Human epidermal growth factor 2 overexpressed alpha-fetoprotein-producing-gastric cancer

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清水　広子

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指導教員　岡村　行泰

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Human epidermal growth factor 2 overexpressed alpha-fetoprotein-producing-gastric cancer

Hiroko Shimizu[1] · Mitsugu Kochi[1] · Masashi Fujii[2] · Megumu Watabe[1] · Yoritaka Matsuno[1] · Takaharu Kawai[1] · Hiroshi Suda[1] · Tomoyuki Tanino[3] · Yoko Nakanishi[3,4] · Shinobu Masuda[3] · Yukiyasu Okamura[1]

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Abstract

Purpose This study aimed to elucidate the clinicopathological characteristics of α-fetoprotein (AFP)-producing gastric carcinoma (AFP-GC) with human epidermal growth factor receptor (HER)2 overexpression to extend the treatment strategy for AFP-GC.

Methods We analyzed 41 patients with AFP-GC who underwent surgical resection or chemotherapy from 1989 to 2019, and who had over 20ng/mL of serum AFP or positive immunohistochemical AFP expression. HER2 expression status was investigated by immunohistochemistry (IHC) for all patients and by fluorescence in situ hybridization (FISH) for cases with an IHC score of 2+. AFP-GC with an IHC score of 3 + or 2 + and FISH positivity was defined as HER2 overexpressed AFP-GC. The correlation between HER2 status and clinicopathological characteristics and prognosis in AFP-GC was analyzed.

Results HER2 overexpression was detected in 17.1% of AFP-GC patients. The prognosis of the patients with HER2 overexpressed AFP-GC was not significantly different compared to HER2 non-overexpressed AFP-GC. HER2 overexpressed AFP-GC consisted of heterogeneous histology with a higher proportion of mixed-type tumors ($p = 0.002$). The clinical outcome of AFP-GC with mixed-type of histology tended to be better than other intestinal or diffuse types ($p = 0.05$).

Conclusion HER2 overexpressed AFP-GC consisted of a mixed type of histology, which showed a better prognosis. The results presented that HER2 status in AFP-GC is one of the molecular candidates to improve the prognosis.

Keywords AFP-producing gastric cancer · HER2 · Mixed histological subtype

1 Introduction

Alpha-fetoprotein (AFP)-producing gastric cancer (AFP-GC) was first reported by Bourreille et al. in 1970 [1]. Alpha-fetoprotein is a glycoprotein produced in fetal hepatocytes and yolk sacs [2]. It is rarely produced in the tissues of healthy adults but is produced by tumor cells such as hepatocellular carcinoma, hepatoblastoma, and York-Sack tumor, and, rarely, by lung and gastric cancer. AFP-GC has subsequently been followed by many reports [3–6]. Studies have shown that liver metastases frequently occur as a pathophysiology of AFP-GC [6–10], and even if the tumor is diagnosed in the early phase, the prognosis is poor [9–16]. To date, there is still much controversy regarding AFP-GC treatment.

✉ Yoko Nakanishi, nakanishi.youko@nihon-u.ac.jp | [1]Division of Digestive Surgery, Department of Surgery, Nihon University School of Medicine, Tokyo, Japan. [2]Japan Clinical Cancer Research Organization, Tokyo, Japan. [3]Division of Oncologic Pathology, Department of Pathology and Microbiology, Nihon University School of Medicine, Tokyo, Japan. [4]Division of Oncologic Pathology, Department of Pathology and Microbiology, Nihon University School of Medicine, 30-1 Ohyaguchi-Kamicho, Itabashi-ku, Tokyo 173-8610, Japan.

Many studies have shown that human epidermal growth factor receptor (HER) 2 overexpression is detected in 7–34% of gastric cancer cases [17–25]. The effectiveness of trastuzumab and lapatinib has been demonstrated in different gastric cancer models and has led to clinical studies. Trastuzumab, a monoclonal antibody against HER2 (also known as ERBB2), in combination with chemotherapy is considered a new standard for patients with advanced gastric or gastroesophageal junction cancer with HER2 overexpression [25]. In AFP-GC, however, the population of HER2 overexpressed tumors and their clinicopathological characteristics are still unclear.

This study aimed to clarify the population of HER2 overexpressed AFP-GC and their clinicopathological characteristics, in real-world patients, to extend the treatment strategy for AFP-GC using anti-HER2 agents.

2 Materials and methods

2.1 Patients

From January 1989 to December 2019, stomach adenocarcinoma patients who had been diagnosed and undergone surgical resection or chemotherapy at Nihon University Itabashi Hospital were retrospectively reviewed. Formalin-fixed and paraffin-embedded (FFPE) tissue specimens of the primary region without any anti-cancer treatment were obtained by resection or biopsy. We analyzed 41 patients who had over 20ng/mL of serum AFP or whose FFPE tissue sections were positive immunohistochemical AFP expression. Serum AFP levels were determined using a commercial enzyme immunoassay kit (Fujirebio Inc., Tokyo, Japan), and a cut-off value of 20 ng/mL were used. Tumor AFP expression was analyzed by immunohistochemistry (IHC) using primary antibodies against AFP (IR500, rabbit polyclonal, Agilent Technologies, Santa Clara, CA, USA) and Simple Stain MAX-PO (Multi) (Nichirei Bioscience Inc., Tokyo, Japan). AFP expression was evaluated as positive when > 5% of tumor cells were stained. The summary of the patients is shown in Table 1. All 41 patients were not treated with trastuzumab. All procedures in our study were performed following the ethical standards of the institutional and national research committees, and the Declaration of Helsinki. This study was approved by the institutional review board of Nihon University Itabashi Hospital (RK-150609-07).

2.2 Immunohistochemistry for HER2

To investigate immunohistochemical HER2 expression status, FFPE tissue specimens of 41 patients were cut into 4-μm-thick sections and mounted on silane-coated glass slides. After deparaffinization, HER2 expression was analyzed using HercepTest (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's instructions. HER2 expression was evaluated by certified board pathologists according to the scoring system by Hofmann et al. [26] and the 2018 ASCO/CAP guidelines [27] as follows. Positive; strongly positive and completely membranous staining (3+) in ≥ 10% of tumor cells. Equivocal; moderately positive staining for complete membranous staining (+ 2) in ≥ 10% of tumor cells. Negative; no reactivity or membranous staining in < 10% of tumor cells, or faint and partial membrane reactivity (1+) in ≥ 10% of tumor cells [26, 27].

2.3 Fluorescence in situ hybridization for HER2

When the HER2 expression of the tested samples was evaluated as equivocal by IHC, we determined whether HER2 DNA was amplified by fluorescence in situ hybridization (FISH) methods using in vitro diagnostics (IVD) kit Histra HER2 FISH (JOKOH CO., LTD., Tokyo, Japan). FFPE tissue specimens were cut into 4-μm-thick sections and mounted on silane-coated glass slides. After deparaffinization, FISH analysis was performed according to the manufacturer's instructions. Fluorescence signals of HER2 and CEP17 were acquired with an Axio Imager Z2 Upright Microscope (Carl Zeiss, Oberkochen, Germany) and ZEN 2 pro software (Carl Zeiss). The HER2 DNA amplification was determined when the signal counts of HER2/CEP17 were ≥ 2.0.

Table 1 Summary of the patients

Clinicopathological factors	Total N = 41
Sex	
Male	31 (75.6)
Female	10 (24.4)
Age (Year)	
Median [Range]	69 [52–81]
52–59	8 (19.5)
60–69	14 (34.1)
70–79	17 (41.5)
80–81	2 (4.9)
Location	
Upper	15 (36.6)
Middle	16 (39.0)
Lower	10 (24.4)
Clinical Stage	
Stage I-III	16 (39.0)
Stage IV	25 (61.0)
Operation	
Non-operated	16 (39.0)
Operated	25 (61.0)
Histologic subtype†	
Intestinal	17 (41.5)
Diffuse	10 (24.4)
Mixed	14 (34.1)
Serum AFP level (ng/mL)	
Median [Range]	605 [7.7-273000]
7.7–19.9 (normal)	3 (7.3)
20.0-99.9	10 (24.4)
100–499	3 (7.3)
500–999	8 (19.5)
1000–9999	12 (29.3)
10,000–273,000	5 (12.2)

†Lauren's classification

2.4 Statistical analysis

The association between HER2 status and clinical and clinicopathological factors was evaluated using the chi-squared test. Survival assays were performed using the Cox proportional hazards and Kaplan–Meier models.

Significance was set at $p < 0.05$. The SAS software package for Windows, version 8.02 (SAS Institute Inc., Cary, NC, USA) and Microsoft Excel 2016 (Microsoft Co., Ltd., Japan) were used for statistical analysis and data calculation.

3 Results

3.1 Her2 overexpression in AFP-GC

The photos of HER2 overexpression by IHC and HER2 amplification by FISH are shown in Fig. 1. Table 2 shows that HER2 overexpression was detected in seven (17.1%) of the 41 AFP-GC patients. The breakdown was 3 + of HER2 score was detected in five (12.2%) patients, and 2 + with gene amplification confirmed by FISH was observed in two (4.9%) patients.

