Monoclonal Antibody 2-152a Suppresses Hepatitis C Virus Infection Through Betaine/GABA Transporter-1

Masaaki Satoh,1 Makoto Saito,1 Takashi Takano,1,2 Yuri Kasama,1 Tomohiro Nishimura,1,3 Yasumasa Nishito,4 Yuichi Hirata,2 Masaaki Arai,2 Masayuki Sudoh,5 Chieko Kai,6 Michinori Kohara,2 and Kyoko Tsukiyama-Kohara1

1Department of Experimental Phylaxiology, Faculty of Life Sciences, Kumamoto University, Honjo Kumamoto City; 2Department of Microbiology and Cell Biology, Tokyo Metropolitan Institute of Medical Science, Kamikitaawa, Setagaya-ku; 3KAKETSUKEN, Kyokushi, Kikuchi, Kumamoto; 4Center for Microarray Analysis, Tokyo Metropolitan Institute of Medical Science; 5Kamakura Research Laboratories, Chugai Pharmaceutical Co., Ltd., Kajiwara, Kamakura-City, Kanagawa; and 6Laboratory of Animal Research Center, Institute of Medical Science, University of Tokyo, Shirokane-dai Minato-Ku, Japan

Background. We recently established a monoclonal antibody (2-152a MAb) that binds to 3β-hydroxysterol-D24-reductase (DHCR24) by immunizing mice with cells (RzM6-LC) persistently expressing hepatitis C virus (HCV). Here, we aimed to analyze the activity of 2-152a MAb against HCV replication and explore the molecular mechanism underlying the antiviral activity.

Methods. We characterized the effects of 2-152a MAb on HCV replication and performed a microarray analysis of antibody-treated HCV replicon cells. The molecules showing a significant change after the antibody treatment were screened to examine their relationship with HCV replication.

Results. The antibody had antiviral activity both in vitro and in vivo (chimeric mice). In the microarray analysis, 2-152a MAB significantly suppressed the expression of betaine/GABA transporter-1 (BGT-1) in 2 HCV replicon cell lines but not in HCV-cured cells. Silencing of BGT-1 expression by small interfering RNA (siRNA) revealed significant suppression of HCV replication and infection without cytotoxicity. Further, BGT-1 expression was significantly increased in the presence of HCV (P < .05).

Conclusions. Our results suggest that 2-152a MAb suppresses HCV replication and infection through BGT-1. These findings highlight important roles of BGT-1 in HCV replication and reveal a possible target for anti-HCV therapy.

Hepatitis C virus (HCV) causes chronic hepatitis and hepatocellular carcinoma (HCC) [1–3]. Chronic HCV infection is a major global public health concern because it affects at least 170 million people worldwide [2]. The most effective treatment against HCV currently comprises a combination therapy of PEGylated α-interferon (IFN-α) and ribavirin [4, 5]. However, considering that sustained virological responses develop in only approximately half of the patients infected with HCV genotype 1, the clinical efficacy of this therapy is limited [6, 7]. Efforts to develop therapies against HCV are further hindered by the high level of viral variation and capacity of the virus to cause chronic infection. Therefore, there is an urgent need to develop effective treatments against chronic HCV infection.

In a previous study, we established a cell line expressing HCV (RzM6-LC) to investigate the effects of persistent HCV expression on cell growth [8]. We also established a monoclonal antibody (2-152a MAb) against the RzM6-LC cell line to produce clones that recognize both cell surface and intracellular molecules. Using this method, we identified 3β-hydroxysterol-D24-reductase (DHCR24) as the recognition molecule of this antibody.
DHCR24 (also termed seladin-1) is an enzyme that catalyzes the conversion of desmosterol to cholesterol in the postqualene cholesterol biosynthetic pathway [9, 10]. DHCR24 also acts as a hydrogen peroxide scavenger [11]. Therefore, DHCR24 may play a crucial role in maintaining cell physiology through cholesterol synthesis and oxidative stress. We previously demonstrated that HCV infection upregulates DHCR24 expression, and overexpression of DHCR24 inhibits apoptosis and inactivates the tumor suppressor gene p53 [12]. Moreover, silencing of DHCR24 suppressed HCV replication [13]. However, the precise mechanisms through which DHCR24 affects the HCV life cycle are unclear. In this study, we aimed to analyze the activity of 2-152a MAb against HCV replication and explore the molecular mechanism underlying the antiviral activity.

