could achieve a significant HBsAg seroconversion rate without inducing drug-resistant YMDD mutants. The important point to note is that after the transient use of lamivudine, some of the recipients who had been naive against HBV infection before transplantation maintained anti-HBs positivity despite withdrawal of HBIG even during immunosuppressive therapy, indicating that they were likely to acquire endogenous immunity to HBV infection.

Passive immunoprophylaxis with HBIG has been used to prevent *de novo* HBV recurrence after liver transplantation in HBV-naive recipients who received liver grafts from anti-HBc-positive donors (8). However, not only the efficacy, but also the safety of long-term HBIG treatment after liver transplantation has not been determined, including the risk of development of hepatitis B 'surface' escape mutants during passive immunoprophylaxis. In this study, we demonstrated that a considerable number of patients developed *de novo* HBV reactivation during the long-term course of HBIG prophylaxis. Our findings suggested that factors affecting the recurrence of *de novo* HBV in those recipients included non-compliance of HBIG, an immunosuppressive condition, and possible emergence of 'surface' escape mutants.

Lamivudine is a potent inhibitor of HBV replication, and several investigators have reported that lamivudine therapy is effective for hepatitis B treatment following liver transplantation (12,13). The present data show that early initiation of lamivudine treatment for post-transplant HBV *de novo* reactivation efficiently induced a sustained loss of HBsAg, suggesting that the effect of lamivudine on *de novo* HBV reactivation is stronger than its effect on chronic HBV infection. In general, HBV replication decreases immediately after administration of lamivudine; however, it has been shown that lamivudine treatment achieves clearance of HBsAg in only a very small proportion of cases. Indeed, loss of HBsAg occurred in only 0–2% of patients with chronic HBV infection that were treated with lamivudine (14). In transplant recipients with HBV-associated liver disease, lamivudine treatment induced the disappearance of HBsAg in only 3 of 52 cases (6%) by week 52, 2 of whom acquired anti-HBs (12). The better results observed in this study can be attributed to the fact that lamivudine treatment was commenced during the acute phase of *de novo* HBV reactivation when the number of HBV was expected to be very small. The beneficial effect of early lamivudine therapy for acute hepatitis has been reported previously (15,16). We suggest that the timing of the lamivudine administration in patients with HBV activation, specifically in the acute phase of HBV reactivation, is important to achieve complete viral suppression and successful seroconversion from HBsAg to anti-HBs.

It is well recognized that prolonged administration of lamivudine can lead to viral breakthrough because of the emergence of viral variants with reduced sensitivity to the drug resulting from one or more mutations in the YMDD locus of the HBV polymerase gene (17). Many investigators have reported a high rate of virological breakthrough with prolonged lamivudine therapy in liver transplant recipients with active HBV infection (17,18). In contrast, we confirmed the beneficial effect of lamivudine on *de novo* HBV reactivation and showed that it does not cause the emergence of YMDD mutants. Reduced risk for virological breakthrough during lamivudine therapy may be attributed to the short-term use of the drug and the low levels of HBV-DNA at the acute phase of *de novo* HBV reactivation, as prolonged use of lamivudine and high HBV-DNA levels before treatment were shown to be associated with the emergence of the drug-resistant mutants (19). Prevention of drug-resistant viral clones by transient use of lamivudine has also been demonstrated in patients with chronic HBV infection (20). Although short-term lamivudine therapy could reduce the incidence of YMDD mutants, the relapse rate was high after the withdrawal of lamivudine in HBV-related chronic liver disease patients (20). Thus, short-term lamivudine treatment might be applicable specifically to the acute phase of *de novo* HBV reactivation.

In conclusion, we have shown in this study that short-term use of lamivudine resulted in complete clearance of HBsAg in the majority of patients with *de novo* HBV reactivation, and that the effects of lamivudine were stronger in patients with *de novo* HBV reactivation than in patients with HBV-related chronic liver disease. More importantly, sustained clearance of HBV was obtained in two cases who stopped both HBIG and lamivudine administration, suggesting acquired immunity against HBV; however, a study of a large number of patients with *de novo* HBV reactivation is needed to reach any definitive conclusion. Thus, further studies with greater patient numbers are required to determine whether short-term lamivudine administration induces clearance of HBsAg in the sera, as well as acquired immunity against HBV in HBV-naive recipients receiving anti-HBc-positive allografts.

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Case Report

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Liver Transplantation from an Identical Twin without Immunosuppression, with Early Recurrence of Hepatitis C

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Hepatitis C virus reinfection after liver transplantation is universal and more severe than in nontransplant patients. Rejection episodes and immunosuppressive agents are considered risk factors for deterioration of recurrent hepatitis C. We report 2 cases of living donor liver transplantation for patients with hepatitis C-related cirrhosis who received right-lobe grafts from an identical twin. Thanks to genetic identity, no immunosuppressive drugs were administered during or after transplantation without rejection. Hepatitis C virus RNA kinetics showed a rapid increase following transplantation and liver biopsies 1 month after transplantation showed acute lobular hepatitis in both cases. Antiviral therapy using interferon α and ribavirin was started immediately, and both cases showed virological and histological response. In conclusion, avoidance of immunosuppression did not delay hepatitis C recurrence following transplantation, while early antiviral therapy without risk of rejection or immunosuppression led to successful viral eradication.

Key words: Hepatitis C, immunosuppression, liver transplantation, living-related liver donors, reinfection

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Introduction

The first successful kidney transplantation between identical twins was performed by Murray in 1954 (1). Solid-organ transplantation between identical twins has been successfully performed with the small intestine (2), kidney, pancreas (3), combined pancreas and kidney (4), parathyroid gland (5) and liver (6,7). This historical success has proved that for solid-organ transplantation from a syngeneic donor, allografts can be transplanted without immunosuppressive therapy. We performed living donor liver transplantation (LDLT) for two patients with hepatitis C virus (HCV)-related cirrhosis, using a right-lobe graft from an identical twin in each case without any immunosuppression.

HCV-related disease is one of the leading indications for liver transplantation worldwide. However, reinfection with HCV is immediate and universal following surgery, and jeopardizes both graft and patient survival (8). The spectrum of allograft injuries related to HCV recurrence ranges from no evidence of injury to graft failure requiring retransplantation in a subset of patients. Several factors have been proposed to explain these variable outcomes, including HCV viral load, genotype, rejection episodes and immunosuppression (9). Immunosuppression accounts for a major part of the accelerated progression. Steroid pulse therapy for rejection reportedly aggravates recurrent HCV (10). Furthermore, intraoperative bolus steroid injection has been shown to increase serum HCV RNA immediately after transplantation (11).

Liver transplantation for HCV is analogous to acute HCV infection, in that a never-infected liver is placed into a viremic host and inevitably becomes infected. Human liver transplantation thus offers a unique opportunity to study viral kinetics and the immunopathogenic mechanisms of acute HCV hepatitis. In the present cases of LDLT between identical twins, the influences of rejection and immunosuppression can also be excluded. We report herein the kinetics of serum HCV RNA levels, clinical course with antiviral therapy and pathological features for acute hepatitis C after LDLT free from both alloimmune response and immunosuppressive therapy.

Case 1

The recipient was a 51-year-old man diagnosed with HCV cirrhosis and multiple hepatocellular carcinoma. Genotype was 1b and HCV RNA level was 160 kIU/mL, while Child-Pugh classification was A. LDLT was performed using a right-lobe graft (graft-to-recipient weight ratio, 1.25%) from his identical twin. No immunosuppressive agent was administered, including intraoperative bolus steroid injection.

