Establishment and gene analysis of an oxaliplatin-resistant colon cancer cell line THC8307/L-OHP

Hua Tang, Yuan-Jun Liu, Min Liu and Xin Li

Oxaliplatin is widely used for chemotherapy of several malignancies, especially of colon cancer. As the mechanism of resistance to oxaliplatin is unclear, we established an oxaliplatin-resistant cell line, THC8307/L-OHP, from an oxaliplatin-sensitive colonic cancer cell line, THC8307. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium assay indicated that THC8307/L-OHP has 30.99-fold greater resistance to oxaliplatin than THC8307. Analyzing its gene expression profile using an in-house oligomicroarray, a number of genes were differentially expressed in the THC8307/L-OHP cells, compared with parental cells (THC8307). Proapoptotic genes such as *STK17A* and *BNIP3* were significantly downregulated, whereas the genes *PSAP* and *GDIA1*, which were involved in antiapoptosis, were overexpressed. Moreover, the THC8307/L-OHP cells are also resistant to the other anticancer drug 5-fluorouracil, and the expression levels of the differentially regulated genes such as *S100P*, *CA*η, *STA15*, *TCF8* are constantly maintained. These results provide clues for understanding the oxaliplatin-resistant mechanisms and imply markers to predict drug sensitivities for 'personalized chemotherapy'. *Anti-Cancer Drugs* 18:633–639 © 2007 Lippincott Williams & Wilkins.

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Keywords: colon cancer, drug resistance, gene expression profile, microarray, oxaliplatin

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Introduction

Previous studies have revealed the correlations between gene expression and drug activity, as well as identification of genes differentially expressed in drug-sensitive and drug-resistant cancer cells, such as LRP, MDR1, MRP and p53 [1–4]. Elucidating the molecular mechanisms of this complex process may lead to the identification of novel molecules, which in turn may serve as targets for therapeutics, diagnostic tests or alternatively predictive markers. Therefore, understanding the basis of drug resistance is a principal goal of molecular oncology.

Oxaliplatin [L-OHP, oxalato(*trans*-l-1,2-diaminocyclohexane) platinum (II)], as a member of the family of platinum (Pt)-containing chemotherapeutic agents, is distinguished from two older drugs cisplatin and carboplatin by its different spectrum of activity both in preclinical models [5] and in clinical trials. L-OHP has shown activity alone or in combination with 5-fluorouracil (5-FU)/leucovorin in colon cancer, a disease that was previously considered to be unresponsive to Pt drugs [6]. Nevertheless, the majority of patients with colon cancer are either intrinsically resistant to this drug or become resistant during therapy. Pt resistance is multifactorial, and includes mechanisms that limit the formation of Pt–DNA adducts and mechanisms that decrease drug uptake, increase drug inactivation and increase repair of Pt–DNA adducts [7–9].

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In this study, the goal was to characterize transcriptional changes associated with L-OHP resistance. Specifically, the L-OHP-sensitive colon cancer cell line THC8307 was exposed to incrementally increasing concentrations of L-OHP. This procedure resulted in the establishment of L-OHP- resistant cell line THC8307/L-OHP. The individual expression profiles of the parental cell line and its resistant cell line were determined using the oligomicroarray technique, which had made it possible to examine the mRNA levels for very large numbers of genes simultaneously. Transcriptional changes associated with acquired L-OHP resistance in colon cancer were identified.

Methods

Cell lines and cell culture

The colon carcinoma cell line (THC8307) was from the Institute of Hematology (CAMS & PUMC, Tianjin, PRC). An L-OHP-resistant colonic cancer cell line (THC8307/L-OHP) created from colon carcinoma cell line (THC8307) is described below. Both the cell lines were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (Institute of Hematology, CAMS & PUMC), 20 mmol/l *N*-2-hydroxyl piperazine-*N*′-2-ehane sulfonic acid (Gibco, Gland Island, New York, USA) and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin) (Invitrogen, Carlsbad, California, USA) in

a humidified incubator at 37°C in an atmosphere of 5% CO_2 until 80–90% confluence was achieved.

