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Original contribution

# Combination of hepatocellular markers is useful for prognostication in gastric hepatoid adenocarcinoma☆

**Mikako Osada MD[a], Shinichi Aishima MD, PhD[a], Minako Hirahashi MD, PhD[a], Nobuyoshi Takizawa MD[a], Shunsuke Takahashi MD[a], Kazuhiko Nakamura MD, PhD[b], Masao Tanaka MD, PhD[c], Yoshihiko Maehara MD, PhD[d], Ryoichi Takayanagi MD, PhD[b], Yoshinao Oda MD, PhD[a,*]**

[a]*Department of Anatomic Pathology, Graduate School of Medical Sciences, Kyushu University, Higashi-ku, Fukuoka 812-8582, Japan*
[b]*Department of Medicine and Bioregulatory Science, Graduate School of Medical Sciences, Kyushu University, Higashi-ku, Fukuoka 812-8582, Japan*
[c]*Department of Surgery and Oncology, Graduate School of Medical Sciences, Kyushu University, Higashi-ku, Fukuoka 812-8582, Japan*
[d]*Department of Surgery and Medical Science, Graduate School of Medical Sciences, Kyushu University, Higashi-ku, Fukuoka 812-8582, Japan*

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**Summary** Hepatoid or α-fetoprotein (AFP)–producing adenocarcinomas of stomach growing in a solid pattern are highly aggressive tumors. It is difficult to detect hepatoid differentiation solely based on findings from hematoxylin and eosin stainings, especially in small biopsy specimens. Gastric adenocarcinomas with hepatoid differentiation should be distinguished from solid-type gastric adenocarcinoma because of their different biological behavior. We immunohistochemically analyzed hepatocellular markers (AFP, glypican 3, and Hepatocyte paraffin 1 [HepPar-1]) and possible markers of gastric hepatoid adenocarcinoma (Sal-like protein 4 [SALL4] and palate, lung, and nasal epithelium carcinoma–associated protein [PLUNC]) to detect hepatoid differentiation in 45 gastric hepatoid adenocarcinomas and 47 nonhepatoid solid-type poorly differentiated adenocarcinomas. There were a higher incidence of vascular invasion ($P$ = .0055) and distant metastasis ($P$ = .0458) in hepatoid adenocarcinoma than in nonhepatoid adenocarcinoma. AFP, SALL4, HepPar-1, and glypican 3 were significantly higher in hepatoid adenocarcinoma than in nonhepatoid adenocarcinoma. All 5 markers were positive in both the hepatoid/solid and the tubular component. In hepatoid adenocarcinoma, the frequency of distant metastasis was significantly higher in SALL4-negative cases than in SALL4-positive cases ($P$ = .0381). HepPar-1 was associated with liver metastasis ($P$ = .0452). PLUNC was correlated with lymph node metastasis ($P$ = .0375). There was a significant difference in the survival rate between HepPar-1–positive and HepPar-1–negative groups ($P$ = .0437). The coexpression of

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* Corresponding author. Department of Anatomic Pathology, Pathological Sciences, Graduate School of Medical Sciences, Kyushu University, Higashi-ku, Fukuoka 812-8582, Japan.
*E-mail address:* oda@surgpath.med.kyushu-u.ac.jp (Y. Oda).

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PLUNC and SALL4 and the other coexpression of HepPar-1 and PLUNC were associated with poorer prognosis ($P = .0181$ and $P = .0443$, respectively). AFP, SALL4, HepPar-1, and glypican 3 are useful for the detection of hepatoid differentiation. A combination of PLUNC, HepPar-1, and SALL4 could be a reliable prognostic indicator in hepatoid adenocarcinoma of the stomach.


