Based on HMGB1-PTEN pathway to explore the difference of T lymphocyte function in chronic hepatitis B with representative TCM syndromes

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Research Article

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Abstract

Background and Aims. Liver depression and spleen deficiency syndrome (LDSDS) and spleen-gastric damp-heat syndrome (SGDHS) are two representative syndromes of chronic hepatitis B (CHB) based on our early researches, and both of them exhibit significant differences in the pathogenesis and prognosis, which is closely related to immune regulation. However, the underlying mechanisms are largely unknown. This study aimed to better understand the immunoregulatory mechanisms of the two syndromes and promote the differentiation precision of the two syndromes in the clinical.

Methods. We studied the content of T lymphocytes by Flow cytometry and the expression levels of HMGB1-PTEN pathway proteins by enzyme-linked immunosorbent assay (Elisa) in the two syndromes and healthy controls, then constructed a protein association network through STRING database and the GeneMANIA database to analyze the functional expression correlation among the HMGB1, PTEN, PI3K, PDK1, and Akt. The correlations between T lymphocytes and proteins were analyzed by constructing multiple regression equations and Pearson test.

Results. The $CD8^+T$ cell levels in the two syndromes were lower than that in healthy controls, and the levels of Th17, Treg cells, Th17/Treg and HMGB1, PI3K, PDK1, Akt were higher than those of the healthy controls ($P<0.05$). Moreover, compared with the SGDHS, the levels of $CD4^+T$, Th17 cells and HMGB1, PTEN, PI3K in LDSDS were higher ($P<0.05$). Protein interaction network indicated that HMGB1 can regulate PI3K/Akt pathway through multiple pathways and has strong relevance. The EGFR may be a key target in HMGB1-PTEN protein network. The regression analysis showed that there was a linear correlation between the HMGB1-PTEN pathway axis and the level of immune cells, and the linear correlation factors of the two syndromes were inconsistent.

Conclusion. HMGB1-PTEN pathway may play an important role in regulating the formation of immune differences between the two syndromes. $CD4^+T$ and Th17 are two representative immune cells which may serve as two potential biological markers of LDSDS and SGDHS in CHB patients.

1. Introduction

At present, Hepatitis B Virus (HBV) infections causes many attentions because of high morbidity, and the prevalence of the disease greatly varies in different regions. In 2017, the number of HBV infections worldwide reached about 2 billion worldwide, of which 257 million were chronic infections [1]. About 1 million people die of chronic liver failure, cirrhosis, or primary hepatocellular carcinoma in which HBV infection is the main pathogenesis every year [2]. Before the wide use of hepatitis B vaccine, the rate of hepatitis B virus surface antigen (HBsAg) positivity was close to 10% in China [3]. Recently, the HBsAg positive rate has been declining, which is about 5-6%, however, there were still approximate 70 million people with chronic HBV infection, in which 20-30 million are CHB patients [4]. Therefore, the current epidemic situation of HBV infection is severe, which has seriously threatened human life and health.

It is well known that HBV does not directly cause liver cell injury in CHB, but the immune response caused by pathogens promotes the development of the disease. The removal of HBV in the body is primarily completed by the specific immune response that is mainly induced by cellular immunity [5-6]. The immune response happened at the beginning of the infection, and the number and ratio of lymphocytes changed, which resulted in the disorder of immune status [7-8]. $CD4^+T$ and $CD8^+T$ cells, as the two main forces of T lymphocytes, involved in the process of the disease, which revealed the immune status and serve as a direct indicator [9]. Some studies have shown that Th17 cells and Treg cells play an important role in chronic and severe HBV infection [10-15]. Treg cells control immune responses, including maintaining immune tolerance, regulating lymphocyte proliferation, and antagonizing the Th17 cells proinflammatory effect. The complementation of the two functions constitutes the balance axis of the body's immune regulation [16]. However, up to date, the mechanisms of immune regulation that cause the disorder of the balance of T lymphocyte subsets in chronic hepatitis B are still unclear, so we studied the $CD4^+T$, $CD8^+T$ and Th17, Treg cells to achieve further exploration.

