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Clinical significance of intratumoral HER2 heterogeneity in gastric cancer

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Abstract ***Aim:*** To evaluate the clinical significance of intratumoral HER2 heterogeneity in gastric cancer (GC).
Methods: A total of 322 GC tissues were evaluated by HER2 immunohistochemistry (IHC), of which 73 with IHC 2+ or 3+ were subjected to fluorescence in situ hybridisation (FISH). Also, 3–5 distinct spots in each case showing different HER2 staining intensities were evaluated individually by comparing IHC staining intensity with gene copy number (GCN). Minimum, average and maximum FISH scores were generated for each case.
Results: Intratumoral heterogeneity of HER2 overexpression and gene amplification were 54 and 30 of 73 cases with IHC 2+ or 3+, respectively. These cases were characterised by diffuse or mixed Lauren type, HER2 IHC 2+, and low-level amplification. Kaplan–Meier survival analysis revealed that the heterogeneous overexpression was significantly associated with longer disease-free survival times than the homogeneous, and the high average GCN was most associated with poor outcome. Also, there was a strong correlation between the IHC and FISH results for each spot. Quantitative polymerase chain reaction (PCR) analysis of the cancer tissues and the cell-free plasma showed that *HER2* gene copy by quantitative PCR on tissue correlated well with those by FISH, but plasma *HER2* level was not.
Conclusions: Considering the high incidence of intratumoral HER2 heterogeneity in GC, accurate HER2 assessment would require larger tissues and more detailed guidelines. The guidelines

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should include the recommendation that FISH-scoring areas be selected with reference to a corresponding IHC slide. Also, the definition of HER2-positive tumours should be reassessed considering the intratumoral heterogeneity.

1. Introduction

HER2 is a transmembrane receptor tyrosine kinase that is a member of the epidermal growth factor receptor (EGFR) family, and it has important roles in cell growth, differentiation and survival.[1] Oncogenic activation of HER2 mainly occurs through gene amplification (GA), which leads to protein overexpression on the cell membrane and subsequent abnormal cell signalling.[1] The importance of HER2 is well established in breast cancer, where HER2 testing has become a standard approach for identifying patients who may benefit from HER2-targeted agents such as trastuzumab and lapatinib in the metastatic setting and, more recently, in adjuvant and neoadjuvant settings.[2,3]

In gastric cancer (GC), HER2 overexpression and/or GA rate was reported to be 7–34%,[4] and was found to be associated with more aggressive disease and poorer survival.[5,6] Preclinical studies have indicated that trastuzumab exerts growth inhibitory effects in HER2-overexpressing human GC cell lines and xenograft models.[6–9] In addition, phase III clinical trials for gastric or gastroesophageal junction cancer patients (ToGA trial) showed that the median overall survival time was longer in patients receiving trastuzumab plus chemotherapy than in patients treated with chemotherapy alone.[4] Moreover, the study indicated that patients with immunohistochemistry (IHC) 2+/fluorescence in situ hybridisation (FISH)+ and IHC 3+ had an increased benefit from trastuzumab treatment.[4] Based on these results, the European Medicines Agency (EMEA) very recently approved trastuzumab for the treatment of metastasised adenocarcinomas of the stomach and the gastroesophageal junction and pointed out that trastuzumab should only be used in patients with metastatic GC whose tumours have HER2 overexpression as defined by IHC 2+ and a confirmatory FISH+ result, or IHC 3+.[10] Thus, accurate assessment of HER2 status by IHC and/or FISH is very important for clinical decision-making in the treatment of patients with GC.

One important issue in HER2-targeted therapy is therapeutic resistance. In fact, a significant number of breast cancer patients acquire therapeutic resistance during trastuzumab treatment.[11,12] One of the possible causes of therapeutic resistance is intratumoral HER2 heterogeneity, which can lead to treatment failure because of selection of subclones lacking *HER2* GA.[13] In addition, the heterogeneity may result in the inaccurate assessment of the HER2 status and the consequent mischoice of patients for trastuzumab treatment.

However, there are few studies on intratumoral HER2 heterogeneity in GC,[14–16] and its clinical significance has not been determined. Furthermore, even the definition is not clear. Recently, the College of American Pathologists (CAP) defined *HER2* genetic heterogeneity as the presence of tumour cells with *HER2*/chromosome enumeration probe (CEP) 17 signal ratios greater than 2.2 in 5–50% of the tumour cells tested in breast cancer.[17] However, only a few studies have addressed the validity of the definition.[13,18,19]

The present study aimed to describe the HER2 heterogeneity of GC in detail by performing HER2 IHC and FISH on whole sections and evaluating the gene copy number (GCN) individually in distinct areas with different IHC staining intensity. Subsequently, the clinical significance of intratumoral HER2 heterogeneity was analysed. In addition, the *HER2* GCN was assessed in tumour tissues and cell-free plasma by real-time polymerase chain reaction (PCR) and compared with the FISH results.

2. Materials and methods

2.1. Patients

This retrospective study was conducted in a cohort of 322 GC patients who underwent surgical resection of primary gastric carcinoma at the Seoul National University Bundang Hospital, Seongnam, Korea. Formalin-fixed, paraffin-embedded tumour tissues were used. Matching cell-free plasma samples of 47 out of 322 patients were obtained by collecting the remnants of pre-operative blood samples. Age, gender, World Health Organisation (WHO)[20] and Lauren's classification and pathologic TNM (pTNM) stage (by Union for International Cancer Control/American Joint Committee on Cancer (UICC/AJCC))[21] were evaluated by reviewing medical charts, pathological records and glass slides. The median follow-up period of patients was 15 (range, 1–82) months. None of the patients received preoperative chemoradiotherapy, and patients with stage II, III or IV disease received postoperative chemotherapy with a fluorouracil (5-FU)-based regimen (5-FU alone, 5-FU plus mitomycin C or 5-FU plus cisplatin). The study protocol was reviewed and approved by the Institutional Review Board of Seoul National University Bundang Hospital.

2.2. IHC

Immunohistochemical staining was performed using an automatic immunostainer (BenchMark® XT,