



SHORT TAKE

Non cell autonomous upregulation of CDKN2 transcription linked to progression of chronic hepatitis C disease

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Summary

Chronic hepatitis C virus infection (C-HC) is associated with higher mortality arising from hepatic and extrahepatic disease. This may be due to accelerated biological aging; however, studies in C-HC have thus far been based solely on telomere length as a biomarker of aging (BoA). In this study, we have evaluated CDKN2 locus transcripts as alternative BoAs in C-HC. Our results suggest that C-HC induces non-cell-autonomous senescence and accelerates biological aging. The CDKN2 locus may provide a link between C-HC and increased susceptibility to age-associated diseases and provides novel biomarkers for assessing its impact on aging processes in man.

Key words: biological aging; CDKN2 locus; chronic HCV infection; telomere length.

Introduction

Acute hepatitis C virus (HCV) infection is largely asymptomatic, and chronic infection is often identified years later, following the development of liver disease (Seeff, 2009). Due to the cumulative nature of fibrosis and cirrhosis, HCV-related morbidity and mortality increase with age (Grebely & Dore, 2011). Chronic HCV infection (C-HC) is also associated with increased risk of extrahepatic diseases in the absence of other comorbidities or risk behaviours (Grebely & Dore, 2011; Lee *et al.*, 2012), suggesting that C-HC alters the capacity of an individual to resist the development of disease. It has been postulated that individuals with C-HC undergo premature biological aging, with increased cellular senescence, coincident with chronic inflammation and oxidative stress

(Hoare *et al.*, 2010a), although the nature of any mechanistic link remains to be determined. This hypothesis parallels observations in human immunodeficiency virus (HIV)-infected patients who have a higher than expected risk of complications typically associated with aging (Deeks, 2011). While an independent association between age-related diseases and HIV-induced aging has not been defined, the elevated risk is consistent with the observation that infection increases biomarkers associated with senescence commonly found in elderly populations (Deeks, 2011).

How biomarkers of aging (BoA) differ between HCV-infected and uninfected patients remains to be conclusively determined. Non-cell-autonomous senescence as a consequence of viral infection could explain the greater risk of age-related diseases in patients with C-HC. In patients with C-HC, shorter telomere lengths are found in circulating leucocytes (Kitay-Cohen *et al.*, 2008), T cells (Hoare *et al.*, 2010b) and liver biopsies (Sekoguchi *et al.*, 2007). However, recent studies highlight methodological issues surrounding measurement of telomere length (Shiels, 2010; Aviv *et al.*, 2011). The cyclin-dependent kinase inhibitor (CDKN)2A has been validated as an alternative BoA, which may represent a more reproducible measure of biological age (Shiels, 2010). Elevated CDKN2A transcription occurs with increasing age in both peripheral blood leucocytes (Liu *et al.*, 2009) and solid organs, where it correlates with organ function (Koppelstaetter *et al.*, 2008). In this study, we investigated the correlation between C-HC and BoAs including telomere length, CDKN2A and the related transcripts CDKN2B and ARF. We hypothesize that viral persistence induces non-cell-autonomous senescence, which may accelerate development of age-related diseases.

Study participants were recruited into two groups: healthy controls ($n = 24$) who were negative for HCV-specific antibodies and patients with C-HC ($n = 43$) who were positive for both HCV-specific antibodies and viral RNA (Table S1). No significant differences were observed in gender, ethnicity, age or body mass index of healthy controls and patients with C-HC. Alanine transaminase values were significantly higher in the C-HC group compared with controls consistent with the presence of liver disease.

Higher expression of CDKN2A ($P = 0.0002$), ARF ($P = 0.0008$) and CDKN2B ($P = 0.0002$) was observed in peripheral blood mononuclear cells (PBMCs) of patients with C-HC, compared with healthy controls (Fig. 1A and B, and Table S1). Elevated expression of CDKN2A transcripts coincided with increased p16 protein levels in PBMCs from patients with C-HC (Fig.S1). By contrast, no statistical difference was found in relative telomere length ($P = 0.2176$, Fig. 1C). To investigate whether these biomarkers were altered due to disease state or viral genotype, the C-HC group was divided according to the presence or absence of liver cirrhosis (measured by transient elastography) and viral genotype (either genotype 1 or genotype 3). In patients with liver cirrhosis ($n = 14$), expression of CDKN2A and ARF was significantly higher compared with those without liver cirrhosis ($n = 27$) (Fig. 2A). CDKN2B expression and p16 protein levels showed the same trend although they failed to reach statistical significance (Fig. 2A and Fig. S1C). No significant differences were noted in relative telomere

