

## Relationship between Telomere Maintenance and Liver Disease

Abbey Barnard<sup>1</sup>, Ashley Moch<sup>2</sup>, and Sammy Saab<sup>1,2</sup>

Departments of <sup>1</sup>Medicine and <sup>2</sup>Surgery, University of California at Los Angeles, Los Angeles, CA, USA

Previous studies have established a correlation between increasing chronological age and risk of cirrhosis. This pattern raised interest in the role of telomeres and the telomerase complex in the pathogenesis of liver fibrosis and cirrhosis. This review aims to summarize and analyze the current understanding of telomere regulation in hepatocytes and lymphocytes and how this ultimately relates to the development of liver fibrosis. Notably, in chronic viral hepatitis, telomere shortening in hepatocytes and lymphocytes occurs in such a way that may promote further viral replication while also leading to liver damage. However, while telomere shortening occurs in both hepatocytes and lymphocytes and ultimately results in cellular death, the mechanisms of telomere loss appear to be initiated by independent processes. The understanding of telomere maintenance on a hepatic and immune system level in both viral and non-viral etiologies of cirrhosis may open doors to novel therapeutic strategies. (**Gut Liver 2019;13:11-15**)

**Key Words:** Telomere; Cirrhosis; Hepatitis

### INTRODUCTION

Liver cirrhosis is a significant source of morbidity, mortality and health care spending with recent estimates reporting a prevalence of 0.27% in the United States.<sup>1</sup> There are a variety of disease states and processes which can lead to end stage liver disease including viral infections and alcohol consumption. However, there are common factors which correlate with disease progression and clinical outcome pointing toward potential common mechanisms and corresponding treatment targets. A number of studies have established a correlation between increasing age and risk of cirrhosis.<sup>2,3</sup> This pattern has raised interest in the role of telomeres and telomerase in the pathogenesis of liver fibrosis and cirrhosis.

Telomeres are the natural ends of chromosomes and protect them from damage and degradation by distinguishing chromosome ends from double stranded DNA breaks.<sup>4</sup> Telomerase complex is responsible for telomere maintenance and is active to different degrees in different cellular populations. This complex prevents the “end replication problem” which would result in significant telomere shortening during every round of DNA replication.<sup>4,5</sup> The telomerase complex is comprised of telomerase reverse transcriptase (TERT), telomerase RNA component (TERC) and other assisting factors.<sup>4,5</sup>

Telomeres are known to shorten with age and this shortening has been associated with increased incidence of a number of disease processes.<sup>6</sup> It has been observed that in the healthy aging liver, cholangiocytes and hepatocytes had preserved telomere length relative to other intrahepatic lineages.<sup>7</sup> Age-related telomere length decline was restricted to Kupffer cells and stellate cells.<sup>7</sup> This is in contrast to the cirrhosis disease state in which hepatocytes are found to have shorter telomeres as compared to stellate cells in fibrotic regions.<sup>8</sup> Data is limited in regard to telomere length in cholangiocytes as cirrhosis progresses, but existing data from a study of primary biliary cirrhosis (PBC) demonstrated shorter telomeres in diseased bile ducts and ductules as compared to normal bile ducts in PBC, chronic viral hepatitis and healthy livers.<sup>9</sup>

A number of studies and case reports have been published to date characterizing or quantifying the relation between both hepatic or immune cell telomeres and liver fibrosis. This review aims to summarize and analyze the current understanding of telomere regulation at the hepatic and immune system level and how this relates to the progression of liver fibrosis.

### METHODS

#### 1. Search strategy and identification of studies

We searched the databases MEDLINE and PubMed for studies

Correspondence to: Sammy Saab

Pflegler Liver Institute, UCLA Medical Center, 200 Medical Plaza, Suite 214, Los Angeles, CA 90095, USA

Tel: +1-310-206-6705, Fax: +1-310-206-4197, E-mail: Ssaab@mednet.ucla.edu

Received on February 3, 2018. Revised on March 9, 2018. Accepted on March 14, 2018. Published online October 30, 2018

pISSN 1976-2283 eISSN 2005-1212 <https://doi.org/10.5009/gnl18081>

© This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

regarding telomeres and cirrhosis from 1995 to the present day. We used a combination of the key words “telomere,” “telomerase,” “Hepatitis B virus,” “Hepatitis C virus,” “chronic viral hepatitis,” and “cirrhosis.” Bibliographies of all identified studies were searched for relevant articles for additional studies.