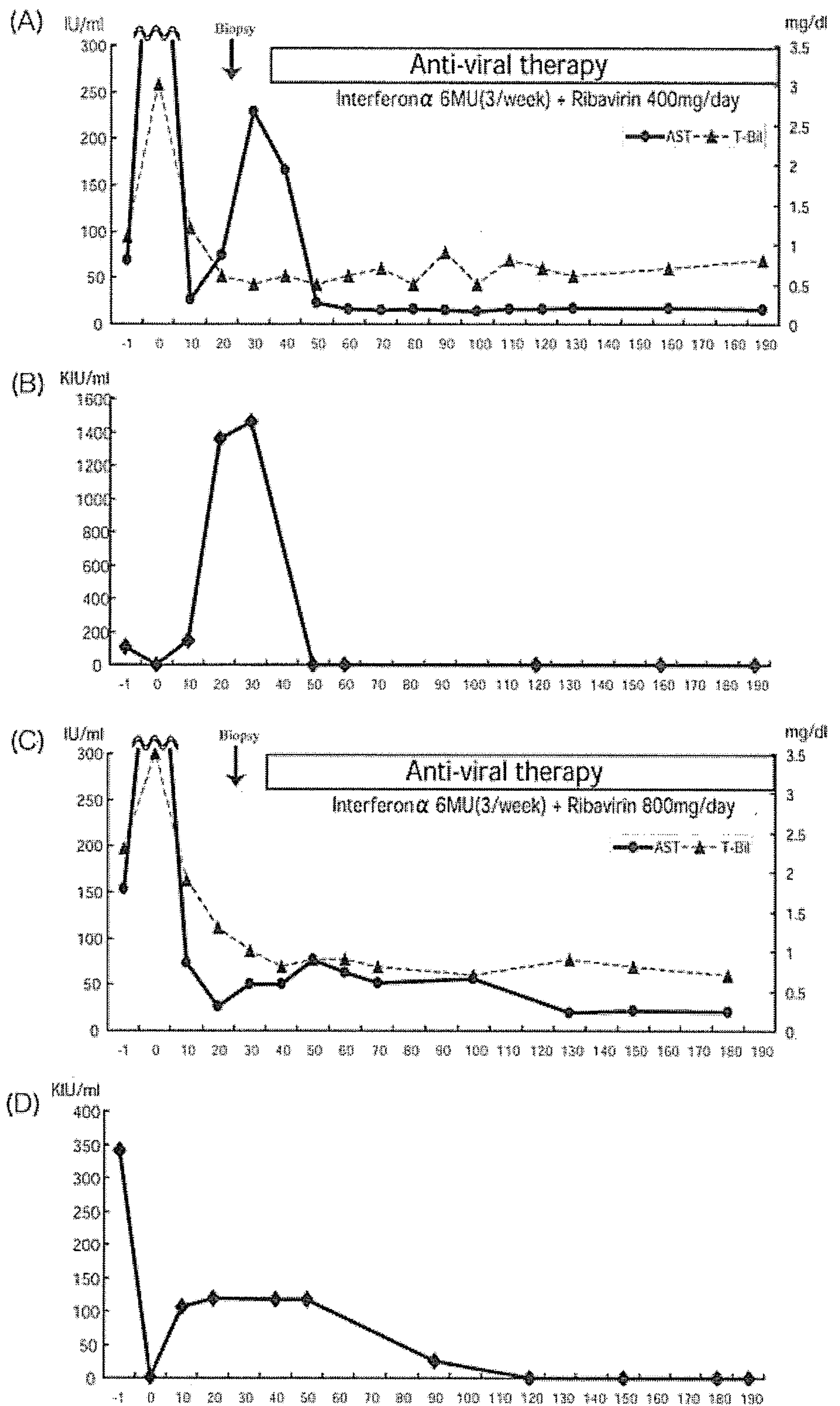

Figure 1: Results of liver function tests, serum HCV RNA level and clinical courses of Cases 1 and 2. (A) AST and total bilirubin (T-Bil) levels in Case 1. AST and T-Bil levels were elevated on POD 1 and decreased to within normal ranges by POD 10. On POD 17, AST elevated again and liver biopsy was performed on POD 24. Combined antiviral therapy was started from POD 31. (B) Kinetics of serum HCV RNA levels in Case 1. After reperfusion, HCV RNA could not be detected from serum. On POD 1, serum HCV RNA became positive and elevated continuously until peaking on POD 21 at 1600 kIU/mL. After antiviral therapy, serum RNA level decreased rapidly to be undetectable within 2 weeks. (C) AST and T-Bil levels in Case 2. AST and T-Bil elevated on POD 1 and gradually decreased. T-Bil gradually decreased to normal range after POD 20, but AST level remained abnormal until 3 months after antiviral therapy. T-Bil and AST have since remained within normal range. (D) Kinetics of serum HCV RNA level in Case 2. After reperfusion, serum HCV RNA became negative and continued until POD 7. On POD 10, serum HCV RNA level became positive and elevated to a peak of 100 kIU/mL on POD 27. After antiviral therapy started, serum HCV RNA gradually decreased and became negative on POD 120.

AST level was elevated to 1000 IU/mL on postoperative day (POD) 1, but subsequently improved before again elevating after POD 17 (Figure 1A). HCV RNA became negative after reperfusion, then turned positive on POD 1 and elevated continuously to a peak of 1600 kIU/mL on POD 21 (Figure 1B). Liver biopsy on POD 24 demonstrated the following findings: mild portal inflammation with infiltration of lymphocytes and eosinophils, but without bile duct

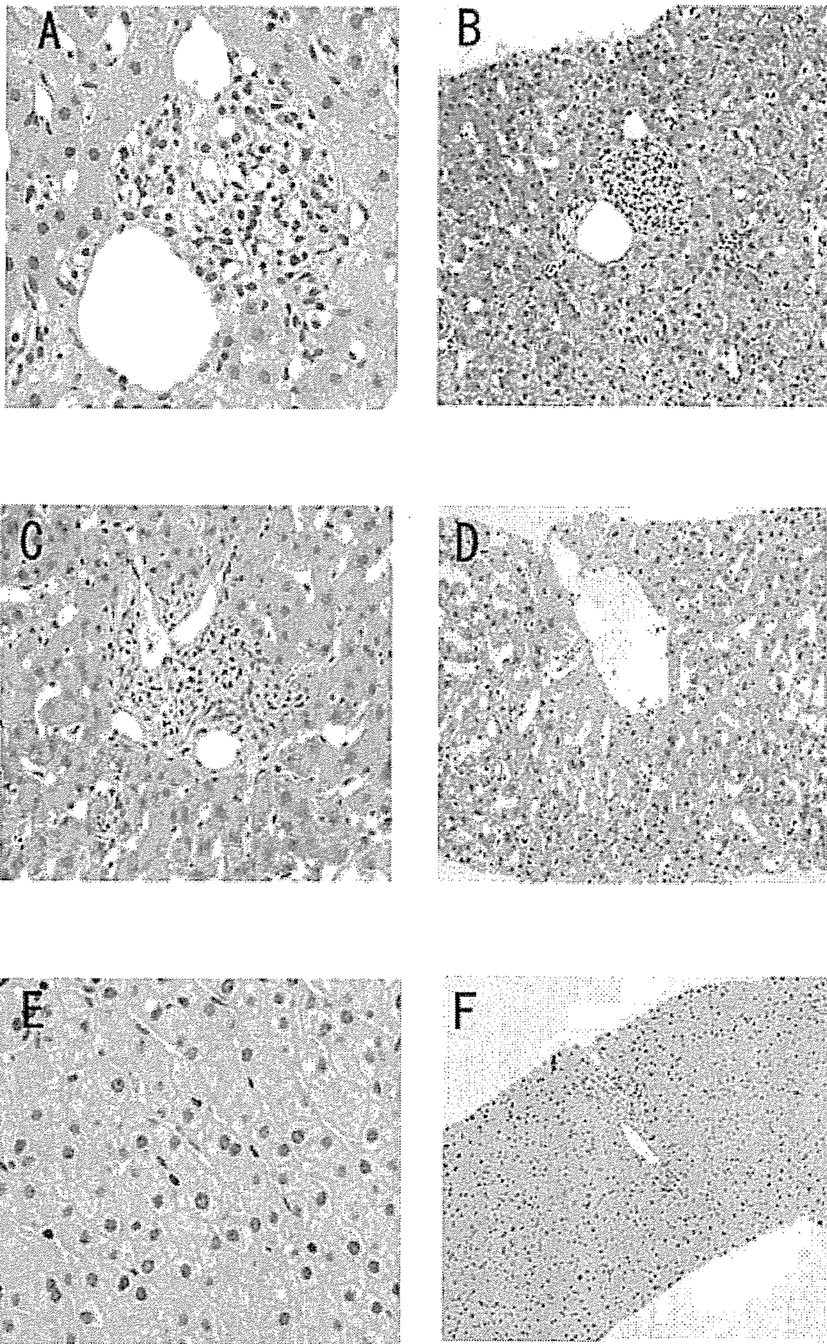

Figure 2: Histopathological findings from liver posttransplant biopsy. (A, B) Liver biopsy was obtained on POD 24 in Case 1 after LFTs elevated. (A) Mild portal inflammation with lymphocytes and eosinophils and no bile duct damage or endothelialitis. (B) Hepatocyte degeneration, steatosis and sinusoidal dilatation. (C, D) Liver biopsy was obtained on POD 27 in Case 2 after LFTs elevated. These biopsies show similar findings to Case 1. (E, F) Liver biopsy in Case 1 was obtained 7 months after transplantation, when HCV RNA was not detected in peripheral blood. These biopsies showed no signs of hepatitis.

damage or endothelialitis; lobular inflammation with acidophilic bodies; hepatocyte degeneration and steatosis and sinusoidal dilatation (Figure 2A, B). With a histological diagnosis of lobular hepatitis (A1, F0, according to METAVIR score (12)), antiviral therapy was started using interferon α-2b (6 MU 3 times/week) and ribavirin (400 mg/day) from POD 31. Ribavirin dose was reduced because the patient was anemic (Hb, 9.4 g/dL). HCV RNA turned negative within 2 weeks after starting treatment. Treatment was continued for 48 weeks, and sustained virological response has been maintained for >1 year after the cessation of therapy with good liver function. Liver biopsy results as of 8 months after LDLT showed no sign of hepatitis (Figure 2E, F).

