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Reversal effect of quercetin on multidrug resistance via FZD7/β-catenin pathway in hepatocellular carcinoma cells

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ABSTRACT

Background: Chemotherapy has been widely used to treat cancer, but the appearance of multidrug resistance (MDR) is the biggest obstacle to successful chemotherapy. One of the conventional mechanisms of MDR is overexpression of ATP-binding cassette (ABC) transporters such as P-glycoprotein (P-gp/ABCB1) and multidrug resistance-associated proteins (MRPs/ABCCs) that limits the prolonged and efficient use of chemotherapeutic drugs. To enhance the chemosensitivity of tumor cells, attentions have been focused on effective MDR modulators.

Purpose: This study aimed to investigate the reversal effect of quercetin on MDR, and explored its mechanism of action in vitro.

Study design/methods: The effect and mechanism of quercetin on MDR was examined by using MTT assay, flow cytometry, real-time PCR and western blot analysis in human hepatocellular carcinoma cells.

Results: Our data found that the intracellular accumulation of rhodamine-123 (Rh123) and doxorubicin (ADR) were increased, the sensitivity of BEL/5-FU cells to chemotherapeutic drugs were increased, and the expressions of ABCB1, ABCC1 and ABCC2 were all down-regulated, which indicated that the functions and expressions of ABCB1, ABCC1 and ABCC2 efflux pump were inhibited by quercetin treatment. Moreover, the suppression of ABCB1, ABCC1 and ABCC2 by quercetin was dependent on the FZD7 through the Wnt/β-catenin pathway. Further research revealed that reduction of FZD7 by RNA interference (siFZD7) enhanced the sensitivity to chemotherapeutic drugs, increased the cellular accumulation of Rh123 and ADR, and induced inhibitory effects on the expression of FZD7, ABCB1, ABCC1, ABCC2 and β-catenin, similar to quercetin. In the meanwhile, overexpression of FZD7 showed the inversely effect on the expressions. Interesting, it was confirmed that quercetin could inhibit the expression levels of FZD7, ABCB1, ABCC1, ABCC2 and β-catenin in BEL-7402 cells; furthermore, treatment by quercetin combined with siFZD7 in BEL/5-FU cells, the expressions of these genes were effectively decreased in comparison to quercetin combined with siRNA negative control (sncRNA).

Conclusion: Overall, these data suggested the effectiveness of using quercetin, at least in part, via inhibiting FZD7 to combat chemoresistance and showed that quercetin could be developed into an efficient natural sensitizer for resistant human hepatocellular carcinoma.

Introduction

Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related mortality worldwide and also one of the most common malignant liver tumors in China (Torre et al., 2015). The available front-line treatment for HCC is surgical resection and liver transplantation, but only a minority of the patients are eligible because HCCs typically present at advanced-stage with macrovascular invasion or

Abbreviations: 5-FU, 5-fluorouracil; ABC, ATP-binding cassette; ATP, adenosine triphosphate; ADR, doxorubicin; FZD7, Frizzled homolog protein 7; GV144-FZD7, lentivirus-FZD7 vector; HCC, hepatocellular carcinoma; IC50, 50% inhibition concentration; MDR, multiple drug resistance; MRP1/ABCC1, multidrug resistance-associated protein 1; MRP2/ABCC2, multidrug resistance-associated protein 2; MMC, Mitomycin C; MTT, thiazolyl blue tetrazolium blue; NC-GV144, empty GV144 vector; P-gp/ABCB1, P-glycoprotein; quercetin, Q; qRT-PCR, quantitative real-time polymerase chain reaction; Rh123, rhodamine-123; SDS-PAGE, sulphate-polyacrylamide gel electrophoresis; siRNA, small interfering RNA; siFZD7, FZD7 interfering small RNA; sncRNA, siRNA negative control; VRP, verapamil

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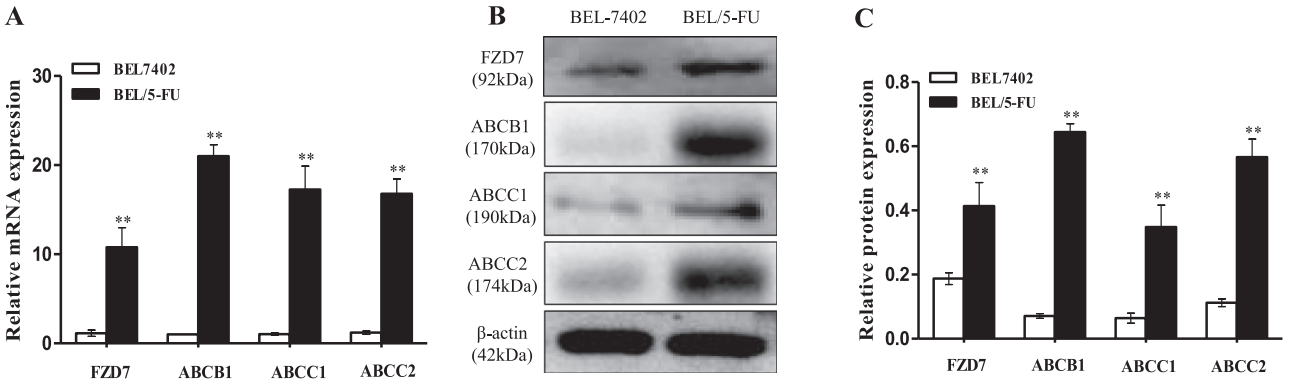

Fig.1. The relative expressions FZD7, ABCB1, ABCC1 and ABCC2 in BEL-7402 and BEL/5-FU cells. (A) Expressions of FZD7, ABCB1, ABCC1 and ABCC2 were detected by qRT-PCR. (B) Expressions of FZD7, ABCB1, ABCC1 and ABCC2 were detected by western blot. The assays were performed at least three times with similar results. Data are shown as the mean ± SD (n = 3). $^{*}P < 0.05$, $^{**}P < 0.01$, versus control.

