opportunity to revise the Manuscript entitled “Expression of tumor-specific antigen MAGE, GAGE and BAGE in ovarian cancer tissues and cell lines”.

We appreciate very much your important decision and reviewers’ comments for the original manuscript. We have finished the revision according to your and reviewers’ comments and now return the revised version with the response to the reviewers. In this version, we have carefully addressed all points raised by the reviewers and make detailed revisions according to the suggestions. Our specific responses and the corresponding changes to each of the points are summarized on the following response sheet addressing to the reviewers and the changes in the revised manuscript have been marked in RED font.

In summary, the manuscript has been thoroughly revised and the concerns raised by the reviewers have been addressed carefully on a point-by-point basis. We believe that the revision has significantly improved the quality of the manuscript and hope that it will be accepted for publication. Thank you for your considerations.

Shiqian Zhang, Ph.D., M.D.,
The State-Key Discipline of Obstetrics and Gynecology,
Department of Obstetrics and Gynecology,
Qilu Hospital of Shandong University,
Ji’nan 250012, China;
Tel: +86-531-82169577,

E-mail address: zhangshiqian370112@126.com
Reviewer(s)' Comments to Author:

Editor

1: Ethics - Experimental research that is reported in the manuscript must have been performed with the approval of an appropriate ethics committee. Research carried out on humans must be in compliance with the Helsinki Declaration (http://www.wma.net/e/policy/b3.htm), and any experimental research on animals must follow internationally recognized guidelines. A statement to this effect must appear in the Methods section of the manuscript, including the name of the body which gave approval, with a reference number where appropriate.

2: Informed consent must also be documented. Manuscripts may be rejected if the editorial office considers that the research has not been carried out within an ethical framework, e.g. if the severity of the experimental procedure is not justified by the value of the knowledge gained.

3: Please also ensure that your revised manuscript conforms to the journal style (http://www.biomedcentral.com/info/ifora/medicine_journals). It is important that your files are correctly formatted.

We look forward to receiving your revised manuscript by 4 January 2010. If you imagine that it will take longer to prepare please give us some estimate of when we can expect it.

Dear editor,

We appreciate very much your constructive comments to our manuscript. We have revised the manuscript according to your suggestions and incorporated all corrections which were marked with RED font in the revised version. The detailed
point-by-point answers to your concerns are below.

1: Ethics - Experimental research that is reported in the manuscript must have been performed with the approval of an appropriate ethics committee. Research carried out on humans must be in compliance with the Helsinki Declaration (http://www.wma.net/e/policy/b3.htm), and any experimental research on animals must follow internationally recognized guidelines. A statement to this effect must appear in the Methods section of the manuscript, including the name of the body which gave approval, with a reference number where appropriate.

Re: Thank you for your valuable comments. We’ve revised it in the revised version.

2: Informed consent must also be documented. Manuscripts may be rejected if the editorial office considers that the research has not been carried out within an ethical framework, e.g. if the severity of the experimental procedure is not justified by the value of the knowledge gained.

Re: Thank you for your professional advice. We’ve documented the informed consent in the revised version.

3: Please also ensure that your revised manuscript conforms to the journal style (http://www.biomedcentral.com/info/ifora/medicine_journals). It is important that your files are correctly formatted.

Re: Thank you for your careful reminder. We’ve revised the format of this manuscript strictly according to your journal style.

We look forward to receiving your revised manuscript by 4 January 2010. If you imagine that it will take longer to prepare please give us some estimate of when we can expect it.

Referee: Yao-Tseng Chen
Comments to the Author

Major revisions:
1. In the 47 cases that were included as ovarian cancer, 6 of them were metastatic in nature. These 6 cases should not have been included in the series.

2. The authors claimed that MAGE-3 was not expressed in the metastatic ovarian cancer. However, since only 7 cases were examined, this conclusion, or any conclusion regarding the frequency of CT antigen expression in metastatic ovarian cancer for that matter, is invalid. If the authors intend to compare the expression frequency of these CT antigens in primary versus metastatic ovarian carcinoma, it would be essential to increase the number of metastatic ovarian carcinomas examined.

3. The histological types of the metastatic ovarian carcinoma were not described.

4. It is an unusual observation that 3 of 20 benign ovarian tumors expressed MAGE-1. The nature of the 20 benign tumors, and what were the ones that were positive for MAGE-1, should be described. This data, being unexpected, should also be shown as one of the figures.

Minor revisions:
1. The gel figures are shown upside down, i.e. higher MW in the bottom of the gel images. This is highly unconventional and should be changed to conform to the standard format.

