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Gastric adenocarcinoma with enteroblastic differentiation should be differentiated from hepatoid adenocarcinoma: A study with emphasis on clear cells and clinicopathologic spectrum

Mi Jung Kwon[a], Sunju Byeon[b], So Young Kang[b], Kyoung-Mee Kim[b,*]

[a] Department of Pathology, Hallym University Sacred Heart Hospital, Anyang, Republic of Korea
[b] Department of Pathology and Translational Genomics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

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ABSTRACT

Background: In gastric cancer, clear cells are preferentially found in gastric adenocarcinoma with enteroblastic differentiation (GAED) and hepatoid adenocarcinoma (HAC). The distinction between GAED and HAC is difficult because of their rarity and histologic overlap.

Methods: To elucidate identification of gastric adenocarcinoma with clear cells as GAED or HAC, survival analyses were performed in 28 GAED, 26 HAC, and 1107 conventional adenocarcinoma cases. Cells of origin were assessed by investigating the expression of oncofetal proteins (α-FP, glypican-3, SALL4), in addition to gastric (MUC5AC, MUC6), and intestinal (MUC2, CD10, CDX-2) cell markers.

Results: Clinically, HAC showed frequent (57.5%) distant metastasis (mostly in the liver) at the time of diagnosis compared to GAED ($P < 0.001$). On pathology, all 28 GAED had a predominantly tubulopapillary growth pattern while 24 HAC displayed a predominantly hepatoid growth pattern. In survival analyses, patients with HAC had significantly shorter overall and recurrence-free survival (mean: 25 months, and 53 months, respectively) compared to those with GAED (mean: 107 months, and 118 months, respectively) ($P < 0.001$).

HAC with clear cells showed diffuse and strong expression of all oncofetal proteins (α-FP, glypican-3, and SALL4), were highly positive for CDX-2, and were negative for CD10, MUC6, MUC5AC, and MUC2, suggesting an intestinal mucin phenotype and hepatoid features. In contrast, GAED showed focal expression of one or two oncofetal proteins and commonly expressed CD10, CDX-2, and MUC6 but not MUC2 and MUC5AC, suggesting both gastric antral/intestinal mucin phenotype and focal enteroblastic differentiation. SALL4 was diffusely and strongly positive in HAC, while it was heterogeneously expressed in GAED.

Conclusions: In conclusion, although rare, HAC with clear cells should be differentiated from GAED based on the poor prognosis, diffuse and strong oncofetal protein expression, and intestinal mucin phenotype.

1. Introduction

Primary gastric adenocarcinoma with clear cells is rarely encountered in the stomach [5,6]. Gastric adenocarcinoma with enteroblastic differentiation (GAED; also known as clear cell carcinoma or pylorocardiac carcinoma), hepatoid adenocarcinoma (HAC), and papillary adenocarcinoma are representative histologic subtypes of gastric adenocarcinoma with clear cells [2,16,18,20,21]. Unlike papillary adenocarcinoma showing easily recognizable and well-formed papillae, differential diagnosis between GAED and HAC is challenging for many pathologists. Although a high level of serum α-fetoprotein (α-FP) and expression of oncofetal proteins such as α-FP, glypican-3, and SALL4 have been used to diagnose HAC [9,21], oncofetal proteins are also variably expressed in GAED [3,5,16,21].

GAED is a rare variant of well-differentiated gastric adenocarcinoma and usually arises either in the cardia or the pylorus/antrum of the stomach, and accounts for 2.24% of gastric cancers [5,6,12]. Histologically, GAED or clear cell carcinoma of the stomach is a variant of well-differentiated gastric adenocarcinoma exhibiting a tubulopapillary growth pattern with predominantly clear cells and luminal eosinophilic secretions [4–6,12,17,20]. In GAED, solid areas are composed of large cells with hyperchromatic nuclei and abundant clear cytoplasm with intracytoplasmic PAS-positive droplets, which closely resemble clear hepatoid areas [6,15,20]. HAC is also an uncommon entity of primary

* Corresponding author at: Department of Pathology and Translational Genomics, Samsung Medical Center, Sungkyunkwan University School of Medicine, 50 Ilwon-dong, Gangnam-gu, Seoul, 135-710, Republic of Korea.
E-mail address: kkmkys@skku.edu (K.-M. Kim).

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gastric adenocarcinoma harboring distinct hepatoid area mimicking hepatocellular carcinoma, frequently associated with elevated serum α-FP, and accounts for 0.17%–0.9% of gastric adenocarcinoma [3,17]. Since its introduction in 1985 [14] HAC is defined by the minimum criteria of distinct hepatoid area regardless of its proportion within the tumor mass or AFP production in WHO classification [9,10,13]. HAC is characterized by hepatoid features arranged in a trabecular or solid nested growth pattern with large polygonal hepatocyte-like neoplastic cells [11,14,21]. HACs are not totally composed of eosinophilic cytoplasm and often contain varying degrees of clear cells mimicking fetal gut epithelium [1,7,8,10,13,16,20]. However, lack of sinusoid-like vascular channel in solid pattern of GAED is the distinguishable characteristic from HAC with clear cells [12]. Although these tumors are generally distinguishable, they display a substantial degree of histologic overlap resulting in diagnostic challenges [8,13,16,20].

Recently, Murakami et al. [18] reported 29 GAED cases as a distinct pathologic entity showing expression of oncofetal proteins (α-FP, glypican-3, and SALL4) and aggressive clinical behavior. They regarded GAED as equivalent to clear cell carcinoma and a subset of (α-FP-expressing) HAC. However, given that GAED does not always express oncofetal proteins [5,6], controversies remain, warranting larger scale studies with clinical data.

