

Case Series

Alpha-Fetoprotein-Producing Early Gastric Cancer with Intramucosal Hepatoid and Fetal Enteric Differentiation

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Keywords

Alpha-fetoprotein-producing gastric cancer · Hepatoid adenocarcinoma · Early gastric cancer · SALL4

Abstract

Alpha-fetoprotein (AFP)-producing gastric carcinomas (AFPGCs) are relatively rare tumors known to have a poor prognosis and commonly found as advanced lesions. Histologically, AFPGCs have been described as having hepatoid and fetal enteric (enteroblastic) morphology and are associated with conventional adenocarcinomas. Prior studies reported a hepatoid component present only in invasive areas and hypothesized that AFPGCs may develop hepatoid features during the process of tumor invasion. We report three cases of AFP-producing early gastric cancer which had an intramucosal hepatoid component. Immunohisto-

chemistry showed that the hepatoid component was diffusely immunoreactive for SALL4, AFP, arginase-1, and HepPar1, and focally for CDX2 and PDX1. An intramucosal transition between the hepatoid component and conventional intramucosal adenocarcinoma was identified. Two patients also had a coexistent fetal enteric component, which was admixed with a hepatoid component. Although at an early stage one patient subsequently developed liver metastasis and a second patient was suspected of having liver metastasis, these were not biopsy-proven. The latter patient had a previous history of hepatocellular carcinoma (HCC) and SALL4 was used on the HCC to distinguish metastatic/further HCC from a gastric metastatic primary with hepatoid differentiation.

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Introduction

Extrahepatic alpha-fetoprotein (AFP)-producing carcinomas are relatively rare tumors and have been reported in various organs such as stomach, lung, gallbladder, ovary, colon, ampulla of Vater, and pancreas [1–5]. The stomach is one of the more common sites for these cancers and typically they are found as advanced lesions and have a high incidence of liver metastasis and a poor prognosis [6, 7]. Histologically, AFP-producing gastric carcinomas (AFPGCs) have also been described as having both hepatoid and fetal enteric (enteroblastic) morphology and concurrent conventional adenocarcinoma [8–12], which is most frequently of the intestinal type [8]. Prior studies reported that a hepatoid component is only present in invasive areas and suggested that AFPGCs might acquire hepatoid differentiation and AFP-producing ability during the process of tumor invasion [8, 13]. Here, we report three patients with AFP-producing early gastric cancer (GC) having an intramucosal hepatoid and additional fetal enteric components.

One patient had a previous history of hepatocellular carcinoma (HCC) in which SALL4 was not immunoreactive, suggesting that this was not the source of the metastatic disease. To the best of our knowledge, these are the first reported cases of early GC with an intramucosal hepatoid and additional enteroblastic components.

Patients and Methods

Case 1

A 72-year-old male was found to have an early incidental GC during an annual health examination. He had undergone a partial hepatectomy for HCC 7 years before. Serum AFP level was slightly elevated (14.9 ng/mL), serum carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) levels were within the normal range. The patient underwent distal gastrectomy, and 3 years after the surgery multiple liver lesions had developed, but no liver biopsies were performed. The patient died of disease 77 months after the gastric surgery.

Case 2

A 77-year-old male was found to have an early GC during a periodic health examination that included endoscopy. His past medical history was unremarkable except for an aortic

aneurysm and a skull fracture. Serum CEA and CA19-9 levels were within normal range. Serum AFP level was not evaluated. Distal gastrectomy was performed, and 48 months after the surgery the tumor recurred as liver metastasis. Serum AFP level was markedly elevated (119,190 ng/mL). After undergoing chemotherapy, the serum AFP level was reduced to within the normal range and no obvious liver lesions were detected on computed tomography scan. Amazingly, 106 months after the surgery, the patient is alive and well with no recurrence.

Case 3

A 66-year-old female who had had a distal gastrectomy for early GC 23 years before was found to have an additional early GC during a routine health checkup that included endoscopy. Serum CEA and CA19-9 levels were within normal range, but serum AFP level was not measured. The patient underwent total residual gastrectomy. No symptoms or abnormal findings suggesting recurrence have been detected in the 60 months since her surgery.

