Combination of histoculture drug response assay and qPCR as an effective method to screen biomarkers for personalized chemotherapy in esophageal cancer

BIN WEI[1*], JIRU WANG[1*], XIAOHUI ZHANG[2*], ZHAOYE QIAN[1], JINGJING WU[3], YUAN SUN[1], QIN HAN[1], LI WAN[1], JING ZHU[1], YONG GAO[1] and XIAOFEI CHEN[1]

[1]Department of Medical Oncology, Huai'an First People's Hospital, Nanjing Medical University, Huai'an, Jiangsu 223300; [2]Department of Medical Oncology, The Second Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu 210011; [3]Department of Hematology, Huai'an First People's Hospital, Nanjing Medical University, Huai'an, Jiangsu 223300, P.R. China

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Abstract. Personalized chemotherapy with the use of biomarkers helps to maximize clinical efficiency. Therefore, the present study aimed to identify a potential method for identifying biomarkers in esophageal cancer. A total of 49 freshly resected tumor tissues and 72 paraffin-embedded specimens from patients with esophageal cancer were obtained. mRNA expression levels of ERCC1, BRCA1, TUBB3, FBW7, RRM1, MDM2, TS and TOP1 were measured quantitative reverse transcription polymerase chain reaction (RT-qPCR). *In vitro* chemosensitivity to cisplatin, docetaxel, gemcitabine, etoposide, fluorouracil and irinotecan were tested using histoculture drug response assay (HDRA). BRCA1 mRNA levels were positively correlated with resistance to cisplatin (P=0.027) and sensitivity to docetaxel (P=0.002). TS mRNA levels were inversely correlated with fluorouracil sensitivity (P=0.044), and TOP1 mRNA expression was positively correlated with irinotecan sensitivity (P=0.008). In addition, high BRCA1 mRNA levels correlated with decreased median overall survival (mOS; P<0.001) and response rate (RR; P=0.002) in cisplatin-fluorouracil chemotherapy group and also correlated with increased mOS (P<0.001) and RR (P=0.023) in docetaxel-fluorouracil chemotherapy group. Overall, these results suggested that HDRA combined with RT-qPCR may serve as an effective method for screening biomarkers in personalized chemotherapy for esophageal cancer.

Correspondence to: Dr Yong Gao or Dr Xiaofei Chen, Department of Medical Oncology, Huai'an First People's Hospital, Nanjing Medical University, 6 Beijing West Road, Huai'an, Jiangsu 223300, P.R. China
E-mail: hayy_gy@163.com
E-mail: hayycxf@163.com

*Contributed equally

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Introduction

Esophageal cancer is one of the leading causes of cancer-associated mortalities in China (1,2). Chemotherapy has been considered an essential method to treat esophageal cancer. However, the efficacy of the chemotherapeutic agents has been limited with rates between 11 and 35% (3-5). Furthermore, to date, there is no 'gold standard' chemotherapy for esophageal cancer. With the development of pharmacogenomics and pharmacogenetics, tumor heterogeneity is considered to be a significant factor that is responsible for the failure of conventional chemotherapeutics (6,7). In predictive biomarker studies, a number of genes have been reported to predict response to chemotherapy for solid tumors, including excision repair cross-complementation group 1 (ERCC1), breast cancer type 1 gene (BRCA1) or ADP ribosylation factor like GTPase 6 interacting protein 5 (JWA) for cisplatin (8), BRCA1, tubulin β-3 class III (TUBB3) or F-box and WD repeat domain containing 7 (FBW7) for docetaxel (9-11), thymidylate synthetase (TS) for fluorouracil (12), and ribonucleotide reductase catalytic subunit M1 (RRM1) for gemcitabine (13), murine double minute 2 (MDM2) for etoposide (14) and DNA topoisomerase 1 (TOP1) for irinotecan (15). Additionally, a few of the genes, including BRCA1, JWA and TS, have been validated for their clinical value in esophageal cancer (8,12). However, the clinical value of the aforementioned biomarkers for chemotherapeutic agents such as irinotecan (16), gemcitabine (5) and etoposide (17), which are not commonly used but exhibit moderate activity for treating esophageal cancer, remains unclear. Therefore, it is important to investigate practical methods, which can be used to screen and identify appropriate biomarkers for personalized therapy of esophageal cancer with chemotherapeutic agents of which there are limited clinical application data available.

