Original Research

HER2 copy number of circulating tumour DNA functions as a biomarker to predict and monitor trastuzumab efficacy in advanced gastric cancer

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Abstract Background: HER2 status is significant to trastuzumab therapy; however, it is difficult to determine HER2 status accurately with few pieces of biopsies from advanced gastric cancer (AGC) due to highly heterogeneity and invasive behaviour, which will be investigated in this study.

Methods: Fifty-six patients with AGC were included in this study. Primary tumour tissues and matched plasmas before medication from 36 patients were retrospectively collected, and the other 20 patients with primary tumour tissues and paired plasmas were prospectively collected. HER2 expression and amplification in 56 tumour tissues were determined by immunohistochemistry (IHC) and dual in situ hybridisation (DISH), and HER2 copy number in 135 circulating tumour DNAs (ctDNAs) was judged by next-generation sequencing.

Results: For tumour tissues, HER2 amplification by DISH was most commonly found in patients with HER2 score 3+ by IHC. For plasmas, HER2 amplification defined as HER2 copy number >2.22 was identified in 26 of 56 patients. There was a high concordance of HER2 amplification between ctDNA and tumour tissues, suggesting that ctDNA could function as an alternative to screen HER2-targeted population. Moreover, the changes of HER2 copy

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number in ctDNA could efficiently monitor trastuzumab efficacy, the power of which was superior to commonly used markers carcinoembryonic antigen (CEA) and CA199, suggesting its potential role in clinical practice.

Conclusion: ctDNA for HER2 analysis was strongly recommended to serve as a surrogate to screen trastuzumab-suitable population and monitor trastuzumab efficacy.

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1. Introduction

Gastric cancer is one of the most lethal cancers worldwide with poor prognosis due to the advanced clinical stage at diagnosis and the resistance to traditional chemotherapy [1]. Therefore, more effective therapeutic options are needed for patients with advanced gastric cancer (AGC). Trastuzumab shows a great promising in the antineoplastic treatment of gastric cancer. Interestingly, the trastuzumab for gastric cancer (ToGA) trial demonstrated a significant survival benefit of trastuzumab for AGC patients with HER2 positive, followed by the approval of trastuzumab as the first-line treatment [2]. HER2 assessment in histological cases of gastric cancer is essential to identify patients suitable for trastuzumab. Based on the guideline, only HER2-positive patients defined as HER2 score 3+ by immunohistochemistry (IHC) or amplification by fluorescence in situ hybridization (FISH)/dual in situ hybridisation (DISH) in tumour tissues were eligible for trastuzumab therapy.

For AGC patients, endoscopic biopsy is the major method to obtain tumour tissues for HER2 evaluation. However, a single or multiple biopsies can provide spatiotemporally limited information due to highly heterogeneous HER2 expression and induce unpleasant side-effects due to invasiveness [3–8]. Also, patients with false negative results of HER2 evaluation or unavailable HER2 status would miss the chance for trastuzumab treatment. Moreover, HER2 status could change along with disease evolution or therapeutic process [9,10], but it is infeasible to re-evaluate HER2 status by repeated tissue biopsies in clinical practice. Therefore, non-invasive methods were urgently needed to screen patients suitable for anti-HER2 therapy and to monitor the therapeutic efficacy for better management of AGC.

Plasma circulating tumour DNA (ctDNA) has been increasingly reported to carry genomic or epigenomic information of tumours, suggesting its potential role in clinical practice [11–13]. Gene variations including mutations, copy number variations and so forth could be successfully identified from ctDNA, which laid the foundation of extensive use, especially in the era of next-generation sequencing (NGS). Regarding HER2 status in ctDNA, previous studies demonstrated the identification of HER2 amplification in plasma ctDNAs by droplet digital PCR (ddPCR) from AGC patients, but the concordance of HER2 status with tumour tissues was not ideal [14,15]. According to our preliminary results, HER2 amplification could be determined by NGS from tumour tissues and ctDNAs in AGC patients with high consistency (data not shown). In this study,
Fig. 1. The heterogeneity of HER2 amplification between plasma ctDNAs and tumour tissues. Plasma HER2 copy numbers were assessed via NGS and compared with the HER2 amplification status in matched tumour tissues by DISH. The X-axis demonstrates the 10,000 kb region adjacent to HER2. Copy numbers of leucocyte cells from the patients were also calculated and displayed as control (blue lines); plasma copy numbers were shown in red lines. Each circle indicates the copy number of a 200 kb segment. The enlarged circles represent
HER2 status from ctDNAs and tumour tissues of AGC patients was evaluated by NGS and IHC/DISH, respectively, and the clinical application of ctDNAs was also confirmed.

