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Loss of F-Box and Leucine Rich Repeat Protein 5 (FBXL5) Expression Is Associated With Poor Survival in Patients With Hepatocellular Carcinoma After Curative Resection: A Two-institute Study

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Abstract. *Background/Aim: Alteration of F-box and leucine-rich repeat protein 5 (FBXL5), an iron-sensing ubiquitin ligase, might be related with carcinogenesis of hepatocellular carcinoma (HCC), by disturbing cellular iron homeostasis. However, the clinical implications of FBXL5 expression using patient samples need to be elucidated. Patients and Methods: We collected HCC tissue samples from two institutes: Samsung Medical Center (n=259) and Hallym University Sacred Heart Hospital (n=115) and evaluated FBXL5 expression using immunohistochemistry. Using cut-off values determined by X-tile software, association between FBXL5 expression and several clinicopathological parameters was investigated. For external validation, the Cancer Genome Atlas (TCGA) cohort was used. Results: The best cutoff value for FBXL5 IHC expression associated with recurrence-free survival (RFS) was 5%. Low FBXL5 expression was found in 18.7% of the total 374 HCCs and was associated with non-viral etiology (p=0.019). Low FBXL5 expression was related with inferior disease-specific survival (DSS, p=0.002) and RFS (p=0.001) and also was an independent prognostic factor for DSS and RFS. In addition, cases with low FBLX5 mRNA levels showed inferior DSS and RFS (p<0.001 and p=0.002, respectively) compared to high FBLX5 mRNA levels in the TCGA cohort. Conclusion: Down-regulation of FBXL5 expression in HCCs might be associated with poor prognosis. FBXL5 might be a prognostic biomarker of HCCs and a potential therapeutic target in conjunction with iron homeostasis.*

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Key Words: Hepatocellular carcinoma, prognosis, FBXL5, disease-specific survival, The Cancer Genome Atlas.

Owing to high tumor recurrence, metastasis, and lack of treatment options, patients with hepatocellular carcinoma (HCC) show poor prognosis (1). Advances in molecular biology techniques (2-7) and multiple clinical trials have led to the approval of multiple drugs, including multikinase inhibitors and immunotherapy, by the United States Food and Drug Administration (FDA) (8). However, the limited treatment effect of targeted therapy and immune inhibitors necessitates the investigation of novel therapeutic targets and reliable biomarkers for more effective HCC treatments (8, 9).

Disturbances in cellular iron homeostasis are known to be related to hepatocarcinogenesis (10). Individuals with hereditary hemochromatosis have a 200-fold greater risk of developing HCC than the general population (11). Excessive hepatic iron is a risk factor for HCC development in patients with various etiologies, such as non-alcoholic fatty liver disease

(12, 13), hepatitis C virus (HCV) infection (14), hepatitis B virus infection (15, 16) and alcoholic cirrhosis (17).

F-box and leucine-rich repeat protein 5 (FBXL5), along with iron regulatory protein 2 (IRP2), is known to be involved in regulating cellular iron levels (18). Previous studies using FBXL5 knockout mice revealed iron accumulation in the liver and steatohepatitis (18), higher HCC incidence, and increased tumor size compared to FBXL5 intact mice, indicating that deficiency of FBXL5 is involved in hepatic carcinogenesis (19). Alterations in iron metabolism-related proteins seem to be related with the prognosis of diverse cancers, such as prostate, breast, pancreatic and esophageal cancer, and renal cell carcinoma (20-24). However, FBXL5 expression and its clinical implications have not yet been reported in samples from patients with HCC.

In this study, we investigated FBXL5 expression in HCC patient samples collected from two institutes and evaluated its prognostic effect as well as its association with clinicopathological parameters.

Patients and Methods

Patients and samples. Samples were collected from two independent institutes under identical inclusion criteria: 1) no history of prior treatment for HCC before surgery, 2) histologically confirmed HCC, and 3) complete tumor resection with clear resection margins.

In Samsung Medical Center (SMC), Seoul, Republic of Korea, between July 2000 and May 2006, a total of 291 patients had curative resection for primary HCC. After excluding eight patients with preoperative local treatment, such as radiofrequency ablation, transarterial chemoembolization, or radiotherapy, and 24 patients with insufficient tissue on tissue microarray, 259 patients were included in this study. In Hallym University Sacred Heart Hospital (HUMC), Anyang, Republic of Korea, 115 patients treated with surgical resection as first-line treatment for HCC between January 2011 and December 2015, satisfied all the inclusion criteria and were all selected.