**2. Inclusion and exclusion criteria**

We included all studies published in scientific journals discussing the relationship between cirrhosis in humans and telomere length and function.

**HEPATOCTE TELOMERES**

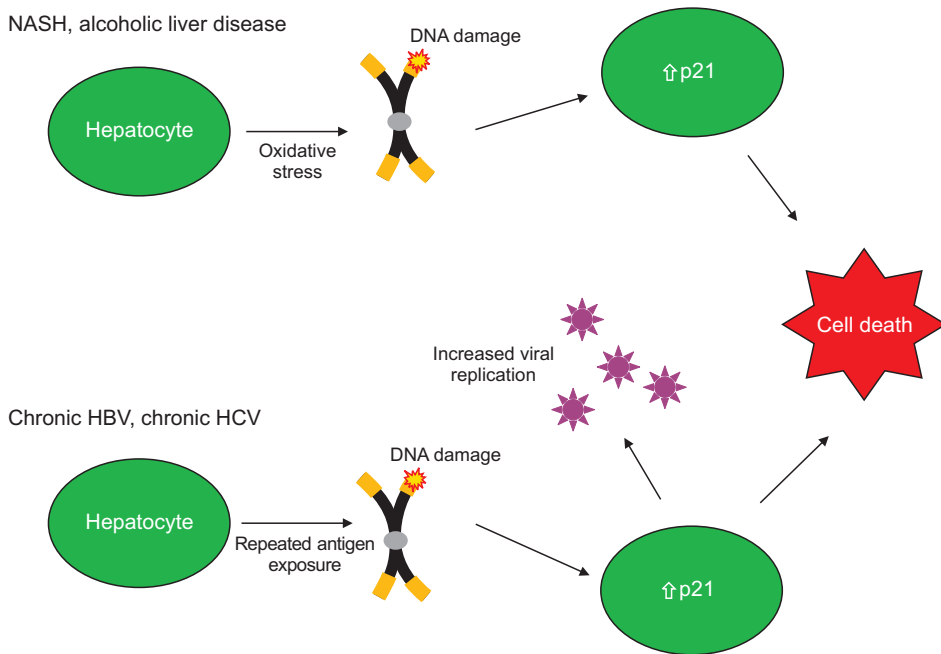
**1. Hereditary syndromes**

The theory of telomere dysfunction as causative in liver fibrosis and, ultimately, cirrhosis was initially posited on the basis of hereditary syndromes involving mutations in the telomerase complex. For example, short telomere syndrome presents as idiopathic pulmonary fibrosis (IPF) and cryptogenic cirrhosis. This is driven by a defect within the telomerase complex secondary to a mutation in TERT or TERC.<sup>10</sup> Other familial syndromes of synchronous IPF and cryptogenic cirrhosis have been described. In one family a heterozygous TERT mutation (L153M) and a heterozygous hTERT polymorphism (A305A) were implicated.<sup>11-13</sup> These inherited syndromes pointed to a role for telomere shortening in the progression of liver fibrosis and subsequent organ failure. These findings raised the question whether telomere maintenance and telomerase function play any role in the pathogenesis of other etiologies of liver fibrosis.

**2. Viral hepatitis**

Hepatocyte telomeres have been shown to be significantly shorter as compared to non-cirrhotic controls regardless of primary etiology and patient age.<sup>14</sup> This telomere shortening and senescence have been linked to the progression of fibrosis proposing a causative role.<sup>14</sup> Clinically this has been observed in the context of hepatitis C viral infection (HCV). Elderly patients with chronic HCV have a more rapid progression to cirrhosis suggesting a protective role of telomeres.<sup>3</sup> Prior studies demonstrated increased expression of p21 (cell cycle inhibitor) in hepatocytes in nonalcoholic steatohepatitis (NASH), alcoholic cirrhosis and chronic HCV.<sup>15-17</sup> Some have conjectured that cell cycle arrest may directly lead to cellular senescence (also known as telomere shortening). This was supported by data demonstrating that the expression of p21 correlates with fibrosis stage in subjects with HCV.<sup>15</sup>

