Cytokeratin 8 is increased in hepatitis C virus cells and its ectopic expression induces apoptosis of SMMC7721 cells

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Abstract

AIM: To investigate cytokeratin 8 (CK8) overexpression during hepatitis C virus (HCV) infection and its pathogenesis, and the effect of ectopic CK8 expression on hepatoma cell lines.

METHODS: We successfully established an in vitro HCV cell culture system (HCVcc) to investigate the different expression profiles of CK8 in Huh-7-HCV and Huh-7.5-HCV cells. The expression of CK8 at the mRNA level was determined by real-time polymerase chain reaction (RT-PCR). The expression of CK8 at the protein level was evaluated by Western blotting. We then constructed a eukaryotic expression combination vector containing the coding sequence of human full length CK8 gene. CK8 cDNA was amplified by reverse transcription-PCR and inserted into pEGFP-C1 and the positive clone pEGFP-CK8 was obtained. After confirming the sequence, the recombinant plasmid was transfected into SMMC7721 cells with lipofectamine2000 and CK8 expression was detected using inverted fluorescence microscopy, RT-PCR and Western blotting. Besides, we identified biological function of CK8 on SMMC7721 cells, including cell proliferation, cell cycle and apoptosis detection.

RESULTS: RT-PCR showed that the expression level of CK8 in Huh-7-HCV and Huh-7.5-HCV cells was 2.88 and 2.95 times higher than in control cells. Western blot showed that CK8 expression in Huh-7-HCV and Huh-7.5-HCV cells was 2.53 and 3.26 times higher than that in control cells, respectively. We found that CK8 at mRNA and protein levels were both significantly increased in HCVcc. CK8 was up-regulated in SMMC7721 cells. CK8 expression at the mRNA level was significantly upregulated in SMMC7721/pEGFP-CK8 cells. CK8 expression in SMMC7721/ pEGFP-CK8 cells was 2.69 times higher than in SMMC7721 cells, and was 2.64 times higher than in SMMC7721/pEGFP-C1 cells. CK8 expression at the protein level in SMMC7721/pEGFP-CK8 cells was 2.46 times higher than in SMMC7721 cells, and was 2.29 times higher than in SMMC7721/pEGFP-C1 cells. Further analysis demonstrated that forced expression of CK8 slowed cell growth and induced apoptosis of SMMC7721 cells.

CONCLUSION: CK8 up-regulation might have a functional role in HCV infection and pathogenesis, and could be a promising target for the treatment of HCV infection.

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Key words: Hepatitis C virus cell culture system; Cytokeratin
INTRODUCTION

Hepatitis C virus (HCV) infection is a significant global healthcare burden[1]. Current estimation suggests that a minimum of 3% of the world’s population is chronically infected, with a prevalence of up to 170 million people[2,3]. However, the mechanism of HCV infection is not fully understood. Recently, the development of HCV replicon technology has accelerated the understanding of the mechanism underlying HCV infection[4,5]. It has been reported that there were more than 100 abnormal expression proteins in HCV infected cells and hepatitis C patients[6-10]. Studies determining the changes in protein expression associated with HCV infection will help elucidate host/virus interactions, and provide further insight to HCV pathogenesis.

Cytokeratin 8 (CK8) is the major component of the intermediate filament cytoskeleton, belonging to the type-II keratin, and is primarily expressed in the epithelia of liver, intestine, and exocrine pancreas[11,12]. CK8 plays a crucial role in maintaining the structural integrity and the mechanical properties of cells[13]. Recent studies have suggested that CK8 is involved in several liver diseases. CK8 knock-out mice develop liver hemorrhage and are more susceptible to liver injury[14,15]. Some variants of CK8 are associated with disease severity and progression in patients with chronic liver diseases[16,17]. Thus, we hypothesized that CK8 contributed to cellular pathological processes and the infection and pathogenesis of HCV, leading to liver injury and chronic liver diseases.

In this study, we established an in vitro HCV cell culture system (HCVcc) and investigated whether HCV affects CK8 levels. Simultaneously, we established eukaryotic expression recombination vector containing the full length coding sequence of CK8, then transfected into hepatoma cells in vitro and investigated the biological and functional role of CK8 in hepatoma cells.

