Plasma Expression Levels of Mannan-Binding Lectin and Mannan-Binding Lectin-Associated Serine Protease-2 in Patients with Hepatocellular Carcinoma (Postprint)

Authors: Li Jie, 朱玲燕 2, Zuo Daming1, Zhang Liyun1, Lu Xiao1, Chen Zhengliang1, Zhou Jia 1

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Abstract

Objective: To detect the expression levels of mannose-binding lectin (MBL) and MBL-associated serine protease-2 (MASP-2) in plasma of patients with hepatocellular carcinoma (HCC), and to investigate their correlation with the occurrence and progression of HCC. Methods: The plasma expression levels of MBL and MASP-2 were detected by enzyme-linked immunosorbent assay (ELISA) in 64 HCC patients and 30 healthy individuals, and statistical methods were used to analyze the correlation between plasma concentrations of MBL and MASP-2 and various clinical parameters. Results: The plasma concentrations of both MBL and MASP-2 were significantly higher in HCC patients than in healthy individuals (P=0.014, 0.002). There was no statistically significant difference in the MBL/MASP-2 ratio between healthy individuals and HCC patients. Additionally, there was no correlation between MBL and MASP-2 in patient plasma. In HCC patients, plasma MBL levels were positively correlated with tumor vascular invasion (r=0.253, P=0.047) and total bilirubin levels (r=0.283, P=0.024). Plasma MASP-2 levels were positively correlated with TNM stage of HCC (r=0.276, P=0.027) and negatively correlated with plasma albumin (r=-0.317, P=0.015). ROC curve analysis showed that the areas under the curve for MBL and MASP-2 were 0.665 (P=0.010) and 0.694 (P=0.003), respectively, with diagnostic sensitivities for HCC of 50% and 89.1%. Conclusion: The key molecules of the complement lectin pathway, MBL and MASP-2, are associated with inflammatory status and disease progression in HCC patients and are involved in the occurrence and development of liver cancer.

Full Text

Preamble

LI Jie[1],[3], ZHU Lingyan[2], ZUO Daming[1], ZHANG Liyun[1], LU Xiao[1], CHEN Zhengliang[1], ZHOU Jia[1]

[1]Department of Immunology, School of Basic Medical Sciences, Southern Medical University, Guangzhou 510515, China;
[2]School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, China;
[3]Third Affiliated Hospital of Southern Medical University (Guangdong Academy of Orthopaedics), Guangzhou 510000, China

Abstract

Objective: To detect the plasma levels of mannan-binding lectin (MBL) and MBL-associated serine protease-2 (MASP-2) in patients with hepatocellular carcinoma (HCC) and explore their role in the tumorigenesis and progression of HCC.

Methods: The plasma levels of MBL and MASP-2 were measured by enzyme-linked immunosorbent assay (ELISA) in 64 HCC patients and 30 healthy control subjects. Statistical methods were used to analyze the correlation between plasma concentrations of MBL and MASP-2 and various clinical parameters.

Results: Plasma levels of both MBL and MASP-2 were significantly higher in HCC patients than in healthy controls (P=0.014 and P=0.002, respectively). However, the MBL/MASP-2 ratio showed no significant difference between the two groups, and no correlation was found between MBL and MASP-2 in HCC patients. In HCC patients, plasma MBL level was positively correlated with vascular invasion (r=0.253, P=0.047) and total bilirubin level (r=0.283, P=0.024). Plasma MASP-2 level was positively correlated with TNM stage (r=0.276, P=0.027) and negatively correlated with plasma albumin level (r=-0.317, P=0.015). ROC curve analysis revealed an area under the curve of 0.665 for MBL (P=0.010) and 0.694 for MASP-2 (P=0.003), with diagnostic sensitivities of 50% and 89.1%, respectively.

Conclusion: MBL and MASP-2, key molecules of the complement lectin pathway, are associated with inflammatory status and disease progression in HCC patients and participate in the development of liver cancer.

