Effect of OATP1B1 genetic polymorphism on the uptake of tamoxifen and its metabolite, endoxifen

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Abstract. Overexpression lentivirus platform was established of OATP1B1 (organic anion transporting polypeptides 1B1) wild-type and mutant type genetic polymorphism *in vitro*, and using this platform we investigated and compared the uptake of tamoxifen and its metabolites by mutating the 388 and the 521 bases. The overexpression lentivirus cell platforms were successfully constructed, including OATP1B1*1a-HEK293T and OATP1B1*1b-HEK293T and OATP1B1*5-HEK293T cell model, the infection efficiency is not less than 80%. It shows a high level of gene expression at the mRNA and protein level. The tamoxifen and endoxifen can be taken up into the cells through organic anion transporter polypeptide 1B1, and OATP1B1521T>C inhibits the function of the transport protein, resulting in the content of drug in cell lysis liquid in OATP1B1*5-HEK293T group is lower than in OATP1B1*1a-HEK293T group (tamoxifen or endoxifen), with statistical significance. The content of the drug in cell lysis liquid in OATP1B1*1b-HEK293T group and the OATP1B1*1a-HEK293T group, similar with no statistical significance. These results suggest that tamoxifen and endoxifen can be transported by OATP1B1. However, OATP1B1 521T>C can inhibit the effects of OATP1B1 on tamoxifen and endoxifen in the cells.

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Introduction

Drugs enter into hepatocytes through influx transporters in addition to the diffusion into blood flow, and will be metabolized by hepatic enzymes and then be transported from hepatocytes into blood flow or into bile to complete the process of drug clearance (1). The absorption, distribution, excretion and transportation of drugs by cell membranes are inseparable from drug transporters (2). Drug transporters are divided into influx transporters and efflux transporters, and influx transporters include organic anion transporters, organic cation transporters, organic anion transporting polypeptides and Na^+/taurocholate co-transporting polypeptides; and efflux transporters consist of multidrug resistance proteins, multi-drug resistance-associated proteins, breast cancer resistance proteins and bile salt efflux pumps (3). Organic anion transporting polypeptides (OATPs) of influx transporter family plays an important role in the transport of endogenous and exogenous substances, especially in the transport of drugs (4).

Organic anion transporting polypeptides 1B1 (OATP1B1), one of the important proteins of OATPs family, is specially distributed in the basolateral membrane of the liver (5). OATP1B1 gene chromosome is located in 12p12, and the encoding gene of its cDNA contains 2073 bases and encodes 691 amino acids, composed of 15 exons and 14 introns. Endogenous substances responsible for transport include bile acids, thyroid hormones, free bilirubin, sulfate and glucuronic acid complexes as well as estradiol-17β-glucuronide (5). Drugs transported by OATP1B1 include statins (pitavastatin, pravastatin and atorvastatin), methotrexate, rifampicin, olmesartan, nateglinide and repaglinide (5). OATP1B1 has genetic polymorphism with high mutation frequency in alleles and racial differences. At present, more than forty mutation sites have been found in people, of which 388A>G, 2000A>G and 1463G>C most frequently occur in the United States blacks, with the frequencies of 74%, 34% and 9%; 388A>G, 463C>A

and 521T>C most commonly occur in the United States whites with mutation frequencies of 30%, 16% and 14%; and two kinds of single nucleotide polymorphisms most commonly occurring in Chinese population are 388A>G and 521T>C with mutation frequencies of 73.4 and 14.0%, and the four main haplotypes of OATP1B1 include SLCO1B1*1a (388A, 521T), SLCO1B1*1b (388G, 521T), SLCO1B1*5 (388A, 521C) and SLCO1B1*15 (388G, 521C), among which SLCO1B1*1a belongs to wild-type. Most studies showed that the mutation of 521 T>C decreased the transport function of OATP1B1 and the efficiency of drugs entering into hepatocytes, therefore improving the concentration of drugs in blood and therapeutic effects (6). There has been no agreement on the role of the mutation of bases located at the mutation of 388A>G in the transport of OATP1B1, some studies suggested that mutation occurring at the site did not affect the transport of proteins, but some hold that it enhanced the transport function of proteins (7).

National Comprehensive Cancer Network (NCCN) recommends that patients with breast cancer whose expression of estrogen receptor are positive, regardless of menstruation, age, tumor size and lymph node status, should be treated with adjuvant endocrine therapy after surgery (8). Tamoxifen, the basic drug of endocrine therapy for breast cancer, is taken up into hepatocytes by OATP1B1 and then turns into active metabolite-endoxifen (4-hydroxy-N-desmethyl tamoxifen) through the metabolism by liver enzyme *in vivo*. Tamoxifen, an estrogen receptor antagonist, has two isoforms such as Z-type which antagonizes the effect of estrogen and E-type which has weak estrogen activity (5). Clinically, tamoxifen inhibits the effect of estrogen by competing for estrogen binding receptors, and thereby inhibiting the growth of breast cancer cells (9). However, tamoxifen also showed significantly different effects in the treatment of breast cancer, which could not be completely explained by the differences in liver and kidney function, age and lifestyles, so we reveal the effects of gene polymorphisms of drug transporters and drug metabolizing enzymes on pharmacokinetics and pharmacodynamics of drugs from the perspective of pharmacogenetics (10).

Tamoxifen is metabolized mainly by two pathways *in vivo*, one is to metabolize tamoxifen into primary metabolite N-desmethyl-tamoxifen (NDT) by CYP3A enzyme, which will be further turned into endoxifen by CYP2D6, accounting for near 90% of all metabolic pathways; the other is to metabolize tamoxifen into 4-hydroxy-tamoxifen (4-OH-TAM) by CYP2D6, which will be turned into endoxifen eventually through CYP3A, so CYP2D6 and CYP3A are key enzymes to the metabolism of tamoxifen (10). FDA and FDA Advisory Committee also proposed to use the genotyping results of CYP2D6 as the reference of tamoxifen treatment, as patients with breast cancer who carry the mutation of CYP2D6 genes speed up the metabolism of tamoxifen into active metabolite-endoxifen and thereby decrease blood concentration in the drug, and the effect of treatment. On the contrary, patients carrying the mutation of CYP2D6 have low metabolism and lead to the accumulation of drugs, thus causing toxicity (11). The latest *in vivo* experimental results showed that the concentration of endoxifen was the main standard to predict whether tamoxifen could achieve desired treatment effect in standard tamoxifen treatment. Similar to the gene polymorphisms of drug metabolizing enzymes affect the metabolism of drugs *in vivo*, the gene polymorphisms of drug transporters influence the metabolism of drugs *in vivo* (12).