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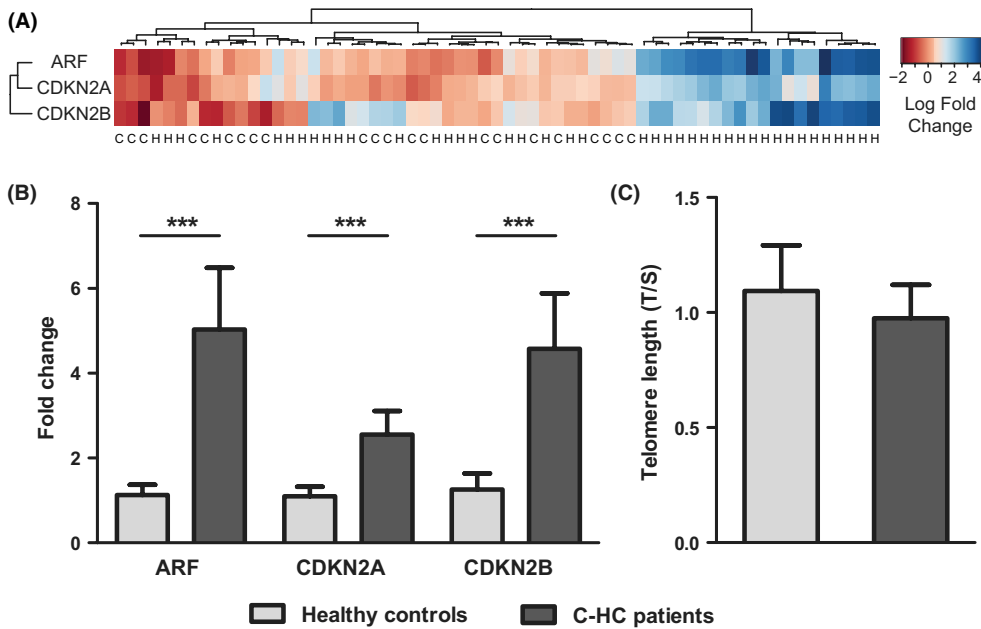


Fig. 1 Expression of CDKN2 locus transcripts and relative telomere length in patients with C-HC and healthy controls. Heat map (A) and expression profile (B) of CDKN2 locus transcripts from patients with C-HC (H; *n* = 41) and healthy controls (C; *n* = 22). (C) Relative telomere length in whole blood from patients with C-HC (*n* = 42) compared with healthy controls (*n* = 24). Data are presented as mean with 95% confidence intervals; ***denotes *P* < 0.001.

length between C-HC patients with or without liver cirrhosis (Fig. 2B). Separating the patients with C-HC according to viral genotype (genotype 1 (*n* = 23) and genotype 3 (*n* = 18)) showed no difference in telomere length or expression of CDKN2 locus transcripts (Supplementary Fig. 2).

Elevated transcription of CDKN2A, ARF and CDKN2B in PBMC is consistent with C-HC-associated non-cell-autonomous senescence, resulting in increased biological aging in infected patients. This finding supports reports linking accelerated aging and HCV (Kitay-Cohen *et al.*, 2008; Hoare *et al.*, 2010b) and implies that C-HC indirectly drives the development of senescence in PBMCs. While a trend towards decreasing telomere length in C-HC patients with cirrhosis was seen, unlike previous findings, HCV infection was not associated with a significant decrease in telomere length (Kitay-Cohen *et al.*, 2008; Hoare *et al.*, 2010b). This difference is likely due to the use of different techniques and highlights the issues surrounding the use of any single measure as a definitive BoA (Shiels, 2010; Aviv *et al.*, 2011).

Our findings indicate that increased CDKN2 locus transcription in PBMCs is a surrogate marker for the degree of liver damage, with the highest expression levels of these BoAs noted in C-HC patients with cirrhosis. Whether this is due solely to the progression of HCV infection or alternatively whether liver cirrhosis independently induces senescence is unknown. Elevated liver fibrosis and cirrhosis have been reported in mice where the p53-dependent senescence programme had been ablated (Lujambio *et al.*, 2013). In this study, the induction of p53-dependent non-cell-autonomous senescence acts to maintain organ integrity and suppress liver damage; our findings are consistent with this model and provide biomarkers for exploring this in *in vivo* human studies.

The observation of altered CDKN2 locus transcription, in association with HCV infection, is consistent with non-cell-autonomous senescence. Our study provides biomarkers for addressing the role of senescence in C-HC infection and may aid patient management as prognostic tools for the progression of liver disease and the development of extrahepatic complications.

