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Original Research Article

Identification of key genes and active components of *Astragalus membranaceus* in the treatment of colorectal cancer based on bioinformatics analysis

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Abstract

Purpose: *To investigate the bio-active components and key targets of Astragalus membranaceus (AM) in the treatment of colorectal cancer (CRC) through bioinformatics analysis.*
Methods: *An AM action network was constructed after taking intersection of the target genes in AM and CRC-related genes. After that, Protein-Protein Interaction analysis, Gene Ontology enrichment analysis and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis were performed on the intersected genes. Then, the differential genes in GSE70772 and GSE113513 datasets were screened separately and intersected with genes from the PPI analysis to obtain the key targets.*
Results: *After intersection with potential targets of AM and CRC-related genes, 105 genes were screened by PPI analysis. Then, 20 genes were identified as potential target genes of AM in GSE70772 and GSE113513 datasets. These genes were intersected with the 105 genes. Finally, a key gene called MET was identified as the key target of action of AM in the treatment of CRC, and methylnissolin (MOL000380) was the active component of AM.*
Conclusion: *MET a potential key target gene of AM, whose active component is methylnissolin, in the treatment of CRC. Thus, the findings of this study may provide a scientific basis for the further development of AM for the treatment of CRC.*

Keywords: *Astragalus membranaceus, colorectal cancer, tumor, bioinformatics analysis, target gene*

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INTRODUCTION

Colorectal cancer (CRC) is a malignant tumor of the gastrointestinal tract. In the early stage, it is often accompanied by symptoms such as changes in bowel habits, blood in the stool and diarrhoea, while in the later stages, it is always accompanied by systemic symptoms such as fatigue and weight loss[1]. CRC is the third most common malignancy worldwide, and its mortality rate ranks second among all malignancies [2]. However, the pathogenesis of CRC is still poorly understood. Therefore, further research has begun to focus on how to effectively treat CRC [3].

Surgical interventions, radiotherapy and chemotherapy are traditional treatments for CRC,

but these treatments are frequently accompanied by side effects that have greatly affected the prognosis of the disease [4]. A rising number of studies have shown that combining traditional Chinese medicine (TCM) with radiotherapy or chemotherapy may reduce side effects and improve treatment outcomes [5]. In this context, TCM is widely used in the treatment of CRC due to its minimal side effects and effectiveness in treating various cancers [6]. *Astragalus membranaceus* (AM) is a commonly used herbal medicine that contains a large number of bioactive components and it has a wide range of pharmacological effects [7]. Studies have shown that the bio-active components of AM can not only modulate the immune system, but also reduce the side effects caused by radiotherapy and chemotherapy, thereby enhancing the anti-tumor efficacy of cancer patients [8]. Therefore, AM has significant research implications in the treatment of gastrointestinal cancers [9]. The mechanism of action of AM in CRC treatment has already been explored by some studies using bioinformatics and network pharmacology [10,11], but the active components and target genes of AM that play key roles in CRC treatment still need to be further explored.

Tseng *et al*, by transplanting the human *CRC* cell line, HCT116, into subcutaneous tumors of nude mice, completed GSE70772 expression profile dataset that included eight CRC samples and eight AM-treated CRC samples [12]. Shen *et al* collected cancerous and non-cancerous tissue samples from 14 CRC patients for the completion of the GSE113513 expression profile dataset [13]. In present study, the target genes of AM were intersected with CRC-related genes, and the pharmacological action network of AM w constructed. Then, the possible active target genes of AM in GSE70772 and GSE113513 datasets were screened by PPI analysis, GO and KEGG enrichment analysis and difference analysis, and their expressions were analyzed in the original dataset to finally obtain the key targets and active components of AM for CRC.

METHODS

Analysis of the active components and targets of AM

The main components of AM were first identified in the TCMSP database (Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform), and based on oral bioavailability (OB) and drug similarity (DL), the bio-active compounds in AM were further screened. And values of OB ≥ 30 % and DL ≥ 0.18 were considered as thresholds for screening potential drug candidates. Then, target genes were identified for the action of AM in the TCMSP database, and the UniProt database (http://www.uniprot.org/) was used to convert the names of these genes. In addition, the Genecards database (http://www.genecards.org/) was used for screening CRC-related genes, and the selected genes with score >10 were identified as CRC-related genes. Finally, after taking intersection of the target genes of AM and the CRC-related genes, the AM action network of the intersected genes obtained and their bio-active components were constructed using the cytoscape software.