Case 2

The recipient was a 38-year-old man diagnosed with HCV cirrhosis (Child-Pugh grade B, genotype 1b, HCV-RNA 340 kIU/mL). He had been treated using interferon

monotherapy for HCV-related chronic hepatitis at 20-years old, but this was ineffective and liver disease progressed to cirrhosis. The patient underwent LDLT using a right-lobe graft (graft-to-recipient weight ratio, 1.28%) from his identical twin without any immunosuppression. Serum HCV RNA rapidly decreased to undetectable levels after reperfusion and remained negative until POD 7. On POD 10, HCV RNA became positive and elevated to a peak of 100 kIU/mL on POD 25 (Figure 1D). Liver biopsy on POD 27 showed lobular hepatitis (A1, F0) with similar findings to those in Case 1 (Figure 2C, D). Combined antiviral therapy comprising interferon α-2b (6 MU 3 times/week), and ribavirin (800 mg/day) was administered from POD 34. Biochemical and virological responses were attained. After 12 weeks of therapy, HCV RNA was 0.57 kIU/mL. By 7 months after LDLT, the patient showed normal results on liver function test (LFTs) and HCV RNA was not detectable in serum. Liver biopsy 7 months after LDLT showed no signs of hepatitis. The patient remains on this treatment.

Discussion

These cases revealed three key findings. First, increased serum HCV RNA levels and histological findings of acute lobular hepatitis accompanied by mild elevation of LFT results were observed soon after LDLT despite the absence of immunosuppression. This absence of immunosuppression was expected to delay or alleviate HCV reinfection, if immunosuppression represents one of major factors in the progression of HCV recurrence. However, recurrence of hepatitis C proved inevitable after liver transplantation for HCV-infected patients, even without immunosuppression. A possible explanation for the early reinfection and progression of acute hepatitis was the genetic identity between donor and recipient. As HCV demonstrates cell tropism (13), a grafted liver from an identical twin would be more easily infected than an allograft. However, whether sharing the same HLA loci represents a risk factor for recurrence of HCV remains controversial. Another explanation is the graft type used in these cases. Right-lobe grafts inevitably undergo regeneration immediately after transplantation, and hepatocyte proliferation promotes viral replication (14). Whether hepatitis C recurrence occurs earlier and with greater severity for LDLT than for deceased donor liver transplantation has recently become a subject of debate (15,16).

The second key finding was that in the present 2 cases, liver biopsy results 1 month after LDLT showed similar features of acute lobular hepatitis, even though viral kinetics differed. Generally, early histopathological features of recurrent hepatitis C may be modified by immunosuppressive therapy and can be difficult to differentiate from acute rejection. The presence of eosinophils in portal inflammatory infiltrate is reportedly a significant variable associated with acute rejection (17,18). In the present cases where the possibility of acute rejection could be excluded, eosinophils were seen in areas of portal inflammation in both patients. Interestingly, this suggests that eosinophil infiltration is not necessarily specific to acute rejection, but also appears in early acute hepatitis. Sinusoidal dilatation, which is reported as the only specific feature of recurrent hepatitis C (17), was identified in both cases.

The third key finding was that, though sustained virological response could not be assessed in Case 2, antiviral therapy was effective in both cases. Antiviral therapy can reportedly stimulate immune responses and may in turn increase the risk of allograft rejection (19). However, in the present cases, treatment could be started without risk of rejection as soon as recurrent hepatitis was confirmed. Early treatment of acute hepatitis C with interferon is reportedly more effective compared to that for chronic infection (20). In terms of early introduction of antiviral therapy and remaining free from immunosuppression, the rapid response of these 2 patients with genotype 1b viruses may display some analogy to acute hepatitis C in nontransplant recipients.

In conclusion, we have reported 2 cases of LDLT between identical twins for HCV cirrhosis without any immunosuppressive drugs. Despite avoidance of immunosuppression, rapid increases in serum HCV RNA levels and histological recurrence of HCV by 1 month after LDLT were observed. However, antiviral therapy for acute hepatitis yielded good responses in both cases.

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

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See Editorial, pages 1–5

***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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***Keywords*: Immortalization; Primary hepatocytes; HCV infection; IRF-7; IRF-3; HPV18/E6E7; Innate immune response**

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 standard 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 pIFNβ promoter-luc and pIFNα promoter-luc plasmids were gifts from Dr. Taniguchi of the Tokyo University. The psiRNA-hIRF-3 and psiRNA-hIRF-7 plasmids were purchased from Invivogen (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 μg/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 μg/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,

Table 1
Primer sequences and RT-PCR parameters

Genes	Primer sequence 5′–3′	PCR parameters[a]
HGF	F: AGGAGCCAGCCTGAATGATGA R: CCCTCTGATGTCCCAAGATTAGC	95, 56, 72 1 min, 45 s, 1 min
TGFα	F: ATGGTCCCCTCGGCTGGA R: GGCCTGCTTCTTCTGGCTGGCA	95, 59, 72 45 s, 30 s, 1 min
TGFβ1	F: GCCCTGGACACCAACTATTGCT R: AGGCTCCAAATGTAGGGGCAGG	95, 58, 72 45 s, 30 s, 1 min
TGFβ2	F: GATTTCCATCTACAAGACCACGAGGGACTTGC R: CAGCATCAGTTACATCGAAGGAGAGCCATTCG	95, 58, 72 45 s, 30 s, 1 min
HGFR	F: TGGTCCTTGGCGTCGTCCTC R: CTCATCATCAGCGTTATCTTC	95, 54, 72 30 s, 45 s, 1 min
EGFR	F: CTACCACCACTCTTGAACTGGACCAAGG R: TCTATGCTCTCACCCCGTTCCAAGTATCG	95, 58, 72 45 s, 30 s, 1 min
TGFβ1R	F: CGTGCTGACATCTATGCAAT R: AGCTGCTCCATTGGCATAC	95 s, 54, 72 30 s, 45 s, 1 min
TGFβ2R	F: TGCACATCGTCCTGTGGAC R: GTCTCAAACTGCTCTGAAGTGTTC	95, 58, 72 45 s, 30 s, 1 min
FGFR	F: ATGTGGAGCTGGAAGTGCCTC R: GGTGTTATCTGTTTCTTTCTCC	95, 54, 72 30 s, 45 s, 1 min
IGF-1R	F: ACCCGGAGTACTTCAGCGCT R: CACAGAAGCTTCGTTGAGAA	95, 54, 72 30 s, 45 s, 1 min
HNF1α	F: GTGTCTACAACTGGTTTGCC R: TGTAGACACTGTCACTAAGG	95, 52, 72 45 s, 30 s, 1 min
HNF1β	F: GAAACAATGAGATCACTTCCTCC R: CTTTGTGCAATTGCCATGACTCC	95, 52, 72 1 m, 45 s, 1 min
HNF3β	F: CACCCTACGCCTTAACCAC R: GGTAGTAGGAGGTATCTGCGG	95, 56, 72 1 m, 45 s, 1 min
HNF4	F: CTGCTCGGAGCCACAAAGAGATCCATG R: ATCATCTGCCACGTGATGCTCTGCA	95, 58, 72 45 s, 30 s, 1 min
Albumin	F: AGTTTGCAGAAGTTTCCAAGTTAGTG R: AGGTCCGCCCTGTCATCAG	95, 55, 72 45 s, 30 s, 1 min
Apolipoprotein-a	F:AGGCTCGGCATTTCTGGCAG R: TATCCCAGAACTCCTGGGTC	95, 55, 72 45 s, 30 s, 1 min
HTF	F: TCGCTACAGCCTTTGCAATG R: TTGAGGGTACGGAGGAGTTCC	95, 55, 72 45 s, 30 s, 1 min
E-cadherin	F: TCCATTTCTTGGTCTACGCC R: TTTGTCCTACCGACTTCCAC	95, 55, 72 45 s, 30 s, 1 min
CYP 1B1	F: CACCAAGGCTGAGACAGTGA R: GCCAGGTAAACTCCAAGCAC	94, 57, 72 30 s, 30 s, 1 min
CYP 2C9	F: GGACAGAGACGACAAGCACA R: TGGTGGGGAGAAGGTCAAT	94, 57, 72 30 s, 30 s, 1 min
CYP 2B	F: GGCACACAGCCAAGTTTACA R: CCAGCAAAGAAGAGCGAGAG	94, 57, 72 30 s, 30 s, 1 min
CYP 3A4	F: TGTGCCTGAGAACACCAGAG R: GCAGAGGAGCCAAATCTACC	94, 57, 72 30 s, 30 s, 1 min
CYP 2E1	F: CCGCAAGCATTTTGACTACA R: GCTCCTTCACCCTTTCAGAC	94, 57, 72 30 s, 30 s, 1 min
CYP 1A1	F: AGGCTTTTACATCCCCAAGG R: GCAATGGTCTCACCGATACA	94, 57, 72 30 s, 30 s, 1 min
GAPDH	F: CCATGGAGAAGGCTGGGG R: CAAAGTTGTCATGGATGACC	95, 8, 72 45 s, 30 s, 1 min

Table 1 (*continued*)

