RESEARCH ARTICLE

Is Her-2 Status in the Primary Tumor Correlated with Matched Lymph Node Metastases in Patients with Gastric Cancer Undergoing Curative Gastrectomy?

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Abstract

Background: HER2 expression in the primary tumor and its lymph node metastases vary in gastric cancer, reflecting intratumoral heterogeneity. This finding also suggests that proliferation of a different clone in metastatic nodes is possible. In the current study, we aimed to determine the cause of discordance in HER-2 expression in the primary tumor and lymph node metastases for patients with gastric cancer. Materials and Methods: Eighty-one patients with gastric cancer who had undergone radical gastrectomy and were found to have lymph node metastasis upon pathological examination were included. Histopathological samples were obtained from biopsies obtained during patient gastrectomies and lymph node dissection. HER2 status was evaluated by both immunohistochemistry (IHC) and silver *in situ* hybridization (SISH). Results: Sixty-four (79%) patients were SISH (-), while 17 (21%) were SISH (+) in the primary tumor. However, in metastatic lymph nodes, HER2 status was SISH positive in 5 (28.3%) of the 64 SISH (-) primary tumor specimens. One of the 17 SISH (+) primary tumors was SISH (-) in the metastatic lymph nodes. Thus, SISH results for HER2 in both primary tumors and lymph node metastases were comparable, showing a concordance of 92.5%. In total, six patients demonstrated discordance between the primary tumor and lymph node metastases. The prevalence of HER2 discordance was significantly higher for patients in the pN2 and N3 stages (p=0.007). Although discordant patients had worse survival rates than concordant patients, the differences were not significant (p>0.05). Conclusions: Our study indicates that the frequency of concordance in HER2 status, as determined by IHC or SISH, is high in primary tumors and their corresponding lymph node metastases for patients with gastric cancer. If there is a discrepancy in HER2 status, its evaluation by both IHC and SISH may be useful for detecting patients who would benefit from trastuzumab, and it would therefore help guide decision-making processes in administering treatment.

Keywords: Immunohistochemistry - HER2 - silver *in situ* hybridization - gastric cancer - trastuzumab

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Introduction

Despite important advances in the diagnosis and treatment of gastric cancer, this type of cancer is still the second highest cause of cancer mortality worldwide. Approximately 65% of patients present with locally advanced or metastatic disease (Jemal et al., 2011). The median overall survival is approximately 3 months with the best supportive care in metastatic disease; however, this time increases to 9 to 14 months with effective combination chemotherapy regimens (Wagner et al., 2006). Recent advances in molecular pathogenesis of have made targeted therapy possible. Trastuzumab is the most widely studied agent in this regard. Currently, trastuzumab in combination with chemotherapy constitutes a new option for treating HER2-overexpressing gastric and gastroesophageal junction (GEJ) carcinomas (Bang et al., 2010). HER2 is an important oncoprotein that plays a key role in the pathogenesis and progression of many tumors and is also a poor prognostic marker. The HER2 gene is amplified in approximately 20% of breast cancer cases (Marx et al., 2009) while gene amplification or protein overexpression is reported in 7-34% of gastric cancers (Marx et al., 2009; Bozzetti et al., 2011). HER2 overexpression is also associated with shorter survival in gastric cancer, suggesting a potential survival benefit from anti-HER2 strategies (Marx et al., 2009; Sawaki et al., 2012). HER2 overexpression is usually studied in primary tumor specimens. Overexpression of HER2 in the metastatic lymph nodes is also important because there is a possibility that a different clone from the primary tumor constitutes the dominant proliferative cell type in the metastatic niche thanks to intratumoral heterogeneity. Although the efficacy of trastuzumab is well established,

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HER2 status in gastric cancer is usually characterized based on its status in the primary tumor and metastatic lymph nodes are rarely studied. Only a limited number of studies have looked into both the primary tumor and metastatic lymph nodes regarding overexpression of HER2 (Marx et al., 2009; Sawaki et al., 2012).

