Metabolic landscape of advanced gastric cancer according to HER2 and its prognostic implications

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Abstract

Background In advanced gastric cancer (AGC), HER2 is a validated therapeutic target. However, the metabolic landscape of AGC based on HER2 status has not been reported. Furthermore, the prognostic value of HER2 in AGC is under debate. The purpose of this study was to determine the metabolic landscape and prognosis on the basis of HER2 status in AGC.

Methods We analyzed 866 AGC patients treated with palliative chemotherapy and whose HER2 status was evaluated. HER2 positivity was defined as HER2 IHC 3+ or *HER2/CEP17* ratio ≥ 2 . Among them, 363 patients were evaluated with ¹⁸F FDG-PET before chemotherapy. We analyzed mSUV (maximal standardized uptake value) according to HER2 status and clinical outcomes.

Results Among 866 patients, 225 (26.0 %) had HER2+ GC. The mSUV of HER2+ GC was significantly higher than that of HER2- GC (12.6 vs. 8.7, $p < 0.001$). Increased HER2 IHC positivity was correlated with increased mSUV (IHC-: 8.1, IHC 1+: 8.2, 2+: 11.4, 3+: 13.2, $p < 0.001$). Excluding HER2+ patients who received HER2-targeting agents, OS of patients was not different by HER2 status (12.5 vs. 11.9 months, $p = 0.688$). However, according to tumor metabolism, patients with higher

mSUV showed worse OS regardless of HER2 positivity (mSUV < 12.8 : 14.8, ≥ 12.8 : 8.6 months, $p < 0.001$).

Conclusion Tumor metabolism of AGC adversely influenced OS under treatment with cytotoxic chemotherapy. Tumor metabolism was higher in HER2+ AGC than HER2-. However, HER2 was not a prognostic factor in patients who received chemotherapy without HER2-targeting agents.

Keywords Gastric cancer · HER2 · c-erbB2 · Prognosis · Metabolism

Introduction

Gastric cancer (GC) is the second leading cause of cancer deaths worldwide. Although there has been progress in the development of cytotoxic chemotherapy, overall survival (OS) of advanced gastric cancer (AGC) patients remains at 10–12 months [1]. Recently, the benefit of second-line chemotherapy has been proven by phase III trials [2, 3].

Gastric cancer is not a single disease. Deng et al. [4] performed a comprehensive genomic analysis of gastric cancer and identified 22 recurrent genetic alterations in gastric cancer. Interestingly enough, they found that five distinct gastric cancer subgroups could be defined by specific alterations, that is, amplifications of *HER2*, *FGFR*, *KRAS*, *EGFR*, and *MET*. Dulak et al. [5] also reported similar results in somatic copy number aberration analysis using high-density genomic profiling arrays in gastric/esophageal tumors. These genes can be targeted for the development of new targeted agents. Among these molecular targets, the first successfully validated one was HER2. Efficacy of an anti-HER2 treatment was demonstrated by the ToGA trial [6]. In contrast to breast cancer in

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which HER2 is known as a poor prognostic factor [7], the prognostic value of HER2 in gastric cancer is still controversial. Even though some studies have shown that HER2-positivity in gastric cancer is associated with poor outcomes and less sensitive to cytotoxic chemotherapy [8–11], others have demonstrated that HER2 expression does not influence overall prognosis in gastric cancer [12–15]. Moreover, it has been getting harder to determine the prognostic value of HER2 per se in AGC after routine prescription of trastuzumab for HER2-positive patients, which can significantly prolong OS [6].

Even though several subtypes of gastric cancer can be classified according to the molecular characteristics [4, 5], the metabolic activities of each subtype including HER2 have not been determined so far. Fluorine-18 (^{18}F) fluorodeoxyglucose positron emission tomography (FDG-PET) is a functional imaging method for the measurement of tumor glucose utilization, using a radioactive tracer bound to FDG, a glucose analog.

The purpose of this study was to evaluate the metabolic landscape of gastric cancer using ^{18}F FDG-PET considering HER2 and to determine the prognostic value of HER2 in advanced gastric cancer.

