ORIGINAL RESEARCH

Mechanism of Quercetin as a Multidrug-resistant Reversing Compound in Oxaliplatin-resistant Gastric-cancer Cell Lines

Guodong Zhao, MMed; Siwei Xue, BS

ABSTRACT

Context • Treatment failure due to multidrug resistance (MDR) is a crucial hurdle during chemotherapy. MDR is generally correlated with an upregulation of adenosine triphosphate (ATP)-binding cassette (ABC) transport proteins. Also, aberrant activation of the phosphoinositide 3-kinase (PI3K)/ protein kinase B (Akt) pathway can counteract chemotherapeutic induction. Identification of safe and functioning MDR-reversing compounds is necessary in gastric-cancer therapy.

Objective • The study intended to examine the role of Quercetin (Qur) in the mediation of osmotic glycoprotein (P-gp) expression and activity as an ABC transporter in the PI3K/Akt/ P-gp cascade in the oxaliplatin (OxR)-resistant, gastric-cancer cell line KATOIII/OxR.

Design • The research team performed a laboratory study.

Setting • The study took place at Nantong Haimen People's Hospital.

Outcome Measures • The research team: (1) determined the impact of OxR on cell viability after treatment with Qur using trypan blue and "3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide" (MTT) assays; (2) employed a rhodamine 123 (Rh123) assay to detect the activity of P-gp; (3) used quantitative reverse transcription polymerase chain reaction (RT-qPCR) to measure mRNA expression of P-gp; and (4) detected apoptosis using an enzyme-linked immunoassay (ELISA) cell-death assay.

Results • Qur: (1) increased the cytotoxicity of OxR; (2) downregulated the expression level and activity of P-gp and reversed MDR through the enhancement of the cytotoxicity of intracellular OxR in KATOIII/OxR cells; and (3) enhanced the apoptosis rate in KATOIII/OxR cells.

Conclusions • Qur induced a dramatic reduction in the survival rate of KATOIII/OxR cells and may reverse OxR resistance through a decrease in P-gp expression and activity. These data imply that exposure of KATOIII/OxR cells in the dose-dependent manner to Qur can circumvent MDR by improving the intracellular accumulation of OxR. Qur might provide a new treatment strategy and improve patients' survival after chemotherapy for gastric cancer. (*Altern Ther Health Med.* 2023;29(8):54-59)

Guodong Zhao, MMed, Physician, Department of General Surgery, Nantong Haimen People's Hospital. Nantong City, Jiangsu Province, China. **Siwei Xue, BS,** Physician, Department of Gastroenterology, People's Hospital of Cangnan County, Wenzhou City, Zhejiang Province, China.

Corresponding author: Siwei Xue, BS
E-mail: 2008xsw@163.com

INTRODUCTION

Gastric cancer is the second most frequent type of carcinoma in women and men, which makes it one of the most lethal human malignancies worldwide.[1,2] Oxaliplatin (OxR) is the most commonly used chemotherapeutic agent for patients. It's a third-generation platinum compound, which adheres to plasma proteins and moves to all of the body's tissues.[3]

Although medical practitioners have achieved critical advances in diagnostic approaches, chemotherapies, and surgical procedures, they often find that patients at stages III and IV of gastric cancer have acquired drug resistance to common therapeutic agents.[4]

Chemotherapy Resistance

Nanayakkara et al and Breier et al found that multidrug resistance (MDR) represents a big hurdle to the ability of chemotherapeutic agents to achieve positive effects.[5,6] OxR-resistance is one of the greatest hindrances in chemotherapy, including in gastric-cancer treatment.

Most clinically practical chemotherapy agents have become ineffective because of the effects of the mechanisms underlying chemotherapy resistance. MDR is generally correlated with an upregulation of adenosine triphosphate (ATP)-binding cassette (ABC) transport proteins, which can

reduce the intracellular accumulation of chemotherapeutic agents through elevation of drug extrude on the cells' surfaces.[7] Zhao et al found that the upregulation of these transporter proteins can lead to intrinsic and/or acquired drug resistance in the majority of tumors.[8]

Gastric-cancer cells acquire resistance to OxR through modifications of ABC transport proteins, diminished cellular uptake, or elevation of drug efflux.[9] Costea et al classified49 recognized ABC genes into subfamilies, including 12 ABCA, 11 ABCB, 13 ABCC, four ABCD, one ABCE, three ABCF, and five ABCG families.[10]

Ahmed found that osmotic glycoprotein (P-gp)/ MDR1/ ABCB1 and multidrug-resistant associated protein 1 (MRP1)/ ABCC1 were the main transporters of ABC.[9] P-gp is a cell surface transporter, which has a very broad substrate spectrum that mediates the extrusion of a diversity of chemotherapeutic agents; therefore, overexpression of P-gp can contribute to the resistance of gastric-cancer cells to chemotherapy.

