Expression of FGF21 and β-Klotho regulate hepatic fibrosis through NF-κB and JNK pathways

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Expression of FGF21 and β-Klotho regulate hepatic fibrosis through NF-κB and JNK pathways

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인생의 동반자이면서 나태해질 때마다 채찍질을 해준 아내에게 고맙다는 말 전합니다. 그리고 낳아주시고 잘 길러주신 어머니께 감사드리며 항상 믿어 주고 지지해주신 장인어른, 장모님께 감사드립니다. 마지막으로 하늘에서 가장 기뻐하실 아버지께 이 박사학위 책자를 바칩니다.

2017년 12월

이경주 올림

CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Expression of FGF21 and β-Klotho Regulate Hepatic Fibrosis Through NF-κB and JNK Pathways

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Fibroblast growth factor (FGF) 21 is a member of the FGF19 subfamily that regulates glucose and lipid metabolism. β-Klotho is a co-receptor that enables FGF21 binding to FGF receptor. FGF21 is also known to be related to hepatic inflammation and fibrosis. However, little is known about the effects of inflammation and fibrosis on the β-Klotho and FGF21 pathway in the liver. Enrolled patients had biopsy-proven viral hepatitis and alcoholic hepatitis. The levels of FGF19, FGF21 and β-Klotho were evaluated using ELISA and real-time polymerase chain reaction, western blotting. Furthermore, we explored the

underlying mechanisms for this process, evaluating the involvement of the NF-κB and JNK pathway in Huh-7 cells. In the present study, we found that the serum levels and mRNA levels of FGF19 and FGF21 in biopsied liver tissue gradually increased and correlated with fibrosis stage. Inflammatory markers (IL-1β, IL-6, and TNF-α) were also positively correlated with degree of fibrosis, whereas β-Klotho expression was negatively correlated with degree of fibrosis. In Huh-7 cells, IL-1β increased the level of FGF21 and decreased the level of β-Klotho. NF-κB and JNK inhibitors abolished the effect of IL-1β on both FGF21 and β-Klotho expression. FGF21 showed a protective effect on IL-1β-induced growth retardation in Huh-7 cells. These results suggest that the inflammatory response occurring during fibrogenesis increases the level of FGF21 and suppresses β-Klotho via the NF-κB and JNK pathway. In addition, FGF21 is probably able to protect hepatocytes in hepatic inflammation and fibrosis.

Key Words: Fibroblast growth factor 21, β-Klotho, IL-1β, NF-κB, JNK

I. INTRODUCTION

The fibroblast growth factor (FGF) 19 subfamily includes FGF19, FGF21, and FGF23. FGF19 subfamily members have a poor affinity for the classic heparin-binding domain,[1] whereas most FGFs bind to and activate cell surface FGF receptors (FGFRs) via a high affinity interaction with heparin.[2, 3] This difference makes the conventional FGFs function in a paracrine/autocrine manner to induce cell proliferation and differentiation; however, members of the FGF19 subfamily are secreted into the bloodstream and function as hormones.[1, 2, 4] FGF19 subfamily members require a coreceptor named Klotho to activate FGFRs due to their low affinity for heparin sulfate.[1, 5, 6] Klotho is a transmembrane protein family whose members take one of two forms, α-klotho and β-Klotho.[7] β-Klotho enables FGF19 and FGF21 binding to FGFR1c, -2c, -3c and FGF19 binding to FGFR4.[5, 6, 8]

Many studies have revealed that the FGF19 subfamily is involved in various biological activities. FGF19 regulates the enterohepatic circulation of bile acid, and FGF21 regulates glucose and lipid metabolism.[9] FGF23 is important for maintaining phosphate/vitamin D homeostasis.[9] Among the FGF19 subfamily, FGF19 and FGF21 are known to have a role in the liver. Both β-Klotho and FGFR4 are highly expressed in the liver. This distinct feature allows FGF19 to act primarily on the liver.[5, 6] FGF19 is found in the liver of patients with cholestasis[10]

and is highly expressed in patients with hepatocellular carcinoma.[11] FGF21 is primarily expressed in the liver, white and brown adipose tissue, and the pancreas.[12] FGF21 is increased in several liver diseases, such as alcoholic liver disease, viral hepatitis, and hepatocellular carcinoma.[13-15] Recently, a few studies have shown that FGF19 and FGF21 are related to hepatic inflammation and fibrosis. However, little is known as to how FGF19, FGF21, and β-Klotho are regulated in hepatic inflammation and fibrosis.

In our study, we evaluated the levels of FGF19, FGF21, and β-Klotho according to severity of liver fibrosis in human samples. In addition, we tried to find pathways through which β-Klotho and FGF21 are regulated by hepatic inflammation in Huh-7 cells.

II. MATERIALS AND METHODS

1. Patients

Liver biopsies and blood samples were obtained (n=35) from patients suspected to have fibrosis. Table 1 shows baseline characteristics of enrolled patients (Table 1). Patients between 19 and 65 years of age with biopsy proven viral hepatitis or alcoholic hepatitis who visited Wonju Severance Christian Hospital between Dec 2008 and Dec 2012 were recruited for this study. Fibrosis level was determined by an expert pathologist and was classified as F0, F1, F2, F3, F4A, F4B, and F4C according to the Laennec fibrosis scoring system. We grouped these into three classes of G1 (F0 and F1), G2 (F2 and F3), and G3 (F4a to F4c). Liver biopsies and blood samples were collected, immediately snap-frozen, and stored at −80°C until analysis. This protocol was approved by the International Review Board for Human Research (CR107059) of Yonsei University Wonju College of Medicine. Written consent was received from all patients.

