MCPIP1 Suppresses Hepatitis C Virus Replication and Negatively Regulates Virus-Induced Proinflammatory Cytokine Responses

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MCPIP1 Suppresses Hepatitis C Virus Replication and Negatively Regulates Virus-Induced Proinflammatory Cytokine Responses


Human MCP-1–induced protein 1 (MCPIP1, also known as ZC3H12A and Regnase-1) plays important roles in negatively regulating the cellular inflammatory response. Recently, we found that as an RNase, MCPIP1 has broad-spectrum antiviral effects by targeting viral RNA. In this study, we demonstrated that MCPIP1 expression was induced by hepatitis C virus (HCV) infection in Huh7.5 hepatoma cells. MCPIP1 expression was higher in liver tissue from patients with chronic HCV infection compared with those without chronic HCV infection. Knockdown of MCPIP1 increased HCV replication and HCV-mediated expression of proinflammatory cytokines, such as TNF-α, IL-6, and MCP-1. Overexpression of MCPIP1 significantly inhibited HCV replication and HCV-mediated expression of proinflammatory cytokines. Various mutants of functional domains of MCPIP1 showed disruption of the RNA binding and oligomerization abilities, as well as RNase activity, but not deubiquitinase activity, which impaired the inhibitory activity against HCV replication. On immunocytochemistry, MCPIP1 colocalized with HCV RNA. Use of a replication-defective HCV John Cunningham 1/AAG mutant and in vitro RNA cleavage assay demonstrated that MCPIP1 could directly degrade HCV RNA. MCPIP1 may suppress HCV replication and HCV-mediated proinflammatory responses with infection, which might contribute to the regulation of host defense against the infection and virus-induced inflammation. The Journal of Immunology, 2014, 193: 4159–4168.

Hepatitis C virus (HCV), the only member of the genus Hepacivirus in the Flaviviridae family, is the major cause of chronic hepatitis with persistent liver inflammation. More than 170 million people are infected with HCV worldwide.
IL-12p40, IL-1β, and IL-2, via AU-rich element–independent mechanisms (9, 13, 14). As an RNAse, MCPIP1 could suppress a broad range of biosynthesis and activity of microRNAs (miRNAs) by cleaving the terminal loops of precursor miRNAs (12). The DUB function of MCPIP1 inhibits LPS-, IL-1β, and TNF-α–mediated NF-κB– and JNK-signaling pathways by removing ubiquitin moieties of TNFR-associated factors (TRAFs), including TRAF2, TRAF3, and TRAF6 (11). Zc3h12a/MCPIP1-deficient mice exhibited severe immune syndrome disorders characterized by severe anemia, autophagosome response, and severe inflammation, and most mice died within 12 wk of birth (9).

TNF-α, MCP-1, and IL-1β can induce MCPIP1 to negatively regulate cellular inflammatory responses (8, 9). TNF-α was elevated in plasma from HCV patients with chronic hepatitis, which is associated with the HCV-induced inflammatory state and pathogenesis (15–18). HCV infection leads to IL-1β induction, activation, and secretion via the caspase-1–inflammation complex in Huh7.5 hepatoma cells (19). Thus, MCPIP1 may be induced with the host response to HCV infection via proinflammatory cytokines, such as TNF-α and IL-1β. However, the relationship between MCPIP1 and HCV infection remains to be explored.

In this study, we investigated the expression of MCPIP1 in HCV-infected cells and in patients with HCV infection. We used gene overexpression and knockdown to explore the antiviral activity against HCV infection and regulation of the inflammatory response during HCV infection. Moreover, the use of MCPIP1 mutants with functional defects in RNA binding, RNase activity, and oligomerization clarified the anti-HCV mechanism of MCPIP1. We show that the induction of MCPIP1 by HCV infection can contribute to the host defense against HCV and the suppression of cellular inflammatory responses induced by HCV.

Materials and Methods

Cell lines, chemicals, Abs, and patient samples

Huh7.5 cells (20), tetracycline-regulated expression embryonic kidney 293 cells (T-REX-293 (Invitrogen)) (21), and HCV subgenomic replicon, HCV-EV71I-Luc cells (22) were grown in DMEM containing 10% FCS with selective antibiotics (5 μg/ml blasticidin and 250 μg/ml hygromycin for T-REX-293; 0.5 mg/ml G418 for HCV-EV71I-Luc). Abs for rabbit monoclonal anti-hemagglutinin (HA) (Covance), rabbit polyclonal anti-MCPIP1 (GeneTex), mouse monoclonal anti-dsRNA (J2 mAb; English Scientific Consulting), mouse monoclonal anti-HCV core (Thermo Scientific), and mouse monoclonal anti-HCV NSSA (Biodesign) were used in this study.

