

# Altered epidermal fatty acid-binding protein expression in hepatocellular carcinoma predicts unfavorable outcomes

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**Objective:** Hepatocellular carcinoma (HCC) is a rapidly proliferating malignancy that requires large amounts of fatty acids to synthesize cellular membranes and provide energy. Epidermal fatty acid-binding protein (EFABP) is uniquely expressed in epidermal cells, but its role and expression in HCC are not clear.

**Subjects and methods:** A total of 804 HCC specimens were collected to construct a tissue microarray (TMA) and for immunohistochemistry (IHC) analysis. The relationship between EFABP expression and clinical features of patients with HCC was analyzed.

**Results:** The EFABP IHC score for HCC tissue was  $0.76 \pm 0.69$ , being significantly higher than that for matched nontumorous tissue ( $0.48 \pm 0.55$ ;  $P < 0.001$ ). Using the median IHC score (ie, 0.8) in the tumorous tissue, a high level of EFABP expression was found in 57.3% (461/804) of the cases. Patients with HCC displaying high EFABP expression had poorer tumor differentiation ( $P = 0.029$ ), more vascular invasion ( $P = 0.006$ ), and a higher proportion of late TNM stage disease ( $P = 0.042$ ). Kaplan–Meier analysis revealed that the patients with high EFABP expression had significantly worse outcomes in terms of overall survival ( $P = 0.003$ ), worse disease-free survival ( $P = 0.021$ ), and a higher probability of recurrence ( $P = 0.014$ ). Multivariate analysis indicated that EFABP expression was an independent prognostic variable for overall survival ( $P = 0.021$ ) and disease-free survival ( $P = 0.044$ ). For HCC recurrence, only vascular invasion ( $P = 0.020$ ) and EFABP expression ( $P = 0.026$ ) were independent risk factors.

**Conclusion:** Our data revealed that EFABP expression was increased in HCC samples. High EFABP expression was correlated with shorter survival times in patients with HCC and served as an independent factor for worse outcomes. Our study therefore provides a promising biomarker for the prognostic prediction of HCC and a potential therapeutic target for the disease.

**Keywords:** epidermal fatty acid-binding protein, lipid metabolism, hepatocellular carcinoma, prognostic biomarker

## Introduction

Hepatocellular carcinoma (HCC), the most common type of hepatic cancer,<sup>1</sup> is best cured by surgical resection and transplantation.<sup>2</sup> However, the rates of recurrence and metastasis are still high even after curative hepatectomy.<sup>3</sup> The rate of HCC recurrence after curative surgical or regional therapy is 75% at the fifth year, whereas the rate of recurrence is 86.5% for intrahepatic metastasis and 13.5% for extrahepatic metastasis.<sup>4,5</sup> At present, serum biomarkers, such as alpha-fetoprotein (AFP), and many clinicopathological factors are used as prognostic markers of HCC, but they are not adequate for predicting survival or recurrence after curative hepatectomy.<sup>6,7</sup> Hence, new biomarkers that are effective for predicting the prognosis and recurrence of HCC are still greatly needed.

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An excellent biomarker should have unique characteristics, along with high sensitivity and specificity.<sup>8</sup> In order to explore HCC-specific markers, it is practical to explore the unique biological characteristics of the HCC cells first, to subsequently better identify the related molecular markers. However, the extensive heterogeneity in tumor biology is an important aspect of HCC cells.<sup>9</sup> The main characteristic of HCC is its rapid proliferation, which means that HCC cells must depend excessively on lipids.<sup>10,11</sup> This is because lipids not only serve as a component for synthesizing the cell membrane (which is necessary for cell proliferation) but also provide the energy needed for rapid proliferation.<sup>9</sup> However, the source of lipids in HCC cells is a question worth pondering. Previous studies have suggested that hyperactivation of lipid synthesis by HCC cells implies that these cells may convert glucose-derived carbon into lipids via the glycolytic pathway.<sup>12</sup> However, increasingly more studies have suggested that HCC can absorb peripheral lipids for their own needs.<sup>10,13</sup> As a result of tumor metabolic reprogramming, this may be one of the specific manifestations of HCC cell heterogeneity. For example,<sup>13</sup> primary ovarian cancer cells undergo highly activated lipogenesis to supply the lipids required for uncontrolled cell proliferation. However, when ovarian cancer metastasizes to omental fat, which contains a microenvironment abundant in the adipocytes, the cancer cells are metabolically reprogrammed to favor lipid oxidation using the adipocyte-derived fatty acids.<sup>13</sup> In patients with HCC, lipolysis of subcutaneous adipocytes occurs, and cachexia appears to be a proven clinical phenomenon.<sup>14</sup> The fatty acid released into the circulatory system by the adipocytes is taken up by the HCC cells; this is also a well-known phenomenon.

