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Reduced MUTYH, MTH1 and OGG1 expression and *TP53* mutation in diffuse-type adenocarcinomas of gastric cardia

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Oxidative stress in diffuse-type adenocarcinoma of gastric cardia

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Conflicts of Interest Disclosures

The authors have no potential conflicts of interest to disclose.

Abstract

The effects of oxidative stress in adenocarcinomas of gastric cardia (AGCs) have not been fully elucidated. With a strict definition of AGC, we examined the immunohistochemical expressions of inducible nitric oxide synthase, 8-hydroxy-deoxyguanosine, the base excision repair enzymes such as MUTYH, MTH1 and OGG1, and *TP53* mutational status. Sixty-three cases of AGC were characterized by younger patient age (p=0.0227) and more frequent venous invasion (p=0.0106) compared to the adenocarcinomas of pylorus (APs). 8-hydroxy-deoxyguanosine was accumulated (p=0.0011) whereas MUTYH (p=0.0325) and OGG1 (p=0.0007) were decreased in the AGCs compared to the adjacent mucosa, but these differences were not detected in the APs. Among the AGCs, lower expression of MUTYH (p=0.0013) and MTH1 (p=0.0059) were each significantly associated with diffuse-type histology. A lower expression of OGG1 was correlated with higher T-stage (p=0.0011), lymphatic invasion (p=0.004) and lymph node metastasis (p=0.0094). In addition, the presence of *TP53* mutation was associated with diffuse-type histology (p=0.0153) and a lower level of MUTYH (p=0.0221). The AGCs also showed a relatively high rate of a transversion-type mutation of *TP53* (50%), whereas all *TP53* mutations in the APs were transition type. Age 62 years or older (p=0.0073), diffuse-type histology (p=0.0020) and *TP53* mutation (p=0.0066) were each associated with worse survival in the AGC patients. Our results indicate that oxidative stress accumulation and a downregulation of base excision repair enzymes may play an important role in the pathogenesis of AGC, in particular diffuse-type AGCs. Diffuse-type AGC might involve molecular pathways different from those of other subsets of gastric cancer.

1. **Introduction**

Gastric cancer is a major public health issue as the sixth most common cancer and the fourth leading cause of cancer-related death in the world [1]. Although the incidence of gastric cancer overall is reported to have decreased nowadays, cancers of the esophagogastric junction or gastric cardia have been increasing, especially in Western countries [2].

The definitions of “esophagogastric junction (EGJ)” and “EGJ cancer” have been a matter of some controversy due to the difficulty of identifying the EGJ. Some divergent classification systems for EGJ carcinoma thus exist (Fig.1). Even the widely used Siewert criteria can be heterogeneous, and the same is the case with the term “adenocarcinoma of the cardia” [3].

The stomach is exposed to many kinds of oxidative or nitrosative stresses, many of which are recognized as risk factors for gastric cancer, including diet, tobacco habit, alcohol intake, and *Helicobacter pylori* infection [4, 5]. Under such stress conditions, excessive reactive oxygen species (ROS) and reactive nitrogen species (RNS) may produce iNOS, one of the inducible isoforms of nitric oxide synthase (NOS), or 8-hydroxy-deoxyguanosine (8-OHdG), an oxidized form of deoxyguanosine.

Nitric oxide (NO), synthesized by iNOS, is a free radical gas that acts as a signaling molecule in the body [6] and iNOS thus plays an important role in growth, invasion, and metastasis in some human cancers (including gastric cancer) [7, 8]. 8-OHdG can form a mismatch pair with adenine as well as with cytosine, leading to a G:C to T:A transversion mutation [9, 10]. To prevent or minimize this replication error due to 8-OHdG accumulation, base excision repair (BER) enzymes such as MTH1, OGG1 and MUTYH are involved in error-avoiding mechanisms against oxidative damage in nucleic acids.

MTH1 protein exists in the nucleotide pool and functions to prevent the misincorporation of 8-OHdG opposite adenine and cytosine [11]. OGG1 cleaves the mutagenic 8-OHdG base in the DNA and MUTYH protein has the ability to excise both adenine opposite 8-OH-G and 2-OH-A incorporated opposite any normal base [9].

Concerning gastric cancers, no target gene due to oxidative stress has been clearly revealed to date, but one of the candidates could be *TP53*, whose point mutation rate in gastric cancer is reported to reach 65% [12]. There have been many studies of these BER enzymes in gastric cancer, but their sample subsites have been unclear, although the internal environment of the stomach is known to vary [13, 14]. In addition, because of the difficulty of defining EGJ cancer as mentioned above, previous studies of gastric cardia may have included a heterogeneous group of tumors; some might have included patients with gastric corpus tumors and others with tumors of esophageal origin.

