Author’s response to reviews

Title: RNA-binding protein RNPC1: acting as a tumor suppressor in breast cancer

Authors:

Jin-Qiu Xue (xuejinqiu_1986@163.com)
Tian-Song Xia (xiatsswms@163.com)
Xiu-Qing Liang (517644520@qq.com)
Wenbin Zhou (zhouwenbin_1984@foxmail.com)
Lin Cheng (694427729@qq.com)
Liang Shi (337039607@qq.com)
Ying Wang (602184984@qq.com)
Qiang Ding (dingqiang@njmu.edu.cn)

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Author’s response to reviews: see over
Dear Editors and Reviewers:

Thank you for the editor’s contribution and the reviewers' comments concerning to our manuscript entitled "RNA-binding protein RNPC1: acting as a tumor suppressor in breast cancer" (ID: 6460663561151965). Those comments are valuable and very helpful for improving our paper, as well as our further study. We have addressed every comment carefully and made correction which we hope could meet the approval.

We appreciate for Editors/Reviewer's warm work earnestly, and I revised my paper point-by-point.

Once again, thank you very much and the review again for your help.

Best Regards,
Qiang Ding
Jiangsu Breast Disease Center, The First Affiliated Hospital with Nanjing Medical University, 300 Guangzhou Road, 210029 Nanjing, China.
Email: dingqiang@njmu.edu.cn
Responds to the editor’s and reviewer’s comments

All the corrections and the responds to the review's comments were displayed point-by-point as follow:

Responds to the editor’s comments

Q: This is an interesting article that can be considered for publication on BMC Cancer if the authors address satisfactorily the reviewer’s questions.
Response: We are appreciated for the editor’s contribution to our manuscript. We are very appreciated for the editor’s positive comments on our manuscript. According to your suggestions, we have addressed the reviewer’s questions. The detailed descriptions about these modifications were presented in response to reviewers.

Responds to the reviewer's comments

Response to Reviewer #1 (the changes according to reviewer 1’comments are marked in blue in the Revised Manuscript with Tracked Changes)

Reviewer#1
Q1: On the technical side, off-target effects must be addressed with additional independent RNPC1 shRNA sequences.
Response: We are very appreciated for the reviewer’s positive comments on our manuscript. As the reviewer mentioned, we also cared about the additional independent RNPC1 shRNA sequences. In fact, we have transfected the additional independent RNPC1 shRNA sequences to MCF-7 and MDA-MB-231 cell lines. In the previous manuscript, we focused on the $\geq 85\%$ knockdown efficiency of MCF-7 and MDA-MB-231 cell lines, and did not show the off-target effects data. We showed this data in the Revised Manuscript with Tracked Changes. (Page 6, Line 150-151, 158; Page 25, Line 620-629; Additional Figure S3 and Table 1)

Q2: The Western blot results need to be quantified to show the relative fold change of proteins.
Response: We are appreciated for the reviewer’s suggestion and showed the Figure 1A and D; Figure 2 A and E; Figure 5; Figure S3A and C and related descriptions on Figure
1A and D; Figure 2 A and E; Figure 5; Figure S3A and C in Revised Manuscript with Tracked Changes. (Page 23, Line 540-542; Page 23, Line 548-550; Page 23, 556-557; Page 23, Line 564-565; Page 25, Line 598-600; Page 25, Line 620-629)
Response to Reviewer #2 (the changes according to reviewer 2’ comments are marked in red in the Revised Manuscript with Tracked Changes)

Reviewer #2

Q1: Response to comment: The authors should reorganize the text and figures.
Response: We are very appreciated for the reviewer’s advice. We have checked the text and figures carefully. We have reorganized the text and figure1 in the Revised Manuscript with Tracked Changes. (Page 2, Line 39-43; Page 5, Line 123-135; Page 6, Line 141-144; Page 7, Line 181, 189-194; Page 9-10, Line 224-251; Page 10, Line 261-262, 264-272; Page 13, Line 332-333; Page 14, Line 365-370; Page 14, Line 372-375; Page 23, Line 553-554; Page 23, Line 557-563; Page 24, Line 570-583, 586-591; Page 25, Line 620-629)

Q2: In Fig 1A-B, the authors should use several normal mammary cell lines as control to rule out the cell line-specific expression of RNPC1.
Response: We are very appreciated for the reviewer’s suggestion. We have added other normal mammary cell lines 184A1 as control and displayed on Figure 1A in the Revised Manuscript with Tracked Changes. (Page 8, Line 143-144; Page 9, Line 217, 218, 220; Page 13, Line 319)

Q3: There are two bands in Western blot of RNPC1. It is unclear which band is RNPC1 and used to quantify the expression of RNPC1.
Response: We are very appreciated for the reviewer’s suggestion. We have marked RNPC1 with added arrows, and the fold change of RNPC1a is shown below each lane. Changes have made in Figure 1A and D; Figure 2 A and E; Figure 5 and Figure S3A and C related descriptions on Figure 1A and D; Figure 2 A and E; Figure 5 and Figure S3A and C in Revised Manuscript with Tracked Changes. (Page 23, Line 540-542; Page 23, Line 548-550; Page 23, 556-557; Page 23, Line 564-565; Page 25, Line 598-600; Page 25, Line 620-629)

Q4: Response to comment: In Fig 1E, due to the significant difference of loadings, it is
Response: We are very appreciated for the reviewer’s comments on the Figure 1E. We re-adjusted the expression of loadings and denoted RNPC1. We compared the expression of gene between tumors and normal tissues by calculating the expression ratio between band and GAPDH (ref: PMID 23289900). We have re-organized previous Figure 1E. Moreover, Figure D and E have re-organized according to the reviewer’s suggestion in the Revised Manuscript with Tracked Changes. (Page 9, Line 238-240; Page 23, Line 547-548)

Q5: Since RNPC1 is one of targets of p53, the level of RNPC1 in breast tumors may just depend on the p53 status. This possibility is demonstrated in Fig 1B. Breast cancer cell lines with a wild-type p53 (MCF7 and ZR75) or a temperature-sensitive mutant p53 (BT474) express higher level of RNPC1 compared to mutant p53 cell lines (MDA-MB231 and SUM1315). In this study, at least, 52/121 of breast tumors have a mutant p53. Thus, it is also possible that the correlation between RNPC1 expression and clinical stages actually represents the correlation between the p53 status and clinical stages. RNPC1 is just an accompanying factor of p53 status-dependent clinical stages in patients with breast cancer.

Response: We are appreciated for the reviewer’s suggestions and comments. We have carefully thought about reviewer’s comments and agreed with reviewer’s interpretation. In fact, it is a really great encouragement to us. Just like ER regulating PR pathway, and both of them making the most important molecular markers of breast cancer, RNPC1 could develop to a novel molecular maker as a downstreaming factor of p53. We have stated this valuable suggestion according to reviewer’s suggestion in the Revised Manuscript with Tracked Changes (Page 14, Line 365-370)

Q: Quality of written English: Needs some language corrections before being published.
Response: We are very appreciated for the reviewer’s comments on quality of written English. We have made carefully correction according to the Review's comments. We tried our best to improve some language corrections in the manuscript. These changes
will not influence the content and framework of the paper. And here we did not list the changes but marked in red in revised paper (Page 3, Line 76; Page 7, Line 186)

Q: Statistical review: Yes, but I do not feel adequately qualified to assess the statistics. Response: We are very appreciated for the reviewer’s positive comments on our statistical analysis. We have re-written this part according to the Reviewer's suggestion in the Revised Manuscript with Tracked Changes. (Page 8, Line 210-214)