Genes	Primer sequence 5′–3′	PCR parameters[a]
CD81	F: CTCAACTGTTGTGGCTCCAAC R: CCAATGAGGTACAGCTTCCC	95, 55, 72 45 s, 30 s, 1 min
TLR3	F: GATCTGTCTCATAATGGCTTG R: GACAGATTCCGAATGCTTGTG	95, 55, 72 45 s, 30 s, 1 min
TLR7	F: CCAGACATCTCCCCAGCGTC R: GGCAAAACAGTAGGGACGGC	95, 55, 72 45 s, 30 s, 1 min
TLR8	F: CTGTGAGTTATGCGCCGAAG R: CGGGATTTCCGTTCTGGTGC	95, 55, 72 45 s, 30 s, 1 min
Myd88	F: GGTCTCCTCCACATCCTCCC R: CCAGCTTGGTAAGCAGCTCG	95, 55, 72 45 s, 30 s, 1 min
IRF3	F: GAACCCCAAAGCCACGGATC R: CCTCCCGGGAACATATGCAC	95, 55, 72 45 s, 30 s, 1 min
IRF7	F: GTGCTGTTCGGAGAGTGGCTC R: CAGCCCAGGCCTTGAAGATG	95, 55, 72 45 s, 30 s, 1 min

CYP, cytochrome P450; EGFR, epidermal growth factor receptor; F, forward primer; FGFR, fibroblast growth factor receptor; GAPDH, glyceraldehyde phosphate dehydrogenase; HGF, hepatocyte growth factor; HGFR, hepatocyte growth factor receptor; HNF, hepatocyte nuclear factor; HTF, human transferrin; IGF-1R, insulin-like growth factor-type I receptor; IRF, interferon regulatory factor; R, reverse primer; TGF, transforming growth factor; TGFR, transforming growth factor receptor; TLR, toll like receptor.

[a] Temperatures are tabulated in the first lane in degrees celsius and the corresponding times in the second lane. Performing one-step RT-PCR, reverse transcription was carried out at 42 °C for 20 min with a pre-PCR denaturation at 95 °C for 10 min.

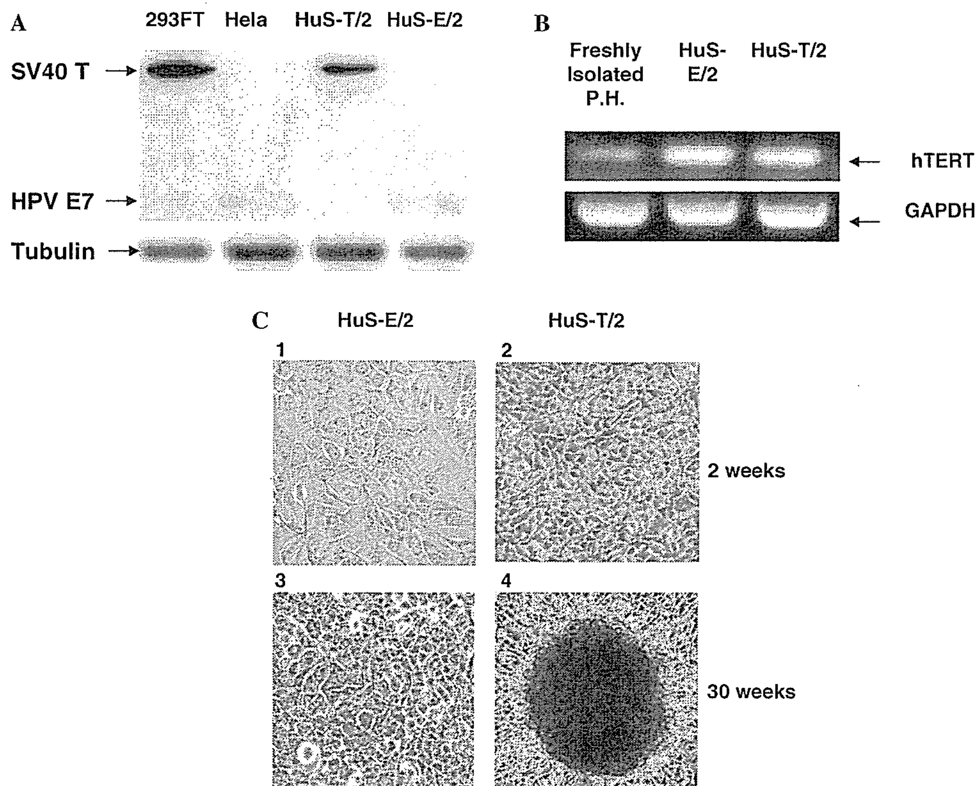

Fig. 1. (A) Immunoblot detection of SV40 T and HPV E7 expression in HuS-T/2 and HuS-E/2 cells, respectively. 293-FT and HeLa cells were used as positive controls for SV40 T and HPV E7 expression, respectively. The specific bands representing the targets are indicated. Detection of tubulin expression in all cells served as an internal control. (B) Human Telomerase Reverse Transcriptase (hTERT) expression was examined by RT-PCR in freshly isolated hepatocytes and the HuS-E/2 and HuS-T/2 cell lines. GAPDH expression was used as an internal control. The hTERT-specific bands are shown. (C) Morphological characteristics of HuS-E/2 and HuS-T/2 cells after two (panels 1 and 2) and 30 (panels 3 and 4) weeks in culture. [This figure appears in colour on the web.]

while the HPV18/E6E7-immortalized clones were named HuS-E cells and given numbers from 1 to 4. Expression of SV40 T and HPV E7 proteins was detected in the appropriate cells by immunoblot analysis (Fig. 1A). In both immortalized cell lines, expression of hTERT-mRNA was enhanced in comparison to non-transduced, freshly isolated hepatocytes as determined by RT-PCR (Fig. 1B). HuS-E cells were larger in size and exhibited slower growth than HuS-T cells (Fig. 1C).

3.2. Characterization of HuS-E and HuS-T immortalized hepatocytes

The HuS-E/2 and HuS-T/2 clones demonstrated the highest expression of hepatocyte-specific markers and transcription factors by RT-PCR (data not shown); these cells were used as representative for each group in this study. To address if HuS-E/2 and HuS-T/2 maintained similar characteristics as primary hepatocytes, they were both cultured continuously for 30 weeks and the expression profiles of a variety of growth factors (Fig. 2A), growth factor receptors (Fig. 2B), hepatocyte-specific nuclear factors (Fig. 2C), albumin, apolipoprotein-A1, transferrin (Fig. 2D), cytochrome p450 (CYP) genes (Fig. 2E), and GAPDH were compared with freshly isolated primary hepatocytes after isolation or two weeks of culture, Huh-7.5 cells, and 293 cells. After two weeks in culture, the expression of nearly all examined genes was similar between freshly isolated hepatocytes and the HuS-E/2 cell line. HuS-E/2 cells, however, exhibited higher expression of TGFβ2 (Fig. 2A), TGFβ2R, and HGFR (Fig. 2B) and lower expression of CYP 3A4 and 2C9 (Fig. 2E) in comparison to freshly isolated hepatocytes. Primary hepatocytes displayed reduced expression of TGFβ1 and TGFβ2 (Fig. 2A) and a loss of CYP1A1 expression (Fig. 2E) after two weeks of culture. HuS-E/2 cells exhibited higher expression of HGF (Fig. 2A), HGF receptor (Fig. 2B), HNF-4, (Fig. 2C), albumin, apolipoprotein-A1, HTF, and E-cadherin (Fig. 2D) in comparison to HuS-T/2 cells. Expression of CYP 3A4 (Fig. 2E) was lost from both HuS-T/2 and HuS-E/2 cells, while HuS-T/2 cells also lost the expression of HNF-1α (Fig. 2D), and CYPs 2B, 2E1 (Fig. 2E).

