·Clinical Research·

Correlation of HER-2/neu gene amplification and protein expression with the prognosis of advanced gastric cancer patients

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[**Abstract**] **Background and Objective:** Evidences proved that HER2 overexpression is correlated to poor prognosis and high invasiveness of gastric cancer. This study was to investigate the correlation of the expression of HER-2/neu to the clinical characteristics of advanced gastric carcinoma, especially the survival. **Methods:** The clinical data of 83 patients with advanced gastric cancer treated in Cancer Hospital, Chinese Academy of Medical Science from 2006 to 2008 were reviewed. The expression of HER-2/neu in the 83 specimens of advanced gastric cancer was detected using immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH). The survival rate was calculated by the Kaplan-Meier method and compared by the log-rank test using SPSS13.0 software. **Results:** HER-2/neu overexpression and amplification were found in 25 (30.1%) and 29 (34.9%) advanced gastric carcinomas. The patients with HER-2/neu amplification and overexpression had poor prognosis. The median survival of the patients without HER-2/neu amplification was 12.6 months, while that of those with HER-2/neu amplification was 5.5 months. **Conclusion:** HER-2/neu expression may be used to predict the prognosis of advanced gastric cancer.

Key words: Gastric neoplasm, HER2/neu, prognosis

HER-2/neu gene, also known as c-erbB-2 gene, encodes the 185 kDa transmembrane tyrosine kinase receptor which is a member of epidermal growth factor receptor (EGFR) family. Recent studies suggested that HER-2/neu plays an important role in the development of many malignant tumors. HER-2/neu gene amplification and protein overexpression were found in 10%–34% of the patients with invasive breast carcinoma, and were associated with poor prognosis and short survival.[1] In recent years, it was also found that HER-2 gene amplification and protein overexpression widely existed in other cancers such as ovarian cancer, endometrial cancer, gastric cancer, esophageal cancer, colon cancer, lung cancer, and so on.[2] It has been reported that, in patients with gastric cancer, HER-2 protein overexpression was found in 5.2% –22.6% of the patients by immunohistochemistry (IHC), and HER-2 gene amplification was found in 3.8% –12.2% of the patients by fluorescence in situ hybridization (FISH).[3-5] It was demonstrated that HER-2/neu overexpression might foreshow the poor prognosis of gastric cancer.[6,7] In order to explore the influence of HER-2/neu gene on the prognosis of advanced gastric cancer, we analyzed the data of 83 advanced gastric cancer patients who were treated in Cancer Hospital of Chinese Academy of Medical Sciences (CAMS).

Materials and Methods

Clinical data

Between January 2006 and October 2008, 83 patients with pathologically confirmed advanced gastric cancer were treated in Cancer Hospital of CAMS. All patients had distant metastasis at initial diagnosis or after operation, and did not receive first-line chemotherapy. These patients were 27–76 years old with a median age of 60 years, 49 were no more than 60 years old and 34 were more than 60 years old. ECOG score ranged from 0 to 1. Of these 83 patients, 62 were men and 21 were women, with a ratio of man to woman of 2.95:1; 38 had gastric antrum cancer,

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27 had cardiac cancer, 13 had gastric body cancer, 4 had gastric whole cancer, and 1 had gastric remnant cancer; 63 had abdominal cavity or retroperitoneal lymph node metastases, 23 had liver m etastasis, 16 had abdominal cavity implantation metastasis, 10 had lung metastasis, 5 had supraclavicular lymph node metastasis, 3 had ovarian metastasis, 3 had spleen metastasis, 3 had mediastinal lymph node metastases, 3 had gastric remnant recurrence, 2 had pancreas metastasis and 2 had abdominal wall metastases, and 16 of them had metastasis above the diaphragm; 2 had well differentiated adenocarcinoma, 16 had moderately differentiated adenocarcinoma, 53 had lowly differentiated adenocarcinoma, 6 had poorly differentiated adenocarcinoma and 6 had signet ring cell cancer.