Construction of PPI networks and enrichment analysis

The PPI database was used for gene analysis, and the top 30 genes with the largest node degrees were statistically plotted while those with node degrees ≥10 were screened for the GO and KEGG enrichment analyses.

Analysis of differential gene expressions

CRC-related expression datasets were downloaded from the GEO database (http://www.ncbi.nlm.nih.gov/geo/). GSE70772 and GSE113513 datasets were selected for this study. The former one contains 8 CRC samples and 8 AM-treated CRC samples, and their differences were analyzed using the limma package for R, with p-value < 0.05. The later contains 14 CRC samples and 14 control samples, and their differential genes were screened using the limma package, with adj. p-value < 0.05 and |logFC |> 1.

Analysis of key targets

Genes up-regulated in GSE70772 dataset and down-regulated in GSE113513 dataset, or down-regulated in GSE70772 dataset and up-regulated in GSE113513 dataset were screened out as possible target genes for the action of AM. Thereafter, these identified key genes were intersected with genes screened with PPI analysis to obtain the key targets. Then the expression of the key targets in GSE70772 and GSE113513 datasets was analyzed.

RESULTS

Analysis of the active components and targets of AM

20 target components were obtained from AM (Supplementary Table S1). By searching the target genes in the TCSMP database and leaving

only those that could be converted into symbols in the Uniprot database, 16 target components and 177 potential targets were identified for AM (Supplementary Table S2). Subsequently, the 177 potential targets associated with AM were intersected with the 2740 CRC-related genes obtained in the Genecards database, and a total of 132 intersected genes were obtained (Figure 1 A). A pharmacological network was constructed based on the above results (Figure 1 B).

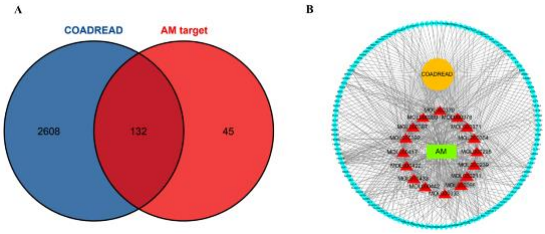

Figure 1: (A) A total of 132 intersected genes were obtained with 177 potential targets for AM and 2740 CRC-related genes. (B) The AM action network of the intersected genes

Construction of PPI networks and enrichment analysis

PPI networks for the 132 intersected genes were constructed (Figure 2 A). These genes were classified according to their node degrees from the largest to the smallest, and the top 30 genes were shown in the node degree bars (Figure 2 B), from which a total of 105 genes with node degrees ≥10 were selected for subsequent analysis. Thereafter, GO and KEGG enrichment analysis were conducted on the 105 genes (Figure 2 C and D).

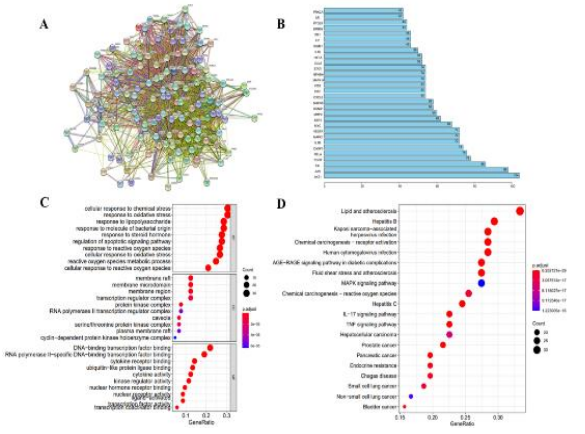

Figure 2: (A) PPI networks for the 132 intersected genes. (B) The top 30 genes with the largest node degrees were shown in the node degree bars. (C-D) GO and KEGG enrichment analysis were performed on the 105 genes with node degrees ≥10. (BP, Biological Process: CC: Cellular Component: MF, Molecular Function)

