ANALYSIS OF EXPRESSIONS AND CLINICAL SIGNIFICANCE OF CXCR1, CXCR2 AND CXCL8 IN PATIENTS WITH PRIMARY HEPATIC CARCINOMA

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ABSTRACT

Purpose*: To investigate the expressions and significance of CXC chemokine receptor 1 (CXCR1), CXC chemokine receptor 2 (CXCR2) and CXC chemokine ligand 8 (CXCL8) in primary hepatic carcinoma (PHC).*

Methods*: A total of 62 PHC patients and 62 CHB patients seen from June 2016 to December 2017 at Guizhou Provincial People's Hospital, Guiyang City were selected, and randomly divided into PHC group and CHB group. The control group consisted of 62 randomly selected healthy people (62, normal group). The mRNA expressions of CXCR1, CXCR2 and CXCL8 were determined in the three groups using real-time fluorescent quantitative method in peripheral blood mononuclear cells (PBMC). The correlation of mRNA expression of CXCL8 with those of CXCR1 and CXCR2 were analyzed by Pearson linear correlation analysis, while the predictive values of mRNA expressions of CXCL8, CXCR1 and CXCR2 for PHC were analyzed by ROC curve.*

Results*: In the PHC group, the mRNA expressions of CXCR1, CXCR2 and CXCL8 were higher than in CHB group and normal group, and in the CHB group, the mRNA expressions of CXCR1 and CXCR2 were higher than those in the normal group ($p < 0.05$). The mRNA expression of CXCL8 was positively correlated with those of CXCR1 and CXCR2 ($r = 0.694, 0.704$; $p < 0.05$). For the prediction of PHC, ROC curve model showed that the area under curve of mRNA expressions of CXCR1, CXCR2 and CXCL8 were 0.864, 0.852 and 0.887, respectively; the sensitivities were 0.871, 0.855 and 0.887 respectively; and the specificities were 0.871, 0.839 and 0.871 respectively.*

Conclusion*: These results indicate that the mRNA expressions of CXCR1, CXCR2 and CXCL8 have high predictive value for PHC.*

Keywords*: Primary hepatic carcinoma, CXC chemokine receptor 1, CXC chemokine receptor 2, CXC chemokine ligand 8.*

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Introduction

Primary hepatic carcinoma (PHC) is a malignant tumor with global high prevalence, the patients of which are mostly between 40 and 50 years. Clinical manifestations of PHC patients include fever, nausea and vomiting, subcutaneous hemorrhage, edema in the legs, abdominal distension, decreased appetite, and weight loss. At present, the specific pathogenesis of PHC is still unclear. However, studies suggest that PHC with generally-poor prognosis may be related to multiple factors, such as viral hepatitis, hepatocirrhosis, heredity, living environment and long-term exposure to carcinogens[1, 2].

Therapies such as radiotherapy, chemotherapy and surgery, are typically employed for PHC patients in clinical practice. However, their overall efficacies are not ideal because of high metastasis and recurrence rates[3, 4].

In recent years, some studies[5, 6] found that pathogenesis of PHC is associated with expressions of multiple chemotactic factors and their receptors, some of which can promote wound healing, but others aggravate infection and injury by enhancing inflammatory response. For example, CXC chemokine ligand 8 (CXCL8) may regulate mechanisms of inflammation and immunologic response, but its receptors, CXC chemokine receptor 1 (CXCR1) and CXC chemokine receptor 2

(CXCR2), are involved in progression of PHC by promoting inflammatory response. This suggests that CXCL8, CXCR1 and CXCR2 may be associated with PHC progression. The present study was carried out to analyze expressions of CXCR1, CXCR2 and CXCL8 in PHC patients, and investigate the correlations between CXCL8 expression and the expressions of CXCR1 and CXCR2. The study was also aimed at determining the correlations of the three indexes with PHC by analyzing their diagnostic values, in order to understand more disease attributes of PHC patients which can provide bases for clinical diagnosis and treatment.