All the included samples were histologically confirmed and showed negative resection margins. Curative resection was defined as the absence of residual tumor one month after surgery. The American Joint Committee on Cancer (AJCC) staging system, 8th edition (25) and Barcelona Clinic Liver Cancer (BCLC) staging classification (26) were used for tumor staging. Definition for intrahepatic metastasis and multicentric occurrence were determined by previously reported criteria (27). All patients were followed-up every 3 months after the operation, with three-phase dynamic computed tomography scans or magnetic resonance imaging and serum alpha-fetoprotein (AFP) levels. Optimal treatment was administered when tumor recurrence was confirmed by these examinations. Patient death was established based on death certificates or telephone follow-up. Recurrence-free survival (RFS) or disease-specific survival (DSS) was defined as the difference between the date of surgery and the date of recurrence or HCC-related death, respectively, as previously described (28).

The Institutional Review Boards of SMC (2021-04-151) and HUMC (2021-09-008-001) approved this study and waived the requirement for informed consent.

Immunohistochemical studies. Using tissue microarray consisting of two 2 mm cores of HCC tissue, immunohistochemistry (IHC) was performed as previously described (29). After antigen retrieval using 100 ml of ER1 buffer (Leica Biosystems, Melbourne, Australia), the sections were incubated with rabbit anti-FBXL5 antibody (ab140175, 1:200, Abcam, Cambridge, USA) for 60 min in a Bond-max autoimmunostainer (Leica Biosystems). Antigen-antibody chromogenic reactions were developed for 10 min using the BondTM Polymer Refine Detection Kit DS9800 (Vision Biosystems, Melbourne, Australia). Normal muscular tissue was used as a positive control. Immunostaining intensity was evaluated as negative, faint, moderate (light brown) and strong (dark brown), as previously described (30, 31). Representative pictures of each staining intensity are presented in Figure 1. Moderate to strong cytoplasmic intensity of FBXL5 IHC staining was considered positive, and the proportion of positive staining among the tumors was assessed.

mRNA expression of FBXL5 using the Cancer Genome Atlas (*TCGA) data*. To investigate the relationship between mRNA expression of FBXL5 and various clinicopathological factors, public data from TCGA database were used. The association of FBXL5 expression with DSS and RFS was evaluated by the Kaplan-Meier (KM)-plotter database in liver cancer (http://kmplot.com/analysis) (32).

Statistical analysis. The cut-off value of FBXL5 expression with the most significant difference in RFS was calculated using the X-tile bioinformatics software (Yale University, New Haven, CT, USA) (33). For analyzing the relationships between FBXL5 expression and clinicopathological parameters, Pearson's chi-square tests, Fisher's exact tests, or Cochran Armitage test were used, as appropriate. The Kaplan–Meier method was used to analyze survival rates, and differences were compared using the log-rank test. Cox proportional hazards model was applied for multivariate regression analysis. Statistical significance was set at $p<0.05$. The IBM SPSS software for Windows (IBM Corp., Armonk, NY, USA) was used for statistical analysis.

Results

FBXL5 IHC staining in HCC in conjunction with clinico-pathologic features. FBXL5 IHC showed cytoplasmic expression, and its intensity and proportion of expression varied among the HCC cases. No staining or faint intensity of FBXL5 staining was considered negative (Figure 1A and B), whereas moderate to strong intensity of FBXL5 staining was considered positive (Figure 1C and D). The proportion of positive staining in HCCs ranged from 0 to 100%, with a mean of 56.59% and a median of 60.00%. Using X-tile analysis (33), the best cutoff value for FBXL5 IHC expression associated with RFS was 5%. Low FBXL5 IHC expression was observed in 18.7% of the 374 HCCs and was more frequently observed in HCC with non-viral etiology than in HCC with viral etiology (30% *vs*. 16.6%, $p=0.019$, Table I). This correlation was also significant when analyzed separately by institutes (Table II).

Impact of FBXL5 expression on the survival of HCC patients Patients with low expression of FBXL5 IHC showed shorter RFS ($p=0.001$, Figure 2A) and DSS ($p=0.002$,

