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Expression of fibroblast growth factor 21 in patients with biliary atresia

Dawei Li [a,1], Tianfei Lu [a,1], Conghuan Shen [a,1], Yuan Liu [a], Jiang Zhang [a], Yuhua Shan [a], Yi Luo [a], Zhifeng Xi [a], Bijun Qiu [a], Qimin Chen [b], Jianjun Zhang [a], Qiang Xia [a,*]

[a] Department of Liver Surgery and Liver Transplantation Center, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China
[b] Shanghai Children's Medical Center, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

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ABSTRACT

Fibroblast growth factor 21 is a critical circulating adipokine involving in metabolic disorders and various liver diseases. This study was performed to investigate whether FGF21 is also associated with the pathophysiology of biliary atresia. Serum FGF21 levels were measured in 57 BA patients and 20 age matched healthy controls. We also examined hepatic FGF21 mRNA expression and FGF21 protein levels in liver tissues obtained from 15 BA patients undergoing liver transplantation and 5 cases of pediatric donation after cardiac death donor without liver diseases by RT-PCR and Western blotting. Patients with BA showed significantly higher serum FGF21 levels than those without BA (554.7 pg/mL [83–2300] vs. 124.5 pg/mL [66–270], $P < 0.05$). Patients with BA also had significantly higher FGF21 mRNA and protein levels in hepatic tissues than control subjects. Serum FGF21 expression increased corresponding to the severity of liver fibrosis. Furthermore, serum FGF21 levels dropped significantly in BA patients within 6 months after liver transplantation and approached baseline in healthy controls ($P > 0.05$). In vivo, FXR knockout could significantly abrogate cholestasis induced FGF21 expression. FGF21 levels in serum and liver tissue increased significantly in BA patients. In vivo, cholestasis could induce FGF21 expression in FXR dependent manner.

1. Introduction

BA (biliary atresia) is the most common cause of cholestatic liver injury, which could progress to fibrosis, cirrhosis and end-stage liver disease (ESLD) [1,2]. ESLD is the consequence of cholestatic liver injury leading to irreversible liver dysfunction [3,4]. The presenting symptoms of ESLD include impaired synthesis of serum proteins and coagulation factors (quantified by INR), imbalance of glucose, lipid and protein metabolism, and impaired bile excretion [5]. Malnutrition is the most common complication occurring in BA and has important prognostic implications [6,7]. Poor nutritional status before liver transplantation is associated with prolonged hospital stay, increased complications and mortality and increased costs of medical care [8,9]. 60–80% of patients with ESLD have moderate to severe malnutrition prior to liver transplantation [4]. BA induced cholestatic liver injury is associated with fat and protein malabsorption and the related complications of fat-soluble vitamin deficiencies [10,11]. Based on the above evidences, we conclude that the BA patients are in a condition of malnutrition induced by nutrient malabsorption, which is similar in pathophysiology to nutrient restriction.

Nutrition restriction engages a variety of metabolic adaptations, and such as in energy restriction, these adaptations mainly include alterations in feeding behavior, insulin sensitivity, and energy expenditure [12,13]. In fact, metabolic risk factors including adipocyte-secreted proteins (adipokines) are associated with the pathogenesis of BA. Here, circulating concentrations of the adipokine leptin were lower in BA and suggested that the leptin might play a critical role in maintaining bone mineral density of BA patients [14]. More and more studies showed important associations between adipokines and pathogenesis of BA [14–17].

Fibroblast growth factor 21 (FGF21) has recently been reportedas a novel adipokine regulating glucose and lipid metabolism and increasing insulin sensitivity in animals [18], acting as endocrine factor implicated in hormone-like metabolic effects by activating FGF receptors [19,20]. FGF21 is predominantly synthesized by the liver, pancreas, muscle and adipose tissue [21]. Previous

Abbreviations: FGF21, fibroblast growth factor 21; BA, biliary atresia; DCD, donation after cardiac death; PPARα, peroxisome proliferator-activated receptors α; FXR, farnesoid X receptor; BDL, bile duct ligation.

* Corresponding author at: 1630 Dongfang Road, Pudong New District, Shanghai 200127, China.
E-mail address: xiaqiang@shsmu.edu.cn (Q. Xia).

[1] These authors contributed to this work equally.

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studies had shown that increased FGF21 expression may be a metabolic adaptation to fasting, starvation, protein restriction and ketogenic diet [22,23]. Recently, several studies indicated that circulating FGF21 level is correlated with various liver diseases, such as acetaminophen induced liver injury, nonalcoholic fatty liver disease, viral infection and carcinogenesis [24–28]. Furthermore, in analogy with insulin and leptin biology, high expression of FGF21 may suggest either a state of hormonal resistance or an adaptive response to fight against metabolic disturbances [29]. These findings highlight the potential role of FGF21 in cholestatic liver disease, leading us to explore its role in pathogenesis of BA. This study was therefore designed to determine the circulating and tissue levels of FGF21 in patients with BA in comparison with healthy control subjects. Our results demonstrated a significant increased expression of FGF21 in patients with BA, suggesting that FGF21 may play a critical role in cholestatic liver disorder. The pathophysiological mechanism of these findings needs to be further elucidated.

