Significant serum proteinic and metabolic alterations in hepatitis B cirrhosis patients treated with umbilical cord mesenchymal stem cells

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Research Article

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Abstract

Stem cells based-treatment is considered as an effective regenerative therapy for liver cirrhosis patients. Data about impact of stem cell transplantation on circulating metabolites in liver cirrhosis patients remains limited. In this study, eligible participants with hepatitis B cirrhosis were injected with 10E7 umbilical cord mesenchymal stem cells (uc-MSCs) through ultrasound-guided percutaneous liver puncture and portal vein catheterization. Changes of serum samples after treatment were measured by performing proteomics and metabolomics. As illustrated, proteins enriched in ECM remodeling were significantly altered, such as 20S proteasome subunits and laminin. Types of circulating phosphatidylcholine were augmented in patients after uc-MSCs treatment compared to original levels (14:0, 16:1, 18:1, 18:2, 20:1, 20:4), whereas phosphatidylethanolamine was diminished (20:4). Changes of serum profiles demonstrated that uc-MSC treatment is efficient in repairing tissue damage and metabolic disorders, revealing early biomarkers in clinical evaluation of uc-MSC treatment in liver cirrhosis patients.

Introduction

Cirrhosis is the late stage of liver fibrosis and the initial stage of HCC, which is caused by multi-forms of liver diseases and conditions, such as viral hepatitis and chronic alcoholism. Anti-virus and symptomatic treatment are recommended in management of liver cirrhosis patients, while liver transplantation is considered in severe cases. To date, stem cells based-treatment has been investigated as an effective regenerative therapy for liver cirrhosis patients. Various types of stem cells from embryonic, induced pluripotent, hematopoietic and mesenchymal stem cells (MSCs), have shown capacity of differentiation into hepatocyte-like cells, laying the foundation of their clinical application. Once homing to impaired liver tissue after infusion, repairment function of MSC is elicited by suppressing inflammatory response to recover damaged hepatic function and their immune-modulatory properties. Therapeutic efficacy of MSC or their bioactive derivates in patients with liver cirrhosis have also been confirmed in clinical trials(1). For example, intravenous injection of umbilical cord MSCs (uc-MSCs) was proved to be clinically safe, improved liver function and reduced ascites in patients with decompensated liver cirrhosis(2). However, data on the impact of stem cell transplantation on circulating metabolites in liver cirrhosis patients remains limited.

Patients And Methods

In this study, 7 eligible participants received 10E7 uc-MSCs injection through ultrasound-guided percutaneous liver puncture-based portal vein catheterization. Changes in serum proteins and metabolites at the timepoint of 72 hours after treatment were measured by proteomics and metabolomics respectively.

Tandem Mass Tag (TMT) based proteomic was performed using serum samples from liver cirrhosis patients prior to or 72 hours after uc-MSCs treatment. After removing high abundant proteins, each sample underwent reduction and alkylation, being digested and labeled with TMT for peptides

preparation. 2ug of total peptides were separated and analyzed with a nano-UPLC (EASY-nLC1200) coupled to Q Exactive HF-X Orbitrap instrument (Thermo Fisher Scientific) with a nano-electrospray ion source. Files were processed for peptide identification and quantification (Supplementary Table 1).

Untargeted metabolomics analysis was performed using ultra-high performance liquid chromatography systems with multiple reaction monitoring mass spectrometry (UHPLC-MRM-MS) coupled to identification with an internal library of authentic chemical standards. Files were processed for peptide identification and quantification (Supplementary Table 2).

Results

A total of 1209 proteins were identified and processed to taken logarithm modeled for principal component analysis (PCA) (Fig. 1A). With the threshold of Fold change > 1.2 and $p < 0.05$, 35 proteins were identified as differentially expressed protein (DEP) between these two groups (Fig. 1B). As presented in Fig. 1C, 28 proteins were upregulated while 7 proteins were down-regulated in patients underwent uc-MSCs injection. Dramatic elevation in certain proteins associated with extracellular matrix (ECM) remodeling was observed, such as proteasome 20S subunits (PSMA/PSMB) and ECM components (LAMC1, POSTN). Gene ontology analysis also suggested these altered proteins were enriched in ECM remodeling and metabolic processes (Fig. 1D.) A cluster of coregulated features strongly are relevant to the ECM components identified, which might be a potential symbol of recovered hepatic tissue (Fig. 1E).

