Original paper

Clinicopathologic study of 10 cases of gastric adenocarcinoma with hepatoid or enteroblastic differentiation

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Gastric adenocarcinoma with hepatoid or enteroblastic differentiation (GAHED), known also as AFP-producing carcinoma, is a rare neoplasm. Ten cases with GAHED and 209 cases without GAHED were selected. Clinicopathological features of GAHED were investigated. The disease-free survival (DFS) of the GAHED group was compared with that of the non-GAHED group. Grossly, the tumours consisted of two early types and eight advanced types. Histologically, all tumours were composed of various proportions of tubular, cribriform, papillary, solid, and/or trabecular growth patterns of clear to slightly eosinophilic tumour cells. Hyaline globules were observed in all tumours. AFP and Hep-Par1 were immunoreactive in all tumours. In fluorescence *in situ* hybridisation of *HER2* gene/chromosome 17, the amplification of *HER2* gene was observed in two cases that showed positive reaction for HER2 protein. Clinical follow-up was available in nine cases. Regarding the clinical outcome, 3 and 6 patients were alive without disease and alive with disease, respectively. In a statistical analysis, the DFS of the GAHED group was significantly worse than that of the non-GAHED group.

GAHED is morphologically characterised by various growth patterns of clear to slightly eosinophilic tumour cells and intracytoplasmic possession of hyaline globules. This tumour may have the potential to behave in an aggressive clinical fashion.

Key words: stomach, hepatoid, enteroblastic, clinicopathological study, differentiation, adenocarcinoma.

Introduction

Gastric hepatoid adenocarcinoma was reported for the first time by Ishikura *et al*. [1, 2, 3, 4]. Subsequently, Matsunoh *et al.* proposed the gastric carcinoma with enteroblastic differentiation [5]. Nowadays, AFP-producing gastric adenocarcinoma is histologically classified into three categories, namely yolk sac, hepatoid, and enteroblastic types [6]. Among them, enteroblastic type seems to be associated with hepatoid type in some tumours. Gastric adenocarcinoma with hepatoid or enteroblastic differentiation (GAHED) is a rare neoplasm, and reported cases have been limited to date. In this article, we performed a clinicopathological study of 10 cases with GAHED.

Material and methods

This study was approved by the ethical committee of Kochi Red Cross Hospital (No.238). Two hundred and nineteen cases were selected from pathology files of surgically resected gastric cancers by partial or total

Table I. Clinical summary of gastric adenocarcinoma with hepatoid or enteroblastic differentiation (GAHED)

Case	Age	Sex	Symptom or sign	cStage	Therapy	Follow-up	Outcome (metastasis)
1	66	M	Vomiting blood	IIA	Residual gastrectomy	37 months	AWOD
2	82	M	Anaemia	IB	Distal gastrectomy	60 months	AWD (lung)
3	81	F	Incidentally found	IB	Distal gastrectomy	Lost	–
4	63	M	Incidental found	IA	Distal gastrectomy	44 months	AWOD
5	52	M	Epigastralgia	IIIA	Distal gastrectomy	56 months	AWD (lung, LN)
6	84	M	Anaemia	IV	Total gastrectomy	1 month	AWD (liver, peritoneum)
7	73	M	Tarry stool	IIIA	Distal gastrectomy	23 months	AWD (liver, peritoneum, LN)
8	64	M	Incidentally found	IIIB	Distal gastrectomy	50 months	AWOD
9	64	M	Dysphagia	IV	Total gastrectomy, CT	16 months	AWD (liver, lung, bone)
10	66	M	Anaemia	IIIB	Total gastrectomy	5 months	AWD (liver, peritoneum)

M – male; F – female; AWOD – alive without disease; AWD – alive with disease; LN – lymph node

