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Overcoming cisplatin resistance by targeting the MTDH-PTEN interaction in ovarian cancer with sera derived from rats exposed to Guizhi Fuling wan extract

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Abstract

Background: The well-known traditional Chinese herbal formula Guizhi Fuling Wan (GFW) was recently reported to improve the curative effects of chemotherapy for ovarian cancer with few clinical side effects. The present study aimed to investigate the reversal mechanism of sera derived from rats exposed to Guizhi Fuling Wan extract (GFWE) in cisplatin-resistant human ovarian cancer SKOV3/DDP cells; the proteins examined included phosphatase and tensin homolog (PTEN) and metadherin (MTDH), and the possible protein interaction between PTEN and MTDH was explored.

Methods: GFWE was administered to healthy Wistar rats, and the sera were collected after five days. The PubMed and CNKI databases were searched for literature on the bioactive blood components in the sera. The systemsDock website was used to predict potential PTEN/MTDH interactions with the compounds. RT-qPCR, western blotting, and immunofluorescence analyses were used to analyze the mRNA and protein levels of MTDH and PTEN. Laser confocal microscopy and coimmunoprecipitation (co-IP) were used to analyze the colocalization and interaction between MTDH and PTEN.

Results: Sixteen bioactive compounds were identified in GFWE sera after searching the PubMed and CNKI databases. The systemsDock website predicted the potential PTEN/MTDH interactions with the compounds. RT-qPCR, western blotting, and immunofluorescence analyses showed decreased MTDH expression and increased PTEN expression in the sera. Laser confocal microscopy images and coimmunoprecipitation (co-IP) analyses demonstrated that a colocalization and interaction occurred between MTDH and PTEN, and the addition of the sera changed the interaction status.

Conclusions: GFWE restored sensitivity to cisplatin by inhibiting MTDH expression, inducing PTEN expression, and improving the interaction between MTDH and PTEN in SKOV3/DDP cells, and these proteins and their interaction may serve as potential targets for cancer treatment. The sera may represent a new source of anticancer compounds that could help to manage chemoresistance more efficiently and safely.

Keywords: Guizhi Fuling wan, Ovarian Cancer, Chemotherapy resistance, MTDH, PTEN

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Background

Ovarian cancer (OC) is the seventh most common cancer and the eighth cause of cancer-related death among women with a range of 30–50% of the age-standardized 5-year net survival rate [1, 2]. OC is generally advanced when it is diagnosed because there are typically no apparent symptoms during the early stages. The standard of care usually consists of platin-based drugs combined with nonplatins, such as paclitaxel or docetaxel, for advanced-stage OC patients or high-risk surgical candidates [3]. More than 70% of patients who initially respond to chemotherapy, including platins and nonplatins, ultimately have recurrence and develop chemoresistance [4]. To this end, exploring the molecular mechanism associated with chemoresistance and finding strategies to improve the therapeutic effect of OC is exceptionally urgent for researchers.

The function of PI3K/Akt/mTOR pathway in OC chemoresistance has received increased attention across a number of disciplines in recent years [5, 6]. Inactivation of PI3K/Akt/mTOR signaling is an effective strategy to re-sensitize chemoresistant OC to chemotherapeutic drugs. Although promising results in several phase I and II studies suggest that mTOR inhibitors may overcome chemoresistance for OC patients, the clinical practice is limited by the toxicity profile of mTOR inhibitors [7]. More broadly, researches are needed to explore other possible targeting as new therapeutic strategies. The role of metadherin (MTDH), also known as astrocyte elevated gene-1 (AEG-1) or lysine-rich CEACAM1 coisolated (LYRIC), in cancer chemoresistance is noted in a number of studies, and downstream molecular mechanisms implicated in chemoresistance include NF- κ B, AMPK/ATG5, PI3K/AKT/mTOR, and Wnt/ β -catenin signaling [8–12]. MTDH overexpression was strongly associated with poor prognosis and cisplatin resistance in advanced serous OC [13], while a loss of MTDH could lead to the inhibition of cell migration and invasion and restore sensitivity to chemotherapeutics. Therefore, inhibition of MTDH could be a useful therapeutic target for reversing the chemoresistance of OC.

Phosphatase and tensin homolog (PTEN), a natural inhibitor of the PI3K/AKT pathway, is one of the most commonly lost tumor suppressors in human cancer. Recently, researchers have shown an increased interest in the role of PTEN in chemoresistance. It was reported that up to 50% of castration-resistant prostate cancer cases are estimated to have a loss of PTEN [14]. There are similarities between the loss of PTEN and chemoresistance in other diseases, such as colorectal cancer, glioma, and non-small-cell lung cancer [15–17]. Previous studies have also shown that low expression or loss of PTEN was a significant risk factor for the chemoresistance of OC patients [18, 19]. Inducing PTEN also plays

a critical role in restoring the sensitivity of anticancer drugs to OC. What remains unknown, however, is whether MTDH interacts with PTEN and whether the interaction serves a role in chemoresistance. Modulation of protein-protein interactions (PPIs), which are the physical contacts of high specificity established between two or more protein molecules as a result of biochemical events, is receiving increasing attention by the scientific community [20]. It is known that disruptions of the normal patterns of PPIs and multiprotein complexes can be causative or indicative of a disease state in humans. For instance, MTDH can interact with CREB-binding protein (CBP) and then prevent its ubiquitin-mediated degradation, licensing the transcriptional activation of TWIST1 [21], which has been shown to contribute to OC cisplatin resistance [22].

Guizhi Fuling Wan (GFW; Gyejibokryeong-hwan in Korean and Keishi-bukuryo-gan in Japanese) is a well-known Chinese classic herbal formula, which is a combination of herbs used in Chinese herbology for greater efficiency than individual herbs, and has been used extensively throughout Asia for the treatment of blood stasis; this formula has been shown to be very safe and effective with few harmful side effects and was established more than 2000 years ago. GFW was developed by Zhang Zhongjing (150–219 A.D.) at the end of the Eastern Han Dynasty. Numerous studies have also confirmed the safety of GFW for genotoxic effects [23], adverse reactions [24], and sub-chronic toxicity [25]. Several researchers have reported that GFW has various therapeutic effects on conditions, such as climacteric syndrome [26], arteriole vasodilation [27], inflammatory skin disorders [28], endometriosis [29], diabetes-mellitus-induced neuropathology [30], and atherosclerosis [31]. Recent evidence also suggests that GFW has an antitumor effect on bladder cancer [32] and breast cancer [33]. Chinese clinicians have shown that GFW could improve the curative effect of chemotherapy for cervical carcinoma [34] and oophoroma [35] with few clinical side effects; however, the underlying mechanism is unclear. We previously demonstrated that GFW could sensitize cisplatin-resistant SKOV3/DDP cells by inhibiting the protein level and function of P-glycoprotein (P-gp) and by inactivating the PI3K/AKT/mTOR pathway; in the resistant SKOV3/DDP xenograft tumour mice, GFW could enhance anticancer efficacy of cisplatin and paclitaxel without any mouse deaths during a 21-day administration at a dose of $4 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ [36]. We have also shown that paeonol, one of the bioactive constituents of GFW, has an effect on the inhibition of MTDH and the induction of PTEN in SKOV3/DDP cells [37].

In light of the knowledge of the above facts, we evaluated whether GFW inhibits MTDH and induces PTEN to address the chemoresistance issues in OC treatment. We hypothesized that MTDH would interact with

PTEN, and the interaction would contribute to chemoresistance. We then further explored the role of GFWE in disturbing the interaction between MTDH and PTEN in SKOV3/DDP cells; this study thus presents a promising strategy for the clinical practice of GFWE in restoring sensitized cisplatin-resistant cells.

Methods

Plant materials and preparation of the GFWE

GFWE consists of five herbs, and the mixed proportion of herbs is illustrated in Table 1. All botanical names can be checked and validated using <http://www.theplantlist.org>. The herbs used in this study were purchased from Nanyang Zhongjing Baixin Medical Technology Co., Ltd. (Nanyang, China). The plant materials were authenticated based on their morphological characteristics by Prof. Chaoyun Zhang, a plant taxonomist from the Zhang Zhongjing College of Chinese Medicine, Nanyang Institute of Technology, where the voucher specimens were deposited (Additional file 1). Assurance of quality control for all the materials was validated according to the Chinese Pharmacopoeia (China Pharmacopoeia Committee, 2015).

GFWE was prepared following the process as stated in the Chinese Pharmacopoeia as previously described [36, 38]. Briefly, the water-soluble ingredients, liposoluble ingredients and volatile oil of GFWE were extracted, and the extracts were combined uniformly. The concentration was adjusted to 1.5 g/mL corresponding to the crude herbs. The mixed solution was used for animal studies and preparing the medicated rat sera.