To clarify the effects of the oxidative stress and the BER system in adenocarcinomas of definitive gastric cardia (AGCs), we evaluated the levels of 8-OHdG and iNOS together with those of the BER enzymes MUTYH, MTH1 and OGG1, by applying the strict definition of gastric cardia. We also evaluated the mutational status of one of the gastric cancer-related genes, *TP53*, to determine whether it is a target gene of repair enzymes.

2. Materials and methods

2.1. Patients and preparation of specimens

We retrieved specimens of 63 cases of AGC from the files of gastric cancers registered in the Department of Anatomic Pathology, Kyushu University, Japan. All of the specimens were free of presurgical chemotherapy or medication and were surgically

resected at Kyushu University or an affiliated institution between 2000 and 2011. Specimens from patients with any evidence of malignancy in other organs or a history of previous treatment of cancer were excluded.

When the center of the tumor was located within 2 cm on the gastric side from the EGJ, we define it as an AGC, which is a narrower definition than that included within Siewert type II or esophago-gastric junctional cancer by the Japanese Classification of Esophageal and Gastric Cancer (Fig.1). We also analyzed the non-cancerous adjacent mucosa around the cancer as the background gastric mucosa [4] and 27 cases of adenocarcinomas of the pylorus (APs), whose centers were within 2 cm from the gastroduodenal junction, together with their surrounding non-cancerous gastric mucosa. The specimens were fixed in 10% formalin solution, embedded in paraffin and cut into 5-μm-thick slices. We divided all of the AGC and AP cases by their histopathology into two groups: intestinal type and diffuse type. This retrospective study was approved by the institutional review board of Kyushu University (No. 25-191).

2.2. Immunohistochemistry

8-OHdG immunostaining was carried out as described [15]. The sections were deparaffinized in xylene and rehydrated in a descending series of ethanol concentrations, then the endogenous peroxidase was neutralized using methanol with 0.3% H_2O_2 for 30 min. Next, the sections were incubated with 5% bovine serum albumin and 2% dry milk in phosphate-buffered saline to block the nonspecific binding of the immuno-reagents. After being reacted with 1:20-diluted monoclonal anti-8-OHdG (N45.1, Japan Institute for the Control of Aging, Shizuoka, Japan) as the first antibody overnight, the sections were subjected to the labeled streptavidin-biotin technique. Color was developed with

3,3-diamino-benzidine, and the sections were counter-stained with hematoxylin.

The iNOS, MUTYH, OGG1, MTH1 and p53 immunohistochemical analyses were carried out as follows. After deparaffinization and rehydration, sections were treated with 3% H_2O_2 with methanol. Antigen retrieval was performed by microwave heating in citrate buffer (pH6.0) for 20 min (iNOS and p53) or in Target Retrieval Solution (pH 9.0) for 20 (OGG1 and MTH1) or 30 (MUTYH) min. Incubation at 4°C overnight with the primary antibodies was carried out, followed by incubation with the second antibody.

The following were used as primary antibodies: polyclonal anti-OGG1 antibody (NB100-106, Novus Biologicals, Littleton, CO, 1:250), polyclonal anti-MUTYH antibody (ab13698, Abcam, Tokyo, 1:200), polyclonal anti-MTH-1 antibody(D-2, Abcam, 1:400), monoclonal anti-iNOS antibody (nos-typeII, BD Biosciences, Franklin Lakes, NJ, 1:100), monoclonal anti-p53 antibody (PAb1801, Calbiochem, Darmstadt, Germany, 1:500). The color-developing procedures were the same as those used for the staining for 8-OHdG.

The staining for iNOS, MUTYH, MTH1 and OGG1 was observed primarily in the cytoplasm, whereas the staining for 8-OHdG and p53 was observed in the nucleus (Fig. 2). The grading of 8-OHdG staining was based on the German Immunoreactive score [15, 16]. The staining intensity was rated on a scale indicating 0=no staining, 1=weak, 2=moderate and 3=strong staining. For the staining proportion, after the numbers of positive and negative cells were counted among no less than 500 cells, the percentage of positive cells was scored as 0 (no staining), 1 (1%–50%), or 2 (51%–100%). The final score was obtained by multiplying the intensity score with the proportional score, ranging from 0 to 6. A final score of 0–3 was regarded as low and 4–6 was regarded

as high.

The staining intensity for MUTYH was rated on a scale of 0 (no staining), 1 (weak) or 2 (strong) [17, 18]. The staining intensity for MTH1 and that for OGG1 were rated on a scale of 0 (no staining), 1 (weak), 2 (moderate) or 3 (strong) [19]. The scores 0 (for MUTYH) or 0 and 1 (for MTH1 and OGG1) were classified as low immunoreactivity, and the scores 1 and 2 (MUTYH) or 2 and 3 (MTH1 and OGG1) were classified as high immunoreactivity. For iNOS and p53, the protein immunoreactive-positive cells were counted regardless of their staining intensity, and if there were ≥ 10% positive cells the immunoreactivity was classified as high [20–22].