2. Materials and methods

2.1. Demographic data of patients and study design

A cohort of 56 patients with pathologically confirmed AGC treated in our department at Peking University Cancer Hospital from June 2013 to June 2016 were included. Among them, 36 patients with primary tumour biopsies and matched plasmas before medication were retrospectively collected, and the other 20 were prospectively collected, who had primary tumour biopsies and paired plasmas together with trastuzumab treatment. Totally, 56 tumour biopsies were analysed for HER2 expression and amplification by IHC and DISH, respectively, and 135 plasmas were used for evaluating HER2 amplification by NGS. All patients received at least two cycles of first-line oxaliplatin-based chemotherapy with/without trastuzumab. The evaluation of clinical response was performed following Response Evaluation Criteria in Solid Tumours 1.1 criteria classified as complete response, partial response, stable disease and progressive disease. The last follow-up was performed in June 2017, and the progression-free survival (PFS) and overall survival (OS) were calculated from the initiation of treatment to disease progression or death, respectively. This study was approved by the Medical Ethics Committee of Peking University Cancer Hospital, and written informed consents were obtained from all patients.

2.2. HER2 expression and amplification in tumour tissues by IHC and DISH

HER2 protein expression was detected using anti-HER2/neu antibody (4B5; Roche, Basel, Switzerland) by IHC staining followed by interpretation of HER2 scores according to the reported procedure [16]. HER2 amplification defined as a ratio HER2/CEP17 ≥ 2.0 was determined using Ventana HER2 dual-colour in situ hybridization (ISH) assay (DISH; BenchMark XT). HER2 expression and amplification were judged by two independent specialists blinded to the study. Briefly, patients with HER2 score 3+ by IHC or HER2 amplification by DISH were defined as HER2 positive.

2.3. Isolation of ctDNA from plasma and NGS

Whole blood from patients was centrifuged at 1600×g for 10 min at 4°C. The supernatant was transferred into a fresh tube, and centrifuged at 16,000×g for 10 min at 4°C. To detect the gene copy number variation in ctDNA, 500 µl of plasma was subjected to DNA isolation. We used at least 3 ng of the ctDNA to generate NGS library and sequenced on an Illumina HiSeq 2500 sequencer. The quality of library was examined by quantitative polymerase chain reaction (PCR) and 2100 Bioanalyzer (Agilent, USA). About 5 million sequencing reads were obtained from each case. The raw data (in.bcl format) were demultiplexed and converted to FASTQ format using a perl script configure Bcl To Fastq.pl in CASAVA (Consensus Assessment of Sequence and Variation, version 1.8.2) package. Illumina adapters and low-quality bases were
removed from the FASTQ file using Trimmomatic (version 0.35) [17]. High-quality reads were mapped to hg19 reference genome [18] using BWA (version 0.7.12-r1039) [19] with default parameters. The mapped reads were sorted and converted to binary format .bam using Sam to Bam.jar in Picard (version 1.119) package.

2.4. HER2 copy number analysis of ctDNA

Unique mapped reads were extracted from the alignment .bam file. The whole reference genome was divided into non-overlapped observation windows (bins) with the size of 1 Mb/200 Kb. Reads number and guanine-cytosine (GC) content were calculated in each bin. GC bias correction was processed for every 1% GC content. We used R (version 3.0.0) to graph the GC-corrected relative reads number (RRN) of each bin to visualise copy number variations.

To determine aberrant HER2 copy number in patients, 102 plasma samples from healthy people were chosen as a reference data set, from which RRN mean and standard deviation (SD) of each bin were obtained. A RRN between the value of mean ± 2 * SD was defined as a normal copy number. Specifically, the cutoff for normal diploid HER2 copy number was 1.78–2.22 accordingly. A RRN >2.22 for the bin covering HER2 locus indicates the HER2 amplification.

2.5. Statistical analysis

Kappa and McNemar’s tests were used to assess the consistency of HER2 amplification between tumour tissues and ctDNA. The chi-square test was performed to investigate the association of HER2 expression with amplification and the correlation of HER2 expression/amplification with other clinicopathological factors. The difference on the response rate to trastuzumab treatment between patients with and without HER2 amplification was also studied by chi-square test. Kaplan–Meier survival analysis was performed to compare outcomes of patients with and without plasma HER2 amplification subjected to trastuzumab treatment. Differences with P < 0.05 were considered statistically significant. All analyses were performed with SPSS 22.0.

