American Journal of Aging Science and Research

Timchenko, Am J Aging Sci Res
2021; 2(1): 1-3.

Mini Review

Age-associated hepatic steatosis and liver proliferation in NAFLD

Nikolai Timchenko*

Professor of Pediatric General and Thoracic Surgery, Director of Liver Tumor Program, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, 45229, United States

*Author for correspondence:
Email: Nikolai.Timchenko@cchmc.org

Received date: July 13, 2020
Accepted date: September 07, 2020

Citation: Timchenko N. Age-associated hepatic steatosis and liver proliferation in NAFLD. Am J Aging Sci Res 2021; 2(1): 1-3.

Abstract

Non-Alcoholic Fatty Liver Disease (NAFLD) is the growing epidemic which is rapidly increasing in USA. Elderly people are the most affected population which suffers from NAFLD. The earliest stage of NAFLD, hepatic steatosis has no evidence of liver injury, but is characterized by an accumulation of triglycerides in hepatocytes. Hepatic steatosis progresses in age-dependent manner to non-alcoholic steatohepatitis (NASH) and cirrhosis. Mechanisms of development of age associated NAFLD are not well understood and approaches to treat this disease are not developed. Recent studies within last three years; however, provided several lines of evidence showing that although hepatic steatosis strongly correlates with development of NAFLD, it might be not a cause of next steps of NAFLD: fibrosis and NASH. Moreover, some reports suggest that hepatic steatosis might play a protective role in development of fibrosis and NASH. Recent studies of NAFLD in animal models showed that the increase of liver proliferation is the first event in high-fat diet-induced NAFLD. Consistent with these findings, several studies showed that inhibition of liver proliferation in animal models of NAFLD inhibits age-associated hepatic steatosis, fibrosis and cancer. This review summarizes these studies with the focus on relationships of liver proliferation, hepatic steatosis and fibrosis in NAFLD patients and in animal models of NAFLD. The review also discusses potential molecular mechanisms by which liver proliferation contributes to development of NAFLD.

Keywords: NAFLD, Steatosis, Fibrosis, Liver proliferation, Oncogenes, Tumor suppressors

Hepatic Steatosis and Fibrosis in NAFLD

NAFLD is characterized by several stages with different severity of liver disease [1]. Based on the timing of appearance, it has been postulated that the first stage of NAFLD is hepatic steatosis that progresses to fibrosis and then to non-alcoholic steatohepatitis (NASH) which is a high risk for hepatocellular carcinoma (HCC). The first evidence that hepatic steatosis might not be the cause of fibrosis in NAFLD came from the early report by Dr. Diehl's group [2]. The authors found that inhibition of a key enzyme of triglycerides synthesis Diacylglycerol Acyltransferase 2 (DGAT2) by antisense oligonucleotide in methionine choline-deficient (MCD) diet-fed mice decreased hepatic steatosis, but exacerbated liver damage suggesting that the accumulation of triglycerides and development of steatosis do not cause fibrosis. Given the increase of liver damage in mice with inhibited steatosis, the authors suggested that steatosis might play a protective role [2]. Recent studies in mice with the hepatocyte-specific deletion of DGAT2 confirmed that the reduction of steatosis does not reduce fibrosis [3]. Examination of saturated fat and cholesterol and different steps of NAFLD in DGAT2-deficient mice, exposed to a diet rich in fructose, showed that the genetic deletion of DGAT2 reduces expression of de novo lipogenesis genes and lowered TGs levels; however, this inhibition of steatosis did not reduce fibrosis [3]. Thus, these reports demonstrated that the progressive liver damage in NAFLD is not caused by hepatic steatosis. In agreement with these findings, Hayashida and colleagues showed that the deficiency of an extracellular matrix protein vitronectin is sufficient to inhibit fibrosis but does not affect development of steatosis in genetically modified mice under conditions of choline-deficient high fat diet [4]. Additional evidence showing that steatosis is not causal for fibrosis has been obtained by examination of high fat diet-fed mice with the hepatocyte-specific deletion of an oncogene Gankyrin (Gank LKO mice) [5]. The authors have shown that high fat diet-fed Gank LKO mice develop stronger hepatic steatosis than high fat diet-fed WT mice; however, no fibrosis has been observed in these mice; while age-matched high fat diet-fed WT mice developed fibrosis [5]. Important, high fat diet-fed Gank LKO mice (with high level of hepatic steatosis) appeared much healthier than age-matched high fat diet-fed WT mice suggesting that hepatic steatosis might have a

beneficial effect on the health of these mice [5]. In addition to these recent reports which directly showed that hepatic steatosis does not cause fibrosis, several early studies did not find correlations of hepatic steatosis and fibrosis [6,7]. Consistent with the studies in animal models of NAFLD, a recent human galactose positron emission tomography study in patients with NAFLD showed that a simple steatosis does not disturb the functional homogeneity of the liver; while NASH patients had reduced regional metabolic liver functions [8]. Taken together, the growing number of studies suggests that hepatic steatosis alone is not a cause of fibrosis and NASH and that there should be an additional early event that triggers liver damage.

