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The expression of human epidermal growth factor receptor 2 in peritoneal metastasis of gastric cancer

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Abstract

Background

The prognosis of gastric cancer patients with peritoneal metastasis (PM) is very poor. In recent report suggested that a monoclonal antibody that targets human epidermal growth factor receptor 2 (HER2) improve the prognosis of gastric cancer with HER2 overexpression and/or gene amplification. However, HER2 overexpression and/or gene amplification of gastric patients with PM is unclear.

Methods

HER2 expression of primary gastric lesion and peritoneal metastasis was investigated immunohistologically and by fluorescence in-situ hybridization. Specimens were obtained from 15 patients with gastric cancer.

Results

There was no difference about the results of HER2 overexpression and gene amplification between primary gastric lesion and peritoneal metastasis. Fifteen gastric carcinoma tissue samples were classified as follows: 12 (80%) were scored as 0, 1 (6.7%) as 1, 2 (13.3%) as 2, and 0 (0%) as 3. Two specimens 2+ for HER-2 expression with immunohistological staining were analyzed by fluorescence in-situ hybridization. HER-2 gene amplification was not determined in these samples

Conclusion

Primary gastric lesion and peritoneal metastasis of gastric cancer did not show HER2 amplification and/or overexpression. Further, prospective, larger-scale studies are needed to evaluate the efficacy of monoclonal antibody that targets HER2 to GC patients with PM.

Key words: gastric cancer, peritoneal metastasis, human epidermal growth factor receptor 2

Background

Gastric cancer (GC) affects about one million people per year and is the second leading cause of cancer-related mortality worldwide.[1] One of the most frequent causes of death from GC is peritoneal metastasis (PM).[2] For GC patients with PM, the median survival time was only 3.1 months in a multicenter prospective study.[2]

In recent large clinical Phase III trials, oral fluoropyrimidine-based regimens were generally accepted as the standard regimen against advanced GC,[3,4] however, looking at just PM, a few trials have been reported, e.g. methotrezate and 5-Fu sequential therapy has been reported to decrease malignant ascites in GC patients[5] and another trial has shown the efficacy of modified FOLFOX-4 for GC patients with malignant ascites,[6] although the one-year survival rates were only 16.2% and 27.2%, respectively. Despite such low survival rates, both regimens have been accepted as the standard chemotherapy. Additionally, a multidisciplinary method, including intraperitoneal chemotherapy, hyperthermia and

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aggressive surgery has been used to treat PM,[7-9] however, these trials could not show a satisfactory clinical outcome. Therefore, new therapeutic strategies, treatment options and novel therapeutic targets are desperately needed to enhance the current management of GC with PM.

Human epidermal growth factor receptor 2 (HER2) is a member of the epidermal growth factor receptor family (EGFR), which comprises HER1 (EGFR), HER2, HER3 (ErbB-3), and HER4 (ErbB-4). The binding of a ligand to aHER receptor family member leads to receptor dimerization and activation of intracellular signaling through receptor tyrosine kinases.[10] HER2 amplification and/or overexpression have also been observed in colon,[11] bladder,[12] prostate,[13] pancreatic,[14] and esophageal[15] and gastric[16] carcinomas. Investigations of HER2 overexpression and the gene amplification rate in gastric and esophageal adenocarcinomas reported 20-30%,[17,18] however, there have been few reports about HER2 overexpression and gene amplification in GC with PM. In this study, we investigated HER2 overexpression and gene amplification in GC with PM.

Materials and Methods

Surgical Specimens

Biopsy samples and specimens of PM were obtained from 15 patients with GC between 2008 and 2010 in our department at the time of upper gastrointestinal endoscopy and staging laparoscopy. In accordance with Department of Surgery Kinki University Faculty of Medicine policy, written informed consent was obtained from the patients at the time of operation.

Immunohistochemical (IHC) study

Freshly obtained tissues were fixed with 4% paraformaldehyde in 0.1 M PBS at 4°C overnight, dehydrated in graded alcohols and then embedded in paraffin. Then, 4 μm-thick serial sections were processed for immunohistochemical study, in addition to routine hematoxylin and eosin staining.