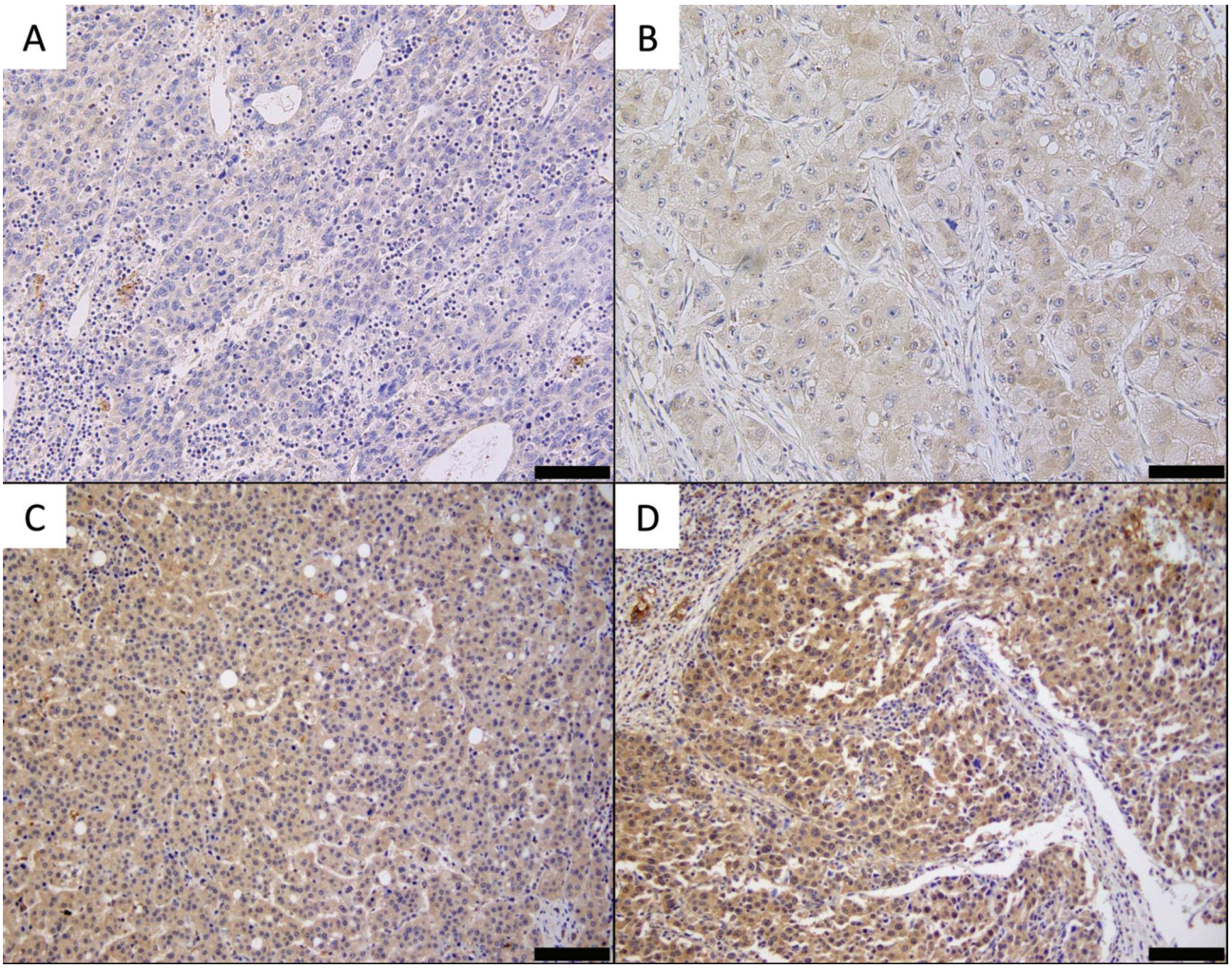

Figure 1. *Representative figures of FBXL5 immunohistochemistry. Tumor cells show cytoplasmic staining: negative (A), faint (B), moderate (C), and strong staining (D). The scale bar indicates 500 μm.*

Figure 2B) in a total of 374 cases. This result was consistent when each cohort was analyzed separately (p<0.05, Figure 2C-F). In univariate analysis, low FBXL5 expression was an independent prognostic factor for DSS [hazard ratio (HR)=1.934, 95% confidence interval (CI)=1.259-2.970, p=0.003] and RFS (HR=1.728, 95% CI=1.271-2.349, p<0.001) among tumor size, microvascular invasion, major portal invasion, intrahepatic metastasis, pathologic T stage, BCLC stage, serum albumin level, and serum AFP level (Table III). In multivariate analysis, low FBXL5 expression was an independent prognostic factor for DSS (HR=2.014, 95% CI=1.287-3.153, p=0.002) and RFS (HR=1.749, 95% CI=1.270-2.409, p=0.001) in the time-dependent Cox model, in addition to intrahepatic metastasis (Table III).

When analyzing HCC patients of the TCGA data set using KM-plotter (using 'autoselect best cut-off'), cases with low *FBXL5* mRNA expression showed shorter RFS and DSS than those with high *FBXL5* mRNA expression (Figure 3A and B; p<0.001 for RFS and DSS).

Discussion

In this study, we demonstrated that low FBXL5 expression was associated with shorter RFS and DSS in a large cohort of HCC patients from two different institutes with long-term follow-up. In addition, low *FBXL5* mRNA expression was associated with lower RFS and DSS in the TCGA dataset.

