Oncology and Translational Medicine
DOI 10.1007/s10330-018-0267-7

April 2018, Vol. 4, No. 2, P48–P53

ORIGINAL ARTICLE

Establishment and characterization of an oxaliplatin-resistant hepatic cancer cell line*

Ping Qiu[1], Gang Chen[2], Yuhong Dai[3], Hong Qiu[3] (✉)

[1] Department of Oncology, Jingzhou Central Hospital, Jingzhou 434020, China
[2] Integration Traditional Chinese Medicine and Western Medicine Department, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China
[3] Department of Oncology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

Abstract

Objective The aim of the current study was to establish an oxaliplatin-resistant hepatoma cell line (HepG2/OXA) and investigate the potential mechanisms of its drug resistance.

Methods The hepatoma cell subline, HepG2/OXA, resistant to oxaliplatin (OXA), was established from a parent cell line HepG2, by stepwise exposure to gradually increasing concentrations of OXA over a half-year period. Chemosenstivity of the cytotoxic drugs, OXA, cisplatin (CDDP), adriamycin (ADM), and 5-fuorouracil (5-FU), was determined in HepG2 and HepG2/OXA cells, by the Cell counting kit-8 (CCK8) assay. Cell cycle distribution of HepG2 and HepG2/OXA cells was analyzed by Flow cytometry (FCM). The expression levels of several drug resistance-related proteins, such as P-glycoprotein (P-gp), multidrug resistant protein 1 (MRP1), and excision repair-cross complementing 1 (ERCC1) protein in the two cell lines were tested by the western blot assay.

Results The IC50 of OXA in HepG2/OXA and HepG2 were 136.84 μmol/L and 23.86 μmol/L, respectively. The resistance index (RI) was 5.34. HepG2 was also demonstrated to be cross-resistant to other anti-tumor agents, such as 5-FU, ADM, and CDDP. The percentage of HepG2/OXA cells in the S phase was significantly decreased compared to HepG2 cells (25.58% ± 2.36% vs 14.37% ± 2.54%, $P < 0.05$), while the percentage of cells in the G0/G1 and G2/M phases showed no statistical difference (respectively 55.29% ± 4.98% vs 56.73% ± 4.56%, $P > 0.05$, and 24.63% ± 4.81% vs 28.26% ± 3.82%, $P > 0.05$). The ERCC1 was found to be over expressed in HepG2/OXA cells, while there was no difference in the expressions of P-gp and MRP1 between the multiple drug resistance (MDR) phenotype cell line and its parental cell line.

Conclusion HepG2/OXA showed an MDR ability; the over expression of ERCC1 might be associated with the platinum resistance of the cells, but P-gp and MRP1 are not.

Received: 20 March 2018
Revised: 1 April 2018
Accepted: 13 April 2018

Key words: hepatocellular carcinoma (HCC); multidrug resistance (MDR); excision repair-cross complementing 1 (ERCC1); oxaliplatin

In China, hepatocellular carcinoma (HCC) is the fourth most common malignancy in men and the eighth in women, and the fourth most common cause of cancer-related mortality in men and women [1]. Chronic HBV infection is thought to be the most important risk factor for the pathogenesis of HCC, especially in China. Unfortunately, the majority of the patients are surgically unresectable at the time of diagnosis, and the risk of recurrence for those postoperative cases is considerably high. As a result, even though the diagnosis and comprehensive treatments are improved, the prognosis of HCC remains extremely poor [2].

Due to the lack of powerful research data from evidence-based medicine, the effect of chemotherapy on the overall survival remains to be controversial. However, chemotherapy is still an important strategy for most

✉ Correspondence to: Hong Qiu. Email: tjqiuhong@163.com
* Supported by grants from the National Natural Sciences Foundation of China (No. 81001067), Ministry of Science and Technology International Cooperation Project (No. S2010GR0991) and Astrazeneca Special Research Foundation for Targeted Therapy of Wu Jieping Medical Foundation (No. 320.6700.09068)

unresectable and recurrent cases. Platinum drugs, such as cisplatin (DDP) and oxaliplatin (OXA) have been used as the common chemotherapeutic agents in the treatments for HCC. The international multi-center randomized phase III study (EACH) confirmed the therapeutic value of FOLFOX4 (OXA, CF plus 5-FU) for advanced HCC, and it was the first time that a combined regimen of oxaliplatin was proved to have survival benefits for patients with advanced HCC [3]. However, the effect of oxaliplatin is limited due to the intrinsic or acquired drug resistance of HCC cells [4–5]. Additionally, the potential mechanism of OXA resistance remains unclear.