Therefore, curbing the activity or lowering the expression of efflux transporters are extremely warranted strategies to promote good results for gastric-cancer chemotherapy.

PI3K/Akt Pathway

Aberrant activation of the phosphoinositide 3-kinase (PI3K)/ protein kinase B (Akt) pathway can counteract chemotherapeutic induction.[10] The PI3K/ Akt -activated pathway can enhance a tumor's biological basis by effectively expressing P-gp and MRP1, the activation of which can decrease the reaction of a tumor to chemotherapy agents and heighten the extrusion of chemotherapeutics drugs from cells.[11]

In addition, some of the nuclear steroid receptors, including peroxisome proliferator-activated receptors (PPARs), regulate various biological processes, such as proliferation, apoptosis, and neoplastic transformation.[10] PPAR-gamma (PPARγ) agonists can have valuable impacts on various types of cancer and can actuate the expression of the "phosphatase and tensin homolog deleted on chromosome 10" (PTEN) protein.

PTEN is a tumor-suppressor protein and hinders the activation of the PI3K/Akt pathway through dephosphorylation of the position-3 phosphate in phosphatidylinositol-3,4,5-trisphosphate (PIP3), which leads to production of PIP2[12]. Montazami *et al* found that downregulation of PTEN in the majority of cancers was associated with poor neoplasm prognosis and MDR.[13] Targeting PPARγ might be an effective adjunct treatment for reversing MDR in gastric-cancer cells through PTEN upregulation.[14]

The PTEN/PI3K/Akt survival pathway serves a complicated and essential role in various aspects of tumor growth, including cell proliferation, migration, angiogenesis, and apoptosis.[15] For example, Li *et al* found that upregulation of PTEN in cancer cells is negatively correlated with reversal of MDR and with an increased reaction of those cells to chemotherapeutic drugs, through the repression of the PI3K/ Akt cascade.[16]

Inhibiting the PI3K/Akt axis with a specific inhibitor such as LY294002 or Akt siRNA might retrieve drug sensitivity by downregulating the expression of P-gp and MRP1. However, the accurate mechanisms that underlie the role of the activation of the PI3K/Akt pathway remain uncertain.

Quercetin (Qur)

Some previous studies found that combined chemotherapy can be effective in reversing MDR and in inducing chemosensitization.[17,18] Although physicians have used various agents, including cyclosporin A (CsA) and verapamil, to reverse the MDR of cancers, their side effects have restricted their application clinically.[19]

Interest in the therapeutic potential of natural compounds has had a long history due to their low side effects and specific pharmacological activities.[20] One of the most successful of these compounds is Qur, which the International Union of Pure and Applied Chemistry (IUPAC) has named "3,5,7,3',4'-pentahydroxyflavone, C15H10O7."

Qur is a permanent-component flavonoid and is present in many vegetables and fruits. Because of its lipophilic nature, Qur can traverse the cell membrane and trigger synergistic effects for some chemotherapeutic agents, including OXR.[21,22,23] Lee et al found that Qur could upregulate PPARγ expression levels to control biological function.[24] For overcoming chemotherapy resistance, Qur is a natural supplement of flavonoids with anticancer and antiproliferative activities.[25,26]

Current Study

The current study intended to examine the role of Qur in the mediation of P-gp expression and activity as an ABC transporter in the PI3K/Akt/P-gp cascade in the OxR-resistant, gastric-cancer cell line KATOIII/OxR.

METHODS

The research team performed a laboratory study, which took place at Nantong Haimen People's Hospital.

Biological material. The research team obtained: (1) OxR from Cayman Chemicals (Michigan, USA); (2) Qur, trypan blue dye, and dimethyl sulfoxide (DMSO) from Sigma USA (Therm, USA), (3) penicillin G/streptomycin (pen/ strep) from Sigma-Aldrich (Therm, USA); (4) fetal bovine serum (FBS), phosphate buffered saline (PBS), Roswell Park Memorial Institute 1640 (RPMI-1640), and Trypsin/ ethylenediaminetetraacetic acid (EDTA) from Gibco Life Technologies (Beijing, China); (5) "3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide" (MTT) from Bio Basic (Beijing, China); (6) RNeasy with a mini kit from Qiagen (Shanghai, China); (7) SYBR master mix from TaKaRa Bio (Dalian, China); (8) radio immune precipitation assay lysis buffer (RIPA) from Beyotime (Beijing, China; (9) a BCA (bicinchoninic acid) assay kit from Thermo Fisher Scientific (Waltham, USA); and (10) antibodies from Abcam (Cambridge, United Kingdom).

