

Serum-derived hepatitis C virus infectivity in interferon regulatory factor-7-suppressed human primary hepatocytes

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Background/Aims: The development of an efficient in vitro infection system for HCV is important in order to develop new anti-HCV strategy. Only Huh7 hepatocyte cell lines were shown to be infected with JFH-1 fulminant HCV-2a strain and its chimeras. Here we aimed to establish a primary hepatocyte cell line that could be infected by HCV particles from patients' sera.

Methods: We transduced primary human hepatocytes with human telomerase reverse transcriptase together with human papilloma virus 18/E6E7 (HPV18/E6E7) genes or simian virus large T gene (SV40 T) to immortalize cells. We also established the HPV18/E6E7-immortalized hepatocytes in which interferon regulatory factor-7 was inactivated. Finally we analyzed HCV infectivity in these cells.

Results: Even after prolonged culture HPV18/E6E7-immortalized hepatocytes exhibited hepatocyte functions and marker expression and were more prone to HCV infection than SV40 T-immortalized hepatocytes. The susceptibility of HPV18/E6E7-immortalized hepatocytes to HCV infection was further improved, in particular, by impairing signaling through interferon regulatory factor-7.

Conclusions: HPV18/E6E7-immortalized hepatocytes are useful for the analysis of HCV infection, anti-HCV innate immune response, and screening of antiviral agents with a variety of HCV strains.

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1. Introduction

Infection with Hepatitis C virus (HCV) is a serious problem worldwide since 3% of the world's population is chronically infected [1]. Chronic HCV may lead to liver cirrhosis and hepatocellular carcinoma. Current stan-

dard therapy utilizes the combination of pegylated interferon- α and ribavirin, which results in a sustained response in only 30–60% of patients [2–5]. Many patients, however, do not qualify for or tolerate standard therapy [6]. Thus, it is important to develop an efficient in vitro infection system for HCV to facilitate the discovery of new anti-HCV strategies. Only Huh7 cell line is permissive for replication, infection and release of the fulminant hepatitis-derived HCV-2a (JFH-1) strain and its chimeric derivatives [7–9]. No other hepatocyte cell lines are able to support HCV replication efficiently.

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Normal human hepatocytes are the ideal system in which to study HCV infectivity. When cultured in vitro, however, they proliferate poorly and divide only a few times [10]. Continuous proliferation could be achieved however by introducing oncogenes, such as Simian virus large tumor antigen (SV40 T) [11]. This often resulted in tumor development [12] together with numerical (aneuploidy) and structural (aberrations) chromosome abnormalities [13]. The human papilloma virus E6E7 genes (HPV/E6E7) immortalized multiple cell types that were phenotypically and functionally similar to the parental cells [14–20]. As yet, no human hepatocytes have been immortalized with HPV18/E6E7.

We established a human primary non-neoplastic hepatocyte cell line transduced with the HPV18/E6E7 that retained primary hepatocyte characteristics even after prolonged culture, and were more prone to HCV infection than those cells immortalized with SV40 T antigen. We further improved the susceptibility of HPV18/E6E7-immortalized hepatocytes to HCV infectivity by impairing interferon regulatory factor-7 (IRF-7) expression. These cells are useful to assay infectivity of HCV strains other than JFH-1, HCV replication, innate immune system engagement of HCV, and screening of anti-HCV agents. This infection system using non-neoplastic cells also suggested that IRF-7 plays an important role in eliminating HCV infection.

2. Materials and methods

2.1. Cell cultures

We obtained the approval of the Ethical Committee of Kyoto University for the use of human hepatocytes and sera obtained from HCV-positive patients. Informed consent was obtained from both the hepatocyte donor and HCV-positive patients. Primary hepatocytes (P.H.) were cultured as described [21]. HeLa, 293, Huh-7.5, and PH5CH8 cells were cultured as previously described [22]. For three-dimensional (3D) cultures, Mebiol Gel (Mebiol Inc.) was prepared according to the manufacturer's instructions.