The histoculture drug response assay (HDRA) is one of a number of *in vitro* tests for chemosensitivity, which allows the characteristics of the three-dimensional tissue structure to be maintained (18). HDRA has the advantage of being able

to maintain three-dimensional tissue structure and may be able to more accurately mimic the *in vivo* response compared with a cell culture model (18,19). The clinical reliability and utility of HDRA have been examined in several clinical studies for various solid tumors, including oral squamous cell carcinoma, head and neck, gastric, colorectal and ovarian cancer (20-22). Furthermore, HDRA has gradually been applied to identify candidate genes or gene sets with the capacity to predict efficiency of chemotherapeutic and targeted agents. Therefore, in the present study, HDRA was employed to evaluate the sensitivity of chemotherapeutic agents (cisplatin, docetaxel, gemcitabine, etoposide, fluorouracil and irinotecan) in tumor tissues, and the quantitative reverse transcription polymerase chain reaction method was performed to detect the mRNA expression of ERCC1, BRCA1, TUBB3, FBW7, RRM1, MDM2, TS and TOP1. Additionally, the present study verified the predictive value of a potential biomarker in patients with advanced esophageal cancer.

Patients and methods

Patients and sample collection. All patients and relevant clinical data were obtained from the Huai'an First People's Hospital, Nanjing Medical University (Huai'an, China) from May 2012 to June 2013. The median age was 62, and the majority of patients were male. Written informed consent for the use of tissue specimens was obtained from all patients, and the protocols for the present study were approved by the Ethics Committee of Huai'an First People's Hospital, Nanjing Medical University.

The surgically resected tumor specimens were obtained from 49 patients. Each specimen was divided into three parts. One part of the specimens was kept in 4˚C Hanks' balanced salt solution with 1% penicillin/streptomycin, and HDRA was employed to measure inhibition rates of chemotherapeutic agents *in vitro* within 15 min. Another part of the specimen was fixed by 10% formalin for 24 h at room temperature and embedded with paraffin for pathological observation. The rest of the tissue was stored in -80˚C for further detection of gene expression.

The paraffin-embedded tumor materials were collected from 72 cases with advanced esophageal cancer that received cisplatin-fluorouracil (cisplatin 25 mg/m^2 on day 1-3; fluorouracil 500 mg/m^2 on day 1-5) or docetaxel-fluorouracil (docetaxel 60-75 mg/m^2; fluorouracil 500 mg/m^2 on day 1-5) chemotherapy. Chemotherapy was repeated every 3-4 weeks for a maximum of six cycles unless patients had disease progression or in unsupportable adverse reactions.

HDRA. HDRA procedures were performed as previously described by Furukawa *et al* (18). Cancerous portions of specimens were washed three times with Hank's balanced salt solution and divided into ~10 mg pieces. Then, the tissue fragments were placed on prepared collagen sponge surfaces (Health Design, Rochester, NY, USA) in 24-well plates and incubated for 6 days in RPMI 1640 medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 20% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) at 37˚C in 5% CO_2. The concentration of each agent was determined by a preliminary experiment with 25% growth inhibition (IC_{25} value) as follows: 100 μg/ml for cisplatin, 30 μg/ml for docetaxel (Jiangsu Hengrui Medicine Co., Ltd., Nanjing, China), 10 μg/ml for fluorouracil, 30 μg/ml for gemcitabine, 10 μg/ml for etoposide and 10 μg/ml for irinotecan (Jiangsu Haosen Medicine Company, Nanjing, China).

Following histoculture, 100 μl Hank's balanced salt solution containing 0.1 mg/ml type I collagenase (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) and 100 μl MTT solution (2 mg/ml) were added to each well. The plates were incubated at 37˚C for another 24 h. Following extraction with dimethyl sulfoxide, the absorbance of the solution in each well was measured with microplate reader at 540 nm. Absorbance per gram of cultured tumor tissue was calculated from the mean absorbance from 8 parallel culture wells, and the weight of tumor tissue was determined prior to culture. The inhibition rate (IR) was calculated using the following formula: IR=(1-T/C) x100%, where T is the mean absorbance of the treated tumor/weight, and C is the mean absorbance of the control tumor/weight.

Quantitative reverse transcription polymerase chain reaction (RT-qPCR). Frozen tissues (~10 mg/per sample) were grinded in liquid nitrogen, and the total RNA was extracted by using the TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.). For paraffin-embedded tumor specimens, micro-dissection was performed to ensure serial sections of 7 mm in thickness with >80% of tumor cells. The pellet of micro-dissected cells was resuspended in Trizol reagent supplemented with proteinase K to extract RNA. Then RNA was reverse-transcribed with FastQuant RT kit (Tiangen Biotech Co., Ltd., Beijing, China). Each sample was detected in triplicate with RNase-free water, and commercial RNA as negative and positive control. Template cDNA was amplified with specific primers for different genes with the SuperReal PreMix Plus (Tiangen Biotech Co., Ltd., Beijing, China) by using the Real-Time PCR Detection system (Roche Applied Science Madison, WI, USA). The sequences of the primers are provided in Table I. Relative gene expression quantification was calculated using the Cq method. Final values were determined by the formula $2^{-\Delta\Delta Cq}$ and were analyzed with the Stratagene analysis software (version Mx3000P; Agilent Technologies, Inc., Santa Clara, CA, USA).