Since HCC cells take up fatty acids in the peripheral system, it is unavoidable that an increase in fatty acid-binding proteins (FABPs) would be needed.<sup>15-17</sup> The FABP family comprises proteins with a high affinity to fatty acids.<sup>18</sup> Members of this family express tissue specificity. For example, liver FABP (LFABP) is expressed only in the hepatocytes. In the adipocytes, both adipocyte FABP and epidermal FABP (EFABP, FABP5) can be expressed. EFABP expression has been reported to be increased in various cancers.<sup>19,20</sup> Ohata et al reported that FABP5 plays a significant role in HCC progression and metastasis through the induction of epithelial-to-mesenchymal transition.<sup>21</sup> Wang et al reported that HSP 90-beta, FABP5, and alcohol dehydrogenase 4 are potential clinically used biomarkers for HCC.<sup>22</sup> Similarly, Jeong et al found that FABP5 is significantly overexpressed in intrahepatic cholangiocarcinoma combined lymph node metastasis

and is involved in cell proliferation and invasion *in vitro*.<sup>23</sup> Interestingly, the expression of LFABP was reported to be decreased in HCC.<sup>24</sup> Therefore, it remains to be elucidated whether ectopic EFABP expression occurs to transport fatty acid within HCC cells, and subsequently whether EFABP can be regarded as a marker of the peripheral lipid uptake ability of the HCC cells as well as a potential prognostic marker for the disease.

In this study, we investigated the role of EFABP in human HCC. We showed that EFABP expression is positively correlated with the overall survival of patients with HCC and, therefore, is a promising biomarker for the prognostic prediction of HCC and a potential therapeutic target for the clinical management of this disease.

## Subjects and methods

### Subjects

From January 2000 to December 2010, a total of 804 paraffin-embedded HCC specimens were collected from the Department of Pathology, Sun Yat-sen University Cancer Center. None of the patients from whom the samples were retrieved had received any chemotherapy or radiotherapy prior to surgery. The follow-up period was defined as the interval between the date of operation and the date of death or the last follow-up. This study was approved by the Medical Ethics Committee of Sun Yat-sen University Cancer Center. Because all specimens used were anonymous, the Medical Ethics Committee of Sun Yat-sen University Cancer Center waived the need for informed patient consent.

### Tissue microarray (TMA) construction and immunohistochemistry (IHC)

The TMA slices comprised tumorous tissue and matched adjacent normal tissues from 804 cases of HCC. Using a tissue arrayer (MiniCore, Excilone, UK), each tissue core (diameter: 0.6 mm) was perforated and re-embedded from the marked area. All specimens were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer for 24 hours and embedded in paraffin. The paraffin-embedded tissues were then sectioned into 4- $\mu$ m sections and mounted on glass slides. After dewaxing, the slides were treated with 3% hydrogen peroxide in methanol and blocked with a biotin blocking kit (Dako Denmark A/S, Glostrup, Denmark). After blocking, the slides were incubated overnight with EFABP antibody (ab84028, 1:1,000; Abcam, Cambridge, MA, USA) in a humid chamber at 4°C, washed three times with PBS, incubated for 1 hour with biotinylated goat anti-mouse antibody, and then stained with 3,3'-diaminobenzidine

tetrahydrochloride. Finally, the sections were stained with hematoxylin and observed under a microscope.

Semi-quantitative IHC was used to detect the protein expression levels of EFABP, according to the following standard scores: “0” (negative staining), “1” (weak staining), “2” (moderate staining), and “3” (strong staining). The final score was calculated as the percentage of positive expression multiplied by the intensity score. The score was independently determined by two pathologists. The median IHC score was used as the cutoff for judging high and low expression levels.

## Statistical analysis

Statistical analysis was performed using SPSS software (version 16.0; SPSS Inc., Chicago, IL, USA). A Student's *t*-test and Pearson's chi-squared test or Fisher's exact test were chosen for examining the correlations between the EFABP expression level and the clinical and pathological variables. Survival curves were constructed using the Kaplan–Meier method (log-rank test). A multivariate Cox proportional hazards regression model was used to evalu-

ate the independence of EFABP in predicting outcomes. Differences were defined as significant for *P*-values less than 0.05.