2.3. ***TP53 Mutational analysis***

Polymerase chain reaction (PCR) amplification and a sequencing analysis were carried out for assessing mutations. Genomic DNA was extracted from macrodissected formalin-fixed, paraffin-embedded tissue using the standard proteinase K digestion and phenol/chloroform extraction methods. A PCR and a mutational analysis were performed for exons 5 to 8 of *TP53*. The primer sequences are summarized in Supplementary Table 1. If the quality of the DNA or the level of PCR amplification was insufficient for a mutation analysis, the cases were excluded from the molecular study.

Finally, 51 cases of AGC and 24 cases of AP were available.

2.4. Statistical analysis

The statistical analyses were carried out with the JMP 11.0 Statistical Software Package (SAS Institute, Cary, NC). The correlations among the clinicopathological factors were assessed by Fisher’s exact test and the Mann-Whitney U-test.

Immunohistochemical expression levels were evaluated by the Mann-Whitney U-test, McNemar test and Wilcoxon signed-ranks test. Disease-specific survival curves were constructed using the Kaplan-Meier method, and differences in the curves were evaluated using the log-rank test. A P-value <0.05 was considered significant.

3. Results

3.1. Clinicopathological findings

Table 1 summarizes the clinicopathological features of the AGCs and APs. The AGC patients were 49 males and 14 females, ranging in age from 39 to 89 years (mean 65.8 years). According to the UICC classification, 14 patients were classified as early-stage (pTis, three cases; pT1b, 11 cases) and 49 as advanced-stage (pT2, seven cases; pT3, 26 cases; pT4a, 13 cases; pT4b, three cases). There were 36 (57.1%) cases of lymph vessel invasion and 36 (57.1%) of venous invasion, and 37 (58.7%) patients had lymph node metastasis. A review of the hematoxylin and eosin sections revealed 52 intestinal-type adenocarcinomas (pure intestinal type, 38 cases; mixed type, 14 cases) and 11 diffuse-type adenocarcinomas.

3.2. Immunohistochemical analysis of AGC

Among the AGCs, the level of 8-OHdG expression (Fig. 2a) was significantly higher in the cancerous area (82.5%) than that in the non-cancerous mucosa (58.7%) (p=0.0011), whereas the positivities for MUTYH (Fig. 2b) and OGG1 (Fig. 2c) were significantly lower in the cancerous areas (77.8% and 38.1%) than those in the non-cancerous mucosa (90.5% and 65.1%, p=0.0325 and p=0.0007, respectively, Table 2). The MTH1 (Fig. 2d) and iNOS (Fig. 2e) expression in the AGCs showed no significant differences compared

to the non-cancerous mucosa. The positive rate of p53 (Fig. 2f) in the AGCs was 39.7%, and none of the non-cancerous mucosa showed p53 positivity.

Table 3 shows the results of the comparison of the immunohistochemical expression of BER enzymes with the clinicopathological factors of the AGC patients. The MUTYH and MTH1 protein expressions were lower in the diffuse-type tumors (36.4% and 9.1%) than in the intestinal-type tumors (86.5% and 57.7%, respectively). Low OGG1 expression was significantly associated with the more advanced tumors (pT2-4, 26.5%; pTis-pT1, 78.6%; p=0.0011), positive lymph vessel invasion (positive, 22.2%; negative, 59.3%; p=0.004), and lymph node metastasis (positive, 24.3%; negative, 57.7%; p=0.0094). No correlation was found between the iNOS, 8-OHdG or p53 protein expression levels and any clinicopathological factors in AGC (Suppl. Table 2). No association was found between the expressions of 8-OHdG and the expressions of iNOS, BER enzymes, or p53 protein in the AGCs (Suppl. Table 3).

3.3. Mutational analysis of TP53 in AGC

There were six *TP53* mutations in six of 51 (11.8%) AGC cases; four were missense mutations, two were silent mutations and three of the six (50%) were the transversion type (Suppl. Table 4). As shown in Table 4, *TP53* mutations were significantly more frequently observed in the diffuse-type tumors (36.4%, 4/11 cases) than in the intestinal-type tumors (5%, 2/40 cases, p=0.0153) and they were also significantly more frequently observed in the low MUTYH expression tumors (33.3%, 4/12 cases) compared to the high MUTYH expression tumors (5.13%, 2/39 cases, p=0.0221).

3.4. Analysis of the AGC patients' survival

Kaplan-Meier analysis of the cases of the patients with an advanced AGC deeper than pT2 revealed that the patients with an diffuse-type tumor (p=0.0022) and those who were aged ≥ 62 years (p=0.0073) had significantly shorter disease-specific survival (Fig. 3). In addition, although the case number was very small (n=3), the patients with a positive *TP53* mutation also had significantly poorer prognoses (p=0.0066). All of the patients with a *TP53*-positive mutation, including the pTis- and pT1-stage patients, also tended to show worse prognoses compared to the patients with a negative mutation, but the tendency was not significant (p=0.0638, data not shown).