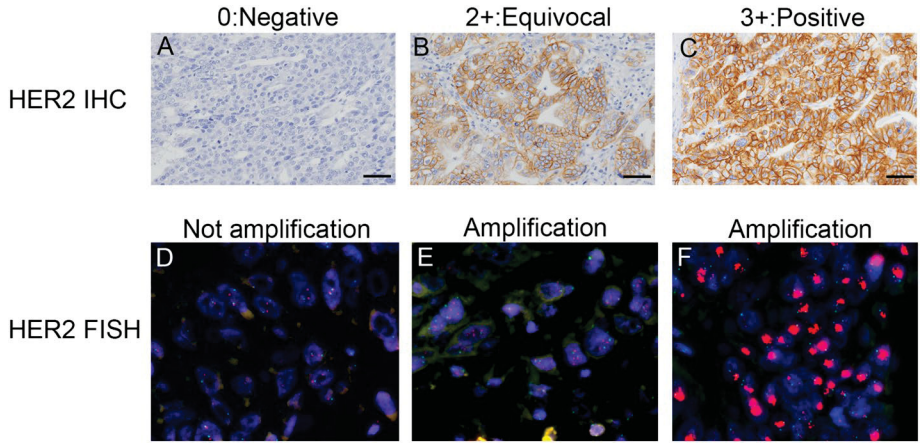


Fig. 1 HER2 status by IHC and FISH. **A** Negative expression (0), **B** equivocal expression (2+), and **C** positive expression (3+) by immunohistochemistry. Each bar shows 50 µm. Equivocal samples need additional FISH analysis to determine whether their HER2 DNA was no amplification **D** or amplification **E**, **F**. The green signals show CEP17 and the red signals show HER2. The HER2 DNA amplification was determined when the signal counts of HER2/CEP17 were ≥ 2.0 in 20 tumor cells

Table 2 HER2 status assessed by IHC and FISH

HER2 status	Number	%
Positive		
IHC 3+	5	12.2
IHC 2+ and FISH positive	2	4.9
Total	7	17.1
Negative		
IHC 0, 1+	31	75.6
IHC 2+ and FISH negative	3	7.3
Total	34	82.9

3.2 Clinicopathological features of HER2 overexpressed AFP-GC

We compared the clinicopathological features of HER2 overexpressed patients to non-overexpressed patients within AFP-GC. Table 3 shows the correlation between clinicopathological features and HER2 status in AFP-GC, and that the proportion of histologic subtypes evaluated by Lauren's classification [28] was significantly different between the HER2 overexpressed group and the non-overexpressed group ($p = 0.005$). By additional residue analysis, Fig. 2 shows that mixed histology was detected at a significantly higher proportion in the HER2 overexpressed group ($p = 0.002$), and intestinal histology was significantly higher in the HER2 non-overexpressed group ($p = 0.01$). Other factors, including sex, age, tumor location, operation, clinical stage, and serum AFP level, were not significantly different between HER2 overexpressed AFP-GC and HER2 non-overexpressed AFP-GC are shown in Table 3.

Histological findings of a representative case of mixed type of histology are shown in Fig. 3. Figure 3A shows H&E stained whole tissue section. Figure 3B shows the details of mixed histology types contained in a single tissue section. In this tumor, different tubular, as shown in Fig. 3B-a, e, i, papillary, as shown in Fig. 3B-b, f, j, hepatoid, as shown in Fig. 3B-c, g, k, and solid, as shown in Fig. 3B-d, h, l, pattern structures consisted. Each component showed a different immunohistochemical phenotype for AFP (Fig. 3B-e, f, g, h) and HER2 (Fig. 3B-I, j, k, l).

3.3 Relationship between HER2 overexpression and patients' prognosis in AFP-GC

We next investigated the prognostic implication of HER2 expression in AFP-GC patients, compared to other clinicopathological factors. Survival analysis was performed by classifying the present AFP-GC patients into two groups according to the clinicopathological factors as follows; HER2 overexpressed and non-overexpressed groups, female

Table 3 Correlation between HER2 status and clinicopathological features in AFP-GC

Clinicopathological factors	Total	HER2 status		P value
		Overexpression	Non-overexpression	
	N = 41	N = 7 (16.6%)	N = 34 (83.3%)	
Sex				
Male	31	5 (71.4)	26 (76.5)	0.78
Female	10	2 (28.6)	8 (23.5)	
Age (Year)				
<70	22	4 (57.1)	18 (52.9)	0.59
≥70	19	3 (42.9)	16 (47.1)	
Location				
Upper	15	3 (42.9)	12 (35.3)	0.71
Middle	16	4 (57.1)	12 (35.3)	
Lower	10	0 (0.0)	10 (29.4)	
Clinical stage				
Stage I-III	16	3 (42.9)	13 (38.2)	0.82
Stage IV	25	4 (57.1)	21 (61.8)	
Operation				
Non-operated	16	2 (28.6)	14 (41.2)	0.53
Operated	25	5 (71.4)	20 (58.8)	
Histologic subtype†				
Intestinal	17	0 (0.0)	17 (50)	0.005*
Diffuse	10	1 (14.3)	9 (26.5)	
Mixed	14	6 (85.7)	8 (23.5)	
Serum AFP level (ng/mL)				
< 500	16	3 (42.9)	13 (38.2)	0.57
≥ 500	25	4 (57.1)	21 (61.8)	

†Lauren's classification, *P < 0.01, chi-square test

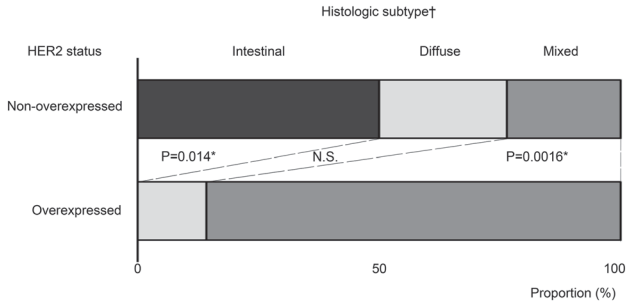


Fig. 2 The proportion of histologic subtype by Lauren's classification in the HER2 overexpressed and the HER2 non-overexpressed AFP-GC groups
†, Lauren's classification. ** P < 0.01, * P < 0.05, by residue analysis

and male, < 70 years and ≥ 70years, lower and upper/middle of tumor location, clinical stage I – III and stage IV, operated and non-operated, < 500ng/ml and ≥ 500ng/ml of serum AFP level, and intestinal/diffuse type and mixed type of histologic subtype classified by Lauren's classification.

Figure 4A, H show the results of the Kaplan-Meyer analysis that showed the differences in the overall survival rate of each AFP-GC group. Overall survival in HER2 overexpressed group and HER2 non-overexpressed group was not significantly different (p = 0.52, log-rank) as shown in Fig. 4A. Patients with clinical stage IV showed significantly worse prognosis than those with stage I - III (p = 0.045) as shown in Fig. 4E. Operated patients had a significantly better prognosis than non-operated patients (p < 0.001) as shown in Fig. 4F. Patients with ≥ 500ng/mL of serum AFP levels had a significantly worse prognosis than those with < 500ng/mL (p = 0.007) as shown in Fig. 4H. Patients with mixed histology tended to have a better prognosis than those with intestinal or diffuse histology (p = 0.05) as shown in Fig. 4G. Other clinicopathological factors, sex, age, and tumor location status were not significantly correlated with clinical outcomes as shown in Fig. 4B C, and D, respectively.

Multivariate survival analysis was performed by Cox proportional hazards model. These results are shown in Table 4. The one- and 3-year survival rates were 42.9% and 28.6% in the HER2 overexpressed group, and 46.7% and 17.6% in the HER2 non-overexpressed group. The hazards of the HER2 overexpressed group were 0.95 times those of the HER2 non-overexpressed group, and 95% confidence interval (CI) was 0.26–3.48 (p = 0.94).

In comparison by other clinicopathological factors, the one- and 3-year survival rates were 24.0% and 8.0% in the patients with ≥ 500ng/mL of serum AFP levels, and 68.8 and 37.5% in those with < 500ng/mL of serum AFP levels (HR, 3.20; 95%CI 1.04–9.82; p = 0.04). The one- and 3-year survival rates were 64.0% and 32.0% in the operated group, and 6.3% and 0.0% in the non-operated group (HR, 0.24; 95%CI 0.078–0.73; p = 0.01). In the patients with mixed histology, the one- and 3-year survival rates were 64.3% and 35.7%, and 37.0% and 18.5% in those with intestinal or diffuse histology (HR, 0.42; 95%CI 0.15–1.20; p = 0.11). Other factors, age, sex, clinical stage, and tumor location, were also not significant.