Table 1. Baseline characteristics

	G1 (n=10)	G2 (n=10)	G3 (n=15)	P value
Gender; Men/Women	6/4	7/3	12/3	0.451
Age (years)	46 (19-63)	50.5 (37-65)	51 (24-70)	0.331
Etiology				0.562
Viral	4 (40%)	6 (60%)	9 (60%)	
Alcohol	6 (60%)	4 (40%)	6 (40%)	
AST, IU/L	65.5 (42-350)	58.5 (24-146)	40 (17-202)	0.237
ALT, IU/L	101 (47-312)	58.5 (13-185)	22 (9-338)	0.002
Albumin, g/dL	4.4 (3.8-4.8)	4.1 (3.3-4.9)	3.4 (2.3-4.9)	0.003
Total bilirubin, mg/dL	0.6 (0.3-1.4)	0.6 (0.3-1.0)	1.2 (0.3-17.2)	0.013
INR	0.9 (0.8-1.0)	1.0 (0.8-1.1)	1.2 (0.9-1.6)	<0.001
Child-Pugh Score	5	5 (5-6)	7 (5-10)	<0.001

AST, aspartate aminotransferase; ALT, alanine aminotransferase; INR, international normalized ratio

2. Determination of serum FGF19 and FGF21

Serum samples were stored at -80℃ until analysis. Quantification of human FGF19 and FGF21 levels was performed using an enzyme-linked immunosorbent assay kit (BioVendor, Brno, Czech Republic), following the manufacturer's instructions. The absorbance of each well was measured at a 450 nm wavelength using a microplate reader ELX 800 (Bio-Tek Instruments, Inc., Winooski, VT, USA).

3. Cell culture

Human hepatoma Huh-7 cells were cultured in DMEM (GibcoBRL, Grand Island, NY, USA) supplemented with 10% FBS. Cells were starved in serum-free medium for 12 h before being treated with IL-1β (R&D Systems, Minneapolis, MN, USA), NK- κB inhibitor (Bay 11-7082, Sigma-Aldrich, St. Louis, MO, USA), c-Jun N-terminal kinase (JNK) inhibitor (SP600125, Sigma-Aldrich), phosphatidylinositol 3-kinase (PI3-K) inhibitor (LY294002, Sigma-Aldrich), mitogen-activated protein kinase kinase (MAPKK) inhibitor (PD98059, Sigma-Aldrich), and FGF21 (R&D Systems).

4. Cell proliferation and MTT assay

Huh-7 cells were seeded into 96-well plates at a density of 1 x 10^4 cells/well. Twenty-four hours later, IL-1β-treated cells (1 ng/ml) were cultured with or without FGF21 in a dose-dependent manner for 24 h, and then methylthiazolyldiphenyl-tetrazolium bromide (MTT, Sigma-Aldrich) dissolved in PBS was added to each well (final 5 mg/ml) and incubated at 37℃ for 2 h. MTT formazan was dissolved in 100 μl DMSO and incubated for a further 15 min with shaking before the optical density of each well was analyzed at 570 nm on a microplate reader (Bio-Tek Instruments).

5. RNA isolation and real-time PCR analysis

Total RNA was isolated from liver specimens using TRIzol reagent (Thermo Fisher Scientific Life Sciences, Waltham, MA) according to the manufacturer's protocol. In addition, total RNA was isolated from Huh-7 cells using the Pure Link RNA mini kit (Ambion). RNA purity and concentration were determined using a spectrophotometer (Ultrospec 2100 pro UV/Visible, GE Healthcare Life Sciences, Piscataway, NJ, USA). cDNA was synthesized from total RNA (1 μg) using the PrimeScript RT reagent Kit (TAKARA). Transcript levels were measured by real-time polymerase chain reaction (PCR) using sequence-specific primers for FGF19, FGF21, IL-1β, IL-6, TNF-α, and β-Klotho (Table 2).

Amplification reactions contained SYBR Green PCR Master Mix (Applied Biosystems) and were performed in an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems) according to the manufacturer's instructions. Data were analyzed using SDS 2.2.2 software (Applied Biosystems). The cycle threshold (Ct) values of the target genes were normalized to those of the endogenous control gene (GAPDH). Relative changes were calculated using the equation $2^{-\Delta\Delta Ct}$.