This result was subsequently reproduced in patients with chronic hepatitis B viral infection (HBV) demonstrating that in this case as well increased expression of p21 correlated with degree of liver fibrosis.<sup>18</sup> Chronic HBV patients had shorter telomeres than controls despite the fact that hepatocyte telomeres do not shorten with age in healthy liver.<sup>18</sup> The results of this study also highlighted that when hepatitis B core antigen (HBcAg) is in the nucleus in the early phase of infection, longer telomeres are observed as opposed to the more advanced phase of infection at which time HBcAg is in the cytoplasm. These findings and similar patterns observed across other human viruses have led to the understanding that cell cycle arrest may enhance viral replication in patients with a variety of viral illness including HBV and HCV.<sup>15,19</sup> In addition, the results of a



**Fig. 1.** Pathways of telomere hepatocyte damage. Telomere shortening in hepatocytes is observed in multiple etiologies of cirrhosis. A stressor, either repeated antigen stimulation or oxidative stress, leads to the dysfunction of telomerase and the shortening of telomeres. These shortened telomeres are then recognized as damaged DNA. Increased markers of DNA damage, such as p21, are observed and result in increased cell cycle arrest. In viral hepatitis this cell cycle arrest leads to increased viral replication. NASH, nonalcoholic steatohepatitis; HBV, hepatitis B viral infection; HCV, hepatitis C viral infection.

recent study demonstrated that in the setting of chronic HCV hepatocyte telomere shortening leads to chromosomal instability, ultimately potentially promoting the development of hepatocellular carcinoma (HCC).<sup>20</sup>

### 3. NASH and alcoholic liver disease

While the mechanism of cell cycle arrest to promote viral replication and subsequent hepatocyte senescence has been accepted in regard to viral hepatitis, current data would suggest that while the ultimate pathway is common in NASH, the inciting mechanisms for telomere damage may differ. Previously studies have proposed that cell cycle arrest and senescence in NASH and alcoholic liver disease are the direct result of oxidative stress on hepatocytes (Fig. 1).<sup>18</sup> This stress in turn leads to telomere attrition and cellular senescence.<sup>21</sup> Ultimately, p21 and other markers of senescence are elevated in both hepatocytes populations and this cascade ultimately leads to fibrosis (Fig. 1).

While oxidative stress certainly plays a role in cellular damage and subsequent senescence in NASH, new data gathered regarding TERT inhibitors has deepened our understanding of telomere attrition in this disease process.<sup>20,21</sup> TERT inhibitors such as Tenofovir are commonly used in the long term treatment of human immunodeficiency virus (HIV) and studies have demonstrated that such use is an independent risk factor for nonalcoholic fatty liver disease (NAFLD).<sup>22</sup> Subsequent murine studies support a role for TERT in hepatic steatosis and hepatic injury.<sup>23</sup>

## T CELL TELOMERES

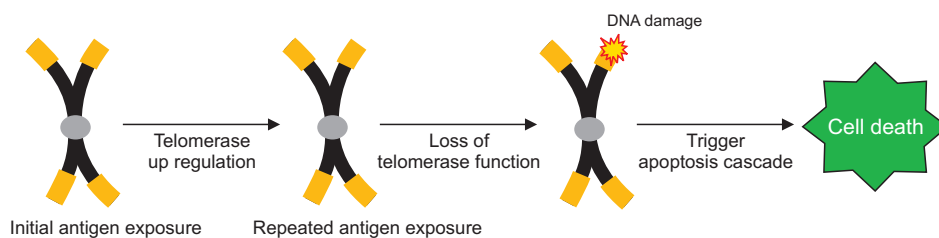
### 1. Viral hepatitis

A separate pathway of fibrosis formation and ultimate liver cirrhosis has been proposed through telomere shortening in T cells. Chronic viral hepatitis has been shown to lead to a phenomena of “T cell exhaustion” characterized by shortened telomeres in T cells.<sup>24</sup> Over time repetitive antigenic stimulation leads to the inhibition of telomerase expression. Subsequently, as immune cells divide in order to respond to continued stimulus, telomere length decreases and ultimately a DNA damage signal triggers the apoptosis cascade (Fig. 2).<sup>21</sup> Immune cells are distinct in that they can reactivate telomerase following cellular

