

# High interleukin-8 and/or extracellular signal-regulated kinase 2 expression predicts poor prognosis in patients with hepatocellular carcinoma

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**Abstract.** Interleukin (IL)-8 and extracellular signal-regulated kinase (ERK) 2 play key roles in tumor progression, but the relationship between IL-8 and/or ERK2 expression in hepatocellular carcinoma (HCC) tissues and postoperative recurrence or survival is unclear. The expression levels of IL-8 and ERK2 in both HCC tissues and non-tumor liver tissues were analyzed using the Oncomine™ database and immunohistochemistry assay. Reverse transcription-quantitative PCR was then used to evaluate the expression levels of IL-8 and ERK2 in the tumor tissues of 67 patients with HCC undergoing radical hepatectomy. Pearson's correlation, Kaplan-Meier, Cox univariate and multivariate survival analyses were utilized to determine the correlation between IL-8 and ERK2 expression in HCC tissues, and their potential prognostic significance. As indicated by the data from the Oncomine™ database, and the patient samples, IL-8 and ERK2 were expressed at significantly higher levels in HCC tissues than in non-tumor liver tissues ( $P<0.05$ ). The rates of high IL-8 and ERK2 expression in HCC tissues were 43.28 (29/67) and 34.33% (23/67), respectively, and the IL-8 and ERK2 expression levels were positively correlated ( $r=0.764$ ;  $P<0.001$ ). Both ERK2 expression and IL-8/ERK2 co-expression were significantly associated with tumor size and differentiation ( $P<0.05$ ). Additionally, high expression levels of IL-8, ERK2 and IL-8/ERK2 co-expression were all significantly associated with poor overall survival (OS;  $P<0.05$ ) and disease-free survival (DFS;  $P<0.05$ ). Multivariate Cox regression analysis also showed that high expression levels of IL-8, ERK2, and IL-8 and ERK2 were independent

prognostic factors for OS and DFS ( $P<0.05$ ). The results of the present study indicate a significant increase in the risk of recurrence and mortality in HCC patients with high expression levels of IL-8 and/or ERK2, compared with patients with low expression. Therefore, IL-8 and ERK2 may be predictors of postoperative prognosis in patients with HCC.

## Introduction

Hepatocellular carcinoma (HCC) is one of the most prevalent primary malignancies of the liver (1). It is the fourth leading cause of cancer-associated mortality worldwide (2), and the third leading cause in China (3), where chronic hepatitis B virus infection and aflatoxin exposure are major risk factors (2). Surgical resection is the primary method of treating HCC, but due to rapid disease progression, most patients exhibit extensive metastasis before the time of surgery. Moreover, only ~20% of HCC patients undergo radical surgical resection (4); following resection, the 2-year HCC recurrence rate is as high as 55%, and most patients develop unresectable metastatic disease (5). The 5-year survival rate of HCC patients is <50% (6). Therefore, identification of early indicator molecules for postoperative recurrence and survival, and the improvement of long-term survival, are urgently required.

Interleukins (ILs)-6, IL-8, IL-17 and IL-10, as well as other inflammation- or immunity-related cytokines, have received attention for their association with tumors. These cytokines can either promote or inhibit the development of tumors (7), and are critical for assessing the risk of postoperative recurrence and long-term survival. IL-8, also known as CXC chemokine 8, has received increasing interest as a tumor inflammatory factor. IL-8 is a member of the chemokine family (8) and plays an important role in the tumor micro-environment, influencing tumor progression and regulating neovascularization, tumor cell growth, apoptosis and cell migration (9,10). It is expressed in a variety of tumors such as lung (11), breast (12) and colon cancer (13); a study by Wang *et al* (14) demonstrated that IL-8 expression is elevated in HCC. IL-8 promotes tumor cellular proliferation and neovascularization, either directly or indirectly, through tumor vascular endothelial cell receptors, thereby promoting tumor growth and metastasis (14,15).

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IL-8 also induces the activation of the classical mitogen-activated protein kinase (MAPK) signaling cascade, and subsequent downstream phosphorylation of both extracellular signal-regulated kinase (ERK)1 and ERK2 in neutrophils and tumor cells (16). ERK1/2 are key members of the MAPK family, whose activation is closely associated with the occurrence and development of various tumors (17,18). Furthermore, Schmitz *et al* (19) detected high expression levels of ERK1/2 in HCC tissues, and ERK1/2 activation in HCC also constitutes an independent prognostic factor. Although these data reveal a possible role for IL-8 and ERK1/2 in tumor progression, the relationship between IL-8 and/or ERK2 expression in HCC tissues, and postoperative recurrence and survival, remains unclear.

To further study the relationship between IL-8 and/or ERK2 levels in HCC, and recurrence and survival after hepatectomy, the expression levels of IL-8 and ERK2 in non-tumor liver tissues and HCC tissues were determined using the Oncomine™ database and immunohistochemistry (IHC). Subsequently, reverse transcription-quantitative (RT-q) PCR was used to quantify IL-8 and ERK2 expression in the tumor tissues of 67 patients with HCC, and their relationship with HCC clinical pathological features was then determined. This in-depth study of the risk factors of HCC recurrence and survival provides a theoretical basis for improving the long-term survival of patients with HCC.

## Materials and methods

**Data retrieval from the Oncomine™ database.** To investigate the clinical importance of IL-8 and ERK2 in HCC, Oncomine™ [https://www.oncomine.org/resource/main.html; GSE14323 (GPL571) and GSE14520 (GPL3921)] was searched for published data to analyze the mRNA expression levels of IL-8 and ERK2 in HCC tissues and non-tumor liver tissues (20,21).

