Analysis of the mechanism of berberine against stomach carcinoma based on network pharmacology and experimental validation

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Background: Although the therapeutic effects of berberine have received some attention in recent years, its potential mechanisms underlying its action against stomach carcinoma (SC) remain unclear. In this study, we aimed to elucidate the mechanisms underlying the effects of berberine against SC using a network pharmacology and experimental verification approach.

Methods: Several publicly available databases were used to collect the targets of berberine and SC. Protein-protein interaction (PPI) network, enrichment analyses and molecular docking were performed based on the potential targets of berberine against SC. The potential clinical significance and prognostic value of the targets were predicted by using nomogram and receiver operating characteristic (ROC) analyses. Then the viability and apoptosis of SC cells treated with berberine were determined. Moreover, reactive oxygen species (ROS), mitochondrial membrane potential (MMP) and adenosine triphosphate (ATP) measurements and western blot assay were carried out to validate the predicted mechanisms.

Results: Seventy-six potential targets of berberine against SC were identified. The construction of PPI network enabled the identification of hub targets, such as AKT1, TP53, IL6, JUN and MAPK1. Enrichment analyses showed that berberine was involved in apoptosis, mitophagy, ROS metabolic process, AMPK and MAPK signaling pathway. The expression levels of hub targets also contributed to the clinical prognosis of patients with SC. Molecular docking revealed the possible patterns of direct interaction between berberine and target proteins, including AMPK, TP53 and MAPK1. Experimental results showed that berberine reduced SC cell viability, promoted apoptosis and ROS generation, and contributed to reductions in MMP and ATP levels. Western blot assay demonstrated that berberine increased AMPK and TP53 expression, while decreased phosphorylated-MAPK3/1 expression.

Conclusions: We elucidated the potential action mechanisms of berberine against SC using a network pharmacology approach. Some predicted mechanisms underlying the anti-SC effects were verified based on experimental approaches. Our findings provide a meaningful foundation for berberine as a cellular apoptosis-

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inducing and energy metabolism-regulating agent against SC. However, *in vivo* experiments and clinical studies need to be further carried out. Moreover, it is necessary to study the potential negative effects of berberine thoroughly.

Introduction

Stomach carcinoma (SC) is one of the most aggressive tumors worldwide and remains the most prevalent type of cancer in East Asia. In 2022, there were more than 968 thousand new cases and close to 660 thousand deaths of SC, ranking fifth in the world in terms of incidence rate and mortality. The prognosis of SC is closely related to the timing of diagnosis and treatment. The 5-year survival rate of early SC can be more than 90% after treatment, but advanced SC even if receives comprehensive treatment mainly through surgery, the 5-year survival rate is still less than 30% (1). Despite numerous advances in diagnosis and treatment, its prognosis remains relatively poor in most countries, owing to tumor metastasis and recurrence (2).

Berberine is a compound isolated from medicinal plants such as *Coptis chinensis*. Recent studies revealed its anticancer properties against several high-risk cancers, including SC (3,4). Berberine was found to suppress *Helicobacter pylori* (*H. pylori*) infection, reverse the precancerous stomach lesions, and regulate SC cell proliferation, migration, angiogenesis, apoptosis and autophagy (4). Given the potential benefits of berberine in the treatment of SC, the mechanisms underlying its therapeutic properties warrant further examination.

Based on database retrieval, omics data analysis, and computer simulation technologies, network pharmacology integrates multidisciplinary information and constructs protein-protein and drug-target-disease interaction networks to achieve research objectives such as predicting potential action mechanisms of natural or multicomponent products. It combines the latest advances in computational biology, omics, and systems biology to overcome the problem of low efficiency in studying individual gene or protein in the past (5). In this study, we adopted a network pharmacology-based approach to elucidate the mechanisms underlying the effects of berberine against SC and experimentally validated the predicted mechanisms. We present this article in accordance with the MDAR reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-668/rc).

Methods

Collection of targets of berberine

We obtained canonical simplified molecular input line entry system (SMILES) and two- and three-dimensional (2- and 3-D) structural models of berberine from PubChem (6). We used either the SMILES string or the 2D and 3D models of berberine structure for dataset searching. Specific filtering parameters were set to enhance data stability. The predicted berberine targets were obtained by searching the Integrative Pharmacology-based Research Platform of Traditional Chinese Medicine (TCMIP) v2.0 (Tanimoto >0.8) (7), Comparative Toxicogenomics Database (CTD, interaction

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Highlight box

Key findings
- Berberine exhibits anti-gastric-cancer properties, and its mechanism involves cell apoptosis and energy metabolism.

What is known and what is new?
- Berberine, a compound isolated from medicinal plants, has been revealed to exert anti-cancer properties; however, its potential mechanisms underlying its action against stomach carcinoma (SC) remain unclear.
- This study has elucidated the potential action mechanisms of berberine against SC using a network pharmacology approach and several experimental assays have been conducted to verify the predicted targets and pathways.

What is the implication, and what should change now?
- The study provides a meaningful foundation for berberine as a cellular apoptosis-inducing and energy metabolism-regulating agent against SC. *In vivo* experiments and clinical studies need to be further carried out in this field.

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count ≥3) (8), and STITCH Database (interaction score ≥0.7) (9). All targets were merged and converted to unique names through UniProt (10). Enrichr platform (11) was used to uncover the potential disease spectrum of berberine. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Collection of targets related to SC

The targets predicted to be associated with SC were identified using DisGeNet [the Concept Unique Identifier of the Unified Medical Language System (UMLS CUI): C0699791, Score ≥0.04] (12) and CTD [Medical Subject Heading (MeSH) ID:D013274, Inference Score ≥40] (8). All targets were merged and converted to unique names through UniProt (10).

Establishment of a PPI network

To determine the potential SC-related targets of berberine, the intersections of targets identified through searches of the aforementioned berberine target and SC target databases were examined using Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/index.html). Berberine-SC targets were analyzed using the STRING database (13) to construct a protein-protein interaction (PPI) network (interaction score ≥0.70). Then, a network of the hub targets was constructed using Cytoscape-Network Analyzer (14).

Pathway and process enrichment analyses

Enrichment analyses were carried out using Metascape database with the following ontology sources: Kyoto Encyclopedia of Genes Genomes (KEGG), Gene Ontology (GO) biological processes, GO cellular component, and GO molecular function (15). In addition, top 20 terms according to log10(P) obtained from these analyses were visualized based on Metascape (parameter settings: min overlap: 3, P value cutoff: 0.01, min enrichment: 1.5).

