# Hepatitis C virus core protein regulates OCT4 expression and promotes cell cycle progression in hepatocellular carcinoma

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**Abstract.** Hepatitis C virus (HCV) core protein plays an important role in the development of hepatocellular carcinoma. Octamer-binding protein 4 (OCT4) is critically essential for the pluripotency and self-renewal of embryonic stem cells. Abnormal expression of OCT4 has been detected in several human solid tumors. However, the relationship between HCV core and OCT4 remains uncertain. In the present study, we found that HCV core is capable of upregulating OCT4 expression. The effect of HCV core-induced OCT4 overexpression was abolished by RNAi-mediated scilencing of HCV core. In addition, HCV core-induced OCT4 overexpression resulted in enhanced cell proliferation and cell cycle progression. Inhibition of OCT4 reduced the CCND1 expression and induced G0/G1 cell cycle arrest. Furthermore, OCT4 protein directly binds to CCND1 promoter and transactivates CCND1. These findings suggest that HCV core protein regulates OCT4 expression and promotes cell cycle progression in hepatocellular carcinoma providing new insight into the mechanism of hepatocarcinogenesis by HCV infection.

## Introduction

Liver cancer, predominantly hepatocellular carcinoma (HCC), is the fifth most deadly cancer worldwide, and considered as the third most common cause of cancer mortality. Hepatitis C virus (HCV) infection is a major etiological factor for developing HCC. Studies showed that HCC patients with HCV infection usually had a poorer prognosis associated with higher rate of intrahepatic recurrence and extrahepatic metastasis, and shorter patient survival than those without HCV infection (1,2). Increasing evidence indicates that HCV encoded viral proteins play a critical role in the modulation of HCV-induced HCC malignancy. HCV core protein has attracted special attention, numerous studies demonstrated the ability of HCV core protein in regulating gene transcription, cell proliferation, apoptosis, transformation and also immortalization of human hepatocytes, which contributes largely to the pathogenesis of HCC (3,4). Furthermore, it has been reported that in transgenic mice, HCV core protein directly induces hepatic steatosis and finally HCC, indicating a direct involvement of HCV core protein in tumorigenesis of HCC (5). Although there is a strong correlation between HCV infection and HCC development, the molecular mechanism of HCV core protein in hepatocarcinogenesis remains to be elucidated.

Octamer-binding protein 4 (OCT4, also known as OCT3/4 or POU5F1), is a nuclear transcription factor belonging to the POU-homeodomain family of DNA binding proteins. OCT4, together with the transcription factor NANOG and SOX2, form a core transcriptional network to modulate the maintenance of pluripotency and self-renewal in undifferentiated embryonic stem cells (ESCs) (6,7). OCT4 also plays a key role in the induced pluripotent stem cell (iPSC) process, which could reprogram human somatic fibroblasts and hepatocytes into embryonic stem cell-like pluripotent cells (8,9). At first, OCT4 was only found to be expressed in germline and pregastrulation embryos and in embryonal carcinomas, but not expressed in mature somatic cells (10). Recently, accumulating evidence shows that OCT4 is abnormally expressed in numerous types of human solid tumors and cell lines, such as breast (11), glioma (12) and lung cancer (13), suggesting that OCT4 may play a potential role in tumorigenesis. Moreover, recent studies demonstrated that hepatitis B virus X protein, a potential oncoprotein of hepatitis B virus, was associated with the modulation of OCT4 expression in HCC (14), indicating that aberrant OCT4 expression and function may be involved in hepatic tumorigenesis of virus-related HCC. However, any

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*Abbreviations:* HCV, hepatitis C virus; OCT4, octamer-binding protein 4; HCC, hepatocellular carcinoma; ESCs, embryonic stem cells; iPSC, induced pluripotent stem cell; siRNAs, small interfering RNAs; CCND1, cyclin D1; ChIP, chromatin immunoprecipitation, CSCs, cancer stem cells

*Key words:* hepatocellular carcinoma, hepatitis C virus core protein, OCT4, CCND1, cell cycle, proliferation

correlation of HCV core protein and OCT4 expression in HCC remains unclear.

In the present study, we investigated the relationship between HCV core protein and OCT4 expression and found that HCV core protein upregulated OCT4 expression and promoted cell cycle progression in hepatocellular carcinoma cells. Our findings provide new insight into the mechanism of hepatocarcinogenesis by HCV infection.

## Materials and methods

*Cell culture and plasmids*. Human hepatoma cell line HepG2 was purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA) and stored in our laboratory. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS; (HyClone Laboratories, Inc., Logan, UT, USA). The full-length HCV core sequence (genotype 1b) was cloned into pcDNA3.1 to construct the HCV core-expressing plasmid pcDNA-core (pCore), and pcDNA-vector (pVector) was used as control. HepG2 stable cell lines were established by transfection with either pCore or pVector, followed by selection with 500 $\mu$g/ml G418 (Gibco), as previously described (15).

