Author's response to reviews

Title: Mapping the interactome of overexpressed Raf kinase inhibitor protein in a gastric cancer cell line

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Version: 2  Date: 13 October 2013

Author's response to reviews: see over
October 13, 2013

Editors of BMC Cancer

Dear Editors:

We would like to resubmit online our manuscript entitled “Mapping the interactome of overexpressed Raf kinase inhibitor protein in a gastric cancer cell line”, written by Huan Gu, Xianquan Zhan, et al., for publication in the journal *BMC Cancer*.

Enclosed are the point-by-point responses to the reviewer comments.

We would like to hearing from you.

Sincerely,

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Responses to the reviewer comments.

Many thanks for the very helpful comments on our manuscript. We took all comments into consideration and revised our manuscript in bold font. Enclosed are the point-by-point responses to each comment.

Reviewer: Dominic M. Desiderio
“Reviewer’s report:
The authors studied the overexpression of Raf kinase inhibitor protein (RKIP) and its interactome in a gastric cancer cell line. RKIP fusion proteins were purified, and RKIP-interacting proteins were analyzed with electrospray ionization (ESI) tandem mass spectrometry (MS/MS) to obtain amino acid sequence data. Interaction complexes were further analyzed with Western blot and co-immunoprecipitation to corroborate MS/MS data. The authors found 72 RKIP-interacting proteins, and an interaction complex among RKIP, HSP90, 14-3-3#, and keratin 8. Those data clarify some of the molecular events that participate in gastric cancers. Three separate databases were used to develop interaction network diagrams, and three levels of interactions were found. In all
three database analyses, 35 proteins consistently associated with RKIP. Those data, plus significant changes found previously, led to the finding that HSP90, 14-3-3#, and keratin 8 interact with RKIP. This study could lead to the use of those RKIP-interacting proteins as early-stage biomarkers and therapeutic targets for gastric cancer. This manuscript is well-written, contains solid experimental data, is easy to read, and presents useful clinical data to detect and treat gastric cancers. It can be published without revision.”

Response: Thanks for the positive comments.

“Level of interest: An article of outstanding merit and interest in its field
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests.”

Response: Thanks for the positive comments.

Reviewer: Zhousheng Xiao
“Reviewer's report:
Minor Essential Revisions:
Raf kinase inhibitory protein (RKIP) has been reported to play an important role in cancer development. However, how RKIP interacts with other proteins in gastric cancer remains unclear. The authors applied tandem mass spectrometry (MS/MS) to identify a total of 72 RKIP-interacting proteins using sequential experimental protocols. The manuscript provides us a first evidence for interactome of RKIP in gastric cancer. It is an interesting and novel paper in this field, but there are some concerns about this research article.”

“1, Figure 2 needs to be clarified. Where is blank carrier group? Where does RKIP transfect group come from? This group was not mentioned in the method section. Also, please mark the figure with anti-RKIP and anti-β-actin if you use the dilution of antibodies.”

Response: Thanks for the comments on the experimental details. Our experiments had a total of four groups: SGC7901 cells transfected with pcDNA3.1-RKIP-3xFLAG plasmid (RKIP-3xFLAG group), SGC7901 cells transfected with pcDNA3.1-3xFLAG plasmid (3xFLAG group), SGC7901 cells transfected with pcDNA3.1-RKIP plasmid (RKIP group), and SGC7901 cells (Blank group). The BLANK in the Figure 2 was actually 3xFLAG group. It was revised in the Figure 2 and in the corresponding text. Also the label in the
Figure 3 was revised.

“2, The manuscript needs professional English editing and check the spelling errors (for instance, RAS/RAF/ERK/MEK signal) to improve the quality of the paper.”

Response: It was checked and edited carefully through the whole manuscript.

“3, The discussion section needs to be revised. It is too long and not organized very well. Authors should emphasize their findings on Ras/Raf/MEK/ERK pathway in this RKIP interactome and elucidate the significance from reviewing the literature reports since they don’t have functional data to support this hypothesis.”

Response: We would prefer the format of the current discussion. RAS/RAF/MEK/ERK is a pathway, but not only one pathway in the RKIP interactome. Also, other reviewer such as Dr. Desiderio agrees with the format of this discussion.

“Level of interest: An article of outstanding merit and interest in its field
Quality of written English: Needs some language corrections before being published
Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.
Declaration of competing interests:
I declare that I have no competing interests”

Response: Thanks for the positive comments.

Reviewer: Josip Blonder
“Reviewer's report:
Manuscript ID: 1408846617101466_article August 9, 2013

General comments
In the manuscript titled “Mapping the interactome of overexpressed Raf kinase inhibitor protein (RKIP) in a gastric cancer cell line”, Gu et al report identification of a total of 72 RKIP-interacting proteins identified by MS/MS. Of these RKIP, HSP90, 14-3-3#, and keratin 8) were confirmed by Western blot analysis and co-immunoprecipitation after a total of 3 RKIP-interaction protein network diagrams were constructed using different software to map molecular pathways of the functional activity of RKIP. Although interesting, this study suffer from ambiguities mentioned in specific comments that should be address before the decision of suitability for publication is made”
Specific comments:
1. Page 2, abstract; the manuscript reads: “The MS/MS-characterized components of the existing interaction complex (RKIP, HSP90, 14-3-3#, and keratin 8) were confirmed by Western blot analysis and co-immunoprecipitation.” The MS/MS spectra of the corresponding peptides identifying these four proteins should be the part of the manuscript or shown as supplementary data.

Response: The MS/MS spectra of the corresponding peptides identifying these proteins were provided as Supplemental Table 2.

2. Page 13, purification of RKIP fusion proteins; the manuscript reads: “After the affinity-magnetic bead purification, with anti-flag M2, of the total protein from the cells, most of the protein sample was pre-separated by 1D-SDS-PAGE using a 10% acrylamide gel. The experiment was repeated three times with the same test conditions and parameter settings, and then the gel images were obtained with clear backgrounds, high resolution, and good reproducibility. A total of 14 RKIP interacting protein bands were identified (Figure 3A).” It is intriguing that heavy or light Ab chain is not visualized in the FLAG line.

Response: We used the anti flag M2 magnetic beads (not anti-flag antibody), therefore, no heavy or light Ab is visualized in the FLAG line. The corresponding text was added in the Figure 3 legend.

3. Page 15, RKIP-interaction protein networks and validation of the RKIP-protein complex.; the manuscript reads: “In the protein network diagram that was derived from the database retrieval with MiMI, among the 72 RKIP-related proteins, 16 proteins were classified as the 1st level neighbors of RKIP, 19 proteins were classified as the 2nd level neighbors of RKIP, 29 proteins were classified as the 3rd level neighbors of RKIP, and 8 proteins were found to not interact with RKIP (Figure 5A, Table 2).” Since all of the three validated proteins were labeled at least once as the 2nd level neighbors a cross validation of well known 1st level neighbors is required.

Response: As Figure 5 and Table 2 shown, Predictome analysis revealed that three validated proteins were all located as the 1st level neighbors; Functional lineage network analysis revealed that two of three validated proteins were located as the 1st level neighbors, one of three validated proteins was located as the 2nd level neighbors; MiMI analysis revealed that three validated proteins were all located as the 2nd level neighbors. Because Predictome, Functional Linage network, and MiMI are three independent software, they are actually a cross validation. Therefore, the results from Predictome and from Functional lineage network analysis can further confirm three validated protein were
interacted with RKIP. Moreover, the discussion has been addressed in the last paragraph in the section of Results.

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

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests: I declare that I have no competing interests”

Response: Thanks for the positive comments.