2.2. Plasmids construction

The SV40 T, hTERT and HPV/E6E7 fragments from pAct-SVT, PCX4neo/hTERT, and pLXSN-E6E7 plasmids were inserted into pCSII-EF-RFA plasmid creating the pCSII-EF-SVT, pCSII-EF-hTERT, and pCSII-EF-E6E7 plasmids, respectively. The full-length IRF-3 and IRF-7 genes were cloned by RT-PCR using total RNA isolated from 293 cells as a template and were inserted into pcDNA3 vector. Dominant-negative forms of IRF-3 (DNIRF-3) and IRF-7 (DNIRF-7) were constructed by PCR amplification of the coding region for amino acid residues 108–427 of IRF-3 and 237–514 of IRF-7, respectively. The amplified IRF-3 fragment was cloned into pcDNA3 in frame with a FLAG epitope tag generating pcFLAG-DNIRF-3. The amplified IRF-7 fragment was cloned into pLXSH in frame with HA epitope tag generating pLXSH-HA-DNIRF-7. The pIFN β promoter-luc and pIFN α promoter-luc plasmids were gifts from Dr. Taniguchi of the Tokyo University. The psiRNA-hIRF-3 and psiRNA-hIRF-7 plasmids were purchased from Invivo-gen (USA).

2.3. Immunoblot analysis

Immunoblot analysis was performed as described previously [22]. We used anti-SV40 T (Santa Cruz), anti-HPV18/E7 (Santa Cruz), anti-tubulin (Sigma), anti-FLAG (Sigma), and anti-HA (Sigma) antibodies.

2.4. Transfection, small interfering RNA silencing and luciferase assays

Transfection of plasmid DNA was performed using Effectene transfection reagent (Qiagen) as recommended by the manufacturer. The pLXSH-HA-DNIRF-7 plasmid was transfected into the HuS-E/2 clone; transfectants were selected in 100 μ g/ml hygromycin B (Gibco). The psiRNA-hIRF-3 and psiRNA-hIRF-7 plasmids were separately transfected into HuS-E/2 cells followed by Zeocin (250 μ g/ml) selection. After two weeks of continuous selection, cells were infected with HCV. Luciferase assays were conducted as previously described [22]. The results are presented as relative light units (RLU) normalized to the total content of protein in the cell lysates.

2.5. Reverse transcriptase polymerase chain reaction (RT-PCR) and real-time RT-PCR

Using 250 ng of total RNA as a template, we performed RT-PCR with a one-step RNA PCR kit (Takara) according to the manufacturer's instructions. The primer sets and reaction conditions used are detailed in Table 1. To measure HCV-RNA titers by real-time RT-PCR, we collected RNA from infected wells. Five hundred nanograms of total cellular RNA was analyzed for the quantity of HCV-RNA as previously described [23].

2.6. HCV infection experiment

HCV infection experiment from serum was done as mentioned before [22]. HCV-infected-serums were titrated and 1×10^5 HCV-RNA copies/ml were used for each infection experiment. Concentrated culture medium for HCV/JFH1-producing cells was prepared as previously described [7]. HCV titer in the concentrated medium was measured, adjusted and added to the cells as mentioned above.

2.7. Blocking of HCV infectivity by anti-CD81

Inhibition of HCV infectivity was performed by blocking CD81 as previously described [7].

3. Results

3.1. Establishment of immortalized primary human hepatocytes

Primary hepatocytes were isolated from liver tissue obtained from a 9-year-old male patient with Primary Hyperoxaluria who had undergone liver transplantation. Hepatocytes were left unmanipulated or transduced with CSII-EF-hTERT alone or in combination with CSII-EF-SVT or CSII-EF-E6E7 to enhance the efficiency of immortalization. After six weeks only cells transduced by the combination of hTERT and either LT or HPV18/E6E7 continued to proliferate. Initially appearing colonies with a growth advantage were picked up and expanded. SV40 T-immortalized cell clones were named HuS-T cells and given numbers from 1 to 7,

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