Analysis of clinical prognostic value of hub targets

RNA sequencing (RNAseq) data of tumor tissues and paracancer tissues of patients with SC were obtained from The Cancer Genome Atlas (TCGA) database (16) to analyze the differential expression of hub targets in tumor and paracancer tissues. The data were collected from 414 tumor tissues and 210 paracancer tissues, and log2 transformation

was performed on the data. Because the datasheets were acquired from TCGA database, no ethics approval was required. Receiver operating characteristic (ROC) analysis was carried out to evaluate the prognostic value of hub targets, and the area under the curve (AUC) indicates the accuracy of prediction. Nomogram analysis was conducted to explore the effect of hub target expression on the survival rate of patients with SC based on the multivariable Cox regression model (17). The analyses were carried out using the "survival" and "pROC" R language packages, which can automatically retain data with clinical information and correct the outcome by default (18,19).

Molecular docking

To predict the candidate protein targets with which berberine tends to interact, we used the Mcule platform (20). The crystalline structures of the proteins were obtained from the Protein Data Bank (PDB) (21). We analyzed the docking scores of berberine, with the following potential targets: AMPK (PDB: 2UV7), TP53 (PDB: 3LH0), and MAPK1 (PDB: 3ERK), which were selected from the results of PPI network and enrichment analyses.

Cell culture and treatment

Human gastric adenocarcinoma (AGS) cells were obtained from the Cell Bank, Chinese Academy of Sciences located in Shanghai, China. AGS cells were cultured in F-12K (Gibco, USA) media with 10% fetal bovine serum (Gibco) and 1% penicillin-streptomycin (Gibco) and incubated in a humidified atmosphere containing 5% CO_2 at 37 ℃. Berberine (MedChemExpress, Shanghai, China) was dissolved in dimethyl sulfoxide and subsequently diluted to the working concentration with the culture media.

Cell viability and apoptosis assay

To assess cell viability, AGS cells were seeded in 96-well plates and incubated with different concentrations of berberine for 24 or 48 h, respectively. Then, cell counting kit-8 reagent (Dojindo, Shanghai, China) was added to each well, followed by further incubation for 1 to 4 h. During incubation, the absorbance of cells in each well at 450 nm was measured using a microplate reader. Then AGS cells were seeded in 6-well plates and cultured overnight prior to exposure to 50 µM berberine or vehicle for 48 h. Apoptotic cells were identified by staining with annexin V-fluorescein
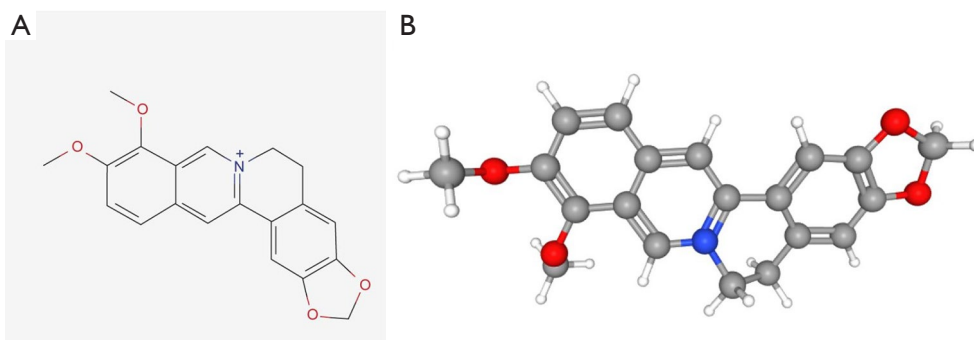
Figure 1 Structural models of berberine acquired from PubChem. (A) Two-dimensional and (B) three-dimensional structural models.

isothiocyanate (FITC)/propidium iodide (PI) (Beyotime, Shanghai, China) following the instructions. The number of apoptotic cells was then immediately assessed using a FACSort flow cytometer (BD Biosciences, USA). Three biological replicates were used.

Measurement of ROS, mitochondrial membrane potential (MMP), and adenosine triphosphate (ATP) levels

AGS cells were seeded in multi-well plates and cultured overnight prior to exposure to berberine or vehicle for 48 h. Thereafter, the intracellular reactive oxygen species (ROS) levels were evaluated using a ROS assay kit (Beyotime). The fluorescence in cells was examined using a fluorescence microscope (LEICA, Germany). The mean fluorescence intensity was calculated using ImageJ software (NIH, USA). The MMP was measured using an assay kit with JC-1 probe (Beyotime). Red fluorescence (excitation/emission: 525 nm/590 nm) and green fluorescence (490 nm/530 nm) in cells were detected, and the ratio of red to green fluorescence was calculated. Total intracellular ATP levels were measured using an ATP assay kit (Beyotime). The obtained data were normalized to the cell total protein content, which was quantified using a bicinchoninic acid (BCA) protein assay kit (ProbeGene, Xuzhou, China).

Western blot assay

To validate the predicted targets or pathways, western blot assay was carried out. Cells were lysed with protease inhibitor to extract total proteins, which were subsequently denatured and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes

(Millipore, USA). After blocking, the membranes were incubated with anti-AMPK (Abcam, Cambridge, UK), anti-p53 [Cell Signaling Technology (CST), USA], anti-Erk1/2 (CST), anti-P-Erk1/2 (Thr202/Tyr204) (CST) or anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibodies (CST) at 4 ℃ overnight, followed by horseradish peroxidase (HRP)-conjugated anti-rabbit or anti-mouse secondary antibodies. GAPDH antibody was used as a loading control. The signals were detected with enhanced chemiluminescence reagents (Thermo, USA) and visualized with an Amersham Imager system (Cytiva, USA).

Statistical analyses

Experimental data were presented as the means ± standard error of the mean (SEM). The data were analyzed by Student's two-tailed t-test using GraphPad Prism 8.0 (www.graphpad.com). P values <0.05 represented statistical significance.

Results

Predicted targets of berberine against SC

The canonical SMILES structure of berberine is COC1=C(C2=C[N+]3=C(C=C2C=C1)C4=CC5=C(C=C4CC3)OCO5)OC, and its 2D and 3D structural models obtained from PubChem are shown in *Figure 1*. We identified 114 targets of berberine and 1,368 targets related to SC. Diseases in which berberine has a potential role include colorectal, pancreatic, ovarian, breast, lung and gastric cancer (*Table 1*). Among these, 76 potential targets of berberine against SC were obtained from the intersection of berberine-SC targets (*Figure 2*).