MATERIALS AND METHODS

Construction and identification of HCVcc

Huh-7 and Huh-7.5 cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% heat-inactivated fetal bovine serum, 0.1 mmol/L nonessential amino acids and 1 × penicillin-streptomycin-glutamine. Plasmid pFL-J6/JFH, containing a chimeric full length HCV genome, was kindly provided by Professor Charles M Rice from Rockefeller University. Plasmid pFL-J6/JFH, containing a single Xba I restriction site and T7 RNA polymerase start site, is the chimera of HCV J6 strain (5’-NCR-NS2) and JFH strain (NS3-3’-NCR). Subsequently, plasmid pFL-J6/JFH encoding the full length HCV chimeric genome was transcribed to HCV RNA in vitro. HCVcc was established by electroporation of HCV RNA into Huh-7 and Huh-7.5 cells.

Huh7 and Huh-7.5 were used as negative controls of HCVcc. Huh-7-HCV and Huh-7.5-HCV cells were maintained under the same condition as Huh-7 and Huh-7.5 cells. Cells were cultured in an incubator at 37 ℃ supplemented with 5% CO2. During the cell culture, the supernatant of cell culture was collected at 24, 48, 72 and 96 h after electroporation in order to determine the HCV copies. Quantitative real-time polymerase chain reaction (qRT-PCR) was used to determine HCV copy number. At approximately 72 h after transfection, cells were washed three times with 1 × phosphate-buffered saline (PBS) and then harvested. In addition, indirect immunofluorescence was used to observe the expression of HCV core protein. Mouse monoclonal HCV core protein antibody (Novus Biologicals, United States) was used as the primary antibody, and goat anti-mouse conjugated with Fluorescein Isothiocyanate (FITC) was used as the secondary antibody. The harvested cells were fixed with 3% glutaraldehyde at 4 ℃ for 24 h, then washed twice by 0.1 mol/L arsenic acid dimethyl sodium buffer (pH 7.4) at 4 ℃, fixed by 1% osmium tetroxide for 1 h, gradient acetone dehydration, embedded by Epon812, sliced by ultra-thin LKB-V slicer. H-7650 transmission electron microscope (HITACHI, Japan) was also used to observe the morphology of the viral particles and intracellular ultrastructure changes.

Total RNA isolation, cDNA synthesis and RT-PCR

Total RNA was extracted from cells by TRIzol reagent (Invitrogen, United States) according to the manufacturer’s protocol. A two-step reverse transcription PCR was performed. The first-strand cDNA was synthesized from 1 μg of total RNA with AMV Reverse Transcriptase (TAKARA, Japan). To investigate the expression of CK8 at the mRNA level, the expression of CK8 and glyceraldehydes-3-phosphate dehydrogenase (GAPDH) genes was quantified by RT-PCR, and GAPDH was used as an internal control. A total of 20 ng cDNA was used as template in the reaction. All RT-PCR assays were performed.
Table 1  Primers used for real-time polymerase chain reaction and high fidelity

| Name       | Forward primer (5’-3’) | Reverse primer (5’-3’) |
|------------|------------------------|-----------------------|
| CK8 (172 bp) | AGCTGGAGTCTGGCCTGGAA   | TGCCCTTGAACCTCAAGCAATG |
| GAPDH (138 bp) | GCACCCCTCAAGGCTGAGAAC | TGGTGAAGAGGCCAGTGAAGAC |
| CK8 (1465 bp) | ATGGTCGACATGTCCATCAGGGTGAC | TAGATGCTCCCTGGCAGGAGCAGTC |

CK8: Cytokeratin 8; PCR: Polymerase chain reaction; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

in triplicate using SYBR green incorporation method with Bio-Rad iQ5 Multicolor RT-PCR Detection System (Bio-Rad, United States) based on the manufacturer’s protocol. Table 1 shows the sequences of the primer sets for CK8 and GAPDH. Briefly, following a denaturation at 95 °C for 5 s, RT-PCR was carried out with 50 cycles at a melting temperature of 95 °C for 30 s, an annealing temperature of 65 °C for 30 s, and an extension temperature of 72 °C for 10 s. Data analysis was performed using the Sequence Detector System software. The relative quantification was calculated by the 2ΔΔCt method with GAPDH as the housekeeping gene and the control cells as the baseline, and the results were expressed as fold-change.

