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ORIGINAL RESEARCH

Expression and Correlation of MIF and ERK1/2 in Liver Cirrhosis and Hepatocellular Carcinoma Induced by Hepatitis B

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Objective: To detect expression and phosphorylation level of macrophage migration inhibitor (MIF) and extracellular-regulated kinases 1 and 2 (ERK1/2) in hepatitis B-induced liver cirrhosis (HBILC) and hepatocellular carcinoma (HCC) with a background of HBILC and analyze the correlation of MIF and ERK1/2 with HBILC and HCC.

Methods: Twenty cases of normal liver tissues were collected as a control group, and 48 specimens of HBILC tissues and 48 specimens of HCC tissues were collected as the experimental group, which were assigned as the HBILC group and HCC group, respectively. All tissue specimens were processed into tissue chips. The expressions of MIF, ERK1/2, and their phosphorylated proteins were detected via immunohistochemistry, and MIF and ERK1/2 nucleic acid expressions were detected by in situ hybridization. The results were statistically analyzed using the chi-square test.

Results: Proteins and nucleic acids of MIF and ERK1/2 presented low expression in the control group and high expression in the HBILC group and HCC group. MIF expression in the three groups was 25.0%, 75.0%, and 79.17%, respectively, while that of the nucleic acids was 25.0%, 70.83%, and 68.75%, respectively. Expression of ERK1/2 in the three groups was 40.0%, 60.42%, and 81.25%, respectively, and that of nucleic acids was 40.0%, 79.17%, and 77.08%. Expression of pERK1/2 was low in the control and HBILC group and high in the HCC group. Expression of pERK1/2 in the three groups was 20%, 45.83%, and 75%, respectively. Expression of pERK1/2 in the HCC group was significantly different from that in the HBILC and control group ($P<0.05$), but the difference between the HBILC group and control group was not statistically significant ($P>0.05$).

Conclusion: Occurrence and development of HBILC and HCC are not only related to the high expression of MIF but also closely related to the activation of the ERK1/2 signaling pathway.

Keywords: MIF, MAPK signaling pathway, hepatitis B-induced liver cirrhosis, hepatocellular carcinoma

Background

Hepatocellular carcinoma (HCC) is one of the most malignant tumors with a poor prognosis. One reason for this is that the pathogenesis of HCC is complex, especially considering hepatitis B-induced liver cirrhosis (HBILC) is the primary pathological basis of HCC. In the process of evolution to HCC, coupled with the role of the hepatitis B virus (HBV), some proteins and signal pathways closely related to inflammation and tumors play an important regulatory role. Our research team has found that macrophage migration inhibitor (MIF) is gradually over-expressed in chronic hepatitis B, HBILC, and HCC,[1,2] suggesting that MIF may play a crucial role in the evolution of chronic hepatitis B to cirrhosis and HCC. According to the literature, MIF, an inflammatory factor, plays a role in promoting inflammation and carcinogenesis. This is closely related to the activation of related signal pathways,[3–6] while the signaling pathway of the

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extracellular-regulated kinases 1 and 2 (ERK1/2), an important sub-pathway of the mitogen-activated protein kinase (MAPK) signaling pathway, is closely related to the occurrence and development of a variety of tumors. For example, the up-regulation of ERK1/2 expression can promote the formation and development of gastric cancer, melanoma, esophageal cancer, and colon cancer.[7–10] Many studies have also reported that abnormal activation of the ERK1/2 signaling pathway is closely related to HCC.[11,12] However, there is no apparent research on whether a correlation exists between the ERK1/2 signaling pathway and MIF at the tissue level. Therefore, the research team collected normal liver tissues, HBILC tissues, and HCC tissues to detect the expression of proteins and nucleic acids of MIF and ERK1/2, as well as phosphorylated ERK1/2 protein, to understand the correlation between these two proteins and the occurrence and development of HBILC and HCC.

Subjects and Methods

Specimen Collection and Preparation

Forty-eight HCC tissue specimens with HBILC and corresponding cirrhosis tissues 5 cm away from HCC were collected as the experimental group. Tissue specimens were collected from 36 male and 12 female patients with ages ranging from 35–65 years old and an average age of 41.05 ± 3.81 years old. Normal liver tissues from 20 patients with gallstones or hemangiomas were collected as the control group. The diagnostic criteria of all HCC patients were based on the Guidelines for the Diagnosis and Treatment of Primary Liver Cancer (2022 version), which was revised and updated again by the National Health Commission in 2022.[13] All tissue specimens were fixed with 10% formaldehyde, embedded in paraffin, and made into paraffin sections of tissue chip with a section thickness of about 4 μm.