The studies on the effect of the gene polymorphism of organic anion transporting polypeptides 1B1 on the metabolism of tamoxifen *in vivo* are very few, and the influence of the gene polymorphism of OATP1B1 on the metabolism of tamoxifen into endoxifen *in vivo* has not been reported. In China, up to 200,000 people are newly diagnosed of breast cancer each year, and 4-5 million people died of the disease (13). Tamoxifen, the gold standard drug for the treatment of breast cancer, has more than 100 years of history, but the age distribution and financial situations of patients with breast cancer are varied. As OATP1B1 has polymorphism with racial differences, it is necessary to combine the endocrine therapy of patients with breast cancer in China based on racial differences of OATP1B1, with OATP1B1*1a, OATP1B1*1b and OATP1B1*5 as research subjects of OATP1B1 polymorphism, to analyze OATP1B1 gene polymorphism on the concentration of tamoxifen and endoxifen, the major metabolite of tamoxifen, thus to provide pharmacogenetic basis for the prediction of the drug effect, and screen out patients who benefit from the therapy, so as to avoid excessive treatment and ensure reasonable and individualized administration with drugs (5,6). Therefore, our objection is to investigate whether or not OATP1B1 polymorphism affect the metabolism of tamoxifen and its metabolite endoxifen.

Materials and methods

Overexpression of lentivirus cloning by PCR and carrier enzyme. GV358 vector was used in this study, we designed PCR primers with *Age*I and *Age*I, OATP1B1-F, 5'-GAGGAT CCCCGGGTACCGGTCGCCACCATGGACCAAAATCAA CATTTG-3'; OATP1B1-R, 5'-TCCTTGTAGTCCATACCAC AATGTGTTTCACTATCTGC-3'. Total RNA was extracted from liver tissue using TRIzol method (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Total RNA (1 μg) was reverse-transcribed using First Strand cDNA Synthesis kit (Promega Biotech Co., Ltd., Beijing, China). PCR was performed using GeneAmp PCR (2720 Thermal Cycler; Applied Biosystems) by Taq polymerase (SinoBio, E001-02B) according to the manufacturer's instructions. The qPCR steps were as follows: initial denaturation at 98˚C for 5 min, 30 cycles (98˚C for 10 sec, 55˚C for 10 sec, and 72˚C for 90 sec), final extension at 72˚C 8 min and hold at 4˚C. The outcome of PCR and GV358 vector were performed with dual-enzyme digestion and T4 ligase at 37˚C overnight.

Preparation of competent cells and transformation. DH-5α *Escherichia coli* (1:100-1:50) was inoculated with 1 ml of LB nutrient solution and cultivated at 37˚C for 2-3 h. Then, bacteria solution was placed in ice for 20 min and centrifuged at 2,000 g for 10 min. After discarding the supernatant, bacteria solution was incubated with 0.1 ml of pre-cooling $CaCl_2$ (100 mM) in ice for 20 min. After centrifugation at 2,000 g for 10 min, bacteria solution was resuspended with 0.1 ml of pre-cooling $CaCl_2$ (100 mM) in ice. Synthetic plasmid (10 μl) was added into 0.1 ml of competence bacteria solution and incubated in ice for 30 min. Then, bacteria solution was activated at 42˚C

Table I. Mass spectrometry conditions.

Compound	Pair ion	DP	EP	CE	CXP
Tamoxifen	372.1/72.2	65	6	40	4
Endoxifen	374.3/58.1	65	5	45	3

for 90 sec and incubated in ice for 2 min. Next, bacteria solution was added into 0.5 ml of LB and incubated at 37˚C for 1 h. Lastly, bacteria solution was seeded at solid state LB with antibiotic and incubated at 37˚C for 12 h. Bacterial colony was seeded into 3-5 ml of LB at 37˚C for 12 h.

HEK293T cell culture and grouping. HEK293T cells were divided into six groups: Control group (A group); HEK293T cells + tamoxifen (6, 30 and 150 ng/ml) or endoxifen (3, 15 and 75 ng/ml) (B group); HEK293T cells + negative plasmid + tamoxifen (6, 30 and 150 ng/ml) or endoxifen (3, 15 and 75 ng/ml) (C group); HEK293T cells + OATP1B1*1a plasmid + tamoxifen (6, 30 and 150 ng/ml) or endoxifen (3, 15 and 75 ng/ml) (D group); HEK293T cells + OATP1B1*1b (388 GG) plasmid + tamoxifen (6, 30 and 150 ng/ml) or endoxifen (3, 15 and 75 ng/ml) (E group); HEK293T cells + OATP1B1*5 (521 CC) plasmid + tamoxifen (6, 30 and 150 ng/ml) or endoxifen (3, 15 and 75 ng/ml) (F group).

Real-time quantitative RT-PCR. Total RNA from cells using TRIzol method (Invitrogen) according to the manufacturer's protocol. Total RNA (1 μg) was reverse-transcribed using First Strand cDNA Synthesis kit (Promega). PCR was performed using GeneAmp PCR (2720 Thermal Cycler; Applied Biosystems) by Taq polymerase (SinoBio, E001-02B) according to the manufacturer's instructions. Real-time PCR assay was performed by SYBR Premix Ex Taq™ (Takara, Japan). Real-time PCR, denaturation at 95˚C for 2 min; 40 cycles at 95˚C for 15 sec and 60˚C for 30 sec; and a final dissociation stage (95˚C for 5 min).

