

Fascin overexpression correlates with positive thrombospondin-1 and syndecan-1 expressions and a more aggressive clinical course in patients with gallbladder cancer

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Abstract

Background/purpose This study was conducted to evaluate the prognostic role of fascin expression in gallbladder (GB) cancer and to define the relationship of thrombospondin-1 (TSP-1) and syndecan-1 in fascin expression.

Methods We performed immunohistochemical detection of fascin, TSP-1, and syndecan-1 in 43 tissue samples from GB cancer patients who underwent macroscopic complete resection.

Results There were 19 (44%) and 24 (56%) cases having low- and high-grade fascin expression, respectively. The tumors with high-grade fascin expression tended to more frequently show poorer differentiation, deeper invasion depth, lymph node metastasis, a higher American Joint Committee on Cancer stage, and recurrence (each $P < 0.05$). The patients with high-grade fascin expression had significantly shorter survival periods than those with low-grade fascin expression ($P < 0.05$). The frequency of positive TSP-1 or syndecan-1 expression in the cases with high-grade fascin expression was significantly higher than that in the cases with low-grade fascin expression (each $P < 0.05$).

Conclusions These results suggest that a subset of advanced GB cancers revealed a marked overexpression of fascin, which was associated with aggressive clinicopathologic findings and poor overall survival. Furthermore, fascin, TSP-1, and syndecan-1 may act in concert to mediate a more aggressive clinical course through enhanced tumor cell motility.

Keywords Fascin · Thrombospondin-1 · Syndecan-1 · Prognosis · Gallbladder cancer

Introduction

Gallbladder (GB) cancer is the most common biliary tract malignancy [1] and is an aggressive and lethal cancer [2]. GB cancer has a great propensity to directly invade the liver and, to a lesser extent, the stomach and duodenum, and it also frequently metastasizes to the liver, the pericholedochal lymph nodes in the lesser omentum, and the lymph nodes behind the first portion of the duodenum [3]. However, the underlying mechanisms responsible for the characteristic invasive growth and metastasis of GB cancer have not yet been established.

Fascin is a cytoplasmic protein that functions to bundle cytoplasmic actin filaments; this is a process that is critical for determining the distribution and activity of the actin cytoskeleton [4]. Fascin expression is usually absent or very low in normal epithelial cells, but it is often upregulated in several types of human neoplasms, such as ovarian, breast, pancreatic, colon, lung, skin, stomach, esophagus, and urinary bladder tumors [5–13]. A high level of fascin in these neoplastic cells is associated with progressive high-grade tumors and also tumors with great metastatic potential [5–13].

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Fascin expression is under complex cellular regulation, as determined by other actin-binding proteins and by extracellular cues that are provided by the extracellular matrix and polypeptide factors [14]. A few studies have reported that the formation of fascin and actin bundles in response to thrombospondin-1 (TSP-1) depends on syndecan-1 and signaling by the guanosine triphosphate (GTP)ases Cdc42 and Rac [15, 16]. In this regard, TSP-1 and syndecan-1 may have distinct activities for inducing cells to form fascin-containing protrusions.

To the best of our knowledge, the expression and role of fascin in GB cancer are still unknown. Furthermore, the precise mechanisms of fascin expression in association with regulatory molecules such as TSP-1 or syndecan-1 have not yet been studied in human cancers, including GB cancer. In the present study, we performed immunohistochemical detection of fascin in GB cancer tissue samples to determine whether the immunohistochemical detection of fascin could provide useful information as a novel therapeutic or prognostic option for treating primary GB cancer. We also performed additional immunohistochemical detection of TSP-1 and syndecan-1 to define their relationship and role in the fascin expression associated with the progression of GB cancer.

Materials and methods

Patients and tissue samples

Forty-three tissue samples from GB cancer patients who underwent macroscopic complete resection for gallbladder carcinoma were retrospectively selected from the surgical pathology records of the Department of Pathology at Dong-A University Medical Center from 1998 to 2002. No preoperative chemotherapy or radiotherapy had been performed in any of these patients. The surgical treatment for the 43 patients was as follows: cholecystectomy with lymph node dissection in 17; laparoscopic cholecystectomy in 10, in whom the GB cancer was incidentally identified during surgery for GB polyp; cholecystectomy with concomitant hepatic wedge resection in 7; cholecystectomy with concomitant hepatic lobectomy in 5; Roux-en-Y choledochojejunostomy in 3; and hepatopancreaticoduodenectomy in 1. All patients, except for the 10 who underwent laparoscopic cholecystectomy, underwent regional lymph node dissection. In the 10 patients who underwent laparoscopic cholecystectomy, no significantly enlarged lymph nodes were detected during surgery. Therefore, nine patients with T1 disease who underwent simple cholecystectomy with no detectable nodes on the preoperative radiological evaluation or during surgery were classified as stage IA and one patient with T2 disease with

