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Clinicopathological Significance of Fatty Acid Synthase Expression in Extrahepatic Cholangiocarcinoma

Maekawa Hiroshi[1]*, Ito Tomoaki[2], Tomoyuki Kushida[1], Orita Hajime[1], Mutsumi Sakurada[1], Sato Koichi[1] and Wada Ryo[2]

[1]*Department of Surgery, Juntendo University School of Medicine Shizuoka Hospital, Izu-no-kuni City, Shizuoka, Japan*
[2]*Department of Pathology, Juntendo University School of Medicine Shizuoka Hospital, Izu-no-kuni City, Shizuoka, Japan*

Abstract

Objective: Here, we investigated whether FAS expression in extrahepatic cholangiocarcinoma is related to clinicopathological features.

Methods: We studied 37 patients surgically treated for extrahepatic cholangiocarcinoma in our hospital. We compared FAS immunohistochemical staining with other pathological features, including Ki-67, p53 staining, and clinical findings using univariate analysis.

Results: Of 37 cases, 20 cases were considered FAS-positive under our criteria. Pathologically, FAS positivity was not correlated with either Ki-67 positivity or p53 positivity (p: 0.1099, 0.0878). FAS expression was not related to differentiation of adenocarcinoma (p: 0.9350). However, a higher rate of FAS-positive cases was found in lymph node metastases (p: 0.0102). Clinically, FAS-positive cases showed earlier recurrence compared to FAS-negative cases (p: 0.0238); though there was no significant difference in overall survival rate between FAS-positive and FAS-negative cases (p: 0.1398).

Conclusion: FAS expression may be related to lymph node metastasis and clinical behavior in extrahepatic cholangiocarcinoma, and may be useful as a prognostic marker of this cancer.

Keywords: Extrahepatic; Cholangiocarcinoma; Fatty acid synthase; Immunohistochemistry

Introduction

Fatty acid synthase (FAS) contributes to cellular proliferation by producing middle chain fatty acids [1]. FAS expression is regulated and inhibited by several hormones in normal tissues [2]. In cancer cells, FAS expression is often up-regulated without suppression, and de novo biosynthesis of fatty acids is increased under FAS up-regulation. Middle chain fatty acids are used for various cellular activities such as an energy source for metabolism [3], in building cellular membranes, and in intracellular second messengers [4,5]. We previously reported that immunohistochemical FAS expression was correlated with clinical outcomes in patients with pancreatic cancer and cancer of the papilla of Vater [6,7]. Patients with high FAS expression also showed shorter survival.

Extrahepatic cholangiocarcinoma has poor prognoses in clinical practice. However, the relationship between FAS expression and clinical outcome in extrahepatic cholangiocarcinoma is still unclear.

Aims

- To investigate clinicopathological significance of FAS expression in extrahepatic cholangiocarcinoma in our hospital.

- The aim of this study is to investigate the relationship between the immunohistochemical expression of FAS and clinicopathological features in extrahepatic cholangiocarcinoma. In addition, we investigated whether FAS expression could be used as a prognostic marker for extrahepatic cholangiocarcinoma.

Materials and Methods

We studied 37 patients with carcinoma of the distal biliary tract between January 2002 and January 2015 in our hospital. Clinical data, such as gender, age, recurrence, and prognoses, were obtained from clinical records. Pathological diagnoses were performed using hematoxylin and eosin staining. We investigated immunohistochemical expression of FAS, Ki-67, and p53 in formalin-fixed and paraffin-embedded tissue sections from carcinoma specimens.

We compared FAS expression with clinical information. Pathological characteristics, vessel involvement, node metastasis, tumor differentiation, and Ki-67 and p53 staining were also compared with FAS expression. The study protocol conformed to the ethical guidelines of the World Medical Association Declaration of Helsinki, and has been approved by the ethical committee in our hospital.