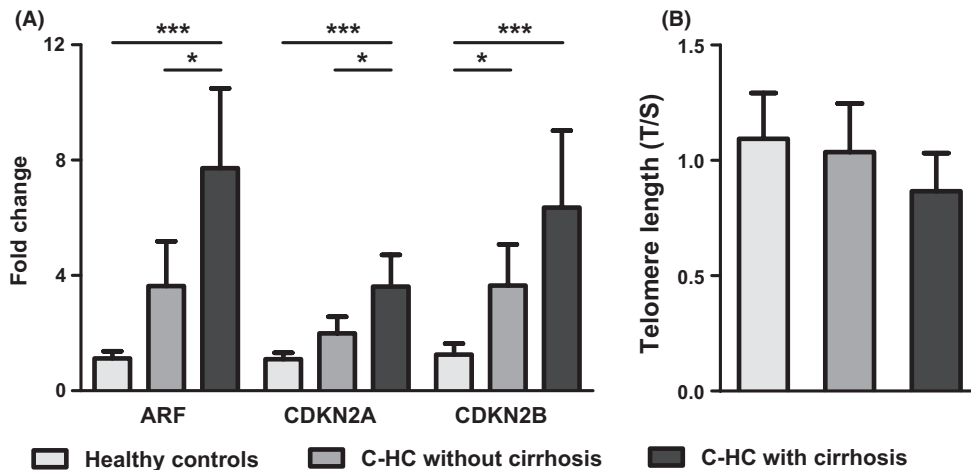


Fig. 2 Expression of CDKN2 locus transcripts and relative telomere length in C-HC patients with/without liver cirrhosis and healthy controls. (A) Expression of CDKN2 locus transcripts from C-HC patients with (*n* = 14) or without (*n* = 27) liver cirrhosis compared with healthy controls. (B) Relative telomere length in whole blood from C-HC patients with (*n* = 15) or without (*n* = 27) liver cirrhosis compared with healthy controls. Data are presented as mean with 95% confidence intervals; **P* < 0.05, ****P* < 0.001.

Author contributions

MWR and DM were involved in study design, data analysis and drafting of manuscript; RS, SB and PRM were involved in patient recruitment; AHP, JM and PGS were involved in study design and critical review of the manuscript.

Disclosures

All authors declare no conflict of interests.

References

- Aviv A, Hunt SC, Lin J, Cao X, Kimura M, Blackburn E (2011) Impartial comparative analysis of measurement of leukocyte telomere length/DNA content by Southern blots and qPCR. *Nucleic Acids Res.* **39**, e134.
- Deeks SG (2011) HIV infection, inflammation, immunosenescence, and aging. *Annu. Rev. Med.* **62**, 141–155.
- Grebely J, Dore GJ (2011) What is killing people with hepatitis C virus infection? *Semin. Liver Dis.* **31**, 331–339.
- Hoare M, Das T, Alexander G (2010a) Ageing, telomeres, senescence, and liver injury. *J. Hepatol.* **53**, 950–961.
- Hoare M, Gelson WT, Das A, Fletcher JM, Davies SE, Curran MD, Vowler SL, Maini MK, Akbar AN, Alexander GJ (2010b) CD4 + T-lymphocyte telomere length is related to fibrosis stage, clinical outcome and treatment response in chronic hepatitis C virus infection. *J. Hepatol.* **53**, 252–260.
- Kitay-Cohen Y, Goldberg-Bittman L, Hadary R, Fejgin MD, Amiel A (2008) Telomere length in Hepatitis C. *Cancer Genet. Cytogenet.* **187**, 34–38.
- Koppelstaetter C, Schratzberger G, Perco P, Hofer J, Mark W, Ollinger R, Oberbauer R, Schwarz C, Mitterbauer C, Kainz A, Karkoszka H, Wiecek A, Mayer B, Mayer G (2008) Markers of cellular senescence in zero hour biopsies predict outcome in renal transplantation. *Aging Cell* **7**, 491–497.
- Lee MH, Yang HI, Lu SN, Jen CL, You SL, Wang LY, Wang CH, Chen WJ, Chen CJ (2012) Chronic hepatitis C virus infection increases mortality from hepatic and extrahepatic diseases: a community-based long-term prospective study. *J. Infect. Dis.* **206**, 469–477.
- Liu Y, Sanoff HK, Cho H, Burd CE, Torrice C, Ibrahim JG, Thomas NE, Sharpless NE (2009) Expression of p16(INK4a) in peripheral blood T-cells is a biomarker of human aging. *Aging Cell* **8**, 439–448.
- Lujambio A, Akkari L, Simon J, Grace D, Tschaharganeh DF, Bolden JE, Zhao Z, Thapar V, Joyce JA, Krizhanovsky V, Lowe SW (2013) Non-Cell-Autonomous Tumor Suppression by p53. *Cell* **153**, 449–460.
- Seeff LB (2009) The history of the “natural history” of hepatitis C (1968–2009). *Liver Int.* **29**(Suppl 1), 89–99.
- Sekoguchi S, Nakajima T, Moriguchi M, Jo M, Nishikawa T, Katagishi T, Kimura H, Minami M, Itoh Y, Kagawa K, Tani Y, Okanou T (2007) Role of cell-cycle turnover and oxidative stress in telomere shortening and cellular senescence in patients with chronic hepatitis C. *J. Gastroenterol. Hepatol.* **22**, 182–190.
- Shiels PG (2010) Improving precision in investigating aging: why telomeres can cause problems. *J. Gerontol. A Biol. Sci. Med. Sci.* **65**, 789–791.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Data S1. Materials and Methods.

Fig. S1. CDKN2A/p16 protein expression in PBMC protein extracts.

Fig. S2. CDKN2 locus transcript expression with samples grouped by HCV genotype.

Table S1. Participants clinical characteristics.