Network Pharmacology-based Anticancer Effect Study of Ardisiacrispin B for Colon Cancer

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Abstract [**Objectives**] To study the network pharmacology-based anticancer effect of Ardisiacrispin B for colon cancer (CRC). [**Methods**] The chemical structure and molecular properties of Ardisiacrispin B were assessed via the PubChem resource, while the putative target genes of Ardisiacrispin B were predicted using the PharmMapper Database. Moreover, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Gene Ontology (GO) enrichment analyses were conducted via the WebGestalt platform. Finally, a drug-target-pathway network was built via Cytoscape to show the visual representation. [**Results**] Ardisiacrispin B exhibited exceptional druggability with 25 putative targets. Analyses conducted using KEGG, GO, and network methods showed that these target genes were related with inflammatory responses, cancer, and varoius other biological functions. On the basis of these findings, we further screened the correlative biotargets of Ardisiacrispin B in relation to CRC, and explored the anticancer effects of Ardisiacrispin B for the treatment of CRC through CCK8 analysis and colony formation assay. Our results confirmed that Ardisiacrispin B exhibited anti-CRC properties, and suggested 11 candidate targets of Ardisiacrispin B in the treatment of CRC. [**Conclusions**] Ardisiacrispin B has been demonstrated to target multiple proteins/genes and pathways, thereby forming a network that displays systematic pharmacological activities. Moreover, it has potential therapeutic value in tumor treatment, specifically in promoting the proliferation of CRC cells.

Key words Ardisiacrispin B, Target identification, Network pharmacology, Colon cancer (CRC)

1 Introduction

Colon cancer (CRC) is a prevalent malignancy of the digestive system, with its incidence and mortality ranking third and fifth, respectively, among malignant tumors in China, and is rising gradually[1]. The early clinical presentation of CRC is atypical. Due to the potential for misdiagnosis and the high malignancy associated with CRC, this condition is prone to metastasis. A notable characteristic of CRC is that the 5-year survival rate for early-stage CRC is approximately 90%, whereas for advanced CRC, the survival rate is only about 7%[2]. Traditional treatment options for CRC include surgical intervention, radiotherapy, chemotherapy, and targeted treatment. Patients with advanced CRC who undergo surgical treatment are at a high risk of relapse and metastasis. Furthermore, these patients often exhibit resistance to multiple chemotherapeutic drugs, resulting in poor prognosis. Therefore, exploring new treatments for CRC is of utmost importance.

Natural products, including traditional Chinese medicine (TCM), are the most plentiful sources of bioactive compounds and pharmaceutical components for drug development. *Ardisia*, for instance, is an important herbal medicinal species within the Myrsinaceae family[3] and has been extensively used for centuries in China. It possesses a wide spectrum/variety of pharmacological effects, including antifungal[4], cytotoxic[5], anti-inflammatory[6] and apoptosis-inducing[7] properties, *etc.* *Ardisia* is rich in structurally diverse natural products, and one of the most important drugs is Ardisiacrispin B, a utero-contracting triterpenic saponin derived from the root of *Ardisia crispa*[8].

Attention has been closely paid to Ardisiacrispin B owing to its cytotoxic roles in the prevention and clinical therapy of a wide spectrum of cancer cells, such as Hela, A549, PC-3, MCF-7, and so on[5,7,9]. These findings suggest that Ardisiacrispin B serves as a valuable compound due to its role in the incision of complicated pathophysiological processes, identification of therapeutic targets, discovery of potential molecular functions and involved pathways. However, the possible molecular mechanisms induced by Ardisiacrispin B have been rarely investigated. Simultaneously, the utilization of computational approaches to identify the drug target molecules and elucidate underlying mechanisms is becoming the main current for the sake of saving time, money, and effort. Identifying computational targets and understanding the following molecular mechanisms are especially important for accelerating the progress of drug development.

Therefore, we systematically investigated the pharmacological functions of Ardisiacrispin B using computational approaches. An overview of the analytical procedures for identifying target genes and investigating mechanisms related to Ardisiacrispin B is illustrated in Fig. 1a. On this basis, we further identified the candidate targets of Ardisacrispin B for the treatment of CRC and explored its anti-CRC effects *in vitro*.

