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 and Reversal of Imatinib Resistance using a "Drug Target Prediction-Gene Microarray Analysis-Protein Network Construction" Strategy” (ID: BCAM-D-18-01560). 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.

Reviewer reports:

Randolph Arroo (Reviewer 1):

1) The manuscript promises to decipher the mechanism of action of indirubin derivatives in the inhibition and/or reversal of imatinib resistance. The methodology used is data-mining.

The promise is not completely fulfilled: whereas the data indicate that some indirubin derivatives inhibit growth of CML cells, there is no indication that indirubin-derivatives make resistant cells sensitive to imatinib again.

At best we can conclude that some indirubin-derivatives inhibit growth of CML cells (that was known already), and that the mechanism of growth inhibition is different from that of imatinib.

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 and/or reversal 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.

Based on the reviewer's suggestion, we reviewed the ideas of this study and found that we could not conclude that indirubin and its derivatives can reverse imatinib resistance. However, in this study, we identified differentially expressed genes that are sensitive to imatinib in CML patients. Through drug target prediction, gene chip analysis, and protein network construction methods, we finally obtained 11 putative targets, namely It is the target of indirubin and its derivatives, and is also a differentially expressed gene sensitive to imatinib in CML patients. Therefore, our study can conclude that indirubin and its derivatives can inhibit the resistance of imatinib in patients with CML and increase the sensitivity of patients with CML to imatinib.

Therefore, we have changed the manuscript's conclusion that indirubin and its derivatives can reverse imatinib resistance (in these places, remove the Reversal. Title section, line 1; abstract section, lines 20, 26, 28, 33; background section, lines 48, 61; method section, line 145; result section, lines 225, 234; discussion section, lines 207, 215, 226, 233, 237, 273,296; conclusion section, line 308).

2) There are some claims in the introduction that cannot be justified, e.g. "Chinese medicine has made tremendous achievements in the treatment of cancer". True, some formulations have been prescribed traditionally for treatment of cancer, but no clinical data on efficacy are presented. References [8-10] present promising results but are all based on in vitro assays.

Reference [11] is said to describe in some detail what the mechanism of action is of Qingdai (rich in indirubin), but the paper by Hu et al. 2015 does not discuss mechanisms of action at all.
Reply: We would like to thank you for your valuable comments. We have revised some of the unreasonable wording of the introduction. Indeed, the progress of traditional Chinese medicine in the treatment of cancer lacks the support of clinical data. A series of current studies are based on in vitro experiments, so we changed the tremendous achievements to some progress in the 50th line of the introduction, and such changes made the wording of the article more rigorous.

Regarding the 11th document, we have replaced the 11th document with another article that analyzes in detail the mechanism of action of Qingdai in the treatment of CML (References section, line 362). Thank you for your suggestions to make the article's logical thinking and wording more rigorous.

3) The process of the data mining exercise itself is written in sufficient detail, although little explanation is given on the theoretical background of systems biology-based investigations. Some jargon words are used but not defined unambiguously.

Data mining is essentially a desk based exercise used to identify potential drug candidates. Several times in the manuscript, the word 'obtained' is used. This may be a matter of translation, but I would suggest to replace 'obtained' by 'identified' (lines 25, 30, 78, 108, 169, 178, 224) or possibly 'characterised' (line 32, 301).

Reply: Thank you very much for your comments and suggestions. This research was done through data mining and computer-aided simulation. We have explained the terminology of the method part according to your suggestion, including lines 104, 105, 110, 117, 126-133 of the method section, which makes the method logic of the manuscript clearer.

We have modified the manuscript according to your suggestion, and the use of 'obtained' multiple times in the article is not suitable for every place., we replaced 'obtained' (lines 21, 25, 68, 69, 74, 75, 80, 84, 85, 95, 99, 148, 154, 188, 202, 203, 219, 287, 294, ) with 'identified' in multiple places in the manuscript, and replaced 'obtained' with 'characterised' (line 27, 272).
4) GSE2018 was downloaded from the GEO database (line 77), but no explanation is given what GSE2018 is, and no proper reference is given. Only later (line 117) it becomes clear that GSE2810 refers to a gene expression profile, but still no reference is provided, apart from that the profile was submitted by Ohyashiki JH. The appropriate reference should be:


Reply: We would like to thank you for your valuable comments and suggestions. GSE2810 refers to the gene expression profile. I added the GSE2810 based platform on line 104. I added a reference based on your opinion at line 372, 'Nunoda K, Tauchi T, Takaku T, Okabe S et al. Identification and functional signature of genes regulated by structurally different ABL kinase inhibitors. Oncogene, 2007; 26: 4179-88. 'The appropriate reference makes the definition of GSE2810 clearer.

