FGF21 upregulation by hepatitis C virus via the eIF2α-ATF4 pathway : Implications for interferon signaling suppression and TRIM31-mediated TSC degradation

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論文題目
FGF21 upregulation by hepatitis C virus via the eIF2α-ATF4 pathway: Implications for interferon signaling suppression and TRIM31-mediated TSC degradation
（C 型肝炎ウイルス感染により eIF2α-ATF4 シグナル伝達経路を介して誘導される FGF21 のアップレギュレーション：インターフェロンシグナル伝達の抑制および TRIM31 依存的な TSC の分解への影響）

論文の内容の要旨
[Introduction]
Hepatitis C virus (HCV) infection is a major cause of chronic liver diseases and is known to induce endoplasmic reticulum (ER) stress, which is potentially involved in alterations in cellular homeostasis, including metabolic abnormalities. Although ER stress may be associated with the pathogenesis of HCV-related diseases, its relevance has not yet been fully elucidated. This study began with a comprehensive mRNA analysis of host factors whose expression is upregulated by ER stress in HCV-infected cells. Focusing on one of the identified factors, fibroblast growth factor 21 (FGF21), further analyses to elucidate the molecular mechanism by which FGF21 expression is induced by HCV infection and to better understand the potential involvement of FGF21 in HCV-related pathogenesis were carried out.
[Materials and Methods]
Comprehensive comparison of gene expression in human hepatocellular carcinoma Huh7.5.1 cells with and without HCV infection or FGF21 knockdown by siRNA was analyzed by cDNA microarray and RNA-sequencing. Identification of transcriptional regulators for FGF21 and tribbles pseudokinase 3 (TRIB3) and of the key sequences in the promoter regions was performed by promoter reporter assay and chromatin immunoprecipitation. Individual mRNA- and protein expression levels were determined by reverse transcription-quantitative polymerase chain reaction and immunoblotting, respectively. This study was approved by Safety Committee for Recombinant DNA Experiments of Hamamatsu University School of Medicine (27-15, 2-41, 27-28, 30-57).
[Results]
cDNA microarray analysis identified host factors whose expression was upregulated by HCV infection and the treatment with tunicamycin, an ER stress inducer, in cells. Among them, FGF21 mRNA and protein levels were markedly increased in an HCV infection titer-dependent manner. A series of experiments to elucidate the mechanism of HCV-induced enhancement of FGF21 expression revealed that binding of activating transcription factor 4 (ATF4) to the FGF21 promoter is important for FGF21

transcriptional enhancement and that similar effects to those observed with HCV infection were obtained from the viral Core-NS2 protein expression. In general, it is known that ER stress-induced upregulation of ATF4 expression is accompanied by progressive phosphorylation of eukaryotic initiation factor 2α (elF2α). Increased ATF4 expression and phosphorylated elF2α levels were observed upon HCV infection. Knockdown of cyclic AMP-responsive element-binding protein H (CREBH), another ER stress-related transcription factor known to be activated by HCV infection, resulted in decreased levels of ATF4 and phosphorylated elF2α. FGF21 mRNA level was also significantly decreased by knockdown of CREBH and ATF4. In addition, TRIB3 was identified as a negative regulator that is upregulated by HCV-induced ATF4 activation and acts as a suppressor of FGF21 expression. Further comprehensive analysis identified FGF21-dependent upregulation of suppressor of cytokine signaling 2 (SOCS2) and E3 ubiquitin-protein ligase tripartite motif containing 31 (TRIM31) in HCV-infected cells. SOCS2 contributes to the suppression of type 1 interferon signaling, while TRIM31 promotes the degradation of the tumor suppressor protein TSC.

[Discussion]

The hepatokine FGF21 is a versatile metabolic regulator that acts in an endocrine manner and responds to various physiological and pathological stresses. Based on the findings obtained in this study, it is likely that the ER stress caused by HCV infection leads to phosphorylation of elF2α and increased expression of ATF4, which in turn increases recruitment of ATF4 to the FGF21 promoter, and that activation of CREBH triggered by HCV infection also contributes to increased ATF4 expression through induction of elF2α. On the other hand, the suppression of FGF21 induction by increased expression of TRIB3 in HCV infection may serve to prevent rapid changes in the cellular environment by inhibiting FGF21 intervention in various physiological pathways in the infected cells. Functional analyses on SOCS2 and TRIM31 demonstrated that FGF21 induced by HCV infection possibly contributes to the negative regulation of IFN signaling through induction of SOCS2 expression and to the activation of the mammalian target of rapamycin complex1 (mTORC1) via promoting the ubiquitin-dependent degradation of TSC, an upstream repressor of the mTORC1 pathway, induced by TRIM31 expression. Although FGF21 possesses multiple physiological functions, its role in the regulation of cell proliferation and immune mechanisms is relatively unknown compared to its metabolic regulatory actions to date. Elucidating the effects of increased expression of the factors identified in this study on hepatocyte proliferation and immune response to HCV infection will help us to understand the role of FGF21 in the pathogenesis of the virus-related diseases.

[Conclusion]

Activation of the eIF2α-ATF4 axis plays a key role in upregulation of FGF21 induced by HCV infection. The upregulation of ATF4 by the viral infection not only upregulates FGF21 expression but contributes to the negative regulation of FGF21 through the induction of TRIB3. Upregulation of SOCS2 and TRIM31 dependent on high FGF21 expression in HCV-infected cells may be involved in the negative regulation of innate immune responses, persistence of viral infection and cell proliferation in the liver.