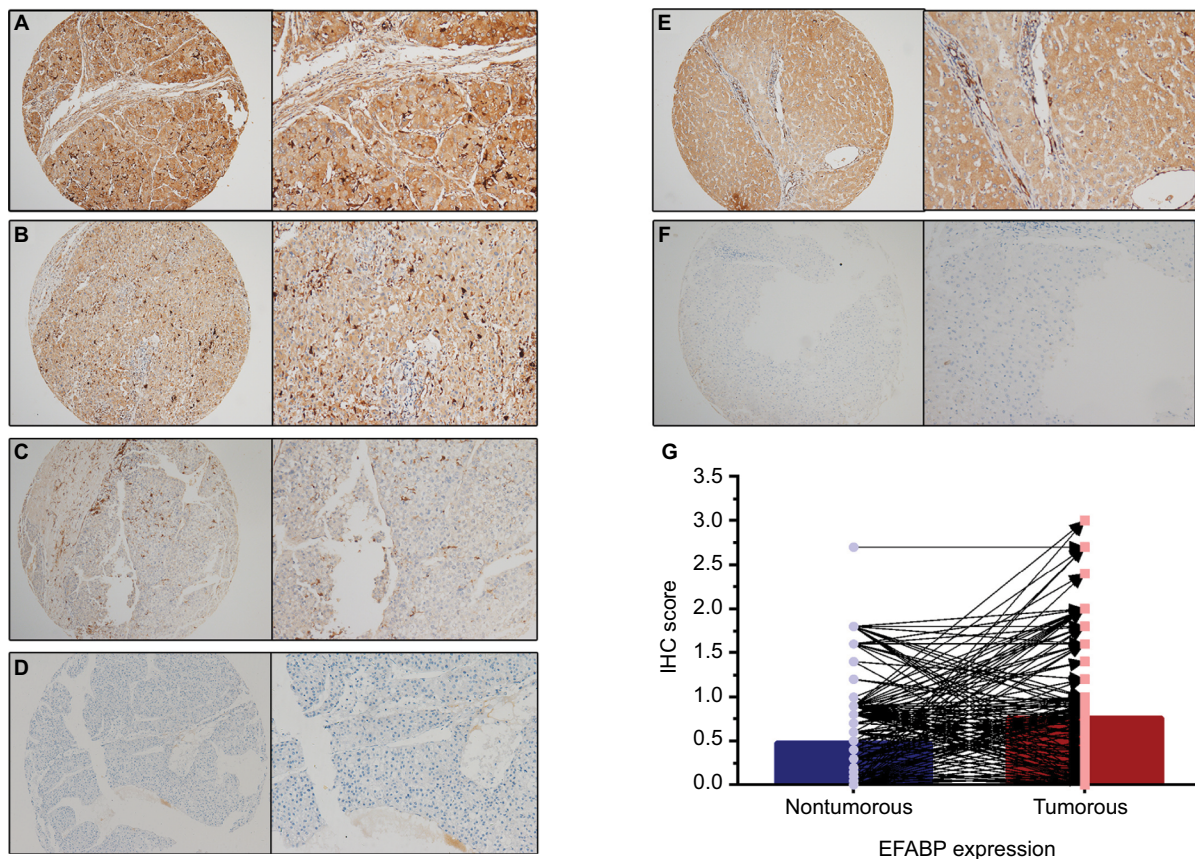
## Results

### Expression of EFABP in the HCC TMA

We used an HCC TMA (n=804) to detect EFABP expression. EFABP is expressed mainly in cytoplasm of HCC cells. The EFABP IHC score for HCC tissue was  $0.76 \pm 0.69$ , which is significantly higher than that for matched nontumorous tissue ( $0.48 \pm 0.55$ ;  $P < 0.001$ ) (Figure 1 and Figure S1). In addition, we also analyzed the difference in the expression levels of EFABP between normal liver tissue and cirrhotic tissue. The results showed no statistical difference between the two (normal liver vs cirrhosis,  $0.79 \pm 0.71$  vs  $0.75 \pm 0.69$ ,  $P = 0.54$ ).