Statistical analysis. The Mann-Whitney U and Kruskal-Wallis tests were used to analyze the association between inhibition rates of agents or gene expression levels and clinical characteristics. The mean value was employed as the cutoff point of gene levels to divide the patients into low or high expression groups. The Mann-Whitney U test was used to compare the inhibition rates between the two groups. Clinical response was evaluated according to the Response Evaluation Criteria in Solid Tumors (23). Overall survival (OS) was calculated from the date of diagnosis to the date of last follow-up or mortality from any cause. The distributions of OS were analyzed using Kaplan-Meier method and compared with the two-sided log-rank test. $P<0.05$ was considered to indicate a statistically significant difference. Statistical analyses were performed by SPSS 19.0 software (IBM Corp., Armonk, NY, USA).

Table I. Primer sequences used for gene analysis.

Genes	Forward primer (5'-3')	Reverse primer (5'-3')
ERCC1	ACCCCTCGACGAGGATGA	GATGGC ATATTCGGCGTAGGT
BRCA1	TCCCATCTGTCTGGAGTTGA	GCCCTTTCTTCTGGTTGAGA
TUBB3	GCAGTCGCAGTTTTCACACTC	GCAGTCGCAGTTTTCACACTC
FBXW7	GGCCAAAATGATTCCCAGCAA	ACTGGAGTTCGTGACACTGTTA
RRM1	AGCAGCCAAAGTATCTAGTTCCA	AGCAGCCAAAGTATCTAGTTCCA
MDM2	TCGTCGGGTGAGGGTACTG	AACCACTTCTTGGAACCAGGT
TS	CTTCAGCGAGAACCCAGACC	TCCAGCCCAACCCCTAAAGAC
TOP1	GAGAGCTGTAGCCCTGTACTTCATC	CAGTGTCCGCTGTTTCTCCTT
β-actin	CTCCATCCTGGCCTCGCTGT	GCTGTCACCTTCACCGTTCC

ERCC1, excision repair cross-complementation group 1; BRCA1, breast cancer type 1 gene; TUBB3, tubulin β-3 class III; FBXW7, F-box and WD repeat domain containing 7; RRM1, ribonucleotide reductase catalytic subunit M1; MDM2, murine double minute 2; TS, thymidylate synthetase; TOP1, DNA topoisomerase 1.

Table II. Clinical characteristics of patients with esophageal cancer.

		Clinical cohort		
Clinical values	HDRA cohort	Total	Cisplatin-fluorouracil	Docetaxel-fluorouracil
Sex, n (%)				
Male	31 (63.3)	45 (62.5)	21 (61.7)	24 (63.2)
Female	18 (36.7)	27 (37.5)	13 (38.2)	14 (36.8)
Age, n (%)				
≤62	27 (55.1)	40 (55.6)	21 (61.7)	19 (50.0)
>62	22 (44.9)	32 (44.4)	13 (38.2)	19 (50.0)
Tumor site, n (%)				
Upper	5 (10.2)	7 (9.7)	4 (11.8)	3 (7.9)
Middle	29 (59.2)	52 (72.2)	23 (67.6)	29 (76.3)
Lower	15 (30.6)	13 (18.1)	7 (20.6)	6 (15.7)
Histological grade, n (%)				
1	10 (20.4)	3 (4.1)	2 (5.9)	1 (2.6)
2	39 (79.6)	49 (68.1)	25 (73.5)	24 (63.1)
3	0 (0.0)	20 (27.8)	7 (20.5)	13 (34.2)
Stage, n (%)				
II	27 (55.1)	0 (0.0)	0 (0.0)	0 (0.0)
III	22 (44.9)	4 (5.6)	2 (5.9)	1 (2.6)
IV	0 (0.0)	68 (94.4)	32 (94.1)	37(97.4)
Response rate, n (%)				
CR + PR			13 (38.2)	14 (36.8)
SD + PD			21 (61.8)	24 (63.2)
mOS (months, 95% CI)			13.2 (11.3-17.0)	10.3 (9.5-14.3)

CI, confidence interval; HDRA, histoculture drug response assay; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; mOS, median overall survival.