Western blotting. HEK293T cells were split with lysis buffer (RIPA, Beyotime) and the supernatant was discarded after centrifugation at 12,000 g for 10 min at 4˚C. Protein concentration was determined by BCA assay (Beyotime). Equivalent protein (50 μg) was loaded for separation into 10% gradient SDS-PAGE and transferred onto nitrocellulose membranes (Santa Cruz Biotechnology, Inc.). Membranes were blocked with 5% non-fat milk in TBST for 1 h at 37˚C and then incubated with primary antibodies: anti-OATP1B1 (1:1,000, Abcam) and β-actin (1:2,000, Beyotime) overnight at 4˚C followed by horseradish peroxidase-conjugated secondary antibodies (1:5,000, Beyotime) for 1 h at 37˚C. The immunoreactive bands were visualized by an ECL Western blotting detection kit (Beyotime) and analyzed semi-quantitatively using Quantity One software (Bio-Rad Laboratories, Milan, Italy).

MTT assay. HEK293T cells (1,000 cell/well) were seeded at 96-well plate and treated with 1-100 μM of tamoxifen or 0.1-1 μM of endoxifen for 48 h. MTT(3-(4,5)-dimethylthiahiazo(-z-yl)-3,5-di-phenytetrazolium bromide, 5 mg/ml, Sigma-Aldrich Co. LLC) was added into cells for 4 h at 37˚C. DMSO (150 μl) was added into cell after removing medium and dissolved for 10 min at 37˚C. Absorbance was measured using microplate reader at 492 nm, the inhibitory rate = (1 - absorbance of experimental group/control of experimental group) x 100%.

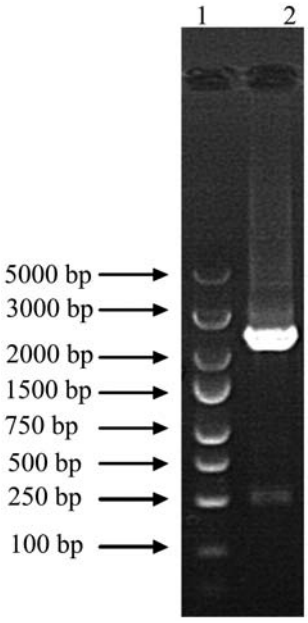

Figure 1. Target gene fragments.

HPLC-MS/MS. Mass spectrometry conditions are shown in Table I.

Liquid condition: A liquid, double distilled water (5 mM of ammonium acetate and 1% formic acid); B liquid, methyl alcohol (1% formic acid), column temperature was 20˚C, and flow velocity was 0.3 ml/min. Injecting time was 8 min, the gradient was eluted.

Sample treatment methods: 10 μl of interior label (Tegretol, 300 ng/ml) was added into 100 μl of samples, then and samples were centrifuged at 1,000 g for 1 min. Methyl alcohol (300 μl) was added into samples and shocked at 1,700 rpm for 2 min. After centrifugation with 1,0000 g for 5 min, clear liquid was absorbed and 5 μl clear liquid was used to analyze tamoxifen and endoxifen levels.

Statistical analysis. All data are expressed as the mean ± standard deviation. Differences between groups were tested by the Student's t-test using the SPSS 18.10 program (Chicago, IL, USA). A p-value of <0.05 was considered statistically significant.

Results

Target gene fragments. Firstly, target fragment of human OATP1B1 is 2,074 bp, and cDNA of this study is 2,117 bp with *Age*I and *Age*I. We used PCR to compound target gene fragments, which is 2,117 bp as shown in Fig. 1.

The construction of a recombinant plasmid. Next, we used PCR to analyze construction of a recombinant plasmid,

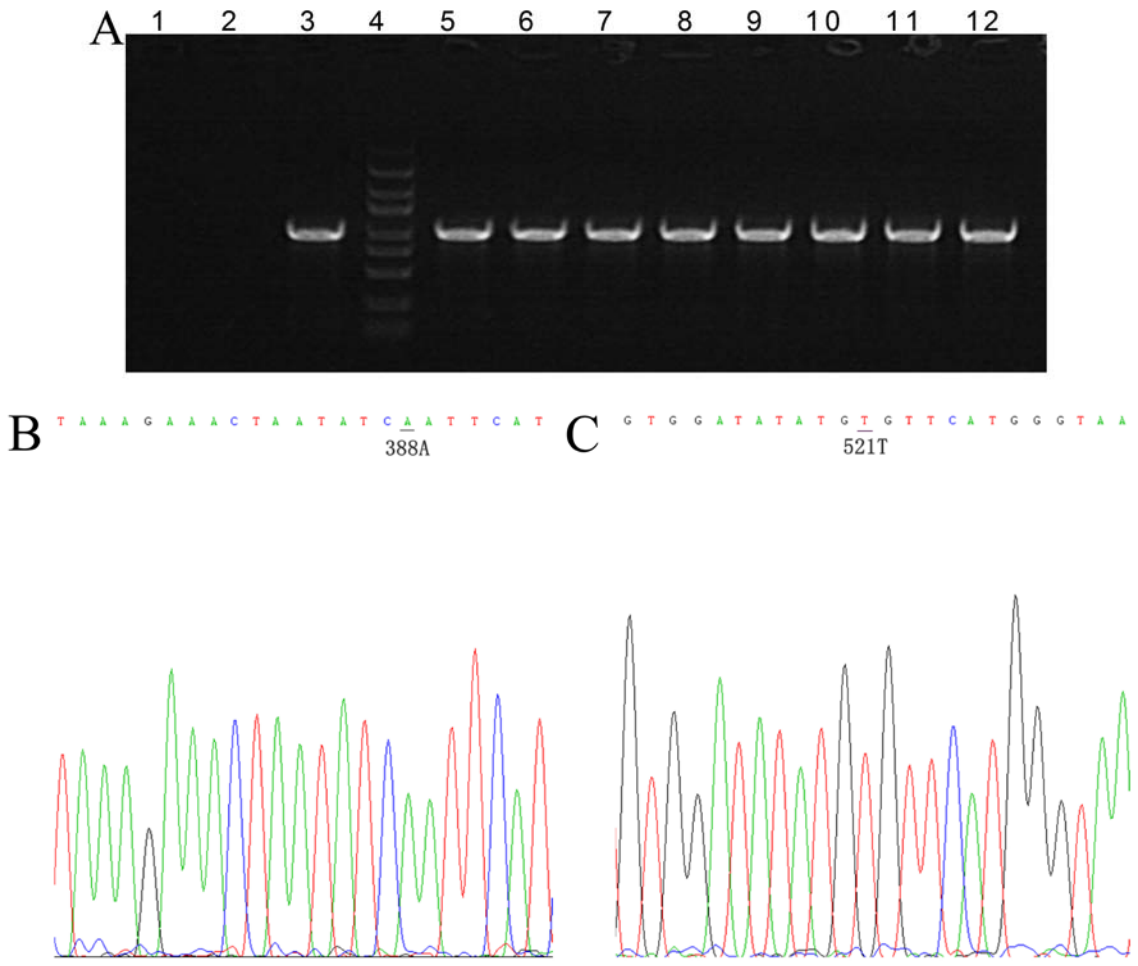

Figure 2. The construction of a recombinant plasmid. Recombinant plasmid monoclonal by PCR (A), sequencing result of OATP1B1*1b (388 AA) (B) and OATP1B1*5 (521 TT) (C).