Cell culture. The research team: (1) obtained KATOIII and KATOIII/OxR, two human gastric-cancer cell lines, from Pasteur Institute (Tehran, Iran); (2) cultured them in the RPMI 1640 medium, supplemented with 100 unit/ml of 10% FBS and 100 µg/ml of pen/strep; (3) incubated these cell lines at 37°C in the presence of 5% carbon dioxide; and (4) determined the viable cells by conducting an exclusion experiment using trypan blue dye.

Cell viability assay. The research team: (1) assessed the induction of Qur's cytotoxicity effects on OxR in the KATOIII/OxR cell line using the MTT assay; (2) plated the cells in 96-well microplates at a density of 2×10^4 cells/200µl/well and exposed them to 20 µM of Qur and concentrations of OxR from 5 to 5 µM for 48 h; (3) subsequently, exposed the cells to 5 mg/mL of 20-µl MTT in a 37°C, humidified incubator (Promega, Madison, WI, USA) for 4 h; (4) dissolved formazan product (Carlsbad, CA, USA) in 175 µL of DMSO; and (5) measured absorbance at 490 nm using a Benchmark Plus microplate spectrometer (Bio-Rad, California, USA).

Analysis of P-gp activity. Rhodamine 123 (Rh 123) serves as a desirable substrate for MDR-correlated P-gp, and previous study have observed that agents that repress P-gp can intensify the retention of Rh123 in drug-resistant cells.[27] The research team: (1) seeded cells into a six-well microplate at a density of 2.5 $\times 10^5$ cells/2 ml/well; (2) after overnight incubation, treated the cells with serum-free media which included 5 µM of Rh 123 in the presence or absence of Qur; and (3) incubated the cells for 90 min. The team used 10 µM of verapamil as the positive control. The team determined the intracellular mean fluorescence intensity (MFI) related to intracellular Rh123 content using a fluorometer (VICAM AflaTest, Beijing, China).

Quantitative reverse transcription polymerase chain reaction (Real-time RT–qPCR). The research team: (1) extracted total RNA from cells using RNAiso plus (Takara Bio, Tokyo, Japan), following the manufacturer's protocols; (2) exposed the RNA to deoxyribonuclease (DNAse). Then, add 0.5 µg total RNA, reversed transcribed using HiScript SuperMix (Vazyme Biotech, Shanghai, China) and converted into complementary DNA; and (3) Using an iCycler iQ multicolor detection system (Bio-Rad, California, USA)to quantified the complementary DNA(cDNA) .

The primers: (1) for MDR1 were 5'- CCCATCATT GCAATAGCAGG -3' (Forward) and 5'- TGTTCAAACT TCTGCTCCTGA -3' (Reverse); (2) for MRP-1 were 5'-GCAGCCCGGCTCTAACATTTTGC -3' (Forward) and 5'-CTCCTCCACCCGCTCCTGCA -3' (Reverse); and (3) for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were 5'-AAGCTGTGTTACGTGGCCCT -3' (Forward) and 5'-GGGCAGCTCGTAGCTCTTCT -3' (Reverse). The team used GAPDH as an internal control.

Molecular Signaling Basis for Qur. To confirm the roles of PTEN and PI3K in the PPARγ/PTEN/PI3K/Akt/MDR1/P-gp molecular pathway, the research team investigated the underlying molecular mechanisms by analyzing the MDR-reversing impact of Qur in the face of specific inhibitors of PTEN (SF1670) and PI3K (LY294002).

PTEN pathway. The cells were transfected with PTEN overexpression vector,qRT-PCR detected the level of PTEN in transfected cells.

Apoptosis assay. The research team used an enzyme-linked immunoassay (ELISA) cell-death kit (Roche, Munich, Germany), following the supplier's protocols, and: (1) seeded the KATOIII and KATOIII/OxR cell lines at a density of 2×10^4 cells per well in twenty-four-well microplates; (2) exposed them to Qur; to OxR, using an IC50 dose; and to their combination in appropriate drug concentrations; and (3) following a 48-h incubation, collected the cells and identified apoptosis using the ELISA kit.