Table 1 Potential diseases targeted by berberine based on Enrichr platform (top 20)

Rank	Disease	Combined score
1	Colorectal cancer	523.35
2	Diabetes mellitus, type 2	267.06
3	Pancreatic cancer	251.21
4	Fibrosis	219.74
5	Long QT syndrome	219.74
6	Ovarian cancer	194.48
7	Rheumatoid arthritis	194.48
8	Malaria	173.82
9	Breast cancer	161.85
10	Cholesterol level	156.63
11	Diabetes	124.39
12	Lung cancer	94.61
13	Diabetes mellitus	52.81
14	Obesity	52.69
15	Gastric cancer	49.21
16	Dementia	43.39
17	Anomalies	38.64
18	Melanoma	38.64
19	Leukemia	32.83
20	Migraine	28.57

Figure 2 The intersection of berberine targets and stomach carcinoma targets was taken to obtain the potential targets of berberine against stomach carcinoma by Venny 2.1.

PPI network of berberine against SC targets

The PPI network of the predicted hub targets of berberine-SC are shown in *Figure 3*. The nodes represent targets, and the edges represent interactions. The nodes changed from small to big, indicating that the degree value gradually increased. Similarly, the nodes changed from blue to red, indicating that the betweenness centrality value gradually increased. The top 20 targets are shown in *Table 2*. The three topological properties include degree, betweenness centrality and closeness centrality. The top 5 targets ranked by degree value include AKT1, TP53, IL6, JUN and MAPK1.

Enrichment analyses for targets of berberine against SC

The top 20 enrichment terms for targets of berberine against SC are depicted in the heatmap shown in *Figure 4*. The
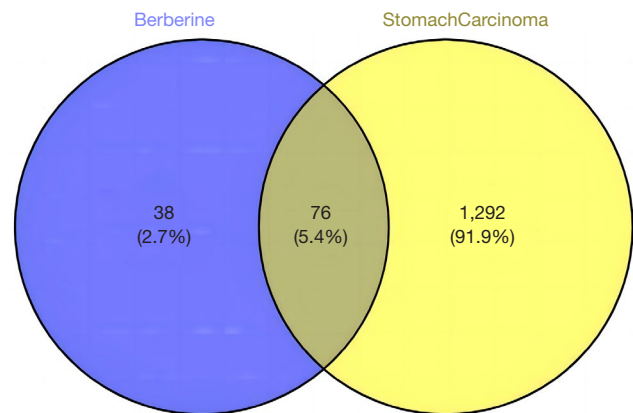
top representative KEGG enrichment results, including terms in apoptosis, mitophagy, FOXO, AMPK, NF-kappa B, MAPK and RIG-I-like receptor signaling pathway, are presented in *Figure 4A*. The top representative GO enrichment results, including terms in cell death, inflammatory response, ROS metabolic process, response to oxygen levels, cellular response to growth factor stimulus, transcription regulator complex, mitochondrial envelope, are presented in *Figure 4B-4D*.

Clinical prognostic value of hub targets

Nomogram analysis of patients' data from TCGA showed that in addition to Tumor Node Metastasis (TNM) stage, the expression levels of hub targets of berberine against SC also affected the 1-, 3- and 5-year survival rates of patients to varying degrees (*Figure 5*). The expression levels of hub targets and corresponding coefficients derived from the multivariable Cox regression model were used to establish the individual-level risk score as following: risk score = $0.7649 \times Age + 0.3685 \times Gender + 0.3617 \times T.stage + 0.5496 \times N.stage + 0.9992 \times M.stage – 0.0482 \times$ expression of AKT1 $– 0.3545 \times$ expression of TP53 $+ 0.1744 \times$ expression of IL6 $+ 0.2154 \times$ expression of JUN $+ 0.2012 \times$ expression of MAPK1 $– 0.0229 \times$ expression of TNF $+ 0.1206 \times$ expression of STAT3 $– 0.0262 \times$ expression of MMP9 $– 0.3215 \times$ expression of CASP3 $+ 0.0198 \times$ expression of PTGS2. A higher risk score indicates a worse prognosis for the patient. Among them, the expression level of TP53 contributed more to the clinical outcome, followed by

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Table 2 The degree, betweenness centrality and closeness centrality of the top 20 targets of berberine against stomach carcinoma were sorted by using Cytoscape

Rank	Degree	Target list 1	Betweenness centrality	Target list 2	Closeness centrality	Target list 3
1	42	AKT1	0.13152219	TP53	0.68932039	AKT1
2	39	TP53	0.1044487	AKT1	0.67619048	TP53
3	36	IL6	0.07779752	IL6	0.6635514	IL6
4	36	JUN	0.07527547	JUN	0.6635514	JUN
5	35	MAPK1	0.0621251	MAPK1	0.64545455	STAT3
6	34	TNF	0.05093833	CYP2E1	0.63963964	MAPK1
7	34	STAT3	0.04952554	STAT3	0.62831858	TNF
8	29	MMP9	0.04482055	CASP3	0.60683761	PTGS2
9	27	CASP3	0.03990954	TNF	0.60169492	MMP9
10	26	PTGS2	0.03835516	CTNNB1	0.59663866	CASP3
11	24	IL1B	0.03670997	CAT	0.58196721	EGFR
12	24	EGFR	0.02936054	EGFR	0.57258065	SRC
13	23	TLR4	0.02764728	MMP9	0.568	SIRT1
14	23	SRC	0.02461845	SIRT1	0.56349206	CTNNB1
15	22	PPARG	0.02441809	PTGS2	0.56349206	HMOX1
16	22	CTNNB1	0.0243843	SRC	0.55905512	PPARG
17	21	SIRT1	0.02381139	PPARG	0.55905512	IL10
18	21	HMOX1	0.01975576	AHR	0.5546875	TLR4
19	21	CCL2	0.01613559	SREBF1	0.5546875	IL1B
20	21	CXCL8	0.0154628	TLR4	0.5546875	CCND1

CASP3, JUN and MAPK1. ROC analysis suggested that the AUC of hub target expression level used to evaluate the prognosis of patients with SC were all >0.5 (*Figure 6*). In predicting the clinical outcome of patients, the predictive ability of MMP9 expression level had high accuracy (AUC =0.943), followed by TP53 (AUC =0.893), CASP3 (AUC =0.867) and MAPK1 (AUC =0.855).

Molecular docking: berberine-protein interaction

The molecular docking modes of berberine and potential target proteins, including AMPK, TP53, and MAPK1, are depicted in *Figure 7*. *Table 3* presents the docking score, which refers to the binding affinity between berberine and its targets. In general, more negative values indicate higher binding affinity. As shown, berberine exhibits the potential to bind to all three of these proteins related to SC.

