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Expression levels of HER2 and MRP1 are not prognostic factors of long-term survival in 829 patients with esophageal squamous cell carcinoma

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Abstract. Esophageal squamous cell carcinoma (ESCC) is the eighth most frequent neoplasm in China. However, the expression levels of human epidermal growth factor receptor 2 (HER2) and multidrug resistance protein 1 (MRP1) in patients with ESCC remain to be determined. In the present study, 829 ESCC cases were evaluated using immunohistochemistry. The association between the expression levels of HER2 and MRP1 and the patient's clinicopathological factors was analyzed using Fisher's exact test or χ^2 test. Univariate analysis was performed via Kaplan-Meier survival curves, while the Cox proportional hazard model was used for multivariate analysis. A significant correlation was observed between the expression levels of HER2 and the patient's gender (P<0.050), tumor size (P=0.013) and venous/lymphatic invasion (P=0.039). However, no significant correlation was identified between the expression levels of MRP1 and the clinicopathological factors of the patients. In univariate analysis, gender, differentiation, depth of invasion, clinical stage, adjuvant radiotherapy or chemotherapy and lymph node metastasis were significantly correlated with progression-free survival (PFS) and overall survival (OS) in patients with ESCC (P<0.050). The graphical representation of the Kaplan-Meier estimate curves suggested that the expression levels of HER2 or MRP1 did not exert any influence on prognosis (log-rank test, P>0.050). In multivariate analysis, tumor location, gender, clinical stage, differentiation and lymph node metastasis were identified as independent factors of prognosis in patients with ESCC (P<0.050). However, the expression levels of HER2 or MRP1 were not independently associated with PFS or OS in these patients. In conclusion, the present large-scale study demonstrates that the protein expression levels of HER2 and MRP1 does not exert any influence on the prognosis of ESCC.

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Introduction

Esophageal carcinoma (EC) is one of the most common malignancies in the world, while esophageal squamous cell carcinoma (ESCC) is the main histopathological subtype of EC in Eastern Asian countries, including China (1). Although the therapeutic treatments for ESCC such as surgery, chemotherapy, radiotherapy and target therapy, have improved in recent years, the five-year survival rate for patients with resectable disease is <40% (2,3). Thus, an increasing interest exist on the prognostic and therapeutic value of biological markers that participate in the carcinogenesis and progression of ESCC.

Human epidermal growth factor receptor (EGFR) 2 (HER2), also known as Erb-B2 receptor tyrosine kinase 2 and c-erbB2, is a member of the EGFR family, whose abnormal activation appears to be involved in tumor development and progression in ESCC (4,5). In addition, HER2 is a therapeutic target in several types of cancer. Previous studies have observed that the oncogene *HER2/neu* was overexpressed in 2.0-19.1% cases of ESCC (6-13), and increased protein expression levels of HER2 have been previously observed in EC (14,15) and ESCC (6,7). However, the role of HER2 in ESCC remains controversial (6,7,9,10,12,16-19). Multidrug resistance protein 1 (MRP1), as a member of the adenosine triphosphate-binding cassette transporter family, has been implicated in resistance to cancer therapeutics (20). High expression levels of MRP1 have been observed in various solid tumors such as lung cancer, and the expression levels of MRP1 have been reported to inversely correlate with prognosis in patients with lung cancer (21-23). In addition, patients with Barrett's carcinoma treated with neoadjuvant chemotherapy who exhibited high messenger RNA expression

levels of MRP1 presented prolonged survival, compared with those patients whose levels of MRP1 were low (24). However the role of MRP1 expression in patients with ESCC remains unclear.

The aim of the present retrospective study was to determine the clinical significance of HER2 and MRP1 expression in a large-scale cohort study involving patients with ESCC who had undergone surgical resection. For that purpose, the protein expression levels of HER2 and MRP1 were detected by immunohistochemistry (IHC), and the association between the clinicopathological features of this disease and the prognostic value of HER2 and MRP1 expression in patients with ESCC was evaluated.

Materials and methods

Patients. Between June 2002 and June 2010, all the consecutive cases of patients with clinical resectable ESCC who had been treated at the Department of Medical Oncology of Zhejiang Cancer Hospital (Hangzhou, China) were retrospectively reviewed. The present study was approved by the institutional review board of Zhejiang Cancer Hospital, and all the patients provided written informed consent for participation in the study. All the patients included in the study had been subjected to complete resection, and none had received neoadjuvant treatment. Those patients who succumbed to the disease within 30 days following surgery were excluded from the study. Cancer stage was determined during the postoperative pathological examination, and included the status of primary tumor invasion, regional lymph nodes and distant metastases, according to the 7th edition of the Cancer Staging Manual published by the American Joint Committee on Cancer (25). Long-term postoperative follow-up consisted of a telephone interview conducted every three months for the first three years, every six months during the fourth and fifth year, and every year thereafter. The date of the last follow-up was April 30, 2014. Overall survival (OS) was defined from the time of diagnosis until the date of mortality or until the date of the last follow-up visit. Progression-free survival (PFS) was measured from the time of completion of the surgery until the time of documented tumor recurrence or mortality. The present study was conducted according to the REporting recommendations for tumor MARKer prognostic studies guidelines (26).