Human liver tissues (n = 44; 22 chronic HCV infection [anti-HCV Ab persisting for >6 mo] and 22 HCV–) were obtained as anonymous surgical specimens (nontumorous portion) from Taipei Medical University. This study received Taipei Medical University–Joint Institutional Review Board approval (protocol no. 201208017). Liver tissues were embedded in paraffin for immunohistochemistry. Scores for MCPIP1 expression were determined by multiplying the proportion of cells positive (P) for MCPIP1 by intensity (I) (0, nonreactive; +1, weak; +2, moderate; +3, strong). Total score = P × I; maximum = 300.

Plasmids and viruses

The HA-tagged mutant constructs of MCPIP1-D141N, -C157A, -C306R, -C312R, -C318R, -H322R, -D225S/226A, -Δ305-325, and -Δ458-536 were generated from a JC1 wild-type construct. The John Cunningham (J1)C1/AAG mutant with a replication-defective mutation (GDD to AAG) in NS5B (23) was generated from a JC1 wild-type construct. The John Cunningham 1 (JC1)/AAG mutant with a replication-defective mutation (GDD to AAG) in NS5B (23) was generated from HCV-EV71I-Luc cells (22) were grown in DMEM containing 10% FCS from HCV 5' UTR was analyzed by 1% agarose gel electrophoresis for RNA-binding assay

Confocal imaging

Confocal images were described previously (25).

Determination of the replication of HCV-EV71I-Luc subgenomic replicons

HCV-EV71I-Luc replicon cells cultured in DMEM without G418 were transduced with the lentiviral vectors expressing the various HA-MCPIP1 proteins for 72 h. The cell lysates were harvested to examine luciferase activity and normalized cell viability by a Bright-Glo Luciferase Assay System (Promega) and a 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium salt assay, respectively.

RNA-binding assay

Briefly, Huh7.5 cells were infected with JC1 for 72 h and then transduced with lentiviral vectors expressing the HA-MCPIP1 protein for 48 h. Cell extracts were mixed with prewashed HA beads (Sigma) and incubated at 4°C overnight. Viral RNA from the complex was extracted using the RNasey Total RNA kit (QIAGEN). RT-PCR involved the primer pair sequences for HCV 5' UTR (forward, 5'-GAGTGTCGTGCAGCCTCCAG-3' and reverse, 5'-CACATGCAAGACCCCTACCA-3'). The PCR product from HCV 5' UTR was analyzed by 1% agarose gel electrophoresis and detected by ethidium bromide staining.

In vitro RNA-cleavage assay

The recombinant HA-tagged MCPIP1 and D141N mutant were pulled down with HA beads from T-REX-293T cells expressing MCPIP1. The full-length viral RNA was transcribed in vitro from JC1 plasmid using the MEGAscript high-yield transcription kit (Ambion). Viral RNA and purified HA-MCPIP1 protein were incubated in RNA cleavage buffer (25 mM HEPES, 50 mM potassium acetate, 5 mM DTT, and 0.5 U RNasi), with or without 5 mM Mg2+2 at 30°C. The integrity of viral RNA was analyzed as described (21).

Establishment of short hairpin RNA–expressing Huh7.5 cell lines

To generate MCPIP1 and control knockdown cells, we used short hairpin RNA (shRNA) targeting MCPIP1 (shMCPIP1) lentiviral particles (Santa Cruz Biotechnology; sc-78944-V) and control shRNA lentiviral particles (sc-108080), respectively. Briefly, Huh7.5 cells were transduced with lentivirus for 24 h and selected with puromycin (2.5 μg/ml) for 72 h.