Keywords: hepatocellular carcinoma; mannan-binding lectin; MBL-associated serine protease-2; complement

Introduction

The pathogenesis of hepatocellular carcinoma is closely related to the hepatic macroenvironment, particularly persistent liver inflammation [1]. The complement system, a crucial component of innate immunity and the first line of im-

mune defense, also participates in various pathophysiological processes including adaptive immunity, inflammatory responses, and coagulation [2]. Mannan-binding lectin (MBL), a soluble pattern recognition receptor, recognizes specific carbohydrate structures on microbial or cellular surfaces to mediate opsonization. Upon binding to these structures, MBL can interact with MBL-associated serine proteases (MASPs) to activate the complement lectin pathway [3]. Immunodeficiency caused by mutations in the structural genes of MBL or MASP places patients at high risk of infection and predisposes them to autoimmune diseases and coronary artery disease [4-5].

Accumulating evidence demonstrates that complement is involved in the development and progression of various tumors, and its role and mechanisms in cancer diagnosis and treatment have attracted widespread attention [6]. Studies suggest that MBL and its downstream MASP are associated with the development of multiple tumors [7], though their role and mechanisms in liver cancer remain to be elucidated. Currently, few studies have examined the relationship between MBL, MASP and HCC development, and these have focused primarily on polymorphisms in the MBL and MASP coding genes [7-8]. No studies have reported simultaneous detection of peripheral MBL and MASP-2 level changes in primary liver cancer patients. This study investigates the correlation between MBL and MASP-2 levels in HCC patient plasma and HCC development by analyzing their relationship with clinical parameters, providing a foundation for understanding the mechanisms of MBL and MASP-2 in HCC pathogenesis and their potential diagnostic and therapeutic applications.

Methods

1.1 General Data

Sixty-four patients who underwent surgical resection or interventional therapy at the Sun Yat-sen University Cancer Center between 2009 and 2012 were randomly selected. Patients who underwent surgical resection were confirmed by pathological examination, while those who received interventional therapy were diagnosed according to the European Association for the Study of the Liver criteria via ultrasound, CT, or ultrasound-guided biopsy. HCC staging was performed according to the TNM classification system established by the International Union Against Cancer and the American Joint Committee on Cancer. None of the patients had prior radiotherapy, chemotherapy, or biological therapy, nor did they have autoimmune disease history or immunotherapy. Thirty healthy subjects with similar age and gender distribution served as controls, all testing negative for HCV, HBV, HIV, and syphilis.

1.2.1 Sample Collection and Processing

Preoperative venous blood was collected in heparin-anticoagulant tubes, left to stand for 10 minutes, then centrifuged at 3000 rpm for 10 minutes. The supernatant was collected and stored at -80°C for MBL and MASP-2 concentration

measurement.

1.2.2 Instruments and Reagents

Human MBL ELISA kit (R&D Systems, USA); human MASP-2 ELISA kit (USCN, Wuhan, China); multifunctional microplate reader (PerkinElmer, USA); centrifuge (Eppendorf, Germany).

1.2.3 Detection Method

Plasma samples were thawed and brought to room temperature. All procedures were performed strictly according to the kit instructions. Absorbance (A450) was measured using a microplate reader, and plasma MBL and MASP-2 concentrations were calculated based on standard curves generated from standard samples.

1.3 Statistical Analysis

SPSS 16.0 software was used for statistical analysis. Measurement data are expressed as mean ± standard deviation. Inter-group comparisons were performed using independent two-sample t-tests for non-homogeneous variance. Pearson correlation analysis was used to examine correlations between MBL, MASP-2 and various variables. $P<0.05$ was considered statistically significant.

Results

2.1 Clinical Parameters of HCC Patients

Preoperative routine examinations revealed the clinical parameters of the HCC patient group and healthy control group as shown in Table 1 .

Table 1 Characteristics of HCC patients

Index	Healthy Control	HCC Patients
Age (year, Mean±SD)	51.6±1.5	48.9±2.2
Male/Female	-	25/>25)
Cirrhosis (+/-)	-	5/>5)
HBsAg (+/-)	-	-
AFP (ng/mL)	-	30/35
Tumor size (cm)	-	20/45
Tumor multiplicity (solitary/multiple)	-	32/33
Vascular invasion (+/-)	-	45/19
TNM stage (I+II/III)	-	19/45
ALT (U/L, Mean±SD)	44.9±3.4	18.81±4.2
AST (U/L, Mean±SD)	49.2±4.0	17.8±4.5
TBIL (mol/L)	18.2±1.4	10.2±3.4
ALB (g/L)	44.2±1.7	30.1±3.1

Index	Healthy Control	HCC Patients
CRP (mg/mL)	15.9±3.6	0.59±0.53
Therapy treatment (resection/RFA/TACE/MCT)	-	26/11/24/3

HBsAg: Hepatitis B virus surface antigen; AFP: Alpha-fetoprotein; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; TBIL: Total bilirubin; ALB: Albumin; CRP: C-reactive protein; RFA: Radiofrequency ablation; TACE: Transcatheter arterial chemoembolization; MCT: Microwave coagulation therapy; NA: None available.