no detectable nodes on the radiological evaluation or during surgery was classified as stage IB. Clinical records, pathological reports, and follow-up information were obtained when available. The institutional review board approved our study, and written informed consent was obtained from all patients for the surgery and for the use of their resected tissue samples for research. Hematoxylin-and-eosin-stained slides were reviewed in each case to confirm the original diagnosis, which was based on the World Health Organization criteria [17]. The tumors were postoperatively staged according to the American Joint Committee on Cancer (AJCC) staging system [18].

Immunohistochemistry

Immunohistochemical studies for fascin, TSP-1, and syndecan-1 were performed on formalin-fixed, paraffin-embedded, 4- μ m-thick tissue sections using the avidin-biotin-peroxidase complex method. Mouse monoclonal antibodies were used as the primary antibody; they were directed against fascin (clone 55K-2; DakoCytomation, Carpinteria, CA, USA; 1:50), against TSP-1 (clone 8A6b; Novocastra, Newcastle, UK; 1:50), and against syndecan-1 (clone 5F7; Novocastra; 1:50). Deparaffinization of all sections was performed through a series of xylene baths, and rehydration was performed with a series of graded alcohol solutions. To enhance the immunoreactivity, microwave antigen retrieval was performed at 750 W for 30 min in citrate buffer (pH 6.0) for fascin and in tris-ethylenediaminetetraacetate (EDTA) buffer (pH 9.0) for TSP-1 and syndecan-1. After the blocking of endogenous peroxidase activity with 5% hydrogen peroxidase for 10 min, primary antibody incubation was performed for 1 h at room temperature. An EnvisionChem Detection Kit (DakoCytomation) was used for the secondary antibody at room temperature for 30 min. After washing the tissue samples in Tris buffered saline for 10 min, 3, 3'-diaminobenzidine was used as a chromogen and then Mayer's hematoxylin counterstain was applied.

Interpretation of immunohistochemical staining

Fascin immunoreactivity in each tumor was verified by the labeling of endothelial cells of the microvessels in every specimen. Fascin-positive samples were defined as those samples showing a cytoplasmic staining pattern in the cancer cells. The distribution of fascin labeling was measured according to the percentage of fascin-positive cells: less than 50% was considered low grade and 50% or more was considered high grade.

TSP-1 immunoreactivity was observed in the stroma. Syndecan-1 immunoreactivity was observed on the cancer cell membrane and/or in the cytoplasm. Evaluation of TSP-1

or syndecan-1 expression was classified according to the percentage of positively stained stroma or cancer cells, respectively. The immunostaining intensity was visually scored and stratified into four groups; negative, weak, moderate, and strong. A moderate staining intensity of more than 10% of the stroma or cancer cells was required for a positive result of TSP-1 or syndecan-1, respectively; all other scores were considered to be negative.

All slides were evaluated independently by two investigators (Y. H. R., M. S. R.) without any prior knowledge of each patient's clinicopathological information. When the opinions of the two evaluators were different, agreement was reached by careful discussion.

Statistical analysis

Associations between the fascin grade and the clinicopathological characteristics and TSP-1 and syndecan-1 expression patterns were analyzed using contingency tables. Statistical significance was evaluated with the χ^2 test. Univariate survival analysis for the influence of fascin expression on the overall survival was estimated according to the Kaplan–Meier method. Patients who died of unrelated causes were treated as censored cases. Statistically significant differences in survival distribution between the prognostic groups were evaluated by log-rank tests. Multivariate Cox proportional hazard regression was used to assess the prognostic significance of fascin expression and other clinicopathological characteristics on survival. Overall, 95% confidence intervals were used throughout. For all statistical tests, differences were considered significant when the *P* value was less than 0.05. The data were analyzed with the Statistical Package, SPSS 12.0 for Windows (SPSS, Chicago, IL, USA).