Immunohistochemical procedure for FAS expression

We used 4 μm tissue sections from formalin-fixed and paraffin-embedded specimens. Sections were deparaffinized by treatment with xylene for 3 min twice and rehydrated by passage through 100% ethanol for 30 sec (x3). Antigen retrieval was performed by microwaving slides in 10 mM sodium citrate buffer (T.R.S) obtained from Dako (Carpinteria, CA, USA) for 8 min. Endogenous peroxidase activity was quenched by incubating slides in 3% H_2O_2 for 5 min. Slides were then washed twice in tris buffered saline -0.05% Tween 20 (TBST) for 5 min each time and blocked with a solution containing 5% goat serum for 15 min at room temperature. Samples were incubated with the primary antibody for 60 min at room temperature. Anti-human FAS rabbit IgG

***Corresponding author:** Maekawa Hiroshi, Associate Professor, Department of Surgery, Juntendo University School of Medicine Shizuoka Hospital, 1129 Nagaoka, Izu-no-kuni City, Shizuoka, 410-2295 Japan, Tel: +81 55.948.3111; E-mail: hmaekawa0201@gmail.com

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was obtained from Immuno-Biological Laboratories Co., Ltd. (Fujioka, Gunma, Japan). Slides were washed three times with TBST for 5 min each time and incubated with secondary antibody, anti-rabbit IgG (Envison+/HRP from DAKO), for 60 min at room temperature. Slides were washed three times in TBST for 5 min each time and incubated with diaminobenzidine solution containing 20 mg/ml DAB-4HCl in 0.01M PB and 30% H_2O_2 until color production developed. Slides were then stained with hematoxylin, dehydrated, and mounted with coverslips.

Immunohistochemical procedure for Ki-67 and p53 expressions

Immunoperoxidase assays for p53 and Ki-67 analysis were performed using a commercially available kit obtained from Roche Diagnostics Co., Ltd. (Tokyo, Japan) in the same manner as described for the immunohistochemical analysis of FAS. Monoclonal mouse anti-human Ki-67 and p53 antigens were obtained from Dako (Carpinteria, CA, USA).

Scoring of immunoreactivity

We compared FAS staining of carcinoma tissue with staining of adipose tissue using the following criteria. Classification of cellular FAS positivity was as follows: negative for staining or stained less than adipose cells, or positive for staining to the same degree as adipose or stained more than adipose cells.

Tissue FAS expression was scored using the following scale: 0 for less than 10% of tumor cells were positive, 1 for 10% to 30% of tumor cells were positive, 2 for 30% to 50% of tumor cells were positive, 3 for over 50% of tumor cells were positive. Finally, scores of 0 and 1 were combined to represent negative FAS expression, and scores of 2 and 3 were combined to represent positive FAS expression (Figure 1).

Expression of p53 and Ki-67 were similarly classified into the same 4 groups as described for FAS expression in tissue sections (Figures 2 and 3). We compared FAS scores with Ki-67 scores or p53 scores.

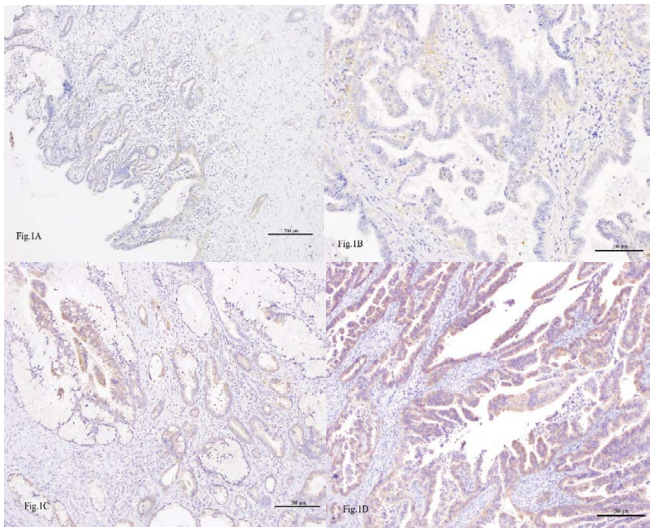

Figure 1: Sample of FAS expression in immunohistochemical staining. (A) In FAS-negative cases, (FAS score 0) cellular cytoplasm were weakly stained and less than 10% of cells were positively stained. Scale bar shows 200 μm. (B) (FAS score 1) 10%-30% of cancer cells were positively stained. Scale bar shows 200 μm. (C) In FAS-positive cases, (FAS score 2) 30%-50% of cancer cells were positively stained. Scale bar shows 200 μm. (D) (FAS score 3) Over 50% of cancer cells were positively stained. Scale bar shows 200 μm.

