Author’s response to reviews

Title: Establishment and characterization of pleomorphic adenoma cell systems: an in-vitro demonstration of carcinomas arising secondarily from adenomas in the salivary gland.

Authors:

Satoshi Maruyama (maru@dent.niigata-u.ac.jp)
Jun Cheng (jun@dent.niigata-u.ac.jp)
Susumu Shingaki (shingaki@dent.niigata-u.ac.jp)
Takashi Tamura (ttamura@kyorin-u.asc.jp)
Shuichi Asakawa (asa@dmb.med.keio.ac.jp)
Shinsei Minoshima (mino@hama-med.ac.jp)
Yoshiko Shimizu (yshimizu@kyorin-u.asc.jp)
Nobuyoshi Shimizu (shimizu@dmb.med.keio.ac.jp)
Takashi Saku (tsaku@dent.niigata-u.ac.jp)

Version: 4 Date: 27 June 2009

Author’s response to reviews: see over
June 27, 2009

Dr. Melissa Norton, Editor-in-chief
Dr. Diana Marshall, PhD, Scientific Editor

MS: 1860293842287409

Dear Editors:

Thank you for your e-mail of June 10, 2009 with comments from the reviewers. We are grateful to the reviewers for their helpful remarks. Following the advice, we have modified our manuscript. I am resubmitting our revised version of MS: 1860293842287409 entitled “Establishment and characterization of pleomorphic adenoma cell systems: an in-vitro demonstration of carcinomas arising secondarily from adenomas in the salivary gland.”

Our incorporation of the reviewers’ suggestions is on next pages

I believe the manuscript has been improved satisfactorily and hope it will be accepted for publication in BMC Cancer.

Sincerely yours,

Takashi Saku, DDS, PhD
Division of Oral Pathology
Department of Tissue Regeneration and Reconstruction
Niigata University Graduate School of Medical and Dental Sciences
2-5274 Gakkocho-dori, Chuo-ku, Niigata 951-8514, Japan
Phone: (+81) 25 227 2832
Fax: (+81) 25 227 0805
e-mail: tsaku@dent.niigata-u.ac.jp
Establishment and characterization of pleomorphic adenoma cell systems: an in-vitro demonstration of carcinomas arising secondarily from adenomas in the salivary gland.
Satoshi Maruyama, Jun Cheng, Susumu Shingaki, Takashi Tamura, Shuichi Asakawa, Shinsei Minoshima, Yoshiko Shimizu, Nobuyoshi Shimizu and Takashi Saku

1) Ethics - Please state that ethical approval was obtained for the work in mice reported in the manuscript. Experimental research on animals must follow internationally recognized guidelines.

   We have added the sentence “The experimental research of animals was also reviewed and approved by the Niigata University Graduate School of Medical and Dental Sciences Ethical Board.” (current version: page 13, lines 16-18).

2) Competing interests - please reword the Competing interests declaration so it reads 'The authors declare that they have no competing interests'.

   Following the scientific editor’s suggestion, we have changed the words for the declaration. (current version: page 25, lines 2).

3) We recommend that you copyedit the paper to improve the style of written English. If this is not possible, you may need to use a professional copyediting service. Examples are those provided by the Manuscript Presentation Service (http://www.biomedes.co.uk), International Science Editing (http://www.internationalscienceediting.com/) and English Manager Science Editing (http://www.sciencemanager.com/). BioMed Central has no first-hand experience of these companies and can take no responsibility for the quality of their service.

   We have checked spelling carefully throughout the manuscript. In addition, we have had this revised version checked by a native professional editor. We believe it has been improved satisfactorily.

We would also request that you go through the manuscript formatting checklist one more time and ensure that your revised manuscript conforms to all of the points. The link to the formatting checklist is provided at (http://www.biomedcentral.com/info/ifora/medicine_journals).

Yes, we have done this.
Reviewer 2: Ivana Magnani

Although most of the revisions have been considered by the authors some interventions in the DISCUSSION and CONCLUSIONS are needed:

Discussion:
line 3: delete ?have?  
We have deleted the words as pointed by the reviewer (current version: page 20, line 3).

line 21: consider to start with ?Interestingly??  
We have added the word “Interestingly” (page 20, line 21).

line 24 to 38: the authors attempt to discuss that squamous cell carcinoma observed in xenografts are different from those of the oral mucosal origin supporting a pleomorphic adenoma origin. This point should be more concise.  
Following the reviewer’s suggestion, we have shortened the paragraph from 18 lines to 12 lines.

line 40: delete ?not only? and ?but?  
We have deleted the words and replaced them with just “and” as pointed by the reviewer (current version: page 21, line 9).

line 45: delete ?for? and put ?of?  
We have replaced the word as pointed by the reviewer (current version: page 21, line 13).

Conclusions:
line 3 to 9: the text contains some too speculative sentences : adenoma-carcinoma sequence ??; some cell groups containing cancer stem cells??..;  
Consider to simplify as follows:  
The present data suggest that pleomorphic adenoma contains cells with genetic alterations even when its histology is benign and that carcinoma cells may develop from some of the population of benign forms. If the atypical cells within benign pleomorphic adenoma (3) can be the direct source for malignant transformation is hard to say. Since??????..
We have revised the paragraph exactly as suggested by the reviewer. (current version: page 23, line 18 to line 22).
Reviewer 3: William C Kisseberth

Major compulsory revisions:

The authors have added the requested Table summarizing "tumor take" for the xenograft experiment; however, they do not address the lack of corresponding xenograft data for the primary tumor (data which they do have for the karyotyping studies). Presumably, this was not done originally and viable tissue is no longer available for transplantation? This, coupled with the very limited number of xenografts done per cell "system" does seriously limit the conclusions that can be made.

Following the reviewer’s previous advice, we have added one table for the result of xenograft experiments of the five pleomorphic adenoma cell systems (Table 3). However, we cannot understand what the reviewer meant by “xenograft for the primary tumor” for the second version. We did not transplant the primary tumor tissue, but only tried xenografts by the five established pleomorphic adenoma cell systems. The histology in the transplanted tumors suggested their pleomorphic adenoma origin as described in the result "Xenografts of SM-AP cells in nude mice." The conclusion must be reasonable enough based on the obtained results.