### Association of cytoplasmic EFABP with HCC clinical features

To determine the potential clinical significance of EFABP in HCC, the relationship between EFABP and the clinical



**Figure 1** Epidermal fatty acid-binding protein (EFABP) is expressed mainly in the cytoplasm.

**Notes:** Representative images of hepatocellular carcinoma (HCC) tissues showing strong (A), moderate (B), weak (C), and negative (D) EFABP expression. Representative images of positive and negative EFABP expression in a nontumorous sample (E, F) (left panel: magnification 100 $\times$ ; right panel: magnification 400 $\times$ ). EFABP expression was increased in HCC tissues compared with that in the corresponding nontumorous tissue, as assessed by immunohistochemistry (IHC) ( $P < 0.001$ ) (G).



features of patients with HCC was evaluated. Using the median IHC score (0.8) of the tumorous tissues, a high level of EFABP expression was found in 57.3% (461/804) of the cases. Patients with high levels of EFABP expression had poorer tumor differentiation ( $P=0.029$ ), more vascular invasion ( $P=0.006$ ), and a higher proportion of late TNM stage disease ( $P=0.042$ ), as shown in Table 1. We also analyzed the level of EFABP expression in liver tissue with liver steatosis.

**Table 1** Association between EFABP expression and clinical features of hepatocellular carcinoma

Variable	EFABP expression		P-value
	High expression	Low expression	
Sample size	461	343	
Age, years	48.61±11.86	49.22±12.04	0.476
Gender			0.335
Male	412 (89.4%)	299 (87.2%)	
Female	49 (10.6%)	44 (12.8%)	
HBsAg			0.823
Positive	383 (83.1%)	287 (83.7%)	
Negative	78 (16.9%)	56 (16.3%)	
AFP, ng/mL			0.334
<20	97 (21.0%)	82 (23.9%)	
≥20	364 (79.0%)	261 (76.1%)	
Cirrhosis			0.465
Yes	371 (80.5%)	283 (82.5%)	
No	90 (19.5%)	60 (17.5%)	
Tumor size, cm			0.058
<5	125 (27.1%)	73 (21.3%)	
≥5	336 (72.9%)	270 (78.7%)	
Tumor multiplicity			0.759
Single	303 (65.7%)	229 (66.8%)	
Multiple	158 (34.3%)	114 (33.2%)	
Differentiation			0.029
Well-moderate	31 (6.7%)	38 (11.1%)	
Poor-undifferentiated	430 (93.3%)	625 (88.9%)	
TNM stage			0.042
I–II	178 (38.6%)	157 (45.8%)	
III–IV	283 (61.4%)	186 (54.2%)	
Vascular invasion			0.006
Yes	101 (21.9%)	49 (14.3%)	
No	360 (78.1%)	294 (85.7%)	
Involucrum			0.203
Complete	185 (40.1%)	153 (44.6%)	
Incomplete	276 (59.9%)	190 (55.4%)	
Lymph node metastasis			0.673
Positive	25 (5.4%)	21 (6.1%)	
Negative	436 (94.6%)	322 (93.9%)	
Distant metastasis			0.122
Positive	41 (8.9%)	42 (12.2%)	
Negative	420 (91.1%)	301 (87.8%)	

**Abbreviations:** AFP, alpha-fetoprotein; EFABP, epidermal fatty acid-binding protein; HBsAg, hepatitis B virus surface antigen.

The results showed no difference in EFABP expression levels in tissues with or without steatosis (non-steatosis vs steatosis,  $0.69±0.65$  vs  $0.80±0.67$ ,  $P=0.19$ ).

## Association of EFABP expression with clinical outcomes in patients with HCC

To determine the prognostic effect of EFABP expression on patients with HCC, we conducted a Kaplan–Meier survival analysis using data from the 804 patients enrolled in the study. For the patients with high EFABP expression, Kaplan–Meier analysis revealed that they had significantly worse outcomes in terms of overall survival ( $P=0.003$ ). Similarly, compared with the patients with low EFABP expression, those with high EFABP expression had a significantly worse disease-free survival ( $P=0.021$ ) and a higher probability of recurrence ( $P=0.014$ ), as shown in Figure 2.

