Individualized chemotherapy guided by the expression of *ERCC1*, *RRM1*, *TUBB3*, *TYMS* and *TOP2A* genes versus classic chemotherapy in the treatment of breast cancer: A comparative effectiveness study

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Abstract. *ERCC1*, *RRM1*, *TUBB3*, *TYMS* and *TOP2A* genes have been shown to be associated with drug resistance in various types of tumors; however, their roles in breast cancer chemotherapy have not been fully validated. In the present study, 140 well-matched patients with breast cancer, comprising 70 patients receiving individualized chemotherapy and 70 receiving classic chemotherapy, were analyzed. In the individualized chemotherapy group, the mRNA expression levels of *ERCC1*, *RRM1*, *TUBB3*, *TYMS* and *TOP2A* in breast cancer tissues were measured using multiplex branched DNA liquidchip technology prior to chemotherapy; an individualized chemotherapy regimen was developed for each patient according to the results. As a control, patients in the classic chemotherapy group received a docetaxel + epirubicin + cyclophosphamide regimen. Survival analyses were performed using the Kaplan-Meier method. The prognostic factors for disease-free survival (DFS) and overall survival (OS) in the patients were identified via Cox's proportional hazards regression model. Adverse reactions were evaluated according to the National Cancer Institute Common Toxicity Criteria 4. Compared with the classic chemotherapy group, the DFS and OS of the individualized chemotherapy group were significantly longer (DFS, 77.4 vs. 67.1 months, P=0.039; OS, 81.4 vs. 75.4 months, P=0.031), and the incidence of grade 2 or 3 palpitations and chest tightness was lower (12.9 vs. 27.1%, P=0.035). The chemotherapy strategy guided by genetic detection was an independent protection factor for DFS [hazard ratio (HR)=0.389, 95% confidence interval (CI): 0.153, 0.989, P=0.047], but not an independent protection factor for OS (HR=0.340, 95% CI: 0.107, 1.078, P=0.067). The results indicate that the combined detection of *ERCC1*, *RRM1*, *TUBB3*, *TYMS* and *TOP2A* gene expression and use of the results to guide individualized chemotherapy can improve treatment efficacy and reduce unnecessary toxicity.

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Abbreviations: ERCC1, excision repair cross complementing 1; RRM1, ribonucleoside reductase M1; TUBB3, β-tubulin III; TYMS, thymidylate synthase; TOP2A, topoisomerase IIα; MBL, multiplex branched DNA liquidchip; T, docetaxel; E, epirubicin; C, cyclophosphamide; DFS, disease-free survival; OS, overall survival; P, cisplatin; G, gemcitabine; X, capecitabine; BMI, body mass index; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor 2; BCS, breast conserving surgery; SLNB, sentinel lymph node biopsy; T-ALND, total axillary lymphadenectomy; HR, hazard ratio; CI, confidence interval; NER, nucleotide excision repair; RT-qPCR: Reverse transcription-quantitative polymerase chain reaction

Key words: breast cancer, individualized chemotherapy, ERCC1, RRM1, TUBB3, TYMS, TOP2A, prognosis

Introduction

Breast cancer is the most frequently diagnosed type of tumor and the second most common cause of mortality among women worldwide (1). As breast cancer is considered a systemic disease, comprehensive treatment with surgery as the main component, in combination with chemotherapy, radiotherapy, endocrine therapy, molecular targeted therapy and other auxiliary interventions, has become the standard for breast cancer treatment. Clinically, chemotherapy serves crucial roles in the control and reduction of lesions before surgery and the prevention of recurrence and metastasis after surgery. For advanced and triple-negative breast cancer, chemotherapy remains the main means of reducing recurrence and metastasis following surgery (2,3). However, as highly heterogeneous tumors, breast cancers with identical pathological and molecular types may differ in their sensitivity to the same chemotherapy regimen. Thus, not all patients will benefit from the same chemotherapy regimen. This variation may be due to the differential expression of certain genes associated with chemotherapy. Consequently, detecting the expression of these genes to guide the selection of chemotherapeutic drugs is of great significance

for improving the efficacy of chemotherapy and reducing the associated toxicity.