Materials and methods

General data

In this study, 62 PHC patients and 62 CHB patients in Guizhou Provincial People's Hospital, Guiyang City from June 2016 to December 2017 were selected, and randomly divided into PHC group and CHB group. In addition, 62 healthy people who were physically examined in the hospital at the same time were randomly selected to serve as control (normal group). This study was approved by the Independent Medical Ethics Committee of Guizhou Provincial People's Hospital, Guiyang City.

Inclusion criteria and exclusion criteria

Inclusion criteria:

• Patients in PHC group whose diagnosis conformed with the diagnostic criteria under Guidelines for the Standardized Pathological Diagnosis of PHC (2015)[7], and patients in CHB group whose diagnosis conformed with the diagnostic criteria under Guidelines for Prevention and Treatment of Chronic Hepatitis B (2015)[8]. These criteria were confirmed by physical examinations and laboratory tests;

• Patients aged 18 and older;

• Patients with normal coagulation function

• Patients without injuries in organs such as heart and brain;

• Patients with alert consciousness and normal cognition;

• Patients who signed the informed consents.

Exclusion criteria:

• Pregnant and lactating patients;

• Patients with coexistent malignant tumor;

• Patients with coexistent hematologic diseases such as leukemia and leukocyte-related diseases.

• Patients with coexistent cardiovascular incidents or cerebrovascular accident;

• Patients with any history of mental disorder and cognitive handicap.

Instruments and reagents

This study was carried out using centrifugal machine (Model: 5430R; German Eppendorf Biotech Company), real-time fluorescence quantitative PCR instrument (Model: 7900; American Applied Biosystems Inc.); light microscope (Model: KYKY--EM3200; KYKY Technology Co., Ltd.); super-clean bench (Model: EVL-5S; Zhuhai Tsao Hsin Enterprise Co., Ltd.); gradient PCR amplification instrument (Model: iCycler; American Bio-Rad Laboratories Company), and electronic balance (Model: AL104; Swiss METTLER TOLEDO Company). The reagents used in this study included absolute alcohol, isopropyl alcohol, PCR Kits (Chinese Beijing Baiaolaibo Technology Co., Ltd.), Total RNA Kit (Chinese Wuhan MSK Biotech Co., Ltd.), and primer (Chinese Sangon Biotech (Shanghai) Co., Ltd.).

Collection of specimens

Peripheral fasting venous blood (5 mL) was collected in anticoagulant tubes from patients in the three groups, and diluted with Hank's solution. Next, the diluted blood specimen was put in a tube with 2 mL of Ficoll-Hypaque cell separation medium, and centrifuged at 2500 rpm for 20 min to obtain cloud-like mononuclear cell layer. Subsequently, the cells were re-suspended in RPMI1640 cell culture fluid, centrifuged again at 2000 rpm for 10 min, and then rinsed twice. The precipitated cells were collected, re-suspended and counted in RPMI1640 cell culture fluid. Finally, the total RNA was isolated from peripheral blood mononuclear cells (PBMCs) when the density of suspended cells was adjusted to 1×106 cells/ml.

Methods

Each parameter was determined by real-time fluorescent quantitative method, and amplified by real-time fluorescent quantitative PCR. In the course of the assays, GAPDH was used as reference gene. The primer sequences used were as follows.

(1) Forward Primer (5'→3'): CXCL8 sequence: CTTTGTCCATTCCACTTCT; CXCR1

sequence: CAGATCCACAGATGTGGA;

CXCR2 sequence: CTTTTCTACTAGCCGC.

(2) Reverse Primer (5'→3'): CXCL8 sequence: TCCCTAACGGTGCCTTGT; CXCR1 sequence: AGCAGCCAGACAACAAA; CXCR2 sequence: AGATGCTGAGACATATGA.