*RNA interference*. HCV core- and OCT4-specific small interfering RNAs (siRNAs) were employed to knock down expression of HCV core and OCT4. The target mRNA sequences for siRNAs were as follows: HCV core, 5'-AAG GCG ACA ACC TAT CCC CAA-3' and OCT4, 5'-CCC TCA CTT CAC TGC ACT G-3'. Non-targeting scrambled siRNAs was used to control for non-specific effects. siRNAs at 100 picomolar/ml medium were transfected into cells using Lipofectamine™ 2000 (Invitrogen), according to the manufacturer's protocols. The Mock transfected cells were transfected with Lipofectamine™ 2000 without siRNAs.

*Real-time PCR assay*. Total RNA isolation, reverse transcription and PCR were as previously described (16). All gene-specific mRNA expression values were normalized to GAPDH expression levels. Primer sequence used in the present study were as follows: OCT4: forward, 5'-TCT CGC CCC CTC CAG GT-3' and reverse, 5'-GCC CCA CTC CAA CCT GG-3'; CCNDl: forward, 5'-CCG CCT CAC ACG CTT CCT CTC-3' and reverse, 5'-CGG CCT TGG GGT CCA TGT TCT-3'; GAPDH: forward, 5'-GCA CCG TCA AGG CTG AGA AC-3' and reverse, 5'-TGG TGA AGA CGC CAG TGG A-3'.

*Western blotting assay*. Cells were lysed with Laemmli's sample solution. The lysates were electrophoresed, transferred onto nitrocellulose membranes, and immunoblotted with antibodies against core, OCT4 (Abcam), CCND1 (Cell Signal Technology) and GAPDH (Sigma). After membranes were incubated with HRP-conjugated secondary antibody (Bio-Rad Laboratories), the SuperSignal West Pico Chemiluminescent Substrate (Thermo Fisher Scientific, Waltham, MA, USA) was used to visualize protein bands on X-ray film.

*Cell proliferation assay*. Cell proliferation assay was performed by using the CCK-8 method. Cells were seeded in 96-well plates (Corning Costar, Corning, NY, USA) and incubated under the indicated conditions. CCK-8 assay was used to detect the viability of cells following the manufacturer's instructions. The absorbance at 450 nm was measured using a microplate reader.

*Cell cycle analysis*. Cell cycle profile was analyzed using flow cytometry. Briefly, $1x10^6$ cells were harvested and fixed with ice-cold 75% ethanol overnight, and incubated with propidium iodide (PI) (50 $\mu$g/ml; Sigma) in the dark at room temperature for 30 min. Flow cytometric analysis was performed on a Beckman Coulter EPICS analyzer.

*Chromatin immunoprecipitation assay*. The ChIP kit (Millipore, Billerica, MA, USA) was used according to the manufacturer's instructions. Cells were bathed in 1% formaldehyde for cross-linking of proteins and DNA, and then the chromatin samples were prepared. The prepared samples were immunoprecipitated with anti-Oct4 antibody (Abcam) or negative control rabbit IgG (Cell Signal Technology). DNA released from precipitated complexes was amplified by PCR using the CCND1 primers (forward, 5'- AGA TTC TTT GGC CGT CTG TC-3' and reverse, 5'-GCA GCG AGG GGC AGA GCC CA-3'). The PCR reaction conditions were as follows: denaturation at 50˚C for 2 min, followed by 40 cycles of 95˚C (15 sec), 60˚C (15 sec) and 72˚C (32 sec), and a final extension at 72˚C for 2 min.

*Statistical analysis*. Results are presented as means of three independent experiments (± SD). Statistical analyses were performed with the two-tailed Student's t-test or ANOVA using the SPSS 13.0 software. $P<0.05$ was considered statistically significant.

## Results

*HCV core regulates OCT4 expression*. To explore whether HCV core has a potential role in modulating OCT4 expression in HCC, we first established human HepG2 cells stably expressing HCV core protein (HepG2-core) to investigate the functional effect of HCV core on OCT4 expression regulation. We found that stable expression of HCV core increased the expression level of OCT4 mRNA (Fig. 1A-a and -b), as determined by real-time PCR, and western blot results showed that the expression level of OCT4 protein in HepG2-Core cells was similarly enhanced by HCV core expression (Fig. 1A-c and -d). To further confirm the correlation between HCV core and OCT4 expression, we measured OCT4 expression following knockdown of HCV core by using specific siRNAs (Fig. 1B-a). The loss-of-function analysis revealed that OCT4 expression at both the mRNA and protein level was decreased following the inhibition of HCV core (Fig. 1B-b-d). Taken together, these results suggested that HCV core upregulated OCT4 expression at both the mRNA and protein level.