Differential Gene Expression Analysis

First, 1215 differential genes (546 up-regulated genes and 669 down-regulated genes) were obtained by performing differential analysis on the GSE70772 dataset, and heat and volcano plots were drawn accordingly (Figures 3 A and B). Besides, the above differential genes were also subjected to enrichment analysis, and enrichment analysis network plots were drawn (Figure 3 C). Secondly, a total of 1540 differential genes (622 up-regulated and 918 down-regulated genes) were determined by carrying out differential analysis on the GSE113513 dataset, and heat map as well as volcano map were plotted (Figures 4 A and B). In addition, the differential genes were enriched and analyzed, and an enrichment analysis network map was plotted (Figure 4 C).

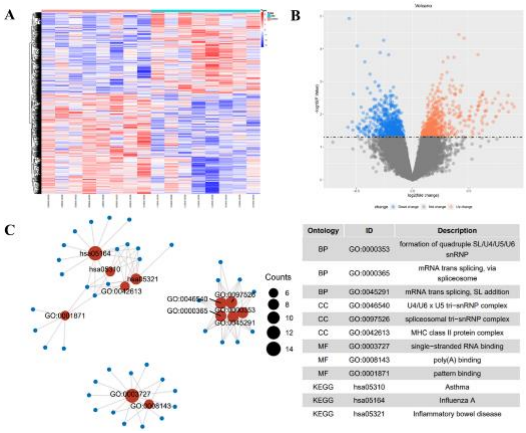

Figure 3: (A-B) Heat map and volcano plot showing 1215 differential genes in GSE70772 dataset. (C) GO and KEGG enrichment analyses of DEGs in GSE70772 dataset.

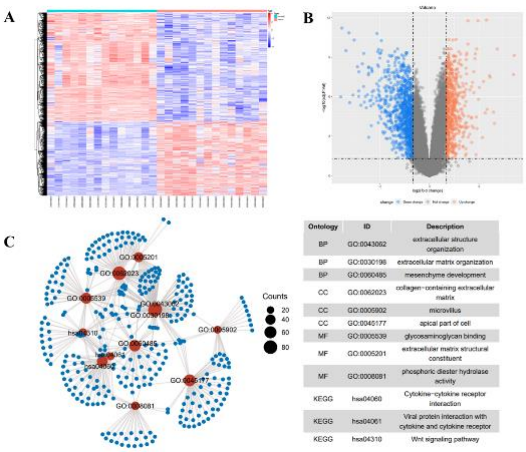

Figure 4: (A-B) Heat map and volcano plot showing 1540 differentially expressed genes in GSE113513 dataset. (C) GO and KEGG enrichment analysis of DEGs in GSE113513 dataset

Screening of AM in CRC

The differential genes from the two datasets were analyzed in order to screen out related genes up-regulated in GSE70772 dataset and down-regulated in GSE113513 dataset, or down-regulated in GSE70772 dataset and up-regulated in GSE113513 dataset. 20 DEGs were finally obtained (Figure 5 A). By taking intersection of the 20 DEGs with the 105 genes obtained via PPI analysis, a key gene named MET was obtained (Figure 5 B). Then the gene expression of MET in GSE70772 dataset versus GSE113513 dataset was analyzed. The results showed that MET was highly expressed in the tumor group, and lowly expressed in the control and AM groups (Figures 5 C and D). Finally, Methylnissolin (MOL000380), an active component of AM targeting MET in CRC, was obtained and its chemical structure is shown in Figure 5 E.