5) Several times in the text, quotation marks are used, but it is not clear why: "if the degree of a node is more than two fold the median degree of all the nodes in a network, the node may function as a big hub." suggested by Li et al[19].

But, the actual quote by Li et al. is: "If the degree of a node is more than 2 fold of the median degree of all nodes in a network, such gene or CM [chemical messenger] is believed to play a critical role in the network structure, and we treat it as a hub gene or a hub CM."

Why use quotation marks, when it is not a literal word-by-word quote?

In this case, there is no strict need to quote previous authors, since it refers to a simple definition of the terms used in this paper.
Reply: We would like to thank you for your valuable comments and suggestions. We reviewed the manuscript according to your suggestion and found that in the manuscript we took 'If the degree of a node is more than 2 fold of the median degree of all nodes in a network, such gene hub is believed to play a critical role in the network, and we treat it as major hub', and the theory of Li et al is not strictly cited. Therefore, we removed Li et al's theory based on your suggestion and made a simple definition of the 'major hub' in the manuscript of line 126 of the method section. This modification makes the wording of the article more rigorous.

6)Line 250: we used a system of "pharmacological computer simulation and gene chip technology of molecular biology" to explore...

Is that a quote? But then, who is quoted?

The authors suggest they used gene chip technology, but the only gene chips that were used are the ones that gave rise to gene expression profile GSE2810 (Nunoda et al. 2007). The manuscript only reports data mining and computer simulation.

Reply: We would like to thank you for your valuable comments and suggestions. As you said, our manuscript uses data mining and computer simulation. We didn't quote here, sorry that we used the quotes incorrectly. And there is a mistake in speech here. In this study, we did not use gene chip technology, but only analyzed the data spectrum of the gene chip, Therefore, At lines 226-227, we hereby replace 'pharmacological computer simulation and gene chip technology of molecular biology' with 'Drug Target Prediction-Gene Microarray Analysis-Protein Network Construction model'. This modification can better reflect the research method used in this study. The idea of the manuscript is more rigorous.

7)The name "QingHuang San" is between quotation marks. However, other mentions of TCM are not in quotation marks (e.g. Bu-Zhong-Yi-Qi, Qingdai).
8) Terms used in network analysis are all in quotation marks, e.g. "degree", "node betweenness", "closeness" and "K value" (line 144, line 223-224).

The quotation suggest that these are very specific jargon words with a precise definition. However, the definition is not given in this manuscript. Reference is made to ElHady et al. 2017 [20] (lines 143-147). However, in reference [20] these terms are not mentioned at all, let alone defined.

As it is, the terms remain undefined, which makes it impossible for a reader to assess the network analysis that is presented here.

Reply: We would like to thank you for your valuable comments and suggestions. In the manuscript, we use the four indicators of degree, betweenness, closeness and K-coreness to screen key genes in the network. According to your comment, we have defined four indicators in the method section, 126-131, degree (the number of links to node ), betweenness (the number of shortest paths between pairs of nodes which run through node ), Closeness (the sum of the distances of node to all other nodes), and K-coreness (a measure of the centrality of node ). The degree, betweenness and closeness can be used to assess the topological importance of a node in a network, and the larger a node's degree, betweenness, and closeness centrality, the more important that node is in the PPI network Better analysis. And we replaced the 20th reference, 'Zhang YQ, Guo QY, Li QY et al. Main active constituent identification in Guanxinjing capsule, a traditional Chinese medicine, for the treatment of coronary heart disease complicated with depression. Acta Pharmacol. Sin 2018; 39: 975-987.' In this article, four key features, including degrees, medium, proximity, and K-core, were also calculated to determine the key genes of the network.
9) Figures 2, 3, 4, and 5 cannot be read properly. In an on-line version, the networks and their connections can be evaluated, but on paper there is very little value in these figures. I would suggest to leave them out, or find other ways to visually present the data (e.g. heat maps).

Reply: We would like to thank you for your valuable comments and suggestions. Figure 2 is a network diagram of differentially expressed genes. In order to make the image more visible, according to your suggestion, we have uploaded the heat map of the differentially expressed gene as an attachment. And the protein network map of 125 differentially expressed genes was constructed, which showed the up-regulated and down-regulated differentially expressed genes, which made the pictures more visible. In order to enhance the visualization and resolution of the Figure 3,4, we have re-edited all the images and increased the size of the labels in the images to make the images more visible. A pie chart is an introduction to the various pathways and biological processes in the image, consistent with the colored labels in the image. Since we have clearly presented the labels in the chart, we removed the low resolution pie chart in the image. Make the visualization of the image enhanced. Regarding Figure 5, due to the visibility of the original image and the poor resolution, we used the data to recreate the protein interaction network.