Metabolomic data was assessed for clustering using PCA (Fig. 2A). 16 increased and 27 decreased metabolites were screened out (Fig. 2B, 2C). The altered metabolites in patients after uc-MSC treatment include a batch of phospholipid especially glyceryl phosphatide and their derivates. Several types of phosphatidylcholine (PC) were augmented after uc-MSC injection compared to baseline (PC14:0, PC16:1, PC18:1, PC18:2, PC20:1, PC20:4), whereas phosphatidylethanolamine (PE) was diminished (PE20:4). GO annotation was performed using 43 changed metabolites, leading with reduced glycerophospholipid metabolism and Nicotinate/Nicotinamide metabolism (Fig. 2D). Those altered metabolites were processed to demonstrate areas-under-the-curve (AUCs) for treatment status. 4 types of PCs (14:0, 18:1, 18:2, 20:1) are considerably valuable to reflect treatment status (AUC = 1) (Fig. 2E).

Discussion

Alteration of serum proteins demonstrated a repairment of uc-MSC on cirrhotic liver. Previous research has unearthed the major role of hepatic stellate cells (HSCs) in development of liver cirrhosis. Upon continuously stimulated by PDGF, TGF-β, ECM proteins secreted by HSCs are accumulated to repair damaged tissue, leading to occurrence of cirrhosis. Normally, conserved degradation machinery such as the 20S proteasome, can maintain proteostasis by cleaving excessive proteins. However, compared to healthy people, plasma proteasome levels are lower in cirrhosis patients, failing to degrade redundant deposits(3). In our study, uc-MSC treatment elevated subunits of 20S proteasome in serum of cirrhosis patients, reflecting regeneration of liver from damaged status, which is consistent with relative findings in

mice models(4). Besides, ability of MSC in self-renew and differentiating into hepatic progenitor cells is critical. Laminin is glycoprotein constituent of basement membrane, augmented LAMC1 was found in hepatic cells originated from uc-MSC compared to original uc-MSCs(5). Increased LAMC1 was also observed in patients after treatment of uc-MSCs. In contrast, Leukocyte cell-derived chemotaxin-2 (LECT2), a contributor of liver fibrogenesis by inhibiting portal angiogenesis, was significantly reduced following with uc-MSC treatment (6).

Alteration in serum metabolites demonstrated a restored liver function after uc-MSC injection. Under viral hepatitis or other pathological conditions, metabolic function of liver especially in lipid storage or transport is out of balance to aggravate liver fibrogenesis. Expression of lipid metabolic genes are found to be influenced during chronic HBV or HCV, which is associated with frequent hepatic steatosis and altered circulating lipid levels(7). PC and PE are the most abundant phospholipids distributed in almost plasma membrane bilayers, serving as major structural components and regulating fluidity of membrane. PC can be conversely produced from PE catalyzed by phosphatidylethanolamine N-methyltransferase (PEMT) via the CDP-choline pathway (8). PC is participated in packaging fat and cholesterol into very low density lipoprotein (VLDL) particles, and secreting them out to regulate their distribution in extrahepatic tissues. Impaired PC synthesis results in inadequate VLDL secretion, leading to an accumulation of fat and cholesterol in the liver. Moreover, PC breaks down scar tissue in the liver and may be able to reverse tissue changes that cause cirrhosis. Compared to healthy people and hepatitis patients, both cirrhosis patients showed downregulated PC level (9). In this study, we observed uc-MSCs transplantation upregulated several species of PC in serum of cirrhosis patients, indicating PC metabolic ability was recovered after treatment. Increasement of PC/PE ratio after treatment reflects a healthier liver status, since a lower hepatic PC/PE ratio is more frequent in patients with NAFLD and NASH than healthy subjects(10).