OXA resistance is often accompanied by cross-resistance to other chemotherapy agents, called multidrug resistance (MDR), which is thought to be the critical reason for the failure of chemotherapy treatment [6]. One of the possible mechanisms of MDR is the drug efflux pumps which lead to the decrease of the intracellular concentration of the chemotherapeutic agents. The drug pumps mainly include ATP-binding cassette (ABC) transporters, such as P-gp, MRP1, and lung resistant-related protein (LRP). However, since the cytotoxicity of platinum is mediated by DNA damage, nucleotide excision repair (NER) plays a critical role in the occurrence of platinum-related resistance [7–8]. ERCC1 over-expression enhances the rapid DNA damage repair ability and is an important factor for platinum resistance [9].

In the current study, we established an OXA-resistant cell line, HepG2/OXA, from its parental cell line HepG2, analyzed its biological characteristics, and explored its potential molecular mechanism of MDR *in vitro*.

Materials and methods

Compounds

Chemotherapeutic drugs, oxaliplatin (OXA; St. Louis, MO, USA), 5-fluorouracil (5-FU; Jiangsu Hen Rui Medicine Co. Ltd, Lianyungang, China), cisplatin (CDDP; Jiangsu Hen Rui Medicine Co. Ltd, Lianyungang, China), doxorubicin (ADM; Jiangsu Hen Rui Medicine Co. Ltd, Lianyungang, China) were used in this study. All the agents were used according to the protocol provided by the manufacturers.

Cell lines and culture conditions

Two human hepatoma cell lines (HepG2 and SMMC-7721) were used in the present study. HepG2 and SMMC-7721 cells were high-malignancy hepatocarcinoma cell lines established from surgically excised specimens of Chinese hepatocellular carcinoma patients, and derived from the China Center for Type Culture Collection. HepG2 and SMMC-7721 cells were cultured in Dulbecco's modified Eagle's medium/high glucose (DMEM/H, Invitrogen Carlsbad, CA, USA) supplemented with 10% (v/v) fetal bovine serum (Invitrogen Carlsbad, CA, USA), 200 IU/mL penicillin (ICN Biomedical, Costa Mesa, CA, USA), 100 mg/mL streptomycin (ICN Biomedical), and 0.5 mmol/L sodium pyruvate (Cambrex, Walkersville, MD, USA). The cells were cultured at 37 °C in a humidified atmosphere, under conditions of 5% CO_2.

Establishment of OXA-resistant subline *in vitro*

The parental cell lines HepG2 and SMMC-7721 were exposed to medium containing OXA at an initial concentration of 25 μM for 24 h. The dead cells were removed with 0.01 mol/L phosphate-buffered saline (PBS), and the remaining cells were cultured in OXA-free culture medium. Once the growth of treated cells reached 70%–80% confluence, the above protocol was repeated. After half a year, a resistant sub-clone of HepG2/OXA was obtained, which could grow exponentially in the medium containing 10 μM OXA. The proliferation of the parental cell line SMMC-7721 was poor; the OXA-resistant sub-line could not be achieved even when the culturing time was prolonged to one year.

Proliferation assay after co-exposure to chemotherapeutic drugs

The proliferation rate of cells under the conditions of exposure to the chemotherapeutic drugs was determined by the CCK-8 assay (Dojindo Molecular Technology, Dojindo, Japan), according to the manufacturer's instructions. We compared the proliferation rates at different concentrations of the drugs to clarify the characteristics of MDR in HepG2/OXA cells. Before the assay, HepG2/OXA cells were maintained in OXA-free medium and cultured for three generations. Cancer cells were seeded into two 96-well plates at a concentration of 8000 cells/well with 100 μL of medium and cultured for 12 h. Then, experiment groups (EG) were cultured in 100 μL of fresh medium containing various concentrations of the anticancer drugs for 24 h, while the control group (CG) was cultured in 100 μL of medium free of anticancer drugs. The concentrations of OXA were 3.125, 6.25, 12.5, 25, 50, and 100 μM; those of CDDP were 2.5, 5, 10, 20, 40, and 80 μM; those of 5-FU were 12.5, 25, 50, 100, 200, and 400 μg/mL; and those of ADM were 1, 2, 4, 8, 16, and 32 μM. The medium was discarded after 24 h, and cells were incubated for 2 h in 110 μL of DMEM/H solution with CCK-8 (10 μL of CCK-8 and 100 μL of DMEM/H). Blank groups (BG) were made in wells without cells. Optical density (OD) was measured for each well at a wavelength of 450 nm. Inhibitory ratio (IR) in different concentrations was calculated by the following formula: $1 - (OD_{EG} - OD_{BG}) / (OD_{CG} - OD_{BG})$. According to the IRs in different concentrations of the anticancer drugs, the 50% inhibitory dose (IC50) was measured by SPSS 19.0 (SPSS Inc, Chicago, IL, USA). Resistant indexes ($RI = IC50_{HepG2/}$

