Chaperonin TRiC/CCT participates in replication of hepatitis C virus genome via interaction with the viral NS5B protein

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ABSTRACT

To identify the host factors implicated in the regulation of hepatitis C virus (HCV) genome replication, we performed comparative proteome analyses of HCV replication complex (RC)-rich membrane fractions prepared from cells harboring genome-length bicistronic HCV RNA at the exponential and stationary growth phases. We found that the eukaryotic chaperonin T-complex polypeptide 1 (TCP1)-ring complex/chaperonin-containing TCP1 (TRiC/CCT) plays a role in the replication possibly through an interaction between subunit CCT5 and the viral RNA polymerase NS5B. siRNA-mediated knockdown of CCT5 suppressed RNA replication and production of the infectious virus. Gain-of-function activity was shown following co-transfection with whole eight TRiC/CCT subunits. HCV RNA synthesis was inhibited by an anti-CCT5 antibody in a cell-free assay. These suggest that recruitment of the chaperonin by the viral nonstructural proteins to the RC, which potentially facilitate folding of the RC component(s) into the mature active form, may be important for efficient replication of the HCV genome.

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Introduction

Hepatitis C virus (HCV) is a major cause of chronic liver diseases, such as chronic hepatitis, hepatic steatosis, cirrhosis, and hepatocellular carcinoma (Hoofnagle, 2002; Manns et al., 2006; Saito et al., 1990; Seeff and Hoofnagle, 2003). HCV is an enveloped positive-strand RNA virus belonging to the *Hepacivirus* genus of the Flaviviridae family. Its genome of ~9.6 kb encodes a polyprotein precursor of ~3000 amino acids (aa) (Suzuki et al., 2007; Taguwa et al., 2008). The precursor polyprotein is post- or cotranslationally processed by both viral and host proteases into at least ten viral products. The nonstructural (NS) proteins NS3–NS5B are necessary and sufficient for autonomous HCV RNA replication. They form a membrane-associated replication complex (RC), in which NS5B is the RNA-dependent RNA polymerase (RdRp) that is responsible for copying the RNA genome of the virus during replication. The HCV RC has been detected in detergent-resistant membrane (DRM)

structures, possibly in a lipid-raft structure (Aizaki et al., 2004; Shi et al., 2003). Cell-free RC replication activity has also been demonstrated in crude membrane fractions of HCV subgenomic replicon cells (Aizaki et al., 2004; Ali et al., 2002; Hara et al., 2009; Hardy et al., 2003; Yang et al., 2004); these cell-free systems provide semi-intact RdRp assays for biochemical dissection of viral replication.

In general, any process that occurs during viral replication is dependent on the host cell machinery and requires close interaction between viral and cellular proteins. Although evidence that host cell factors interact with HCV NS proteins and are involved in viral replication is accumulating (Moriishi and Matsuura, 2007), the cellular components of HCV RC and their functional roles in viral replication are not fully understood.

Recently, using comparative proteome analysis, we identified 27 cellular proteins that were highly enriched in the DRM fraction of HCV replicon cells relative to parental cells. Subsequent analyses demonstrated that one of the identified proteins, creatine kinase B, a key ATP-generating enzyme, is important for efficient replication of the HCV genome and for production of the infectious virus (Hara et al., 2009).

In this study, to extend our investigation and to increase our understanding of the precise components of HCV RC and the

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mechanisms of viral genome replication, we designed another comparative proteomic approach in which cells harboring genome-length bicistronic HCV RNA at the exponential growth phase (showing rapid replication of viral RNA) were compared with cells at the confluent-growth phase (showing poor replication of viral RNA). This strategy revealed that the chaperonin T-complex polypeptide (TCP1)-ring complex/chaperonin-containing TCP1 (TRiC/CCT) participates in HCV RNA replication and virion production possibly through an interaction between CCT5 (chaperonin-containing TCP1, subunit 5) and NS5B.

Results

CCT5 and Hsc70 are enriched in the DRM fraction containing the HCV RC

Recently, we analyzed the protein content of DRM fractions prepared from HCV subgenomic replicons and parental Huh-7 cells and identified 27 cellular proteins that were enriched in the DRM fraction prepared from the replicon cells (Hara et al., 2009). These were identified as factors that may be involved in the HCV RC and in viral replication. In fact, subsequent silencing of several genes coding for these proteins resulted in the inhibition of HCV RNA replication (Hara et al., 2009). However, it is likely that proteins unrelated to HCV replication are also included in the identified groups because long-term culture of the replicon cells under the selective pressure of G418 selects for a subpopulation of the parental cells and may induce changes in their protein expression profiles. Thus, to minimize interline differences in culture background, we further designed a comparative proteome analysis using a single cell line as follows.

HCV replication efficiency is dependent on the conditions of host cell growth. High cell density of the replicon culture has a reversible inhibitory effect on viral replication (Nelson and Tang, 2006; Pietschmann et al., 2001). Fig. 1A demonstrates that a high level of HCV RNA was detected in cells harboring the genome-length bicistronic HCV RNA, Con1 strain of genotype 1b (RCYM1) in the growth phase, whereas the RNA level declined sharply when the cells reached the stationary phase. We further compared the synthesis of HCV RNA in cell-free reaction mixtures containing the viral RC isolated from the RCYM1 cells at various cell densities (Fig. 1B). Replication activity was highest at the mid-log phase of cell growth (day 4 after seeding). By contrast, little or no RNA synthesis was observed under the confluent-growth cell culture (day 8), confirming the critical role of host cell growth conditions in the replication of the HCV genome.

Thus, to identify the host cell proteins required for HCV replication, we designed a two-dimensional fluorescence difference gel electro-phoresis (2D-DIGE)-based comparative proteomics analysis of RC-rich DRM fractions prepared from RCYM1 cells at the mid-log and confluent-growth phases. Protein spots that reproducibly showed a greater than 1.5-fold difference in the mid-log growth- and the confluent phases were excised and digested by trypsin or lysylendo-peptidase. Matrix-assisted laser desorption ionization–time-of-flight (MALDI-TOF) mass spectrometry (MS), which allows identification of the corresponding proteins in 9 cases (Table 1). Two increased spots that showed an increase in levels (their stereoscopic images are shown in Fig. 2A) were identified as CCT5 and Hsc70. CCT5, an epsilon subunit of chaperonin TRiC/CCT, is a 900-kDa toroid-shaped complex consisting of eight different subunits (Valpuesta et al., 2002; Yaffe et al., 1992). Hsc70, a member of the HSP70 family, is a 71-kDa heat shock cognate protein (Dworniczak and Mirault, 1987). Independent of the proteome analyses, DRM fractions and whole cell lysates were prepared from RCYM1 cells at two different growth phases (as above) and were analyzed by immunoblotting (Fig. 2B). Steady-state levels of CCT5 and Hsc70 were obviously higher in the DRM fraction prepared from the cells that were at the mid-log growth phase compared with those at the confluent phase. However, in the whole cell analyses, they were shown to be present at comparable levels during the two different growth phases. These results suggest that expression of CCT5 and Hsc70 is not enhanced in proliferating cells and that the enrichment of these proteins in the DRM fraction is possibly due to their post-translational modification. It should be noted that in the previous proteome analysis, CCT5 and other TRiC/CCT subunits, such as CCT1 and CCT2, were identified as proteins that were enriched in the DRM fraction prepared from subgenomic replicon-containing cells compared with that prepared from parental cells (Hara et al., 2009). We showed that CCT5 and CCT1 were enriched in the DRM fractions of cells transfected with the HCV genomic RNA derived from JFH-1 isolate as well as of subgenomic replicon cells (Fig. 2C).

TRiC/CCT participates in replication of the HCV genome

We investigated gain- and loss-of-functions of TRiC/CCT and Hsc70 with respect to the replication of HCV RNA. Seventy-two hours after RCYM1 cells were transfected with eight plasmids corresponding to each of the TRiC/CCT subunits, the level of HCV RNA in the cells (determined by quantitative RT-PCR) significantly increased to 2-fold that observed in the control cells. However, exogenous expression of Hsc70 in the RCYM1 cells showed no effect on the viral RNA (Fig. 3A). siRNAs targeted to CCT5 or Hsc70 and consisting of pools of three target-specific siRNAs or control nonspecific siRNAs were transfected
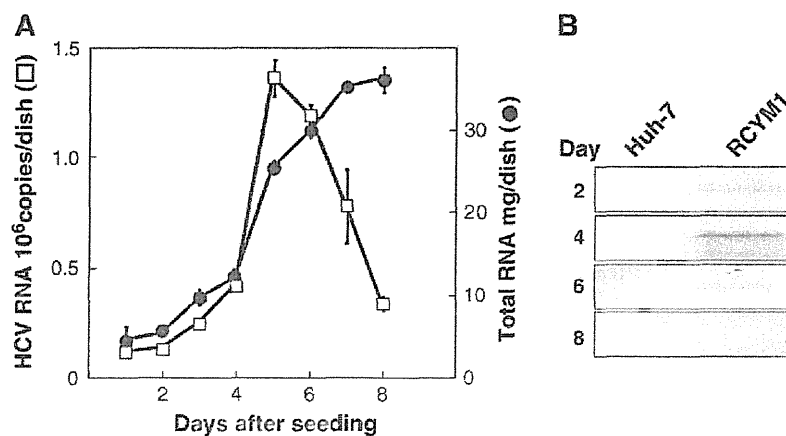

Fig. 1. Effect of cell growth on HCV RNA replication. (A) Measurement of HCV RNA (open squares) and total cellular RNA (closed circles) in RCYM1 cells at the time of harvest (days after seeding). (B) DRM fractions obtained from RCYM1 and parental Huh-7 cells harvested as indicated (day) were analyzed by cell-free RNA replication assay. RNA extracted from each sample was analyzed by agarose gel electrophoresis and autoradiograph.