HER-2/neu detection

The HER-2/neu gene amplification and protein expression in paraffin-embedded gastric cancer tissues were detected by FISH and IHC, respectively. The assessment of IHC results was performed as follows: 0 represented no staining or less than 10% cells with membrane staining, 1+ represented more than 10% cells with incomplete membrane staining, 2+ represented more than 10% cells with weakly complete membrane staining, 3+ represented more than 10% cells with strongly complete membrane staining; 0 and 1+ indicated no or low expression, 2+ and 3+ indicated overexpression. Using the double fluorescence labeling probes of Her-2 and chromosome 17 to detect HER-2/neu gene amplification, once the ratio of total red signals to total green signals was ⩾ 2, it was considered as HER2 gene amplification.

Treatment methods

Of the 83 patients, 61 received 1–11 cycles (median, 5 cycles) of chemotherapy: 30 were treated with capecitabine/5-fluorouracil (5-FU) plus cisplatin (DDP)/oxaliplatin; 26 were treated with docetaxel, oxaliplatin plus 5-FU; 2 were treated with adriamycin (ADM) plus 5-FU; 2 were treated with paclitaxel plus 5-FU; 1 was treated with oxaliplatin plus hydroxycamptothecine.

Follow-up

The follow-up time was counted since the date of confirmed diagnosis of advanced gastric cancer. Till April 2009, the patients were followed up for 1.38–36.2 months with a median of 8.9 months.

Statistical analysis

Statistical analysis of all data was performed with SPSS13.0 statistical software. Patients' survival was analyzed by Kaplan-Meier method and Cox regression model. Survival rates were compared by log-rank test. A P value of <0.05 was considered significant.

Results

HER-2/neu gene amplification and its protein expression

The amplification rate of HER-2/neu gene was 34.9%, and the overexpression rate of HER-2/neu protein was 30.1% (Table 1). The number of organs with metastasis had significant influence on HER-2/neu gene amplification (P = 0.026) and protein expression (P =0.046); the organ metastasis above the diaphragm had significant effect on HER-2/neu gene amplification (P = 0.041), but had no significant influence on protein expression (P =0.103); gender, age, primary site and pathologic type had no significant effects on HER-2/neu gene amplification and protein expression (P > 0.05) (Table 2).

Table 1 Relationship between HER-2/neu gene amplification and its protein overexpression

HER-2/neu amplification	HER-2/neu protein expression				
	IHC 0	IHC 1+	IHC 2+	IHC 3+	Total
FISH+	5 (12.8)	5 (26.3)	3 (37.5)	16 (94.1)	29 (34.9)
FISH–	34 (87.2)	14 (73.7)	5 (62.5)	1 (5.9)	54 (65.1)
Total	39(100)	19(100)	8(100)	17(100)	83(100)

IHC, immunohistochemisty; FISH, fluorescent in situ hybridization.

Patients' survival and its influence factors

The median survival of the 83 patients was 9.4 months (range, 1.4–37.2 months). The 3-, 6-, 12- and 24-month survival rates were 91.6%, 63.9%, 41.6% and 13.9%, respectively (Figure 1). The median survival was significantly shorter in the patients with HER-2/neu protein overexpression than in those without protein overexpression (5.9 months vs. 12.6 months, P =0.021) (Figure 2), significantly shorter in the patients with HER-2/neu gene amplification than in those without gene amplification (5.5 months vs. 12.6 months, P =0.028) (Figure 3), and significantly longer in the patients treated with chemotherapy than in those received no chemotherapy (10.8 months vs. 4.8 months, P = 0.018) (Figure 4). No significant differences in the proportion of patients receiving chemotherapy were seen between the patients with and those without HER-2/neu gene amplification (P=0.098), and between the patients with and those without protein overexpression (P=0.179) (Table 3). The median survival was 11.7 months for the 30 patients treated with capecitabine/5-FU plus DDP/oxaliplatin, 16.6 months for the 26 patients treated with docetaxel, oxaliplatin plus 5-FU, and 3.0 months for the 5 patients treated with other chemotherapy regimens, without significant differences between each two groups (P = 0.096). Gender and age had no significant influence on survival (Table 4).