The aims of the present study were to 1) classify gastric adenocarcinoma with clear cells as GAED or HAC based on histology and oncofetal protein expression, 2) identify the clinical significance of GAED and HAC compared to conventional tubular adenocarcinoma, and 3) determine the mucin profiles of clear cells that may help trace the origin of clear cells.

2. Materials and methods

2.1. Patients and data collection

This study was approved by the Institutional Review Board of Samsung Medical Center. A computer search of patient electronic charts and pathology reports was performed to identify surgically resected cases with pathology reports. As a result, 26 HACs and 30 GAEDs diagnosed in Samsung Medical Center between January 2002 and March 2012 were identified. The resected gastric specimens were fixed with 10% neutral buffered formalin for 10–48 h and the representative tumor mass (6–10 sections) was embedded in paraffin blocks. All hematoxylin and eosin-stained sections of specimens were thoroughly reviewed and confirmed by two gastrointestinal pathologists (Kim, K-M and Kwon, MJ).

The diagnoses of GAED and HAC were primarily based on typical morphologic features. GAED was based on the criteria described previously [5,15,18] and diagnosed when cells with clear cytoplasm, tubulopapillary growth pattern, and luminal eosinophilic secretions comprised more than 30% of the total tumor population without definite clear hepatoid areas. Focal clear cells within the tumor were excluded to strictly analyze GAED.

HAC was diagnosed with distinct observation of a typical hepatoid component with solid or trabecular growth pattern separated by sinusoidal vascular channels [11,14]. In total, 26 HACs and 28 GAEDs were analyzed.

Tumor stage was classified based on the 7th edition of the American Joint Committee on Cancer (AJCC) [14]. Clinical information including age, sex, and survival or recurrence follow-up data was obtained retrospectively from medical charts. Tubular adenocarcinomas surgically resected from 1996 to 2005 were used as controls and were divided into well differentiated (WD-TA), moderately differentiated (MD-TA), and poorly differentiated (PD-TA) tubular adenocarcinoma according to histologic differentiation. A total of 1107 control group patients included 725 men and 382 women from 23 to 74 years of age (median 52 years) and consisted of 808 PD-TA and 299 WD/MD-TA.

2.2. Immunohistochemical analyses

Immunohistochemistry (IHC) was performed on 4 μm sections of formalin-fixed, paraffin-embedded tissue from all 54 cases. Bond-max autoimmunostainer (Leica Biosystem, Melbourne, Australia) with Bond™ Polymer refine detection (DS9800, Vision Biosystems, Melbourne, Australia) was used according to the manufacturer's protocol. The primary antibodies were mouse monoclonal antibodies for MUC2 (1:100, Novocastra, Newcastle, UK), MUC5AC (1:100, Novocastra), MUC6 (1:100, Novocastra), CD10 (1:100, Novocastra), CDX-2 (1:50, BioGenex, San Ramon, CA), α-FP (pre-diluted, Dako, Glostrup, Denmark), glypican-3 (1:100, BioMosaics, Burlington, VT, USA), and SALL4 (1:200; Cell Marque, Rocklin, CA, USA).

Staining status was defined as positive when ≥1% of tumor cells showed staining; staining was scored "focal" positive when 1–30% of tumor cells showed staining and "diffuse" positive when staining was observed in > 30% of tumor cells, as previously described [9,11]. Cytoplasmic and/or membranous staining was interpreted for α-FP, glypican-3, MUC2, MUC5AC, MUC6, and CD10 status assessment [11]. For SALL4 and CDX-2, only nuclear staining was interpreted as positive [11,18,19].

2.3. Statistical analysis

Pearson's χ^2 test and Fisher's exact test were used to compare clinicopathologic variables and expression of CD10, CDX2, MUC2, MUC5AC, MUC6, α-FP, and glypican-3 in HAC and GAED. Recurrence-free and overall survival outcomes were estimated by the Kaplan-Meier method, and differences between survival curves were analyzed by log-rank test. Recurrence-free survival was defined as the interval between the first day of surgery and tumor progression or the end of follow-up. Overall survival was defined as the interval between the first day of surgery and death or the end of follow-up. Survival analysis was performed in June 2012. All calculations were performed using the Statistical Package for the Social Sciences (SPSS) software (version 18; SPSS Inc., Chicago, IL, USA), and results were considered statistically significant when P values were < 0.05.

3. Results

3.1. Clinicopathologic findings of HAC and GAED

The clinical and pathologic features of patients with HAC and GAED are listed in Table 1 and Fig. 1. HACs showed a high proportion (57.5%) of distant metastasis (mostly in the liver (42.3%)) at the time of diagnosis ($P < 0.001$), which was significantly different from that of GAEDs ($P = 0.001$). In addition, HACs were diagnosed at a more advanced disease stage than GAEDs: 61.5% of HACs were diagnosed at stage III or IV; 82.1% of GAED were diagnosed at stage I or II ($P = 0.002$). Vascular invasion was frequently identified in HACs (96.2%, 25/26) but was less frequently identified in GAEDs (71.4%, 20/28) ($P = 0.025$). There was no statistical difference in lymphatic or perineural invasion between HAC and GAED ($P = 0.18$ and $P = 0.7$, respectively).

