Author's response to reviews

Title: Deciphering the Mechanism of Indirubin and Its Derivatives in the Inhibition of Imatinib Resistance using a "Drug Target Prediction-Gene Microarray Analysis-Protein Network Construction" Strategy.

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Response letter

Dear Editors:

Thank you very much for your letter and for Reviewers’ comments concerning our manuscript entitled “Deciphering the Mechanism of Indirubin and Its Derivatives in the Inhibition of Imatinib Resistance using a "Drug Target Prediction-Gene Microarray Analysis-Protein Network Construction" Strategy” (ID: BCAM-D-18-01560R2). We believe this manuscript will be interesting to general readers of BMC Complementary and Alternative Medicine. Currently, imatinib resistance presents a major challenge to the treatment of chronic myelogenous leukaemia. Identifying strategies to suppress imatinib resistance is of great significance. Through
the methods of drug target prediction, gene microarray analysis, and protein network construction, 15 small molecule compounds screened from indirubin and its derivatives can pass the cytokine-cytokine receptor signalling pathway and the JAK-stat pathway, the NF-κB signalling pathway inhibits imatinib resistance, indicating that indirubin and its derivatives may be used as new drugs to antagonize imatinib resistance. We obtained imatinib-resistant biomarkers from gene microarray data.

We have carefully evaluated the Reviewers’ comments and suggestions, and responded to these suggestions point-by-point which we hope to meet with approval. We adopted the comments of the reviewers and revised the manuscript in accordance with the reviewers’ comments to make the content of the article more rigorous and richer. Here are my responses to the reviewers’ comments.

Randolph Arroo (Reviewer 1):

(1) In response to reviewer 3, the first part of the conclusion section was rewritten. However, in my opinion lines 306 - 310 are not much clearer than the same section in the previous version of the manuscript.

I suggest to rephrase this section again, e.g.:

Definition of a potential drug target is an important first step in the process of drug discovery and drug design. Gene microarray analysis and protein network mapping can be key tools for identification of the factors that play a role in disease progression and thus are the potential drug targets. Subsequently, molecular docking experiments in silico can be used to predict putative interaction of small molecule compounds with the identified targets. In this study, the mechanism of action of...
Reply: We would like to thank you for your valuable comments and suggestions. In this study, through the methods of drug target prediction, gene microarray analysis, and protein network construction, we explored indirubin and its derivatives in the inhibition of imatinib resistance. By analyzing the microarray data of the gene chip, we identified differentially expressed genes that are sensitive to imatinib in CML patients. Subsequently, through the prediction of drug targets, protein network construction and molecular docking, 15 small molecules that may inhibit imatinib resistance were initially screened. And 15 small molecules were found to inhibit 11 putative targets of imatinib resistance.

Therefore, this study used the "drug target prediction - gene microarray analysis - protein network construction" strategy. Use data mining and computer simulation aids to narrow down the number of potential drug candidates for a particular target. However, this is the first step in drug discovery, followed by structure-activity relationships, in vitro experiments and other in-depth studies to further improve drug screening. This is also the ongoing research of our team and we look forward to new research progress.

Thank you again for your comments. Based on your comments, we have revised the conclusions to make the general statements in the conclusions more logical. The main changes are in lines 306-310.

(2) Also, lines 278-288 are and amended section in the text, again in response to reviewer 3.

Why the sudden change from 'imatinib' to 'IMA' halfway this section? It is confusing.

In the list of abbreviations (line 43, 319), 'IM' is given as abbreviation for imatinib. However, the abbreviation 'IM' is not used anywhere in the text.

It will be best not to use any abbreviation for imatinib, and just use the full word throughout the text (as already is the case in >90% of the current manuscript anyway).
Reply: We would like to thank you for your valuable comments. Based on your comments, we have conducted a comprehensive review of the full text of imatinib. As you said, 90% of the full text of Imatinib is not used for short names, so we removed the abbreviations Abbreviation for imatinib (line 43, 317). And the imatinib format in the whole text was unified, and the modification was mainly in lines 19, 281, and 282.

(3) Finally, reviewer 3 requested that the discussion should be more focused on practical application.

In response, we find lines 233 - 235: "Our findings can help clinicians treat patients with CML more effectively. Effective early prevention of patients who are likely to develop imatinib resistance, thereby improving the overall survival rate of CML patients."

That is not true; clinicians will find little help in the information or in the techniques described in this paper. The information is used in Drug Discovery and Development (line 300, line 306).

The manuscript is an example of data mining, i.e. using existing data to highlight any connections or links in large datasets. The manuscript shows the application of the String online website (https://string-db.org/) and the freeware programme Cytoscape 3.5.1. Data mining is not necessarily expected to lead to new and original conclusions, but the method described in the paper may show an objective and rapid way to spot connections and identify potential drug targets.

It should be emphasized that the methods described are only a first step in the process of drug discovery and development. Any further claims, e.g. use as a tool for clinicians, are exaggerations.
Reply: We would like to thank you for your valuable comments and suggestions. In this study, the "drug target prediction - gene chip analysis - protein network construction" strategy was used to investigate the inhibitory effect of indirubin and its derivatives on imatinib resistance. By analyzing microarray data from gene chips, we identified differentially expressed genes that are sensitive to imatinib in CML patients. Subsequently, by predicting drug targets, protein network construction and molecular docking, 15 small molecules that might inhibit imatinib resistance were initially screened. It was also found that 15 small molecules inhibited the putative targets of 11 imatinib resistance. Therefore, the primary application of this study is the use of data mining and computer simulation aids to reduce the number of potential drug candidates for a particular target. However, this is the first step in drug discovery, followed by structure-activity relationships, in vitro experiments and other in-depth studies to further improve drug screening. This is also the ongoing research of our team and we look forward to new research progress.

Our aim was to investigate the inhibitory effect of indirubin and its derivatives on imatinib resistance. Therefore, as you said, the most important practical application of this research is mainly based on existing databases for data mining, objectively and quickly discovering connections and identifying potential drug targets, and facilitating the discovery of drugs that inhibit imatinib resistance. We removed the original content and added it to the actual application in lines 233-235.

(4) Minor corrections:

Line

25 ...of which 15 affected 11 putative targets...

212 ...compounds that affected 11 major hubs...

213 ...compounds affected these putative targets.

Reply : We would like to thank you for your valuable comments. According to your suggestion, we have modified these places in the manuscript to make the manuscript more clear. The changes are mainly on lines 25, 212 and 213.
We appreciate for Editors/Reviewers’ warm work earnestly, and hope that the reply will meet with approval. Once again, thank you very much for your comments and suggestions.

Best regards

Yours sincerely,

Changgang Sun