Biochemical and Biophysical Research Communications 457 (2015) 614–620

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Cholesterol reduces the sensitivity to platinum-based chemotherapy via upregulating ABCG2 in lung adenocarcinoma

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ARTICLE INFO

Article history:
Received 8 January 2015
Available online 17 January 2015

Keywords:
Cholesterol
Chemosensitivity
Lung adenocarcinoma
ABCG2

ABSTRACT

Inoperable lung adenocarcinoma is currently treated with platinum-based chemotherapy. However, the effectiveness of these chemotherapeutic agents is not the same for all patients. Patients either show quick chemoresistance (QCR) or delayed chemoresistance (DCR), which are defined by 87 and 242 days of progression-free survival (PFS) after initial platinum-based treatment, respectively. We found that QCR patients displayed an elevated level of serum cholesterol and that their tumors showed upregulated ABCG2 expression. We propose that chemoresistance may be attributed to cholesterol-induced ABCG2 expression and hypothesize that blocking ABCG2 may increase the efficacy of platinum-based chemotherapeutic agents. Using the MTT cell viability assay, we observed that cotreatment with ABCG2 blocker Nicardipine and platinum-based drugs Cisplatin, Oxaliplatin or Carboplatin significantly decreased cell viability of tumor cells. Importantly, our results also showed that incubating cells with cholesterol prior to chemotherapy treatment or cotreatment increased cell viability of tumor cells relative to the controls.
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1. Introduction

Lung cancer is the leading cause of cancer morbidity and mortality worldwide, with almost 1.6 million new cases of lung cancer per year (13% of total cancer morbidity) and 1.4 million deaths per year (18% of total cancer mortality) [1]. In case of patients with inoperable lung cancer, platinum-containing regimens have now been clinically validated as effective therapies for advanced lung adenocarcinoma [2]. However, the relatively rapid acquired chemotherapeutic resistance to such therapies significantly limits their effects and remains a substantial obstacle to the clinical management of lung adenocarcinomas [3].

While the majority of lung adenocarcinoma respond to initial chemotherapy [typically with 4–6 cycles of a platinum agent: cisplatin (CP), carboplatin (CB) or Oxaliplatin (OXA)], up to 75% of lung adenocarcinoma patients will relapse within 6 months with chemotherapeutic-resistant disease [4]. But, clinically, a few of lung adenocarcinoma patients with chemoresistance had recurrence of disease less than 3 months after initial treatment (quickly chemoresistance, QCR).

Recently, retrospective epidemiological studies have suggested that elevated serum levels of cholesterol increase the rate of breast cancer among women in developed countries [4–6], and statins, which inhibit the rate-limiting step in cholesterol synthesis [7], decrease overall breast cancer prevalence [8]. Sok M. suggested that preoperative total serum cholesterol levels may be an important prognostic factor for overall survival after lung cancer resection [9]. Furthermore, Kucharska-Newton observed low HDL-cholesterol levels were associated with a higher incidence of lung cancer [10].

Cholesterol levels have been shown to be involved in the regulation of various membrane proteins, including ABCG2 [11]. Membrane cholesterol was also found to modulate ABCG2 activity in a selective and reversible manner [12]. Kenneth et al. suggested that statins may downregulate ABCG2 or ABCB1 expression and function through reducing low-density lipoprotein cholesterol levels [13,14].

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http://dx.doi.org/10.1016/j.bbrc.2015.01.035
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However, there have been no published data yet on the effect of cholesterol on chemosensitivity of lung cancer.

The present study was designed to address this question by firstly examining cholesterol levels of lung adenocarcinoma with QCR and DCR (Delayed chemoresistance) and analyzing whether there is a difference in serum cholesterol levels; secondly, evaluating the correlation between high levels of cholesterol with chemosensitivity to platinum-based drugs *in vitro*; thirdly, investigating different mRNA expression of those genes that are widely reported as the chemoresistance-related genes in tumors and discovering target genes and verifying by protein analyzed; and lastly, examining whether the cholesterol effects are suppressed by target gene inhibitors in comparison with the statins.

