我校附属普陀医院科研团队在GUT发表研究成果

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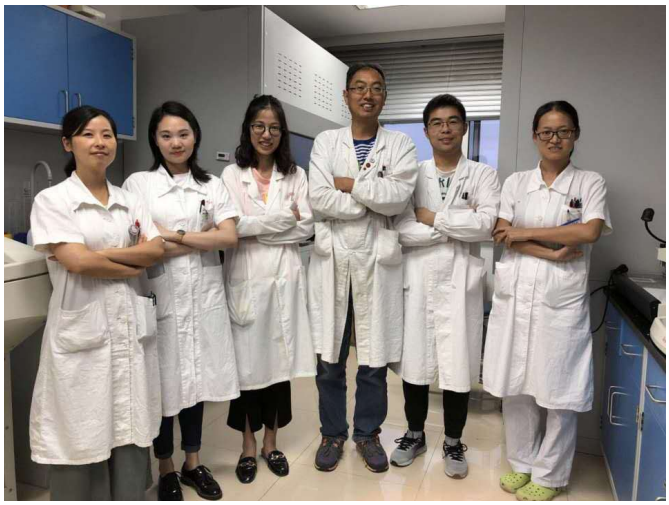
我校附属普陀医院中心实验室/肝病实验室刘成团队联合我校附属曙光医院肝病研究所刘平教授、德国海德堡大学及美国西达-赛奈医学中心科研团队在肝星状细胞活化及肝纤维化研究上取得重要突破。研究成果*Glial cell line-derived neurotrophic factor (GDNF) mediates hepatic stellate cell activation via ALK5/Smad signaling*（胶质细胞源性神经营养因子（GDNF）通过ALK5/Smads信号介导肝星状细胞活化）6月6日在线发表于消化病国际知名期刊*GUT*（IF:17.016）。

GDNF（TGF-β超家族一员）在神经系统（如帕金森和癫痫）及发育等领域发挥重要作用，然而在肝纤维化及肝星状细胞活化方面则罕见报道。刘成团队领衔的研究团队从临床样本出发，发现肝纤维化患者血清及肝组织GDNF显著上调且与HSC活化标志物α-SMA呈显著性正相关；运用肝穿多重连续冰冻切片联合免疫组化双染技术，发现GDNF主要表达于HSC，并随纤维化进展上调，此结果也在小鼠纤维化

模型中得到验证，过表达GDNF病毒的纤维化小鼠较对照病毒小鼠炎症和纤维化程度显著加重。研究团队通过分析人和小鼠原代肝脏细胞，发现相对肝细胞、肝窦内皮及肝脏库普弗细胞，GDNF在HSC表达较高，且GDNF刺激HSC可诱导胶原表达显著增高。研究运用基因沉默和抑制剂等方法，发现GFRα1（GDNF经典受体）并不参与GDNF诱导的HSC活化，此结果也在人肝穿mRNA得到印证。与此同时，用TGF-β受体1抑制剂（ALK5）处理后GDNF刺激，HSC产生胶原显著被抑制，证实GDNF/ALK5/Smads所介导的HSC活化。研究最后利用表面等离子共振、分子对接、基因点突变及蛋白质免疫共沉淀技术证实ALK5的His^{39}及Asp^{76}在与GDNF结合中发挥关键作用，表明TGF-β可诱导GDNF上调。

该研究为深入研究中医药抗肝纤维化的作用机制提出了新的靶标通路，在肝纤维化领域具有原创性，对纤维化患者的分期诊断及临床治疗具有重要的指导意义。（普陀医院）



Hepatology

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ORIGINAL ARTICLE

Glial cell line-derived neurotrophic factor (GDNF) mediates hepatic stellate cell activation via ALK5/Smad signalling

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ABSTRACT

Objective Although glial cell line-derived neurotrophic factor (GDNF) is a member of the transforming growth factor-β superfamily, its function in liver fibrosis has rarely been studied. Here, we investigated the role of GDNF in hepatic stellate cell (HSC) activation and liver fibrosis in humans and mice.

Design GDNF expression was examined in liver biopsies and sera from patients with liver fibrosis. The functional role of GDNF in liver fibrosis was examined in mice with adenoviral delivery of the GDNF gene, GDNF sgRNA CRISPR/Cas9 and the administration of GDNF-blocking antibodies. GDNF was examined on HSC activation using human and mouse primary HSCs. The binding of activin receptor-like kinase 5 (ALK5) to GDNF was determined using surface plasmon resonance (SPR), molecular docking, mutagenesis and co-immunoprecipitation.

Results GDNF mRNA and protein levels are significantly upregulated in patients with stage F4 fibrosis. Serum GDNF content correlates positively with α-smooth muscle actin (α-SMA) and Col1A1 mRNA in human fibrotic livers. Mice with overexpressed GDNF display aggravated liver fibrosis, while mice with silenced GDNF expression or signalling inhibition by GDNF-blocking antibodies have reduced fibrosis and HSC activation. GDNF is confined mainly to HSCs and contributes to HSC activation through ALK5 at His^{39} and Asp^{86} and through downstream signalling via Smad2/3, but not through GDNF family receptor alpha-1 (GFRα1). GDNF, ALK5 and α-SMA colocalise in human and mouse HSCs, as demonstrated by confocal microscopy.

Conclusions GDNF promotes HSC activation and liver fibrosis through ALK5/Smad signalling. Inhibition of GDNF could be a novel therapeutic strategy to combat liver fibrosis.

Significance of this study

What is already known on this subject?
- Glial cell line-derived neurotrophic factor (GDNF) is a member of the transforming growth factor-β superfamily and an important player in neuronal survival.
- GDNF ligands canonically signal by binding to glycosyl-phosphatidylinositol (GPI)-anchored receptors, termed GDNF family receptor alpha-1 (GFRα1), in collaboration with signalling receptor subunits, such as the Ret tyrosine kinase.

What are the new findings?
- GDNF in the serum increased significantly in human liver fibrosis compared with healthy controls.
- Serum levels of GDNF are positively associated with hepatic stellate cell (HSC) activation and liver fibrosis progression.
- GDNF promotes HSC activation through activin receptor-like kinase 5 at His^{39} and Asp^{86} and downstream signalling via Smad2/3, but not through GFRα1.
- Mice with overexpression of GDNF display aggravated liver fibrosis, while mice with silenced GDNF expression or treated with GDNF-blocking antibodies have reduced fibrosis and HSC activation in carbon tetrachloride and bile duct ligation (BDL) disease models.

How might it impact on clinical practice in the foreseeable future?
- GDNF upregulation presents as a new biomarker for HSC activation and liver fibrosis progression that is measurable in blood and thus has potential for clinical diagnosis of liver fibrosis; moreover, inhibition of GDNF can be developed as novel therapeutic approach for liver fibrosis.

INTRODUCTION

Liver fibrosis, which is characterised by excessive extracellular matrix (ECM) deposition, results from chronic liver injury of different aetiologies and represents a major health problem worldwide.[1–3] The progression of liver fibrosis to cirrhosis gives rise to severe complications including portal hypertension, liver failure and hepatocellular carcinoma.[4] Hepatic stellate cells (HSCs) reside in the space of Disse in the liver,[5] and on liver injury, quiescent HSCs undergo a phenotypic and metabolic switch that leads to the acquisition of a myofibroblast (MFB)-like phenotype. Such HSC-derived MFBs are the predominant liver cell type that produces ECM in response to various damaging insults.[6 7] Moreover, activated HSCs release cytokines and chemokines