Preparation of rat sera after GFWE exposure

The rat sera after GFWE exposure were made according to the published protocols described previously with a slight modification [36]. Briefly, 60 healthy Wistar female rats, aged between 6 and 8 weeks old and weighing 220~250 g, were randomly divided into four groups with 15 rats per group: the control group administered normal saline by gavage and the low-dose (LD) GFWE group, middle-dose (MD) GFWE group and high-dose (HD) GFWE group administered the mixed GFWE solution by gavage at dosages of 4 g·kg⁻¹·d⁻¹, 8 g·kg⁻¹·d⁻¹, and 16 g·kg⁻¹·d⁻¹, respectively, based on clinical dosage of GFWE. The animals were supplied by the Henan Experimental Animal Center (Zhengzhou, China). This

study was carried out in accordance with the accepted standards of humane animal care as outlined in ethical guidelines on the care and use of laboratory animals in an air-conditioned room with a controlled temperature of 22 ± 2 °C, a humidity level of 45 to 65%, and a 12 h/12-h light/dark cycle. All animal procedures were conducted with protocol approval from the Ethics Committee of Nanyang Institute of Technology (Nanyang, China), and all efforts were made to minimize animal suffering. After five days of administration, the rats were anesthetized with 1.5% isoflurane in an anesthesia mask, and the blood was collected from the jugular vein; all blood samples from the same group from the experimental rats were combined into one tube to eliminate individual variability. No rats died, and no body weight loss was observed during the 5-day administration of the 3 doses. At the end of the study, the animals were euthanized with 30% CO₂. The samples of different groups were centrifuged, and the collected sera were filtered through a Millipore filter for sterilization, aliquoted into 1-mL Eppendorf tubes and preserved at -80 °C for future use.

Blood bioactive ingredients identification and molecular docking

As GFWE is widely used in China, Japan, and Korea, the bioactive compounds in the sera containing GFWE have already been demonstrated elsewhere. After searching the PubMed and CNKI databases to collect the literature on the blood bioactive ingredients of the sera, the SystemsDock website (<http://systemsdock.unit.oist.jp>, Version 2.0) [39], a web server for compound-protein docking simulation with good prediction accuracy, was used to predict potential PTEN/MTDH interactions with the compounds. The PI3K/AKT inhibitor LY294002, which has been shown to modulate PTEN/MTDH [40, 41], was used as a positive compound when the docking simulation was applied.

Cell culture and cytotoxicity assay

The human OC SKOV3 cell line and its cisplatin-resistant SKOV3/DDP cell line were obtained from the Chinese Academy of Medical Sciences and Peking Union Medical College. The cells were treated as previously described [36].

Table 1 The composition and proportion of herbs in GFWE

Botanical name	Herbal name	Chinese name	Voucher number	Dosage
<i>Cinnamomum cassia</i> (L.) J. Presl	<i>Ramulus Cinnamomi cassia</i>	Gui-Zhi	170,801	15 g
<i>Smilax glabra</i> Roxb.	<i>Sclerotium Poriae Cocos</i>	Fu-Ling	170,901	15 g
<i>Prunus persica</i> (L.) Batsch	<i>Semen Pruni Persicae</i>	Tao-Ren	170,801	15 g
<i>Paeonia suffruticosa</i> var. <i>papaveracea</i> (Andrews) A. Kern.	<i>Cortex Radicis Moutan</i>	Mu-Dan-Pi	170,801	15 g
<i>Paeonia lactiflora</i> Pall.	<i>Radix Albus Paeoniae Lactiflorae</i>	Shao-Yao	170,601	15 g

The ability of the medicated rat sera containing GFWE to potentiate cisplatin cytotoxicity was evaluated in SKOV3/DDP and SKOV3 cells using the xCELLigence Real-Time Cell Analysis (RTCA) S16 instrument (ACEA Biosciences, San Diego, CA, USA), which is an impedance-based platform for monitoring cell viability; the instrument was placed in a humidified incubator at 37 °C with 5% CO₂. Cytotoxicity assays were performed using E-plate 16 (ACEA Biosciences, San Diego, CA, USA), which was inserted into the platform during experiments. The cells were harvested in the exponential growth phase and seeded into the plate at a density of 5.0×10^3

cells per well with fetal bovine serum (FBS)-free RPMI 1640 medium. Six hours after seeding, LD sera plus cisplatin or cisplatin alone at final concentrations of 16.7, 8.3, 5.0 and 2.5 µg/ml were added to the culture. The sera volume ratio for incubation was 10%. All incubation was performed at a final volume of 200 µL. The impedance was recorded at 15-min intervals. The amount of growth area covered in the E-plate 16 due to cell adhesion was represented as the cell index (CI). A high CI indicates high levels of cell adhesion and high cell viability and vice versa. The inhibition growth by 50% (IC₅₀ value) was calculated based on the CIs.

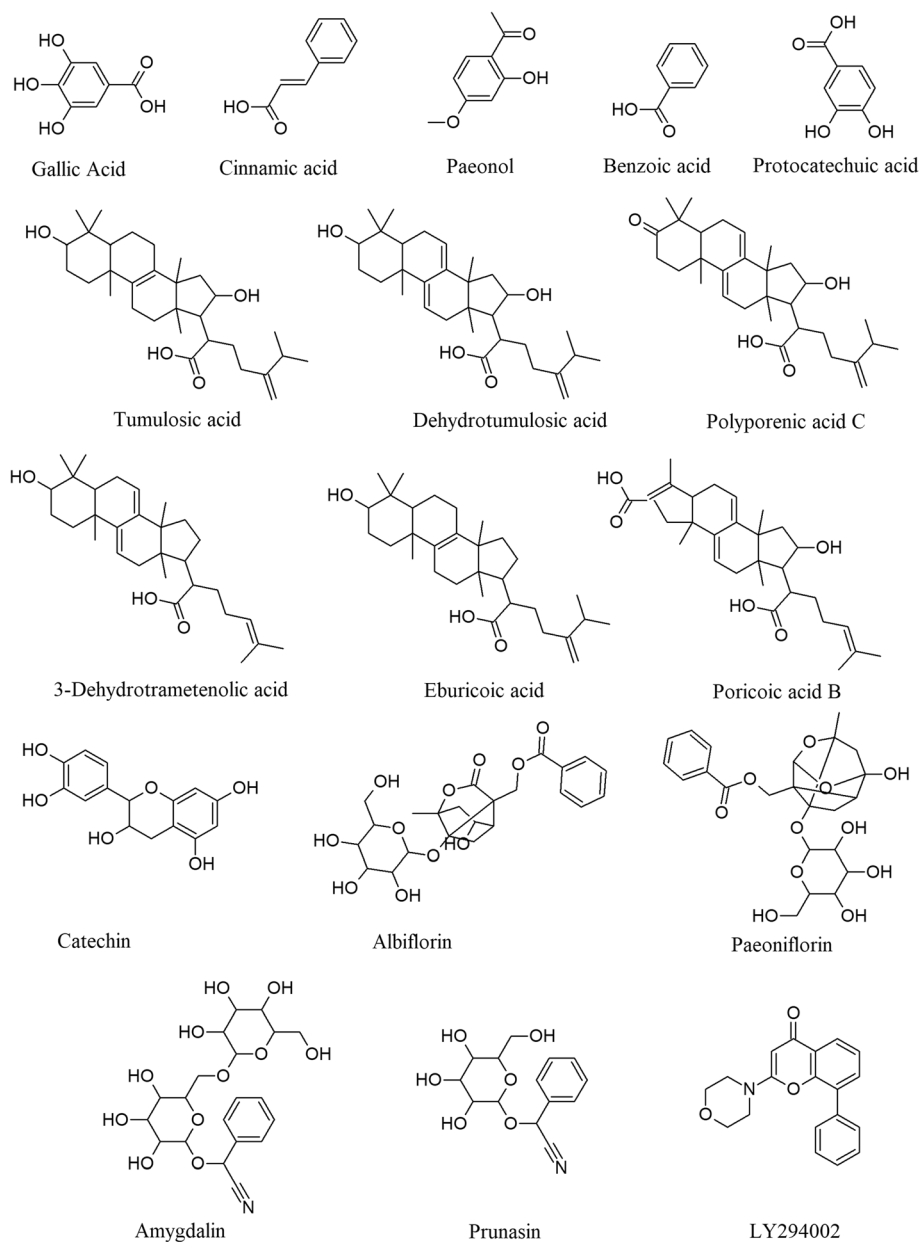


Fig. 1 Main active ingredients from rat sera containing GFWE and the positive control compound LY294002

RT-qPCR analysis

To analyze the mRNA expression, SKOV3/DDP cells were seeded into 12-well plates at a density of 2.0×10^5 cells per well and were treated with medicated rat control sera, LD, MD, HD sera or LY294002 ($10 \mu\text{mol}\cdot\text{L}^{-1}$; MedchemExpress, USA) for 48 h. Total RNA was extracted using the UNIQ-10 Column TRIzol Total RNA Isolation Kit (Sangon Biotech, China) according to the manufacturer's instructions. Extracted RNA was assessed for purity and quantity using a Nanodrop 2000 Spectrophotometer (Thermo Fisher, USA). A total of 500 ng of total RNA was used for cDNA synthesis using the ReverAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific Inc., USA). A quantitative comparison of the mRNA levels was performed using FastStart Universal SYBR Green Master with Rox (Merck KGaA, Germany) in an Applied Biosystems ViiA7 Real-Time PCR System by the manufacturer's recommended thermal cycling conditions. The forward and reverse primers for amplifying MTDH are as follows: 5'-CCTGGCCTTGCTGAAG AATC-3' and 5'-GGCTGCTTTGCTGTTACTACT-3', and the length of the amplified fragment was 150 bps. The forward and reverse primers for amplifying PTEN were as follows: 5'-ATGTTTCAGTGGCGGAACTTG-3' and 5'-CACACAGGTAACGGCTGAGG-3', and the length of the amplified fragment was 137 bps. A 196-bp fragment of GAPDH was used as the internal control and was amplified with the following forward and reverse primers: 5'-GAGTCAACGGATTTGGTCGT-3' and 5'-GACAAGCTTCCCGTTCTCAG-3'. The relative gene expression analysis was performed using the $2^{-\Delta\Delta C_t}$ method as described previously [42].