2. Methods

2.1. Subjects & study design

24 Patients (age range: 53–76 years) with histologic or cytologic confirmed inoperable non-metastatic (stage IIIA or IIIB according to American Joint Committee on Cancer (AJCC) staging system 6th edition) lung adenocarcinoma and chemoresistance occurred within 3 months after receiving platinum-based chemotherapy were eligible for the QCR group. Likewise, 40 lung adenocarcinoma patients (age range: 57–78 years) with chemoresistance occurred at least 7 months after the therapies were recruited for the DCR group. Patients were excluded if they had 1) a history of hepatic, renal, biliary, cardiovascular, gastrointestinal, hematologic and other chronic and acute diseases within 3 months prior to the study or had other comorbidities known to affect lipid metabolism (for example, patients with diabetes mellitus and thyroid disorders); 2) participated in any clinical drug study within 2 months prior to the study, or if they were taking any drugs or supplements that could influence the activity of drug transporters unless on stable dose for at least 6 weeks prior to the study and throughout study. All patients received initial chemotherapy.

Blood samples were collected from all subjects to examine the lipid profile, and drug transporters in tissues were tested from these subjects. All patients were advised to maintain their usual diet and other aspects of lifestyle during the study. Anthropometric measurements, including bodyweight, body height, waist circumference and percentage of total body fat were obtained at the study visits.

The study protocol was approved by the Joint Clinical Research Ethics Committee of Affiliated Cancer Hospital of Zhengzhou University (Zhengzhou, P.R. China). All subjects provided a written informed consent.

2.2. Chemicals & reagents

Water soluble cholesterol and Nicardipine were obtained from Sigma-Aldrich (MO, USA). Cisplatin, Oxaliplatin, Carboplatin and Pravastain were purchased from Dalian Meilun Biology Technology Co., Ltd. (Dalian, P.R. China). Other reagents are described below.

2.3. Lipid profile measurement

Plasma concentrations of total cholesterol, high-density lipoprotein cholesterol and triglycerides were measured using standard enzymatic methods. The levels of LDL-C were calculated according to the Friedewald formula [15]. All the biochemistry tests were performed in the Central Laboratory of Shanghai Tenth People's Hospital, the Shanghai Key Laboratory (Shanghai, P.R. China).

2.4. Cell culture

The A549 tumor cell line was kindly provided by Dr. Hong Tu (Shanghai Cancer Institute, Shanghai, P. R. China) and was maintained in complete medium consisting of DMEM (Hyclone, Logan, UT) with 10% fetal bovine serum (Invitrogen, Carlsbad, CA), and the medium contained 2 mM glutamine, 100 μg/ml streptomycin and 100 U/ml penicillin in a humidified incubator at 37 °C with 5% CO_2.

To investigate the effect of Cholestrol on the A549 growth, cells were incubated with DMEM containing 180 ng/μL of water soluble cholesterol (Sigma Aldrich, Shanghai, P. R. China) at 37 °C for 48 h before the experiments. Nicardipine (10 μM) and Pravastain (10 μM) were administered in A549 cells for 15 min, and then the drug sensitivity test was implemented as described below.

2.5. Cell sensitivity to anti-cancer drugs treatment

To assess the chemosensitivity of tumor cells, cell viability was measured by the MTT assay (Colorimetric CellTiter^{96} AQueous One Solution Cell Proliferation Assay) (Promega, Beijing, P. R. China). Cell suspension was cultured in 96-well flat-bottomed microtiter plates at a concentration of 1×10^4 cells/per well and incubated overnight. Cisplatin, Carboplatin or Oxaliplatin treatments were carried out as follows: 10^{-8} M, 10^{-7} M, 10^{-6} M, 10^{-5} M, 10^{-4} M, 10^{-3} M. Each drug was tested in triplicate, and A549 tumor cells with only complete medium were used as blank controls. Cells were incubated for 48 h before the addition of MTT solution (1 mg/ml per well), and OD values were read at 490 nm using a spectrophotometric microplate reader (Bio-Rad; CA, USA). The percent cell viability to different drug concentrations was calculated as the inhibition rate of (mean absorbance of treated wells/mean absorbance of control wells) × 100%. IC_{50} was calculated by GraphPad Prism v5.0 (GraphPad Software, Inc; P. R. China).