differentiation and thereby extend their replicative capacity.<sup>21</sup> This function is of particular importance when combating a viral infection. CD8+ memory cells from the initial acute phase of viral infection must retain the capacity to replicate in order to allow for antigen recall and appropriate immune response to the infection throughout the host’s life.<sup>21</sup> In chronic hepatitis telomerase activity is up regulated in acute viral infection, but this is not maintained in the chronic phase of infection.<sup>25</sup> This is consistent with the model of “T cell exhaustion” which has also been observed in other human viral infections. For example, in HIV loss of telomere length in immune cells is accelerated during the chronic phase of infection. In both HBV and HCV peripheral blood lymphocytes have been observed to have lower telomerase RNA levels.<sup>19</sup> Shorter telomeres are found in both CD8+ and CD4+ cell populations.<sup>26,27</sup> Specifically in HCV, CD8+ T cells have elevated markers of DNA damage, for instance p53 serine phosphorylation, and shorter telomeres as compared to controls.<sup>28</sup> All of this data taken together points to an impaired cytotoxic T cell response. It has been proposed that this is a form of immunosuppression, which allows HBV and HCV infections to persist ultimately leading to liver dysfunction and disease.<sup>22</sup>

### 2. NASH and alcoholic liver disease

Data regarding immune telomere maintenance in NASH and alcoholic liver disease are unfortunately not as robust as in viral hepatitis. Despite this there exists data and observations which imply a potential role for immune senescence in the pathogenesis of NASH. For instance, accelerated immune senescence has been demonstrated in type 2 diabetes mellitus which is strongly associated with the development of NAFLD.<sup>29</sup> In addition, as discussed above, TERT knockouts experienced accelerated hepatic steatosis likely in keeping with impaired hepatocyte telomerase. However, given that the murine models in question were global knockouts, a role of enhanced immune senescence may have played a role in the steatosis observed.<sup>21,30</sup> Further studies will be needed to clarify the extent to which impaired immune telomere maintenance fuels the development of NAFLD.



**Fig. 2.** T cell exhaustion. Upon initial antigen encounter T cells can reactivate telomerase allowing for rapid proliferation in response to acute infection. However, if the infection and antigenic stimulus persist telomerase expression will cease. This leads to a reduction in telomere length followed by activation of a DNA damage signal, which triggers apoptosis.

## DISCUSSION

The study of telomere maintenance in both hepatocytes and immune cells furthers our understanding of the mechanisms which promote cellular senescence and ultimately liver fibrosis, thus opening the door to new potential therapy targets. While there remains room for further study in regard to hepatocyte telomere shortening in the non-viral etiologies of cirrhosis, such as NASH, current data suggest that in viral hepatitis cell cycle arrest and cellular senescence may directly promote viral replication.<sup>15</sup> It is possible that disruption of this process might allow for reduced viral replication and immune clearance of an often chronic and damaging infection. In addition, studies have demonstrated higher levels of cellular senescence at the hepatocyte level leading to an increased risk of HCC, with this being compounded by the impaired clearance of said senescent hepatocytes by immune cells.<sup>31,32</sup> These findings demonstrate the complex interplay between hepatic and immune telomerase maintenance while highlighting the telomerase complex as a possible therapeutic target.<sup>29</sup>

As outlined above, the dynamics of telomere maintenance and telomerase function within immune cells in the setting of chronic viral infections offer many potential points of intervention. There is the question of whether an exhausted T cell population could be reactivated against the chronic viral infection in question. This concept has led to research into the possible utility of PD-1 inhibitors in viral hepatitis with the goal of reactivating exhausted T cells when this inhibitory pathway within the immune system is blocked. It appears that in HBV this may be the case. In an *in vivo* model blocking PD-1/PD-L1 interactions, there was increased effector immune cell response and viral clearance.<sup>33</sup> Further studies are necessary to see whether the results of these studies can be replicated on a clinically meaningful level. From another angle there is also ongoing work to develop therapeutic vaccines targeted at stimulating the CD8+ T cell response in patients with chronic HBV.<sup>22</sup> Both strategies offer exciting possibilities for the millions of patients impacted by this disease.