Drugs and selection of drug-resistant cells

For establishment of the drug-resistant cell line, the colon carcinoma cell line (THC8307) as parent cells was initially exposed to the drug L-OHP at concentration 0.108 μg/ml. The drug concentration was increased after 2 weeks of continuous drug exposure (0.144, 0.2, 0.3, 0.480 and 0.6 μg/ml). Fresh drug was added by gradually increasing to the final concentration of 6.000 μg/ml. Stable drug-resistant cell line was selected and cultured in the presence of the final drug concentration for 4 months. The establishment period of the drug-resistant cell line was 9 months. Before analyzing the expression profile of genes by microarray experiments, the resistant cancer cell line was maintained for 1 week without drug to eliminate acute effects.

Cytotoxicity assay

Sensitivities (IC_{50}) of the drug-resistant and parent cell lines to 5-FU and L-OHP were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay. Briefly, single-cell suspension was obtained by trypsinization of monolayer cultures. The number of cells (5×10^4) plated into 96 wells was determined by reference. An equal number of cells was inoculated into each well in RPMI 1640 supplemented with 10% fetal bovine serum. For each drug, five or four concentrations were used on the next day, covering a 5- or 4-log concentration range that was chosen to span the 50% inhibitory concentration determined by preliminary assays. After 2 days of culture, 20 μl of MTT (Sigma, St. Louis, Missouri, USA) at concentration 5 mg/ml was added to each well and was incubated at 37°C for an additional 4 h. The medium was discarded from plates in each well after a 5-min centrifugation. Care was taken not to disturb the formazan crystals at the bottom of the wells. One hundred microliters of dimethyl sulfoxide (Sigma) was added to each well and the plates were placed on a shaker for 15 min to solubilize the formazan crystals. The plates were then read immediately at 570 nm on a μQuant Universal Microplate Spectrophotometer (Bio-tek Instruments, Winooski, Vermont, USA). All data points represent the mean value of a minimum of three replicates.

Cell growth curve and doubling time

Single-cell suspension was obtained by trypsinization of monolayer cultures. An equal number of cells (1×10^4) was inoculated into each well of 24 wells in RPMI 1640 supplemented with 10% fetal bovine serum. Both cell lines were cultured in a humidified incubator at 37°C in an atmosphere of 5% CO_2. Every day, the cells of three wells were counted from the first day to the seventh day. Cell doubling time was calculated by the Patterson formula:

$$T_D 280280 = T \times \log 2 / (\log N_t - \log N_0)$$

where N_0 is the corresponding number of cells at time zero, N_t is the cell number at the seventh day. A cell growth curve was drawn, with the number of cells as ordinate and cultivation time as abscissa.

RNA preparation and GeneChip (microarray) hybridization

Total RNA was extracted using the Trizol reagent (Molecular Research, Cincinnati, Ohio, USA) according to the manufacturer's instructions. Genes expressed in drug-resistant and parental cell lines were analyzed on a high-density oligonucleotide microarray, containing 7267 transcripts. Target preparation and microarray processing procedures were performed as described by the TSA Labeling and Detection Kit (Perkin-Elmer, Boston, Massachusetts, USA). Briefly, 10 μg of total RNA was used to synthesize single-strand cDNA with Avian myeloblastosis virus reverse transcriptase. In the reverse transcription, fluorescein is incorporated in the drug-resistant cancer cell line and biotin is in the parent colon cancer cell. Then the purified cDNA was hybridized with our lab oligonucleotide microarray, which was washed and coupled with anti-FL–horseradish peroxidase conjugate and streptavidin–horseradish peroxidase conjugate, respectively, and stained with cyanine 3 tyramide and cyanine 5 tyramide, respectively. Scanning was performed with a ScanArray Express (Perkin-Elmer).

Microarray data analysis

All the genes represented on the GeneChip were subtracted from background and globally normalized. Fold changes were calculated by comparing transcripts between parental and acquired drug-resistant cell line. The equation of the ratio is as follows:

$$\text{Ratio} = \frac{\text{CH2 median} - \text{CH2 background median}}{\text{CH1 median} - \text{CH1 background median}}.$$

An eight-fold or greater change in intensity was used as the criterion.