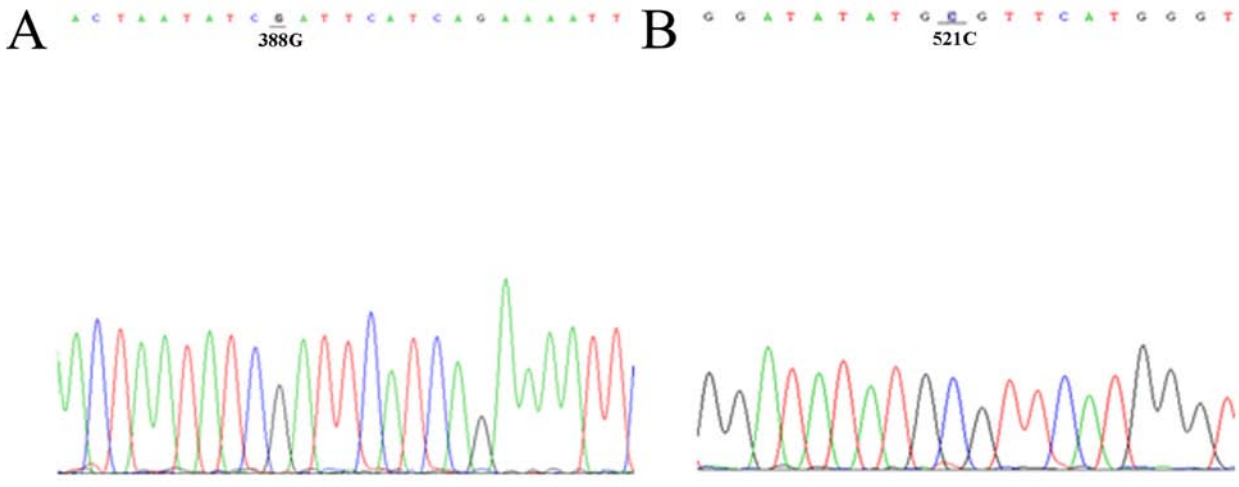

Figure 3. Site-specific mutagenesis of recombinant plasmids. Sequencing result of OATP1B1*1b (388 GG) (A) and OATP1B1*5 (521 CC) (B).

1,004-bp fragment is shown at Fig. 2A. As showed in Fig. 2B and C, 388 site is adenine (A), and 521 site is thymine (T).

Site-specific mutagenesis of recombinant plasmids. We used site-specific mutagenesis to induce mutation at 388 and 521 sites, respectively; site-specific mutagenesis production was DNA sequenced. As showed in Fig. 3, 388 site is guanine (G), and 521 site is cytosine (C).

HEK293T cell infection. OATP1B1*1a, OATP1B1*1b (388 GG) and OATP1B1*5 (521 CC) plasmids were infected into HEK293T cells with Polybrene. After infection for 72 h,

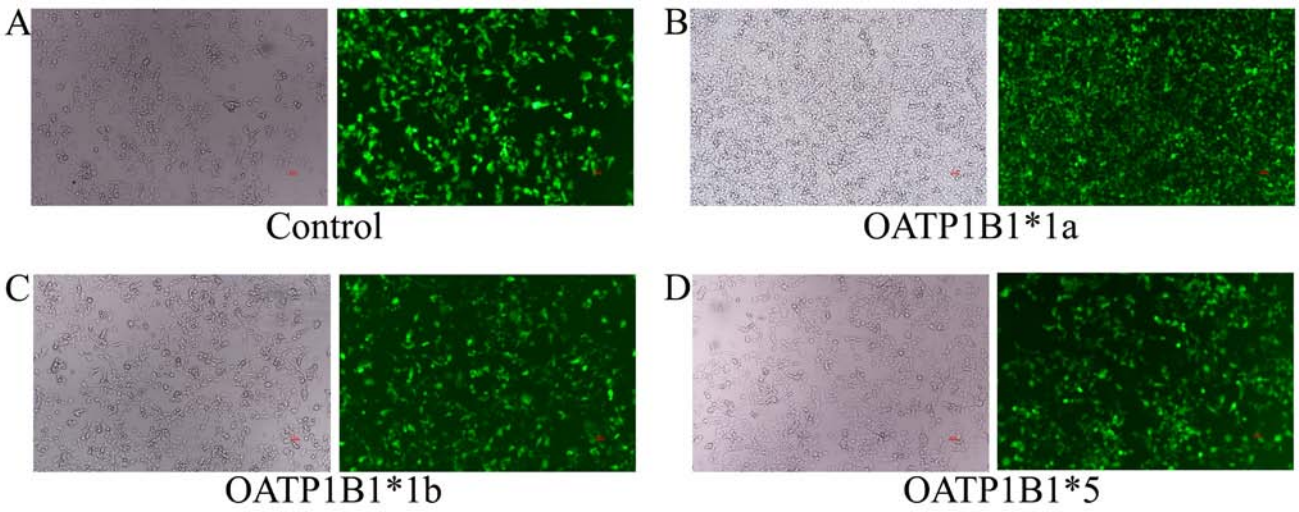

Figure 4. HEK293T cell infection. Control (A), OATP1B1*1a (B), OATP1B1*1b (388 GG) (C) and OATP1B1*5 (521 CC) (D). Control, negative group; OATP1B1*1a, OATP1B1*1a group; OATP1B1*1b (388 GG) OATP1B1*1b group; OATP1B1*5 (521 CC) group.