Table 1 Determination of IC50 and resistance index (RI) according to the inhibition ratio of different concentrations of OXA of HepG2/OXA cells and HepG2 cells (mean ± SD; n = 5)

	Inhibition ratio of different concentrations of OXA (μmol/L)						IC50	RI
	3.125 μM	6.25 μM	12.5 μM	25 μM	50 μM	100 μM		
HepG2/OXA	10.18 ± 1.81	12.49 ± 2.68	13.01 ± 2.07	13.92 ± 3.43	26.36 ± 6.27	63.32 ± 7.26	136.84 ± 8.46	5.74
HepG2	12.89 ± 1.76*	16.59 ± 2.97*	21.14 ± 2.29*	28.13 ± 4.15**	51.31 ± 6.89**	96.68 ± 8.24**	23.86 ± 3.78	

* P < 0.05, ** P < 0.01

$_{OXA}$ / $IC50_{HepG2}$) of each tested drug were calculated. Five replicate wells were used for each drug concentration and the testing was carried out independently for three times.

Cell cycle analysis

The HepG2/OXA cells and the parental HepG2 cells were harvested after digestion by trypsinogen (Invitrogen, Carlsbad, CA, USA) without ethylene diamine tetraacetic acid (Invitrogen, Carlsbad, CA, USA), washed two times with ice-cold PBS (pH 7.2), and immobilized using dehydrated alcohol (–20 °C) and PBS (3:1) at 4 °C for 12 h. The immobilized cells were then washed twice with ice-cold PBS and incubated in the presence of 25 mg/mL RNase (Invitrogen, Carlsbad, CA, USA) at 37 °C for 30 min, stained with 50 mg/mL PI (Invitrogen, Carlsbad, CA, USA), and then placed on ice for 30 min in the dark. Cell cycle phase distribution was analyzed using a flow cytometer (Becton Dickinson, Biosciences, San Jose, CA, USA). Data from 10 000 cells were collected and analyzed using the Modofit software program (Becton Dickinson, Biosciences, San Jose, CA, USA).

Expression of P-gp, MRP1, ERCC1 by western blotting

Total protein was collected from the cultured HepG2/OXA cells and the parental HepG2 cells. The protein concentration was measured by a BCA Protein Assay Kit (Beyotime Institute of Biotechnology, Jiangsu, China). Before electrophoresis, the protein was denatured in lithium dodecyl sulfate (LDS) sample buffer (106 mmol/L Tris-HCl, 141 mmol/L Tris base; pH 8.5, 0.51 mmol/L EDTA, 10% glycerol, 2% LDS, 0.22 mmol/L SERVA blue G250, 0.175 mmol/L, phenol red, 0.1 mmol/L 2-mercaptoethanol) for 10 min at 95 °C. Total protein (20 μg per lane) was electrophoresed on an 8% SDS-PAGE gel and transferred onto a 0.45-μm nitrocellulose filter membrane (NC) (Roche, Indianapolis, IN, USA). Membranes were blocked with 5% (w/v) nonfat dry milk in PBST (phosphate-buffered saline containing 0.05% Tween 20) for 2 h at room temperature and incubated overnight at 4 °C with antibodies against P-gp (1:200), or MRP1 (1:200), or ERCC1 (1:100) (Santa Cruz, CA, USA). The membranes were then incubated with Dylight™ 800-Labeled antibodies (Gaithersburg Biotechnology, MD, USA) for 1 h after being washed four times with PBST for 5 min. In the end, the immunoblot signals were scanned and analyzed by the Odyssey Infrared Imaging System (Li-Cor Biosciences Nebraska USA).