Effect of berberine on SC cells

In cell viability assay, we detected notable reductions in AGS cell viability with an increase in berberine dose and treatment duration (*Figure 8A*). Treatment with 50 μM berberine for 48 h induced an increase in the number of apoptotic AGS cells (*Figure 8B*). Treatment with 20, 50, and 80 μM berberine for 48 h increased ROS generation in AGS cells in a concentration-dependent manner (*Figure 9A,9B*). Berberine treatment for 48 h was also associated with reductions in MMP (*Figure 9C*), intracellular ATP levels (*Figure 9D*), and normalized ATP levels based on the protein concentration (*Figure 9E*).

Effect of berberine on target protein expression in SC cells

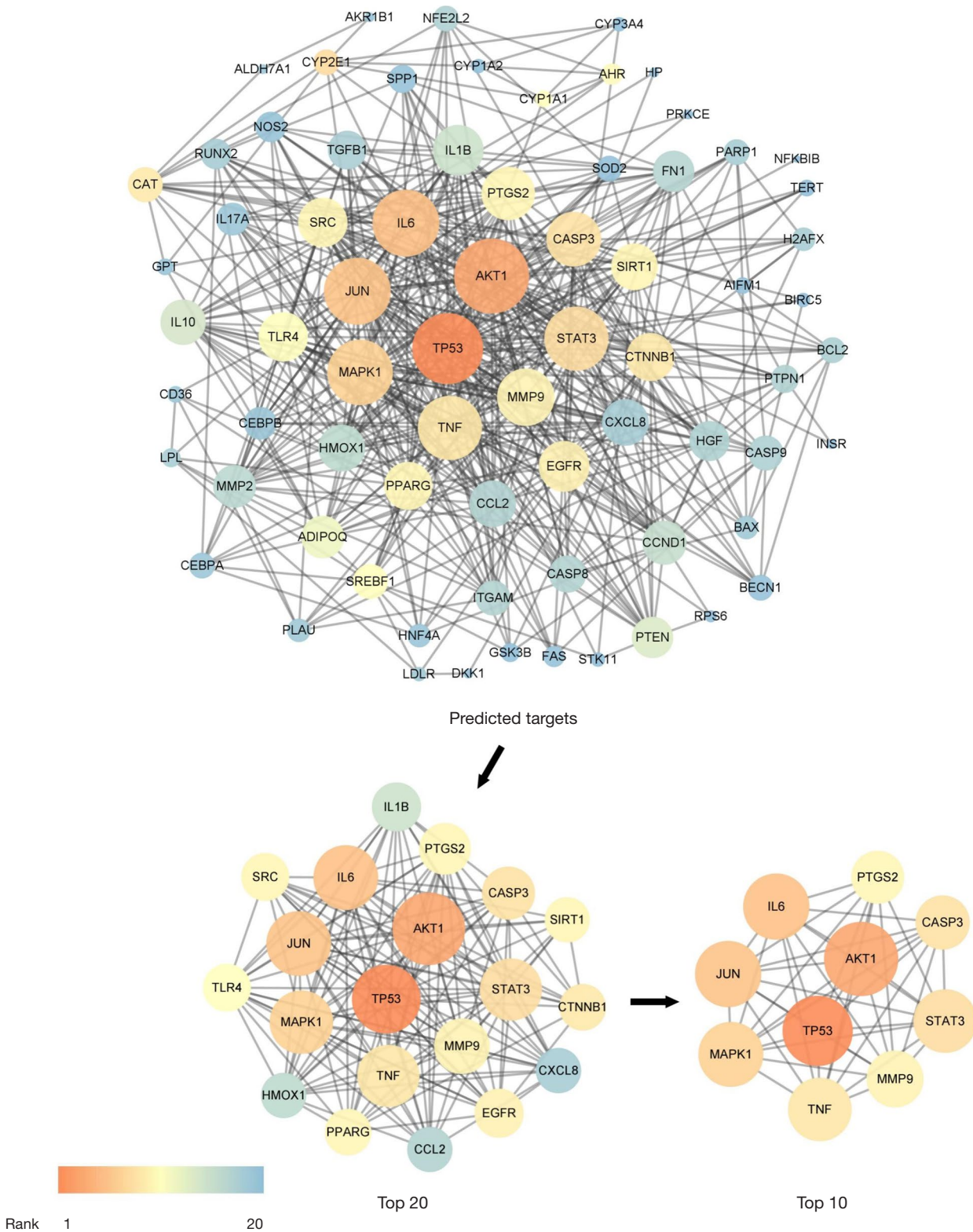Western blot assay was also conducted to validate the