(3) Product size (bp). CXCL8: 306; CXCR1: 468; CXCR2: 417. The PCR reaction conditions were 95 °C for 5 min; 95 °C for 30 sec; 55 °C for 30 sec; 72 °C for 30 sec (30 cycles); and 72 °C for 5min. At the end of reactions, the relative expression of the target DNA was calculated by using 2 - △△ct Method.

Statistical analysis

Numerical data are expressed as percentage (%), and χ^2 test was used for group comparison. Measurement data are expressed as mean ± standard deviation (mean ± SD). Single-measurement variance analysis was used for three-group comparisons, while Student's *t*-test was used for paired comparison among three groups. The statistical analyses were performed using SPSS 19.0 statistical software. Correlations between variables were determined using Pearson linear correlation method. The predictive values of mRNA expressions of CXCR1, CXCR2 and CXCL8 for PHC were analyzed by ROC curve. Differences were considered statistically significant when *p* value was less than 0.05.

Results

Baseline data of the three groups

The baseline data of all subjects are listed in Table 1. There were no significant differences in baseline data (e.g. sex, age and body mass index) among the three groups ($p > 0.05$).

Group	Sex		Age (Year)	Body mass index (kg/m²)	TNM staging			Depth of infiltration		
	Male	*Female*			*Stage I*	*Stage II*	*Stage III*	*T1*	*T2*	*T3*
PHC (n=62)	33	29	49.28±10.53	23.21±2.14	16	29	17	15	31	16
CHB (n=62)	35	27	47.51±9.17	23.18±2.09	-	-	-	-	-	-
Normal (n=62)	30	32	47.42±9.05	22.86±2.11	-	-	-	-	-	-
F/χ^2	0.82		0.739	0.522	-	-	-	-	-	-
p	0.664		0.479	0.594	-	-	-	-	-	-

Table 1: Comparison of baseline data among the three groups.

Comparison of mRNA expressions of CXCR1, CXCR2 and CXCL8 among the three groups

The RNA expressions of CXCR1, CXCR2 and CXCL8 were highest in the PHC group, and significantly higher than in the other two groups (CHB group and normal group) ($p < 0.05$). In the CHB group, the mRNA expressions of CXCR1 and CXCR2 were higher than corresponding values in the normal group ($p < 0.05$; Table 2).

Group	CXCR1	CXCR2	CXCL8
PHC group (n=62)	0.93±0.13	0.86±0.16	1.74±0.24
CHB group (n=62)	0.68±0.11*	0.69±0.13*	0.81±0.26*
Normal group (n=62)	0.41±0.11*#	0.40±0.11*#	0.74±0.19*
F	306.0.78	184.297	359.354
P	0	0	0

Table 2: Comparison of mRNA expressions of CXCR1, CXCR2 and CXCL8 among the three groups (mean ± SD, logcDNA/logGAPDH).

**p < 0.05, compared with the PHC group; #p < 0.05, compared with the CHB group*

Correlation of CXCL8 expression with CXCR1 and CXCR2 expressions in PHC patients

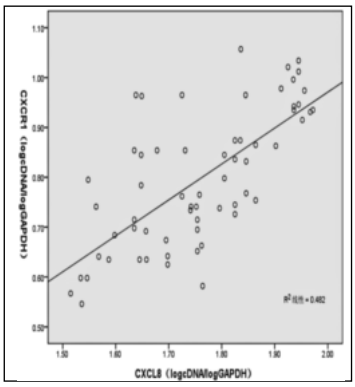

Fig: 1: Pearson linear correlation analysis of CXCL8 and CXCR1 expressions.

The three indexes were subjected to Pearson linear correlation analysis, and it was found that the mRNA expression of CXCL8 was positively correlated with those of CXCR1 and CXCR2 ($r = 0.694, 0.704$; $p < 0.05$). These results are shown in Figure 1 and Figure 2.