3.5. Comparison of the AGC and AP results

Unlike the AGCs, no significant association was observed between the clinicopathological data and the immunohistochemical results in the AP cases (data not shown). In the cancerous areas, the OGG1 expression was significantly decreased in the AGCs (38.1%) compared to that in the APs (77.8%, p=0.0011, Suppl. Table 5). In the non-cancerous areas, significantly higher iNOS expression (AGCs, 85.7%; APs, 63.0%; p=0.0235) and significantly lower MTH1 expression (AGCs, 34.9%; APs, 59.3%; p=0.0385) were detected in the AGCs (Suppl. Table 5).

In the molecular analysis, six *TP53* mutations were detected in four of 25 (16.0%) AP cases (Suppl. Table 4). All of them were the transition type; three were missense mutations, and the other three were silent mutations. No significant correlation was found between the *TP53* mutational status and clinicopathological or immunohistochemical results in the AP group (data not shown).

4. Discussion

The results of the present study demonstrated that AGCs have the characteristics of younger patient age and more frequent venous invasion compared to APs; these characteristics are consistent with previously reported features [23].

Elevated levels of 8-OHdG have been reported in various types of human tumors [24, 25]. In the preset study we observed the accumulation of 8-OHdG in most of the AGCs, and this accumulation was much more prevalent than in the paired non-cancerous mucosa. However, no association was observed between 8-OHdG expression and aggressive clinicopathological factors such as the histopathology, tumor depth, vessel invasion or lymph node metastasis. These results suggest that 8-OHdG may be more involved with the initiation of AGC rather than the progression.

We also observed that the OGG1expression was significantly decreased in the tumors that were pT2 or deeper, the tumors with positive lymphatic invasion, and the tumors with positive lymph node metastasis. This study provides the first report showing the correlation of low OGG1 expression and T-stage, lymphatic invasion and lymph node metastasis in AGC. Karihtla et al [19] showed that the aggressive phenotype of breast cancer presented a low expression of OGG1 with worse prognosis. In contrast, our results do not show a disease-specific survival difference between the patients with OGG1-positive tumors and those with OGG1-negative tumors.

Kubo et al [15] showed frequent 8-OHdG accumulation and infrequent nuclear OGG1 expression in esophageal squamous cell carcinomas. In contrast, in the present study, the low expression of OGG1 was not correlated with the 8-OHdG expression. OGG1 in gastric cancer has not been described in detail to date, and future studies of the correlation between OGG1 and 8-OHdG in AGCs are expected.

The present study also demonstrated the down-regulation of MUTYH in AGC

compared with its non-cancerous mucosa, and its association with diffuse-type histology as well. In their study of human gastric cancers, Shinmura et al [26] showed a reduced expression of MUTYH protein and its association with diffuse-type histology, advanced T-stage and poor prognosis, but the sample subsites were not mentioned. The samples of the present study were limited to the cardia, and the AP samples did not show a reduction in the MUTYH protein level. To the best of our knowledge, this is the first report that shows a significant reduction of MUTYH protein in diffuse-type AGC. In addition, similar to MUTYH, the MTH1 protein expression was also decreased in the AGCs — in particular in the diffuse type — compared to their non-cancerous mucosa. As *MUTYH* is speculated to be one of the genes whose expression is reduced in diffuse-type gastric cancer in addition to *CDH1* or *GKN1* [26], our result supports the hypothesis that *MTH1* can also be a candidate decreased gene in diffuse-type AGC.

We observed that the diffuse-type AGCs also had more frequent *TP53* mutations (36.4%) with poorer prognosis compared to the intestinal type. The reported incidence of *TP53* mutations in primary gastric cancer varies from 3% to 65% [12], and mutations have been more frequently identified in intestinal-type tumors [27]. Considering our results regarding MUTYH, MTH1 and *TP53* mutation, we suggest that the malfunction of these BER enzymes may fail to remove the effect of oxidative stress (shown as 8-OHdG accumulation) and result in the *TP53* mutation in AGCs, especially in the diffuse type. However, further experimental study using in vitro or animal models is needed to clarify this hypothesis.

The G:C to A:T transition is reported to be the most common type of mutation in primary gastric cancer [28], whereas a transversion mutation accounts for approximately 30% [12, 27]. However, our present findings revealed that three out of the six mutations

in the AGCs (50%) were transversion mutations. This *TP53* transversion mutation rate in AGC is relatively high and is similar to that of esophageal cancer [27]. The esophagus is thought to be exposed more frequently to external possible carcinogens such as those in the diet, alcohol or tobacco — which are related to oxidative or nitrosative stresses — compared to the stomach and intestine. Another possible reason for this high transversion mutation rate in AGC may be based on the anatomical vicinity of the cardia to the esophagus. Since the number of *TP53*-positive mutation cases in the present study was limited, it is hoped that further investigations with larger numbers of lesions or in vitro experimental models will elucidate the role and effects of *TP53* mutation in the carcinogenesis of AGC.