2.6. Immunohistochemistry

For immunohistochemistry, 2 μm sections were de-paraffinized in xylene, followed with 2 × 100% ethanol, 95%, 75%, 50% and 30% ethanol, 3 × $_{dd}H_2O$, rehydrated for each. Subsequently, sections were placed in 1× retrieval solution (10 mM citrate buffer, pH 6.0) and boiled for 100 °C for 10 min and were then allowed to cool to the room temperature, followed by treatment with 3% H_2O_2 to block endogenous peroxidase. Then the sections were incubated with primary Ab at 4 °C overnight. Primary antibody staining was visualized using the ImPress Universal kit (Vector Laboratories, Burlingame, CA) with NovaRed (Vector Laboratories) as a substrate. The sections were then counterstained with hematoxylin, dehydrated, and mounted. To quantify immunohistochemical staining, ImageJ software (http://rsbweb.nih.gov/ij/) was used to quantify the percentage of tissues that showed immunoreactivity for ABCG2 in microscopically and acquired JPEG images (20× microscope field with 10 random fields per subject).

2.7. Real-time quantitative PCR

Total RNA was extracted and subjected to reverse transcription using FastQuant RT kit (with gDNase) (Tiangen Biotech, Beijing, China). Quantitative real-time PCR was performed with FastStart Universal SYBR Green Master (Roche Diagnostics, Mannheim, Germany) on a StepOne Plus real-time PCR system (Applied Biosystems, Foster City, CA). The relative expressions of target genes were calculated after normalization against a reference gene (GAPDH). The primer sequences used for quantitative PCR are given in Table 1.

Table 1
Sequence of primers for mRNA quantification.

Gene name	Sequence of primers	Reference sequence (amplicon size)
ABCB1	Forward: 5′-CCCATCATTGCAATAGCAGG-3′ Reverse: 5′-GTTCAAACTTCTGCTCCTGA-3′	NM_000927.4 (156 bp)
ABCC1	Forward: 5′-TCTGGTCAGCCCAACTCTCT-3′ Reverse: 5′-ACTAGGGCTACCAGCCAGAA-3′	NM_004996.3 (123 bp)
ABCC2	Forward: 5′-TCTGGTCAGCCCAACTCTCT-3′ Reverse: 5′-ACTAGGGCTACCAGCCAGAA-3′	NM_000392.4 (148 bp)
ABCG2	Forward: 5′-TTTCCAAGCGTTCATTCAAAAA-3′ Reverse: 5′-TACGACTGTGACAATGATCTGAGC-3′	NM_004827.2 (73 bp)
ABCC9	Forward: 5′-ACGTATGCTGGAACTGACGG-3′ Reverse: 5′-GCAGTGAGGACAATGCAAGC-3′	NM_005691.3 (114bp)
PPARa	Forward: 5′-GCCTCCTTCGGCGTTCG-3′ Reverse: 5′-GCTCCAAGCTACTGTGGTGA-3′	NM_001001928.2 (146 bp)
DPYD	Forward: 5′-GGACAGAGTCCAGCTACTGTG-3′ Reverse: 5′-TGCGCTGTTCCAGATAAGGT-3′	NM_000110.3 (109bp)
GST-p1	Forward: 5′-TCCAATACCATCCTGCGTCAC-3′ Reverse: 5′-CGGGCAGTGCCTTCACATA-3′	NM_000852.3 (178 bp)
GSTm1	Forward: 5′-GGACGCCTTCCCAAATCTGA-3′ Reverse: 5′-TACTTGTTGCCCCAGACAGC-3′	NM_000561.3 (132bp)

2.8. Western blot

Cell or tumor lysates were obtained and equal amounts of protein lysates from each sample were diluted with loading buffer, denatured, and separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by protein transfer to polyvinylidene fluoride membranes (PVDF).

After incubation in a blocking solution (5% nonfat milk powder) in the TBST buffer (10 mM Tris–HCl, pH8.0, 150 mM NaCl, and 0.1% Tween20) for 1 h at room temperature, the membranes were immunoblotted overnight with primary monoclonal antibodies against either ABCG2 or actin at 1:1000 dilution at 4 °C, and were then incubated for 2 h at room temperature with secondary antibody (1:1000 dilution). The protein antibody complex was detected by enhanced chemiluminescence detection system. The protein expression was quantified by ImageJ software (http://rsbweb.nih.gov/ij/).

2.9. Statistical analysis

Statistical analyses were performed using the SPSS software version 19.0 (SPSS Inc., Chicago, IL). The significance of difference between groups was assessed by Student's *t* test for single comparisons or by analysis of variance (ANOVA) with Student–Newman–Keuls tests for multiple comparisons. A value of $P < 0.05$ (two-tailed) was considered statistically significant. *, ** and *** indicates statistical significance relative to $P < 0.05$, $P < 0.01$ and $P < 0.001$.