Predictive values of mRNA expressions of CXCR1, CXCR2 and CXCL8 for PHC

Using the ROC curve model, this study found that the AUCs of mRNA expressions

of CXCR1, CXCR2 and CXCL8 were 0.864, 0.852 and 0.887 respectively for the prediction of PHC. For PHC prediction, the sensitivities of mRNA expressions of CXCR1, CXCR2 and CXCL8 were 0.871, 0.855 and 0.887, respectively, and the specificities of the three indexes were 0.871, 0.839 and 0.871, respectively. These results are shown in Table 3, and in Figures 3 - 5.

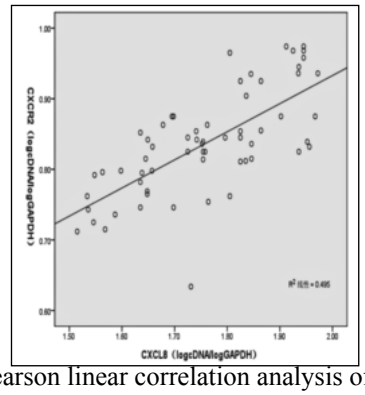

Fig: 2: Pearson linear correlation analysis of CXCL8 and CXCR2 expressions.

Index	Area under curve	Standard error	*p*	*95 % CI*	Optimal cut-off value (logcDNA/log GAPDH)	Sensitivity	Specificity
CXCR1	0.864	0.036	0	0.794-0.934	0.815	0.871	0.871
CXCR2	0.852	0.037	0	0.780-0.924	0.702	0.855	0.839
CXCL8	0.887	0.033	0	0.822-0.951	1.223	0.887	0.871

Table 3: Predictive values of mRNA expressions of CXCR1, CXCR2 and CXCL8 for PHC.

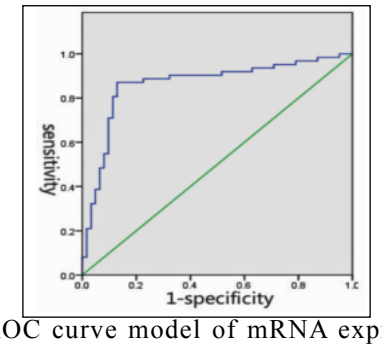

Fig: 3: ROC curve model of mRNA expression of CXCR1 for prediction of PHC.

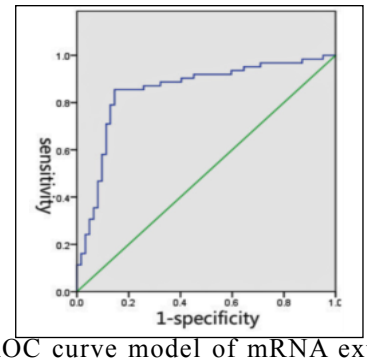

Fig: 4: ROC curve model of mRNA expression of CXCR2 for PHC prediction.

Discussion

PHC is a malignant tumor that typically occurs in intrahepatic bile duct epithelial cells and/or liver cells, the clinical manifestations of which include pain of hepatic region, enlargement of liver and symptoms associated with the digestive system (e.g. nausea, diarrhea and fever)[9, 10]. It is common in middle-aged and elderly people, and affects more men than women. Some studies[11, 12] have shown that liver cancer with complex pathological processes is induced and caused by a combination of multiple etiologies, multiple stages and multiple factors, which may involve many different mechanisms. These mechanisms include hemodynamics, clinical histopathology on hepatocarcinoma, pathological anatomy, and molecular biology of cirrhotic liver. Vascular proliferation occurs in PHC patients, and provides favorable conditions for tumor growth and metastasis. In addition, tumor progression is closely related to inflammation, and can promote proliferation and metastasis of cancer cells. Studies have suggested that CXCL8, CXCR1 and CXCR2 secreted by neutrophilic granulocytes can activate recipient cells, induce inflammatory reactions, and are highly expressed in liver injury, suggesting that they are important for evaluating severity of liver injury[13].

A comparison of the related parameters among the three groups showed that the mRNA expressions of CXCL8, CXCR1 and CXCR2 in the PHC group were significantly higher than those in the other two groups (CHB group and normal group), indicating that the three indexes were higher in PHC patients. It is known that CXCL8 is an inflammatory mediator which regulates expression of inflammatory factors and promotes proliferation of capillary vessels[14]. Its mRNA expression is low in normal people, and high in tumor patients[15].