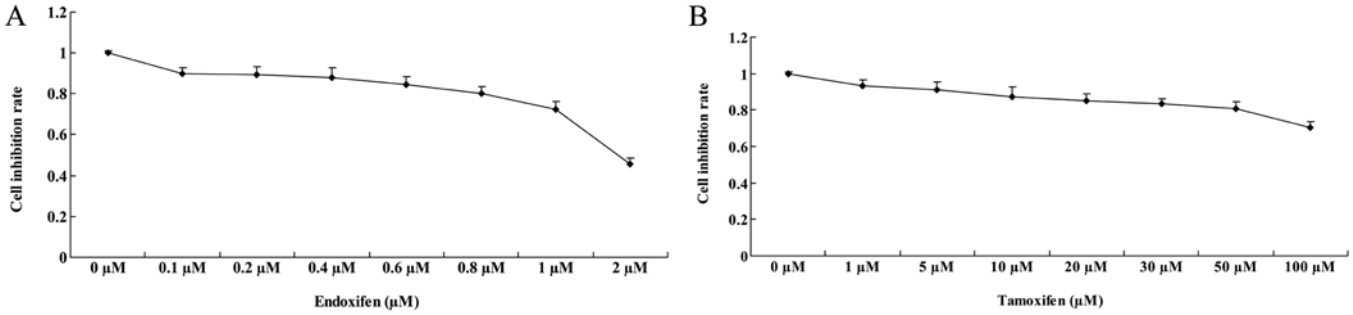

Figure 5. Cytotoxicity of HEK293T cells. Cytotoxicity of HEK293T cells by endoxifen (A) and tamoxifen (B).

fluorescent expression was observed using a fluorescence microscope. There was an outstanding increase of fluorescent expression in control, OATP1B1*1a, OATP1B1*1b (388 GG) and OATP1B1*5 (521 CC) plasmids (Fig. 4).

Cytotoxicity of HEK293T cells. Tamoxifen (1-100 μM) and 0.1-1 μM of endoxifen treated in HEK293T cells for 48 h. Fig. 5A shows the inhibition rate of >80% treated with 0.1, 0.3 and 1 μM of endoxifen for 48 h in HEK293T cells. The inhibition rate of HEK293T cell treated with 2 μM of endoxifen was 0.423±0.02% (Fig. 5A). Inhibition of the rate was >80% treated with 10, 25 and 50 μM of tamoxifen for 48 h (Fig. 5B). The inhibition rate of HEK293T cells treated with 100 μM of tamoxifen was 0.785±0.04% (Fig. 5B).

mRNA and protein expression in HEK293T cells. Control, negative, OATP1B1*1a, OATP1B1*1b (388 GG) and OATP1B1*5 (521 CC) plasmids were infected into HEK293T for 72 h. As showed in Fig. 6A and B, there was marked increase of OATP1B1 mRNA expression in OATP1B1*1a, OATP1B1*1b (388 GG) and OATP1B1*5 (521 CC) plasmids, compared with negative group. The protein expression of OATP1B1 was significantly induced in OATP1B1*1a, OATP1B1*1b (388 GG) and OATP1B1*5 (521 CC) plasmid groups, compared with negative group (Fig. 6C).

Table II. Precision and accuracy of tamoxifen in interblock.

Concentration (ng/ml)	6	30	150
Measured concentrations	6.11	29.4	146
	5.98	30.2	147
	6	29.9	145
	6.03	29.4	159
	5.51	29.6	156
Average value (mean ± SD)	5.93±0.24	29.70±0.35	150.6±6.43
RSD (%)	4.01	1.17	4.27
RE (%)	98.83	99.04	100.38

RSD, relative standard deviation; RE, relative error.

The methodology of tamoxifen level using HPLC-MS/MS. Tamoxifen at 6, 30 and 150 ng/ml was added into the lysate of HEK293T cells. In interblock, HPLC-MS/MS was used to analyze tamoxifen levels, and the RSD (relative standard deviation) of tamoxifen was 4.01, 1.17 and 4.27% in 6, 30 and 150 ng/ml of tamoxifen group, respectively (Table II). RE (relative

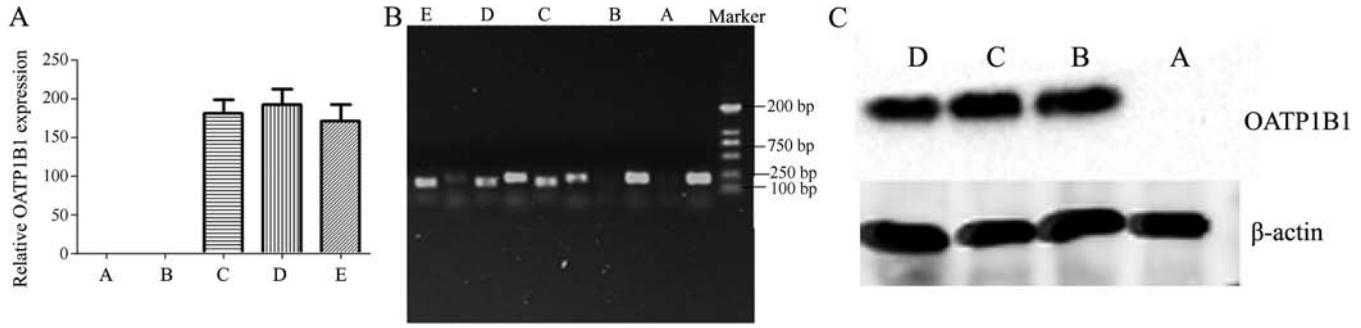

Figure 6. mRNA and protein expression in HEK293T cells. OATP1B1 mRNA expression by QPCR (A), PCR (B) and protein expression (C) in HEK293T cells. A, control group; B, negative group; C, OATP1B1*1a group; D, OATP1B1*1b (388 GG) group; E, OATP1B1*5 (521 CC) group.

Table III. Precision and accuracy of tamoxifen in interclass.

Concentration (ng/ml)	6	30	150
Measured concentrations (mean ± SD)	5.97±0.05	28.83±0.40	148.73±2.45
RSD (%)	0.90	1.38	1.65
RE (%)	99.50	96.05	99.15

RSD, relative standard deviation; RE, relative error.