3. Results

3.1. Clinical characteristics of study subjects

The median progression-free-survival (PFS) of QCR group was 87 days, meanwhile DCR group was 242 days (Fig. S1, $P < 0.0001$). The demographic and clinical characteristics of the patients of QCR (n = 24) or DCR (n = 40) are summarized in Table 2. Results revealed that patients of the QCR group had significantly higher baseline levels of total cholesterol ($P < 0.0001$), LDL-C ($P = 0.0006$) and Triglyceridesthan ($P < 0.0001$) than the DCR group. For other characteristics, there were no significant differences between two groups.

3.2. Cholesterol increased chemoresistance of A549 cells

In order to investigate whether cholesterol affect A549 cell proliferation, the water soluble cholesterol was added to the cell culture medium (180 ng/μl). A549 cell proliferation was analyzed by MTT every 12 h. As shown in Supplement Fig. S2, cholesterol had no significant toxicity on cell proliferation.

Before test chemosensitivity, A549 cells per culture in medium with cholesterol for 24 h. As shown in Fig. 1A, cell chemosensitivity was significantly decreased in A549-Cholesterol group after exposure to Cisplatin. As expected, the half maximal inhibitory concentration (IC_{50}) of A549 cells cultured with cholesterol was obviously increased (A549-CHO 51.12 ± 3.37 μmol/L v.s. A549-Ctrl 4.23 ± 1.28 μmol/L, $P < 0.001$, Table 2).

Meanwhile, Chemosensitivity of A549-Cholesterol cells to Oxaliplatin and Carboplatin was less but also significantly decreased. For Oxaliplatin, cholesterol group demonstrated an IC_{50} of 6.75 ± 1.29 μmol/L, whereas IC_{50} for normal medium was 1.19 ± 1.40 μmol/L ($P < 0.01$). For Carboplatin, IC_{50} was 135.12 ± 1.34 μmol/L for cholesterol group and 36.75 ± 1.25 μmol/L for normal medium group ($P < 0.001$).

3.3. ABCG2 mediates the resistance to chemotherapy

In understand the mechanisms, 8 candidate genes were chosen for further validation: *ABCB1, ABCC2, ABCG2, ABCC9, PPARa, DPYD, GST-P1* and *GSTM1*. We examined 8 candidate genes' mRNA expression of lung adenocarcinoma tumors between DCR and QCR

Table 2
General characteristics.

Variables	Quickly chemoresistance (n = 24)	Delayed chemoresistance (n = 40)	P-value
Age, years	66.2 ± 6.3	65.3 ± 7.1	0.5903
Male, n (%)	14 (58.3)	22 (55.0)	0.854
BMI (kg/m^2)	23.2 ± 3.35	23.7 ± 3.27	0.2499
Waist circumference (cm)	78.5 ± 8.6	78.8 ± 7.4	0.4741
Body fat (%)	28.4 ± 12.5	28.6 ± 13.3	0.3997
Cigarette smoking, n (%)	6 (25.0)	10 (25.0)	0.8900
Hypertension, n (%)	2 (8.3)	4 (10.0)	0.8756
Total cholesterol (mmol/l)	6.37 ± 1.02	4.32 ± 0.76	**<0.0001*****
LDL-C (mmol/l)	3.53 ± 1.10	2.28 ± 0.69	**0.0006*****
HDL-C (mmol/l)	1.52 ± 0.35	1.53 ± 0.45	0.3666
Triglycerides (mmol/l)	1.32 ± 0.31	0.51 ± 0.19	**<0.0001*****

Data are presented as mean ± standard deviation.
To convert lipids from mmol/l to mg/dl: for total cholesterol, HDL-C and LDL-C multiply by 38.67; for triglycerides multiply by 88.57. ***P < 0.001.