Immunohistochemistry. Immunohistochemical analyses were performed by the avidin-biotin peroxidase method, using ultraView Universal DAB Detection Kit (Ventana Medical Systems, Inc., Tucson, AZ, USA). For this purpose, paraffin sections of 4 mm thickness were excised from paraffin blocks, and subsequently immunostained with rabbit monoclonal primary antibodies against HER2 (catalogue no. 4B5; dilution, 1:100; Roche Diagnostics GmbH, Mannheim, Germany) and MRP1 (catalogue no. H-70; dilution, 1:100; Santa Cruz Biotechnology, Inc., Dallas, TX, USA). The primary antibodies were detected with an automated staining system (BenchMark XT; Roche Diagnostics GmbH).

Next, the slides were washed with phosphate-buffered saline (PBS) three times, and incubated with the secondary antibody provided in the ultraView DAB Detection Kit (Fuzhou Maixin Biotech. Co., Ltd, Fujian, China). The colorimetric reaction was developed with 3,3'-diaminobenzidine. Counterstaining was performed with hematoxylin. All steps were conducted at room temperature. For the negative control, the primary antibody was replaced with PBS.

The immunostaining results were independently examined by two clinical pathologists who were blinded to the patients' information, using a DM4000B light microscope (Leica, Wetzlar, Germany). For each sample, five high-power fields (magnification, x400) were randomly selected. The percentage of positive cells and the intensity of the staining were assessed, and a semi-quantitatively score ranging from 0 to 3 was assigned to the samples accordingly. The staining intensity was scored as follows: i) 0, no staining; ii) 1, weak staining; iii) 2, moderate staining; and iv) 3, strong staining. This score was then multiplied by the percentage of positively stained cells present in the sample, as follows: i) 0, no staining; ii) 1, ≤10% of stained cells; iii) 2, 11-25% of stained cells; iv) 3, 26-50% of stained cells; and v) 4, ≥51% of stained cells. The resulting scores were used to classify the samples as follows: i) 0, negative staining; ii) 1-4, weak positive staining; iii) 5-8, moderate staining; and iv) 9-12, strong positive staining.

Statistical analysis. The correlation between the expression levels of HER2 and MRP1 and the patient's clinicopathological factors was analyzed using Fisher's exact test or χ^2 test. Univariate and multivariate analyses were performed with Kaplan-Meier survival curves and Cox proportional hazard model, respectively. Log-rank test was used to evaluate the significance of the differences observed between pairs of survival probabilities. $P<0.050$ was considered to indicate a statistically significant difference. Statistical data were calculated with SPSS version 18.0 software (SPSS Inc., Chicago, IL, USA).

Results

Patient's characteristics. The cohort of 829 patients with ESCC whose expression levels of MRP1 were analyzed in the present study exhibited a female:male ratio of 1.0:4.5 and a median age of 59 years (range, 34-78 years). Among the 829 patients, 768 patients also detected HER-2 expression. ESCC was observed to be well differentiated in 110 cases (13.3%), moderately differentiated in 561 cases (67.7%), poorly differentiated in 153 cases (18.4%) and undifferentiated in 5 cases (0.6%). The enrolled patients were staged pathologically as I (n=61), II (n=335) and III (n=433). Complete resection was confirmed histologically in all patients. Locoregional lymph node metastasis was observed in 477 patients (57.5%). A total of 260 patients who presented poor prognostic factors, metastatic disease or recurrence following operation, received adjuvant radiotherapy and/or chemotherapy subsequently to surgery. A total of 762 patients were available for survival analysis, with a median duration of postoperative follow-up of 32 months (range, 1.03-102.00 months). Among them, 733 patients were followed-up for >3 years. The clinicopathological factors, staging and survival of the patients included in the present study have been previously reported (27,28).