3. Results

3.1. The clinicopathological characteristics of the patients

We enrolled 56 AGC patients with the median age of 57 years, ranging from 29 to 80 years. Forty-two (75%) patients were men, and fourteen (25%) patients were women. Among all patients, 36 patients were retrospectively included, and the other 20 were prospectively collected. All patients received first-line oxaliplatin-based chemotherapy with or without trastuzumab for at least two cycles. Fifty-three patients had progressive disease with a median PFS of 7.1 months (95% confidence interval [CI]: 6.0–8.2), and 46 patients died with a median OS of 14.4 months (95% CI: 12.7–16.1) at the last follow-up. The median PFS (8.0 vs. 5.8 months) and OS (16.0 vs. 13.5 months) in patients treated with trastuzumab plus chemotherapy were higher than patients treated with chemotherapy alone. The clinicopathological information including primary tumour site, Lauren classification, metastasis, and trastuzumab treatment were detailed in Table 1.

3.2. HER2 expression and amplification in tissues and plasma ctDNAs and its correlation with patients’ characteristics

In total, 56 tumour tissues and 135 plasmas were obtained from 56 patients; among them, 36 had single tumour biopsy and paired plasma before medication, and 20 provided single tumour biopsy before treatment and multiple plasmas along with trastuzumab treatment. For tumour tissues, HER2 scores 3+, 2+ and 1+/0 by IHC were found in 19, 22 and 15 patients, among which 17, 7 and one patient had HER2 amplification by DISH. For plasmas with the matching tumour tissues, HER2 amplification was identified in 26 out of 56 patients (Table 2). Tissue HER2 status by IHC and DISH and plasma HER2 copy number by NGS of all patients were listed in Table S1. HER2 protein expression was significantly positively correlated with HER2 amplification in tumour tissues or ctDNAs; however, HER2 status was not significantly associated with characteristics of patients.

3.3. HER2 amplification in plasma ctDNAs could overcome HER2 heterogeneous amplification in tumour tissues

Since HER2 protein expression was significantly correlated with HER2 amplification, we also analysed the consistency of HER2 amplification in tumour tissues and plasma ctDNAs in this study. Consistent with our previous result [20], a high concordance (91.07%, kappa index = 0.820, P < 0.001; Table 3) was also found between tumour tissues and plasmas in this study, suggesting a potential role of ctDNAs as a surrogate to screen trastuzumab-suitable patients.
Studies had confirmed that not all patients with HER2 overexpression carried HER2 amplification and vice versa [21], because there were other factors such as mutations rather than HER2 amplification that could activate HER2 overexpression (cases 6 and 7 in Table S1). As presented in Table 3 and Table S1, patients had inconsistency of HER2 amplification in tumour tissues and plasmas. Cases 49 and 50 carried HER2 amplification in tumour tissues but not in plasmas (Table S1), and this might be explained by that HER2 amplification was unevenly distributed in tumour tissues (Fig. 1), which resulted in the negative results derived from pooled ctDNAs. For cases 9, 22 and 25 with only HER2 amplification in plasmas (Fig. 1), a logical interpretation was that tumour biopsies containing HER2-amplified cells were not captured.

3.4. The roles of plasma HER2 copy number in predicting and monitoring trastuzumab efficacy

The consistency of HER2 amplification in tumour tissues and ctDNAs was very high, suggesting ctDNAs could serve as a surrogate to predict trastuzumab efficacy, which was confirmed in our cohort. Among patients who received trastuzumab plus chemotherapy (N = 22), 13 of 15 with plasma HER2 amplification achieved partial response, which was higher than patients without HER2 amplification in ctDNA (P = 0.032).

Moreover, the role of plasma HER2 amplification in dynamic monitoring was investigated in 20 patients treated with trastuzumab, and in total, 99 plasmas were analysed. In plasmas before trastuzumab, 15 patients were identified with HER2 amplification and five patients were not. The fluctuation of HER2 copy number in five patients without initial HER2 amplification was not predominant (Figure S1) versus significant changes in response to trastuzumab treatment in most patients with initial HER2 amplification (Fig. 2). Compared to HER2 copy number at baseline, most patients exhibited a decrease in HER2 copy number when patients benefited from trastuzumab. However, HER2 copy number at progressive disease increased again compared to beneficial points except for cases 4, 1 and 39. Furthermore, the power of HER2 copy number was superior/comparable to commonly used monitoring markers CEA or CA199 (Fig. 3), suggesting its potential role in clinical practice.