Materials and methods

Patients

This study is a retrospective analysis of de-identified patient-level data from collected medical charts. The study was designed to compare the metabolic features and prognostic value of HER2 in AGC. Patients who received palliative cytotoxic chemotherapy for AGC and whose HER2 status was evaluated at Seoul National University Hospital, Republic of Korea, from 2004 to 2013 were included.

The overall survival (OS) was estimated from the date of diagnosis of inoperable locally advanced, metastatic, or recurrent gastric cancer to the date of death or last known follow-up date. HER2 positivity was defined as HER2 IHC 3+ or FISH-positive [$HER2/CEP17$ (centromere enumerator probe 17) ratio ≥ 2], according to the ToGA study [6]. We used PathVysion HER-2 DNA probe kit (Vysis) for assessing the $HER2/CEP17$ ratio and $HER2$ gene copy number (GCN). All computerized tomography (CT) scans were reviewed. According to the standard protocol of our hospital, FDG was injected after fasting for 8 h, and PET scans were then started 1 h after injection, using dedicated PET scanners (Gemini, Philips; Biograph 40, Siemens; or mCT, Siemens). PET images were reconstructed using an iterative algorithm (ordered-subset expectation maximization). Standardized uptake value (SUV) was calculated as tissue concentration of radioactivity (kBq/ml) divided by

injected dose per weight (kBq/g). To measure the maximal SUV (mSUV) of lesion, a volume of interest was placed on PET/CT fusion images to cover the whole tumor volume, and mSUV was automatically measured using an analysis software package (Syngo.via, Siemens) [16]. Tumor response was evaluated with RECIST 1.1 [17].

Statistical analysis

Pearson's chi-square test was performed to analyze the categorical variables including clinical characteristics. Analysis of variance (ANOVA) or t test was applied to continuous parameters. Kaplan-Meier estimates with a log-rank test of OS were done. Subgroup analyses were also performed using a Cox regression model, summarizing the hazard ratio (HR) and 95 % confidence interval (CI) of each group. The optimal cutoff of mSUV discriminating between a positive or negative result, in terms of response to treatment and OS, was determined using receiver-operating characteristic (ROC) curve analyses. The cutoff of mSUV that maximized the sum of sensitivity and specificity was determined [18]. All reported p values were two-sided. Analyses were done with STATA version 12 (StataCorp LP; College Station, TX, USA).

Ethics

The study protocol was reviewed and approved by the Institutional Review Board of Seoul National University Hospital (H-1306-007-493). All studies were conducted according to guidelines (Declaration of Helsinki) for biomedical research.

Fig. 1 Flow chart of patients in the study. AGC advanced gastric cancer, PET positron emission tomography, HER2 human epidermal growth factor receptor 2