Table 1
The sensitivity of BEL-7402 and BEL/5-FU cells treated with chemotherapeutic drugs.

	IC50(μg/ml)		
	BEL/5-FU	BEL-7402	Resistance index
5-FU	69.648 ± 1.23	4.076 ± 0.27	17.08
MMC	4.151 ± 0.30	2.065 ± 0.25	2.01
ADR	8.882 ± 1.18	4.021 ± 0.13	2.21

metastases (Ling et al., 2017). Systemic chemotherapy is adopted as an effective nonsurgical therapeutic strategy for most HCCs. However, the emergence of multidrug resistance (MDR) to pharmacologically and structurally distinct class of clinical drugs severely blocks the successful management of HCC (Bruix et al., 2014; Wu et al., 2016). The well recognized mechanism underlying MDR is the significant overexpression of adenosine triphosphate (ATP)-binding cassette (ABC) super-family of transporters, such as P-glycoprotein (P-gp/ABCB1), multidrug resistance-associated protein 1 (MRP1/ABCC1) and multidrug resistance-associated protein 2 (MRP2/ABCC2), et al., which acts as an efflux pump on cell surface (Chen et al., 2016). Hence, the strategy consists in inhibiting the activity, or lowering the expression of the efflux transporter, which is highly warranted to improve the outcomes of HCC chemotherapy.

Quercetin, a typical flavonol-type flavonoid compound found ubiquitously distributed in many plants and vegetables, which has been exhibited to possess antioxidative, anti-inflammatory, immunomodulatory, and vasodilating activities (Chen et al., 2010). In recent years, increasing evidence suggests that quercetin can be useful in cancer treatment by inducing cell apoptosis in vitro and in vivo (Hashemzaei et al., 2017; Lee et al., 2015; Nguyen et al., 2017). Quercetin could be suggested as a potential modulator of ABCB1 which inhibits the ABCB1 expressions in a concentration-dependent manner in the KB/VCR oral cancer resistant cell lines (Yuan et al., 2015). Besides, quercetin might target the MAPK/ERK/JNK pathway and transcriptionally down-regulate ABCB1, suggesting that quercetin may reverse MDR by targeting the MAPK/ERK/JNK/ABCB1 pathway in multidrug resistant leukemia K562 cells (Chen et al., 2015). Furthermore, quercetin effectively inhibited tumor growth and enhanced the sensitivity to chemotherapy in a xenograft mouse model of HCC (Dai et al., 2016). Research carried out in the past few decades has shown that quercetin plays a vital role in the development of HCC. However, studies on the function and mechanisms of quercetin on MDR in HCC are still limited.

Our previous study firstly revealed that the activation of FZD7/β-catenin pathway plays an important role in the MDR (Chen et al., 2013), but whether or not quercetin reverses MDR by suppressing FZD7/β-catenin pathway in HCC is not yet clear. Therefore, the present study aimed to investigate the effect and the underlying molecular mechanisms of quercetin on human hepatocellular carcinoma MDR BEL-7402/5-fluorouracil (BEL/5-FU) cells.

Material and methods

Cell culture

The parental sensitive human hepatocellular carcinoma cell line BEL-7402 and multidrug resistant cell line BEL/5-FU (all obtained from keygen biotech, Nanjing, China) were grown in RPMI-1640 medium (Gibco, KeyGen Biotech, Nanjing, China) containing 10% fetal calf serum (Sijiqing, Zhejiang Tianhang, China), 100 U/ml penicillin and 100 mg/ml streptomycin at 37 °C under a humidified atmosphere of 5% CO_2. The BEL/5-FU cells were cultured in the media mentioned above that additionally contained 20 μg/ml 5-FU until at least two weeks before beginning experimentation to maintain its MDR phenotype.

Table 2
Enhancement of quercetin on the cytotoxicity of chemotherapeutic drugs in BEL/5-FU cells. RF represents the reversal effect of modulator, the greater the RF magnitude, the more significant the effect.

	Reversal resistance of 5-FU		Reversal resistance of MMC		Reversal resistance of ADR	
	IC50(μg/ml) of 5-FU	RF	IC50(μg/ml) of MMC	RF	IC50(μg/ml) of ADR	RF
Quercetin (0 μM)	69.65 ± 1.23		4.15 ± 0.30		8.88 ± 1.18	
Quercetin (40 μM)	42.49 ± 0.72	1.63	3.04 ± 0.12	1.36	6.67 ± 0.49	1.33
Quercetin (80 μM)	34.28 ± 1.69	2.03	2.63 ± 0.20	1.58	5.00 ± 0.21	1.78
Quercetin (160 μM)	20.42 ± 1.52	3.41	1.65 ± 0.28	2.51	3.18 ± 0.35	2.79
Verapamil (5 μM)	22.94 ± 0.68	3.03	1.97 ± 0.06	2.11	3.46 ± 0.42	2.57
MK571 (5 μM)	23.82 ± 1.57	2.92	2.04 ± 0.24	2.03	3.65 ± 0.34	2.43