Results

Clinicopathological characteristics of the study group

The patients consisted of 14 (33%) men and 29 (67%) women, and they ranged in age from 40 to 80 years (median age: 64 years). The tumor size ranged from 0.5 to 10 cm (median size: 2.5 cm): 23 (53%) patients had tumors of 2.5 cm or less, while 20 (47%) patients had tumors of more than 2.5 cm. Histologically, the 43 tumors included 2 (5%) papillary adenocarcinomas, 36 (84%) tubular adenocarcinomas (15 well-differentiated, 14 moderately differentiated, and 7 poorly differentiated carcinomas), and 5 (11%) other histological types (1 mucinous adenocarcinoma, 1 signet ring cell carcinoma, 1 adenosquamous cell carcinoma, 1 squamous cell carcinoma, and 1 pleomorphic

giant cell carcinoma). According to the depth of invasion, 2 (5%) tumors invaded the mucosa, 7 (16%) tumors invaded the muscle layer, 25 (58%) tumors invaded the perimuscular tissue, and 9 (21%) tumors invaded beyond the serosa. There were 32 (74%) negative cases and 11 (26%) positive cases for lymphovascular invasion. There were 31 (72%) negative cases and 12 (28%) positive cases for lymph node metastases. The tumors were staged according to the TNM staging system: 9 (21%) were stage IA, 19 (44%) were stage IB, 3 (7%) were stage IIA, and 12 (28%) were stage IIB.

Immunohistochemical findings

Fascin immunoreactivity was not detected in the normal GB epithelial tissues, but positive staining was observed in the endothelial cells, lymphocytes, and stromal cells in the underlying lamina propria and muscle layer. In the GB cancer, fascin expression was localized in the cytoplasm of the tumor cells. There were 19 (44%) cases and 24 (56%) cases having low-grade and high-grade fascin expression, respectively (see Fig. 1a, b for examples).

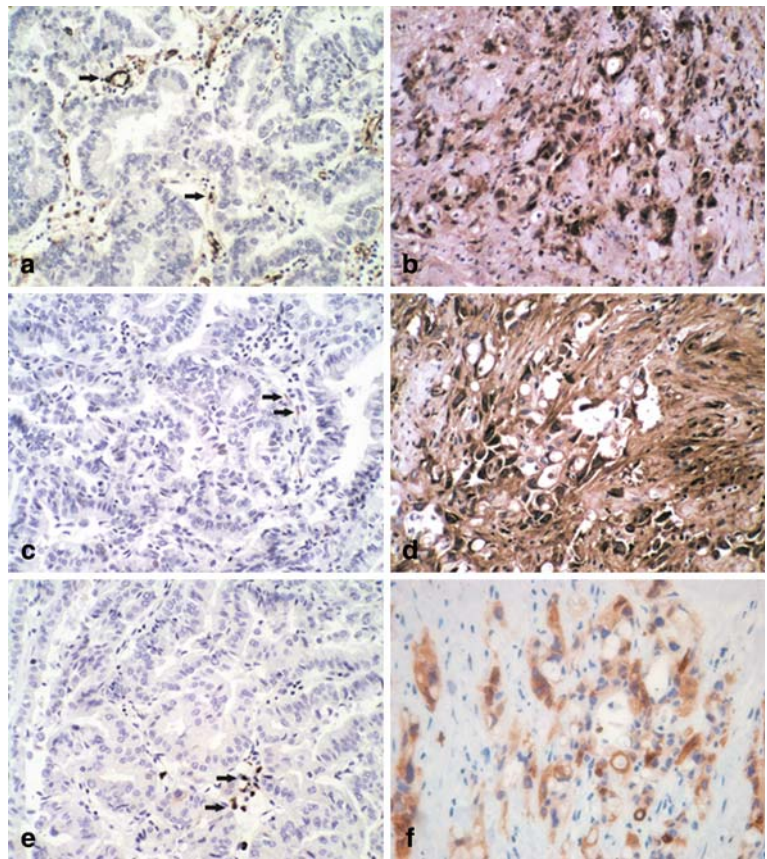
TSP-1 immunoreactivity was not detected in the non-neoplastic GB tissues including the mucosa and connective tissue, except for focal weak staining of stromal cells. In the GB cancer tissues, TSP-1 immunostaining was found in the stroma. TSP-1 immunoreactivity was observed in 29 (67%) of the 43 GB cancer cases (see Fig. 1c, d for examples).