Notably absent from the current body of literature regarding T cell telomeres and the development of cirrhosis is discussion of the non-viral etiologies of end stage liver disease, such as NASH and alcoholic cirrhosis. Our current model proposes that in viral hepatitis repeated antigenic stimulation of immune cells leads to the transcriptional silencing of the telomerase promoter which ultimately results in telomere shortening and senescence.<sup>22</sup> It may be the case that repetitive oxidative stress in NASH triggers senescence by a similar pathway in immune cells leading to a common targeted therapy. Further studies are necessary to model and clarify these theoretical pathways.

## CONCLUSIONS

Telomere preservation and telomerase function in both hepatic and immune cell populations play key roles in the development of liver fibrosis and ultimate cirrhosis. However, the mechanisms which initiate telomere loss appear to be independent. There remains work to be done to further elucidate the mechanism or mechanisms of hepatic telomere and immune telomere shortening in non-viral etiologies such as NASH and alcoholic cirrhosis. Further clarification of mechanisms of telomere shortening and telomerase impairment is necessary if we are to be successful in clinically targeting this pathway.

## CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

## ACKNOWLEDGEMENTS

Role in the study: study concept and design (A.B., S.S.); acquisition of data (A.B., A.M.); analysis and interpretation of data (A.B., A.M., S.S.); drafting of the manuscript (A.B., A.M., S.S.); critical revision of the manuscript for important intellectual content (A.B., S.S.); statistical analysis (not applicable); administrative or technical, support; study supervision (A.B., A.M., S.S.).

## REFERENCES

- Scaglione S, Kliethermes S, Cao G, et al. The epidemiology of cirrhosis in the United States: a population-based study. *J Clin Gastroenterol* 2015;49:690-696.
- Yim HJ, Lok AS. Natural history of chronic hepatitis B virus infection: what we knew in 1981 and what we know in 2005. *Hepatology* 2006;43(2 Suppl 1):S173-S181.
- Poynard T, Ratziu V, Charlotte F, Goodman Z, McHutchison J, Albrecht J. Rates and risk factors of liver fibrosis progression in patients with chronic hepatitis C. *J Hepatol* 2001;34:730-739.
- Blackburn EH. Switching and signaling at the telomere. *Cell* 2001;106:661-673.
- Evans SK, Bertuch AA, Lundblad V. Telomeres and telomerase: at the end, it all comes together. *Trends Cell Biol* 1999;9:329-331.
- Shammas MA. Telomeres, lifestyle, cancer, and aging. *Curr Opin Clin Nutr Metab Care* 2011;14:28-34.
- Verma S, Tachtatzis P, Penrhyn-Lowe S, et al. Sustained telomere length in hepatocytes and cholangiocytes with increasing age in normal liver. *Hepatology* 2012;56:1510-1520.
- von Zglinicki T. Oxidative stress shortens telomeres. *Trends Biochem Sci* 2002;27:339-344.
- Sasaki M, Ikeda H, Haga H, Manabe T, Nakanuma Y. Frequent cellular senescence in small bile ducts in primary biliary cirrhosis: a possible role in bile duct loss. *J Pathol* 2005;205:451-459.