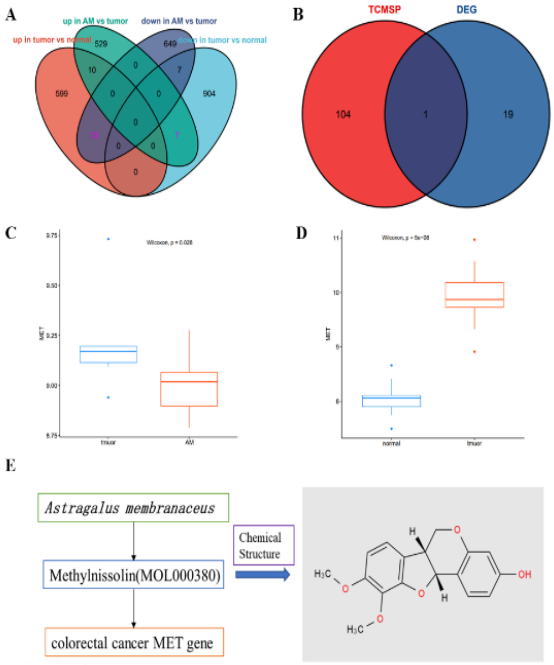

Figure 5: (A) Intersection of DEGs in GSE70772 and GSE113513 datasets. The 20 DEGs up-regulated in GSE70772 dataset and down-regulated in GSE113513 dataset, or down-regulated in GSE70772 dataset and up-regulated in GSE113513 dataset were screened out. (B) Intersection of the 20 DEGs and 105 genes obtained by PPI analysis. (C) Expression of MET in GSE70772 dataset. (D) Expression of MET in GSE113513 dataset. (E) Chemical structure of methylnissolin, the bio-active components of AM targeting MET in CRC

DISCUSSION

CRC is one of the most common malignancies worldwide, and its etiology involves a variety of factors, such as genetic alterations in cancer cells and in the tumor cell microenvironment [14]. As a result, the incidence and mortality rate of CRC remain high [15]. At the same time, the side effects caused by conventional treatment of CRC, such as radiotherapy and chemotherapy, contribute to its poor prognosis [16]. TCM has been shown by some studies to alleviate the side effects of conventional therapies for CRC, so TCM has been recognized worldwide as one of the treatment options for CRC [17]. Meanwhile, AM, as one of the most widely used herbal medicines, has been used for treating many malignancies including CRC. However, the active components and key targets of AM in CRC treatment need to be further explored [18]. Therefore, this study was conducted to further screen the active components and key targets of AM in the treatment of CRC through bioinformatics. The active compound components of AM in the TCMSP database were screened based on OB and DL. OB is particularly important in the clinical treatment of most herbal medicines as one of the most important pharmacokinetic characteristics of orally administered drugs [19]. DL is commonly used as a qualitative method of analysis for drug design to assess whether a compound is suitable for use as a drug [20]. In this study, based on OB ≥ 30 % and DL ≥ 0.18, 16 target components and 177 potential targets of AM were screened out, and they were subsequently intersected with 2740 CRC-related genes. Then, a pharmacological network of 132 intersected genes for AM in CRC therapy was constructed.

Subsequently, by conducting PPI analysis on the 132 intersected genes mentioned above, 105 genes with node degree ≥10 were further screened out. And the 105 genes mentioned above were further analysed for GO and KEGG enrichment. Relevant target genes of AM were revealed to be enriched in biological processes such as chemical stress, and oxidative stress response, and equally enriched in lipid and atherosclerosis, hepatitis, MAPK, IL-17, TNF, etc. Interestingly, AKT1, with the highest node degree in the PPI network, has been shown to influence cancer progression by regulating biological functions such as cell proliferation, metabolism and growth [21]. Numerous studies have also shown that signalling pathways, such as MAPK, IL-17 and TNF, have an important role in cancer development [22-24]. The results of these studies not only confirm the influence of AM in the course of CRC by participating in the above-mentioned tumor-related signalling pathways, but also provide a scientific basis for the good efficacy of AM in cardiovascular and infectious diseases to a certain extent [25,26].

After bioinformatics analysis, one key gene, named MET, was finally obtained, which the active component was methylnissolin (MOL000380). MET, as a proto-oncogene, has been studied and confirmed to play an essential role in the proliferation, migration and invasion of tumor cells [27]. It has also been demonstrated as an independent therapeutic target in CRC since it is overexpressed in CRC [28]. Methylnissolin has been identified as one of the active compounds in Danggui Buxue Decoction (DBD) and may play an active part in treating CRC [29]. In this study, methylnissolin was found to be one of the effective active compounds of AM in the treatment of CRC. This study may provide a potential target for the treatment of CRC and may inform the further development of AM in the treatment of CRC patients.