*HCV core promotes cell cycle progression and elevates CCND1 expression*. In order to investigate the biological function of enhanced expression of OCT4 in HCV core-expressing HepG2 cells, we first examined whether HCV core promotes cancer cell growth and proliferation. Cell

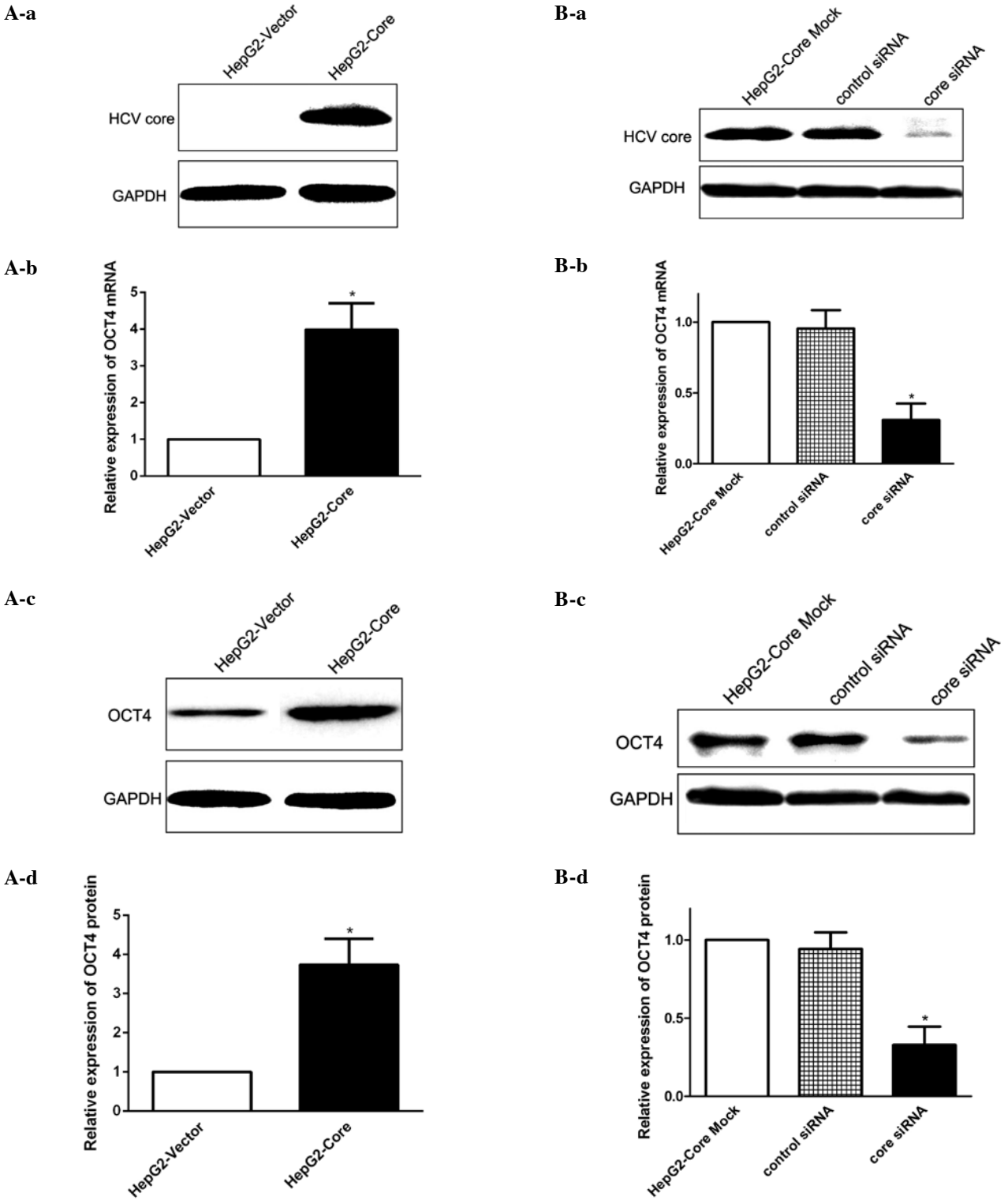


Figure 1. HCV core regulates OCT4 expression. (A-a) Western blot analyses showed that HCV core was highly expressed in HepG2 cells stably transfected with pCore (HepG2-core), as compared to the HepG2 cells transfected with pVector (HepG2-vector). (b, c and d) Real-time PCR and western blot analyses showed that expression of OCT4 mRNA and protein were significantly upregulated in HepG2-core cells as compared to HepG2-vector cells. GAPDH was used as a loading control for real-time PCR and western blotting. (B-a) Western blot analyses showed that expression of HCV core in HepG2-Core cells was significantly downregulated by RNAi-mediated knockdown of the HCV core. (b, c and d) Real-time PCR and western blot analyses showed that expression of OCT4 mRNA and protein in HepG2-Core cells were significantly downregulated by RNAi-mediated knockdown of the HCV core. GAPDH was used as a loading control for real-time PCR and western blotting. *P<0.05.

proliferation assay showed that cell growth capacity of HCV core-expressing cells (HepG2-core) was much stronger than that of the parental HepG2 cells without HCV core expression (Fig. 2A). FCM analysis revealed that HCV core effectively stimulated cell cycle progression from G1 to S phase (Fig. 2B). The experimental data suggest that HCV

