Virology 522 (2018) 177–192

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/virology

Recombinant hepatitis C virus genotype 5a infectious cell culture systems expressing minimal JFH1 NS5B sequences permit polymerase inhibitor studies

Daryl Humes[1], Santseharay Ramirez, Tanja B. Jensen, Yi-Ping Li[2], Judith M. Gottwein, Jens Bukh*

Copenhagen Hepatitis C Program (CO-HEP), Department of Infectious Diseases and Clinical Research Centre, Hvidovre Hospital and Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

ARTICLE INFO

Keywords:
Hepatitis C virus
HCV
Genotype 5a
Liver Cancer
Replication
Infectious cell-culture
Direct acting antivirals
DAA
Polymerase inhibitors

ABSTRACT

The six major epidemiologically important hepatitis C virus (HCV) genotypes differ in global distribution and antiviral responses. Full-length infectious cell-culture adapted clones, the gold standard for HCV studies in vitro, are missing for genotypes 4 and 5. To address this challenge for genotype 5, we constructed a consensus full-length clone of strain SA13 (SA13fl), which was found non-viable in Huh7.5 cells. Step-wise adaptation of SA13fl-based recombinants, beginning with a virus encoding the NS5B-thumb domain and 3′UTR of JFH1 (SA13/JF372-X), resulted in a high-titer SA13 virus with only 41 JFH1-encoded NS5B-thumb residues (SA13/JF470-510cc); this required sixteen cell-culture adaptive substitutions within the SA13fl polyprotein and two 3′UTR-changes. SA13/JF372-X and SA13/JF470-510cc were equally sensitive to nucleoside polymerase inhibitors, including sofosbuvir, but showed differential sensitivity to inhibitors targeting the NS5B palm or thumb. SA13/JF470-510cc represents a model to elucidate the influence of HCV RNA elements on viral replication and map determinants of sensitivity to polymerase inhibitors.

1. Introduction

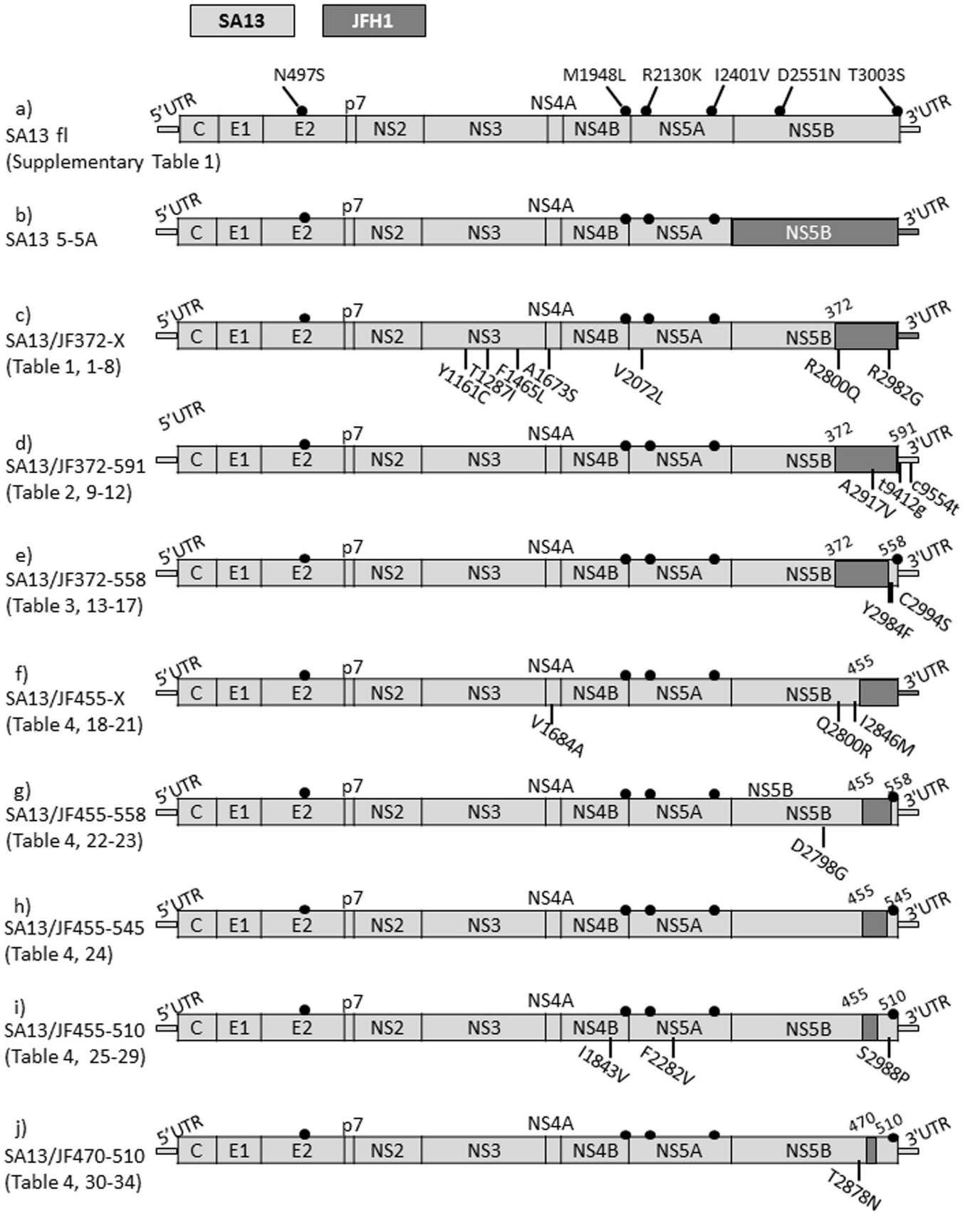
Hepatitis C virus (HCV) infections cause chronic liver diseases, including hepatocellular carcinoma (HCC), associated with about 500 thousand annual deaths (Bukh, 2016; Webster et al., 2015). More than 70 million people are estimated to be chronically infected with HCV, however, the infection status has been established in < 20% of these individuals. Annually, over 2 million new acute infections occur, with a chronicity rate of > 70%. This poses significant scientific challenges to optimize treatment regimens, and to develop a prophylactic vaccine (Webster et al., 2015). Therefore, it is important to develop efficient experimental culture systems for various HCV variants, which have been classified into six epidemiologically important genotypes (genotypes 1–6) and numerous epidemiologically important subtypes (a, b, etc.) differing in global distribution and antiviral responses (Bukh, 2016; Smith et al., 2014).

