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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Author’s response to reviews:

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-18-01560R1). 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.

Editor Comments:

Overall, the reviewers agreed that your manuscript is a good example of data mining as a tool to narrow down the number of potential drug targets and potential drug candidates, despite the manuscript does not contain new information since all data used was already publicly available.

Would it be possible for you to address reviewers' comments as minor amendments to improve the manuscript?

Thank you very much!

Reply: We would like to thank you for your valuable comments, as you said, our manuscript is to use the data that is now publicly available, using data mining methods to achieve the goal of reducing the number of potential drug candidates. In this study, in order to explore the possible mechanism of inhibition of imatinib resistance by indirubin and its derivatives, drug target prediction, gene chip data analysis and protein network construction methods were mainly used. Finally, a total of 15 drug small molecules were identified that could act through 11 putative targets. The results were verified using computer-aided simulation of molecular docking.
We have been evaluated the Reviewers’ comments and suggestions, and responded to these suggestions point-by-point which we hope to meet with approval.

Thank you again for your reply!

Randolph Arroo (Reviewer 1): All my comments on the previous version of the manuscript have been adequately addressed.

Reply: We would like to thank you for your valuable comments and suggestions. We adopt your comments and revised the manuscript to make the content of the article more rigorous and richer. Your opinion makes the manuscript's method clearer; the logic is more rigorous. Thanks again for your valuable comments.

Cornelia Braicu (Reviewer 3):

(1)The first part of the conclusion section need to be rewritten " Drug target prediction is an important approach for drug discovery and drug design. Gene microarray analysis is an important method for discovering disease progression and new therapeutic targets. Network construction is a quick means to evaluate the relationship between targets and genes, and molecular docking is the verification method for the interaction of small molecule compounds with disease targets."

It is not clear the connection between first and second sentence.
What is the practical relevance of the present study?

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.

In this study, first, we obtained predictive target genes for drugs through drug target prediction. Subsequently, differentially expressed genes were obtained by data analysis of the gene chip. Finally, the two parts of the content were linked by the construction of the protein network, and the inhibition of imatinib resistance by indirubin and its derivatives was found by this method. Therefore, the first sentence and the second sentence are all part of our research method, and the first two sentences are connected by a third sentence. Make the research logic of the manuscript clearer.

According to the reviewer's suggestion, we have reorganized and filled out the first paragraph of the conclusion section to make the article's logical thinking and research ideas clearer (The parts that are rearranged and rewritten are mainly in lines 302 – 307).
(2) It is not clear what was the reason of using some specific programs for network generation instead of others?

Reply: We would like to thank you for your valuable comments. In this study, our network generation and analysis methods: (1) to obtain the interaction between genes through the String online website, (2) Cytoscape to visualize and analyze the network. String (https://string-db.org/) is an online website that analyzes the interactions between genes. Cytoscape 3.5.1 (http://www.cytoscape.org/) is an open software application for visualizing, Integrating, modeling and analyzing interactive networks. First, we use the String online software to obtain the interaction between genes. Subsequently, Cytoscape was imported to visualize the network, and through topology analysis, the importance and role of each gene node in the network was obtained to obtain key genes. Finally, through a series of analysis, the results of the study are obtained. String and Cytoscape are the most commonly used software for building protein networks. And our Go and KEGG analysis is also implemented through the plugin of Cytoscape. Therefore, in this study, we used String and Cytoscape to generate the network and analyze the gene nodes in the network.

(3) Additional connection with target miRNA transcripts using miRNet or miRtargetlink should be considered.

Reply: We would like to thank you for your valuable comments. In our research, we mainly studied the DNA level, and have not yet touched the level of miRNA. The main reason is that we are temporarily at the molecular docking level. We obtained the protein structure for docking through the RCSB PDB online website, and this site has no miRNA structure yet. Therefore, our research is temporarily related to the DNA level. However, in our current work, we have been involved in miRNA-mRNA pairs associated with chronic myeloid leukemia to further acquire the deeper pathogenesis of the disease, thereby better studying the mechanisms associated with imatinib resistance. I look forward to uploading the latest research results to the magazine again.
(4) An overlapping with the drug resistance from NCBI-gene will be also usefully.

Reply: We would like to thank you for your valuable comments. Based on your suggestion, we compared the 11 indirubin and its derivatives that we obtained to inhibit the imatinib-resistant target gene and NCBI-gene. We found that in chronic myeloid leukemia, EGFR inhibitors bind efficiently to the allosteric site of Bcr-Abl. And EGFR signaling increases the expression of SNAIL and its targets MMP9 and IL8, thereby maintaining the survival of CML cells. EGFR inhibits or reverses imatinib resistance by enhancing the ability of imatinib to bind at the ATP-binding site of Bcr-Abl kinase. The study found that JAK2 and JAK3 had antiproliferative effects on imatinib-resistant BCR-ABL(+) cells, and the administration of IMA plus a JAK inhibitor reduced expression of stem cells markers, enhancing the antitumour effects of IMA in CML cells. Human ERBB2 is a proto-oncogene that codes for the erbB-2 epithelial growth factor receptor. CHUK plays an important role in the NF-κB signalling pathway; indirubin and its derivatives inhibited CML cell proliferation by inhibiting CHUK activation of the NF-κB signalling pathway. A study showed that NF-κB represents a potential target for molecular therapies in CML. KIF11 inhibited cell proliferation by blocking the cycle of CML cells. The data showed that KIF11 was overexpressed in BCR-ABL+ CML cells. Administration of the imatinib plus JAK inhibitor reduces the expression of stem cell markers, such as ABCG2 and ALDH1A1. Blocking JAK3 with imatinib and JAK3 inhibitors may represent a new therapeutic strategy for eradicating LSCs and preventing CML recurrence. Therefore, in our study, the role of some genes in imatinib resistance has been elucidated, which proves the feasibility of our research method. Genes that have not yet been developed can provide new ideas for inhibiting imatinib resistance. And we added this part of the 278-288 line in the discussion section.

(5) Also the discussion should be more focused on practical application. Until present still lack the originality of the paper. A workflow for further studies, focused on drug resistance will be welcome.

Reply: We would like to thank you for your valuable comments. Based on your comments, the discussion section of our manuscript was verified.
The aim of our study was to investigate the inhibitory effect of indirubin and its derivatives on imatinib resistance. It can help clinicians to inhibit/delay the patient's resistance to imatinib, thereby increasing the therapeutic effect of imatinib and prolonging the overall survival rate of patients. Added content on practical applications in the discussion section (paragraphs 233-235 of the discussion section).

Our next step is to perform more sophisticated molecular docking and validate our conclusions in vitro and in vivo to investigate the intrinsic molecular mechanisms by which indirubin and its derivatives inhibit imatinib resistance. We look forward to some new research results. And upload the latest research results to your magazine again.

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