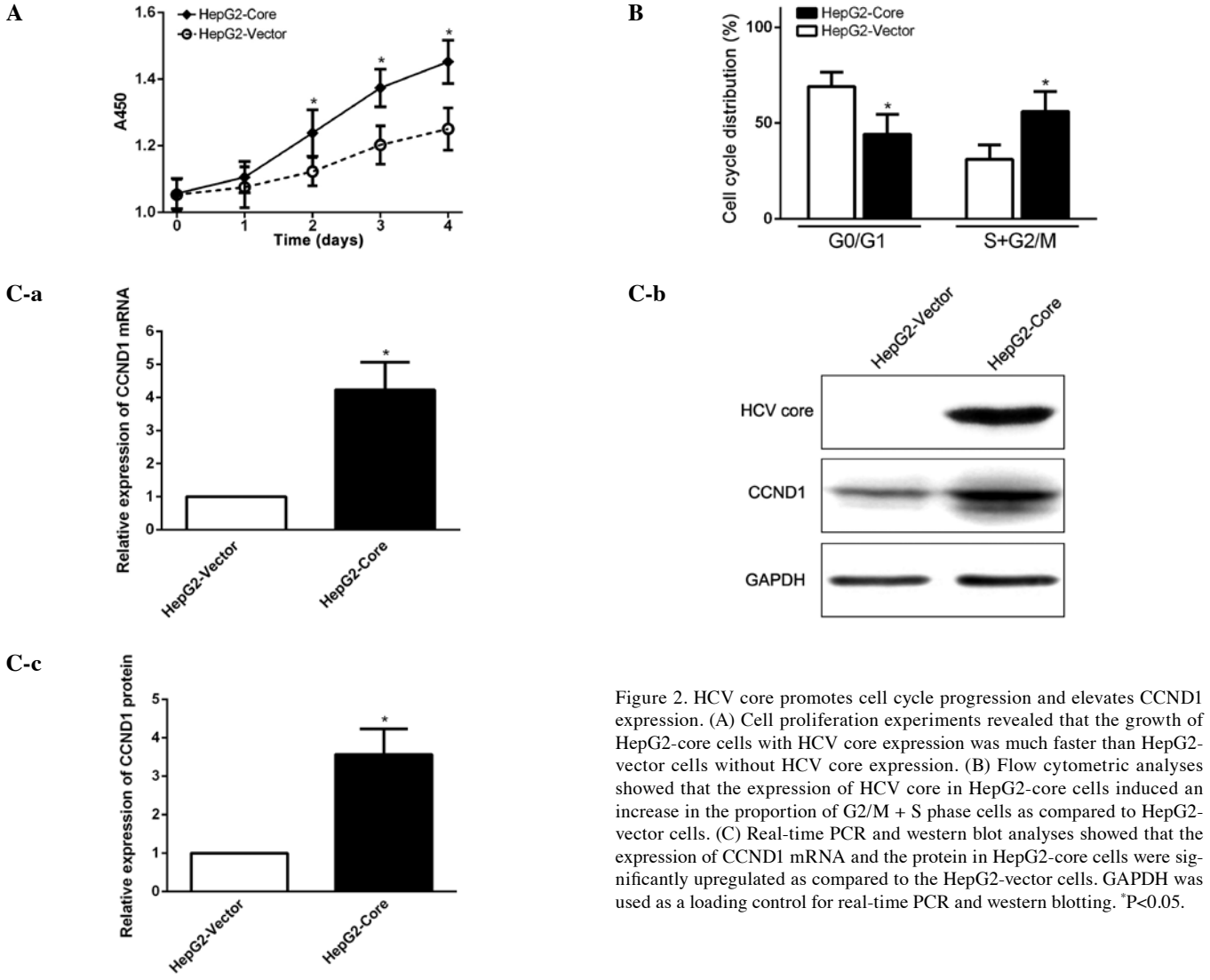


Figure 2. HCV core promotes cell cycle progression and elevates CCND1 expression. (A) Cell proliferation experiments revealed that the growth of HepG2-core cells with HCV core expression was much faster than HepG2-vector cells without HCV core expression. (B) Flow cytometric analyses showed that the expression of HCV core in HepG2-core cells induced an increase in the proportion of G2/M + S phase cells as compared to HepG2-vector cells. (C) Real-time PCR and western blot analyses showed that the expression of CCND1 mRNA and the protein in HepG2-core cells were significantly upregulated as compared to the HepG2-vector cells. GAPDH was used as a loading control for real-time PCR and western blotting. *P<0.05.

core promotes cell growth by enhancing cell cycle progression from G1 to S phase. Western blot analysis demonstrated that cell cycle related protein CCND1 (cyclin D1) expression was significantly elevated in HCV core-expression cells (Fig. 2C), indicating that CCND1 might be a downstream target gene, regulated by the HCV core, to promote cell cycle progression in HCV-associated HCC.

*Knockdown of OCT4 blocks cell cycle progression and inhibits CCND1 expression*. OCT4 is a pivotal transcription factor involved in maintaining pluripotency and self-renewal in ESCs (7). However, the biological function of OCT4 in HCV associated HCC remains unclear. Using RNA interference technology to silence OCT4 mRNA, we examined the effect of OCT4 on cell cycle progression and proliferation in HepG2-core cells. We found that knockdown of OCT4 inhibited cell growth of HCV core-expressing cells (Fig. 3A). FCM analysis revealed that cell cycle was mainly blocked in the G0/G1 phases in the OCT4-inhibited cells (Fig. 3B), therefore, the G0/G1 ratio was much higher and the proportion of S + G2/M was much lower than that in HCV core-expressing cells (Fig. 3B). Q-PCR and western blot analysis demonstrated that CCND1 expression was significantly decreased in OCT4-inhibited cells as compared to parental cells (Fig. 3C). These results indicated that enhanced OCT4 expression by HCV core, may modulate cell growth and promote cell proliferation at least in part via the OCT4/CCND1 pathway.