Experimental Reagents and Methods

The MIF monoclonal antibody was procured from Santa Cruz (USA) with a dilution ratio of 1:80, the monoclonal antibody of Rabbit Anti-human Thymocyte Immunoglobulin ERK1/2 and the goat anti-rabbit secondary antibody were procured from Abcam (UK) with a dilution ratio of 1:80, and the phosphorylated rabbit anti-human thymocyte immunoglobulin ERK1/2 monoclonal antibody was procured from CST (USA) with a dilution ratio of 1:100. MIF, ERK1/2 in situ hybridization kit (person) was procured from Roche (Shanghai). Immunohistochemical technique was used for detecting the expression of MIF, ERK1/2 protein and phosphorylated ERK1/2 protein in the liver tissues of the experimental group and the control group. After adding the first and second antibodies, they had been incubated at 37°C for 50 min. The expression of MIF and ERK1/2 nucleic acids in the experimental group and the control group was detected by in situ hybridization. In this step, 20% hydrogen peroxide was added to inactivate the activity of endogenous peroxidase. It was necessary to incubate the MIF and ERK1/2 nucleic acids in a 37°C incubator for 10 min, expose the MIF and ERK1/2 nucleic acids in a 37°C incubator for 20 min, and then fix the 37°C incubator for 10 min, pre-hybridize in a 37°C incubator for 4h, hybridize in a 37°C incubator for 24h, and then add the sealing solution for 30 min, the biotinylated rabbit anti-digoxin was incubated at 37°C for 1h, and SABC and biotinylated peroxidase were incubated at 37°C for 30 min respectively.

Result Interpretation Criteria

The protein is mainly expressed in the cytoplasm, while the phosphorylated protein is primarily expressed in the nucleus. The presence of brownish-yellow particles indicates a positive result. Figures were read at high magnification (× 400). For each section, a non-repeating field was chosen in the upper, middle, lower, left, and right sites. Both the percentage and staining intensity of positive cells among all cells of the same type were observed. Results were calculated based on the combination of these two factors. The scoring standard for the percentage of positive cells was as follows: <10% recorded as 0 points, 10–40% recorded as 1 point, 41–70% recorded as 2 points, and >70% recorded as 3 points. The scoring standard for staining intensity was as follows: 0 points for no visible staining, 1 point for light yellow, 2 points for brownish-yellow, and 3 points for yellow-brown. A comprehensive score was calculated from the sum of the two scores, where cells with a score ≤2 points were negative and cells with a score >2 points were positive.

Statistical Methods

The data were processed and analyzed using SPSS 17.0. Count data were evaluated using Pearson's X^2 test, and P<0.05 was considered statistically significant.

Results

Expression of MIF and ERK1/2 Proteins

The positive expression of MIF protein was mainly in the cytoplasm, which was highly expressed in tissues of HCC and HBILC, while the positive rate in normal liver tissue was low (Figure 1A–C). The positive expression of ERK1/2 protein was primarily in the cell membrane and cytoplasm; MIF expression in the HCC and HBILC groups was also higher than that in the control group (Figure 2A–C). There were significant differences in MIF and ERK1/2 protein expression in different liver tissues between all groups (P<0.05). However, there was no significant difference between the HCC group and the HBILC groups (P>0.05; Table 1).

Expression of MIF and ERK1/2 Nucleic Acids

MIF mRNA and ERK1/2 mRNA were mostly positively expressed (present) in the HCC group and HBILC group and negatively expressed (absent) in the control group (Figures 3A and 4C); that is, there were significant differences in the expression of the two nucleic acids between the HCC group and the control group and between the HBILC group and the control group (P<0.05). There were no significant differences in the expressions of the two nucleic acids between the HCC and HBILC groups (P>0.05, Table 2).