Table II. Gross features of GAHED

Case	Location	Gross type	Tumour size
1	B-40-A (residual)	Type 3	50×30 mm
2	M, Post	Type 0-I	40 × 21 mm
3	L, Post	Type 0-III	20 × 12 mm
4	L, Gre	Type 1	54 × 20 mm
5	M, Less	Type 2	34 × 32 mm
6	M, Ant	Type 3	70 × 58 mm
7	L, Ant	Type 3	28 × 23 mm
8	MU, Ant∼Less	Type 5	85 × 76 mm
9	U, Less	Type 3	42 × 38 mm
10	MU, Post	Type 1	106 × 70 mm

U – upper; M, – middle; L – lower; Ant – anterior wall; Post – posterior wall; Less – lesser omentum; Gre – greater omentum; B – benign; A – anastomosing site; 40 – 40 years after the first operation

Gross type was determined according to the classification in the text

gastrectomy from January 2011 to December 2015 in my hospital. The patients consisted of 10 cases with GAHED and 209 cases without GAHED. Surgically resected gastric tissues for light microscopy were fixed in 10% buffered formalin, processed by conventional methods, and stained with haematoxylin and eosin stain and periodic acid-Schiff stain with diastase pretreatment (d-PAS) for the identification of hyaline globules. Immunohistochemistry was performed using an Ventana Benchmark Ultra autostainer (Roche). Antibodies against AFP (C3, 1 : 50, Novocastra Laboratories Ltd, Newcastle, UK), Hep Par1(Hepatocyte) (OCHI1E5, 1 : 200, DAKO, Glostrup, Denmark), Glypican-3 (IG12, pre-diluted, Nichirei, Tokyo, Japan) and SALL4 (6E3, prediluted, Roche, Tokyo, Japan), D2-40 (D2-40, 1 : 50, DAKO, Glostrup, Denmark), HER2 (4B5, pre-diluted, Roche, Tokyo, Japan) were employed in the present study. EBER *in situ* hybridisation (Roche, Tokyo, Japan) was performed in all tumours. Appropriate positive control specimens were used as positive controls for immunohistochemistry and EBER *in situ* hybridisation. Clinicopathological features (sex, gross findings, lymphatic invasion, and venous invasion) of the GAHED and non-GAHED groups were investigated and compared with each other, based on pathologic reports and clinical information from electronic medical reports. The gross findings were subdivided into five categories, namely Type 0 (superficial), 1 (protruding), 2 (ulcerative and localised), 3 (ulcerative and infiltrating type), 4 (diffusely infiltrating), and 5 (unclassified). Type 0 (superficial) was divided into three categories, namely 0-I (superficial and protruding), 0-II (superficial and flat), and 0-III (superficial and encavated). The frequency of metastasis to lymph node, liver, lung, peritoneum, and bone was compared between both groups within the time from initial diagnosis to end of follow-up. The disease-free survival (DFS) of the GAHED group was also compared with that of the non-GAHED group using Kaplan-Meier method and the long-rank test. All p values were two sided and $p < 0.05$ was

Table III. Histological features of GAHED

Case	Ratio of GAHED	GAHED morphology	HG	Other histological types	pStage
1	10%	Tubular, cribriform, solid, papillary	+	tub2 90%	IIIA
2	60%	Papillary, tubular	+	tub1 40%	IB
3	80%	Tubular, cribriform, papillary	+	tub1 20%	IB
4	10%	Tubular, cribriform, papillary	+	tub1 90%	IB
5	20%	Tubular, cribriform, trabecular, papillary	+	tub2 80%	IIIB
6	20%	Tubular, cribriform, papillary	+	tub2 70%, por1-2 8%, small 2%	IV
7	80%	Tubular, papillary, solid	+	tub1 20%	IIIC
8	50%	Tubular, cribriform, solid, papillary, trabecular	+	tub2 30%, por1-2 20%	IIIA
9	20%	Solid, tubular, cribriform, papillary	+	tub2 80%	IV
10	100%	Solid, trabecular, cribriform, tubular, giant cells	+	0%	IV