Table 2 Results of IC50 and resistance index (RI) of different anticancer drugs of HepG2/OXA cells and HepG2 cells (mean ± SD)

Drugs	IC50 (n = 5)		RIs
	HepG2 (μmol/L)	HepG2/OXA (μmol/L)	
OXA	23.86 ± 3.78	136.84 ± 8.46**	5.74
DDP	7.56 ± 1.71	12.33 ± 2.29*	1.63
5-FU	109.84 ± 6.84	154.01 ± 8.93*	1.40
ADM	4.45 ± 1.12	8.67 ± 1.97*	1.94

* P < 0.05, ** P < 0.01

Statistical analysis

All digital results were displayed as mean ± SD. The quantitative ratios of different groups were compared by Student's t-test with SPSS 13.0. Probability values of P < 0.05 were regarded as statistically significant. All statistical tests were two-sided. All the experiments were repeated at least three times.

Results

Establishment of the OXA-resistant cell line HepG2/OXA and determination of its MDR

The HepG2/OXA cell line was established by pulsed exposure to a high concentration of OXA. The cytotoxicity assay (CCK8) showed that HepG2/OXA cells were resistant not only to OXA, but also to other anticancer drugs. The IC50 of HepG2/OXA for OXA was (136.84 ± 8.46) μmol/L, which was 5.74 times that of the parental cells HepG2 [(23.86 ± 3.78) μmol/L]. In addition, the RIs of HepG2/OXA cells were 1.63, 1.40, and 1.93 for DDP, 5-FU, and ADM, respectively (Tables 1 and 2).

Cell cycle distribution of HepG2/OXA and HepG2

The cell cycle distribution in the HepG2/OXA cell line exhibited a significantly decreased percentage of cells in the S phase (25.58% ± 2.36% vs 14.37% ± 2.54%, P < 0.05) in comparison with the HepG2 cells, while the percentage of cells in the G0/G1 phase (55.29% ± 4.98% vs 56.73% ± 4.56%, P > 0.05) and G2/M phase (24.63% ± 4.81% vs 28.26% ± 3.82%, P > 0.05) showed no statistical differences (Fig. 1).

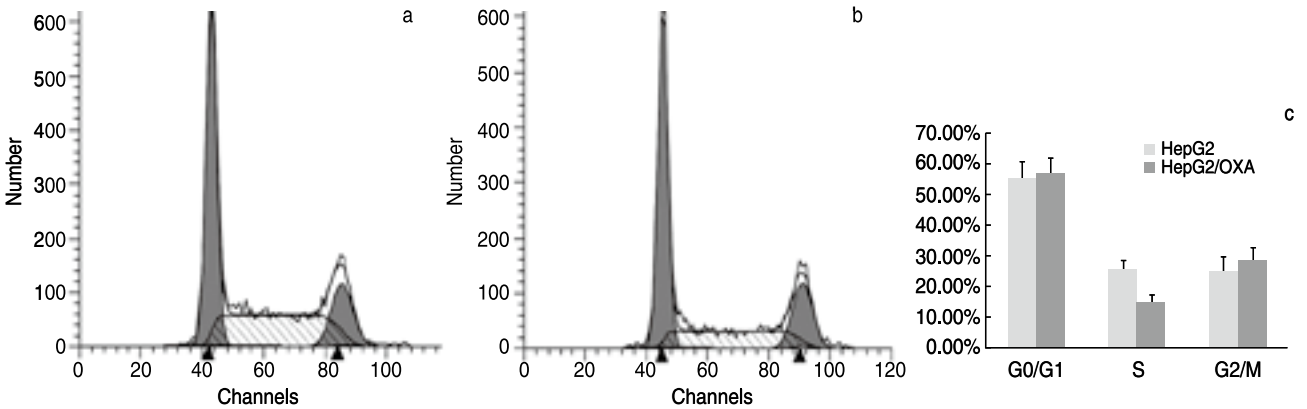

Fig. 1 (a) Cell cycle distribution of HepG2 cells; (b) Cell cycle distribution of HepG2/OXA cells; (c) The percentage of G0/G1, S, G2/M cells of HepG2 and HepG2/OXA

The distribution of HepG2/OXA cells in the S phase exhibited a significant decrease, in comparison with HepG2 cells, while the percentages of the cells in the G0/G1 and G2/M phases showed no statistical differences.