This promotes cancer cell mobility and enhances infiltration of capillary vessels which then aggravates pathological conditions of patients. It has also been shown that CXCL8 can enhance renewing and turnover of cancer cells, by influencing contiguous cells through paracrine/autocrine action, thereby promoting tumor progression[16]. The combination of CXCR1 and CXCR2 (members of CXC Chemokine receptor family) with CXCL8 (a common ligand of CXCR1 and CXCR2), can cause inflammatory immunological response which is important for progression of tumor.

It is known hat CXCR1 and CXCR2 are members of CXC Chemokine receptor family, and CXCL8 is their common ligand. The combination of CXCR1 and CXCR2 with CXCL8 can cause inflammatory immunological response which is important for progression of tumor. Studies have demonstrated that CXCR2 is highly expressed in hepatocellular carcinoma[18]. This is in agreement with the findings in the present study.

In some studies[19, 20], chronic infection from hepatitis B virus (HBV) is generally considered as an independent risk factor for liver cancer, because local body tissues present with cellular inflammatory infiltration after being infected by HBV. At this stage, CXCL8, CXCR1 and CXCR2 are involved in the course of liver injury through a specific mechanism involving a buildup of inflammatory mediators in hepatic vascular system. In addition, it involves high expression of CXCR1 and CXCR2 which reach the site of hepatic injury by chemotactic migration and blood circulation. This shows that high expressions of CXCR1 and CXCR2 are important factors in the aggravation of liver injury.

The present study found that the mRNA expressions of CXCR1, CXCR2 and CXCL8 were increased in PHC patients. Pearson correlation analysis revealed that the mRNA expression of CXCL8 was positively correlated with those of CXCR1 and CXCR2, suggesting that the mRNA expressions of CXCR1 and CXCR2 increased correspondingly with that of CXCL8. This can provide a basis for diagnosis of PHC.

In another study, it was reported that CXCL8 can control inflammatory immunological responses and promote progression of liver cancer by up-regulating mRNA expressions of CXCR1 and CXCR2 during the progression of liver cancer[21]. This is in agreement with the results obtained in the present study. In addition, the mRNA expressions of CXCR1, CXCR2 and CXCL8 showed high predictive values for PHC because of good sensitivities and specificities, indicating that the three indexes could be used as important markers for PHC diagnosis.

Study Limitations

This study has two main limitations:

• the sample size was small;

• the correlations between the three indexes and clinic-pathological features of PHC were not analyzed further. Therefore, further studies with large sample size need to be performed in the future.

Conclusion

This study has confirmed that CXCL8, CXCR1 and CXCR2 are involved in the progression of PHC, which could provide a basis for the clinical diagnosis of PHC. The mRNA expressions of CXCR1, CXCR2 and CXCL8 can reflect changes in pathological conditions of PHC patients, which help to improve prognosis by clinically-targeted therapeutic measures.

References

1) Chassagne F, Rojas TR, Bertani S, Bourdy G, Eav S, et al. A 13-year retrospective study on primary liver cancer in cambodia: a strikingly high hepatitis C occurrence among hepatocellular carcinoma cases. Oncol 2016; 91: 106-116.

2) Broutier L, Mastrogiovanni G, Verstegen MM, Francies HE, Gavarró LM, et al. Human primary liver cancer-derived organoid cultures for disease modeling and drug screening. Nat Med 2017; 23: 1424-1435.

3) Sun C, Liao W, Deng Z, Li E, Feng Q, et al. The diagnostic value of assays for circulating tumor cells in hepatocellular carcinoma: A meta-analysis. Med 2017; 96: 7513.

4) Chayanupatkul M, Omino R, Mittal S, Kramer JR, Richardson P, et al. Hepatocellular carcinoma in the absence of cirrhosis in patients with chronic hepatitis B virus infection. J Hepatol 2017; 66: 355-362.