Table 1

Selected cellular proteins that reproducibly increased and decreased in membrane fraction of RCYM1 cells at exponential growth phase.

Av. ratio	T-test	Coverage (%)	Protein name	Molecular function	GI
Increased proteins					
1.58	0.017	31	CCT5	Protein folding	33879913
1.54	0.005	35	HSPA8 (Hsc70)	Protein folding	24657660
Decreased proteins					
−1.95	0.028	44	Creatine kinase isozyme CK-B gene, exon 8	Energy pathway/metabolism	180568
−1.53	0.011	16	Chain C, Human Sirt2 Histone deacetylase	Cell cycle control	15826438
−2.14	0.001	33	Proteasome regulatory particle subunit p44S10	Metabolism	15341748
−1.71	0.004	21	Aldehyde dehydrogenase	Metabolism	178388
−1.85	0.004	40	Aminoacylase 1	Metabolism	12804328
−2.77	0.003	15	Eukaryotic translation initiation factor 3, subunit 3 gamma	Metabolism (translation regulator activity)	6685512
−2.43	0.014	20	Intraflagellar transport protein 74 homolog (Coiled-coil domain-containing protein 2)	Cell growth and/or maintenance	10439078

Three paired samples of RC-rich membrane fractions at the exponential- and confluent-growth phases of RCYM1 cultures were analyzed. The proteins representing a more than 1.5-fold increase or decrease (−) reproducibly and significantly are indicated.

Coverage (%): the ratio of the portion of protein sequence covered by matched peptides to the whole sequence.

GI: GenInfo Identifier number.

into RCYM1 cells. After 72 h, the HCV RNA level was reduced by 42% and 27% in the cells transfected with siRNAs against CCT5 and Hsc70, respectively, compared with controls (Fig. 3B). TRiC/CCT possibly interacts with Hsc70, and its complex formation contributes to increasing the efficiency of protein folding (Cuéllar et al., 2008). Our results suggest the involvement of TRiC/CCT and Hsc70 in the HCV life cycle. In particular, TRiC/CCT may play an important role in the replication of the viral genome.

To verify the specificity of the knockdown of CCT5 siRNA, we further synthesized two siRNAs targeted to different regions used in the above CCT5 siRNA and assessed their knockdown effect on HCV genome replication (Fig. 3C, upper panel). As expected, transfection of
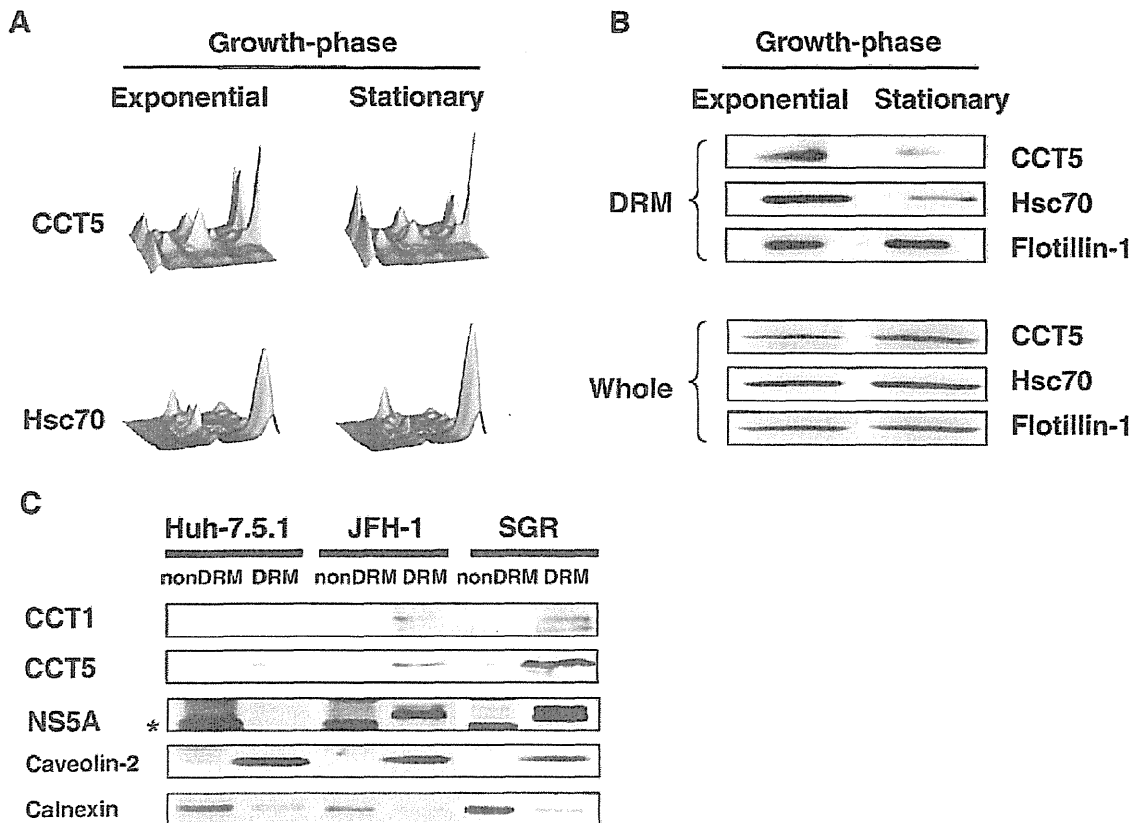

Fig. 2. Comparison of protein levels in DRM fractions prepared from RCYM1 cells at the exponential and stationary growth phases. (A) Three-dimensional images of CCT5 and Hsc70 analyzed by Ettan DIGE (GE Healthcare). Spots corresponding to CCT5/Hsc70 at exponential and stationary growth phases of the cells, respectively, are shown in green and red. (B) Equal amounts of protein in the DRM fractions prepared from RCYM1 cells at the exponential and stationary growth phases or corresponding whole cell lysates were analyzed by immunoblotting with Abs against CCT5, Hsc70 or flotillin-1. (C) Enrichment of CCT1 and CCT5 in the DRM fractions of HCV RNA replicating cells. Equal amounts of DRM or non-DRM fractions from full-length JFH-1 RNA transfected cells (JFH-1), subgenomic replicon cells (SGR) and parental Huh-7.5.1 cells were analyzed by immunoblotting with antibodies against CCT1, CCT5, NS5A, caveolin-2 or calnexin. *Non-specific bands.
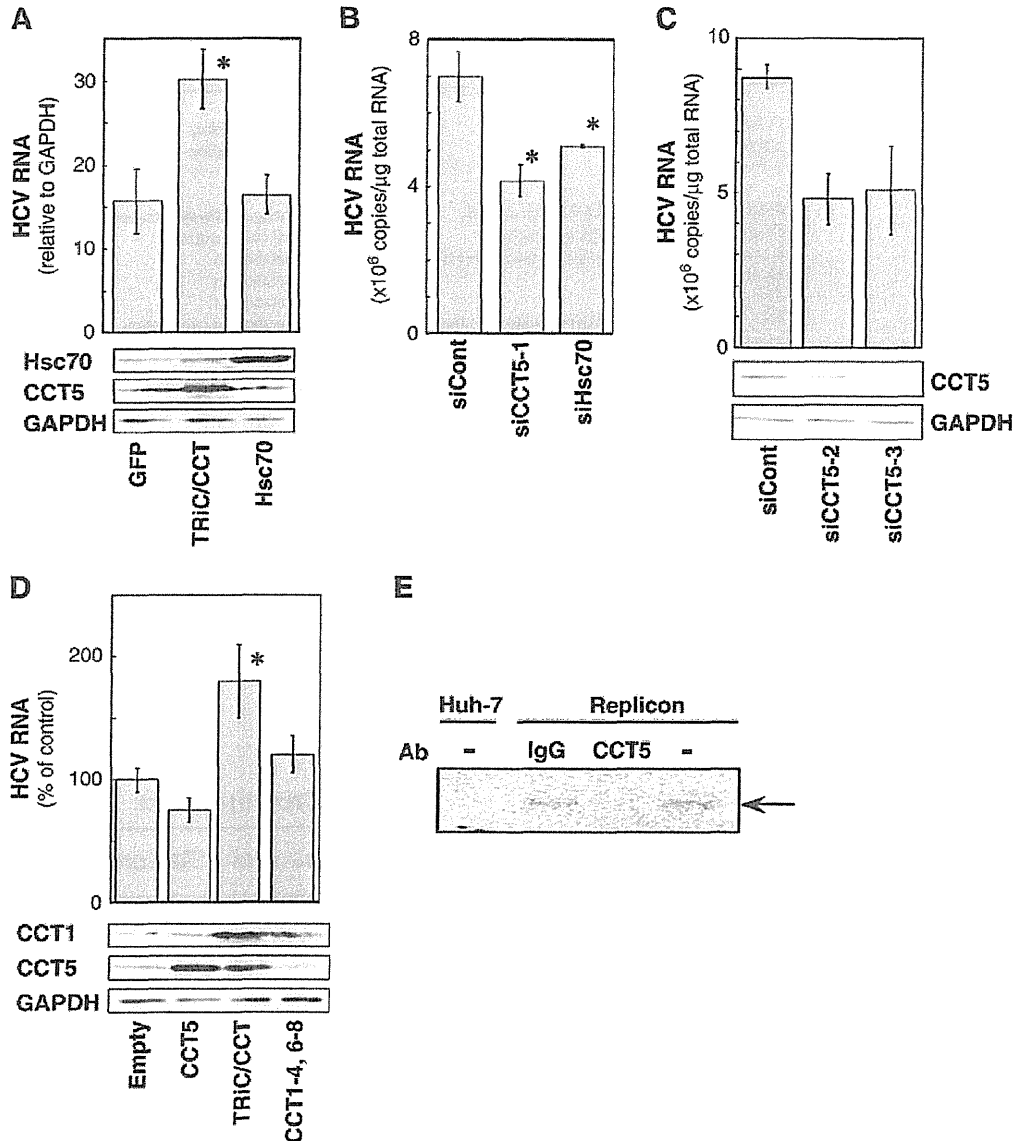
Fig. 3. Involvement of TRiC/CCT in HCV replication (A and D). Overexpression of all eight subunits of TRiC/CCT (TRiC/CCT); seven subunits, CCT1, 2, 3, 4, 6, 7, and 8 (CCT1–4, 6–8); subunit CCT5 only (CCT5); Hsc70; or control GFP in RCYM1 cells. HCV RNA levels were determined 48 h post-transfection (B and C). Knockdown of endogenous CCT5 or Hsc70 in RCYM1 cells, which were transfected with three types of siRNAs against CCT5 (siCCT5-1, -2, and -3), siRNA against Hsc70 (siHsc70), or control siRNA (siCont), and were harvested at 72 h post-transfection. siCCT5-1 and siHsc70 consisted of pools of three target-specific siRNAs. Immunoblotting for CCT1, CCT5, Hsc70 and GAPDH was performed (A, C and D; lower). (E) Cell-free de novo viral RNA synthesis assays were performed in the presence of anti-CCT5 Ab or control mouse IgG. Cytoplasmic fractions from SGR-N (replicon) and parental Huh-7 cells were used. An arrow indicates the synthesized HCV RNA. Error bars denote standard deviations with asterisks indicating statistical significance (*P<0.01).