ERCC1, P-gp, and MRP1 protein expression in HepG2/OXA and HepG2 cells

P-glycoprotein (P-gp), multidrug-resistant protein 1 (MRP1), and ERCC1 protein expression in HepG2/OXA cells compared with that in the parental cells was determined by western blotting. ERCC1 was overexpressed in HepG2/OXA cells in comparison with HepG2 cells. The ratio of ERCC1 and β-actin grey intensity was 0.099 ± 0.014 and 0.396 ± 0.040, respectively, in the HepG2 and HepG2/OXA cells; the difference was statistically significant ($P < 0.0001$). However, the protein expression levels of P-gp (0.788 ± 0.085 vs 0.740 ± 0.063, $P = 0.367$) and MRP1 (0.374 ± 0.060 vs 0.350 ± 0.073, $P = 0.468$) between the MDR phenotype cells and the parental cells showed no significant difference. Data is shown in Table 3 and Fig. 2.

ERCC1 was over-expressed in the HepG2/OXA cells. The ratio difference of ERCC1 and β-actin grey intensity in HepG2/OXA and HepG2 was significant ($P < 0.0001$). However, there was no significant difference between the P-gp and MRP1 protein expression levels in the MDR phenotype cells and the parental cells.

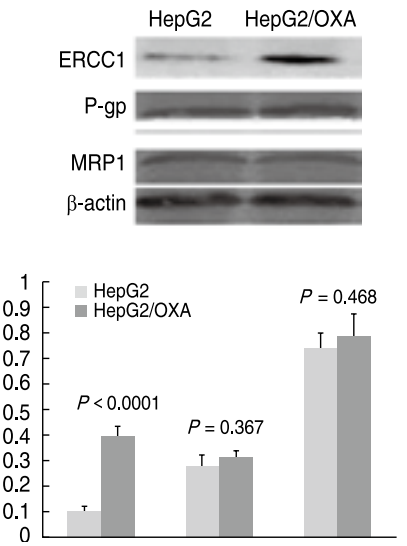

Fig. 2 ERCC1, P-gp, and MRP1 protein expression levels in HepG2/OXA and HepG2 cells were assayed by western blot

Table 3 ERCC1, P-gp, and MRP1 protein expression levels in HepG2/OXA and HepG2 cells (mean ± SD, $n = 5$)

Cell lines	ERCC1	P-gp	MRP1
HepG2	0.099 ± 0.013	0.740 ± 0.063	0.350 ± 0.073
HepG2/OXA	0.396 ± 0.040**	0.788 ± 0.085	0.374 ± 0.060

** $P < 0.01$

Discussion

The effect of chemotherapy on the overall survival of most HCC patients has been constantly controversial. However, a randomized phase III trial (EACH) presented in the 2010 ASCO meeting indicated that FOLFOX4 significantly improved the median progression-free survival from 1.77 to 2.93 months ($P = 0.0002$), and overall survival from 4.90 to 6.47 months ($P = 0.00425$). Response rates (complete and partial responses), as calculated by RECIST (Response Evaluation Criteria in Solid Tumors) were 8.7% for FOLFOX4 and 2.7% for doxorubicin ($P = 0.0142$). Disease control rates (complete and partial responses and stable disease) were 53% for FOLFOX4 vs 32% for doxorubicin ($P < 0.0001$). It is the first survival advantage report for HCC for any chemoregimen [10]. Drug resistance is the main reason for the failure of chemotherapy in the treatment of malignant tumors [11]. Undoubtedly, reducing or reversing the drug resistance

may improve the benefits of chemotherapy and overall survival. As OXA is one of the most useful drugs for the treatment of malignant tumors including HCC, which has a different mechanism of drug resistance than CDDP [12], it is necessary to elucidate the mechanism of OXA resistance.

Once malignant tumor cells gain resistance against one drug, they may be resistant to other chemotherapy drugs as well [13]. In this study, an OXA-resistant hepatoma cell line (HepG2/OXA) was established by discontinuously exposing the cell line to high concentrations of OXA for half a year. The results of the CCK8 assay showed that the IC50 of the HepG2/OXA cells was 5.34 times more than that of the HepG2 cells. The OXA-resistant cells also developed cross-resistance to other anti-tumor agents like 5-FU, ADM, and CDDP. The growth rate of HepG2/OXA cells was lower than that of the parental cells. This result is similar to that of a study on a CDDP-resistant hepatoma cell line SK-Hep-1/CDDP, which was established by Zhou *et al* [14]. As far as the cell cycle distribution of the HepG2/OXA cell line was concerned, there was a difference between HepG2/OXA and SK-Hep-1/CDDP cell lines. There was a significantly decreased percentage of HepG2/OXA cells in the S phase, when compared to the case for HepG2 cells. Conversely, the proportion of SK-Hep-1/CDDP cells in both the G2/M and S phases increased significantly, when compared with the parental cells. The reason for this dissimilarity might be the difference between the drugs and cell lines.