However, this study has some limitations. Firstly, since the public dataset used in this study is still being updated, all bioactive components and target genes included in AM in this study are not comprehensive. Secondly, this study only analyzed the data through bioinformatics without experimental verification, and hence the clinical significance of this study needs to be confirmed.

CONCLUSION

The findings of this study indicate that MET is a key target gene of AM in the treatment of CRC, with the active component being methylnissolin. Thus, results may provide a scientific basis for the further development of AM for the treatment of CRC.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

REFERENCES

1. *Kanth P, Inadomi JM. Screening and prevention of colorectal cancer. Bmj. 2021; 374: n1855.*
2. *Zou J, Xiao Z, Wu Y, Yang J, Cui N. Noninvasive fecal testing for colorectal cancer. Clin Chim Acta 2022; 524: 123-131.*
3. *Si H, Yang Q, Hu H, Ding C, Wang H, Lin X. Colorectal cancer occurrence and treatment based on changes in intestinal flora. Semin Cancer Biol 2021; 70: 3-10.*
4. *Kishore C, Bhadra P. Current advancements and future perspectives of immunotherapy in colorectal cancer research. Eur J Pharmacol 2021; 893: 173819.*
5. *Huang S, Zhang Z, Li W, Kong F, Yi P, Huang J, Mao D, Peng W, Zhang S. Network pharmacology-based prediction and verification of the active ingredients and potential targets of Zuojinwan for treating colorectal cancer. Drug Des Devel Ther 2020; 14: 2725-2740.*
6. *Xiang Y, Guo Z, Zhu P, Chen J, Huang Y. Traditional Chinese medicine as a cancer treatment: Modern perspectives of ancient but advanced science. Cancer Med 2019; 8(5): 1958-1975.*
7. *Li S, Sun Y, Huang J, Wang B, Gong Y, Fang Y, Liu Y, Wang S, Guo Y, Wang H, et al. Anti-tumor effects and mechanisms of Astragalus membranaceus (AM) and its specific immunopotentiation: Status and prospect. J Ethnopharmacol 2020; 258: 112797.*
8. *Sheik A, Kim K, Varaprasad GL, Lee H, Kim S, Kim E, Shin JY, Oh SY, Huh YS. The anti-cancerous activity of adaptogenic herb Astragalus membranaceus. Phytomed 2021; 91: 153698.*
9. *Auyeung KK, Han QB, Ko JK. Astragalus membranaceus: A review of its protection against*

inflammation and gastrointestinal cancers. Am J Chin Med 2016; 44(1): 1-22.