*OCT4 directly binds to the promoter of CCND1 and regulates its expression*. Since OCT4 is known as a DNA binding protein to directly activate its target gene transcription and expression, and we also demonstrated that CCND1 expression was significantly decreased via OCT4 inhibition, we hypothesized OCT4 might regulate CCND1 expression directly. To test the hypothesis, we first analyzed the 5'-UTR of CCND1 gene and found an octamer motif for OCT4 in the CCND1 promoter region (Fig. 4A). Then, we employed chromatin immunoprecipitation (ChIP) to investigate whether OCT4 protein can bind to the CCND1 promoter region. After gathering the crosslink product, we used ultrasound to shear the chromatin DNA into an average size of 100-1000 bp, and then ChIP assay was carried out according to the manufacturer's instructions. Our PCR amplification experiment revealed that the OCT4 protein can directly bind to the CCND1 promoter region (Fig. 4B). These results indicated that OCT4 regulates CCND1 transcription and expression directly. Together with

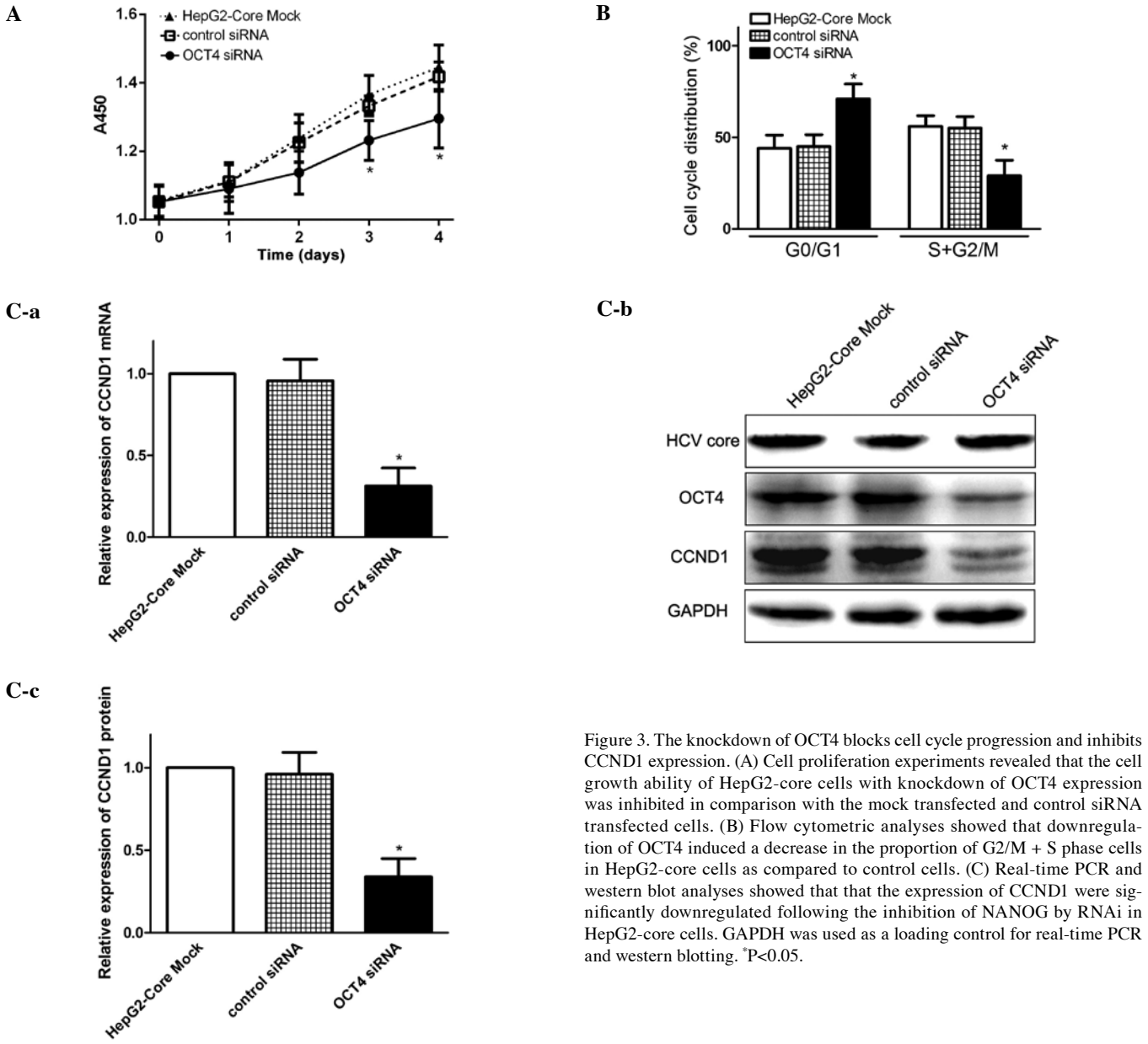


Figure 3. The knockdown of OCT4 blocks cell cycle progression and inhibits CCND1 expression. (A) Cell proliferation experiments revealed that the cell growth ability of HepG2-core cells with knockdown of OCT4 expression was inhibited in comparison with the mock transfected and control siRNA transfected cells. (B) Flow cytometric analyses showed that downregulation of OCT4 induced a decrease in the proportion of G2/M + S phase cells in HepG2-core cells as compared to control cells. (C) Real-time PCR and western blot analyses showed that that the expression of CCND1 were significantly downregulated following the inhibition of NANOG by RNAi in HepG2-core cells. GAPDH was used as a loading control for real-time PCR and western blotting. *P<0.05.

the results described above, our findings demonstrate a novel positive link between the HCV core expression, the OCT4 activation and the CCND1 transcription.

## Discussion

Accumulating evidence has demonstrated that OCT4 may be a type of human oncogene involved in the tumor initiation and development (17). Aberrant expression of OCT4 was associated with proliferation, invasiveness, recurrence, metastasis and unsatisfactory clinical outcome in a variety of human solid tumors, including HCC (18). Ectopical expression of OCT4 positively regulated survivin expression and promoted cancer cell proliferation in esophageal squamous cell carcinoma (19), knockdown of OCT4 inhibits colorectal cancer cell migration and invasion (20), and reduced liver cancer cell resistance to chemotherapeutic drugs (21). These findings indicated that OCT4 may play a particularly important role in HCC carcinogenesis.