Materials And Methods

Cell Lines and Reagents

Human hepatoma cell line HuH-7 cell-based HCV replicon-harboring cell lines [14] R6FLR-N (genotype 1b) [15], FLR3-1 (genotype 1b) [16], and JFH-1 (genotype 2a) [17] were maintained in Dulbecco's modified Eagle's medium (DMEM) GlutaMAX (Invitrogen) containing 10% fetal calf serum (FCS; Sigma-Aldrich) in the presence of G418 (500 mg/mL for R6FLR-N and FLR3-1, 300 mg/mL for JFH-1; Invitrogen). Cured/HuH-7 histone H3 lysine 4 (K4) cells cured off HCV by interferon treatment [18] were maintained in DMEM GlutaMAX containing 10% FCS without G418. The JFH/K4 cell line persistently infected with the HCV JFH-1 strain and HuH-7 cell lines were maintained in DMEM containing 10% FCS [19]. The human hepatoblastoma HepG2 cell line was also maintained in DMEM containing 10% FCS.

Generation of 2-152a MAb

BALB/c strain of mice was immunized with 7–8 intraperitoneal injections of RzM6-LC cells (5 × 10^6) in RBI adjuvant (trehalose dimycolate + monophosphoryl lipid A emulsion; RBI ImmunoChem Research). After completion of the immunization regimen, their spleens were excised and splenocytes were fused with mouse myeloma plasminogen activator inhibitor (PAI) cells by using PEG1500 (Roche). Hybridoma cells were then selected with hypoxanthine, aminopterin, and thymidine (HCR6) [8, 21], in accordance with the Declaration of Helsinki. The protocols for the animal experiments were preapproved by the local ethics committee, and the animals were maintained in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. HCV genotype 1b RNA levels were established at 0.96–1.84 × 10^7 copies/mL in mouse serum samples before the antibody treatment. The antibody (2-152a MAb) and normal immunoglobulin G (IgG, 400 mg/20 g body weight) were intraperitoneally injected into the mice (n = 4) at 2-day intervals over a period of 14 days. IFN-α (30 mg/kg) was administered subcutaneously at 2-day intervals over a period of 2 weeks. Human serum albumin in the blood of chimeric mice was measured by using an Alb-II kit according to the manufacturer’s instructions (Eiken Chemical). HCV RNA levels in serum and JFH/K4 cells were measured by real-time detection polymerase chain reaction (real-time detection [RTD]-PCR) as described previously [22]. HCV RNA in the cell cultures and supernatants was extracted by using Isogene and Isogene LS (Nippon Gene), respectively.

Replication Assay Using HCV Replicon Cells

We used 3 HCV subgenomic replicon cell lines: R6FLR-N, FLR3-1, and JFH-1. They were seeded at a density of 5 × 10^3 cells/well in 96-well tissue culture plates in DMEM GlutaMAX (Invitrogen) containing 5% fetal bovine serum (Thermo Scientific). Following incubation for 24 hours at 37°C (in 5% CO2), the medium was removed and serial dilutions of antibody were added. Luciferase activity was determined by using a Bright-Glo luciferase assay kit (Promega) after 72 hours according to the manufacturer’s instructions. The results were calculated as the average percentage relative to the reactivity in untreated cells, which was set at 100%. The viability of the replicon cells was measured by using a WST-8 cell counting kit (Dojindo) according to the manufacturer’s instructions.

