

## VARIANTS OF JFH1 STRAIN OF HEPATITIS C VIRUS, WHICH HAVE ADAPTED TO CULTURED Huh7 CELLS

Lijuan Yu<sup>1</sup>, Chie Aoki<sup>1,2</sup>, Yoko Shimizu<sup>3</sup>, Wei Hou<sup>1</sup>, Fumihiro Yagyu<sup>1,2</sup>, Oshima Masamichi<sup>4</sup>, Aikichi Iwamoto<sup>2</sup>, Bin Gao<sup>1</sup>, Frank Wenjun Liu<sup>1</sup>, George Fu Gao<sup>1</sup>, and Yoshihiro Kitamura<sup>1,2</sup>

<sup>1</sup>China-Japan Joint Laboratory of Molecular Immunology and Molecular Microbiology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China; <sup>2</sup>Research Center for Asian Infectious Diseases, Institute of Medical Science, University of Tokyo, Tokyo, Japan; <sup>3</sup>Genome Center, Nihon University School of Medicine, Tokyo, Japan; <sup>4</sup>Department of Immunology, National Institute of Infectious Diseases, Tokyo, Japan

Replication of infectious hepatitis C virus in Huh7 cells, a human hepatocyte cell line, has become possible due to the unique properties of the JFH1 isolate. Mimicking chronic hepatitis, we have been culturing infected Huh7 cells over two years to follow evolution of JFH1. We have obtained Huh7-adapted JFH1 variants (JFH1a) capable of yielding up to 1000-fold higher titers of infectious particles. Sequence analysis of variant genome RNA revealed a complex pattern of mutations and suggested that this adapted population consisted of mainly two variants (JFH1a -A and -B). The mutations that resulted in amino acid substitutions were observed both in the structural and in the nonstructural regions. The silent mutations in the open reading frame region as well as the mutations in the 5'-untranslated region were also observed. The majority of the substitution mutations were found in the NS5A region. Joining the obtained representative viral complementary DNA fragments, we are constructing a series of chimera genomes. So far, 5'JFH1a-A/3'wt, a chimera that carries the 5'-half of JFH1a-A and 3'-half of the wild-type JFH1, has been characterized. Transfection of Huh7 cells with in-vitro-transcribed 5'JFH1a-A/3'wt genome RNA has been found to start producing progeny infectious particles up to three weeks earlier than the wild-type JFH1 as assayed by focus titration of the culture supernatants. Furthermore, infection of Huh7 cells with these produced viral particles has been found to produce up to 1000 times more infectious viral particles than the wild-type. This suggests that at least some mutations in the 5'-half of JFH1a-A are involved in the adaptive replication. The results of similar experiments with authentic JFH1a-A and JFH1a-B as well as some other chimera genomes will be presented and the possible mechanism of adaptation will be discussed.

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