Although there was no statistical significance, patients with GAED tended to display lower pT stages than those with HAC; 60.7% (17/28) of GAED were either in pT1 or pT2, whereas 65.4% (17/26) of HAC were in pT3 or pT4 ($P = 0.064$). Five of 28 GAED cases (17.9%) exhibited tumor multicentricity ($P = 0.052$). Other parameters such as sex, age, tumor location, tumor size, and pN category were not significantly different between HAC and GAED ($P > 0.05$).

On detailed pathologic examination, significant differences were observed in dominant morphologic pattern, presence and proportion of clear cells, eosinophilic secretions, geographic coagulation necrosis, height (columnar) of glandular epithelial cells, and presence of multinucleated giant cells between HAC and GAED (Supplementary Table 1).

Table 1
Clinicopathologic features of hepatoid adenocarcinomas and clear cell gastric carcinomas.

	HAC n = 26 (%)	GAED n = 28 (%)	*P*
Sex			0.884
Male	20 (76.9)	22 (78.6)	
Female	6 (23.1)	6 (21.4)	
HBV/HCV			0.699
Present	4 (15.4)	3 (10.7)	
Absent	22 (84.6)	25 (89.3)	
Family gastric cancer Hx			1.000
Present	2 (7.7)	3 (10.7)	
Absent	24 (92.3)	25 (89.3)	
Age (y)[a]	59.77 ± 1.18	63.11 ± 1.04	0.263
Tumor location			
Upper	1 (3.8)	2 (7.1)	0.866
Middle	8 (30.8)	8 (28.6)	
Lower	17 (65.4)	18 (64.3)	
Size (cm)[a]	5.75 ± 2.94	5.44 ± 2.79	0.808
T classification			0.064
pT1-T2	9 (34.6)	17 (60.7)	
pT3-T4	17 (65.4)	11 (39.3)	
N classification			0.260
pN0-1	14 (53.8)	20 (71.4)	
pN2-3	12 (46.2)	8 (28.6)	
M classification			< 0.001
pM0	11 (42.3)	27 (96.4)	
pM1	15 (57.7)	1 (3.6)	
Lymphatic invasion			0.179
Present	23 (88.5)	20 (71.4)	
Absent	3 (11.5)	8 (28.6)	
Vascular invasion			0.025
Present	25 (96.2)	20 (71.4)	
Absent	1 (3.8)	8 (28.6)	
Perineural invasion			0.706
Present	3 (11.5)	5 (17.9)	
Absent	23 (88.5)	23 (82.1)	
AJCC stage			0.002
I-II	10 (38.5)	23 (82.1)	
III-IV	16 (61.5)	5 (17.9)	
Multicentricity			0.052
Present	0 (0)	5 (17.9)	
Absent	26 (100)	23 (82.1)	
Liver metastasis			0.001
Present	11 (42.3)	1 (3.6)	
Absent	15 (57.7)	27 (96.4)	
Recurrence			< 0.001
Present	21 (80.8)	6 (21.4)	
Absent	5 (19.2)	22 (78.6)	
Survival			< 0.001
Alive	11 (42.3)	26 (92.9)	
Dead	15 (57.7)	2 (7.1)	
Treatment			0.955
Surgery only	11 (42.3)	13 (46.4)	
Surgery + CTx	8 (30.8)	8 (28.6)	
Surgery + CCRT	7 (26.9)	7 (25.0)	

[a] Two-tailed *t*-tests of mean ± SD. *HAC* hepatoid adenocarcinoma, *GAED* clear cell gastric carcinoma, *HBV/HCV* hepatitis B virus and hepatitis C virus, *Hx* history, *AJCC* American Joint Committee on Cancer, *CTx* chemotherapy, *CCRT* concomitant chemoradiation therapy.

All 28 cases of GAED (100%) had a predominantly tubulopapillary growth pattern with prominent eosinophilic secretions ($P < 0.001$) (Fig. 1a–c). Twenty-four HACs (92.3%) displayed a predominantly hepatoid growth pattern, while only two HAC cases (7.7%) showed a dominantly glandular morphological pattern (Fig. 1d–f).

Eosinophilic secretions were more frequently observed in GAED (89.3%) than in HACs (23.1%) ($P < 0.001$). Geographic coagulation necrosis was observed in 19 HACs (73.1%) (Fig. 1g) but was not observed in GAED ($P < 0.001$). Multinucleated giant cells were frequently detected in HAC (16 cases, 61.5%) (Fig. 1h), while they were infrequent in GAED (5 cases, 17.9%) ($P = 0.002$). Clear cells frequently seen in the glandular portion of GAED were columnar and usually stratified, while those in HAC were single-layered cuboidal cells. The average clear cell percentage in GAED (67.0 ± 18.9%) was much higher than that in HAC (14.2 ± 20.1%) ($P < 0.001$) (Fig. 1i). Eighteen cases (69.2%) of HACs harbored clear cells, occupying < 10% of tumor volume in 50% of the cases. In contrast, 71.4% of GAEDs showed clear tumor cells occupying more than 50% of the tumor volume. This difference between HAC and GAED was statistically significant ($P = 0.002$).