10. Al-Issa K, Tolle LB, Purysko AS, Hanouneh IA. Short telomere syndrome and fibrosis. *QJM* 2016;109:125-126.
11. Carulli L, Dei Cas A, Nascimbeni F. Synchronous cryptogenic liver cirrhosis and idiopathic pulmonary fibrosis: a clue to telomere involvement. *Hepatology* 2012;56:2001-2003.
12. Carulli L. Telomere shortening as genetic risk factor of liver cirrhosis. *World J Gastroenterol* 2015;21:379-383.
13. Carulli L, Anzivino C. Telomere and telomerase in chronic liver disease and hepatocarcinoma. *World J Gastroenterol* 2014;20:6287-6292.
14. Wiemann SU, Satyanarayana A, Tshuridu M, et al. Hepatocyte telomere shortening and senescence are general markers of human liver cirrhosis. *FASEB J* 2002;16:935-942.
15. Marshall A, Rushbrook S, Davies SE, et al. Relation between hepatocyte G1 arrest, impaired hepatic regeneration, and fibrosis in chronic hepatitis C virus infection. *Gastroenterology* 2005;128:33-42.
16. Aravinthan A, Pietrosi G, Hoare M, et al. Hepatocyte expression of the senescence marker p21 is linked to fibrosis and an adverse liver-related outcome in alcohol-related liver disease. *PLoS One* 2013;8:e72904.
17. Aravinthan A, Scarpini C, Tachtatzis P, et al. Hepatocyte senescence predicts progression in non-alcohol-related fatty liver disease. *J Hepatol* 2013;58:549-556.
18. Tachtatzis PM, Marshall A, Arvinthan A, et al. Chronic hepatitis B virus infection: the relation between hepatitis B antigen expression, telomere length, senescence, inflammation and fibrosis. *PLoS One* 2015;10:e0127511.
19. Satra M, Dalekos GN, Kollia P, Vamvakopoulos N, Tsezou A. Telomerase reverse transcriptase mRNA expression in peripheral lymphocytes of patients with chronic HBV and HCV infections. *J Viral Hepat* 2005;12:488-493.
20. Miura N, Horikawa I, Nishimoto A, et al. Progressive telomere shortening and telomerase reactivation during hepatocellular carcinogenesis. *Cancer Genet Cytogenet* 1997;93:56-62.
21. Nakajima T, Moriguchi M, Katagishi T, et al. Premature telomere shortening and impaired regenerative response in hepatocytes of individuals with NAFLD. *Liver Int* 2006;26:23-31.
22. Vodkin I, Loomba R. Editorial: NAFLD in HIV infection--call for action. Authors' reply. *Aliment Pharmacol Ther* 2015;41:591.
23. Alves-Paiva RM, Kajigaya S, Feng X, et al. Telomerase enzyme deficiency promotes metabolic dysfunction in murine hepatocytes upon dietary stress. *Liver Int* 2018;38:144-154.
24. Bellon M, Nicot C. Telomere dynamics in immune senescence and exhaustion triggered by chronic viral infection. *Viruses* 2017;9:10:E289.
25. Plunkett FJ, Soares MV, Annels N, et al. The flow cytometric analysis of telomere length in antigen-specific CD8+ T cells during acute Epstein-Barr virus infection. *Blood* 2001;97:700-707.
26. Bellon M, Nicot C. Regulation of telomerase and telomeres: human tumor viruses take control. *J Natl Cancer Inst* 2008;100:98-108.
27. Hoare M, Gelson WT, Das A, et al. CD4+ T-lymphocyte telomere length is related to fibrosis stage, clinical outcome and treatment response in chronic hepatitis C virus infection. *J Hepatol* 2010;53:252-260.
28. Hoare M, Shankar A, Shah M, et al. Gamma-H2AX+CD8+ T lymphocytes cannot respond to IFN-alpha, IL-2 or IL-6 in chronic hepatitis C virus infection. *J Hepatol* 2013;58:868-874.
29. Sampson MJ, Winterbone MS, Hughes JC, Dozio N, Hughes DA. Monocyte telomere shortening and oxidative DNA damage in type 2 diabetes. *Diabetes Care* 2006;29:283-289.
30. Aravinthan AD, Alexander GJ. Telomere, telomerase and liver disease. *Liver Int* 2018;38:33-34.
31. Rey S, Quintavalle C, Burmeister K, et al. Liver damage and senescence increases in patients developing hepatocellular carcinoma. *J Gastroenterol Hepatol* 2017;32:1480-1486.
32. Kang TW, Yevsa T, Woller N, et al. Senescence surveillance of premalignant hepatocytes limits liver cancer development. *Nature* 2011;479:547-551.
33. Tzeng HT, Tsai HF, Liao HJ, et al. PD-1 blockage reverses immune dysfunction and hepatitis B viral persistence in a mouse animal model. *PLoS One* 2012;7:e39179.