10) Overall, the manuscript shows how datamining can be used to narrow down the number of potential drug candidates for a specific target. Arguably, this is a sensible first step in a drug discovery programme. However, the data, as presented in the current manuscript, are just that: a first step. A total of 42 indirubin analogues is whittled down to 15 potential drug candidates. No further analysis is given (e.g. structure-activity relationship) that would give the reader an insight into the properties of an ideal indirubin-based drug for use in CML therapy.

Reply: We would like to thank you for your valuable comments and suggestions. As you said, this study uses 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, as you said, this is the first step in drug discovery, followed by structural-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.
Mokrish Ajat, Ph.D. (Reviewer 2): General

1) No flow in writing the report especially at the introduction part.

Reply: We would like to thank you for your valuable comments. Based on your comments, we reviewed the full text of the manuscript. In the background, we first introduced the latest research results on the molecular mechanism of chronic myeloid leukemia. Subsequently, the molecular mechanism of imatinib in CML treatment was introduced, and imatinib resistance was introduced. Finally, the research progress of traditional Chinese medicine in tumor treatment is put forward, which leads to the topic of this paper, the possible role of indirubin and its derivatives in inhibiting imatinib resistance. In terms of method, we used the method of 'Drug Target Prediction-Gene Microarray Analysis-Protein Network Construction' to explore, and a total of 15 small molecules of drug were applied to CML cells through 11 putative targets. Finally, the results of the study were verified by computer-aided simulation of molecular docking. In order to make the idea of the article clearer, we reviewed and revised the article. In the background section, lines 63-65 add the summative language of the Background, making the logic of the background part clearer. At lines 104, 110, 126-131, remarks on the terms used are explained in the Methods section. In the results and discussion sections, at lines 153, 154, 226-228, the irrational statements in the article were also modified to make the logic of the article clearer.

2) There were some spelling/punctuation errors (page 4, 8 and 12).

Reply: We would like to thank you for your valuable comments. Based on your suggestion, we reviewed the full spelling and punctuation of the manuscript and corrected it. The scope of the revision mainly focused on the introduction, methods and results, including 23, 58, 88, 126-133, 140, 202, 221, 222, 259. The revision made the manuscript more rigorous in writing.
3) Method

Some methods are not clearly explained and elaborated.

Reply: We would like to thank you for your valuable comments and suggestions. 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. 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. In order to make the logic of the method part clearer, we have modified it in the method section and modified it in lines 104, 110, 117, 126-133.

4) Results and discussion

The discussion was presented well with exception on the provided illustrations/images which were all in poor quality, not sharp and distorted in general.

Reply: We would like to thank you for your valuable comments and suggestions. According to your suggestion, in order to enhance the visualization of the images in the manuscript, we have modified all the images. Including Figure 1-6.

We modified the tables and images. The main modifications include Table 1. We magnified the 2D structure of the small molecules in the table to make it more visible.
Regarding Figure 1, Figure 1 is deleted because of poor visibility and all the pictures involved in the Figure 1 have been modified.

Regarding Figure 2, we have uploaded the heat map of the differentially expressed genes as an attachment, and constructed a protein interaction network map as Figure 1 of the manuscript using 125 genes that were differentially expressed.

Regarding the original Figure 3, 4, due to the visibility and resolution difference of the original image, we re-map the data in the manuscript and delete the pie chart with poor visibility in the original image.

Regarding Figure 5, due to the visibility of the original image and the poor resolution, we used the data to recreate the protein interaction network.

Regarding Figure 6, since this image was first edited by Word document, the image clarity is poor. Therefore, we replace the original document with the image. Therefore, replace Figure 6 with Table 4 to make the image more visible.

5) Images/diagrams/charts

1. 2D structure images on page 28 to 36 were all not clear, poor quality and too small to be appreciated.

   Reply: We would like to thank you for your valuable comments and suggestions. Table 1 mainly describes the small molecular structures and predicted targets of the indirubin and its derivatives. We magnified the 2D structure of the small molecules in the table to make it more visible.
2. Flow charts on page 41 were not consistent in spacing, alignment, of poor quality and not sharp.

   Reply: We would like to thank you for your valuable comments and suggestions. Due to the visibility and poor resolution of Figure 1, and the images contained in Figure 1 have been modified, we have removed Figure 1 from the reviewer's comments.