**Patient information.** The use of patient samples in the present study was approved by The Hunan Normal University Medical Ethics Committee, and accords with the provisions stated in The Declaration of Helsinki, as revised in 2013. A total of 67 frozen HCC specimens were collected from patients who underwent surgery at the Department of General Surgery, Affiliated Changsha Hospital, Hunan Normal University between January 2002 and December 2012. An additional 60 paraffin-embedded HCC tissues and adjacent non-tumor liver tissues were collected. The inclusion criteria were: i) R0 tumor resection and ii) postoperative pathology confirmed as HCC. The exclusion criteria were: i) Administration of any anti-cancer treatment before surgery; ii) serious complications or death within 30 days post-surgery; iii) non-tumor related mortality; and iv) incomplete clinical, pathological or surgical data. The 67 HCC patients were aged between 36 and 83 years, with a median age of 55.0 years. There was a total of 55 men and 12 women, with a male-to-female ratio of 4.58:1. According to the TNM staging detailed in the eighth edition of the American Joint Committee on Cancer (AJCC) Cancer Staging Manual (22), 42 patients had stage I tumors, 18 had stage II tumors, and 7 had stage III tumors.

**IHC.** All patient specimens were fixed in 10% neutral formalin, embedded in paraffin, cut into 4-μm-thick serial sections and

Table I. Association between mRNA expression levels of IL-8 and ERK2.

ERK2 expression	IL-8 expression		Pearson's contingency coefficient	P-value
	High	Low		
High	22	1	0.764	<0.001 <sup>a</sup>
Low	7	37		

IL-8, interleukin-8; ERK2, extracellular regulated protein kinases 2.  
<sup>a</sup>P<0.05.

stained as previously described (23). The primary antibody against IL-8 was purchased from R&D Systems, Inc., (1:500; cat. no. AF-208-NA), and the primary antibody against ERK2 was purchased from Santa Cruz Biotechnology, Inc., (1:200; cat. no. SC-1647). Expression levels were scored as the proportion of the immuno-positive staining area (0, 0%; 1, 1-25%; 2, 26-50%; and 3, 51-100%) multiplied by the staining intensity (0, negative; 1, low; 2, medium; 3, high), and ranged from 0 to 9. The scores were independently evaluated by two pathologists.

**RT-qPCR.** Total RNA was extracted from the frozen HCC tissues using TRIzol® (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Subsequently, reverse transcription was performed using the PrimeScript™ RT kit (Takara Bio, Inc.) according to the manufacturer's protocols. SYBR Premix EX Taq™ (Takara Bio, Inc.) was used for qPCR (according to the manufacturer's protocol) on an ABI 7900 Prism HT (Applied Biosystems; Thermo Fisher Scientific, Inc.). Relative gene expression was quantified using the 2<sup>-ΔΔC<sub>q</sub></sup> method (24), and the patients were divided into high- and low-IL-8 and -ERK2 expression groups using the median expression value as the cut-off point. The PCR primers were as follows: IL-8 forward, 5'-AAGAAACCACCGGAA GGAAC-3', and reverse, 5'-ACTCCTTGCCAAAACCTGC AC-3'; ERK2 forward, 5'-GAAGGTGCCTACGGGATGG-3', and reverse, 5'-GGTCAATGGTTGGTGTGCGG-3'; and GAPDH forward, 5'-AACAGCCTCAAGATCATCAGCA-3', and reverse, 5'-CATGAGTCCTTCCACGATACCA-3'. The thermocycling conditions were as follows: Initial denaturation for 30 sec at 98°C, followed by 24 cycles of 98°C for 15 sec and 72°C for 30 sec, and lastly 72°C for 5 min to allow final extension before cooling to 4°C.

**Case follow-up.** Follow-ups in the form of outpatient visits or telephone calls were conducted for all cases that met the study criteria. The regular follow-up plan was as follows: i) Review every 3 months within 2 years after surgery; ii) review every 6 months after 2-5 years; and iii) review every year after 5 years. The review included an analysis of liver function, an abdominal ultrasound and a chest radiograph. If required, enhanced computed tomography, magnetic resonance imaging and needle biopsy were performed. Recurrence was defined as confirmation of the presence of new lesions in or outside of the liver via imaging studies or biopsy. Overall survival (OS) was defined as the time between the date of surgery to death