Numerous studies have suggested that the differential expression of several genes, including excision repair cross complementing 1 (*ERCC1*), ribonucleotide reductase M1 (*RRM1*), thymidylate synthetase (*TYMS*), β-tubulin III (*TUBB3*) and topoisomerase IIα (*TOP2A*), in tumor tissues is closely associated with chemoresistance and prognosis in patients with cancer. For example, the expression level of *ERCC1*, which is crucial for the repair of platinum-DNA adducts, has been reported to negatively affect the effectiveness of platinum drugs and suggested to be a major predictor of the response of cancer to platinum-based chemotherapy (4,5). Furthermore, a randomized prospective clinical study confirmed that customized cisplatin chemotherapy based on quantitative *ERCC1* mRNA expression improved the survival of patients with non-small-cell lung cancer (6). These studies indicate that the assessment of *ERCC1* mRNA expression is feasible in a clinical setting and is able to predict the response to cisplatin-based treatment. The expression level of *RRM1*, which is the main target of gemcitabine, has been reported to be negatively correlated with the efficacy of gemcitabine (6,7). *TUBB3* is thought to be a marker of taxane resistance, and high expression levels of *TUBB3* are reported to correlate with low response rates in patients treated with taxane-containing regimens (8,9). The expression level of *TYMS*, which is a central enzyme in the folate metabolic pathway and a major target for cytotoxic antifolate chemotherapeutic agents, such as 5-fluorouracil and capecitabine, is negatively associated with the efficacy of antimetabolic drugs (10,11). *TOP2A* is an essential nuclear enzyme that changes DNA topology and is the primary molecular target of various cytotoxic agents, including anthracyclines. The expression level of *TOP2A* has been demonstrated to be positively correlated with the efficacy of anthracycline drugs (12,13). Therefore, the assessment of the expression levels of these drug-associated genes in the tumor tissues of patients prior to chemotherapy is useful for therapeutic decision-making.

Although mounting evidence indicates their important roles in the evaluation of chemoresistance, to the best of our knowledge, no study on the combined detection of *ERCC1*, *RRM1*, *TUBB3*, *TYMS* and *TOP2A* gene expression for the guidance of chemotherapy in breast cancer patients has yet been reported. Therefore, the present prospective study was carried out to with the aim of providing new suggestions and clinical evidence for the individualized treatment of breast cancer.

Materials and methods

Data collection. All 140 breast cancer patients, who were treated by the same medical team from January 1, 2012 to December 31, 2013 at the Department of Thyroid and Breast Surgery, the General Hospital of Western Theater Command (Chengdu, China) were enrolled in the study. The patients included an individualized chemotherapy group (n=70) and a classic chemotherapy group (n=70). The mechanism, cost and expected efficacy of the two chemotherapy methods were explained in detail to the patients, and each patient decided which method of treatment to receive. All patients had complete medical records and none of them had received neoadjuvant therapy prior to surgery. All patients had primary operable breast cancer with no distant metastasis. Details of multiple clinicopathological parameters were collected, including age, body mass index (BMI), menstrual status, histological grade, tumor size, axillary lymph node status, TNM stage, estrogen receptor status, progesterone receptor status, human epidermal growth factor receptor 2 status, Ki67 index, molecular classification, type of surgery, and hormonal and radioactive therapy status. All patients provided written informed consent for tissue sample retention and analysis for research purposes and publication in the present article. This retrospective study was approved by the ethics committee of the General Hospital of Western Theater Command (registration no. 2011ky020).

Detection of mRNA expression levels. The mRNA expression levels of *ERCC1*, *RRM1*, *TUBB3*, *TYMS* and *TOP2A* in the breast cancer tissues were measured simultaneously using multiplex branched DNA liquidchip (MBL) technology (Guangzhou SurExam Bio-Tech Co., Ltd.) as previously reported (14-16). The main steps in this analysis were as follows: i) Samples were lysed in buffer at 56˚C for 2 h; ii) the lysed product was added to each well of a 96-well plate containing blocking reagent, target gene-specific probe sets and capture beads; iii) the plate was sealed, and then incubated for 18 h at 54˚C on a shaker, followed by the addition of hybridization mixture; iv) the unbound mRNA and other debris in each well were removed by washing three times with buffer; v) signals for bound target mRNA were amplified with streptavidin-phycoerythrin at 50˚C for 30 min; vi) the fluorescence value of each sample was measured and analyzed using the Luminex® 200 system™ (Luminex Corporation) to determine the mRNA expression level of each gene. Compared with the cut-off value of each gene, the mRNA expression level was categorized as low (<25%), low-to-medium (25-49%), medium (50%), medium-to-high (51-75%) and high expression (>75%) (17).

Reverse transcription-quantitative (RT-q)PCR. Total RNA was extracted from cryopreserved tissue using TRIzol reagent (Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. Total RNA was reverse transcribed into cDNA using the RevertAid™ First Strand cDNA Synthesis kit (cat. no. k1622; Fermentas, Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. The following thermocycling conditions were used for qPCR: 50˚C for 2 min, 95˚C for 10 min, 40 cycles at 95˚C for 20 sec, and 60˚C for 1 min. A total of 40 cycles of nucleic acid amplification were applied using Fast SYBR™ Green Master Mix 4385612 (Applied Biosystems; Thermo Fisher Scientific, Inc.) in an ABI PRISM® 7900HT Sequence Detection System (Applied Biosystems; Thermo Fisher Scientific, Inc.), and the cycle threshold (CT) value of the target gene was identified. Target genes were normalized to the internal reference gene *GAPDH*, and quantified using the comparative $2^{-\Delta\Delta Cq}$ method (18). Gene expression levels were measured in triplicate, with a good reproducibility, and the average was calculated. The following primer sequences were used for qPCR: *ERCC1* forward, 5'-GGGAATTTGGCGACGTAATTC-3', and reverse, 5'-GCG GAGGCTGAGGAACAG-3'; *RRM1* forward, 5'-TGGCCT

Table I. Implementation of chemotherapy regimens.