Results

Patient characteristics. Characteristics of all patients are shown in Table II. In the HDRA cohort, all patients were in stages II-III (24) at the time of diagnosis. In the clinical cohort, the patients were restricted to stages III-IV. In the clinical cohort, 34 patients treated with cisplatin-fluorouracil-based chemotherapy with response rate (RR) of 38.2%

Table III. Association between inhibition rates of chemotherapy agents and clinical characteristics.

	Inhibition rates (%) of chemotherapy agents (mean and 95% CI)[a]					
Clinical values	Cisplatin	Docetaxel	Gemcitabine	Etoposide	Fluorouracil	Irinotecan
Sex						
Male	39.1 (31.2-46.9)	21.3 (15.9-26.7)	18.4 (13.7-23.0)	20.1 (14.9-25.1)	17.5 (13.9-21.2)	20.5 (14.7-26.3)
Female	45.9 (36.3-55.5)	14.9 (9.6-20.3)	28.7 (19.3-38.0)	19.7 (12.9-26.5)	22.9 (14.6-31.3)	22.4 (14.2-30.5)
Age						
≤62	42.0 (34.5-49.5)	21.3 (16.5-26.2)	21.5 (15.5-27.4)	22.3 (16.6-28.1)	21.4 (15.9-26.8)	25.1 (19.0-31.2)
>62	41.1 (30.8-51.4)	16.1 (9.4-22.7)	23.0 (15.4-30.6)	16.9 (11.6-22.3)	17.3 (11.9-22.6)	16.4 (9.6-23.2)
Tumor site						
Upper	39.0 (12.3-65.8)	33.8 (12.4-55.3)	30.4 (0.0-61.2)	26.8 (12.1-41.4)	29.1 (14.8-43.3)	22.9 (5.1-50.9)
Middle	43.9 (35.8-52.2)	15.4 (11.2-19.5)	21.4 (16.7-26.1)	18.9 (13.6-24.3)	19.5 (14.2-24.7)	20.0 (15.0-25.0)
Lower	37.8 (27.0-48.7)	21.0 (13.1-28.9)	20.9 (10.6-31.3)	19.5 (12.0-29.9)	16.4 (10.4-22.5)	23.0 (12.5-33.4)
Histological grade						
1	39.7 (20.1-59.3)	22.6 (14.6-30.7)	21.7 (8.9-34.5)	27.4 (15.4-39.4)	22.2 (11.1-33.4)	26.2 (10.8-41.5)
2	42.1 (35.9-48.3)	18.1 (13.5-22.6)	22.3 (17.2-27.3)	18.0 (14.0-21.9)	18.8 (14.8-22.8)	19.9 (15.4-23.5)
Stage						
II	44.4 (35.9-52.9)	17.2 (11.9-22.4)	24.2 (16.7-31.6)	21.4 (15.8-26.9)	22.0 (16.1-27.9)	21.5 (13.6-29.4)
III	38.1 (29.4-46.8)	21.2 (15.0-27.4)	19.7 (14.8-24.6)	18.1 (12.3-23.9)	16.4 (12.3-20.6)	20.9 (17.0-24.7)

[a]All P>0.05. CI, confidence interval.

Table IV. Association between gene expression and clinical characteristics.

	Genes mRNA expression levels (mean ± standard error)[a]							
Clinical values	ERCC1	BRCA1	TUBB3	FBW7	RRM1	MDM2	TS	TOP1
Sex								
Male	4.4±0.5	1.1±0.1	1.0±0.2	5.0±0.9	1.7±0.2	5.5±0.9	1.7±0.4	3.4±0.9
Female	5.2±1.0	1.4±0.3	1.3±0.4	9.0±3.8	1.9±0.5	7.1±1.4	1.2±0.2	4.0±0.8
Age								
≤62	4.5±0.6	1.0±0.1	0.7±0.1	7.0±2.6	1.7±0.2	5.2±0.9	1.5±0.4	3.6±1.0
>62	4.2±0.8	1.5±0.3	1.5±0.4	5.9±1.0	1.9±0.4	7.2±1.3	1.6±0.3	3.7±0.7
Tumor site								
Upper	3.5±0.4	1.0±0.2	0.6±0.3	2.6±1.7	1.8±0.5	4.0±1.6	2.6±1.7	1.9±0.7
Middle	5.3±0.7	1.4±0.2	1.2±0.3	8.0±2.5	2.0±0.3	7.4±1.1	1.6±0.3	4.3±1.0
Lower	4.0±0.8	1.0±0.1	0.9±0.3	4.9±0.8	1.4±0.3	4.2±0.9	1.1±0.3	2.9±0.6
Histological grade								
1	4.8±1.4	1.2±0.2	0.8±0.3	4.4±1.1	1.3±0.3	4.4±1.1	1.3±1.1	3.4±1.3
2	4.7±0.5	1.2±0.2	1.1±1.2	7.0±1.9	1.9±0.3	6.5±0.9	1.6±0.3	3.7±0.7
Stage								
II	5.1±0.7	1.3±0.2	1.1±0.3	6.1±1.1	1.9±0.3	7.2±1.2	1.5±0.2	4.1±1.0
III	4.2±0.7	1.1±0.1	1.0±0.3	7.0±3.1	1.6±0.2	4.7±0.9	1.6±0.5	3.1±0.6