Expression of PERK1/2

The expression of pERK1/2 was localized in the nucleus but was also detected in the cell membrane and cytoplasm. The present study revealed that pERK1/2 had a high expression rate in the HCC group and a low expression rate in the HBILC and control groups (Figure 5A–C). There was a significant difference between the HCC group and HBILC group

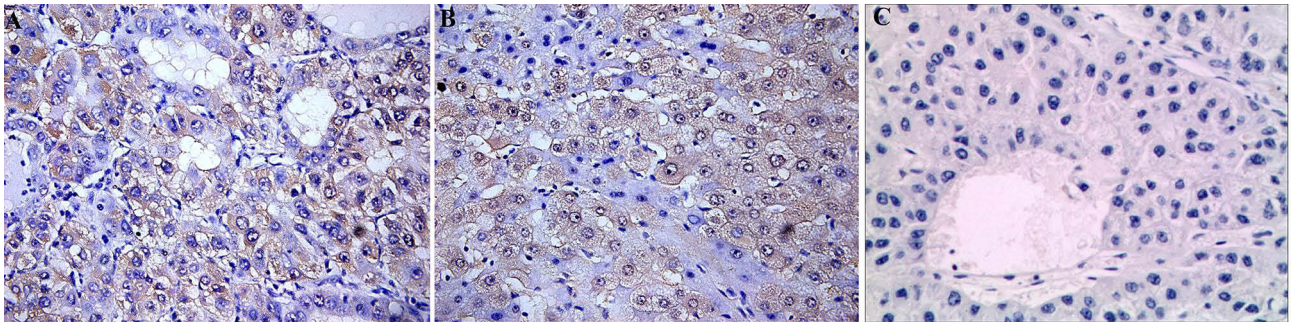

Figure 1 Expression of MIF. (**A**) Positive expression of MIF in HCC×200. (**B**) Positive expression of MIF in HBV-LC×200. (**C**) Negative expression of MIF in normal liver tissue×200.

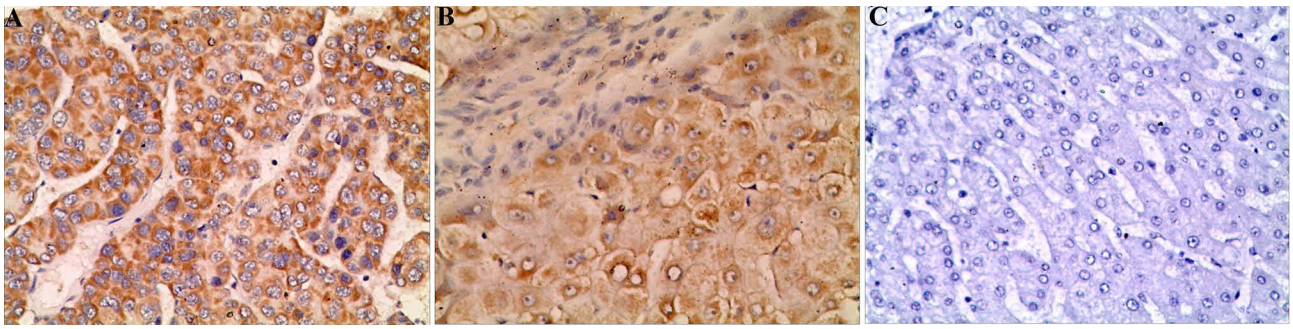

Figure 2 Expression of ERK1/2. (**A**) Positive expression of ERK1/2 in HCC×200. (**B**) Positive expression of ERK1/2 in HBV-LC ×200. (**C**) Negative expression of ERK1/2 in normal liver tissue×200.

Table 1 Expressions of MIF and ERK1/2 Proteins in Experimental Groups and Control Group

Groups	Number of Cases	Positive Expression Rate (%)	
		MIF	ERK1/2
HCC group	48	38/79.17	39/81.25
HBILC group	48	36/75.0	29/60.42
Control group	20	5/25.0	8/40.0
$\chi 2$ value		20.861	11.576
P value		<0.05	<0.05

Notes: There are significant differences in the expressions of MIF and ERK1/2 proteins in different liver tissues among all groups ($P<0.05$), Comparison of expression of MIF and ERK1/2 protein between the HCC group and control group ($P<0.05$), comparison of expression of MIF and ERK1/2 proteins between the HBILC group and control group ($P<0.05$), comparison of expression of MIF and ERK1/2 proteins between the HCC group and HBILC group ($P>0.05$).

and between the HCC group and control group ($P<0.05$). However, there was no significant difference between the HBILC and control groups ($P>0.05$, Table 3).