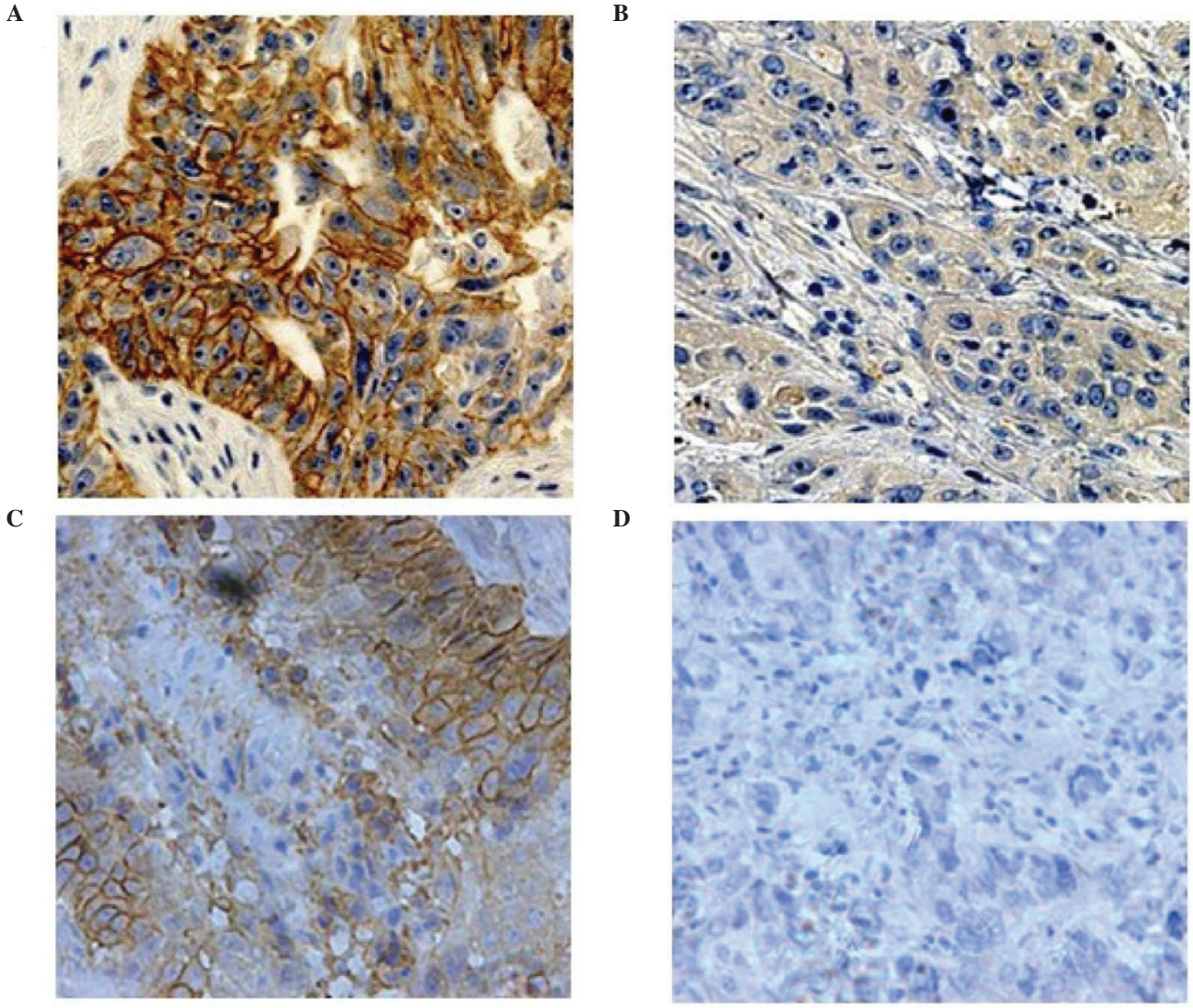

Figure 1. Immunohistochemical staining of HER2 and MRP1 in esophageal squamous cell carcinoma tissue. (A and C) Positive and (B and D) negative protein expression of HER2 and MRP1, respectively (magnification, x400). HER2, human epidermal growth factor receptor 2; MRP1, multidrug resistance protein 1.

Association between HER2 and MRP1 expression and clinicopathological features. In ESCC tissues, HER2 exhibited membrane staining, while MRP1 displayed membrane and cytoplasmic staining (Fig. 1). A total of 768 patients were available for evaluation of HER2 expression. Among them, 543 cases (70.7%) were observed to be negative, 170 cases (22.1%) were scored as 1, 37 cases (4.8%) as 2 and 18 cases (2.3%) as 3. For statistical analysis, samples with a final score of 0 were considered to exhibit low expression levels of HER2, whereas those with a final score of 1-3 were considered to display high expression levels of HER2. Among the 829 cases who were assessed for MRP1 expression by IHC, 326 (39.3%), 291 (35.1%), 144 (17.4%) and 68 (8.2%) cases were scored as 0, 1, 2 and 3, respectively. For statistical analysis, samples with a final score of 0 or 1 were considered to exhibit low expression levels of MRP1, while those with a final score of 2 or 3 were considered to display high expression levels of MRP1.

The associations between the expression levels of HER2 and MRP1 and the clinicopathological features of the patients are presented in Table I. A significant correlation was observed between the expression levels of HER2 and gender (P<0.050), tumor size (P=0.013) and venous/lymphatic invasion (P=0.039). However, no significant correlation was identified between MRP1 expression and any of the clinicopathological factors analyzed. Furthermore, high expression levels of HER2 were positively correlated with high expression levels of MRP1 (P=0.001).