Immunostaining and Antibodies

Cells were cultured on glass coverslips (1.0 cm diameter) and fixed with 1% paraformaldehyde in phosphate-buffered saline (PBS) at room temperature for 10 minutes in 24-well plates. To permeabilize the cell membranes, the cells were treated with 1% Triton X-100 in PBS at room temperature for 10 minutes. After washing with 0.05% Tween-20 in PBS, the cells were incubated with 2-152a MAb, antiprotein disulfate isomerase (PDI) rabbit polyclonal antibody (Stressgen Bioreagents) or normal mouse IgG for 1 hour and washed with 0.05% Tween-20 in PBS. Alexa Fluor 488-labeled goat antimouse IgG was used as the secondary antibody.

Anti-NSSA antibody was provided by Dr Yoshiharu Matsuura (Osaka University). Anti-myc mouse monoclonal antibody BGT-1 Plays a Role in HCV Replication • JID 2011:204 (15 October) • 1173
Figure 1. Anti-DHCR24 monoclonal antibody (2-152a MAb) suppresses HCV replication in vitro and in vivo. A, The effects of 2-152a MAb on HCV replication were measured by the luminescence activity and cell viability in FLR3-1 cells. The replicon cell line was incubated with IgG from normal mice or 2-152a MAb at 1 or 10 μg/mL for 24, 48, and 72 hours. The mean values from triplicate wells are indicated, and the vertical bars represent the standard deviation. The medium control (2% FCS-DMEM) without IgG is indicated as 0. B, The JFH/K4 cells were treated with cholesterol synthesis inhibitor U18666A (1 mM, 10 mM), IFN-α (250 IU/mL), Cyclosporin A (25 μM) and its solvent Cremophor, normal mouse IgG (10 μg/mL), and 2-152a IgG (10 μg/mL). HCV core and actin proteins were detected. C, HCV RNA copies were measured in JFH/K4 cells after treatment with normal or 2-152a IgG.
(9E10; Cell Signaling Technology) and antiactin mouse monoclonal antibodies (Sigma-Aldrich) were utilized for detecting myc-fusion protein and normalization of the results, respectively.

**cDNA Synthesis and Quantitative Reverse Transcriptase PCR**

cDNA was synthesized from 0.5 or 1 mg of total RNA with a Superscript II kit (Invitrogen). TaqMan gene expression assays were custom designed and manufactured by Applied Biosystems. The expression was quantified with the ABI 7500 real-time PCR system (Applied Biosystems).

**Microarray Analysis**

For microarray analysis, total RNAs were extracted using RNAeasy kit (Qiagen), and RNA integrity was assessed using a Bioanalyzer (Agilent Technologies). cRNA targets were synthesized and hybridized with Whole Human Genome Oligo Microarray (G4112F; Agilent) according to the manufacturer’s instructions.

**RNA Interference, Expression Vector Construction, Transfection, and Rescue Experiments**

Small interference RNA (siRNA) targeting betaine/GABA transporter-1 (BGT-1; nucleotides 120–144) was designed by using a program (https://rnaidesigner.invitrogen.com/) based on registered sequences in GenBank (5′-CACCAAGATGGAGTAGTTGTGCTGTCA-3′). Alternative siRNA (BGT-1-siRNA-362; nucleotides 362–386) was similarly designed. The HCV-siRNA (R7) sequence was 5′-GUCUCGUAGACCGUGCACCAATTGGG-3′ (the mutations introduced by mutagenesis are underlined).

The coding region of the BGT-1 gene was obtained from RNA of R6FLR-N cells by reverse transcription–polymerase chain reaction (RT-PCR). The PCR products were inserted in EcoRV–XhoI sites of pcDNA6-myc His, version A (Invitrogen) after digestion of EcoRV–XhoI. To generate mutant plasmids that contained nucleotide substitutions in the siRNA-targeted site, we introduced point mutations into pcDNA-BGT-1 by using site-directed mutagenesis with a QuickChange multisite-directed mutagenesis kit (Stratagene), according to the manufacturer’s instructions, and the following oligonucleotide primer: BGT-1-mut, 5′-CCAATGGAGACAAAGATTGGAATTCTGTCTATCGGTGGCCGGAGACTCATGTGGG-3′ (the mutations introduced by mutagenesis are underlined).