Discussion

HER-2 gene amplification and protein overexpression not only were found in breast cancer but also existed widely in other cancers.[2] It has been reported that, in gastric cancer patients, the overexpression rate of HER-2/neu gene was 5.2%–22.6%, and the amplification rate of HER-2/neu gene was 3.8%–12.2% by FISH detection.[3-5] In our study, the amplification rate and protein overexpression rate of HER-2/neu gene were 34.9% and 30.1%, respectively, which were higher than those reported in literature. In addition, all cases at stages I-IV were analyzed in previous reports,[3-5] but only the cases at stage IV were analyzed in our study. Therefore, the high rates of amplification and protein overexpression of HER-2/neu gene could reflect the malignant

Table 2 The status of HER-2/neu gene amplification and its protein overexpression in 83 patients with advanced gastric cancer

Characteristic	Patient No.	HER-2/neu amplification (No.)		*P*	HER-2/neu overexpression (No.)		*P*
		Positive	Negative		Positive	Negative	
Sex							
Male	62	22	40	0.806	19	43	0.677
Female	21	6	15		6	15	
Age (years)							
≤60	49	16	33	0.686	14	35	0.714
>60	34	13	21		11	23	
Tumor location							
Sinus ventriculi	38	12	26	0.368	11	27	0.169
Cardia	27	10	17		9	18	
Body of stomach	13	5	8		2	11	
Gastric stump	1	1	0		1	0	
Linitis plastica	4	1	3		1	3	
Numbers of metastatic organ							
1	7	0	7	0.026	1	6	0.046
2	57	23	34		19	38	
3	10	2	8		3	7	
4	6	4	2		4	2	
5	3	3	0		2	1	
Metastatic sites							
Above diaphragm	16	10	6	0.041	8	8	0.103
Below diaphragm	67	22	45		17	50	
Pathological status							
Well-differentiated	2	2	0	0.270	2	0	0.060
Moderately-differentiated	16	6	10		6	10	
Poorly-differentiated	53	16	37		14	39	
Undifferentiation	6	5	1		3	3	
Signet-ring cell carcinoma	6	0	6		0	6	

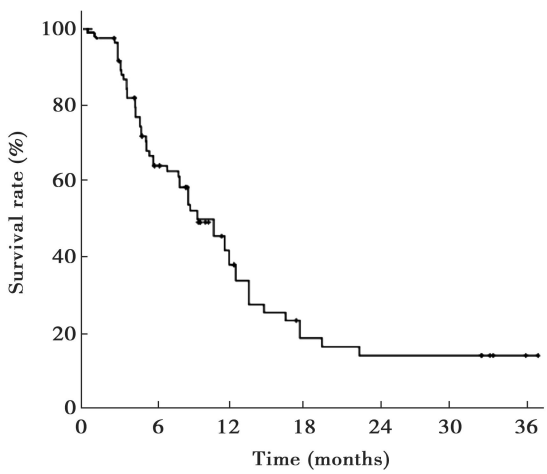

Figure 1 Overall survival curve of advanced gastric cancer patients

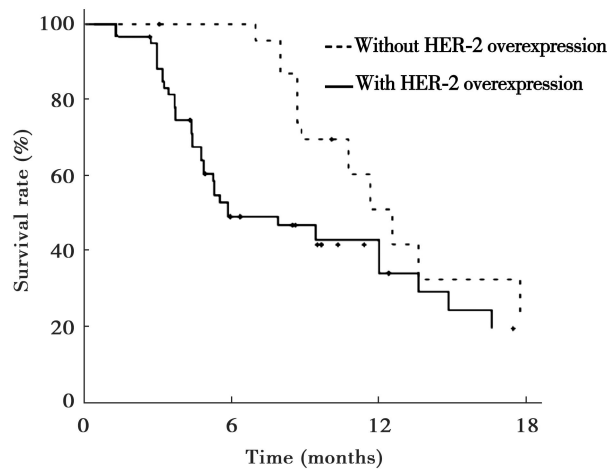

Figure 2 Survival curves of advanced gastric cancer patients with or without HER-2/neu overexpression

progression of gastric cancer to some extent.