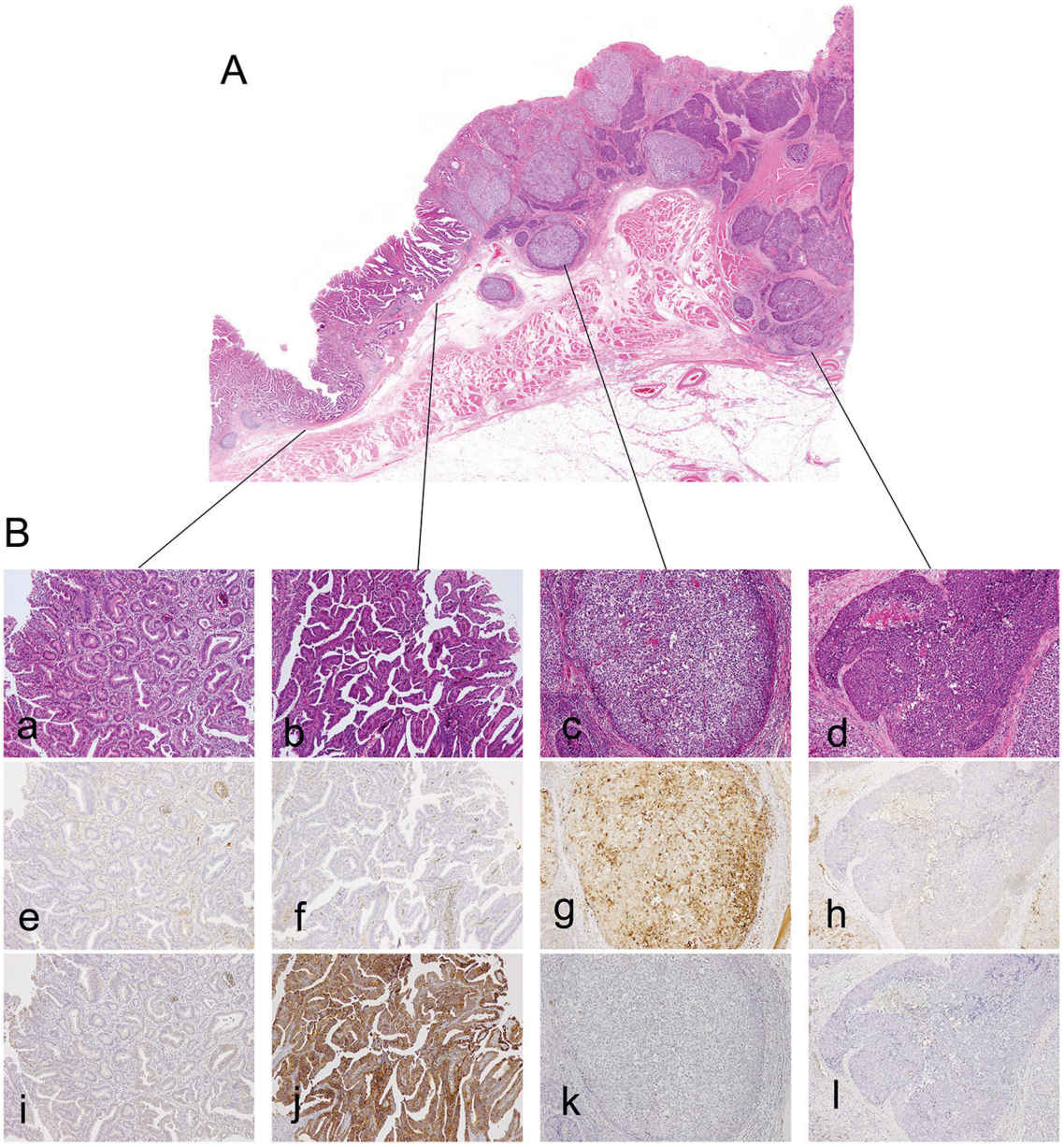


Fig. 3 Histopathological findings of a representative case of mixed type of histology. Whole H&E stained tissue section **A** and detailed structures **B**. This gastric tumor consists of heterogeneous cancer cells with different structural features: tubular (a, e, i), papillary (b, f, j), hepatoid (c, g, k), and solid (d, h, l) patterns. The tumor was diagnosed as a mixed type when both intestinal and diffuse types were detected. Each component showed a different immunohistochemical phenotype for AFP (e, f, g, h) and HER2 (i, j, k, l)

4 Discussion

Even if AFP-GC is diagnosed in an earlier phase, its prognosis is poor due to the susceptibility to liver metastasis and the lower radical resectability [6–10], and no treatment strategy has been established as yet. In contrast, HER2 overexpressed gastric cancer has been established in clinical trials. The ToGA trial was the first phase III trial to add trastuzumab to standard chemotherapy and included patients with HER2-overexpressing advanced gastric or gastroesophageal junction cancer who were randomized to receive 5-fluorouracil/capecitabine and cisplatin alone or in combination with trastuzumab. The results demonstrated the efficacy of HER2-targeted molecular therapy for gastric cancer [25].

Both AFP-GC and HER2 overexpressed gastric cancer has been known for aggressive clinical behavior and poor prognosis. However, no study has addressed HER2 overexpression in AFP-GC. This study revealed the incidence of HER2 overexpression in 17.1% of AFP-GC. The percentage is within the scope of the frequency of all gastric cancer [17–25]. It was also revealed that the prognosis of HER2 overexpressed AFP-GC was not worse than HER2 non-overexpressed AFP-GC, contrasted to the worse prognosis of the only HER2 overexpressed gastric cancer that had been reported [21, 24]. All patients in this study were not treated with trastuzumab, therefore, we don't have to consider the influence of trastuzumab on the prognosis for HER2 overexpressed AFP-GC in this study. Instead, HER2 overexpressed AFP-GC consisted of a mixed type of histology, and its clinical outcome tended to be better. These results suggested that HER2 overexpressed cancer cells may occur in AFP-GC, consisting of heterogeneous subtypes with a better clinical outcome, compared with HER2 non-overexpressed AFP-GC, which consists of homologous cancer cells with aggressive clinical behavior. To address the relationship between HER2 expression status and the therapeutic effects including trastuzumab and prognosis, a larger number of cohorts are required to study in the future. The cancer genome atlas [29] categorized gastric cancers as Epstein–Barr virus-positive, microsatellite instability, genomic instability, and chromosomal instability (CIN). AFP-GC and HER2 overexpressed gastric cancer were categorized into the CIN subtype, which was the largest category, comprising approximately 50% of gastric cancers. The present study showed that the CIN category has the potential to be sub-grouped according to AFP and/or HER2 overexpression.

Several AFP-producing gastric cancers have been reported to be successfully treated with combined neoadjuvant chemotherapy with epirubicin (EPI), 5-fluorouracil (5-FU), and leucovorin (LV) [30]. We performed combination chemotherapy with 5-FU, LV, etoposide (VP-16), and cis-diamminedichloroplatinum (CDDP) specified by Nakajima et al. as the FLEP regimen for patients with stage IV gastric cancer who were not candidates for surgery [31]. The purpose of the FLEP therapy, which consisted of a combination of local delivery of VP-16 and CDDP to the aorta and systemic delivery of 5-FU and LV, was the control of both local and disseminated disease in the intra- and extra-abdominal regions. In our previous observational study of FLEP chemotherapy, the median survival time in the group with AFP-GC was 15.8 months compared to 10.3 months in the non-AFP-GC group. The cumulative survival of stage IV patients with AFP-GC was significantly higher than those with non-AFP-GC [16]. This finding has suggested that AFP-GC has high chemosensitivity. Based on our results, heterogeneity of cancer cells with different susceptibility or resistance to chemotherapy may affect the prognosis. Furthermore, evidence for novel therapies targeting HER2 or AFP molecules has accumulated. For example, lapatinib was shown to be more effective for HER2-positive AFP-GC [15, 32], and ramucirumab was reported to be more effective for AFP-positive hepatocellular carcinoma (HCC) than for AFP-negative HCC [33].

The clinical aggressiveness of HER2 non-overexpressed AFP-GC may also apply to other new anticancer medicines. Genetic testing is becoming more widely available for various carcinomas [34]. Currently, the AFP-GC needs to select the appropriate drug using a panel test. Some investigators have reported that the higher expression of c-Met may explain the poorer prognosis of AFP-GC [35]. When the different biochemical mechanisms and oncogenes of AFP-GC are revealed, it may be possible to use this information in the therapeutic management of this cancer.