Protein extraction, SDS-PAGE and Western blotting
Total proteins were prepared by RIPA cell lysis. Proteins of interest were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) with a 10% polyacrylamide gel, and 1 μg/mL protein was loaded onto a SDS-PAGE gel. Proteins were transferred to nitrocellulose membranes and then detected by Western blotting under the recommended conditions. Mouse anti-human CK8 IgG (Abcam, United States) was used as the primary antibody, goat anti-mouse IgG conjugated with horseradish peroxidase (HRP) was used as the secondary antibody, and GAPDH was used as the control. The antigen-antibody complex was detected by an enhanced chemiluminescence (ECL) kit following the manufacturer’s protocol. The experiments were repeated in triplicate. The chemiluminescent signal of each band was analyzed by gel image analysis system (Syngene, United States).

Construction of pEGFP-CK8 recombination vector
The BamHI and SalI restriction sites were introduced into the CK8 coding sequence (CDS) by high fidelity PCR (Thermo, United States). Sequences for the primers are listed in Table 1, with the amplified product being 1465 bp. The CK8 CDS was purified by gel extraction. CK8 CDS and pEGFP-C1 vector (TAKARA, Japan) were digested respectively by the restriction enzyme SalI and BamHI I (TAKARA, Japan). The digestion products were examined on 1% agarose gel by electrophoresis. The ligation reaction (Ligation Kit, TAKARA, Japan) was carried out between both of the DNA fragments, followed by transformation into competent Escherichia coli DH5α cells at 37 °C overnight (12-16 h). Colony selection was performed by PCR, and the amplicons were examined on 1% agarose gel by electrophoresis. Plasmid extraction (E.Z.N.A.® Endo-Free Plasmid Midi Kit, Omega, United States) was carried out for positive colonies, and then sequenced and matched by Blast method.

Transfection of pEGFP-CK8 vector into SMMC 7721 cells
SMMC7721 cells were seeded in 6-well plates in 4 mL of growth medium for 24 h prior to transfection. In each well, 0.8 × 10^5-4.0 × 10^6 adherent cells were seeded. Four microgram (4.0 μg) of DNA (pEGFP-CK8 vector or pEGFP-C1 vector) was diluted in 250 μL of serum-free DMEM. Lipofectamine2000 (Millipore, United States) was added (10 μL) to the diluted DNA and mixed immediately by pipetting. The mixture was incubated for 25 min at room temperature. The lipofectamine2000/DNA mixture (500 μL) was added dropwise to the four wells containing the pEGFP-CK8 plasmid, and another two wells to control cells containing the pEGFP-C1 plasmid. The plate was then gently rocked to achieve even distribution of the complexes and incubated at 37 °C in a 5% CO2 incubator.

Detection assay
The expression and distribution of CK8 was observed under an inverted fluorescence microscope (Nikon eclipse Ti, Japan) 24 h after transfection. Forty-eight hours after transfection, cellular RNA and total cellular proteins were determined by RT-PCR and Western blotting, respectively. Total RNA was extracted from SMMC7721, SMMC7721/pEGFP-C1, and SMMC7721/pEGFP-CK8 cells by TRIzol reagent. Total proteins were prepared by RIPA cell lysis. Real time PCR assays (SYBR® Premix Ex Taq™ II, TAKARA, Japan) were performed in triplicate with Bio-Rad iQ5 Multicolor RT-PCR Detection System according to the manufacturer’s protocol. Rabbit anti-human IgG (Santa, United States) was used as the primary antibody, goat anti-rabbit IgG conjugated with HRP was used as the secondary antibody and β-actin (Abcam, United States) was used as control. Cells were collected after 24, 48 and 72 h transfection to perform a proliferation assay by MTT reaction (MTT cell proliferation Assay kit, Trevigen, United States). Cells were also collected 48 h after transfection to detect apoptosis (Annexin V-FITC Apoptosis Detection Kit, Abcam, United States) using Flow Cytometry (guava easyCyte HT, Millipore, United States).

Statistical analysis
All experiments were performed in triplicate. Representative graphical data are presented as mean ± SD. Statistical analyses were performed using the SPSS 10.0 software.
revealed a large number of enveloped or unenveloped virus-like particles (VLPs) in HCVcc. Some characteristic structures of *Flaviviridae* virus infection were observed, including an increased number of endoplasmic reticulum, mitochondrial swelling, cristae disappearance, and cytoplasmic vacuolar structures. Also, a large number of HCV nucleocapsid-like particles of inclusion body were presented in HCVcc cells (Figure 2). Viral-like particles were not seen in the control cells. Moreover, hyperplasia, vacuolar membrane structure, and formation of inclusion bodies were not observed in the control cells.