Semiquantitative reverse transcription-polymerase chain reaction

We selected seven genes for semiquantitative reverse transcription–polymerase chain reaction (RT-PCR) for validation of the microarray data. We used the primers designed by Soft Primer Premier 5.0 (Palo Alto, California, USA). The expression of seven genes (*S100P*, serine/threonine kinase 17α (*STK17A*), *CA*η, *STA15*, *TCF8*, *ARHGDIA* and *AAMP*) was verified by semiquantitative RT-PCR. The primer sets for PCR amplification were as in Table 1.

For reverse transcription, 5 μg of total RNA was used for creation of single-stranded cDNA using reverse transcriptase M-MLV (Promega, Madison, Wisconsin, USA) in 20 μl. PCR was carried out with 1 μl of the RT products as follows: initial denaturation at 94°C for 5 min followed

by 35 cycles of 94°C for 1 min, 50–65°C for 1 min, 72°C for 1 min, followed by a final elongation at 72°C for 8 min. PCR products were visualized on a 1.5% agarose gel.

Results

THC8307/L-OHP was oxaliplatin resistant

When the drug concentration was increased to 2.4 μg/ml, the majority of cells died, the surving cells were maintained in RPMI 1640 supplemented with 20% fetal bovine serum, without L-OHP. Fifteen days later, the colonies were observed as in Fig. 1, and the colonies were propagated with the higher drug concentration. After 9 months of continuous culture in L-OHP, the L-OHP-resistant cell line was generated. The resistant phenotype was found to be stable after 4 months of continuous culture a in medium containing the final concentration of 6 μg/ml L-OHP. No obvious changes of morphology in the cells were observed using an optical microscope. MTT cytotoxicity experiments demonstrated that THC8307/L-OHP was 30.99-fold resistant to L-OHP as compared with its sensitive parental cell line and 6.69-fold cross-resistant to 5-FU (Fig. 2).

Table 1 Primer sequences for reverse transcription-polymerase chain reaction

Gene	Primer sequence (5–3)	Product (bp)	Annealing (°C)
β-actin			
Sense	CGTGACATTAAGGAGAAGCTG	500	54
Antisense	CTAGAAGCATTTGCGGTGGAC		
S100P			
Sense	AATCTAGCACCATGACGGA	376	54
Antisense	GGGAATAATTGCCAACAAAC		
STAK17A			
Sense	TTTGTGGAGTTAGGTGGAA	590	65
Antisense	TGAGTGTGGTGTGATGAGG		
*CA*η			
Sense	TCTGTGCTGCCTTCTCA	448	61.7
Antisense	TGCTGTCTCGCTTGGA		
AAMP			
Sense	GGAGACCACAAAGCGAAAG	368	65
Anti-sense	AAAGAACGGGAACCTCAAAC		
GDIA1			
Sense	TCGCTGTCGGTCCCGTCTAA	547	63.9
Antisense	GCACTTGGTCCCTTGTTTGTTC		
STA15			
Sense	AATGCCCTGTCTTACTGTC	387	54
Antisense	CTGGCTGGGATTATGCT		
TCF8			
Sense	TGCCAATAAGCAAACG	468	50
Antisense	CAGTAGGAGTAGCGATG		

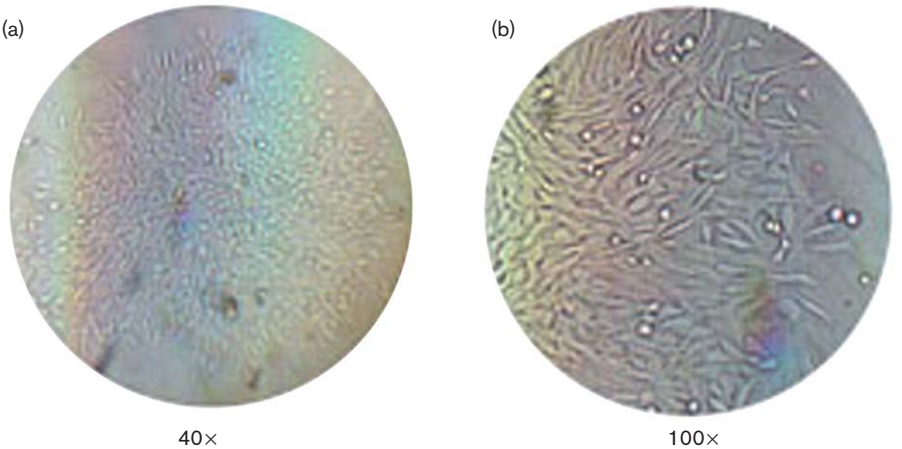
The growth of THC8307/L-OHP cell was suppressed