Chemotherapy regimens	No. of cycles					n
	Four	Five	Six	Seven	Eight	
Individualized chemotherapy						
E (90 mg/m^2) + P (80 mg/m^2)	1	1	2			4
E (90 mg/m^2) + G (1,000 mg/m^2)			1			1
E (90 mg/m^2) + X (950 mg/m^2)			1			1
T (75 mg/m^2) + P (80 mg/m^2)		1	4			5
T (75 mg/m^2) + C (500 mg/m^2)			1			1
T (75 mg/m^2) + G (1,000 mg/m^2)	1	2	14	3	4	24
T (75 mg/m^2) + X (950 mg/m^2)	1		8	1		10
T (75 mg/m^2) + E (90 mg/m2) + C (500 mg/m^2)	1	2	17	1	3	24
Classic chemotherapy						
T (75 mg/m^2) + E (90 mg/m^2) + C (500 mg/m^2)	4	6	60			70

E, epirubicin; P, cisplatin; G, gemcitabine; X, capecitabine; T, docetaxel; C, cyclophosphamide.

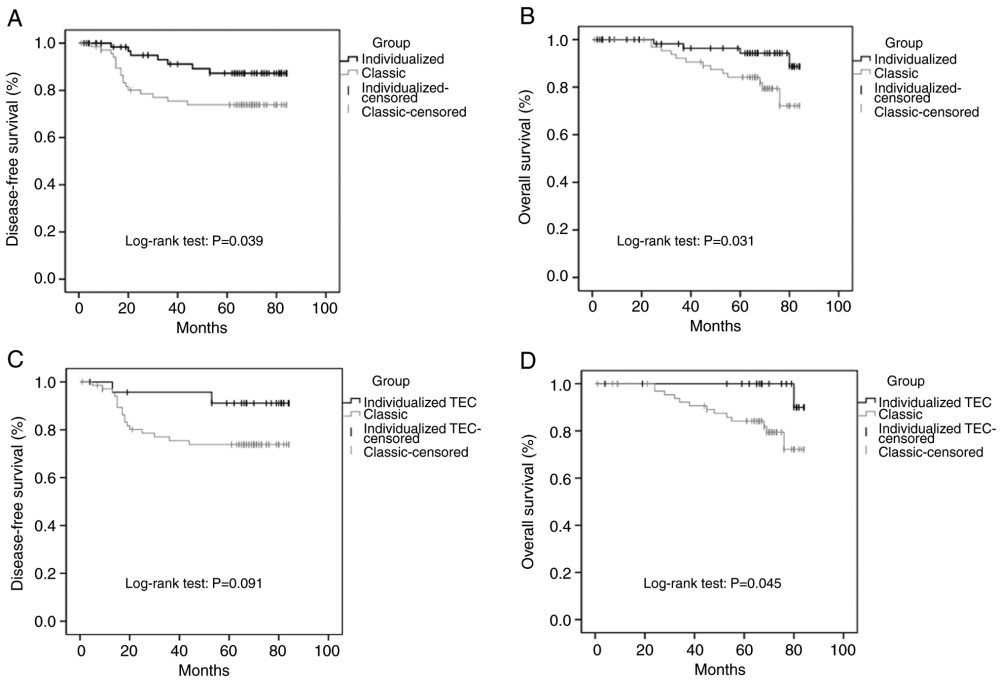

Figure 1. Kaplan-Meier curves showing the association of chemotherapy strategy with DFS and OS in patients with breast cancer. (A) Average DFS was 77.4 months in the individualized group compared with 67.1 months in the classic group. (B) Average OS was 81.4 months in the individualized group compared with 75.4 months in the classic group. (C) Average DFS was 79.5 months in the individualized TEC group compared with 67.1 months in the classic group. (D) Average OS was 83.6 months in the individualized TEC group compared with 75.4 months in the classic group. DFS, disease-free survival; OS, overall survival; TEC, docetaxel + epirubicin + cyclophosphamide.

TGTACCGATGCTG-3' and reverse, 5'-GCTGCTCTTCCT TTCCTGTGTT-3'; *TUBB3* forward, 5'-AGTCGCCCACGT AGTTGC-3' and reverse, 5'-CGCCCAGTATGAGGGAGA T-3'; *TYMS* forward, 5'-GCCTCGGTGTGCCTTTCA-3' and reverse, 5'-CGTGATGTGCGCAATCATG-3'; *TOP2A* forward, 5'-CATTGAAGACGCTTCGTTATGG-3' and reverse, 5'-CCAGTTGTGATGGATAAAATTAATCAG-3'; and *GAPDH* forward, 5'-GCCACATCGCTCAGACACC-3', and reverse, 5'-GATGGCAACAATATCCACTTTACC-3'.