The assay specifies the value of mono- and oligonucleosomes released into the cytosol throughout apoptosis. The team: (1) used an enzyme to digest the cells and centrifuged them at 150 × g for 12 min; (2) after the addition of 30 µl of the cell supernatant and 70 µl of a mixture of anti-DNA-peroxidase and anti-histone-biotin to each well, incubated the plate coated with horseradish peroxidase (HRP)-streptavidin for 1.5 h; (3) after washing the plates with 150 µl of 2, 2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) solution (Beyotime ,Beijing,China.), transferred the cells in each well; and (4) measured absorbance at 405 nm using a microplate auto-reader (MR-T00B,Hongkong,China).

Outcome measures. The research team: (1) determined the impact of OxR on cell viability after treatment with Qur, using trypan blue and MTT assays; (2) employed an Rh123 assay to detect the activity of P-gp; (3) used RT-qPCR to measure mRNA expression of P-gp; and (4) detected apoptosis using an ELISA cell-death assay.

Outcome Measures

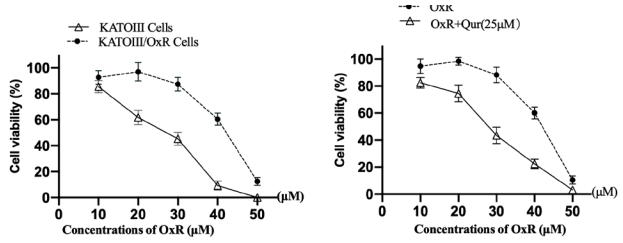
Reversal of MDR. The research team determined the cytotoxic impact of Qur and OxR on KATOIII and KATOIII/OxR cells using an MTT assay. In addition, the team evaluated the effects of Qur on the OxR-resistant cells. In the presence of a regulator, the team determined the folding reversal (FR) of resistance by dividing the IC50 value of the control cells by the IC50 value of the treated cells.

P-gp and MRP-1 activity. The research team determined P-gp activity using the intracellular Rh123 accumulation assay. Verapamil was the positive control.

Expression of P-gp gene. The reversal of MDR may be achieved by regulating P-gp levels by controlling the down-regulation of Qur expression and affecting the activity of PTEN. The research group detected P-gp expression levels by qRT-PCR.

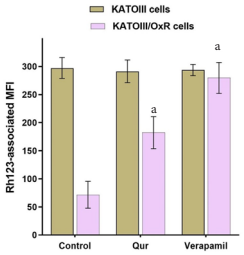
Reversal of Qur's Effects. To clarify the molecular signaling basis for Qur, the research team speculated that Qur may play a dominant role in enhancing the cytotoxic effects of OxR by repression of P-gp expression through downregulating the PI3K/Akt pathway. The team investigated the MDR-reversing effects of Qur in the presence of specific blockers, including PTEN—SF1670, PI3K—LY294002, and mitogen-activated protein kinases (MAPK)—PD098059.

Figure 1. Cytotoxic Impact of OxR on KATOIII and KATOIII/OxR Cells and of OxR Only Compared With Qur Combined With OxR on KATOIII/OxR Cells. Figure 1A showed the differences between the KATOIII and KATOIII/OxR cells in the cytotoxicity toward MDR of the five concentrations of OxR, from 10 to 50 μM. Figure 1B showed the differences in the changes in cell viability of the KATOIII/OxR cells between treatment with OxR only and treatment with OxR combined with Qur.



Abbreviations: OxR, oxaliplatin; Qur, quercetin.

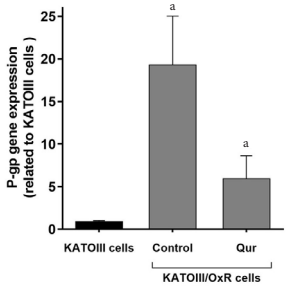
Figure 2. Determination of P-gp Activity Using Intracellular Rh123 Accumulation Assay. Figure 2 shows Qur's enhancement of Rh123 accumulation in the KATOIII/OxR cells after 1.5 h compared to that in the KATOIII cells, which displayed no enhancement.



[a]$P < .05$, indicating that Qur's and verapamil's incubation with RH123 led to a significant enhancement of MFI in the KATOIII/OxR cells compared to the control while no accumulation of RH123 occurred in the KATOIII cells

Abbreviations: MFI, mean fluorescence intensity; P-gp, permeability-glycoprotein; Rh123, rhodamine 123.

Figure 3. A qRT-PCR Analysis Using the P-gp Gene



[a]$P < .05$, indicating that expression of the P-gp gene (MDR-1) was significantly higher in the control and Qur than that in the KATOIII cells

Abbreviations: P-gp, permeability-glycoprotein; qRT-PCR, quantitative reverse transcription polymerase chain reaction.

Overexpression of the PTEN pathway. To assess the association between Qur and PTEN, the research team evaluated the PTEN expression in KATOIII parental and KATOIII/OxR cells.

