HER2 status in gastric cancers: a retrospective analysis from four Chinese representative clinical centers and assessment of its prognostic significance

W. Q. Sheng1,2,†, D. Huang1,2,†, J. M. Ying3, N. Lu3, H. M. Wu4, Y. H. Liu4, J. P. Liu5, H. Bu5, X. Y. Zhou1,2 & X. Du1,2*

1Department of Pathology, Fudan University Shanghai Cancer Center, Shanghai; 2Department of Oncology Fudan University Shanghai Medical College, Shanghai; 3Department of Pathology Cancer Institute and Hospital, Chinese Academy of Medical Science, Beijing; 4Department of Pathology Guangdong General Hospital, Guangzhou; 5Department of Pathology West China Hospital of Sichuan University, Chengdu, China

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Background: HER2 has a predictive value in gastric cancer. However, its association with prognosis remains uncertain. The aim of our study was to estimate the HER2-positive rate in Chinese gastric cancers, compare the classical fluorescence in situ hybridization (FISH) method with the novel bright-field dual color silver-enhanced in situ hybridization (DSISH) detection system, and evaluate the relationship between the HER2 status and prognosis.

Patients and methods: Seven hundred and twenty-six resected gastric cancers separately from four clinical centers in China were examined for HER2 by immunohistochemistry (IHC), FISH, and DSISH.

Results: The HER2-positive rate was 13%. The consistency between FISH and DSISH results was high (99%; = 0.958; P < 0.001). Tumor heterogeneity and polysomy were the main reasons for inconsistency. There was no significant difference in the 3-year overall survival (OS) between HER2-positive and -negative patients (P = 0.959). Multivariate analysis showed that HER2 was not an independent prognostic factor.

Conclusion(s): HER2 overexpression and amplification occur in a significant number of Chinese gastric cancer patients. Given the obvious advantages and high consistency with FISH, DSISH was superior for evaluating HER2 amplification in gastric cancer. HER2 was not a prognostic factor for gastric cancer in our study.

Key words: dual SISH, FISH, gastric cancer, HER2, immunohistochemistry, prognosis

introduction

Gastric cancer, including the gastro-esophageal junction (GEJ) adenocarcinoma, is still one of the most common and aggressive carcinomas throughout the world with a high mortality, especially in China. The Shanghai Municipal Center for Disease Control and Prevention ranked gastric cancer second in males and fourth in females for highest cancer incidence, and the second in males and third in females for mortality in 2008 [1]. Despite concerted efforts for treating this disease, the overall survival (OS) remains poor [2]. The trastuzumab for gastric cancer (ToGA) trial, have assessed HER2-targeting agents for treating advanced gastric cancer [3, 4]. HER2 evaluation...
becomes an important approach for predicting patient response to HER2-targeting agents. The HER2 status of Chinese gastric cancer patients currently remains uncertain. On the other hand, the association between HER2 status and prognosis in gastric cancer remains much less clear. Therefore, in this study, we investigated the HER2 status by immunohistochemistry (IHC) and in situ hybridization (ISH) in a series of consecutive Chinese gastric cancer patients from four major clinical centers located in China and analyzed the relationship with survival and clinicopathological characteristics. The two currently recommended methods for evaluating HER2 gene amplification, fluorescence in situ hybridization (FISH) and bright-field dual-color silver in situ hybridization (DSISH), were conducted simultaneously and the concordance of the results was evaluated.

materials and methods

patient collection and sample preparation

A total of 726 consecutive cases of gastric cancer treated by radical surgery without any preoperative therapy in 2008 were retrieved from the Departments of pathology of Shanghai Cancer Center of Fudan University (189 cases), West China Hospital of Sichuan University (170 cases), Guangdong General Hospital (175 cases), and The Cancer Institute and Hospital of the Chinese Academy of Medical Science located in Beijing (192 cases). The clinicopathological characteristics of each patient were retrieved from the four hospital information systems. The pathologic diagnosis, including pT/NM stage and the Lauren classification, was confirmed by two experienced and independent pathologists. For each sample, 3 μm sections were cut from the selected representative paraffin-embedded tumor blocks for IHC and ISH analyses. A paraffin-embedded breast cancer tissue sample (HER2 3+ by IHC and amplified by FISH) was mounted on each slide as a positive control. The study was approved by the Ethical Committee for Clinical Research at Fudan University Shanghai Cancer Center.