## 1. Introduction

Hepatoid adenocarcinoma is an extrahepatic tumor characterized by morphologic similarities to hepatocellular carcinomas [1]. It often produces α-fetoprotein (AFP) and shows aggressive features of extensive vascular invasion and frequent liver metastases [1-3]. The stomach is the organ in which hepatoid adenocarcinoma has most commonly been identified. The histologic features of gastric hepatoid adenocarcinoma typically consist of a tubular adenocarcinoma component and solid growth of the carcinoma component, the latter of which is similar to a feature of hepatocellular carcinomas [4,5]. Thus, solid-type gastric adenocarcinoma has the potential to contain an area of hepatoid features or of an AFP-producing component. Because gastric hepatoid adenocarcinoma is thought to be an aggressive tumor, we should detect hepatoid differentiation when we encounter gastric adenocarcinoma growing in a solid pattern. Although hepatoid adenocarcinoma is morphologically defined by hematoxylin and eosin (HE) staining, hepatoid differentiation and definite diagnosis are difficult to achieve solely based on histologic findings, especially in small biopsy specimens. Further immunohistochemical staining is necessary for differential diagnosis.

Hepatocellular differentiation can be detected by multiple immunohistochemical markers. Oncofetal proteins, such as AFP and glypican 3, are reliable diagnostic markers for yolk sac tumors, hepatocellular carcinoma, and a special subgroup of gastric carcinoma that includes AFP-producing type, hepatoid type, and fetal phenotype of gastric carcinoma [6-11]. Hepatocyte paraffin 1 (HepPar-1) is a monoclonal antibody specific for normal and neoplastic hepatocytes [12]. HepPar-1 was expressed in hepatoid components and tubular components in gastric hepatoid adenocarcinoma [13]. Sal-like protein 4 (SALL4) is a zinc finger transcription factor that plays a role in maintaining self-renewal and pluripotency in embryonic stem cells and has been used as a marker of germ cell tumor and AFP-producing gastric carcinoma [14,15]. The palate, lung, and nasal epithelium carcinoma–associated protein (PLUNC) is also expressed in gastric hepatoid adenocarcinoma [16,17]. However, the prognostic value of the expression of these proteins in gastric carcinoma has not been fully investigated.

We compared the immunohistochemical expression of 5 markers (AFP, SALL4, HepPar-1, glypican 3, and PLUNC) between hepatoid adenocarcinoma and nonhepatoid adenocarcinoma and examined the prognostic implications.

## 2. Materials and methods

### 2.1. Case selection

Solid-type poorly differentiated adenocarcinoma is composed of neoplastic cells growing in a solid- or sheet-like pattern and has a well-defined boundary [18,19]. Hepatoid adenocarcinoma was morphologically defined as a tumor composed of large polygonal eosinophilic hepatocyte–like neoplastic cells in a sheet-like pattern based on the World Health Organization system [20]. AFP production was not needed to define hepatoid adenocarcinoma. Nonhepatoid adenocarcinoma was defined as a solid-type poorly differentiated adenocarcinoma without prominent lymphoid stroma associated with Epstein-Barr infection and components of neuroendocrine tumor. We collected 45 cases of hepatoid adenocarcinoma with hepatocellular morphology and 47 cases of nonhepatoid solid-type poorly differentiated adenocarcinoma in this study. Lymph node metastasis was assessed in 90 of 92 cases. Distant (liver) metastasis was assessed in 85 of 92 cases. AFP-producing gastric carcinoma was defined if the serum AFP was elevated and/or the results of immunohistochemical staining for AFP were positive. These samples were histologically diagnosed at the Department of Anatomic Pathology of Kyushu University and its affiliated hospitals between 1979 and 2013. All patients had undergone curative resection, without preoperative chemotherapy or radiation therapy preoperatively. The research protocol was approved by the Kyushu University Medical Human Investigation Committee (Institutional Review Board no. 25-213).

### 2.2. Immunohistochemical staining

Immunohistochemical staining was performed using mouse monoclonal antibodies against HepPar-1 (diluted at 1:200, clone OCH1E5; Dako, Glostrup, Denmark), glypican 3 (diluted at 1:200, clone 1G12; BioMosaics, Burlington, VT), SALL4 (diluted at 1:1000, clone 6E3; Abnova, Taipei, Taiwan), AFP (diluted at 1:400, rabbit polyclonal; Dako), and PLUNC (diluted at 1:500, goat polyclonal; R&D Systems, Abingdon, UK). Sections were cut to a thickness of 4 μm, deparaffinized in xylene, and dehydrated in ethanol. Endogenous peroxidase activity was blocked by 30 minutes of incubation with 0.3% hydrogen peroxidase in absolute methanol. Antigens were retrieved by microwave heating in citrate buffer (pH 6.0) for 20