The above-mentioned molecular alterations were not significant in the AP cases, which also supports the hypothesis that AGC, especially the diffuse type, may have a unique molecular pathogenic pathway that is different from that of AP. Some clinicopathological and epidemiological data also support this hypothesis. For example, cardia adenocarcinomas seem to have a more aggressive character with deeper invasion, greater lymph node involvement, and worse survival rate compared to distal adenocarcinomas [29]. Cardial cancer patients' risk factors are obesity, hiatal hernia, and gastro-esophageal reflux, whereas those of distal cancer patients' are *H. pylori* infection, high salt intake, and high nitrate intake [30]. *H. pylori* infection in particular, which is recognized as a carcinogen, is closely related to distal cancer of the stomach but not to proximal cancer of the stomach [31].

A recent study showed that the background gastric mucosa of Siewert type III carcinoma patients is related to gastric inflammation such as intestinal metaplasia and atrophy whereas the background gastric mucosa of Siewert type I patients is not, and that

type II shows an intermediate position, showing less severe preneoplastic conditions [14]. Interestingly, Clarke et al. reported that the cardia 5.5–27.5 mm distal from the squamocolumnar junction increases in acidity to become the most acidic lesion throughout the postprandial period, whereas the rest of the stomach showed a drop in acidity [13]. These findings suggest that the environment of the cardia is unique; it may be different from that of the esophagus, and from those of other subsites of the stomach as well.

In conclusion, our study characterized the correlations among the expressions of MUTYH, MTH1, OGG1, and 8-OHdG, *TP53* mutational status and clinicopathological factors in AGC. Our results suggest that particularly in diffuse-type AGCs, the BER enzymes MUTYH and MTH1 are down-regulated, and oxidative stress as shown by 8-OHdG accumulation may play a role in the pathogenesis via the induction of *TP53* transversion mutation. These abnormalities might result in the poor prognosis. In addition, these phenomena were not evident in AP, suggesting that diffuse-type AGC might involve unique molecular pathways that are different from other subsets of gastric cancer.

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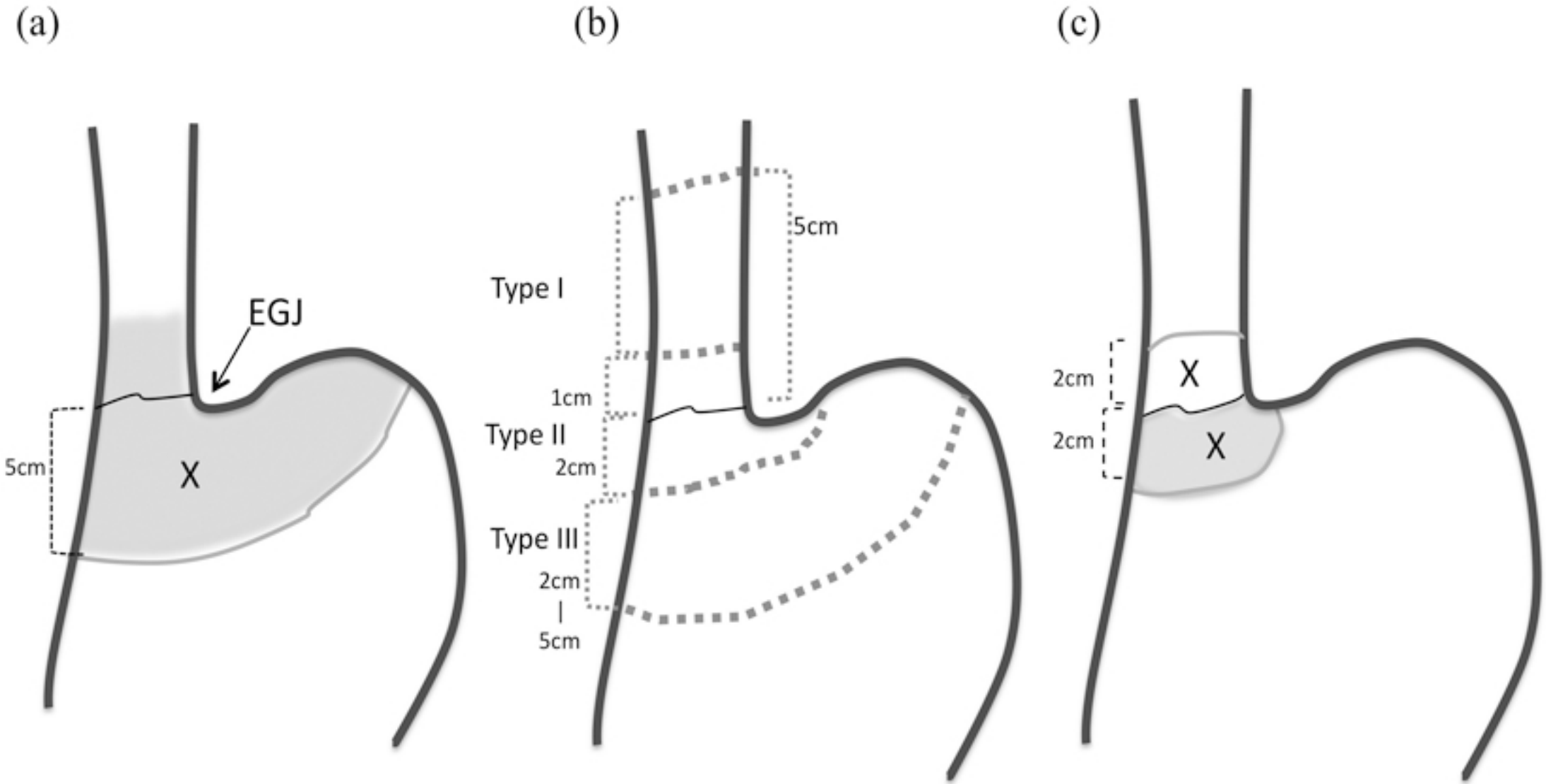
Figure legends