3.2. Comparison of HAC and GAED with conventional tubular adenocarcinoma

The clinicopathologic findings of HAC were compared with those of PD-TA, and the clinicopathologic findings of GAED were compared with those of WD/MD-TA (Table 2). There were statistically significant differences in age, vascular invasion, pN stage, pM stage, lymphatic invasion, vascular invasion, perineural invasion, liver metastasis, recurrence rate, and mortality rate between HACs and PD-TAs. The median age of the PD-TA group (50 years) was younger than that of the HAC group (58 years) ($P < 0.001$). Higher rates of advanced pN stage and perineural invasion were noted in PD-TAs compared to HACs ($P = 0.037$ and $P = 0.030$, respectively). Lymphatic invasion, vascular invasion, and distant metastasis were more frequently identified in HACs than in PD-TAs ($P < 0.001$, $P < 0.001$, and $P < 0.001$, respectively).

Liver metastasis was noted in 42.3% of HACs but was found in only 5.8% of PD-TAs ($P < 0.001$). The average follow-up period was 77.9 ± 31.9 months, and mortality and recurrence occurred more frequently in HACs than in PD-TAs ($P = 0.033$ and $P < 0.001$, respectively). Finally, patients with HAC had much worse overall and recurrence-free survival rates (mean: 53 months, and 24 months, respectively) than patients with PD-TAs (mean: 108 months, and 107 months, respectively) ($P < 0.001$, and $P < 0.001$, respectively).

Direct comparison between GAEDs and WD/MD-TAs showed significant differences in age, vascular invasion, pT stage, pN stage, AJCC stage, multicentricity, and mortality rates. GAED occurred more frequently in older patients (median age of 63 years) than WD/MD-TA (median age of 58 years) ($P = 0.002$). Vascular invasion was more frequently identified in GAED than in WD/MD-TA ($P < 0.001$). However, GAED was significantly associated with lower pT stage, pN stage, and AJCC stage than WD/MD-TA ($P < 0.001$, $P < 0.001$, and $P < 0.001$, respectively). Overall, the clinical outcomes of HAC and GAED were different from those of PD-TA and WD/MD-TA, respectively.

3.3. Prognostic impact of clear cells in HAC and GAED

Patients with HAC had significantly shorter overall and recurrence-free survival (mean: 25 months, and 53 months, respectively) compared to those with GAED (mean: 107 months, and 118 months, respectively) ($P < 0.001$) (Fig. 2). The 5-year survival rates for GAED, tubular adenocarcinoma, and HAC were 79%, 64%, and 14%, respectively ($P < 0.001$). Sub-group analysis was performed to understand differences in survival rates according to the proportion of clear cell histology within the tumor. Based on a cut-off value of 10% of clear cells within the tumor, there was no statistically significant survival difference in HAC. For GAED with a cut-off of 50%, there were no significant differences in recurrence-free and overall survivals. These results indicate that clear cell histology does not affect prognosis in patients within HAC and GAED.

3.4. Immunohistochemical findings of clear cell in HAC and GAED

To identify distinctive features of clear cells in HAC and GAED, the expression pattern of oncofetal proteins (α-FP, glypican-3, SALL4), gastric mucin (MUC6, MUC5AC), and intestinal mucin phenotype