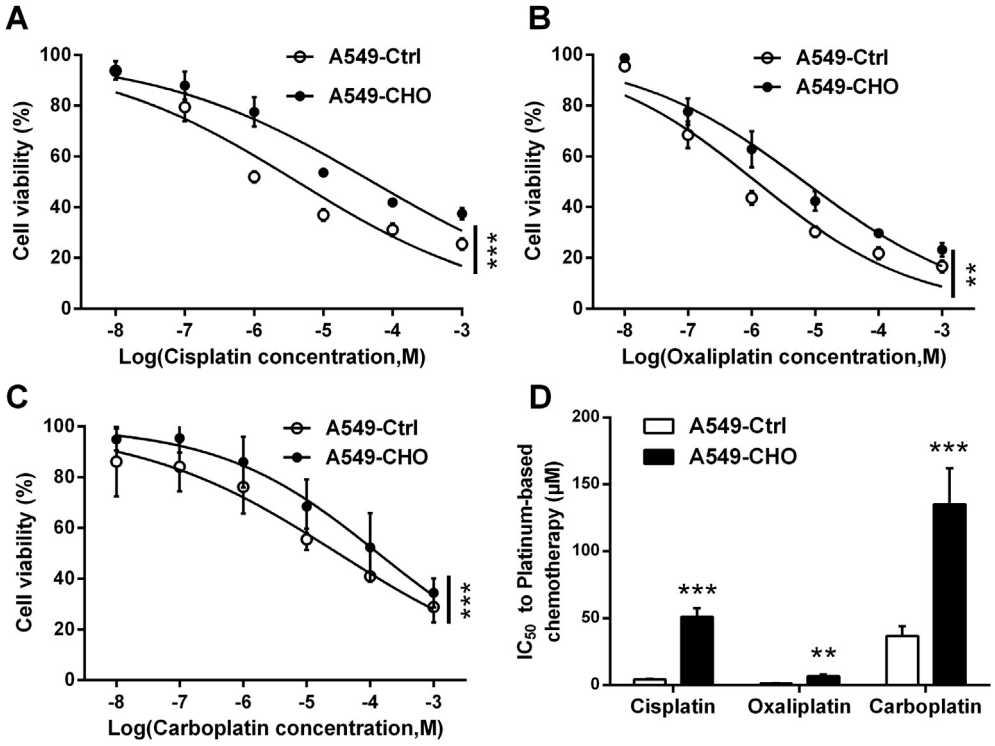

Fig. 1. Cholesterol enhance chemoresistance of A549 cell to platinum-based drugs. Chemosensitivity was significantly *increased* in A549-Cholesterol group after exposure to Cisplatin (A), carboplatin (B) and oxaliplatin (C). IC_{50} of A549 cells cultured with cholesterol were obviously increased than control cells (D). (**P < 0.01, ***P < 0.001).