In our study, HER-2/neu gene amplification existed in 76% of the cases with HER-2/neu protein overexpression, which was basically consistent with that reported by Yano *et al*.[8] In gastric cancer, the status of protein expression is not absolutely consistent with that of gene amplification, especially in IHC++ and IHC+ cases. The cases without gene amplification may have protein overexpression, while the cases with high gene amplification may have low or no protein expression. In our study, HER-2/neu gene amplification existed in 12.8% of the cases

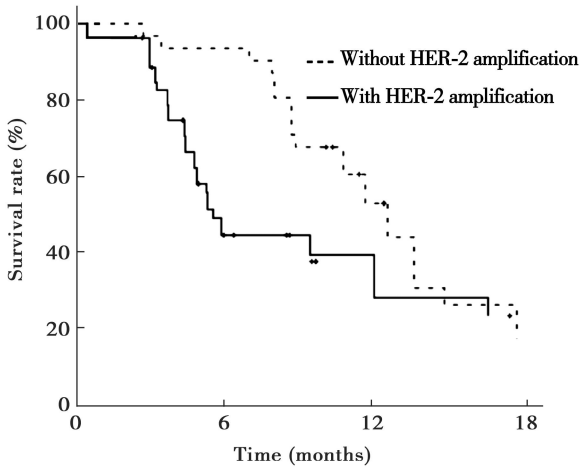

Figure 3 Survival curves of advanced gastric cancer patients with or without HER-2/neu gene amplification

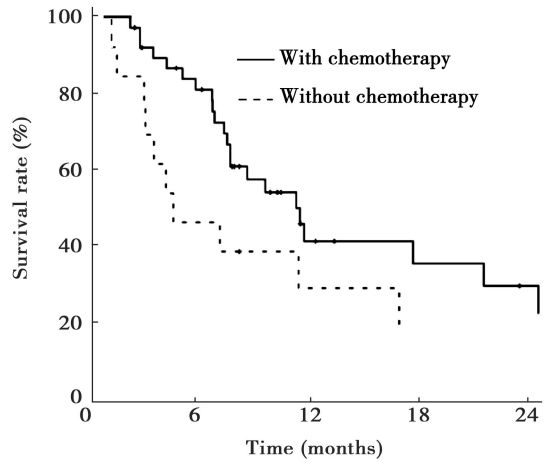

Figure 4 Survival curves of advanced gastric cancer patients treated with or without chemotherapy

Table 3 Relationship between chemotherapy and HER-2/neu gene amplification and its protein overexpression

Status of HER2/neu	Patient No.	Chemotherapy		*P*
		Yes	No	
Amplification				
Yes	29	25	4	0.098
No	54	36	18	
Overexpression				
Yes	25	20	5	0.179
No	58	41	17	

without protein expression and in 26.3% of the cases with protein expression. This may be associated with polyploid of chromosome 17, fragment length of HER-2 mRNA non-translational region (UTR), post-transcriptional regulation, IHC false positive, and so on.[9,10] It can be speculated that post-transcriptional or post-translational regulation of HER-2 gene probably plays a certain role in gastric cancer.