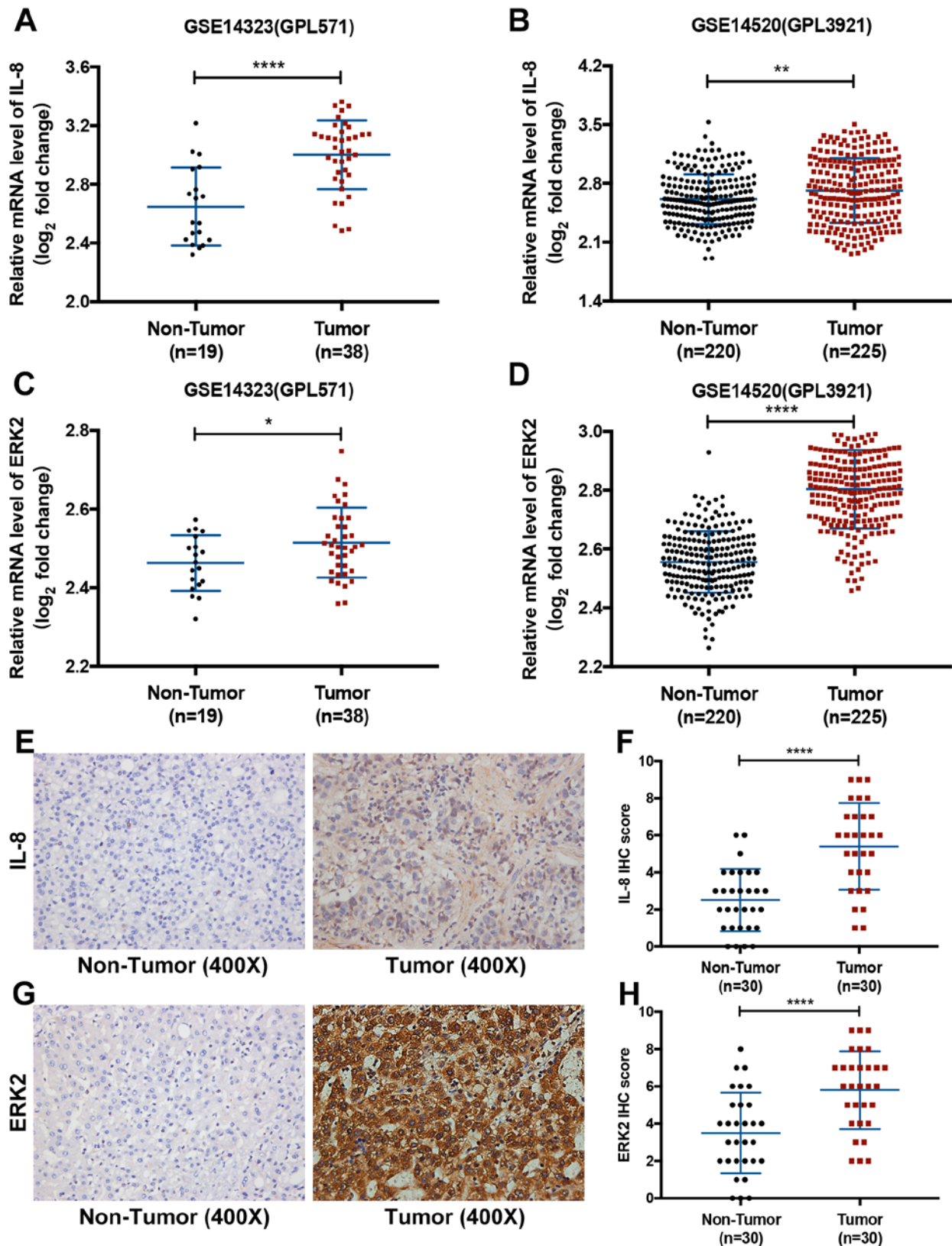


Figure 1. IL-8 and ERK2 expression in HCC patients. IL-8 mRNA expression level data in HCC vs. non-tumor liver tissues were extracted from the (A) GSE14323 (GPL571) and (B) GSE14520 (GPL3921) HCC datasets. ERK2 mRNA expression levels in HCC vs. non-tumor liver tissues were retrieved from the (C) GSE14323 (GPL571) and (D) GSE14520 (GPL3921) HCC datasets. Representative images of (E) IL-8 and (G) ERK2 protein expression. Quantitative analysis of (F) IL-8 and (H) ERK2 IHC score. IL-8, interleukin-8; ERK2, extracellular signal-regulated kinase 2; HCC, hepatocellular carcinoma; OS overall survival; DFS, disease-free survival; IHC, immunohistochemistry. \*P<0.05, \*\*P<0.01 and \*\*\*\*P<0.0001

or follow-up, and disease-free survival (DFS) was defined as the date of surgery to relapse or follow-up. Both OS and DFS

were calculated on a monthly basis, and the follow-up deadline was December 2018.

Table II. Correlations of IL-8 and ERK2 mRNA expression with clinicopathological characteristics.

Parameter	IL-8		P-value	ERK2		P-value	IL-8 and ERK2		P-value
	High	Low		High	Low		Both high	Others	
Sex			0.158			0.678			0.765
Male	26	29		20	35		19	36	
Female	3	9		3	9		3	9	
Age			0.372			0.940			0.747
<50, years	7	13		7	13		6	14	
≥50, years	22	25		16	31		16	31	
Alcoholism			0.438			0.180			0.325
Absence	18	27		13	32		13	32	
Presence	11	11		10	12		9	13	
HBV			0.623			1.000			1.000
Positive	23	33		19	37		18	38	
Negative	6	5		4	7		4	7	
Cirrhosis			0.502			1.000			1.000
Absence	0	2		1	1		0	2	
Presence	29	36		22	43		22	43	
AFP			0.660			0.634			0.958
≤400 μg/l	22	27		16	33		16	33	
>400 μg/l	7	11		7	11		6	12	
Albumin			0.669			1.000			1.000
<35 g/l	2	5		2	5		2	5	
≥35 g/l	27	33		21	39		20	40	
ALT			0.401			0.848			0.435
≤60 U/l	26	30		20	36		20	36	
>60 U/l	3	8		3	8		2	9	
AST			0.839			0.923			0.728
≤42 U/l	22	28		17	33		17	33	
>42 U/l	7	10		6	11		5	12	
PLT			0.352			0.923			0.803
<100x10 <sup>9</sup> /l	9	8		6	11		6	11	
≥100x10 <sup>9</sup> /l	20	30		17	33		16	34	
Bilirubin			0.623			1.000			1.000
≤22 μmol/l	23	33		19	37		18	38	
>22 μmol/l	6	5		4	7		4	7	
Tumor number			0.662			1.000			1.000
Single	24	34		20	38		19	39	
Multiple	5	4		3	6		3	6	
Tumor size			0.117			0.013 <sup>a</sup>			0.019 <sup>a</sup>
≤5 cm	19	32		13	38		12	39	
>5 cm	9	6		9	6		9	6	
Tumor margin			0.981			0.627			0.762
≤2 cm	10	13		7	16		7	16	
>2 cm	19	25		16	28		15	29	
Pathological differentiation			0.134			0.014 <sup>a</sup>			0.008 <sup>a</sup>
High	19	31		13	37		12	38	
Middle and low	10	7		10	7		10	7	
Microvascular tumor thrombus			0.469			0.524			0.415
Yes	10	10		8	12		8	12	
No	19	28		15	32		14	33	