HG – hyaline globules; tub1 – tubular adenocarcinoma, well differentiated; tub2 – tubular adenocarcinoma, moderately differentiated; por1 – poorly differentiated adenocarcinoma, solid type; por2 – poorly differentiated adenocarcinoma, non-solid type; small – small cell carcinoma; (+) – present

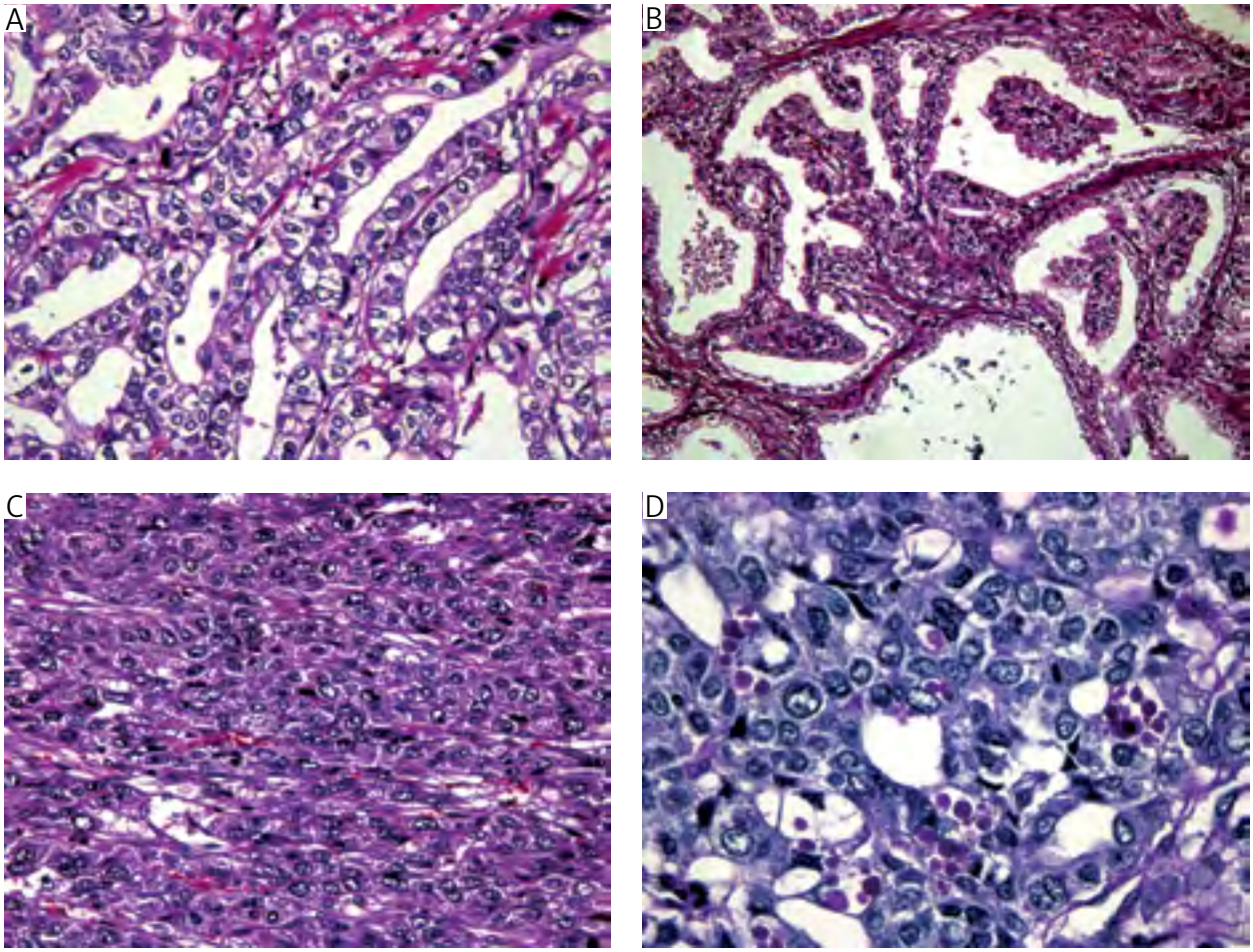

Fig. 1. Histological pattern of gastric adenocarcinoma with hepatoid or enteroblastic differentiation (GAHED). A) Tubular pattern. B) Papillary pattern. C) Trabecular pattern suggesting hepatoid differentiation. D) Hyaline globules are observed in the cytoplasm of tumour cells (periodic acid-Schiff stain with diastase pretreatment)