Table 1 Patient characteristics

	Total <i>N</i> = 866	HER2− All [A] <i>N</i> = 641	HER2+ All [B] <i>N</i> = 225	<i>p</i> value [A] vs. [B]	HER2+ Tmab− [C] <i>N</i> = 57	<i>p</i> value [A] vs. [C]
Age						
Median (range)	60 (20–89)	59 (20–89)	63 (22–85)	0.003	63 (37–77)	0.084
<60 years old, <i>n</i> (%)	403 (46.5)	314 (49.0)	89 (39.6)		22 (38.6)	
≥60 years old, <i>n</i> (%)	463 (53.5)	327 (51.0)	136 (60.4)	0.015	35 (61.4)	0.132
Sex						
Male, <i>n</i> (%)	620 (71.6)	435 (67.9)	185 (82.2)		47 (82.5)	
Female, <i>n</i> (%)	246 (28.4)	206 (32.1)	40 (17.8)	<0.001	10 (17.5)	0.022
ECOG						
0–1, <i>n</i> (%)	705 (90.5)	538 (91.3)	167 (87.9)		41 (83.7)	
2–3, <i>n</i> (%)	74 (9.5)	51 (8.7)	23 (12.1)	0.159	8 (16.3)	0.075
Palliative setting						
Initially metastatic, <i>n</i> (%)	608 (70.2)	444 (69.3)	164 (72.9)		40 (70.2)	
Recurrent, <i>n</i> (%)	258 (29.8)	197 (30.7)	61 (27.1)	0.307	17 (29.8)	0.887
Tumor location						
GEJ, <i>n</i> (%)	75 (8.7)	53 (8.3)	22 (9.8)		6 (10.5)	
Other stomach, <i>n</i> (%)	792 (91.3)	588 (91.7)	203 (90.2)	0.489	51 (89.5)	0.557
Pathology type						
Adenocarcinoma, <i>n</i> (%)	699 (80.7)	494 (77.1)	205 (91.1)		55 (96.5)	
Signet ring cell, <i>n</i> (%)	152 (17.6)	136 (21.2)	16 (7.1)		2 (3.5)	
Other types, <i>n</i> (%)	15 (1.7)	11 (1.7)	4 (1.8)	<0.001	0 (0)	0.003
Contains signet ring cell feature, <i>n</i> (%)	222 (25.6)	193 (30.1)	29 (12.9)	<0.001	5 (8.8)	0.001
Lauren						
Intestinal, <i>n</i> (%)	108 (36.7)	66 (28.3)	42 (68.9)		14 (82.4)	
Diffuse, <i>n</i> (%)	149 (50.7)	138 (59.2)	11 (18.0)		2 (11.8)	
Mixed, <i>n</i> (%)	37 (12.6)	29 (12.5)	8 (13.1)	<0.001	1 (5.9)	<0.001
Baseline PET						
Total, <i>n</i> (%)	363 (41.9)	279 (43.5)	84 (37.3)	0.105	17 (29.8)	0.045
mSUV						
Mean ± SE	9.6 ± 0.4	8.7 ± 0.4	12.6 ± 0.9	<0.001	9.1 ± 1.3	0.809
HER2 IHC						
Negative, <i>n</i> (%)	276 (34.5)	274 (42.8)	2 (1.0)		0 (0)	
1+, <i>n</i> (%)	191 (23.9)	182 (28.4)	9 (4.3)		3 (5.7)	
2+, <i>n</i> (%)	184 (23.0)	136 (21.2)	48 (23.0)		13 (24.5)	
3+, <i>n</i> (%)	150 (18.7)	0 (0)	150 (66.7)	<0.001	37 (69.8)	<0.001
Total, <i>n</i> (%)	801 (92.5)	592 (92.4)	209 (92.9)	0.794	53 (93.0)	0.864
HER2/CEP17 ratio						
Median (95 %CI)	1.2 (0.9–10.8)	1.1 (0.8–1.6)	5.8 (1.2–13)	<0.001	4.6 (1.2–11.6)	<0.001
Total, <i>n</i> (%)	321 (37.1)	205 (31.9)	116 (51.6)	<0.001	22 (38.6)	0.307
HER2 GCN						
Median (95 %CI)	2.9 (1.7–20.7)	2.2 (1.6–4.1)	10 (2.4–30)	<0.001	11.1 (1.9–49.7)	<0.001
Total, <i>n</i> (%)	293 (33.8)	194 (30.3)	99 (44.0)	<0.001	18 (31.6)	0.836

Bold values indicate statistically significant correlations with *p* values less than 0.05

CEP17 centromere enumerator probe 17, *CI* confidence interval, *GCN* gene copy number, *GEJ* gastroesophageal junction, *ECOG* Eastern Cooperative Oncology Group performance status, *HER2* human epidermal growth factor receptor 2, *IHC* immunohistochemistry, *PET* positron emission tomography, *Tmab* trastuzumab, *mSUV* maximal standardized uptake value, *SE* standardized error

Results

Patient characteristics

A total of 866 AGC patients were analyzed (Fig. 1). The patient characteristics according to HER2 status are shown in Table 1. Among all patients, 225 (26.0 %) were HER2 positive. HER2 positivity was observed more frequently with advanced age and in males as well as non-signet-ring cell and intestinal-type pathology. Among all patients, 363 (279 HER2−, 84 HER2+) were evaluated with FDG-PET before palliative chemotherapy, and it was evenly distributed by HER2-status (Table 1). Of all cases, *HER2* FISH was done in 321 cases (205 HER2−, 116 HER2+). *HER2*/CEP17 ratio and *HER2* GCN according to HER2 IHC are summarized in Supplementary Table 1.