In this study, we aimed to investigate the discordance of HER2 overexpression in the primary tumor and metastatic lymph nodes by IHC and silver *in situ* hybridization (SISH).

Materials and Methods

This study included a total of 81 patients with gastric cancer who had undergone radical resection with a lymph node dissection and were found to be lymph node positive upon pathologic examination at the Istanbul University Cerrahpasa Medical Faculty Department of Medical Oncology. The eligibility criteria consisted of a histologically confirmed R0 gastric resection and lymph node metastasis and postoperative survival expectancy longer than 3 months. Patients with distant metastases and peritoneal metastases at diagnosis were excluded from the study.

The following patient characteristics were obtained from patients' charts after written informed consent had been obtained from patients or their relatives: age, gender, resection type, tumor location, histopathology, TNM stage, lymph node involvement, the depth of tumor invasion, lymphatic vessel and perineural invasion, type of adjuvant chemotherapy and radiation therapy, responses to treatment and survival. The Local Ethics Committee of our hospital approved the study. All patients received their adjuvant treatments in our outpatient clinic and were evaluated at periodic follow-up visits.

IHC analysis for HER2

All histologic sections were obtained from formalin-fixed, paraffin wax-embedded gastrectomy and lymph node dissection specimens. Gastric resection specimens and positive lymph nodes were simultaneously studied by IHC and SISH for HER2 overexpression. HerceptTest was used for IHC. No staining or membrane staining in less than 10% of invasive tumor cells was deemed as IHC 0, barely perceptible partial membrane staining in more than 10% of tumor cells as IHC 1+, weak to moderate complete or basolateral membrane staining in more than 10% of invasive tumor cells as IHC 2+ and moderate to strong complete or basolateral membrane staining in more than 10% of tumor cells as IHC 3+.

SISH analysis for HER2

SISH analysis was carried using a PathVysion HER-2 DNA probe kit. HER2/CEP17 ratios ≥2.0 were considered positive.

Statistical analysis: Patient and tumor characteristics were reported as medians and percentages, respectively. The clinicopathological factors of SISH negative and SISH positive tumors in both the primary tumor and lymph nodes were compared by Chi-squared test and Fisher's exact test. The relationship between HER2 overexpression for IHC and SISH in the primary tumor and the lymph node metastasis were also reported as percentages and evaluated by Chi-squared test and Fisher's exact test. Concordance between HER2 SISH status in primary gastric tumor specimens and matched lymph node metastases were calculated as the ratio of concordant cases to total cases using McNemar's test. Survival analysis and curves were established according to the Kaplan-Meier method and compared by the log-rank test. Disease-free survival (DFS) was defined as the time from curative surgery to disease progression or recurrence, or to the date of death or discontinued follow-up. Overall survival (OS) was described as the time from diagnosis to the date of the patient's death or discontinued follow-up. All statistical analyses were performed with SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

Results

Fifty patients (62%) were male, and 31 (38%) were male, with a median age of 58 years (range; 29-87 years). Fifty-eight patients (71.6%) were older than 50 years. Of the study group, postoperatively, 33 patients (41%) were classified as stage II and 48 (59%) as stage III. The majority of patients (n=42, 52%) were at the pT3 stage. Based on the number of lymph node metastases, 28 (34.6%) patients were classified as pN1, 32 (39.4%) as pN2 and 21 (26%) as pN3. The primary tumor location was in the upper third of the stomach in 36 patients (44.4%), in the middle third in 11 (13.6%) and in the lower third in 34 (42%). Forty-eight (59%) patients underwent total gastrectomy, while subtotal resection was performed in 33 patients (41%). Helicobacter pylori were found in 16% of patients.