Survival analysis. During the follow-up, 397 patients succumbed to ESCC, while recurrence or metastasis occurred in 470 patients. The metastatic areas included the supraclavicular lymph node, mediastinal lymph node, liver, lung, skeleton and brain.

In univariate analysis, gender, differentiation, depth of invasion, clinical stage, adjuvant radiotherapy or chemotherapy and lymph node metastasis were significantly correlated with the patients' PFS and OS (P<0.050) (Table II). However, the graphic pattern of the Kaplan-Meier estimate curves (Fig. 2) suggested that the expression levels of HER2 or MRP1 did not

Table I. Analysis of the association between the expression levels of HER2 and MRP1 and the clinicopathological features of patients with esophageal squamous cell carcinoma.

	HER2 expression levels (no. of patients)				MRP1 expression levels (no. of patients)			
Characteristics	Total	Low	High	P-value	Total	Low	High	P-value
Gender				0.000				0.516
Male	627	466	161		678	499	179	
Female	141	77	64		151	115	36	
Age (years)				0.760				0.702
≥55	491	349	142		527	388	139	
<55	277	194	83		302	226	76	
Smoking				0.163				0.532
Never	179	134	45		202	153	49	
Ever	589	409	180		627	461	166	
Alcohol consumption				0.178				0.755
Never	256	189	67		282	207	75	
Ever	512	354	158		547	407	140	
Differentiation				0.216				0.055
Intermediate/well	622	447	175		671	507	164	
Poor/undifferentiated	144	96	48		158	107	51	
Tumor size (cm)				0.013				0.671
<5	442	297	145		464	341	123	
≥5	326	246	80		365	273	92	
Depth of invasion				0.678				0.362
T1+T2	160	111	49		171	122	49	
T3	608	432	176		658	492	166	
Lymph node metastasis				0.347				0.126
N0	321	232	89		352	273	79	
N1	214	148	66		232	162	70	
N2	159	106	53		169	120	49	
N3	74	57	17		76	59	17	
Clinical stage				0.647				0.456
I+II	358	256	102		396	298	98	
III	410	287	123		433	316	117	
Venous/lymphatic invasion				0.039				0.094
No	622	450	172		672	506	166	
Yes	146	93	53		157	108	49	
Perineural invasion				0.050				0.219
No	588	426	162		638	466	172	
Yes	180	117	63		191	148	43	
Adjuvant radio/chemotherapy				0.269				0.196
No	517	359	158		569	429	140	
Yes	251	184	67		260	185	75	

HER2, human epidermal growth factor receptor 2; MRP1, multidrug resistance protein 1.

have any impact on prognosis (log-rank, P>0.050). In multivariate analysis, tumor location, clinical stage and lymph node metastasis were identified as independent factors of prognosis in patients with ESCC (Table III). However, the expression levels of HER2 or MRP1 were not observed to be independently associated with PFS and OS in these patients (Table III).

Discussion

HER2 is a therapeutic target in several tumors such as breast cancer (29-32), and a predictive factor of response to chemotherapy in various solid malignancies (33-36). In order to identify improved prognostic indicators for ESCC, the

Table II. Univariate analysis of factors that influence the survival of patients with esophageal squamous cell carcinoma.

	Progression-free survival		Overall survival	
Factors	No. of patients	P-value	No. of patients	P-value
Gender		0.009		0.018
Male	554		645	
Female	108		129	
Age (years)		0.339		0.843
≥55	416		491	
<55	246		283	
Smoking		0.315		0.177
Never	156		186	
Ever	506		588	
Alcohol consumption		0.869		0.199
Never	218		266	
Ever	444		508	
Location		0.400		0.149
Upper/middle	274		338	
Lower	388		436	
Differentiation		0.000		0.000
Intermediate/well	528		618	
Poor/undifferentiated	134		155	
Tumor size (cm)		0.164		0.023
<5	369		444	
≥5	293		330	
Depth of invasion		0.000		0.000
T1+T2	131		168	
T3	531		606	
Lymph node metastasis		0.000		0.000
N0	261		331	
N1	178		212	
N2	152		160	
N3	71		71	
Clinical stage		0.000		0.000
I+II	297		374	
III	365		400	
Venous/lymphatic invasion		0.473		0.373
No	531		629	
Yes	131		145	
Perineural invasion		0.203		0.919
No	531		604	
Yes	131		170	
Adjuvant radio/chemotherapy		0.049		0.002
No	425		524	
Yes	237		250	
Human epidermal growth factor receptor 2		0.373		0.196
Low expression levels	432		515	
High expression levels	178		194	
Multidrug resistance protein 1		0.338		0.661
Low expression levels	423		496	
High expression levels	239		278	