RCYM1 cells with each CCT5 siRNA resulted in a reduction in viral RNA to a level of about 50% of that observed in cells treated with control siRNAs. Immunoblotting confirmed the efficient reduction in expression of endogenous CCT5 and the lack of cytotoxic effect exerted by the CCT5 siRNAs (Fig. 3C, middle and lower panels).

Having confirmed the upregulation of HCV RNA by ectopic expression of all the TRiC/CCT subunits, we further addressed the possibility that CCT5, independent of the complete TRiC/CCT complex, might have a role in promoting replication of HCV RNA. Transfection with either a CCT5 expression plasmid alone or with seven plasmids expressing all the TRiC/CCT subunits except CCT5 resulted in no or only a slight increase in the level of HCV RNA, indicating that all CCT subunits are required for HCV replication (Fig. 3D).

TRiC/CCT is generally known as a cytosolic chaperone (Valpuesta et al., 2002). However, it is enriched in the DRM fraction of HCV-

replicating cells during the exponential growth phase (Fig. 2B). We used immunofluorescence staining to investigate whether TRiC/CCT is localized in the intracellular membrane compartments where replication of the viral genome occurs (Fig. 4). The de novo-synthesized RdRp was labeled by bromouridine triphosphate (BrUTP) incorporation in the presence of actinomycin D, and brominated nucleotides were detected with a specific antibody (Ab). Fluorescence staining in distinct speckles of various sizes was found in the cytoplasm of the HCV subgenomic replicon cells, whereas no signal was detected in the control cells, indicating that the observed BrUTP-incorporating RNA is mostly viral, newly synthesized viral RNA (Fig. 4A). Double immunofluorescence staining showed that a certain section of CCT5 co-distributed with the BrUTP-labeled RNA (Fig. 4A), which is known to co-exist with HCV NS proteins in viral replicating cells (Shi et al., 2003). We further observed that CCT5 was at least partially colocalized

A

BrUTP **CCT5** **Merge**

Huh-7

Replicon

B

NS5A **CCT5** **Nucleus**

Merge **High magnification**

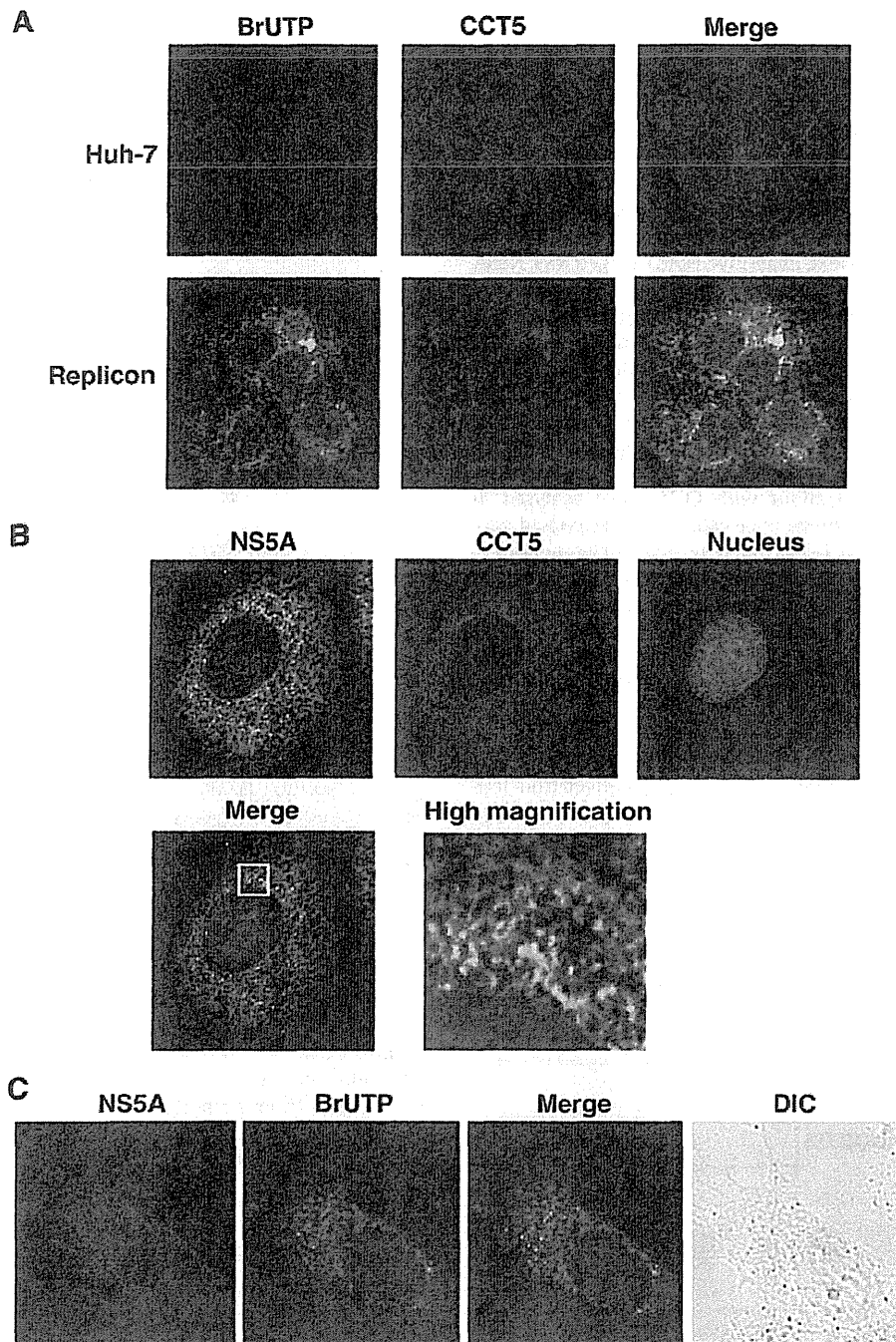C

NS5A **BrUTP** **Merge** **DIC**

Fig. 4. Immunofluorescence analysis of CCT5 in SGR-N and Huh-7 cells (A) and HCVcc-infected cells (B). The primary Abs used were anti-CCT5 goat polyclonal Ab (red), anti-BrUTP monoclonal Ab (green), and anti-NS5A monoclonal Ab (green). Merged images of red and green signals (A) or of red, green and blue (nucleus) signals (B) are shown. The high magnification panel is an enlarged image of a white square of the merge panel. (C) Colocalization of NS5A protein with the viral RNA. The replicon cells were permeabilized with lysolecithin and labeled with BrUTP, followed by staining with anti-NS5A rabbit polyclonal Ab (red) and the anti-BrUTP monoclonal Ab (green). DIC, differential interference contrast.

with the viral NS protein in certain compartments sharing a dot-like structure in Huh-7 cells infected with HCV JFH-1 infectious HCV (HCVcc) derived from HCV genotype 2a (Fig. 4B) as well as in the replicon cells (data not shown). Fig. 4C indicated co-localization of BrUTP-labeled RNA with NS5A.

To further address the role of TRiC/CCT in HCV genome replication, we performed immunodepletion and in vitro replication analyses, which have been used for studying the genome replication of several

viruses (Daikoku et al., 2006; Garcin et al., 1993; Liu et al., 2009). Cell extracts prepared from the HCV-replicating cells were reacted with either a mouse monoclonal Ab against CCT5 or mouse IgG derived from preimmune serum, followed by cell-free synthesis of HCV RNA. Fig. 3E shows that treatment with anti-CCT5 Ab inhibited viral RNA synthesis, whereas the control IgG did not affect the process, suggesting that TRiC/CCT participates directly in HCV RNA replication.

CCT5 interacts with HCV NS5B

The genome replication machinery of HCV is a membrane-associated complex composed of multiple factors including viral NS proteins. Given the involvement of TRiC/CCT in HCV RNA synthesis, we next examined its possible interaction with HCV NS proteins. A first attempt to immunoprecipitate the viral proteins with antibodies against TRiC/CCT subunits in the replicon cells was unsuccessful (data not shown), suggesting that endogenous levels of TRiC/CCT is not sufficient to pull out NS5B. Next, dual (myc/FLAG)-tagged NS3, NS5A, or NS5B proteins derived from the genotype 1b NIHJ1 strain were co-expressed with CCT5 in Huh-7 cells and then subjected to two-step immunoprecipitation with anti-myc and anti-FLAG Abs (Ichimura et al., 2005; Shirakura et al., 2007). An empty plasmid was used as a negative control in the analyses. As shown in Fig. 5A, CCT5 specifically interacted with NS5B. Little or no interaction was found between CCT5 and NS3 or NS5A. To determine the NS5B region required for the interaction with CCT5, various deletion mutants of HA-NS5B were constructed and their interactions with CCT5 were analyzed as described above. CCT5 was shown to be coimmunoprecipitated with either a full-length NS5B (aa 1–591), an N-terminal deletion (aa 71–591) or a C-terminal deletion (aa 1–570), but not with deletions aa 215–591 or aa 320–591 (Fig. 5B), suggesting that aa 71–214 of NS5B are important for its interaction with CCT5.