Table IV. Precision and accuracy of endoxifen in interblock.

Concentration (ng/ml)	3	15	75
Measured concentrations	2.85	14.3	72.6
	2.82	15	70.3
	3.12	14.4	75.2
	2.72	15.3	70.2
	3.26	15.3	73
Average value (mean ± SD)	2.95±0.23	14.86±0.48	72.26±2.09
RSD (%)	7.66	3.25	2.89
RE (%)	98.33	99.04	96.28

RSD, relative standard deviation; RE, relative error.

Table V. Precision and accuracy of endoxifen in interclass.

Concentration (ng/ml)	3	15	75
Measured concentrations (mean ± SD)	2.88±0.07	13.86±0.10	70.40±0.89
RSD (%)	2.38	0.71	1.26
RE (%)	95.96	92.4	93.87

RSD, relative standard deviation; RE, relative error.

error) of tamoxifen was 98.83, 99.04 and 100.38% in 6, 30 and 150 ng/ml of tamoxifen group, respectively (Table II). Next, in interclass, HPLC-MS/MS was used to analyze tamoxifen levels, and the RSD (relative standard deviation) of tamoxifen was 0.90, 1.38 and 1.65% in 6, 30 and 150 ng/ml of tamoxifen group, respectively (Table III). RE of tamoxifen was 99.50, 96.05 and 99.15% in 6, 30 and 150 ng/ml of tamoxifen group, respectively (Table III).

The methodology of endoxifen level using HPLC-MS/MS. Endoxifen (3, 15 and 75 ng/ml) was added into the lysate of HEK293T cell. In interblock, HPLC-MS/MS was used to analyze endoxifen levels, and the RSD (relative standard deviation) of endoxifen was 7.66, 3.25 and 2.89% in 3, 15 and 75 ng/ml of endoxifen group, respectively (Table IV). RE of endoxifen was 98.33, 99.04 and 96.28% in 3, 15 and 75 ng/ml of endoxifen group, respectively (Table IV). Next, in interclass, HPLC-MS/MS was used to analyze endoxifen levels, and the RSD of endoxifen was 2.38, 0.71 and 1.26% in 3, 15 and 75 ng/ml of endoxifen group, respectively (Table V). RE of endoxifen was 95.96, 92.4 and 93.87% in 6, 30 and 150 ng/ml of endoxifen group, respectively (Table V).

Special properties of the methods. Under the condition of the above experiments, chromatographic peak of tamoxifen, endoxifen and Tegretol (interior label) showed sharp and good separation, and this methods had good specificity (Fig. 7). The retention time of tamoxifen and Tegretol were 3.66 and 3.65 min, respectively (Fig. 7A and B). Fig. 7C and D show that the retention time of endoxifen and Tegretol are 3.57 and 3.65 min, respectively.

The content of tamoxifen using HPLC-MS/MS. We explored the function of OATP1B1*1a, OATP1B1*1b (388 GG) and OATP1B1*5 (521 CC) in the metabolism of tamoxifen using HPLC-MS/MS. After infection at 24 and 48 h, there were significant increases of tamoxifen levels in OATP1B1*1a, OATP1B1*1b (388 GG) and OATP1B1*5 (521 CC) groups, compared with negative group. However, the tamoxifen level of OATP1B1*5 (521 CC) group was remarkably lower than that of OATP1B1*1a or OATP1B1*1b (388 GG) (Fig. 8). However, the tamoxifen level of OATP1B1*1a group was similar to that of OATP1B1*1b (388 GG) group (Fig. 8).

The content of endoxifen using HPLC-MS/MS. We further explored the function of OATP1B1*1a, OATP1B1*1b (388 GG)