Iron is inevitable in many physiological phases, such as DNA synthesis and mitochondrial oxidative metabolism, and

Table I. *Clinicopathological features of the total 374 hepatocellular carcinoma cases associated with FBXL5 expression.*

Category	Variables	No. of cases (n=374)	FBXL5 IHC expression		*p*-Value
			High (%) (n=304)	Low (%) (n=70)	
Age (year)	≤55	193	160 (52.6)	33 (47.1)	0.407
	>55	181	144 (47.4)	37 (52.9)	
Sex	Male	310	252 (82.9)	58 (82.9)	0.994
	Female	64	52 (17.1)	12 (17.1)	
Tumor size (cm)	≤5.0	258	212 (69.7)	46 (65.7)	0.512
	>5.0	116	92 (30.3)	24 (34.3)	
Edmondson grade	I-II	282	229 (75.3)	53 (75.7)	0.946
	III-IV	92	75 (24.7)	17 (24.3)	
Microvascular invasion	Absent	186	151 (49.7)	35 (50.0)	0.960
	Present	188	153 (50.3)	35 (50.0)	
Major portal vein invasion	Absent	358	294 (96.7)	64 (91.4)	0.092
	Present	16	10 (3.3)	6 (8.6)	
Intrahepatic metastasis	Absent	301	245 (80.6)	56 (80.0)	0.910
	Present	73	59 (19.4)	14 (20.0)	
Multicentric occurrence	Absent	346	282 (92.8)	64 (91.4)	0.702
	Present	28	22 (7.2)	6 (8.6)	
AJCC T stage	1	102	87 (28.6)	15 (21.4)	0.116
	2	170	136 (44.7)	34 (58.6)	
	3	84	70 (23.0)	14 (20.0)	
	4	18	11 (3.6)	7 (10.0)	
BCLC stage	0-A	247	203 (66.8)	44 (62.9)	0.395
	B	108	88 (28.9)	20 (28.6)	
	C	18	12 (3.9)	6 (8.6)	
	D	1	1 (0.3)		
Albumin level (g/dl)	>3.5	322	261 (85.9)	61 (87.1)	0.779
	≤3.5	52	43 (14.1)	9 (12.9)	
AFP level (ng/ml)*	≤200	238	192 (64.6)	46 (69.7)	0.435
	>200	125	105 (35.4)	20 (30.3)	
Smoking	Non-smoker	188	153 (50.3)	35 (50.0)	0.960
	Smoker	186	151 (49.7)	35 (50.0)	
Alcohol	≤20g/d	227	187 (61.5)	40 (57.1)	0.500
	>20g/d	147	117 (38.5)	30 (42.9)	
Etiology	Non-viral	60	42 (13.8)	18 (25.7)	0.019
	Viral	314	262 (86.2)	52 (74.3)	
Non tumor liver pathology	Cirrhosis	198	159 (52.3)	39 (55.7)	0.606
	Others	187	145 (47.7)	31 (44.3)	

Values are presented as number (%). AJCC, American Joint Committee on Cancer; BCLC, Barcelona Clinic Liver Cancer; AFP, α-fetoprotein; HBV, hepatitis B virus; HCV, hepatitis C virus. *Values are not available in some cases at the time of study.

some cancers have alternative pathways to control cellular iron balance (19). An *in vivo* study revealed that cellular iron levels are regulated by FBXL5 and IRP2 (18). Under iron-replete conditions, ubiquitylation, and degradation of IRP2 are mediated by the SCFFBXL5 E3 ubiquitin ligase complex, and FBXL5 is used as a substrate recognition component for IRP2. FBXL5 is stabilized when iron binds to the hemerythrin domain of FBXL5; otherwise, FBXL5 is unstable under iron-deficient conditions. FBXL5 controls IRP2 expression in an iron-dependent manner (34, 35). FBXL5 deficient mice show failure to sense increased cellular iron availability, leading to consecutive accumulation of IRP2 and aberrant expression of its target genes. In addition, FBXL5-null mice show early embryonic mortality due to extreme oxidative stress, suggesting that FBXL5 is an essential factor in cellular iron homeostasis during early embryogenesis (18, 36).