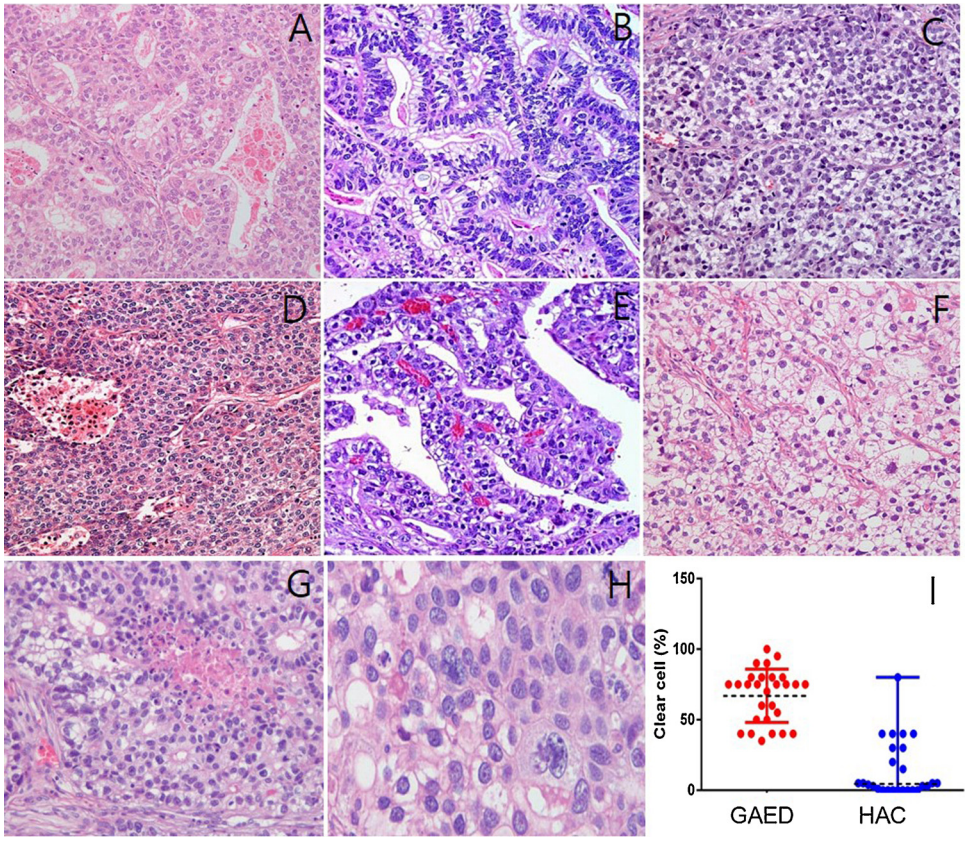

Fig. 1. Histologic features of clear cell gastric carcinoma (GAED) and hepatoid adenocarcinoma (HAC). (A) GAED is typically a well-differentiated gastric adenocarcinoma exhibiting a tubulopapillary growth pattern with predominantly clear cells and luminal eosinophilic secretions. (B) Clear cells contain abundant cytoplasm and are arranged in well-differentiated tubules or papillae with basally-located hyperchromatic nuclei. (C) Solid pattern is also observed in GAED, and the clear cytoplasm is vacuolated. (D) Typical hepatoid area of HAC is composed of eosinophilic granular hepatocyte-like neoplastic cells arranged in cords of variable thickness. (E) Clear cells growing in a papillary pattern are frequently found in HAC. (F) Clear cells of HAC with vacuolated cytoplasm are arranged in a trabecular or nested pattern. Coagulation necrosis (G) and multinucleated giant cells (H) are noted in HAC with clear cells. (I) The proportion of clear cells is predominant in GAED compared to HAC.

(CD10, CDX-2, MUC2) markers were compared by histologic pattern (hepatoid areas of HAC vs. solid areas of GAED; clear glandular areas of HAC vs. tubulopapillary areas of GAED) (Table 3). There were statistically significant differences in α-FP and glypican-3 expression between hepatoid areas of HAC and solid areas of GAED ($P = 0.014$). All (100%) hepatoid areas of HAC showed diffuse and strong positive expression of glypican-3, while half of the solid areas of GAED displayed heterogeneous and weak glypican-3 expression ($P < 0.001$). SALL4 was diffusely positive in 94.7% of clear cells with HAC, whereas it showed heterogeneous expression in all cases of GAED (100%) ($P < 0.001$). In clear cell areas in both HAC and GAED, SALL4 was the most sensitive marker (positivity, 100%), followed by glypican-3 (66%) and α-FP (53.2%).

Expression of CD10, CDX2, and MUC6 was more frequently observed in tubulopapillary areas of GAED than in clear cell glandular areas of HAC. CD10 expression was identified in 71.4% (20/28) of GAED ($P < 0.001$). CDX2 expression was found in 96.4% of GAED and 38.5% of HAC ($P < 0.001$). The CDX-2 expression pattern in GAED was diffuse (19/27), while it tended to be focal in HAC (9/10). Higher MUC6 expression was identified in GAED than in HAC ($P = 0.007$), and its expression was focal (9/9) in GAED. Conversely, α-FP expression was more frequently observed in HAC with clear cell glandular areas (78.9%, 15/19) than in GAED with tubulopapillary areas (35.7%, 10/28) ($P = 0.007$). Glypican-3 expression was more frequently identified in HAC than in GAED ($P < 0.001$). HAC with clear cell glandular areas (73.3% and 77.8%) displayed diffuse α-FP and glypican-3 expression, in contrast to GAED (10% and 7.7%). Representative IHC findings for HAC and GAED are summarized in Fig. 3.

Expression of α-FP, glypican-3, and SALL4 was more frequently observed in HAC with clear cells than in GAED. HAC with clear cells displayed a diffuse α-FP, glypican-3, and SALL4 expression pattern, in contrast to GAED. Expression of CD10, CDX2, and MUC6 was more frequently observed in GAED than in clear cells of HAC. MUC5AC and MUC2 were negative in both HAC with clear cells and GAED.

4. Discussion

Because of their rarity and similar histology, the differential diagnosis of GAED and HAC is often challenging. To prove the need to separate gastric adenocarcinoma with clear cells into GAED and HAC, we evaluated the survival differences of 28 GAED and 26 HAC cases compared to 1107 conventional adenocarcinoma cases. We also investigated the expression of oncofetal proteins (α-FP, glypican-3, SALL4) and mucins with their impact on prognosis. To the best of our knowledge, this is the largest case series from a single institute.

The present study revealed that HAC and GAED were clinically distinct from PD-TA and WD/MD-TA. GAED tended to occur in the older age group and was more strongly linked to vascular invasion and multicentricity than WD/MD-TA. HAC was associated with older age and higher frequencies of lymphatic invasion, vascular invasion, distant metastasis, liver metastasis, and frequent recurrences than PD-TA, whereas PD-TA showed higher incidence of advanced pN stage and perineural invasion than HAC. Similar to our results, Murakami et al. [18] compared GAED with 100 samples of conventional gastric cancers. However, they could not investigate the clinical outcome of GAED compared to WD/MD-TA. In the present study, HAC with clear cells showed the worst prognosis, followed by HAC without clear cells, PD-TA, WD/MD-TA, and GAED. Although more than half of the patients with HAC or GAED received adjuvant chemotherapy, the 5-year survival rates for GAED, conventional gastric cancer, and HAC were 79%, 64%, and 14%, respectively. Our results provide evidence that patients with HAC with clear cells truly displayed higher recurrence rates or more unfavorable survival outcomes than patients with GAED. However, clear cell histology itself and high proportion of clear cells within either HAC or GAED are not related to aggressiveness or prognosis. Therefore, HAC should be distinguished from GAED and PD-TA.

Thorough histologic analyses revealed that geographic necrosis, multinucleated giant cells, and clear glandular areas lined by single-layered cuboidal cells are pathologic findings suggestive of HAC,

Table 2
Comparison between clinicopathologic features of hepatoid adenocarcinomas and poorly-differentiated tubular adenocarcinomas (PD-TA) and between clear cell gastric carcinomas and well-differentiated/moderately-differentiated tubular adenocarcinomas (WD/MD-TA).