Table IV. Immunohistochemical findings of GAHED

Case	AFP	Hep Par1	Glypican-3	SALL4	HER2	EBER
1	f+	f+	–	f+	1+	–
2	f+	d+	–	–	0	–
3	f+	f+	–	f+	3+	–
4	f+	f+	–	f+	2+	–
5	f+	f+	–	f+	0	–
6	f+	d+	–	f+	3+	–
7	f+	f+	–	f+	3+	–
8	f+	f+	f+	f+	1+	–
9	f+	f+	–	–	0	–
10	d+	d+	d+	d+	0	–

f – focal; d – diffuse; (+) – positive; (–) – negative

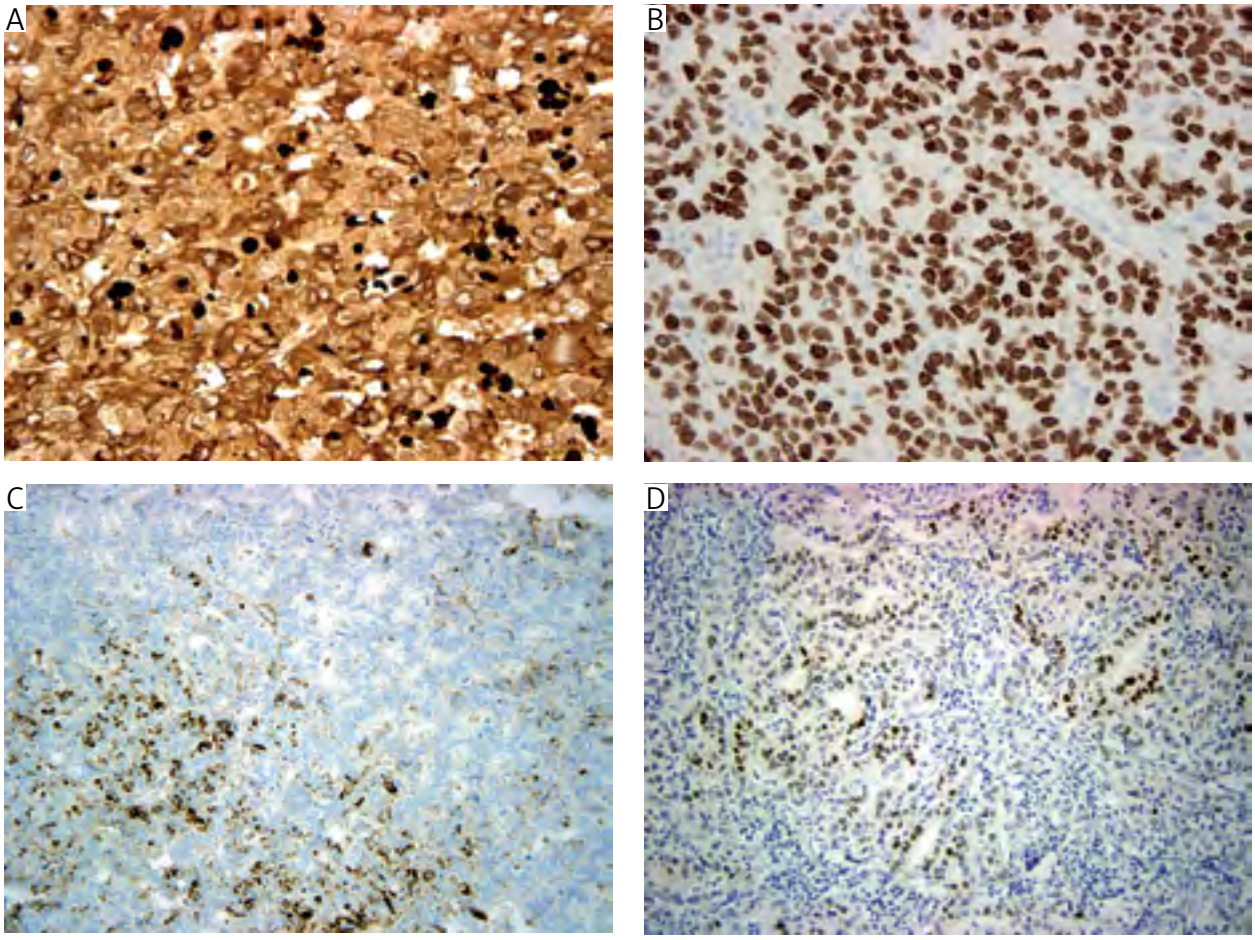

Fig. 2. Immunohistochemical results of GAHED. A) AFP is diffusely immunoreactive in hepatoid carcinoma (No. 10). B) Neoplastic cells diffusely express SALL4 in hepatoid carcinoma. C) Tumour cells show the focal reactivity for AFP in enteroblastic carcinoma. D) SALL4 is focally positive in enteroblastic carcinoma