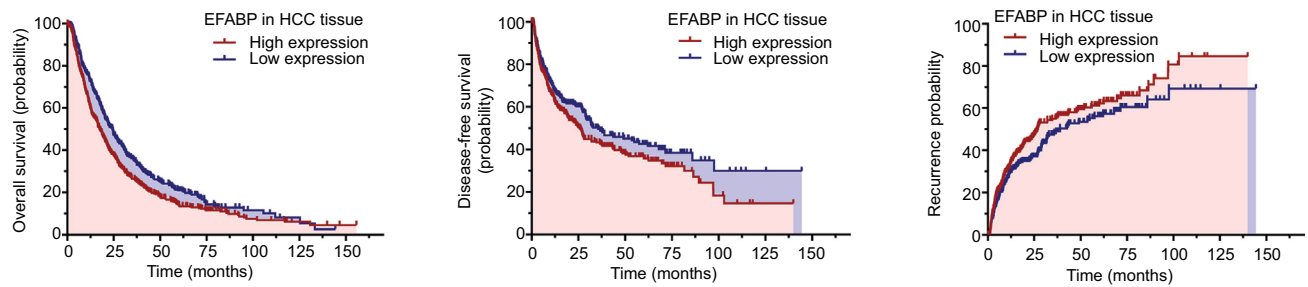
## Univariate and multivariate analyses of prognostic variables in HCC

To evaluate whether EFABP expression was an independent risk factor for outcomes in HCC, both univariate and multivariate analyses were conducted. The serum AFP level, tumor size, tumor multiplicity, tumor differentiation, TNM stage, vascular invasion, involucrum, and EFABP expression were all shown to be prognostic variables for overall survival in patients with HCC. In the multivariate analysis, only tumor size ( $P=0.001$ ), TNM stage ( $P<0.001$ ), vascular invasion ( $P<0.001$ ), involucrum ( $P=0.044$ ), and EFABP expression ( $P=0.021$ ) were found to be independent prognostic variables for overall survival (Table 2).

We further explored the risk factors associated with disease-free survival (Table 3) and HCC recurrence (Table 4). Univariate analysis showed that age, serum AFP level, TNM stage, vascular invasion, and EFABP expression were risk factors associated with disease-free survival. In the multivariate analysis, vascular invasion ( $P=0.002$ ) and EFABP expression ( $P=0.044$ ) were independent risk factors associated with disease-free survival. For HCC recurrence, only vascular invasion ( $P=0.020$ ) and EFABP expression ( $P=0.026$ ) were the associated independent risk factors.

## Subgroup analyses of the prognostic value of EFABP expression in the cytoplasm in HCC

A stratified survival analysis was conducted to further reveal the prognostic significance of EFABP expression among patients with HCC. Kaplan–Meier survival analysis showed



**Figure 2** High epidermal fatty acid-binding protein (EFABP) expression is correlated with an unfavorable prognosis in 804 patients with hepatocellular carcinoma (HCC). **Notes:** Kaplan–Meier analysis showed significant differences in postoperative overall survival between patients with high EFABP expression and those with low EFABP expression ( $P=0.003$ ). A similar trend was observed in both patient groups when comparing disease-free survival ( $P=0.021$ ) and the probability of recurrence ( $P=0.014$ ).

**Table 2** Univariate and multivariate analyses of prognostic variables for overall survival

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	P-value	HR	95% CI	P-value
Age, years	0.871	0.749–1.013	0.073	0.974	0.935–1.137	0.740
Sex	0.867	0.680–1.106	0.251	0.913	0.714–1.167	0.467
HBsAg	1.182	0.961–1.453	0.114	1.108	0.896–1.369	0.343
AFP	1.258	1.050–1.506	0.013	1.046	0.867–1.263	0.638
Cirrhosis	0.978	0.802–1.192	0.822	1.051	0.859–1.286	0.627
Tumor size, cm	1.648	1.373–1.977	<0.001	1.458	1.210–1.757	<0.001
Tumor multiplicity	1.628	1.390–1.908	<0.001	1.150	0.955–1.384	0.140
Differentiation	1.633	1.244–2.144	<0.001	1.235	0.928–1.644	0.148
TNM stage	2.063	1.762–2.416	<0.001	1.661	1.402–1.967	<0.001
Vascular invasion	2.583	2.137–3.121	<0.001	1.843	1.502–2.262	<0.001
Involucrum	1.358	1.163–1.585	<0.001	1.179	1.005–1.383	0.044
EFABP expression	1.265	1.085–1.474	0.003	1.201	1.028–1.403	0.021

**Abbreviations:** AFP, alpha-fetoprotein; EFABP, epidermal fatty acid-binding protein; HBsAg, hepatitis B virus surface antigen.

