Author's response to reviews

Title: A microRNA Molecular Modeling Extension for Prediction of Colorectal Cancer Treatment

Authors:

Jian Li (simon_li82@hotmail.com)
Ulrich Robert Mansmann (mansmann@ibe.med.uni-muenchen.de)

Version: 3 Date: 1 April 2015

Author's response to reviews: see over
Dear Editor,

sincerest thanks for your response and reviewers comments. We have modified the manuscript in response to the extensive and insightful reviewer comments and addressed all the changes recommended by the reviewers. We also have rewritten many parts of manuscript to comply with referees' remarks. With this new version of manuscript, we are confident that it is easier to be understand and has a more fluent scientific discourse. The revisions, starting with the last submission, are addressed below:

**First Reviewer: Malay MB Bhattacharyya**

**Requirements**

1. Please rewrite the portion “Since clinical outcome data for these patients is not available” in the Abstract as “Since clinical outcome data for these patients are not available”.

   **Answer:** this sentence has been rewritten. We performed a general language polishing.

2. In the third sentence of page 8 “to includemiRNA” should be “to include miRNA”.

   **Answer:** error corrected.

3. In the last line (subsection header) of page 7: give a space between “(NSAID)Model”.

   **Answer:** error corrected

4. The first complete sentence (“The p-value to show ...”) in page 7 should be rewritten. It is erroneous.

   **Answer:** this sentence has been rewritten.

5. In page 8: “The Model Availability” can be written as “The Availability of the Model” or simply “Availability”.

   **Answer:** modified

6. when you write "cancer stage (III) did not respond" then it appears like a separate point number (III). Why don't you write "stage III" or "stage 3" only?

   **Answer:** changed

7. The Conclusion (with too much details) can be separated into Discussion and Conclusion portions

   **Answer:** the conclusion part has been improved in the revised manuscript.
Second Reviewer: Yajun Yi

I. MAJOR COMPULSORY REVISIONS

1. Data presented in the study is not sufficient to support the conclusion as indicated in the manuscript, e.g., “miRNA expression data contribute to making clinical decisions, including identification of the optimal drug with regard to individual patients”.

Answer: Thank you for this suggestion. We improved the section in the new version accordingly. We were too enthusiastic about our model and did rewrite the paper with more caution regarding clinical applications. This is an aspect of our ongoing research. We try to work on real patient data to be able to compare prospectively our predictions with the patient’s clinical response. We hope to be able to finish this study in one or two years.

2. In the study, miRNA results of CRC cell lines seems independent of the miRNA data in CRC patients.

Answer: We performed Spearman's rank correlation for miRNA expression profile from CRC cell lines and CRC patients. The rho correlation is 0.58 (p<0.0001). Therefore, we could assume that both types of profiles share certain similarity. However, the translational link for the CRC cell lines to CRC patient treatment is not only the similarity of miRNA profiles, but also the entire molecular mechanism including signaling pathway, other transcriptional regulation and the effects of targeted therapy.
3. Most drug agents used in CRC cell lines are tyrosine inhibitors, and there are only two out of 10 drug agents in CRC cell line model (Everolimus and Temsirolimus) targeting to mTor protein that are the same type of chemotherapy agents for CRC patients. Can authors further calculate the specificity and sensitivity of prediction in CRC cell line model for these two related agents (Everolimus and Temsirolimus) by comparing predicted sensitivity with actual experimental response (GI50 cutoff at 1)?