Immunohistochemistry and Staining Evaluation

The tissue specimens were fixed in 10% buffered formalin, embedded in paraffin serial tissue sections (thickness 3 μ m) were prepared from paraffin blocks and stained with H&E for histological examination or used for immunohistochemical staining. Immunohistochemical staining was performed using the following primary antibodies in accordance with the manufacturer's instructions: SALL4 (clone: 6E3; Anova, Taipei City, Taiwan), AFP (A0008; Agilent, Santa Clara, CA, USA), arginase-1 (HPA003595; Atlas Antibodies AB, Bromma, Sweden), HepPar1 (OCH1E5; Agilent), CDX2 (CDX2-88; BioGenex, San Ramon, CA, USA), PDX1 (guinea pig polyclonal antibody; generously provided by Prof. Christopher V.E. Wright, Vanderbilt University Medical School, Nashville, TN, USA), MUC2 (Ccp58; Novocastra, Newcastle upon Tyne, UK), MUC5AC (CLH2; Novocastra), and MUC6 (CLH5; Novocastra). MUC2, MUC5AC, and MUC6 specifically expressed in goblet cells of the intestine, in the gastric foveolar mucous cells, and in the gastric gland mucous cells, respectively. In well-developed gastrointestinal tract, PDX1 is expressed in gastric antrum and duodenum with decreased expression in the distal small intestine [14, 15].

The results of immunostaining were evaluated as the percentage of positively stained cells. The degree of positive staining was graded into four categories as follows: (i) 0, negative, no positive cells; (ii) +, focal expression, less than one-third of cells were positive; (iii) ++, one-third to two-thirds were positive; and (iv) +++, diffuse expression, more than two-thirds of the cells were positive.

Results

In case 1, a polypoid solid lesion measuring 15 \times 15 mm was detected in the distal third of the stomach. While there was no submucosal invasion, lymphovascular invasion was identified in the submucosa (Fig. 1). The lesion was composed of tumor cells with abundant eosinophilic cytoplasm, centrally located vesicular nucleus, and prominent nucleolus arranged in trabecular or solid nests with a sinusoidal arrangement of vascular channels. The features were those of hepatoid carcinoma. The tumor cells were diffusely immunoreactive for arginase-1, HepPar1, and SALL4, moderately for AFP, and focally for PDX1 and CDX2 (Fig. 2). A

small portion of conventional intramucosal adenocarcinoma was also present. An intramucosal transition from conventional type to hepatoid component was identified (Fig. 2). The conventional component showed a gastric-type immunoprofile (MUC5AC+, MUC6+, CDX2–, MUC2–) and was diffusely immunoreactive for PDX1. Given the previous history of HCC in case 1, SALL4 immunohistochemistry was performed for the resected HCC specimen and no SALL4 expression was seen.

In case 2, a 16 × 8 mm depressed lesion was found in the lower third of the stomach. Microscopically, tumor invaded into the superficial submucosa, and dysplastic mucosal glands which had clear cytoplasm resembling fetal gut were seen (fetal enteric/enteroblastic component). This component was diffusely immunoreactive for SALL4, AFP, PDX1, and CDX2, and patchily for MUC2 and HepPar1 (Fig. 3). Both an intramucosal hepatoid component and conventional component were present, the hepatoid component showing the same immunoprofile as in case 1 (Fig. 3). In the submucosa as well as the hepatoid and fetal enteric components were admixed and difficult to clearly distinguish. The hepatoid component focally showed arginase-1 positivity. The conventional component showed an intestinal predominate immunophenotype (CDX2+++ , MUC2+, MUC5AC+, MUC6+) and was focally immunoreactive for SALL4 (Fig. 3).

In case 3, a 15 × 10 mm slightly depressed lesion was detected in the upper third of the residual stomach. The tumor invaded the submucosa and intramucosal hepatoid, and fetal enteric and conventional components were identified (Fig. 4).

The immunohistochemical profiles observed in these three cases are summarized in Table 1.

Discussion

Here, we report three cases of early GC with an intramucosal hepatoid component, suggesting that this component can evolve very early in the development of these tumors even in the lamina propria. Nevertheless, there was still an intramucosal transition between conventional-type component and hepatoid component, suggesting that “de novo” hepatoid carcinomas do not always require a conventional invasive component, even intramucosal, to initiate those patterns of differentiation.