3. The flow charts were poorly arranged.

   Reply: We would like to thank you for your valuable comments and suggestions. Based on your comments, we reviewed the flow chart we presented, found that the flow chart was improperly arranged, and the visibility and resolution were poor. Therefore, since the first paragraph of the method section clearly stated the research ideas in words, We have removed the flowchart to make the manuscript more rigorous.

4. The provided images on molecules structures on page 41 were distorted, poor quality and resolution.

   Reply: We would like to thank you for your valuable comments and suggestions. Figure 2 on page 41 is a network diagram of differentially expressed genes. In order to make the image more visible, according to your suggestion, we have uploaded the heat map of the differentially expressed gene as an attachment. And the protein network map of 125 differentially expressed genes was constructed, which showed the up-regulated and down-regulated differentially expressed genes, which made the pictures more visible.

5. Images/illustrations provided from page 42 onwards were all distorted, not sharp, the labelled fonts were too small and hard to read.
Reply: We would like to thank you for your valuable comments and suggestions. According to your suggestion, images starting on page 42 include Figures 3, 4, 5, and 6. We have all modified it. In order to enhance the visualization and resolution of the image, we have re-edited all the images and increased the size of the labels in the images to make the images more visible.

6. The fonts colours used to label diagrams from page 42 onwards were too bright.

   Reply: We would like to thank you for your valuable comments and suggestions.

Based on your suggestion, we reviewed Figure 3, 4, 5, and 6. In order to make the image more visible and more detailed, and the labels in the image are easier to see, we have re-edited Figures 3, 4, and 5. However, since the image was made by a specific plug-in in the specific software Cytoscape 3.5.1, the color of the specific label in Figure 3, 4 cannot be modified, but I have tried my best to make the label clear. The labels in the image in Figure 5 have been modified to make the visualization stronger. In order to make the picture clearer, we have replaced Table 4 with Figure 6. The labels are all in black.

7. The pie charts given are too small to be appreciated, the labels are too small.

   Reply: We would like to thank you for your valuable comments and suggestions. For Figure 3 and Figure 4, we have re-edited the image in order to make the image more visible and more transparent. A pie chart is an introduction to the various pathways and biological processes in the image, consistent with the colored labels in the image. Since we have clearly presented the labels in the chart, we removed the low resolution pie chart in the image. Make the visualization of the image enhanced.
8. Images of gene sequences on page 46 are not sharp, the resolution and quality are very poor.

Reply: We would like to thank you for your valuable comments and suggestions. According to your suggestion, we have modified Figure 6. Since this image was first edited by Word document, the image clarity is poor. Therefore, we replace the original document with the image. Therefore, replace Figure 6 with Table 4 to make the image more visible.

Cornelia Braicu (Reviewer 3):

1) Please include all comments for the authors in this box rather than uploading your report as an attachment. Please only upload as attachments annotated versions of manuscripts, graphs, supporting materials or other aspects of your report which cannot be included in a text format.

Please overwrite this text when adding your comments to the authors.

Reply: We would like to thank you for your valuable comments and suggestions. 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.
2) Figures 2-6 need to be simplified.

Additional Venn diagrams and a heatmap for imatinib resistant genes.

Reply: We would like to thank you for your valuable comments and suggestions. Figure 2 is a network diagram of differentially expressed genes. In order to make the image more visible, according to your suggestion, we have uploaded the heat map of the differentially expressed gene as an attachment. And the protein network map of 125 differentially expressed genes was constructed, which showed the up-regulated and down-regulated differentially expressed genes, which made the pictures more visible. In order to enhance the visualization and resolution of the Figure 3,4, we have re-edited all the images and increased the size of the labels in the images to make the images more visible. A pie chart is an introduction to the various pathways and biological processes in the image, consistent with the colored labels in the image. Since we have clearly presented the labels in the chart, we removed the low resolution pie chart in the image. Make the visualization of the image enhanced. Regarding Figure 5, due to the visibility of the original image and the poor resolution, we used the data to recreate the protein interaction network. we have modified Figure 6. Since this image was first edited by Word document, the image clarity is poor. Therefore, we replace the original document with the image. Therefore, replace Figure 6 with Table 4 to make the image more visible.

3) Some minor issues related to: ... new drugsthat can combat..

2.1 Data preparation

Go analysis

Reply: We would like to thank you for your valuable comments and suggestions. Currently, imatinib resistance presents a major challenge to the treatment of chronic myelogenous leukaemia. Identifying strategies to suppress imatinib resistance is of great significance. 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 and/or reversal 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 found that 15 small molecules can act on 11 related targets of imatinib resistance.

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