HCV is the prototype member of the Hepacivirus genus within the Flaviviridae family of viruses (Simmonds et al., 2017; Smith et al., 2016). Its ~9.6-kb positive single-stranded RNA genome contains a single open reading frame (ORF) that is translated into an ~3000 amino acid (aa) polyprotein (Gottwein and Bukh, 2008). At the termini, the HCV genome has highly structured 5′ and 3′ untranslated regions (UTRs); the 3′UTR consists of a short variable region, a poly(U/UC) tract of various length and a highly conserved 3′ terminal X region 98 nucleotides (nts) in length (Bukh, 2016; Gottwein and Bukh, 2008). The polyprotein is processed into the structural proteins Core, envelope 1 (E1), and envelope 2 (E2), and the nonstructural proteins p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B. The NS3/4A-protease, the NS5A protein, and the NS5B RNA polymerase are the major targets for direct acting antivirals (DAAs), which recently have revolutionized the

Abbreviations: HCV, hepatitis C virus; DAA, direct-acting antivirals; ORF, open reading frame; UTR, untranslated region; E, envelope; nt, nucleotide; aa, amino acid; FFU/ml, focus forming units per milliliter; NI, nucleos(t)ide inhibitors; NNI, non-nucleoside inhibitors

* Correspondence to: Department of Infectious Diseases, Hvidovre Hospital, Kettegaard Alle 30, DK-2650 Hvidovre, Denmark.
E-mail address: jbukh@sund.ku.dk (J. Bukh).
[1] **Present address:** Fred Hutchinson Cancer Research Center, Seattle, WA 98109-1024, USA.
[2] **Present address:** Institute of Human Virology and Key Laboratory of Tropical Disease Control of Ministry of Education, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou 510080, China.

https://doi.org/10.1016/j.virol.2018.05.020
Received 4 March 2018; Received in revised form 24 May 2018; Accepted 25 May 2018



(caption on next page)