Table 2. Primer sequences for quantitative PCR

Gene	Forward/reverse	Primer sequence
GAPDH	Forward	5′-AATGAAGGGGTCATTGATGG-3′
	Reverse	5′-AAGGTGAAGGTCGGAGTCAA-3′
FGF19	Forward	5′-GTGCGGTACCTCTGCATGG-3′
	Reverse	5′-CCTCCGAGT ACTGAAGCAGCC-3′
FGF21	Forward	5′-GCCCAGCAGACAGAAGCCCAC-3′
	Reverse	5′-CAGCTGCAGGAGACT TTCGGGG-3′
β-Klotho	Forward	5′-ACGGCGACATGGACATTTAC-3′
	Reverse	5′-CATCCTCCAGAGCCTGGTC-3′
IL-1β	Forward	5′-GCACGATGCACCTGTACGAT-3′
	Reverse	5′-CACCAAGCTTTTTTGCTGTGAGT-3′
IL-6	Forward	5′-AAAGAGGCACTGGCAGAAAA-3′
	Reverse	5′-TTTCACCAGGCAAGTCTCCT-3′
TNF-α	Forward	5′- ACTTTGGAGTGATCGGCC-3′
	Reverse	5′- GCTTGAGGGTTTGCTACAAC-3′

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; FGF, fibroblast growth factor; IL, interleukin; TNF-α, tumor necrosis factor-α

6. Western blotting

Huh-7 was lysed in 1X Laemmli sample buffer (62.5 mM Tris-HCl, pH 6.8, 1% SDS, 10% glycerol, and 5% ß-mercaptoethanol) and boiled for 5 min. The lysates were subjected to Western blotting analysis under reducing conditions using previously validated human β-Klotho antibodies (R & D Systems), anti-phospho-Erk1/2, total Erk1/2, phosphor-AKT, total AKT, phospho-JNK, and total JNK antibodies (Cell Signaling Technology, Danvers, MA, USA), phospho-IκBα, total IκBα, proliferating cell nuclear antigen (PCNA), and GAPDH antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and FGF21 antibody (Boster Biological Technology, Pleasanton, CA, USA).

7. Statistical analysis

All values are presented as mean ± S.E.M. Data were analyzed by the Kruskal-Wallis H test and the Mann-Whitney U test using SPSS software version 23.0 (IBM, Armonk, NY, USA). Fisher's exact test was used for categorical data. Correlation was measured by Spearman rank method. For all analyses, P values < 0.05 were considered statistically significant.

III. RESULTS

1. Analysis of serum FGF19 and FGF21

The serum levels of FGF19 and FGF21 were significantly increased in cirrhosis (G3 group) compared with G1 and G2 groups ($P<0.01$; Figure 1A and 1B). We observed that the serum levels of FGF19 and FGF21 were correlated with fibrosis stage in liver biopsies.

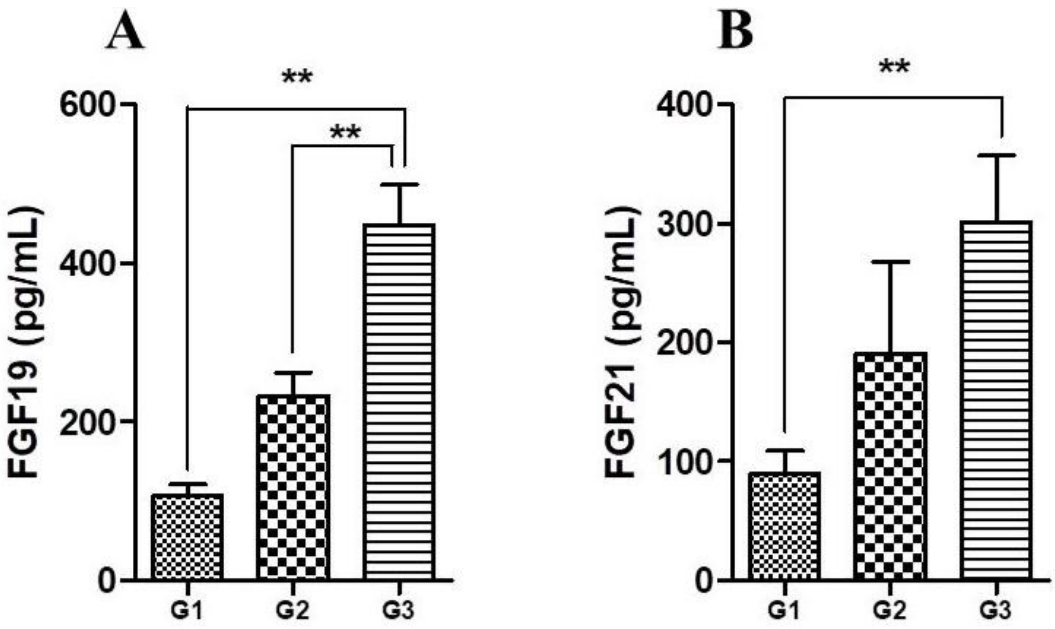

Figure 1. Analysis of serum FGF19 and FGF21 levels in patients with viral hepatitis and alcoholic hepatitis. Serum levels of FGF19 (A) and FGF21 (B) were determined by ELISA. *P<0.05, **P<0.01. G1 (F0~F1, n=10), G2 (F2~F3, n=10), G3 (F4A~F4C, n=15).

2. Analysis of FGF19, FGF21, β-Klotho, and inflammatory markers in liver tissue

FGF19 and FGF21 mRNA levels were significantly increased and associated with fibrosis stage ($P<0.01$; Figure 2A and B), whereas β-Klotho level was significantly decreased and associated with fibrosis stage ($P<0.01$; Figure 2C). Furthermore, inflammatory markers IL-1β, IL-6, and TNF-α were positively correlated with degree of fibrosis ($P<0.01$; Figure 2D, 2E, and 2F). The mRNA levels of FGF19, FGF21 and inflammatory markers were positively correlated with fibrosis stage and that of β-Klotho was negatively correlated with fibrosis stage (Table 3).