	HAC n = 26 (%)	PD-TA n = 808 (%)	*P*	GAED n = 28 (%)	WD/MD-TA n = 299 (%)	*P*
Sex			0.106			1.000
Male	20 (76.9)	491 (60.8)		22 (78.6)	234 (78.3)	
Female	6 (23.1)	317 (39.2)		6 (21.4)	65 (21.7)	
Age (y)[a]	59.77 ± 1.18	49.99 ± 10.90	< 0.001*	63.11 ± 1.04	56.23 ± 8.91	0.002*
Tumor location			0.428			0.801
Upper	1 (3.8)	88 (10.9)		2 (7.1)	25 (8.4)	
Middle	8 (30.8)	273 (33.8)		8 (28.6)	69 (23.1)	
Lower	17 (65.4)	447 (55.3)		18 (64.3)	205 (68.5)	
Size (cm)[a]	5.75 ± 2.94	6.01 ± 3.05	0.665	5.44 ± 2.79	5.50 ± 2.23	0.896
T classification			0.079			< 0.001*
pT1-T2	9 (34.6)	159 (19.7)		17 (60.7)	73 (24.4)	
pT3-T4	17 (65.4)	649 (80.3)		11 (39.3)	226 (75.6)	
N classification			0.037*			< 0.001*
pN0-1	14 (53.8)	271 (33.5)		20 (71.4)	110 (36.8)	
pN2-3	12 (46.2)	537 (66.5)		8 (28.6)	189 (63.2)	
M classification			< 0.001*			0.334
pM0	11 (42.3)	680 (84.2)		27 (96.4)	266 (89.0)	
pM1	15 (57.7)	128 (15.8)		1 (3.6)	33 (11.0)	
Lymphatic invasion			< 0.001*			0.642
Present	23 (88.5)	409 (50.6)		20 (71.4)	151 (50.5)	
Absent	3 (11.5)	399 (49.4)		8 (28.6)	148 (49.5)	
Vascular invasion			< 0.001*			< 0.001*
Present	25 (96.2)	33 (4.1)		20 (71.4)	18 (6.0)	
Absent	1 (3.8)	775 (95.9)		8 (28.6)	281 (94.0)	
Perineural invasion			0.030*			0.642
Present	3 (11.5)	257 (31.8)		5 (17.9)	69 (23.1)	
Absent	23 (88.5)	551 (68.2)		23 (82.1)	230 (76.9)	
AJCC stage			1.000			< 0.001*
I-II	10 (38.5)	309 (38.2)		23 (82.1)	132 (44.1)	
III-IV	16 (61.5)	499 (61.8)		5 (17.9)	167 (55.9)	
Multicentricity			1.000			< 0.001*
Present	0 (0)	5 (0.6)		5 (17.9)	1 (0.3)	
Absent	26 (100)	803 (99.4)		23 (82.1)	298 (99.7)	
Liver metastasis			< 0.001*			0.225
Present	11 (42.3)	47 (5.8)		1 (3.6)	39 (13.0)	
Absent	15 (57.7)	761 (94.2)		27 (96.4)	260 (87.0)	
Recurrence			< 0.001*			0.148
Present	21 (80.8)	322 (39.9)		6 (21.4)	108 (36.1)	
Absent	5 (19.2)	486 (60.1)		22 (78.6)	191 (63.9)	
Survival			0.033*			0.002*
Alive	11 (42.3)	508 (62.9)		26 (92.9)	198 (66.2)	
Dead	15 (57.7)	300 (37.1)		2 (7.1)	101 (33.8)	

[a] Two-tailed t-tests of mean ± SD; *HAC* hepatoid adenocarcinoma, *PD-TA* poorly-differentiated tubular adenocarcinoma, *GAED* clear cell gastric carcinoma, *WD/MD-TA* well- and moderately-differentiated tubular adenocarcinoma.

* Statistical significance: *P* value < 0.05.