Barros-Silva *et al.*[11] reported that HER-2/neu gene amplification was not significantly associated with gender, age, cancer embolus, lymph node metastasis, clinical stage and operation pattern. In our study, HER-2/neu gene amplification and

Table 4 Univariate analyses for the prognosis of the 83 gastric cancer patients

Characteristic	Patient No.	Survival rate (%)				*P*
		3-month	6-month	12-month	24-month	
Age(years)						0.648
≤60	49	87.8	61.8	41.8	14.1	
>60	34	97.1	66.9	42.6	14.2	
Sex						0.493
Male	62	88.9	69.0	43.8	14.5	
Female	21	95.0	50.0	30.0	10.0	
HER-2/neu overexpression						0.021
Negative	58	100.0	100.0	51.0	23.2	
Positive	25	88.2	49.1	41.6	6.5	
HER-2 amplification						0.028
Negative	54	96.8	93.5	53.0	17.7	
Positive	29	88.5	44.7	37.8	11.8	
Chemotherapy						0.018
Yes	61	93.3	73.8	42.3	21.2	
No	22	87.0	39.1	39.1	16.8	
Chemotherapy regimen						0.096
X+C/L[a]	30	100.0	83.3	46.8	15.6	
X+L+5-FU[b]	26	92.0	69.0	55.2	0	
Others	5	50.0	50.0	0	0	

[a]X+C/L, capecitabine/5-fluorouracil (5-FU) plus cisplatin (DDP)/oxaliplatin;

[b]X+L+5-FU, docetaxel, oxaliplatin plus 5-FU.

protein overexpression also were not significantly associated with gender, age, primary site and pathologic type, but the number of distant metastasis sites was positively correlated with HER-2/neu gene amplification and protein overexpression. Moreover, the rate of HER-2/neu gene amplification in the patients with metastasis above the diaphragm was higher than that in the patients with metastasis only below the diaphragm. Wolf-Yadlin *et al*.[12] considered that different statuses of HER-2/neu gene amplification in tumor tissues led to different growth pattern of tumor tissues, and HER-2/neu gene amplification significantly promoted the migration of tumor cells under the action of epidermal growth factor, but only minimally promoted tumor cell proliferation. Therefore, wider range and more quantity of distant metastasis occurred in the patients with HER-2/neu gene amplification. It has been reported that HER-2/neu gene amplification was more common in the gastroesophageal junction, and the positive rate was 24%–32%, which was far higher than that (9.5%–18%) in other parts of the stomach.[13,14] However, the related mechanism remains unclear. In our study, the rate of HER-2/neu gene amplification in the patients with primary cancer in the gastric cardia was a little higher than that in other gastric parts (37.0% vs. 33.9%), but the difference was not significant, which might be associated with the limited sample.

In most studies, it has been reported that HER-2/neu gene amplification and protein overexpression in gastric cancer were not correlated with clinicopathologic parameters such as depth of tumor invasion and lymph node metastasis, but were associated with the the poor prognosis of patients.[15,16] In our study, the median survival of the patients without HER-2/neu protein overexpression or gene amplification was over two times of that of the patients with protein overexpression or gene amplification, indicating that HER-2/neu gene expression has a negative effect on the survival time of advanced gastric cancer patients.

By chemotherapy, the survival of advanced gastric cancer patients can be significantly extended. In our study, the median survival was significantly longer in the patients treated with chemotherapy than in those treated without chemotherapy. Because there were No significant differences in the proportion of patients receiving chemotherapy were seen between the patients with and without HER-2/neu gene amplification and between the patients with and without protein overexpression, excluding the influence of the proportion on survival. Currently, the regimens of capecitabine/5-FU plus DDP/oxaliplatin and docetaxel, oxaliplatin plus 5-FU, which are widely used in clinic, can prolong the survival. It was proved in our study.

In summary, HER-2/neu gene amplification may foreshow the poor prognosis in advanced gastric cancer patients. Currently, the role of HER-2/neu gene amplification in therapeutic field of gastric cancer has been further confirmed by prospective comparative studies. TOGA BO18255 trial revealed that, for the advanced gastric cancer patients with HER-2/neu gene amplification, the median survival of those treated with DDP, capecitabine plus herceptin as first-line chemotherapy reached 13.8 months, but that of those treated without herceptin was only 11.1 months. Further study is needed to explore HER-2/neu protein-targeted drug therapy for gastric cancer. Due to high correlation between HER-2/neu gene amplification and protein overexpression, especially in IHC 3+ patients, IHC detection can also be carried out to guide the treatment when FISH detection can not be performed.