[a]All P>0.05. ERCC1, excision repair cross-complementation group 1; BRCA1, breast cancer type 1 gene; TUBB3, tubulin β-3 class III; FBXW7, F-box and WD repeat domain containing 7; RRM1, ribonucleotide reductase catalytic subunit M1; MDM2, murine double minute 2; TS, thymidylate synthetase; TOP1, DNA topoisomerase 1.

and median OS (mOS) of 13.2 [95% confidence interval (CI), 11.3-17.0] months, while the other 38 patients received the docetaxel-fluorouracil-based chemotherapy with RR of 36.8% and mOS of 10.3 (95% CI, 9.5-14.3) months.

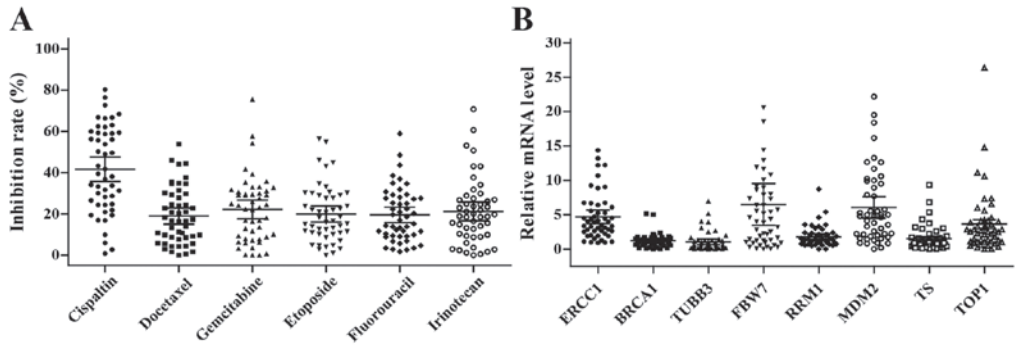

Figure 1. Chemosensitivity and gene expression levels in esophageal cancer tissues. (A) *In vitro* chemosensitivity to cisplatin, docetaxel, gemcitabine, etoposide, fluorouracil and irinotecan were tested using histoculture drug response assay. (B) The levels of ERCC1, BRCA1, TUBB3, FBW7, RRM1, MDM2, TS and TOP1 mRNA expression in tumor tissues were analyzed by quantitative PCR. The bars indicate the mean and 95% confidence interval. ERCC1, excision repair cross-complementation group 1; BRCA1, breast cancer type 1 gene; TUBB3, tubulin β-3 class III; FBXW7, F-box and WD repeat domain containing 7; RRM1, ribonucleotide reductase catalytic subunit M1; MDM2, murine double minute 2; TS, thymidylate synthetase; TOP1, DNA topoisomerase 1.