Among 225 HER2+ patients, 142 were treated with trastuzumab and cytotoxic chemotherapy combination, and 57 were not exposed to any HER2-targeting agents. Most commonly used first-line chemotherapy was

fluoropyrimidine (5-FU, capecitabine, or TS-1) and platinum (cisplatin or oxaliplatin) combination.

Metabolic landscape and metastatic pattern based on HER2 status in AGC

The mSUV of HER2+ GC was significantly higher than that of HER2− GC (HER2−: 8.7, HER2+: 12.6, $p < 0.001$, Fig. 2a), although differences in mSUV were not evident, excluding patients who were exposed to trastuzumab (HER2−: 8.7, HER2+ without trastuzumab: 9.1, $p = 0.809$, Table 1; Supplementary Fig. 1).

Increased HER2 IHC positivity was correlated with increased mSUV (IHC−: 8.1, IHC 1+: 8.2, 2+: 11.4, 3+: 13.2, $p < 0.001$, Fig. 2b), but a high *HER2*/CEP17 ratio (HCR) as well as high *HER2* GCN was not correlated with increased mSUV (HCR < 2:10.1, HCR ≥ 2:10.6, $p = 0.660$, GCN < 6:10.7, GCN ≥ 6:10.7, $p = 0.965$, Fig. 2c, d).

Figure 3 shows the spatial distribution of tumor metabolism according to HER2 status. The SUVs of stomach

Fig. 2 Metabolic activity based on HER2 status. Maximal standardized uptake value (mSUV) according to HER2 status (a), HER2 immunohistochemistry (IHC) status (b), *HER2*/CEP17 ratio (c), and *HER2* gene copy number (d). Mean ± standard error

Fig. 3 Metabolic activity of metastatic lesions based on HER2 status. Standardized uptake value (SUV) of stomach, lymph node, peritoneal seeding, and liver according to HER2 status (a), SUV of metastatic and recurrent setting according to HER2 status (b). Percentage of

recurred stomach or metastasis to lymph node, peritoneal seeding, and liver were compared according to HER2 status (c). Percentages of multiple metastases, more than 3 lesions, were compared according to HER2 status (d)

and lymph nodes were significantly higher in HER2+ AGC than HER2- AGC (mean stomach SUV, HER2-: 9.2, HER2+: 11.0, $p = 0.042$, mean lymph node SUV, HER2-: 7.3, HER2+: 9.7, $p = 0.003$, Fig. 3a). SUV of peritoneal seeding lesions was slightly higher in HER2- AGC, but that of liver was higher in HER2+ AGC, although those trends were not statistically significant (mean peritoneal seeding SUV, HER2-: 6.7, HER2+: 5.7, $p = 0.681$, mean liver SUV, HER2-: 10.2, HER2+: 12.5, $p = 0.224$).

We further analyzed the SUV of lesions in initially metastatic cases compared with those in recurrent cases. In HER2- AGC, the mSUV of initially metastatic cases was significantly higher than that of recurrent cases, and this trend was similarly observed in HER2+ AGC but failed to achieve statistical significance (HER2-, metastatic: 10.3, recurrent: 6.1, $p < 0.001$, HER2+, metastatic: 13.8, recurrent: 10.4, $p = 0.08$, Fig. 3b). Incidences of initial

metastasis by organ are summarized in Fig. 3c. Incidences of lymph node and liver metastasis were higher in HER2+ AGC (lymph node, HER2-: 44.5 %, HER2+: 64.0 %, $p < 0.001$, liver, HER2-: 20.6 %, HER2+: 46.2 %, $p < 0.001$), but peritoneal seeding was more frequently observed in HER2- AGC (HER2-: 62.6 %, HER2+: 40.4 %, $p < 0.001$). The number of metastatic lesions was different according to HER2 status. The portion of multiple metastases (more than three organs) was higher in HER2+ AGC (HER2-: 10.5 %, HER2+: 16.9 %, $p = 0.011$, Fig. 3d).

