Author's response to reviews

Title: The Effect of Proteoglycans Inhibited by RNA Interference on Metastatic Characters of Human Salivary Adnoid Cystic Carcinoma

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Author's response to reviews: see over
Dear PhD. Sabina Alam:

Thank you very much for your E-mail of Aug. 24, 2009, with regard to my manuscript (MS: 1869934628276378) together with the comments from the reviewers. We really appreciate the constructive comments of the reviewers. Overall, the referees are enthusiastic about the significance of our work. Their concerns are mostly about our unclear presentations. We have tried our best to review the manuscript and got some help in editing. We are grateful for these valuable comments and feel that after incorporating the reviewers’ advice, the revised manuscript has been significantly strengthened and the significance of our findings is now more evident. A detailed point-by-point response to reviewers’ comments is listed below.

Thank you for your time and effort! We look forward to hearing your decision.

Sincerely yours,

Hong Shi
Response to Reviews

Response to Reviewers’ Comments
We would like to thank the reviewers for the constructive and positive comments.

Reviewer: 1
In this manuscript, the authors describe the effects of RNAi-mediated inhibition of XTLY-I on the biological activities (i.e., cell adhesion, migration, invasion, and lung metastasis) of a human salivary gland tumor cell line. The study is well designed and the methodological approaches used are appropriate. Results from this study could contribute, at least in part, to furnish new idea for the treatment of the salivary gland tumors, and add new information to the present knowledge of the role of proteoglycans in the metastasis mechanism of this malignancy. However, in order to further strengthen the authors’ conclusions and improve the clarity of the presentation, a few points need to be thoroughly addressed.

Minor points:
1. The authors base their conclusions only on one human salivary gland cancer cell line. Although the results are corroborated by relevant controls for specificity, it would be important to confirm similar results at least in another cell line to assess the generalizability of these findings. If feasible, a transient transfection approach may be appropriate to address this point without embarking in the generation of a stable cell line.
2. Table 2. Replace “shRNA-HK” with “SACC-M-HK”.
4. Figure 3A. The quality of the picture is very poor and it should be changed with a better one.
5. Figure 3B. Verify the label.
6. Figure 4A. The quality of the picture is very poor and it should be changed with a better one.

We thank the reviewer for the thorough review and helpful advice. According to your advice, we have amended the relevant part in manuscript. All of your questions were answered below.

Comments:
1 The authors base their conclusions only on one human salivary gland cancer cell line. Although the results are corroborated by relevant controls for specificity, it would be important to confirm similar results at least in another cell line to assess the generalizability of these findings. If feasible, a transient transfection approach may be appropriate to address this point without embarking in the generation of a stable cell line.

Response: We appreciate the reviewer’s constructive suggestion. It would be important to confirm similar results in another cell line to assess the generalizability of these findings. In fact, our group
is continuing to carry out this work in other cell lines such as SACC-83, primary culture cell of salivary pleomorphic adenoma. In this study, cell adhesion, invasion and metastasis of SACC-M need to be investigated in vivo and vitro. Especially, the experimental lung metastasis of nude mice was designed, so a stable cell line is necessary. A transient transfection approach may be used in other cell lines. Thanks a lot for this valuable advice!

2. Table 2. Replace “shRNA-HK” with “SACC-M-HK”.

Response: We have made the correction in the revised manuscript. We feel sorry for this mistake.


Response: We appreciate the reviewer sincerely for raising this question and have replaced the wrong words in the revised manuscript. It was a careless mistake we have made in the original manuscript. We are so sorry!

4. Figure 3A. The quality of the picture is very poor and it should be changed with a better one.

Response: We have changed Figure 3A with a better one. We thank the reviewer’s helpful comment!

5. Figure 3B. Verify the label.

Response: We thank the reviewer for raising the question and have deleted the useless label. We are so sorry for our carelessness.

6. Figure 4A. The quality of the picture is very poor and it should be changed with a better one.

Response: We thank the reviewer for pointing out this shortcoming and have changed it with a better one. We are grateful for these valuable comments that made the revised manuscript significantly strengthened.

Reviewer: 2

Comments to the Author
The manuscript present interesting data. However, some corrections and clarifications need to be made.
Major:
1) To address physiological relevance, expression of proteoglycans and XTLY-I in vivo in salivary glands should be demonstrated.
2) Characterization of SACC-M cells. What salivary tumor markers do this cell line express?

3) Immunoblot of XTLY-I is included in results (Fig. 4) but is barely mentioned in material and methods. Why? What protocol was used?

4) Fig 8 is really poor. It must be replaced by a black and white, high contrast illustration.

