Author's response to reviews

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

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Version: 2 Date: 18 July 2014

Author's response to reviews: see over
Munich, July 11th 2014

Dear editor,

Please find enclosed the manuscript (previous submission ID: 1336707998124490): **A microRNA Molecular Modeling-Extension for Therapeutic Prediction of Colorectal Cancer Treatment**, by Jian Li and Ulrich R. Mansmann, to be submitted as an Original Research Article to BMC Cancer. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report. We certify that the submission is original work and is not under review at any other publication.

We sincerest thank 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 and hope that this comply with the referee's remarks. We are confident that the new version of the manuscript is easier to understand and has a more fluent scientific discourse. The revisions, starting with the last submission, are addressed below.

**Manuscript Format Correction:**

1. changed abstract format according to the guideline in BMC Cancer.
2. changed manuscript format according to the guideline in BMC Cancer.
3. formatted the reference list and order them after citation in the manuscript.
4. Listed the author contribution section.

**First Reviewer:**

Major requirements

1) Additional data is available (TCGA) where overlapping mRNA and miRNA profiles exist and should be utilized to determine whether these results are repeatable.

Answer: With information in the “Methods and Materials” and in the “Supplementary Information” from the revised manuscript, the results should be repeatable.

2) This method needs to be tested on a sample set where response to drug is known (Gold Standard) and the predictive capabilities should be better than those predicted by MSS and MSI status alone, allowing identification of a subsets of MSS and MSI patients with better and worse outcomes to be identified in a statistically meaningful manner.
Answer: A sample set where different drug responses were experimentally measured by the study of Holbeck et al. (2010), has been applied in the revised manuscript to validate the predictive ability of the current approach and the result is promising.

3) It is not clear whether the incorporation of miRNA transcript level and regulation led to improvements or even changes in the model. Showing improvement of the model by incorporation of miRNA is essential.

Answer: In the result part of the revised manuscript, it is shown that without incorporation of miRNA transcript data, the prediction rate for drug-response is clearly reduced, which indicates the essential value of this type of data for better understanding the molecular mechanisms.

4) It is not clear whether the drug treatments are modeled appropriately, to address this test a few well known nodes to determine how these generally affect "hallmark" proliferation (like MYC). Clear differences exist in the miRNA and mRNA transcript levels between dMMR and pMMR samples. These differences may be responsible for the differential responses rather than the drug mediated FCA.

Answer: the primary goal of this study is to utilize diverse types of information including genetic information in order to perform therapeutic response prediction. The hallmark “proliferation” directly summarizes the signal flux from MAPK-, WNT-, MTOR-, HIF1-signaling, which is shown in the revised manuscript to be able to reflect the growth-strength of different CRC cell lines under different types of drug treatments. This result provides an indication that signal flux from a large-scale molecular network could be able to present dynamic property of underlying cellular system.

5) It is not clear how many "hallmarks" were tested and thrown away due to lack of expected correlation.

Answer: In the revised manuscript, it is explained that only the hallmark “proliferation” is currently in use and represents the growth-strength that the model system reflects. The main reason for only using the “proliferation” as readout component of FCA analysis is that this study is focusing on therapeutic response prediction, which is mainly related to this hallmark. This case is similar as the case that the GI50 value (indicates the maximal drug concentration to achieve the 50% cell growth inhibition) has been used to clarify how well a cell line can response to a type of drug treatment. Other three hallmarks represent corresponding aspects of tumor development that can not provide so clear indication of therapeutic responses as the hallmark “proliferation” does.

6) The paper is conceptually difficult to read and needs clear separation into method, supported results and hypothetical discussion right now the majority of the results are hypothesis which are not tested. Fundamental factors are not labeled on the figures MSS, MSI state, ethnic background. Labels are to small to be readable in Figure 4.

Answer: In the revised manuscript, improved concept of the experiment-design has been performed with validation of simulation data. For instance, the simulated sensitivity-scores of different CRC cell lines are compared to the experimentally measured sensitivity-scores of the same CRC cell lines and reached high correlations. This result provides a validation evidence for the hypothesis. The labels of
figure 4 are improved, too.

(This study has found many important molecular components (protein, miRNA) that are highly deregulated to be the main reason for the pathogenesis, which are also in agreement with other independent studies).

Second Reviewer:

Major comments:

1. The Result portion of the Abstract is very poorly written. The single long sentence is also not grammatically correct. It should be improved. Try to include more technical details.

Answer: The result portion of the abstract is improved accordingly.