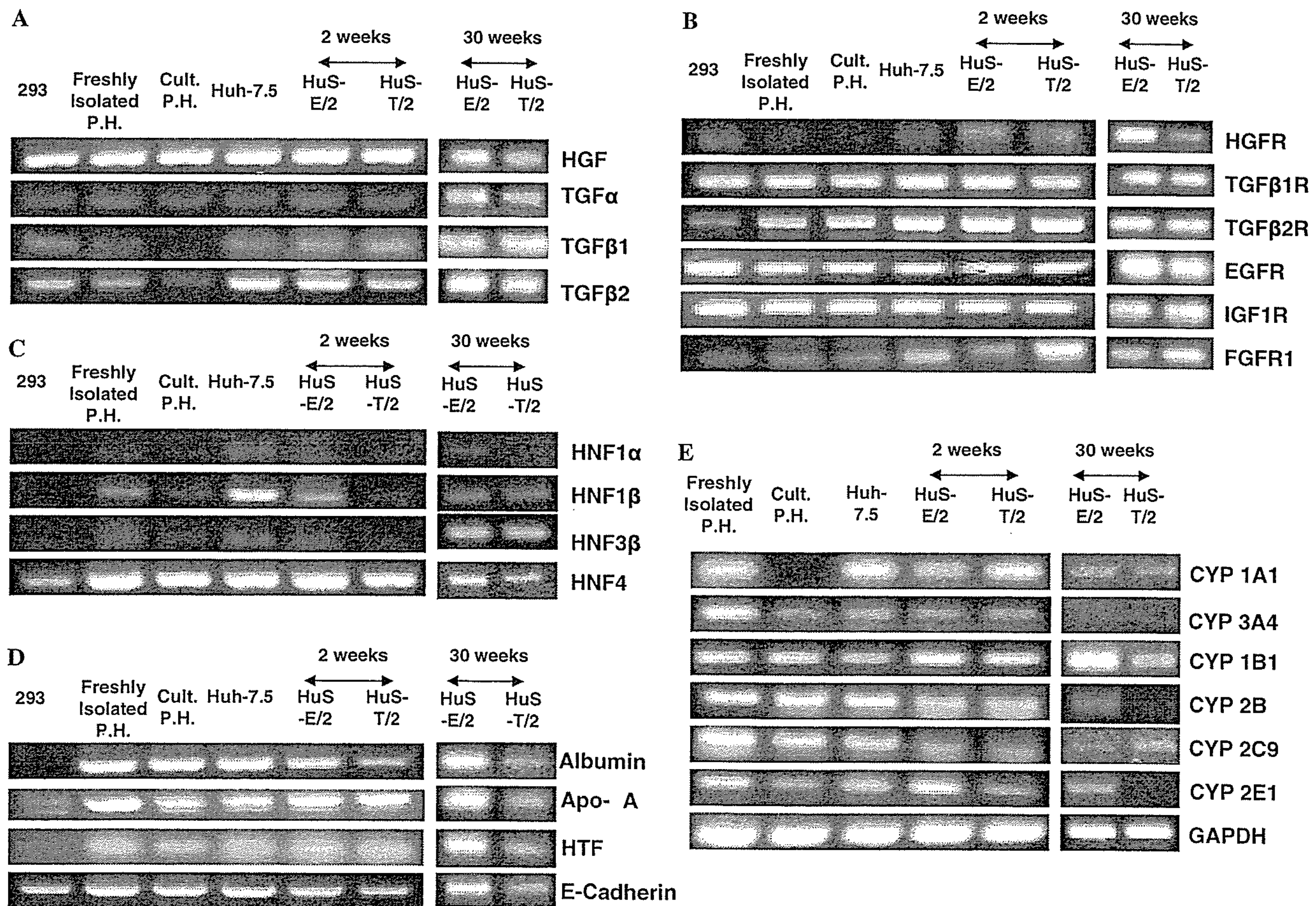

Fig. 2. Expression of the genes encoding growth factors (A), growth factor receptors (B), hepatocyte-specific nuclear factors (C), hepatocyte differentiation and functional markers (D), and CYP enzymes (E) in 293 cells, freshly isolated primary hepatocytes (P.H.), primary hepatocytes cultured for two weeks (Cult. P.H.), Huh-7.5 cells, and HuS-E/2 and HuS-T/2 cells cultured for two and 30 weeks were investigated by RT-PCR. The bands representing specific targets are indicated in the representative reactions.

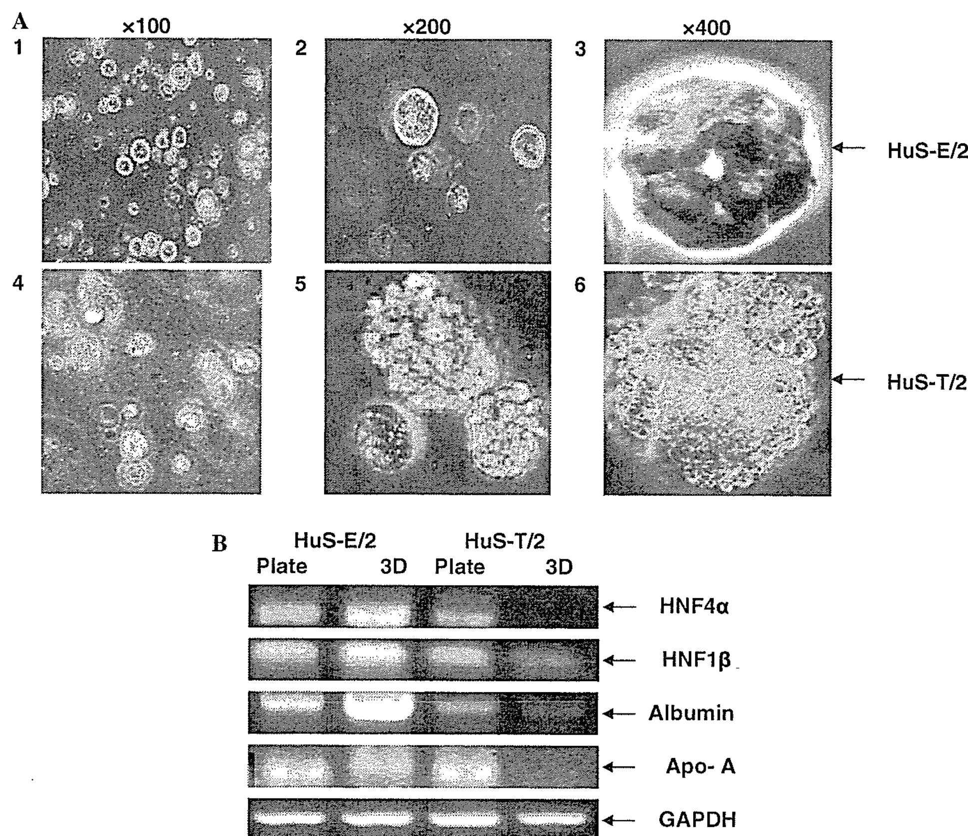

Fig. 3. (A) The morphology of HuS-E/2 and HuS-T/2 cells in 3D culture. HuSE/2 and HuS-T/2 cells were cultured in Mebiol Gel in 12-well plates at a concentration of 5×10^5 cells/well. The microscopic characteristics of these cells after one week of 3D culture are shown. (B) The expressions of HNF4α, HNF1β, albumin, and apo-A by HuS-E/2 and HuS-T/2 cells in both flat and 3D cultures are detailed. After one week of culture of HuS-E/2 and HuS-T/2 cells in flat and 3D cultures, the expressions of HNF4α, HNF1β, albumin, and apo-A were measured by RT-PCR in 250 ng total RNA.

HuS-T/2 but not in HuS-E/2 cells showed a transformed-like character starting from the 13th week of culture. This was demonstrated by continuing proliferation after confluence, pile-up formations (Fig. 1C), and proliferating in serum-deprevied condition. However, HuS-E/2 cells did not show any transformed-like characters even after 30 weeks of culture.

3.3. The characteristics of HuS-E and HuS-T immortalized hepatocytes in 3D culture

After one week in 3D culture, HuS-E/2 (Fig. 3A, panels 1, 2, and 3) cells adopted a donut-shaped structure with a central pore, while HuS-T/2 cells (Fig. 3A, panels 4, 5, and 6) displayed irregular mass formations (similar to the growth pattern of Huh-7.5 cells in 3D culture (data not shown)). In 3D culture, while the expression of HNF4, HNF1β, and albumin was enhanced in HuS-E/2, it was decreased in HuS-T/2 cells (Fig. 3B).

3.4. HCV infection to HuS-E/2

We further assessed the HCV infectivity of HuS-E- and HuS-T-derived clones by infection with HCV-1b-infected serum. Of the three HuS-E clones examined, HuS-E/2 clone demonstrated the highest infectability with HCV genotype 1b in comparison to Huh-7.5, PH5CH8 (Fig. 4A), and HuS-T cells (data not shown), which were excluded from further experiments.

3.5. Anti-CD81 blocked HCV infectivity

CD81 is involved in the entry of HCV pseudoparticles [24] and in vitro-synthesized JFH-1 [7]. To determine if authentic viral particles follow the same route of entry when infecting HuS-E/2 cells, we first examined the CD81 expression by RT-PCR. Both HuS-E/2 and HuS-T/2 cells expressed similar amounts of CD81 as freshly isolated hepatocytes and Huh-7.5 cells (Fig. 4B). Antibodies against CD81 reduced HCV infectivity of HuS-E/2 cells from the levels seen using a non-specific control antibody, confirming the importance of CD81 in HCV infectivity (Fig. 4C).

