of intracellular Ca^{2+} induced by CoCl2 was significantly blocked, which proved that IRBIT may promote the occurrence and development of liver fibrosis by regulating intracellular Ca^{2+} level under hypoxia stress.

Conclusion: Hypoxia stress can stimulate the up regulation of IRBIT expression in hepatic stellate cells, increase IRBIT movement to open corresponding calcium channels on the cell membrane, mediate the intracellular Ca^{2+} remodeling, promote the continuous activation of HSCs cells and the development of liver fibrosis.

The results of this study have found a new way to affect the activation mechanism of HSC, which provides theoretical basis for targeted treatment of liver fibrosis targeting IRBIT.

Disclosure: no conflict interest.

P0633

DOPAMINE REGULATES AUTOPHAGY DURING TGF-B1-INDUCED ACTIVATION OF HEPATIC STELLATE CELLS

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Introduction: The activation of hepatic stellate cells (HSCs) is the key factor in the pathogenesis of liver fibrosis. Studies have confirmed that autophagy is involved in the activation of HSCs, but the regulatory mechanism of autophagy is unclear. With the development of brain gut axis and neurotransmitters in the study of digestive system diseases, our preliminary results show that neurotransmitter dopamine (DA) can significantly inhibit the expression of autophagy gene and liver fibrosis marker α-SMA during the activation of HSCs.

Further studies suggest that DA stimulates the activation of TRPV1 calcium channel on the cell membrane of HSCs, which leads to intracellular calcium remodeling, and then inhibits autophagy and activation of HSCs, and finally plays an anti-fibrosis role. This project aims to clarify the role and mechanism of DA in the autophagy of HSCs activation from animal experiments in vivo and cell experiments in vitro, that provides a theoretical basis for the targeted therapy of autophagy during the process of liver fibrosis.

Aims & Methods: The expression of a-SMA, autophagy gene and TRPV1 in human and liver fibrosis model mice were detected by immunohistochemistry. The effect of DA on the expression of α - SMA and autophagy gene before and after activation of HSCs was detected by Western-blot.

The inhibition and construction of TRPV1 interference and overexpression plasmids were transfected into HSCs to observe the effect of DA on the expression of autophagy gene Flux variation. Calcium imaging was used to detect the changes of intracellular Ca^{2+} during DA stimulation on HSCs.

Results: 1. Compared with normal liver tissue, the expression of α-SMA and autophagy gene LC3 in liver fibrosis tissue increased, while the expression of TRPV1 decreased. Compared with the normal group, the expression of α - SMA increased and TRPV1 decreased in the liver fibrosis model group, which was reversed after DA treatment

2. Compared with activated HSCs, DA can significantly reduce the proliferation of HSCs (stimulated by TGF-β1). Flow cytometry showed that DA had no significant effect on the apoptosis of HSCs.

3. Western-blot showed that DA and capsaicin could significantly reduce the expression of α - SMA, autophagy gene LC3 protein and autophagy flow in activated HSCs, which were eliminated by TRPV1 inhibitor sb-705498 or by interfering with the function of TRPV1. Overexpression of TRPV1 could enhance the effect of DA. It is suggested that DA may play a protective role by activating TRPV1.

4. Through calcium imaging experiments, it was found that DA stimulation was accompanied by the increase of intracellular Ca^{2+} in HSCs. At the same time, the intracellular Ca^{2+} in HSCs stimulated by capsaicin was increased, while using the intracellular Ca^{2+} chelators BAPTA-AM, sb-705498 and calcium free environment could not induce the change of Ca^{2+} in HSCs.

5. Mechanism studies confirmed that DA significantly inhibited the expression of p-smad3 protein in activated HSCs, and this effect disappeared after inhibition and interference of TRPV1.

Conclusion: Dopamine may act as a protective factor in the process of liver fibrosis. Dopamine may activate TRPV1 calcium channel on cell membrane to mediate extracellular calcium influx, leading to intracellular calcium remodeling, and then inhibit the autophagy and activation of HSCs mediated by TGF-β1/Smad3 signaling pathway, and finally play an anti-fibrosis role.

Disclosure: no conflict interest.

P0634

KALLISTATIN AS A BIOMARKER OF NON-ALCOHOLIC FATTY LIVER DISEASE PROGRESSION IN PATIENTS WITH HYPERTENSION

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Introduction: The prevalence of nonalcoholic fatty liver disease (NAFLD) ranges from 17% to 46% worldwide [3]. Searching for non-invasive diagnostic methods of the NAFLD severity and progression becomes a central objective, especially, in patients with combination of NAFLD and arterial hypertension (HT) [1].

Kallistatin is an tissue kallikrein inhibitor, endogenous protein, which is predicted to play an important role in anti-inflammatory protection and prevention of the chronic liver diseases progression [2].

Aims & Methods: The aim of the study was to determine the role of kallistatin as a diagnostic biomarker of NAFLD progression in patients with concomitant HT.

We examined 115 patients with NAFLD in non-alcoholic steatohepatitis (NASH) stage. They were divided into two groups: the main group consisted of 63 patients with NAFLD on the background of HT and the comparison group consisted of 52 patients with isolated NAFLD. The control group was composed of 20 relatively healthy volunteers. Anthropometric parameters were obtained using standard methods.

Plasma kallistatin levels were measured using the Human SERPINA4 (Kallistatin) ELISA Kit (Elabscience, USA). The level of C-reactive protein (CRP) was determined using the hs-CRP ELISA Kit (Biomerica USA). The data was statistically processed using standart PC-programmes.

Results: The kallistatin level in patients with comorbidity of NAFLD and HT averaged 65.98 ng/ml (95% CI 62.85; 69.12), that was less, than in group of isolated NAFLD (83.42 ng/ml (95% CI 81.89; 84.94)) and control group (111.70 ng/ml (95% CI 106.14; 113.22)) in 1.3 ($p < 0.001$) and 1.7 times ($p < 0.001$), respectively.

The levels of kallistatin were decreased in patients on condition of increasing body mass index (BMI) both in the group with NAFLD and HT and in the group with isolated NAFLD ($r = -0.58$, $p < 0.001$; $r = 0.54$, $p = 0.002$, respectively).

The content of kallistatin decreased with the progression of HT in patients from the main group: in patients with HT I stage the level of biomarker averaged 73,38 ng/ml (95% ДІ 70,24; 78,19) while in patients with HT II stage its values were 61,87 ng/ml (95% ДІ 58,12; 65,62), $p < 0.001$.

At the same time, the biomarker levels were significantly different in patients with HT II, depending on hypertension grade and declined with increase of blood pressure (BP) numbers (Table 1).