Knockdown of CCT5 results in the reduction of propagation of infectious HCV

We further examined whether the knockdown of CCT5 would abrogate the production of infectious HCV (HCVcc), derived from JFH-1 (Fig. 6). At 72 h post-transfection with each CCT5 siRNA, HCV RNA

Fig. 6. Knockdown of endogenous CCT5 in HCVcc-infected cells. The cells were transfected with siRNAs against CCT5 (siCCT5-2, -3) or with control siRNAs (siCont). At 72 h post-transfection, the viral core protein levels in cells were determined (upper panel). Collected culture supernatants were inoculated into naïve Huh7.5.1 cells and intracellular core proteins were determined at 72 h post-infection (middle panel). Cells transfected with siRNAs were analyzed by immunoblotting with anti-CCT5 or anti-GAPDH Ab (lower panel). Error bars denote standard deviations with asterisks indicating statistical significance (*P<0.05; **P<0.01).

levels in Huh-7 cells infected with HCVcc were reduced by 25–35% compared with controls. Accordingly, virion production from CCT5 siRNA-transfected cultures was significantly decreased, as determined by intracellular HCV core protein levels at 72 h after the infection of naïve cells with culture supernatants taken from transfected cells. These results demonstrate that reduction of the HCV RNA replication by siRNA-mediated knockdown of CCT5 results in reduction of the propagation of the infectious virus.

Discussion

The chaperone-assisted protein-folding pathway is a process in living cells that results from coordinated interactions between multiple proteins that often form multi-component complexes. Several steps in the viral life cycle, such as protein processing, genome replication, and viral assembly, are regulated by cellular chaperones. Hsp90, one of the most abundant proteins in unstressed cells, has been implicated in HCV RNA replication (Nakagawa et al., 2007; Okamoto et al., 2006, 2008; Taguwa et al., 2008, 2009; Ujino et al., 2009). FKBP8, a member of the FKBP506-binding protein family, and hB-ind1, human butyrate-induced transcript 1, play key roles through their interaction with HCV NS5A and Hsp90 (Okamoto et al., 2006, 2008; Taguwa et al., 2008, 2009). Hsp90 has also been implicated in viral enzymatic activities including those of the influenza virus (Momose et al., 2002; Naito et al., 2007), herpes simplex virus (Burch and Weller, 2005), Flock house virus (Kampmueller and Miller, 2005), and hepatitis B virus (Hu et al., 2004).

In our former study, comparative proteome analyses of the viral RC-rich DRM fractions prepared from subgenomic replicon cells and Huh-7 cells were carried out to identify host factors involved
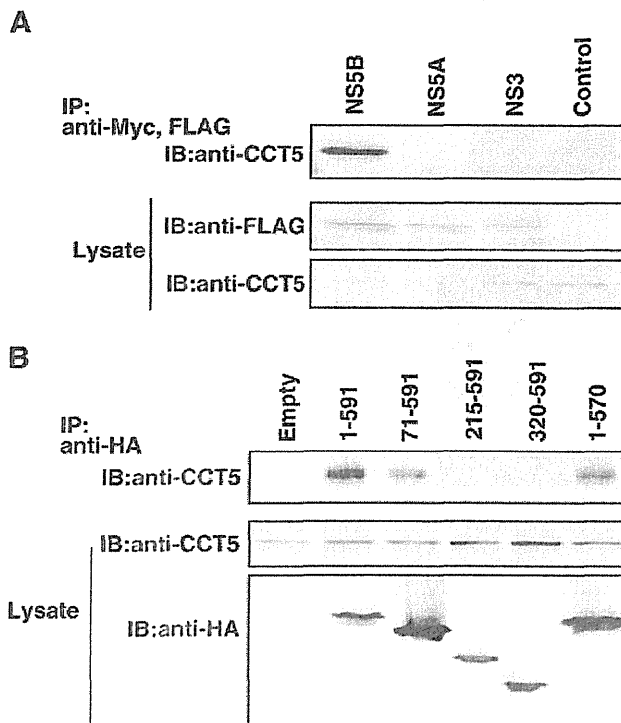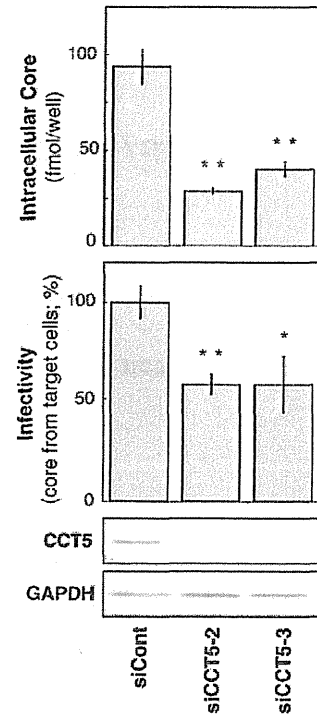

Fig. 5. CCT5 interacts with HCV NS5B. (A) CCT5 was co-expressed with MEF-tagged-NS5B, -NS5A, or -NS3 protein of strain NIHJ1 in cells, followed by two-step immunoprecipitation (IP) with anti-FLAG and anti-myc Abs. Immunoprecipitates were subjected to immunoblotting with anti-CCT5 Ab (IB). (B) Full-length NS5B (1–591) or its deletions (71–591, 215–591, 320–591, 1–570) along with a HA tag were co-expressed with CCT5. IP and IB were performed as described above.

in HCV replication (Hara et al., 2009). We extended the proteomics by modifying our protocol of the analysis to reduce the interline differences in culture background and analyzed the DRM samples derived from the mid-log and confluent-growth phases of single cell line. Here, we identified two proteins, CCT5 and Hsc70, showing an increase in levels at the mid-log growth phase. Although CCT5 was also identified in the former study as expected, Hsc70 was not included in the list of proteins identified in the study (Hara et al., 2009). This difference may be due to the use of cells carrying the full-length replicon RNA in this study.

In this study, we demonstrated that TRiC/CCT participates in HCV RNA replication and virion production possibly through its interaction with NS5B. TRiC/CCT is a group II chaperonin that assists in protein folding in eukaryotic cells and forms a double-ring-like hexadecamer complex. Although relatively little is known about its function compared with that of the group I chaperonins such as bacterial GroEL, several mammalian proteins whose folding is mediated by TRiC/CCT have been identified, such as actin, tubulin, and von Hippel–Lindau tumor suppressor protein (Farr et al., 1997; Feldman et al., 2003; Frydman and Hartl, 1996; Meyer et al., 2003; Tian et al., 1995). With regard to viral proteins, the Epstein–Barr virus nuclear antigen, HBV capsid protein, and p4 of M-PMV have been identified as TRiC/CCT-interacting proteins (Yam et al., 2008). However, the functional significance of their interactions in the viral life cycles has yet to be determined. Here we demonstrated that the reduction in CCT5 expression in HCV replicon cells and in virus-infected cells inhibits HCV RNA replication (Figs. 3B and C) and virus production (Fig. 6) respectively. Gain-of-function was also shown by co-transfection of the replicon cells with eight constructs corresponding to all the TRiC/CCT subunits (Figs. 3A and D).

A recent study of the three-dimensional structure of the TRiC/CCT and Hsc70 complex has demonstrated that the apical domain of the CCT2 (CCT-beta) subunit is involved in the interaction with Hsc70 (Cuéllar et al., 2008). The complex formation created by the TRiC/CCT and Hsc70 interaction may promote higher efficiency in the folding of certain proteins (Cuéllar et al., 2008). In our comparative proteome analyses, both CCT subunits and Hsc70 were enriched in the HCV RC-rich membrane fraction of the replicon cells that showed high viral replication activity (Fig. 2B). Transfection of Hsc70 siRNA into the replicon cells moderately inhibited viral RNA replication (Fig. 3B). However, upregulation of HCV replication was not observed by ectopic expression of Hsc70 (Fig. 3A), and little or no interaction was observed between Hsc70 and HCV NS proteins in the co-immunoprecipitation analysis (data not shown). Thus, it is likely that TRiC/CCT acts as a regulator of HCV replication through participating in the de novo folding of NS5B RdRp, and Hsc70 might serve to assist in folding through its interaction with TRiC/CCT. It was recently reported that Hsc70 is associated with HCV particles and modulates the viral infectivity (Parent et al., 2009). Here we showed an additional role of Hsc70 in the HCV life cycle.