Figure 3 Protein-protein interaction network of the targets of berberine against stomach carcinoma, which was constructed using Cytoscape-Network Analyzer.
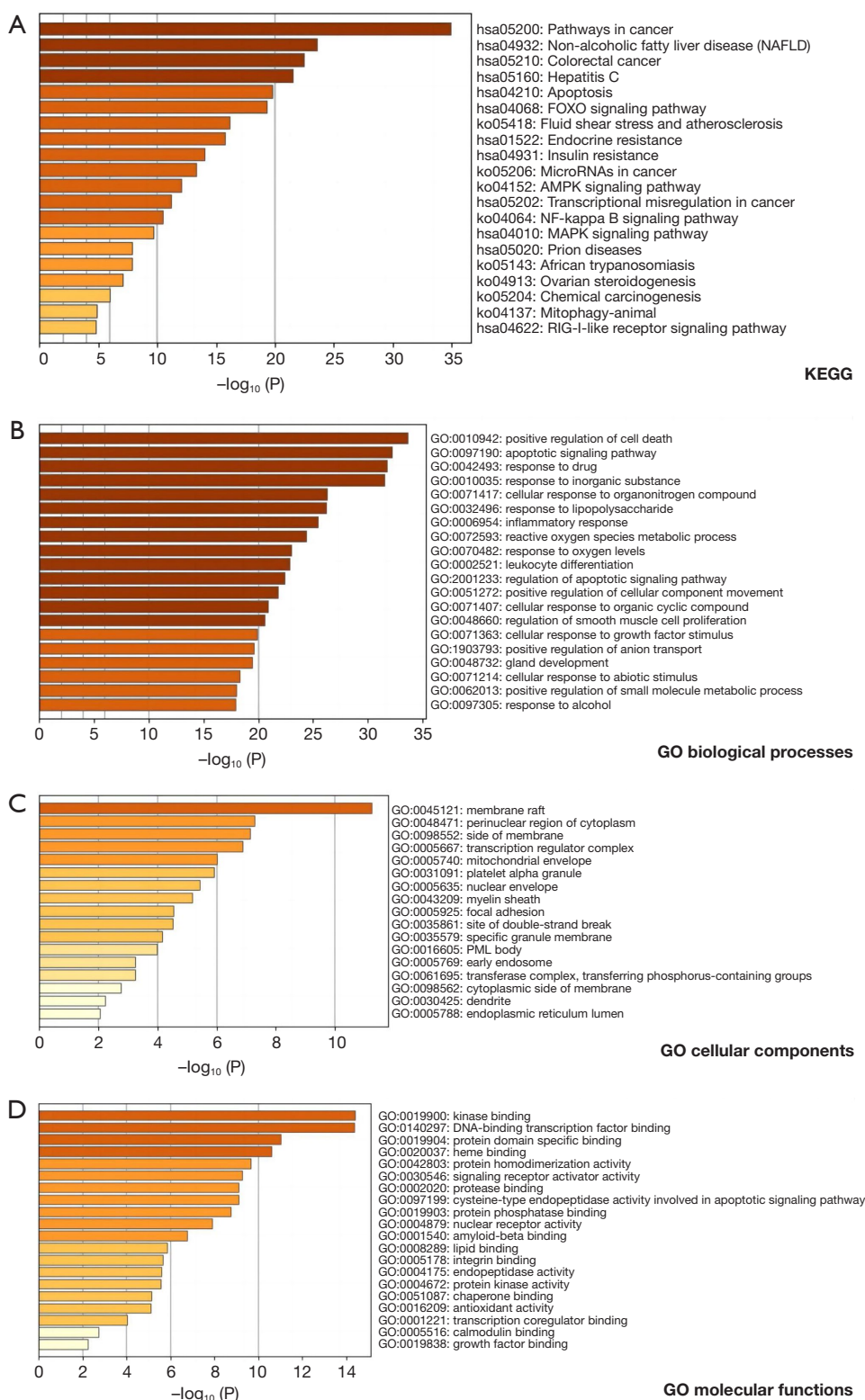
Figure 4 Top 20 terms identified from enrichment analyses. (A) KEGG and (B-D) GO enrichment analyses for targets of berberine against stomach carcinoma based on Metascape. Log10(P) is the multi-test adjusted P value in log base 10. KEGG, Kyoto Encyclopedia of Genes Genomes; GO, Gene Ontology.
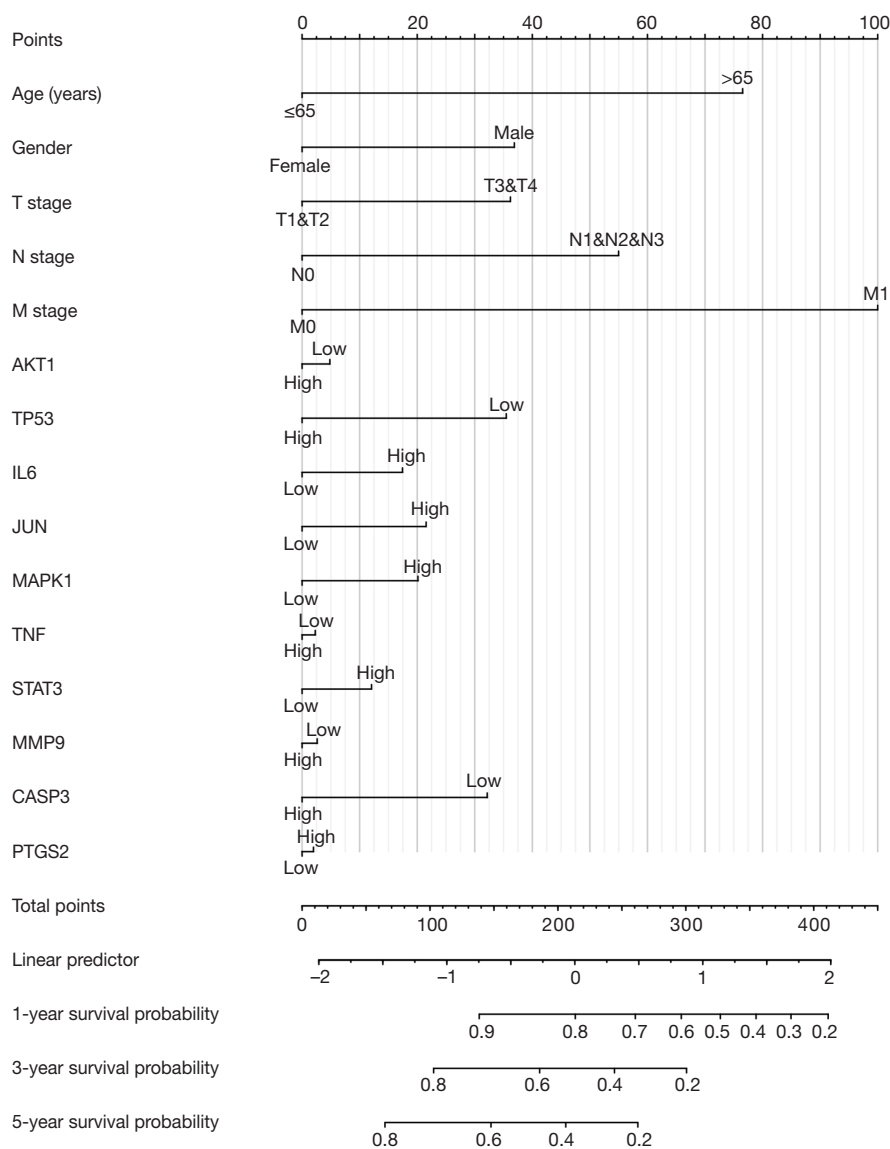
Figure 5 Nomogram analysis of the effect of hub target expression levels on the prognosis of patients with stomach carcinoma.

predicted targets and pathways. Treatment with 50 µM berberine for 48 h increased the protein expression of AMPK and p53 in AGS cells (*Figure 10A,10B*). The results also indicated that there was no significant difference in the expression of Erk1/2 (MAPK3/1) after treatment with 50 µM berberine for 48 h (*Figure 10C*), while the expression of phosphorylated-Erk1/2 (P-MAPK3/1) was decreased (*Figure 10D*). The relative intensity ratio of target proteins/GAPDH is calculated using ImageJ.

Discussion

With the increasing application of multi-target strategies in tumor treatment, the accumulated knowledge of natural products has become a valuable resource. In this study, network pharmacology analysis and *in vitro* experiments show that berberine has the potential to become a therapeutic agent against SC.