Because of the history of partial hepatectomy for HCC in case 1, SALL4 immunohistochemistry was performed on the resected HCC, but no SALL4 expression was identified. SALL4 is an embryonic stem cell marker and a transcription factor playing an important role in maintaining self-renewal [16–18]. It is diffusely expressed in both the hepatoid and fetal enteric components, but can also be expressed focally in conventional adenocarcinoma. Ushiku et al. [13] reported that SALL4 is diffusely expressed in fetal gastric epithelium and AFPGCs, whereas SALL4 expression is not identified in HCC. Although other studies reported SALL4 expression in HCC, diffuse expression is relatively rare [19, 20]. Based on the differential expression of SALL4 and the presence of an intramucosal transition with the conventional component, the gastric lesion and HCC were considered as separate primary cancers. After gastrectomy, multiple liver lesions were clinically diagnosed as recurrent HCC; however, some radiological studies reported that liver metastasis from hepatoid adenocarcinoma of the stomach shared many imaging similarities with HCC [21, 22]. Although the gastric lesion was

limited to the lamina propria, submucosal lymphovascular invasion was identified. In this patient, liver metastases from AFPGCs were thought to be the most likely explanation. SALL4 is a useful biomarker to distinguish primary gastric hepatoid and enteroblastic adenocarcinoma from HCC.

While all three AFPGCs were early stage at resection (pT1N0M0), one case developed liver metastasis after gastrectomy (case 2) and case 1 had had liver metastases as well, but these were not biopsied.

AFPGC has a higher intrinsic malignant potential than conventional GC in pT1 stage [23]. The present cases also indicate high potential for metastases even at an early (intramucosal) stage. Vigilant clinical follow-up (imaging and serology) should be considered for AFPGCs.

The hepatoid component showed diffuse arginase-1 and HepPar1 expression. Arginase-1, a binuclear manganese metalloenzyme that catalyzes the hydrolysis of arginine to ornithine and urea, is specifically expressed in normal hepatocytes and HCCs [24]. Although HepPar1 has been used to identify hepatoid differentiation, it recognizes the urea cycle enzyme carbamoyl phosphate synthetase 1, which is present in small intestinal epithelial cells as well as in hepatocytes, so its expression is expected whenever there is intestinal differentiation [25]. HepPar1 has been reported to react with Barrett esophagus, gastric intestinal metaplasia, and adenocarcinomas of stomach and small intestine [26–28]. In our cases 2 and 3, the conventional component showed CDX2 expression which also suggests intestinal differentiation as well as HepPar1 expression; however no arginase-1 expression was identified. The features are interpreted as intestinal-phenotype adenocarcinoma and suggest that arginase-1 is a more sensitive biomarker for hepatic differentiation than HepPar1.

In the present cases, the tumor showed hepatoid and fetal enteric differentiation. Embryologically stomach, proximal duodenum, and liver are derived from fetal foregut. The liver bud arises as an outpouching of the lumen of the distal foregut. In the present cases, the hepatoid component showed focal CDX2 and PDX1 expression. CDX2 is one of the homeobox genes which encodes an intestinal-specific transcription factor, expressed throughout the intestine, from duodenum to rectum [29]. Pancreatic-duodenal homeobox 1 gene (PDX1) is one of the transcription factors from the homeodomain gene family which regulates the development of the pancreas, duodenum, and gastric antrum [14]. Interestingly, during the early phase of embryogenesis in mice, both CDX2 and PDX1 show restricted expression in the same region of liver and pancreas bud, and the same as Hlx9 (Hb9), Hnf4a, Nkx2-2, Onecut1, Prox1, and Sox2 [30]. These findings suggest that the hepatoid component may share characteristics with the liver and pancreas buds.

In conclusion, we first demonstrated intramucosal hepatoid adenocarcinoma in three patients with AFP-producing early gastric hepatoid and fetal enteric adenocarcinoma, and that these lines of differentiation can evolve early in tumor development. The hepatoid and fetal enteric components may evolve very early on from conventional GC, and even when still intramucosal.

Statement of Ethics

Study approval was obtained from the Research Ethics Board at Shinshu University. Consent for publication was obtained from the patients and/or their families.

Conflict of Interest Statement

All authors declare that they have no conflicts of interest related to this study.

Funding Sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author Contributions

M. Iwaya mainly executed the project and wrote most of the manuscript. K. Asano and K. Kobayashi provided clinical data. H. Ota and T. Uehara contributed to the diagnostic process. R. Riddell was involved in the diagnostic process and suggested the property of the manuscript.