There is a strong relationship between CHB and proinflammatory factors. High mobility group box-1 protein (HMGB1) is a ubiquitous DNA binding protein secreted by immune cells. Endotoxin and various inflammatory factors induce the secretion of HMGB1 and promote the inflammatory response [17], HMGB1 is speculated to be a potential inflammatory mediator and a "risk signal" of tissue damage because of early increases and longer duration [18-19]. The results of a Meta-Analysis showed HMGB1 serum levels were higher in severe hepatitis B or acute-on-chronic liver failure patients [20]. The phosphatase and tension homology deleted on chromosome ten (PTEN) is a gene on chromosome 10 which regulates some cellular processes, including proliferation, survival, energy metabolism, cellular architecture, and motility [21]. The expression of PTEN is affected by HMGB1, and the HMGB1-PTEN pathway plays an important role in tissue injury, inflammation, and cell necrosis [22]. Recruiting 3-phosphoinositide dependent kinase-1(PDK1) to the cell membrane is regulated by PI3K, which promotes the activation of protein kinase B(Akt), and forms PI3K/PDK1/Akt signaling pathway [23-24]. Research indicated that the lack or decrease of PTEN caused the increase of PI3K [25], thus, elevating the level of PDK1 and Akt in the downstream [26-27]. PTEN negatively regulates PI3K/Akt pathway, inhibits cell growth, and accelerates cell apoptosis [28]. Since PTEN is another gene closely related to tumorigenesis, p53 gene, PTEN/PI3K/Akt pathway seems to be related to some cancers, such as liver cancer, lung cancer, gastric cancer, lymphatic cancer [29-32]. Nevertheless, such a pathway has been recognized as an inflammatory regulator, which has been confirmed to regulate the proliferation of mouse mesangial cells [33]. Recently, a number of works have demonstrated an important role for the PI3K/Akt pathway in Treg cell development, function, and stability to affect disease progression and therapeutic effect [34-35]. As a result, PI3K/PDK1/Akt signaling pathway, which is regulated by HMGB1-PTEN signaling axis, demonstrates a significant effect on the differentiation of Treg cells (Figure 1). Thus, we proposed a hypothesis: HMGB1-PTEN pathway affects the balance of immune cell subtypes by activating of T lymphocytes and inducing the differentiation of cell subtypes to regulate the immune response of HBV infection.

Nowadays, antiviral is still the main treatment method to achieve the purpose of suppressing the virus, reducing liver damage, and alleviating the disease, but it can't completely achieve the functional cure of chronic HBV infection [36]. Traditional Chinese medicine (TCM) can prevent and cure CHB by regulating the body's immune function, anti-hepatocyte fibrosis, suppressing virus, and improving liver function [37]. Clinical surveys revealed that over 90% of patients with CHB in China received TCM therapy [38]. TCM takes syndrome differentiation as the basic principle that guides TCM to use medicine in clinical practice. "Syndrome" is a comprehensive manifestation of various clinical symptoms at a certain stage. The same disease may exhibit different syndromes owing to individual differences. Nevertheless, the objective diagnosis of syndrome differentiation in clinical TCM is affected by various factors such as experience and subjective judgment, which is not conducive to the improvement of traditional Chinese medicine in CHB. LDSDS and SGDHS are the two most representative syndromes [39]. The two syndromes have great differences in pathogenesis, condition, and prognosis, and have different immune functions that affect the progression and outcome of the disease [40-41]. In this study, we can provide syndrome differentiation evidence for the treatment of chronic hepatitis B from the perspective of immunology and HMGB1-PTEN pathway.

2. Materials And Methods

2.1 Patients and sample collection

The ethics committee of the Affiliated Hospital of Chengdu University of Traditional Chinese Medicine has approved the study. A total of 36 CHB patients (18 LDSDS and 18 SGDHS) were enrolled in the Affiliated Hospital of Chengdu University of Traditional Chinese Medicine. The diagnostic criteria for CHB referred to the *Guidelines for the Prevention and Treatment of Chronic Hepatitis B (2019)* [42]: with serum HBsAg positive for at least half a year and/or HBV-DNA positive, with serum ALT levels continue or repeatedly increase within 1 year or liver biopsy showing chronic hepatitis. The diagnostic criteria for chronic hepatitis B with LDSDS referred to the *Guidelines for Chronic Hepatitis TCM Diagnosis and Treatment (2018)* [43]; The diagnostic criteria of SGDHS referred to the *consensus of experts in TCM diagnosis and treatment (2012)* [44] (Supplementary Table S1). Another 14 healthy people were also recruited as controls. All subjects should be satisfied with the inclusion and exclusion criteria (Supplementary Table S2) and gave informed consent. The anticoagulant blood was collected from these participants by using EDTA tubes, 2 copies per person. One specimen was sent to flow cytometry within 6 hours. Another was centrifuged, then the supernatant was taken and stored in a sterile dry cryotube, and placed at -80°C.

2.2 Serological and biochemical tests

Biochemical analyzer was used to detect liver function indexes such as ALT, AST, TB, ALB. Real-time PCR was used to detect HBV-DNA load. The above were all completed in the hospital biochemical laboratory.

2.3 Flow cytometry

The fluorescent antibodies and reagents required for flow cytometry testing are listed in Supplementary Table S3. Add antibodies (CD3 APC-Cy7 antibody dosage is 0.5μL, the remaining antibody dosage is 2.5μL) according to the experimental groups, mix the blood sample, and add 50μL whole blood into each tube. Stain for 30min, add 600μL erythrocyte lysate, lyse for 10-15min, if the cell lysis is not clean, centrifuge at 1000r for 4min, then add 200μL lysate, lyse for 3min, after centrifugation at 1000r for 4min, add 300μL of PBS and test $CD4^+T$, $CD8^+T$, Treg cells by flow cytometer (CytoFLEX). Th17 cells are rarely activated and are usually difficult to detect, so PBMC cell extraction is required. Add 1μL PMA, 1μL Ionomycin, and 2μL Brefeldin A to stimulate and incubate for 4h, and measure the levels of $CD8^-T$ and IL-17 in the cytoplasm for detection Th17 cell level.