3.6. IFNα blocked HCV infectivity

We treated HuS-E/2 cells with HCV-containing serum. Cells were then cultured in fresh medium supplemented

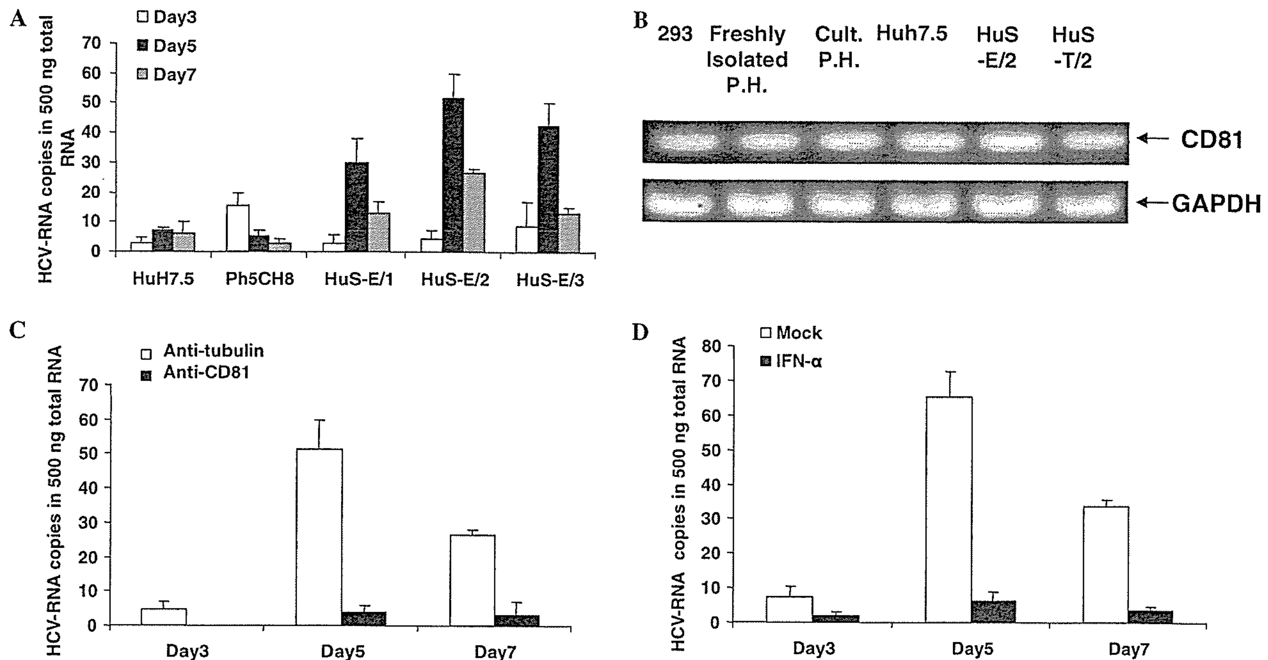

Fig. 4. (A) Serum from an HCV patient was used to infect Huh-7.5 cells, PH5CH8 cells, and three HPV E6E7-immortalized clones (HuS-E/1-3) for 24 h. After washing three times in phosphate-buffered saline (PBS), cells were cultured in fresh medium. Cells were then harvested and lysed at the indicated time points. The quantity of HCV genome RNA per 500 ng total RNA was determined by real-time RT-PCR analysis. (B) HuS-E/2 and HuS-T/2 cells both expressed CD81. Expression of CD81 (upper panel) and GAPDH as an internal control (lower panel) in 293 cells, freshly isolated P.H., cultured P.H., and Huh-7.5, HuS-E/2, and HuS-T/2 cells was investigated by RT-PCR. (C) Anti-CD81 antibodies blocked HCV infectivity. HCV infection was performed as described in (A) with the addition of CD81-specific (black bar) or anti-tubulin antibodies (control, white bar). (D) IFNα inhibits HCV multiplication in HuS-E/2 cells infected with HCV-containing serum. HuS-E/2 cells were infected with HCV as described in (A). After washing three times with PBS, cells were cultured in fresh medium supplemented with (black bar) or without (white bar) 100 U/ml IFNα.

without or with 100 U/ml IFNα. The enhancement of the HCV-RNA genome titers on the fifth day (about 10-fold) was not observed in cells treated continuously with IFNα (Fig. 4D). This result suggests that IFNα inhibited HCV replication in infected HuS-E/2 cells.

3.7. The effect of blocking IRF-3 and IRF-7 signaling on HCV infectivity

Production of interferon-alpha (IFNα) and interferon-beta (IFNβ) limits viral replication and spread, providing one of the most effective innate antiviral responses [25]. Signaling through IRF-3 and IRF-7 plays important roles in the stimulation of IFN-α/β production [25]. To determined which molecules (IRF-3 or IRF-7) play an important role in modulation of the innate immune response against HCV infection in these cells, we first detected intrinsic expression of double-stranded RNA-stimulated Toll-like receptor (TLR) 3, the downstream effector IRF-3, single-stranded RNA-stimulated TLR7, and 8, and the downstream effectors MyD88 and IRF-7 by RT-PCR. TLR3 exhibited very low expression in freshly isolated hepatocytes, Huh-7.5, HuS-E/2, and HuS-T/2 cells, while TLR7, TLR8, MyD88, and IRF-7 were easily detectable in both freshly isolated and immortalized cell lines (Fig. 5A).

The abilities of DNIRF-3 and DNIRF-7 to inhibit IFNβ and IFNα production by HuS-E/2 cells infected with Sendai virus were confirmed using assays of IFNβ or IFNα promoter-driven luciferase reporters. DNIRF-3 exhibited strong inhibition of IFNβ production (Fig. 5B) and weaker inhibition of IFNα transcription (Fig. 5C), while DNIRF-7 strongly inhibited IFNα production (Fig. 5C) and only weakly inhibited IFNβ production (Fig. 5B).

We then assessed the inhibition of HCV infectivity by DNIRF-3 and DNIRF-7. Transient transfection with DNIRF-3, DNIRF-7, or an empty vector was performed prior to HCV infection. Using Effectene reagent, the efficiency of plasmid transfection into HuS-E/2 cells was approximately 70% (data not shown). While there was no significant effect of DNIRF-3 on HCV infectivity, DNIRF-7 demonstrated a marked increase in HCV titers on days 3 and 5 after infection in comparison to control cells (Fig. 5D). To confirm that the enhancement of HCV replication by DNIRF-7 is not mediated by the impairment of IRF-3 signaling by heterodimeric interactions between IRF-3 and DNIRF-7, we performed siRNA inhibition of IRF-3 and IRF-7. The reduction of IRF-3 and IRF-7 expression by siRNA was obvious by RT-PCR (Fig. 5E). siRNA-mediated suppression of either IRF-3 or IRF-7 inhibited IFNβ and IFNα production