Syndecan-1 immunoreactivity was not detected in the non-neoplastic GB tissues, including the mucosa and connective tissue, but the positively stained plasma cells acted as an internal positive control. In the GB cancer tissues, syndecan-1 was clearly expressed on the cell membranes and in the cytoplasm of the tumor cells. Syndecan-1 immunoreactivity was observed in 25 (58%) of the 43 GB cancer cases (see Fig. 1e, f for examples).

Correlation of fascin expression with clinicopathological characteristics

A variety of clinicopathological characteristics of the patients and their tumors were compared based on whether their tumors had low-grade fascin expression or high-grade fascin expression. The analysis revealed that fascin expression was correlated with the histological grade, invasion depth, lymph node metastasis, and AJCC stage, according to the grade of fascin expression. Tumors with high-grade fascin immunoreactivity tended to more frequently show poorer differentiation ($P = 0.044$), deeper invasion depth ($P = 0.008$), lymph node metastasis ($P = 0.023$), and a higher AJCC stage ($P = 0.019$). There was no significant association of fascin expression with

Fig. 1 Immunohistochemical findings of fascin (a, b), thrombospondin-1 (c, d), and syndecan-1 (e, f) in gallbladder cancer. A case of well-differentiated adenocarcinoma of gallbladder shows low-grade fascin expression (a), negative expression of thrombospondin-1 (c) and negative expression of syndecan-1 (e), whereas a case of poorly differentiated adenocarcinoma of gallbladder shows high-grade fascin expression (b), positive stromal and epithelial expression of thrombospondin-1 (d), and positive expression of syndecan-1 (f). Arrows internal positive controls. a Endothelial cells and lymphocytes between tumor glands are positive for fascin, c a few stromal cells are weakly positive for thrombospondin-1, and e plasma cells are positive for syndecan-1. a, b, c, d, e $\times 200$, f $\times 400$



age, gender, tumor size, or lymphovascular invasion (Table 1).

Influence of fascin expression on survival

Adequate clinical follow-up information was available for all 43 patients. The mean follow-up in the 43 patients was 38 months, and the range was 13–68 months. Sixteen patients (37%) were still alive, but 27 patients (63%) died during the follow-up period. Twenty-nine patients (67%) had disease recurrences during the follow-up period: 12 patients had distant metastasis to the lung, bone, and brain; 17 patients had locoregional recurrences. The mean duration from surgery to recurrence in these 29 patients was 17 months, with a range of 2–48 months. The patients with high-grade fascin expression had more frequent disease recurrence than those with low-grade fascin expression ($P = 0.026$).

The overall 5-year survival of the 43 patients was analyzed based on the endpoint of cancer-related death. Univariate survival analysis showed that the 5-year survival in the patients with low- or high-grade fascin expression was 63 and 17%, respectively; this difference was statistically significant. The Kaplan–Meier survival

curves demonstrated that patients with high-grade fascin expression had a significantly shorter survival than those with low-grade fascin expression ($P = 0.001$; Fig. 2).

Multivariate analysis using the Cox proportional hazard model revealed that there were no independent poor prognostic clinicopathological factors. Although high-grade fascin expression was not an independent poor prognostic factor for survival in patients with GB cancer, it was marginally significant ($P = 0.055$; Table 2).

Relationship between fascin expression and TSP-1 and syndecan-1 expression

Eight (42%) of the 19 cases with low-grade fascin expression and 21 (88%) of the 24 cases with high-grade fascin expression showed positive stromal TSP-1 expression ($P < 0.001$). Eight (42%) of the 19 cases with low-grade fascin expression and 17 (71%) of the 24 cases with high-grade fascin expression showed positive syndecan-1 expression ($P = 0.011$). The frequency of positive TSP-1 expression or positive syndecan-1 expression in the cases with high-grade fascin expression was significantly higher than that in the cases with low-grade fascin expression (Table 3).