2 Materials and methods

2.1 Chemical structure evaluation of Ardisiacrispin B by PubChem PubChem is an open-access resource for chemical molecules and their activities in biological assays[10]. It contains

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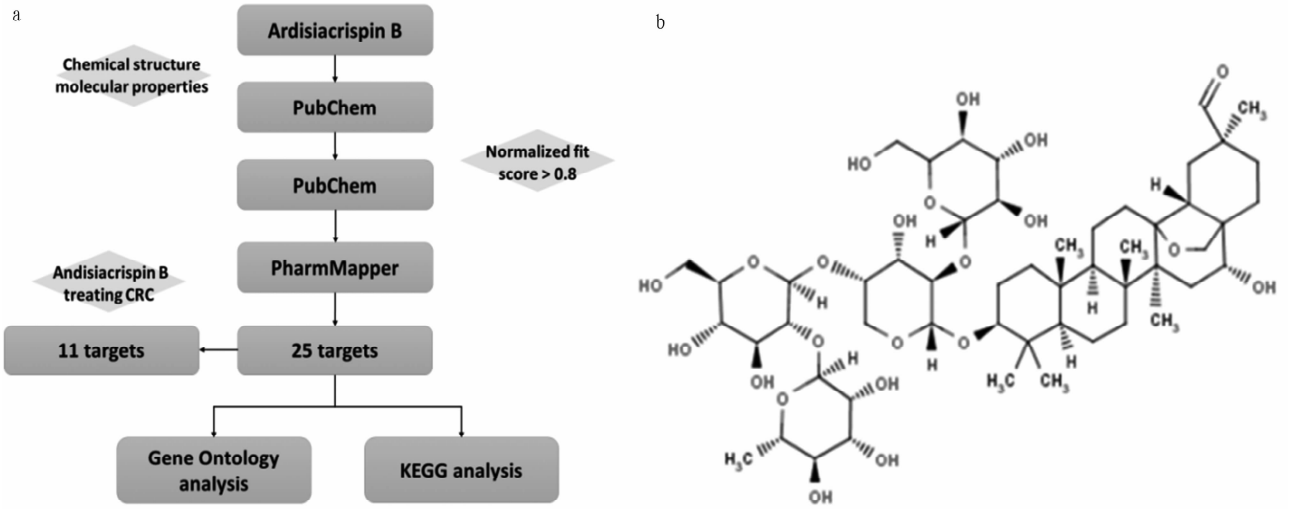


NOTE a. Workflow for the identification of potential Ardisiacrispin B target genes that integrates PubChem, TCMSP, GO and KEGG pathway analyses, and network construction; b. Chemical structure of Ardisiacrispin B downloaded from PubChem. Normalized fit score > 0.8.

Fig. 1 Analytical procedures for target gene identification and mechanism investigation and chemical structure of Ardisiacrispin B

millions of compound structures and descriptive datasets that can be freely accessed through a web interface. In this study, "Ardisiacrispin B" was input to the PubChem database, and its pharmacokinetic activities were assessed at the molecular level. The chemical structure and molecular SDF format were downloaded from the PubChem database for further investigation.

2.2 Targets identification via the PharmMapper database PharmMapper (http://www.lilab-ecust.cn/pharmmapper/) is an online reverse docking database designed for the identification of potential targets for small molecules[11]. Given a small molecule (such as a drug, natural product, or other newly discovered compound) in Mol2 or SDF format, it provides the predicted targets ordered in descending order by the normalized fit score. In the present study, the SDF format file of Ardisiacrispin B was downloaded from PubChem and input into PharmMapper. Only human protein targets were selected, while all other parameters were set to their default values. The targets with a normalized fit score greater than 0.8 were selected for further investigation.

2.3 Gene function and KEGG pathway enrichment analyses We employed the Web-based Gene Set Analysis Toolkit (WebGestalt) to systematically investigate the function and pathway enrichment information associated with the target genes we predicted[12]. The gene of interest was entered into the WebGestalt web server utilizing the overrepresentation enrichment analysis (ORA) approach with Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Oncology (GO) databases and other default parameters. A *P*-value of less than 0.05 was considered statistically significant. GO is an extensively employed resource for annotating gene functions, which include biological processes, molecular functions, and cellular components[13]. In contrast, KEGG is a widely used database for systematically investigating information on gene-involved pathways[14-15].