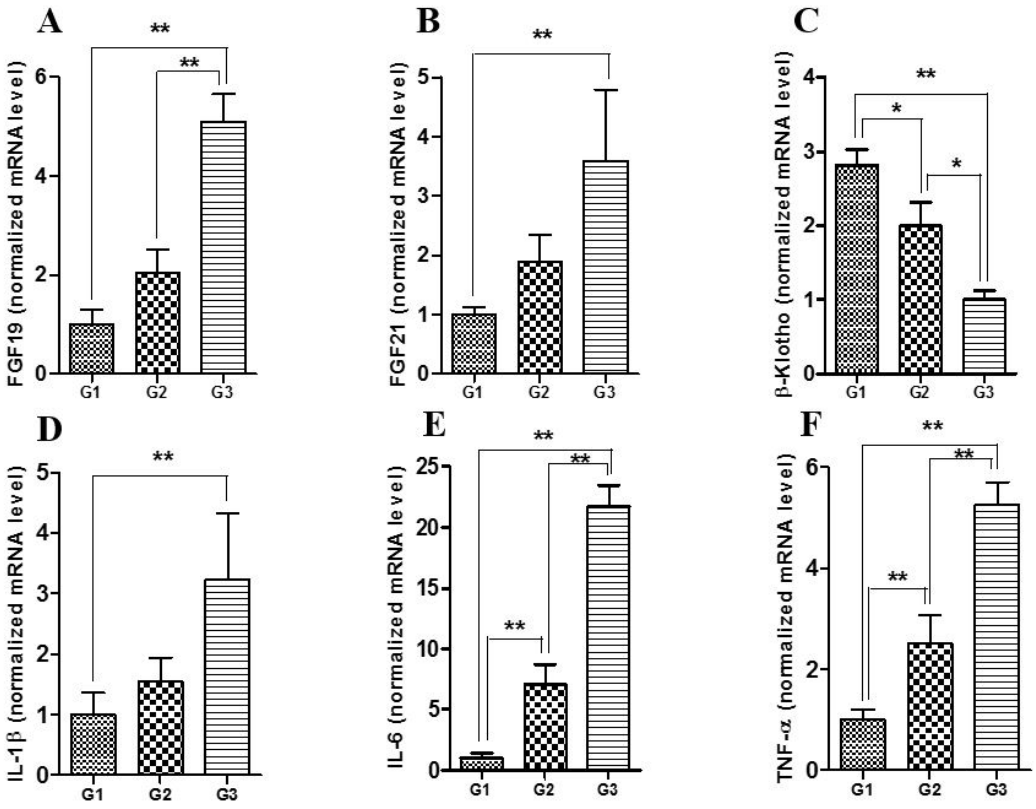

Figure 2. Analysis of FGF19, FGF21, and β-Klotho expression and inflammatory markers in liver tissue. Expressions levels of mRNA for FGF19 (A), FGF21 (B), β-Klotho (C), IL-1β (D), IL-6 (E),and TNF-α (F) were determined by real-time PCR. *P<0.05, **P<0.01. G1 (F0~F1, n=10), G2 (F2~F3, n=10), G3 (F4A~F4C, n=15).

Table 3. Correlation between fibrosis stage and mRNA expressions of FGF19/21, β-Klotho, and inflammatory markers

	Statistics	FGF19	FGF21	β-Klotho	IL-1β	IL-6	TNF-α
Fibrosis stage	*R*	0.739	0.315	-0.767	0.446	0.887	0.842
	P value	<0.001	0.046	<0.001	0.007	<0.001	<0.001

Spearman rank method was used.

3. Effects of inflammatory cytokines on β-Klotho expression in Huh-7 cells

We investigated the effects of pro-inflammatory cytokines IL-1β, IL-6, and TNF-α on hepatic β-Klotho expression. All pro-inflammatory cytokines used in this experiment inhibited β-Klotho expression in a dose-dependent manner (Figure 3).

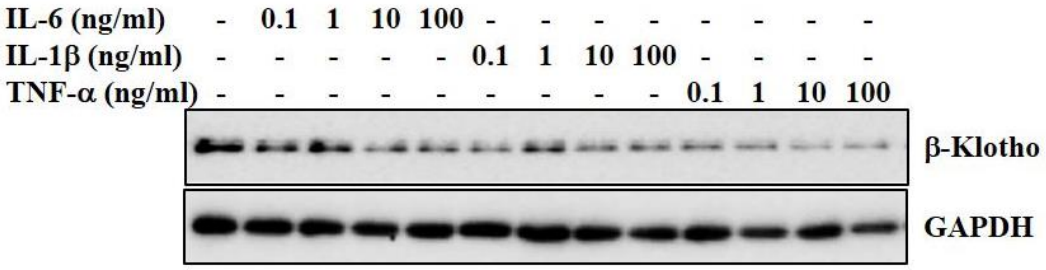

Figure 3. Effects of inflammatory cytokines on β-Klotho expression in Huh-7 cells. Huh-7 cells were incubated with increasing amounts of IL-6, IL-1β, and TNF-α for 6 hours. Expression level of β-Klotho was determined by immunoblotting.