Qur and OxR-induced apoptosis. To better show the sensitizing effects of Qur on OxR-resistance in the gastric-cancer cell line KATOIII/OxR, the research team assessed the effects of Qur in OxR-induced apoptosis for 48h using the ELISA cell-death kit.

Statistical Analysis

The research team analyzed the data analysis using GraphPad Prism 7.04 (GraphPad Software, San Diego, USA). The team: (1) expressed continuous variables as means ± standard deviations (SDs) and compared the groups using one-way analysis of variance (ANOVA), followed by Bonferroni's and Sidak's tests and replicated at least three independent times. $P < .05$ indicated statistical significance.

RESULTS

Reversal of MDR

Figure 1A shows the differences between the KATOIII and KATOIII/OxR cells in the cytotoxicity toward MDR of the five concentrations of OxR, from 10 to 50 μM. Figure 1B showed the differences in the changes in cell viability of the KATOIII/OxR cells between treatment with OxR only and treatment with OxR combined with Qur. The IC50 values for OxR in the KATOIII cell line compared with KATOIII/OxR cells were almost three fold, indicating a high resistance to OxR in the KATOIII/OxR cells. These findings demonstrated that Qur can sensitize KATOIII/OxR cells to OxR and decrease OxR IC50 against KATOIII/OxR cells.

P-gp and MRP-1 Activity

As Figures 2 shows, Qur enhanced the Rh123 accumulation in the KATOIII/OxR cells after 1.5 h, while KATOIII cells displayed no enhancement. The incubation of the Qur-treated KATOIII/OxR cells with Rh123 led to a significant time-dependent enhancement in the MFI compared to the control ($P < .05$). This impact was comparable to that observed in the KATOIII/OxR cells treated with verapamil, which was also significant compared to the control. However, no accumulation of substrate occurred in the KATOIII cells over the same amount of time. Therefore, Qur limited the activity of P-gp in the KATOIII/OxR cells.

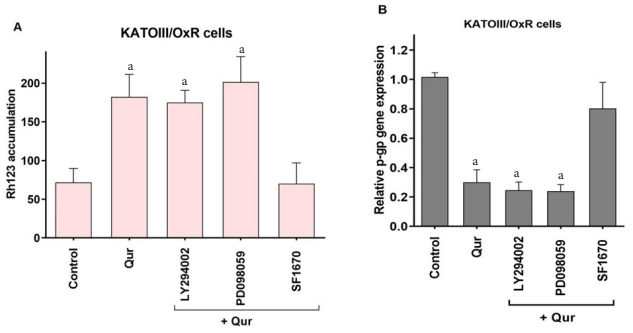
Expression of P-gp Gene

qRT-PCR analysis found that the expression of the P-gp gene (MDR-1) was significantly lowe in the KATOIII/OxR cells after treatment with the Qur than control ($P < .05$). These results indicated that Qur could reverse drug resistance in KATOIII/OxR cells.

Reversal of Qur's Effects

As Figure 4A shows, treatment with 10 μM of SF1670 almost abrogated the benefits of Qur. Rh123 accumulation

Figure 4. Clarification of the Molecular Signaling Basis for Qur. Figure 4A shows the effects of the control, of Qir alone, and of Qur plus 10 μM of SF1670, 10 μM of PD098059, or LY294002 on Rh123-associated MFI. Figure 4B shows the effects of the control, of Qir alone, and of Qur plus 10 μM of SF1670, 10 μM of PD098059, or LY294002 on P-gp gene expression.



[a]$P < 0.05$, indicating that the Rh123 accumulation and P-gp gene expression were significantly higher in the KATOIII/OxR cells with treatment using Qur, Qur+LY294002, and Qur+PD098059 than those of the control

Abbreviations: OxR, oxaliplatin; Qur, quercetin.

was significantly higher in the KATOIII/OxR cells after treatment using Qur, Qur+LY294002, and Qur+PD098059 than that of the control (all $P < .05$).

As Figure 4B shows, the P-gp mRNA levels were significantly lower in the KATOIII/OxR cells at 48 h after treatment using Qur, Qur+LY294002, and Qur+PD098059 than those of the control (all $P < .05$). In addition, the SF1670 suppressed the effects of Qur.

Overexpression of the PTEN Pathway

As Figure 5 shows, PTEN expression fold change in the KATOIII/OxR cells was significantly higher for the Qur than for the control ($P < .05$), which confirmed the findings that Qur can upregulate PTEN expression after 48 h incubation in a dose-dependent manner. This finding suggested that Qur-induced lower expression of P-gp might related with PTEN expression in KATOIII/OxR cells.