Western blot assay

Western blotting analyses of the MTDH and PTEN proteins were performed using a slight modification of the method described previously [43]. Briefly, SKOV3/DDP cells were treated as above for 48 h, and then the cells were lysed using a Minute™ Total Protein Extraction Kit (Invent Biotechnologies, USA). The protein concentrations were determined using the Pierce Rapid Gold BCA Protein Assay Kit (Thermo Fisher, USA). A total of 20 μg of protein per sample was electrophoresed via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto PVDF membranes (Millipore, USA), which were then blocked with 5% skim milk for 1 h at room temperature (RT). The membranes were then incubated with a rabbit polyclonal antibody against MTDH (1:600 dilution; Proteintech Group, China), a mouse monoclonal antibody against PTEN (1:1000 dilution; Proteintech Group, China), and a mouse monoclonal antibody against GAPDH (1:4000 dilution; Proteintech Group, China) at 4 °C overnight. After three washes with TBST, the primary antibodies were then

detected using species-matched secondary antibodies (Proteintech Group, China) with a 1.5 h incubation at RT. The membranes were incubated with Pierce ECL Western Blotting Substrate (Thermo Fisher, USA) for 5 min in the dark. The bands were detected using the Tanon 5200 Blots Imaging System (Tanon Science & Technology Co., Ltd., China).

Immunofluorescence and laser scanning confocal microscopy

SKOV3/DDP cells in the different groups were seeded in 20 mm diameter NEST confocal glass-bottom cell culture dishes at 37 °C and 5% CO₂ for 48 h. The cells were then fixed with 4% paraformaldehyde for 5 min and kept stable in 0.5% Triton X-100 for 10 min to rupture the cell membranes. Following three PBS washes, nonspecific antigen-binding sites were blocked with 5% goat serum for 1 h at RT. The cells were then incubated with a rabbit polyclonal antibody (pAb) against MTDH (1:300 dilution; Proteintech Group, China) and a mouse monoclonal antibody (mAb) against PTEN (1:500 dilution; Proteintech Group, China) overnight at 4 °C. After washing, the cells were incubated with the corresponding species-matched Alexa Fluor-488/647-conjugated secondary antibodies (Proteintech Group, China) for 1 h at RT. After washing, the nuclei were stained with DAPI for 2 min, and the slides were washed with PBS. The

Table 2 Docking scores of blood bioactive ingredients of GFWE and LY294002 to PTEN/MTDH

Compounds	Docking Scores (pKd/pKi)	
	MTDH	PTEN
Cinnamic acid	3.06	2.82
Paeonol	3.78	3.90
LY294002	4.47	4.02
Benzoic acid	5.67	4.96
Prunasin	5.73	5.59
Albiflorin	6.14	5.98
Protocatechuic acid	6.14	5.18
Amygdalin	6.18	5.33
Paeoniflorin	6.26	6.21
Gallic acid	6.42	5.43
Catechin	6.99	6.16
Tumulosic acid	7.95	7.67
Polyporenic acid C	8.01	7.68
Dehydrotumulosic acid	8.04	6.21
Poricoic acid B	8.16	7.46
Eburicoic acid	8.16	8.02
3-Dehydrotrametenolic acid	8.17	8.08

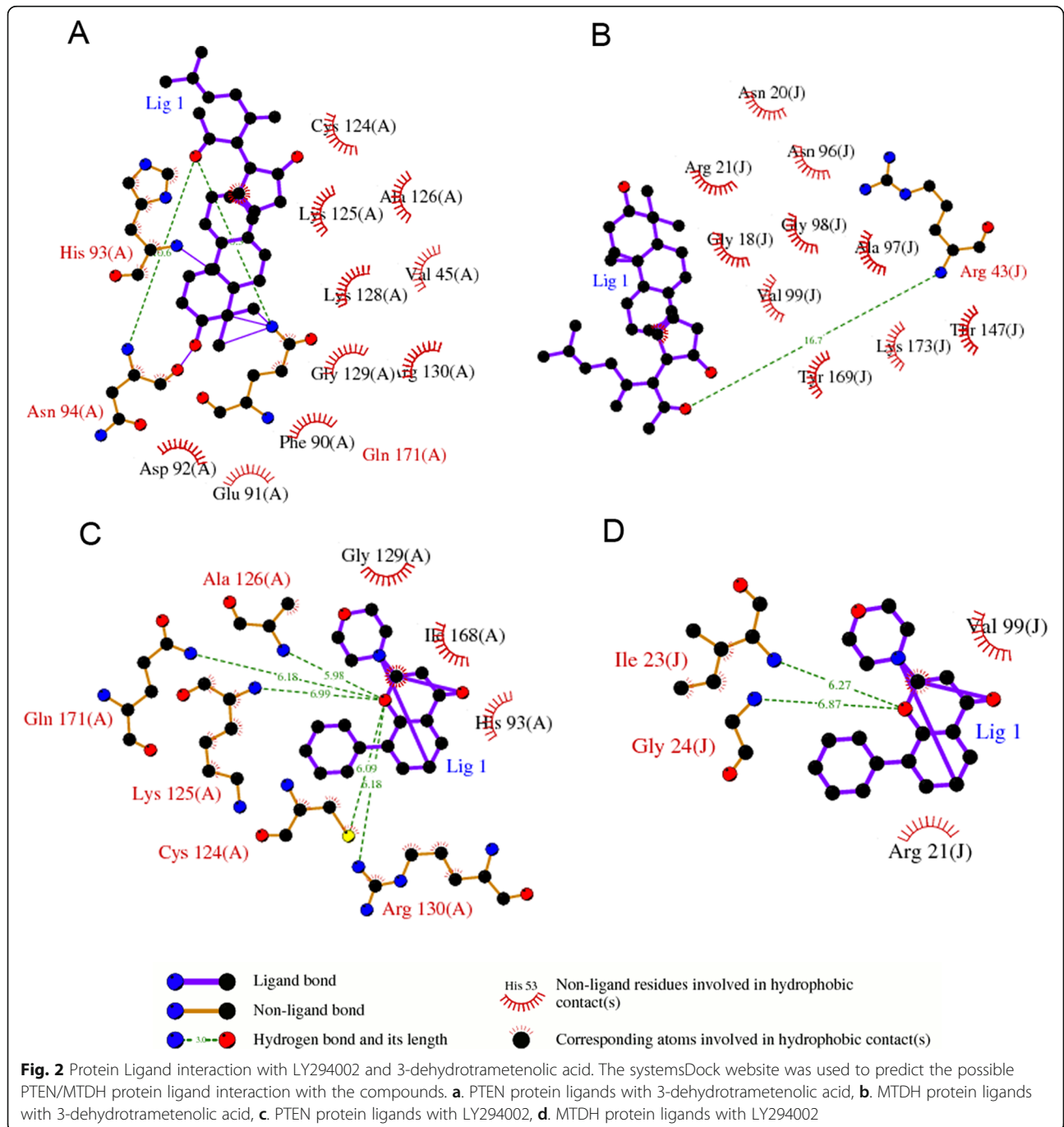
Note: The docking score represents the weak to strong binding activity ranging from 0 to 10

dishes were kept from light before being observed with a fluorescence microscope. The observation was made using a Zeiss LSM800 Laser Scanning Confocal Microscope (Carl Zeiss AG, Germany). For the analysis of protein level, images were acquired using the 40× Plan-Apochromat objective (numerical aperture, NA: 0.95), and mean fluorescence intensities (MFI) were measured after background subtraction with the graphics module of Zeiss Zen software (version 2.3). For the analysis of cellular protein localization, images were acquired using

the 63× Plan-Apochromat oil objective (numerical aperture, NA: 1.40) with Airyscan, and the colocalization coefficients of MTDH/PTEN were analyzed by the Confocal Topography module of Zeiss Zen software.

Coimmunoprecipitation (co-IP) assay

The SKOV3/DDP cells in different groups as above were seeded in a Corning 25 cm² Cell Culture Flask at 37 °C and 5% CO₂ for 48 h. SureBeads™ Starter Kit Protein A/G Beads (Bio-Rad Laboratories, Inc., USA) were



precleaned with 1.5 μg of pAb-MTDH or pAb-IgG for 20 min at RT before co-IP. The cells were lysed using a Minute™ Total Protein Extraction Kit (Invent Biotechnologies, Inc., USA), and the protein concentrations were determined using a Pierce Rapid Gold BCA Protein Assay Kit (Thermo Fisher, USA). A total of 300 μg protein per sample was incubated with the beads and rotated for 1 h at RT. After washing 3 times with PBS buffer containing 0.1% Tween-20, the beads were incubated with Laemmli Sample Buffer containing β -mercaptoethanol for 10 min at 70 °C. After the beads were magnetized, the eluent was boiled for 5 min at 95 °C before being loading onto an SDS-PAGE gel and performing western blotting analysis with the mAb-PTEN.

Statistics

Data were expressed as the mean \pm SD of at least three independent experiments. Statistical evaluation of the data was performed using one-way ANOVA with Tukey's multiple comparisons test. The colocalization of MTDH with PTEN was investigated using both Pearson's correlation coefficient and Manders' colocalization coefficient. Statistical analysis was performed with GraphPad Prism version 6.01 for Windows. Differences were considered to be statistically significant for $P < 0.05$.