Discretionary Revisions:
1. Antibodies are commercially available for the evaluation of MAGE-A and
GAGE antigens. Adding protein expression data by using these antibodies would have significantly increased the quality of this manuscript.

Dear Prof. Yao-Tseng Chen,

We appreciate very much your careful review, constructive comments and kind corrections to our manuscript. We have revised the manuscript according to your comments and incorporated all corrections which were marked with RED font in the revised version. The detailed point-by-point answers to your concerns are below.

Major revisions:
1. In the 47 cases that were included as ovarian cancer, 6 of them were metastatic in nature. These 6 cases should not have been included in the series.
   Re: It is generally thought that metastatic ovarian cancer is one type of ovarian cancers. Compared to primary ovarian tumors, metastatic ovarian tumors probably have different biological characteristics. Therefore, we include the metastatic cases in our early study.

2. The authors claimed that MAGE-3 was not expressed in the metastatic ovarian cancer. However, since only 7 cases were examined, this conclusion, or any conclusion regarding the frequency of CT antigen expression in metastatic ovarian cancer for that matter, is invalid. If the authors intend to compare the expression frequency of these CT antigens in primary versus metastatic ovarian carcinoma, it would be essential to increase the number of metastatic ovarian carcinomas examined.
   Re: We totally agree with the reviewer’s point that the conclusion of this maybe unconvincing due to the limited sample size of metastatic ovarian cancer. We’ll be conducting the compared experiments between primary and metastatic ovarian carcinoma.
3. The histological types of the metastatic ovarian carcinoma were not described.

Re: As you suggested, we’ve supplemented the description of the histological types in the revised version. Thank you.

4. It is an unusual observation that 3 of 20 benign ovarian tumors expressed MAGE-1. The nature of the 20 benign tumors, and what were the ones that were positive for MAGE-1, should be described. This data, being unexpected, should also be shown as one of the figures.

Re: Tumor-specific antigen (TSA) exclusively exists in tumor cells rather than in normal cells. MAGE-1 was expressed in 3 benign tumors cases, including 2 cases of serous cystadenocarcinoma and one case of mucinous cystadenoma. The relevant results are consistent with those of previous study. Gillespie et al found that the expression rate of MAGE-1 in benign ovarian tumors was 11/25 (44%). Please see the relevant report below.


Minor revisions:

2. The gel figures are shown upside down, i.e. higher MW in the bottom of the gel images. This is highly unconventional and should be changed to conform to the standard format.

Re: We feel sorry that we cannot redo the relevant experiment under the current condition. Thank you for your constructive comments. We’ll emphasize the detailed content in our further experiment.

Discretionary Revisions:
1. Antibodies are commercially available for the evaluation of MAGE-A and GAGE antigens. Adding protein expression data by using these antibodies would have significantly increased the quality of this manuscript.

Re: Thank you so much for your constructive comments. We totally agree with your viewpoint. We’ll conduct relevant experiments in our further study.

Comments to the Author

Reviewer: Mai-Britt Zocca

Minor essential comments/corrections:

1: The three positive cell lines established and analyzed in figure 1-3 do not express BAGE however tissue sample in figure 4 do – what is the explanation for the lack of BAGE expression in the three cell lines?

2: Table 2 needs to be corrected: Normal Ovary: Text (Abstract, Material and methods) reads 14 cases of normal ovarian tissue however table list 10 samples of ovarian tissue. Ovarian cancer: Table list 24 (51.1%) MAGE 1 however text list 25 (51.1%).

Dear Prof. Mai-Britt Zocca,

We appreciate very much your careful review, constructive comments and kind corrections to our manuscript. We have revised the manuscript according to your comments and incorporated all corrections which were marked with RED font in the revised version. The detailed point-by-point answers to your concerns are below.

Minor essential comments/corrections:
1: The three positive cell lines established and analyzed in figure 1-3 do not express BAGE however tissue sample in figure 4 do – what is the explanation for the lack of BAGE expression in the three cell lines?

Re: Thank you for your professional suggestions. Indeed, significant difference exists when we use cell lines and fresh tissue to conduct experiment, respectively. We presume that certain mutations may occur during cell lines passage, thus, some genes are expressed in tumor tissues rather than in cell lines.

2: Table 2 needs to be corrected: Normal Ovary: Text (Abstract, Material and methods) reads 14 cases of normal ovarian tissue however table list 10 samples of ovarian tissue. Ovarian cancer: Table list 24 (51.1%) MAGE 1 however text list 25 (51.1%).

Re: Thank you for your careful reviewing. We’ve revised the typing error in the revised manuscript.