Transfection of siRNAs was carried out by reverse transfection using Lipofectamine RNAiMAX according to the manufacturer’s protocol (Invitrogen). Transfection of the expression vector was undertaken by using Lipofectamine LTX with Plus reagent (Invitrogen).

The rescue experiment was performed after reverse transfection of BGT-1 siRNA (1.5 nM) into R6FLR-N cells by using RNAiMAX reagent. After 48 hours, wild-type (wt) and mutant (mut) BGT-1 expression vectors (10 ng) were transfected by using Lipofectamine LTX, and the luciferase activity and cell viability were assessed by WST-8 assay (Dojindo) after 24 hours.

**Analysis of HCV Infection and BGT-1 Expression**

For infection assays, Cured/HuH-7 K4 cells were incubated with JFH/K4 cell-derived HCV (2.0 × 10^6 copies/mL). At 72 hours after incubation, HCV infection and BGT-1 expression were analyzed by real-time detection (RTD)-PCR and TaqMan expression assay, respectively, as described earlier.

**Statistical Analysis**

The Student t test was used to test the statistical significance of the results. P values < .05 were considered statistically significant.

**Results**

**Inhibitory Effect of 2-152a MAb on HCV Replication In Vitro**

We examined the effects of 2-152a MAb on HCV replication and the viability in HCV replicon cell lines. The treatment with 2-152a MAb significantly decreased HCV replication after 48 hours and cell viability after 72 hours (Figure 1A). To determine the recognition site of 2-152a MAb, we performed epitope mapping by using serial overlapping deletion mutants of the DHCR24 fusion protein (Supplementary Figure 1A). The recognition site was identified within amino acid residues 259–314 (Supplementary Figure 1B) and the predicted “Di-minuto-like protein” homologous region [23] indicated in Supplementary Figure 1A.

**Suppression of HCV Infection by 2-152a MAb**

To determine the effects of 2-152a MAb on HCV infection, we inoculated the antibody into a persistently HCV-infected cell line (JFH/K4; Figure 1B and C) or uPA-SCID chimeric mice previously transplanted with human hepatocytes [20] and...
Figure 2. A, Genes that showed significant changes in expression after the 2-152a MAb treatment. HCV replicon cells (FLR3-1 and R6FLR-N) and K4 cells were treated with 2-152a MAb. The symbols shaded in gray indicate the genes that showed significantly changed expression commonly in R6FLR-N and FLR3-1 cells, and those shaded in orange indicate the genes that showed significantly changed expression in K4 cells. The amount of labeled probe for microarray analysis was 7-fold higher than that in the first experiment (Supplementary Table 1). Each value indicates the number of ratios of signal 2-152a MAb/normal IgG treatment.

B, TaqMan expression assay of BGT-1 in samples of R6FLR-N cells treated with 2-152a MAb or normal IgG. BGT-1 mRNA (0.5 μg) samples treated with 2-152a MAb or normal IgG were transcribed by reverse transcriptase, and synthesized cDNAs were used for TaqMan.
infected with HCV (Figure 1D and F). We detected viral protein (core) (Figure 1B) or viral RNA in cells (Figure 1C) and mouse blood by using RTD-PCR (Figure 1D). There was a significant reduction in the viral titers with 2-152a MAb treatment compared with that in normal IgG treatment (control) (P < .005, Figure 1C and D). No significant effects on body weight were observed by the inoculation of 2-152a MAb (Figure 1E). Further, no significant differences were found among the levels of human albumin in the sera of the normal IgG- and 2-152a MAb-inoculated mice (Figure 1F).