Table II. Continued.

Parameter	IL-8		P-value	ERK2		P-value	IL-8 and ERK2		P-value
	High	Low		High	Low		Both high	Others	
Capsule invasion			0.971			0.846			0.951
Yes	23	30		19	34		18	35	
No	6	8		4	10		4	10	
TNM stage			0.236			0.078			0.061
I+II	24	36		18	42		17	43	
III	5	2		5	2		5	2	

IL-8, interleukin-8; ERK2, extracellular signal-regulated kinase 2; HBV, hepatitis B virus; AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, alanine aminotransferase; PLT, platelet; TNM, tumor, lymph node, metastasis. <sup>a</sup>P<0.05.

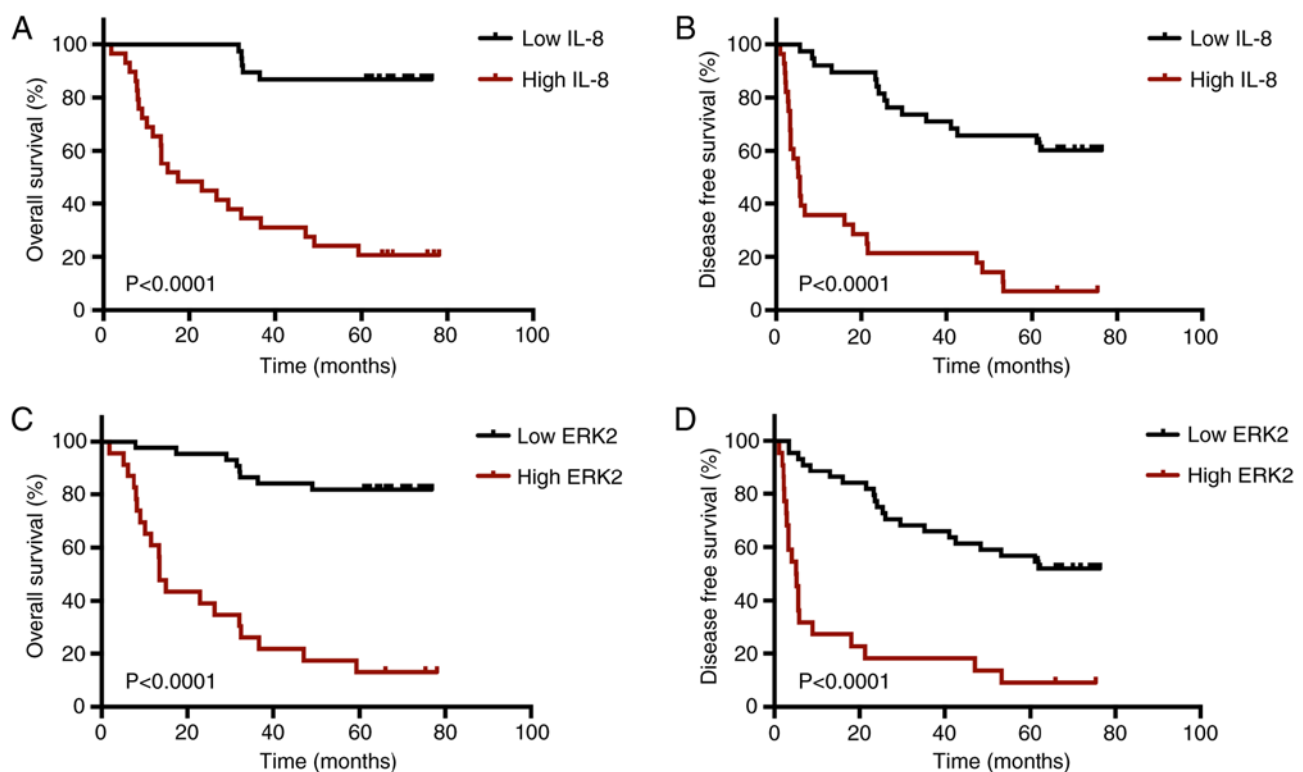


Figure 2. Prognostic values of IL-8 and ERK2 expression in HCC tissues. (A-D) Kaplan-Meier analysis of overall survival and disease-free survival among different tumor types stratified by (A and B) IL-8 and (C and D) ERK2. IL-8, interleukin-8; ERK2, extracellular signal-regulated kinase 2; HCC, hepatocellular carcinoma.