HCV genomic single-stranded RNA serves as a template for the synthesis of the full-length minus strand that is used for the overproduction of the virus-specific genomic RNA. NS5B RdRp is a single subunit catalytic component of the viral replication machinery responsible for both of these processes. It is known that the in vitro RdRp activity of recombinant NS5B expressed in and purified from insect cells and Escherichia coli is low in many cases. This could be due to the lack of a suitable cellular environment for favorable RdRp activity, although the particular conformational features dependent on the viral isolates may also be involved (Lohmann et al., 1997; Weng et al., 2009). In fact, besides interacting with HCV NS proteins, NS5B has been reported to interact with several host cell proteins. For example, human vesicle-associated membrane protein-associated protein subtype A (VAP-A) and subtype B (VAP-B), which are involved in the regulation of membrane trafficking, lipid transport and metabolism, and the unfolded protein response, interact with NS5B and NS5A and

participate in HCV replication (Hamamoto et al., 2005). Recently, VAP-C, a splicing variant of VAP-B, was found to act as a negative regulator of viral replication through its interaction with NS5B but not with VAP-A (Kukihara et al., 2009). Cyclophilin A and B, peptidyl-prolyl isomerases that facilitate protein folding by catalyzing the cis–trans interconversion of peptide bonds at proline residues, play a role in stimulating HCV RNA synthesis through interaction with NS5B (Liu et al., 2009; Watashi et al., 2005). SNARE-like protein (Tu et al., 1999), eIF4AII (Kyono et al., 2002), protein kinase C-related kinase 2 (Kim et al., 2004), nucleolin (Kim et al., 2004; Hirano et al., 2003; Shimakami et al., 2006), and p68 (Goh et al., 2004) are also known to associate with NS5B and are possibly involved in HCV RNA replication.

We found that the aa 71–214 region in NS5B is important for interaction with TRiC/CCT. The catalytic domain of HCV RdRp has a "right-hand" configuration similar to other viral polymerases, such as HIV-1 reverse transcriptase (Huang et al., 1998) and poliovirus RdRp (Hansen et al., 1997), and is divided into the fingers, palm, and thumb functional subdomains (Lohmann et al., 2000). The region required for the interaction with TRiC/CCT has been mapped in a part of the fingers and palm domains of NS5B RdRp. To address how TRiC/CCT assists in the correct folding or disaggregation of NS5B through their interaction, leading to the formation of a functional RdRp, work based on an in vitro reconstitution system using purified proteins is under way. As all the TRiC/CCT subunits possess essentially identical ATPase domains, their protein-recognition regions are apparently divergent, allowing for substrate-binding specificity. It has recently been reported that TRiC/CCT interacts with the PB2 subunit of the influenza virus RNA polymerase complex and TRiC/CCT binding site is located in the central region of PB2, suggesting involvement of TRiC/CCT in the influenza virus life cycle (Fislová et al., 2010). Eukaryotic RNA polymerase subunit has also been identified as a binding partner of TRiC/CCT from interactome analysis (Yam et al., 2008). It would be interesting to examine how conserved the mechanisms of TRiC/CCT action that result in enhanced replication are among RNA polymerases.

The recruitment of a chaperonin by viral NS proteins may be important for understanding regulation of the viral genome replication. In this study, we demonstrated the involvement of TRiC/CCT in HCV RNA replication possibly through its interaction between TRiC/CCT and HCV NS5B. Although possible interaction of subunit CCT5 with NS5B was shown, considering involvement of whole TRiC/CCT complex in its chaperonin function, whether CCT5 directly interacts with NS5B is unclear. Further detailed studies are needed to make clear the manner of TRiC/CCT-NS5B interaction. NS5B RdRp is one of the main targets for HCV drug discovery. The search for NS5B inhibitors has resulted in the identification of several binding sites on NS5B, such as the domain adjacent to the active site and the allosteric GTP site (De Francesco and Migliaccio, 2005; Laporte et al., 2008). The findings obtained here suggest that disturbing the interaction between NS5B and TRiC/CCT may be a novel approach for an antiviral chemotherapeutic strategy.

Materials and methods

Cell culture, transfection, and infection

Human hepatoma Huh-7 and Huh-7.5.1 cells (kindly provided by Francis V. Chisari from The Scripps Research Institute) and human embryonic kidney 293T cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum. Huh-7-derived SGR-N (Shi et al., 2003) and RCYM1 (Murakami et al., 2006) cells, which possess subgenomic replicon RNA from the HCV-N strain (Guo et al., 2001; Ikeda et al., 2002) and genome-length HCV RNA from the Con 1 strain (Pietschmann et al., 2002), were cultured in the above medium in the presence of 1 mg/ml G418. Cells were transfected with plasmid DNAs using FuGENE transfection reagents

(Roche Diagnostics, Tokyo, Japan). Culture media from Huh-7 cells transfected with in vitro-transcribed RNA corresponding to the full-length HCV RNA derived from the JFH-1 strain (Wakita et al., 2005) were collected, concentrated, and used for the infection assay (Aizaki et al., 2008).

Ab

Primary Abs used in this study were mouse monoclonal Abs against FLAG (Sigma-Aldrich, St. Louis, MO), c-myc (Sigma-Aldrich), CCT5 (Abnova Corporation, Taipei City, Taiwan), flotillin-1 (BD Biosciences, San Jose, CA), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Chemicon, Temecula, CA), BrdU (Caltag, CA) and HCV NS5A (Austral Biologicals, San Ramon, CA), a rabbit polyclonal Ab against hemagglutinin (HA; Sigma-Aldrich), a sheep polyclonal Ab against bromodeoxyuridine (Biodesign International, Saco, ME), and goat polyclonal Abs against the individual subunits of CCT (Santa Cruz Biotechnology, Santa Cruz, CA) and Hsc70 (Santa Cruz Biotechnology). Anti Hsc70 and CCT5 monoclonal rat Abs were obtained from Abcam (Tokyo, Japan) and AbD serotec (Oxford, UK). Rabbit polyclonal antibody to NS5A was described previously (Hamamoto et al., 2005). Anti NS5B monoclonal Ab was kindly provided by D. Moradpour (Centre Hospitalier Universitaire Vaudois, University of Lausanne; Moradpour et al., 2002).

Plasmids

To generate expression plasmids for the NS proteins with dual epitope tags, DNA fragments encoding the NS3, NS5A, or NS5B proteins were amplified from HCV strain NIHJ1 (Aizaki et al., 1998) by PCR and cloned into the EcoRI–EcoRV sites of pcDNA3-MEF, which includes the MEF tag cassette containing the myc tag, TEV protease cleavage site, and FLAG tag sequences (Ichimura et al., 2005; Shirakura et al., 2007). To create a series of NS5B truncation mutants, each fragment was amplified by PCR and cloned into the EcoRI–XhoI site of pCMV-HA (Clontech, Mountain View, CA). To generate expression plasmids for the individual CCT subunits, cDNA fragments encoding human CCT1 through CCT8 were amplified from the total cellular RNA by RT-PCR and then cloned into the SmaI site of pCAGGS (Niwa et al., 1991). All PCR products were confirmed by nucleotide sequencing.

Proteome analysis

RC-rich membrane fractions from the cells were isolated as described previously (Aizaki et al., 2004). Briefly, cells were lysed in hypotonic buffer. After removing the nuclei, the supernatants were mixed with 70% sucrose, overlaid with 55% and 10% sucrose, and centrifuged at 38,000 rpm for 14 h. Proteins from the membrane fractions were then analyzed by 2D-DIGE as described previously (Hara et al., 2009). Briefly, protein samples were resolved in protein solubilization buffer (Bio-Rad Laboratories, Tokyo, Japan) and washed with pH adjustment buffer (7 M urea, 2 M thiourea, 4% CHAPS, 30 mM Tris-HCl [pH 10.0]), before being labeled with fluorescent dyes; the dyes used were Cy3 for RCYM1 cells samples taken at the exponential growth phase, Cy5 for cells samples taken at the confluent phase, and Cy2 for a protein standard containing equal amounts of both cell samples. Aliquots of the labeled samples were pooled and applied to Immobiline DryStrip (GE Healthcare, Tokyo, Japan) for first-dimension separation and to 12.5% polyacrylamide gels for second-dimension separation. Images of the 2-D gels were captured on a Typhoon scanner (GE Healthcare), and analyzed quantitatively using DeCyder v5.0 software (GE Healthcare). Samples were analyzed in triplicate as independent cultures and the Student's t-test was applied using the DeCyder biological variation analysis

module to validate the significance of the differences in spot intensity detected between the samples.

In vitro RNA replication assay

In vitro replication of HCV RNA was performed as described previously (Hamamoto et al., 2005). Briefly, cytoplasmic fractions of subgenomic replicon cells were treated with 1% NP-40 at 4 °C for 1 h, followed by being incubated with 1 mM of ATP, GTP, and UTP; 10 µM CTP; [^{32}P]CTP (1 MBq; 15 TBq/mmol); 10 µg/ml actinomycin D; and 800 U/ml RNase inhibitor (Promega, Madison, WI) for 4 h at 30 °C. RNA was extracted from the total mixture by using TRI Reagent (Molecular Research Center, Cincinnati, OH). The RNA was precipitated, eluted in 10 µl of RNase-free water, and analyzed by 1% formaldehyde-agarose gel electrophoresis. For the immunodepletion assay, the cytoplasmic fractions were incubated with anti-CCT5 Ab in the presence of NP-40 for 4 h before NTP incorporation.

MALDI-TOF MS analysis

Target spots were cut and collected from gels under UV luminescence and rechecked with Typhoon scanner. The spot gels of the target proteins were subjected to in-gel trypsin digestion and analyzed by MALDI-TOF MS meter (Voyager-DE STR, Applied Biosystems, Tokyo, Japan) as described previously (Yanagida et al., 2000). All proteins were identified by peptide mass fingerprinting.

Immunoblot analysis and immunoprecipitation

Immunoblot analysis was performed essentially as described previously (Aizaki et al., 2004). The membrane was visualized with SuperSignal West Pico chemiluminescent substrate (Pierce, Rockford, IL). For immunoprecipitation, cells transfected with plasmids expressing epitope-tagged HCV protein or CCT5 were lysed and then subjected to two-step precipitations with anti-myc and anti-FLAG Abs according to the procedures described previously (Ichimura et al., 2005). In some experiments, HA-tagged full-length NS5B (aa 1–591) or its deletion mutants (aa 71–591, 215–591, 320–591, 1–570) were co-expressed with CCT5 in cells, followed by single-step immunoprecipitation and immunoblotting.

