Research Progress Report

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Title: Role of liver stiffness in the pathophysiology of portal hypertension

1) Brief outline of the topic

Cirrhosis is the common final stage of advanced chronic liver disease (ACLD), caused by chronic inflammation and injury that stimulates a continuous extracellular matrix (ECM) accumulation and remodeling, increasing both liver stiffness and hepatic vascular resistance. Currently, there is no approved therapeutic agent for ACLD. Increased liver stiffness values were found to predict an increased risk of clinical complications, increasing mortality. Moreover, patients with similar clinical background and with similar stages of cirrhosis showed different liver stiffness, measured with ultrasound-based techniques or magnetic resonance. The role of liver stiffness in the progression of portal hypertension remains unclear, making it a crucial topic for developing novel therapeutic targets.

In the past years, we focused on stiffness-induced LSECs dysfunction, which is the key mediators of hepatic vascular function. We selected calcium and integrin-binding protein 1 (CIB1) to further study its potential role in ACLD and mechanobiology. We found CIB1 was significantly upregulated in human cirrhotic LSECs and high stiffness induced the upregulation of CIB1 in HUVECs and LSECs which suggested CIB1 plays a critical role in mechanotransduction. In this year, we aimed at characterizing: i) the transcriptomic de-regulation depends on high stiffness on LSECs ii) the effect of CIB1 in high stiffness, as well as identifying mechanotransduction pathways involved, downstream to CIB1.

2) Results

CIB1 is necessary to maintain nuclear morphology.

Our previous data have validated that matrix stiffness modulates the phenotype of LSECs by altering the nuclear morphology through cytoskeleton-derived mechanical forces. As CIB1 was upregulated on high stiffness, we then knocked down CIB1 by siRNA transfection on LSECs at high stiffness. Notably, reduction in nuclear area coincides with the downregulation of CIB1(Fig1).

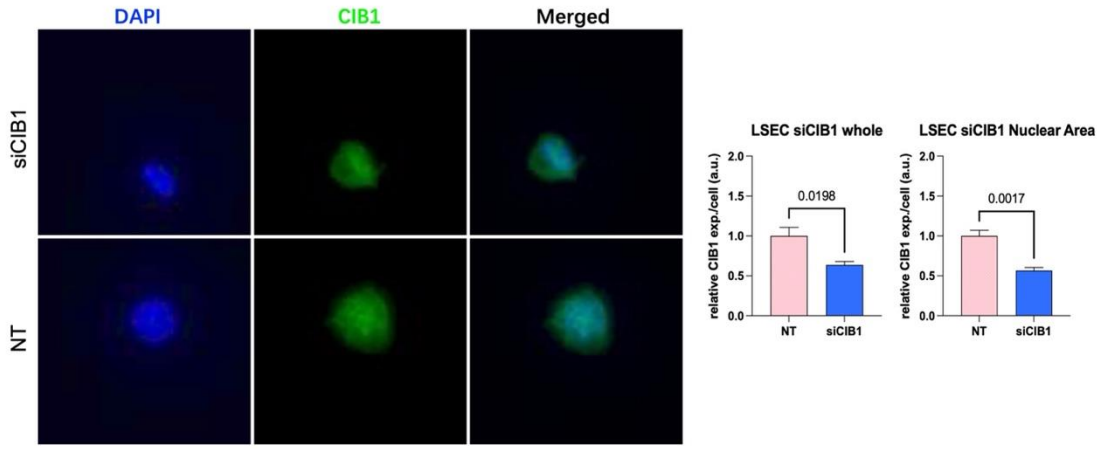

Figure 1. Immunofluorescence of CIB1 (green) in healthy rat LSECs on 30kPa treated with siCIB1 or vehicle for 24hours.

CIB1 knockdown reduces internal stiffness, improving LSECs phenotype

RNA sequencing of LSECs isolated from the same experimental conditions revealed 157 differentially expressed genes (DEGs) ($p<0.05$ and fold-change >1.5). The majority of DEGs were downregulated (78.3%) while 21.6% were upregulated (Fig. 2A). Gene Set Enrichment Analysis (GSEA) revealed downregulation of cytoskeleton actin-related gene sets, as previously studied (Fig. 2B).

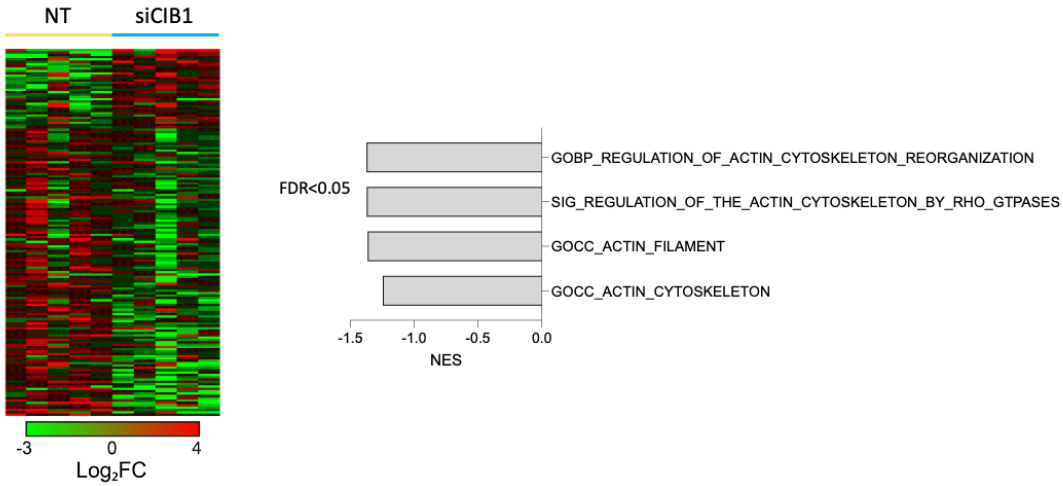

Figure 2. Stiffness modulates the transcriptome of LSECs. A) Heatmap of LSECs transcriptomics at control vs siRNA. B) Top dow regulated pathways from GSEA.

3) Discussion

Mechanical forces, including ECM stiffness or mechanical stretch, can regulate cellular phenotype[1].This year, we selected CIB1 from nucleoproteomic data, which interacts with several proteins, playing a critical role in mechanotransduction. Above results showed CIB1 in LSECs is translocated from the nucleus to the cytoplasm due to high stiffness. Besides, inhibitory effects of CIB1 in LSEC on high stiffness showed a reduction of nuclear area and downregulation of actin cytoplasm. Taken together, all these observations support that CIB1 stabilization and translocation regulate cytoskeleton remodeling due to LSECs nuclear stretching, suggesting that CIB1 may represent a new target for drug therapy.

4) Outlook

Considering the results of CIB1 involved in mechanotransduction in LSEC have shown CIB1 is a promising target in pathological pathway of cirrhotic liver. Currently, we are pleased to announce the completion of our manuscript, which is now ready for submission.