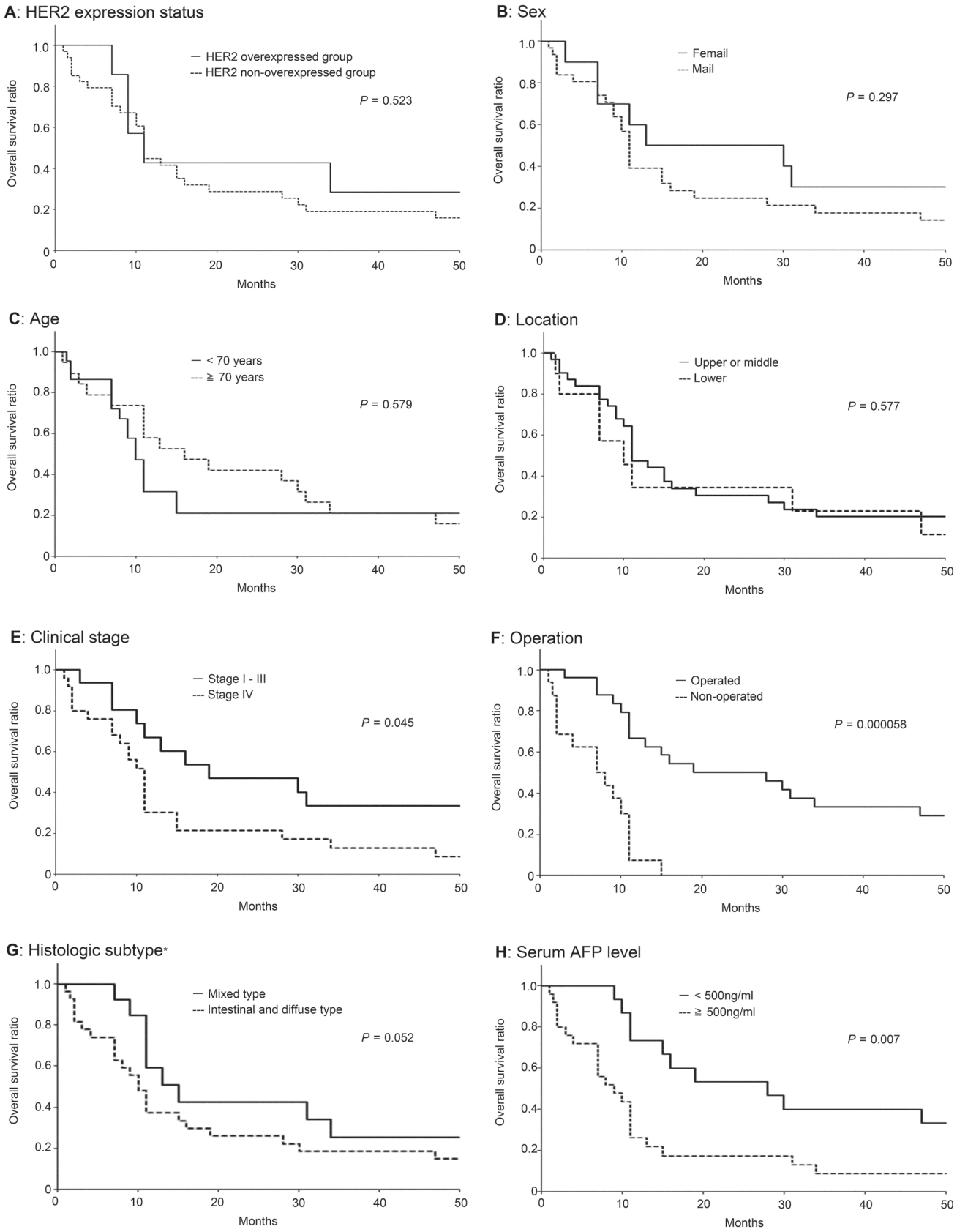
In conclusion, HER2 overexpression was detected in 17.1% of AFP-GC. HER2 overexpressed AFP-GC consisted of a mixed type of histology, which showed a better prognosis. These results showed that HER2 status in patients with

Fig. 4 Overall survival of AFP-GC. Overall survival of HER2 overexpressed and non-overexpressed groups **A**, female and male **B**, < 70 years ▶ and ≥ 70years **C**, lower and upper/middle of tumor location **D**, clinical stage I – III and stage IV **E**, operated and non-operated **F**, intestinal/diffuse type and mixed type of histology classified by Lauren's classification **G**, and < 500ng/ml and ≥ 500ng/ml of serum AFP level **H**, analyzed using the Kaplan–Meier methods (log-rank), are shown

Table 4 Multivariate survival analysis by Cox proportional hazards model

Covariate	1-year survival rate (%)	3-year survival rate (%)	HR	95% CI	P value
Sex					
Female	60.0	30.0			
Male	35.5	16.1	1.94	0.64–5.94	0.24
Age					
< 70 years	57.9	21.1			
≥ 70 years	27.3	18.2	1.55	0.64–3.73	0.33
Location					
Upper or middle	46.7	20.0			
Lower	30.0	20.0	1.35	0.54–3.37	0.52
Clinical stage					
Stage I-III	66.7	33.3			
Stage IV	29.2	12.5	1.21	0.39–3.76	0.74
Operation					
Non-operated	6.3	0.0			
Operated	64.0	32.0	0.24	0.078–0.73	0.01*
Serum AFP level					
< 500ng/ml	68.8	37.5			
≥ 500ng/ml	24.0	8.0	3.20	1.04–9.82	0.04*
Histologic subtype[†]					
Intestinal or Diffuse type	37.0	18.5			
Mixed type	64.3	35.7	0.42	0.15–1.20	0.11
HER2 status					
Non-overexpressed	46.7	17.6			
Overexpressed	42.9	28.6	0.95	0.26–3.48	0.94

[†]Lauren's classification; *P < 0.05; HR, hazard ratio; CI, confidential interval


A: HER2 expression status
HER2 overexpressed group
HER2 non-overexpressed group
P = 0.523
B: Sex
Femail
Mail
P = 0.297
C: Age
< 70 years
≥ 70 years
P = 0.579
D: Location
Upper or middle
Lower
P = 0.577
E: Clinical stage
Stage I - III
Stage IV
P = 0.045
F: Operation
Operated
Non-operated
P = 0.000058
G: Histologic subtype*
Mixed type
Intestinal and diffuse type
P = 0.052
H: Serum AFP level
< 500ng/ml
≥ 500ng/ml
P = 0.007
Overall survival ratio
Months

AFP-GC should be examined to investigate its unique characteristics from clinical, pathological, and molecular aspects and improve the prognosis of patients with AFP-GC by providing optional treatments for molecular targets.

Author contributions All authors contributed to the study's conception and design. Study designs were performed by MK, MF, and SM. Material preparation and analysis were performed by HS and YN. Clinical data collection and analysis were performed by MK, MW, YM, TK, and HSU. Histological analyses were performed by TT, YN, and SM. The first draft of the manuscript was written by HS, MK, and YN. The manuscript was revised by SM and YO. All authors read and approved the final manuscript.

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Availability of data and materials The authors confirm that the data supporting the findings of this study are available within the article.

Declarations

Ethics approval and consent to participate This retrospective observational study was approved by the Ethics Committee of Nihon University Itabashi Hospital (RK-150609-07). Informed consent was obtained from all individual participants that their pathology specimens were used.

Consent for publication Information is anonymized and the submission does not include images that may identify the person.

Competing interests The authors declare that they have no competing interests.

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Human epidermal growth factor 2 overexpressed alpha-fetoprotein-producing- gastric cancer

(上皮成長因子受容体 2 型 (HER2) を過剰発現している α-フェトプロテイン (AFP) 産生胃癌)

日本大学医学部　外科学系消化器外科学分野　清水　広子

【背景】

AFP 産生胃癌は非常にまれな疾患で、偶発的に診断される場合が多く、全胃癌の 1.2～6.6%と報告されている [1)]。その特徴として脈管侵襲を高度に認め、肝転移の頻度が高いことから、一般的に予後不良である [2)3)]。進行速度の速さ、高率な遠隔転移、組織型の多様性を考えれば、通常の胃癌と考えるより、希少癌の一種ととらえて治療を行うことが臨床上重要であると考えるが、まれな疾患であることから AFP 産生胃癌に対する定型的な治療法は確立されていない。

一方、HER2 の過剰発現を示す HER2 陽性胃癌の存在が認められており、全胃癌の 7～34%とされている [4)-12)]。HER2 陽性胃癌に対する分子標的薬であるトラスツズマブの有効性が確認され、化学療法との併用で、HER2 陽性の胃癌や食道胃接合部癌の新しい標準治療となっている [13)]。

これまでの報告、文献から、AFP 産生胃癌は組織多様性の癌であり、個々の AFP 産生胃癌の薬剤感受性はすべて異なる。[14)-20)]その為個々の感受性を探り当て、治療を行う必要性がある。しかし多くの AFP 産生胃癌は手術適応にはならず、内視鏡による腫瘍生検のみでの結果をもとに治療法を決定しなければならないが、多様性の癌腫のごく一部の生検結果では正確性を欠く結果になることが危惧される。したがって、AFP 産生腫瘍において共通したバイオマーカーの発見は、今後 AFP 産生胃癌治療方針の一路になると考えた。学会報告で AFP 陽性胃癌の HER2 陽性率が 40%と高率であったという報告があり [21)]、AFP 産生胃癌において HER2 の陽性率が高いということを証明することができれ