10. *Sun R, Gu J, Chang X, Liu F, Liang Y, Yang X, Liang L, Tang D. Metabonomics study on orthotopic transplantation mice model of colon cancer treated with Astragalus membranaceus-Curcuma wenyujin in different proportions via UPLC-Q-TOF/MS. J Pharm Biomed Anal 2021; 193: 113708.*
11. *Chu XD, Zhang YR, Lin ZB, Zhao Z, Huangfu SC, Qiu SH, Guo YG, Ding H, Huang T, Chu XL, et al. A network pharmacology approach for investigating the multi-target mechanisms of Huangqi in the treatment of colorectal cancer. Transl Cancer Res 2021; 10(2): 681-693.*
12. *Tseng A, Yang CH, Chen CH, Chen CH, Hsu SL, Lee MH, Lee HC, Su LJ. An in vivo molecular response analysis of colorectal cancer treated with Astragalus membranaceus extract. Oncol Rep 2016; 35(2): 659-668.*
13. *Shen A, Chen Y, Liu L, Huang Y, Chen H, Qi F, Lin J, Shen Z, Wu X, Wu M, et al. EBF1-mediated upregulation of ribosome assembly factor PNO1 contributes to cancer progression by negatively regulating the p53 signaling pathway. Cancer Res 2019; 79(9): 2257-2270.*
14. *Wang H, Tian T, Zhang J. Tumor-Associated Macrophages (TAMs) in colorectal cancer (CRC): From mechanism to therapy and prognosis. Int J Mol Sci 2021; 22(16).*
15. *Liang F, Wang S, Zhang K, Liu TJ, Li JN. Development of artificial intelligence technology in diagnosis, treatment, and prognosis of colorectal cancer. World J Gastrointest Oncol 2022; 14(1): 124-152.*
16. *Huang XM, Yang ZJ, Xie Q, Zhang ZK, Zhang H, Ma JY. Natural products for treating colorectal cancer: A mechanistic review. Biomed Pharmacother 2019; 117: 109142.*
17. *Lin T, Liang C, Peng W, Qiu Y, Peng L. Mechanisms of core Chinese herbs against colorectal cancer: A study based on data mining and network pharmacology. Evid based complement Alternat Med 2020; 2020: 8325076.*
18. *Guo Z, Lou Y, Kong M, Luo Q, Liu Z, Wu J. A systematic review of phytochemistry, pharmacology and pharmacokinetics on Astragali Radix: Implications for aRadix as a personalized medicine. Int J Mol Sci 2019; 20(6).*
19. *Gao K, Song YP, Song A. Exploring active ingredients and function mechanisms of Ephedra-bitter almond for prevention and treatment of Corona virus disease 2019 (COVID-19) based on network pharmacology. BioData Min. 2020 Nov 10;13(1):19.*
20. *Sun JH, Sun F, Yan B, Li JY, Xin L. Data mining and systematic pharmacology to reveal the mechanisms of traditional Chinese medicine in Mycoplasma pneumoniae pneumonia treatment. Biomed Pharmacother 2020; 125: 109900.*
21. *Alwhaibi A, Verma A, Adil MS, Somanath PR. The unconventional role of Akt1 in the advanced cancers and in diabetes-promoted carcinogenesis. Pharmacol Res 2019; 145: 104270.*
22. *García-Hernández L, García-Ortega MB, Ruiz-Alcalá G, Carrillo E, Marchal JA, García M. The p38 MAPK components and modulators as biomarkers and molecular targets in cancer. Int J Mol Sci 2021; 23(1).*
23. *Li X, Bechara R, Zhao J, McGeachy MJ, Gaffen SL. IL-17 receptor-based signaling and implications for disease. Nat Immunol 2019; 20(12): 1594-1602.*
24. *Dash S, Sahu AK, Srivastava A, Chowdhury R, Mukherjee S. Exploring the extensive crosstalk between the antagonistic cytokines- TGF-β and TNF-α in regulating cancer pathogenesis. Cytokine 2021; 138: 155348.*
25. *Su HF, Shaker S, Kuang Y, Zhang M, Ye M, Qiao X. Phytochemistry and cardiovascular protective effects of Huang-Qi (Astragali Radix). Med Res Rev 2021; 41(4): 1999-2038.*
26. *Ko PH, Huang CW, Chang HH, Chuang EY, Tsai MH, Lai LC. Identifying the functions and biomarkers of Codonopsis pilosula and Astragalus membranaceus aqueous extracts in hepatic cells. Chin Med 2019; 14: 10.*
27. *Lai X, Dong Q, Xu F, Wu S, Yang D, Liu C, Li Y, Li Z, Ma D. Correlation of c-MET expression with clinical characteristics and the prognosis of colorectal cancer. J Gastrointest Oncol 2021; 12(5): 2203-2210.*
28. *Huang CM, Huang MY, Chen YC, Chen PJ, Su WC, Chang TK, Li CC, Huang CW, Tsai HL, Wang JY. miRNA-148a Enhances the treatment response of patients with rectal cancer to chemoradiation and promotes apoptosis by directly targeting c-Met. Biomed 2021; 9(10).*
29. *Feng SH, Zhao B, Zhan X, Motanyane R, Wang SM, Li A. Danggui buxue decoction in the treatment of metastatic colon cancer: network pharmacology analysis and experimental validation. Drug Des Devel Ther. 2021; 15:705-720.*