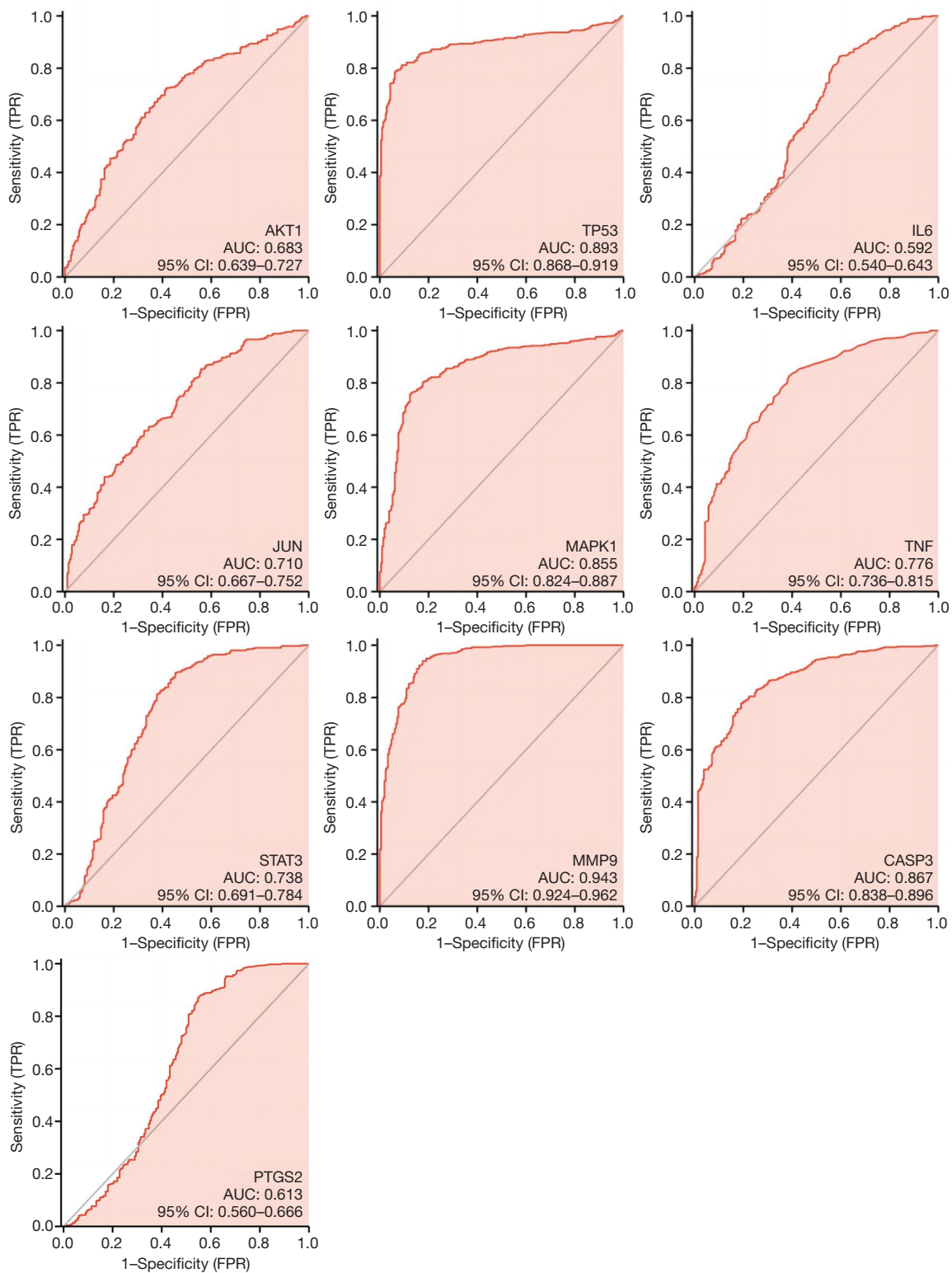Enrichment analyses showed that berberine was involved

Figure 6 Receiver operating characteristic analysis of hub target expression levels in predicting the accuracy of clinical outcome. AUC, area under the curve; CI, confidence interval; FPR, false positive rate; TPR, true positive rate.
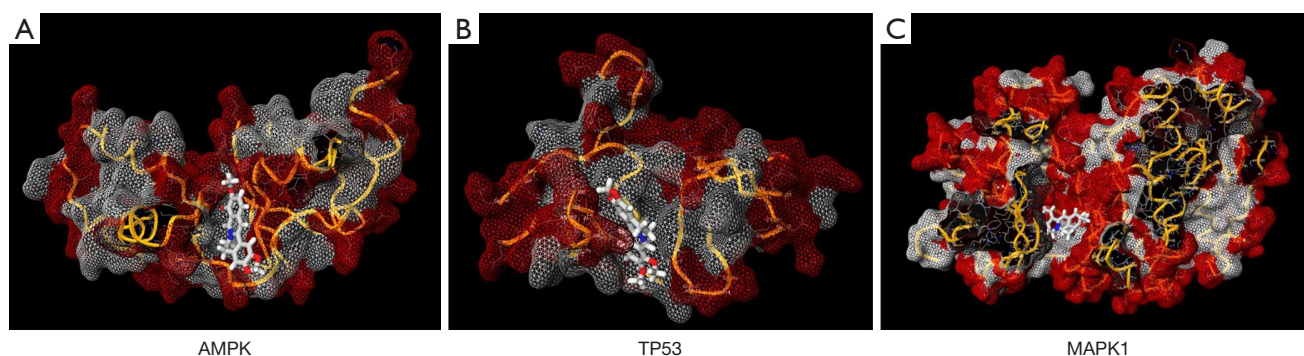
Figure 7 Partial view of molecular docking modes, which were visualized using the Mcule platform. (A) Berberine and AMPK, (B) berberine and TP53, and (C) berberine and MAPK1.