5) Many graphs are not properly described. For instance Fig 6 has a Y axis named "The adhesion rate". What does it mean? Fig 4 has a Y axis named "The protein level". Is it absorbance?

Minor

Manuscript will benefit from professional language editing.

Major:

1) To address physiological relevance, expression of proteoglycans and XTLY-I in vivo in salivary glands should be demonstrated.

Response: Thanks a lot for the thorough review. We have tried our best to add the demonstration in the revised manuscript. In vivo in salivary gland, the acinus and duct cells do not produce and secrete proteoglycans, so they do not express proteoglycans and XTLY-I. Recently fifteen more years, Prof. Jie Wang et al. demonstrated that the normal myoepithelial cells do not synthesize and secrete proteoglycans. When they transform to tumor cells, the neoplastic myoepithelial cells in salivary gland tumors have the ability to synthesize and secrete proteoglycans. (1. Jie Wang, et al. An electron microscopic histochemical study on proteoglycans in salivary gland myoepithelioma. Zhonghua Kouqiang Yixue Zazhi 1995; 30(4): 215. Article in Chinese; 2. Jie Wang, et al. The relationship between the secretion of proteoglycans and the histological type of adenoid cystic carcinoma of salivary gland. Zhonghua Yixue Zazhi 1994; 74(7): 434. Article in Chinese). The similar results are also demonstrated by other researches (1. Kimura S, et al. Perlecan (heparan sulphate proteoglycan) gene expression reflected in the characteristic histological architecture of salivary adenoid cystic carcinoma. Virchows Arch 2000; 437: 122. 2. Kimrua S, et al. Basement membrane heparan sulfate proteoglycan (perlecan) synthesized by ACC3, adenoid cystic carcinoma cells of human salivary gland origin. J Biochem 1999; 125: 406.) Xylosyltransferase-I (XTLY-I) catalyzes the initial step of proteoglycans biosynthesis, and it was inhibited in SACC-M cells in our study, the tumor cells showed that the biosynthesis and secretion of proteoglycans were suppressed. The detailed content can be found in page 26 (lane: 2-9) of revised manuscript. Thanks a lot for this helpful advice!

2) Characterization of SACC-M cells. What salivary tumor markers do this cell line express?

Response: We thank the reviewer for this good suggestion. We have added this content in the revised manuscript. SACC-M with high metastasis of lung in this study is derived from SACC-2 and mainly has the differentiated and biological features of the neoplastic myoepithelial cells. The neoplastic myoepithelial cells in adenoid cystic carcinoma and other salivary gland tumors could express smooth muscle actin, myosin, S-100 protein and GFAP (1. Jie Wang, et al. Immunoelectron microscopic and immunohistochemical study of adenoid cystic carcinoma of
3) Immunoblot of XTLY-I is included in results (Fig. 4) but is barely mentioned in material and methods. Why? What protocol was used?

Response: Thanks a lot for this carefully review. This is a poor mistake we had made. It might have been made in the process of copy the manuscript from the original document to the BMC template. We feel really sorry for that carelessness! We have added the immunoblot protocol in the revised manuscript. Detailed content can be found in page 14.

4) Fig 8 is really poor. It must be replaced by a black and white, high contrast illustration.

Response: We thank the reviewer for pointing out this shortcoming and have tried our best to replace the poor one with a black and white, high contrast illustration. Detailed content can be seen in Fig 8 in the revised manuscript. We are grateful for these valuable comments that made the revised manuscript significantly strengthened.

5) Many graphs are not properly described. For instance Fig 6 has a Y axis named "The adhesion rate". What does it mean? Fig 4 has a Y axis named "The protein level". Is it absorbance?

Response: We have overlooked these points in the original manuscript. The adhesion rate in Fig.6 means the cell adhesion rate. In detail, cells were seeded onto the Matrigel-coated wells, respectively. After being incubated, the wells were washed and the cells adhered to the Matrigel firmly were lysed in MTT. Then DMSO was added into each well and spectrometric absorbance was measured at wavelength of 490 nm, the cell adhesion rate was determined as follow: (value of experimental group/value of blank control −1)×100%. "The protein level" in Fig.4 is not the accurate name, it should be replaced with "value (XTLY/GAPDH) ", which means the band integrated optical density (IOD) ratio of XTLY and GAPDH. We have modified it in the revised manuscript. Thanks a lot for this valuable advice!

Minor Manuscript will benefit from professional language editing.

Response: About the English writing of the manuscript, we have carefully revised the paper before it was resubmitted to the magazine and invited a teacher who had worked in U.S.A. for a long
time to amend this manuscript. I don’t know whether it has reached to your magazine’s standard.