2.4 Protein Quantification

Enzyme-linked immunosorbent assay was used to detect the expression of signal proteins (HMGB1, PTEN, PI3K, PDK1, and Akt). These proteins were detected by using human protein ELISA kits (Jianglai Biological, China). The operation protocols were according to the manufacturer's instructions.

2.5 Construction of association network

To better understand the interaction between proteins in HMGB1-PTEN pathway, a protein association map was built. The STRING database(https://string-db.org/), and the GeneMANIA database(http://genemania.org/) were used to analyze and construct the functional expression correlation between HMGB1, PTEN, PI3K, PDK1, and Akt.

2.6 Statistical Analysis

Statistical analysis was performed using SPSS22.0 software (IBM, Chicago IL, USA). Since this experiment was a small sample study, Shapiro Wilk method was used for the normal distribution test of all measurement data. For normal distribution, two groups of samples were tested by independent sample t-test, three groups of samples were further tested for homogeneity of variance, single factor analysis of variance (Bonferroni method) was used for homogeneity of variance, Tamhane method was used for non-homogeneity of variance, Mann Whitney U test was used for two groups of samples, and Kruskal Wallis test was used for three groups of samples; Chi square test was used to compare categorical variables such as gender. Multiple stepwise regression analysis and Pearson or Spearman test were used to explore the correlation between the HMGB1-PTEN pathway axis and immune cells. For all tests, $P<0.05$ was considered statistically significant.

3. Results

3.1 Patients Characteristics

A total of 50 participants were recruited in this study, including 18 LDSDS, 18 SGDHS, and 14 healthy subjects. Among the 18 LDSDS, 8 were males and 10 were females; among the 18 SGDHS, 12 were

males and 6 were females; among the 14 healthy participants, there were 11 males and 3 females. There were significant differences in gender and age among the three groups (P⩽0.05). However, there was no significant difference in age and gender between the LDSDS and SGDHS(P>0.05). Serum aspartate aminotransferase (AST) levels, alanine aminotransferase (ALT) levels, total bilirubin (TBIL) levels, albumin (ALB) and HBV-DNA had no significant difference between the LDSDS and SGDHS(*P*>0.05) (Table 1).

Table 1 Patients characteristics.

Variables	LDSDS(n=18)	SGDHS(n=18)	HC(n=14)	*P* Value
Age(*y*) [†]	39.00±9.15	41.00±9.15	24.57±0.85	0.000
Female(%)[‡]	10(55.56%)	6(33.33%)	11(78.57%)	0.038
ALT(u/L) [§]	23.5(17.75~47.25)	25.5(20.25~36.23)	NA	0.696
AST(u/L) [§]	21.5(16~29.25)	25(20.75~28.25)	NA	0.323
TBIL(umol/L) [§]	13(10.38~14.55)	14(9.55~18.68)	NA	0.228
ALB(g/L) [§]	45(42.48~47.15)	44.85(42.18~47.2)	NA	0.729
HBV DNA(≥2000IU/ml,n(%)[‡]	7 (38.89%)	6 (33.33%)	NA	0.383

LDSDS, liver depression and spleen deficiency syndrome; SGDHS: spleen-gastric damp heat syndrome; HC: healthy controls; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; ALB, albumin; NA, not available; [†]Means ± SD; [‡]Numbers and percentages; [§]Median and interquartile range.

3.2 Distribution of T lymphocytes in subjects

The results of flow cytometry showed that the $CD8^+$T cell level was significantly decreased in LDSDS and SGDHS compared with the healthy controls (*P*<0.05), however, there were no significant difference observed (*P*>0.05) between LDSDS and SGDHS (Figure 2A). Significant decrease of $CD4^+$T cells was observed only in the SGDHS, but not in LDSDS when compared with healthy controls (*P*<0.05). Moreover, the expression of $CD4^+$T cell in LDSDS were increased compared with SGDHS (*P*<0.05) (Figure 2A). There were significant differences in Th17 cell expression among the three groups, and expression level of LDSDS was the highest(P<0.05) (Figure 2B). For Treg, both LDSDS and SGDHS exhibited significantly increased levels compared with the healthy controls (*P*<0.01), but there were no significant difference observed (*P*>0.05) between LDSDS and SGDHS (Figure 2C). In addition, the ratio of $CD4^+$T to $CD8^+$T cells and the ratio of Th17 to Treg cells reflect the immune balance of T cells. The specific percentage of each T lymphocyte was shown in Table 2. It can be seen that there was no significant difference in the ratio of $CD4^+$T to $CD8^+$T cells among the three groups. The ratio of Th17 to Treg cells of LDSDS and SGDHS was significantly higher than that of healthy controls(P<0.05)(Table 2). What has most interesting is that among the four T lymphocytes, only Th17 showed statistical differences not only between these two syndromes but also between the syndromes and healthy controls, suggesting that Th17 may serve as one of the potential biochemical indicators of the TCM-syndrome of CHB, which needs further exploration.