The relative mechanism of the phenomenon of multidrug resistance is considered to be related with drug efflux pumps mediated by ATP-binding cassette (ABC) transporters such as P-gp, MRP1, and lung resistant-related protein (LRP) [15]. Inhibiting MDR transporter function can reverse the MDR phenotype and improve the sensitivity of chemotherapy [16–17]. In case of HCC, some MDR cell lines like HepG2/ADM [18] and SMMC7721/ADM [19] overexpressed the ABC protein, compared to their parental cell lines. Nevertheless, in our experiment, neither P-gp nor MRP1 expression was significantly increased in HepG2/OXA cells, when compared to the case for HepG2 cells. However, ERCC1, one of the rate-limiting enzymes in NER, was observed to be overexpressed in the MDR phenotype cells. This demonstrated that there was another mechanism for OXA resistance in HepG2/OXA cells, besides the decrease of drug concentration in the cytoplasm due to the overexpression of ABC [20]. It is well-known that more than 85% of HCC cases are accompanied with cirrhosis [21]. Increased DNA repair activity in cirrhosis with inflammatory activity may reflect increased DNA damage as a consequence of chronic liver injury [22]. Meanwhile, ERCC1, which is involved in the early steps of the NER process, is associated with liver fibrogenesis and cancer, and it could be related to the well-recognized resistance of HCC to chemotherapeutics [23]. In addition, increased ERCC1 expression is associated with CDDP resistance in HCC specimens and cell lines. Immunohistochemical analysis for resected HCC tissues may be a useful predictor for the effectiveness of adjuvant chemotherapy using CDDP [24–25]. Therefore, ERCC1 overexpression plays an extremely important role in HCC chemotherapeutic drug resistance, especially for platinum resistance.

In summary, although a chemotherapeutic regimen including OXA was proved to have survival benefits for HCC, the need to establish drug resistance models, especially for platinum resistance, is urgent, so as to elucidate its mechanism and surmount drug resistance. An OXA-resistant human hepatoma cell line, HepG2/OXA, which was established by discontinuously exposing the parental cell line, HepG2, to a high dose of OXA, provided a stable cell model for the further study of platinum resistant mechanisms and the reversal of clinical HCC drug resistance.

Conflicts of interest

The authors indicated no potential conflicts of interest.

References

1. Chen W, Zheng R, Baade PD, *et al*. Cancer statistics in China, 2015. CA Cancer J Clin, 2016, 66: 115–132.
2. Nault JC, Galle PR, Marquardt JU. The role of molecular enrichment on future therapies in hepatocellular carcinoma. J Hepatol, 2018, Mar 2. doi: 10.1016/j.jhep.2018.02.016 [Epub ahead of print].
3. Qin S, Bai Y, Lim HY, *et al*. Randomized, multicenter, open-label study of oxaliplatin plus fluorouracil/leucovorin versus doxorubicin as palliative chemotherapy in patients with advanced hepatocellular carcinoma from Asia. J Clin Oncol, 2013, 31: 3501–3508.
4. Wakamatsu T, Nakahashi Y, Hachimine D, *et al*. The combination of glycyrrhizin and lamivudine can reverse the cisplatin resistance in hepatocellular carcinoma cells through inhibition of multidrug resistance-associated proteins. Int J Oncol, 2007, 31: 1465–1472.
5. Folmer Y, Schneider M, Blum HE, *et al*. Reversal of drug resistance of hepatocellular carcinoma cells by adenoviral delivery of anti-ABCC2 antisense constructs. Cancer Gene Ther, 2007, 14: 875–884.
6. Mohammad IS , He W, Yin L. Understanding of human ATP binding cassette superfamily and novel multidrug resistance modulators to overcome MDR. Biomed Pharmacother, 2018, 100: 335–348.
7. Dip R, Camenisch U, Naegeli H. Mechanisms of DNA damage recognition and strand discrimination in human nucleotide excision repair. DNA Repair (Amst), 2004, 3: 1409–1423.
8. Park CJ, Choi BS. The protein shuffle. Sequential interactions among components of the human nucleotide excision repair pathway. FEBS J, 2006, 273: 1600–1608.
9. Altaha R, Liang X, Yu JJ, *et al*. Excision repair cross complementing-group 1: gene expression and platinum resistance. Int J Mol Med, 2004, 14: 959–970.
10. Qin S, Bai Y, Ye S, *et al*. Phase III study of oxaliplatin plus 5-fluorouracil/leucovorin (FOLFOX4) versus doxorubicin as palliative systemic chemotherapy in advanced HCC in Asian patients. J Clin