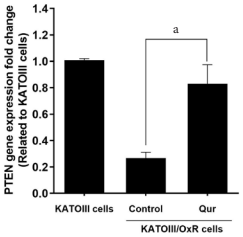
Qur and OxR-induced apoptosis

The Qur enhanced the extent of apoptosis, after a 48h exposure of the cells, by 17.83-fold relative to the control group. As Figure 6 showed that apoptosis with Qur group alone or OxR alone group was significantly higher in KATOIII/OxR than that with no treatment ($P < .05$) and the apoptosis with Qur and OxR group was significantly higher in KATOIII/OxR than other all groups ($P < .01$). These findings showed that Qur may enhance the synergistic effects of OxR-induced apoptosis of KATOIII/OxR cells.

DISCUSSION

The current study showed that Qur combined with OxR can consistently reverse MDR through the PPARγ/PTEN/PI3K/Akt/MDR1/P-gp molecular pathway, with lower

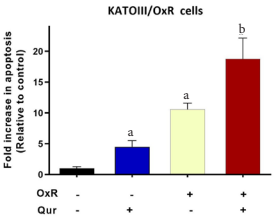
Figure 5. PTEN Expression in KATOIII Parental and KATOIII/OxR Cells



[a]$P < .05$, indicating that the PTEN expression fold change was significantly higher for the Qur than for the control

Abbreviations: OxR, oxaliplatin; PTEN, phosphatase and tensin homolog deleted on chromosome 10; Qur, quercetin.

Figure 6. Sensitizing Effects of Qur on OxR Resistance in the Gastric-cancer Cell Line KATOIII/OxR



[a]$P < .05$, indicating that apoptosis with Qur alone or OxR alone was significantly higher in KATOIII/OxR than that with no treatment
[b]$P < .01$, indicating that apoptosis with Qur+OxR was significantly higher in KATOIII/OxR than that with Qur alone or OxR alone

Abbreviations: OxR, oxaliplatin; Qur, quercetin.

expression and activity of P-gp in the KATOIII/OxR cells of gastric cancer.

Regarding the role of the PTEN pathway in the process of P-gp regulation, the current study's findings implied that exposure to Qur in a dose-dependent manner can lead to enhancement in the expression levels of PTEN. Some previous studies have also found that some mi-RNAs had similar effects in various tumor cells such as miR-205 reversed P-gp Mediated doxorubicin resistance via PTEN in human liver cancer HepG2 Cells.[28] Shi et al found miR-29a can significantly down-regulate the expression and activity of P-gp in colon cancer cell line HT29/DOX, and increased intracellular DOX to reduce drug resistance. In addition, after transfection of miRNA-29a, the expression of PTEN was restored in drug-resistant cells.[29] Loong et al found PPARγ activation may upregulated PTEN to inhibit cell signaling downstream of PI3K to mediate apoptosis in lung cancer cells.[30] Therefore, the influence of drugs or genes on the pathway may be through the PI3K/Akt pathway to regulate drug resistance, proliferation and apoptosis in cells.

Although the accurate mechanisms that underlie the role of the PI3K/Akt pathway activation remain uncertain, the current study revealed that the PI3K/Akt survival cascade played a major role in the pathogenesis of MDR. Its findings imply that the antiproliferative outcome of the combination of Qur and OxR in treatment of KATOIII/OxR cells was

associated with the PTEN/PI3K/AKT survival pathway. The mechanism underlying generating the MDR phenotype was the upregulation of drug efflux transporters.

Rhodamine123 is commonly used to identify the activity of common drug efflux via the membrane transporter P-glycoprotein (P-gp).[30] The current study also demonstrated that Qur in combination with OxR can prevent extrusion of Rh123 in KATOIII/OxR cells. In a sense, incubation of Qur-treated KATOIII/OxR cells with Rh123 led to an enhancement of the MFI.

The current study's results illustrated that exposure of resistant cells to Qur combined with OxR can circumvent MDR through promoting PTEN expression, limiting P-gp activity, and increasing the intracellular accumulation of OxR in KATOIII/OxR cells. However, we only conducted the corresponding detection in vitro, and the future research direction was to conduct more in-depth detection in vivo or animal models, which was also the limitation of our experiment.

CONCLUSIONS

In summary, Qur induced a dramatic reduction in the survival rate of KATOIII/OxR cells and may reverse OxR resistance through a decrease in P-gp expression and activity. These data implied that exposure of KATOIII/OxR cells in the dose-dependent manner to Qur can circumvent MDR by improving the intracellular accumulation of OxR. Qur might provide a new treatment strategy and improve patients' survival after chemotherapy for gastric cancer.