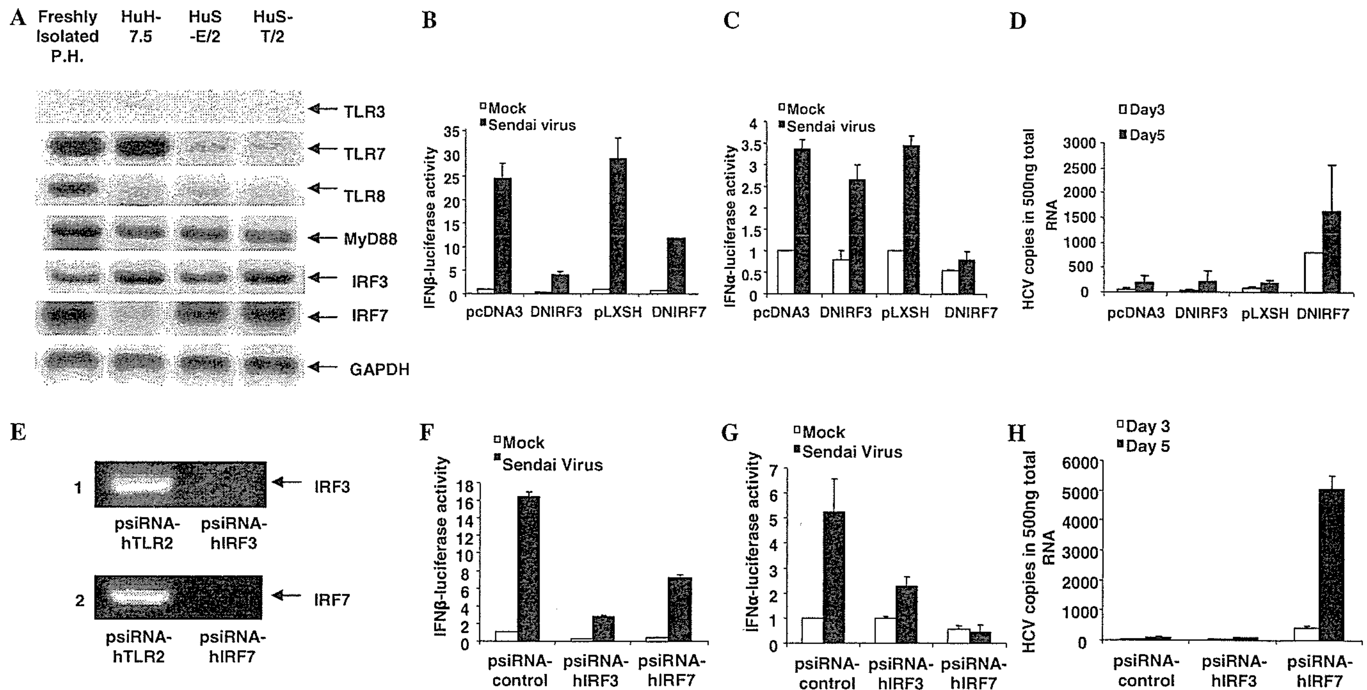

Fig. 5. (A) We examined the expression of TLR3, TLR7, TLR8, MyD88, IRF-3, and IRF-7, as well as GAPDH as an internal control in freshly isolated primary hepatocytes and Huh-7.5, HuS-E/2, and HuS-T/2 cells was investigated by RT-PCR. (B and C) HuS-E/2 cells were cotransfected with pIFNβ-luc (B) or pIFNα-luc (C) with an expression plasmid encoding DNIRF-3, DNIRF-7, or the appropriate empty vector (pcDNA3 and PLXSH, respectively). Twenty-four hours later, cells were infected (black bar) with Sendai virus or mock-infected (white bar), then analyzed for luciferase activity after 12 h. (D) IRF-7, but not IRF-3, suppression enhanced HCV infectivity of HuS-E/2 cells. HuS-E/2 cells were transiently transfected with empty pcDNA3, DNIRF-3, empty pLXSH, or DNIRF-7 plasmids. Twenty-four hours later, serum from a patient with HCV was used to infect transfected cells for 24 h. After washing, cells were cultured in fresh medium. The cells were then harvested and lysed at the indicated time points. The quantity of HCV genome RNA per 500 ng total RNA was determined by real-time RT-PCR analysis. (E) IRF-3 and IRF-7 levels were suppressed by specific siRNAs. HuS-E/2 cells were transfected with control psiRNA-hTLR2, psiRNA-hIRF-3, or psiRNA-hIRF-7, then selected with Zeocin at 250 μg/ml. Two weeks later, cells were harvested and assessed for the expression of IRF-3 and IRF-7 by RT-PCR. (F and G) HuS-E/2 cells were transfected with control psiRNA-hTLR2, psiRNA-hIRF-3, or psiRNA-hIRF-7, followed by selection in Zeocin at 250 μg/ml. Two weeks later, cells were cotransfected with pIFNβ-luc (F) or pIFNα-luc (G). Twenty-four hours later, cells were infected (black bar) with Sendai virus or mock-infected (white bar), then analyzed for luciferase activity after 12 h. (H) Transfected cells were infected with serum from HCV patient; HCV infectivity was assessed as described above.

in HuS-E/2 cells infected with Sendai virus in patterns similar to the effects seen following DNIRF-3 and DNIRF-7 expression, respectively (Figs. 5F and G). Blockade of IRF-7 expression resulted in a significantly higher titer of HCV after infection, while IRF-3 down-regulation did not have any significant effect on HCV titers (Fig. 5H). The enhancement of IRF-7 silencing by siRNA improved the infectivity of HCV (data not shown). These results suggest that IRF-7 plays the major role in the innate immune response to HCV in HuS-E/2 cells.

3.8. Establishment of stable DNIRF-7 expressing clones derived from HuS-E/2 cells

Since DNIRF-7 enhanced HCV infectivity, we transduced the plasmid encoding DNIRF-7 and a hygromycin-B resistance gene, into HuS-E/2 cells. Following selection with hygromycin-B, we obtained the HuS-E7/DN22 and HuS-E7/DN24 clones. As detected by RT-PCR, both clones demonstrated similar expression levels of albumin, apolipoprotein-A1, and HNF4 as the parental HuS-E/2 cells (Fig. 6A). The HuS-E7/DN24 clone exhibited stronger expression of DNIRF-7 than the HuS-E7/DN22 clone by immunoblotting (Fig. 6B). The induction of IFNα in HuS-E7/DN24 in response to infection with an RNA virus (Sendai virus) was low in comparison to the parental HuS-E/2 and HuS-E7/DN22 clones, as detected by IFNα-luciferase reporter assay (Fig. 6C). HuS-E7/DN24 also exhibited a higher HCV infectability in comparison to parental HuS-E/2 cells and the HuS-E7/DN22 clone (Fig. 6D).

3.9. Infection of HuS-E7/DN24 cells with different HCV genotypes

Huh7.5 and HuS-E7/DN24 cells were separately infected with serums derived from 3 different HCV-patients or by JFH-1 concentrated medium (HCV-2a). Two serums were infected by HCV-1b, while the third by HCV-2b. Inoculated virus titer was adjusted to be the same in all cases. Except for JFH-1, which efficiently

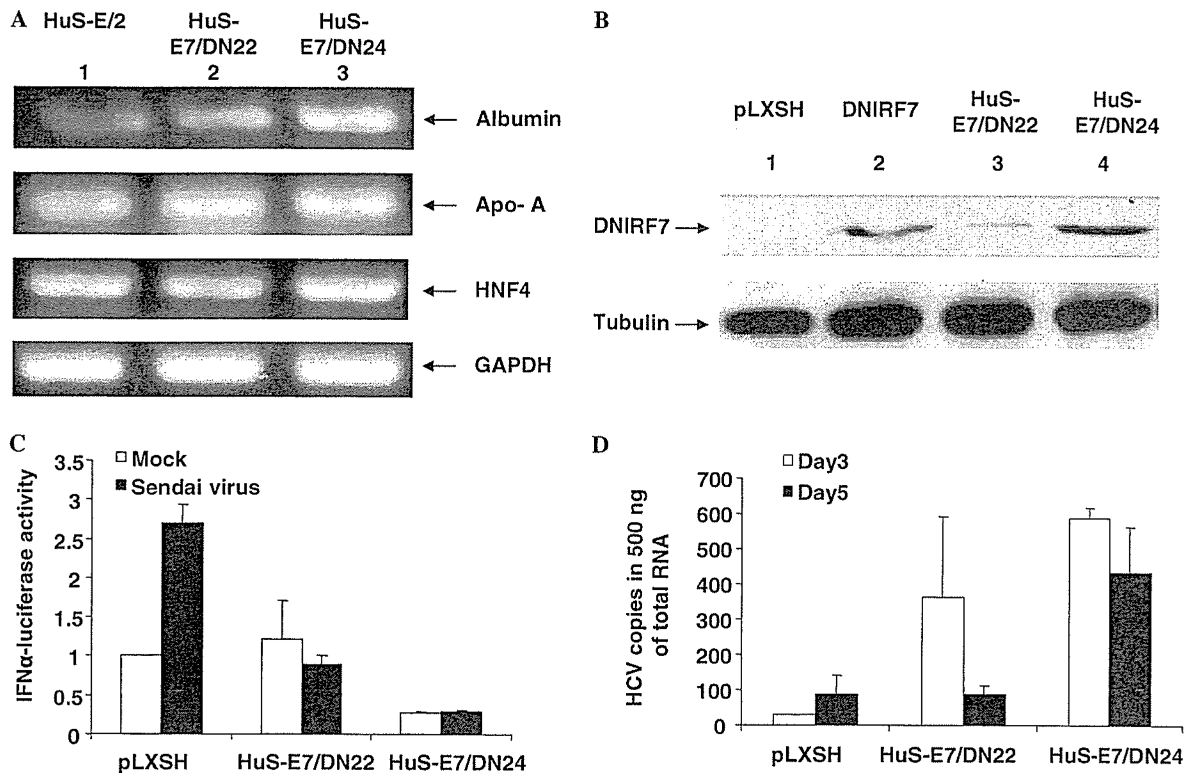

Fig. 6. (A) The pLXSH-HA-DNIRF-7 plasmid was transfected into HuS-E/2 cells, followed by selection in 100 μg/ml Hygromycin B. Two clones, HuS-E7/DN22 (lane 2) and HuS-E7/DN24 (lane 3), were obtained. We investigated the expression of albumin, apo-A, HNF4, and GAPDH as an internal control in parental HuS-E/2, HuS-E7/DN22, and HuS-E7/DN24 hepatocytes cultured for two weeks by RT-PCR. (B) Expression of HA-tagged DNIRF-7 (upper panel) and tubulin (control, lower panel) was detected by immunoblotting analysis. HuS-E/2 cells transiently transfected with either empty pLXSH vector (lane 1) or pLXSH-HA-DNIRF-7 (lane 2) were used as negative and positive controls, respectively, after 48 h. (C) HuS-E/2, HuS-E7/DN24, and HuS-E7/DN22 cells were transfected with IFNα-luc. HuS-E/2 cells were also cotransfected with pLXSH. All of these cells were then infected (black bar) or with Sendai virus or mock-infected, then analyzed for luciferase activity after 12 h. (D) HuS-E7/DN24 cells exhibited high infectivity to HCV samples derived from patient serum. HuS-E/2 cells were transiently transfected with empty pLXSH. Twenty-four hours later, serum from a recurrently transplanted HCV patient was used to infect transfected cells and HuS-E7/DN22 and HuS-E7/DN24 cells for 24 h. After washing three times, cells were cultured in fresh medium. Cells were then harvested and lysed at the indicated time points.