Table 3 Molecular docking scores of berberine and its candidate targets

Component	Putative target	Docking score
Berberine	AMPK	−6.3
	TP53	−6.2
	MAPK1	−7.1

in apoptosis, mitophagy and ROS metabolic process (*Figure 4*). Our experimental results *in vitro* also revealed that berberine significantly inhibited the viability of SC cells and promoted cell apoptosis (*Figure 8*). Dysregulation of cellular energy metabolism is implicated in multiple types of disorders, including age-related diseases, diabetes and cancers (*22*). Oxidative stress in tumor cells is mainly attributed to the accumulation of ROS. A certain ROS level is essential for cell survival, but excess ROS generation can trigger cell death. It has been demonstrated that the ROS levels in tumor cells are generally considerably higher than those in normal cells (*23*). Mitochondria, the main source of intracellular ROS, are the cell components most vulnerable to be attacked by ROS, which are powerful inducers of mitochondrial permeability transition pore (mPTP) opening. At elevated ROS levels, prolonged opening of mPTPs promotes ROS burst leading to mitochondrial destruction, which if propagated from one mitochondrion to another, leads to cell death (*24,25*). In cells undergoing apoptosis, MMP disruption is a significant event in the early stage of cell death. Under such circumstances, there is a reduction in MMP, decoupling of oxidative phosphorylation, and suppression of ATP production (*26*). In this regard, our experimental results demonstrated

that berberine not only induced ROS generation but also reduced MMP and ATP levels in SC cells (*Figure 9*). The above findings suggested that berberine takes part in ROS-dependent apoptosis in SC cells. These mitochondria-related mechanisms warrant further study with respect to the effects of berberine against SC.

The construction of PPI network enabled the identification of hub targets, such as TP53 and MAPK1 (*Figure 3*). Enrichment analyses also showed that berberine was involved in AMPK and MAPK signaling pathway (*Figure 4*). Molecular docking indicated that berberine may interact with AMPK, TP53 and MAPK1 to perform its biological functions (*Figure 7*). Previous experiments have also confirmed that berberine can directly bind to TP53 (*27*). Our western blot assay demonstrated that berberine increased AMPK and TP53 expression, while decreased phosphorylated-MAPK3/1 expression in AGS cells (*Figure 10*).

AMPK activity is induced in response to energy-related stress factors, such as low ATP levels and AMP/ADP ratios, which promote AMPK-mediated phosphorylation of many cellular targets, upregulation of the ATP production pathway, and inhibition of energy-consuming processes. Previous studies have demonstrated that berberine is a potent AMPK activator (*28,29*). p53-mediated control of apoptosis has been reported before. p53 can directly bind to BCL2-associated X (BAX, an apoptosis regulator), thereby increasing the permeability of mitochondrial membrane and leading to cytochrome c efflux followed by caspase activation and apoptosis (*30*). p53 also functions to lower or enhance ROS levels according to different cellular contexts (*31*). p53 protein has also been demonstrated to interact with MAPK pathways. MAPK-dependent p53 phosphorylation following
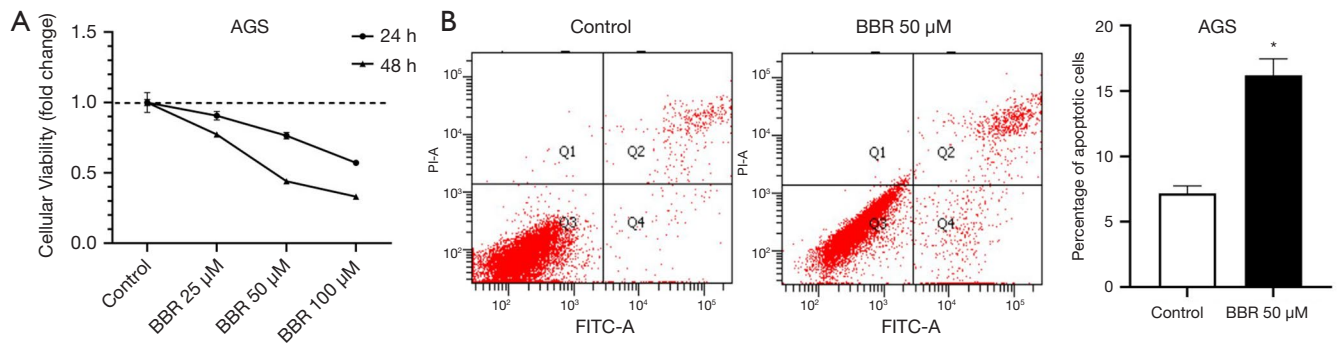
Figure 8 The effects of berberine on AGS cell viability and apoptosis. (A) The viability of AGS cells decreased with increase in berberine dose and treatment duration. (B) Berberine induced apoptosis in AGS cells. The bar graph shows the mean percentage of AGS cells that underwent apoptosis after a 50-µM berberine treatment for 48 h. *, P<0.05. BBR, berberine; FITC, fluorescein isothiocyanate; PI, propidium iodide.
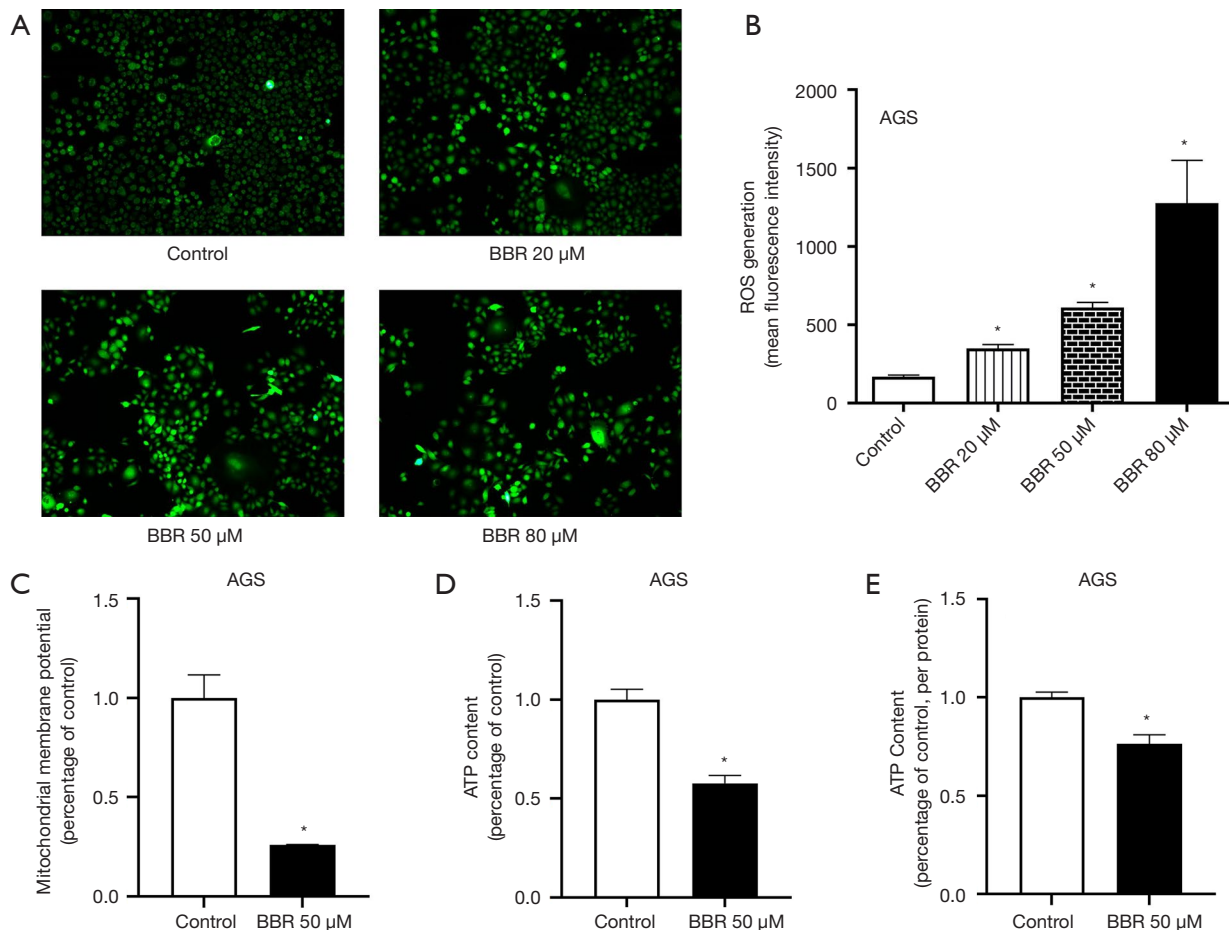