Previous studies using FBXL5 knockout mice revealed iron accumulation in the liver and steatohepatitis (18), higher HCC incidence, and bigger tumor size compared to FBXL5 intact mice, indicating that deficiency of FBXL5 is involved in hepatic carcinogenesis (19). In addition, hepcidin expression is down-regulated in FBXL5-deficient mice (18). FBXL5 also plays role in epithelial-mesenchymal transition

Table II. *Clinicopathological characteristics of FBXL5 expression in the Samsung Medical Center and Hallym University Sacred Heart Hospital cohort.*

Category	Variables	No. of SMC cases (n=259)	FBXL5 IHC expression		*p*-Value	No. of HUMC cases (n=115)	FBXL5 IHC expression		*p*-Value
			High (%) (n=209)	Low (%) (n=50)			High (%) (n=95)	Low (%) (n=20)	
Age (year)	≤55	156	128 (61.2)	28 (56.0)	0.523	37	32 (33.7)	5 (25.0)	0.450
	>55	103	81 (38.8)	22 (44.0)		78	63 (66.3)	15 (75.0)	
Sex	Male	211	170 (81.3)	41 (82.0)	>0.999	99	82 (86.3)	17 (85.0)	>0.999
	Female	48	39 (18.7)	9 (18.0)		16	13 (13.7)	3 (15.0)	
Tumor size (cm)	≤5.0	171	139 (66.5)	32 (64.0)	0.742	87	73 (76.8)	14 (70.0)	0.569
	>5.0	88	70 (33.5)	18 (36.0)		28	22 (23.2)	6 (30.0)	
Edmondson grade	I-II	238	194 (92.8)	44 (88.0)	0.386	44	35 (36.8)	9 (45.0)	0.495
	III-IV	21	15 (7.2)	6 (12.0)		71	60 (63.2)	11 (55.0)	
Microvascular invasion	Absent	113	91 (43.5)	22 (44.0)	>0.999	73	60 (63.2)	13 (65.0)	0.876
	Present	146	118 (56.5)	28 (56.0)		42	35 (36.8)	7 (35.0)	
Major portal vein invasion	Absent	246	201 (96.2)	45 (90.0)	0.139	112	93 (97.9)	19 (95.0)	>0.999
	Present	13	8 (3.8)	5 (10.0)		3	2 (2.1)	1 (5.0)	
Intrahepatic metastasis	Absent	194	157 (75.1)	37 (74.0)	>0.999	107	88 (92.6)	19 (95.0)	>0.999
	Present	65	52 (24.9)	13 (26.0)		8	7 (7.4)	1 (5.0)	
Multicentric occurrence	Absent	242	197 (94.3)	45 (90.0)	0.336	104	85 (89.5)	19 (95.0)	0.686
	Present	17	12 (5.7)	5 (10.0)		11	10 (10.5)	1 (5.0)	
AJCC T stage	1	40	37 (17.7)	3 (6.0)	0.024	62	50 (52.6)	12 (60.0)	0.837
	2	128	100 (47.9)	28 (56.0)		42	36 (37.9)	6 (30.0)	
	3	77	64 (30.6)	13 (26.0)		7	6 (6.3)	1 (5.0)	
	4	14	8 (3.8)	6 (12.0)		4	3 (3.2)	1 (5.0)	
BCLC stage	0-A	146	120 (57.4)	26 (52.0)	0.350	101	83 (87.4)	18 (90.0)	0.716
	B	98	79 (37.8)	19 (38.0)		10	9 (9.5)	1 (5.0)	
	C	15	10 (4.8)	5 (10.0)		3	2 (2.1)	1 (5.0)	
	D					1	1 (1.1)		
Albumin level (g/dl)	>3.5	229	186 (89.0)	43 (86.0)	0.622	93	75 (78.9)	18 (90.0)	0.356
	≤3.5	30	23 (11.0)	7 (14.0)		22	20 (21.1)	2 (10.0)	
AFP level (ng/ml)*	≤200	151	119 (58.9)	32 (66.7)	0.332	87	73 (76.8)	14 (77.8)	>0.999
	>200	99	83 (41.1)	16 (33.3)		26	22 (23.2)	4 (22.2)	
Smoking	Non-smoker	121	99 (47.4)	22 (44.0)	0.753	67	54 (56.8)	13 (65.0)	0.620
	Smoker	138	110 (52.6)	28 (56.0)		48	41 (43.2)	7 (35.0)	
Alcohol	≤20g/d	150	122 (58.4)	28 (56.0)	0.873	77	65 (68.4)	12 (60.0)	0.602
	>20g/d	109	87 (41.6)	22 (44.0)		38	30 (31.6)	8 (40.0)	
Etiology	Non-viral	29	19 (9.1)	10 (20.0)	0.043	31	21 (22.1)	10 (50.0)	0.011
	Viral	230	190 (90.9)	40 (80.0)		84	74 (77.9)	10 (50.0)	
Non tumor liver pathology	Cirrhosis	132	105 (50.2)	27 (54.0)	0.641	66	54 (56.8)	12 (60.0)	0.795
	Others	127	104 (49.8)	23 (46.0)		49	41 (43.2)	8 (40.0)	

Values are presented as number (%). AJCC, American Joint Committee on Cancer; BCLC, Barcelona Clinic Liver Cancer; AFP, α-fetoprotein; HBV, hepatitis B virus; HCV, hepatitis C virus. *Values are not available in some cases at the time of study.