Hepatic Steatosis and Liver Proliferation in NAFLD

Previously, the increase of liver proliferation in NAFLD patients and in animal models of NAFLD has been found at late stages of NAFLD: NASH and HCC. Therefore, until recently, the increase of liver proliferation was considered as a consequence of hepatic steatosis and/or fibrosis. However, recent studies found that high fat diet-fed WT mice have increased liver proliferation at early stages of NAFLD prior or at the time of appearance of hepatic steatosis [6,7]. The initial rationale for the studies of liver proliferation in NAFLD was associated with elevation of a cell proliferation promoter cdk4, which phosphorylates C/EBPα at Ser193 (Ser190 in human C/EBPα), leading to subsequent accumulation of C/EBPα-p300 complexes that activate enzymes of TG synthesis in animal models of NAFLD and in patients with NAFLD [6,7,9]. Searching for the potential treatments of NAFLD, Jin and colleagues detected the increased rate of liver proliferation in WT mice at 3 weeks after initiation of high fat-diet treatments, while hepatic steatosis was detected at 4-5 weeks [6]. It has been further shown that inhibition of cdk4-C/EBPα-p300 axis in WT mice by specific inhibitor of cdk4 PD-033291 inhibited not only production of enzymes of TG synthesis, but also liver proliferation which leads to inhibition of NAFLD [7]. Moreover, generation and examination of two mouse models C/EBPα-S193D and C/EBPα-S193A with reduced and increased liver proliferation correspondingly showed that liver proliferation is involved in development of age-associated NAFLD [7,10-12]. In agreement with these reports, a recent paper from Michalopoulos's group has investigated the role of key promoter of liver proliferation and regeneration, epidermal growth factor receptor (EGFR) in NAFLD using a pharmacological inhibition strategy. The authors have found that the inhibition of liver proliferation by inhibiting EGFR suppresses NAFLD [13]. Another study demonstrated that the strong promoter of liver proliferation E2F1 is elevated in animal models of NAFLD and in NAFLD patients and that the deletion of E2F1 completely abrogates progression of NAFLD in animal models [14]. Thus, these studies revealed that the increase of liver proliferation is a significant part of the NAFLD and that the inhibition of liver proliferation reduces NAFLD. Taking together data with NAFLD patients, pharmacological inhibition data and genetic mutations/deletions studies, it becomes apparent that the increase of liver proliferation should be considered as an essential driver of NAFLD.

The Role of Oncogene Gankyrin and Tumor Suppressors in Age-related NAFLD

In addition to the evidence for the role of liver proliferation in NAFLD, the above-mentioned studies provided mechanistic knowledge on how key regulators of liver proliferation (oncogenes and tumor suppressor proteins) are involved in regulation of NAFLD. The most convincing observations have been collected for the oncogene Gank which was initially identified as a protein that is elevated in patients with hepatocellular carcinoma (HCC) [15,16]. In liver cancer, Gank eliminates several tumor suppressor proteins (TSPs) including p53, Rb, C/EBPα, HNF4α and CUGBP1 [17-19]. Further examinations of Gank expression in NAFLD showed that Gank is elevated in animal models of NAFLD [6,15,16]. Sakurai and colleagues examined expression of Gank in liver biopsies from patients with non-alcoholic steatohepatitis and found an increase of Gank in these patients [20]. These findings suggested that Gank is involved in the development of NAFLD. Consistent with these observations, the generation of a zebrafish model with overexpression of Gank in the liver showed that Gank causes development of hepatic steatosis, cholestasis, fibrosis and hepatic tumors [21]. Recently, Cast and colleagues have evaluated the role of Gank in NAFLD using mice with liver-specific deletion of Gank, Gank LKO mice [5]. The authors showed that the high fat diet-mediated increase of Gank in NAFLD reduces tumor suppressors, which leads to subsequent development of fibrosis. In this study, the authors also showed that Gank-mediated degradation of the tumor suppressor CUGBP1 develops age-associated fibrosis and liver tumor (at 17 months of age); while age-matched WT mice had no fibrosis or liver tumor [5]. Considering therapeutic approaches for patients with NAFLD, it is important to note that obeticholic acid, OCA, is successfully used in ongoing Phase II/III clinical trials (called FLINT) [23]. In this regard, it has been shown that OCA inhibits liver proliferation via down-regulation of Gank and subsequent stabilization of the tumor suppressor C/EBPα [22] suggesting that the positive effects of OCA treatments in FLINT trials might be related to a rescue of C/EBPα. In support of this suggestion, the studies from Habib's group showed that the rescue of C/EBPα (target of Gank) by a short activating RNA reversed changes associated with hepatosteatosis in MCD-induced NASH [24]. Taken together, these studies showed an essential role of Gank-TSPs axis in development of NAFLD.