References

[1] Ross JS, Fletcher JA. HER-2/neu (cerb-B2) gene and protein in breast cancer [J]. Am J Chn Pathol 1999, 112(Suppl 1):S53–S67.

[2] Menard S, Casalini P, Campiglio M, et al. HER2 overexpression in various tumor types, focusing on its relationship to the development of invasive breast cancer [J]. Ann Oncol, 2001,12(Suppl 1):S15–S19.

[3] Varis A, Zaika A, Puolakkainen P, et al. Coamplified and overexpressed genes at ERBB2 locus in gastric cancer [J]. Int J Cancer, 2004,109:548–553.

[4] Park DI, Yun JW, Park JH, et al. HER-2/neu amplification is an independent prognostic factor in gastric cancer [J]. Dig Dis Sci, 2006,51: 1371–1379.

[5] Kim MA, Jung EJ, Lee HS, et al. Evaluation of HER-2 gene status in gastric carcinoma using immunohistochemistry, fluorescence in situ hybridization, and real-time quantitative polymerase chain reaction [J]. Hum Pathol, 2007,38:1386–1393.

[6] Uchino S, Tsuda H, Maruyama K, et al. Overexpression of c-erbB-2 protein in gastric cancer. Its correlation with long-term survival of patients [J]. Cancer, 1993,72:3179–3184.

[7] Mizutani T, Onda M, Tokunaga A, et al. Relationship of C-erbB-2 protein expression and gene amplification to invasion and metastasis in human gastric cancer [J]. Cancer, 1993,72:2033–2038.

[8] Yano T, Ochiai A, Doi T, et al. Expression of HER2 in gastric cancer: comparison between protein expression and gene amplification using a new commercial kit [J]. J Clin Oncol, 2004,22:14S (Abstr 4053).

[9] Tubbs RR, Pettay JD, Roche PC, et al. Discrepancies in clinical laboratory testing of eligibility for trastuzumab therapy: apparent immunohistochemical false-positives do not get the message [J]. J Clin Oncol, 2001,19:2714–2721.

[10] Bae CD, Juhnn YS, Park JB. Post-transcriptional control of cerb B-2 overexpression in stomach cancer cells [J]. Exp Mol Med,2001,33:15–19.

[11] Barros-Silva JD, Leitão D, Afonso L. Association of ERBB2 gene status with histopathological parameters and disease-specific survival in gastric carcinoma patients [J]. Br J Cancer, 2009,100:487–493.

[12] Wolf-Yadlin A, Kumar N, Zhang Y, et al. Effects of HER2 overexpression on cell signaling networks governing proliferation and migration [J]. Mol Syst Biol, 2006,2:54.

[13] Lordick F, Bang YJ, Kang YK, et al. HER2-positive advanced gastric cancer: similar HER2-positivity levels to breast cancer [J]. Eur J Cancer, 2007, 5(4):271.

[14] Tanner M, Hollmen M, Junttila TT, et al. Amplification of HER-2 in gastric carcinoma: association with topoisomerase IIa gene amplification, intestinal type, poor prognosis and sensitivity to trastuzumab [J]. Ann Oncol, 2005, 16:273–278.

[15] Tanner M, Hollmen M, Junttila TT, et al. Amplification of HER-2 in gastric carcinoma: association with Topoisomerase II alpha gene amplification, intestinal type, poor prognosis and sensitivity to trastuzumab [J]. Ann Oncol, 2005,16:273–278.

[16] Ross JS, McKenna BJ. The HER-2/neu oncogene in tumors of the gastrointestinal tract [J]. Cancer Invest, 2001,19:554–568.