Oncol, 2010, 28: 7s (suppl; abstr 4008).
11. Lønning PE. Molecular basis for therapy resistance. Mol Oncol, 2010, 4: 284–300.
12. Tozawa K, Oshima T, Kobayashi T, *et al*. Oxaliplatin in treatment of the cisplatin-resistant MKN45 cell line of gastric cancer. Anticancer Res, 2008, 28: 2087–2092.
13. Baguley BC. Multiple drug resistance mechanisms in cancer. Mol Biotechnol, 2010, 46: 308–316.
14. Zhou Y, Ling XL, Li SW, *et al*. Establishment of a human hepatoma multidrug resistant cell line *in vitro*. World J Gastroenterol, 2010, 16: 2291–2297.
15. Elliott A, Adams J, Al-Hajj M. The ABCs of cancer stem cell drug resistance. IDrugs, 2010, 13: 632–635.
16. Angelini A, Iezzi M, Di Febbo C, *et al*. Reversal of P-glycoprotein-mediated multidrug resistance in human sarcoma MES-SA/Dx-5 cells by nonsteroidal anti-inflammatory drugs. Oncol Rep, 2008, 20: 731–735.
17. Yan F, Jiang Y, Li YM, *et al*. Reversal of P-glycoprotein and multidrug resistance-associated protein 1 mediated multidrug resistance in cancer cells by HZ08 Isomers, tetrataisohydroquinolin derivatives. Biol Pharm Bull, 2008, 31: 1258–1264.
18. Yan F, Wang XM, Pan C, *et al*. Down-regulation of extracellular signal-regulated kinase 1/2 activity in P-glycoprotein-mediated multidrug resistant hepatocellular carcinoma cells. World J Gastroenterol, 2009, 15: 1443–1451.
19. Yan F, Wang XM, Liu ZC, *et al*. JNK1, JNK2, and JNK3 are involved in P-glycoprotein-mediated multidrug resistance of hepatocellular carcinoma cells. Hepatobiliary Pancreat Dis Int, 2010, 9: 287–295.
20. Martin LP, Hamilton TC, Schilder RJ. Platinum resistance: the role of DNA repair pathways. Clin Cancer Res, 2008, 14: 1291–1295.
21. Sanyal AJ, Yoon SK, Lencioni R. The etiology of hepatocellular carcinoma and consequences for treatment. Oncologist, 2010, 15 Suppl 4: 14–22.
22. Zindy P, Andrieux L, Bonnier D, *et al*. Upregulation of DNA repair genes in active cirrhosis associated with hepatocellular carcinoma. FEBS Lett, 2005, 579: 95–99.
23. Fautrel A, Andrieux L, Musso O, *et al*. Overexpression of the two nucleotide excision repair genes ERCC1 and XPC in human hepatocellular carcinoma. J Hepatol, 2005, 43: 288–293.
24. Ueda S, Shirabe K, Morita K, *et al*. Evaluation of ERCC1 expression for cisplatin sensitivity in human hepatocellular carcinoma. Ann Surg Oncol, 2011, 18: 1204–1211.
25. Liu XR, Liang DH, Jiang T, *et al*. Low-dose radiation enhances susceptibility to cisplatin in resistant ovarian cancer cells via downregulation of ERCC1 and Bcl-2. Oncol Transl Med, 2016, 2: 84–89.

DOI 10.1007/s10330-018-0267-7
Cite this article as: Qiu P, Chen G, Dai YH, *et al*. Establishment and characterization of an oxaliplatin-resistant hepatic cancer cell line. Oncol Transl Med, 2018, 4: 48–53.