Table 2 Expression of T lymphocyte subsets (n=50, Mean ±SD)

T lymphocyte	LDSDS (%)	SGDHS (%)	HC (%)
CD4$^+$T	33.64±5.08$^{\#}$	28.70±7.11**	36.58±4.76
CD8$^+$T	25.64±6.66*	22.71±8.25**	32.07±6.27
CD4$^+$T /CD8$^+$T	1.38±0.36	1.37±0.34	1.18±0.30
Th17	13.01±5.11$^{**\#}$	9.12±3.74**	0.49±0.24
Treg	9.17±2.15**	10.35±1.86**	4.67±3.08
Th17/Treg	1.46±0.58**	0.89±0.39**	0.20±0.22

HC represents healthy controls. "*" represents statistically significant difference compared with the healthy control ($P<0.05$), "**" represents statistically significant difference compared with the healthy control ($P<0.01$); "#" represents statistically significant difference compared with SGDHS($P<0.05$).

3.3 Analysis of HMGB1-PTEN pathway protein expression level

Through the Elisa, the HMGB1-PTEN proteins were quantified. The results showed the levels of HMGB1, PI3K, PDK1, and Akt in LDSDS and SGDHS were significantly higher than healthy controls ($P<0.01$) (Figure 3). Moreover, the expression levels of HMGB1, PI3K in LDSDS were even higher than that in SGDHS ($P<0.01$) (Figure 3). For PTEN, compared with healthy controls, it was significantly increased in LDSDS but significantly decreased in SGDHS($P<0.01$), and exhibited a significant difference between the two syndromes($P<0.01$) (Figure 3). Collectively, HMGB1, PTEN, and PI3K were highly expressed in LDSDS compared with the SGDHS($P<0.05$) (Figure 3), indicating that the expression level of these three proteins may be related to the formation of TCM syndromes to some extent, but this need to be further studied in the following research.

3.4 Correlation analysis of HMGB1-PTEN pathway protein

To further elucidate the correlation strength among the HMGB1-PTEN pathway proteins, the protein-protein interaction networks between five proteins were constructed through the STRING database (https://www.string-db.org/). As shown in Figure 4, we found that HMGB1 was connected with PTEN, PI3K, PDK1 through Akt from the aspects of literature mining, experiments, databases, co-expression, gene neighborhood, gene fusion, co-occurrence, etc. The data demonstrated that the combined score of evidence suggesting a functional link in PI3K, PDK1, and Akt were above 0.8, and they had positive regulation. The combined scores of PTEN, PI3K, and Akt were above 0.9, but PTEN negatively regulates PI3K/Akt. Although the combined score of HMGB1 and Akt was 0.554, it can be seen from the number of lines in the figure, there was not only one way of connection between them.

The GeneMANIA database (http://genemania.org/) was used to further construct the network map related with HMGB1, PTEN, PI3K, PDK1, and Akt. As shown in Figure 5, there are many interaction modes such as physical interactions (67.64%), co-expression (13.50%), co-localization (6.17%), pathway-mediated (4.35%) and other aspects. Further analysis of the interaction mechanism revealed that HMGB1 is co-expressed with PDK1, which is associated with PI3K/Akt pathway, and can also co-express PTEN through

CENPC, while PTEN directly negatively regulates PI3K/Akt pathway. That is to say, HMGB1 can regulate PI3K/Akt pathway by direct or indirect means. In the PI3K/Akt pathway, EGFR is the key target factor of the pathway and has a certain impact on mTOR signaling molecules that is the classic factor in liver disease research. These remind us that EGFR may be a key factor in HMGB1-PTEN protein network, which provides a target gene for further research.

3.5 Correlation analysis of HMGB1-PTEN pathway protein and immune cell levels

The proteins of HMGB1-PTEN pathway were, respectively, subjected to stepwise regression analysis on T lymphocyte subsets to construct a regression model. Next, take the LDSDS as an example: The adjusted R^2 of all proteins were good fit. The results showed that it was highly consistent, mainly linearly correlated with $CD4^+T$ cells and Th17/Treg (Table 3). Similarly, in the SGDHS, the regression results showed that the HMGB1-PTEN pathway axis could significantly affect the differentiation of T lymphocytes and was highly consistent, all of which were significantly correlated with Treg cells. In healthy controls, the HMGB1-PTEN pathway proteins were significantly associated with $CD4^+T$, $CD8^+T$ cells, and $CD4^+T/CD8^+T$. It can be seen that in the two syndromes, the immune cells affected by pathway regulation were different, in other words, linearly related to different lymphocytes, indicating that the physiological and pathological state of the body may affect the internal relationship between HMGB1-PTEN and immune cells.

Table 3 Multiple stepwise regression analysis of HMGB1-PTEN pathway and T lymphocytes in LDSDS

Pathway	$CD4^+T$	$CD8^+T$	$CD4^+T/CD8^+T$	Th17	Treg	Th17/Treg
HMGB1	10.855/0.983**	—	—	—	—	2.408/0.983*
PTEN	4.845/0.974**	—	—	—	—	—
PI3K	10.742/0.982**	—	—	—	—	2.390/0.982*
PDK1	10.824/0.983**	—	—	—	—	2.397/0.983*
Akt	10.808/0.983**	—	—	—	—	2.419/0.983*

The numbers on the left and right sides of each slash line represent the regression coefficient and coefficient of determination, respectively. *, **: p=0.05,0.01, respectively.