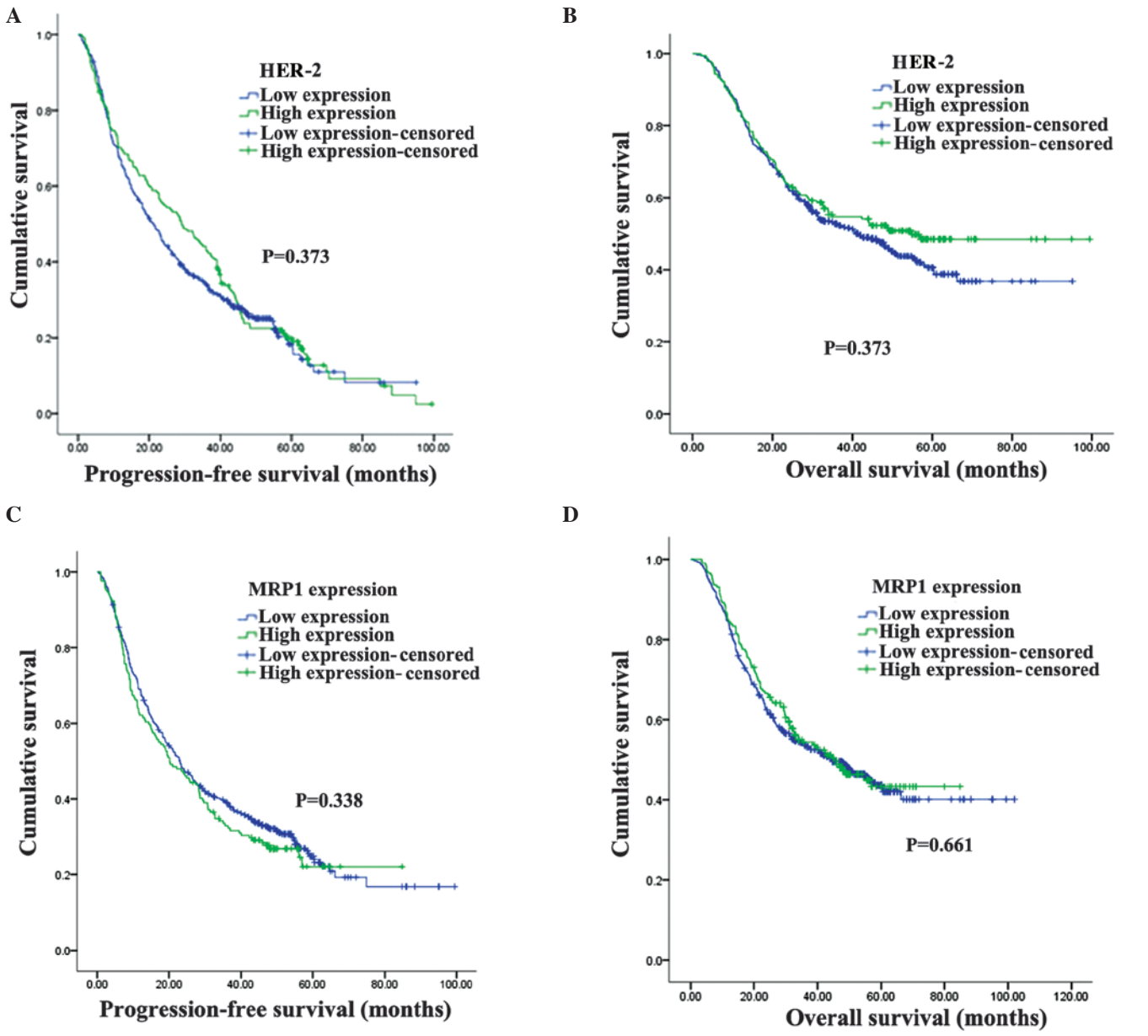

Figure 2. Kaplan-Meier estimate curves of (A and C) progression-free survival and (B and D) overall survival in patients with esophageal squamous cell carcinoma, according to the protein expression levels of HER2 and MRP1, respectively. HER2, human epidermal growth factor receptor 2; MRP1, multidrug resistance protein 1.

clinicopathological features of patients with ESCC were investigated in the present study. In previous studies, the number of copies of the *HER2/neu* gene was analyzed by fluorescence or chromogenic *in situ* hybridization, and the protein expression levels of HER2 were evaluated by IHC (6,7,9,10,12,16-19). Previous studies examining the association between the expression levels of HER2 (as determined by IHC) and prognosis in patients with ESCC have reported conflicting results (Table IV). These discrepancies may be due to the following reasons: i) Different quality of the studies; ii) multiple factors that may influence prognosis were not considered in all the studies; iii) different laboratory techniques were employed in different studies, including the use of different antibodies to detect HER2 and MRP1; and iv) small size of the samples used in the studies (≤250 patients; Table IV). The association between HER2 expression and prognosis in EC has not been reported thus far.