immunohistochemistry (IHC)

IHC staining was carried out using the anti-HER-2/NEU (4B5) antibody (Ventana Medical Systems, Inc. Tucson, Arizona) as the primary antibody against HER2 on a Ventana Benchmark XT automatic staining system according to the optimized manufacturer’s instructions. The amended HER2 IHC scoring system for gastric cancer proposed by Hoffmann et al. [5] was used as criteria for scoring the stained slides.

in situ hybridization

HER2 amplification levels were detected by both of FISH and DSISH assays, and the results between these two different methods were compared. FISH analysis was carried out according to the manufacturer’s protocol using the PathVysion®HER2 DNA Probe kit (LSI®HER2/neu Spectrum OrangeTM/CEP®17 Spectrum GreenTM), and DSISH was carried out on a Ventana Benchmark XT automatic staining system according to the optimized manufacturer’s instruction using the INFORM® HER2 DNA Probe and INFORM® Chromosome 17 (CHR17) Probe (Ventana Medical Systems SA). The way of ISH scoring is displayed in supplementary S1, available at Annals of Oncology online. Polysomy was defined as an average of three or more CHR17 signals per nucleus. The DSISH and FISH were assessed by different pathologists separately who were blinded to the IHC scores.

Any case with IHC 3+ and IHC2+/FISH+ was considered to be HER2 positive, while the cases with IHC 0 and IHC 1+ whether FISH + or −, and IHC 2+/FISH− were considered to be HER2 negative according to the European Medicines Agency [6].

inter-observer and inter-laboratory reproducibility

A tutorial study and two episodes of centralized training were conducted before and during the study for all pathologists. Of the cases, 5% from each center were double tested in the same laboratory for inter-quality control, while an additional 5% of cases were interchanged for detection in two laboratories for inter-laboratory quality control.

follow-up and statistical analysis

According to the study protocol, the institutional ethic committee approved the collection of survival information from three centers, which totalled 556 patients. Patients were asked to return for follow-up every 6 months for oncological assessment. Between January and April 2012, all patients were followed up by telephone or mail to obtain patients’ survivorship.

Data analysis was conducted using SPSS statistical software (version 16, SPSS Inc). The chi-squared test and Kruskal–Wallis test were carried out to compare the distributions of HER2 status and clinicopathological factors in patient populations from the four geographic locations tested in China. The chi-squared test and the logistic regression test were used to investigate the association between HER2 status and each clinicopathological variable. The kappa statistic was calculated to compare the measures of FISH and SISH tests. The Spearman rank correlation was used to assess the correlation between the IHC and FISH measurements.

Survival analysis was carried out using the Kaplan–Meier method and the multivariate survival analysis was in proportion hazards regression models described by COX. The significance tests were two-sided and a P value of <0.05 were considered statistically significant.

results

HER2 status and the results of IHC, FISH, and DSISH for patients from four regions of China

The clinicopathological features, HER2 status, and the results of IHC, FISH, and DSISH in 726 cases of gastric cancer patients from four medical centers representing four different geographic locations in China are summarized in supplementary Table S2, available at Annals of Oncology online, supplementary Figure S3, available at Annals of Oncology online. The HER2 positive rate was 13% (91 of 726). There was no significant difference in HER2 status among patients from the four different regions of China regardless of the detection method (IHC or FISH and DSISH).