HER2 IHC and SISH results

In terms of the primary tumor, 44 patients (54.3%) were classified as IHC (-), 18 (22.2%) as IHC (1+), 8 (9.9%) as IHC (2+) and 11 (13.6%) as IHC (3+) based on IHC. In terms of the lymph nodes, 44 patients were also found to be IHC negative, while 10 patients (12.4%) were IHC (1+), 4 (4.9%) IHC (2+) and 23 (28.4%) IHC (3+). According to SISH HER2 amplification, SISH was negative in 64 primary tumors (79%), while it was positive in 17 patients (21%). However, in the metastatic lymph nodes, 60 specimens (74%) were found to be SISH negative and 21 (26%) SISH positive.

Significant differences were detected between *H. pylori* status and SISH HER2 status in both primary tumors and lymph nodes. The prevalence of SISH positivity was significantly higher for *H. pylori* (+) than *H. pylori* (-) tumors ($p<0.001$). The relationships between SISH status and clinicopathological factors are summarized in Tables 1 and 2.

Discordance of HER2 expression in the primary tumor and metastatic lymph nodes

Sixty-four (79%) patients were SISH (-), while 17 (21%) were SISH (+) in the primary tumor. On the other hand, of the SISH negative group, IHC was negative in 61 patients (95.3%), but the remaining 3 patients (4.7%)

showed equivocal IHC (2+). In the SISH (+) group, there was 1 (5.9%) patient classified as IHC (-), 5 (29.4%) as IHC (2+) and 11 (64.7%) as IHC (3+). Table 3 shows HER2 status according to IHC and SISH in the primary gastric cancer. Based on HER2 IHC analysis, 53 patients (88.3%) were classified as negative, 3 (5%) as IHC (2+) and 4 (6.7%) as IHC (3+) for SISH (-) patients in metastatic lymph nodes. Furthermore, 21 lymph node specimens were found to be SISH positive and 19 of these 21 patients (90.5%) were IHC (3+). The remaining 2 specimens were IHC (-) and (2+), respectively (Table 3). This finding suggests that staining is stronger in the lymph nodes than in the primary tumors.

Thereafter, when HER status by SISH was compared between primary tumors and matched lymph nodes, in metastatic lymph nodes, HER2 status was observed to be SISH positive in 5 (28.3%) of 64 SISH (-) primary tumor specimens. On the other hand, 1 of the 17 SISH (+) primary tumors indicated as SISH (-) in the metastatic lymph nodes. Thus, SISH results for HER2 in both primary tumors and lymph node metastases were comparable (rate of concordance: 92.5%). The results of HER2 status with respect to SISH on primary gastric tumor specimens and matched lymph node metastases are listed in Table 2. The most striking result was the discrepancy in HER2 expression between the primary tumor and the lymph nodes in 5 patients. In these patients, SISH results were negative in the primary tumor but positive in their matched metastatic lymph nodes.

Table 1. Clinical and Pathological Features of Patients

		Patient (n)	%
Sex	Male	47	64
	Female	27	36
	Age, range	58 (29-87)	
Type of surgery	Total gastrectomy	45	60
	Subtotal gastrectomy	29	40
Localization of tumor	Antrum	30	40
	Corpus	23	31
	Cardia	21	29
Histological type	Intestinal	48	65
	Diffuse	26	35
Stage	Stage II a	9	12
	Stage II b	20	28
	Stage IIIa	16	21
	Stage IIIb	12	16
	Stage IIIc	17	23

Table 2. SISH Status of Primary and Lymph Node

		primary		lymph node	
		SISH (-)	SISH (+)	SISH (-)	SISH(+)
General		59(80)	15(20)	56(75)	18(25)
Histology	Diffuse	21	5	20	6
	Intestinal	38	10	36	12
Stage	StageII	22	7	19	10
	StageIII	37	8	37	8
Localization	Antrum	24	4	24	4
	Corpus	17	7	14	10
	Cardia	18	4	18	4

*Primary SISH (-) histology p 0.87, primary SISH (-) stage p 0.5, primary SISH (-) localization p 0.39, primary SISH (+) histology p 0.1, primary SISH (+) stage p 0.5, primary SISH (+) localization p<0.05

Correlation of clinicopathologic characteristics and HER2 discordance

A significant difference was found between HER2 discordance and the presence of lymph node metastasis (pN stage). The prevalence of HER2 discordance was significantly higher for patients in the pN2 and N3 stages (p=0.007). No relation was detected between other clinicopathological factors and HER2 discordance (p>0.05).