considered to be significant. The statistical analysis was performed with BellCurve (Tokyo, Japan). Fluorescence *in situ* hybridisation (FISH) of the *HER2* gene was performed according to the manufacturer's protocol in two cases. Pretreatment was carried out using a VP-2000 Processor (Abbott Molecular, Tokyo, Japan), and hybridisation was performed using ThermoBrite (Abbott Molecular Tokyo, Japan). FISH signals were observed using an Axio Imager 2 Upright Microscope (Zeiss, Tokyo, Japan) and analysed using MetaCyte Lite (MetaSystems, Altlussheim, Germany) with Isis (Zeiss). When the ratio of HER2/CEP17 was more than 2.0, we considered it to be positive for *HER2* gene amplification.

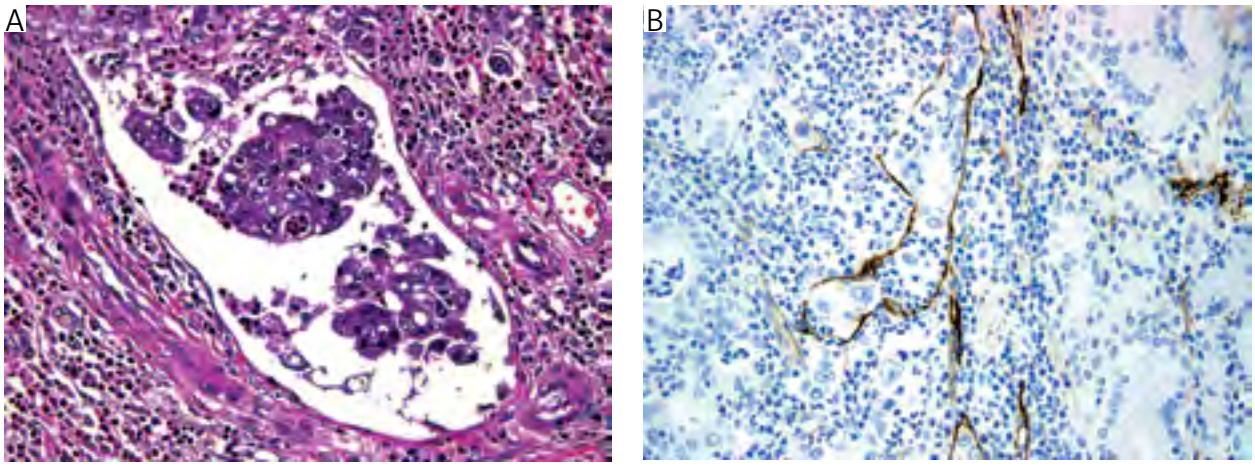

Fig. 3. A) Lymphatic invasion of GAHED. B) D2-40 immunohistochemistry highlights lymphatic invasion of carcinoma cells