The inter-laboratory trial for assessing HER2 status in samples was carried out at the four centers, and each center tested 36 different samples. Good inter-laboratory agreement was obtained not only for the IHC method (average Spearman’s rho = 0.83; P < 0.01), but also for the ISH tests (average kappa statistic = 0.89; P < 0.01).

assessments of HER2 status by IHC, FISH, and DSISH

The results of IHC, FISH, and DSISH tests and the consistency between FISH and DSISH are summarized in supplementary Table S4, available at Annals of Oncology online. There was a highly significant correlation between IHC and FISH results (ρ = 0.6; P < 0.001), especially in those cases with an IHC score
of 0 or 1+. Most of the cases with IHC 3+ (93%) showed HER2 gene amplification (supplementary Figure S5, available at Annals of Oncology online). Tumor heterogeneity was observed in 57% of the cases. CHR 17 polysomy was found in 8 of 726 samples (1%), all of which occurred in the 13 IHC/FISH discordant cases, including the five cases of IHC 3+/FISH− (supplementary Figure S5, available at Annals of Oncology online). For those three cases of IHC 0/FISH+, although an IHC 3+ area was identified in one case and an IHC 2+ area was identified in the other two cases, but the percentage of stained tumor cells was <10%.

A very high consistency in the results between FISH and DSISH (99%; κ = 0.958; P < 0.001) was obtained. No significant differences were obtained in 428 cases of IHC 0 (P > 0.05). Three discordant IHC 3+ cases and two out of three discordant IHC 2+ cases were polysomic. The HER2/CEP17 ratio of the remaining two cases was 1.97 and 2.08 by FISH versus 2.12 and 1.93 by DSISH, respectively.

correlation of HER2 status with clinicopathological features

The correlation between HER2 status and the clinicopathological features is shown in supplementary Table S6, available at Annals of Oncology online. The frequency of HER2 positivity correlated with gender, tumor location, histological grade, and the Lauren classification. HER2 positivity was more common in intestinal-type, well- or moderately-differentiated tumors, GEJ carcinomas, and males. The positive rate of HER2 was similar between stage I and stage II–IV diseases (12% versus 13%, respectively; P = 0.416). There were no statistically significant associations with advanced age, lymph node metastasis, pT stage, pN stage, or pM stage, respectively.

A multivariate logistic regression analysis found that HER2 positivity correlated with tumor location (OR 2.12; 95% CI 1.32–3.42; P = 0.002), tumor grade (OR 2.94; 95% CI 1.61–5.38; P = 0.000) and the Lauren classification (OR 1.90; 95% CI 1.03–3.49; P = 0.04).

correlation of HER2 status with survival

Based on the study protocol, a total of 454 patients were successfully followed up for survival analysis (82% for all patients at the three centers receiving IRB approval). The median follow-up time was 26 months (range: 2–45 months), and 156 patients (34%) died of cancer, while the remaining 298 patients (66%) were still alive at the end of the study period. The 3-year OS rates were 63% and 65% in HER2-positive and HER2-negative patients, respectively (P = 0.959; supplementary Figure S7, available at Annals of Oncology online). The mean survival time was 32 months [standard error (SE) 1.67; 95% CI 29.0–35.6] in HER2-positive patients, compared with 34 months (SE 0.79; 95% CI 32.7–35.7) in HER2-negative patients.

Tumor location, Lauren classification, stage, grade, gender, HER2 status, and lymph node metastasis were included in univariate analyses and a multivariate analysis. Only the tumor stage was identified as an independent risk factor for OS, with hazard ratios of 4.08 (95% CI 2.73–6.10; P = 0.000). Moreover, HER2 positivity was not found to be an independent prognostic factor for gastric cancer (HR 1.04; 95% CI 0.66–1.66; P = 0.854).

discussion

Since the overexpression of HER2 in gastric cancer was first published in 1986 [7], many studies have reported the frequency of HER2 positivity in gastric cancer patients from various regions throughout the world. According to the review by Jørgensen et al. [8], the positive rate ranged from 4% to 53% by IHC alone and from 9% to 18% when ISH was included. Detected by both of IHC and ISH, the HER2-positive rate in our study was 13%. There was no significant difference between the cases from different Chinese populations. In addition, there was no significant difference in the positive rate in cases based on the clinical stage. Similar to the ToGA study [4], the positive rate was much higher in GEJ tumors and the intestinal subtype by the Lauren classification. Furthermore, in agreement with previous studies [9–12], HER2 positivity was correlated with male gender, moderate- to well-differentiated histology, but not associated with lymph node metastasis, pT, pN, or pM staging.