AUTHORS' DISCLOSURE STATEMENT

The authors have no conflicts of interest related to the study to declare.

REFERENCES

1. Siegel RL, Miller KD, Goding Sauer A, et al. Colorectal cancer statistics, 2020. *CA Cancer J Clin*. 2020;70(3):145-164. doi:10.3322/caac.21601
2. Lauby-Secretan B, Vilahur N, Bianchini F, Guha N, Straif K; International Agency for Research on Cancer Handbook Working Group. The IARC perspective on colorectal cancer screening. *N Engl J Med*. 2018;378(18):1734-1740. doi:10.1056/NEJMsr1714643
3. Comella P, Casaretti R, Sandomenico C, Avallone A, Franco L. Role of oxaliplatin in the treatment of colorectal cancer. *Ther Clin Risk Manag*. 2009;5(1):229-238. doi:10.2147/TCRM.S3583
4. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. *CA Cancer J Clin*. 2015;65(1):5-29. doi:10.3322/caac.21254
5. Nanayakkara AK, Follit CA, Chen G, Williams NS, Vogel PD, Wise JG. Targeted inhibitors of P-glycoprotein increase chemotherapeutic-induced mortality of multidrug resistant tumor cells. *Sci Rep*. 2018;8(1):967. doi:10.1038/s41598-018-19325-x
6. Breier A, Gibalova L, Seres M, et al. New insight into p-glycoprotein as a drug target. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents) 2013;13(1):159-70. doi:10.2174/187152013804487380
7. Song H, Liu D, Dong S, et al. Epitranscriptomics and epiproteomics in cancer drug resistance: therapeutic implications. *Signal Transduct Target Ther*. 2020;5(1):193. doi:10.1038/s41392-020-00300-w
8. Zhao BX, Sun YB, Wang SQ, et al. Grape seed procyanidin reversal of p-glycoprotein associated multi-drug resistance via down-regulation of NF-κB and MAPK/ERK mediated YB-1 activity in A2780/T cells. *PLoS One*. 2013;8(8):e71071. doi:10.1371/journal.pone.0071071
9. Krishna R, Mayer LD. Multidrug resistance (MDR) in cancer. Mechanisms, reversal using modulators of MDR and the role of MDR modulators in influencing the pharmacokinetics of anticancer drugs. *Eur J Pharm Sci*. 2000;11(4):265-283. doi:10.1016/S0928-0987(00)00114-7
10. Costea T, Vlad OC, Miclea L-C, Ganea C, Szöllősi J, Mocanu MM. Alleviation of Multidrug Resistance by Flavonoid and Non-Flavonoid Compounds in Breast, Lung, Colorectal and Prostate Cancer. *Int J Mol Sci*. 2020;21(2):401. doi:10.3390/ijms21020401
11. Ahmed FE. Molecular markers that predict response to colon cancer therapy. *Expert Rev Mol Diagn*. 2005;5(3):353-375. doi:10.1586/14737159.5.3.353
12. Yuan P, Zheng A, Tang Q. Tripartite motif protein 25 is associated with epirubicin resistance in hepatocellular carcinoma cells via regulating PTEN/AKT pathway. *Cell Biol Int*. 2020;44(7):1503-1513. doi:10.1002/cbin.11346
13. Silva A, Yunes JA, Cardoso BA, et al. PTEN posttranslational inactivation and hyperactivation of the PI3K/Akt pathway sustain primary T cell leukemia viability. *J Clin Invest*. 2008;118(11):3762-3774. doi:10.1172/JCI34616
14. Hu M, Zhu S, Xiong S, Xue X, Zhou X. MicroRNAs and the PTEN/PI3K/Akt pathway in gastric cancer (Review). [Review]. *Oncol Rep*. 2019;41(3):1439-1454. doi:10.3892/or.2019.6962
15. Montazami N, Kheir Andish M, Majidi J, et al. siRNA-mediated silencing of MDR1 reverses the resistance to oxaliplatin in SW480/OxR colon cancer cells. Cellular and molecular biology (Noisy-le-Grand, France) 2015;61(2):98-103.
16. Teng Y-N, Wang Y-H, Wu T-S, Hung HY, Hung CC. Zhankuic acids A, B and C from taiwanofungus camphoratus act as cytotoxicity enhancers by regulating p-glycoprotein in multi-drug resistant cancer cells. *Biomolecules*. 2019;9(12):759. doi:10.3390/biom9120759