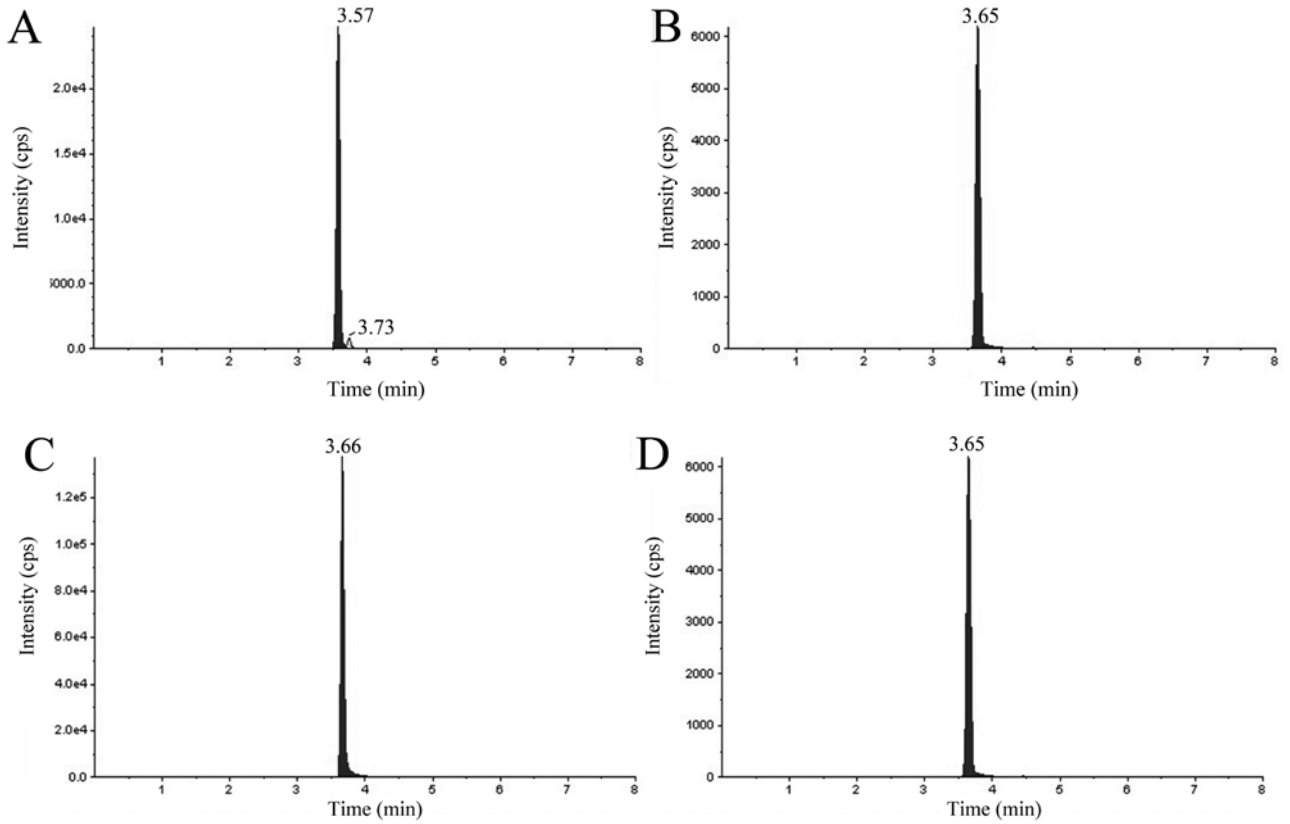

Figure 7. The methodology of tamoxifen and endoxifen level using HPLC-MS/MS. The methodology of tamoxifen (A), Tegretol (B and D) and endoxifen (C) level using HPLC-MS/MS.

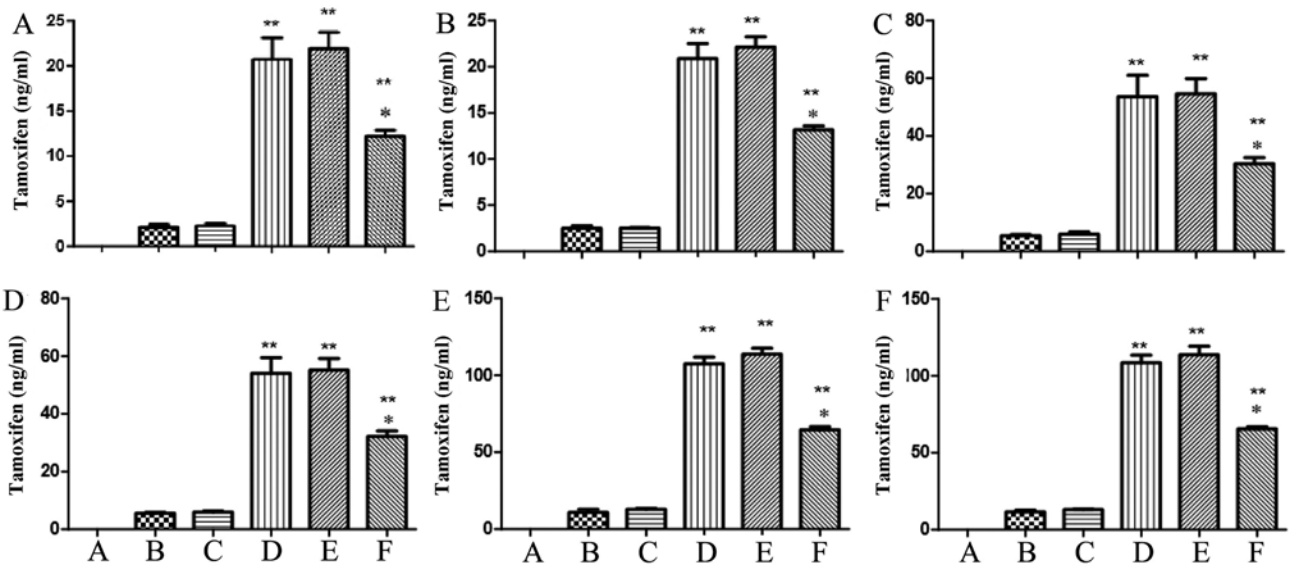

Figure 8. The content of tamoxifen using HPLC-MS/MS. The content of tamoxifen in HEK293T by 6, 30 and 150 ng/ml at 24 (A, C and E) and 48 h (B, D and F) using HPLC-MS/MS. A, control group; B, negative group; C, OATP1B1*1a group; D, OATP1B1*1b (388 GG) group; E, OATP1B1*5 (521 CC) group. **p<0.01 versus negative group; *p<0.01 versus OATP1B1*1a group.

and OATP1B1*5 (521 CC) in the metabolism of endoxifen using HPLC-MS/MS. After infection at 24 and 48 h, significant increases of tamoxifen levels in OATP1B1*1a, OATP1B1*1b (388 GG) and OATP1B1*5 (521 CC) groups were observed, compared with negative group. However, the tamoxifen level of OATP1B1*5 (521 CC) group was prominently lower than that of OATP1B1*1a or OATP1B1*1b (388 GG) (Fig. 9). However, the tamoxifen level of OATP1B1*1a group was also similar to that of OATP1B1*1b (388 GG) group (Fig. 9).

Discussion

Endocrine therapy plays an important role in the treatment of breast cancer, and tamoxifen is used as the major drug of endo-