Increased CK8 levels in HCVcc cells by RT-PCR

Extracted total cellular RNA was examined by electrophoresis on a 0.8% non-denaturing agarose gel. A 172 bp fragment of *CK8* was successfully amplified by PCR without unspecific amplification. The melting and amplification curves of *CK8* expression indicated that the primers were properly designed. *CK8* expression in Huh-7-HCV cells was 2.88 times higher than that in Huh-7 cells, and *CK8* expression in Huh-7.5-HCV cells was 2.95 times higher than that in Huh-7.5 cells (Figure 3). Therefore, *CK8* was significantly highly expressed in HCVcc cells.

**RESULTS**

**Detection of HCV RNA copies, HCV core protein, and HCV particles**

We determined HCV RNA copy number by performing qRT-PCR of viral supernatants obtained from HCV-transfected cells. High-level viral copies in the supernatant of transfected cells were observed at different time-points and reached its peak value at 48 h after transfection (Table 2). Indirect immuno-fluorescence also showed high expression of HCV core protein in the HCV-transfected cells. Huh-7-HCV and Huh-7.5 HCV cells were also labeled with GFP, further indicating that HCV core protein has been expressed in these cells compared to control cells (Figure 1). Transmission electron microscopy (TEM) revealed a large number of enveloped or unenveloped virus-like particles (VLPs) in HCVcc. Some characteristic structures of *Flaviviridae* virus infection were observed, including an increased number of endoplasmic reticulum, mitochondrial swelling, cristae disappearance, and cytoplasmic vacuolar structures. Also, a large number of HCV nucleocapsid-like particles of inclusion body were presented in HCVcc cells (Figure 2). Viral-like particles were not seen in the control cells. Moreover, hyperplasia, vacuolar membrane structure, and formation of inclusion bodies were not observed in the control cells.

**Table 2 Detection of hepatitis C virus at RNA level in transfected cellular supernatant**

| HCV RNA in supernatant of Huh-7-HCV cells | HCV RNA in supernatant of Huh-7.5-HCV cells |
|-------------------------------------------|---------------------------------------------|
| 24 h                                      | 5.73 × 10^5                                 |
|                                           | 9.48 × 10^5                                 |
| 48 h                                      | 1.38 × 10^6                                 |
|                                           | 6.40 × 10^6                                 |
| 72 h                                      | 3.00 × 10^6                                 |
|                                           | 9.29 × 10^6                                 |
| 96 h                                      | 6.62 × 10^7                                 |
|                                           | 1.43 × 10^7                                 |

HCV: Hepatitis C virus.

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**Increased CK8 levels determined by Western blotting of HCVcc cells**

By Western blotting, we showed that the ratio of *CK8*/GAPDH was 0.079 ± 0.004 and 0.031 ± 0.003 in Huh-7-HCV cells and Huh-7 cells, respectively, which was 2.53
times higher. Furthermore, the ratio of CK8/GAPDH was $0.105 \pm 0.004$ in Huh-7.5-HCV cells, which was significantly higher than in Huh-7.5 cells ($0.032 \pm 0.002$) and expression was 3.26 times higher (Figure 4). Therefore, we confirmed that HCVcc cells do have increased CK8 expression.
We next ectopically expressed CK8 in SMMC7721 cells. We confirmed the overexpression of CK8 in cells under inverted fluorescence microscope 24 h after transfection. Since the CK8 expression vector contains an EGFP marker, we observed that SMMC7721/pEGFP-C1 and SMMC7721/pEGFP-CK8 cells appeared bright green compared to control SMMC7721 cells (Figure 5). This data indicated that ectopic expression of CK8 was achieved in SMMC7721 cells.

**CK8 mRNA expression by qRT-PCR**

The $2^{-\Delta\Delta C_t}$ value of CK8 mRNA levels in SMMC7721, SMMC7721/pEGFP-C1, and SMMC7721/pEGFP-CK8
cells are shown in Figure 6. Beta-actin was used as the housekeeping gene, while SMMC7721 cells were used for baseline detection. The results were expressed as fold-change. CK8 expression at the mRNA level was significantly upregulated in SMMC7721/pEGFP-CK8 cells. CK8 expression in SMMC7721/pEGFP-CK8 cells was 2.69 times higher than in SMMC7721 cells, and was 2.64 times higher than in SMMC7721/pEGFP-C1 cells.