Survival analysis

In 4 of the 6 patients (66.7%) with HER2 discordance, recurrence was detected. At the median follow-up of 16 months (range; 6-30 months), the median DFS and OS times for patients with HER2 discordance were worse than those for patients without HER2 discordance (DFS: 12 *vs* 17 months; OS: 21 *vs* 24 months, respectively). However, these differences were not significant (p=0.39 and 0.69, respectively). Univariate and multivariate analyses could not be performed because of small sample size of HER2 discordant patients.

Discussion

HER2 is a glycoprotein transmembrane receptor with intracellular receptor tyrosine kinase activity. It dimerizes with the other members of the ERB family to form heterodimers and initiate signal transduction (Khasraw and Bell 2012). It has been reported to be overexpressed in 20% of breast and 22% of gastric cancers, although different studies have reported varying results (Bang et al., 2010; Arteaga et al., 2011). In a study investigating 200 patients, HER2 overexpression was reported in 23% of patients, while another study that analyzed 248 patients showed overexpression of HER2 mRNA and/or proteins

Table 3. IHC Distribution in Primary and Lymph Node

		primary				lymph node			
		IHC (-)	IHC 1+	IHC 2+	IHC 3+	IHC (-)	IHC 1+	IHC 2+	IHC 3+
General		42(57)	15(20)	7(10)	10(13)	43(58)	9(12)	3(4)	19(26)
Histology	Diffuse	14	7	2	3	13	4	2	7
	Intestinal	28	8	5	7	30	5	1	12
Stage	StageII	14	8	2	5	15	5	1	8
	StageIII	28	7	5	5	28	4	2	11
Localization	Antrum	19	5	1	3	18	5	-	5
	Corpus	9	5	5	5	9	2	2	11
	Cardia	14	5	1	2	16	2	1	3

*Primary IHC histology p 0.75, primary IHC stage p 0.4, primary IHC localization p 0.184, lymp node IHC histology p 0.546, lymph node IHC stage p 0.5, lymph node IHC localization p<0.05

and amplification of genes in 22.6% of patients (Yano et al., 2006; Kim et al., 2007).

In routine clinical practice, HER2 status is usually assessed by IHC or FISH/CISH/SISH techniques in gastric cancer. IHC is used to determine protein expression and FISH/CISH/SISH are utilized to analyze gene amplification (Wang et al., 2011). In addition, HER2 status is generally studied in the primary tumor (Bozzetti et al., 2011). The discrepancy in HER2 status between the primary tumor and metastatic site has not been sufficiently investigated. Bozzetti et al. reported that a high concordance was found between HER2 status obtained by both IHC and FISH on primary tumors and matched metastases. Thus, the authors concluded that HER2 status remains unchanged in most patients during the metastatic process in gastric cancer (Bozzetti et al., 2011). In a similar study by Marx et al., the authors showed that HER2 status by FISH was similar between 49 primary tumors and their metastatic lymph nodes (Marx et al., 2009).