Breakdown products of PC and PE such as LysoPC and lysoPE can be formed under cleavage by PLA2, which can be converted back to PC of PE in the presence of lysoPC acyltransferase. LysoPC expressed in hepatocytes is involved in regulating biosynthesis of cholesterol and hepatic fatty acid oxidation(11), while LysoPC released into serum contributes to inflammatory reaction or lipid accumulation in vascular system(12). In previous literature, types of lysoPCs(e.g., 16:0, 18:2, 22:6) were reported to be ascended in cirrhotic patients (13), some of them are even higher in serum of HCC patients(14). Therefore, reduced serum LysoPC level by ucMSC treatment symbolled blocked deterioration of cirrhosis.

Conclusion

In summary, we illustrated changes of serum proteins and metabolites under uc-MSC treatment in cirrhotic patients, corroborating that uc-MSC alleviated dysregulation of metabolic function. Expression profiling of glyceryl phosphatides, especially PC and lysoPC, provides a mechanistic insight into the efficacy of uc-MSC in patients with hepatitis B cirrhosis.

Abbreviations

DEP, differentially expressed protein; ECM, extracellular matrix; HBV, hepatitis B virus; HCV, hepatitis C virus; HSC, hepatic stellate cells; MSC, mesenchymal stem cell; PCA, principal component analysis; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PEMT, phosphatidylethanolamine N-methyltransferase; TMT, Tandem Mass Tag; uc-MSC, umbilical cord MSCs; VLDL, very low density lipoprotein.

Declarations

Ethics approval and consent to participate

The clinical trial (ChiCTR2100052843) is entitled “A prospective, single center clinical study of umbilical cord mesenchymal stem cell transplantation for end-stage cirrhosis after hepatitis B”, which is approved by Ethics Committee of Clinical Research Institute of Cell Biotherapy in Sichuan Provincial People's Hospital (Approval number:2019001, Approval date: 30th, August 2019). This project is conducted by Sichuan Academy of Medical Sciences and Sichuan Provincial People’s Hospital. Written informed consents from patients have been obtained according to the 1964 Declaration of Helsinki protocol and its later amendments.

Consent for publication

We consent for publication.

Availability of data and material

The proteomic and metabolomic datasets used and/or analyzed during the current study are provided as the supplementary table 1 and 2 respectively.

Competing interests

The company "Asia Cellular Therapeutics (Zhejiang) Co., Ltd." provided product (stem cell) for this study.

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Authors' contributions

TF, CL, DZ, SW contributed to sample collection. TF, YY and QY contributed to statistical analysis. TF, JS and YS drafting of the manuscript and interpretation of data. XH and YS had full access to all of the data

in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. XH and YS contributed to study concept and design, and critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript.