2.2 Plasma MBL and MASP-2 Levels in HCC Patients and Healthy Controls

ELISA results showed that plasma MBL levels were higher in HCC patients than in healthy controls (1471±106 ng/mL vs. 1029±124 ng/mL, P=0.014). Plasma MASP-2 levels were also higher in HCC patients compared to healthy controls (382.7±16.3 ng/mL vs. 290.7±24.7 ng/mL, P=0.002) [Figure 1: see original paper].

2.3 Correlation Between MBL and MASP-2 in HCC Patients

Pearson correlation analysis revealed no correlation between plasma MBL and MASP-2 levels in HCC patients (r=0.044, P=0.727) [Figure 2: see original paper]. Additionally, the MBL-to-MASP-2 ratio in individual subjects showed no significant difference between healthy controls and HCC patients (4.4±0.6 vs. 4.3±0.4, P=0.870) [Figure 3: see original paper].

2.4 Correlation of Plasma MBL and MASP-2 with Clinical Variables

2.4.1 Correlation of MBL with Clinical Variables Pearson correlation analysis between plasma MBL concentration and clinical variables in HCC patients (Table 2) showed that plasma MBL level was positively correlated with tumor vascular invasion (r=0.253, P=0.047) and total bilirubin level (r=0.283, P=0.024). MBL levels were higher in patients with vascular invasion than in those without (1880±186 ng/mL vs. 1306±122 ng/mL, P=0.011) [Figure 4: see original paper].

2.4.2 Correlation of MASP-2 with Clinical Variables Plasma MASP-2 level was positively correlated with TNM stage (r=0.276, P=0.027) and negatively correlated with plasma albumin level (r=-0.317, P=0.015) (Table 2). MASP-2 levels were higher in patients with advanced TNM stages than in those with early stages (422.7±26.2 ng/mL vs. 350.4±19.1 ng/mL, P=0.027) [Figure 5: see original paper].

2.5 ROC Curve Analysis of Plasma MBL and MASP-2 in HCC Patients

The areas under the ROC curve were 0.665 for MBL (P=0.010) and 0.694 for MASP-2 (P=0.003) [Figure 6: see original paper]. Using the Youden index formula (sensitivity + specificity - 1 = sensitivity - (1 - specificity)), the optimal cut-off values were determined to be 1422.14 ng/mL for MBL and 253.22 ng/mL for MASP-2, with sensitivities of 50% and 89.1%, respectively.

Discussion

MBL and MASP-2 are key molecules in the complement lectin pathway. MBL is a C-type lectin-like acute-phase protein synthesized by the liver that functions in opsonophagocytosis, complement activation, and inflammatory responses, playing an important role in immune defense. MASP-2 is the critical protease of the lectin pathway; when MBL or ficolin binds to ligands, the associated MASP-2 is activated, triggering downstream complement cascade reactions [3]. Studies have shown that MBL and MASP-2 are associated not only with infectious diseases [9] but also with inflammatory conditions such as rheumatoid arthritis progression [10]. Research on tumors has further revealed that MBL and MASP-2 in serum and tissues are tumor-associated [11-12]. HCC typically develops on the basis of chronic liver injury, which can activate innate immune function and maintain persistent inflammatory responses that promote hepatocyte regeneration, thereby facilitating liver cancer formation and progression [1]. Therefore, we hypothesized that MBL and MASP-2 are associated with HCC development and participate in tumor pathogenesis.