It is well accepted that HCV infection may lead to the development of HCC and the core protein of HCV induces HCC in transgenic mice, however, whether HCV infection, especially HCV core protein modulates OCT4 expression and activity was not well understand. It is therefore imperative to investigate whether HCV core regulate OCT4 expression in liver cancer cells and to better understand the biological function of OCT4 in HCV-associated HCC. In the present study, we found that HCV core induced upregulation of OCT4 expression in HepG2 cells. The loss-of-function analysis confirmed that knockdown of HCV core downregulates the expression of OCT4 at both the mRNA and protein level. Furthermore, inhibition of OCT4 expression in HCV core-expressing cells attenuated the HCV core-induced cell proliferation and migration. These results established a novel link between HCV core and OCT4 in liver cancer cells, and indicated that OCT4 may play a role in HCV core associated hepatocellular carcinogenesis. Moreover, recent studies showed that OCT4 was highly expressed in cancer stem cells (CSCs) (21,22), a

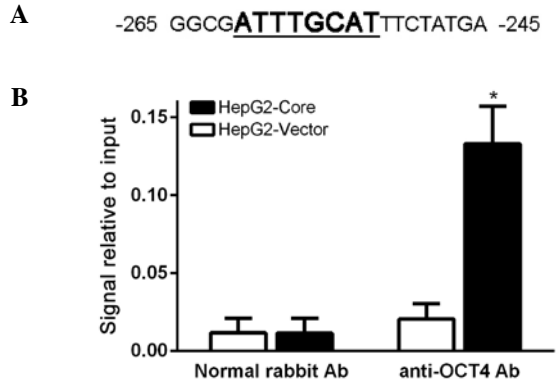


Figure 4. OCT4 protein can directly bind to the promoter of CCND1. (A) The promoter region of CCND1 contains an octamer motif (bold) for OCT4 protein at the nucleotide positions of -265 to -245. (B) A marked increase of OCT4 binding on the upstream of transcriptional start site of CCND1 gene was found in HepG2-core cells as compared with HepG2-vector cells in ChIP assay. Normal rabbit antibody served as a negative control. Error bars represent standard deviation (SD) from at least three independent experiments.

sub-population of heterogeneous cancer cells with the capacity of self-renewal and differentiation into non-tumorigenic cells, contribute to cancer development, metastasis, recurrence and multidrug resistance. The CSCs properties of $OV6^+$ HCC cells such as self-renewal, tumorigenicity and chemoresistance were modulated partly by elevated OCT4 expression (23). In addition, exogenous expression of OCT4 was sufficient to directly reprogram adult neural stem cells to pluripotency (24), and exogenous expression of OCT4 in cancer cells could promote expansion of CSCs and mediate tumor initiating properties (25,26). Therefore, our finding of HCV core induced OCT4 expression in HCC cells suggested that HCV core might play a particularly potential role in promoting the appearance of CSCs and consequently promoting HCC development and progression.