Expression of DHCR24 in Carcinoma Cells and on the Surface of HuH-7-Derived Cells
We observed abundant intracellular expression of DHCR24 in hepatoma cell lines in the previous study [12]; therefore, we characterized its expression on the surface of various carcinoma cell lines by flow cytometric analysis to clarify the mechanism of 2-152a MAb antiviral effects. In this analysis, DHCR24 expression was localized to the surface of the HuH-7 and HuH-7-based cell lines, HCV replicon cell lines (R6FLR-N, FLR3-1, and JFH-1), HCV persistently infected cell line (JFH/K4), and K4 cells; on the other hand, DHCR24 was not significantly expressed on the surface of the HepG2, Hep3B, RZM6-0d, RZM6- LC, WRL68, and PLC/PRF/5 cell lines (Supplementary Figure 1C). To confirm the expression of DHCR24 on the cell surface, we performed immunofluorescence staining (Supplementary Figure 1D). DHCR24 expression was detected in the HuH-7 cells without permeabilization.

Suppression of BGT-1 mRNA Expression in HCV Replicon Cell Lines After Treatment With 2-152a MAb
To determine the molecular mechanism underlying the effects of 2-152a MAb, we performed microarray analysis twice with different amounts of probes and evaluated the changes in gene expression associated with the 2-152a MAb treatment, which were specific to the HCV replicon cells rather than to the HCV-cured K4 cells. Using this methodology, we identified approximately 3–14 genes as upregulated and about 17–20 genes as downregulated following the treatment with 2-152a MAb, compared with the expressions in normal IgG-treated R6FLR-N, FLR3-1, and K4 cells (Figure 2A). Among these genes, the expression level of SLC6A12 (BGT-1; GenBank accession number NM_003044) showed significant downregulation in both the R6FLR-N and the FLR3-1 cell lines but not in the K4 cells (Figure 2A; Table 1). To validate this result, we tested BGT-1 mRNA expression in R6FLR-N cells treated with 2-152a MAb and normal IgG by using TaqMan expression assay. This assay demonstrated that the relative expression of BGT-1 was significantly suppressed by the treatment with 2-152a MAb (P < .001, Figure 2B). Significant downregulation of BGT-1 was also observed by treatment with 2-152a MAb in HCV-JFH-1-infected cells (Figure 2C).

We further addressed the mechanism of action of 2-152a MAb. Treatment with 2-152a MAb did not decrease the level of cholesterol (Figure 2D), and silencing of DHCR24 did not influence BGT-1 significantly (Figure 2E).

Inhibition of HCV Replication and Infection by siRNA Directed Against BGT-1
Because BGT-1 expression was suppressed by the treatment with 2-152a MAb, which had antiviral activity, we attempted BGT-1 silencing in HCV replicon cell lines by using designed siRNAs to examine the potential role of BGT-1 in HCV replication. BGT-1 silencing was confirmed by RT-PCR (Figure 3A). The effect of the siRNAs on HCV replication was examined by Western blotting with anti-NS5A antibody (Figure 3B) and measured by the luminescence level (Figure 3C) and cell viability (Figure 3C, right panel) in FLR3-1 cells. We also examined the effect of these siRNAs in R6FLR-N and JFH-1 cells (Supplementary Figure 2A) and observed similar inhibitory effects as

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**Table 1. Screened Genes in HCV Replicon Cell Lines After Treatment of IgG**