2.4 Network construction To gain a comprehensive understanding of the complex associations among the drug, target genes and related pathways, we used Cytoscape 3.7.2 to build and analyze the drug-target-pathway networks.

2.5 Identification of candidate targets of Ardisiacrispin B in the treatment of CRC CRC-associated genes were obtained from the DisGeNET database, a discovery platform that houses one of the largest publicly available collections of genes and variants related to human diseases[16]. Candidate targets of Ardisiacrispin B for the treatment of CRC were identified through the overlap of targets associated with Ardisiacrispin B and CRC-associated genes. Furthermore, the STRING database[17] was employed to investigate the protein-protein interactions (PPI) of the targets associated with Ardisiacrispin B in the treatment of CRC.

2.6 Colony formation assay and CCK-8 assay Each group of CRC cells, specifically SW620 and HCT116, treated with Ardisiacrispin B during the logarithmic growth phase was digested with 0.25% trypsin to form a single cell suspension, which was subsequently seeded into a 12-well plate. They were incubated for 2-3 weeks in a cell incubator maintained at 37°C with 5% CO_2. Once colonies became visible to the naked eye, the culture process was terminated, the supernatant was discarded, and the cells were washed twice with PBS. Then, the cells were fixed with 1 mL of 4% paraformaldehyde for 15 min, followed by staining with a 0.1% crystal violet staining solution for 30 min. The plate was then washed with water prior to the acquisition of photographs and the execution of colony counts.

To analyze the cell viability of SW620 and HCT116 cells, a Cell Counting Kit-8 (CCK-8, Dojindo, Japan) was used. SW620 and HCT116 cells were treated at a density of 5 000 cells/well in 96-well plates for 24, 48 and 72 h. Cells were then incubated with 10 μL of CCK-8 for 4 h at 37 ℃. Cell viability was measured using a Multiskan GO Microplate Reader (Thermo Scientific, USA) as previously described.

2.7 Statistical analysis All statistical analyses were performed using Graphpad Prism 5.0. Experimental data, derived from a minimum of three independent experiments, were presented as

mean ± *SD*. A *P*-value of less than 0.05 was considered as statistically significant.

3 Results and analysis

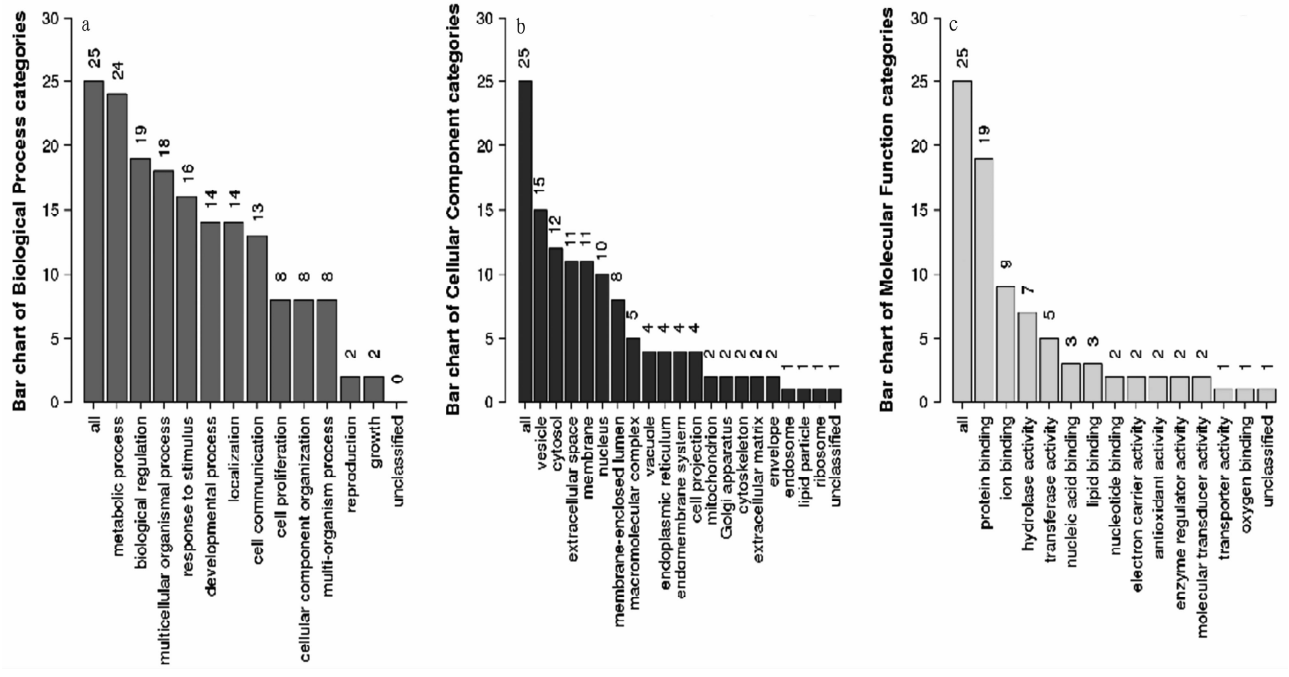
3.1 Chemical structure and molecular properties of Ardisiacrispin B PubChem serves as a vital resource for the biomedical research community by providing key chemical information. The chemical structure and molecular properties of Ardisiacrispin B were obtained from the PubChem database. The molecular formula of Ardisiacrispin B is $C_{53}H_{86}O_{22}$ (Fig. 1b), and its molecular weight is 1 075.2 g/mol. The molecular SDF format file was downloaded for further investigation.