Figure 9 The effects of berberine on the ROS, MMP, and ATP levels in AGS cells. (A,B) Berberine induced ROS generation in AGS cells in a concentration-dependent manner after a 48-h treatment, which was measured by staining the cells with DCFH-DA (magnification, ×100). (C) Berberine promoted a reduction in MMP in AGS cells after a 48-h treatment. (D,E) Berberine induced reductions in intracellular ATP levels and normalized ATP levels based on the protein concentration in AGS cells after a 48-h treatment. *, P<0.05. ROS, reactive oxygen species; BBR, berberine; ATP, adenosine triphosphate; MMP, mitochondrial membrane potential; DCFH-DA, 2',7'-dichlorofluorescin diacetate.
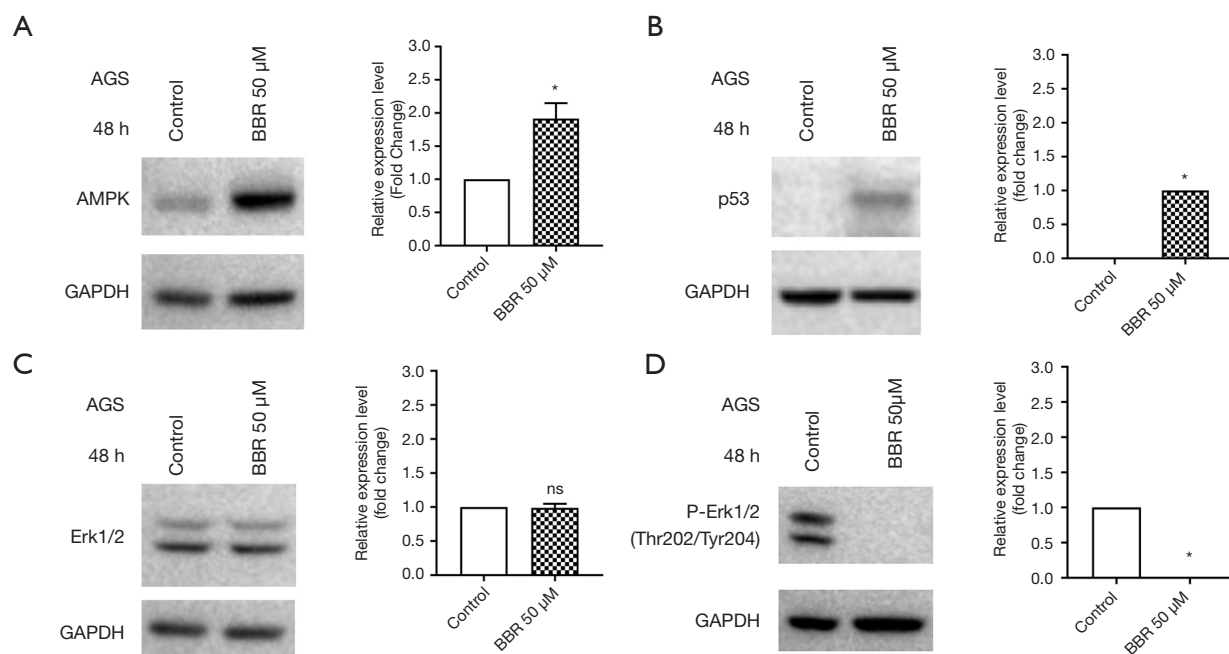
Figure 10 Effect of berberine on target protein expression in AGS cells. Effect of 50 μM berberine or vehicle for 48 h on (A) AMPK, (B) p53, (C) Erk1/2 and (D) P-Erk1/2 protein expression in AGS cells. The bar graph shows the mean of the relative intensity ratio of target proteins/GAPDH. *, P<0.05; ns, no significance. BBR, berberine; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

DNA damage can induce p53-dependent cellular reactions such as apoptosis and cycle arrest (32). The MAPK cascades, including ERK1/2, are principal intracellular pathways in diverse biological processes in cells (33).

Therefore, these mechanisms warrant further experiments *in vivo* and *in vitro*. It should, nevertheless, be emphasized that these mechanisms do not occur in isolation but are interconnected to differing extents, highlighting the significant benefits of conducting network pharmacology analyses.

Conclusions

Our findings highlight that network pharmacology approach can be used to effectively explore the multi-target effects of berberine against SC, which can provide a valuable basis for further studies. Collectively, the findings of our PPI network, hub target, pathway enrichment, and molecular docking analyses revealed the potential effects of berberine as a therapeutic agent against SC. The experimental results demonstrated that berberine can reduce SC cell viability, enhance apoptosis and ROS generation, and contribute to

reductions in MMP and ATP levels. Western blot assay suggested that berberine increased the expression of TP53, and AMPK, while decreased the expression of P-MAPK3/1. However, it should be recognized that this study also has some limitations, for example, the lack of non-neoplastic stomach cells as a control when evaluating the cell response to berberine. Berberine is commonly used in clinical practice to treat gastrointestinal diseases, but its anti-tumor mechanism and clinical application alone or in combination with other treatment options still need further research to confirm. Moreover, it is necessary to study the potential negative effects of berberine thoroughly.

Acknowledgments

Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-668/rc

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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