**Table 3** Univariate and multivariate analyses of prognostic variables for disease-free survival

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	P-value	HR	95% CI	P-value
Age, years	0.818	0.670–0.997	0.047	0.865	0.707–1.060	0.162
Sex	0.875	0.635–1.206	0.268	0.855	0.619–1.180	0.340
HBsAg	0.992	0.763–1.289	0.951	0.960	0.733–1.258	0.766
AFP	1.281	1.007–1.630	0.044	1.191	0.927–1.529	0.171
Cirrhosis	1.013	0.784–1.308	0.921	1.061	0.818–1.377	0.655
Tumor size, cm	1.224	0.976–1.534	0.080	1.174	0.927–1.486	0.183
Tumor multiplicity	1.200	0.968–1.488	0.097	1.106	0.856–1.429	0.440
Differentiation	1.294	0.925–1.811	0.132	1.092	0.767–1.556	0.624
TNM stage	1.225	1.002–1.499	0.048	0.998	0.771–1.292	0.987
Vascular invasion	1.555	1.195–2.025	0.001	1.512	1.160–1.971	0.002
Involucrum	1.170	0.957–1.431	0.126	1.062	0.859–1.313	0.576
EFABP expression	1.267	1.035–1.552	0.022	1.233	1.006–1.511	0.044

**Abbreviations:** AFP, alpha-fetoprotein; EFABP, epidermal fatty acid-binding protein; HBsAg, hepatitis B virus surface antigen.

that EFABP expression was associated with overall survival in both small and large HCCs (small HCCs:  $P=0.011$ ; large HCCs:  $P=0.007$ ), in serum hepatitis B virus surface antigen (HBsAg)-positive and -negative HCCs (HBsAg-negative HCCs:  $P=0.017$ ; HBsAg-positive HCCs:  $P=0.030$ ), in vascular invasion-positive and -negative HCCs and TNM stage III–IV HCCs (vascular invasion-positive HCCs:  $P=0.043$ ;

vascular invasion-negative HCCs:  $P=0.044$ ), and in HCCs with and without cirrhosis (HCCs with cirrhosis:  $P=0.013$ ; HCCs without cirrhosis:  $P=0.035$ ), as shown in Figure 3.

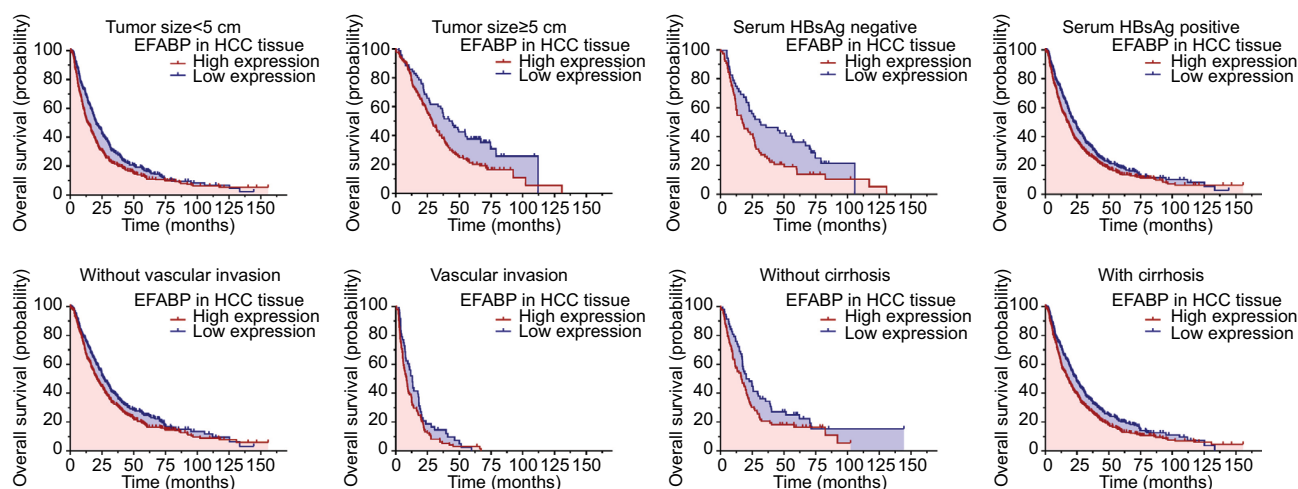
## Discussion

HCC is an end-stage liver disease. Chronic viral infection accounts for most of the global etiology of the disease,