Results

The potential modulating effect of GFWE on PTEN/MTDH

The PubMed and CNKI databases were searched for the UHPLC-MS/MS, UPLC/Q-TOF-MS/MS, or ^1H NMR

with UPLC-MS methods for detecting sera bioactive ingredients after rats were administered GFWE orally, and 16 bioactive compounds were identified in the sera (Fig. 1): cinnamic acid, paeonol, benzoic acid, prunasin, albiflorin, protocatechuic acid, amygdalin, paeoniflorin, gallic acid, catechin, tumulosic acid, polyporenic acid C, dehydrotumulosic acid, poricoic acid B, eburicoic acid, and 3-dehydrotrametenolic acid [44–47]. Three of the 16 bioactive compounds, paeonol, gallic acid and paeoniflorin, were also identified in the sera samples using HPLC/QqQ MS in our previous study [36]. The molecular docking results showed that 14 of 16 compounds have a higher binding activity with PTEN (PDB ID: 1D5R)/MTDH (PDB ID: 1H5Q) compared to LY294002. 3-Dehydrotrametenolic acid has the highest docking score with PTEN/MTDH, and cinnamic acid has the lowest docking score with PTEN/MTDH. The docking score of paeonol with PTEN/MTDH was slightly weaker than that of LY294002, suggesting that most of the blood bioactive ingredients of GFWE have a potential modulating effect on PTEN/MTDH (Table 2). Further analysis by systemsDock showed that the interactions between the docking scores of PTEN/MTDH and the tested compounds may be related to the number of interacting residues, e.g., PTEN has 13 residues (Arg A 130, His A 93, Lys A 125, Cys A 124, Gln A 171, Ala A 126, Gly A 129, Asn A 94, Lys A 128, Asp A 92, Phe A 90, Val A 45, and Glu A 91) that interact with 3-dehydrotrametenolic, but 8 residues (Arg A 130, His A

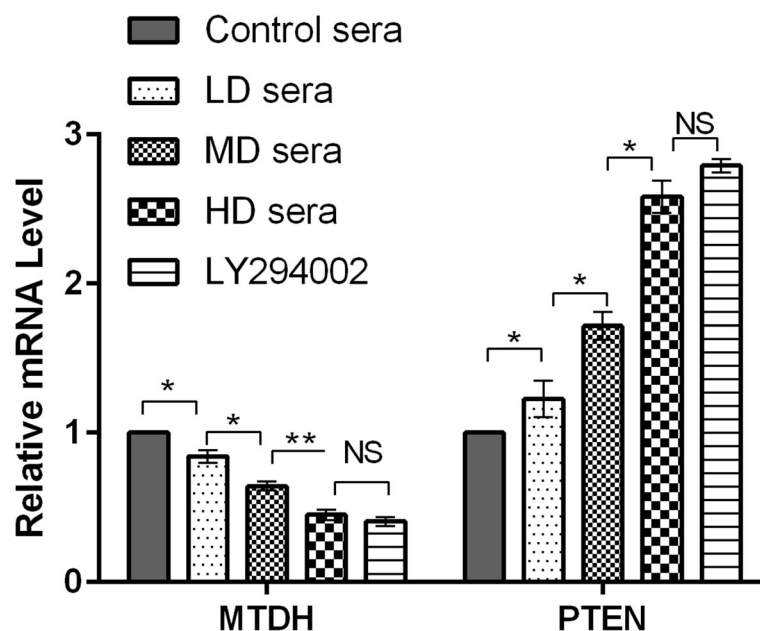


Fig. 3 Relative quantification of MTDH and PTEN mRNA in SKOV3/DDP cells. A $2^{-\Delta\Delta\text{Ct}}$ method was used to analyze the mRNA expression changes after treatment with sera containing GFWE or LY294002, and GAPDH was used as an internal standard. LD sera = low-dose sera containing GFWE, MD sera = middle-dose sera containing GFWE, HD sera = high-dose sera containing GFWE. Error bars represent the SD. * $P < 0.05$, ** $P < 0.01$

93, Lys A 125, Cys A 124, Gln A 171, Ala A 126, Gly A 129, and Ile A 168) interacted with LY294002; MTDH has 11 residues (Ala J 97, Gly J 98, Val J 99, Thr J 147, Asn J 96, Gly J 188, Asn J 20, Arg J 21, Arg J 43, Tyr J 169, and Lys J 173) that interact with 3-dehydrotrametenolic, but 4 residues (Arg J 21, Ile J 23, Val J 99, and Gly J 24) interacted with LY294002 (Fig. 2).

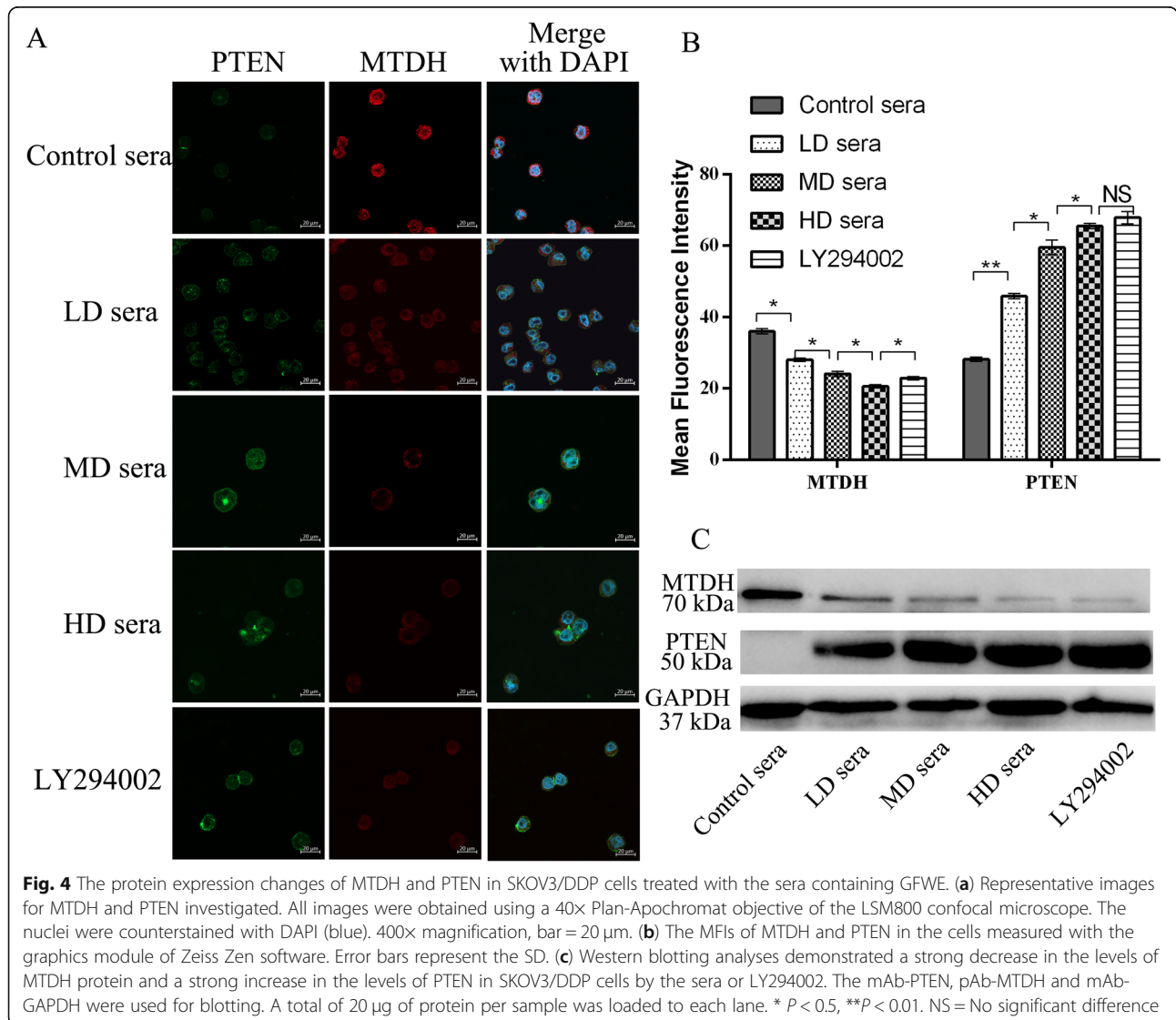
Sera containing GFWE strengthen the potency of cisplatin

xCELLigence RTCA showed that the IC₅₀ of SKOV3/DDP and SKOV3 cells to cisplatin was 15.53 ± 0.11 mg·L⁻¹ and 3.40 ± 0.14 mg·L⁻¹, respectively. The LD, MD and HD sera alone had a less than 30% growth inhibition effect on SKOV3 and SKOV3/DDP cells, and the LD sera alone had the lowest inhibition rate (<5%). Treatment with the LD sera induced a significant reduction in the IC₅₀ of cisplatin against SKOV3/DDP cells to

3.04 ± 0.18 mg·L⁻¹. However, no such activity was observed in SKOV3 cells (3.40 ± 0.14 vs. 3.28 ± 0.21 mg·L⁻¹, P > 0.05). These findings indicate that the sera containing GFWE enhance the potency of cisplatin against SKOV3/DDP cells, whereas it had little effect on SKOV3 cells, supporting the notion that the sera containing GFWE reverse the resistance of SKOV3/DDP cells. These results are similar to those of our previously conducted study using CCK-8 kits [36].