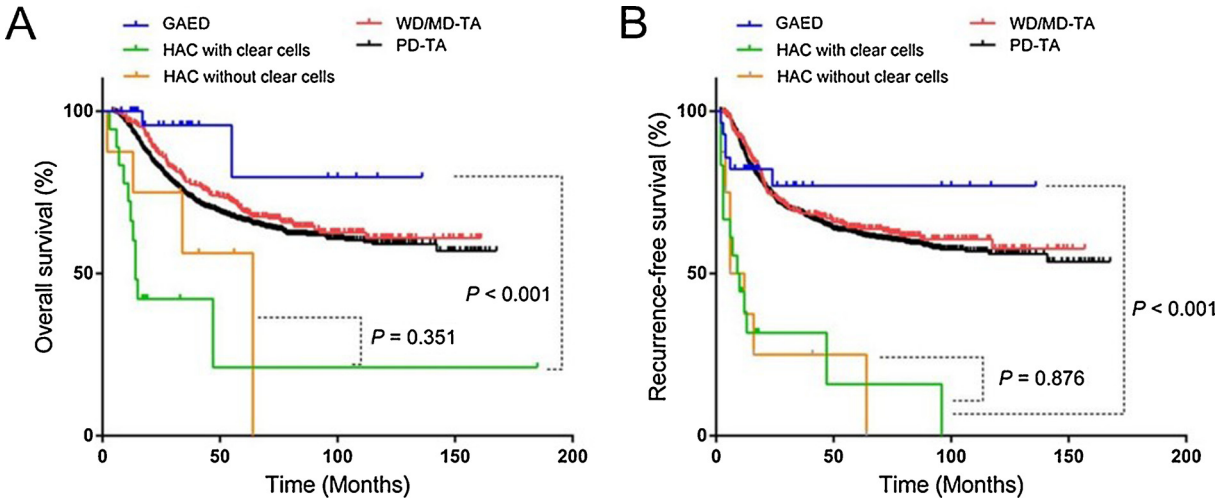

Fig. 2. Kaplan-Meier plots of overall survival and recurrence-free survival of hepatoid adenocarcinoma (HAC) and clear cell gastric carcinoma (GAED) compared with conventional gastric cancers. Overall survival (A) and recurrence-free survival (B) outcomes were significantly worse in patients with HAC with clear cells than in patients with GAED or conventional gastric cancers.

Table 3
Comparison of immunohistochemical results of hepatoid adenocarcinomas and clear cell gastric carcinomas according to hepatoid and clear cell areas.

	HAC Clear cell area n = 19(%)	GAED TP area n = 28(%)	*P*	HAC Non-clear hepatoid area n = 26(%)	GAED Solid area n = 12(%)	*P*
α-FP			0.007*			0.014*
Positive	15 (78.9)	10 (35.7)		20 (76.9)	4 (33.3)	
Diffuse	11	1		18	0	
Focal	4	9		2	4	
Negative	4 (21.1)	18 (64.3)		6 (23.1)	8 (66.7)	
Glypican-3			< 0.001*			< 0.001*
Positive	18 (94.7)	13 (46.4)		26 (100)	6 (50)	
Diffuse	14	1		21	0	
Focal	4	12		5	6	
Negative	1 (5.3)	15 (53.6)		0 (0)	6 (50)	
SALL4			< 0.001*			< 0.001*
Positive	19 (100)	28 (100)		25 (96.2)	12 (100)	
Diffuse	18	0		24	0	
Focal	1	28		1	12	
Negative	0 (0)	0 (0)		1 (3.8)	0 (0)	
CD10			< 0.001*			0.850
Positive	0 (0)	20 (71.4)		5 (19.2)	2 (16.7)	
Diffuse	0	9		0	1	
Focal	0	11		5	1	
Negative	19 (100)	8 (28.6)		21 (80.8)	10 (83.3)	
CDX-2			0.001*			0.060
Positive	10 (52.6)	27 (96.4)		11 (42.3)	1 (8.3)	
Diffuse	1	19		2	1	
Focal	9	8		9	0	
Negative	9 (47.4)	1 (3.6)		15 (57.7)	11 (91.7)	
MUC6			0.007*			–
Positive	0 (0)	9 (32.1)		0 (0)	0 (0)	
Diffuse	0	0		0	0	
Focal	0	9		0	0	
Negative	19 (100)	19 (67.9)		26 (100)	12 (100)	
MUC5AC			–			–
Positive	0 (0)	0 (0)		0 (0)	0 (0)	
Negative	19 (100)	28 (100)		26 (100)	12 (100)	
MUC2			–			–
Positive	0 (0)	0 (0)		0 (0)	0 (0)	
Negative	19 (100)	28 (100)		26 (100)	12 (100)	

HAC hepatoid adenocarcinoma, *GAED* clear cell gastric carcinoma, *TP* tubulopapillary, *α-FP* alfa-fetoprotein, *CD10* cluster of differentiation 10, *MUC6* mucin 6, *MUC5AC* mucin 5AC, *MUC2* mucin 2.

* Statistical significance: *P* value < 0.05.

whereas eosinophilic secretions and stratified columnar clear cells lining glandular portions favored GAED. In particular, geographic necrosis was exclusively observed in HAC. Moreover, the clear cells of HAC showed diffuse and strong expression of α-FP, glypican-3, and SALL4, whereas GAED showed focal/heterogeneous expression. SALL4 was the most sensitive marker, followed by glypican-3 and α-FP, to discriminate clear cells between HAC and GAED. Murakami et al. [18] suggested that glypican-3 is the most sensitive marker for GAED, followed by SALL4 and α-FP [18]. Ushiku et al. [19,20] reported that SALL4 is a sensitive marker for α-FP-producing gastric carcinomas and is especially useful to diagnose HAC [19,20]. In the present study, although the positivity of glypican-3 was lower than previously reported [1], SALL4 and AFP expression levels were similar. Moreover, we found that SALL4 expression was heterogeneous within GAED, in contrast to diffuse expression within HAC.

Detailed mucin profiles of HAC and GAED have not been intensively studied [11,18]. GAED commonly expressed CD10 (71.4%), CDX-2 (96.4%), and MUC6 (32.1%) but not MUC2 and MUC5AC, suggesting a higher tendency for both gastric antral mucin phenotype and intestinal mucin phenotype. In contrast, HAC with clear cells was commonly positive for CDX-2 but negative for CD10, MUC6, MUC5AC, and MUC2, suggesting a phenotype that is intestinal rather than gastric. Similarly, Kumashiro et al. [11] also reported that the histogenesis of HAC is strongly associated with the intestinal phenotype, and its hepatoid component is associated with reduced CDX2 expression. We also observed that the non-clear, hepatoid areas of HAC showed low expression of CDX-2, whereas clear cell areas of HAC were strongly positive for CDX-2. In this context, the expression patterns of α-FP, glypican-3, SALL4, CD10, CDX-2, and MUC6 are important clues to differentiate HAC with clear cells from GAED. Strong expression of CD10 and CDX-2 with MUC6 positivity in tubulopapillary clear cells may support a diagnosis of GAED rather than HAC with clear cells.