Table 1 Relationship between fascin expression and conventional clinicopathological factors in 43 gallbladder cancer patients

Factors	Grade of fascin expression		P value
	Low, n = 19 (%)	High, n = 24 (%)	
Age (years)			0.086
≤64	11 (46)	13 (54)	
>64	8 (42)	11 (58)	
Gender			0.902
Male	6 (43)	8 (57)	
Female	13 (45)	16 (55)	
Tumor size			0.258
≤ 2.5 cm	12 (52)	11 (48)	
> 2.5 cm	7 (35)	13 (65)	
Histological type			0.044
Papillary	1 (50)	1 (50)	
Well	10 (67)	5 (33)	
Moderately	7 (50)	7 (50)	
Poorly	1 (14)	6 (86)	
Others	0 (0)	5 (100)	
Depth of tumor invasion			0.008
Mucosa	2 (100)	0 (0)	
Muscle layer	6 (86)	1 (14)	
Perimuscular connective tissue	10 (40)	15 (60)	
Beyond serosa	1 (11)	8 (89)	
Lymphovascular invasion			0.402
Negative	15 (47)	17 (53)	
Positive	4 (36)	7 (64)	
Lymph node metastasis			0.023
Negative	17 (55)	14 (45)	
Positive	2 (17)	10 (83)	
TNM stage			0.019
I (IA + IB)	16 (57)	12 (43)	
II (IIA + IIB)	3 (20)	12 (80)	
Recurrence			0.026
No	11 (79)	3 (21)	
Yes	8 (28)	21 (72)	

Others one mucinous adenocarcinoma, one signet ring cell carcinoma, one adenosquamous cell carcinoma, one squamous cell carcinoma, and one pleomorphic giant cell carcinoma

Discussion

In the present investigation, we have shown that fascin overexpression frequently occurred in GB cancer, and a subset of advanced GB cancers revealed a marked overexpression of fascin, which was associated with aggressive clinicopathological findings and poor overall survival. Although the multivariate analysis revealed that high-grade fascin expression was not an independent poor prognostic

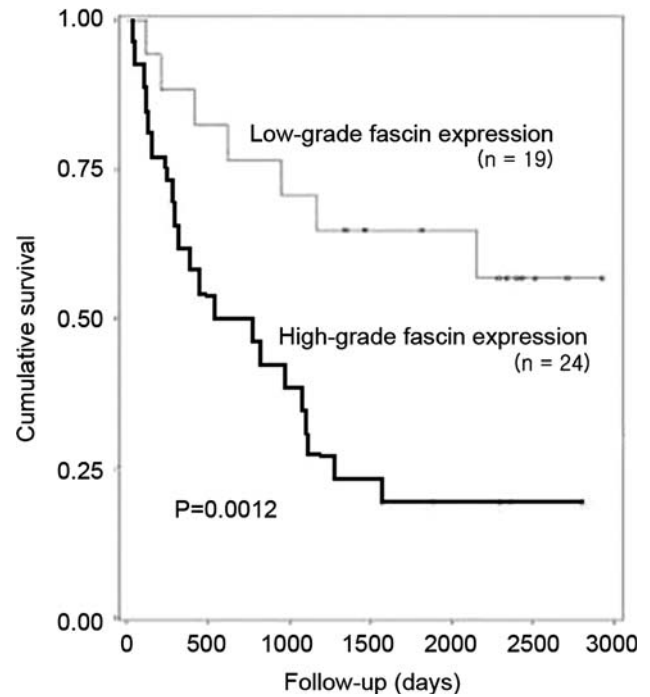


Fig. 2 Overall survival curves after surgical therapy, grouped by fascin expression, calculated by the Kaplan–Meier method. The high-grade fascin expression group (n = 24; thick line) had significantly worse survival than the low-grade fascin expression group (n = 19; thin line; P = 0.001)

factor, it was marginally significant. These findings suggest that high-grade fascin expression may contribute to the development or progression of GB cancer, and that this could be a novel prognostic marker. To the best of our knowledge, this is the first report about the relationship between fascin expression and prognosis in patients with GB cancer.

However, the precise underlying mechanism of fascin expression in the progression of primary GB cancer is still unclear. Yamashiro et al. [19] have reported that fascin transfection or protein microinjection into normal epithelial or mesenchymal cell lines induced remarkable changes in cell morphology: an increased number of longer and thicker microvilli on apical surfaces, extended lamellipodia-like structures at basolateral surfaces, and disorganization of cell–cell contacts. This finding supports the notion that fascin overexpression may affect the differentiation of GB cancers via the remodeling of cell shape and volume, and the aggressive status via changes in cell motility.