**Statistical analysis.** Statistical processing was performed using SPSS software v.19.0 (IBM Corp.). Spearman's correlation analysis was used to calculate the correlation between IL-8 and ERK2 expression levels. The  $\chi^2$  test and Fisher's exact probability test were used to analyze the correlation between IL-8 and ERK2 expression in HCC tissues, and patient clinicopathological features. Survival analysis was performed using Kaplan-Meier curves, and the relationship between IL-8 and/or ERK2 expression and postoperative recurrence, and survival in patients with HCC was determined using the log-rank test. Univariate and multivariate analyses of HCC recurrence and survival were performed using the Cox proportional hazard model to screen for variables. The

data are presented as the mean  $\pm$  standard deviation, and P<0.05 was considered to indicate a statistically significant difference.

## Results

**Expression of IL-8 and ERK2 in non-tumor liver tissues and HCC tissues.** The mRNA expression levels of IL-8 and ERK2 were retrieved from two published HCC datasets published in the Oncomine<sup>TM</sup> database. It was observed that the mRNA levels of both IL-8 and ERK2 were significantly higher in HCC tissues compared with non-tumor liver tissues from both datasets (P<0.05; Fig. 1A-D). Subsequently, this result

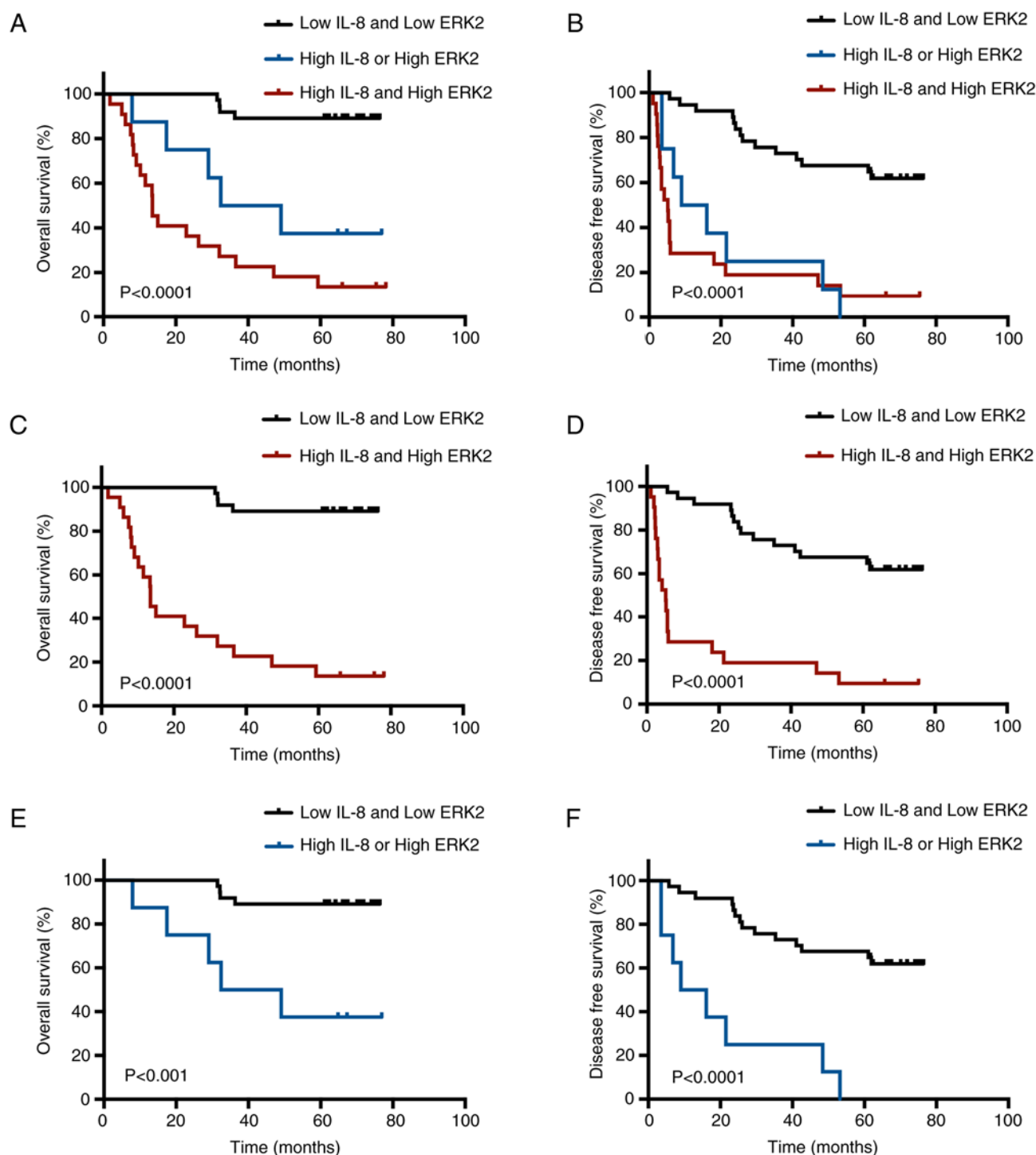


Figure 3. Prognostic values of IL-8 and/or ERK2 expression in HCC tissues. (A-F) Kaplan-Meier analysis of overall survival and disease-free survival among different tumor types stratified by IL-8 and/or ERK2 expression: Both high vs. all others (A and B) both high vs. both low (C and D) IL-8 or ERK2 high vs. both low (E and F) IL-8, interleukin-8; ERK2, extracellular signal-regulated kinase 2; HCC, hepatocellular carcinoma; OS overall survival; DFS, disease-free survival.

was validated in patient samples using IHC. The protein expression levels of IL-8 and ERK2 were significantly higher in HCC tissues compared with non-tumor liver tissues ( $P < 0.05$ ; Fig. 1E-H).