Fig. 1. The definitions of EGJ carcinoma. (a) UICC Classification (2010). The location of the tumor epicenter is in stomach within 5 cm from the EGJ, and the tumor extends to the esophagus. (b). Siewert classification. Type I: 1–5 cm proximal to the EGJ; Type II (true junction): 1 cm proximal, 2 cm distal to the EGJ; Type III: 2–5 cm distal to the EGJ. (c) The 14th Japanese classification of gastric carcinoma (2010) and the 10th Japanese classification of esophageal carcinoma (2008). The location of the tumor epicenter is in either the stomach or the esophagus within 2 cm from the EGJ. In the present study, AGC was defined as a tumor whose epicenter is located in the gray shaded area of (c).

Fig. 2. Representative immunohistochemical images of AGCs (original magnification ×400) b: MUTYH, c: OGG1, d: MTH1 and e: iNOS show cytoplasmic staining positivity, whereas a: 8-OHdG, and f: p53 showed nuclear staining positivity.

Fig. 3. Kaplan-Meier survival curves for the disease-specific survival of the patients with advanced stage (≥ pT2) AGCs. (a) Patients with diffuse-type tumors had a significantly shorter survival rate (p=0.002). (b) Patients aged ≥ 62 years had a significantly shorter survival rate (p=0.0073). (c) Patients with a *TP53*-positive mutation had a significantly shorter survival rate (p=0.0066).