4. Discussion

HER2 evaluation in tumour tissues by IHC/DISH before trastuzumab administration is essential for clinical practice [2]. Owing to the confirmed intra-tumoural heterogeneity of HER2 expression, Ahn S et al. demonstrated that at least four biopsy fragments were sufficient to precisely predict HER2 expression in AGC patients [22]. Moreover, HER2 assessment was recommended in real-time manner due to HER2 changes along with the treatment [9,10]. However, several issues were raised and needed to be solved clinically. Some patients with only metastatic lesions had difficulties in tumour biopsies, and some had risks in obtaining multiple biopsies. Also, it was infeasible in clinical practice to perform repeated biopsies.

Therefore, this study was designed to propose an alternative and non-invasive method based on NGS for HER2 assessment in ctDNA. HER2 amplification was identified successfully from plasma ctDNAs in our cohort, and its concordance with tumour tissues by DISH was 91.07%, which was obviously higher than that reported by ddPCR [14], suggesting its potential significance in clinical utility. Technically, NGS maps the whole sequence of HER2 but ddPCR detects partial fragment of HER2, and NGS is more suitable for batch analysis.

Despite the high consistency of HER2 amplification in ctDNAs and tumour tissues, five patients had inconsistent amplification. For amplification found only in tumour tissues but not in paired ctDNAs, it might be caused by dilution of ctDNAs released from cells without HER2 amplification due to uneven distribution. For patients with HER2 amplification only in ctDNAs (cases 9, 22 and 25), tumour biopsies containing HER2-amplified cells might not have been captured. These three patients did not receive trastuzumab. Cases 9 and 22 died at our last follow-up; however, case 25 carrying a plasma HER2 copy number 16.44 would be followed up closely to receive trastuzumab if possible.

A total of 22 patients in this study received trastuzumab treatment; owing to the limited number, the solid statistical conclusion could not be made. However, 13 of 15 patients with plasma HER2 amplification achieved partial response, which was higher than patients without amplification (P = 0.032). Since HER2 amplification in tumour tissues was a definite marker to predict trastuzumab efficacy and the high concordance of HER2 amplification between tumour tissues and ctDNAs was confirmed, it was reasonable to use plasma HER2 amplification as a predictive surrogate. It was demonstrated that patients with plasma HER2 amplification had a more effective response to trastuzumab [15]. In future, the correlation of HER2 amplification in ctDNAs to prognosis would be determined in large samples.

Based on the advantage of non-invasiveness, the role of ctDNAs in dynamic monitoring was more closely focused on. ctDNAs had been demonstrated to monitor the recurrence and progression in gastric cancer [14], and our previous studies revealed that HER2 phenotype of circulating tumor cell (CTC) could change following targeted therapy. In this study, a total of 20 patients treated with trastuzumab were prospectively included.
generating 99 plasmas for HER2 copy number assessment. For 15 patients with HER2 amplification in plasmas before trastuzumab, HER2 copy number fluctuated along with trastuzumab treatment and presented a trend of decreasing first and then increasing in most patients, which corresponded to benefit from treatment and disease progression, respectively (Fig. 2), suggesting its potential role in efficacy monitoring. CEA and CA199 are the frequently used monitoring markers at present; however, in patients treated with trastuzumab, the monitoring power of HER2 copy number was superior or comparable to CEA or CA199 (Fig. 3). Conceivably, HER2 copy number analysis in plasmas was strongly recommended to monitor efficacy in patients treated with trastuzumab.

We also explored whether HER2 copy number was influenced in patients without trastuzumab treatment. No significant correlation was found due to the limited samples (data not shown), which needs to be further confirmed in large samples. Moreover, the results from other five patients without initial HER2 amplification further confirmed that HER2 evaluation in ctDNAs by NGS was reliable and stable.

In conclusion, we employed a NGS method to assess HER2 copy number in plasma ctDNA and confirmed the high concordance of HER2 amplification in tumour tissues by DISH and ctDNAs by NGS. Also, HER2 copy number could serve as a surrogate marker to screen HER2-targeted therapy, and more interestingly, monitor the trastuzumab efficacy. Based on our results, in addition to tumour tissues, ctDNA for HER2 analysis was strongly recommended in clinical practice to guide precision medicine.

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Conflict of interest

None declared.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ejca.2017.10.032.

References