ば、多角的な組織検査を推奨することで的確な治療薬の選択につながると考え、HER2 との関連性に注目した。

本研究では、AFP 産生胃癌の治療戦略を確立するために、AFP 産生胃癌の HER2 過剰発現とその臨床病理学的特徴についての検討を行った。

【対象と方法】

1989 年 1 月から 2019 年 12 月までに日本大学医学部附属板橋病院において胃腺癌の診断で治療を受けた患者 2799 例について後ろ向きに検討した。

AFP 産生胃癌は、AFP 免疫染色で癌細胞に陽性像が証明されるか、もしくは胃癌以外の要因がなく、血清 AFP 値の異常高値が示されることで診断される [22]が、具体的な診断基準は存在しない。その為血清 AFP 値については当施設の基準値 20ng/mL 以上で高値と判断した。AFP 免疫染色に関しては、1%程度でも癌細胞に明確な陽性像が得られれば AFP 産生胃癌と考えてよいとの報告 [23]があるが、本研究ではより厳密に症例を選択するために 5%を閾値とした。

本研究期間中、胃腺癌の全症例 2799 例に対し血清 AFP 値を測定した。免疫染色については、血清 AFP 値が高値の症例のほか、従来通り病理専門医が必要と判断する症例について施行された。その結果、血清 AFP 値が 20ng/mL 以上の症例が 38 例、血清 AFP 値が基準値内で、AFP 染色陽性の症例が 3 例、計 41 例が AFP 産生胃癌と診断され、本研究の対象となった (Table 1) 。

対象症例の病理組織の内訳は、生検検体が 19 例 (術前化学療法施行の 2 例を含む) 、手術検体が 22 例であった。対象症例 41 例の AFP と HER2 について、免疫組織化学 (IHC) 法を用いて発現を調べ、その結果、HER2 の発現が HER2 スコア 2＋と評価された検体については蛍光 in situ ハイブリダイゼーション (FISH) 法で HER2 遺伝子の増幅を確認した (Fig. 1) 。これらの結果をもとに AFP 産生胃癌における HER2 過剰発現群と HER2 非過剰発現群について、臨床病理学的特徴を比較検討した。血清 AFP 値については、中央値である 605ng/mL をカットオフ値として 2 群に分け、統計解析を行った。

病理組織型の分類は基本的に胃癌取り扱い規約 (第 15 版) [24]に基づき行った。しかし、本研

究で検討した AFP 産生胃癌には特殊型を含む混合型が多く、胃癌取り扱い規約 (第 15 版) の定義が当てはまらなかったため、混合型については WHO 分類を参考に独自に定義した。最初に組織形態毎に胃癌取り扱い規約 (第 15 版) により、高分化型 (pap,tub1,tub2) 、低分化型 (sig, por1, por2) 、特殊型(肝様腺癌、胎児消化管類似腺癌)に分類し、特殊型を含めて複数の組織型カテゴリー(高分化型、低分化型、特殊型)が混在するものを混合型として分類した (Table 2) 。

統計解析は、HER2 発現と臨床病理学的要因について、Fisher の正確確率検定で解析後、組織学的特徴について残差分析を行った。AFP 産生胃癌の予後因子については、カプランマイヤー法による生存曲線とログランク検定を用いて解析を行った。

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【結果】

当科における全胃癌 2799 症例中 AFP 産生胃癌は 41 例、1.46%であった。AFP 産生胃癌 41 例のうち、HER2 の過剰発現を認めたものは 7 例 (17.1%) であった。そのうち、IHC 法で HER2 スコア 3＋が 5 例 (12.2%) 、HER2 スコア 2＋かつ FISH 法陽性が 2 例 (4.9%) であった (Table 3)。

HER2 過剰発現群と HER2 非過剰発現群の比較検討において、性別、年齢、腫瘍の位置、手術の有無、化学療法の有無、臨床病期、血清 AFP 値を含むその他の因子については差を認めなかった (Table 4) 。組織型の割合が有意に異なっており (p=0.03) (Table 4) 、追加の残差分析では、HER2 過剰発現群で混合型の割合が有意に高く (p=0.0015) 、HER2 非過剰発現群では高分化型の割合が有意に高かった(p=0.010) (Fig. 2) 。

次に、AFP 産生胃癌の予後について、臨床病理学的要因について比較検討した。HER2 過剰発現群と HER2 非過剰発現群で、全生存率に差は認めなかった (p=0.523) (Fig. 3A) 。性別 (Fig. 3B) 、年齢 (Fig. 3C) 、腫瘍の位置 (Fig. 3D) 、血清 AFP 値の高低 (Fig. 3G)についても有意差を認めなかった。臨床病期分類IV期 (p=0.045) (Fig. 3E) とともに、手術治療なし群 (p=0.000058) (Fig. 3F) が有意に予後不良であった (Fig. 2) 。組織型別の生存曲線については、低分化型 (sig, por1, por2) と混合型の 2

群間に有意差を認め、混合型の予後が良好であった (p=0.044) (Fig. 4) 。

混合型を構成する組織亜型と、各領域における免疫組織学的 AFP ならびに HER2 陽性所見に関するデータを Table 2 に示した。HER2 過剰発現が指摘された 7 例 (症例 3, 6, 14, 20, 25, 29, 39) のうち、5 症例については、AFP 陰性あるいは低発現 (<5%) であったが (Fig. 5) 、症例 3 については HER2 陽性病変の 80%に (Fig. 6) 、症例 6 については 30%の癌細胞に AFP 陽性所見が指摘された。AFP と HER2 は共発現が認められた症例は 2/7 例 (28.6%)であり、共発現が認められなかった症例が多かった (5/7 例, 71.4%)。また、組織亜型別の免疫組織学的 AFP 陽性率を Table 6 に示した。肝様腺癌 (hep) と胎児消化管類似癌 (ent) が 100%と高く、次いで充実型 (por1) が 77.8%、乳頭型 (pap) が 58.3%であった (Table 6)。

【考察】

AFP 産生胃癌も、HER 2 陽性胃癌も、進行が早く予後不良の胃癌として知られているが、AFP 産生胃癌の HER2 過剰発現について、単施設での多数の症例を集めた研究はされていない。

AFP 産生胃癌の診断は、その特殊性に気付いた時に、血清 AFP を測定したり組織の AFP の免疫染色を行うところから糸口が見つかるものであり、AFP 産生胃癌と診断されずに治療が行われる症例も少なくないと考える。その点において、本研究は、胃腺癌の全症例で AFP 値を測定したという系統立てた症例集積を行なっており、AFP 産生胃癌全体の実態を忠実に反映する研究結果として意義のあるものと考える。

本研究では、腫瘍全体としてとらえた場合には、AFP 産生胃癌の 17.1%に HER2 の過剰発現を認め、全胃癌の HER2 の過剰発現率と大きな差はなく、これまでの報告 [4)-12)] と同様の結果である。一般的に HER2 陽性胃癌が、全胃癌と比べて予後不良であること [11)25)] と対照的に、今回、HER2 過剰発現のある AFP 産生胃癌と HER2 非過剰発現の AFP 産生胃癌の予後に有意差は認めなかった。2011 年 3 月にトラスツズマブが本邦で承認され、本研究ではそれ以降の HER2 陽性症例が 2 例あるが、いずれも化学療法の適応がない症例であったため、結果的に今回の研究の対象症例は、トラスツズマブによる治療を行っていない。したがって、HER 2 の過剰発現のある AFP 産生胃癌のトラスツズマブ治

療の影響を受けていない予後を反映しているものと考えられる。

腫瘍を癌細胞単位で考えた場合、HER2 過剰発現をともなう胃癌は、乳癌と比較して、同一腫瘍内での HER2 過剰発現の不均一性が高いと報告されている [26)-29)]。本研究の AFP 産生胃癌のなかでも、HER2 過剰発現群は混合型の割合が高く、形質多様性 (ヘテロジェナイティ) を示している。HER2 過剰発現を示す組織亜型領域 7 か所中 5 か所は AFP 陰性あるいは低発現であり、AFP 発現と HER2 過剰発現とは相互排他的傾向がみられた。これは、AFP 産生が免疫組織学的に確認される胃癌においては、HER2 が分子標的となる可能性が低いことを示唆している。

胃癌のガイドライン上は HER2 陽性､陰性で一次治療が規定されているが､以上の結果より、必ずしも有効性の高い治療法が選択できていない可能性がある。HER2 陰性胃癌で有効性が認められ [30)31)]､標準治療となっている免疫チェックポイント阻害剤の治療が奏効する可能性もあるが､HER2 陽性胃癌では 4 次治療以降の適応となり､予後不良の AFP 産生胃癌においてはその治療の機会は失われている。これについては、既報にて HER2 陽性の胃癌や食道胃接合部癌に対する一次治療としてのペムブロリズマブとトラスツズマブを併用した化学療法の有用性が示され、今後 HER2 陽性胃癌に対しても免疫チェックポイント阻害剤が適応となる可能性がでてきている [32)]。このような現状から、個々の症例に応じた治療選択が必要であり、個々の治療の方向性を見極めるためのバイオマーカーは今後ますます重要となると考える。

本研究の limitation としては、AFP 産生胃癌はまれな疾患の為、症例数の集積が困難で、単施設の解析には限界がある。また、本研究では化学療法のレジメン毎の生存解析や、HER2 過剰発現のある AFP 産生胃癌に対してトラスツズマブの治療を行っていない為､その有用性については検証できていない。HER2 過剰発現の AFP 産生胃癌における、トラスツズマブを含む化学療法の治療効果、予後についての関係性については、さらなる研究が必要である。