Sera containing GFWE modulate MTDH/PTEN at the mRNA level

To examine whether MTDH/PTEN was downregulated by the sera containing GFWE at the mRNA level, we analyzed MTDH and PTEN mRNA expression by RT-qPCR. After 48 h of sera treatment, the expression of PTEN mRNA in SKOV3/DDP cells increased in a dose-



effect pattern. The expression of MTDH mRNA in SKOV3/DDP cells was significantly inhibited with increasing sera dose ($P < 0.05$, $P < 0.01$). Compared to the HD sera group, the LY294002 group showed a similar inhibition of MTDH mRNA and induction of PTEN mRNA expression (Fig. 3). Nevertheless, these data indicated that the sera modulate MTDH/PTEN at the mRNA level.

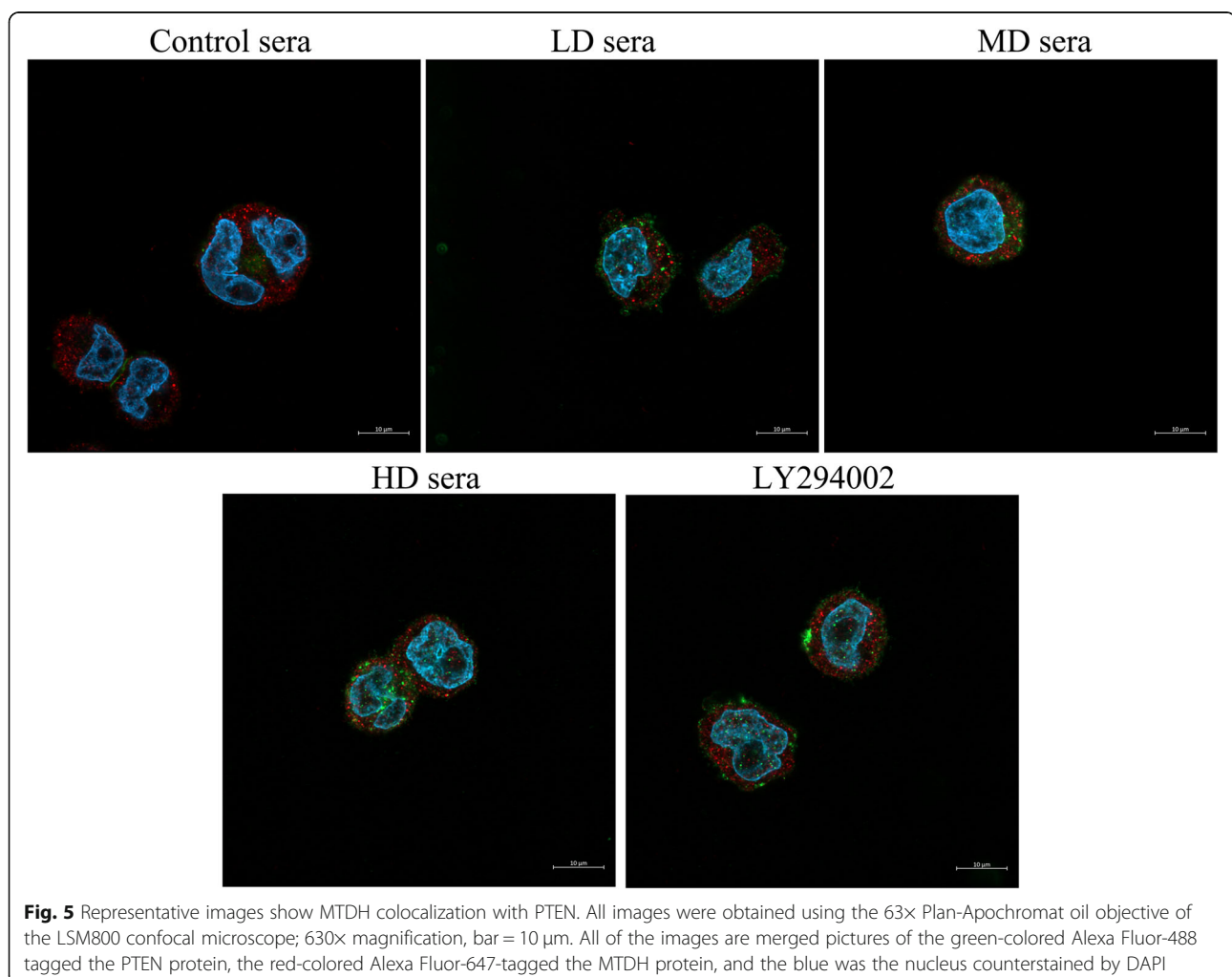
Sera containing GFWE modulate MTDH/PTEN at the protein level

Laser confocal microscopy images showed that the MFI values of the PTEN protein significantly increased and that the MFI values of the MTDH protein significantly decreased in the SKOV3/DDP cells after treatment with the sera containing GFWE in a dose-dependent manner. The effect of the HD sera on the PTEN protein was similar to that of LY294002 (Fig. 4 a and b). Western blotting analyses demonstrated the highest MTDH protein expression and undetectable PTEN expression in SKOV3/DDP cells treated with the control sera,

suggesting a stronger resistance of SKOV3/DDP cells. The observed difference between western blotting and laser confocal microscopy for detecting PTEN in the control sera-treated cells may be due to the latter being more sensitive than the former. By western blotting, the sera containing GFWE showed a similar dose-effect pattern to the inhibition of the MTDH protein and the induction of the PTEN protein by laser confocal microscopy (Fig. 4 c). These findings support earlier conclusions that MTDH overexpression and the loss of PTEN were strongly associated with cisplatin resistance, and the sera restored the cisplatin sensitivity to SKOV3/DDP cells by inhibiting MTDH and inducing PTEN.

Sera containing GFWE enhance the colocalization of MTDH with PTEN

Combined staining of MTDH with PTEN revealed that the sera containing GFWE and LY294002 changed the protein colocalization with each other, which is most clearly visible on the larger magnification images (Fig. 5). Analyses of these images using Pearson's coefficient and



Manders' coefficient, which generally indicate that the proteins colocalize when they are more significant than 0.5, showed that the colocalization of MTDH with PTEN occurred to a greater extent in the cells treated with the sera than in the cells treated with the blank control sera. The MD and HD sera have the highest effect on the colocalization of MTDH with PTEN, while the coefficients have no significant difference between the MD and HD sera-treated cells ($P > 0.05$, Fig. 6).

Sera containing GFWE facilitated the interaction of MTDH with PTEN

STRING database analysis indicated that there was no known direct interaction between MTDH and PTEN; only text mining has shown that there was a possible interaction between the two proteins (Fig. 7 a). To further investigate the association of MTDH with PTEN, coimmunoprecipitation was performed. PTEN was detected in immune complexes immunoprecipitated with the anti-MTDH antibody. As demonstrated in Fig. 6 c, the PTEN protein was discovered in the fractions of approximately 50 kDa, corresponding to the monomeric form of the PTEN protein and is in line with Shen's previous results [48]. Compared with that in the control sera, the GFWE-containing sera had increased PTEN expression, while LY294002 demonstrated the highest PTEN expression in the complexes (Fig. 7 b). This

experiment indicates that there is a physical interaction between MTDH and PTEN, and the interaction increased by the sera containing GFWE and LY294002, suggesting that the interaction may serve a role in chemoresistance and targeting the interaction may be helpful to restore cancer cell sensitivity to anticancer drugs.

Discussion

Acquired resistance is common in OC, especially in advanced-stage OC, and has been defined as a failure to respond to subsequent treatment after having previously responded to first or more lines of therapy with a platinum-free interval of 6 months or longer [49]. The mechanisms underlying platin chemoresistance and how resistance occurs after chemotherapy are not fully understood. The known main mechanisms of platin resistance reported to date include cancer stem cells (CSCs) [50], epithelial-to-mesenchymal transition (EMT) [51], abnormal DNA repair-related pathways [52], and the NF-κB and PI3K/AKT/mTOR signaling pathways [53]. Some of the previously reported mechanisms provide valuable targets for overcoming platin resistance in OC. Developing anticancer drugs to avoid known resistance mechanisms is an effective strategy for the management of OC. For example, sorafenib, an antiangiogenic agent, showed a statistically and clinically significant improvement in progression-free survival in women with

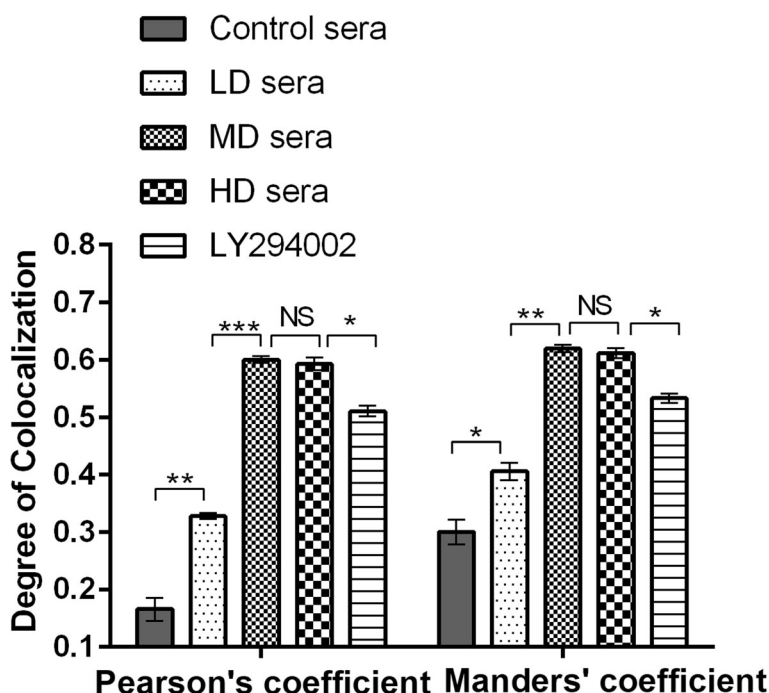


Fig. 6 Degree of MTDH colocalization with PTEN in SKOV3/DDP cells. This bar graph represents the corresponding mean Pearson's coefficient and Mander's coefficient for MTDH and PTEN affected by the sera containing GFWE and LY294002. Error bars represent the SD. All calculations for Pearson's and Mander's coefficient have been analyzed by the confocal topography module of Zeiss Zen software. *** $P < 0.001$. NS = No significant difference