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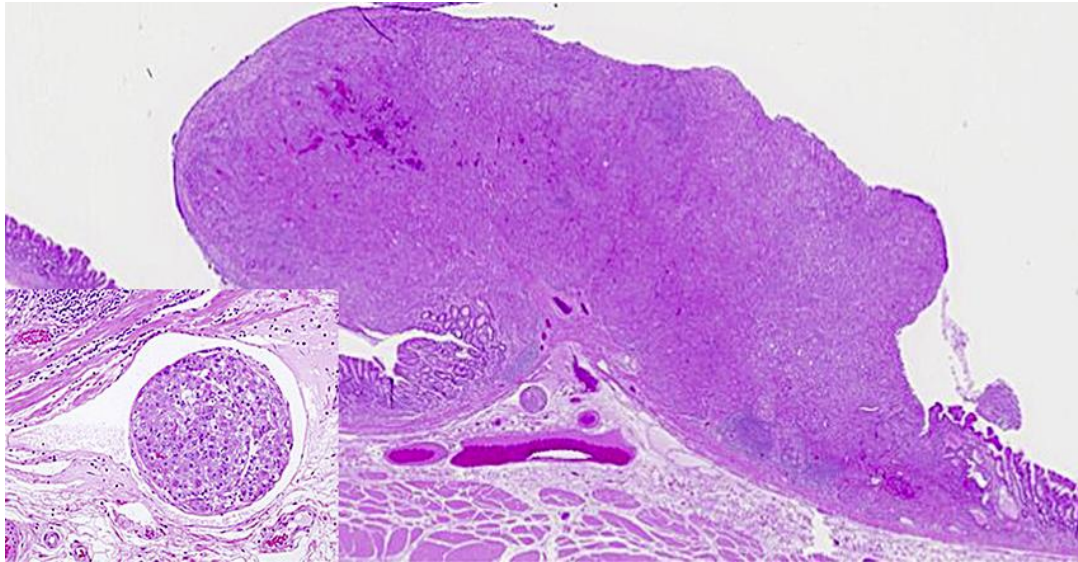


Fig. 1. Case 1 showed a polypoid solid intramucosal lesion. Submucosal lymphovascular invasion is present ($\times 5.7$).

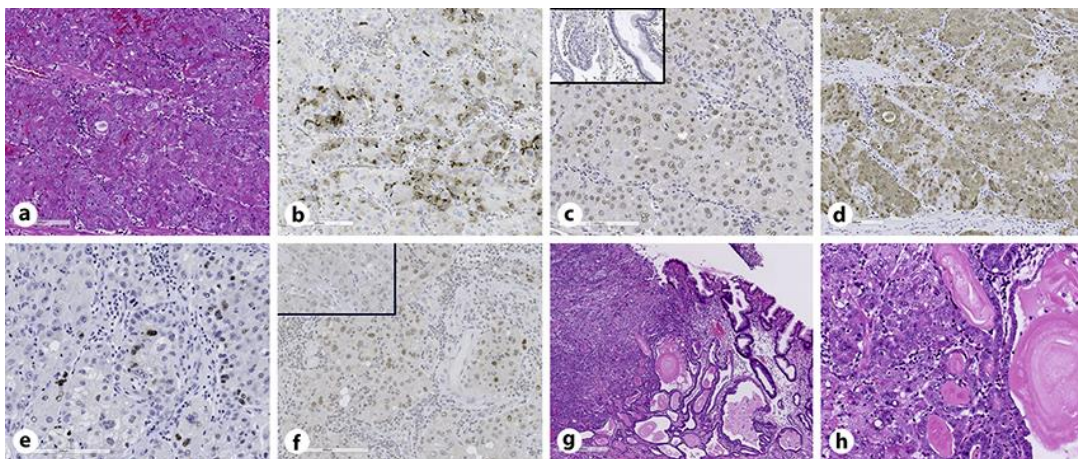


Fig. 2. Detail of case 1. The tumor showed intramucosal hepatoid appearance (a), tumor cells were immunoreactive for AFP (b), SALL4 (inset: negative control) (c), and arginase-1 (d), focally for CDX2 (e) and PDX1 (inset: negative control) (f). An intramucosal transition between the hepatoid and conventional component was seen (g, h). AFP, alpha-fetoprotein.