Unlike in a previous study [18], we found more favorable prognosis in patients with GAED compared to conventional adenocarcinomas. The presence of many patients with early stage (pT1-2 in 60.7%) GAED may have led to this discrepant result. Although there are few reported cases and no direct comparison studies, it is generally accepted that gastric HAC and GAED have similar clinicopathologic features [18]. The presence of clear cells in most cases of HAC also suggests that HAC may originate from GAED, or vice versa. However, our results suggest that GAED should be differentiated from HAC by histology, focal or less frequent oncofetal protein expression, and distinct mucin profile. Based on these observations, we created a flow chart detailing the suggested diagnostic and decision-making process for differential diagnosis of HAC and GAED in gastric adenocarcinoma with clear cells. Given that HAC and GAED are very rare carcinomas, readers will likely be interested in the frequency and method of distinguishing these rare entities.

In conclusion, although rare, HAC with clear cells should be differentiated from GAED based on its poor prognosis, diffuse strong expression of oncofetal proteins, and intestinal mucin phenotype.

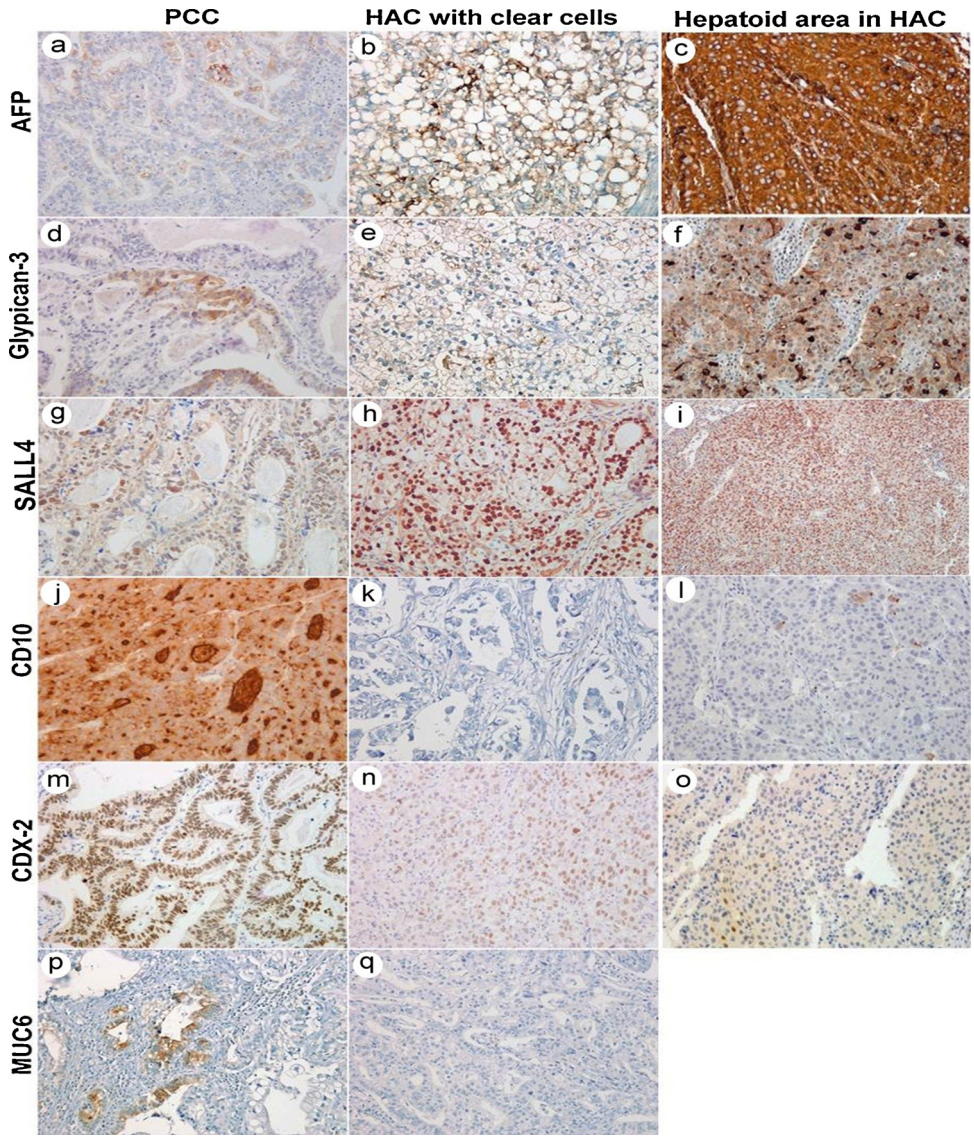

Fig. 3. Immunohistochemical findings of GAED and HAC with clear cells and hepatoid areas. Clear cells of GAED are focally positive for α-FP (a), glypican-3 (d), SALL4 (g), and MUC6 (p) and diffusely positive for CD10 (j) and CDX-2 (m). Clear cells of HAC are positive for α-FP (b), glypican-3 (e), SALL4 (h), and CDX-2 (n) but negative for CD10 (k) and MUC6 (j). Hepatoid areas of HAC are diffusely positive for α-FP (c), glypican-3 (f), and SALL4 (i) but negative for CD10 (E), and focal positive for CDX-2 (I).

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prp.2019.152525.

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