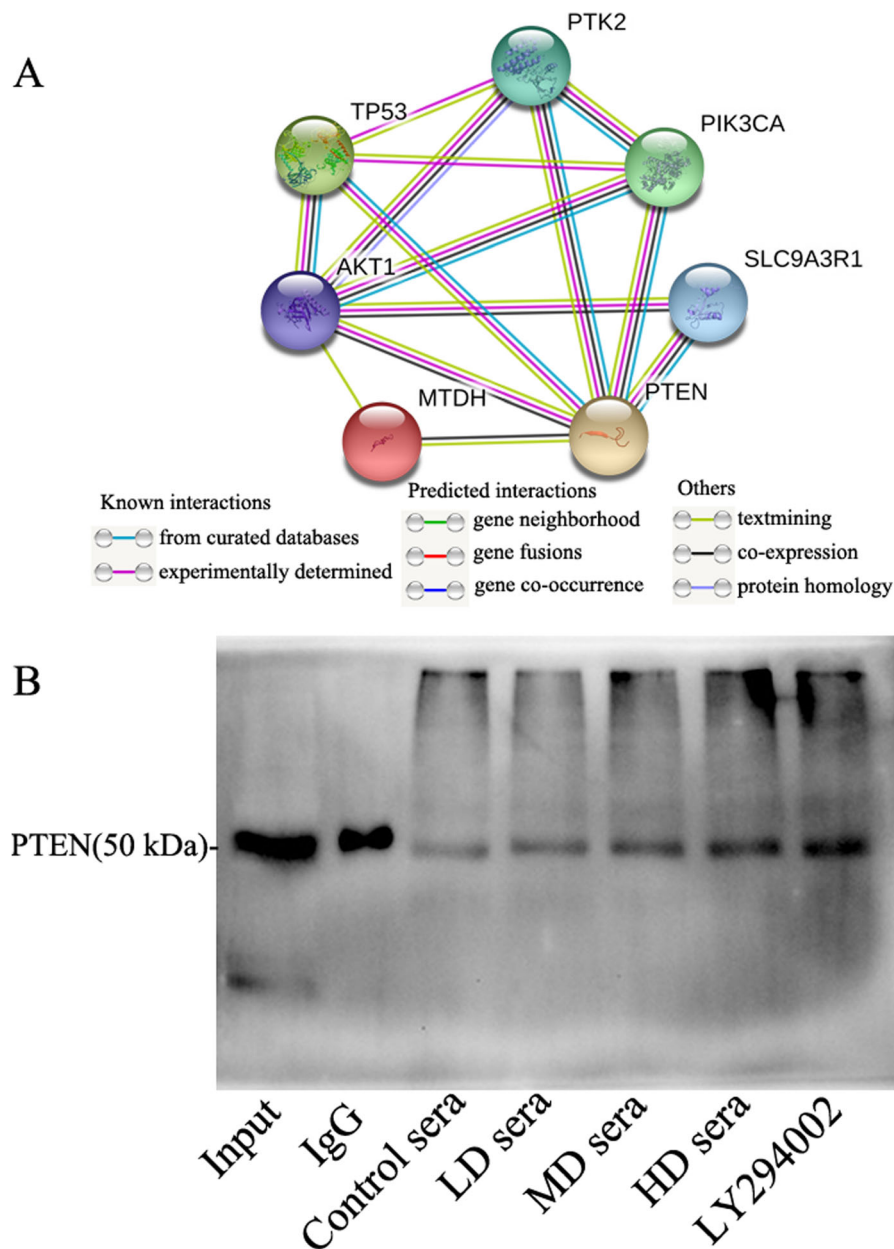


Fig. 7 Identification and characterization of the MTDH-PTEN interaction. **(a)** Analysis of the MTDH-PTEN interaction by the STRING database. The edge indicates an interaction between the two proteins. **(b)** Interaction of PTEN with MTDH detected by co-IP. The pAb-MTDH was used for immunoprecipitation, and a rabbit IgG pAb was used as a control antibody. The mAb-PTEN was used for western blotting. The Input blot of PTEN indicates non-SureBeads-handled cell lysate to prove that there was the protein of interest in the sample to begin with

platin-resistant OC in a phase 2 trial [54]. However, the vital ideal that researchers cannot give up is to develop targeted interventions aimed at the known resistance mechanisms and find customized strategies to assist clinicians and patients in making better-informed treatment decisions. Several studies have revealed some possible correlations between the known resistance mechanisms in cancer. Tsou SH et al. demonstrated that the activation of the NF- κ B signaling pathway was partially attributable to

EMT in MCF-7 cells; thus, the cells acquire stem cell-like properties, which facilitate drug resistance [55]. In another report, it was found that the doxorubicin-resistance of gastric cancer cells was related to EMT, which was induced by activating Akt and inhibiting PTEN [56]. MTDH also plays an essential role in promoting CSC accumulation [21] and inducing EMT [57]. All these observations demonstrated a strong relationship between MTDH/PTEN and known resistance mechanisms, suggesting that

MTDH and PTEN are promising therapeutic targets for the chemoresistance of OC.

Traditional Chinese medicine (TCM) is a style of traditional medicine based on more than 2500 years of Chinese medical practice. TCM practitioners use herbal medicines, including Chinese classical herbal formulas, to treat or prevent health problems, whereas the biological characteristics and molecular mechanisms remain largely unknown. The use of herbal formulas differs from plant-derived drugs in standard pharmacology because it does not isolate or standardize bioactive compounds, which may result in the loss of potential essential compounds at the time of separating others. In the present study, a seropharmacology method arising recently for the pharmacological study was adopted to research TCM in vitro using medicated animal sera [58]. The essence of seropharmacology is to administer herbal formulas to experimental animals (generally rabbits or rats), followed by harvesting the animal sera after a period of time. After searching the PubMed and CNKI databases, 16 bioactive compounds were identified in the sera in the published articles. The molecular docking results confirmed that 14 of 16 compounds have higher binding activity with PTEN/MTDH compared to LY294002. Among them, gallic acid, cinnamic acid, benzoic acid, catechin, and protocatechuic acid are phenolic compounds that have been demonstrated to upregulate PTEN, p53, p21, and p27 and inhibit tumor angiogenesis and metastasis [59]. Paeonol has been shown to inhibit MTDH and induce PTEN expression in SKOV3/DDP cells in our previous study [37]. The downregulation of MTDH by polyporenic acid C was also demonstrated by other researchers [60]. In the present study, we documented that the sera containing GFWE, together with the ample evidence of the bioactive compounds, has an inhibitory effect on MTDH and an inducing effect on PTEN.

The direct interaction between MTDH and PTEN has not been reported elsewhere. However, WASH complex subunit 5 (WASHC5; also known as RTSC, SPG8, RTSC1, and KIAA0196) was reported to be coexpressed with MTDH [61] and to genetically interact with PTEN [62], suggesting that WASHC5 may interact with MTDH and PTEN. Similarly, plectin (PLEC) [63], neural precursor cell expressed developmentally downregulated 4 (NEDD4), and E3 ubiquitin-protein ligase [64] were all reported physical interactions or predicted genetic interactions from the Biological General Repository for Interaction Datasets [65], with MTDH and PTEN, respectively. These findings support the possible view that MTDH may physically interact with PTEN. In the present study, we first demonstrated that MTDH colocalizes and physically interacts with PTEN, and the colocalization and interaction statuses were changed by sera containing GFWE and LY294002. These findings in the

present study provide a robust evidence base for the role of MTDH-PTEN complexes in chemoresistance, and the complexes may be a therapeutic target for cancer treatment. However, further work is required to establish the probability of the MTDH-PTEN complexes interacting with WASHC5, PLEC, or NEDD4. Another important finding from other researchers was that the loss of PTEN was associated with resistance to anti-PD-1 checkpoint blockade therapy in metastatic uterine leiomyosarcoma [66]. MTDH has also demonstrated a role in cancer immunotherapy [67]. A further study with more focus on the MTDH-PTEN complexes in cancer immunity is therefore suggested.

Conclusions

In summary, the present study fills a gap in the literature by demonstrating that MTDH is coexpressed and physically interacts with PTEN in resistant cancer cells. GFWE restored sensitivity to cisplatin by inhibiting MTDH expression, inducing PTEN expression, and changing the interaction status of MTDH and PTEN in SKOV3/DDP cells. Our findings suggest that GFWE may potentially be used alone or in combination with cisplatin-based chemotherapy to provide a therapeutic benefit in OC after further validation. To further develop these findings, additional molecular studies in patients with OC will be required.

Supplementary information

Supplementary information accompanies this paper at <https://doi.org/10.1186/s12906-020-2825-9>.

Additional file 1. Voucher numbers for the five herbs specimens.