【結語】

AFP 産生胃癌の 17.1%に HER2 の過剰発現が見られるが、この割合は胃癌全体と比較しても変わらず、AFP 産生と HER2 の過剰発現との間に特別な関連性は認められなかった。HER2 過剰発現

AFP 産生胃癌は混合型の割合が高く、AFP 発現と HER2 発現の組織は排他的傾向を示していることから、HER2 が必ずしも分子標的とならないことが示唆された。その治療法としては、個々の組織に応じた感受性のある薬剤選択をすることが必要である。

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Table 1. Summary of the patients

Clinicopathological factors	Total N = 41
Sex	
Male	31 (75.6)
Female	10 (24.4)
Age (Year)	
Median［Range］	69［52-81］
52−59	8 (19.5)
60-69	14 (34.1)
70-79	17 (41.5)
80-81	2 (4.9)
Location	
Upper	15 (36.6)
Middle	16 (39.0)
Lower	10 (24.4)
Clinical Stage	
Stage I-III	16 (39.0)
Stage IV	25 (61.0)
Operation	
Non-operated	16 (39.0)
Operated	25 (61.0)
Chemotherapy	
Non-treated	24 (58.5)
Treated	17 (41.5)
Histologic subtype*	
Well differentiated type	16 (39.0)
Poorly differentiated type	6 (14.6)
Hepatoid adenocarcinoma	4(9.8)
Mixed type	15 (36.6)
Serum AFP level (ng/mL)	
Median［Range］	605［7.7-273000］
7.7-19.9 (normal)	3 (7.3)
20.0-99.9	10 (24.4)
100-499	3 (7.3)
500-999	8 (19.5)
1000-9999	12 (29.3)
10000-273000	5 (12.2)

*Well differentiated type(pap,tub1,tub2), Poorly differentiated type(por1, por2,sig), and Hepatoid adenocarcinoma classified by Japanese Classification of Gastric Carcinoma(15th edition)
Mixed type : 独自の分類によるmixed type

Table2. Clinicopathological summary of patients‘ with AFP-producing gastric cancer

症例No.	年齢	性別	血清AFP値 (ng/mL)	規約分類*	本研究での分類**	免疫組織化学		Stage	手術術式***	化学療法****	転帰
						AFP陽性率 (%)	HER2ｽｺｱ (FISH判定)				
1	71	M	91.4	pap	W	10	1	Ⅳ	TG		死亡
				tub1		10	0				
2	58	M	7.7	hep	H	10	0	Ⅳ	TG		死亡
3	52	F	535	tub2	M	5	0	Ⅲc	TG		死亡
				tub2		1	0				
				tub2		60	0				
				por1		80	3				
				hep		60	0				
4	71	F	40.8	hep	H	10	0	Ⅲa	TG	術前FLEP	死亡
5	76	M	369.8	pap	W	20	0	Ⅳ	DG		死亡
6	59	M	60.7	por1	M	5	0	Ⅳ	TG		死亡
				hep		30	3				
7	67	M	31110	tub2	W	80	0	Ⅳ	DG		生存
8	71	F	521.1	pap	W	10	2(-)	Ⅰb	TG		死亡
9	56	M	64.2	tub2	W	10	1	Ⅲa	TG	術前FLEP	死亡
10	62	M	1469	tub2	M	10	0	Ⅱa	DG		死亡
				hep		70	0				
11	67	M	681.7	pap	W	1	1	Ⅳ	なし	TAX＋CDDP	死亡
12	69	M	9.8	tub2	W	10	1	Ⅳ	なし	TAX＋CDDP＋5-FU	死亡
13	62	F	7771.1	por2	P	20	0	Ⅱ	TG	術前FLEP	生存
14	61	M	8425.2	por2	P	1	2(+)	Ⅳ	なし	FLEP	死亡
15	54	M	273000	tub1	W	1	1	Ⅳ	なし		死亡
16	56	M	2029.6	tub1	M	1	1	Ⅳ	なし		生存
				tub2		1	1				
				por1		0	1				
17	69	M	845.3	tub1	M	0	0	Ⅳ	TG		死亡
				hep		30	0				
18	80	F	1141	pap	W	1	1	Ⅳ	なし	TS-1	死亡
19	55	M	31800	tub2	W	1	1	Ⅳ	なし	TS-1	死亡
20	71	M	23390	tub1	M	0	1	Ⅳ	なし	FLEP	死亡
				por1		1	2(+)				
21	63	M	1980	pap	M	1	1	Ⅳ	なし		死亡
				tub1		0	1				
				por1		1	1				
22	78	F	3601	tub2	M	0	0	ⅢA	DG		死亡
				por1		80	0				
23	74	M	32.4	por2	P	1	0	ⅢA	DG		死亡
24	71	M	605	por2	P	1	0	Ⅳ	なし		死亡
				sig		0	0				
25	73	M	53.3	tub1	M	0	0	ⅢA	DG		生存
				tub1		70	0				
				por2		0	3				
26	81	M	1448	por2	P	1	0	Ⅳ	なし	TS-1	死亡
27	76	F	15.2	sig	M	1	0	ⅢC	TG	TS-1＋CDDP	生存
				pap		20	1				
28	71	F	595	tub1	W	1	1	Ⅳ	なし	TS-1＋DOC	死亡
29	61	F	33.8	tub2	M	5	3	Ⅳ	TG		死亡
				por1		1	3				
30	61	M	535	tub2	W	1	2(-)	Ⅳ	なし		死亡
31	63	M	3626	tub2	W	1	1	Ⅳ	なし	FLEP	死亡
32	70	M	29.3	ent	M	40	2(-)	ⅢB	TG		死亡
				tub1		0					
				tub2		0					
33	56	M	389.1	hep	H	10	1	ⅢB	TG		死亡
34	62	M	60.3	pap	M	20	0	ⅢB	DG	TS-1+CDDP	生存
				hep		100	0				
35	71	M	273	hep	H	20	0	Ⅳ	TG	5-FU+TXT	死亡
36	73	M	833	por1	P	1	1	Ⅳ	なし	TS-1＋TXT	死亡
37	71	F	1350	pap	M	1	0	ⅢB	TG		死亡
				tub2		0	0				
				hep		30	0				
38	74	M	39.5	pap	W	30	1	Ⅰb	DG		生存
				tub1		0	0				
39	70	M	24940	pap	M	1	3	Ⅳ	DG		死亡
				tub1		0	0				
				hep		50	0				
				por1		0	0				
40	69	M	5035	tub2	W	1	1	Ⅳ	なし	FLEP	死亡
41	65	M	1954	pap	W	10	0	ⅡA	DG		死亡
				tub2		20	0				

*pap: papillary adenocarcinoma, tub1: tubular adenocarcinoma well differentiated, tub2: tubular adenocarcinoma moderately differentiated, por1: poorly differentiated adenocarcinoma solid type, por2: poorly adenocarcinoma non-solid type, sig: signet-ring cell carcinoma, hep: hepatoid adenocarcinoma, ent: adenocarcinoma with enteroblastic differentiation

**W:Well differentiated type(pap,tub1,tub2), P:Poorly differentiated type(por1, por2,sig), H: hepatoid adenocarcinoma, M:独自の分類によるmixed type

***TG: Total gastrectomy, DG: Distal gastrectomy

**** FLEP: 5-FU+LV+VP-16+CDDP, 5-FU: fluorouracil, LV: leucovorin, VP-16: etoposide, CDDP: cisplatin, TXT: docetaxel hydrate, TS-1: tegafur・gimeracil・oteracil potassium

Table 3. HER2 status assessed by IHC and FISH

HER2 status	Number	%
Positive		
IHC 3＋	5	12.2
IHC 2＋ and FISH positive	2	4.9
Total	7	17.1
Negative		
IHC 0, 1＋	31	75.6
IHC 2＋ and FISH negative	3	7.3
Total	34	82.9

Table 4. Correlation between HER2 status and clinicopathological features in AFP-GC

Clinicopathological factors	Total	HER2 status		P value
		Overexpression	Non-overexpression	
	N = 41	N = 7 (17.1%)	N = 34 (82.9%)	
Sex				
Male	31	5 (71.4)	26 (76.5)	0.56
Female	10	2 (28.6)	8 (23.5)	
Age (Year)				
<70	22	4 (57.1)	18 (52.9)	0.59
≥70	19	3 (42.9)	16 (47.1)	
Location				
Upper	15	3 (42.9)	12 (35.3)	0.51
Middle	16	4 (57.1)	12 (35.3)	
Lower	10	0 (0.0)	10 (29.4)	
Clinical Stage				
Stage I-III	16	3 (42.9)	13 (38.2)	0.57
Stage IV	25	4 (57.1)	21 (61.8)	
Operation				
Non-operated	16	2 (28.6)	14 (41.2)	0.43
Operated	25	5 (71.4)	20 (58.8)	
Chemotherapy				
Non-treated	24	5 (71.4)	19 (55.9)	0.37
Treated	17	2 (28.6)	15 (44.1)	
Histologic subtype†				
Well differentiated type	16	0 (0.0)	16 (47.0)	0.03*
Poorly differentiated type	6	1 (14.3)	5 (14.7)	
Hepatoid adenocarcinoma	4	0 (0.0)	4 (11.8)	
Mixed type	15	6(85.7)	9(26.5)	
Serum AFP level (ng/mL)				
<605	20	4 (57.1)	16 (47.1)	0.47
≥605	21	3 (42.9)	18 (52.9)	