Results

The clinical features of 10 cases with GAHED are summarised in Table I. The patients consisted of nine males and one female. The age of patients ranged from 52 to 82 years with a mean age of 69.5 years. The macroscopic characteristics of the 10 cases with GAHED are shown in Table II. Grossly, the tumours consisted of two early types and eight advanced types. The tumour size varied from 28 mm to 106 mm with a mean size of 52.9 mm. The microscopic features of 10 cases with GAHED are demonstrated in Table III. Histologically, all tumours were composed of various proportions of tubular (Fig. 1A), cribriform, papillary (Fig. 1B), solid, and/or trabecular (Fig. 1C) growth patterns of clear to slightly eosinophilic tumour cells. Evident hepatoid differentiation was characterised by trabecular architecture in one case (No. 10). In this tumour, giant cells were also observed. The rate of hepatoid or enteroblastic differentiation in the entire neoplastic volume ranged from 10 to 100% with a mean rate of 45%. Hyaline globules were identified in all tumours and highlighted by d-PAS stain (Fig. 1D). A summary of the immunohistochemical results is shown in Table IV. Immunohistochemically, AFP and Hep-Par1 were positive in all tumours. Glypican-3 and SALL4 were positive in 2 and 8 tumours, respectively. Interestingly, evident hepatoid carcinoma (No. 10) showed diffuse positivity for AFP (Fig. 2A), Hep Par1, Glypican-3, and SALL4 (Fig. 2B). By contrast, enteroblastic carcinoma usually showed focal positivity for AFP (Fig. 2C), Hep Par1, and SALL4 (Fig. 2D). No tumours expressed EBER. The comparison of lymphovascular invasion between both groups in the histological evaluation is shown in Table V. Lymphatic invasion more frequently occurred in the GAHED group than in the non-GAHED group (Fig. 3A), but there was statistically no significant difference of sex ratio, gross findings, and vascular invasion between both groups. Lymphatic invasion of carcinoma cells was highlighted by D2-40 immunostaining (Fig. 3B). Comparison of the metastasis to various anatomic sites is also summarised in Table V. The metastasis to lymph node, liver, or lung was more frequent in the GAHED group than in the non-GAHED group, but the metastasis to peritoneum or bone was not significantly different between both groups. Metastasis to lymph node exceeding 3 cm in maximum diameter was identified in three cases. The clinical follow-up was available in nine cases with GAHED and 189 cases with non-GAHED. The follow-up duration of the GAHED group ranged from 1 to 60 months with a mean duration of 32.4 months. Regarding the clinical outcome, 3 and 6 patients

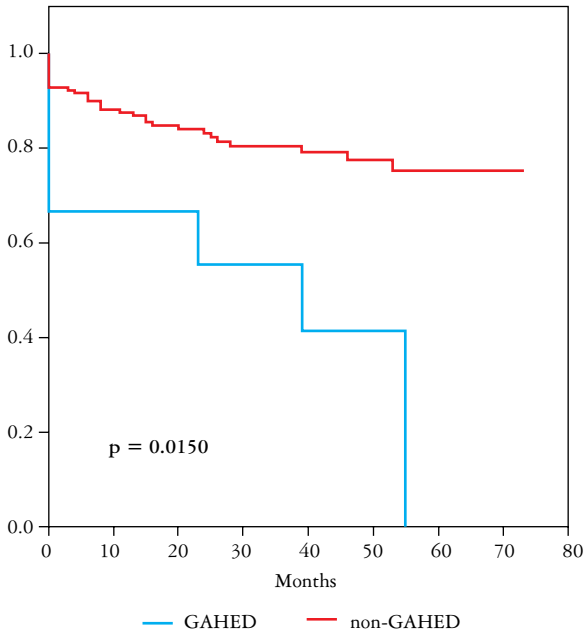

Fig. 4. Comparison of disease-free survival (DFS) between GAHED and non-GAHED groups. DFS of the GAHED group is significantly worse than that of the non-GAHED group