Immunofluorescence staining

Cell permeabilization with lysolecithin and detection of de novo-synthesized viral RNA was performed as described previously (Shi et al., 2003). Briefly, Huh-7 cells were plated on 8-well chamber slides at a density of 5×10^4 cells per well. Cells were incubated with actinomycin D (5 µg/µl) for 1 h and were washed twice with serum-free medium, before being incubated for 10 min on ice. The cells were then incubated in a transcription buffer containing 0.5 mM BrUTP for 30 min. The cells were fixed in 4% formaldehyde for 20 min and then incubated for 15 min in 0.1% Triton X-100 in phosphate-buffered saline (PBS). Primary Abs were diluted in 5% bovine serum albumin in PBS and were incubated with the cells for 1 h. After washing with PBS, fluorescein-conjugated secondary Abs (Jackson Immunoresearch Laboratories, West Grove, PA) were added to the cells at a 1:200 dilution for 1 h. The slides were then washed with PBS and mounted in ProLong Antifade (Molecular Probes, Eugene, OR). Confocal microscopy was performed on a Zeiss Confocal Laser Scanning Microscope LSM 510 (Carl Zeiss MicroImaging, Thornwood, NY).

RNA interference

Small interfering RNAs (siRNAs) targeted to CCT5 or Hsc70 and scrambled negative control siRNAs were purchased from Sigma-Aldrich Japan (Tokyo, Japan). Cells were plated on a 24-well plate with

antibiotic-free DMEM overnight, and each plate was transfected with 10 nM siRNAs by X-tremeGENE (Roche Diagnostics) according to the manufacturer's protocol. Forty-eight hours post-transfection, the total RNA and protein extracts were prepared and subjected to real-time RT-PCR and immunoblot analyses, respectively.

Quantitation of HCV RNA and core protein

Total RNA was extracted from cells using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Real-time RT-PCR was performed using TaqMan EZ RT-PCR Core Reagents (PE Applied Biosystems, Foster City, CA) as described previously (Aizaki et al., 2004; Murakami et al., 2006). HCV core protein levels in the cells and in the supernatant were quantified using an HCV core enzyme-linked immunosorbent assay (Ortho-Clinical Diagnostics, Tokyo, Japan).

Acknowledgments

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「肝胆膵」 別刷

石川県の取り組み

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索引用語：肝炎ウイルス検診，診療連携，IFN療法，フォローアップ事業，専門医療機関受診

抄録：石川県では肝炎ウイルス検診初年度から肝炎協議会をつくり，石川県下の市町村でほぼ統一した形で肝炎ウイルス検診を実施してきた．年ごとに改良を加え，検診の精度，インターフェロン療法施行率をあげてきた．肝炎ウイルス検診のデータベースを解析することにより，性・年齢・地域ごとの問題点が明らかとなり，また保健士などによるフォローアップ事業の有効性，専門医療機関受診がインターフェロン療法に繋がることが確認された．今後の肝疾患診療連携体制の構築にむけて拠点病院・専門医療機関で新たな協議会を立ち上げ，患者が専門医療機関に年1度受診することを勧めるシステムを運営開始した．

1 はじめに

ウイルス性慢性肝疾患が医療上のみならず，国をあげての問題となってきた．ウイルス性肝疾患の診断，治療に関する進歩は目覚しく，抗ウイルス療法により多くの症例が肝硬変への進展，肝がん発生の危険より救われている．しかしながら多くのウイルス性肝炎患者は自覚症状に乏しく，検査を受けなければ自分がウイルスキャリアであることが分からない．そこで行政は平成14年からウイルスキャリアの発掘し，適切な医療が行われることを目的に肝炎ウイルス検診を開始した．石川県では検診初年度よりこの検診の重要性に鑑み，県健康福祉部，保健所などの行政・検診を担当する医師会・学識経験者・検査センターをメンバーとして肝炎協議会を設置しその運営，県下の肝炎診療体制の確立に取り組んできた．本稿では石川県における肝炎ウイルス検診から現在までの取り組みについて述べる．

2 肝炎ウイルス検診初年度と翌年の改善

検診・精密検査医療機関を考える上で，石川県は肝臓専門医のいる総合病院は都市部に集中しており，消化器肝臓専門医のいる医療機関だけに精密検査医療機関を指定してしま

表1 肝炎ウイルス検診問題点の改善

平成14年度 HCV 168人	異常なし	慢性肝炎	肝硬変	肝がん	その他
	18 (10.7%)	120 (71.4%)	8 (4.8%)	1 (0.6%)	21 (12.5%)
	追跡調査の必要なし：6人　画像検査なし：18人				

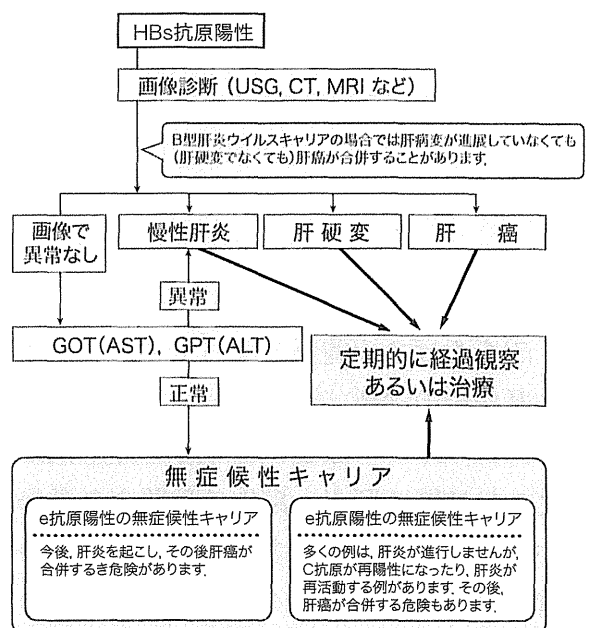| 平成15年度 HCV 221人	無症候性キャリア	慢性肝炎	肝硬変	肝がん	その他
	43 (19.5%)	128 (57.9%)	21 (9.5%)	5 (2.3%)	24 (10.8%)
	追跡調査の必要なし：1人　画像検査なし：1人				
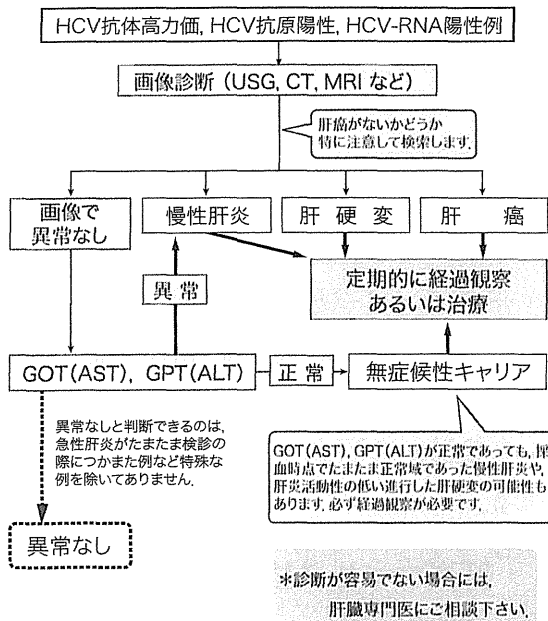
図1 肝炎ウイルス検診要精検者精密検査の手引き

うと精検受診率が低下する懸念があった．また多くの患者はかかりつけ医を持っていることが予想されたので，石川県では精密検査を行う医療機関の指定は行わなかった．このた

め，肝炎ウイルス検診の目的を考えると検診としての精度を上げることはもちろん，慢性疾患であるウイルス性肝炎は事後の経過観察が重要であるとの観点から，以下の7点を石

表2 肝炎ウイルス検診全国および石川県の状況

| | | 節目検診
受診率 | 精密検査
受診率 | 初年度IFN療法施行率 | |
				精検受診者全体	慢性肝炎
全国	平成14年	30.0%	65.4%	13.8%	
	平成15年	29.4%	85.6%	13.3%	
	平成16年	25.1%	81.8%	18.2%	
	平成17年	24.7%	80.8%		
	平成18年	23.2%	79.3%		
石川県	平成14年	41.5%	66.9%	3.0%	3.8%
	平成15年	41.5%	73.1%	5.7%	8.5%
	平成16年	37.8%	68.3%	14.7%	23.5%
	平成17年	36.8%	65.8%	24.5%	35.3%
	平成18年	36.6%	57.4%	23.7%	31.0%

全国の精密検査受診率, IFN療法施行率は日野のデータ[1]より

川県肝炎ウイルス検診の柱とした.

　1)検診への行政が関与することの通知と同意.

　2)精密検査の全県下で統一.

　3)住民, 検診・精密検査担当医に対する手引きの作成.

　4)精密検査での画像検査の義務付け.

　5)全症例に対する事例検討会の開催.

　6)前年度陽性者に対する保健師による事後調査.

　7)保健師などを対象にした研修会の開催.

　検診初年度の精密検査結果(C型)を表1に示す. 平成14年は168人が精密検査受診をし, 120人が慢性肝炎, 8例が肝硬変と診断されている. 問題点のひとつは義務付けていた画像検査を行っていない症例が18人いたこと, もうひとつはC型慢性肝疾患への事後対応として「追跡調査の必要なし」を選択された症例が6人いたことである. 「追跡調査必要なし」が選択された一因は精密検査結果報告書の診断名に「異常なし」があったことであった. このため肝炎協議会で検討し, ①診断名を「異常なし」から「無症候性キャリア」へ変更, ②

一例ごとの事例検討会において画像検査の重要性を強調, ③担当医・陽性者に送付する診断の手引きの作成(図1)を行った. その結果翌年には画像検査なしに診断された症例および「追跡調査必要なし」とされたのは221人中それぞれ1人のみであり, 県下統一して検診の精度が保たれるようになった.