**Relationship between IL-8 and/or ERK2 expression and the clinicopathological features of HCC.** IL-8 and ERK2 exhibited high expression rates in 43.28 (29/67) and 34.33% (23/67) of the HCC tissues, respectively. Additionally,

Pearson's correlation analysis indicated a positive correlation between IL-8 and ERK2 expression ( $r = 0.764$ ;  $P < 0.001$ ; Table I). Although no significant correlation was observed between IL-8 expression and clinicopathological features in HCC tissues, ERK2 expression was significantly associated with both tumor size and differentiation ( $P < 0.05$ ; Table II). Moreover, IL-8 and ERK2 co-expression was also significantly associated with tumor size and differentiation ( $P < 0.05$ ; Table II).



Table III. Univariate analysis of variables with patient overall survival (Cox regression model).

Variable	Univariate analysis		
	Hazard ratio	95% CI	P-value
Sex (male vs. female)	2.143	0.647-7.101	0.212
Age ( $\geq 50$ vs. $< 50$ , years)	1.046	0.460-2.375	0.915
HBV (positive vs. negative)	2.110	0.895-4.975	0.088
Cirrhosis (present vs. absent)	1.053	0.143-7.756	0.960
AFP ( $> 400$ $\mu\text{g/l}$ vs. $\leq 400$ $\mu\text{g/l}$ )	1.205	0.531-2.737	0.655
Albumin ( $> 35$ g/l vs. $\leq 35$ g/l)	1.159	0.350-3.840	0.809
ALT ( $> 60$ U/l vs. $\leq 60$ U/l)	1.352	0.547-3.340	0.513
AST ( $> 42$ U/l vs. $\leq 42$ U/l)	1.859	0.856-4.035	0.117
PLT ( $< 100 \times 10^9/\text{l}$ vs. $\geq 100 \times 10^9/\text{l}$ )	1.992	0.918-4.323	0.081
Bilirubin ( $> 20$ $\mu\text{mol/l}$ vs. $\leq 20$ $\mu\text{mol/l}$ )	1.740	0.704-4.302	0.231
Tumor number (multiple vs. single)	1.799	0.683-4.740	0.235
Tumor size ( $> 5$ cm vs. $\leq 5$ cm)	2.580	1.187-5.606	0.017 <sup>a</sup>
Tumor margin ( $\leq 2$ cm vs. $> 2$ cm)	1.052	0.476-2.326	0.900
Pathological differentiation (middle and low vs. high)	1.800	0.813-3.987	0.147
Microvascular tumor thrombus (yes vs. no)	1.347	0.609-2.979	0.462
Capsule invasion (yes vs. no)	1.063	0.431-2.623	0.894
TNM stage (III vs. I+II)	5.364	2.123-13.554	$< 0.001^a$
IL-8 (positive vs. negative)	11.618	4.373-30.863	$< 0.001^a$
ERK2 (positive vs. negative)	10.090	4.366-23.317	$< 0.001^a$
IL-8 and ERK2 (both vs. others)	3.938	2.424-6.397	$< 0.001^a$

CI, confidence interval; HBV, hepatitis B virus; AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, alanine aminotransferase; PLT, platelet; TNM, tumor, lymph node, metastasis; IL-8, interleukin-8; ERK2, extracellular signal-regulated kinase 2. <sup>a</sup> $P < 0.05$ .

*Relationship between IL-8 and/or ERK2 expression and postoperative prognosis.* In the data from the 67 HCC samples, the median follow-up time was  $49.56 \pm 25.79$  months (range, 1.8-78.1 months). The OS rates of patients at 1, 3 and 5 years were 85.08, 65.67 and 58.21%, respectively, while DFS rates were 67.16, 49.25 and 40.30%, respectively (Data not shown). Kaplan-Meier survival analysis of 67 patients with HCC exhibited significantly shorter OS ( $P < 0.0001$ ; Fig. 2A) and DFS ( $P < 0.0001$ ; Fig. 2B) times in the IL-8 high-expression group compared with the low-expression group. Furthermore, the ERK2 high-expression group had shorter OS ( $P < 0.0001$ ; Fig. 2C) and DFS ( $P < 0.0001$ ; Fig. 2D) times than the corresponding low-expression group. Based on these results, the 67 patients with HCC were divided into three groups for further Kaplan-Meier analysis (low IL-8 and low ERK2 group, high IL-8 or high ERK2 group, and high IL-8 and high ERK2 group). OS ( $P < 0.001$ ; Fig. 3A, C and E) and DFS ( $P < 0.0001$ ; Fig. 3B, D and F) were significantly shorter in the IL-8 and/or ERK2 high-expression group than in the low-expression group.

Given the significant correlation between IL-8 and ERK2 expression, univariate and multivariate Cox proportional hazard analyses were performed on the IL-8 expression group, the ERK2 expression group and the IL-8 and ERK2 expression group. Further multivariate Cox regression analysis was then performed on the significant factors identified from univariate analysis (Tables III and IV). The multivariate Cox