Selection and implementation of chemotherapy schemes. The regimen of each patient in the individualized chemotherapy group was based on their genetic report. The principles of selection were as follows: i) Platinum drugs, such as cisplatin and oxaliplatin, are recommended for patients with low *ERCC1* expression; this regimen can be used in patients with low-to-medium expression but should be avoided in patients with medium-to-high and high expression (6). ii) Gemcitabine is recommended for patients with low *RRM1* expression; this

Table II. Baseline characteristics of the patients.

Characteristic	Group		t/χ^2-value	P-value
	Individualized regimen	Classic regimen		
Age (years)	51.1±8.1	48.5±7.6	1.939	0.055
BMI (kg/m^2)	23.8±2.9	23.8±3.1	0.011	0.991
Menstrual status				
Premenopausal	37 (52.9)	40 (57.1)	0.260	0.610
Postmenopausal	33 (47.1)	30 (42.9)		
Histological grade				
I	9 (12.9)	13 (18.6)	1.098	0.578
II	47 (67.1)	46 (65.7)		
III	14 (20.0)	11 (15.7)		
Tumor size (cm)				
≤2	22 (31.4)	29 (41.4)	3.161	0.182
2-5	45 (64.3)	35 (50.0)		
≥5	3 (4.3)	6 (8.6)		
Nodal status				
Negative	38 (54.3)	33 (47.1)	0.714	0.398
Positive	32 (45.7)	37 (52.9)		
TNM stage				
I	14 (20.0)	14 (20.0)	2.703	0.259
II	43 (61.4)	35 (50.0)		
III	13 (18.6)	21 (30.0)		
ER status				
Positive	47 (67.1)	45 (64.3)	0.127	0.722
Negative	23 (32.9)	25 (35.7)		
PR status				
Positive	34 (48.6)	42 (60.0)	1.842	0.157
Negative	36 (51.4)	28 (40.0)		
HER-2 status				
Positive	32 (45.7)	27 (38.6)	0.732	0.392
Negative	38 (54.3)	43 (61.4)		
Ki67 index				
≤14%	15 (21.4)	9 (12.9)	1.810	0.178
>14%	55 (78.6)	61 (87.1)		
Molecular type				
Luminal A	6 (8.6)	4 (5.7)	0.541	0.910
Luminal B	41 (58.6)	43 (61.4)		
HER-2-enriched	9 (12.9)	8 (11.4)		
Triple-negative	14 (20.0)	15 (21.4)		
Type of surgery				
Modified radical mastectomy	64 (91.4)	67 (95.7)	1.844	0.438
BCS + SLNB/T-ALND	4 (5.7)	1 (1.4)		
Mastectomy + SLNB	2 (2.9)	2 (2.9)		
Radiotherapy				
Yes	48 (68.6)	41 (58.6)	1.511	0.219
No	22 (31.4)	29 (41.4)		
Endocrine therapy				
Yes	42 (60.0)	35 (50.0)	1.414	0.234
No	28 (40.0)	35 (50.0)		

Values are presented as mean ± standard deviation or n (%). BMI, body mass index; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor 2; BCS, breast conserving surgery; SLNB, sentinel lymph node biopsy; T-ALND, total axillary lymphadenectomy.

Table III. Expression of five mRNAs in the individualized group.

Gene	Low	Low-to-medium	Medium	Medium-to-high	High
ERCC1	32 (45.7)	20 (28.6)	0 (0.0)	15 (21.4)	3 (4.3)
RRM1	45 (64.3)	14 (20.0)	0 (0.0)	7 (10.0)	4 (5.7)
TUBB3	17 (24.3)	19 (27.1)	0 (0.0)	15 (21.5)	19 (27.1)
TYMS	15 (21.4)	21 (30.0)	0 (0.0)	18 (25.7)	16 (22.9)
TOP2A	27 (38.6)	20 (28.6)	0 (0.0)	11 (15.7)	12 (17.1)

Values are presented as n (%). ERCC1, excision repair cross complementing 1; RRM1, ribonucleoside reductase M1; TUBB3, β-tubulin III; TYMS, thymidylate synthase; TOP2A, topoisomerase IIα.

regimen can be used in patients with low-to-medium expression but should be avoided in patients with medium-to-high and high expression (6). iii) Anti-microtubule drugs, such as docetaxel and paclitaxel, are recommended for patients with low *TUBB3* expression; this regimen can be used in patients with low-to-medium expression but should be avoided in patients with medium-to-high and high expression (9). iv) Capecitabine is recommended for patients with low *TYMS* expression; this regimen can be used in patients with low-to-medium expression but should be avoided in patients with medium-to-high and high expression (11). v) Anthracycline drugs, such as epirubicin and doxorubicin, are recommended for patients with high *TOP2A* expression (13); this regimen can be used in patients with medium-to-high expression but should be avoided in patients with low-to-medium expression and low expression. Although multiple treatments may be recommended based on these principles, only treatments that meet the guideline for diagnosis and treatment of breast cancer (version 2011) will be used for individualized chemotherapy (19). For the classic chemotherapy group, the docetaxel + epirubicin + cyclophosphamide (TEC) regimen was used. Details of the implementation of the chemotherapy regimens are shown in Table I.