The immunohistochemical reactions were realized by the streptavidin-biotin-peroxidase complex technique (StreptABC ; DAKO, Denmark). The tissue sections were deparaffinized and incubated in citrate buffer in a pressure cooker for antigenic retrieval, and then endogenous peroxidase activity was blocked with 3% H_2O_2. The sections were then incubated with polyclonal primary antibodies against HER2 (1 : 500, A0485 ; DAKO). Subsequently, they were incubated with the secondary biotinylated antibody from the LSAB+ peroxidase kit (K0690, DAKO), followed by incubation with Streptavidin HRP (DAKO), and then counterstained with hematoxylin.

Immunohistochemical analysis

Immunohistochemical analyses of HER2 expression describe the intensity and staining pattern of tumor cells. As in the ToGA trial,[19] immunohistochemistry scoring for HER2 classified four categories : no staining, or weak staining in fewer than 10% of the tumor cells (0) ; weak staining in part of the membrane in more than 10% of the tumor cells (1+) ; complete staining of the membrane with weak or moderate intensity in more than 10% of the neoplastic cells (2+) ; and strong staining in more than 10% (3+).

Fluorescence in-situ hybridization (FISH)

The HER2 gene was amplified with dual-color FISH using a Passvision HER2 DNA probe kit (Vysis Inc ; Downers Grove, IL, USA) according to the manufacturer's instructions. Briefly, hybridization buffer, DNA probe, and purified water were centrifuged, and heated to 73°C for 5 min in a water bath. Slides were immersed in a denaturing bath (70% formamide 2×SSC) for 5 min at 73°C, followed by dehydration in increasing ethanol concentrations, and then dried. The probe mixture was applied to each slide. The slides were placed in a 42°C incubator for 30 min, washed with 0.4×SSC/0.3% NP-40 for 2 min, air-dried in darkness, counterstained with 4',6-diamidino-2-phenylindole (DAPI), and covered with a cover-slip. HER-2/neu-spectrum orange probe contains a DNA sequence specific for the HER-2 human gene locus and hybridized to the region 17q11.2-q12 of human chromosomes. CEP17 (chromosome enumeration probe 17)/spectrum green probe containing alpha-satellite DNA that hybridizes to the D17Z1 locus (centromere region of chromosome 17) was used as a control. Nuclei were counterstained with DAPI.

FISH analysis

The slides were observed under a B×60 fluorescence microscope equipped with a digital camera (DP50 ; Olympus, Tokyo, Japan) and the images were captured on a Windows PC with Viewfinder Lite software. A cell was considered to be amplified when a definite cluster or more

than 10 signals for HER-2 were found. Known positive and negative cells were used as controls for each FISH. Gene amplification was scored when a minimum of 20 cancer cell nuclei exhibited a HER-2/CEP17 ratio≧2, or when a HER-2 signal cluster was observed.

GC were classified and interpreted according to the evaluation protocol recommended by the American Joint Committee on Cancer (AJCC) and International Union against Cancer (IUCC). For histological type, gastric cancers were also classified as intestinal type or diffuse type using The Laurens system.

Results

Patient characteristics

Patients had a median age of 59 years (range 47-75 years). There were 6 female and 9 male patients. Borrmann III and IV types accounted for the majority. The details of the main clinicopathological features of patients are presented in Table 1.

Table 1 Clinicopathological features of patients

Clinicopathological factors	No. of cases
Gender	
Male	9
Female	6
Average age (range; years)	59 (47-75)
Borrmann type	
I	0
II	1
III	5
IV	9
Laurens system	
intestinal type	4
diffuse type	11

Expression of HER-2 protein in gastric cancer

HER-2 protein status in 15 GC specimens from gastric cancer was determined with immunohistochemical staining. Figure 1 demonstrates a representative case. There was no difference about the results of HER2 overexpression and gene amplification between primary gastric lesion and peritoneal metastasis. Fifteen CG tissue samples were classified as follows; 12 (80%) were scored as 0, 1 (6.7%) as 1, 2 (13.3%) as 2, and 0 (0%) as 3 (Fig. 2).

HER-2 gene amplification in gastric cancer

Two specimens 2+ for HER-2 expression with IHC staining were analyzed by FISH (Fig. 2). HER-2 gene amplification was not determined in these samples (Fig. 2). Finally, the HER2 overexpression and gene amplification rate was 0% (0/15) in all PM specimens from GC.