Ectopic expression of CK8 determined by Western blot analysis
Using Western blotting, we compared the chemiluminescent signals of CK8 and beta-actin in SMMC7721, SMMC7721/pEGFP-C1, and SMMC7721/pEGFP-CK8 cells. The ratio between CK8 and beta-actin were reflective changes in CK8 expression. CK8 expression in SMMC7721/pEGFP-CK8 cells was 2.46 times higher than in SMMC7721 cells, and 2.29 times higher than in SMMC7721/pEGFP-C1 cells. This demonstrated that ectopic expression of CK8 was observed at the protein level in SMMC7721/pEGFP-CK8 cells (Figure 7). Therefore, we confirmed that CK8 expression was increased in SMMC7721 cells after transfection with pEGFP-CK8 vector.

**Effects of ectopic CK8 overexpression on cell proliferation**
Using MTT detection, we determined the effects of ectopic CK8 expression on SMMC7721 cells 72 h after transfection. CK8 overexpression decreased the growth and proliferation of SMMC7721 cells compared to control cells and mock-transfected cells (Figure 8). This data indicated that ectopic CK8 expression decreased cell growth and proliferation of SMMC7721 cells.

**Effects of ectopic CK8 expression on the apoptosis of SMMC7721 cells**
We determined the effects of ectopic CK8 expression on the apoptosis of SMMC7721 cells 48 h after transfection. Using flow cytometry, ectopic CK8 expression increased the apoptotic rate of SMMC7721 cells, compared to untransfected and mock-transfected cells (Figure 9).

**DISCUSSION**
In this study, we established a full-length HCV genomic replication in Huh-7 and Huh-7.5 cells. Lohmann et al.\(^{18}\) reported that subgenomic HCV RNA replicons are capable of autonomously replicating in Huh7 cells. These dicistronic replicons include the neomycin-resistant gene, making them selectable by G418, and most or all of the viral nonstructural genes\(^{19,20}\). This system provides a novel and powerful tool for the study of HCV replication mechanisms and for study of the interaction between host and viral factors involved in viral progression\(^{21,22}\). In our study, we transfected Huh-7 and Huh-7.5 cells to express HCV RNA and generated the HCVcc cell line. We used qRT-PCR, immunofluorescence, and TEM to detect HCV RNA, HCV core protein, and HCV particles, respectively. The results confirmed that HCV expression in Huh-7 and Huh-7.5 cells led to the production of HCV particles.

CK8 is a cytoskeletal intermediate filament protein that abundantly expresses in hepatocytes to maintain cell integrity, and prevent mechanical and non-mechanical cell injury\(^{23,24}\). Previous studies showed that CK8 was upregulated in HBV-infected liver tissues from p21-HBx mice\(^{25}\).
and that its upregulation contributed to the development and progression of HCC-induced HBV. Tai DI found that CK8 was focally positive in a patient with a malignant liver patient infected with HCV[26]. Toivola et al[27] found that in chronic HCV infection, CK8 phosphorylation is a progression marker during HCV progression and regression. Furthermore, Strnad et al[28] found that a number of CK8 gene variants are increased in patients with chronic HCV infection. However, it is unclear about the relation between CK8 expression and HCVcc cells. We observed a concomitant increase in CK8 levels, which was confirmed by RT-PCR and Western blot analysis. CK8 mRNA expression in Huh-7-HCV and Huh-7.5-HCV cells was 2.88 and 2.95 times higher than in Huh-7 and Huh-7.5 cells, respectively. At the protein level, CK8 expression was 2.53 and 3.26 times higher in Huh-7-HCV and Huh-7.5-HCV cells, respectively, than Huh-7 and Huh-7.5 cells. This suggests that HCV up-regulates CK8 expression in HCVcc cells, and that CK8 expression is significantly associated with HCV.

CK8 plays a role in maintaining cellular structural integrity, signal transduction, and cellular differentiation[34-36]. Snider NT demonstrated that acetylation of CK8 was up-regulated in diabetic human livers[37]. We showed that HCV up-regulates CK8 expression in HCVcc cells. However, the biological function of ectopic CK8 in tumor cells is not fully elucidated. To further investigate the biological function of aberrant CK8 expression, we cloned the full length CDS of CK8 to establish the eukaryotic expression recombination vector pEGFP-CK8. To study the biological function of increased CK8 on cells independently, we chose another cell line called SMMC7721 cells in our laboratory. SMMC7721 cells were transfected by pEGFP-CK8 recombination vector, and under an inverted fluorescence microscope we observed the expression and distribution of GFP-tagged CK8. In addition, by RT-PCR and Western blot analysis, we found that CK8 mRNA levels in SMMC7721/pEGFP-CK8 cells was 2.69 and 2.64 times higher than in SMMC7721 cells and SMMC7721/pEGFP-C1 cells, respectively. At the protein level, CK8 expression in SMMC7721/pEGFP-CK8 cells was 2.46 and 2.29 times higher than in SMMC7721 and SMMC7721/pEGFP-C1 cells, respectively. These observations showed that CK8 gene was transcribed and expressed in SMMC7721 cells.