(EMT), in relation to Snail polyubiquitination, decreasing Snail protein stability (37, 38). One study showed microRNA miR-1306-3p targets FBXL5 and inhibits Snail degradation to promote EMT, leading to metastasis in HCC (39).

In this study, we first report that low expression of FBXL5 is related to inferior prognosis of HCC in cohorts from two institutes, as well as in the TCGA cohort. These results are consistent with previous *in vivo* mouse experiments, and our study provides clinical evidence suggesting that FBXL5 is a potential therapeutic target for HCC.

Interestingly, low FBXL5 expression was more frequently found in HCC with non-viral etiology than in those with viral etiology. The association between FBXL5 expression and specific etiology is provided in Table IV. There have been only a few studies on FBXL5 expression in specific liver diseases. It has been reported that FBXL5 depletion induced steatohepatitis (18) and hepatocellular carcinoma (19). Several studies have revealed *FBXL5* mRNA or hepcidin levels are reduced in HCV patients (40-42), suggesting that HCV infection is associated with FBXL5 dysregulation. In

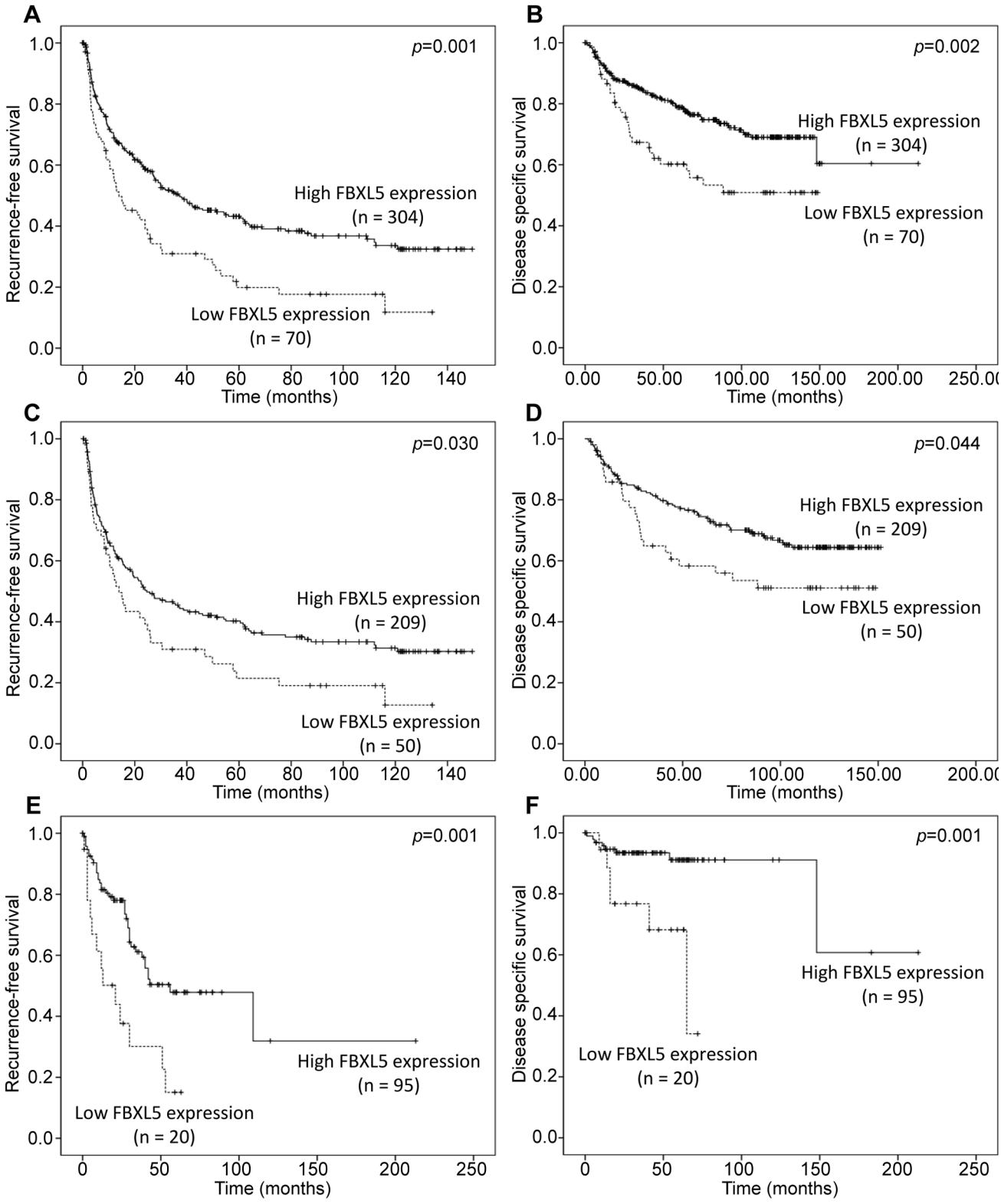

Figure 2. *Kaplan–Meier survival curves according to FBXL5 expression in the total cohort (A-B). Kaplan–Meier survival curves according to FBXL5 expression in the Samsung Medical Center cohort (C-D). Kaplan–Meier survival curves according to FBXL5 expression in the Hallym University Sacred Heart Hospital cohort (E-F).*

Table III. *Univariate and multivariate analysis of 374 hepatocellular carcinoma cases associated with FBXL5 expression.*

Category	Variables	Disease specific survival				Recurrence free survival			
		Univariate		Multivariate		Univariate		Multivariate	
		HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value
Age (y)	≤55	1		-		1		-	
	>55	0.858 (0.583-1.260)	0.434	-	-	1.044 (0.803-1.357)	0.750	-	-
Sex	Female	1		-		1		-	