**Table 4** Univariate and multivariate analyses of prognostic variables for hepatocellular carcinoma recurrence

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	P-value	HR	95% CI	P-value
Age, years	0.888	0.722–1.093	0.262	0.937	0.759–1.156	0.543
Sex	0.828	0.589–1.164	0.278	0.815	0.579–1.147	0.240
HBsAg	1.073	0.811–1.419	0.621	1.048	0.785–1.399	0.749
AFP	1.279	0.996–1.642	0.054	1.247	0.961–1.618	0.097
Cirrhosis	0.982	0.754–1.279	0.892	0.999	0.763–1.307	0.992
Tumor size, cm	1.077	0.857–1.353	0.526	1.038	0.817–1.318	0.761
Tumor multiplicity	1.192	0.952–1.493	0.126	1.122	0.858–1.468	0.399
Differentiation	1.165	0.835–1.627	0.368	1.012	0.711–1.440	0.947
TNM stage	1.193	0.968–1.471	0.098	0.997	0.762–1.304	0.981
Vascular invasion	1.446	1.090–1.919	0.011	1.400	1.054–1.861	0.020
Involucrum	1.089	0.884–1.341	0.424	1.007	0.808–1.255	0.948
EFABP expression	1.301	1.054–1.607	0.015	1.273	1.030–1.574	0.026

**Abbreviations:** AFP, alpha-fetoprotein; EFABP, epidermal fatty acid-binding protein; HBsAg, hepatitis B virus surface antigen.



**Figure 3:** High epidermal fatty acid-binding protein (EFABP) expression is associated with overall survival in both small and large hepatocellular carcinomas (HCCs) (small HCCs:  $P=0.011$ ; large HCCs:  $P=0.007$ ).

**Notes:** Similar trends were observed in serum hepatitis B virus surface antigen (HBsAg)-positive and -negative HCCs (HBsAg-negative HCCs:  $P=0.017$ ; HBsAg-positive HCCs:  $P=0.030$ ), in vascular invasion-positive and -negative HCCs and TNM stage III–IV HCCs (vascular invasion-positive HCCs:  $P=0.043$ ; vascular invasion-negative HCCs:  $P=0.044$ ), and in HCCs with and without cirrhosis (HCCs with cirrhosis:  $P=0.013$ ; HCCs without cirrhosis:  $P=0.035$ ).

especially in Asia.<sup>25–28</sup> Recently, nutritionally related liver diseases, such as non-alcoholic fatty liver disease (NAFLD), have been found to be associated with HCC, and energy metabolism reprogramming is also one of the markers of cancer.<sup>29–31</sup> Cancer cells rely on non-glucose carbon sources and increased expression of enzymes involved in the synthesis of fatty acids to biosynthesize cell membrane, which promotes tumor aggressiveness by increasing cell proliferation.<sup>12,32</sup> However, lipid absorption is another potential way for cancer cells to increase their lipid content for cell biosynthesis.<sup>11,17</sup> EFABP has high affinity for fatty acids, and its content is directly proportional to the lipid content.<sup>17</sup> Therefore, we evaluated the expression of EFABP in HCC and explored the relationship between EFABP and HCC prognosis.

EFABP, also known as psoriasis-associated FABP or skin FABP, is an isomer of FABPs that are small and soluble

intracellular lipid-binding proteins that bind fatty acids.<sup>15,33</sup> FABPs transport lipids to the cell compartment to be stored as lipid droplets, to the endoplasmic reticulum for membrane synthesis, and to the nucleus for lipid-mediated transcriptional regulation.<sup>34,35</sup> The function of EFABP is to enhance the transcriptional activity of the nuclear receptors PPAR $\beta/\delta$  and promote cell migration, proliferation, and survival.<sup>36–38</sup> EFABP is overexpressed in many human cancers, including prostate cancer,<sup>39,40</sup> esophageal squamous cell carcinoma,<sup>41,42</sup> and breast cancer.<sup>43,44</sup> Previous studies have found that EFABP may play an important role in liver cancer. Ohata et al reported that FABP5 promotes HCC progression by epithelial-to-mesenchymal transition.<sup>21</sup> Wang et al reported that FABP5 can be regarded as a potential clinically used biomarker for HCC.<sup>22</sup> Similarly, Jeong et al found that FABP5 is significantly overexpressed in intrahepatic cholangiocarcinoma and is involved

in cell proliferation and invasion *in vitro*.<sup>23</sup> Interestingly, in our study, we found that patients with HCC displaying high EFABP expression had poorer tumor differentiation, more vascular invasion, and a higher proportion of late TNM stage disease. More importantly, the patients with high EFABP expression had significantly worse outcomes than those with low EFABP expression, with worse disease-free survival and a higher probability of recurrence. In addition, high EFABP expression was an independent prognostic variable for overall survival, disease-free survival, and HCC recurrence.