Uncontrolled cell proliferation is one of the essential hallmarks of tumor malignancy, which may be associated with cell cycle dysregulation. The CCND1 gene, encoding cyclin D1 protein, plays a critical role in controlling G1/S phase transition of cell cycle (27). Overexpression of CCND1 is commonly found in several human cancers, including HCC (28). A variety of carcinogenic factors including HCV infection contribute to aberrant expression of CCND1 (29). In the present study, we found that HCV core-stimulated cell proliferation of HepG2 cells by increasing CCND1 expression, and downregulation of OCT4 inhibited HCV core-induced cell proliferation, indicating enhanced cell growth capacity and proliferative ability of core-expressing cells may be partially associated with upregulation of OCT4 expression. OCT4 can activate the transcription of its target gene by binding to the octamer motif (5'-ATGCAAAT-3') within the promoter regions. We found that there is an octamer motif for OCT4 in the CCND1 promoter, indicating that OCT4 may directly participate in the regulation of CCND1 transcription and expression. Our ChIP assay confirmed that OCT4 protein can directly bind to the promoter of CCND1. Therefore, this study presents, for the first time, evidence that HCV core protein induces CCND1 expression in an OCT4-dependent manner, raising the possibility that OCT4 could serve as a new target for inhibiting HCV induced proliferation. Furthermore, a previous study demonstrated that silencing of NANOG, another core regulator for stem cell self-renewal and stemness, reduces cell proliferation and induces G0/G1 cell cycle arrest by inhibiting CCND1 expression in breast cancer cells, and CCND1 promoter also contain the ATTA binding motifs for NANOG (30), suggesting CCND1 as one of the co-occupied target genes for both OCT4 and NANOG. In addition, it has been reported that OCT4 and NANOG, together with SOX2, collaborate to form regulatory circuitry consisting of autoregulatory and feed-forward loops and co-occupy a substantial portion of their target genes (7,31), indicating a complex regulatory network in regulating the CCND1 expression and consequently modulating cell proliferation of cancer cells or CSCs.

In summary, we found that HCV core upregulated OCT4 expression and subsequently promoted CCND1 expression and cell cycle progression in HepG2 cells. In addition, inhibition of OCT4 expression in HCV core-expressing cells led to decreased CCND1 expression and cell cycle arrest at the G0/G1 phases. Collectively, the present findings provide new insight into the mechanism of HCV-induced hepatocarcinogenesis, and highlight OCT4 as a novel potential molecular target for HCV-related HCC.

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## References

1. Caselmann WH and Alt M: Hepatitis C virus infection as a major risk factor for hepatocellular carcinoma. J Hepatol 24 (Suppl): 61-66, 1996.
2. Bartosch B, Thimme R, Blum HE and Zoulim F: Hepatitis C virus-induced hepatocarcinogenesis. J Hepatol 51: 810-820, 2009.
3. Yamashita T, Honda M and Kaneko S: Molecular mechanisms of hepatocarcinogenesis in chronic hepatitis C virus infection. J Gastroenterol Hepatol 26: 960-964, 2011.
4. Arzumanyan A, Reis HM and Feitelson MA: Pathogenic mechanisms in HBV- and HCV-associated hepatocellular carcinoma. Nat Rev Cancer 13: 123-135, 2013.
5. Moriya K, Fujie H, Shintani Y, Yotsuyanagi H, Tsutsumi T, Ishibashi K, Matsuura Y, Kimura S, Miyamura T and Koike K: The core protein of hepatitis C virus induces hepatocellular carcinoma in transgenic mice. Nat Med 4: 1065-1067, 1998.
6. Pan GJ, Chang ZY, Schöler HR and Pei D: Stem cell pluripotency and transcription factor Oct4. Cell Res 12: 321-329, 2002.
7. Boyer LA, Lee TI, Cole MF, Johnstone SE, Levine SS, Zucker JP, Guenther MG, Kumar RM, Murray HL, Jenner RG, *et al*: Core transcriptional regulatory circuitry in human embryonic stem cells. Cell 122: 947-956, 2005.
8. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K and Yamanaka S: Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131: 861-872, 2007.