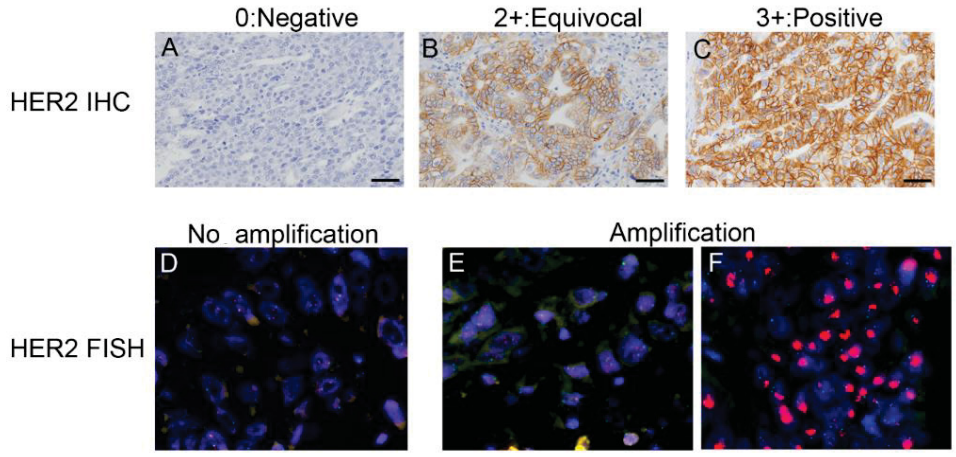
* $p<0.05$.Fisher's exact test

Table 5. Immunohistochemical estimation of AFP on each subtype of histology

Histology component	number	AFP positive	AFP negative	(%)
Well differentiated type				
pap	12	7	5	58.3
tub1	12	2	10	16.7
tub2	17	7	10	41.2
Poorly differentiated type				
sig	2	0	2	0.0
por2	6	1	5	16.7
por1	9	7	2	77.8
Specific type				
ent	1	1	0	100.0
hep	11	11	0	100.0

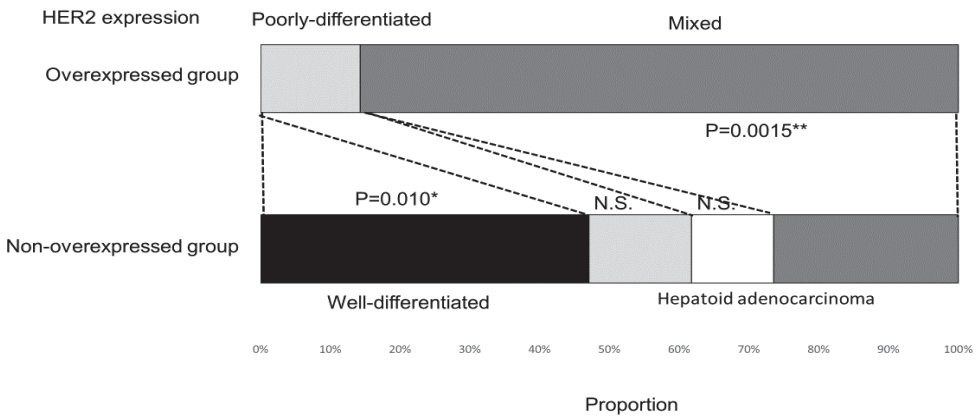
*pap: papillary adenocarcinoma, tub1: tubular adenocarcinoma well differentiated, tub2: tubular adenocarcinoma moderately differentiated, por1: poorly differentiated adenocarcinoma solid type, por2: poorly adenocarcinoma non-solid type, sig: signet-ring cell carcinoma,
hep: hepatoid adenocarcinoma, ent: adenocarcinoma with enteroblastic differentiation

Fig. 1 HER2 status by IHC and FISH



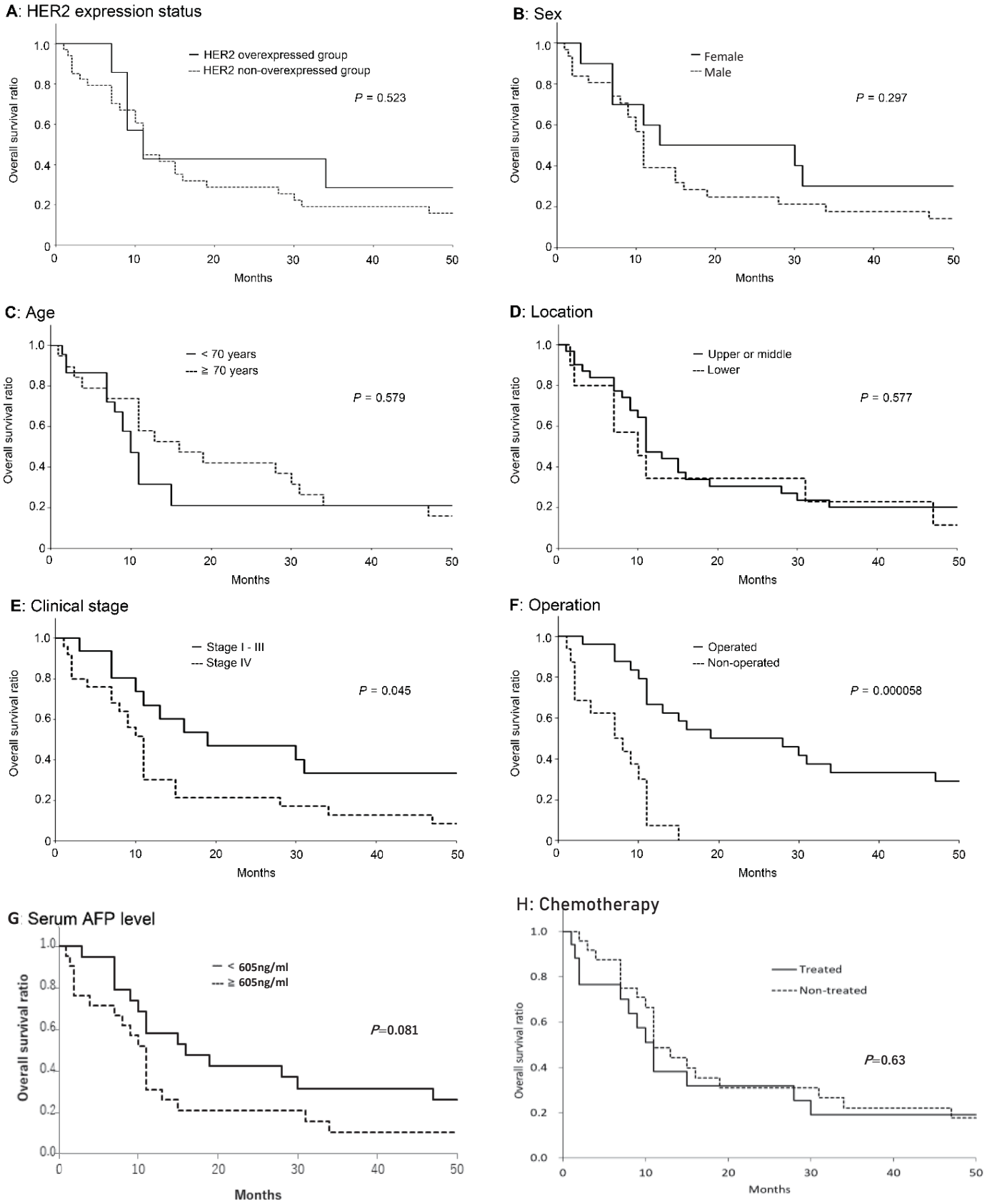
(A) Negative expression (0), (B) equivocal expression (2+), and (C) positive expression (3+) by immunohistochemistry. Each bar shows 50μm. Equivocal samples need additional FISH analysis to determine whether their HER2 DNA was no amplification (D) or amplification (E, F). The green signals show CEP17 and the red signals show HER2. The HER2 DNA amplification was determined when the signal counts of HER2/CEP17 were ≥2.0 in 20 tumor cells.