4. Signaling pathways inhibiting β-Klotho by IL-1β in Huh-7 cells

We tried to find the pathways of inhibition of hepatic β-Klotho expression by IL-1β. We incubated Huh-7 cells with IL-1β for 6 hours. IL-1β inhibited β-Klotho expression in a time-dependent manner (Figure 4A and 4G). We investigated the effects of IL-1β on inflammatory signaling pathways in Huh-7 cell. IL-1β activated the AKT pathway (Figure 4B), ERK pathway (Figure 4C), JNK pathway (Figure 4D) and NF-κB pathway (Figure 4E). We then examined which signaling pathway mediates IL-1β-induced suppression of β-Klotho expression. We used NF-κB inhibitor (Bay 11-7082), JNK inhibitor (SP600125), phosphatidylinositol 3-kinase (PI3-K) inhibitor (LY294002), and mitogen-activated protein kinase kinase (MAPKK) inhibitor (PD98059). Huh-7 cells were pretreated with these four inhibitors for 20 min before being treated with IL-1β. The inhibitory effect of IL-1β on β-Klotho expression was attenuated by the NF-κB inhibitor (Bay 11-7082) in Huh-7 cells (Figure 5A, 5E, 5I and 5K). The same results were shown by the JNK inhibitor SP600125 (0.5 and 1 μM) (Figure 5B, 5F, 5J and 5L). These results suggest that the NF-κB pathway and the JNK pathway have inhibitory effects on IL-1β action on β-Klotho expression. However, AKT inhibitor (LY294002) and ERK inhibitor (PD98059) have no effect on IL-1β-induced inhibition of β-Klotho (Figure 5C, 5D, 5G and 5H).

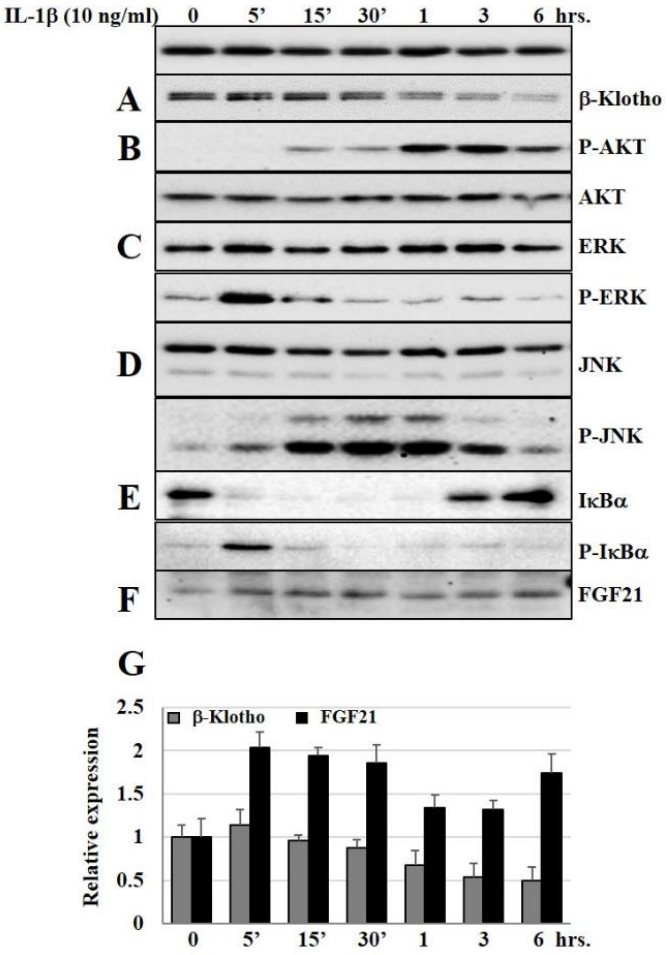

Figure 4. Effects of IL-1β on β-Klotho and FGF21 in Huh-7 cell. Huh-7 cells were incubated with IL-1β for 6 hours. Expression levels of β-Klotho (A and G), AKT (B), ERK (C), JNK (D), IκBα (E), and FGF21 (F and G) were determined by immunoblotting.