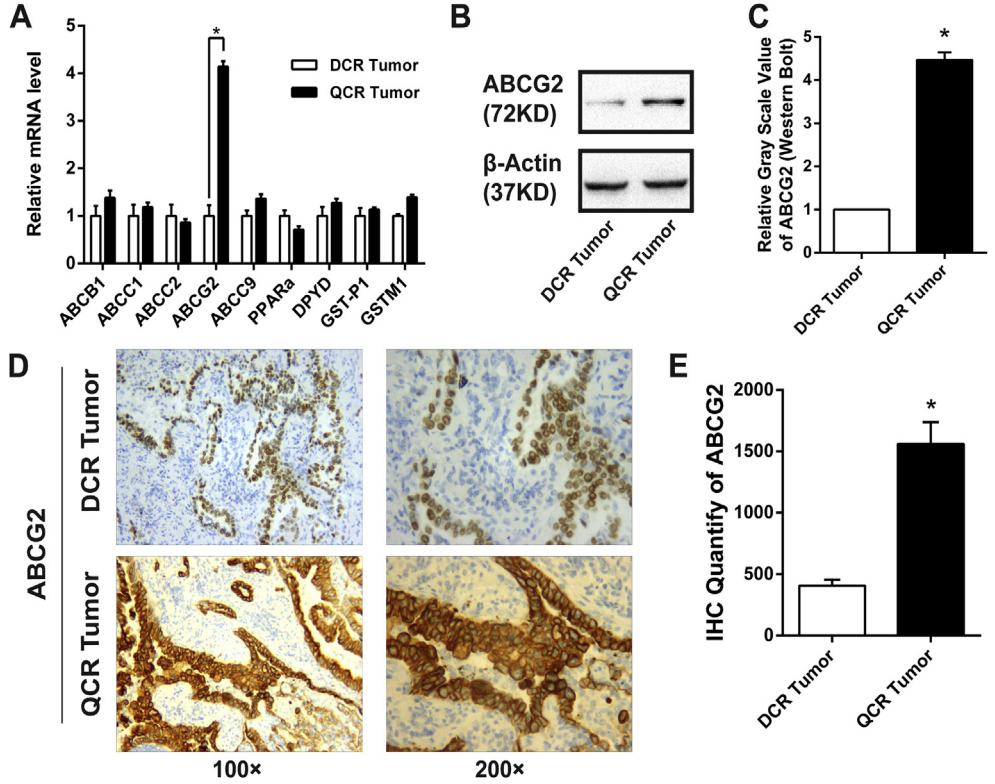

Fig. 2. ABCG2 of QCR tumor was significantly higher than the DCR group. The mRNA expression of ABCG2 in QCR tumor was significantly higher than the DCR group (A); ABCG2 expression of tumor shown by Western blot (B); Western blot relative quantify of ABCG2 expression in QCR tumor was significantly higher than DCR tumor (C); Immunohistochemical analysis of ABCG2 expression in QCR tumor was significantly higher than DCR group (D); % positive area for ABCG2 in lung cancer of QCR versus DCR group is shown in (E). DCR: delayed chemoresistance; QCR: quickly chemoresistance.

groups. As showed in Fig. 2A, patients with QCR had significantly higher levels of ABCG2 mRNA expression than those with DCR (QCR 4.141 ± 0.342 v.s. DCR 1.000 ± 0.282, $P < 0.01$). Meanwhile, other candidate mRNA had no significant differences between the two groups ($P > 0.05$).

Then we tested the ABCG2 protein in tumor tissues. Western blot analysis revealed that tumors from the QCR group showed a marked increase of ABCG2 expression (Fig. 2B). Grayscale analysis shows, ABCG2 of QCR was 4.32 fold higher than that of DCR (Fig. 2C). We next explored the expression of ABCG2 using immunohistochemistry. The results were in agreement with the western blot data (Fig. 2D), as seen the ImageJ analysis showing strong expression of ABCG2 in tumors from the QCR group.

3.4. Nicardipine reverse chemoresistance caused by cholesterol

To further investigate the mechanism how cholesterol induced chemoresistance, Nicardipine was used as an ABCG2 blocker. First, we examined the effect of Nicardipine on cell proliferation (Supplement Fig. S3A). Lower concentration (10 μM) was chosen and had no significant effect on the proliferation of A549 cells (Supplement Fig. S3B).

Based on ABCG2 protein expression shown in Fig. 3, cholesterol led to an increased intracellular expression (3.423 fold, $P < 0.001$), but Nicardipine significantly downregulated ABCG2 in A549 cells cultured with cholesterol ($P < 0.001$). Interestingly, for non-high-cholesterol cultured cells, Nicardipine did not significantly reduce the expression of ABCG2.

It was important to confirm these results by chemosensitivity test, and for comparison, Pravastain was used as positive control. The MTT assay was implemented, and the observed significantly lower curve of A549-Cholesterol + Nicardipine indicated that cholesterol induced chemoresistance to Cisplatin was significantly inhibited, but not to the extent of Pravastain (Fig. 3C). This phenomenon was also demonstrated, when we use Carboplatin (Fig. 3D) and Oxaliplain (Fig. 3E).

Meanwhile, as the results shown in Fig. 3F and Table 3, IC_{50} of the Cholesterol group for Cisplatin was 42.39 ± 2.32 μM, but Cholesterol + Nicardipine was 4.67 ± 1.19 μM ($P < 0.001$) and 3.05 ± 1.79 μM after adding Pravastain. For Carboplatin, IC_{50} of the Cholesterol group was 139.07 ± 5.25 μM, but Cholesterol + Nicardipine was 46.26 ± 2.34 μM ($P < 0.05$) and 37.71 ± 6.34 μM after adding Pravastain; for Oxaliplatin, the cholesterol group demonstrated an IC_{50} of 8.28 ± 1.13 μM but