3.2 Identification of potential drug targets Potential targets for Ardisiacrispin B were identified using PharmMapper[11], with a selection criterion of a normalized fit score of no less than 0.8. We obtained their official gene symbols and gene IDs from PDB and uniprot and the Gene database of National Center for Biotechnology Information (NCBI), yielding 25 unique target genes after the removal of duplicates. These 25 identified target genes of Ardisiacrispin B were utilized for further exploration (Table 1).

3.3 GO and KEGG pathway analysis To enhance the understanding of the 25 predicted targets, GO and KEGG enrichment analyses were performed using WebGestalt. As demonstrated in Fig. 2, the top seven functions were metabolic processes (24/25), biological regulation (19/25), protein binding (19/25), multicellular organismal processes (18/25), responses to stimulus (16/25), vesicle-related functions (15/25), and developmental processes (14/25). These GO terms were highly relevant to anti-inflammatory, anti-tumor properties. The pathway analysis revealed that 25 target genes were involved in eight KEGG pathways that exhibited significant *P*-values, including pathways related to chemical carcinogenesis, drug metabolism, and so on (Fig. 3).

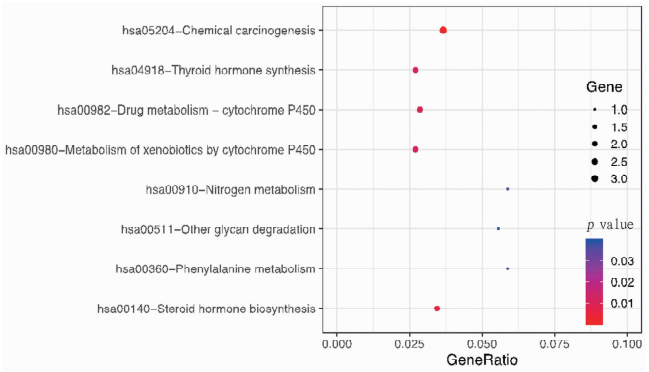
Table 1 Putative drug targets of Ardisiacrispin B

No.	Gene ID	Gene symbol	Target name
1	5478	*PPIA*	Peptidyl-prolyl cis-trans isomerase A
2	650	*BMP2*	Bone morphogenetic protein 2
3	7376	*NR1H2*	Oxysterols receptor LXR-beta
4	9261	*MAPKAPK2*	MAP kinase-activated protein kinase 2
5	412	*STS*	Steryl-sulfatase
6	2629	*GBA*	Glucosylceramidase
7	2191	*FAP*	Seprase
8	4507	*MTAP*	S-methyl-5-thioadenosine phosphorylase
9	1646	*AKR1C2*	Aldo-keto reductase family 1 member C2
10	54210	*TREM1*	Triggering receptor expressed on myeloid cells 1
11	231	*AKR1B1*	Aldose reductase
12	4129	*MAOB*	Amine oxidase (flavin-containing) B
13	213	*ALB*	Serum albumin
14	3384	*ICAM2*	Intercellular adhesion molecule 2
15	4860	*PNP*	Purine nucleoside phosphorylase
16	759	*CA1*	Carbonic anhydrase 1
17	590	*BCHE*	Cholinesterase
18	7276	*TTR*	Transthyretin
19	23173	*METAP1*	Methionine aminopeptidase 1
20	51170	*HSD17B11*	Estradiol 17-beta-dehydrogenase 11
21	2638	*GC*	Vitamin D-binding protein
22	4322	*MMP13*	Collagenase 3
23	6822	*SULT2A1*	Bile salt sulfotransferase
24	2280	*FKBP1A*	Peptidyl-prolyl cis-trans isomerase FKBP1A
25	2950	*GSTP1*	Glutathione S-transferase P