Similar to the published report [5, 9, 13, 14], the concordance rate of HER2 gene amplification and HER2 protein overexpression in our study was generally high. In the ToGA trial, HER2 amplification by FISH was detected in 4.9% of IHC 0 cases and 15.7% of IHC 1+ cases. However, it was only 1% and ∼4% in our IHC 0 and IHC 1+ cases, respectively. Compared with the amplification of HER2 in more than 50% of IHC 2+ cases from the ToGA study, only 20% of the IHC 2+ cases in our study had HER2 amplification as determined by both FISH and DSISH. The sensitivity of the antibody used in the assay may influence the distribution of cases with different immunohistochemical scores.

Heterogeneity of HER2 expression in gastric cancer has been reported in many previous studies [5, 11, 15] and could be an important explanation for divergent results reported by different studies. Moreover, the pattern of heterogeneity not only made the scoring of IHC staining difficult and discrepant, but also caused inconsistencies in the IHC and ISH results based on the current scoring system. In one case, <10% of strong IHC staining tumor cells but enough tumor cells showing HER2 amplified made the results of IHC and ISH inconsistent. The study by Ruchoff et al. [16] suggested that these types of cases can be considered as HER2 positive regardless of the specimen type. However, further assessment with regard to the benefits and hazards of trastuzumab therapy in this population is needed before this approach can be accepted as a universal standard. Lee et al. [15] suggested using larger tissues for accurate HER2 assessment due to the high incidence of intratumoral HER2 heterogeneity in gastric cancer. The US Food and Drug Administration also advises not to rule out potential Herceptin benefit relying on a single method [17]. In this study, a HER2 test was only conducted in one representative block of each case. Double check on another block for those HER2-negative cases might increase the accuracy.

The DSISH is a recently developed method to quantitatively detect amplification of the HER2 gene using two-color chromogenic ISH on a fully automated platform [18]. Excellent concordance and reproducibility in the detection of HER2
amplification have been demonstrated using the assay for breast and gastric cancer cases [18, 19]. We also achieved a high accordance rate (99%) between FISH and DSISH in our study. However, we found that more than 3 CHR17 signals could be clearly observed in FISH slides by a fluorescence microscope, particularly when a single bandpass filter was used, but it was not clearly detected by DSISH. Despite a recent study showing that CHR 17 polysomy is very rare and most likely due to concomitant gain or amplification of the centromeric region [20], we found 8 in 726 (1%) cases of gastric cancer with a CEP 17 copy number of more than 3, which was <4% found in the ToGA trial [21]. In addition, polysomy of CHR17 was the main cause of discrepancy, which occurred in five of seven discrepant cases and similar to what was found in a Spanish study [19]. As suggested by Garcia-Garcia et al. [19], raising the cut-off ratio of HER2/CEP17 to 3 as amplification can avoid the influence of polysomy and achieve complete agreement in the results from FISH and DSISH. However, altering the cut-off ratio did not change the results in our study. Therefore, additional modification and improvements are needed to optimize this method. In addition, authoritative supplementary instructions are needed to instruct the assessment for those cases with a high HER2 gene copy number (i.e. 6 copies), but that have a HER2/CEP17 ratio slightly <2.

