Peer Review File

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Reviewer A

My major concern for this network pharmacology is that the authors did not adequately review the anti-cancer effect of SV for CRC from the perspective of evidence-based medicine. Without such convincing evidence, the current analysis on the pharmacological mechanisms is not necessary.

Reply: From the perspective of traditional Chinese medicine, qi blood is the material basis for the function of internal organs, and qi blood is recognized as the etiology and pathogenesis of malignant tumors. SV has the effect of activating blood circulation and diuresis, and its effect of activating blood circulation and removing stasis is strong, and its anti-tumor effect has also been valued and applied in modern Chinese medicine clinics.

In this revised version, the authors still did not present clinical findings on the effectiveness of SV for CRC so it remains questionable whether this research focus deserves to be done.

Reply: We were really sorry for our careless mistakes. Current Chinese medicine literature reports, SV plays an important role in inhibiting vascular regeneration, cancer metastasis, inflammation and so on in the treatment of cancer. SV has been used in many cancers, including lung cancer, prostate cancer (10.13935/j.cnki.sjzx.210501), bladder cancer, colorectal cancer, leukemia, etc. Unfortunately, these reports are Chinese paper from CNKI and are not cited in this manuscript. The corresponding author has rich clinical experience in TCM, she deduced from the theory of traditional Chinese medicine treatment of CRC and the medicinal properties of SV. This manuscript is the potential therapeutic value of SV in CRC with the help of network pharmacology analysis, and it is also the preliminary theoretical basis for the subsequent verification of SV in the treatment of CRC. In 2004, it was used SV's blood drainage and diuretic effects to assist in the treatment of CRC in Linyi Cancer Hospital and Linyi Hospital of Traditional Chinese Medicine. There is less literature, but with more in-depth research, it will be further explored about the value of SV in cancers.

In addition, the conclusion on the clinical implications is vague. The authors did not have comments on the further experimental work to validating the findings from network pharmacology analysis.

Reply: We combine clinical experience and understanding of Chinese medicine. Network pharmacology is a new and more holistic research mode, and development of a suitable research mode is important in the innovation and development of modern Chinese medicine. Network pharmacology has added a reliable basis for our clinical treatment. Unfortunately, we have no valuable medication treatment statistics about SV treatment CRC, and the further experimental work. Based on the application of SV in cancers, SV may have greater potential value in CRC.

In the methodology of the main text, I suggest the authors to use a flowchart to describe the research procedures of this study.

Reply: The figure 1A is the flowchart about study design and analysis. Thanks.

It is also questionable that whether the network pharmacology is so helpful for identifying the therapeutic targets for CRC. The authors may provide a successful example of network pharmacology analysis for similar work in oncology.

Reply: Thanks for your professional review work. Traditional Chinese Medicine (TCM) has always been based on holistic analysis and treatment of patients, and overall systematic adjustment of the disease is its advantages and characteristics. However, avital concern for the development of TCM is to surmise how to associate medicine with the complex human body, to carry out qualitative and quantitative analysis. Based on the biomolecular network, network pharmacology analyzes the relationship between diseases and medicine, which is in line with the needs of TCM development, thereby initiating the innovation of TCM research methods. The concept of network target was then formally proposed in 2011. The hypotheses, cases, concepts, and methods related to network target originated from TCM research have played a key role in the origin and development of network pharmacology. Considering the network pharmacology analysis of classic, famous, and proven prescriptions from credible doctors, pertaining to network target theory and method with disease-syndrome biological network as the intervention target, traditional efficacy, modern indications, effective substances, and action mechanism of Liuwei Dihuang prescription (nourishing Yin, enhancing body's disease resistance or immunity) and Gegen Qinlian Decoction (clearing heat and removing dampness) were elucidated.

At present, there are some relatively successful cases in the field of traditional Chinese medicine treatment of colorectal cancer, including Integrating network pharmacology deciphers the action mechanism of Zuojin capsule in suppressing colorectal cancer (DOI: 10.1016/j.phymed.2021.153881), A network pharmacology approach for investigating the multi-target mechanisms of Huangqi in the treatment of colorectal cancer (DOI: 10.21037/tcr-20-2596), Network pharmacology and molecular docking study on the mechanism of colorectal cancer treatment using Xiao-Chai-Hu-Tang (10.1371/journal.pone.0252508) and others.

Reviewer B

The manuscript contains fundamental errors. The manuscript (MS) is very thin (in terms of result especially), without any empirical support. Unfortunately, there is no solid evidence of the relationship with statistical significance in this manuscript. More work is needed to add quality. Paper lacks clarity in writing (one example; Disease enrichment analysis of genes was conducted using Harmonizome (https://maayanlab.cloud/Harmonizome/) and construction of the heatmap (23)) and soundness of organization of the paper.

Reply: We tried our best to improve the manuscript and made some changes marked in revised paper. According to your suggestions, there are mainly the following parts: added the part of statistical analyses (see Page 6, line 18-20), added detailed description about Harmonizome (see Page 5, line 2-5), added detailed description about Molecular docking (see Page 6, line 1-

4 & 11-14), added detailed description about results (see Page 8, line 27-31, and Page 9, line 1-6), added detailed description about discussion (see Page 10, line 5-29, and Page 11, line 11-14 & 20-24), added detailed description about conclusions (see Page 11, line 30-34).