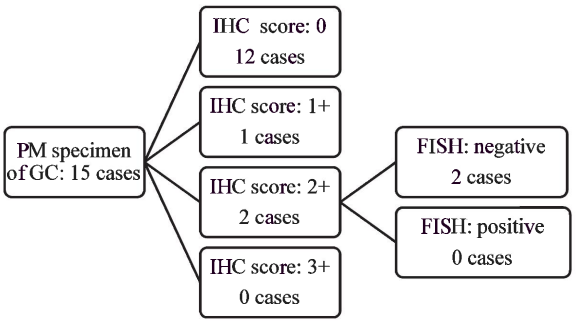

Fig. 2 Analysis of HER2 overexpression and gene amplification
HER2 overexpression and gene amplification cascade in peritoneal metastasis from gastric cancer.
PM: peritoneal metastasis, GC: gastric cancer, IHC: immunohistochemistory, FISH: Fluorescence in-situ hybridization, HER2: human epidermal growth factor receptor

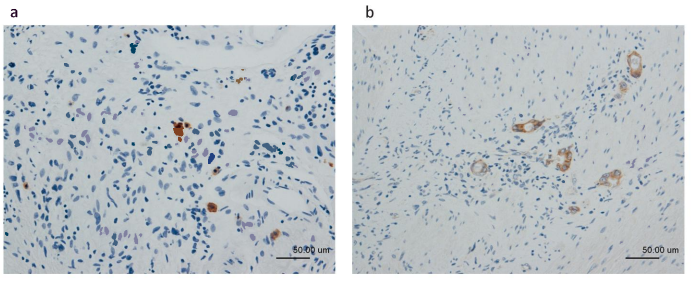

Fig. 1 Representative case of HER2 overexpression
Immunohistochemistry staining of peritoneal metastasis specimen from gastric cancer showing negative HER-2 protein expression.
a: No reactivity of HER2 in tumor cells, classified as 0.
b: Weak basolateral HER2 reactivity of tumor cells, classified as 2+.
HER2: human epidermal growth factor receptor

Discussion

In our data, all biopsy samples from primary lesion and PM specimens did not show HER2 overexpression and/or gene amplification. Bozzetti et al. compared the HER2 status in primary and paired metastatic sites of GC, and concluded that high concordance was observed between HER2 results obtained by both IHC and FISH on primary tumors and metastatic lesions,[20] however, they did not mention PM. Additionally, common GC classified as intestinal type is more likely to be HER2-positive (16-34%) than diffuse (2-7%).[18,21] PM easily developed from GC classified as diffuse type ; therefore, the PM specimen was suspected to not show HER2 overexpression and/or gene amplification. However, there are no data about HER2 overexpression and/or gene amplification of primary lesion and PM. This is the first report about these findings.

The reason for not showing overexpression of HER2 in diffuse-type GC is complex and needs further investigation. The association of HER2 with a specific type suggests that intestinal- and diffuse-type gastric cancers develop along different molecular pathways and supports earlier studies showing distinct patterns of genetic alterations in gastric cancers with differing histopathologic features.[22]

Some similarities can be drawn with breast cancer : low expression of E-cadherin was recognized in lobular invasive breast carcinomas[23,24] and diffuse-type gastric cancers,[25] which correlated with the low frequency of HER2 amplification and/or overexpression.[23,24,26] These results are consistent with our results.

In this study, there were no patients with HER2 overexpression 2+ and amplification positive. In the ToGA study, probably because of 75% patients belonged to the intestinal type, so 27% patients might show HER2 overexpression 2+ and amplification positive. In contrast, 73% of patients in our study belonged to the diffuse type. However, a small number of patients showed HER2 overexpression 1+ or 0 and amplification positive.[27] To determined exact HER2 expression, HER2 expression could be tested by FISH.

ToGA trial suggested that a monoclonal antibody that targets human epidermal growth factor receptor 2 (HER2) improve the prognosis of advanced and/or recurrent gastric cancer patients with HER2 overexpression and/or gene amplification.[19] However, HER2 overexpression and/or gene amplification of gastric patients with PM is unclear. Although our study size was small, PC patients with PM not showed HER2 overexpression and/or gene amplification. Further, prospective, larger-scale studies are needed to evaluate the efficacy of monoclonal antibody that targets HER2 to GC patients with PM.

In conclusion, peritoneal metastasis with gastric cancer did not show HER2 amplification and/or overexpression. This is the first report about these findings. Further, prospective, larger-scale studies are needed to evaluate the efficacy of monoclonal antibody that targets HER2 to GC patients with PM.

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