Functional interactions with extracellular matrix molecules and polypeptide factors may provide further mechanisms for the cellular regulation of fascin [4]. Transfection of COS-7 cells with syndecan-1 was sufficient to stimulate cell spreading, fascin spike assembly, and extensive protrusive lateral ruffling [16]. TSP-1 has distinct

Table 2 Cox multivariate analysis to determine the independent prognostic value of different variables in relation to overall survival in 43 gallbladder cancer patients

Covariate	Risk ratio (95% confidence interval)	P value
Age (≤ 64 vs. >64 years)	1.08 (0.79–1.58)	0.421
Gender (male vs. female)	1.56 (0.78–2.92)	0.210
Tumor size (≤ 2.5 vs. >2.5 cm)	2.74 (0.92–8.16)	0.070
Histological type (papillary/well/moderately/poorly/others)	0.78 (0.49–1.26)	0.313
Depth of tumor invasion (mucosa/muscle layer/perimuscular connective tissue/beyond serosa)	0.78 (0.22–2.76)	0.704
Lymphovascular invasion (– vs. +)	2.62 (0.93–7.45)	0.070
Lymph node metastasis (– vs. +)	1.03 (0.07–1.47)	0.144
TNM stage (I vs. II)	3.39 (1.59–19.56)	0.072
Fascin (low vs. high)	2.64 (1.69–10.05)	0.055

Others one mucinous adenocarcinoma, one signet ring cell carcinoma, one adenosquamous cell carcinoma, one squamous cell carcinoma, and one pleomorphic giant cell carcinoma

Table 3 Relationship between fascin expression and thrombospondin-1 and syndecan-1 expression in 43 gallbladder cancer patients

Factors	Grade of fascin expression		P value
	Low, <i>n</i> = 19 (%)	High, <i>n</i> = 24 (%)	
Thrombospondin-1			<0.001
Negative	11 (58)	3 (12)	
Positive	8 (42)	21 (88)	
			0.011
Syndecan-1			
Negative	11 (58)	7 (29)	
Positive	8 (42)	17 (71)	

activities in inducing cells to form fascin-containing protrusions, and this activity of TSP-1 is dependent on syndecan-1 [15, 16]. However, any data about the interrelationship between fascin, TSP-1, and syndecan-1 are not well-defined. Our analysis of the immunohistochemical expression of fascin, TSP-1, and syndecan-1 in GB cancers is an attempt to reveal a possible relationship between these three molecules. Our findings suggest that fascin, TSP-1, and syndecan-1 may act in concert to mediate a more aggressive clinical course through enhanced tumor cell motility.

GB cancer is one of the most biologically virulent malignancies and it is notoriously difficult to cure. The majority of GB cancer patients are usually diagnosed at an advanced stage, which is so difficult to cure by surgery alone [2]. Thus, it is important to identify new molecular targets for its treatment and for the prognostic factors, in addition to the TNM stage. In the present study, high-grade

fascin expression was significantly associated with shortened 5-year survival in GB cancer patients. In clinical practice, for an individual patient with an advanced GB cancer, if the tumor shows high-grade fascin expression, close follow up should be carried out to detect possible recurrence, and adjuvant therapy may also be beneficial. Hashimoto et al. [8] have reported that they observed fascin downregulation and decreased motility and invasiveness in an esophageal squamous cell carcinoma cell line by using the vector-based siRNA. Although there still remains the question of which pathway contributes to the overexpression of fascin protein in GB cancer, downregulation of tumor-specific fascin may also become a novel therapeutic strategy.

In conclusion, the results of our study suggest that a subset of advanced GB cancers revealed a marked overexpression of fascin, which was associated with an aggressive clinical course and poor overall survival. Furthermore, fascin, TSP-1, and syndecan-1 may act in concert to mediate a more aggressive course through enhanced tumor cell motility. However, our study was limited by the number of study cases, with relatively small and heterogeneous histological and stage subgroups, and by the study being retrospective. Further prospective investigations with a large number of cases would allow us to evaluate fascin in a variety of clinical settings to help us better understand its unique role in GB cancer progression. Further molecular studies, such as reverse transcriptase polymerase chain reaction (RT-PCR) or Western analysis, are needed to substantiate the immunohistochemical findings, and such studies may provide a better understanding of the relationship between the fascin, TSP-1, and syndecan-1 expressions involved in the regulation of the biological behavior of GB cancer.