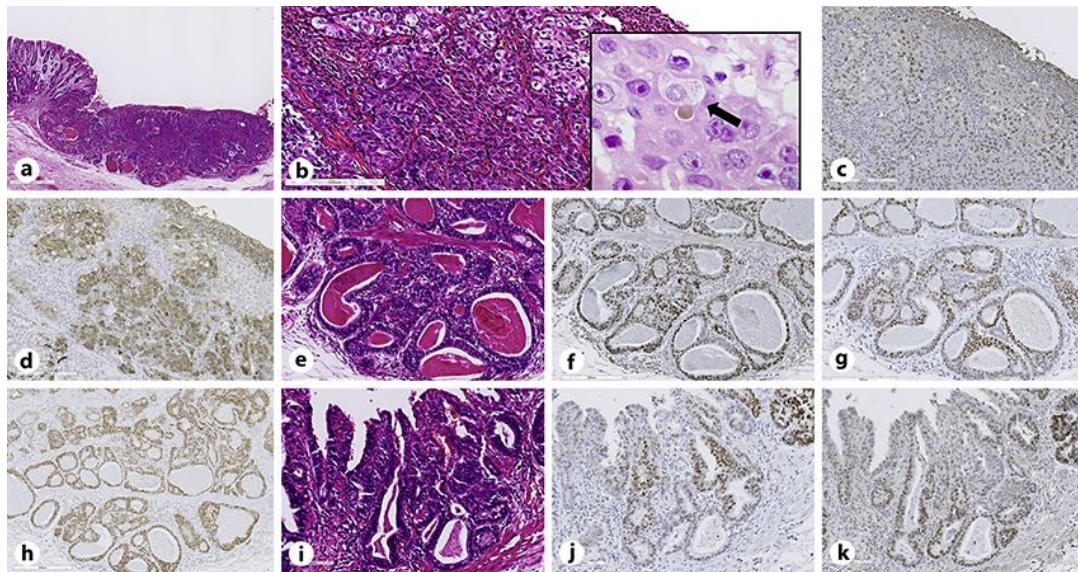


Fig. 3. Case 2 showed a slightly depressed lesion (a) and hepatoid component (b) showed bile production (inset) with SALL4 (c) and arginase-1 (d) expression. Fetal enteric component showed glandular structure with clear cytoplasm (e) and immunoreactivity for SALL4 (f), CDX2 (g), and PDX1 (h). Intramucosal conventional-type component (i) showed focal CDX2 expression (j) and SALL4 expression (k).

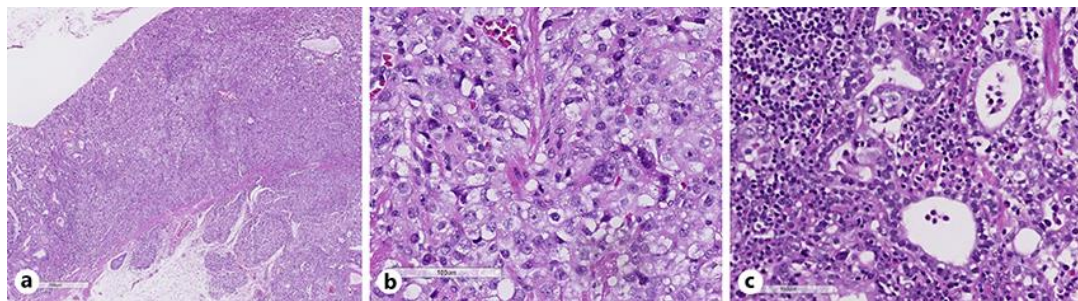


Fig. 4. Case 3 showed an intramucosal-submucosal solid lesion (a) with hepatoid (b) and fetal enteric morphology (c).

Table 1. Immunohistochemical expression patterns observed in each component (cases 1–3)

Antigen	Hepatoid component	Fetal enteric component	Conventional-type component
SALL4	(+++)/(+++)/(+++)	ND/(+++)/(+++)	0/(+)/(+)
AFP	(+)/(+++)/(+++)	ND/(+++)/(+++)	0/0/0
Arginase-1	(+++)/(+++)/(+++)	ND/0/0	0/0/0
HepPar1	(+++)/(+++)/(+++)	ND/(+)/(+)	0/(+)/(+++)
CDX2	(+)/(+)/(++)	ND/(+++)/(++)	0/(+++)/(+++)
PDX1	(+)/(++)/(+)	ND/(+++)/(++)	(+++)/(+++)/(+++)
MUC2	0/0/0	ND/(+)/0	0/(+)/(++)
MUC5AC	0/0/0	ND/0/0	(+)/(+)/(+++)
MUC6	0/0/0	ND/0/0	(+)/(+)/(++)

The extent of immunostaining was scored as follows: (i) 0, negative, no positive cells; (ii) +, focal expression, less than one-third of cells were positive; (iii) ++, one-third to two-thirds were positive; and (iv) +++, diffuse expression, more than two-thirds of the cells were positive. AFP, alpha-fetoprotein; ND, not detected.