9. Aoi T, Yae K, Nakagawa M, Ichisaka T, Okita K, Takahashi K, Chiba T and Yamanaka S: Generation of pluripotent stem cells from adult mouse liver and stomach cells. Science 321: 699-702, 2008.
10. Freberg CT, Dahl JA, Timoskainen S and Collas P: Epigenetic reprogramming of OCT4 and NANOG regulatory regions by embryonal carcinoma cell extract. Mol Biol Cell 18: 1543-1553, 2007.
11. Oskarsson T, Acharyya S, Zhang XH, Vanharanta S, Tavazoie SF, Morris PG, Downey RJ, Manova-Todorova K, Brogi E and Massagué J: Breast cancer cells produce tenascin C as a metastatic niche component to colonize the lungs. Nat Med 17: 867-874, 2011.
12. Du Z, Jia D, Liu S, Wang F, Li G, Zhang Y, Cao X, Ling EA and Hao A: Oct4 is expressed in human gliomas and promotes colony formation in glioma cells. Glia 57: 724-733, 2009.
13. Chiou SH, Wang ML, Chou YT, Chen CJ, Hong CF, Hsieh WJ, Chang HT, Chen YS, Lin TW, Hsu HS, *et al*: Coexpression of Oct4 and Nanog enhances malignancy in lung adenocarcinoma by inducing cancer stem cell-like properties and epithelial-mesenchymal transdifferentiation. Cancer Res 70: 10433-10444, 2010.
14. Arzumanyan A, Friedman T, Ng IO, Clayton MM, Lian Z and Feitelson MA: Does the hepatitis B antigen HBx promote the appearance of liver cancer stem cells? Cancer Res 71: 3701-3708, 2011.
15. Zhou JJ, Chen RF, Deng XG, Zhou Y, Ye X, Yu M, Tang J, He XY, Cheng D, Zeng B, *et al*: Hepatitis C virus core protein regulates NANOG expression via the stat3 pathway. FEBS Lett 588: 566-573, 2014.
16. Zhou JJ, Deng XG, He XY, Zhou Y, Yu M, Gao WC, Zeng B, Zhou QB, Li ZH and Chen RF: Knockdown of NANOG enhances chemosensitivity of liver cancer cells to doxorubicin by reducing MDR1 expression. Int J Oncol 44: 2034-2040, 2014.
17. Hayashi H, Arao T, Togashi Y, Kato H, Fujita Y, De Velasco MA, Kimura H, Matsumoto K, Tanaka K, Okamoto I, *et al*: The OCT4 pseudogene POU5F1B is amplified and promotes an aggressive phenotype in gastric cancer. Oncogene 34: 199-208, 2015.
18. Yuan F, Zhou W, Zou C, Zhang Z, Hu H, Dai Z and Zhang Y: Expression of Oct4 in HCC and modulation to wnt/β-catenin and TGF-β signal pathways. Mol Cell Biochem 343: 155-162, 2010.
19. Li C, Yan Y, Ji W, Bao L, Qian H, Chen L, Wu M, Chen H, Li Z and Su C: OCT4 positively regulates Survivin expression to promote cancer cell proliferation and leads to poor prognosis in esophageal squamous cell carcinoma. PLoS One 7: e49693, 2012.
20. Dai X, Ge J, Wang X, Qian X, Zhang C and Li X: OCT4 regulates epithelial-mesenchymal transition and its knockdown inhibits colorectal cancer cell migration and invasion. Oncol Rep 29: 155-160, 2013.
21. Wang XQ, Ongkeko WM, Chen L, Yang ZF, Lu P, Chen KK, Lopez JP, Poon RT and Fan ST: Octamer 4 (Oct4) mediates chemotherapeutic drug resistance in liver cancer cells through a potential Oct4-AKT-ATP-binding cassette G2 pathway. Hepatology 52: 528-539, 2010.
22. Ikushima H, Todo T, Ino Y, Takahashi M, Saito N, Miyazawa K and Miyazono K: Glioma-initiating cells retain their tumorigenicity through integration of the Sox axis and Oct4 protein. J Biol Chem 286: 41434-41441, 2011.
23. Qian YW, Chen Y, Yang W, Fu J, Cao J, Ren YB, Zhu JJ, Su B, Luo T, Zhao XF, *et al*: $p28^{GANK}$ prevents degradation of Oct4 and promotes expansion of tumor-initiating cells in hepatocarcinogenesis. Gastroenterology 142: 1547-58.e14, 2012.
24. Kim JB, Sebastiano V, Wu G, Araúzo-Bravo MJ, Sasse P, Gentile L, Ko K, Ruau D, Ehrich M, van den Boom D, *et al*: Oct4-induced pluripotency in adult neural stem cells. Cell 136: 411-419, 2009.
25. Beltran AS, Rivenbark AG, Richardson BT, Yuan X, Quian H, Hunt JP, Zimmerman E, Graves LM and Blancafort P: Generation of tumor-initiating cells by exogenous delivery of OCT4 transcription factor. Breast Cancer Res 13: R94, 2011.
26. Tsai LL, Hu FW, Lee SS, Yu CH, Yu CC and Chang YC: Oct4 mediates tumor initiating properties in oral squamous cell carcinomas through the regulation of epithelial-mesenchymal transition. PLoS One 9: e87207, 2014.
27. Hui AM, Makuuchi M and Li X: Cell cycle regulators and human hepatocarcinogenesis. Hepatogastroenterology 45: 1635-1642, 1998.
28. Sundarrajan M, Gupta S and Rao KV: Overexpression of cyclin D1 is associated with the decondensation of chromatin during den-induced sequential hepatocarcinogenesis. Cell Biol Int 26: 699-706, 2002.
29. Sato Y, Kato J, Takimoto R, Takada K, Kawano Y, Miyanishi K, Kobune M, Sato Y, Takayama T, Matunaga T, *et al*: Hepatitis C virus core protein promotes proliferation of human hepatoma cells through enhancement of transforming growth factor alpha expression via activation of nuclear factor-kappaB. Gut 55: 1801-1808, 2006.
30. Han J, Zhang F, Yu M, Zhao P, Ji W, Zhang H, Wu B, Wang Y and Niu R: RNA interference-mediated silencing of NANOG reduces cell proliferation and induces G0/G1 cell cycle arrest in breast cancer cells. Cancer Lett 321: 80-88, 2012.
31. Sharov AA, Masui S, Sharova LV, Piao Y, Aiba K, Matoba R, Xin L, Niwa H and Ko MS: Identification of Pou5f1, Sox2, and Nanog downstream target genes with statistical confidence by applying a novel algorithm to time course microarray and genome-wide chromatin immunoprecipitation data. BMC Genomics 9: 269, 2008.