RNA extraction and real-time RT-PCR

Total cellular and viral RNAs were isolated for generating cDNA, as described (21). Gene expression was determined using the following TaqMan primer/probe mixes (Applied Biosystems) with TaqMan Gene Expression Assay ID: MCPIP1: HS00962356_m1, IL-1β: HS01554510_m1, IL-6: HS00985639_m1, TNF-α: HS01131624_g1, MCP-1: HS00234140_m1, and GAPDH: HS02758991_g1. For HCV RNA
detection, TaqMan primer or probe mixes (sense: 5'-CGGGA-GACGCATAGTGG-3', antisense: 5'-AGTACCACAAGGCCTTTCG-3', probe: 5'-CTGCGGAACCGGTAGTACAC-3') were used (Applied Biosystems), as described (28). Real-time PCR amplification and data analysis were performed using an ABI Prism 7500 Sequence Detector system (Applied Biosystems).

**Statistical analysis**

All data are expressed as mean ± SD of three independent experiments and were compared by the two-tailed Student t test. The Mann–Whitney–Wilcoxon test was used to assess the association between HCV infection and MCPIP1 expression. The p values < 0.05 were considered statistically significant.

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**FIGURE 1.** Induction of MCPIP1 by HCV infection negatively regulates HCV replication and HCV-induced proinflammatory responses. (A) Huh7.5 cells were mock infected or infected with HCV JC1 (multiplicity of infection [MOI] = 1) for 3, 6, or 8 d. Western blot analysis of protein level of MCPIP1, HCV core, and β-actin (loading control) (upper panel). Quantitative RT-PCR analysis of MCPIP1 mRNA level normalized to that of GAPDH (lower panel). (B) Huh7.5 cells were mock infected or infected with HCV JC1 (MOI = 1) for 3 or 6 d. Quantitative real-time RT-PCR of mRNA levels of IL-1β, IL-6, TNF-α, and MCP-1 normalized to that of GAPDH. (C) Huh7.5 cells were transduced with lentiviral particles of shMCPIP1 or control shRNA (shCtrl). Quantitative real-time RT-PCR and Western blot analysis of the knockdown. (D–F) Huh7.5 cells were transduced with lentiviral particles of shMCPIP1 or control shRNA (shCtrl) and then infected with HCV JC1 (MOI = 1) for 6 d. Quantitative real-time RT-PCR analysis of mRNA levels of IL-1β, IL-6, TNF-α, MCP-1, and MCPIP1 (D) and HCV RNA (negative- and positive-sense) (E). (F) The cell culture supernatants were collected to determine the infectious HCV titers (FFU/ml) by focus-formation assays. Data are mean ± SD of three independent experiments. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.
Results

Induction of MCPIP1 in HCV-infected hepatoma cells inhibits both HCV replication and HCV-induced inflammation

To examine whether HCV infection could induce MCPIP1 expression, Huh7.5 cells were infected with HCV JCI strain. The mRNA and protein levels of MCPIP1 were increased over time in HCV-infected cells (Fig. 1A). Furthermore, the mRNA levels of IL-6, TNF-α, and MCP-1, but not IL-1β, were upregulated in HCV-infected cells (Fig. 1B). To assess the role of MCPIP1 in regulating cellular inflammatory responses and HCV replication in HCV-infected cells, shMCPIP1 was used to transiently knock down MCPIP1. Compared with a scramble shRNA control, shMCPIP1 knocked down endogenous MCPIP1 expression by >70% in cells (Fig. 1C). The mRNA levels of IL-6, TNF-α, and MCP-1 were higher with shMCPIP1 than with shRNA in HCV-infected cells (Fig. 1D). Notably, the RNA (negative- and positive-sense) and core protein levels of HCV (Fig. 1E), as well as infectious HCV production (Fig. 1F), were significantly increased with shMCPIP1.

To further verify the ability of MCPIP1 to negatively regulate the HCV-induced inflammatory response and HCV replication, a lentiviral expression vector was used to overexpress HA-tagged MCPIP1 in Huh7.5 cells. Overexpression of MCPIP1 decreased the mRNA levels of IL-6, TNF-α, and MCP-1 induced by HCV (Fig. 2A). HCV replication, detected by HCV RNA level, was reduced in MCPIP1-overexpressed cells (Fig. 2C). Similarly, the expression of HCV core protein was decreased in MCPIP1-overexpressed cells, which showed a slightly lower level of expression of endogenous MCPIP1 (Fig. 2B). Thus, overexpression of MCPIP1 inhibited HCV replication and the HCV-induced inflammatory response. Induction of MCPIP1 by HCV may negatively regulate the HCV-induced inflammatory response and HCV replication.