3 IFN施行率の向上のための施策

　肝炎ウイルス検診でみいだされたC型肝炎症例については全国的にもインターフェロン療法の施行率が低いことが問題となっている. 肝炎ウイルス検診の最終目標はみいだした症例をIFN療法に結びつけることであると考えるが, 平成14〜16年全国集計では[1]主に経口薬が用いられ(平成14年:38%, 平成15年:43%, 平成16年:34%), IFN療法施行率は20%に満たない(表2). 石川県においても要精検者全体で平成14年3.0%, 15年5.7%, また精検診断名として無症候性キャリアでなく慢性肝炎とした症例においても, 平成14年3.8%, 15年8.5%と低率であった(表2). 特に65歳以上の高齢者ではIFN施行

表3 慢性肝炎・肝硬変への初年度 IFN 施行例数(％)

	平成14	平成15	平成16	平成17	平成18
能登北部	2/40 (5.0)	2/12 (16.7)	0/6 (0)	1/6 (16.7)	5/8 (62.5)
能登中部	3/33 (9.1)	2/23 (8.7)	8/24 (33.3)	0/8 (0)	6/20 (30.0)
金沢市	-	5/91 (5.5)	6/33 (18.2)	10/27 (37.0)	4/26 (15.3)
石川中央	1/42 (2.4)	3/18 (16.7)	3/11 (27.3)	3/9 (33.3)	4/10 (40.0)
南加賀	0/16 (0)	1/20 (5.0)	7/28 (25.0)	10/18 (55.6)	3/7 (42.9)
総計	6/131 (4.6)	13/164 (7.9)	24/102 (23.5)	24/68 (35.3)	22/71 (31.0)

率が2.6％と65歳未満の9.6％に対して有意に低いことが問題であった[2]．

インターフェロン療法が行われない理由を検討するために県下の内科標榜している医療機関567施設(平成16年時点)に自記式アンケート調査を行い，回収された279施設にて検討した．回答した医療機関形態は病院63施設(22.6％)，診療所213施設(76.3％)，回答した医師の専門は肝臓29名(10.4％)，消化器84名(30.1％)，消化器肝臓以外165名(59.1％)であった．「インターフェロン療法を一度は説明するか？」という設問には肝臓専門医は82.1％，消化器専門医は62％，消化器肝臓以外は48.5％と専門により症例をえらばずインターフェロン療法を説明する医師の割合が大きく異なっていた．インターフェロン療法を行わない理由としては「高齢」「ALT値正常」「禁忌症例」「患者拒否」が専門を問わずあげられた．しかし「高齢」「ALT値正常」の内容についてみると，年齢によるインターフェロン療法の適応年齢は肝臓専門，消化器専門，消化器肝臓以外でそれぞれ「60歳上限」：7.1％，14.1％，14.9％，「65歳」：21.4％，26.8％，20.1％，「70歳」：42.9％，42.3％，30.6％，「75歳」：21.4％，4.2％，9.7％，「問わない」：7.1％，9.9％，14.9％と肝臓専門医はその上限を高く，専門外医師は低く考えがちであった．またALT値によるイン

ターフェロン療法の適応についてはALT値を「問わない」医師は32.1％，36.6％，26.6％と大きな差はなかったが，ALT値が「100IU/L以上」：7.1％，21.1％，35.1％，「80IU/L以上」：14.3％，22.5％，13.4％，「60IU/L以上」：14.3％，7.0％，9.0％，「40IU/L以上」：32.1％，8.5％，4.5％と肝臓専門医とそれ以外の医師でALT値とそのインターフェロン適応の考え方に差が認められた．

このため平成14～16年の事例検討会で一例ごとに治療についてのサジェスチョンを行い，また県下各3地域での医師会肝炎部会での講演会では，平成17年度「最新のインターフェロン療法」，18年度「高齢C型肝炎に対する考え方」，19年度「ALT正常者に対する考え方」などテーマをしぼり，治療についての知識啓蒙を行ってきた．以後インターフェロン療法施行率はやや上昇するようになり，平成16年102例中24例(23.5％)，平成17年68例中24例(35.3％)，平成18年71例中22例(31.0％)と後半2年間はインターフェロン療法施行率が30％を超えていた(表2)．これは石川県の医療圏ごとに分けてみても大きな地域差無く検診症例へのインターフェロン施行率が上昇していた(表3)．また検診を受けた同年度にインターフェロン療法は受けなくても，以後にインターフェロン療法を受ける症例が増え，施行率の低かった平成14，

表4　石川県肝炎ウイルス検診(C型)精検未受診者のその後の状況

	検診初年度 精検未受診	翌年以降 医療機関受診	翌年以降受診者 IFN療法施行数
能登北部	18 (14.8%)	12 (66.7%)	3 (25.0%)
能登中部	32 (17.5%)	17 (53.1%)	2 (11.8%)
金沢市	71 (31.8%)	45 (63.4%)	7 (15.6%)
石川中央	88 (40.6%)	52 (59.1%)	10 (19.2%)
南加賀	147 (28.1%)	39 (26.5%)	2 (5.1%)
	356 (28.1%)	165 (46.3%)	24 (14.5%)

15年の症例も平成19年にはそれぞれ累計で27%, 32%の症例がインターフェロン療法を受けたことが確認されている. またインターフェロン療法医療費補助制度のデータでは, 石川県では平成20年に533人のC型肝炎症例が同制度を利用したが, うち158人(29.6%)は65歳以上と高齢者に対するインターフェロン療法も普及していることが伺える.

4 フォローアップ事業の重要性

石川県では初年度から肝炎ウイルス検診受診時に, 検診後も保健士・行政が係わってフォローすることの同意を得ている. 保健士・行政は少なくとも年1回は本人あるいは医療機関に受診状況の把握に努め, 医療機関に未受診の時にはパンフレットを用いながら直接受診勧奨している. このようなフォローアップ事業を毎年続けており, 例えば平成14年度HCV精検者は1年後54.8%, 2年後52.5%, 3年後56.7%, 4年後57.5%, 5年後63.2%, が医療機関受診していること, およびその診療内容を把握している. 以後, 医療機関受診率は平成15年症例で1年後63.7%, 2年後56.9%, 3年後55.8%, 4年後48.7%, 平成16年症例で1年後51.9%, 2年後46.4%, 3年後51.1%, 平成17年症例で1年後56.7%, 2年後54.1%, 平成18年症例で1年後53.2%で

あった. 一方その年の状況不明な症例も31, 39, 32, 28, 17%存在している. 精検受診率は全国データ(表2)より低い傾向にあるが, 全国報告は精検受診有無が確認できたのが平均57%ほどのデータから得られたものであり, 行政がフォローできていない地域も多い[1]. 石川県では各年毎の状況把握では受診率は上記のごとくであるが, 実際にフォロー期間中に少なくとも一度は医療機関を受診している率は市町ごとで, C型肝炎で86～100%, B型肝炎でも49～100%とかなりの症例は一度は受診していることがうかがえる. 本県では受診勧奨に務めた結果, 検診初年度には未受診でも翌年以降に46.3%が医療機関を受診し, そのうち14.5%でIFN療法が行われる(表4)など保健士・行政によるフォローアップ事業が医療機関への受診勧奨・適切な医療へ結びつくことがうかがえる.

5 肝炎ウイルス検診データ・住民アンケート調査からみる問題点

キャリアの発見には肝炎ウイルス検診受診率の向上が重要であったし, 未受診者には現在行われている緊急肝炎ウイルス検査の周知が必要である. 石川県では5年間の肝炎ウイルス検診受診率は36.6～41.5%と全国平均[1]と比べると10%ほど受診率が良かったが

精検受診率

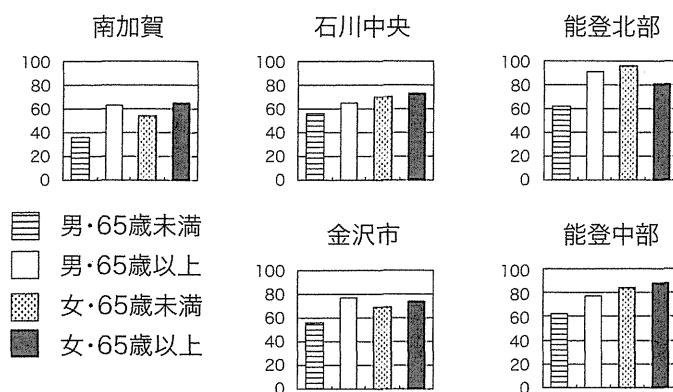|	65歳未満	65歳以上	計(性別)
男性	70/131(53.4%)	216/292(74.0%)	286/423(67.6%)
女性	205/285(71.9%)	418/557(74.0%)	286/423(75.0%)
計	275/416(66.1%)	634/849(74.7%)	909/1,265(72.0%)

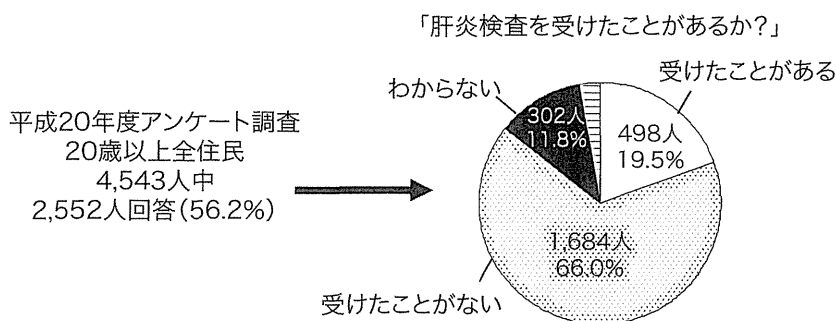図2　石川県C型肝炎ウイルス検診(平成14〜18)精密検査受診状況

図3　石川県K町における全住民に対するアンケート調査
K町における肝炎ウイルス検診対象者(H14〜19年)
906人中744人受診(82.1％)

