Author's response to reviews

Title: Identification of valid reference genes for gene expression studies of human stomach cancer by reverse transcription-qPCR

Authors:

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Version: 3 Date: 24 May 2010

Author's response to reviews: see over
Dear Dr. Sültmann,

Thank you for reviewing our revised manuscript (MS:2063659324330046) entitled “Identification of valid reference genes for gene expression studies of human stomach cancer by reverse transcription-qPCR” and determine it as acceptable after correction of some errors.

Enclosed is our error corrected manuscript in accordance with the reviewers’ comments. In addition, we checked and corrected the format of author list and tables as Editorial Production Team requested for final acceptance in BMC Cancer.

Please find the changes made on the following pages and let us know if you need any further assistance for the processing this manuscript. Thank you.

Sincerely,

Sung-Ho Goh PhD

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Response to Reviewer's report-1

Title: Identification of valid reference genes for gene expression studies of human stomach cancer by reverse transcription-qPCR

Version: 2 Date: 12 April 2010

Reviewer: Ruprecht Kuner

Reviewer's report:
The authors sufficiently answered and corrected all important issues.

Level of interest: An article whose findings are important to those with closely related research interests

Response: Thank you.

Response to Reviewer's report-2

Title: Identification of valid reference genes for gene expression studies of human stomach cancer by reverse transcription-qPCR

Version: 2 Date: 4 May 2010

Reviewer: Olaf J.C. Hellwinkel

Reviewer's report:
The paper from Rho et al. is dedicated to the analysis and discussion of several housekeeping genes for application as internal controls in quantitative RT-PCRs on stomach cancer. As the authors state, the choice of suitable reference transcripts is critical, as it depends on the tissue type analysed; indeed, several studies demonstrated, that transcription activity of various housekeeping genes varies considerably from cell type to cell type. For the normalisation of gene activities in stomach cancer measured by quantitative RT-PCRs, so far no methodical analysis has been published.
Here, the authors test six housekeeping genes selected earlier by a medline-analysis for their applicability as internal controls in stomach cancer and cell lines by partially computer automated comparisons of their Cp-values (determined by real-time RT-PCRs). As a consequence of their study, RPL29 and RPL29-B2M are assigned to be the best reference genes for RT-qPCR on stomach tissues, while B2M and B2M-GAPDH are recommended for stomach cancer cell lines.

The increasing number of molecular analyses on tumours and their potential applicability in clinical diagnostics emphasize the importance of proper internal standardisation of tumour-relevant gene activities. Taking this in mind, it is clear that the findings described by the authors just represent first line results which should be further tested on larger stomach cancer collectives. However, this compact but well designed study applies appropriate analytical tools and thus provides substantial and interesting hypotheses on reference genes putatively appropriate for quantification purposes on stomach tissues and cell lines.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Response:** Thank you.

**Response to Reviewer's report-3**

**Title:** Identification of valid reference genes for gene expression studies of human stomach cancer by quantitative real-time RT-PCR

**Version:** 2 **Date:** 23 April 2010

**Reviewer:** Zoltan Konthur

**Reviewer's report:**
Minor essential revisions:
The manuscript still contains some inconsistency in the definition of RT-qPCR:
Response: Thank you for detailed comments on the manuscript. We addressed every comment on the manuscript.

Please amend these for clarification:
Page 4, second line should read: Reverse transcription quantitative real-time polymerase chain reaction...
Response: We changed the second line on page 4 as “Reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR) is a powerful tool…”

Page 8, line 8 should read: Reverse transcription quantitative real-time PCR (RT-qPCR)
Response: We changed the subtitle on line 8 of page 8 as “Reverse transcription quantitative real-time PCR (RT-qPCR)”

Discretionary revisions:
Page 5, line 7 should read: ... makes early detection very important.
Response: On line 7 of page 5, It was changed as “... which makes early detection very important.”

Page 5, line 12 should read: ... for valid comparisons between expression of ....
Response: On line 11-12 of page 5, it was changed as “But appropriate reference genes have to be identified for valid comparisons between expressions of normal versus cancer genes.”
Page 5, line 23 should read: please include gene name for beta-tubulin in the brackets
Response: On line 23 of page five the gene symbol of beta-tubulin was inserted as “beta-tubulin (TUBB; 1 case; 0.9%)”.

Page 8, line 9 should read: Based on previous reports,…
Response: We changed line 9 of page 8 as “Based on previous reports, we adopted primers…”.

Page 10, line 6 should read: … confirming that...
Response: We changed line 6 of page 10 as “…confirming that…”

Page 19, line 14 should read: …statistical data, …
Response: We corrected line 14 of page 19 as “…by statistical data…”

Page 20, line 8 should read: …higher transcriptional activity...
Response: We corrected line 8 of page 20 as “higher transcriptional activity”

Page 20, line 11 should read: … candidate reference genes under the ...
Response: We changed line 11 of page 20 as “…candidate reference genes under the suggested rules”.

Page 20, line 19 should read: … in terms of the abundance of reference ...
Response: Line 11-12 of page 20 was changed as “Third, in terms of the abundance of reference genes…”

Page 20, last sentence needs rephrasing.
Response: For the clear understanding we rephrased the last sentence of page 20 as “However, in reality it is hard to find out genes showing exactly same amount of expression. Therefore, it is advisable to use the lesser different reference gene to give out the more accurate interpretation.”

Page 21, line 13 should read: ... considered suitable.
Response: We changed the line 13 of page 21 as “…considered suitable one.”

Other changes requested by Editorial Production Team

Major Revisions:
Author List: the list of authors in the manuscript should be written exactly as it is in the submission system, both in style and order. The preferred style is ‘First name Initial Last name’ (e.g. Joe F Bloggs).
Response: We confirmed the order and style of author list and it was correct. None of the author has middle name, so there is no middle name initial. However, there is a hyphen in all of the first name.

Tables: the first table referenced in the manuscript text should be labeled Table 1. The second, Table 2, etc. The tables should be provided in this same order at the end of the manuscript file. Please reorder the table citations numerically.
Response: We changed the order of Table 2 as Table 1, and Table 1 as Table 2, and reordered on the revised manuscript. The caption in the manuscript text was corrected according to the order of Tables.
Minor Revisions:

Email address: For the authors' email address list, please use the following format as an example:

Response: We changed e-mail addresses as follows on title page.

E-mail addresses:
H-WR: skyrkdp@ncc.re.kr
B-CL: id3701@ncc.re.kr
E-SC: chemk01@naver.com
I-JC: cij1224@ncc.re.kr
Y-SL: yslee2@ncc.re.kr
S-HG: andrea@ncc.re.kr

Line numbering: Please remove.

Response: We removed line numbering as it was requested.

Typography:

Response: We checked typographical error and could not find it.