Trastuzumab is a monoclonal antibody targeted against the HER2 protein (Im et al., 2011). The ToGA study confirmed the survival benefits of trastuzumab in combination with chemotherapy compared with chemotherapy alone as a first line of treatment for patients overexpressing HER2 in advanced gastric or GEJ cancer (Bang et al., 2010). IHC 3+, FISH+ and IHC and FISH+ advanced gastric and GEJ cancers were investigated. It is well-known that HER2 positivity is associated with unfavorable prognosis, and its relation to tumor localization and depth of invasion has been extensively studied (Wang et al., 2010; Kim et al., 2008). Two studies failed to correlate histologic subtype, grade, tumor location, TNM stage, depth of invasion, lymph node metastasis, sex and age (Tsigris et al., 2002; Yu et al., 2007). On the other hand, HER2 positivity was found to be associated with peritoneal dissemination, liver metastasis and lymph node involvement (Orita et al., 1997). Motojima et al. (1994) reported a higher incidence of local lymph node involvement and distant metastasis in HER2-positive patients. In the study carried out by Orita et al. (1997) HER2 positivity was associated with poorer survival results in gastric cancer patients. Another study further confirmed the shorter survival of HER2-positive patients, which had a 5-year survival of 29% compared to 47% HER2-negative patients (Im et al, 2011). Other studies reported similar findings (Czyzewska et al., 2009, Nakajima et al., 1999).

Despite the well-reported heterogeneity of HER2 expression in breast cancer, some studies have suggested a somewhat more uniform expression pattern in gastric cancer (Marx et al., 2009). However, one study reported contrary findings (Hofmann et al., 2008). Due to tumor heterogeneity, a different clone might become the predominant subtype in the metastatic lymph nodes. Breast cancer studies have unequivocally shown that metastatic sites might display a different phenotype from the primary tumor as a sign of continuing genomic instability of progressing tumors. Treatment decisions based on the primary tumor characteristics might change with further analysis of the metastatic tumor (Bozzetti et al., 2011). Therefore, HER2 expression in the metastatic lymph nodes may need to be determined in addition to the analysis of the primary tumor samples.

On the other hand, Xu et al. reported 100% concordance regarding HER2 status between the primary tumor and axillary metastases by FISH (Xu et al., 2002). In HER2-negative cases, there is a 95% probability that the axillary lymph nodes will be HER2-negative (Simon R, et al, 2001). Similar findings were also reported for gastric cancer (Marx et al., 2009; Bozzetti et al., 2011).

In light of this information, a HER2 analysis of metastatic lymph nodes or distant metastatic sites might provide us with useful information that can help us in our decision-making process regarding treatment. As HER2 status is a predictive factor for patient response to trastuzumab and given tumor heterogeneity, the receptor is usually decreased in patients less responsive to trastuzumab (Pusztai et al., 2010).

In the present study, we showed that HER2 status is comparable in both the primary tumor and lymph node metastasis when evaluated by IHC and FISH. Moreover, SISH results in the primary tumors were compared with those of matched metastatic lymph nodes, which were found to be concordant with each other. In total, 6 patients had discordant SISH HER2 results. Our results showed a high level of concordance between primary tumors and metastatic lymph nodes by both IHC and SISH, which were similar to reports in literature (Marx et al., 2009, Bozzetti et al., 2011). However, in our study, HER2 status was evaluated by both IHC and SISH in the primary tumors and their corresponding lymph node metastases. Therefore, our study may be useful for determining HER2 status in both primary tumors and their lymph node metastases by IHC and SISH in patients with curative gastrectomy.

We found that *H. pylori* status was significantly correlated with SISH-HER2 status in both primary tumors and lymph node metastases. In other words, the prevalence of SISH positivity was significantly higher for *H. pylori* (+) than *H. pylori* (-) tumors. This finding might be related to the molecular pathology of gastric cancer, especially because it is known that either HER2 or *H. pylori* infection could play a role in carcinogenesis of gastric cancer.

In conclusion, our study indicates that the frequency of concordance in HER2 status by IHC or SISH is high in primary tumors and their corresponding lymph node metastases for patients with gastric cancer who underwent curative gastrectomy. The prevalence of HER2 discordance tends to be high in patients in the pN2 and N3 stages. The evaluation of HER2 status by both IHC and SISH may be useful in detecting patients who could benefit from trastuzumab and help guide our treatment decision-making process.

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