2. It will be better to include the details about NSAID model for the completeness of the paper. The inclusion of NSAID happens suddenly without any background details or justification. Again, better to mention what stands for NSAID.

Answer: Explain the mean of NSAID and summaries the NSAID model's role and implication in the current manuscript.

3. Several portions of the paper are not technically justified with full details. Have the authors included tissue-specificity information? How they might (or not) be useful here? What do the authors mean by writing that the miR- and gene-expression data is available for their study (see lines 263-264 in the materials section), while the additional descriptions appear later. Again, on what basis the regulatory databases (TF to microRNA and microRNA to gene) have been chosen is not clear. TransmiR includes very small amount of validated information about TF to microRNA regulation. What about the large-scale putative databases like PuTmiR? Again, what about the inclusion of tools like TarBase for microRNA gene regulation information? The clinical details are required to be comprehensive. Also, the Fig. 2 is not fully understandable.

Answer: In the “Materials and Methods” part of improved version of manuscript, it is precisely explained that these CRC data contain tissue-specific information and was produced by analyzing high-quality colon tumor tissues of corresponding participants. The reason for applying such high quality tissue-specific data is to give the concept MCIM a high quality input.

The mean of the sentence “miR- and gene-expression data is available for our study” is only intended to explain that these data are made available in the publicly accessible resource.

Although the transcriptional regulation information from the PuTmiR is larger than it from the TransmiR, the information from the PuTmiR contains much less literature references and does not explain which type of transcriptional regulation it involves for each transcription factor. Therefore, the
PuTmiR was not selected for the current modeling process. In the improved version of manuscript, it is shortly explained the reason for choosing the latest version of TransmiR.

The Fig. 2 depicts a reaction pattern to visualize the algorithm of miR-add-on, which adds the information of transcriptional regulation of miRNAs and miRNA-targets into the current model. As its legend explains, this figure visualizes seven types of biochemical reactions.

4. The results should be supported with robust statistical analyses. At no point, the authors have used (or mentioned) statistical tests or adopted robust statistical validation while presenting the results. This weakens their claims.

**Answer:** In the improved version of manuscript, the Spearman correlation tests were performed to evaluate the comparison between simulated sensitivity-score and experimentally measured sensitivity-score in order to validate whether the introduced approach in the manuscript could really predict the drug responses of different CRC cancer cell lines.

5. The quality of English is poor throughout the paper. I strongly suggest revising the paper. It has many grammatical errors, typos, construction problems. Some of these are highlighted in the Minor comments section follows, however, they are not comprehensive.

**Answer:** A native-English speaker has been employed to revise the manuscript regarding language issues.

Minor Comments:

1. Both the terms ‘miR’ and ‘miRNA’ have been used in the paper to abbreviate ‘microRNA’. Please make this consistent.

**Answer:** corrected.

2. Page 1, line 15: Write as “have provided various evidences that”.

**Answer:** corrected.

3. Page 3, line 95: Write as “according to the TransmiR”. Please do mention here the version number of TransmiR used by the authors.

**Answer:** corrected.

4. Page 4, line 100: Write as “NSAID-miR and its statistical summarization”.

**Answer:** corrected.

5. Refer to the tables as Table x, not as table x.
Answer: corrected

6. Page 4, lines 104-105: Better to include and cite an appropriate reference here.

Answer: corrected

7. Page 4, line 122: Write as “performed a Flux Comparative Analysis”.

Answer: corrected

8. Page 5, line 145: Write as “respond to the LY-294002 treatment, show high activity”.

Answer: corrected

9. Page 7, line 190: Write as “is consistently reducing”.

Answer: corrected

10. Page 7, line 220: Rewrite the portion “In sum, during this study we give an application example of”.

Answer: corrected

11. Page 9, line 252: Write as “(such as gene array, mRNA array, protein array, reaction array, etc.)”.

Answer: corrected

12. Page 9, line 264: Write as “Primarily, both the data will be”.

Answer: corrected

13. I suggest including the following relevant references in appropriate places.
   i) Sonia A Melo and Raghu Kalluri, Molecular Pathways: MicroRNAs as Cancer Therapeutics, Clin Cancer Res 2012.
   
   ii) Masanori Hotchi et al., microRNA expression is able to predict response to chemoradiotherapy in rectal cancer, Molecular and Clinical Oncology 1: 137-142, 2013.
   

Answer: included these three references in appropriated places in the improved version of manuscript.

We believe that the improved version of our manuscript could be of interest to the readers of BMC Cancer and we hope that the editorial board and reviewers will agree on the interest of this study.
Sincerely yours,

Jian Li and Ulrich Mansmann

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