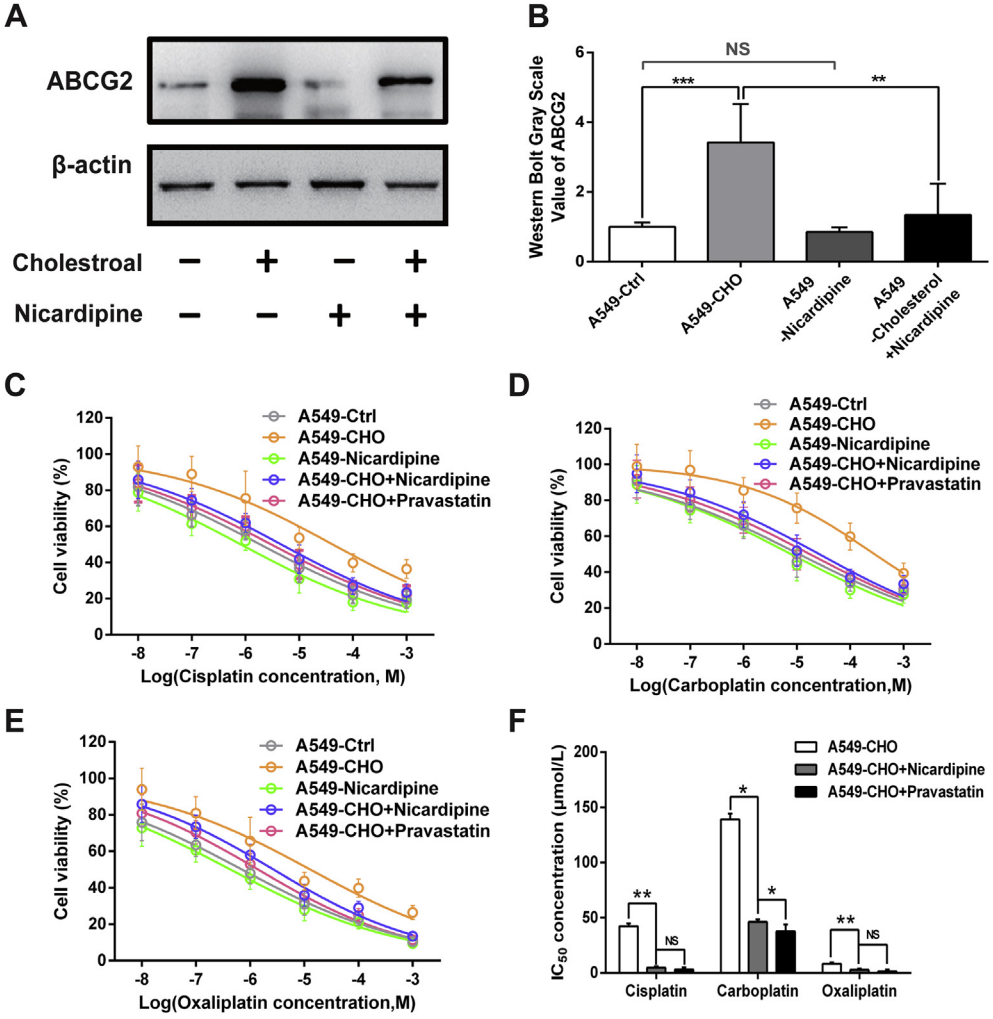

Fig. 3. Nicardipine as ABCG2 blocker downregulate chemoresistance of A549. Nicardipine downregulate ABCG2 protein expression in A549 cultured with cholesterol ($P < 0.01$), but does not significantly decreased ABCG2 of control cells (A. B.). Cholesterol induced chemoresistance was significantly reversed by Nicardipine, the significantly lower curve showed A549 sensitivity to Cisplatin significantly increased (C). This phenomenon is also found when we use Carboplatin (D) and Oxaliplain (E). IC_{50} of Cholesterol group for platinum-based chemotherapy is significantly inhibited (F).

Table 3
IC50 of A549 cultured in high cholesterol.

	IC_{50} (μmol/L)		
	A549-Ctrl	A549-CHO	P-value
Cisplatin	4.23 ± 1.28	51.12 ± 3.37	**<0.001*****
Carboplatin	36.75 ± 1.25	135.12 ± 1.34	**<0.001*****
Oxaliplatin	1.19 ± 1.40	6.75 ± 1.29	**<0.01****

2.80 ± 1.16 μM ($P < 0.01$) after adding Nicardipine and 1.40 ± 1.56 μM after adding Pravastain (Table 4).