The inhibitory effect of MCPIP1 on HCV RNA replication is via an RNase-dependent mechanism

The NYN domain of MCPIP1 has RNase activity, but the D141N mutation in the NYN domain abolish its RNase activity (Fig. 3A). To examine whether RNase activity of the NYN domain involves inhibiting HCV replication, we used a lentiviral vector expressing the HA-tagged MCPIP1 or MCPIP1-D141N mutant. Overexpression of MCPIP1, but not a D141N mutant, inhibited the expression of HCV core protein (Fig. 3B) and RNA (Fig. 3C) in HCV-infected cells. To further assess the anti-HCV effects of MCPIP1 on HCV replication, we used an Huh7 cell line stably harboring a subgenomic HCV replicon and an EV71-IRES-driven luciferase gene (Fig. 3D) (22). The replicon cells were transduced with lentiviruses expressing the HA-tagged MCPIP1 or MCPIP1-D141N mutant. Compared with the MCPIP1-D141N mutant, overexpression of MCPIP1 markedly reduced luciferase activity (Fig. 3E) and HCV NS5A protein expression (Fig. 3F). Because the D141N mutant also lost DUB activity, we examined whether the DUB activity was involved in the anti-HCV activity. Huh7.5 cells were transduced with lentiviruses expressing HA-tagged MCPIP1, MCPIP1-C157A (RNase+/DUB−) mutant, or D225/226A (RNase−/DUB+) mutant (Fig. 3A) and then infected with HCV. The MCPIP1-C157A mutant, but not the D225/226A mutant, inhibited the expression of HCV core protein and RNA (Supplemental Fig. 1), which suggests that RNase, but not DUB, activity of MCPIP1 is required for its anti-HCV activity.

MCPIP1 directly interacts with HCV RNA and then degrades HCV RNA

To determine whether MCPIP1 protein directly interacts with HCV RNA and then degrades HCV RNA, Huh7.5 cells were infected with HCV and transfected with the HA-tagged MCPIP1 or MCPIP1-D141N mutant, and MCPIP1 protein and HCV RNA levels were detected with anti-HA and anti-dsRNA Abs, respectively. Merged images indicated the colocalization of MCPIP1 and HCV RNA. Both MCPIP1- and MCPIP1-D141N-expressing cells showed colocalization, with less colocalization in MCPIP1-D141N-expressing cells (Fig. 4A). These effects might be due to MCPIP1 quickly degrading HCV RNA when they come in contact. In contrast, the D141N mutant can bind to HCV RNA but may not degrade it, accounting for the greater degree of colocalization. To further determine whether MCPIP1 could directly degrade HCV RNA in cells, Huh7.5 cells were overexpressed with MCPIP1 or MCPIP1-D141N, and then transfected with replication-defective HCV (JCI/AAG mutant) RNA. HCV RNA time dependently accumulated with overexpression of MCPIP1-
D141N, but not MCPIP1, which suggests that MCPIP1 could degrade HCV RNA in cells (Fig. 4B). In vitro–transcribed full-length HCV RNA was incubated with recombinant MCPIP1 or MCPIP1-D141N protein in the presence of Mg²⁺; MCPIP1 time dependently degraded HCV RNA, but MCPIP1-D141N lost the ability to degrade HCV RNA (Fig. 4C). Thus, MCPIP1 may directly interact with HCV RNA and then degrade it.

C312 and C318, but not C306 or H322, residues of MCPIP1 are critical for the antiviral activity and HCV RNA-binding activity

To evaluate the importance of the ZF domain in the HCV RNA-binding and anti-HCV activities of MCPIP1, we mutated or truncated the conserved cysteine residues (C306, C312, and C318) and one conserved histidine residue (H322) (red letters). (B and C) Huh7.5 cells were transduced with lentiviral vector (multiplicity of infection [MOI] = 2) expressing the HA-tagged MCPIP1 or MCPIP1-D141N mutant for 48 h and then mock infected or infected with HCV JC1 (MOI = 1) for 72 h. (B) Western blot analysis of indicated protein. (C) Quantitative real-time RT-PCR analysis of HCV RNA. Pretreatment with IFN-α (1000 U/ml) for 24 h was a control.

