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 microenvironment, 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).
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, Schnitz 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 GSE4520 (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 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−ΔΔCq 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 used were as follows: IL-8: forward, 5’-AAGAAACCCAGGAGAA GGAC-3’, and reverse, 5’-ACTCCTTTGCGAAATCTGC AC-3’; ERK2 forward, 5’-GAAGGTGCTACCGGATGG-3’, and reverse, 5’-GGTCAATGGTTGGTGTGGCGG-3’; and GAPDH forward, 5’-AACAGCTCAAGATCATACGCA-3’, and reverse, 5’-CATGATCCTTCCACGATACCA-3’. The thermo cycling 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.

| Table 1. 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* |
| Low                 | 7                   | 37                   |         |         |

IL-8, interleukin-8; ERK2, extracellular regulated protein kinases 2.

*P<0.05.
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       | ERK2       | IL-8 and ERK2 |
|-------------------------------|------------|------------|---------------|
|                               | High | Low | P-value | High | Low | P-value | Both high | Others | P-value |
| Sex                           |       |     |         |       |     |         |           |        |         |
| Male                          | 26   | 29  | 0.158   | 20   | 35  | 0.678   | 19        | 36     | 0.765   |
| 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   |
| <100x10⁹/l                    | 9    | 8   |         | 6    | 11  |         | 6         | 11     |         |
| ≥100x10⁹/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*  |           |        | 0.019*  |
| ≤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*  |           |        | 0.008*  |
| 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     |         |
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 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 ± 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™ 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
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).
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±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. 2A) and DFS (P<0.0001; Fig. 2B) times in the IL-8 high-expression group compared with the 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) <100x10⁹/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<100x10⁹/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 all 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<100x10⁹/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 III. Univariate analysis of variables with patient overall survival (Cox regression model).

| Variable | Hazard ratio | 95% CI | P-value |
|----------|--------------|--------|---------|
| Sex (male vs. female) | 2.143 | 0.647-7.101 | 0.212 |
| Age (≥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 µg/l vs. ≤400 µg/l) | 1.205 | 0.531-2.737 | 0.655 |
| Albumin (≥35 g/l vs. ≥35 g/l) | 1.159 | 0.350-3.840 | 0.009 |
| ALT (≥60 U/l vs. ≤60 U/l) | 1.352 | 0.547-3.340 | 0.513 |
| AST (≥42 U/l vs. ≤42 U/l) | 1.859 | 0.856-4.035 | 0.117 |
| PLT (<100x10⁹/l vs. ≥100x10⁹/l) | 1.992 | 0.918-4.323 | 0.081 |
| Bilirubin (>20 µmol/l vs. ≤20 µmol/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. ≤5 cm) | 2.580 | 1.187-5.606 | 0.017 |
| Tumor margin (≥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 |
| IL-8 (positive vs. negative) | 11.618 | 4.373-30.863 | <0.001 |
| ERK2 (positive vs. negative) | 10.090 | 4.366-23.317 | <0.001 |
| IL-8 and ERK2 (both vs. others) | 3.938 | 2.424-6.397 | <0.001 |

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. *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™ 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.

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

| Variable                                      | Hazard ratio | 95% CI      | P-value |
|-----------------------------------------------|--------------|-------------|---------|
| Sex (male vs. female)                         | 1.567        | 0.660-3.719 | 0.309   |
| Age (≥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 µg/l vs. ≤400 µg/l)                 | 1.537        | 0.798-2.960 | 0.198   |
| Albumin (<35 g/l vs. ≥35 g/l)                 | 1.008        | 0.360-2.826 | 0.988   |
| ALT (>60 U/l vs. ≤60 U/l)                     | 1.064        | 0.472-2.398 | 0.882   |
| AST (>42 U/l vs. ≤42 U/l)                     | 1.209        | 0.607-2.411 | 0.589   |
| PLT (<100x10⁹/l vs. ≥100x10⁹/l)               | 1.940        | 1.015-3.708 | 0.045   |
| Bilirubin (>20 µmol/l vs. ≤20 µmol/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 cm vs. ≤5 cm)                  | 2.070        | 1.075-3.987 | 0.030   |
| Tumor margin (≤2 cm vs. >2 cm)                | 1.157        | 0.601-2.226 | 0.663   |
| Pathological differentiation (middle and low vs. high) | 1.085    | 0.532-2.121 | 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   |
| IL-8 (positive vs. negative)                  | 6.098        | 3.172-11.720| <0.001  |
| ERK2 (positive vs. negative)                  | 5.048        | 2.701-9.433 | <0.001  |
| IL-8 and ERK2 (both vs. others)               | 2.607        | 1.868-3.639 | <0.001  |

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. *P<0.05.

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

| Variable                                      | Hazard ratio | 95% CI      | P-value |
|-----------------------------------------------|--------------|-------------|---------|
| IL-8 (high vs. low)                           | 12.369       | 4.589-33.341| <0.001  |
| ERK2 (high vs. low)                           | 10.011       | 4.268-23.479| <0.001  |
| IL-8 and ERK2 (both vs. others)               | 9.082        | 3.974-20.757| <0.001  |

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

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, 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.
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, 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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