MedCrave
Step into the World of Research

Journal of Human Virology & Retrovirology

Short Communication

Open Access

CrossMark

Induction of C/EBP?-thr266 phosphorylation by HCV infection is indispensable for the zone-3 phenotype and proliferation in cultured human hepatocytes

Abstract

Liver acinar zonation allows for diverse functions to be performed on demand by specialized hepatocytes. Therefore, understanding how hepatitis C virus (HCV) infection may modulate the acinar zone phenotypes should provide insights into the pathophysiology of HCV infection. We assessed the effects HCV infection in the highly-differentiated primary human hepatocyte culture that we have developed for the efficient replication of intact HCV virions.

We found that HCV genotype 1 infection stimulated the zone-3 phenotype after 72 hr since the cultured hepatocytes expressed GS, β-Catenin and HIF-1α , markers of zone-3 hepatocytes. HCV infection also induced phosphorylated-C/EBPβ-Thr266 and proliferation in these cultured hepatocytes. A peptide designed to selectively inhibit phosphorylation of C/EBPβ-Thr266 blocked this phosphorylation, the zone-3 phenotype and hepatocyte proliferation induced by HCV infection in these cultured human hepatocytes. In addition, we found that in normal, uninfected human hepatocytes, unphosphorylated-C/EBPβ-Thr266 was associated with protein von Hippel Lindau (pVHL) and Axin (inhibitors of HIF-1α and β-catenin activation, respectively). These associations were blocked by the HCV infection since phosphorylated C/EBPβ-Thr266 was not associated with either pVHL or Axin. The inhibition of C/EBPβ-Thr266 phosphorylation normalized the association of unphosphorylatable C/EBPβ-Thr266 with pVHL and Axin, which is expected to inhibit the activation of HIF-1α and β−catenin. Collectively, these results strongly suggest that C/EBPβ-Thr266 phosphorylation is indispensable for the activation of GS, HIF-1α and β-catenin, and the induction of zone-3 phenotype in HCV-infected human hepatocytes.

Keywords: Human hepatocytes, Hepatitis C, Liver zone-3, C/EBPβ, Glutamine synthetase, HIF-1, β-catenin

Volume 1 Issue 4 - 2014

Martina Buck,[1,2,3] Mario Chojkier[1,2,3]
[1]Veterans Affairs San Diego Healthcare System, USA
[2]Department of Medicine, University of California, USA
[3]Biomedical Sciences Program, University of California, USA

Correspondence: Martina Buck, Veterans Affairs San Diego Healthcare System, Department of Medicine, Biomedical Sciences Program, University of California, San Diego, 3350 La Jolla Village Drive, San Diego, CA 92161; USA, Tel 858-552-8585, Fax 858-552-4366, Email buckmartina@gmail.com

Received: August 12, 2014 | **Published:** September 25, 2014

Abbreviations: C/EBP, CCAAT/Enhancer Binding Protein; HIF, Hypoxia Inducible Factor; GS, Glutamine Synthetase; HCC, Hepatocellular Carcinoma; pVHL, protein von Hippel Lindau; HCV, Hepatitis C Virus

Introduction

The hepatocytes in different liver acinar zones are under different O_2 tension and perform different metabolic activities.[1] Hepatocytes in acinar zone-1 are under normoxic conditions, which stimulate aerobic (mitochondrial oxidative) metabolism. By contrast hepatocytes in acinar zone-3 are exposed to hypoxia, which induces survival by hypoxic/'anaerobic' (non-mitochondrial) metabolism. Zone-3 hepatocytes are characterized by the expression of Glutamine Synthetase (GS), β-Catenin and Hypoxia-Inducible Factor (HIF)-1.[2-5] Expression of the GS gene in zone-3 hepatocytes is stimulated by C/EBPβ.[6]

We have shown that TGFα induces phosphorylation of mouse C/EBPβ on Thr217 as well as hepatocyte proliferation.[7] The C/EBPβ-Thr217 phosphoacceptor is highly conserved through evolution.[7,8] We reported that expression of a catalytically inactive mutant RSK, which behaves as a dominant negative, blocks hepatocyte proliferation induced by TGFα, indicating that RSK activity is important for this effect.[7]