<table>
<thead>
<tr>
<th>Gene name</th>
<th>R6FLR-N 24 hours</th>
<th>FLR3-1 24 hours</th>
<th>FLR3-1 72 hours</th>
<th>HuH-7/K4 24 hours</th>
</tr>
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<tr>
<td>1st screening</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AKR1C1</td>
<td>0.67</td>
<td>0.62</td>
<td>0.65</td>
<td>NS</td>
</tr>
<tr>
<td>BGT-1</td>
<td>0.53</td>
<td>0.63</td>
<td>0.53</td>
<td>NS</td>
</tr>
<tr>
<td>2nd screening</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AKR1C1</td>
<td>0.74</td>
<td>NS</td>
<td>0.73</td>
<td>NS</td>
</tr>
<tr>
<td>(7-fold)*</td>
<td>or 0.75</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F2RL2</td>
<td>0.72</td>
<td>0.68</td>
<td>0.62</td>
<td>NS</td>
</tr>
<tr>
<td>BGT-1</td>
<td>0.58</td>
<td>0.58</td>
<td>0.61</td>
<td>NS</td>
</tr>
<tr>
<td>Screened in replicon and cured K4 cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st screening</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNN1</td>
<td>2.75</td>
<td>0.6</td>
<td>1.62</td>
<td>1.9</td>
</tr>
<tr>
<td>2nd screening</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CNN1</td>
<td>2.63</td>
<td>2.18</td>
<td>1.39</td>
<td>1.88</td>
</tr>
<tr>
<td>(7-fold)c</td>
<td>TAGLN</td>
<td>1.63</td>
<td>1.52</td>
<td>1.47</td>
</tr>
<tr>
<td>VNN2</td>
<td>0.65</td>
<td>0.63</td>
<td>0.66</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Abbreviations: HCV, hepatitis C virus; IgG, immunoglobin G; NS, not screened.

* Screened genes were significantly changed in HCV replicon cells but not in HuH-7/K4 cells; each value indicates ratio of signal 2-152a MAb IgG/normal IgG treatment.

b Screened genes were significantly changed in all cell lines, including replicon cells and HuH-7/K4 cells.

c Comparing to 1st screening, 7-fold amount of labeled probe was used for microarray.

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Figure 2 continued. gene expression assay. Each value was compensated with values of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA as the internal control and normal IgG. The asterisk indicates P < .001, and the vertical bars indicate the standard deviation. C, Level of BGT-1 and DHCR24 proteins detected in JFH/K4 cells after treatment with 2-152a or normal IgG (10 μg/mL). D, The relative cholesterol amount was measured in R6FLR-N cells treated with 2-152a or normal IgG (10 μg/mL). E, BGT-1 and DHCR24 proteins were detected in normal IgG- or 2-152a IgG-treated R6FLR-N cells.
those in FLR3-1 cells. The median inhibitory concentration (IC₅₀) values of BGT-1 siRNAs in various HCV replicon cell lines were as follows: FLR3-1 cells, 0.93 nM; R6FLR-N cells, 1.37 nM; JFH-1 cells, 5.95 nM. The cell viability was not significantly influenced by the siRNA treatment (Figure 3C, right panel; Supplementary Figure 2A, right panel).

Further, we monitored the levels of HCV RNA in JFH/K4 cells and their supernatants after BGT-1 silencing. Using RTD-PCR, we detected significant suppression in the HCV RNA levels by BGT-1 silencing in these cells (P < .005; Figure 3D, left panel) and their supernatants (P < .05; Figure 3D, right panel). These results were consistent with the strong inhibitory effects of

Figure 3. BGT-1 silencing by siRNA inhibits HCV replication in subgenomic HCV replicon cell lines and the persistently infected cell line. A, The siRNA targeting BGT-1 suppressed the expression of the corresponding mRNA. The mRNA of each sample was extracted 72 hours after siRNA (10 nM) transfection. Total RNA was transcribed and amplified by RT-PCR using primers specific to the open reading frame (ORF) of the BGT-1 (1842 bp) gene. The experiments were performed in triplicate, and the representative data are presented. B, The effects of BGT-1 siRNA (10 nM) on HCV were confirmed by Western blot analysis using an antibody against the HCV NS5A protein (55 kDa). The blots were stripped and reprobed with an antibody directed against actin to examine protein loading in each lane. C, Levels of HCV replication (left panel) and cell viability (right panel) are presented according to serial concentrations of siRNA targeting BGT-1 and control siRNA in FLR3-1 cells (72 hours after transfection). The inhibition of replication or cell viability following siRNA targeting BGT-1 is defined relative to those of the cells that received no treatment (100%). The error bars represent the standard error of triplicate experiments. D, Quantification of HCV RNA by RTD-PCR in HCV persistently infected cells (JFH/K4) after treatment with BGT-1 siRNA. The cells were treated with siRNAs (10 nM) against BGT-1, control, and HCV (HCV R7) and harvested at 72 hours after transfection. TaqMan quantitative RT-PCR was performed for quantitation of HCV RNA in extracted RNA from cells (left panel) and their supernatants (right panel). The single asterisk (*) and double asterisk (**) indicate P < .005 and P < .05 against the control, respectively. The mean values from triplicate wells are indicated, and the vertical bars indicate the standard deviation.
BGT-1 siRNA on HCV replication, as shown in Figure 3C. We designed alternative siRNA targeting BGT-1 (BGT-1-siRNA-362) and observed its significant inhibitory effect on HCV replication without significant cytotoxicity (Supplementary Figure 2B).