17. Ding Z, Ogata D, Roszik J, et al. iNOS Associates With Poor Survival in Melanoma: A Role for Nitric Oxide in the PI3K-AKT Pathway Stimulation and PTEN S-Nitrosylation. *Front Oncol*. 2021;11:631766. doi:10.3389/fonc.2021.631766
18. Li M, Li ZH, Song J, et al. *miR-205* Reverses MDR-1 Mediated Doxorubicin Resistance via *PTEN* in Human Liver Cancer HepG2 Cells. *Cell J*. 2022;24(3):112-119.
19. Li S, Zhao Q, Wang B, Yuan S, Wang X, Li K. Quercetin reversed MDR in breast cancer cells through down-regulating P-gp expression and eliminating cancer stem cells mediated by YB-1 nuclear translocation. *Phytother Res*. 2018;32(8):1530-1536. doi:10.1002/ptr.6081
20. Yousefi B, Azimi A, Majidinia M, et al. Balaglitazone reverses P-glycoprotein-mediated multidrug resistance via upregulation of PTEN in a PPARγ-dependent manner in leukemia cells. *Tumour Biol*. 2017;39(10):1010428317716501. doi:10.1177/1010428317716501
21. Shi X, Valizadeh A, Mir SM, et al. miRNA-29a reverses P-glycoprotein-mediated drug resistance and inhibits proliferation via up-regulation of PTEN in colon cancer cells. *Eur J Pharmacol*. 2020;880:173138. doi:10.1016/j.ejphar.2020.173138
22. Xu Y, Wang S, Chan HF, et al. Dihydromyricetin Induces Apoptosis and Reverses Drug Resistance in Ovarian Cancer Cells by p53-mediated Downregulation of Survivin. *Sci Rep*. 2017;7(1):46060. doi:10.1038/srep46060
23. Lan H, Hong W, Fan P, et al. Quercetin Inhibits Cell Migration and Invasion in Human Osteosarcoma Cells. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology 2017;43(2):553-67. doi:10.1159/000480528
24. Srivastava S, Somasagara RR, Hegde M, et al. Quercetin, a Natural Flavonoid Interacts with DNA, Arrests Cell Cycle and Causes Tumor Regression by Activating Mitochondrial Pathway of Apoptosis. *Sci Rep*. 2016;6(1):24049. doi:10.1038/srep24049
25. Zhang X, Huang J, Yu C, et al. Quercetin enhanced paclitaxel therapeutic effects towards PC-3 prostate cancer through ER stress induction and ROS production. *OncoTargets Ther*. 2020;13:513-523. doi:10.2147/OTT.S228453
26. Lee S-M, Moon J, Cho Y, Chung JH, Shin MJ. Quercetin up-regulates expressions of peroxisome proliferator-activated receptor γ, liver X receptor α, and ATP binding cassette transporter A1 genes and increases cholesterol efflux in human macrophage cell line. *Nutr Res*. 2013;33(2):136-143. doi:10.1016/j.nutres.2012.11.010
27. Chen FY, Cao LF, Wan HX, et al. Quercetin enhances adriamycin cytotoxicity through induction of apoptosis and regulation of mitogen-activated protein kinase/extracellular signal-regulated kinase/c-Jun N-terminal kinase signaling in multidrug-resistant leukemia K562 cells. *Mol Med Rep*. 2015;11(1):341-348. doi:10.3892/mmr.2014.2734
28. Zhang X, Guo Q, Chen J, Chen Z. Quercetin Enhances Cisplatin Sensitivity of Human Osteosarcoma Cells by Modulating microRNA-217-KRAS Axis. *Mol Cells*. 2015;38(7):638-642. doi:10.14348/molcells.2015.0037
29. Dong W, Liao ZG, Zhao GW, et al. Reversal Effect of Oxypeucedanin on P-glycoprotein-mediated Drug Transport. *Molecules*. 2018;23(8):1841. doi:10.3390/molecules23081841
30. Li M, Li ZH, Song J, et al. *miR-205* Reverses MDR-1 Mediated Doxorubicin Resistance via *PTEN* in Human Liver Cancer HepG2 Cells. *Cell J*. 2022;24(3):112-119.
31. Shi X, Valizadeh A, Mir SM, et al. miRNA-29a reverses P-glycoprotein-mediated drug resistance and inhibits proliferation via up-regulation of PTEN in colon cancer cells. *Eur J Pharmacol*. 2020;880:173138. doi:10.1016/j.ejphar.2020.173138
32. To KKW, Wu WKK, Loong HHF. PPARgamma agonists sensitize PTEN-deficient resistant lung cancer cells to EGFR tyrosine kinase inhibitors by inducing autophagy. *Eur J Pharmacol*. 2018;823:19-26. doi:10.1016/j.ejphar.2018.01.036