In the present study, a large cohort of 829 patients with ESCC and long follow-up was analyzed, and no correlation was observed between the expression levels of HER2 or MRP1 and the postoperative survival time exhibited by these patients. To the best of our knowledge, the present study is the first large-scale study conducted to evaluate the prognostic role of MRP1 expression in patients with ESCC who had been treated with primary surgery. In the present study, a significant association was observed between survival and clinical stage, lymph node metastasis and poor differentiation in patients with ESCC. However, the present study is affected by certain limitations, including its retrospective and single-hospital nature. Thus, multicenter and prospective studies are required to further validate the results of the present study. In addition, the protein expression levels of HER2 and MRP1 were solely examined by IHC in the present study, which may not be completely consistent with their gene expression levels.

Table III. Multivariate analysis of progression-free survival and overall survival in patients with esophageal squamous cell carcinoma.

	Progression-free survival			Overall survival		
Factors	Hazard ratio	95% CI	P-value	Hazard ratio	95% CI	P-value
Gender			0.021			0.051
Male	Ref.			Ref.		
Female	0.732	0.563-0.954		0.756	0.567-1.008	
Tumor location			0.027			NR
Upper/middle	Ref.			NR		
Lower	1.235	1.024-1.489		NR	NR	
Clinical stage			0.020			0.014
I+II	Ref.			Ref.		
III	1.507	1.069-2.129		1.640	1.104-2.435	
Lymph node metastasis			0.000			0.000
N0	Ref.			Ref.		
N1	1.170	0.826-1.656		1.342	0.893-2.015	
N2	1.516	1.021-2.251		2.181	1.395-3.409	
N3	2.260	1.463-3.491		1.640	1.104-2.435	
Differentiation			0.061			0.085
Intermediate/well	Ref.			Ref.		
Poor/undifferentiated	1.240	0.999-1.553		1.233	0.972-1.564	

CI, confidence interval; Ref., reference; NR, not reported.

Table IV. Previous studies describing the prognostic significance of the expression levels of human epidermal growth factor receptor 2 in ESCC, as determined by IHC or ISH.

First author, year (reference)	Type of cancer	Prognostic effect	Sample size (no. of patients)	Method
Hardwick, 1997 (19)	ESCC, AC	No effect	205	IHC
Friess, 1999 (12)	ESCC, AC	No effect	39	IHC
Mimura, 2005 (9)	ESCC	Unfavorable	66	IHC, ISH
Reichelt, 2007 (10)	ESCC, AC	No effect	251	IHC, ISH
Sato-Kuwabara, 2009 (6)	ESCC	Unfavorable	188	IHC, ISH
Stoecklein, 2008 (37)	ESCC, AC	No effect	101	ISH
Schoppmann, 2010 (11)	ESCC, AC	No effect	341	IHC, ISH
Birner, 2011 (38)	ESCC, AC	Unfavorable	330	IHC, ISH
Zhan, 2012 (7)	ESCC	Unfavorable	145	IHC, ISH
Kato, 2013 (16)	ESCC	No effect	245	IHC, ISH
Wang, 2013 (39)	ESCC	Unfavorable	72	IHC
Wang, 1999 (18)	ESCC	No effect	117	IHC

ESCC, esophageal squamous cell carcinoma; AC, adenocarcinoma; IHC, immunohistochemistry; ISH, *in situ* hybridization.

In summary, the results of the present study indicated that the expression levels of MRP1 and HER2, as determined by IHC analysis, were not associated with survival rate in patients with ESCC. This result indicates that HER2 or MRP1 expression may not serve as informative prognostic biomarkers. Therefore, further studies are required to clarify the mechanism behind the expression of MRP1 and HER2 in patients with ESCC.