2. Patients and methods

2.1. Patients

Clinical records of patients with BA from January 2014 to December 2014 were reviewed. 57 BA children (age, 9.32 ± 2.3 months; 35 males and 22 females) were recruited as BA group. For the serum study, 20 children (age, 9.1 ± 2.2 months; 13 males and 7 females) were recruited as the healthy controls. It was clinically confirmed that control subjects were free from hepatobiliary or gastrointestinal disease, severe infection, diabetes, or dyslipidemia. All BA cases were diagnosed by operation or pathology. 15 BA children who underwent liver graft surgery were recruited as post-LT group, and their blood samples were collected within 6 months after liver transplantation. All BA patients and healthy controls were prospectively recruited from Ren Ji Hospital and Shanghai Children's Medical Center, Shanghai Jiao Tong University School of Medicine. Written informed consent was obtained in each case before enrollment (signed by the parents of each infant). This study was approved by the Research Ethics Committee of the Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University.

2.2. Human sample collection and preparation

The blood samples for FGF21 detection were prepared by centrifugation at 3000 rpm for 10 min after blood collection and stored at −80 °C until analyzed. Hepatic tissues obtained from 5 pediatric cardiac death donors (DCD) without liver disease served as the control. Once the liver tissues were collected, they were immediately shock-frozen in liquid nitrogen and then stored at −80 °C until analysis. 24 cases of liver biopsies were evaluated for fibrosis stage according to Scheuer classification by two experienced liver pathologists and the primary researcher.

2.3. Measurement of serum FGF21 levels

Serum concentrations of FGF21 were measured by using the enzyme linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, USA) according to the manufacturer's instructions.

2.4. Animal models

Male C57BL/6 mice, 8–10 weeks of age (weight 20–25 g) were obtained from the department of Laboratory Animal Science of Shanghai Jiao Tong University School of Medicine. $FXR^{-/-}$ mice were a generous donation from Dr. Ben He (Renji Hospital) [30]. All animal experiments were approved by the institutional guidelines of Shanghai Jiao Tong University School of Medicine according to the Regulations for Practice of Experimental Animals (issued by Scientific and Technical Committee, P.R. China, 1988). The care and use of the animals in these experiments followed all rules from the Animal Use and Care Committee of Shanghai Jiao Tong University School of Medicine (approval number: SYKX-2008-0050). All mice were anesthetized with sodium pentobarbital by intraperitoneal injection before bile duct ligation (BDL). After making the abdominal midline incision, the common bile duct was triple-ligated with a 8-0 silk and divided between the second and third ligature. Sham operated animals served as controls, underwent laparotomy with exposure of common bile duct without ligation. At days 1, 3, 6, 9 or 14 after BDL, depending on the experimental design, mice were re-anesthetized and blood samples were collected for serum FGF21 determinations; the liver tissue samples were harvested for RNA and protein analyses.

2.5. Quantitative real-time PCR and Western blot

Harvested liver tissues were subjected to transcriptional or immunoblotting analyses. Total RNA from liver tissues was isolated by using Trizol reagent (Invitrogen) in a standard method. RNA quantity and quality were determined by spectrophotometry using a NanoDrop (Thermo Scientific). Quantitative real-time PCR was performed by using SYBR Premix (Takara) on the ABI PRISM® 7900 platform. And β-actin served as an endogenous control. The protein levels of FGF21 were visualized by Western blot and normalized to β-actin levels. Immunoblots were probed with antibodies against FGF21 (Abcam) and β-actin (Santa Cruz) at the recommended dilutions.

2.6. Statistical analysis

All statistical analysis were performed with the SPSS software version 19.0 (IBM, USA). The data were expressed as mean (range) or the mean ± standard deviation (SD). Quantitative data were analyzed using the independent samples *t*-test, as appropriate. Correlations were conducted using Spearman rank correlation. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Characteristics of subjects

The clinical characteristics of patients are shown in Table 1. No significant differences in age and gender were observed between control and BA subjects; however, subjects with BA were inferior in weight and height to those of control subjects (all $P < 0.05$). Concentrations of TB, ALT, AST, GGT, AKP, bile acid in subjects with BA were significantly higher than those in the control subjects (all $P < 0.05$). By contrast, serum levels of albumin in patients with BA were significantly lower than those of control subjects ($P < 0.05$). Significantly greater INR and longer PT were found in BA patients than those in the control group ($P < 0.05$).

3.2. Serum FGF21 levels in BA patients and control subjects

In this study, serum FGF21 concentrations ranged from 64 to 2300 pg/ml (Fig. 1). There were no gender differences in serum FGF21 levels between the two groups. Patients with BA had significantly higher serum FGF21 levels (554.7 pg/ml [83, 2300]) than control subjects (124.5 pg/ml [66, 270]) ($P < 0.05$) (Fig. 1A). Moreover, serum FGF21 significantly decreased in BA patients within