Abbreviations

CBP: CREB-binding Protein; CSC: Cancer stem cell; EMT: Epithelial to mesenchymal transition; GFW: Guizhi Fuling Wan; GFWE: Guizhi Fuling Wan extract; MTDH: Metadherin; NEDD4: Neural precursor cell expressed, developmentally down-regulated 4, E3 ubiquitin protein ligase; P-gp: P-glycoprotein; PLEC: Plectin; PPI: Protein-protein interactions; PTEN: Phosphatase and tensin homolog; TWIST1: Twist-related protein 1; WASHC5: WASH complex subunit 5

Acknowledgments

The authors would like to thank all members of this work for their advice and technical assistance.

Authors' contributions

Conceived and designed the experiments: HL, BH. Performed the experiments: HL, GX. Analyzed the data: CZ and CX. Contributed reagents/materials/analysis tools: ZY and YL. Wrote the manuscript: HL, CX. All authors have read and approved the manuscript.

Funding

This work was supported by the Natural Science Foundation of Henan Province (162300410204). The funders had no role in the study design, performance, data collection and analysis, decision to publish, or preparation/writing of the manuscript.

Availability of data and materials

The datasets and supporting materials during the current study are available from the corresponding authors on reasonable request.

Ethics approval and consent to participate

This study was carried out in accordance with the accepted standards of humane animal care as outlined in ethical guidelines on the care and use of laboratory animals (National Institutes of Health Guide for the Care and Use of Laboratory Animals). All animal procedures were conducted with protocol approval from the Ethics Committee of Nanyang Institute of Technology (NYIT-2017-010), and all efforts were made to minimize animal suffering.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

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Received: 19 August 2019 Accepted: 23 January 2020

Published online: 17 February 2020

References

- Allemani C, Matsuda T, Di Carlo V, et al. Global surveillance of trends in cancer survival 2000–14 (CONCORD-3): analysis of individual records for 37 513 025 patients diagnosed with one of 18 cancers from 322 population-based registries in 71 countries. *Lancet*. 2018;391(10125):1023–75.
- Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68(6):394–424.
- https://www.nccn.org/professionals/physician_gls/pdf/ovarian.pdf. NCCN Guidelines Version 5.2017: Epithelial Ovarian Cancer/Fallopian Tube Cancer/Primary Peritoneal Cancer & Less Common Histopathologies (February 2, 2018).
- Ozols RF, et al. Phase III trial of carboplatin and paclitaxel compared with cisplatin and paclitaxel in patients with optimally resected stage III ovarian cancer: a gynecologic oncology group study. *J Clin Oncol*. 2003;21(17):3194–200.
- Deng J, Bai X, Feng X, Ni J, Beretov J, Graham P, Li Y. Inhibition of PI3K/Akt/mTOR signaling pathway alleviates ovarian cancer chemoresistance through reversing epithelial-mesenchymal transition and decreasing cancer stem cell marker expression. *BMC Cancer*. 2019;19(1):618.
- Gasparri ML, et al. miRNAs and their interplay with PI3K/AKT/mTOR pathway in ovarian cancer cells: a potential role in platinum resistance. *J Cancer Res Clin Oncol*. 2018;144(12):2313–8.
- Gasparri ML, Bardhi E, Ruscito I, Papadia A, Farooqi AA, Marchetti C, Bogani G, Ceccacci I, Mueller MD, Benedetti Panici P. PI3K/AKT/mTOR pathway in ovarian Cancer treatment: are we on the right track. *Geburtshilfe Frauenheilkd*. 2017;77(10):1095–103.
- Li PP, Feng LL, Chen N, Ge XL, Lv X, Lu K, Ding M, Yuan D, Wang X. Metadherin contributes to the pathogenesis of chronic lymphocytic leukemia partially through Wnt/ β -catenin pathway. *Med Oncol*. 2015;32(2):479.
- Pei G, Luo M, Ni X, Wu J, Wang S, Ma Y, Yu J. Autophagy facilitates Metadherin-induced chemotherapy resistance through the AMPK/ATG5 pathway in gastric Cancer. *Cell Physiol Biochem*. 2018;46(2):847–59.
- Sarkar D, Fisher PB. AEG-1/MTDH/LYRIC: clinical significance. *Adv Cancer Res*. 2013;120:39–74. <https://www.ncbi.nlm.nih.gov/pubmed/23889987>
- Yang L, Tian Y, Leong WS, Song H, Yang W, Wang M, Wang X, Kong J, Shan B, Song Z. Efficient and tumor-specific knockdown of MTDH gene attenuates paclitaxel resistance of breast cancer cells both in vivo and in vitro. *Breast Cancer Res*. 2018;20(1):113.
- Zhu GC, et al. Metadherin regulation of vascular endothelial growth factor expression is dependent upon the PI3K/Akt pathway in squamous cell carcinoma of the head and neck. *Med (Baltimore)*. 2015;94(6):e502.
- Li C, et al. Elevated expression of astrocyte elevated gene-1 (AEG-1) is correlated with cisplatin-based chemoresistance and shortened outcome in patients with stages III-IV serous ovarian carcinoma. *Histopathol*. 2012;60(6):953–63.
- Jamaspishvili T, Berman DM, Ross AE, Scher HI, De Marzo AM, Squire JA, Lotan TL. Clinical implications of PTEN loss in prostate cancer. *Nat Rev Urol*. 2018;15(4):222–34.
- Liao Y, Shen L, Zhao H, Liu Q, Fu J, Guo Y, Peng R, Cheng L. LncRNA CASC2 interacts with miR-181a to modulate Glioma growth and resistance to TMZ through PTEN pathway. *J Cell Biochem*. 2017;118(7):1889–99.
- Liu Y, Yang EJ, Zhang B, Miao Z, Wu C, Lyu J, Tan K, Poon T, Shim JS. PTEN deficiency confers colorectal cancer cell resistance to dual inhibitors of FLT3 and aurora kinase a. *Cancer Lett*. 2018;436(2018):28–37.
- Shen H, et al. Alteration in Mir-21/PTEN expression modulates gefitinib resistance in non-small cell lung cancer. *PLoS One*. 2014;9(7):e103305.
- Wang L, Wang C, Jin S, Qu D, Ying H. Expression of NF- κ B and PTEN in primary epithelial ovarian carcinoma and the correlation with chemoresistance. *Int J Clin Exp Pathol*. 2015;8(9):10953–63.
- Ying H, Qu D, Liu C, Ying T, Lv J, Jin S, Xu H. Chemoresistance is associated with Beclin-1 and PTEN expression in epithelial ovarian cancers. *Oncol Lett*. 2015;9(4):1759–63.
- Laraia L, McKenzie G, Spring DR, Venkataraman AR, Huggins DJ. Overcoming chemical, biological, and computational challenges in the development of inhibitors targeting protein-protein interactions. *Chem Biol*. 2015;22(6):689–703.
- Liang Y, et al. Epigenetic activation of TWIST1 by MTDH promotes Cancer stem-like cell traits in breast Cancer. *Cancer Res*. 2015;75(17):3672–80.
- Haslehurst AM, et al. EMT transcription factors snail and slug directly contribute to cisplatin resistance in ovarian cancer. *BMC Cancer*. 2012;12(2012):91.
- Lee MY, Seo CS, Ha H, Park E, Kim JY, Shin HK. The genotoxicity of an aqueous extract of Gyejibokryeong-hwan. *BMC Complement Altern Med*. 2018;18(1):21.
- Zhang LS, Yang FW, Zhang JH, Zheng WK, Zhang MY, Li Y, Zhao HJ. Guizhi Fuling capsule / pill treatment for chronic pelvic inflammatory disease: a systematic review of randomized clinical trials. *Zhongguo Zhong Yao Za Zhi*. 2017;42(8):1500–9.
- Jin SE, Seo CS, Lee MY, Shin HK, Yang MJ, Ha H. Sub-chronic toxicity of Gyejibokryeong-hwan in Sprague-Dawley rats. *J Ethnopharmacol*. 2018;224(18):441–50.
- Kim JE, Cho J, Kwon O, Kim AR, Park HJ, Jung SY, Kim JH, Kim M, Lee HY, Lee JH. Effect of Guizhifulingwan (Keishibukuryogan) on climacteric syndrome: study protocol for a randomized controlled pilot trial. *Trials*. 2017;18(1):135.
- Tomita T, Hirayama A, Matsui H, Aoyagi K. Effect of Keishibukuryogan, a Japanese traditional Kampo prescription, on improvement of microcirculation and Oketsu and induction of endothelial nitric oxide: a live imaging study. *Evid Based Complement Alternat Med*. 2017;2017(1):3620130.
- Jeong SJ, Lim HS, Seo CS, Jin SE, Yoo SR, Lee N, Shin HK. Anti-inflammatory actions of herbal formula Gyejibokryeong-hwan regulated by inhibiting chemokine production and STAT1 activation in HaCaT cells. *Biol Pharm Bull*. 2015;38(3):425–34.
- Hu C, Wang Z, Pang Z, Lu W, Cai X, Yang J, Wang D, Cao P. Guizhi fuling capsule, an ancient Chinese formula, attenuates endometriosis in rats via induction of apoptosis. *Climacteric*. 2014;17(4):410–6.
- Wu KJ, Chen YF, Tsai HY, Wu CR, Wood WG. Guizhi-Fuling-wan, a traditional Chinese herbal medicine, ameliorates memory deficits and neuronal apoptosis in the Streptozotocin-induced hyperglycemic rodents via the decrease of Bax/Bcl2 ratio and Caspase-3 expression. *Evid Based Complement Alternat Med*. 2012;2012(1):656150.
- Nagata Y, Goto H, Hikami H, Nogami T, Fujimoto M, Shibahara N, Shimada Y. Effect of keishibukuryogan on endothelial function in patients with at least one component of the diagnostic criteria for metabolic syndrome: a controlled clinical trial with crossover design. *Evid Based Complement Alternat Med*. 2012;2012(1):359282.
- Lu CC, et al. Guizhi Fuling wan as a novel agent for Intravesical treatment for bladder Cancer in a mouse model. *Mol Med*. 2016;22(16):64–73.
- Shihong J, Lan L, Yaosong W, Leida X, Peixun Z. mechanism of Guizhi Fuling wan in inhibiting proliferation of human breast Cancer MCF-7 cells. *Chin J Exp Tradit Med Formulae*. 2018;24(15):132–6.