regression analysis of the IL-8 expression group showed that TNM stage III [hazard ratio (HR)=6.246; 95% confidence interval (CI), 2.233-17.471;  $P < 0.001$ ] and high IL-8 expression (HR=12.369; 95% CI, 4.589-33.341;  $P < 0.001$ ) were independent risk factors for OS (Table V), while a platelet count (PLT)  $< 100 \times 10^9/\text{l}$  (HR=2.106; 95% CI, 1.071-4.141;  $P = 0.031$ ), TNM stage III (HR=3.477; 95% CI, 1.367-8.847;  $P = 0.009$ ) and high-IL-8 expression (HR=6.620; 95% CI, 3.350-13.084;  $P < 0.001$ ) were all independent risk factors for DFS (Table VI). Multivariate Cox regression analysis of the ERK2 expression group showed that TNM stage III (HR=4.832; 95% CI, 1.760-13.269;  $P = 0.002$ ) and high ERK2 expression (HR=10.011; 95% CI, 4.268-23.479;  $P < 0.001$ ) were independent risk factors for OS (Table V), while PLT  $< 100 \times 10^9/\text{l}$  (HR=2.171; 95% CI, 1.115-4.226;  $P = 0.023$ ), TNM stage III (HR=2.873; 95% CI, 1.153-7.156;  $P = 0.023$ ) and high ERK2 expression (HR=5.263; 95% CI, 2.760-10.036;  $P < 0.001$ ) were independent risk factors for DFS (Table VI). Furthermore, multivariate Cox regression models of the IL-8 and ERK2 expression groups showed that TNM stage III (OS: HR=4.595; 95% CI, 1.682-12.549;  $P = 0.003$ . DFS: HR=2.821; 95% CI, 1.134-7.017;  $P = 0.026$ ) with IL-8 and ERK2 co-expression (OS: HR=9.082; 95% CI, 3.974-20.757;  $P < 0.001$ . DFS: HR=4.918; 95% CI, 2.586-9.355;  $P < 0.001$ ) was an independent risk factor for both OS and DFS (Tables V and VI). PLT  $< 100 \times 10^9/\text{l}$  (HR=2.101; 95% CI, 1.080-4.086;  $P = 0.029$ ) was also shown to be an independent risk factor for DFS (Table VI).

Table IV. Univariate analysis of variables with patient disease-free survival (Cox regression model).

Variable	Univariate analysis		
	Hazard ratio	95% CI	P-value
Sex (male vs. female)	1.567	0.660-3.719	0.309
Age ( $\geq 50$ vs. $< 50$ , years)	1.631	0.867-3.069	0.129
HBV (positive vs. negative)	1.500	0.693-3.244	0.304
Cirrhosis (present vs. absent)	1.380	0.190-10.042	0.750
AFP ( $> 400 \mu\text{g/l}$ vs. $\leq 400 \mu\text{g/l}$ )	1.537	0.798-2.960	0.198
Albumin ( $< 35 \text{ g/l}$ vs. $\geq 35 \text{ g/l}$ )	1.008	0.360-2.826	0.988
ALT ( $> 60 \text{ U/l}$ vs. $\leq 60 \text{ U/l}$ )	1.064	0.472-2.398	0.882
AST ( $> 42 \text{ U/l}$ vs. $\leq 42 \text{ U/l}$ )	1.209	0.607-2.411	0.589
PLT ( $< 100 \times 10^9/\text{l}$ vs. $\geq 100 \times 10^9/\text{l}$ )	1.940	1.015-3.708	0.045 <sup>a</sup>
Bilirubin ( $> 20 \mu\text{mol/l}$ vs. $\leq 20 \mu\text{mol/l}$ )	1.400	0.648-3.028	0.392
Tumor number (multiple vs. single)	1.766	0.783-3.983	0.171
Tumor size ( $> 5 \text{ cm}$ vs. $\leq 5 \text{ cm}$ )	2.070	1.075-3.987	0.030 <sup>a</sup>
Tumor margin ( $\leq 2 \text{ cm}$ vs. $> 2 \text{ cm}$ )	1.157	0.601-2.226	0.663
Pathological differentiation (middle and low vs. high)	1.085	0.532-2.212	0.822
Microvascular tumor thrombus (yes vs. no)	1.064	0.544-2.079	0.856
Capsule invasion (yes vs. no)	1.088	0.520-2.276	0.822
TNM stage (III vs. I+II)	3.071	1.284-7.342	0.012 <sup>a</sup>
IL-8 (positive vs. negative)	6.098	3.172-11.720	$< 0.001^a$
ERK2 (positive vs. negative)	5.048	2.701-9.433	$< 0.001^a$
IL-8 and ERK2 (both vs. others)	2.607	1.868-3.639	$< 0.001^a$

CI, confidence interval; HBV, hepatitis B virus; AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, alanine aminotransferase; PLT, platelet; TNM, tumor, lymph node, metastasis; IL-8, interleukin-8; ERK2, extracellular signal-regulated kinase 2. <sup>a</sup> $P < 0.05$ .

## Discussion

The present study demonstrated that HCC patients with high IL-8 and/or ERK2 expression had significantly higher risks of recurrence and death than those with low expression. The Oncomine<sup>TM</sup> database and IHC were used to show that IL-8 and ERK2 expression were significantly higher in HCC tissues compared with non-tumor liver tissues. A positive correlation was also found between IL-8 and ERK2 expression in tissues from HCC patients using RT-qPCR. Moreover, IL-8 and/or ERK2 expression was significantly associated with tumor size and differentiation, and patients with high IL-8 and/or ERK2 expression had a poorer prognosis than the low expression group. Multivariate survival analysis further supported the high expression of IL-8 and/or ERK2 in HCC as an independent risk factor for OS and DFS.

The important role played by inflammation and the immune response in the occurrence and development of tumors has gradually become more recognized. A previous study reported that IL-8 is closely associated with the occurrence and development of HCC (25). In the present study, it was observed that IL-8 was expressed to a significantly higher level in HCC tissues, compared with non-tumor liver tissues, consistent with the results of a previous study (14). Schmitz *et al* (19) detected high expression levels of ERK1/2 in patient HCC tissues, and this was also detected in the HCC tissues examined in the present study. Waugh and Wilson (16) reported that IL-8 in tumor cells was able to promote ERK1/2 phosphorylation,

Table V. Multivariate analysis of variables with patient overall survival (Cox regression model).