In breast cancer, genomic amplification and overexpression of the HER2 gene were also associated with poor outcomes, higher mortality, and increased higher recurrence and metastasis [22–24]. However, the association between HER2 status and prognosis in gastric cancer remains controversial, and a correlation between HER2 amplification or overexpression and favourable survival has only been shown in a few studies [4, 25, 26]. Most published studies assessing this association have shown a poor prognosis or related clinicopathological features in HER2-positive gastric cancers. Given the fact that only IHC testing was used in most of the studies analyzed without ISH analysis for further determination, and that the IHC result was variable due to a series of factors (e.g. reagents and scoring system used), the conclusion of the relationship between HER2 status and prognosis of gastric cancer remains unclear. In our study, no significant difference in the 3-year OS was found between HER2-positive and negative patients. Moreover, HER2 positivity was not an independent prognostic factor by multivariate analyses. However, we did not carry out a more intensive analysis of HER2 status and prognosis through stratification of stages, use of adjuvant chemotherapy, response, and other parameters, and therefore, it is possible that there is an association if these stratifications are assessed.

In summary, we assessed the HER2 status in 726 samples from consecutive surgical cases of gastric cancer derived from four representative clinical centers in China using three leading methodologies (IHC, FISH, and DSISH). The total HER2-positive rate was 13%. In addition, a high concordance was obtained not only between IHC and FISH, but also between DSISH and FISH. Considering the unique features of HER2 overexpression in gastric cancers and the advantages of DSISH, this new method may be more suitable for routine analysis of HER2 amplification. However, laboratory validation and technical training for both analytical and post-analytical stages are necessary. Moreover, improvements in the technique itself are expected to perfect and refine HER2 assessment, especially in those cases with aneusomy of chromosome 17. In addition, authentic modifications and supplements to the scoring system of both IHC and ISH will require confirmation and approval by regulatory authorities. Although HER2 status was found to be associated with several clinicopathological characteristics related to the invasive behavior of gastric cancer, it was not a prognostic marker for gastric cancer based on the results of our study.

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disclosure

The authors have declared no conflicts of interest.

references

The frequency and impact of ROS1 rearrangement on clinical outcomes in never smokers with lung adenocarcinoma


1Yonsei Cancer Center; 2Department of Internal Medicine, Yonsei University College of Medicine, Seoul; 3JE UK Institute for Cancer Research, Gumi City, Kyungbuk; 4Korea CFC Pathology Laboratory, Seoul; 5Department of Thoracic and Cardiovascular Surgery, Yonsei University College of Medicine, Seoul, Korea; 6Chao Family Comprehensive Cancer Center, University of California Irvine Medical Center, Orange; 7Novartis; 8Abbott Molecular, Inc., Illinois, USA; 9Biostatistics Collaboration Unit, Yonsei University College of Medicine, Seoul, Korea; 10Department of Haematology-Oncology, National University Cancer Institute, National University Health System, Singapore; 11Cell Signaling Technology, Danvers, Massachusetts, USA; 12Department of Pathology, Yonsei University College of Medicine, Seoul, Korea

Background: To determine the frequency and predictive impact of ROS1 rearrangements on treatment outcomes in never-smoking patients with lung adenocarcinoma.

Patients and methods: We concurrently analyzed ROS1 and ALK rearrangements and mutations in the epidermal growth factor receptor (EGFR), and KRAS in 208 never smokers with lung adenocarcinoma. ROS1 and ALK rearrangements were identified by fluorescent in situ hybridization.

Results: Of 208 tumors screened, 7 (3.4%) were ROS1 rearranged, and 15 (7.2%) were ALK-rearranged. CD74-ROS1 fusions were identified in two patients using reverse transcriptase–polymerase chain reaction. The frequency of ROS1 rearrangement was 5.7% (6 of 105) among EGFR/KRAS/ALK-negative patients. Patients with ROS1 rearrangement had a higher objective response rate (ORR; 60.0% versus 8.5%; \(P = 0.01\)) and a longer median progression-free survival (PFS; not reached versus 3.3 months; \(P = 0.008\)) to pemetrexed than those without ROS1/ALK rearrangement. The PFS

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