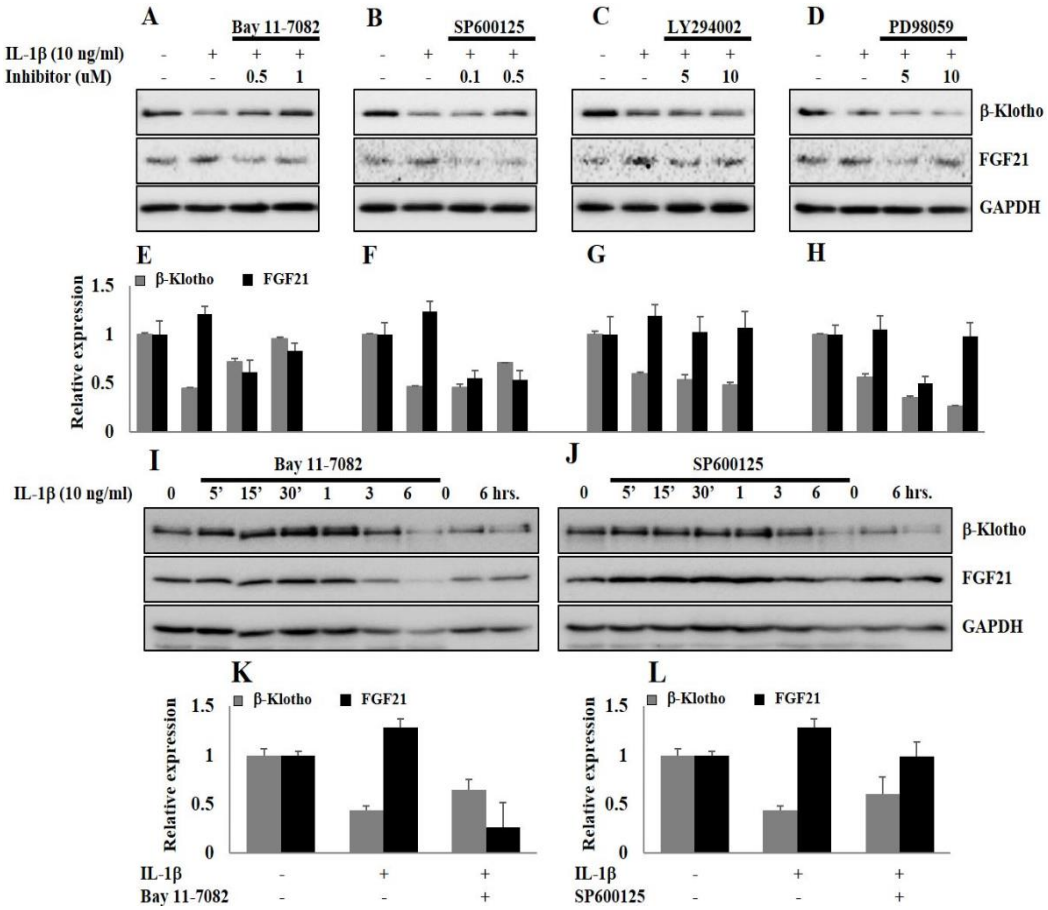

Figure 5. Signaling pathways inhibiting β-Klotho and increasing FGF21 by IL-1β in Huh-7 cells. Huh-7 cells were pretreated with NF-κB inhibitor (Bay11-7082, A and E), JNK inhibitor (SP600125, B and F), AKT inhibitor (LY294002, C and G), or ERK inhibitor (PD98059, D and H) for 20 min and then treated with 10 ng/mL of IL-1β for 6 hours to detect β-Klotho and FGF21. I: Huh-7 cells were pretreated with 1 μM NF-κB inhibitor Bay11-7082, and β-Klotho and FGF21 levels were determined by immunoblotting. J: Huh-7 cells were pretreated with

0.5 μM JNK inhibitor SP600125, and β-Klotho and FGF21 levels were determined by immunoblotting. K: β-Klotho and FGF21 levels were determined six hours after pretreatment of 1 μM NF-κB inhibitor Bay11-7082 in Huh-7 cells. L: β-Klotho and FGF21 levels were determined six hours after pretreatment of 0.5 μM JNK inhibitor SP600125 in Huh-7 cells.

5. Effect of IL-1β on FGF21 in Huh-7 cells

We investigated whether FGF21 protein level was increased by IL-1β in Huh-7 cells. We incubated Huh-7 cells with IL-1β for 6 hours. IL-1β increased the expression of FGF21 proteins, reaching the highest level 5 minutes after IL-1β treatment and gradually decreasing (Figure 4F and 4G). However, FGF19 was not detected in Huh-7 cells treated with IL-1β. We then examined which signaling pathway mediates IL-1β-induced activation on FGF21 expression. Huh-7 cells were pretreated with Bay11-7082 before being treated with IL-1β. NF-κB inhibitor abolished the effect of IL-1β on FGF21 signaling (Figure 5A, 5E, 5I and 5K). Also, JNK inhibitor SP600125 suppressed the effect of IL-1β on FGF21 signaling (Figure 5B, 5F, 5J and 5L).

6. FGF21 inhibited IL-1β-induced growth retardation of hepatocytes

Using MTT assay and immunoblotting assay with PCNA as a marker of proliferation, we determined the effects of IL-1β and FGF21 on hepatocyte cell proliferation. Huh-7 cells were treated with IL-1β and/or FGF21. IL-1β decreased PCNA expression in Huh-7 cells in a time-dependent manner (Figure 6A). FGF21 had no effect on PCNA expression (Figure 6B). However, when IL-1β was co-treated with FGF21, FGF21 inhibited hepatocyte growth retardation, as determined by immunoblotting and MTT assay (Figure 6C and 6D).