Fig. 2 The proportion of histologic subtype in the HER2 overexpressed and the HER2 non-overexpressed AFP-GC groups



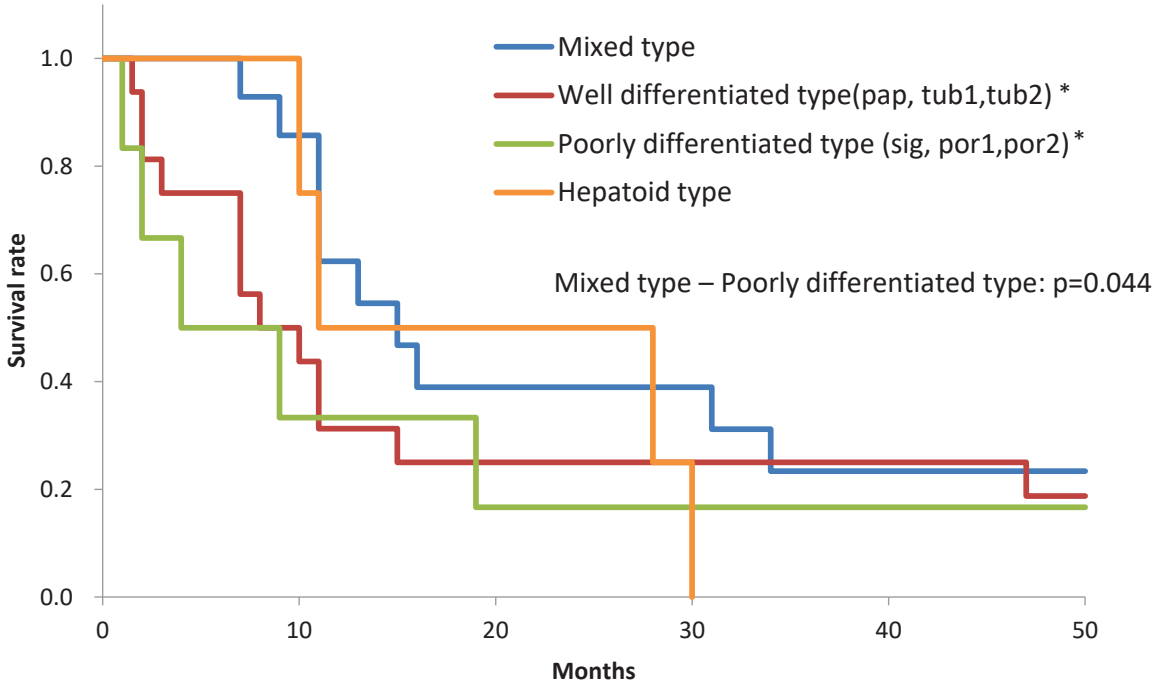
** P < 0.01, * P < 0.05, by residual analysis

Fig. 3 Overall survival of AFP-GC



Overall survival of HER2 overexpressed and non-overexpressed groups (A), female and male (B), <70 years and ≥70years (C), lower and upper/middle of tumor location (D), clinical stage I – III and stage IV (E), operated and non-operated (F), <605ng/ml and ≥ng/ml of serum AFP level using a median split (G), and treated with chemotherapy and non-treated (H), analyzed using the Kaplan–Meier methods (log-rank), are shown.

Fig. 4 Overall survival of AFP-GC

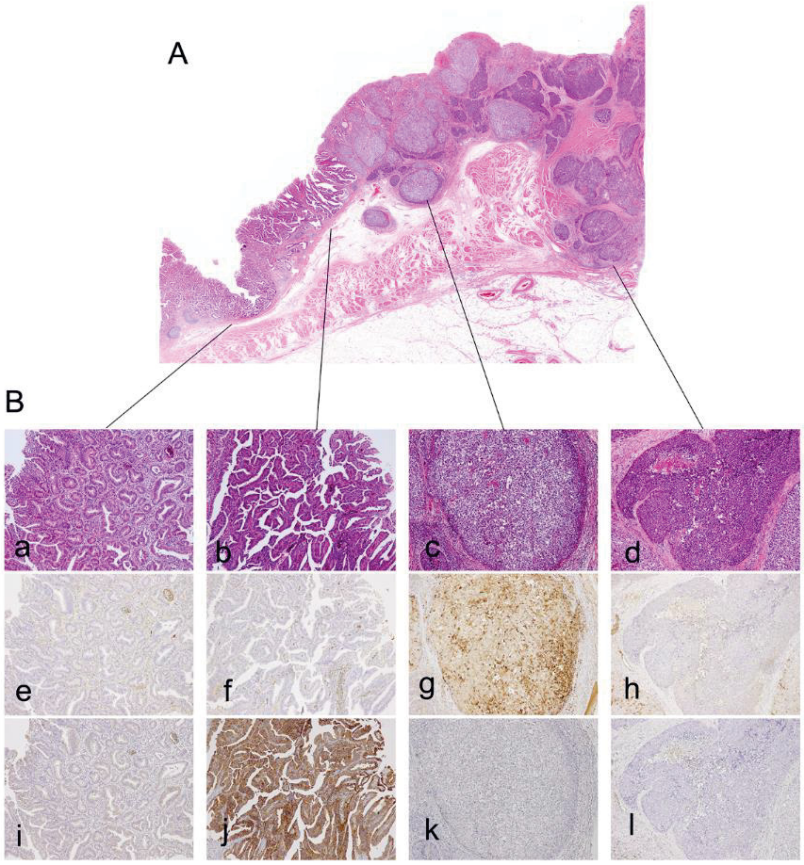


Mixed type , Well differentiated type(pap, tub1,tub2), Poorly differentiated type(sig,por1,por2), and Hepatoid type of histology, analyzed using the Kaplan–Meier methods (log-rank), are shown.

Mixed type classified by our own classification, and Common type and Hepatoid type classified Japanese Classification of Gastric Carcinoma(15th edition)

*pap: papillary adenocarcinoma, tub: tubular adenocarcinoma, sig: signet-ring cell carcinoma, por: poorly differentiated adenocarcinoma

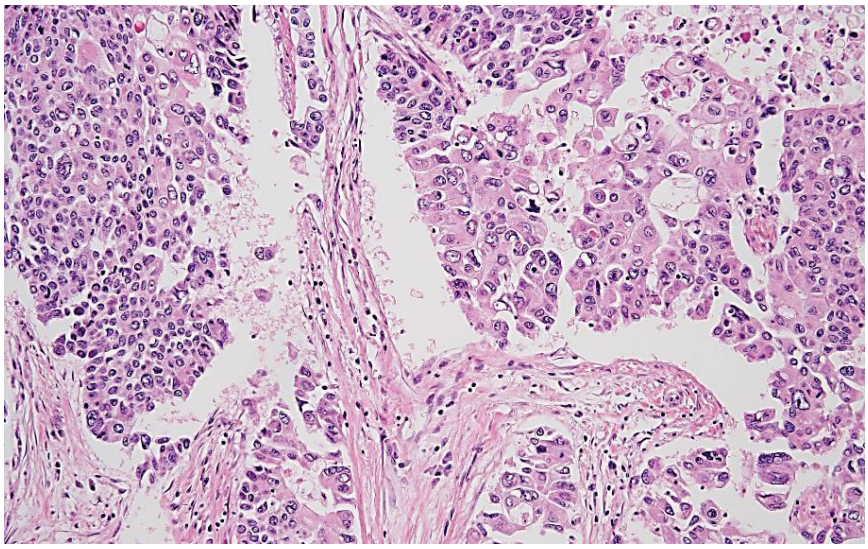
Fig. 5 Histopathological findings of a representative case of mixed type of histology



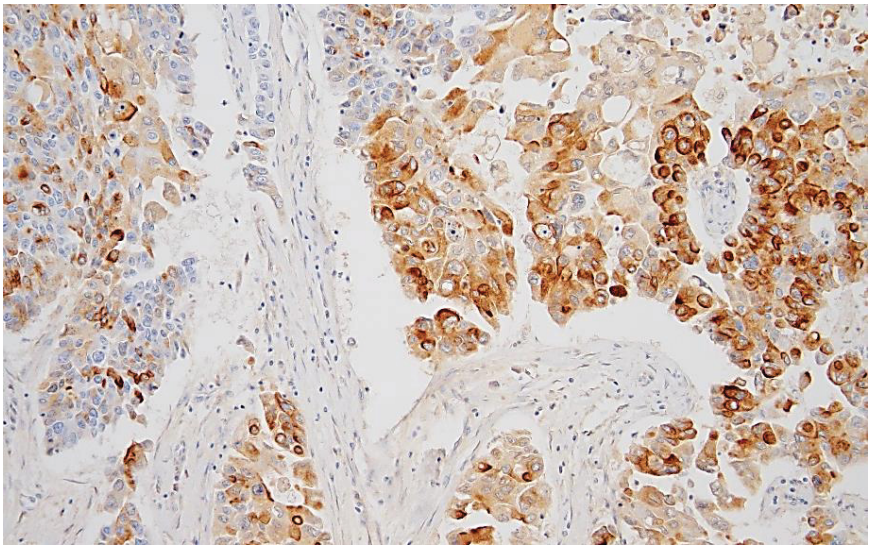
Whole H&E stained tissue section (A) and detailed structures (B). This gastric tumor consists of heterogeneous cancer cells with different structural features: tubular (a, e, i), papillary (b, f, j), hepatoid (c, g, k), and solid (d, h, l) patterns. The tumor was diagnosed as a mixed type when both intestinal and diffuse types were detected. Each component showed a different immunohistochemical phenotype for AFP (e, f, g, h) and HER2 (i, j, k, l).

Fig.6 Histopathological findings of a case of co-expression of AFP and HER2 (case3, ×200)

H＆E



AFP



HER2