NOTE a. Biological process; b. Cellular component; c. Molecular function.

Fig. 2 GO map of putative target genes



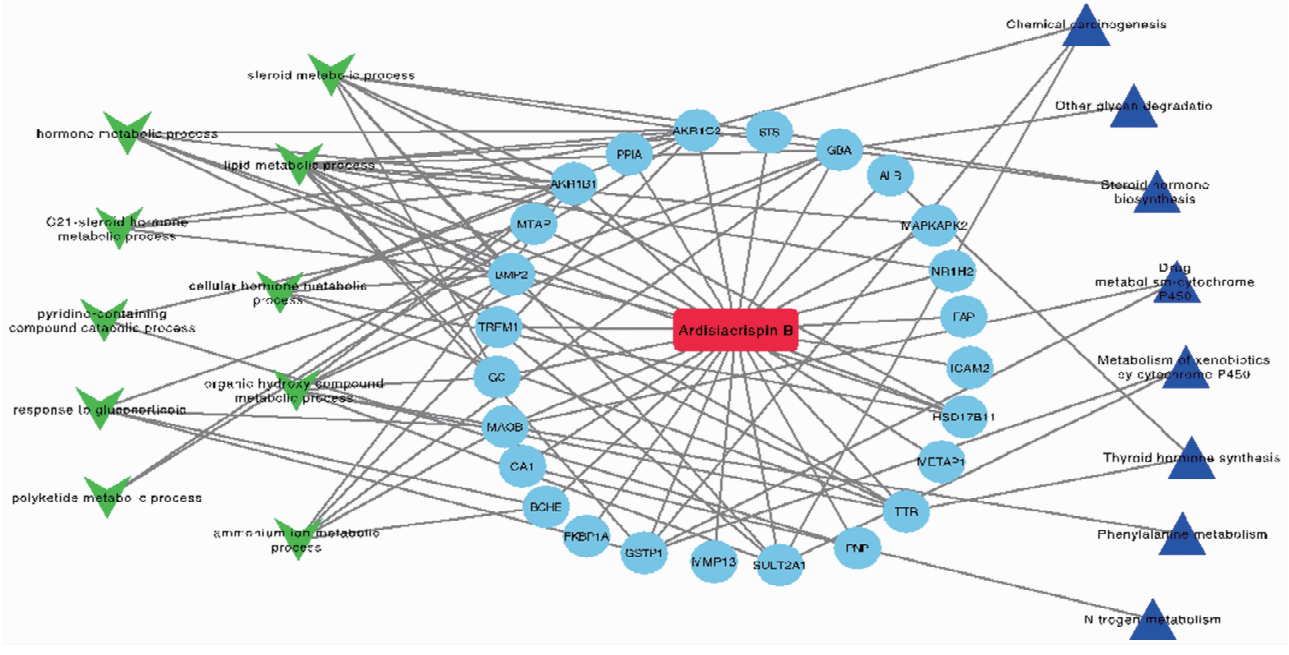
NOTE *X*-axis represents negative $\log_{10}$ of *P* value while *Y*-axis demonstrates the enriched KEGG pathways with ID and name.

Fig. 3 KEGG pathway analysis of putative target genes

3.4 Network Analysis Based on the target identification and pathway analysis, a comprehensive network encompassing the drug, target genes, and associated pathways was constructed via Cytoscape 3.7.2, as demonstrated in Fig. 4.