The PRR domain of MCPIP1 is required for its oligomerization and anti-HCV activity

A PRR domain of MCPIP1 located on the C-terminal region (458–536 aa) (Fig. 3A) for its oligomerization and RNase activity is required for miRNA inhibition and antiviral activity (12, 21). To examine whether the oligomerization of MCPIP1 is involved in inhibition of HCV replication, the PRR domain of MCPIP1 was deleted (designated as the D458-536 mutant). The oligomerization of MCPIP1 was assessed by chemical cross-linking; the D458-536 mutant lost nearly all oligomerization ability (Fig. 6A) and could not inhibit HCV core protein.
PRR domain of MCPIP1 is required for its oligomerization and inhibition of HCV replication.

Expression of MCPIP1 in human liver tissues is associated with HCV infection

MCPIP1 expression in human liver tissues, with and without HCV infection, was compared using the Mann–Whitney–Wilcoxon test. By immunohistochemistry, MCPIP1 expression was strong in the hepatocyte cytosol of nontumorous liver tissue (Fig. 7A). MCPIP1 expression was increased in liver tissue from patients with chronic HCV infection \((p = 0.043)\) (Fig. 7B), which suggests that HCV infection induced MCPIP1 expression in human liver tissue, and MCPIP1 might be involved in the control of HCV replication.

Discussion

MCPIP1, a multifunctional protein with cellular RNase and DUB activities, plays important roles in negatively regulating the cellular inflammatory response by multiple molecular mechanisms \((9, 11, 14)\). Recently, MCPIP1 was discovered to be a host factor inhibiting the activity of various RNA viruses, including Japanese encephalitis virus, dengue fever virus, influenza A virus, Sindbis virus, and encephalomyocarditis virus, as well as DNA viruses, such as adenovirus \((21)\). We found that MCPIP1 mRNA and protein levels were readily induced in HCV-infected Huh7.5 cells (Fig. 1). Moreover, immunohistochemistry revealed significantly greater MCPIP1 expression in liver tissue from patients with chronic HCV infection than in those without chronic HCV infection (Fig. 7). Using knockdown and overexpression of MCPIP1, we demonstrated that MCPIP1 induced by HCV can contribute to host anti-HCV activity and the suppression of HCV-induced proinflammatory cytokines, such as IL-6, TNF-\(\alpha\), and MCP-1. MCPIP1 inhibited HCV replication through viral RNA degradation by its oligomerization, as well as its viral RNA-binding and RNase activities. These findings are similar to the antiviral mechanism of MCPIP1 on JEV and DEN-2 viruses that we reported previously \((21)\). Therefore, MCPIP1 inhibits viral replication of members of the Flaviviridae family, such as JEV and DEN-2, as well as a unique member, HCV, of the genus Hepacivirus.
HCV RNA. Pretreatment with IFN-β blot analysis of protein levels of HA-tagged MCPIP1, HCV core, and control. Data are mean ± SD of three independent experiments. **p < 0.01, ***p < 0.001.

FIGURE 5. Mutation analysis of each ZF residue of MCPIP1 in its anti-HCV and HCV viral RNA-binding activities. (A) Huh7.5 cells were mock infected or infected with HCV JC1 (multiplicity of infection [MOI] = 1) for 72 h and then transduced with lentiviral vectors (MOI = 2) expressing the various types of HA-tagged MCPIP1 for 48 h. HCV viral RNA bound with MCPIP1 protein was pulled down with HA beads and amplified by RT-PCR with HCV 5′ UTR–specific primers. Western blot analysis of HA-MCPIP1 protein expression in cell lysates. (B and C) Huh7.5 cells were transduced with lentiviral vectors (MOI = 2) expressing the various types of HA-tagged MCPIP1 for 48 h and then mock infected or infected with HCV JC1 (MOI = 1) for 72 h. (B) Western blot analysis of protein levels of HA-tagged MCPIP1, HCV core, and β-actin (loading control). (C) Quantitative real-time RT-PCR analysis of HCV RNA. Pretreatment with IFN-α (1000 U/ml) for 24 h was used as a control. Data are mean ± SD of three independent experiments. **p ≤ 0.01, ***p ≤ 0.001.