4. Discussion

The levels of serum cholesterol increased rapidly along with the improvement of living standards. More than half of elderly persons have elevated levels of blood cholesterol and triglyceride, which was not a serious problem for cancer in the past. But recently, several studies showed that cholesterol itself is a risk factor for cancer [16], and patients taking statins were found having a lower breast cancer incidence and decreased recurrence [17]. Moreover, cholesterol can activate substrate-stimulated ABCG2 ATPase activity and direct vesicular substrate transport [13]. On the other hand, because cholesterol accumulation contributes to a wide range of pathologies, cholesterol has emerged as a novel factor regulating cell death pathways and tumor growth and chemoresistance of HCC [18], but there has no report on lung adenocarcinomas. Due to the poor efficacy of current lung adenocarcinoma treatments, understanding the role of cholesterol in lung adenocarcinoma may open up novel avenues for future therapy.

Our results showed that cholesterol levels were correlated with duration of chemoresistance of patients. For example, A549 cells cultured with higher cholesterol had a higher half maximal inhibitory concentration of Cisplatin, Carboplatin and Oxaliplain. This result is consistent with the findings from epidemiological studies of breast cancer [4,6]. Our study suggests that there is an association between dyslipidaemia and lung adenocarcinoma.

ABCG2 transporter protein is one of the most important ABC ubiquitously expressed in different tissues and contributes to the disposition of a wide variety of endogenous substances including platinum-based regimens [19,20] and cholesterol [21]. Recent studies reported an association of ABCG2 expression with the prognosis and survival of cancer patients [22–24]. Moreover, a significant association between increased cholesterol levels and ABCG2 mRNA expression was observed [25]. To the best of our knowledge, there has been no reported study that has examined the role of ABCG2 mRNA expression induced by cholesterol *in vitro* in lung adenocarcinoma treatment.

Some controversial study found that cells exposed to cytotoxic agents increased cellular cholesterol content at the first step and that preventing this increase might sensitize these cells to chemotherapy [26]. To further illustrate this issue, we added the water soluble cholesterol to the cell culture medium in our experiments. Our results showed that cell chemosensitivity was significantly increased in A549-Chol group after exposure to Cisplatin, Carboplatin and Oxaliplain. Unfortunately we did not measure cholesterol levels in the drug-resistant cell lines.

Table 4
IC_{50} of Nicardipine and Pravastatin blocked ABCG2 with cholesterol.

	IC_{50} (μM)		
	A549-CHO	A549-CHO + Nicardipine	A549-CHO + Pravastatin
Cisplatin	42.39 ± 2.32	4.67 ± 1.19**	3.05 ± 1.79
Carboplatin	139.07 ± 5.25	46.26 ± 2.34*	37.71 ± 6.34$^{\triangle}$
Oxaliplatin	8.28 ± 1.13	2.80 ± 1.16**	1.40 ± 1.56

*, $P < 0.05$; **, $P < 0.01$, compared with A549-CHO group.
$^{\triangle}$, $P < 0.05$, compared with A549-CHO + Nicardipine group.

Our experiments suggest that cholesterol increases the expression of ABCG2 in A549 cells, which may be the reason of its enhanced resistance to chemotherapy. This finding will improve patient survival after chemotherapy and help physicians to preferably select more appropriate methods of treatment for patients with unresectable lung adenocarcinoma.

Higher levels of ABCG2 expression driven by cholesterol *in vitro* might be inhibited by Nicardipine [27,28]. In our experiments, Nicardipine was selected to be the next phase of the experiments as a strong inhibitor of ABCG2. As expected, western blot results showed that ABCG2 expression levels was significantly down-regulated by Nicardipine in A549 cells cultured in high-cholesterol, compared with that of normal cells. Interestingly, Nicardipine did not significantly reduce the expression of ABCG2 in cells cultured with non-high-cholesterol. When using Pravastatin as the positive control, we found that the beneficial effect of Pravastain was very strong. Our study showed Nicardipine alone cannot achieve the reverse rate of Pravastatin, considering that Pravastatin inhibits the intracellular levels of cholesterol and ABCG2 [14,29], and so there may be other pathways involved. Our study might provide a helpful method to reverse the potential of acquisition of MDR driven by cholesterol, but Nicardipine may have no significant effect in patients with non-high-cholesterol.

Competing interests

The authors have no conflicts of interests.

Acknowledgments

This work was supported by grants from the National Science Foundation of China (nos. 81272600 to Qiming Wang), the China International Medical Foundation (CIMF-F-H001-057), the Scientific Research Project of Henan Provincial Bureau of Traditional Chinese Medicine (L213236).

Transparency document

The transparency document associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bbrc.2015.01.035.

Appendix ASupplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.01.035.

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