Discussion

At present, the global incidence rate of HCC is still mounting. Although various methods have played a role in the treatment of HCC—especially the development and clinical application of molecular targeting and immune checkpoint inhibitors—and improved the curative effects of HCC to a certain extent, they are not successful in all patients. One reason is that the pathogenesis of HCC is extremely complex. During the evolution from HBILC to HCC, HBV-induced hepatocyte inflammation[14] and abnormal expressions of some genes, proteins, and cytokines (such as the abnormalities

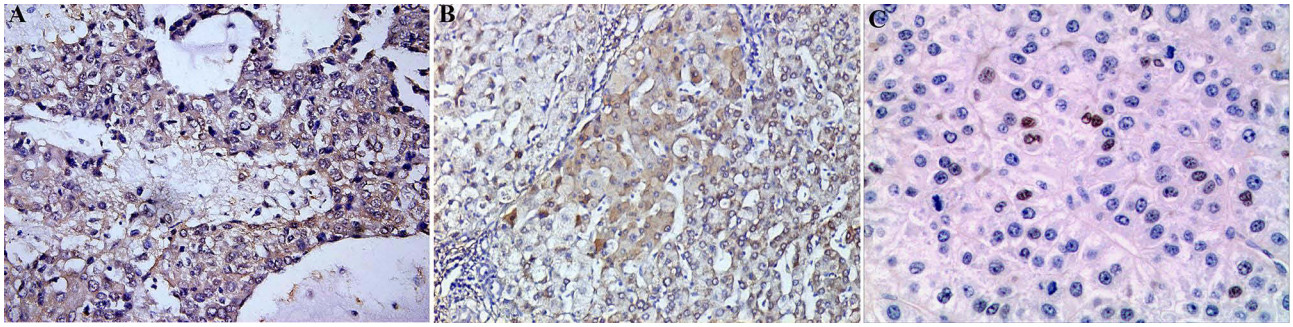

Figure 3 Expression of MIFmRNA. (**A**) Positive expression of MIFmRNA in HCC×200. (**B**) Positive expression of MIFmRNA in HBV-LC×200. (**C**) Negative expression of MIFmRNA in normal liver tissue×200.

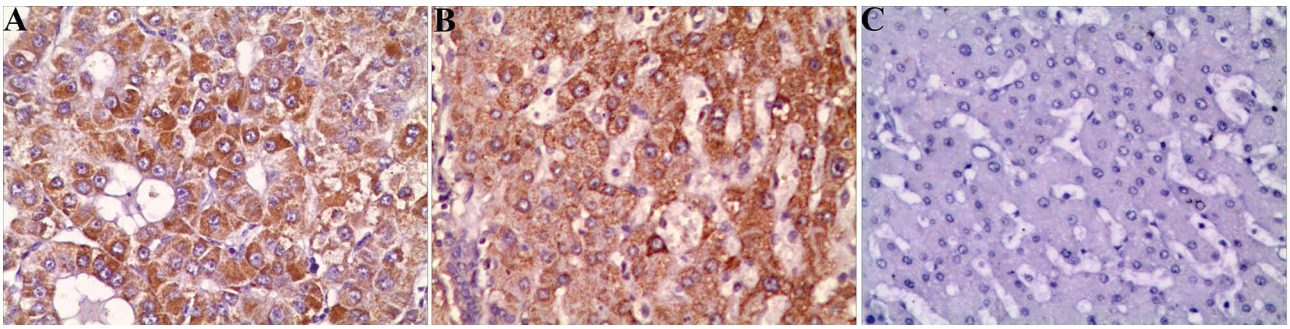

Figure 4 Expression of ERK1/2 mRNA. (**A**) Positive expression of ERK1/2 mRNA in HCC×200. (**B**) Positive expression of ERK1/2 mRNA in HBV-LC ×200. (**C**) Negative expression of ERK1/2 mRNA in normal liver tissue×200.