Compared with the parental THC8307, the growth of THC8307/L-OHP cells was suppressed and the cell doubling time was prolonged (Fig. 3). The cell doubling times of THC8307/L-OHP and THC8307 were 34.17 and 27.67 h, respectively.

Differentially expressed genes in oxaliplatin-resistant colon cancer cell line

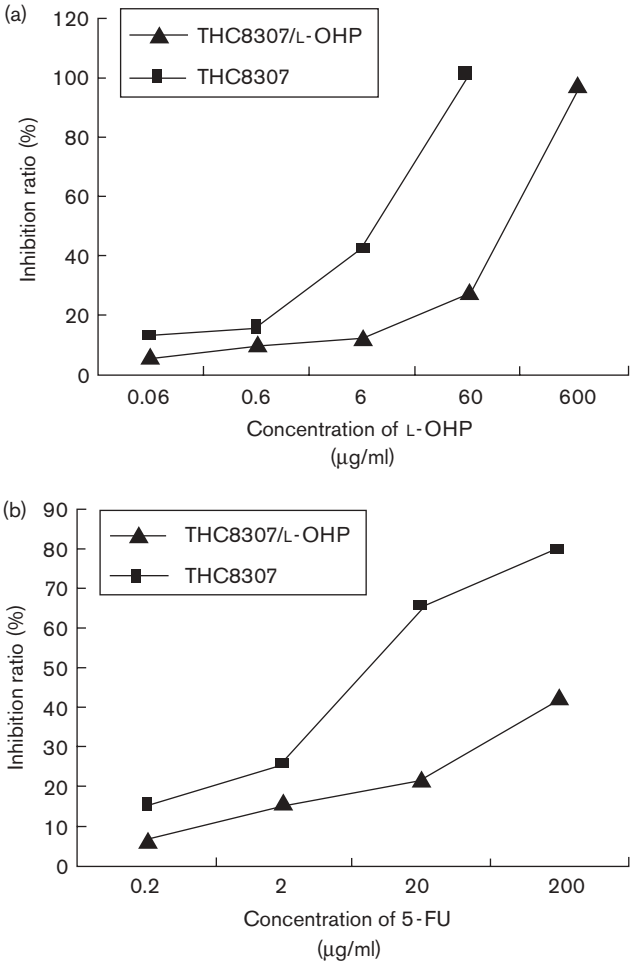
In the L-OHP-resistant colon cancer cell line, we identified 62 differentially expressed genes (Tables 2 and 3), most of which are involved in cell proliferation, metabolic pathways, cell growth and apoptosis. In addition, a subset of differentially expressed genes was associated with cell–cell/matrix interactions (*SDC1*, *PKD1*). In particular, we observed upregulation of DNA repair genes [damage-specific DNA binding protein 1 (*DDB1*) and replication protein A1 (*RPA1*)] whose overexpression was consistent with the Pt–DNA adduct formation from L-OHP. Moreover, the decreased expression of *STK17A* (apoptosis-inducing) and *BNIP3* (BCL2/adenovirus E1B 19-kDa interacting protein 3) was consistent with the action of drug-resistant cells. The signal transducers RAP1B (a member of the RAS

Fig. 1



Oxaliplatin (L-OHP)-resistant cell clone during inducing. After the majority of cells were killed with 2.4 μg/ml L-OHP, the colonies were formed from the few surviving cells, 15 days later.