Table V. Comparison of clinicopathological parameters between GAHED and non-GAHED groups

Parameter	GAHED	non-GAHED	p-value
Sex			
Male	9	144	
Female	1	65	0.02885
Gross findings			
Early	3	85	
Advanced	7	124	0.7433
Lymphatic invasion			
Present	10	125	
Absent	0	84	0.0079*
Vascular invasion			
Present	6	73	
Absent	4	136	0.174
Metastatic site			
Lymph node metastasis			
Present	9	87	
Absent	1	122	0.0057*
Liver metastasis			
Present	4	12	
Absent	6	197	0.0031*
Lung metastasis			
Present	3	3	
Absent	7	206	0.0013*
Peritoneal metastasis			
Present	3	20	
Absent	7	189	0.0746
Bone metastasis			
Present	1	1	
Absent	9	208	0.0894

**statistically significant*

were alive without disease and alive with disease, respectively (Table I). The comparison of DFS in both groups is shown in Fig. 4. In a statistical analysis, the DFS of the GAHED group was significantly worse than that of the non-GAHED group. HER2 overexpression (3+) was identified in three tumours (Fig. 5A). A FSIH study of *HER2* gene /chromosome 17 was performed in two cases (No. 6 and 7). The ratio for each case was 2.9 and 9.5, respectively, in 370 and 414 counting cells. Both cases were considered to be positive for *HER2* gene amplification (Fig. 5B).

Discussion

Grossly, GAHED seems to be distinguished from usual gastric adenocarcinoma, based on the results of the present study. GAHED is morphologically characterised by various proportions of tubular, cribriform, papillary, solid, and/or trabecular growth patterns of clear to slightly eosinophilic tumour cells and intracytoplasmic possession of hyaline globules. Motoyama *et al*. divided AFP-producing gastric cancer into three categories, namely hepatoid, yolk sac tumour-like, and foetal gastrointestinal types [6]. However, it seems to be very difficult to clearly distinguish hepatoid differentiation from enteroblastic differentiation because both tumours have a capacity to produce AFP and some tumours admixed with each other have been describe previously. We confirmed no tumours with yolk sac tumour-like differentiation in the present tumour. Therefore, we unified these differentiations into GAHED. The elevation of the serum AFP may become a vital clue in pathological diagnosis [1, 2]. However, we found