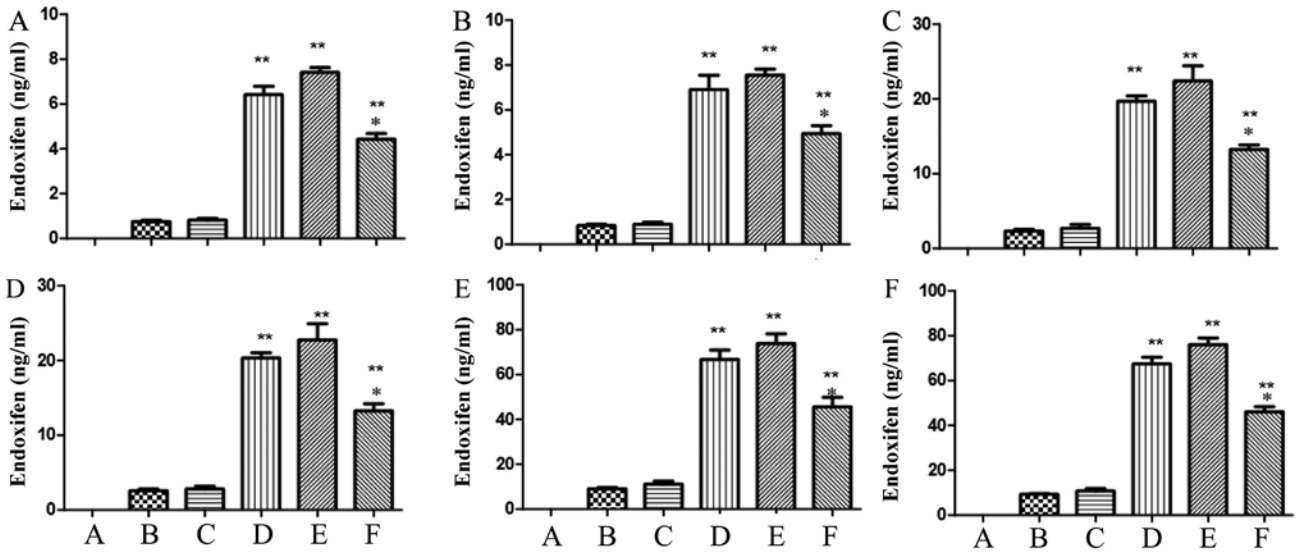

Figure 9. The content of endoxifen using HPLC-MS/MS. The content of endoxifen in HEK293T by 3, 15 and 75 ng/ml at 24 (A, C and E) and 48 h (B, D and F) using HPLC-MS/MS. A, control group; B, negative group; C, OATP1B1*1a group; D, OATP1B1*1b (388 GG) group; E, OATP1B1*5 (521 CC) group. **p<0.01 versus negative group; *p<0.01 versus OATP1B1*1a group.

crine inhibitor therapy in the treatment of patients with breast cancer with positive premenopausal estrogen receptor (ER) and those with postmenopausal breast cancer (14). An *in vitro* experiment showed that tamoxifen inhibited the expression of progesterone receptor in MCF-7 cells (12). However, the individual differences and adverse reactions of tamoxifen is of increasing concern. Tamoxifen's metabolites composed of 4-OH-TAM and N-desmethyl-TAM (NDT) and endoxifen. Lin *et al* compared pharmacokinetic parameters of endoxifen and tamoxifen, finding that endoxifen was more easily absorbed and the concentration of drugs in the blood peaked faster compared with tamoxifen (15). In the first *in vivo* experiment of oral administration with different single doses of endoxifen and single dose of tamoxifen, direct administration with endoxifen reached C_{max} faster, so orally taking smaller dose of endoxifen can achieve the same effect as tamoxifen, so as to avoid the adverse reaction of tamoxifen (16).

Clinical drugs transported by OATP1B1 transporters include angiotensin converting enzyme inhibitors, statins, angiotensin II receptor antagonists, antitumor drugs and antibiotics (17). As OATP1B1 has highly genetic polymorphism with racial differences in specific mutations, its gene polymorphism affects the transport function of transporters. A study of oral administration of 18 cases of Han ethnicity people with 40 mg pravastatin showed that the drug concentration of subjects with genotype OATP1B1*15 (388G, 521C), especially the homozygous subjects increased significantly compared with non-mutation subjects (18). A study showed that the relationships between the gene polymorphism of OATP1B1 transporters and rosuvastatin, finding that the transport of rosuvastatin by mutant OATP1B1*5 was significantly lower than that by wild-type OATP1B1*1a transporter, the difference being statistically significant ($p<0.05$), and the result was consistent with that of the *in vivo* experiment (19). When subjects with genotype OATP1B1*5 and genotype OATP1B1*1a took the same dose of rosuvastatin, the concentration of drug in the blood of the former increased more significantly, which was probably because the function of transporter was weakened due to 521T>C mutations and thereby resulting in the slowing down of drug metabolism (20). In addition, the association between atorvastatin and atorvastatin with OATP1B1521T>C were also reported (21).

There is no agreement to the impact of the mutation of OATP1B1388A>G on drug transporting proteins, but some experiments recently proved that the mutations of the 388 site was related to the expression of proteins (22). Some studies found that OATP1B1*1b could enhance the transport of drugs by transporters, and an *in vivo* experiment reached the same conclusion (22,23). Experimental results showed that area under the curve (AUC) of pravastatin in people carrying genotype SLCO1B1*1B/*1B gene decreased by 35% compared with those carrying wild-type SLCO1B1 gene (18). Some experiments held that OATP1B1*1B inhibited the transport of drugs by transporters.

At present, the studies on the correlation between the gene polymorphism of OATP1B1 and breast cancer are few. The research on the correlation between the gene polymorphism of OATP1B1 and breast cancer conducted showed that the frequency of mutant C allele of OATP1B1521T>C was higher in breast cancer patients with positive estrogen receptor (ER) than those with negative ER (5,6). However, breast cancer patients with positive ER were more sensitive to endocrine therapy, suggesting that the gene polymorphism of OATP1B1 may affect the prognosis of patients (24). Previous studies on the correlation between the gene polymorphism of OATP1B1 with tamoxifen found that the mutation of OATP1B1388A>G and 521T>C resulted in the decreased intake of tamoxifen by OATP1B1, while not showing statistically significant differences compared with wild-type group, mainly because the gene platform was previously constructed by liposome transfection, which had low efficiency with short period of gene expression (5,6,25). The overexpression lentivirus cell platforms have been successfully constructed, including OATP1B1*1a-HEK293T and OATP1B1*1b-HEK293T and OATP1B1*5-HEK293T cell

model, the infection efficiency is not less than 80%. The gene expressions are high at mRNA and protein level. The tamoxifen and endoxifen can be taken up into cells through organic anion transporter polypeptide 1B1, and OATP1B1521T>C inhibited the function of the transport protein, resulting in the content of drug in cell lysis liquid in OATP1B1*5-HEK293T group is lower than in OATP1B1*1a-HEK293T group with statistical significance. OATP1B1388A>G makes the content of drug in cell lysis liquid in OATP1B1*1b-HEK293T group and the wild group+drug similarly, but there is no statistical significance.

In conclusion, our results have demonstrated tamoxifen and endoxifen can be transported by OATP1B1. However, OATP1B1 521T>C can inhibit the function effects of OATP1B1 on tamoxifen and endoxifen into cell. This study determined OATP1B1 participation in transportation of tamoxifen and endoxifen into cells, which may represent a potential therapeutic strategy for treatment of breast cancer.

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