Fig. 2



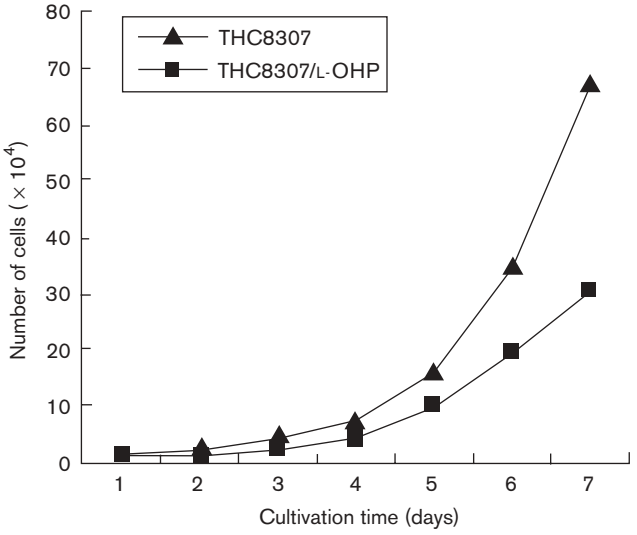
Oxaliplatin (L-OHP) resistance of THC8307/L-OHP versus THC8307. Data are the means of three replicates at each concentration. (a) The IC_{50} values for the THC8307/L-OHP and THC8307 cells to L-OHP are 172.92 and 5.58 μg/ml, respectively. (b) Those to 5-fluorouracil (5-FU) are 215.60 and 32.2 μg/ml, respectively.

oncogene family) and RGS4 (regulator of G-protein signalling 4) were downregulated in the L-OHP-resistant cell line, as was calcium ion-binding molecule (S100P).

Validation of microarray results

To verify the expression of the genes identified in microarray experiments, semiquantitative RT-PCR was performed using the same RNA as that used in the microarray analysis. We tested seven genes (*S100P*, *STK17A*, *CA*η, *STA15*, *TCF8*, *ARHGDIA* and *AAMP*) for semiquantitative RT-PCR, which may be more relative to L-OHP resistance in colon cancer among the 62 differentially expressed genes after we read some references about the functions of every gene. We found that the results (Fig. 4) were in good agreement with those from the microarray data, in which the observed differences were significant.

Fig. 3



Growth curves of THC8307/L-OHP and THC8307. The growth of THC8307/L-OHP cells was suppressed, the doubling time of THC8307/L-OHP was 6.5 h longer than that of THC8307.

Discussion

At present, several studies on the drug sensitivity and drug resistance in either untreated human cancer cell lines or drug-exposed cells have been performed using microarray technologies [10–14]. They have revealed the correlations between gene expression and drug activity, as well as identification of genes differentially expressed in drug-sensitive and drug-resistant cancer cells. Also, several microarray studies on the identification of genes with L-OHP resistance in human ovarian cancer have been performed [15,16]. Although the majority of patients with colon cancer have become resistant to L-OHP during therapy, few microarray studies have sought to identify candidate genes associated with this drug resistance in colon cancer. Here, we established an L-OHP-resistant colon cancer cell line from colon cancer cell line THC8307 by repeated exposure to this drug. The resistant cell line has acquired L-OHP resistance and a little cross-resistance to 5-FU, over the long-term period of 9 months, by increasing drug dosage, and we confirmed stable resistance by repeated drug-sensitivity assays (MTT assay).

In our study, cells were maintained without drugs before microarray experiments to avoid making note of acute transcriptional changes caused by the insult of the drug itself. Thus, this precaution may not be compatible to detect dynamic changes in response to the drug itself, like acquired transcriptional activation [14]. As a result, of 7267 genes analyzed, a total of 62 genes (> 8-fold) were differentially expressed in L-OHP-resistant colon cancer cell line. A few references exist about resistant