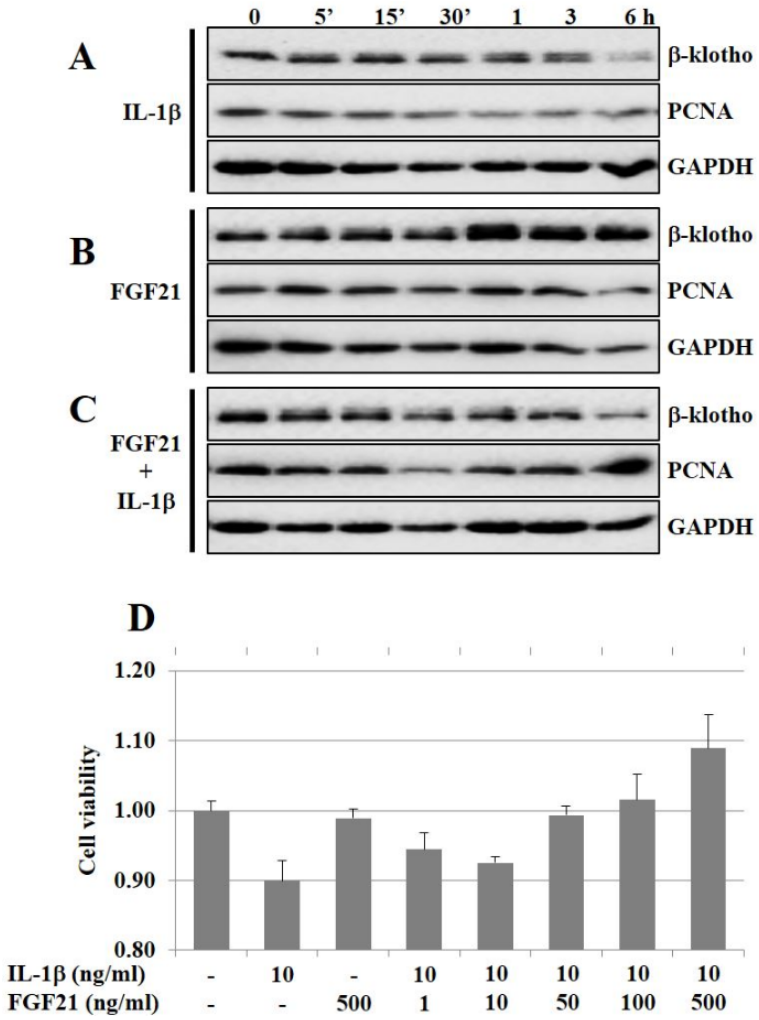

Figure 6. FGF21 inhibits IL-1β-induced growth retardation of hepatocytes. A: Huh-7 cells were incubated with 10 ng/ml of IL-1β (A), 500 ng/ml of FGF21 (B), or IL-1β + FGF21 (C), and expression of β-Klotho and PCNA was determined. D: IL-1β-treated Huh-7 cells were incubated with or without FGF21 in a dose-dependent manner. Cell viability was measured by MTT assay.

IV. DISCUSSION

In the present study, we observed that inflammation and fibrosis increased FGF19 and FGF21 in patients with hepatic fibrosis, with both mRNA and protein levels increasing gradually, whereas mRNA level of β-Klotho was decreased. In Huh-7 cells, pro-inflammatory cytokines including IL-1β inhibited β-Klotho expression but increased FGF21 level. We also showed that IL-1β inhibited β-Klotho expression via JNK and NF-κB pathways in Huh-7 cell. FGF21 inhibited growth retardation of hepatocytes induced by IL-1β.

Hepatocyte death or growth suppression is linked to inflammation and hepatic fibrosis.[16-19] In patients with chronic viral hepatitis and alcoholic hepatitis, hepatocyte death is the initial disease driver and subsequently triggers inflammation and fibrosis.[20-23] Several studies have revealed that pro-inflammatory chemokines and their receptors are positively associated with hepatic fibrogenesis in patients with hepatitis B virus or hepatitis C virus.[16, 24, 25] In alcoholic hepatitis, Toll-like receptor 4 activation and natural killer cell inhibition caused by chronic ethanol exposure lead to liver inflammation and fibrosis.[21, 26] In our study, we enrolled patients with viral hepatitis and alcoholic hepatitis. We first verified the relationships between inflammation levels and the degree of hepatic fibrosis using the inflammatory markers IL-1β, IL-6 and TNF-α.

In this regard, the expression of β-Klotho mRNA gradually decreased as the degree of liver inflammation increased. Previous experimental studies have shown that pro-inflammatory cytokines repress β-Klotho expression.[27, 28] Although heterogeneous etiology is a limitation, this is the first human data to show that the level of β-Klotho was decreased by inflammation and fibrosis.

In this study, we found that the mRNA and protein levels of FGF19 and FGF21 positively increased with the degree of liver inflammation and fibrosis. Consistent with our findings, a recent report has revealed that concentration of FGF19 was increased under cholestatic and cirrhotic conditions as an adaptive hepatic response.[10] However, Zhou et al. reported that, while protecting the liver, prolonged exposure to FGF19 at a circulating level as low as 20 ng/ml induced hepatocellular carcinoma in mice with targeted disruption of the orthologous multidrug resistance 2 gene.[29] FGF19 is supposed to act as a "double-edged sword" that, on the one hand, acts as an adaptive response to the liver injury and, on the other hand, induces hepatocellular carcinoma in patients with chronic liver disease. FGF21 is also increased in inflammatory conditions. Patients with sepsis showed a significant elevation of plasma FGF21 level compared to healthy subjects.[30] In an endotoxemic mouse model, plasma FGF21 level was also increased.[31] Increase in plasma FGF21 level during inflammation might be a protective response as treatment with exogenous FGF21 reduced the rate of death

in a septic mouse model.[31] Rusli et al. reported that plasma FGF21 was negatively correlated with expression of β-Klotho in a non-alcoholic fatty liver disease mouse model.[32] The up-regulated FGF21 level suggests to be the protective response against non-alcoholic fatty liver disease-induced adverse events.[32]