Prognosis and safety evaluation. The endpoints of the study were disease-free survival (DFS) and overall survival (OS). DFS time was calculated as the length of time between the first confirmed diagnosis to tumor recurrence or metastasis. OS time was calculated as the length of time between the first confirmed diagnosis and mortality from any cause. Censoring was defined as being lost to follow-up or alive without relapse (local or distant) or mortality at the end of follow-up. Breast ultrasound, liver-focused abdominal ultrasound, axillary and neck lymph node ultrasound, chest computed tomography (CT), skull enhanced magnetic resonance imaging/CT, bone emission computed tomography, serum tumor markers and pathological examinations were performed as appropriate to detect whether local tumor recurrence or distant metastasis occurred. Survival data were obtained in follow-ups with all patients conducted via telephone contact or outpatient visits; the deadline was January 1, 2019. Adverse events associated with chemotherapy were evaluated and graded according to the National Cancer Institute Common Toxicity Criteria 4 (NCI-CTC version 4.0).

Statistical analysis. Categorical variables are presented as numbers and corresponding percentages, while continuous variables are presented as mean ± standard deviation. Student's t-test was applied to compare differences in age and BMI between the individualized and classic groups. The differences in other baseline characteristics and adverse events between the groups were evaluated using Pearson's χ^2 test. The Kaplan-Meier method was employed for survival analysis, and the curves were compared using the log-rank test. DFS time and OS time were analyzed using Kruskal-Wallis and Dunn's post hoc test. Cox's proportional hazards regression model was used to identify the independent predictors of DFS and OS. Univariate predictors with $P \leq 0.10$ were entered into a stepwise multivariate model to identify factors that independently predicted DFS and OS. For all analyses, a two-tailed $P \leq 0.05$ was considered to indicate a statistically significant result. All statistical analyses were performed using SPSS 17.0 software (SPSS, Inc.).

Results

Comparison of baseline characteristics. A total of 140 well-matched female patients with breast cancer were analyzed. All patients were histologically confirmed as having invasive ductal carcinoma and none of them had received targeted therapy or traditional Chinese medicine prior to surgery. There were no significant differences in baseline characteristics between the individualized chemotherapy and classic chemotherapy groups. Details of the baseline characteristics of the two groups of patients are summarized in Table II.

Gene expression. The mRNA expression levels of *ERCC1*, *RRM1*, *TUBB3*, *TYMS* and *TOP2A* were detected in the individualized chemotherapy group. Table III shows the case distribution according to expression intensity of the five mRNAs in the individualized group. High expression levels of *ERCC1* and *RRM1* were observed in 4.3 and 5.7% of the group, respectively, while high expression levels of *TUBB3* and *TYMS* were observed in 27.1 and 22.9% of the group, respectively. A low expression level of *TOP2A* was observed in 38.6% of the group.

Prognosis comparison. The median follow-up time among the patients included in the study was 67.5 months (range, 1.0-84.0 months). At the deadline, the tumor had progressed

Table IV. Disease-free and overall survival of the patients.

Variable	Group			χ^2/Z-value[a]	*P*-value[a]	χ^2/Z-value[b]	*P*-value[b]
	Individualized (n=70)	Individualized TEC (n=24)	Classic (n=70)				
Recurrence/metastasis [n (%)]	7 (10.0)	2 (8.3)	17 (24.3)	5.029	0.025	2.820	0.140
DFS time [mean (95% CI); months]	77.4 (72.7, 82.1)	79.5 (73.1, 85.9)	67.1 (60.2, 74.1)	4.251	0.039	2.855	0.091
5-year DFS rate (%)	87.3	91.1	73.8	3.609	0.057	4.518	0.034
Mortality [n (%)]	4 (5.7)	1 (4.2)	13 (18.6)	5.423	0.020	2.926	0.107
OS time [mean (95% CI); months]	81.4 (78.6, 84.1)	83.6 (82.9, 84.3)	75.4 (71.1, 79.8)	4.652	0.031	4.020	0.045
5-year OS rate (%)	94.3	90.0	84.2	3.249	0.071	0.302	0.583

[a]Individualized group vs. classic group; [b]individualized TEC group vs. classic group. TEC, docetaxel + epirubicin + cyclophosphamide; DFS, disease-free survival; OS, overall survival.

in 24 (17.1%) patients; 17 patients in the classic group and 7 patients in the individualized group, the latter of which included 2 patients who received TEC (from the individualized TEC group). Moreover, 17 (12.1%) patients had died; 13 patients in the classic group and 4 patients in the individualized group, which included 1 patient in the individualized TEC group. Compared with the classic group, the DFS and OS times of the individualized group were significantly prolonged (DFS, P=0.039; OS, P=0.031) and the OS time of the individualized TEC group was significantly prolonged (P=0.045). Furthermore, the 5-year DFS and OS rates of the patients in the individualized group were higher than those in the classic group (DFS, 87.3 vs. 73.8%; OS, 94.3 vs. 84.2%). The 5-year DFS rate of the individualized TEC group was higher than that of the classic group (91.1 vs. 73.8%; Table IV). The Kaplan-Meier survival curves of the patients are shown in Fig. 1. Compared with the classic group, the cumulative DFS rate and cumulative OS rate of the individualized group were significantly higher (Fig. 1A and B), and the cumulative OS rate of the individualized TEC group was significantly higher (Fig. 1D). However, no statistically significant difference was observed in the cumulative DFS rate between the individualized TEC group and the classic group (Fig. 1C).