Variable	Hazard ratio	95% CI	P-value
IL-8			
TNM stage (III vs. I+II)	6.246	2.233-17.471	$< 0.001^a$
IL-8 (high vs. low)	12.369	4.589-33.341	$< 0.001^a$
ERK2			
TNM stage (III vs. I+II)	4.832	1.760-13.269	0.002 <sup>a</sup>
ERK2 (high vs. low)	10.011	4.268-23.479	$< 0.001^a$
IL-8 and ERK2			
TNM stage (III vs. I+II)	4.595	1.682-12.549	0.003 <sup>a</sup>
IL-8 and ERK2 (both vs. others)	9.082	3.974-20.757	$< 0.001^a$

CI, confidence interval; TNM, tumor, lymph node, metastasis; IL-8, interleukin-8; ERK2, extracellular signal-regulated kinase 2. <sup>a</sup> $P < 0.05$ .

thereby promoting tumor growth and metastasis. A significant positive correlation between IL-8 and ERK2 in HCC tissues was also detected, indicating that high expression of IL-8 influences disease progression by activating ERK2.



Table VI. Multivariate analysis of variables with patient disease-free survival (Cox regression model).

Variable	Hazard ratio	95% CI	P-value
IL-8			
PLT (<100x10 <sup>9</sup> /l vs. ≥100x10 <sup>9</sup> /l)	2.106	1.071-4.141	0.031 <sup>a</sup>
TNM stage (III vs. I+II)	3.477	1.367-8.847	0.009 <sup>a</sup>
IL-8 (high vs. low)	6.620	3.350-13.084	<0.001 <sup>a</sup>
ERK2			
PLT (<100x10 <sup>9</sup> /l vs. ≥100x10 <sup>9</sup> /l)	2.171	1.115-4.226	0.023 <sup>a</sup>
TNM stage (III vs. I+II)	2.873	1.153-7.156	0.023 <sup>a</sup>
ERK2 (high vs. low)	5.263	2.760-10.036	<0.001 <sup>a</sup>
IL-8 and ERK2			
PLT (<100x10 <sup>9</sup> /l vs. ≥100x10 <sup>9</sup> /l)	2.101	1.080-4.086	0.029 <sup>a</sup>
TNM stage (III vs. I+II)	2.821	1.134-7.017	0.026 <sup>a</sup>
IL-8 and ERK2 (both vs. others)	4.918	2.586-9.355	<0.001 <sup>a</sup>

CI, confidence interval; PLT, platelet; TNM, tumor, lymph node, metastasis; IL-8, interleukin-8; ERK2, extracellular signal-regulated kinase 2. <sup>a</sup>P<0.05.

There is increasing evidence that serum IL-8 levels are an effective predictor of prognosis in patients with HCC (26), pancreatic cancer (27) and lymphoma (28), and that high nuclear ERK2 expression is an indicator of poor prognosis in patients with invasive breast cancer (29). However, these studies have been limited to the use of serum IL-8, and the relationship between IL-8 and/or ERK2 expression in HCC tissues and patient prognosis is poorly understood. The present study found that patients with high expression levels of IL-8 and/or ERK2 in HCC tissues had a worse prognosis than those in the low expression group. In addition, high expression levels of IL-8 and/or ERK2 in HCC tissues were shown to be an independent risk factor for OS and DFS in HCC. These data provide new insights for research into HCC progression, which may offer novel targets for the development of anti-tumor drugs.

The cytokine network is a complex system, with cytokine gene expression affected by other cytokines and the existence of synergistic restrictions and inhibition (30,31). As well as elevated IL-8 levels, patients with HCC also exhibit characteristic changes in expression of various cytokines, such as decreased IL-2 or elevated IL-6 and tumor necrosis factor- $\alpha$  (32-34). Therefore, in addition to the application of IL-8 and ERK2 as early indicator molecules, the deregulation of other cytokines in HCC should be examined to advance disease understanding, diagnosis and treatment. Moreover, because the present study is a single-center study of a small sample size, future studies of larger sample populations should be investigated to confirm these findings.

In summary, a significant positive correlation between the expression of IL-8 and ERK2 in HCC tissues was reported.

It was also found that high expression levels of IL-8 and/or ERK2 in HCC tissues were an independent risk factor for OS and DFS, and that HCC patients with high IL-8 and/or ERK2 expression had worse prognoses than those with low expression. The results of the present study support the hypothesis that patients with high expression of IL-8 and/or ERK2 should undergo more frequent follow-ups. Additionally, IL-8 and ERK2 are potential predictors of postoperative prognosis in patients with HCC, and therefore, may be used as therapeutic targets for the development of drugs that prevent HCC recurrence, thereby improving the long-term survival of patients with HCC.

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#### Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author upon reasonable request.

#### Authors' contributions

Study conception and design were conducted by GH, who also provided administrative support. YD, QY and BH provided the study materials. YD, QY, BH and ZH collected and assembled the data, and data analysis and interpretation were undertaken by YD and ZN. The manuscript was written and approved by all of the authors.

#### Ethics approval and consent to participate

The use of materials in the present study was approved by the Hunan Normal University Medical Ethics Committee, and all patients provided written informed consent.

#### Patient consent for publication

Not applicable.

#### Competing interests

The authors declare that they have no competing interests.

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