Wang Bu Liu Xing (Semen vaccariae) has the effect of activating blood circulation and diuresis, and its effect of activating blood circulation and removing stasis is strong, and its anti-tumor effect has also been valued and applied in modern Chinese medicine clinics. In the field of traditional Chinese medicine, Wang Bu Liu Xing (Semen vaccariae) has been used in many cancers, including lung cancer, prostate cancer (10.13935/j.cnki.sjzx.210501), bladder cancer, colorectal cancer, leukemia, etc. Unfortunately, most of the above related articles are published in Chinese journals from CNKI, and the DOI cannot be provided in detail.

Our manuscript is based on theoretical inferences of Chinese medicine, which is then analyzed in combination with network pharmacology and bioinformatics. In order to compensate for the regret that we have no any empirical support, we correlated 5 groups of CRC clinical dates from the GEO database to ensure the accuracy and reliability of our analysis as much as possible. Hope you are satisfied.

Reviewer C

The authors studied the potential pharmacological mechanism of action of Semen vaccariae for colorectal cancer using computational approaches. This work has major concerns which need to be improved before acceptance. As the study is based on computational approaches using bioinformatics and docking there should be in depth analysis for each experiment sections.

1. Only docking is not enough to explain the interaction of the compounds with the targets unless some bioassay results been incorporated to support the result. Perhaps, MD simulation study would give better information and the stability of binding of the compounds with the targets. In this case, MD simulation is highly recommended followed by thermodynamics calculations.

Reply 1: Thanks for your suggestion. AutoDock has proven to be an effective tool capable of quickly and accurately predicting bound conformations and binding energies of ligands with macromolecular targets. The software automatically used default parameters during standard docking, the best-fit pose of docked molecules, the binding energy values, potential conformations, bond distances and types of interactions were predicted. When the binding energy is less than zero, the small-molecule ligand can spontaneously bind to the macromolecular receptor. The 2 displayed greater binding activity when the binding energy was less than −5.0 kcal/mol. The molecular docking is credible. Our results show the number of hydrogen bonds docked, the bond length, the docking site, and the binding energy.

2. The docking study method and result is poorly described. No detailed information was found.

Reply 2: We added detailed description about Molecular docking method (see Page 6, line 1-4 & 11-14), and result (see Page 8, line 27-31, and Page 9, line 1-6). Thanks.

3. No reference compound was used to compare the binding of the tested compounds. It is very important to compare with a positive control.

Reply 3: Molecular docking is a method of drug design through the characteristics of the receptor and the interaction between the receptor and the drug molecule. A theoretical simulation method that mainly studies intermolecular (such as ligands and acceptors) interactions and predicts their binding patterns and affinities. Molecular docking using autodock mainly includes the following steps: 1 receptor preparation, 2 ligand Preparation, 3 set up the grid box, 4 run autodock, 5 analysis result (the number of hydrogen bonds docked, the bond length, the docking site, and the binding energy). We carefully referred to the literature on autodock analysis and found no positive reference reports. If we have overlooked some important information, we hope to get your forgiveness.

4. The comparison with co-crystalized ligand was not given. Was there any co-crystallized ligand for the target proteins? Where did the compounds bind in the target proteins? was it on the active site? where is the active binding site?

Reply 4: We screen protein crystal structures based on species (Homo sapiens), resolution (<3.0 Å), protein acquisition method (X-ray diffraction) from RCSB (https://www.rcsb.org/). We searched the ligand from PDB BANK and PyMol, including CDK2 (PDB:6q4g) has 2 bound ligands: HJK-301(A) and HJK-301(B), BCL2L1 (PDB:7jgw) has a bound ligand: V9S 301(A), RV2 401 bound to chain A_1 of SERPINE1 (PDB:7aqf). We added detailed descriptions about the binding sites of compounds to proteins (see Page 8, line 27-31, and Page 9, line 1-6). For example, five hydrogen bonds are formed between CDK2 and swerchirin, with a binding-energy value of – 7.61 kcal/mol, swerchirin was able to bind with CDK2 at residues ASP-86, HJK-301, GLN-131 and ILE-10 (Figure 4A). We examined the active sites using PyMol, HJK-301 is the active site of CDK2, this is consistent with our docking results. We chose the site with the better binding energy and hydrogen bonds, and missed the docking of the active center about BCL2L1 and SERPINE1.

5. The binding interaction with amino acids, the type of bonds involved, and the bond lengths should be explained and compared with the reference drug.

Reply 5: Thank you for your precious comments and advice. We used the flexible docking method, Figure 4 shows the binding site, hydrogen bond, bond length, and we further describe the binding site, the number of hydrogen bond, the binding energy and protein resolution in the results (see Page 8, line 27-31, and Page 9, line 1-6). We're sorry we're missing the results of compared with the reference drug.

6. Validation of docking experiments should be done with co-crystalized ligand for each target proteins.

Reply 6: Thanks for the comment. The molecular docking is an easily understandable way to finish as refer to other literatures. We are sorry that we cannot give you a satisfactory answer, and we will incorporate it into experiment in the future studies.

7. The discussion need overhaul by providing previous study results and comparing this study is significant.

Reply 7: Thank you for your comment. We have made significant changes to the discussion section around the results (see Page 10, line 5-29, and Page 11, line 11-14 & 20-24).