Prognostic factors. Multivariable regression analyses were performed to identify prognostic factors for DFS and OS (Table V). The results revealed metastasis of axillary lymph nodes as an independent factor that increased the risk of tumor relapse (HR=7.049, 95% CI: 1.813, 27.410, P=0.005). Additionally, poor endocrine therapy compliance (treatment time <5 years) was identified as an independent risk factor that affected DFS (HR=3.378, 95% CI: 1.074, 10.624, P=0.037) and OS (HR=8.140, 95% CI: 1.666, 39.759, P=0.010). Furthermore, the individualized chemotherapy strategy guided by gene detection was shown to be an independent protection factor for DFS (HR=0.389, 95% CI: 0.153, 0.989, P=0.047) but not for OS (HR=0.340, 95% CI: 0.107, 1.078, P=0.067).

Comparison of adverse reactions. There were no significant differences in the incidence rate of dose reduction or reduction in the number of chemotherapy cycles (<6 cycles) due to adverse reactions between the individualized and classic groups (21.4 vs. 25.7%, P=0.550). In addition, there were no mortalities associated with adverse events in either of the treatment groups. It is noteworthy that there was no statistically significant difference in the incidence of other adverse events between the two groups. However, in terms of grade 2 or 3 palpitations and chest tightness, the incidence rate in the individualized group was lower than that in the classic group (12.9 vs. 27.1%, P=0.035). Furthermore, there was no statistically significant difference in the incidence of adverse events between the classic group and the individualized TEC group (Table VI).

Discussion

Individualized therapy has become an intensively pursued approach at the molecular level. Previous studies have indicated the important roles of *ERCC1*, *RRM1*, *TUBB3*, *TYMS* and *TOP2A* gene expression in the pathogenesis, diagnosis and prognosis of various types of carcinomas. Notably, as

Table V. Multivariable Cox's regression analysis of DFS and OS.

	DFS		OS	
Factor	HR (95% CI)	P-value	HR (95% CI)	P-value
Tumor size (cm)				
≤2	1.00			
2-5	2.700 (0.910, 8.008)	0.073		
≥5	1.783 (0.377, 8.443)	0.466		
Nodal status				
Negative	1.00		1.00	
Positive	7.049 (1.813, 27.410)	0.005	3.360 (0.836, 13.504)	0.088
TNM stage				
I	1.00		1.00	
II	0.351 (0.053, 2.330)	0.279	0.704 (0.115, 4.313)	0.704
III	0.420 (0.051, 3.458)	0.420	0.912 (0.119, 6.990)	0.930
ER status				
Positive	1.00		1.00	
Negative	1.258 (0.225, 7.037)	0.794	1.452 (0.071, 29.565)	0.808
PR status				
Positive	1.00		1.00	
Negative	1.727 (0.321, 9.281)	0.524	1.042 (0.050, 21.844)	0.979
Chemotherapy strategy				
Classic	1.00		1.00	
Individualized	0.389 (0.153, 0.989)	0.047	0.340 (0.107, 1.078)	0.067
Endocrine therapy compliance				
Good	1.00		1.00	
Poor	3.378 (1.074, 10.624)	0.037	8.140 (1.666, 39.759)	0.010

DFS, disease-free survival; OS, overall survival; ER, estrogen receptor; PR, progesterone receptor; HR, hazard ratio; CI, confidence interval.

their roles in chemoresistance have been fully confirmed, these genes are suitable markers to provide guidance for individualized cancer chemotherapy. However, to the best of our knowledge, there have been no studies on the combined detection of *ERCC1*, *RRM1*, *TUBB3*, *TYMS* and *TOP2A* gene expression to guide the selection of chemotherapy regimens for patients with breast cancer. The present study was designed to address this issue. The results demonstrated that individualized chemotherapy strategies can prolong DFS and OS, and also reduce adverse cardiovascular reactions, specifically palpitations and chest tightness, in patients with breast cancer.