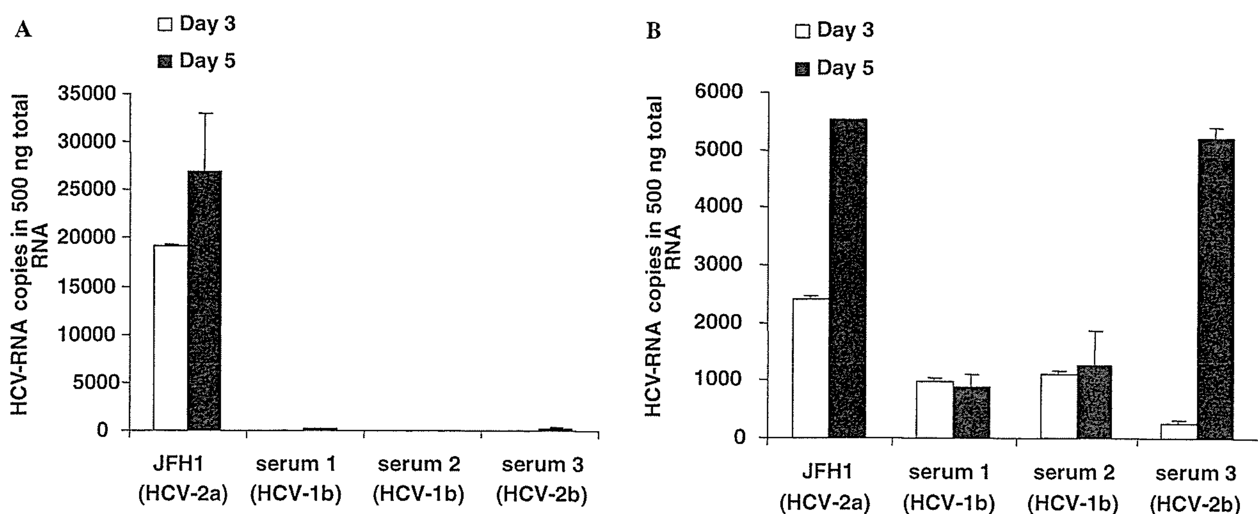

Fig. 7. The infectability of Huh-7.5 and HuS-E7/DN24 cells to different HCV genotypes. Huh-7.5 (A) and HuS-E7/DN24 (B) cells were infected with same titer of JFH1 (HCV-2a), two different HCV-1b serums and one HCV-2b serum. After removing the infected medium, the cells were washed in PBS and recultured in fresh medium. Cells were harvested and lysed at the indicated time points. The quantity of HCV genome RNA per 500 ng RNA was detected by real-time RT-PCR analysis.

replicated in Huh7.5 cells (Fig. 7A), HuS-E7/DN24 cells showed a higher and reproducible infectability for the different HCV strains than Huh7.5 cells (Fig. 7B). Similar higher infectability of HuS-E7/DN24 cells was observed with HCV-4a genotype (unpublished data). These results suggest that the high infectability of Huh-7.5 with JFH-1 is specific among the combinations of HCV strains and cell lines; while HuS-E7/DN24 cells were generally permissive to HCV-infected serum independent of HCV strains.

4. Discussion

This study demonstrates that ectopic expression of the HPV18/E6E7 genes in combination with hTERT could efficiently immortalize mature human hepatocytes, generating a cell line with stable expression of hepatocyte markers and functions for more than 30 weeks in culture. HuS-E/2 cells continuously exhibited higher expression of both HGF and HGFR than HuS-T/2 cells. This result suggests that HPV18/E6E7-immortalized hepatocytes maintain responsiveness to paracrine signals capable of inducing cell differentiation to a greater extent than SV40 T-immortalized hepatocytes. This conclusion is further supported by the increased expression of HNF4 in HuS-E/2 cells in comparison to HuS-T/2 cells. HNF4 is a major hepatocyte transcription factor, required for hepatocyte differentiation and liver-specific gene expression [26]. HNF4 drives hepatocytes differentiation by acting upstream in a transcription factor cascade that included HNF1α [27]. HuS-E/2 cells continued to express HNF1α throughout prolonged culture, while HuS-T/2 cells lost expression completely. Maintenance of hepatocellular functions was demonstrated by continuous and high expression of albumin, apolipoprotein-A, human transferrin, and E-cadherin by HuS-E/2 in comparison to HuS-T/2 cells. These differences became more pronounced in the late passages. In a similar manner, HuS-E/2 cells continued to express all of the examined CYP genes, with the exception of CYP 3A4, while HuS-T/2 cells lost expression of CYP 3A4, 1B, and 2E1 completely and displayed markedly lower expression of CYP 1B1 than HuS-E/2 cells. Thus, human hepatocytes immortalized by HPV E6/E7 transfection are phenotypically similar to primary hepatocytes, even during extended cultures.

Recently, it was reported that the JFH-1 strain and derived chimeras could only infect and propagate efficiently in Huh7.5.1 and Huh7.5 cells, both of which are subclones of Huh7 cells [7–9]. This limitation, however, may be specific to the JFH-1 **strain**, which may not accurately reflect the course of other HCV strains' infection. Thus, usage of HCV particles isolated from patient serum could be more useful to study authentic HCV infection. Using sera from HCV patients as a source of infective virus, HPV18/E6E7-immortalized cell lines exhibited higher reproducible susceptibility to HCV infection than HuS-T, PH5CH8, and Huh-7.5 cell lines.

IRF3 and IRF7 play an important role in the activation of interferon signaling [28]. We suppressed the functions of IRF-3 or IRF-7 to assess their role in HCV infectivity. In fact, we observed significant increase of HCV replication in HuS-E/2 cells bearing dominant-negative IRF7 that impaired IFN signaling. The suppression of IRF-3, however, did not have any significant effect on HCV infectivity or replication in this cell line. This may result from the blockade of IRF-3 activation by an HCV NS3/4A serine protease [29] through at least two independent pathways that inhibit the TLR3-dependent and RIG-I-dependent signaling pathways [29–33]. Although HCV was shown to inhibit basal expression levels of IRF-7 at both mRNA and protein levels and it was shown that NS5A suppresses IRF-7-induced IFNα promoter activation [34], Stimulation of TLR7 was shown to activate IRF-7 and induce suppression of HCV replicon levels in Huh-7 cells [35]. This suggests that the inhibition of IRF7 by HCV is not complete. Using IRF-7-deficient (IRF-7−/−) mice, Honda [36] demonstrated that the transcription factor IRF-7 is essential for the induction of IFNα/β genes. We established a clone stably expressing DNIRF-7 (HuS-7E/DN24), which demonstrated higher infectivity with different HCV strains than the parental HuS-E/2 clone.

In summary, we have established a human hepatocyte-derived cell line that maintains the characteristic features of primary hepatocytes by transduction with HPV18/E6E7. This cell line is highly infectable by HCV, which suggests that these cells may be useful to characterize the molecular mechanisms involved with HCV infection and to develop novel HCV treatment modalities.

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肝炎等克服緊急対策研究事業

生体肝移植後のC型肝炎再発予防を目指したステロイド剤不使用による免疫抑制療法に関する研究

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分担研究報告書

生体肝移植後のＣ型肝炎再発予防を目指したステロイド剤不使用による免疫抑制療法に関する研究

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研究要旨
C型肝硬変患者に対する生体肝移植後の肝炎の再発率は高く、再発後、早期に肝硬変に至る症例と共に、中には胆汁うっ滞による高ビリルビン血症を伴って激烈な経過により死亡する症例もあり、その治療戦略の確立が望まれている。本研究は、肝炎再発防止を目指し、移植後ステロイド剤を使用しない、新しい免疫抑制療法に関する無作為比較試験を行い、その効果を判定しようとするものである。

A. 研究目的

C 型肝炎ウィルス(HCV)感染による肝硬変ならびに合併する肝細胞癌は、現在肝疾患のなかでも死亡原因となる最大の疾患であり、その治療法として肝移植に期待がかけられている。しかし、近年海外における肝移植医療において肝移植後の HCV 肝炎再発が移植肝の予後を左右する重要な問題となっており、肝炎再発に関連するグラフト機能不全のために他の疾患に比べて 5 年以降の長期予後が有意に不良であることが示されている。

肝移植後のHCV肝炎再発の特徴として、ウィルス量が肝移植後に急速に上昇しその値は移植前に比べて非常に高くなること、慢性肝炎から肝硬変への進展が早い、すなわち肝の線維化速度が速いことなどが挙げられ、その原因として移植後免疫抑制療法の影響が考えられている。特に、ステロイド剤は HCV の増殖を促進すると言われ、移植後 HCV 肝炎再発防止のためにはこれまでのステロイドを中心とした免疫抑制療法の見直しが必要である。

本研究本年度の目的は、１）当施設で実施した C 型肝炎への生体肝移植後の肝炎再発について、再発の形式および再発に対する治療成績を検討することにより、その実態を明らかにする、２）生体肝移植後肝炎再発防止を目指したステロイドフリーによる免疫抑制療法を従来法と比較検討し、新しい免疫抑制プロトコールの確立をめざす。