4. Discussion

Chronic hepatitis B is a major medical and health problem that needs to be solved urgently. Antiviral therapy to some extent inhibited HBV replication, but it was still unable to eliminate completely and gradually produced drug resistance and dose-dependent side effects because of administration for the long term [45]. It needs to call for a novel treatment for CHB that could lead to sustained and off-treatment inhibition of viral replication. As an established segment of the public health system, TCM has been widely used in China for more than 2000 years. Because of the unique advantages of TCM in the prevention and treatment of CHB, it is often used as an important adjuvant therapy in China [46]. The "syndrome" is the basis for TCM treatment. Accuracy of syndrome differentiation is the key to the

effectiveness of TCM treatment. Consequently, to carry out objective research on CHB syndrome and explore the biological nature of common syndromes is conducive to promoting the application of TCM in the clinical treatment of CHB. Furthermore, it is crucial to study the differences in the immune level, which can be used as a quantitative index for syndrome differentiation of TCM. The in-depth study of the biological nature of the typical syndromes of CHB is of great significance for the clinical differentiation of CHB in TCM, the analysis of the prognosis of the disease, and the exploration of the targets of prescriptions and drugs.

As is known to all, the process of chronic HBV infection is closely related to the immune system. Previous studies have proved that the main pathogeny of chronic hepatitis B is not HBV infection, but its replication and proliferation promotes the immune response, and finally leading to liver injury [47-48]. On the one hand, the immune response brings the protective function to remove the virus. On the other hand, the immune activities lead to liver cell damage and even induce virus mutation. Unlike acute HBV infection, the cellular immune response decreased in chronic infection. And the chronic cellular immune response caused the persistence of HBV and finally developed the disease [49]. As a helper of cellular immunity, both $CD4^+T$ cells and $CD8^+T$ can directly kill viruses, which provide an important defense line to fight against viruses. However, these T cells significantly decreased in CHB patients, and the SGDHS was more obvious, suggesting that the immune system of the SGDHS was seriously damaged. After the antigen activated the initial $CD4^+T$, they differentiate into different subtypes, such as Th17 and Treg, and perform different functions. Th17 and Treg keep a stable balance, and once the balance is broken, it should take part in the persistence and severity of CHB. Th17 cells mainly participate in the pathological process of various inflammatory reactions by secreting a variety of cytokines. Studies have found that the number of Th17 cells in the peripheral blood of CHB patients significantly increased [50], which suggests that Th17 cells play an immune activation role in chronic HBV infection. Th17 cells abnormally differentiated and secreted inflammatory factors, such as IL-17, which aggravated the inflammation of the body [51]. However, Treg cells are mainly immunosuppressive. In chronic HBV infection, Treg cells inhibit the differentiation and activation of $CD4^+T$, $CD8^+T$, and other effector T lymphocytes. Treg cells decrease the secretion of some cytokines, such as IL-10, TGF - β, which weaken the ability to clear target cells, immune response, and liver inflammation. It also reduces the ability to clear the pathogen, which leads to the chronic progress of HBV infection [52]. This shows that Th17/Treg cell balance is an important component of maintaining the normal cellular immune function, and it has been recognized as the main cause of the development of CHB. The imbalance was a risk factor for the development of CHB in cirrhosis and HCC [53]. In our research, the levels of Th17, Treg cells, and Th17/Treg were increased in CHB with the two syndromes, indicating that long-term chronic HBV infection could stimulate immune inflammation, causing autoimmune disorders. And in the comparison of the two syndromes, the expression of Th17 in LDSDS was higher, which demonstrated that patients with LDSDS could produce a more effective immune response to HBV specific antigens. In other words, patients with LDSDS had more perfect immune function, signifying having more active inflammation simultaneously. The patients with SGDHS had lower cellular immunity, which was difficult to resist the evil. With the pathogen lingering, it was more likely to delay the recovery, which may be easier to lead to the formation of liver cirrhosis, even

liver cancer. The previous research of our team showed that the expression of IL-4, IL-10, and INF-γ in CHB with LDSDS were higher than those in SGDHS [54], while IL-4, IL-10 and INF-γ were mainly secreted by Th1 and Th2 cells, it suggested that the cell immune function of the LDSDS was stronger than that of the SGDHS. The results of this experiment were consistent with the previous results. This study also suggested that the different syndrome types have different degrees of immune response, resulting in changes of T lymphocyte levels.

HMGB1 levels increased in CHB accompanied with the severity of liver inflammation, and closely related to the progress of liver fibrosis after hepatitis [55-56]. During HBV infection, the PI3K/Akt pathway was activated [57]. IL-12, as one of the proinflammatory factors, significantly increases in CHB patients and HBV-induced IL-12 expression involved in the activation of the PI3K-Akt pathway [58]. In addition, evidence has demonstrated that the change of PTEN activity leads to the development of CHB by deregulating the PI3K/Akt pathway [59]. Therefore, the main role of the HMGB1-PTEN mediated PI3K/PDK1/Akt pathway is to promote the progress of inflammation in CHB patients. In contrast, the levels of some HMGB1-PTEN pathway proteins (HMGB1, PI3K, PDK1, and Akt) of CHB patients with two syndromes were higher, revealing that the inflammatory states were activated, which was consistent with the nature of the changes reflected by immune cell levels. Furthermore, the levels of HMGB1, PTEN, and PI3K in LDSDS were significantly higher than those in SGDHS, which revealed that the internal inflammation of the LDSDS was more intense.