Early studies suggested that genetic polymorphisms of both MBL and MASP-2 were unrelated to HCC occurrence [13], though subsequent research indicated that MBL coding gene polymorphisms were associated with HCC development [14]. One study of colorectal cancer patients found that MBL and MASP-2 expression levels did not match their genotypes [15], and similar findings were reported in tuberculosis research, where MBL and MASP-2 genotypes did not reflect protein expression levels [16]. Therefore, this study measured plasma MBL and MASP-2 expression levels in 64 HCC patients and 30 healthy controls and analyzed their correlation with tumor development-related parameters to infer their association with HCC pathogenesis.

ELISA results demonstrated that plasma levels of both MBL (P=0.014) and MASP-2 (P=0.002) were significantly higher in HCC patients than in healthy controls, suggesting their involvement in HCC development—likely related to the fact that HCC typically arises from chronic liver injury and inflammation [1]. Although an early study reported decreased plasma MBL levels in HCC patients and speculated this might be due to reduced hepatic production of complement molecules like MBL resulting from abnormal liver function [14], detailed reports on MASP-2 expression in liver cancer are scarce, with only one domestic study linking it to liver injury [17]. However, CRP, another acute-phase protein pro-

duced by the liver, is elevated in HCC patient plasma and participates in disease pathogenesis [18], and serum MBL levels are higher in HCV-infected patients than in healthy individuals [19]. These findings do not support the aforementioned speculation. The discrepancy with our results may be attributed to advances in detection technology and differences in study populations. Future studies should include additional control groups, such as HBV-infected non-HCC individuals, to address this question more thoroughly. Furthermore, we found no significant difference in the plasma MBL-to-MASP-2 ratio between healthy individuals and HCC patients, indicating consistent elevation trends for both molecules despite the lack of a clear correlation between them. This may be because MASP-2 is a potential acute-phase protein that shares common stress response transcription elements with MBL and CRP, and its expression is up-regulated during inflammation to participate in the inflammatory response [20]. These results suggest that MBL and MASP-2 may be involved in inflammatory reactions that contribute to tumor development and progression.

To further analyze the relationship between plasma MBL and MASP-2 levels and disease progression, we examined their correlations with clinically relevant variables. Common variables associated with HCC development include gender, age, tumor size, tumor number, cirrhosis, alpha-fetoprotein, vascular invasion, HBsAg, ALT, AST, total bilirubin, plasma albumin, and C-reactive protein [21]. Pearson correlation analysis revealed that plasma MBL level was positively correlated with tumor vascular invasion ($r=0.253$, $P=0.047$), with higher MBL levels in patients with vascular invasion than in those without ($P=0.011$). Since vascular invasion is closely related to tumor development and metastasis [21-22], this result suggests that MBL may participate in tumor progression and metastasis by influencing vascular invasion. MBL was also positively correlated with total bilirubin level ($P=0.024$). As elevated total bilirubin is associated with liver inflammation, injury, and tumor progression [23], this finding suggests that MBL increases during liver damage and inflammation—consistent with its role as an acute-phase protein involved in inflammatory responses.

Correlation analysis of plasma MASP-2 with clinical variables showed that MASP-2 level was positively correlated with TNM stage ($r=0.276$, $P=0.027$), with significantly higher levels in advanced-stage patients than in early-stage patients ($P=0.027$), indicating that MASP-2 levels increase with disease progression and suggesting its involvement in tumor advancement. Additionally, plasma MASP-2 level was negatively correlated with plasma albumin level ($P=0.015$). Since decreased plasma albumin is an important indicator of liver injury and is also associated with tumor progression [24], this suggests that MASP-2 expression is upregulated during liver damage and inflammatory responses, participating in hepatic inflammation. Finally, to explore the potential of these molecules as HCC diagnostic markers—following previous studies that analyzed the relationship between MBL and MASP-2 gene polymorphisms and HCC using healthy controls [25-26]—we performed ROC curve analysis. The areas under the curve were 0.665 for MBL and 0.694 for MASP-2, with low diagnostic sensitivities of 50% and 89.1%, respectively, indicating that neither

molecule can serve as an independent diagnostic indicator for HCC.

These findings collectively suggest that MBL and MASP-2, key molecules in the complement lectin pathway, may be involved in the development and progression of HCC, providing a basis for further mechanistic studies and exploration of their clinical applications in HCC diagnosis and treatment. Future research should consider larger sample sizes and optimized sample classification for more in-depth investigation, as well as studies on their role in HCC clinical management.

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Note: Figure translations are in progress. See original paper for figures.