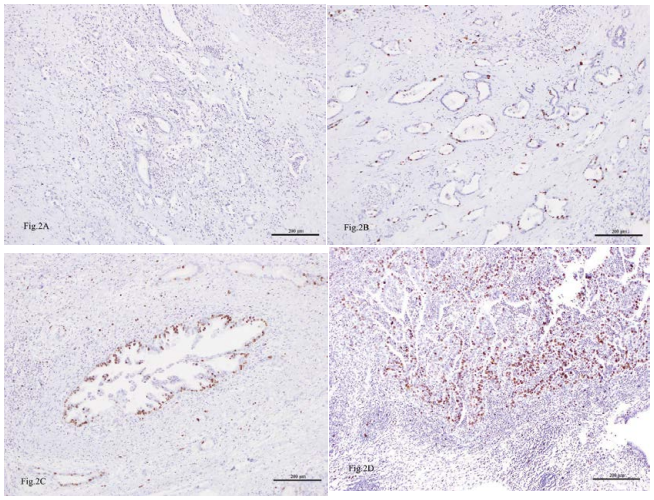

Figure 2: Sample of Ki-67 expression in immunohistochemical staining. (A) In Ki-67-negative cases, (Ki-67 score 0) nuclei were weakly stained and less than 10% of cells were positively stained. Scale bar shows 200 μm. (B) (Ki-67 score 1) 10%-30% of cancer cells were positively stained. Scale bar shows 200 μm. In Ki-67 positive cases, (C) (Ki-67 score 2) 30%-50% of cancer cells were positively stained. Scale bar shows 200 μm. (D) (Ki-67 score 3) Over 50% of cancer cells were positively stained. Scale bar shows 200 μm.

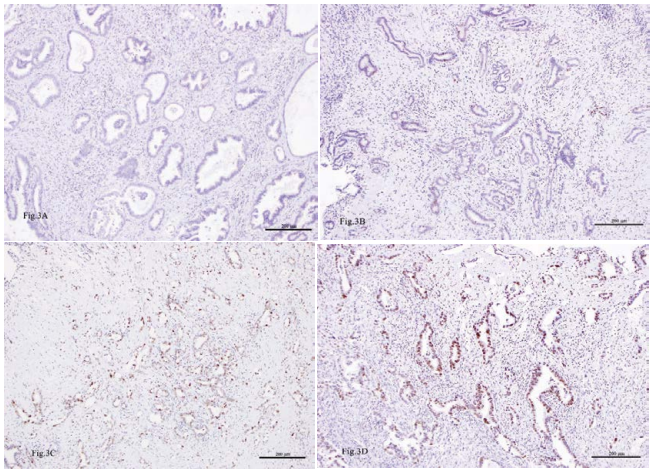

Figure 3: Sample of p53 expression in immunohistochemical staining. (A) In p53 negative cases, (p53 score 0) nuclei were weakly stained and less than 10% of cells were positively stained. Scale bar shows 200 μm. (B) (p53 score 1) 10%-30% of cancer cells were positively stained. Scale bar shows 200 μm. (C) In p53 positive cases, (p53 score 2) 30%-50% of cancer cells were positively stained. Scale bar shows 200 μm. (D) (p53 score 3) Over 50% of cancer cells were positively stained. Scale bar shows 200 μm.

Statistical analysis

The relationship between scores of FAS expression and those of Ki-67 or p53 expression was analyzed using the Spearman's correlation coefficient by rank test. Cut-off end-points were determined according to observed positive and negative immunohistochemical tissue FAS expression levels.

Positive FAS expression levels were tested for association with clinicopathological features using the Fisher's exact test or Mann-Whitney's test. Disease-free survival rate and overall survival rate were estimated using the Kaplan-Meier method and survival between the groups was compared using log-rank tests. All analyses were conducted

Gender (Male/Female)	24 / 13
Age **(**mean)	70.8
Clinical stage (UICC 6th edition) (cases)	IB 12 / IIA 14 / IIB 11
Recurrences (cases)	13
Follow-up (month)	27.9 (2-152)

Table 1: Clinical features of 37 cases of extrahepatic cholangiocarcinoma.