ERCC1 is a key nuclease that regulates the nucleotide excision repair (NER) pathway, which serves an essential role in repair of DNA damage caused by platinum compounds (20,21). High expression of ERCC1 indicates increased NER activity that compromises the efficacy of platinum drugs. Certain studies have demonstrated that resistance to platinum-based chemotherapy is associated with high *ERCC1* expression levels in some advanced cancers, including gastric cancer (22), colorectal cancer (23), urinary tract cancer (5) and non-small cell lung cancer (24). Ribonucleotide reductase consists of two subunits, RRM1 and RRM2, and is the rate-limiting enzyme in the DNA synthesis pathway (25). The RRM1 subunit encoded by the *RRM1* gene is the main target of gemcitabine. Studies have shown that high *RRM1* expression is associated with gemcitabine resistance (6,7). TUBB3 is a major component of the microtubules, a constructive component of spindles and the cytoskeleton, that control mitosis and cellular motility (26). Upregulation of *TUBB3* expression, which may destabilize microtubules and counteract the effects of taxanes (9,27), has been confirmed in various cancer types, including breast (28,29), lung, ovarian, prostate, breast, stomach and pancreatic tumors (30). TYMS is a central enzyme in the synthesis of pyrimidine nucleotides and a major target for antifolate cytotoxic drugs, such as 5-fluorouracil and capecitabine. This enzyme exerts anticancer effects by inhibiting the synthesis of deoxythymidylate and further affecting DNA synthesis and repair (31). In clinical studies of breast cancer (32), colorectal cancer (33) and lung cancer (34), patients with low expression of *TYMS* have exhibited improved chemotherapeutic responses to fluorochemical drugs and a longer median survival time. TOP2A is an essential nuclear enzyme that changes the topology of DNA and is the primary molecular target of various cytotoxic agents, including anthracyclines (35), which stabilize the cleavable complex formed between DNA and topoisomerase II. Stabilization of the DNA-topoisomerase II complex results in increased DNA cleavage and inhibition of

Table VI. Adverse events among the patients.

	Group						
Grade	Individualized (n=70)	Individualized TEC (n=24)	Classic (n=70)	χ^2-value[a]	*P*-value[a]	χ^2-value[b]	*P*-value[b]
Nausea and vomiting							
1	28 (40.0)	8 (33.3)	29 (41.4)	0.478	0.788	0.674	0.784
2	37 (52.9)	14 (58.3)	34 (48.6)				
3	5 (7.1)	2 (8.3)	7 (10.0)				
Diarrhea							
1	62 (88.6)	20 (83.3)	64 (91.4)	0.317	0.573	1.232	0.271
2	8 (11.4)	4 (16.7)	6 (8.6)				
Constipation							
1	63 (90.0)	23 (95.8)	61 (87.1)	0.282	0.595	1.420	0.443
2	7 (10.0)	1 (4.2)	9 (12.9)				
Mucositis							
1	51 (72.9)	19 (79.2)	56 (80.0)	0.991	0.319	0.008	1.000
2	19 (27.1)	5 (20.8)	14 (20.0)				
Leukopenia/neutropenia							
1	23 (32.9)	11 (45.8)	26 (37.1)	0.319	0.853	0.598	0.775
2	29 (41.4)	8 (33.3)	28 (40.0)				
3,4	18 (25.7)	5 (20.8)	16 (22.9)				
Thrombocytopenia							
1	58 (82.9)	21 (87.5)	59 (84.3)	0.052	0.820	0.146	0.758
2	12 (17.1)	3 (12.5)	11 (15.7)				
Anemia							
1	66 (94.3)	22 (91.7)	59 (84.3)	3.659	0.056	0.817	0.504
2	4 (5.7)	2 (8.3)	11 (15.7)				
Liver toxicity							
1	46 (65.7)	16 (66.7)	58 (82.9)	5.351	0.059	3.189	0.144
2	19 (27.1)	7 (29.2)	10 (14.3)				
3	5 (7.1)	1 (4.2)	2 (2.9)				
Fatigue							
1	26 (37.1)	9 (37.5)	20 (28.6)	1.166	0.280	0.668	0.414
2	44 (62.9)	15 (62.5)	50 (71.4)				
Palpitations and chest tightness							
1	61 (87.1)	19 (79.2)	51 (72.9)	4.464	0.035	0.374	0.541
2,3	9 (12.9)	5 (20.8)	19 (27.1)				
Hand-foot syndrome							
1	52 (74.3)	17 (70.8)	58 (82.9)	1.527	0.271	1.602	0.243
2	18 (25.7)	7 (29.2)	12 (17.1)				

Values are presented as n (%). [a]Individualized group vs. classic group; [b]individualized TEC group vs. classic group. TEC, docetaxel + epirubicin + cyclophosphamide.

the rejoining of cleaved DNA, leading to cell death. Studies of the anthracycline chemotherapy of breast cancer showed that patients with low *TOP2A* expression had a poor response to treatment and poor prognosis (12,13,36). These findings led to the hypothesis that the detection of the expression of these genes will be beneficial for guiding the selection of chemotherapeutic drugs and may improve the efficacy of chemotherapy.

In the individualized group, the proportion of patients with medium-to-high and high expression levels of the genes that are negatively correlated with efficacy were as follows: *ERCC1*, 25.7%; *RRM1*, 15.7%; *TUBB3*, 48.6%; and *TYMS*,

48.6%. Low and low-to-medium expression levels of *TOP2A* were observed in 67.2% of the individualized group. As none of the patients received neoadjuvant therapy prior to surgery, the results indicate that some patients had primary resistance to certain chemotherapeutic drugs. Therefore, the regimens used for each patient in the individualized group were selected on the basis of their genetic report. The patients in the classic group all received chemotherapy according to the TEC regimen.