Table 2 Expressions of MIF and ERK1/2 Nucleic Acids in Experimental Groups and Control Group

Groups	Number of Cases	Positive Expression Rate (%)	
		MIFmRNA	ERK1/2mRNA
HCC group	48	33/68.75	37/77.08
HBILC group	48	34/70.83	38/79.17
Control group	20	5/25.0	8/40.0
$\chi 2$ value		14.149	11.870
P value		<0.05	<0.05

Notes: There are significant differences in the expressions of MIFmRNA and ERK1/2mRNA proteins in different liver tissues among all groups ($P<0.05$), Comparison of expression of MIFmRNA and ERK1/2mRNA protein between the HCC group and control group ($P<0.05$), comparison of expression of MIFmRNA and ERK1/2mRNA proteins between the HBILC group and control group ($P<0.05$), comparison of expression of MIFmRNA and ERK1/2mRNA proteins between the HCC group and HBILC group ($P>0.05$).

of signaling pathways related to proteins regulated by cytokines) is one of the mechanisms of HCC occurrence and development. MIF is a common one and is mainly derived from T lymphocytes, which are secreted to the outside of cells in the form of autocrine and paracrine and play a role by binding with membrane receptors.[15] MIF is a pleiotropic cytokine with pro-inflammatory and carcinogenic effects[16,17] and is not only related to the occurrence of many inflammatory diseases[18–20] but also participates in the occurrence and development of a variety of tumors[21–24] and plays a role in the progression of HBILC.[25,26] The MAPK signaling pathway is the main pathway of intracellular signal transduction and is the core link of tumor cell proliferation, differentiation, apoptosis, and migration. It consists of four subfamilies: extracellular signal-regulated kinase (ERK), p38 kinase (p38 MAPK), c-Jun terminal kinase (JNK), and extracellular signal-regulated kinase 5 (ERK5). After extracellular stimulation by cytokines, growth factors, and other elements, related pathway proteins are phosphorylated and transmitted into cells and nuclei through a typical tertiary kinase cascade to activate many transcription factors. Then they regulate many physiological processes such as cell proliferation, differentiation, and apoptosis, as well as promoting tumor growth, invasion, and migration. In recent years, several studies suggest that the occurrence of HBILC and HCC is related to the abnormalities of ERK1/2, p38 MAPK, and JNK1 sub-pathways.[11,27–29] This suggests that the occurrence and development of HBILC and HCC are closely related to the abnormalities of MIF, ERK1/2, p38 MAPK, and JNK1.

In the early stage of this study, it was found that hepatocellular carcinoma and its adjacent tissues highly expressed MIF and ERK1/2, suggesting that the occurrence of hepatocellular carcinoma is closely related to the expression of these two proteins.[30] However, because there may be satellite lesions of cancer in the adjacent tissues of hepatocellular carcinoma, which is different from the simple liver cirrhosis tissues, and liver cirrhosis is the pathological basis of the occurrence of hepatocellular carcinoma. Therefore, to further explore the correlation between the expression of MIF and

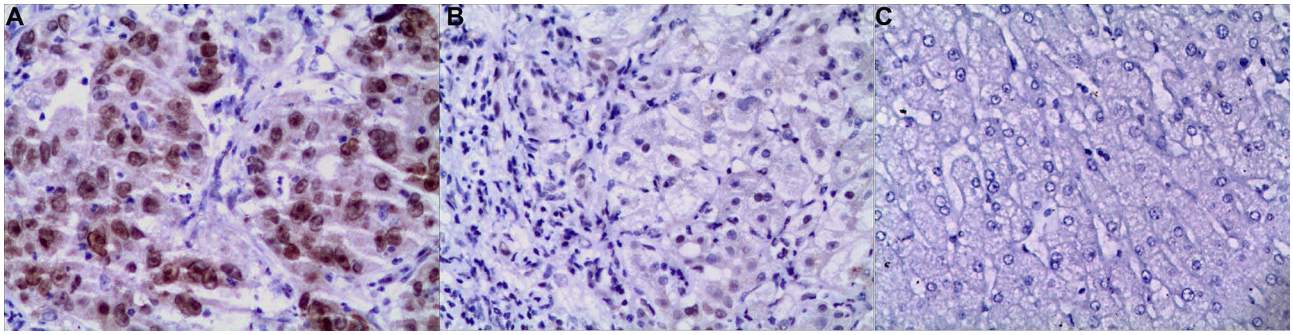

Figure 5 Expression of pERK1/2. (**A**) Positive expression of pERK1/2 in HCC×200. (**B**) Suspected positive expression of pERK1/2 in HBV-LC ×200. (**C**) Negative expression of pERK1/2 in normal liver tissue×200.

Table 3 Expression of Phosphorylated ERK1/2

Groups	Number of Cases	Positive Expression Rate (%)
		ERK1/2
HCC group	48	36/75.0
HBILC group	48	22/45.83
Control group	20	4/20
χ^2 value		19.072
P value		<0.05

Notes: Comparison of expression of pERK1/2 between the HCC group and control group ($P<0.05$), comparison of expression of pERK1/2 between the HCC group and HBILC group ($P>0.05$), comparison of expression of pERK1/2 between the HBILC group and control group ($P<0.05$).