Pathological features	FAS-positive 20 cases	FAS-negative 17 cases	p value
High vessel involvement (%)	45	47.1	1
High perineural invasion (%)	50	58.8	0.7433
Node metastasis (%)	45	5.9	0.0102*
Differentiation: well/moderate/ poor (cases)	12/7/1	11/5/1	0.935
Ki-67 positivity (>30%) (%)	80	52.9	0.1575
P53 positivity (>30%) (%)	65	58.8	0.7447
Gross inspection: nodular/ infiltrative (cases)	6/14	9/8	0.1931
Tumor size: maximum diameter (cm)	2.6	2.8	0.5497
High vessel involvement means ly2,3 or v2,3 grade. High perineural invasion means ne2,3. ly: lymphatic vessel involvement. v: venous involvement. ne: perineural invasion * *p* values less than 0.05 were considered significant.			

Table 2: Pathological features of extrahepatic cholangiocarcinoma cases according to FAS immunohistochemical staining.

Clinical features	FAS-positive 20 cases	FAS-negative 17 cases	*p* value
Gender (M/F)	14/6	10/7	0.5119
Age (mean)	72.7	69.8	0.1835
Preoperative CA19-9 level (IU/L)	762.3	197.7	0.6644
Diabetes Mellitus complication (%)	15	11.8	1.0000
Clinical stage (UICC 6th edition) (cases)	I_B 7/ II_A 4/ II_B 9	I_B 5/ II_A 10/ II_B 2	0.0278*
Recurrence (%)	50	17.6	0.0823
Follow-up time (months)	25.6	37.2	0.4736
* *p* values less than 0.05 were considered significant.			

Table 3: Clinical features of extrahepatic cholangiocarcinoma cases according to FAS immunohistochemical staining.

using the GraphPad Prizm5®software statistic package (GraphPad Software Inc., La Jolla, CA, USA).

P values of less than 0.05 were considered significant.

Results

The clinical features of the 37 patients are shown in Table 1. Twenty-four patients were male and 13 were female. The mean age of patients was 70.8-year-old. Twelve cases were stage IB, 14 were stage IIA, and 11 were stage IIB (UICC classification 6th edition). The 37 cases were adenocarcinoma and recurrences were noted in 13 patients. These 13 patients died due to cholangiocarcinoma. Four other patients died of other diseases. The mean follow-up time was 27.9 months (ranging from 2 to 152 months).

Correlation between FAS expression and Ki-67 or p 53 expression

Correlation between scores of FAS expression and scores of Ki-67 expression in tissues was analyzed. FAS positivity was not correlated with Ki-67 positivity (p: 0.1575). Correlation between scores of FAS expression and scores of p53 expression in tissues was also analyzed. However, there was no relationship between scores of FAS positivity and scores of p53 positivity (p: 0.7447).

Relation between pathological features and FAS expression

The pathological features of both FAS positive cases and FAS negative cases are shown in Table 2. There were no differences in high vessel involvement, high perineural invasion or tumor differentiation (p: 1.000, 0.7433, 0.9350). Gross inspection and tumor size also did not significantly differ in both groups. Lymph node metastasis was often seen in FAS-positive groups compared with FAS-negative groups (p: 0.0102).

Relation between clinical features and FAS expression

The clinical features of the two groups were also analyzed. There were no significant differences between the two groups in gender, age, preoperative levels of CA19-9, diabetes mellitus complication, or follow-up time. Clinical stages were different between FAS positive group and negative group. Recurrences were seen in 50% of cases in the FAS-positive group, and 17.6% of cases in the FAS-negative group (Table 3).

Overall survival and disease-free survival according to FAS expression

Recurrences were seen in both FAS-positive and FAS-negative groups. Disease-free survival curves and overall survival curves were estimated using the Kaplan-Meier method, as shown in Figures 4 and 5. Disease-free survival rate of FAS-positive cases was lower than that of FAS-negative cases (p: 0.0238). However, there was no significant difference in overall survival rate between FAS-positive cases and FAS-negative cases (p: 0.1398). Additionally, we analyzed whether disease-free rate and overall survival rate differed between cases positive or negative for Ki-67 or p53 expression. Both disease-free rate and overall survival rate was not associated with by positivity for Ki-67 or p53 (data not shown).

Discussion

Extrahepatic cholangiocarcinoma is associated with poor prognosis, and its incidence is increasing in clinical practice [8,9].