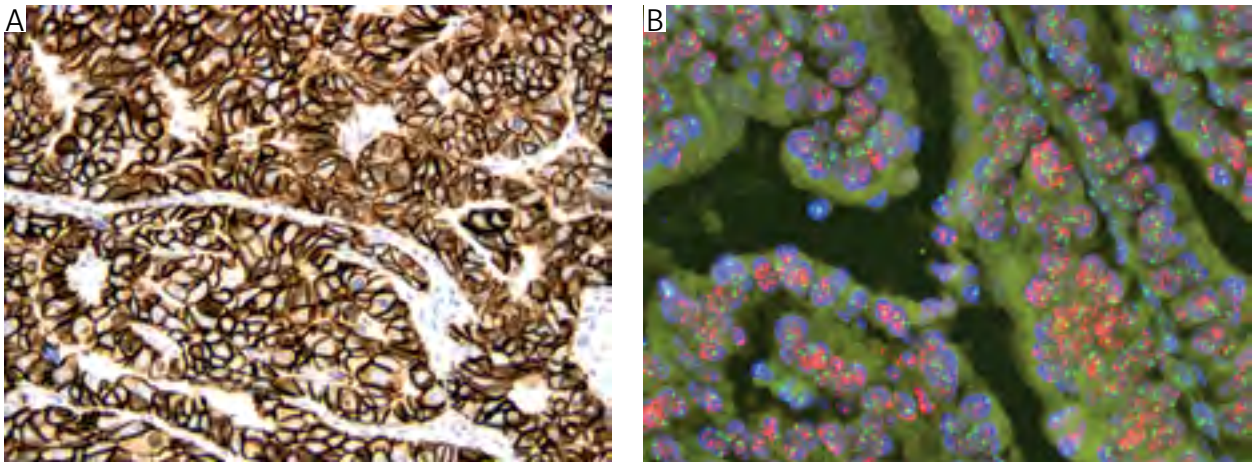

Fig. 5. A) HER2 immunohistochemistry shows the positivity with 3+ score intensity. B) Fluorescence *in situ* hybridisation of *HER2* gene (red)/chromosome 17 (green). The amplification of *HER2* gene is observed

in the present study that clinicians did not measure serum AFP value in most cases. Hence, pathologists should bear in mind that the abovementioned histological features can lead to the pathological diagnosis of GAHED. It is likely that hyaline globules may be an important diagnostic clue. Whenever pathologists encounter cases suspicious for GAHED, they should try the immunostaining of AFP, Hep Par1, Glypican-3, and/or SALL4 to confirm a definite diagnosis [7, 8, 9]. This tumour seems to be associated with intestinal phenotype [1, 4, 10]. Based on the present study results, we particularly recommend the combined immunohistochemical panel of SALL4 as well as AFP and Hep Par1 for the confirmation of GAHED diagnosis because Hep Par1 usually expresses in the intestinal phenotype irrespective of hepatoid or enteroblastic differentiation, and Glypican-3 is low sensitivity (20%) of GAHED diagnosis.

In the present study, the GAHED group had a greater propensity for lymphatic invasion than the non-GAHED group, but there was no significant difference in vascular invasion between both groups. The result of lymphatic invasion is consistent with the previous report [11]. By contrast, the result of vascular invasion is not compatible with the previous result [3, 11].

Previously, the propensity to metastasise to lymph nodes and liver has been frequently described [2, 7, 11, 12]. The frequency of lung metastasis in GAHED accounts for 3.4% and is relatively low [7]. However, this is the first report showing the propensity of metastasis to lung in the GAHED group, compared with the non-GAHED group.

This tumour may have the potential to behave in an aggressive clinical fashion [2, 3, 4, 7, 8, 13, 14, 15]. The results of disease-free survival of GAHED in the present study support the previous descriptions. Motoyama *et al*. and Hwang *et al.* reported a case of gastric adenocarcinoma with choriocarcinomatous and hepatoid differentiation [6, 16]. They suggest that choriocarcinoma or hepatoid carcinoma may show the retrodifferentiation related to the unfavourable pathological differentiation [16]. Gastric neuroendocrine carcinoma also has a propensity to behave in an aggressive clinical fashion [17]. Therefore, neuroendocrine differentiation may be one of retrodifferentiation. However, there was no case with neuroendocrine differentiation in the present study.

To the best of our knowledge, there were no descriptions of amplification of the *HER2* gene in GAHED. Therefore, this is the first report on this topic. This means that GAHED with advanced stage may be effective for molecular targeted therapy for *HER2* gene. From a practical point of view, this result could supply the available information to digestive tract clinicians and patients suffering from GAHED. However, the number of patients in the present study is too small to come to a definite conclusion. Further examination in a large-scale study is required in order to elucidate the utility of molecular targeted therapy for GAHED.

In conclusion, GAHED is histologically characterised by various proportions of tubular, cribriform, papillary, solid, and/or trabecular growth patterns and intracytoplasmic possession of hyaline globules. Immunohistochemical identification of SALL4 as well as AFP and Hep Par1 is useful for the definite diagnosis of GAHED. The tumour of GAHED has a propensity of lymphatic invasion and metastasis to lymph nodes, liver, and lung, compared with the non-GAHED group. Therefore, this tumour usually behaves in an aggressive clinical fashion.

The authors declare no conflicts of interests.

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