3.5 Candidate targets of Ardisiacrispin B for the treatmeng of CRC In the findings derived from web-based tools, a total of 3 297 disease-related genes were obtained from the DisGeNET database. By integrating CRC related genes and Ardisiacrispin B target genes, 11 candidate targets of Ardisiacrispin B for the treatment of CRC were identified. Moreover, these 11 targets were used to construct the PPI network (Fig. 5).

3.6 Inhibitory effect of Ardisiacrispin B on CRC cells: *in vitro* experiments Colony formation assays showed that the number of colonies formed by SW620 and HCT116 cells significantly decreased following treatment with Ardisiacrispin B, with the effect exhibiting a dose-dependent increase (Fig. 6a-b). The survival of SW620 cells over 24, 48, and 72 h exhibited a gradual decline with increasing concentrations of Ardisiacrispin B (Fig. 6c). A similar trend was observed in HCT116 cells (Fig. 6d), indicating that Ardisiacrispin B inhibits the cell viability of both SW620 and HCT116 cells.



NOTE The red oblong shape, blue circles, green inverted triangles, and purple triangles indicate drugs, target genes, and their associated GO terms and pathways, respectively.

Fig. 4 Ardisiacrispin B-target-GO-pathway network

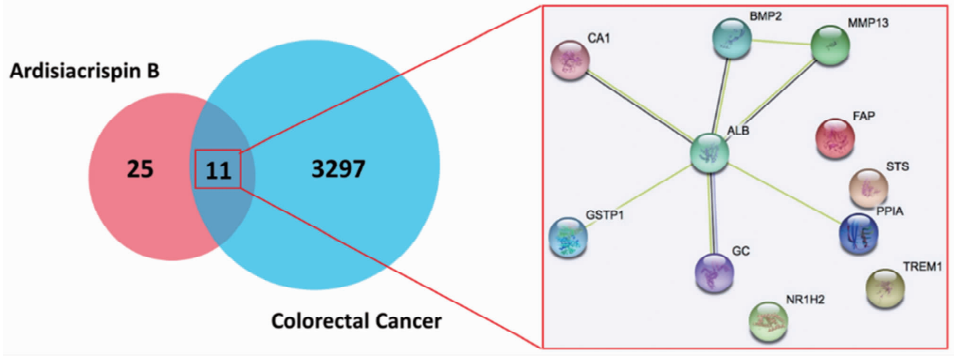
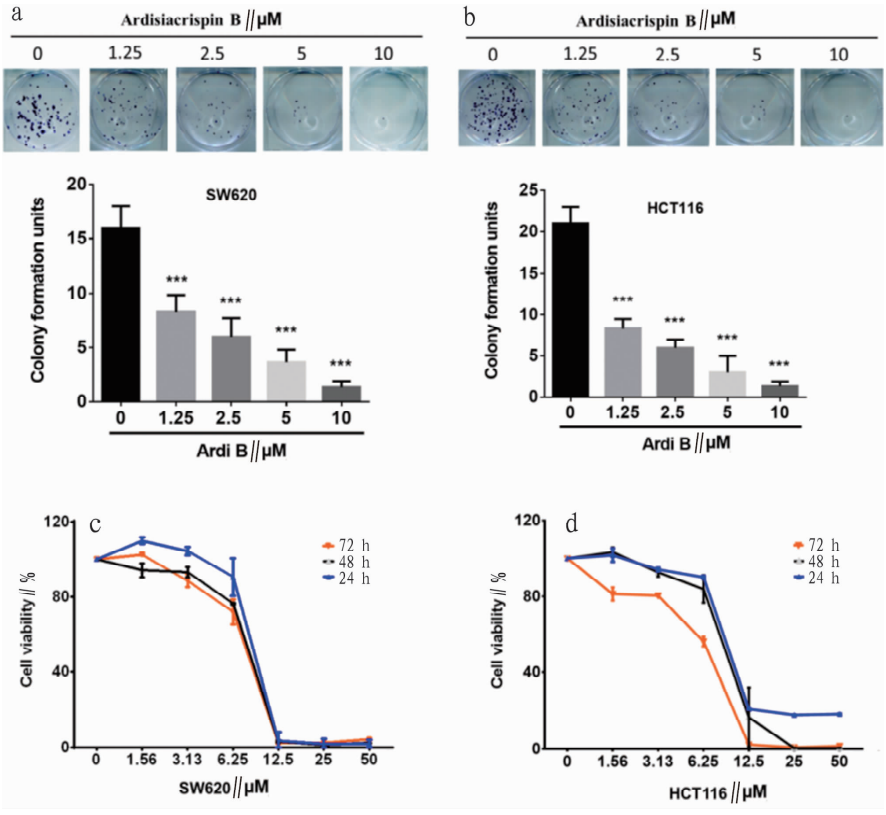