Prognostic value of HER2

The median follow-up duration of all cases was 36.7 months (95 % CI 14.7–90.2). Since trastuzumab definitely influenced survival in HER2+ AGC, we excluded HER2+ AGC patients treated with trastuzumab in

Fig. 4 Prognostic value of HER2. Kaplan-Meier curves for OS according to HER2 status (a), HER2 immunohistochemistry (IHC) status (b), *HER2/CEP17* (c), and *HER2* gene copy number (GCN) (d)

the following analysis. Excluding trastuzumab exposure, median overall survival of patients with intestinal-type Lauren classification was 19.1 months compared to 13.4 months in those with diffuse type (HR 1.58, $p = 0.007$) and 13.1 months in mixed type (HR 1.73, $p = 0.035$), respectively (Supplementary Fig. 2). However, HER2 itself did not significantly alter the prognosis (OS, HER2-: 12.5, HER2+: 11.9 months, $p = 0.688$, Fig. 4a). In parallel, neither increased HER2 IHC positivity (IHC-: 12.3, IHC 1+: 14.2, 2+: 11.7, 3+: 11.9 months, $p = 0.386$, Fig. 4b), nor *HER2/CEP17* ratio (HCR < 2: 12.1, HCR ≥ 2: 13.4 months, $p = 0.181$, Fig. 4c), nor *HER2* gene copy number (GCN < 6: 11.9, GCN ≥ 6: 11.3 months, $p = 0.305$, Fig. 4d) influenced OS.

Prognostic values of mSUV

ROC analysis were used to determine the optimal cutoff value of mSUV that discriminated patients with longer survival and those who respond more to first-line

chemotherapy, excluding patients with trastuzumab exposure (Fig. 5a, b). ROC analysis showed that SUVs of 12.8 and 8.3 were optimal cutoff values for estimating shorter OS (less than 12 months) and response [objective response rate (ORR)] to first-line chemotherapy, respectively. Patients with higher mSUVs of more than 8.3 showed higher ORR (mSUV < 8.3: 24.7 %, mSUV ≥ 8.3: 41.0 %, $p = 0.004$). However, when divided by an SUV of 12.8, patients with higher mSUV showed worse OS (mSUV < 12.8: 14.8, mSUV ≥ 12.8: 8.6 months, $p < 0.001$, Fig. 5c). This trend was in concordance irrespective of HER2 status (HER2- mSUV < 12.8: 14.5, HER2- mSUV ≥ 12.8: 8.6 months, $p < 0.001$, HER2+ mSUV < 12.8: 36.8, HER2+ mSUV ≥ 12.8: 9.4 months, $p = 0.006$, Fig. 5d). Clinicopathologic characteristics of patients grouped by mSUV of 12.8 are shown in Supplementary Table 2. The group of high metabolism (mSUV ≥ 12.8) had more initially metastatic cases and less signet ring feature pathology. Supplementary Table 3 shows the univariate and multivariate Cox analysis of OS, which indicated that

Fig. 5 Prognostic value of metabolic activity assessed by positron emission tomography. Optimal cutoff values of maximal standardized uptake value (mSUV) predicting OS shorter than 12 months (**a**) and

response rate (**b**) were determined by receiver-operating characteristic curves. Kaplan-Meier curves for OS according to an mSUV cutoff value of 12.8 (**c**) and according to mSUV and HER2 status (**d**)

mSUV was an independent poor prognostic factor along with poor performance, peritoneal seeding, and liver metastasis (Table 2).