5) Zhu Y, Gao X M, Yang J, Xu D, Zhang Y, et al. C-C chemokine receptor type 1 mediates osteopontin-promoted metastasis in hepatocellular carcinoma. Cancer Sci 2017; 109: 710-723.

6) Liu J, Chen S, Wang W, Ning BF, Chen F, et al. Cancer-associated fibroblasts promote hepatocellular carcinoma metastasis through chemokine-activated hedgehog and TGF-Î² pathways. Cancer Lett 2016; 379: 49-59.

7) Chinese Society of Liver Cancer. Guidelines for the Standardized Pathological Diagnosis of Primary hepatic carcinoma (2015). Med J Chin PLA 2015; 21: 865-872.

8) Chinese Society of Hepatology of Chinese Medical Association. Guidelines for Prevention and Treatment of Chronic Hepatitis B (2015). J Clin Hepatol 2015; 31: 1941-1960.

9) Marrero JA, Ahn J, Reddy KR. ACG clinical guideline: the diagnosis and management of focal liver lesions. Am J Gastroenterol 2014; 109: 1328.

10) Verne J, Ives A. Use of cancer registration data to examine trends and variation in primary liver cell cancer in England? Implications for public health and health service action. J Hepatol 2016; 64: 461-S461.

11) Gopal R, Selvarasu K, Pandian PP, Ganesan K. Integrative transcriptome analysis of liver cancer profiles identifies upstream regulators and clinical significance of ACSM3, gene expression. Cell Oncol 2017; 40: 1-15.

12) Sia D, Villanueva A, Friedman SL, Llovet JM, et al. Liver cancer cell of origin, molecular class, and effects on patient prognosis. Gastroenterol 2016; 152: 745-761.
13) Joseph P R B, Sawant K V, Rajarathnam K. Heparin-bound chemokine CXCL8 monomer and dimer are impaired for CXCR1 and CXCR2 activation: implications for gradients and neutrophil trafficking. Open Biol 2017; 7: 170168-170168.
14) Amrouche L, Desbuissons G, Rabant M, Sauvaget V, Nguyen C, et al. MicroRNA-146a in human and experimental ischemic AKI: CXCL8-dependent mechanism of action. J Am Soc Nephrol 2017; 28: 479-493.
15) Bie YQ, Li YF, Yin ZF. The relationship of CXCL8 and its receptors to liver metastasis of colorectal cancer. Chin J Cancer Biother 2017; 24: 194-200.
16) Hu SS, Yang H, Zhu YJ, Yao Y, Qian JM, et al. Expressions of CXCL8 and VEGF and their associations with vascularization in pancreas cancer tissues. Anat Res 2017; 39: 185-190.
17) Mossanen JC, Krenkel O, Ergen C, Govaere O, Liepelt A, et al. Chemokine (C-C motif) receptor 2-positive monocytes aggravate the early phase of acetaminophen-induced acute liver injury. Hepatol 2016; 64: 1667-1682.
18) Qiao B, Luo W, Liu Y, Wang J, Liu C, et al. The prognostic value of CXC chemokine receptor 2 (CXCR2) in cancers: a meta-analysis. Oncotarget 2018; 9: 15068-15076.
19) Yoo S, Wang W, Wang Q, Fiel MI, Lee E, et al. A pilot systematic genomic comparison of recurrence risks of hepatitis B virus-associated hepatocellular carcinoma with low-and high-degree liver fibrosis. Bmc Med 2017; 15: 214.
20) Xia Z, Zhu MY, Yu DM, Li W, Zhang DH, et al. Serum WFA+-M2BP levels for evaluation of early stages of liver fibrosis in patients with chronic hepatitis B virus infection. Liver Int 2017; 37: 35-44.
21) Oikawa T. Cancer stem cells and their cellular origins in primary liver and biliary tract cancers. Hepatol 2016; 64: 645-651

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