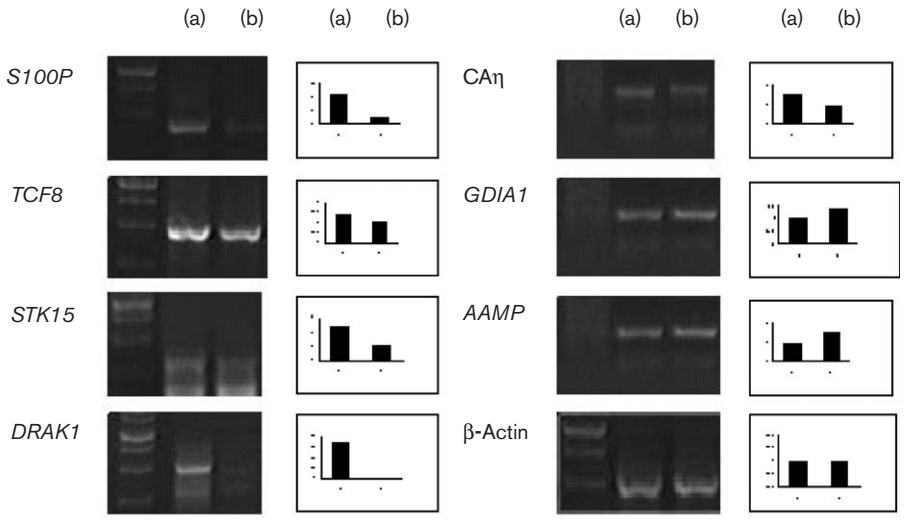
Table 2 Genes upregulated eight-fold or greater in THC8307/L-OHP cells compared with THC8307 cells

GenBank accession	Gene symbol	Description	Ratio
NM_007065	CDC37	CDC37 cell division cycle 37 homolog (*Saccharomyces cerevisiae*)	10.85
NM_002778	PSAP	Prosaposin (variant Gaucher disease and variant metachromatic leukodystrophy)	32.74
AK027274		*Homo sapiens* cDNA FLJ14368 fis, clone HEMBA1001122	30.63
NM_014516	CNOT3	CCR4-NOT transcription complex, subunit 3	10.55
BC010273	PAICS	Phosphoribosylaminoimidazole carboxylase, phosphoribosylaminoimidazole succinocarboxamide synthetase	13.32
NM_004309	ARHGDIA	Rho GDP dissociation inhibitor (GDI) α	8.59
NM_002707	PPM1G	Protein phosphatase 1G (formerly 2C), magnesium-dependent, γ isoform	10.01
NM_002997	SDC1	Syndecan 1	40.78
NM_001087	AAMP	Angio-associated, migratory cell protein	29.07
NM_002945	RPA1	Replication protein A1 (70 kDa)	11.32
NM_001326	CSTF3	Cleavage stimulation factor, 3′ pre-RNA, subunit 3, 77 kDa	9.09
NM_006185	NUMA1	Nuclear mitotic apparatus protein 1	16.53
NM_001923	DDB1	Damage-specific DNA-binding protein 1 (127 kDa)	28.46
NM_005027	PIK3R2	Phosphoinositide-3-kinase, regulatory subunit, polypeptide 2 (p85β)	11.89
NM_023028	FGFR2	Fibroblast growth factor receptor 2 (bacteria-expressed kinase, keratinocyte growth factor receptor)	8.96
NM_003128	SPTBN1	Spectrin, β, nonerythrocytic 1	10.35
NM_006035	CDC42BPB	CDC42-binding protein kinase β (DMPK-like)	21.67
NM_004393	DAG1	Dystroglycan 1 (dystrophin-associated glycoprotein 1)	10.36
NM_005654		Nuclear receptor subfamily 2, group F, member 1	771.3
U49262		Human dishevelled (DVL) mRNA complete cds	10.44

Table 3 Genes down-regulated eight-fold or greater in THC8307/L-OHP cells compared to THC8307 cells