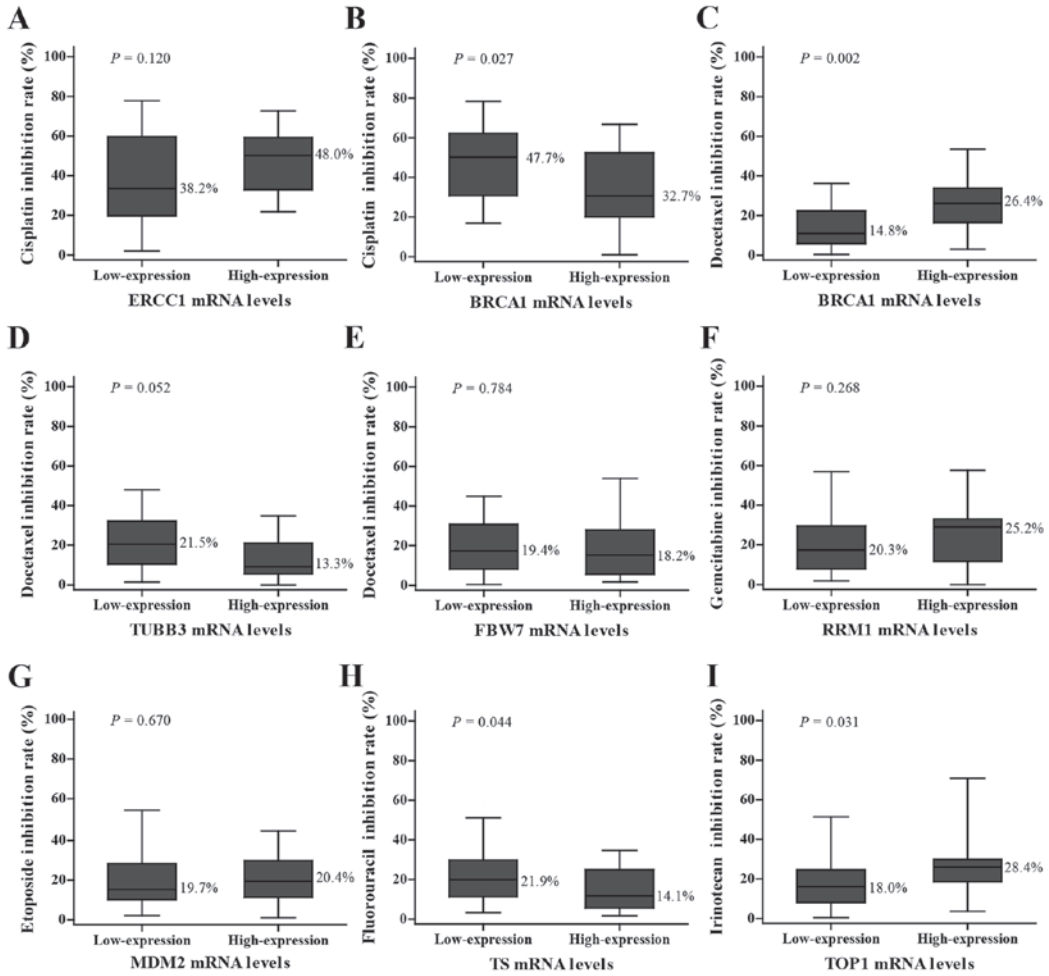

Figure 2. Association between gene expression levels and chemosensitivity. The 49 samples were divided into two subgroups by mean gene expression. The box plots indicate the inhibition rates of chemotherapeutic agents (A) ERCC1 and cisplatin; (B) BRCA1 and cisplatin; (C) BRCA1 and docetaxel; (D) TUBB3 and docetaxel; (E) FBW7 and docetaxel; (F) RRM1 and gemcitabine; (G) MDM2 and etoposide; (H) TS and fluorouracil; and (I) TOP1 and irinotecan. The bars indicate the mean, 5th and 95th percentile. ERCC1, excision repair cross-complementation group 1; BRCA1, breast cancer type 1 gene; TUBB3, tubulin β-3 class III; FBW7, F-box and WD repeat domain containing 7; RRM1, ribonucleotide reductase catalytic subunit M1; MDM2, murine double minute 2; TS, thymidylate synthetase; TOP1, DNA topoisomerase 1.

Inhibition rates of chemotherapeutic agents. The ability of 6 chemotherapeutic agents to inhibit the growth of 49 tumor specimens was successfully tested using HDRA. Not only did the spectrum of sensitive agent vary between individual

Table V. Outcomes in different chemotherapy groups stratified by BRCA1 expression.

			mOS		RR, n (%)		
Treatments	BRCA1	No.	Median (95% CI)	P-value	CR + PR	SD + PD	P-value
Cisplatin/fluorouracil	Low	21	16.5 (14.6-18.4)		38.1	61.9	
	High	13	6.5 (5.4-7.6)	<0.001	7.7	92.3	0.002
Docetaxel/fluorouracil	Low	24	6.8 (5.7-7.9)		20.8	79.2	
	High	14	22.0 (14.0-26.8)	<0.001	57.1	42.9	0.023

BRCA1, breast cancer type 1 susceptibility protein; CI, confidence interval; mOS, median overall survival; RR, response rate; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