Conclusions and Future Molecular and Clinical Studies

As demonstrated in this review, liver proliferation and Gank-TSPs axis are essential drivers of NAFLD including age-associated steatosis and fibrosis [5]. At this stage, precise mechanisms by which liver proliferation causes fibrosis remain unknown. Since the cellular origin of fibrosis are hepatic stellate cells, the main mechanistic question is if Gank-TSPs pathway is activated in hepatic stellate cells or if it is activated in hepatocytes and causes a secretion of systemic factors which activate hepatic stellate cells. Regardless of the exact mechanisms, the inhibition of liver proliferation appears to be a promising direction in development of therapeutic approaches for treatments of patients with NAFLD. It is important to note that studies in animal models revealed that the inhibition of proliferation by inhibitors of cdk4 not only reduces NAFLD, but also corrects other age-associated liver disorders [7] suggesting that the therapeutic approaches, based on the inhibition of liver-proliferation, might be beneficial for elderly people. Interestingly, the ongoing FLINT trial phase III with NAFLD patients uses OCA which is an activator of FXR and the inhibitor of Gank [22]. Two reports from FLINT trial showed that OCA inhibits fibrosis in NAFLD patients [23,25] likely via the OCA-FXR-Gank-TSPs mediated inhibition of liver proliferation.

Conflicts of Interest

The author declares no Conflicts of Interest.

Funding

This work is supported by NIH grant R01DK102597 and by Internal Development Funds from CCHMC (NT).