Excessive immune response causes inflammation but also affects the expression of T lymphocytes. Studies have shown that HMGB1 levels increased in CHB patients, which in turn inhibit the activation of Treg cells through TLR-IL-6 receptor signal transduction, and further promote the activities of Th17 cells [60-61]. PTEN regulates the stability of Treg cells through Akt, and the lack of PTEN activates T cells and leads to spontaneous inflammation [62]. HMGB1 can also regulate the differentiation of Treg cells by deregulating PTEN [63]. Moreover, in vivo and vitro, knocking out the PDK1gene of T cells decreased the number of Th17 and Treg cells [64]. Otherwise, PDK1 gene could mediate Akt levels and elevate Treg cells [65]. The level of Treg cells in the peripheral blood of lung cancer patients significantly increased, which might be related to the PTEN weak expression and PI3K-Akt signaling pathway activation [66]. Therefore, PI3K/PDK1/Akt signaling pathway regulated by HMGB1-PTEN signaling axis could take effect on lymphocytes. The abnormally expression of HMGB1, PTEN, PI3K in LDSDS may be an important reason for the high expression of CD4^{+}T and Th17 cells in LDSDS compared with SGDHS, but further research is still needed.

In summary, we believed that the HMGB1-PTEN pathway could reflect the changes of T lymphocyte levels to a certain extent, not only between CHB patients and the healthy group, but also between different syndrome types of CHB, such as LDSDS and SGDHS. The result was confirmed by regression analysis as well. However, this is only a preliminary exploration, in-depth study of the regulatory relationship between HMGB1-PTEN pathway and lymphocytes is conducive to grasp the internal pathogenesis of different syndrome types of CHB, and can also be used as a specific biological marker between syndrome types, promoting the diagnosis and clinical efficacy of CHB. We can further study the HMGB1-PTEN pathway

targets, such as EGFR, to explore its role on the diagnosis, treatment, and prognosis of CHB, clarify the regulation mechanism, enrich the biological connotation.

5. Conclusion

CD4$^+$T and Th17 are two representative immune cells which may serve as two potential biological markers for the diagnosis of LDSDS and SGDHS in CHB patients, and are related closely to the regulation of HMGB1-PTEN pathway. Chronic hepatitis B patients with SGDHS are more likely to develop into liver cirrhosis, even liver cancer due to imperfect immunological function. Our results, to some extent, reflected the biological essence of CHB with the same disease and different syndromes.

Declarations

Data Availability

All data used to support the findings of this study are available from the corresponding authors upon request.

Ethical Approval

This study was approved by the research medical ethics Committee of the Affiliated Hospital of Chengdu University of Traditional Chinese Medicine (China).

Consent

All participants in this study provided written informed consent.

Conflict of interest

The authors declare no conflict of interest.

Authors' contributions

Xia Li and Chao Liu contributed equally to this work. Xia Li was involved in drafting the manuscript and acquisition of data; Chao Liu completed the analysis and interpretation of the data; Guiyu Li and Yanfeng Zheng helped the acquisition and analysis of data; Jie Mu and Lushuang Xie contributed to the statistical analysis and correction of the manuscript. Quansheng Feng and Cen Jiang designed this study and gave final approval of the version to be published. All authors have read and approved the final version of the manuscript.

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Figures
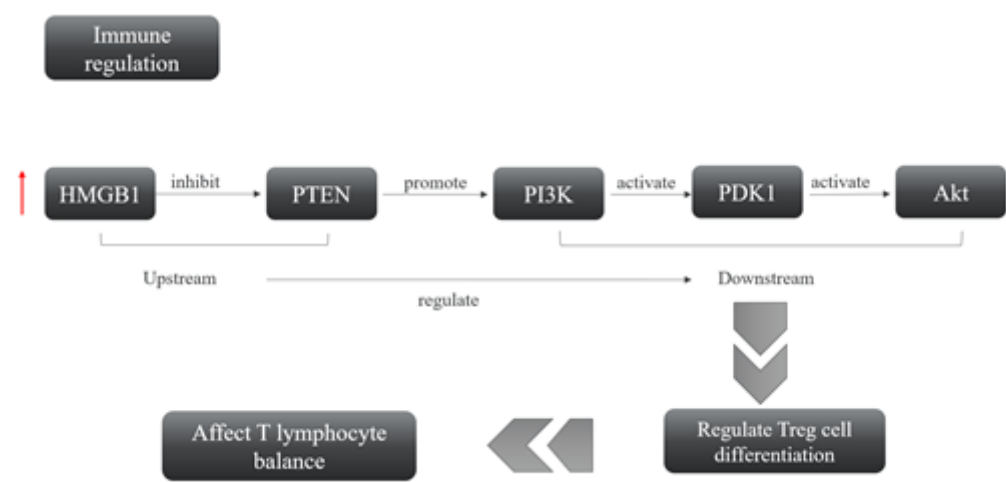
Figure 1