In the present study, an analysis of the survival data of breast cancer patients from the two groups was performed. The results showed that the DFS time in the individualized group was 10.3 months longer than that in the classic group (P=0.039), and the 5-year DFS rate was higher than that in the classic group (87.3 vs. 73.8%). The OS time in the individualized group was 6 months longer than that in the classic group (P=0.031), and the 5-year OS rate was higher than that in the classic group (94.3 vs. 84.2%). Furthermore, the Kaplan-Meier survival curves of DFS and OS showed that the overall prognosis of the patients in the individualized group was better than that in the classic group (log-rank test: P=0.039 and 0.031, respectively). To investigate the potential of selection of the individualized chemotherapy strategy under the guidance of genetic testing as an independent prognostic factor for breast cancer patients, the associations between all baseline variables and survival data were initially investigated in a univariate analysis (data not shown). Those variables with P≤0.10 were entered into the Cox's proportional hazards regression model for multivariable analysis. The regression analysis revealed that this individualized chemotherapy strategy can reduce the risk of recurrence or metastasis (HR=0.389, 95% CI: 0.153, 0.989, P=0.047). Furthermore, it was identified that metastasis of axillary lymph nodes was an independent risk factor for DFS, and poor endocrine therapy compliance was an independent risk factor for DFS and OS. In terms of drug safety, the majority of the patients tolerated and successfully completed 6-8 cycles of chemotherapy. Although various adverse reactions did occur during chemotherapy, they were controlled by symptomatic treatment, reduction of drug dosage, or the interruption or termination of chemotherapy. No grade 5 adverse events were reported in the study. The incidence of grade 2 or 3 palpitations and chest tightness in the individualized group was significantly lower than that in the classic group (12.9 vs. 27.1%, P=0.035). This may be associated with the use of anthracyclines, which were included in the classic regimen but only used selectively in the individualized group according to the patient's level of *TOP2A* gene expression. In addition, no significant differences were detected between the two groups in terms of the incidence of other adverse events, namely nausea and vomiting, diarrhea, constipation, mucositis, myelosuppression, liver toxicity, fatigue and hand-foot syndrome. It is noteworthy that 24 patients in the individualized group were treated using TEC regimens. To avoid the influence of different therapy regimens, the survival and adverse events in the classic group were compared with those in the individualized TEC group. Although the patients in the two groups were treated using the same TEC regimens, the overall prognosis of the individualized TEC group was improved compared with that of the classic group, and there was no significant difference between these two groups in the incidence of adverse events. These findings show that the selection of chemotherapy regimens according to each patient's gene expression characteristics can reduce the occurrence of drug resistance and increase therapeutic effectiveness, as well as providing new ideas and clinical evidence for the individualized treatment of breast cancer patients.

Admittedly, the present study has some limitations. First, this study used a nonrandomized patient cohort and a relatively small sample size, which may be inconsistent with previous studies. Second, gene expression was detected using MBL technology, but not confirmed by other methods using normal breast tissues or paracancerous tissue as a control. However, the reliability of the results is supported by the use of MBL technology, which is a mature gene detection technology that has been widely applied for predicting the prognosis and selecting the individualized treatment regimen for several types of tumors (15,37-40). Additionally, the genes investigated do not perform a single biological function. Further research is essential to explore the associations between the expression of these genes and other chemotherapeutic drugs. Finally, the application of testing technology may increase treatment costs and the benefit-cost ratio should be evaluated for each individual patient. In summary, large-scale, prospective studies with randomized patient cohorts, the addition of control samples and immunohistochemical confirmation are necessary to further investigate the guiding significance of the expression of *ERCC1*, *RRM1*, *TUBB3*, *TYMS*, *TOP2A* and other genes in the individualized therapy of breast cancer.

In conclusion, the findings of the present study indicate that therapeutic decision-making on the basis of *ERCC1*, *RRM1*, *TUBB3*, *TYMS* and *TOP2A* gene expression can prolong DFS and OS, improve prognosis, reduce cardiovascular adverse reactions such as palpitations and chest tightness, enhance the quality of life and benefit patients.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

JCL contributed to the study design as well as data analysis and interpretation, and drafted the manuscript. PS helped conceive the present study. TH helped analyze and interpret the data. SDH and LFL were involved in acquiring data and drafting the manuscript. PS and TH assessed and revised the manuscript critically for important intellectual content. GX participated in the study conception and design, contributed

to quality control of the data and algorithms, and edited and reviewed the manuscript. Each author participated sufficiently in the work to take public responsibility for appropriate portions of the content, and GX agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the General Hospital of Western Theater Command (Chengdu, China; approval no. 2011ky020). Written informed consent was provided by all patients prior to the study start for tissue sample retention and analysis for research purposes.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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