Previous reports have shown the association of high EFABP expression with tumor metastasis and poor prognosis in various types of cancer.<sup>20,45</sup> EFABP overexpression was reported to promote tumor metastasis by matrix metalloproteinase 9 upregulation, tumorigenesis of proteolytic enzymes to promote tumor metastasis, and increased expression of vascular endothelial growth factor, a protein in tumor angiogenesis.<sup>19</sup> According to the results of our study, the patients with a high expression level of EFABP had a higher incidence of vascular invasion. This may indicate the molecular mechanism of EFABP in promoting HCC progression, in that the protein may facilitate epithelial–mesenchymal transition in HCC cells. Fatty acids are peroxisome proliferator-activated receptor- $\alpha$  (PPAR $\alpha$ ) ligands.<sup>46</sup> Whether there is a correlation of EFABP and PPAR expression is an interesting question that is waiting for an answer. In addition, according to our study, EFABP expression is very low in nontumor liver tissues. A previous study suggested that there is a variation in FABPs.<sup>47</sup> Whether or not that there is another variation of EFABP expressed in nontumor liver tissue requires further study to confirm.

Most HCCs occur in patients with chronic liver diseases, such as chronic hepatitis B virus (HBV) infection, chronic hepatitis C virus (HCV) infection, and alcohol abuse.<sup>48–51</sup> However, as a result of worldwide HBV vaccine immunization and antiviral therapy for HBV and HCV, NAFLD and non-alcoholic steatohepatitis have become the higher risk factors for HCC.<sup>29</sup> Recent studies have reported obesity, metabolic syndrome, and type 2 diabetes as important risk factors for HCC.<sup>30</sup> Other recent studies have reported the accumulation of lipids in NAFLD, HCV-related liver steatosis, and HBV-related HCC.<sup>52</sup> Since the role of FABP is in binding fatty acids, the inhibition of EFABP may be a potential way to prevent metabolic liver disease progression to HCC. In addition, EFABP may be regarded as a metabolic-related target in HCC treatment. However, further studies are needed to confirm the potential clinical application of EFABP. In addition, EFABP can be measured in serum.<sup>53</sup> It would be interesting

to clarify that EFABP in serum is derived from HCC cells. However, EFABP in serum is affected by a variety of factors, including ethnicity, metabolic disorders, and cardiovascular disease.<sup>53–55</sup> Further studies with strict enrollment criterion are needed to clarify whether there is a correlation between EFABP in HCC tissue and EFABP in serum.

## Conclusion

In summary, our study results demonstrate a role for EFABP in the development of HCC. Our data revealed that EFABP expression was increased in HCC samples, and such an increase was significantly correlated with poorer tumor differentiation and more vascular invasion. High EFABP expression was also correlated with shorter survival times in patients with HCC and served as an independent factor for worse outcomes. Collectively, our data suggest that EFABP is a promising biomarker for the prognosis of patients with HCC and a potential metabolic-related target in HCC treatment.

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## Disclosure

The authors report no conflicts of interest in this work.

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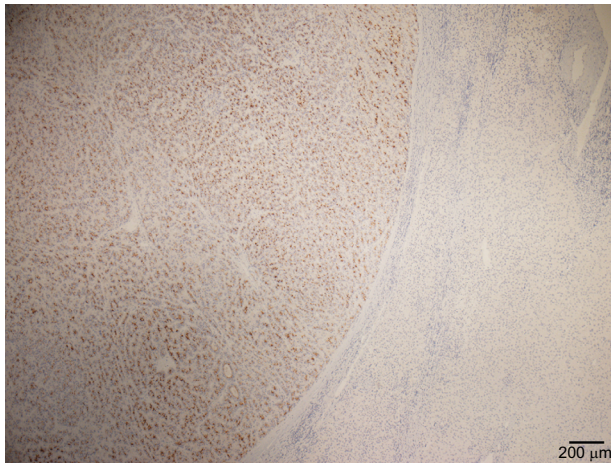


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## Supplementary material



**Figure S1** The EFABP expression in HCC tissues and adjacent non-tumor tissue.

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