ERK1/2 in the process of hepatocarcinogenesis, HBV-related hepatocellular carcinoma and liver cirrhosis tissues were collected in this study and expression of these two proteins and nucleic acids were detected. The present study revealed that the protein and nucleic acid expressions of MIF and ERK1/2 were significantly increased in both the HBILC and HCC groups, and the differences compared with the control group were statistically significant ($P<0.05$ for both). However, there was no significant difference between the HCC group and the HBILC group ($P>0.05$, Figures 1A and 4C, Tables 1–2). The expression of pERK1/2 was increased in the HCC group, which was found primarily in the nucleus. There was a significant difference between the HCC and HBILC groups and between the HCC and control groups ($P<0.05$), but there was no significant difference between the HBILC group and the control group ($P>0.05$, Figure 5A–C and Table 3). These results suggest that the abnormal expressions of MIF and ERK1/2 are closely related to the occurrence and development of HBILC and HCC. Abnormal expression of pERK1/2 could be observed in the nuclei of HCC but not in the nuclei of HBILC, suggesting that ERK1/2 phosphorylation may be one of the mechanisms leading to HCC. Meanwhile, MIF may play an important regulatory role, for which the specific mechanism is worthy of further in-depth research.

Data Sharing Statement

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

This study was conducted with approval from the Ethics Committee of the 940 Hospital of Joint Logistic Support Force of People's Liberation Army. This study was conducted in accordance with the declaration of Helsinki. Written informed consent was obtained from all participants.

Acknowledgment

As one of the members of this study group, some of the pathological specimens involved in Yu Haipeng's study[30] were sourced from the 940th Hospital of the Joint Logistics Support Force of Chinese People's Liberation Army (normal liver tissues, hepatocellular carcinoma tissues and paracancerous tissues), and the others came from the Cancer Hospital affiliated to Tianjin Medical University Cancer Institute & Hospital (normal liver tissues, hepatocellular carcinoma tissues and paracancerous tissues). In his study, he first detected the expression of MIF, ERK1/2 and p-ERK1/2 in normal liver tissues, hepatocellular carcinoma and paracancerous tissues, as well as the expression of MIFmRNA and ERK1/2 mRNA. He found that the expression of these three proteins and two nucleic acids in hepatocellular carcinoma and paracancerous tissues was significantly higher than that in normal liver tissues, and then focused on adding the recombinant gene MIF (rMIF) to liver cancer cells and normal liver cells. He used Western and RT-PCR methods to detect the expression of ERK1/2, p-ERK1/2 and ERK1/2 mRNA in these two cells. The results showed that with the increase of rMIF concentration, the expression of ERK1/2, p-ERK1/2 and ERK1/2 mRNA was significantly higher than

that of normal hepatocytes. The conclusion, MIF may promote the occurrence and development of hepatocellular carcinoma through ERK1/2 signal pathway.