34. Chiwen H, Xiaoqi L. Guizhi Fuling pill in the treatment of cervical carcinoma of kidney deficiency and Duhan syndrome. *Acta Chinese Med.* 2019;34(3):634–7.
35. Min T. Clinical observation on the treatment of 28 cases of oophoroma with Guizhifuling pills and adjuvant. *Anti-tumor Pharm.* 2011;1(6):520–3.
36. Han L, Guo X, Bian H, Yang L, Chen Z, Zang W, Yang J. Guizhi Fuling wan, a traditional Chinese herbal formula, sensitizes Cisplatin-resistant human ovarian Cancer cells through inactivation of the PI3K/AKT/mTOR pathway. *Evid Based Complement Alternat Med.* 2016;16(1):1–11.
37. Han L, Guo XJ, Chen Z, Bian H, Zhang CY, Zang WH, Wang Q, Hu JL. the mechanisms of paeonol reversing multidrug resistance in ovarian cancer SKOV3/DDP cells. *Yao Xue Xue Bao.* 2018;53(9):1511–7.
38. National Commission of Chinese Pharmacopoeia. *Pharmacopoeia of Peoples Republic of China, vol. 1.* Beijing: China medical Science and Technology Press; 2015. p. 1336.
39. Hsin KY, Matsuoka Y, Asai Y, Kamiyoshi K, Watanabe T, Kawaoka Y, Kitano H. systemsDock: a web server for network pharmacology-based prediction and analysis. *Nucleic Acids Res.* 2016;44(W1):W507–13.
40. Dinesh P, Rasool M. Berberine inhibits IL-21/IL-21R mediated inflammatory proliferation of fibroblast-like synoviocytes through the attenuation of PI3K/Akt signaling pathway and ameliorates IL-21 mediated osteoclastogenesis. *Cytokine.* 2018;106(2018):54–66.
41. Ma J, Xie SL, Geng YJ, Jin S, Wang GY, Lv GY. In vitro regulation of hepatocellular carcinoma cell viability, apoptosis, invasion, and AEG-1 expression by LY294002. *Clin Res Hepatol Gastroenterol.* 2014;38(1):73–80.
42. Han L, Guo X, Bian H, Zhou Y, Li T, Yang J. Changed expression and function of P-gp in peripheral blood CD56+ cells predicting chemoresistance in non-Hodgkin lymphoma patients. *Cancer Biomark.* 2015;15(3):289–97.
43. Li H, Hua B, Jingfeng O, Yuefeng B, Lei Y, Songshan Y. Wenyang Huazhuo Tongluo formula, a Chinese herbal decoction, improves skin fibrosis by promoting apoptosis and inhibiting proliferation through down-regulation of survivin and cyclin D1 in systemic sclerosis. *BMC Complement Altern Med.* 2016;16(1):69.
44. Cheng Y, Chu Y, Su X, Zhang K, Zhang Y, Wang Z, Xiao W, Zhao L, Chen X. Pharmacokinetic-pharmacodynamic modeling to study the anti-dysmenorrhea effect of Guizhi Fuling capsule on primary dysmenorrhea rats. *Phytomed.* 2018;48(2018):141–51.
45. Xiong Z, Lang L, Gao X, Xiao W, Wang Z, Zhao L. An integrative urinary metabolomic study of the therapeutic effect of Guizhi Fuling capsule on primary dysmenorrheal rats based 1H NMR and UPLC-MS. *J Pharm Biomed Anal.* 2019;164(2019):750–8.
46. Zhang Y, et al. Systematic screening and characterization of multiple constituents in Guizhi Fuling capsule and metabolic profiling of bioactive components in rats using ultra-high-performance liquid chromatography/quadrupole-time-of-flight mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2017;1061-1062:474–86.
47. Zhao L, Xiong Z, Sui Y, Zhu H, Zhou Z, Wang Z, Zhao Y, Xiao W, Lin J, Bi K. Simultaneous determination of six bioactive constituents of Guizhi Fuling capsule in rat plasma by UHPLC-MS/MS: application to a pharmacokinetic study. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2015;1001(2015):49–57.
48. Shen SM, et al. Nuclear PTEN safeguards pre-mRNA splicing to link Golgi apparatus for its tumor suppressive role. *Nat Commun.* 2018;9(1):2392.
49. Davis A, Tinker AV, Friedlander M. "platinum resistant" ovarian cancer: what is it, who to treat and how to measure benefit. *Gynecol Oncol.* 2014;133(3):624–31.
50. Pylväs-Eerola M, Liakka A, Puistola U, Koivunen J, Karihtala P. Cancer stem cell properties as factors predictive of Chemoresistance in Neoadjuvantly-treated patients with ovarian Cancer. *Anticancer Res.* 2016;36(7):3425–31.
51. Davidson B, Holth A, Helleslyt E, Tan TZ, Huang RY, Tropé C, Nesland JM, Thiery JP. The clinical role of epithelial-mesenchymal transition and stem cell markers in advanced-stage ovarian serous carcinoma effusions. *Hum Pathol.* 2015;46(1):1–8.
52. Freimund AE, Beach JA, Christie EL, Bowtell D. Mechanisms of drug resistance in high-grade serous ovarian Cancer. *Hematol Oncol Clin North Am.* 2018;32(6):983–96.
53. Thakur B, Ray P. Cisplatin triggers cancer stem cell enrichment in platinum-resistant cells through NF- κ B-TNF α -PIK3CA loop. *J Exp Clin Cancer Res.* 2017;36(1):164.
54. Chekerov R, et al. Sorafenib plus topotecan versus placebo plus topotecan for platinum-resistant ovarian cancer (TRIAS): a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet Oncol.* 2018;19(9):1247–58.
55. Tsou SH, Hou MH, Hsu LC, Chen TM, Chen YH. Gain-of-function p53 mutant with 21-bp deletion confers susceptibility to multidrug resistance in MCF-7 cells. *Int J Mol Med.* 2016;37(1):233–42.
56. Xu J, Liu D, Niu H, Zhu G, Xu Y, Ye D, Li J, Zhang Q. Resveratrol reverses doxorubicin resistance by inhibiting epithelial-mesenchymal transition (EMT) through modulating PTEN/Akt signaling pathway in gastric cancer. *J Exp Clin Cancer Res.* 2017;36(1):19.
57. Li W, Zhai L, Zhao C, Lv S. MiR-153 inhibits epithelial-mesenchymal transition by targeting metadherin in human breast cancer. *Breast Cancer Res Treat.* 2015;150(3):501–9.
58. Guo CC, Zheng LH, Fu JY, Zhu JH, Zhou YX, Zeng T, Zhou ZK. Antiosteoporotic effects of Huangqi Sanxian decoction in cultured rat osteoblasts by proteomic characterization of the target and mechanism. *Evid Based Complement Alternat Med.* 2015;2015(1):514063.
59. Anantharaju PG, Gowda PC, Vimalambike MG, Madhunapantula SV. An overview on the role of dietary phenolics for the treatment of cancers. *Nutr J.* 2016;15(1):99.
60. Rajasekaran D, et al. Combination of nanoparticle-delivered siRNA for astrocyte elevated Gene-1 (AEG-1) and all-trans retinoic acid (ATRA): an effective therapeutic strategy for hepatocellular carcinoma (HCC). *Bioconjug Chem.* 2015;26(8):1651–61.
61. Bild AH, et al. Oncogenic pathway signatures in human cancers as a guide to targeted therapies. *Nat.* 2006;439(7074):353–7.
62. Vizeacoumar FJ, et al. A negative genetic interaction map in isogenic cancer cell lines reveals cancer cell vulnerabilities. *Mol Syst Biol.* 2013;1(9):696.
63. Havugimana PC, et al. A census of human soluble protein complexes. *Cell.* 2012;150(5):1068–81.
64. Burington B, Barlogie B, Zhan F, Crowley J, Shaughnessy JD Jr. Tumor cell gene expression changes following short-term in vivo exposure to single agent chemotherapeutics are related to survival in multiple myeloma. *Clin Cancer Res.* 2008;14(15):4821–9.
65. Oughtred R, et al. The BioGRID interaction database: 2019 update. *Nucleic Acids Res.* 2019;47(D1):D529–529D541.
66. George S, et al. Loss of PTEN is associated with resistance to anti-PD-1 checkpoint blockade therapy in metastatic uterine Leiomyosarcoma. *Immun.* 2017;46(2):197–204.
67. Dhiman G, Lohia N, Jain S, Baranwal M. Metadherin peptides containing CD4(+) and CD8(+) T cell epitopes as a therapeutic vaccine candidate against cancer. *Microbiol Immunol.* 2016;60(9):646–52.

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