Previous studies showed that MCPIP1 inhibits LPS-, IL-1β-, and TNF-α–mediated NF-κB– and JNK-signaling pathways by removing ubiquitin moieties of TRAFs via its direct DUB activity (11). A recent report indicated that the DUB activity of MCPIP1 is mediated by interacting with USP10 rather than MCPIP1 itself (29). However, we demonstrated MCPIP1’s anti-HCV activity in the C157A mutant [RNase/DUB] but not the D141N [RNase/DUB+] mutant or the D225/226A [RNase/DUB+] mutant (Fig. 3); therefore, the anti-HCV mechanism of MCPIP1 may not be dependent upon either the direct or indirect DUB activity of MCPIP1.

In the CCCH-type ZF protein family, both MCPIP1 and ZF antiviral protein (ZAP; also known as Zc3h2) have a distinct mechanism of antiviral activity (21, 30–34). The antiviral effect of ZAP directly mediates viral RNA binding through its ZF domain and then recruits the components of the RNA-degradation machinery, such as exosome complexes and p72 DEAD box RNA helicase, to promote viral RNA degradation (32, 35, 36). The target sequences of Moloney murine leukemia virus and Sindbis genomes of ZAP have been mapped to the 3′ long terminal repeat of Moloney murine leukemia virus and multiple fragments in the Sindbis genome but not AU-rich elements (36). In this study, MCPIP1 appeared to function as an antiviral effector through its RNA-binding capacity and RNase activity via the ZF and NYN domains, respectively (21). MCPIP1 exhibits intrinsic RNase activity for directly degrading the mRNA levels of a set of inflammatory genes, including IL-6, IL-12p40, IL-1β, and IL-2, via their 3′ UTRs (9, 13, 14). Further studies showed that the terminal loop structures of precursor miRNAs were preferentially recognized and cleaved by MCPIP1 RNase (12). Recently, MCPIP1/Regnase-1 in T cells also regulated a set of genes, including c-Rel, OxlO, and IL-2, through their 3′ UTR, and the stem-loop structure of c-Rel mRNA was required for the recognition and cleavage of MCPIP1 (37). The 3′ UTR region of the HCV genome, predicted to have a conserved stem-loop structure (38), can be cleaved effectively by MCPIP1 using an in vitro cleavage assay (Supplemental Fig. 2); therefore, the 3′ UTR region of HCV might act as a platform for RNase activity of MCPIP1. Using mutagenesis analysis, we further demonstrated that both C312 and C318, but not C306 or H322, are the important ZF residues for HCV viral RNA-binding and anti-HCV activities.

miR-122, a liver-specific and abundant miRNA, was identified as a host factor in HCV replication in infected cells by several mechanisms. miR-122 directly binds to the 5′ UTR with adjacent sites upstream of the IRES in HCV genomic RNA and activates translation and replication of HCV RNA (28, 39–41). Moreover, HCV in cell culture propagation is enhanced by the direct interaction of miR-122 with HCV 5′ UTR; therefore, miR-122 is required for HCV replication (42). MCPIP1 can suppress miR-122 biosynthesis and activity (12). Of note, the miR-122 level was decreased in our Huh7.5 cells overexpressing MCPIP1 (Supplemental Fig. 3). Thus, MCPIP1, as a broad suppressor of the miRNA pathway, likely interferes with HCV replication by targeting miR-122 biosynthesis and activity. Whether MCPIP1 can suppress HCV replication by inhibiting miR-122 biosynthesis and activity remains to be addressed.

Several inflammatory factors, such as LPS, TNF-α, MCP-1, and IL-1β, can induce MCPIP1 expression, which is mediated by both the NF-κB and ERK pathways (7–10, 14). In this study, we found upregulated mRNA expression of TNF-α, IL-6, and MCP-1 in HCV-infected Huh7.5 cells. Thus, we also clarified whether MCPIP1 induction with HCV infection was mediated by the production of TNF-α or MCP-1. Neutralization of TNF-α by etanercept (Pfizer), a human TNFR2-Fc fusion protein, and MCP-1 by anti-MCP-1 Ab significantly reduced the expression of MCPIP1 in HCV-infected cells (Supplemental Fig. 4). Thus, MCPIP1 expression induced by HCV infection might be triggered, at least in part, by the production and activation of inflammatory factors. However, HCV infection induces the activation of reactive oxygen species by NF-κB and ERK pathways in regulating TGF-β1 production (43); therefore, HCV-induced expression of MCPIP1 may occur by direct activation of the NF-κB and ERK pathways.