HMGB1-PTEN pathway regulates T lymphocytes
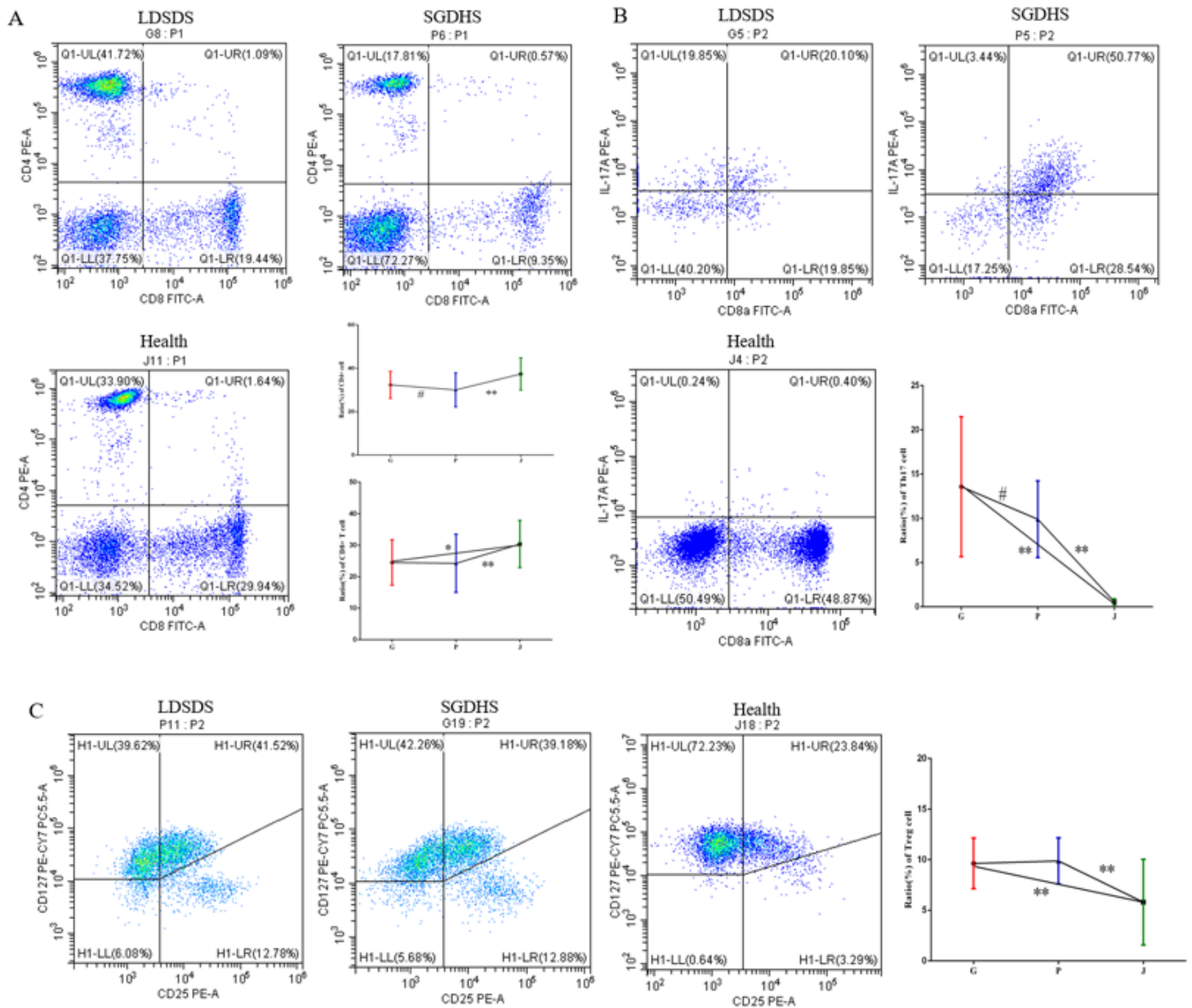
Figure 2