Phosphorylation of C/EBPβ on Thr217 is required for the stimulation of hepatocyte proliferation by TGFα since hepatocytes expressing a C/EBPβ-Ala217 mutant, lacking the critical phosphoacceptor, are refractory to the stimulation of hepatocyte proliferation by TGFα. Also, expression of the phosphorylation-mimic C/EBPβ−Glu217 transgene was sufficient to induce hepatocyte proliferation in the absence of TGFα. In contrast, expression of the C/EBPβ−Ala217 transgene blocked hepatocyte proliferation in the presence of TGFα.[7] Thus, C/EBPβ PhosphoThr217 plays an active role in inducing hepatocyte proliferation after treatment with TGFα.

In this study we asked whether Hepatitis C Virus infection in cultured human hepatocytes stimulates C/EBPβ-Thr266 phosphorylation (the human homologue phosphoacceptor) and whether it is required for hepatocyte proliferation and a zone-3 phenotype.

Conclusion

In this study, we assessed the effects HCV infection in the highly-differentiated primary human hepatocyte culture that we have developed for the efficient replication of intact HCV virions. We used primary human hepatocytes (Invitro technologies; Baltimore, MD) cultured on a collagen matrix and infected on day-3 after plating the cells, with serum from a patient with a chronic HCV genotype 1 infection as described previously.[9] The matrix was rat-tail collagen(

BD Biosciences); the collagen matrix was prepared within 24 hr of hepatocyte plating , at a concentration of 50 mg/ml or greater; the culture plates were coated with polylysine; the suspended hepatocytes were allowed to attach in 20% fetal calf serum for not more than 18 hr; the hepatocyte-specific media was given for at least 24 hr prior to the HCV infection; the hepatocytes were >85% confluent until the time of infection; and hepatocyte media was changed every 72 hr.[9]

This normal human hepatocyte culture system is permissible to the infection with, and physiologically significant amplification of, naturally occurring HCV.[9]

We found that HCV genotype 1 infection after 72 hr (viral load ~ 7 x10^6 IU/uL) stimulated a zone-3 phenotype since the cultured hepatocytes expressed HIF-1α and GS.[2,5] HCV infection also induced phosphorylated-C/EBPβ-Thr266 (the human homologous phosphoacceptor[7,8,10]) in these cultured hepatocytes. Control uninfected, normal quiescent cultured human hepatocytes did not express phosphorylated-C/EBPβ-Thr266, HIF-1α or GS (Figure 1).

We found that HCV infection did not affect the expression of unphosphorylated C/EBPβ-Thr266 compared to control uninfected hepatocytes but stimulated hepatocyte proliferation as determined by the expression of ki67. Hepatocyte proliferation occurred in zone-3 phenotypic hepatocytes, judging by the expression of β-Catenin, another marker of zone-3[3,4] (Figure 2).

To test whether phosphorylated-C/EBPβ-Thr266 is required for zone-3 phenotype and hepatocyte proliferation in HCV infection, we treated 72 hr HCV-infected hepatocytes cultures with a peptide designed to selectively inhibit phosphorylation of C/EBPβ-Thr266.[8] The peptide blocked this phosphorylation (Figure 1) but did not affect the expression of unphosphorylated C/EBPβ-Thr266 (Figure 2). The peptide also blocked the zone-3 phenotype (expression of HIF-1, GS and β-Catenin as well as hepatocyte proliferation induced by HCV infection (Figures 1 and 2).

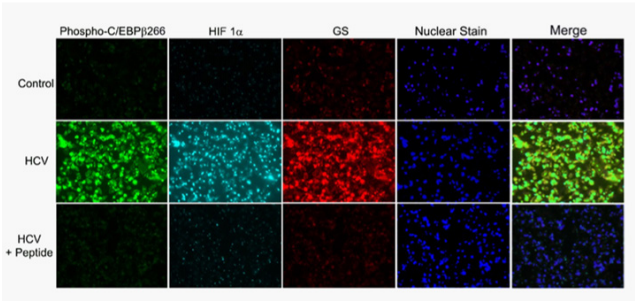

Figure 1 Phosphorylation of human C/EBPβ-Thr266 induced by HCV infection is indispensable for the zone-3 phenotype. Primary human hepatocytes infected with HCV (viral load ~ 7 x106 IU/uL after 72) also express phosphorylation of C/EBPβ-Thr266 and a zone-3 phenotype (HIF-1α; GS). Blocking phosphorylation of C/EBPβ-Thr266 with the dominant negative peptide (100 nM for 24 hr) prevents expression of the zone-3 phenotype. Nuclear stain and the merge images are shown.

