

INHIBITORY ACTIVITY OF INTERFERON-INDUCED PROTEIN 44 AND ITS PARALOG ON HEPATITIS C VIRUS REPLICATION

Chie Aoki^{1,2}, Yoko Shimizu³, Kazufumi Shimizu³, Lijuan Yu², Minako Hijikata⁴, Fumihiro Yagyu^{1,2}, Oshima Masamichi⁵, Aikichi Iwamoto^{2,6}, and Yoshihiro Kitamura^{1,2}

¹Research Center for Asian Infectious Diseases, Institute of Medical Science, University of Tokyo, Japan; ²China-Japan Joint Laboratory of Molecular Immunology and Molecular Microbiology, Institute of Microbiology, Chinese Academy of Sciences, China; ³Genome Center, Nihon University School of Medicine, Japan; ⁴Department of Respiratory Diseases, Research Institute, International Medical Center of Japan, Japan; ⁵Department of Immunology, National Institute of Infectious Diseases, Japan; ⁶Advanced Research Center, Institute of Medical Science, University of Tokyo, Japan

The standard therapy for chronic hepatitis C virus (HCV) infection uses type-I interferons (IFNs) such as IFN-alpha. Although IFNs induce the expression of many IFN-stimulated genes (*ISGs*), few have been found to inhibit some viral replication. Furthermore, most *ISGs* have not been studied. In this study, we are interested in two human genes, "Interferon-Induced Protein 44 (*IFI44*)" and its paralog "*IFI44L*." *Pan troglodytes*' *IFI44* encodes a 44-kilodalton protein originally identified as the 'hepatitis C-associated microtubular aggregate' in the hepatocytes of chimpanzees infected with HCV. Its human ortholog *IFI44* and its paralog *IFI44L* are present in the human genome, but their functions during the course of HCV infection are not known. To help us understand their nature, we have characterized them both biochemically and genetically in this study. A luciferase reporter assay confirmed that human *IFI44* and *IFI44L* genes were induced by IFN-alpha and -beta but not -gamma. Human *IFI44* and *IFI44L* proteins produced by *in vitro* translation showed a guanosine triphosphatase (GTPase) activity. In fact, their C-terminal halves of the amino acid sequences have a Ras-GTPase motif, indicating they are distant relatives of the Ras-GTPase superfamily. Overexpression of human *IFI44* or *IFI44L* protein in Huh7 cells (human hepatocyte cell line) by adenoviral vectors suppressed the replication of JFH-1, which is a replication-competent HCV genotype 2a strain sensitive to IFN-alpha. Moreover, knock-down by siRNAs of *IFI44* or *IFI44L* gene expression followed by IFN-alpha treatment in Huh7 cells showed a reduction in JFH-1's sensitivity to IFN-alpha. Taken together, our results demonstrated that *IFI44* and *IFI44L* are members of a new class of type-I IFN-induced GTPases that inhibit JFH-1 replication in Huh7 cells.