A: Distribution and comparison of CD4+T and CD8+T cells level differences in three groups by flow cytometry. B: After cell stimulation, the membrane was broken, CD4+T was labeled with CD3+CD8-, then IL-17A antibody was added, and finally Th17 cells were labeled with CD8-IL17+. C: The combination of CD4+CD25+CD127low can produce highly purified regulatory T cells, and without breaking the membrane, Treg cell expression level can be determined. In the scatter plot, group G represents LDSDS, group P represents SGDHS, group J represents the healthy controls. "*" represents statistically significant difference compared with the healthy controls (P<0.05), "**" represents statistically significant difference compared with the healthy controls (P<0.01); "#" represents statistically significant difference compared with SGDHS(P<0.05).
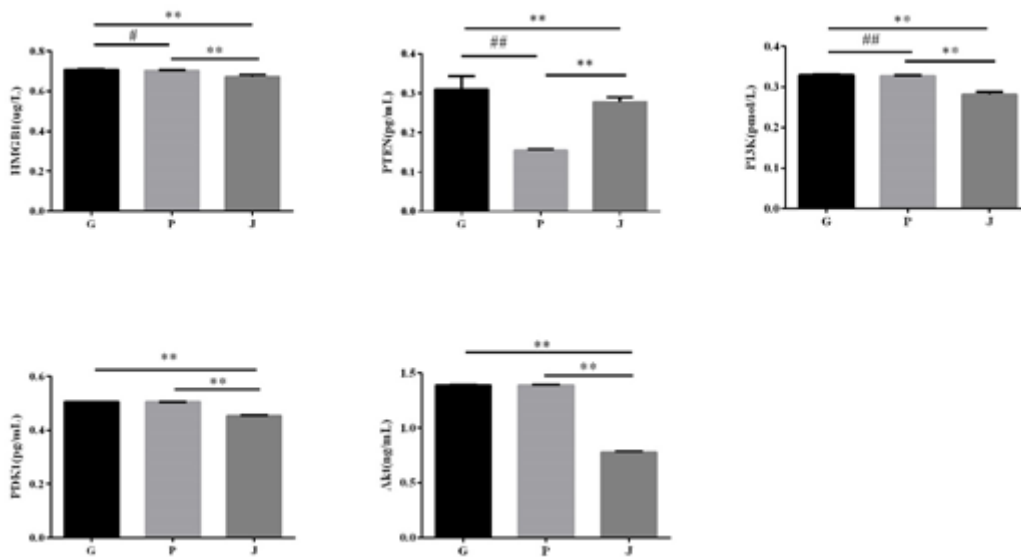
Figure 3

Comparison of the difference of the signal protein level in HMGB1-PTEN pathway, Group G represents LDSDS, group P represents SGDHS, group J represents healthy controls."*" represents statistically significant difference compared with healthy controls (P<0.05), "**" represents statistically significant difference compared with healthy controls (P<0.01); "#" represents statistically significant difference compared with SGDHS(P<0.05), and "##" represents statistically significant difference with SGDHS(P<0.01).
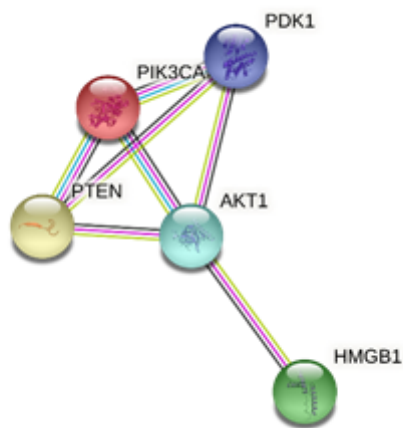
Figure 4

Conducting a HMGB1-PTEN pathway protein interaction network diagram, different connection colors represent different interaction types. The thickness of the line represents the strength of the association. The thicker the line, the stronger the association, and the thinner the line, the weaker the association.
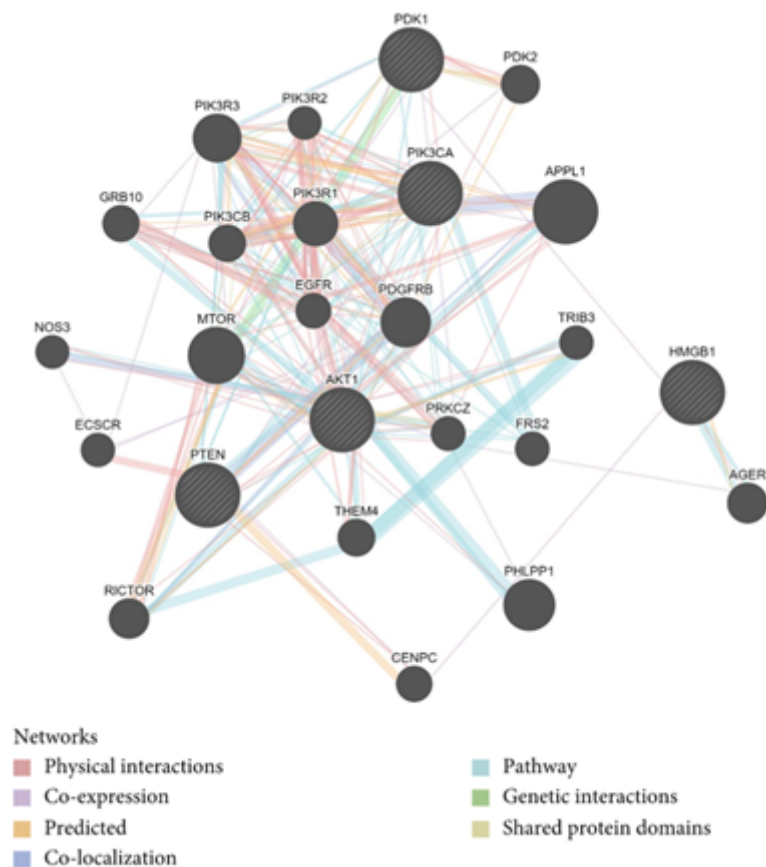
Figure 5

HMGB1-PTEN pathway protein function correlation diagram