GeneBank accession	Gene symbol	Description	Ratio
NM_005475	LNK	Lymphocyte adaptor protein	0.10
NM_000296	PKD1	Polycystic kidney disease 1 (autosomal dominant)	0.07
NM_015646	RAP1B	RAP1B, member of RAS oncogene family	0.07
NM_000126	ETFA	Electron-transfer-flavoprotein, α polypeptide (glutaric aciduria II)	0.07
NM_004494	HDGF	Hepatoma-derived growth factor (high-mobility group protein 1-like)	0.08
NM_002486	NCBP1	Nuclear cap binding protein subunit 1, 80 kDa	0.08
NM_003244	TGIF	TGFB-induced factor (TALE family homeobox)	0.06
NM_002627	PFKP	Phosphofructokinase, platelet	0.08
NM_002497	NEK2	NIMA (never in mitosis gene a)-related kinase 2	0.10
BC000737	RGS4	Regulator of G-protein signalling 4	0.06
NM_030751	TCF8	Transcription factor 8 (represses interleukin 2 expression)	0.08
NM_003600	STK15	Serine/threonine kinase 15	0.11
NM_000609	SDF1	Stromal cell-derived factor 1	0.07
NM_006947	SRP72	Signal recognition particle 72 kDa	0.08
NM_001449	FHL1	Four and a half LIM domains 1	0.09
NM_002577	PAK2	P21 (CDKN1A)-activated kinase 2	0.12
NM_000714	BZRP	Benzodiazapine receptor (peripheral)	0.12
AF113008	RPS20	Ribosomal protein S20	0.01
NM_004760	STK17A	Serine/threonine kinase 17a (apoptosis-inducing)	0.12
NM_004052	BNIP3	BCL2/adenovirus E1B 19 kDa interacting protein 3	0.11
NM_007066	PKIG	Protein kinase (cAMP-dependent, catalytic) inhibitor γ	0.09
NM_003000	SDHB	Succinate dehydrogenase complex, subunit B, iron sulfur (Ip)	0.09
NM_004199	P4HA2	Procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), α polypeptide II	0.03
NM_004563	PCK2	Phosphoenolpyruvate carboxykinase 2 (mitochondrial)	0.06
NM_000917	P4HA1	Procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), α polypeptide I	0.09
NM_005980	S100P	S100 calcium binding protein P	0.01
NM_022358		Potassium channel, subfamily K, member 15 (TASK-5)	0.05
AF104032		Solute carrier family 7 (cationic amino-acid transporter, y+ system), member 5	0.09
NM_012258		Hairy/enhancer-of-split related with YRPW motif 1	0.08
V00530		Human mRNA encoding the c-myc oncogene	0.10
AF282269	GPRK7	*Homo sapiens* G protein-coupled receptor kinase 7 mRNA, complete cds	0.12
NM_002695	POLR2E	Polymerase (RNA) II (DNA directed) polypeptide E (25 kDa)	0.12
NM_014554	SENP1	Sentrin/SUMO-specific protease	0.03
BC010269	PFKFB4	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4	0.05
NM_006931	SLC2A3	Solute carrier family 2 (facilitated glucose transporter), member 3	0.07
M31159	IGFBP3	Human growth hormone-dependent insulin-like growth factor-binding protein mRNA complete cds	0.02
NM_000022	ADA	Adenosine deaminase	0.09
NM_001673	ASNS	Asparagine synthetase	0.04
AB019568		Homo sapiens mRNA expressed only in placental villi, clone SMAP83	0.10
NM_003155	STC1	Stanniocalcin 1	0.06
NM_001216	CA9	Carbonic anhydrase IX	0.10
NM_005165	ALDOC	Aldolase C, fructose-bisphosphate	0.12

Fig. 4



Semiquantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis of mRNA expression. Total RNA was extracted from THC8307 and THC8307/L-OHP cell lines and analyzed by RT-PCR using the primers as–Table 1. The differential expression of selected genes was confirmed by RT-PCR. β-Actin was amplified as an internal control. (a) THC8307 and (b) THC8307/L-OHP.

colon cancer cell lines, but we found only one reference on L-OHP-resistant colon cancer cell line. In this document, the parent cell line is HT-29 and 14 genes were detected [14]; therefore, we did not find any common genes relative to L-OHP-resistant colon cancer.

First, nucleotide excision repair is the only known mechanism by which bulky adducts, including those generated by Pt chemotherapeutic agents, are removed from DNA in human cells. Several previous studies have demonstrated that increased repair of Pt–DNA adducts is one of the L-OHP resistance mechanisms. The gene *DDB1* (127 kDa) encodes the large subunit of DNA damage-binding protein which functions in nucletide excision repair [17] and the other gene *RPA1* (70 kDa) [18] is one of the subunits of replication protein A, which is essential for multiple processes in DNA metabolism, including DNA replication, recombination and DNA repair pathways. In this experiment, genes *DDB1* and *RPA1* are upregulated in the resistant cell line, which makes cell nucletide excision repair increase and augments L-OHP–DNA damage tolerance.