Fig. 1


(a)
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X

Fig. 2

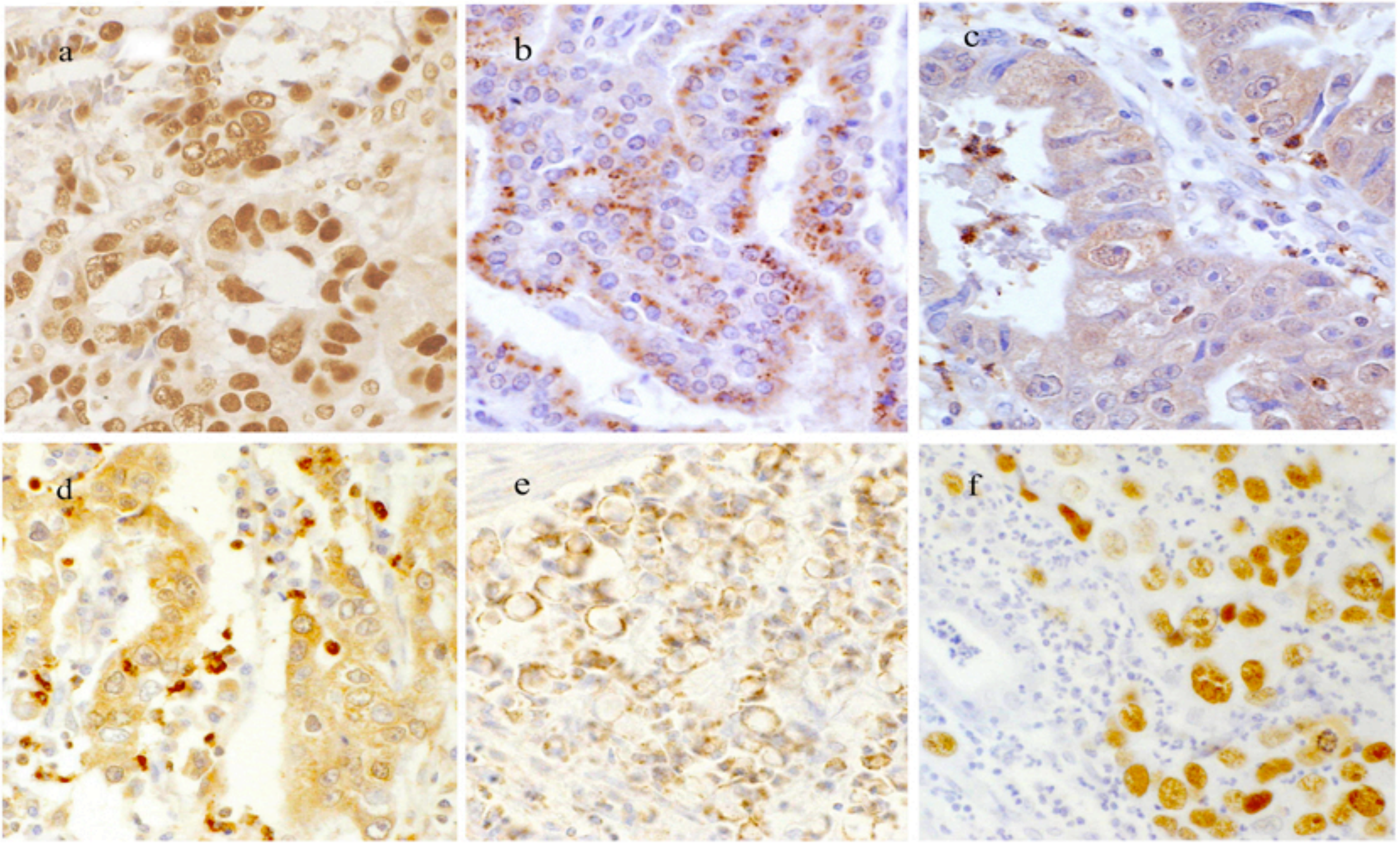

Fig. 3

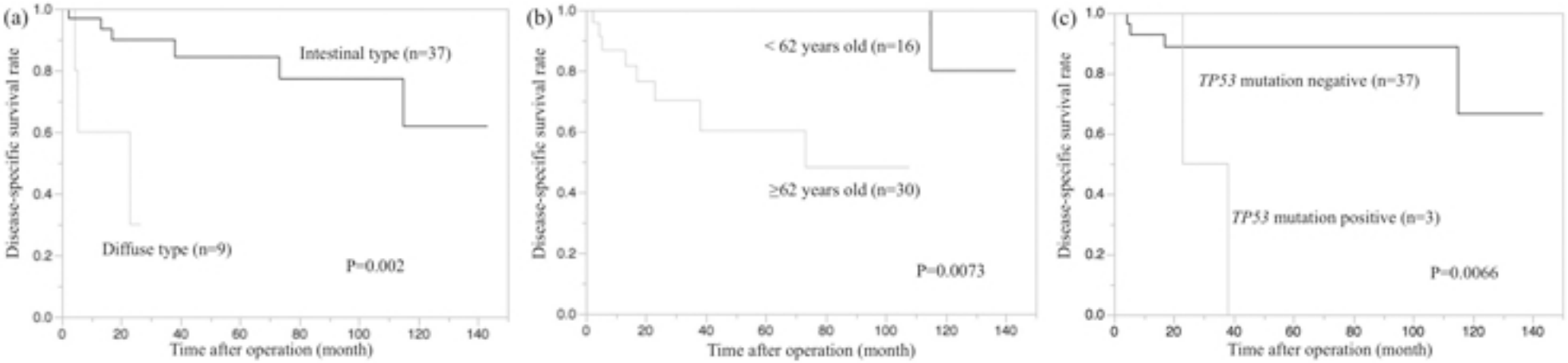
(a)
Disease-specific survival rate
Intestinal type (n=37)
Diffuse type (n=9)
P=0.002
Time after operation (month)
(b)
Disease-specific survival rate
< 62 years old (n=16)
≥62 years old (n=30)
P=0.0073
Time after operation (month)
(c)
Disease-specific survival rate
TP53 mutation negative (n=37)
TP53 mutation positive (n=3)
P=0.0066
Time after operation (month)

Table 1. Summary of clinicopathological findings in adenocarcinoma of gastric cardia and pylorus.