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References

1. Siegel R, Naishadham D and Jemal A: Cancer statistics, 2013. CA Cancer J Clin 63: 11-30, 2013.
2. Cunningham D, Allum WH, Stenning SP, Thompson JN, Van de Velde CJ, Nicolson M, Scarffe JH, Lofts FJ, Falk SJ, Iveson TJ, *et al*; MAGIC Trial Participants: Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. N Engl J Med 355: 11-20, 2006.
3. Omloo JM, Lagarde SM, Hulscher JB, Reitsma JB, Fockens P, van Dekken H, Ten Kate FJ, Obertop H, Tilanus HW and van Lanschot JJ: Extended transthoracic resection compared with limited transhiatal resection for adenocarcinoma of the mid/distal esophagus: Five-year survival of a randomized clinical trial. Ann Surg 246: 992-1001, 2007.
4. Andl CD, Mizushima T, Nakagawa H, Oyama K, Harada H, Chruma K, Herlyn M and Rustgi AK: Epidermal growth factor receptor mediates increased cell proliferation, migration, and aggregation in esophageal keratinocytes in vitro and in vivo. J Biol Chem 278: 1824-1830, 2003.
5. Rowinsky EK: Signal events: Cell signal transduction and its inhibition in cancer. Oncologist 8 (Suppl 3): 5-17, 2003.
6. Sato-Kuwabara Y, Neves JI, Fregnani JH, Sallum RA and Soares FA: Evaluation of gene amplification and protein expression of HER2/neu in esophageal squamous cell carcinoma using Fluorescence in situ Hybridization (FISH) and immunohistochemistry. BMC Cancer 9: 6, 2009.
7. Zhan N, Dong WG, Tang YF, Wang ZS and Xiong CL: Analysis of HER2 gene amplification and protein expression in esophageal squamous cell carcinoma. Med Oncol 29: 933-940, 2012.
8. Chan DS, Twine CP and Lewis WG: Systematic review and meta-analysis of the influence of HER2 expression and amplification in operable oesophageal cancer. J Gastrointest Surg 16: 1821-1829, 2012.
9. Mimura K, Kono K, Hanawa M, Mitsui F, Sugai H, Miyagawa N, Ooi A and Fujii H: Frequencies of HER2/neu expression and gene amplification in patients with oesophageal squamous cell carcinoma. Br J Cancer 92: 1253-1260, 2005.
10. Reichelt U, Duesedau P, Tsourlakis MCh, Quaas A, Link BC, Schurr PG, Kaifi JT, Gros SJ, Yekebas EF, Marx A, *et al*: Frequent homogeneous HER2 amplification in primary and metastatic adenocarcinoma of the esophagus. Mod Pathol 20: 120-129, 2007.
11. Schoppmann SF, Jesch B, Friedrich J, Wrba F, Schultheis A, Pluschnig U, Maresch J, Zacherl J, Hejna M and Birner P: Expression of HER2 in carcinomas of the esophagus. Am J Surg Pathol 34: 1868-1873, 2010.
12. Friess H, Fukuda A, Tang WH, Eichenberger A, Furlan N, Zimmermann A, Korc M and Büchler MW: Concomitant analysis of the epidermal growth factor receptor family in esophageal cancer: Overexpression of epidermal growth factor receptor mRNA but not of c-erbB-2 and c-erbB-3. World J Surg 23: 1010-1018, 1999.
13. Suzuki H, Abo S, Kitamura M, Hashimoto M, Izumi K, Terada K and Sugiyama T: Gene amplification of int-2 and erbB in human esophageal cancer: Relationship to clinicopathological variables. Cancer Invest 15: 411-415, 1997.
14. Nakamura T, Nekarda H, Hoelscher AH, Bollschweiler E, Harbeck N, Becker K, Siewert JR and Harbec N: Prognostic value of DNA ploidy and c-erbB-2 oncoprotein overexpression in adenocarcinoma of Barrett's esophagus. Cancer 73: 1785-1794, 1994.
15. Polkowski W, van Sandick JW, Offerhaus GJ, ten Kate FJ, Mulder J, Obertop H and van Lanschot JJ: Prognostic value of Laurén classification and c-erbB-2 oncogene overexpression in adenocarcinoma of the esophagus and gastroesophageal junction. Ann Surg Oncol 6: 290-297, 1999.
16. Kato H, Arao T, Matsumoto K, Fujita Y, *et al*: Gene amplification of EGFR, HER2, FGFR2 and MET in esophageal squamous cell carcinoma. Int J Oncol 42: 1151-1158, 2013.
17. Thompson SK, Sullivan TR, Davies R and Ruszkiewicz AR: HER2/neu gene amplification in esophageal adenocarcinoma and its influence on survival. Ann Surg Oncol 18: 2010-2017, 2011.
18. Wang LS, Chow KC, Chi KH, Liu CC, Li WY, Chiu JH and Huang MH: Prognosis of esophageal squamous cell carcinoma: Analysis of clinicopathological and biological factors. Am J Gastroenterol 94: 1933-1940, 1999.
19. Hardwick RH, Barham CP, Ozua P, Newcomb PV, Savage P, Powell R, Rahamin J and Alderson D: Immunohistochemical detection of p53 and c-erbB-2 in oesophageal carcinoma; no correlation with prognosis. Eur J Surg Oncol 23: 30-35, 1997.
20. Leonard GD, Fojo T and Bates SE: The role of ABC transporters in clinical practice. Oncologist 8: 411-424, 2003.