**Answer:** Since GI-50 values of CRC cell lines for Everolimus and Temsirolimus range from 4.91 to 7.94, the setting cutoff value of GI50 to 1 would increase sensitivity of prediction and decrease its specificity. But we did compare the calculated response per cell line with the corresponding GI-50 value. This showed a high correlation:
This way we support our claim that the hallmark score does reflect the real fate of the cell under the treatment given. (Black circles: Everolimus treated cell lines; Red Triangles: Temsirolimus treated cell lines; How well Hallmark Score is predictable by GI.50 value is quantified by the R² - Everolimus: Multiple R-squared: 0.9713; Temsirolimus: Multiple R-squared: 0.9824)

4. According to the study, there are about 9~10 out of 22 patients (41%) can be predicted as responders to Sirolimus and LY294002 because of their hallmark proliferation < 1 (Fig. 5). However, CRC Patient clinic outcomes such as pCR/RCB and long-term survival events after Sirolimus and LY294002 treatment are not available in the study. Proliferation' hallmark is not commonly used in clinical settings for justification of chemotherapy response. Thus, there is no easy way to prove that the prediction in CRC patients.

Answer: The hallmark “Proliferation” is actually interpreted as the ability for cellular growth and
proliferation and can be measured as the increase-rate of cell numbers. Like the clinical term “minimal residue disease” (MRD), the MRD is detected number of disease cells after treatment, which is often used in clinical for measure or determine the effect of corresponding treatments. The MRD can be mathematically implemented as the hallmark of “Proliferation”.

What we could calculate was a ROC curve which compares the response prediction between MSI and MSS patients (knowing from literature that MSI should respond better compared to MSS). We did calculate the corresponding AUC value:

![ROC Curve for Sirolimus.MSS and LY294002.MSS](image)

II. MINOR ESSENTIAL REVISIONS

1. Abbreviation for Colorectal cancer (CRC) should be explained when used in the first time (abstract).

   **Answer**: error corrected

2. What miRNA gene records are used in prediction model of CRC cell lines and CRC patients? Are they derived from a common set?

   **Answer**: The prediction model for cell lines and for the patients are the same. There is only one prediction model. We incorporated all miRNA information into our model which was described in the literature and in relevant data bases as influencing CRC. This is using the resources described in the paper by Wang et al. 2010. The data we used to validate the model provided measurements for all these miRNAs which have been implemented into the model.

III. Discretionary Revisions

1. The manuscript could be improved by paragraph of discussion of the limitations of the method.

   **Answer**: the limitation of the method has been discussed in the revised version of manuscript
2. The conclusion section is too long which can be more succinct.

**Answer:** the conclusion section has been improved to be more succinct.

**Third Reviewer: Lorenzo F Sempere**

**Major comments:**

1. Tables and figures are not particularly helpful to illustrate authors’s results and data. Perhaps, displays of miRNA regulatory networks with specific drug treatment that show how miRNA influence response would be a better way to illustrate how these different data sets are integrated.

*Answer:* We created new figures to visualize the treatment effect of the miRNA regulatory network and highlight the key miRNAs whose deregulation contributed to patients responses according to our results.

2. While I don’t fully understand the molecular concept for individualized medicine as described by the authors, it seems to have some resemblance with work by following studies:


*Answer:* Both studies by Ragoussis and colleagues did apply the miRNA and mRNA expression data to reveal possible associations between molecular biomarkers (miRNAs) and novel therapeutic strategy and pathogenesis for breast cancer. In comparison to our study, both studies did not incorporate important biological knowledge into analysis, which is essential for the approach (molecular modeling) of our study. Further, both studies only attempted to discover possible molecular associations, which can hardly provide actionable information towards making medical decision. We did not mention both studies in our manuscript, because the goals and approaches are entirely different from our study.

2b) Mewes and colleagues Nucleic Acids Res. 2014 Dec 1;42(21);

*Answer:* Our manuscript was submitted in the 2014 Jul. to the journal before the publication of this article. Nevertheless, we are appreciated that the reviewer pointed us to this study. This study did have similarity with our study, therefore, we have added the corresponding discussion in our revised manuscript.


*Answer:* goals of these four studies are to present different data sets and tools, therefore they deviated much from the goal of our study, which is to apply an integrated molecular modeling approach for individualized medicine. Thus, these four studies were not mentioned in our manuscript.