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References

1. Parkin DM, Muir C. Cancer incidence in five continents. Comparability and quality of data. IARC Sci Publ. 1992;120:45–173.
2. Benoist S, Panis Y, Fagniez PL. Long-term results after curative resection for carcinoma of the gallbladder. Am J Surg. 1998;175:118–22.
3. Shirai Y, Tsukada K, Ohtani T, Watanabe H, Hatakeyama K. Hepatic metastases from carcinoma of the gallbladder. Cancer. 1995;75:2063–8.
4. Kureishy N, Sapountzi V, Prag S, Anilkumar N, Adams JC. Fascins, and their roles in cell structure and function. Bioessays. 2002;24:350–61.
5. Goncharuk VN, Ross JS, Carlson JA. Actin-bundling protein fascin expression in skin neoplasia. J Cutan Pathol. 2002;29:430–8.

6. Grothey A, Hashizume R, Sahin AA, McCrea PD. Fascin, an actin-bundling protein associated with cell motility, is upregulated in hormone receptor negative breast cancer. *Br J Cancer*. 2000;83:870–3.
7. Hashimoto Y, Shimada Y, Kawamura J, Yamasaki S, Imamura M. The prognostic relevance of fascin expression in human gastric carcinoma. *Oncology*. 2004;67:262–70.
8. Hashimoto Y, Ito T, Inoue H, Okumura T, Tanaka E, Tsunoda S, et al. Prognostic significance of fascin overexpression in human esophageal squamous cell carcinoma. *Clin Cancer Res*. 2005;11:2597–605.
9. Hu W, McCrea PD, Deavers M, Kavanagh JJ, Kudelka AP, Verschraegen CF. Increased expression of fascin, motility associated protein, in cell cultures derived from ovarian cancer and in borderline and carcinomatous ovarian tumors. *Clin Exp Metastasis*. 2000;18:83–8.
10. Jawhari AU, Buda A, Jenkins M, Farthing MJ, Pignatelli M, Adams JC. Fascin, an actin-bundling protein, modulates colonic epithelial cell invasiveness and differentiation in vitro. *Am J Pathol*. 2003;162:69–80.
11. Maitra A, Iacobuzio-Donahue C, Rahman A, Sohn TA, Argani P, Meyer R, et al. Immunohistochemical validation of a novel epithelial and a novel stromal marker of pancreatic ductal adenocarcinoma identified by global expression microarrays: sea urchin fascin homolog and heat shock protein 47. *Am J Clin Pathol*. 2002;118:52–9.
12. Pelosi G, Pastorino U, Pasini F, Maissonneuve P, Frassetto F, Iannucci A, et al. Independent prognostic value of fascin immunoreactivity in stage I nonsmall cell lung cancer. *Br J Cancer*. 2003;88:537–47.
13. Tong GX, Yee H, Chiriboga L, Hernandez O, Waisman J. Fascin-1 expression in papillary and invasive urothelial carcinomas of the urinary bladder. *Hum Pathol*. 2005;36:741–6.
14. Adams JC. Roles of fascin in cell adhesion and motility. *Curr Opin Cell Biol*. 2004;16:590–6.
15. Adams JC, Schwartz MA. Stimulation of fascin spikes by thrombospondin-1 is mediated by the GTPases Rac and Cdc42. *J Cell Biol*. 2000;250:807–22.
16. Adams JC, Kureishy N, Taylor AL. A role for syndecan-1 in coupling fascin spike formation by thrombospondin-1. *J Cell Biol*. 2001;152:1169–82.
17. Albores-Saavedra J, Scoazec JC, Wittekind C, Sripa B. Carcinoma of the gallbladder and extrahepatic bile ducts. In: Hamilton SR, Alatonen LA, editors. World Health Organization classification of tumours. Pathology and genetics of tumors of the digestive system. Lyon: IARC Press; 2004. p. 206–13.
18. Fritz A, Balch C, Haller DG, Morrow M. Gallbladder and extrahepatic bile ducts. In: Greene F, Page D, Fleming I, editors. American Joint Committee on cancer staging manual. 6th ed. Tokyo: Springer; 2002. p. 139–50.
19. Yamashiro S, Yamakita Y, Ono S, Matsumura F. Fascin, an actin-bundling protein, induces membrane protrusions and increases cell motility of epithelial cells. *Mol Biol Cell*. 1998;9:993–1006.