21. Galimberti S, Marchetti A, Buttitta F, Carnicelli V, Pellegrini S, Bevilacqua G and Petrini M: Multidrug resistance related genes and p53 expression in human non small cell lung cancer. Anticancer Res 18: 2973-2976, 1998.
22. Yokoyama H, Ishida T, Sugio K, Inoue T and Sugimachi K: Immunohistochemical evidence that P-glycoprotein in non-small cell lung cancers is associated with shorter survival. Surg Today 29: 1141-1147, 1999.
23. Tews DS, Nissen A, Külgen C and Gaumann AK: Drug resistance-associated factors in primary and secondary glioblastomas and their precursor tumors. J Neurooncol 50: 227-237, 2000.
24. Langer R, Specht K, Becker K, Ewald P, Bekesch M, Sarbia M, Busch R, Feith M, Stein HJ, Siewert JR and Höfler H: Association of pretherapeutic expression of chemotherapy-related genes with response to neoadjuvant chemotherapy in Barrett carcinoma. Clin Cancer Res 11: 7462-7469, 2005.
25. Sobin LH, Gospodarowicz MK and Wittekind C (eds): Title section/chapter. In: TNM Classification of Malignant Tumours. 7th edition. Wiley-Blackwell, Hoboken, NJ, p455, 2009.
26. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M and Clark GM; Statistics Subcommittee of the NCI-EORTC Working Group on Cancer Diagnostics: REporting recommendations for tumour MARKer prognostic studies (REMARK). Br J Cancer 93: 387-391, 2005.
27. Xu XL, Zheng WH, Fu ZX, Li ZP, Xie HX, Li XX, Jiang LH, Wang Y, Zhu SM and Mao WM: Topo2A as a prognostic biomarker for patients with resectable esophageal squamous cell carcinomas. Med Oncol 32: 396, 2015.
28. Xu XL, Zheng WH, Tao KY, Li XX, Xu WZ, Wang Y, Zhu SM and Mao WM: p53 is an independent prognostic factor in operable esophageal squamous cell carcinoma: A large-scale study with a long follow-up. Med Oncol 31: 257, 2014.
29. Konecny G, Pauletti G, Pegram M, Untch M, Dandekar S, Aguilar Z, Wilson C, Rong HM, Bauerfeind I, Felber M, *et al*: Quantitative association between HER2/neu and steroid hormone receptors in hormone receptor-positive primary breast cancer. J Natl Cancer Inst 95: 142-153, 2003.
30. Owens MA, Horten BC and Da Silva MM: HER2 amplification ratios by fluorescence in situ hybridization and correlation with immunohistochemistry in a cohort of 6,556 breast cancer tissues. Clin Breast Cancer 5: 63-69, 2004.
31. Parums DV: Current status of targeted therapy in non-small cell lung cancer. Drugs Today (Barc) 50: 503-525, 2014.
32. Pollock NI and Grandis JR: HER2 as a therapeutic target in head and neck squamous cell carcinoma. Clin Cancer Res 21: 526-533, 2015.
33. Kontopodis E, Kentepozidis N, Christophyllakis CH, Boukovinas I, Kalykaki A, Kalbakis K, Vamvakas L, Agelaki S, Kotsakis A, Vardakis N, *et al*: Docetaxel, gemcitabine and bevacizumab as salvage chemotherapy for HER2-negative metastatic breast cancer. Cancer Chemother Pharmacol 75: 153-160, 2015.
34. Lee HJ, Seo AN, Kim EJ, Jang MH, Suh KJ, Ryu HS, Kim YJ, Kim JH, Im SA, Gong G, *et al*: HER2 heterogeneity affects trastuzumab responses and survival in patients with HER2-positive metastatic breast cancer. Am J Clin Pathol 142: 755-766, 2014.
35. Partridge AH, Rumble RB, Carey LA, Come SE, Davidson NE, Di Leo A, Gralow J, Hortobagyi GN, Moy B, Yee D, *et al*: Chemotherapy and targeted therapy for women with human epidermal growth factor receptor 2-negative (or unknown) advanced breast cancer: American Society of Clinical Oncology Clinical Practice Guideline. J Clin Oncol 32: 3307-3329, 2014.
36. Raben D, Helfrich B, Chan DC, Ciardiello F, Zhao L, Franklin W, Barón AE, Zeng C, Johnson TK and Bunn PA Jr: The effects of cetuximab alone and in combination with radiation and/or chemotherapy in lung cancer. Clin Cancer Res 11: 795-805, 2005.
37. Stoecklein NH, Hosch SB, Bezler M, Stern F, Hartmann CH, Vay C, Siegmund A, Scheunemann P, Schurr P, Knoefel WT, *et al*: Direct genetic analysis of single disseminated cancer cells for prediction of outcome and therapy selection in esophageal cancer. Cancer Cell 13: 441-453, 2008.
38. Birner P, Jesch B, Friedrich J, Riegler M, Zacherl J, Hejna M, Wrba F, Schultheis A and Schoppmann SF: Carbonic anhydrase IX overexpression is associated with diminished prognosis in esophageal cancer and correlates with HER2 expression. Ann Surg Oncol 18: 3330-3337, 2011.
39. Wang G, Zhang W, Jiang W and Luan L: Overexpression of HER2 associated with the progression of esophageal cancer patients. Hepatogastroenterology 60: 1972-1978, 2013.