CK8 abnormal expression and mutations can lead to acute or sub-acute liver injury and promote tumor cells apoptosis[34,35]. The persistent expression of CK8 can induce tumor cell apoptosis through a number of transcription factors that regulate a large number of oncogenes[36]. In SMMC7721 transfected by pEGFP-CK8, we further observed the biological effects of increased CK8 on cells. We detected proliferation and apoptosis by MTT reaction and flow cytometry, respectively. We found that ectopic CK8 expression decreased cell growth and proliferation, and increased apoptosis of SMMC7721 cells. Therefore, we concluded that the abnormal expression of CK8 regulates cellular pathological injury. However, it is unclear what the mechanisms are by which CK8 affects cell cycle and apoptosis. In conclusion, these results suggest CK8 up-regulation might have a functional role during HCV infection and pathogenesis, and it could be a promising target for the treatment of HCV infection.

In summary, we successfully established and identified HCVcc and observed that CK8 is up-regulated in HCVcc. Overexpression of CK8 in SMMC7721 cells inhibited cell proliferation and induced apoptosis. CK8 could be a potential target for the treatment of HCV infection. Future studies will (1) identify the interactions of CK8 with other proteins to mediate its effects; (2) assess how CK8 expression regulates a number of known oncogenes in HCV; and (3) determine how CK8 promotes apoptosis.

** COMMENTS**

**Background**

Currently, several proteins have been identified to be overexpressed during hepatitis C virus (HCV) infection and pathogenesis. Studies have suggested...
that cytokeratin 8 (CK8) is closely related to a number of liver diseases. CK8 knock-out mice develop liver hemorrhage and are more susceptible to liver injury. However, it remains unknown whether HCV affects CK8 levels in their established in vitro HCV cell culture system (HCVcc) and the biological and functional role of CK8 in hepatoma cells.

Research frontiers
It has been reported that there are more than 100 abnormal proteins expressed in HCV-infected cells and hepatitis C patients. Studies determining the changes in protein expression associated with HCV infection will help to elucidate host/ virus interactions, and provide further insight to HCV pathogenesis. CK8 plays a crucial role in maintaining the structural integrity and the mechanical properties of cells. Recent studies have suggested that CK8 is involved in several liver diseases. Much interest is shown to understand CK8 overexpression during HCV infection and to investigate the role of ectopic CK8 expression in hepatoma cell lines.

Innovations and breakthroughs
In this study, the authors transfected HuH-7 and HuH-7.5 cells to express HCV RNA and generated the HCVcc cell line. Previous studies showed that CK8 is upregulated in HBV-infected liver tissues from p21-HBx mice and in a patient with a malignant liver infected with HCV. However, it is unclear what the relation between CK8 expression and HCVcc cells is. The authors observed a concomitant increase in CK8 levels by real-time Polymerase chain reaction and Western blot analysis. The results show that HCV up-regulates CK8 expression in HCVcc cells. However, the biological function of ectopic CK8 in tumor cells is not fully elucidated. The authors found that ectopic CK8 expression decreased cell growth and proliferation, and increased apoptosis of SMMC7721 cells. Therefore, the authors concluded that the abnormal expression of CK8 regulates cellular pathological injury.

Applications
The results of this study suggest that CK8 up-regulation might have a functional role during HCV infection and pathogenesis, and it could be a promising target for the treatment of HCV infection.

Peer review
This is a very well written manuscript. In this paper, the authors show the over-expression of CK8 in an in vitro HCV cell culture system. Large-scale proteome analyses of the in vitro HCV infection model have also been performed. Thus new hopes characterize the HCV field and new advances are reasonably expected. Here, CK8 is found up-regulated in HuH7 and HuH7.5 cells infected with chimeric full length HCV genome. The methodology is acceptable. The conclusion is acceptable. Here, CK8 is involved in several liver diseases. Much interest is shown to understand CK8 overexpression during HCV infection and to investigate the role of ectopic CK8 expression in hepatoma cell lines.

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