	Male	0.783 (0.466-1.317)	0.357	-	-	1.127 (0.808-1.571)	0.481	-	-
Tumor size (cm)	≤5	1		1		1		1	
	>5	3.350 (2.293-4.895)	<0.001	1.244 (0.698-2.217)	0.459	1.877 (1.432-2.462)	<0.001	1.267 (0.838-1.915)	0.263
Microvascular invasion	Absent	1		1		1		1	
	Present	3.221 (2.125-4.880)	<0.001	1.134 (0.631-2.039)	0.674	2.072 (1.591-2.699)	<0.001	1.220 (0.846-1.730)	0.298
Major portal vein invasion	Absent	1		1		1		1	
	Present	5.373 (2.865-10.075)	<0.001	1.188 (0.593-2.382)	0.627	3.834 (2.225-6.608)	<0.001	1.051 (0.576-1.917)	0.872
Intrahepatic metastasis	Absent	1		1		1		1	
	Present	6.348 (4.320-9.327)	<0.001	3.771 (2.111-6.734)	<0.001	3.858 (2.861-5.204)	<0.001	3.811 (2.427-5.984)	<0.001
Multicentricity	Absent	1		-		1		-	
	Present	0.640 (0.297-1.377)	0.254	-	-	0.778 (0.468-1.294)	0.334	-	-
Edmonson grade	I-II	1		-		1		-	
	III	1.198(0.731-1.962)	0.473	-	-	1.118(0.808-1.546)	0.500	-	-
Pathologic T stage	pT1	1		1		1		1	
	pT2- pT4	3.573 (1.915-6.666)	<0.001	1.524 (0.747-3.108)	0.247	1.686 (1.231-2.310)	0.001	1.026 (0.700-1.503)	0.896
BCLC stage	0 to A	1		1		1		1	
	B to C	3.605 (2.445-5.313)	<0.001	1.307 (0.648-2.638)	0.450	1.682 (1.289-2.196)	<0.001	0.769 (0.466-1.269)	0.304
Serum albumin level	>3.5 g/dl	1		1		1		-	
	≤3.5 g/dl	1.685 (1.053-2.695)	0.030	2.150 (1.286-3.593)	0.003	1.084 (0.756-1.555)	0.660	-	-
Serum AFP level	≤200 ng/ml	1		1		1		1	
	>200 ng/ml	1.836 (1.250-2.696)	0.002	1.189 (0.775-1.824)	0.428	1.773 (1.354-2.321)	<0.001	1.252 (0.933-1.679)	0.134
Etiology	Non-viral	1		-		1		1	
	Viral	1.439 (0.789-2.622)	0.235	-	-	1.574 (1.053-2.354)	0.027	1.397 (0.896-2.178)	0.141
Non-tumor pathology	Cirrhosis	1		-		1		-	
	Others	0.932 (0.638-1.362)	0.717	-	-	0.903 (0.695-1.173)	0.446	-	-
FBXL5 IHC expression	High	1		1		1		1	
	Low	1.934 (1.259-2.970)	0.003	2.014 (1.287-3.153)	0.002	1.728 (1.271-2.349)	<0.001	1.749 (1.270-2.409)	0.001

BCLC, Barcelona Clinic Liver Cancer; AFP, α-fetoprotein; IHC, immunohistochemistry.