Second, cell apoptosis deregulation is an important molecular event in the acquisition of drug resistance. It was previously shown that the cell apoptosis gene *STK17A* has an interesting expression pattern in microarray experiments after exposure to etoposide in MeWo cells [10]. Thus, this result suggests that cell apoptosis genes might significantly contribute to etoposide resistance. In our microarray result, the downregulation of *STK17A* was observed in L-OHP-resistant cells. *STK17A* (apoptosis-inducing), also termed death-associated protein kinase-related 1, has a stimulatory effect on apoptosis [19] and locates to chromosome 7p. A positive correlation between STK17A and L-OHP or etoposide resistance in human cancer cell lines was suggested, implying that the lower expression of *STK17A* might be associated with drug resistance. Besides, the gene *BNIP3* (BCL2/adenovirus E1B 19 kDa interacting protein 3), by which the dimeric mitochondrial protein encoded is known to induce apoptosis, even in the presence of BCL2, is lost, too. Moreover, Erkan *et al.* [20] reported that downregulation of *BNIP3* resulted in increased resistance to 5-FU and gemcitabine. Instead, as antiapoptosis factors, the genes Prosaposin (variant Gaucher disease and variant metachromatic leukodystrophy) (*PSAP*) [21] and rho-GDP dissociation inhibitor α (*GDIA1*) are upregulated in our resistant cell line. Thus, the apoptosis induced by *STK17A* and *BNIP3* is decreased, and the antiapoptosis from the genes *PSAP* and *GDIA1* is increased in the resistant cell line; this suggests not only that apoptotic cell reduction is one of the reasons that the cell is resistant to L-OHP but also that the change in apoptotic mechanisms might be responsible for resistance in these cells resulting in decreased sensitivity owing to the structurally unrelated drug 5-FU.

In this study, another important gene is *S100P*. A positive correlation was observed between *S100P* expression and increased sensitivity to L-OHP. The parental cancer cell line contains significantly higher levels of *S100P* mRNA

(100-fold) as compared with its drug-resistant counterpart; the relatively high alteration obtained was due, in part, to tyramine signal amplification in the label, but in RT-PCR experiments the differential expression was obvious, too.

The S100 proteins comprise a multigene family of low-molecular-weight proteins (9–12 kDa). There are at least 21 members of the S100 family and most map closely together on chromosome 1q21, with the notable exception of S100P, which is located on 4p16 [22]. S100 proteins have been reported to serve a number of important functions, many of which are isoform-specific. S100 proteins have recently become of major interest owing to their differential expression in a variety of tumors [23,24] and their putative involvement in the metastatic process [25]. Among the 21 S100 proteins, the S100P isoform was the most highly differentially expressed member of the S100 protein family in malignant tumors compared with their benign counterparts. S100P is a small 95-amino-acid protein first isolated from human placenta. S100P is also expressed in some noncancerous diseases, including inflammatory bowel disease, alopecia areata and psoriasis [22]. Besides, it has metastasis-inducing properties in pancreatic cancer and rat mammary 37-cell line [26,27]. In this study, we observed that S100P is responsible for L-OHP resistance, and in Hio Chung Kang's study [14], they found that S100P was downregulated in 5-FU-resistant gastric cancer cell lines. Thus, we presume this gene may be a marker gene in the drug-resistant cell lines.

In conclusion, using DNA microarray technology, we have identified a set of genes with altered expression in L-OHP-resistant colon cell lines. Some of the identified genes were previously known to be associated with other drug resistance, such as *STK17A* to etoposide, *BNIP3* to 5-FU and gemcitabine, *S100P* to 5-FU, etc. Thus, we suggest that these genes may not only be potential biomarkers of L-OHP resistance but also act as markers of multidrug resistance. These results demonstrate the potential of DNA microarrays to identify novel genes involved in mediating the response of tumor cells to chemotherapy and perhaps to the development of better cancer chemotherapy strategies.

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