満足できるものではない．図2に平成14年肝炎ウイルス検診の性・年齢・医療圏別での精検受診状況を示す．検診自体の受診率は能登地方および南加賀で低い傾向にある．しかしながら能登地方はウイルスキャリアと判明すると医療機関にはきちんと受診する傾向にある．一方南加賀ではウイルスキャリアと判明しても医療機関への受診率が悪い．能登地方ではキャリアの発掘が重要であり，南加賀ではキャリアの発掘と受診勧奨の両面が必要なことがうかがえる．また医療機関受診の時間がとりにくい若年男性の受診率が悪いのは共通しており，受診動機を促す啓蒙活動が必要である．

　自覚症状のないウイルス性肝炎の発見には，一生の間に一度は検査を受けてもらうのが肝炎ウイルス検診の一つの目的である．ウイルス検査陽性の方が医療機関を受診するのはもちろんだが，陰性であった人は「肝炎ウイルス検査」をして「陰性」であったことを意外なほど覚えていない．図3に石川県K町において20歳以上の全住民対象のアンケート

Q 治療内容(n=510)

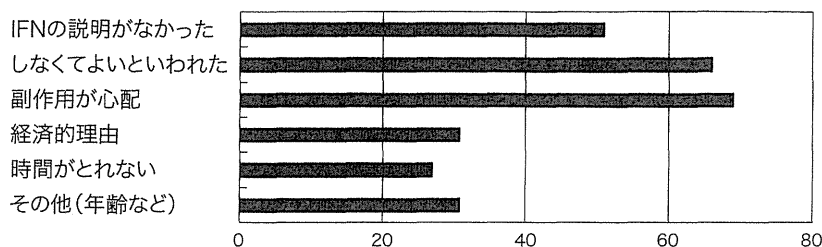| | C型(n=321) | | B以下(n=243) |
	全体	75歳以下(n=243)	
経過観察のみ	98(31%)	66(27%)	154(82%)
経口薬	78(24%)	49(20%)	10(5%)
IFN以外の注射	29(9%)	22(9%)	0(0%)
IFN	116(36%)	106(44%)	6(3%)
抗ウイルス薬(B型)			19(10%)

Q IFNを受けたことがない理由(C型:複数回答)

図4 肝炎ウイルス検診受診者アンケート調査

調査を行った[3]が同町は肝炎検診受診率が高かった(肝炎ウイルス検診受診率82.1%)にもかかわらず,「肝炎検査を受けたことがあるか?」の問いに「受けたことがある」と答えたのは20%未満であった. 患者は検査を受けても,検診の一検査として陰性であれば検査を受けたこと自体を忘れがちで,何度も受診することになり,費用の無駄あるいは肝炎検査受診者の正確な把握を難しくすると予想される.

検診後状況を行政側でフォローしているが,実際の治療状況を把握するために検診陽性者にアンケート調査を行った. ウイルス検診陽性者全員対象のアンケート調査には12市町が協力可能で1,910名にアンケートが送付され780名(B型42.1%, C型53.3%, 回答率40.8%,)から回答が得られた. 検診からの通知後医療機関を受診していないのは67名(8.7%)であり,検診初年度に精密検査を受診していなくても,フォローしていくことで以後に受診している状況が,アンケート調査でも判明した. しかしながら現在通院をやめ

ている症例はB型35%, C型17%存在し,理由として「通院しなくてもよい」といわれたのが6割以上を占めたのが問題であった. 75歳以下のC型肝炎症例は44%がインターフェロン療法を,B型肝炎症例は10%が抗ウイルス薬投与を受けていた(図4). インターフェロンを受けていない理由としては,「副作用が心配」が多く,これは主治医よりインターフェロンの説明を受けているからこその回答と考える. 一方,「しなくてよい」といわれた症例も多く存在しており,前述の医師にとったアンケート結果同様「しなくてよい」と考える,その内容が問題であると考えられた.

6 石川県の肝炎診療の今後の対策

IFN療法の施行率をあげるもう一つの方法は専門医が診ることである. 石川県肝炎ウイルス検診において精検を行った年より経過観察のみでなく何らかの治療を行った症例は185名であった. 41名が非専門医の一般診療所,144名が総合病院あるいは専門医が精検を担当しているが,初年度からのIFN療法施

表5　初年度受診医療機関別Ｃ型肝炎治療法

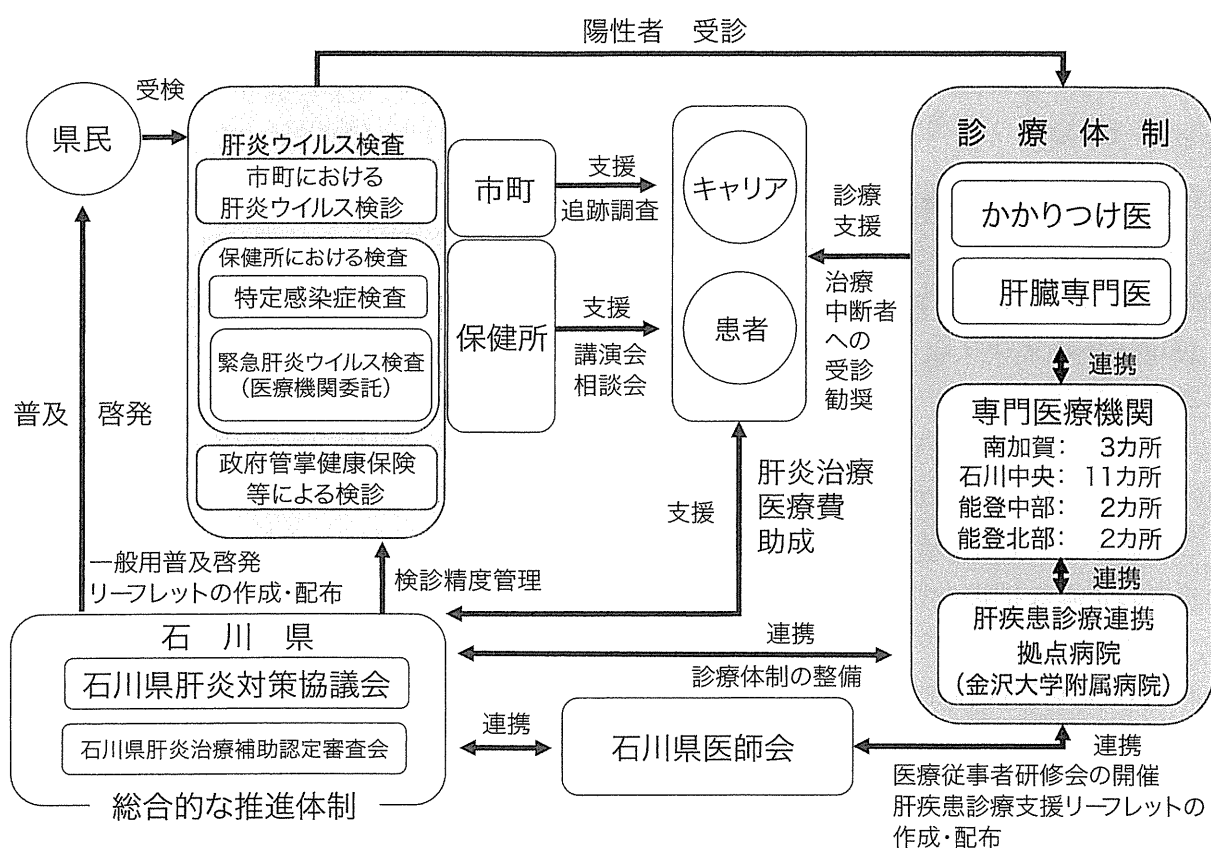| 治療方針	診療所 (n=41)	総合病院・専門医 (n=144)
IFN治療	4（ 9.8%）	53（ 36.8%）
他の注射薬	4（ 9.8%）	3（ 2.1%）
→IFN（移行率）	2（50.0%）	3（100.0%）
内服薬	33（80.5%）	88（ 61.1%）
→IFN（移行率）	2（ 6.1%）	15（ 17.0%）
内服薬・65歳未満	7	28
→IFN（移行率）	0（ 0.0%）	8（ 28.6%）
のべIFN療法	8（19.5%）	79（ 54.9%）

図5　石川県における肝炎総合対策体制

行率は診療所で9.8%，総合病院・専門医では36.8%と総合病院・専門医で高いIFN導入率が示された．診療所では実に80.5%が経口薬にて治療されており，その後のIFN導入率も低い傾向であった．一方総合病院・専門医では経口薬或いはIFN以外の注射にて当初治療されても，その後にIFN導入が行われ，のべIFN導入率は54.9%と診療所の19.5%と比べ明らかに高かった（表5）．現在厚生労働省の肝炎検診後の診療ガイドラインでも少なくとも年1回の専門医受診が推奨されており，IFN施行率を上げるうえでガイドラインに即

した地域診療体制の確立が重要である.

　石川県ではガイドラインを受けて肝疾患診療連携拠点病院,専門医療機関を整備し,図5に示すような県全体での総合対策体制を打ち出している.さらに昨年度より「石川県肝炎診療連携協議会」を拠点病院・専門医療機関で立ち上げ,本年度より医師会・行政の協力のもと,年1回患者に直接専門医療機関受診票を送付し,かかりつけ医を介して専門医療機関を紹介するシステムを運用開始し,今回の解析で有効と考えられた受診勧奨・専門医療機関受診の両面から患者をサポートしている.

7　まとめ

　石川県が肝炎診療連携のために肝炎ウイルス検診から取り組んできたことを紹介した.システム作りは大切であるが,行政・保健所・医師会の協力が不可欠であり,また彼ら

の肝炎診療に対する理解もボトムアップしていかないと地域としてうまく動かないことも痛感している.幸い石川県では各領域での理解と協力が得られており各事業がうまく運営され,成果が期待される.

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