We also investigated the mechanisms involved in inflammation-induced suppression of β-Klotho expression and increase of FGF21 expression using Huh-7 cells. IL-1β, which is a potent pro-inflammatory cytokine, is related to toxicity-, ethanol-, and non-alcoholic steatohepatitis-induced fibrosis.[33, 34] First, we verified that IL-1β phosphorylates the IκB-α pathway and activates the JNK pathway. These pathways produce transcription factors that act as regulators of inflammation and fibrosis.[35, 36] These two pathways are also the main pathways that transduce IL-1β signaling.[37] IL-1β inhibited β-Klotho expression in a time-dependent manner. Zhao et al. reported that IL-1β signaling directly inhibits β-Klotho transcription.[27] Simultaneous inhibition of both the NF-κB and JNK inhibitor pathways suppressed the role of IL-1β to inhibit β-Klotho expression. Interestingly, in our study, each NF-κB and JNK inhibitor alone suppressed the ability of IL-1β to inhibit β-Klotho expression.

We further investigated whether IL-1β has an effect on FGF21 expression in Huh-7 cells. FGF21 expression increased after IL-1β treatment. We again applied NF-κB inhibitor or JNK inhibitor to determine whether the NF-κB and JNK

pathways affect the inhibitory effect of IL-1β on FGF21 expression. Along with the result of IL-1β on β-Klotho, each inhibitor abolished the ability of IL-1β to increase FGF21 expression. One previous study showed that the JNK pathway is involved in the inhibition of β-Klotho expression and FGF21 signaling by TNF-α in 3T3-L1 adipocytes.[28] Our results showed that the NF-κB pathway is also involved in IL-1β induced FGF21 expression.

FGF21 protein is known to be highly expressed in a variety of liver disease, such as viral hepatitis, alcoholic liver disease, and hepatocellular carcinoma.[14, 15] In the present study, we showed that both mRNA and protein of FGF21 increased with the degree of fibrosis in patients with viral hepatitis and alcoholic liver disease. Furthermore, in Huh-7 cells, IL-1β increased FGF21 expression. Elevation of FGF21 level is thought to be a protective effect in liver disease by removing systemic lipids and enhancing insulin sensitivity.[13] In our study, FGF21 inhibited the growth retardation of hepatocytes induced by IL-1β. In this view, FGF21 is considerable as a therapeutic agent for anti-inflammatory and anti-fibrosis roles.

V. CONCLUSION

In conclusion, the present study demonstrates that expression of FGF19 and FGF21 increased in serum and liver tissue in patients with viral hepatitis and alcoholic hepatitis, whereas the expression of β-Klotho decreased. IL-1β inhibited β-Klotho expression via NF-κB and JNK pathways. On the other hand, IL-1β increased FGF21 expression by the NF-κB and JNK pathways. FGF21 showed a protective effect on IL-1β-induced growth retardation of hepatocytes. This mechanism will help to better understand FGF21 signaling and can be applied as a therapeutic agent in inflammatory and fibrotic conditions.

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국문요약

NF-κB와 JNK 경로를 통한 섬유아세포성장인자21과 베타클로토의 간섬유화 조절 기전

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의학과

섬유아세포성장인자(FGF)21은 FGF19 아과의 하나로써 당과 지질대사 조절에 관여한다. 베타클로토는 보조수용체로 FGF21이 FGF수용체에 붙어 기능을 할 수 있도록 도와준다. FGF21은 간염증과 간섬유화에 관여하는 것으로 알려져 있다. 하지만, 베타클로토와 FGF21이 간염증과 간섬유화에서 어떤 경로를 통해 작용하는지 잘 알려져 있지 않다. 이번 연구에서는 조직검사로 증명된 바이러스 간염이나 알코올성 간염 환자를 대상으로 효소면역측정법, 실시간 중합효소 연쇄 반응기, 단백질흡입법을 이용하여 FGF19, FGF21과 베타클로토 수치를 측정하였다. 또한 Huh-7 세포를 이용하여 작용

경로를 찾아보았다. 본 연구를 통해 혈액과 간조직에서 FGF19와 FGF21이 간섬유화 정도에 따라 양의 상관관계가 있고 베타클로토와는 음의 상관관계가 있음을 확인하였다. 염증성 인자 (인터루킨-1베타, 인터루킨-6, 종양괴사인자-6) 또한 간섬유화 정도에 따라 양의 상관관계를 가졌다. Huh-7 세포에서 인터루킨-1베타를 처리했을 때, FGF21은 증가하였고 베타클로토는 감소하였다. NF-κB와 JNK 억제제를 각각 Huh-7 세포에 전처리 후에 인터루킨-1베타를 처리했을 때, 두 경우 모두 FGF21과 베타클로토에 대한 인터루킨-1베타의 효과를 파괴하였다. Huh-7 세포에서 FGF21을 처리했을 때 인터루킨-1베타에 의한 간세포성장 지연을 방어하는 효과를 보였다. 이런 결과를 종합했을 때, 섬유화 과정에서 발생한 염증으로 인해 NF-κB와 JNK 경로를 통해 FGF21은 증가되고 베타클로토는 억제가 된다. 또한, FGF21은 간염증과 간섬유화에서 간세포를 보호한다고 본다.

핵심되는 말: 섬유아세포성장인자21, 베타클로토, 인터루킨-1베타, NF-κB, JNK