References

1. Benedict M, Zhang X. Non-alcoholic fatty liver disease: An expanded review. World Journal of Hepatology. 2017 Jun 8;9(16):715.
2. Yamaguchi K, Yang L, McCall S, Huang J, Yu XX, et al. Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis. Hepatology. 2007 Jun;45(6):1366-74.
3. Gluchowski NL, Gabriel KR, Chitraju C, Bronson RT, Mejhert N, Boland S, et al. Hepatocyte Deletion of Triglyceride-Synthesis Enzyme Acyl CoA: Diacylglycerol Acyltransferase 2 Reduces Steatosis Without Increasing Inflammation or Fibrosis in Mice. Hepatology. 2019 Dec;70(6):1972-85.
4. Hayashida M, Hashimoto K, Ishikawa T, Miyamoto Y. Vitronectin deficiency attenuates hepatic fibrosis in a non-alcoholic steatohepatitis-induced mouse model. International Journal of Experimental Pathology. 2019 Apr;100(2):72-82.
5. Cast A, Kumbaji M, D'Souza A, Rodriguez K, Gupta A, Karns R, et al. Liver proliferation is an essential driver of fibrosis in mouse models of nonalcoholic fatty liver disease. Hepatology Communications. 2019 Aug;3(8):1036-49.
6. Jin J, Valanejad L, Nguyen TP, Lewis K, Wright M, Cast A, et al. Activation of CDK4 triggers development of non-alcoholic fatty liver disease. Cell Reports. 2016 Jul 19;16(3):744-56.
7. Nguyen P, Valanejad L, Cast A, Wright M, Garcia JM, El-Serag HB, et al. Elimination of age-associated hepatic steatosis and correction of aging phenotype by inhibition of cdk4-C/EBPα-p300 Axis. Cell Reports. 2018 Aug 7;24(6):1597-609.
8. Eriksen PL, Thomsen KL, Larsen LP, Grønbæk H, Vilstrup H, Sørensen M. Non-alcoholic steatohepatitis, but not simple steatosis, disturbs the functional homogeneity of the liver–a human galactose positron emission tomography study. Alimentary Pharmacology & Therapeutics. 2019 Jul;50(1):84-92.
9. Jin J, Iakova P, Breaux M, Sullivan E, Jawanmardi N, Chen D, et al. Increased expression of enzymes of triglyceride synthesis is essential for the development of hepatic steatosis. Cell Reports. 2013 Mar 28;3(3):831-43.
10. Wang GL, Shi X, Haefliger S, Jin J, Major A, Iakova P, et al. Elimination of C/EBPα through the ubiquitin-proteasome system promotes the development of liver cancer in mice. The Journal of Clinical Investigation. 2010 Jul 1;120(7):2549-62.
11. Jin J, Wang GL, Iakova P, Shi X, Haefliger S, Finegold M, et al. Epigenetic changes play critical role in age-associated dysfunctions of the liver. Aging Cell. 2010 Oct;9(5):895-910.
12. Jin J, Hong IH, Lewis K, Iakova P, Breaux M, Jiang Y, et al. Cooperation of C/EBP family proteins and chromatin remodeling proteins is essential for termination of liver regeneration. Hepatology. 2015 Jan 1;61(1):315-25.
13. Bhushan B, Banerjee S, Paranjpe S, Koral K, Mars WM, Stoops JW, et al. Pharmacologic Inhibition of Epidermal Growth Factor Receptor Suppresses Nonalcoholic Fatty Liver Disease in a Murine Fast-Food Diet Model. Hepatology. 2019 Nov;70(5):1546-63.
14. Denechaud PD, Lopez-Mejia IC, Giralt A, Lai Q, Blanchet E, Delacuisine B, et al. E2F1 mediates sustained lipogenesis and contributes to hepatic steatosis. The Journal of Clinical Investigation. 2016 Jan 4;126(1):137-50.
15. Fujita J, Sakurai T. The Oncoprotein Gankyrin/PSMD10 as a Target of Cancer Therapy. In: Human Cell Transformation 2019 (pp. 63-71). Springer, Cham.
16. Iakova P, Timchenko L, Timchenko NA. Intracellular signaling and hepatocellular carcinoma. In: Seminars in cancer biology 2011 Feb 1 (Vol. 21, No. 1, pp. 28-34). Academic Press.
17. Timchenko NA, Lewis K. Elimination of tumor suppressor proteins during liver carcinogenesis. Cancer Studies Mol Med Open J. 2015;1:27-38.
18. D'Souza AM, Jiang Y, Cast A, Valanejad L, Wright M, Lewis K, et al. Gankyrin promotes tumor-suppressor protein degradation to drive hepatocyte proliferation. Cellular and Molecular Gastroenterology and Hepatology. 2018 Jan 1;6(3):239-55.
19. Lewis K, Valanejad L, Cast A, Wright M, Wei C, Iakova P, et al. RNA binding protein CUGBP1 inhibits liver cancer in a phosphorylation-dependent manner. Molecular and Cellular Biology. 2017 Aug 15;37(16).
20. Sakurai T, Yada N, Hagiwara S, Arizumi T, Minaga K, Kamata K, et al. Gankyrin induces STAT 3 activation in tumor microenvironment and sorafenib resistance in hepatocellular carcinoma. Cancer Science. 2017 Oct;108(10):1996-2003.
21. Huang SJ, Cheng CL, Chen JR, Gong HY, Liu W, Wu JL. Inducible liver-specific overexpression of gankyrin in zebrafish results in spontaneous intrahepatic cholangiocarcinoma and hepatocellular carcinoma formation. Biochemical and Biophysical Research Communications. 2017 Aug 26;490(3):1052-8.
22. Loomba R, Gindin Y, Jiang Z, Lawitz E, Caldwell S, Djedjos CS, et al. DNA methylation signatures reflect aging in patients with nonalcoholic steatohepatitis. JCI Insight. 2018 Jan 25;3(2).
23. Jiang Y, Iakova P, Jin J, Sullivan E, Sharin V, Hong IH, et al. Farnesoid X receptor inhibits gankyrin in mouse livers and prevents development of liver cancer. Hepatology. 2013 Mar;57(3):1098-106.
24. Reebye V, Huang KW, Lin V, Jarvis S, Cutilas P, Dorman S, et al. Gene activation of CEBPA using saRNA: preclinical studies of the first in human saRNA drug candidate for liver cancer. Oncogene. 2018 Jun;37(24):3216-28.
25. Gawrieh S, Guo X, Tan J, Lauzon M, Taylor KD, Loomba R, et al. A Pilot Genome-Wide Analysis Study Identifies Loci Associated With Response to Obeticholic Acid in Patients With NASH. Hepatology communications. 2019 Dec;3(12):1571-84.