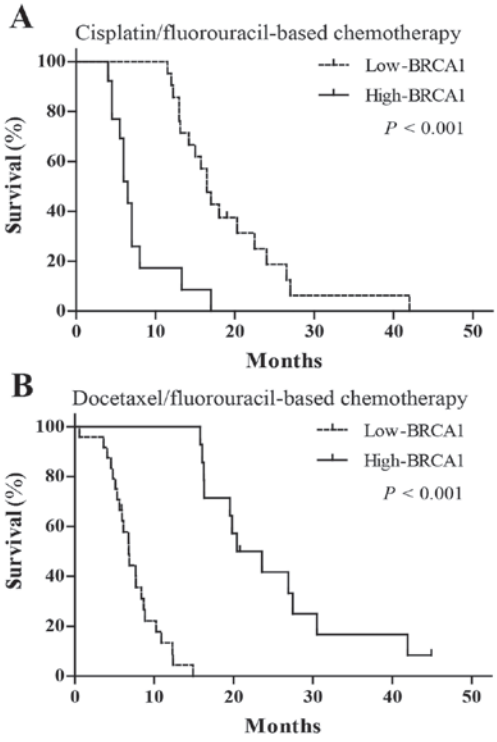

Figure 3. Median overall survival according to BRCA1 mRNA expression in different treatment groups. (A) Cisplatin-fluorouracil chemotherapy group. (B) Docetaxel-fluorouracil chemotherapy group. BRCA1, breast cancer type 1 gene; mRNA, messenger RNA.

patients, but the inhibition rate of each agent also varied and ranged widely. The average inhibition rates were as follows: Cisplatin, 37.6% (95% CI, 35.6-47.5%); docetaxel, 20.0% (95% CI, 15.1-22.9%); gemcitabine, 22.2% (95% CI, 16.0-23.8%); etoposide, 19.9% (95% CI, 16.0-23.8%); fluorouracil, 19.5% (95% CI, 15.8-23.2%); and irinotecan, 21.2% (95% CI, 16.7-25.7%) (Fig. 1A). However, there was no significant association between inhibition rates and clinical characteristics (all P>0.05, Table III).

Gene expression levels. The mRNA expression levels of 8 genes were detected in all tumor tissues, with mean levels of 4.7 (95% CI, 3.7-5.7) for ERCC1; 1.2 (95% CI, 0.9-1.5) for BRCA1; 1.1 (95% CI, 0.7-1.5) for TUBB3; 6.5 (95% CI, 3.5-9.5) for FBW7; 1.8 (95% CI, 1.3-2.2) for RRM1; 6.1 (95% CI, 4.5-7.6) for MDM2; 1.5 (95% CI, 1.0-2.1) for TS; and 3.6 (95% CI, 2.4-4.9) for TOP1 (Fig. 1B). No association between clinical characteristics and gene expression was identified (all P>0.05, Table IV).

Association between gene expression and chemosensitivity. The inhibition rates of various chemotherapeutic agents are indicated in Fig. 2. The inhibition rate of cisplatin in the group with low BRCA1 mRNA expression was higher compared with the group with high expression (44.7 vs. 32.7%; P=0.027; low expression vs. high expression group; Fig. 2B), while the result of docetaxel inhibition rate was the opposite with a higher inhibition rate in the high expression group (14.8 vs. 26.4%, low expression vs. high expression group; P=0.002; Fig. 2C). The tissues with low TS mRNA expression levels had a higher sensitivity to fluorouracil compared with those with high expression levels (21.9 vs. 14.1%; P=0.044; Fig. 2H). The group with high TOP1 mRNA expression levels was more sensitive to irinotecan compared with those with low expression levels (18.0 vs. 28.4%; P=0.031; Fig. 2I). However, statistically insignificant associations between genes levels and inhibition rates were also observed as follows: ERCC1 with cisplatin (38.2 vs. 48.0%, P=0.120; Fig. 2A), TUBB3 with docetaxel (21.5 vs. 13.3%, P=0.052; Fig. 2D), FBW7 with docetaxel (19.4 vs. 18.2%, P=0.784; Fig. 2E), RRM1 with gemcitabine (20.3 vs. 25.2%, P=0.268; Fig. 2F) and MDM2 with etoposide (19.7 vs. 20.4%, P=0.670; Fig. 2G).

Association between BRCA1 expression and clinical outcomes in patients treated with chemotherapy. In order to verify whether the predictive effects of screened biomarkers in personalized chemotherapy is consistent with *in vitro* chemosensitivity, the authors of the present study further investigated the associations of BRCA1 mRNA expression with clinical outcomes in patients with advanced esophageal cancer, who were treated with cisplatin-fluorouracil or docetaxel-fluorouracil chemotherapy. The findings indicated that patients treated with cisplatin-fluorouracil chemotherapy with low BRCA1 expression had increased mOS (16.5 vs. 6.5 months; P<0.001; Fig. 3A, Table V) and RR (38.1 vs. 7.7%; P=0.002; Table V) compared with those with high expression. However, patients treated with docetaxel-fluorouracil chemotherapy with high BRCA1 mRNA expression had increased mOS (22.0 vs. 6.8 months; P<0.001; Fig. 3B, Table V) and RR

(57.1 vs. 20.8%; P=0.023; Table V) compared with those with low BRCA1 expression.