Validation of the Anti-HCV Effects of siRNA Against BGT-1 by Rescue With Expression Vectors

To assess the specificity of BGT-1 silencing, we attempted to rescue HCV replication against the ectopic effects by this silencing. To examine the effect of the rescue, we constructed expression vectors of wild-type and mutant BGT-1 (Figure 4A) and confirmed the expression of each BGT-1-myc-fused protein (Figure 4B). The mutant BGT-1 vector contained 5 base mismatches within the site targeted by the BGT-1 siRNA without a change in the amino acid sequence of the protein (underlined in Figure 4A). We also transfected the pcDNA-BGT-1 plasmid after the siRNA treatment and observed significant recovery of HCV replication with mutant pcDNA-BGT-1 (P < .05; Figure 4C, left panel) without significant cytotoxicity (Figure 4C, right panel). BGT-1 expression was increased significantly in K4 cells in the presence of HCV (P < .05, Supplementary Figure 2C) at 72 hours after infection compared with the absence of HCV, and in RzM6-LC cells, which persistently express HCV [8], compared with RzM6-0d cells, which lack HCV expression (P < .05, Supplementary Figure 2D).

DISCUSSION

In this study, we determined that 2-152a MAb, which binds to but does not affect the activity of DHCR24, suppresses HCV replication and that BGT-1 is highly downregulated in HCV replicon cell lines treated with this antibody. Further, the efficient rescue of viral replication with a mutant expression vector indicates the specific inhibitory effect of BGT-1 silencing on HCV replication. Therefore, we hypothesize that BGT-1 plays an important role in HCV replication through a pathway that is likely independent of DHCR24, which in its own right can regulate the HCV life cycle [13].

BGT-1 is involved in sodium- and chloride-coupled betaine uptake, which helps in maintaining normal cellular conditions. Previous reports have described that the transcription of BGT-1 mRNA is regulated by a tonicity sensitive element (TonE) in response to hypertonic stress, a result that was first identified in the Madin-Darby canine kidney (MDCK) cell line [24]. BGT-1...
is also thought to be responsible for the hyperosmotic stress response and in maintaining cell hydration. Denkert et al. [25] reported that BGT-1 gene expression is induced by hyperosmolarity and inhibited by p38 mitogen-activated protein kinase (p38MAPK) inhibitor SB20358. Further, several reports have evidenced that cell hydration affects viral replication and that viral replication increases during cell shrinkage due to hyperosmolarity, a result that was accompanied by increased BGT-1 mRNA expression [26]. Considering the reduction in HCV replication by the BGT-1 siRNA treatment, this treatment may prevent HCV replication by affecting hypoosmotic conditions in HCV-infected cells. Further studies are required to examine in detail the function of BGT-1 in HCV replication.

In summary, we demonstrated that the 2-152a monoclonal antibody inhibits HCV replication in HCV replicon cells and HCV infection in human hepatocytes transplanted into chimeric mice. The inhibitory effect of the monoclonal antibody on viral replication may be mediated by the suppression of BGT-1 expression. We propose BGT-1 as a key target for anti-HCV therapies.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (Supplementary Data).

Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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