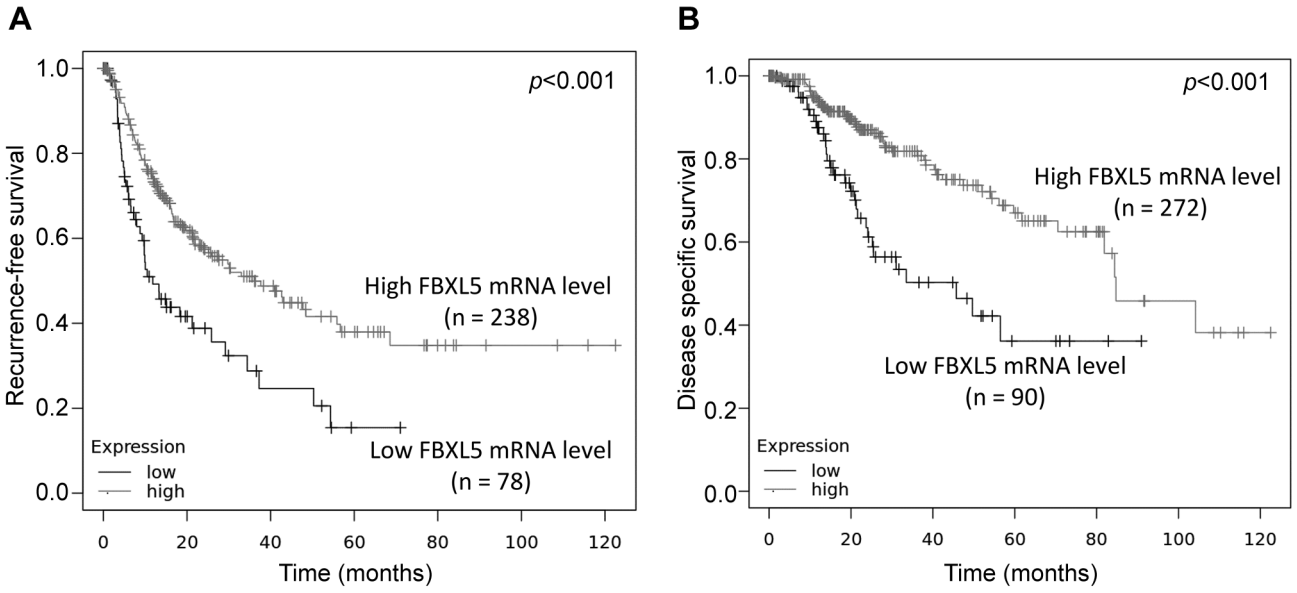

Figure 3. *Kaplan–Meier survival curves according to FBXL5 mRNA expression of the TCGA dataset using KM-plotter (A-B).*

Table IV. *The association between FBXL5 expression and etiology of hepatocellular carcinoma in the total 374 hepatocellular carcinoma cases.*

Category	Variables	No. of cases (n=374)	FBXL5 IHC expression		*p*-Value
			High (%) (n=304)	Low (%) (n=70)	
Etiology	HBV	280	236 (77.7)	44 (62.9)	0.017
	HCV	29	25 (8.2)	4 (5.7)	
	HBV&HCV	5	3 (1.0)	2 (2.8)	
	Alcohol	27	18 (5.9)	9 (12.9)	
	Others	33	22 (7.2)	11 (15.7)	

Values are presented as number (%). HBV, Hepatitis B virus; HCV, hepatitis C virus.

this study, the frequency of low FBXL5 expression was 30.0% in HCC patients with non-viral etiology, 16.6% in those with viral etiology, and 14% in HCV patients. This discrepancy may be due to the relatively small number of patients with HCV infection in our cohort. Down-regulation of hepcidin expression is not a universal mechanism for hepatic iron overload in chronic liver diseases (43, 44). In addition, considering the association between low FBXL5 expression and non-viral etiology in our study, there may be other possible mechanisms causing iron overload in non-viral patients. However, further studies are needed to clarify the importance of FBXL5 in various liver diseases.

Conclusion

In conclusion, our study revealed that low FBXL5 expression was more associated with non-viral etiology HCC and associated with poor DSS and RFS in two separate cohorts. In addition, the prognostic effect of FBXL5 expression was validated in an independent TCGA data set, which was composed of different ethnic groups. FBXL5 might be a prognostic biomarker of HCCs and a potential therapeutic target in conjunction with iron homeostasis.

Conflicts of Interest

None of the Authors have any conflicts of interest to declare regarding this study.

Authors' Contributions

Conception and design: SYH; Acquisition of data: YAC, SEK, SYH; Analysis and interpretation of data: YAC, C-KP, HHK, CKP, SYH; Drafting the article: YAC, CKP, SYH. All Authors read and approved the final manuscript.

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