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Figures

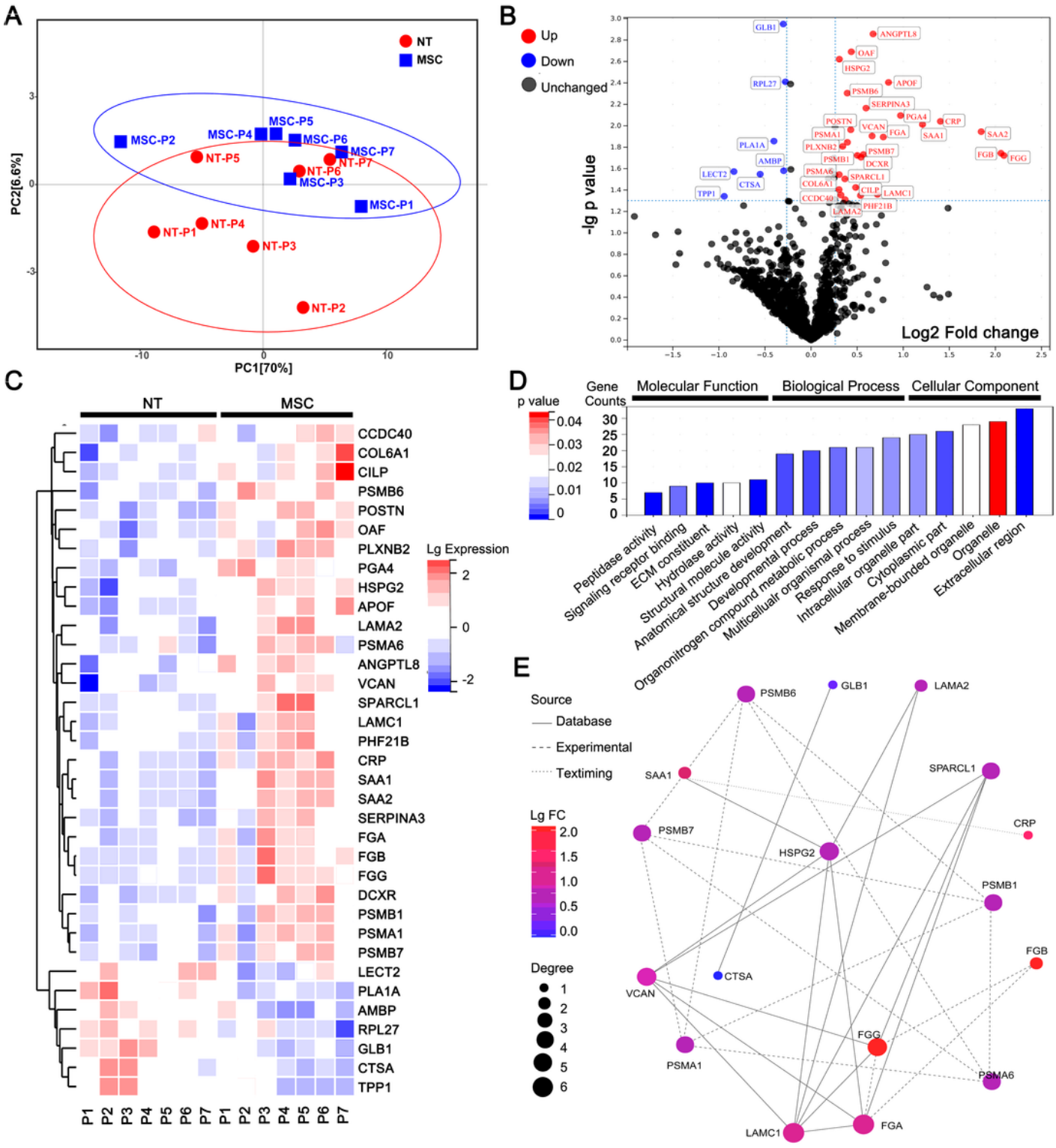

Figure 1

Integrative network analysis of proteomics data of serum samples from hepatitis B cirrhosis patients under un-MSC treatment.

A. The PCA model illustrated discrimination of serum proteins from hepatitis B cirrhosis patients before or after uc-MSC treatment. B. Volcano plot of differential expressed proteins in serum from hepatitis B

cirrhosis patients before or after uc-MSC treatment. C. Heatmap with differentially expressed protein levels in serum from hepatitis B cirrhosis patients before or after uc-MSC treatment. D. GO terms enriched by the key proteins. E. Network analyses illustrating correlation among proteins. Nodes color is set by fold changes. The thickness of the edge represents the correlative evidence. NT-P: non-treated patient; MSC-P: uc-MSC treated patient.