Dr Xiao-Hui Yu is also a member of this subject, and the pathological samples involved in the study were all sourced from the 940th Hospital of the Joint Logistics Support Force of Chinese People's Liberation Army (normal liver tissues, liver cirrhosis tissues and hepatocellular carcinoma tissues). The study mainly focuses on detecting the expression of three proteins (MIF, ERK1/2 and p-ERK1/2) and two nucleic acids (MIF mRNA and ERK1/2 mRNA) in normal liver, liver cirrhosis and hepatocellular carcinoma tissues. The results showed that the protein and nucleic acid of MIF and ERK1/2 were low expressed in normal liver group, high expressed in cirrhosis group and HCC group, while pERK1/2 was low expressed in normal liver tissues and cirrhosis group, and high expressed in HCC group. The expression of pERK1/2 in HCC group was significantly different from that in cirrhosis group and normal control group ($P < 0.05$), the expression of pERK1/2 in the cirrhosis group is not significantly different from the normal expression in the normal liver tissues control group ($P > 0.05$). Thus, the low expression of pERK1/2 in cirrhosis group of this study is different from the high expression in hepatocellular carcinoma tissues of Yu Haipeng, suggesting that the occurrence and development of cirrhosis and hepatocellular carcinoma are not only related to the high expression of MIF, but also may be related to the activation of ERK1/2, a key protein, especially the carcinogenesis of liver cirrhosis is more closely related to the phosphorylation of ERK1/2. Therefore, the study focused on the relationship between the occurrence of canceration of liver cirrhosis and the phosphorylation of ERK1/2.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Yu XH, Zhang FX, Sun YB, et al. 巨噬细胞移动抑制因子在乙型肝炎肝硬化形成中的可能作用 [Possible role of macrophage mobility inhibitory factor in the formation of hepatitis B cirrhosis]. *Chin J Dig*. 2007;27(04):270–272. Chinese.
2. Yu XH. MIF在HBV感染相关性肝病中的表达及其可能机制研究 *[Expression of MIF in HBV Infection-Associated Liver Disease and Its Possible Mechanism]*. SCU (Sichuan University; 2005. Chinese.
3. Ives A, Le Roy D, Theroude C, et al. Macrophage migration inhibitory factor promotes the migration of dendritic cells through CD74 and the activation of the Src/PI3K/myosin II pathway. *FASEB J*. 2021;35(5):e21418. doi:10.1096/fj.202001605R
4. Song H, Zhu Z, Zhou Y, et al. MIF/CD74 axis participates in inflammatory activation of Schwann cells following sciatic nerve injury. *J Mol Histol*. 2019;50(4):355–367. doi:10.1007/s10735-019-09832-0
5. Jager B, Klatt D, Plappert L, et al. CXCR4/MIF axis amplifies tumor growth and epithelial-mesenchymal interaction in non-small cell lung cancer. *Cell Signal*. 2020;73:109672. doi:10.1016/j.cellsig.2020.109672
6. H WT, Saal A, Bergmann I, et al. Macrophage migration inhibitory factor exerts pro-proliferative and anti-apoptotic effects via CD74 in murine hepatocellular carcinoma. *Br J Pharmacol*. 2021;178(22):4452–4467. doi:10.1111/bph.15622
7. Lee H, Kim WJ, Kang HG, et al. Upregulation of LAMB1 via ERK/c-Jun axis promotes gastric cancer growth and motility. *Int J Mol Sci*. 2021;22(2):626.
8. Savoia P, Fava P, Casoni F, et al. Targeting the ERK signaling pathway in melanoma. *Int J Mol Sci*. 2019;20(6):1483. doi:10.3390/ijms20061483
9. Zaparte A, Cappellari AR, Brandao CA, et al. P2Y2 receptor activation promotes esophageal cancer cells proliferation via ERK1/2 pathway. *Eur J Pharmacol*. 2021;891:173687. doi:10.1016/j.ejphar.2020.173687
10. Urosevic J, Blasco MT, Llorente A, et al. ERK1/2 signaling induces upregulation of ANGPT2 and CXCR4 to mediate liver metastasis in colon cancer. *Cancer Res*. 2020;80(21):4668–4680. doi:10.1158/0008-5472.CAN-19-4028
11. Xing Y, Liu Y, Qi Z, et al. LAGE3 promoted cell proliferation, migration, and invasion and inhibited cell apoptosis of hepatocellular carcinoma by facilitating the JNK and ERK signaling pathway. *Cell Mol Biol Lett*. 2021;26(1):49. doi:10.1186/s11658-021-00295-4
12. Min J, Hu J, Luo C, et al. IFITM3 upregulates c-myc expression to promote hepatocellular carcinoma proliferation via the ERK1/2 signalling pathway. *Biosci Trends*. 2020;13(6):523–529. doi:10.5582/bst.2019.01289
13. 中华人民共和国国家卫生健康委员会医政医管局 [Bureau of Medical Administration, National Health Commission of the People's Republic of China]. 原发性肝癌诊疗指南(2022年版) [Guidelines for the diagnosis and treatment of primary liver cancer (2022 edition)]. *Chin J Pract Surg*. 2022;42(03):241–273. Chinese.
14. Chen Y, Tian Z. HBV-induced immune imbalance in the development of HCC. *Front Immunol*. 2019;10:2048. doi:10.3389/fimmu.2019.02048
15. Li L, Li Y, Huang Y, et al. Long non-coding RNA MIF-AS1 promotes gastric cancer cell proliferation and reduces apoptosis to upregulate NDUFA4. *Cancer Sci*. 2018;109(12):3714–3725. doi:10.1111/cas.13801
16. Song S, Xiao Z, Dekker FJ, et al. Macrophage migration inhibitory factor family proteins are multitasking cytokines in tissue injury. *Cell Mol Life Sci*. 2022;79:105. doi:10.1007/s00018-021-04038-8