Fig. 5 PPI network of Ardisiacrispin B for the treatment of CRC



NOTE a – b. Colony formation assay measured after treatment of SW620 and HCT116 cells for 24 h at different concentrations of Ardisiacrispin B (0, 1.25, 2.5, 5, 10 μM); c – d. SW620 and HCT116 cells treated with Ardisiacrispin B (0, 1.56, 3.13, 6.25, 12.5, 25, 50 μM) for 24, 48, 72 h. The daily cell viability was measured.

Fig. 6 Effect of Ardisiacrispin B on the proliferation of SW620 and HCT116 cells *in vitro*

4 Discussion

Network pharmacology can improve pharmacokinetic modeling, prediction, as well as toxicity and metabolic endpoints, all of which streamline and speed up the drug development progress[18]. The identification of target genes is the first step in drug discovery. It has been revealed that an increasing number of active drugs or compounds interact with multiple proteins or genes to exert their pharmacological functions[19-20]. A variety of target prediction methods have been developed and are widely used towards this end. As shown in Table 1, 25 putative target genes of Ardisiacrispin B were predicted using network pharmacology.

We have identified an anti-cancer role for Ardisiacrispin B. Our findings indicate that some of the predicted target genes of Ardisiacrispin B are associated with cancer, including *AKR1C1*, *GSTP1*, and *SULT2A1*[21-23]. These genes are closely related to cell proliferation, differentiation, and migration, and they participant in pathways related to chemical carcinogenesis, drug metabolism, and so on. Consistently, Mbaveng *et al.*[24] have also discovered that Ardisiacrispin B exerts significant cytotoxic effects in nine cancer cell lines, with IC_{50} values of less than 10 uM. These interesting findings may elucidate the potential mechanisms underlying the anti-tumor effects exhibited by Ardisiacrispin B. Furthermore, we screened the pathogenic genes of CRC and therapeutic genes of Ardisacrispin B, which were subsequently integrated to construct a PPI network of Ardisacrispin B for the treatment of CRC. The results indicate that *CA1*, *BMP2*, *MMP13*, *GSTP1*, *ALB*, *GC*, *FAP*, *STS*, *PPIA*, *TREM1*, and *NR1H2* are correlative biotargets of Ardisacrispin B in relation to CRC. All of these genes are potential therapeutic targets of Ardisacrispin B for the treatment of CRC. However, further studies are required to validate this pharmacological hypothesis proposed.

The drug-target network illustrated in Fig. 5 also demonstrates that Ardisiacrispin B interacts with multiple targets, thereby exerting a range of pharmacological effects. Therapeutic agents that engage multiple targets are generally more effective in the treatment of complex diseases, such as tumors or leukemia, and are less vulnerable to drug resistance. Therefore, Ardisiacrispin B may be a promising natural product that could serve as a lead compound, chemical moiety, or active ingredient for further drug development. Based on these analysis results, we particularly explored the anticancer effects of Ardisiacrispin B in the treatment of CRC. The present study showed that Ardisacrispin B significantly increased the inhibition of the proliferation of SW620 and HCT116 cells in a dose-dependent manner compared to the control group, suggesting its potential inhibitory effect on CRC. Nevertheless, we have to acknowledge that there are some limits and biases in our analyses due to the databases we employed, for example, the release, update date and resources used. Based on current results, we hy-

pothesize that Ardisacrispin B may pharmacologically inhibit the development of CRC by regulating 11 correlative biotargets. However, further research is needed to validate this hypothesis.

5 Conclusions

In short, we would like to emphasize that Ardisiacrispin B is a valuable compound for the development of a safe and effective multi-targeted anticancer medicament. Further studies have confirmed its anticancer effect on CRC *in vitro*, and screened 11 correlative biotargets of Ardisacrispin B in relation to CRC. This work provides novel insights into the perspectives and challenges associated with the Ardisiacrispin B research and its clinical application in CRC for future investigations.

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