In addition, we found that in normal, uninfected human hepatocytes, unphosphorylated-C/EBPβ-Thr266 was associated with protein von Hippel Lindau (pVHL) and Axin (inhibitors of HIF-1α and β-catenin activation, respectively)[11,12] (Figure 3).

These associations were blocked by the HCV infection since phosphorylated C/EBPβ-Thr266, which stimulates HIF-1α and β-catenin activation and expansion of zone-3 phenotype (and the resultant increase in hepatocyte proliferation), was not associated with either pVHL or Axin. The inhibition of C/EBPβ-Thr266 phosphorylation normalized the association of unphosphorylatable C/EBPβ-Thr266 with pVHL and Axin (Figure 3), which is expected to inhibit the activation of HIF-1α and β−catenin (Figure 1 and 2). Further, the interaction between β-catenin and HIF-1 promotes cellular adaptation to hypoxia.[13]

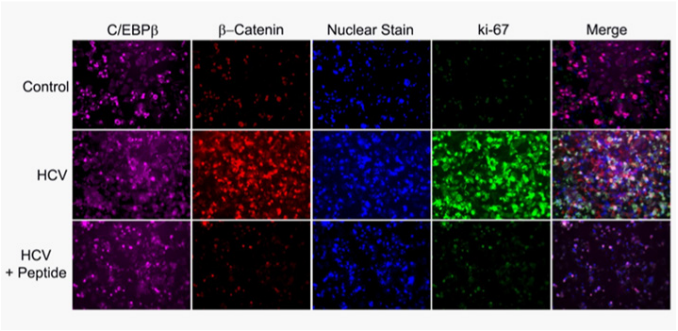

Figure 2 Phosphorylation of human C/EBPβ-Thr266 induced by HCV infection is indispensable for the expression of β-Catenin and hepatocyte proliferation. Experiments were performed as described in Figure 1. Normal or HCV-infected primary human hepatocytes express unphosphorylated C/EBPβ-Thr266. HCV-infected primary human hepatocytes express β-Catenin and a marked increase in zone-3 hepatocyte proliferation (ki67 + hepatocytes). Blocking phosphorylation of C/EBPβ-Thr266 with the dominant negative peptide (100 nM for 24 hr in 72-hr HCV-infected human hepatocytes) prevented expression of the zone-3 β-Catenin and hepatocyte proliferation. Nuclear stain and merge images are shown.

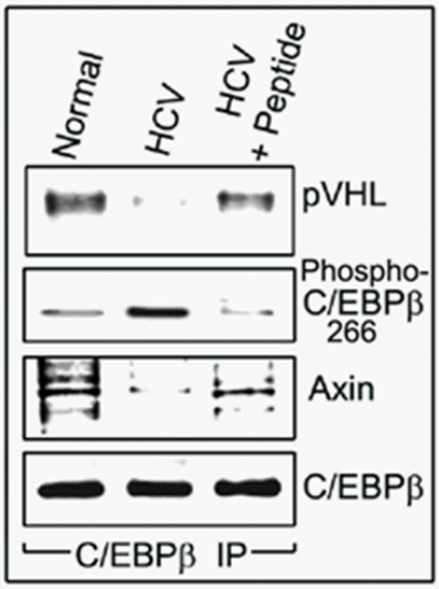

Figure 3 Unphosphorylated-C/EBPβ-Thr217 is physically associated with pVHL and Axin. Experiments were performed as described in Figure 1. C/EBPβ was immunoprecipitated from the 72-hr HCV-infected human hepatocyte's cell lysates. Unphosphorylated-C/EBPβ-Thr217 was physically associated with pVHL and Axin in uninfected normal primary human hepatocytes (Left lane) and in HCV-infected cultures treated with the inhibitory peptide (Right lane). In HCV-infected human hepatocytes not treated with the peptide, phospho-C/EBPβ-Thr266 is not associated with either pVHL or Axin (Center lane).