17. Wang Y, Chen Y, Wang C, et al. MIF is a 3' flap nuclease that facilitates DNA replication and promotes tumor growth. *Nat Commun*. 2021;12(1):2954. doi:10.1038/s41467-021-23264-z
18. Bozza MT, Lintomen L, Kitoko JZ, et al. The role of MIF on eosinophil biology and eosinophilic inflammation. *Clin Rev Allergy Immunol*. 2020;58:15–24. doi:10.1007/s12016-019-08726-z
19. Caltabiano R, De Pasquale R, Piombino E, et al. Macrophage Migration Inhibitory Factor (MIF) and Its Homologue d-Dopachrome Tautomerase (DDT) inversely correlate with inflammation in discoid lupus erythematosus. *Molecules*. 2021;26(1):184. doi:10.3390/molecules26010184
20. Gunther S, Fagone P, Jalce G, et al. Role of MIF and D-DT in immune-inflammatory, autoimmune, and chronic respiratory diseases: from pathogenic factors to therapeutic targets. *Drug Discov Today*. 2019;24(2):428–439. doi:10.1016/j.drudis.2018.11.003
21. Avalos-Navarro G, Munoz-Valle JF, Daneri-Navarro A, et al. Circulating soluble levels of MIF in women with breast cancer in the molecular subtypes: relationship with Th17 cytokine profile. *Clin Exp Med*. 2019;19(3):385–391. doi:10.1007/s10238-019-00559-6
22. Zhang H, Duan J, Wu O. The expression of macrophage migration inhibitory factor in the non-small cell lung cancer. *Saudi J Biol Sci*. 2020;27(6):1527–1532. doi:10.1016/j.sjbs.2020.04.027
23. Qin L, Qin J, Lv X, et al. MIF promoter polymorphism increases peripheral blood expression levels, contributing to increased susceptibility and poor prognosis in hepatocellular carcinoma. *Oncol Lett*. 2021;22(1):549. doi:10.3892/ol.2021.12810
24. Pacheco-Fernandez T, Juarez-Avelar I, Illescas O, et al. Macrophage migration inhibitory factor promotes the interaction between the tumor, macrophages, and T cells to regulate the progression of chemically induced colitis-associated colorectal cancer. *Mediators Inflamm*. 2019;2019:2056085. doi:10.1155/2019/2056085
25. Zhang K, Pan X, Shu X, et al. Relationship between MIF-173 G/C polymorphism and susceptibility to chronic hepatitis B and HBV-induced liver cirrhosis. *Cell Immunol*. 2013;282(2):113–116. doi:10.1016/j.cellimm.2013.04.013
26. Wirtz TH, Reuken PA, Jansen C, et al. Balance between macrophage migration inhibitory factor and sCD74 predicts outcome in patients with acute decompensation of cirrhosis. *JHEP Rep*. 2021;3(2):100221. doi:10.1016/j.jhepr.2020.100221
27. Jeng KS, Lu SJ, Wang CH, et al. Liver fibrosis and inflammation under the control of ERK2. *Int J Mol Sci*. 2020;21(11):3796. doi:10.3390/ijms21113796
28. Zhang C, Xiao C, Ren G, et al. C-terminal-truncated hepatitis B virus X protein promotes hepatocarcinogenesis by activating the MAPK pathway. *Microb Pathog*. 2021;159:105136. doi:10.1016/j.micpath.2021.105136
29. Feng PC, Ke XF, Kuang HL, et al. BMP2 secretion from hepatocellular carcinoma cell HepG2 enhances angiogenesis and tumor growth in endothelial cells via activation of the MAPK/p38 signaling pathway. *Stem Cell Res Ther*. 2019;10(1):237. doi:10.1186/s13287-019-1301-2
30. Yu HP, Zheng Y, Lu LX, et al. 巨噬细胞游走抑制因子在肝细胞癌组织中的表达及其与ERK1/2信号通路关系的初步研究 [Preliminary study on the expression of MIF in HCC tissues and its relationship with ERK1/2 signaling pathway]. *Zhonghua Nei Ke Za Zhi*. 2022;61(11):1228–1233. Chinese. doi:10.3760/cma.j.cn112138-20220502-00334