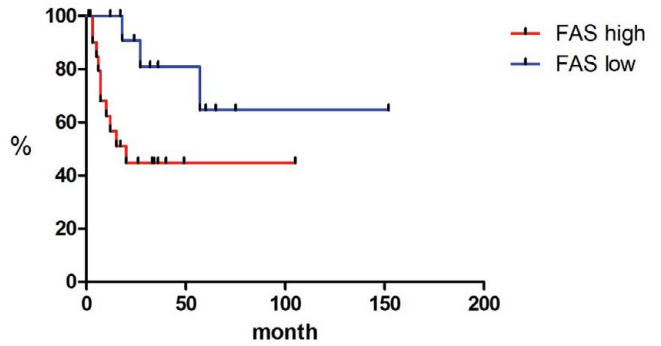

Figure 4: Kaplan-Meier curves related to FAS expression in disease-free survival. Disease survival rate of FAS-positive cases was lower than that of FAS-negative cases (*p*: 0.0238). The red line shows FAS-positive cases and the blue line shows FAS-negative cases.

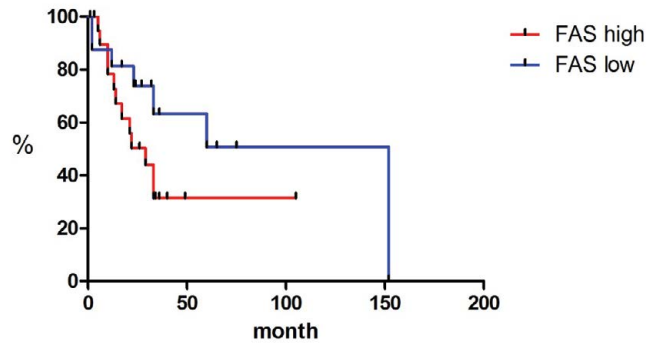

Figure 5: Kaplan-Meier curves related to FAS expression in overall survival. There was no significant difference in overall survival rate between FAS-positive cases and FAS-negative cases (*p*:0.1398). The red line shows FAS-positive cases and the blue line shows FAS-negative cases.

Reported prognostic factors for cholangiocarcinoma include: node metastasis [10-14], lymphovasclar invasion [14] in histology, p53 [15], c-Met [16] or c-erbB-2 [17] expression in immunohistochemistry, and EGFR mutation [18] in molecular pathology. Clinically, margin-negative resection [19,20] and adjuvant therapies after resection improve the prognosis of this cancer [21-23].

FAS is a lipogenic enzyme that facilitates de novo lipid biosynthesis. The products of this process, fatty acids, contribute to cellular proliferation [3]. Therefore, FAS overexpression is related to cancer progression and cancer aggressiveness. In various kinds of cancers, FAS overexpression results in a high incidence of recurrence or shorter survival, even after curative resection [6,7].

Coleman et al. suggested that *in vitro* c-Met protein expression affects FASN activity [24]. Miyamoto et al. demonstrated that c-Met overexpression is associated with EGFR expression and is a poor prognostic factor for cholangiocarcinoma [16]. Thus, it may be concluded that FAS expression is related to the prognosis of cholangiocarcinoma.

In our study, high FAS expression was associated with a high incidence of node metastasis and earlier recurrence, indicating that node metastasis is a prognostic factor for extrahepatic cholangiocarcinoma. FAS expression, c-Met expression, and node metastasis may be related in extrahepatic cholangiocarcinoma.

Despite p53 expression being previously reported to be a prognostic factor for extrahepatic cholangiocarcinoma [15], in the present study, p53 and Ki-67 expression were not found to be related to node metastasis or prognosis of extrahepatic cholangiocarcinoma.

FAS expression was related to lymph node metastasis and prognoses in extrahepatic cholangiocarcinoma. We concluded that FAS positivity was associated with tumor aggressiveness in extrahepatic cholangiocarcinoma. However, we could not find a significant association between p53 and Ki-67 positivity and clinical behavior of extrahepatic cholangiocarcinoma. Due to the small sample size of our study, future studies in more cases will be beneficial for determining the relationship between FAS expression and survival rate for extrahepatic cholangiocarcinoma [16-24].

Conclusion

FAS expression may be related to clinical behavior in extrahepatic cholangiocarcinoma with high incidence of lymph node metastasis. FAS-positive cases of extrahepatic cholangiocarcinoma show earlier recurrence compared with FAS-negative cases. This study Our study contains a small number of samples, so we need to accumulate more cases for further consideration in order to determine FAS expression relates clinical behavior.

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