Discussion

In this study, we found that tumor metabolism, measured by the SUV of ^{18}F FDG-PET, was increased in HER2+ AGC compared with HER2- AGC. HER2 itself did not impact the overall prognosis under cytotoxic chemotherapy without HER2-targeting agents; however, hypermetabolism of tumors was a poor prognostic factor irrespective of HER2 status.

The prognostic value of HER2 in AGC has been variously reported and controversial. Although some studies have reported that HER2 positivity is a poor prognostic factor in gastric cancer [8–11], others have found that HER2 has no influence on prognosis [12–15].

Moreover, a certain report showed that higher HER2 gene amplification was associated with good prognosis [19]. Even though most studies were based on curatively resected cases, the prognostic role of HER2 in AGC is still under debate.

The general characteristics of HER2+ AGC shown in this study were in concordance with previous results [20]. HER2+ was predominantly observed with older patients, males, adenocarcinoma, and intestinal-type pathology. Metastasis to lymph nodes and liver was more prevalent in HER2+ AGC. Maximal SUV and SUVs of stomach and lymph nodes were higher in HER2+ AGC than HER2- AGC. Moreover, SUVs of stomach and lymph nodes were higher in HER2+ AGC than HER2- AGC, with a statistical significance.

As far as we know, this is the first report that compares levels and distributions of SUV in whole metastatic lesions according to HER2 status in AGC. Since HER2 is believed to play a role in cell proliferation and migration [8] and

Table 2 Univariate and multivariate Cox analysis of overall survival

	<i>n</i>	<i>mOS</i>	Univariate of OS		Multivariate of OS	
			HR (95 % CI)	<i>p</i>	HR (95 % CI)	<i>p</i>
<i>mSUV</i>						
<12.8	234	14.8	1.0		1.0	
≥12.8	62	8.6	2.38 (1.72–3.30)	<0.001	2.34 (1.65–3.33)	<0.001
<i>Age</i>						
<60	336	11.8	1.0		1.0	
≥60	362	13.1	0.84 (0.71–1.00)	0.047	0.88 (0.66–1.18)	0.390
<i>Sex</i>						
Male	482	12.6	1.0		–	
Female	216	12.1	1.05 (0.87–1.26)	0.595	–	–
<i>ECOG</i>						
0–1	579	13.0	1.0		1.0	
2–3	59	6.5	2.68 (1.99–3.60)	<0.001	1.88 (1.20–2.93)	0.006
<i>Palliative setting</i>						
Recur	214	13.1	1.0		1.0	
Mets	484	12.1	1.22 (1.01–1.48)	0.036	0.98 (0.72–1.32)	0.875
<i>Tumor location</i>						
Other	639	12.5	1.0		–	
GEJ	59	12.1	0.90 (0.66–1.24)	0.535	–	–
<i>Pathology: SRC</i>						
No	500	13.2	1.0		1.0	
Yes	198	10.5	1.35 (1.12–1.64)	0.002	1.25 (0.90–1.75)	0.187
<i>HER2</i>						
–	641	12.5	1.0		–	
+	57	11.9	0.94 (0.70–1.26)	0.688	–	–
<i>Lymph node metastasis</i>						
No	376	13.0	1.0		–	
Yes	322	12.1	1.01 (0.85–1.21)	0.870	–	–
<i>Peritoneal seeding</i>						
No	274	13.4	1.0		1.0	
Yes	424	11.7	1.32 (1.10–1.58)	0.002	1.61 (1.18–2.18)	0.003
<i>Liver metastasis</i>						
No	540	13.1	1.0		1.0	
Yes	158	11.6	1.31 (1.08–1.61)	0.007	1.48 (1.04–2.10)	0.027

Bold values indicate statistically significant correlations with *p* values less than 0.05

CI confidence interval, *ECOG* Eastern Cooperative Oncology Group performance status, *GEJ* gastroesophageal junction, *HER2* human epidermal growth factor receptor 2, *HR* hazard ratio, *mOS* median overall survival, *mSUV* maximal standardized uptake value, *SRC* signet ring cell