Collectively, these results strongly suggest that C/EBPβ-Thr266 phosphorylation is indispensable for the activation of GS, HIF-1 and catenin, and the induction of zone-3 phenotype in HCV-infected human hepatocytes. These novel findings support the activation of catenin and HIF-1 by phospho-C/EBPβ-Thr217 in zone-3 and their inhibition by the peptide blocking the zone-3 phenotype.

We hypothesize that the induction of hepatocyte proliferation confers a survival advantage to the HCV infection. The HCV-induced

Citation: Buck M, Chojkier M. Induction of C/EBP?-thr266 phosphorylation by HCV infection is indispensable for the zone-3 phenotype and proliferation in cultured human hepatocytes. *J Hum Virol Retrovirol.* 2014;1(4):118–120. DOI: 10.15406/jhvrv.2014.01.00021

hepatocyte proliferation is mediated in cultured human hepatocytes by C/EBPβ-Thr266 phosphorylation and the adoption of a zone-3 hepatocyte phenotype.

Acknowledgements

We thank Daniela Traykova and Caitlin Stalling for their technical support. This study was supported by following grants: RC1 Challenge-DK 087031 (to MB and MC); MERIT R37-DK-46071 (to MC); R01-DK-084139 (to MB), and the Department of Veterans Affairs: Merit Review Award (to MC).

Conflicts of Interset

None.

References

1. Oinonen T, Lindros KO. Zonation of hepatic cytochrome P–450 expression and regulation. *Biochem J*. 1998;329(Pt 1):17–35.
2. Gebhardt R, Baldysiak–Figiel A, Krugel V, et al. Hepatocellular expression of glutamine synthetase: an indicator of morphogen actions as master regulators of zonation in adult liver. *Prog Histochem Cytochem*. 2007;41(4):201–266.
3. Colletti M, Cicchini C, Conigliaro A, et al. Convergence of Wnt signaling on the HNF4alpha–driven transcription in controlling liver zonation. *Gastroenterology*. 2009;137(2):660–672.
4. Burke ZD, Reed KR, Phesse TJ, et al. Liver zonation occurs through a beta–catenin–dependent, c–Myc–independent mechanism. *Gastroenterology*. 2009;136(7):2316–2324.e1–e3.
5. Kietzmann T, Cornesse Y, Brechtel K, et al. Perivenous expression of the mRNA of the three hypoxia–inducible factor alpha–subunits, HIF1alpha, HIF2alpha and HIF3alpha, in rat liver. *Biochem J*. 2001;354(Pt 3):531–537.
6. Hadden TJ, Ryou C, Zhu L, et al. CAAT/enhancer binding protein activates an enhancer in the glutamine synthetase distal 5'–flanking sequence. *Arch Biochem Biophys*. 2002;397(2):258–261.
7. Buck M, Poli V, van der Geer P, et al. Phosphorylation of rat serine 105 or mouse threonine 217 in C/EBPb is required for hepatocyte proliferation induced by TGF alpha. *Mol Cell*. 1999;4(6):1087–1092.
8. Buck M, Chojkier M. A ribosomal S6K–mediated signal to C/EBPb is critical for the development of liver fibrosis. *PLoS One*. 2007;2(12):e1372.
9. Buck M. Direct infection and replication of naturally occurring hepatitis C virus genotype 1, 2, 3, and 4 in normal human hepatocyte cultures. *PLoS One*. 2008;3(7):e2660.
10. Trautwein C, Caelles C, van der Geer P, et al. Transactivation by NF–IL6/LAP is enhanced by phosphorylation of its activation domain. *Nature*. 1993;364(6437):544–547.
11. Clevers H, Nusse R. Wnt/β–catenin signaling and disease. *Cell*. 2012;149(6):1192–1205.
12. Bruick RK. Oxygen sensing in the hypoxic response pathway: regulation of the hypoxia–inducible transcription factor. *Genes Dev*. 2003;17(21):2614–2623.
13. Kaidi A, Williams AC, Paraskeva C. Interaction between b–catenin and HIF–1 promotes cellular adaptation to hypoxia. *Nat Cell Biol*. 2007;9(2):210–217.