Discussion

Treatment options in esophageal cancer have advanced over the last several years with the introduction of effective chemotherapeutics. However, personalized therapy is far from being implemented due to the lack of effective predictive biomarkers (25). Currently, the prediction of response to chemotherapy at the molecular level is primarily based on data derived from *in vitro* experiments (26-28). Furthermore, studies, which utilize cell culture model of tumors, organoid cultures or xenografts currently best mimic the characteristics of an *in vivo* tumor (29). An *in vitro* histoculture system is able to maintain the structure of the three-dimensional tissue and the natural tumor environment (18,19). Despite mouse xenograft models having the advantages of being able to mimic the micro-environmental conditions, tumor architecture, angiogenesis and metastasis present in a real patient, the *in vitro* histoculture system has relative advantages of good availability, low cost, ease of handling and short intervention time. Therefore, in the present study the HDRA histoculture system was selected to determine chemosensitivity. In addition, 8 parallel culture wells were designed to test the chemosensitivity of different parts of tumor specimen to avoid the issue of tumor heterogeneity.

The efficacy rate for an individual agent using HDRA *in vitro* has a considerable good correlation with clinical response rate to each agent (30,31). Previous studies have reported that TS and DPD expression are correlated with fluorouracil sensitivity (32-35). It was also reported that ERCC1 expression and SULF2 methylation are correlated with platinum sensitivity (33,36). Furthermore, CXCR4 and TUBB3 expression are correlated with docetaxel sensitivity (37,38). Aprataxin (APTX) expression also has been correlated with irinotecan sensitivity (29,33), and MET expression has been correlated with crizotinib sensitivity (39) using HDRA. These results suggest that HDRA can be used in predictive biomarker studies. In addition, the qPCR method, which may be more clinically useful with the benefits of being able to provide more quantitative and accurate measurement compared with fluorescence *in situ* hybridization and immunohistochemistry, has been widely employed to detect the expression levels of candidate genes in the aforementioned studies. Therefore, in the present study, a total of 8 candidate biomarkers were selected based on literature review, and the gene expression patterns were analyzed using qPCR. A number of potential biomarkers for chemotherapy in esophageal cancer were identified, including BRCA1 for docetaxel or cisplatin, TS for fluorouracil and TOP1 for irinotecan. The authors then considered whether combining HDRA with qPCR for biomarker discovery may provide novel opportunities for prediction of individual response to chemotherapy. Despite the identification of a number of candidate biomarkers for esophageal cancer in the present study, whether the predictive functions of these genes may be reproduced in clinical practice still requires validation.

Previous studies have evaluated the predictive value of potential biomarkers generated from HDRA in xenograft model and in clinical settings. Shen *et al* (29) established different mice models with patient-derived gastric cancer xenografts and demonstrated that tumor growth is significantly suppressed in the cohort with sensitive-signature based on the expression of APTX, BRCA1 and TOP1. Yang *et al* (39) have reported that patient-derived tumor xenograft models with higher MET expression exhibited high sensitivity to crizotinib, and tumor shrinkage was observed in a patient with advanced gastric cancer and MET overexpression following crizotinib treatment. However, differences in metabolism, body size and genetic background between the host species and humans may have an impact on the predictive value of biomarkers.

Therefore, in the present study, a total of 72 patients with advanced esophageal cancer, who were treated with cisplatin-fluorouracil or docetaxel-fluorouracil chemotherapy, were recruited, and the predictive function of BRCA1 in personalized treatment was analyzed. Fluorouracil was used in both regimens, as the presence of BRCA1 did not confer resistance or sensitivity to fluorouracil.

High expression of BRCA1 mRNA was negatively associated with RR and mOS in patients treated with cisplatin-fluorouracil chemotherapy. Conversely, high BRCA1 expression was also positively associated with clinical outcomes in those who received docetaxel-fluorouracil chemotherapy. As a dual predictive biomarker, the results were consistent with previous findings by the present authors (40,41). The findings supported the hypothesis that the use of a combination of HDRA and qPCR is able to effectively distinguish biomarkers in their ability to evaluate response to chemotherapy.

In summary, the present study observed that the level of BRCA1, TS and TOP1 mRNA present make these genes suitable as predictable biomarkers to assess the response of cisplatin, docetaxel, fluorouracil or irinotecan in patients with esophageal cancer. Furthermore, the combination of HDRA and qPCR may be an effective method for screening biomarkers to assess chemosensitivity in personalized chemotherapy for esophageal cancer.

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