SUV correlates with tumor cell metabolism [21], HER2+ AGC would be a more hypermetabolic tumor compared with HER2– AGC on the basis of this analysis. Several factors may influence these results. First of all, the proportion of signet ring cell pathology with an SUV lower than that of adenocarcinoma [22–25] was significantly low in HER2+ AGC. However, when we performed subgroup analysis of the *mSUV* difference based on pathologic type, the mean *mSUV* of patients with the signet ring cell feature was also significantly increased in HER2+ AGC compared to HER2– AGC (data not shown). Second, the proportion

of patients with initially metastatic stage IV, which generally had more tumor burden than recurrent cases, was slightly higher in HER2+ AGC in our cohort, but it did not differ significantly (HER2–: 69.3 % vs. HER2+: 72.9 %). Moreover, the rate of availability of ¹⁸F FDG-PET data was well balanced according to HER2 status. Third, other markers associated with HER2 would also influence the hypermetabolism of the HER2+ tumor. Recently, The Cancer Genome Atlas (TCGA) genomic analysis of gastric cancer reported recurrent co-amplifications of the receptor tyrosine kinase family, such as *EGFR*, *FGFR2*, *VEGFR2*,

and *MET*, as well as cell cycle regulators, such as *CCNE1*, *CDK6*, and *CCND1* along with *HER2*, especially in the chromosomal-unstable (CIN) subtype of gastric cancer [26, 27]. Hence, the hypermetabolism of *HER2*+ tumor might be derived from *HER2* itself or other markers including the receptor tyrosine kinase family and cell cycle regulators associated with *HER2*. The clarification of tumor metabolism by those factors should be further investigated. Taken together, we interpret our current data macroscopically as showing a difference in tumor metabolism based on *HER2* status, although it needs to be further validated.

In our cohort, although mSUV was positively correlated with *HER2* protein expression seen by IHC and the *HER2*/CEP17 ratio was increased with a higher IHC positivity (Supplementary Table 1), the *HER2*/CEP17 ratio and *HER2* GCN were not related to mSUV. Therefore, *HER2* protein expression rather than *HER2* gene amplification may play a more crucial role in representing influences on tumor metabolism.

Irrespective of *HER2* status, tumor hypermetabolism negatively influenced OS. It also suggests that other factors besides *HER2* influence hypermetabolism of gastric cancer. For example, tumor metabolism is consistently influenced by the mutation status of the *MYC*, *TP53*, and LKB1-AMPK-PI3 K pathway [28]. Therefore, cancer metabolism seems far more pleiotropic than it is expected to be.

Tumor hypermetabolism in gastric cancer was correlated with a higher response rate to conventional cytotoxic chemotherapy in our study. Recent data showed that the tumor turnover rate is dependent on glucose metabolism [29]. Therefore, cytotoxic chemotherapy could be more effective in tumors with hypermetabolism, but tumor progression would be more rapid because of its aggressiveness.

Surprisingly, although *HER2*+ AGC is a hypermetabolic tumor and generally hypermetabolism is associated with poor prognosis, OS of *HER2*+ AGC was not worse than that of *HER2*- AGC in the current study. This effect would be explained by exclusion of *HER2*+ AGC patients with higher mSUV when performing survival analysis, because of trastuzumab exposure. Uneven trastuzumab treatment to *HER2*+ AGC according to the level of mSUV may be an incidental finding, since the decision for trastuzumab treatment was not based on the mSUV in clinical practice. Moreover, the number of patients with AGC without exposure to *HER2*-targeting agents is relatively small, since trastuzumab treatment in *HER2*+ AGC is the standard of care after the ToGA study [6], where the statistical significance could be underpowered.

In conclusion, tumor metabolism is higher in *HER2*+ AGC, and this metabolic activity adversely influences OS. However, *HER2* itself is not a prognostic factor in AGC patients who receive cytotoxic chemotherapy excluding

HER2-targeting agents. Further evaluation should be focused on measuring tumor metabolism with other methods besides ^{18}F FDG-PET to understand the biologic roles of *HER2* in AGC.

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