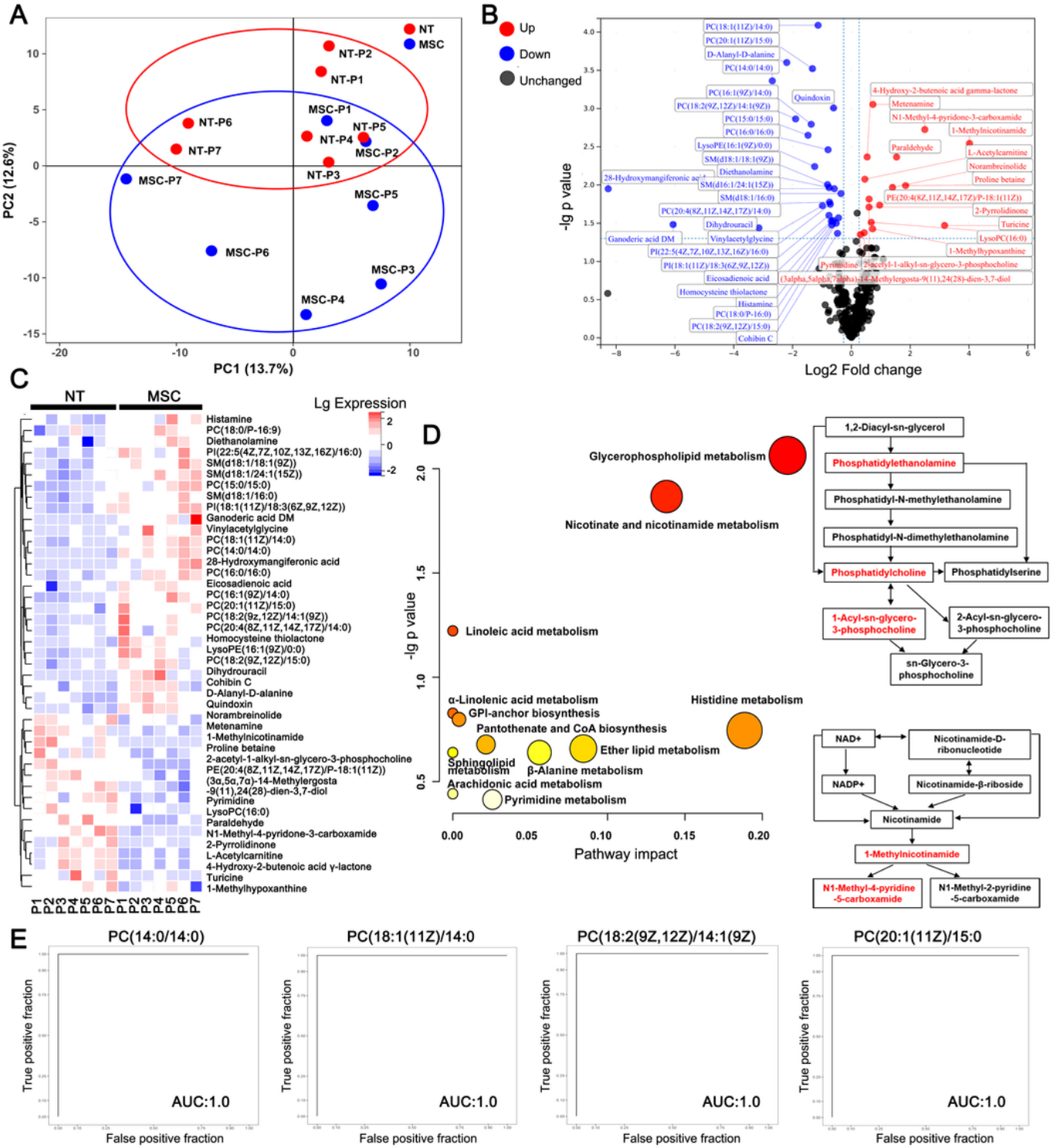

Figure 2

Integrative network analysis of untargeted metabolomics data of serum samples from hepatitis B cirrhosis patients under un-MSC treatment.

A. The PCA model illustrated discrimination of serum metabolites between hepatitis B cirrhosis patients before and after uc-MSC treatment. B. Volcano plot of differential expressed serum metabolites in hepatitis B cirrhosis patients before and after uc-MSC treatment. C. Heatmap with differentially expressed serum metabolites levels in hepatitis B cirrhosis patients before and after uc-MSC treatment. D. Metabolic pathway analysis of differentially expressed metabolites. The color of each pathway is based on p-values, while the size is based on pathway impact values. E. ROC curves showed level of major serum metabolites are predictive of treatment status. NT-P: non-treated patient; MSC-P: uc-MSC treated patient.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable1.Proteomicdata.txt
- SupplementaryTable2.Metabolomicdata.txt