Our findings indicate that the induction of MCPIP1 by viral infection can be a host innate defense, via its RNase activity, for...
targeting and degrading viral RNA. In addition to showing innate immune responses, MCPIP1 plays an important role in negatively regulating adaptive immune responses. MCPIP1/Regnase-1 expressed in various immune cells functions as a negative regulator required for controlling CD4+ T cell generation and T cell activation through its T cell–intrinsic inhibitory function (37). However, the HCV-specific T cell immune response is critical for the control of HCV infection and HCV clearance (44, 45). A case report indicated that HCV-specific CD4+ T cell responses are weak in chronic HCV infection (46). Moreover, the recurrence of persistent HCV viremia was associated with the depletion of CD4+ T cell responses (47–49). Thus, whether the induction of MCPIP1 during HCV infection is involved in HCV escape from the host adaptive immune system by depletion of the HCV-specific CD4+ T cell responses remains to be explored.

A recent study demonstrated that commensal microbiota contributed to the development and pathogenesis of inflammatory disease in MCPIP1-deficient mice: therefore, MCPIP1, as a novel anti-inflammatory protein, may be critical for tolerance of commensal bacteria and maintenance of cellular immune homeostasis (50). Inflammatory cytokines and chemokines, such as TNF-α, IL-1β, IL-6, and MCP-1, have been evaluated in patients with chronic HCV, especially those with liver cirrhosis and hepatocellular carcinoma and were implicated in the progression from chronic inflammation to cancer (51–54). The induction of TNF-α and IL-6 by HCV or HCV protein has been found involved in resistance to IFN-induced anti-HCV therapy by inducing suppressors of cytokine signaling 3 or protein-tyrosine phosphatase 2 (17, 55–57). HCV patients who were resistant to IFN therapy show increased plasma levels of IL-6 and TNF-α.

FIGURE 6. Oligomerization of MCPIP1 mediated by PRR domain is essential for its anti-HCV activity. (A) Huh7.5 cells were transduced with lentiviral vectors (multiplicity of infection [MOI] = 2) expressing the HA-tagged MCPIP1 or MCPIP1–Δ458-536 mutant for 72 h. Cell lysates were cross-linked with disuccinimidyl suberate, and HA-MCPIP1 proteins were pulled down with HA beads and analyzed by Western blot with anti-HA Ab. (B and C) Huh7.5 cells were transduced with lentiviral vectors (multiplicity of infection [MOI] = 2) expressing HA-tagged MCPIP1 or MCPIP1–Δ458-536 mutant for 48 h and then mock infected or infected with HCV JC1 (multiplicity of infection [MOI] = 1) for 72 h. (B) Western blot analysis of indicated protein. (C) Quantitative real-time RT-PCR analysis of HCV RNA. Pretreatment with IFN-α (1000 U/ml) for 24 h was used as a control. Data are mean ± SD of three independent experiments. **p ≤ 0.01.

FIGURE 7. Expression of MCPIP1 in human liver tissues is associated with HCV infection. (A) Representative immunohistochemistry staining for MCPIP1 negative (left panel) and strong positive (brown color, +3; right panel) in liver tissues. Original magnification ×400. (B) Distribution of MCPIP1 scores in 22 HCV+ liver tissues and 22 HCV− liver tissues.
(17, 58); therefore, the production of TNF-α and IL-6 may play an important role in the tolerance and therapeutic potential of IFN in HCV. MCPIP1, as a negative regulator of cellular inflammatory responses, blocked TNF-α-induced signaling and IL-6 mRNA degradation (9, 11), which might support a role for it in attenuating IFN tolerance by TNF-α and IL-6 during HCV infection.

More work is needed to fully understand the relationships among the expression and activity of MCPIP1, production of TNF-α and IL-6, IFN sensitivity, and the progression and development of HCV-related hepatocellular carcinoma in HCV. MCPIP1 induction by HCV infection may be beneficial to the host by inducing a host defense against HCV infection and negatively regulating the inflammatory response, which might help to decrease the risk for hepatocellular carcinoma in patients with chronic HCV. MCPIP1 might be a potential therapeutic target for treating HCV-related diseases and improving the efficacy of IFN therapy.

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Disclosures
The authors have no financial conflicts of interest.

References