	Cardia (%) (n=63)	Pylorus (%) (n=27)	p-value
Age	65.8 (39 − 89)	72.4 (38 − 88)	0.0227*
Gender			
Male	49 (77.8)	19 (70.4)	0.4148
Female	14 (22.2)	8 (29.6)	
Histology			
Intestinal	52 (82.5)	19 (70.4)	0.0583
Diffuse	11 (17.5)	8 (29.6)	
Tumor depth			
≤pT1	14 (22.2)	6 (22.2)	1
≥pT2	49 (77.8)	21 (77.8)	
Lymph vessel invasion			
Negative	27 (42.9)	11 (40.7)	1
Positive	36 (57.1)	16 (59.3)	
Venous invasion			
Negative	27 (42.9)	20 (74.1)	0.0106*
Positive	36 (57.1)	7 (25.9)	
Lymph node metastasis			
Negative	26 (41.3)	11 (40.7) [a]	1
Positive	37 (58.7)	15 (55.6)	

*Statistically significant

[a] Data of one case was unavailable

Table 2. Immunohistochemical findings of repair enzyms, iNOS, 8OHdG and p53 in adenocarcinoma of gastric cardia and pylorus compared with their adjacent non-cancerous mucosa.

		Cardia			Pylorus		
		Cancer (%)	Non-cancer (%)	p-value	Cancer (%)	Non-cancer (%)	p-value
iNOS	High	46 (73.0)	54 (85.7)	0.0736	22 (81.5)	17 (63.0)	0.0253*
	Low	17	9		5	10	
8-OHdG	High	52 (82.5)	37 (58.7)	0.0011*	21 (77.8)	18 (66.7)	0.2568
	Low	11	26		6	9	
MUTYH	High	49 (77.8)	57 (90.5)	0.0325*	24 (88.9)	20 (74.1)	0.1025
	Low	14	6		3	7	
MTH1	High	31 (49.2)	22 (34.9)	0.0719	15 (55.6)	16 (59.3)	0.6547
	Low	32	41		12	11	
OGG1	High	24 (38.1)	41 (65.1)	0.0007*	21 (77.8)	18 (66.7)	0.4054
	Low	39	22		6	9	
p53	High	25 (39.7)	0 (0)		10 (37.1)	0 (0)	
	Low	38	63		17	27	

*Statistically significant

Table 3 The correlations between immunohistochamical findings of BER enzymes and clinicopathological features in AGC.

	MUTYH			MTH1			OGG1		
	High (%) (n=49)	Low (n=14)	p-value	High (%) (n=31)	Low (n=32)	p-value	High (%) (n=24)	Low (n=39)	p-value
Histology									
Intestinal	45 (86.5)	7	0.0013*	30 (57.7)	22	0.0059*	21 (40.4)	31	0.5094
Diffuse	4 (36.4)	7		1 (9.1)	10		3 (27.3)	8	
Tumor depth									
≤pT1	12 (85.7)	2	0.7163	7 (50.0)	7	1	11 (78.6)	3	0.0011*
≥pT2	37 (75.5)	12		24 (49.0)	25		13(26.5)	36	
Lymph vessel invasion									
Negative	21 (77.8)	6	1	15 (55.6)	12	0.45	16 (59.3)	11	0.004*
Positive	28 (77.8)	8		16 (44.4)	20		8 (22.2)	28	
Venous invasion									
Negative	20 (74.1)	7	0.5571	11 (40.7)	16	0.3114	14 (51.9)	13	0.0685
Positive	29 (80.6)	7		20 (55.6)	16		10 (27.8)	26	
Lymph node metastasis									
Negative	20 (76.9)	6	1	15 (57.7)	11	0.3114	15 (57.7)	11	0.0094*
Positive	29 (78.4)	8		16 (43.2)	21		9 (24.3)	28	

*Statistically significant

Table 4. The correlations between *TP53* mutational status and cliniopathological factors or immunohistochemical features of AGC.

		TP53 mutation		
		Positive (%) (n=6)	Negative (n=45)	p-value
Age		72.8±11.5	64.4±12.8	0.193
Gender	Male	5 (13.5)	32	1
	Female	1 (7.1)	13	
Histology	Intestinal	2 (5.0)	38	0.0153*
	Diffuse	4 (36.4)	7	
Tumor depth	≤pT1	2 (22.2)	7	0.2836
	≥pT2	4 (9.5)	38	
Lymph vessel invasio	Negative	4 (21.1)	15	0.1792
	Positive	2 (6.3)	30	
Venous invasion	Negative	4 (17.4)	19	0.3902
	Positive	2 (7.1)	26	
Lymph node metastas	Negative	3 (15.0)	17	0.668
	Positive	3 (9.7)	28	
Immunohistochemistry				
iNOS	High	4 (10.3)	35	0.6164
	Low	2 (16.7)	10	
8-OHdG	High	5 (11.6)	38	1
	Low	1 (12.5)	7	
MUTYH	High	2 (5.1)	37	0.0221*
	Low	4 (33.3)	8	
MTH1	High	2 (9.5)	19	1
	Low	4 (13.3)	26	
OGG1	High	3 (15.8)	16	0.6586
	Low	3 (9.4)	29	
p53	High	2 (10.5)	17	1
	Low	4 (12.5)	28	

*Statistically significant