

Available online at www.sciencerepository.org

Science Repository



Research Article

The Roles of Epigenetic Regulators and Inflammasome in Hepatocellular Carcinoma Tumorigenesis in Patients with Alcoholic Steatohepatitis (ASH) vs Non-Alcoholic Steatohepatitis (NASH)

Yue Jia*, Ping Ji, Luan Nguyen, Barbara French, Brittany Tillman, Askalu Iyasu and Samuel W. French

Harbor-UCLA Medical Center, Department of Pathology, Torrance, CA, 90502 USA

ARTICLE INFO

Article history:

Received: 21 May, 2019

Accepted: 2 July, 2019

Published: 29 July, 2019

Keywords:

Alcoholic Steatohepatitis (ASH)

non-Alcoholic Steatohepatitis (NASH)

hepatocellular Carcinoma (HCC)

tumorigenesis

epigenetic regulators

inflammasome

G9a

LHPP

ASC

ABSTRACT

As the fifth most common cancer and the second leading cause of cancer related deaths worldwide, hepatocellular carcinoma (HCC) is an aggressive tumor type with poor prognosis causing 250,000 to one million deaths annually. Alcoholic steatohepatitis (ASH) and non-alcoholic steatohepatitis (NASH) are the two major growing risk factors and both may develop liver fibrosis or even HCC. However, compared with the rate of patients with ASH progressing to HCC annually, it is much lower in NASH patients. The present study is to clarify the protein expression of epigenetic regulators and Inflammasome components in the oncogenesis pathway between ASH and NASH. By using the immunofluorescence method and morphometrically quantitating the fluorescence intensity in liver biopsied specimens from NASH and ASH patients, we studied the protein expression within hepatocytes cytoplasm of candidate epigenetic regulators G9a and LHPP and inflammasome component ASC. Compared with the control group patients, the expression levels of all three proteins were upregulated in the ASH and NASH group of patients ($p < 0.001$ in all molecules). While compared with the ASH group of patients, only the expression levels of G9a were statistically lower in the NASH group of patients ($p < 0.01$). The other two molecules, LHPP and ASC did not change. These results are consistent with our previous work that there are significant differences of many molecules including epigenetic modulators and the inflammasome pathway in both NASH and ASH compared to the control group. Thus, we conclude that there are significantly different molecules and pathways involved during the pathogenesis of HCC development in NASH compared to ASH which may shed some light on developing prevention methods and targeted treatments in NASH and ASH patients.

© 2019 Yue Jia. Hosting by Science Repository.

Introduction

Hepatocellular carcinoma (HCC) is a very common cancer and the second leading cause of cancer related deaths worldwide which causes 250,000 to one million deaths annually, therefore HCC is a major global healthcare burden and the major risk factors include Hepatitis B (HBV) and hepatitis C (HCV), obesity/metabolic syndrome, and alcoholism [1]. In the last twenty years, the incidence of the non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) increased significantly due to the prevalence of overweight, obesity, and metabolic syndrome. The relationship between NASH and HCC development has been proved by both experimental and epidemic studies. In addition to

NAFLD and NASH, Alcoholic liver disease (ALD) and ASH are also becoming the major causes of HCC in the US [2-4]. NASH and ASH specimens have similar histological features, such as fat droplets storage and significant lipid deposition in the hepatocytes. Although both NASH and ASH may progress to fibrosis, cirrhosis, and eventually hepatocellular carcinoma (HCC), the ratios of progressing are different. NASH progresses to cirrhosis and to HCC in about 7–10% and 0.5% victims, respectively, while ASH progresses to cirrhosis and to HCC in 10–20% patients, annually [5-8].

Although there are many theories including genetic alterations of tumor suppressor genes, oxidative stress, and decreased liver retinoic acid level, the detailed mechanisms underlying tumorigenesis of HCC in

*Correspondence to: Yue Jia; E-mail: YJia@dhs.lacounty.gov

NASH and ASH patients are not completely understood [2, 9, 10]. Our previous work found different gene and protein changes in ASH compared with NASH, such as the TLR/NFkB/CXCR4/7, PI3K/AKT/mTORC1 signaling pathways; Ras/RASSF1A/P53 and RUNX3/p53/GSTP1 cross links together in molecular change both in ASH and NASH [11-20]. The present study attempted to identify the role of epigenetic modulator proteins and inflammasome components in ASH and NASH patients. Using liver biopsy specimens from NASH and ASH patients and control groups, we found that the G9a (histone-lysine N-methyltransferase G9a), LHPP (Phospholysine Phosphohistidine Inorganic Pyrophosphate Phosphatase), and ASC (Apoptosis-associated speck-like protein containing a CARD) play important roles in tumorigenesis of HCC for both ASH and NASH groups.

Methods

Formalin-fixed paraffin-embedded biopsies of ASH liver (n=39 in G9A, 50 in LHPP, 40 in ASC), NASH liver (n=40), and normal liver (n=23) were collected from Harbor-UCLA Medical Center and from the Long Beach Veterans Affairs' clinical trial in treatment of alcoholic hepatitis. The study followed the principals of the Declaration of Helsinki and was designated as exempt by our institutional ethics review board and the data was analyzed anonymously. The primary antibodies rabbit anti-G9A, anti-LHPP, and anti-ASC were purchased (Abcam, Cambridge, MA). For each protein studied, the biopsy sections were stained together first with protein-specific primary antibody followed by a secondary fluorescence antibody. Either donkey anti-mouse or anti-rabbit Alex Fluor (Jackson Labs, West Grove, PA) was used as the second antibody. The slides were double stained for ubiquitin (to identify MDBs) using Texas Red (Millipore, Temecula, CA) and nuclear stained by DAPI. All the specimens were stained under the same situation to provide accurate comparison between groups. The intensity of the fluorescent staining of each candidate molecule in the liver cells was measured in at least three different areas on each section with 40× magnifications and 800 ms standard exposure time by using a Nikon 400 fluorescent microscope. Ten peak fluorescence intensities on each of 3 sections were collected and the fluorescent intensity was quantitated by using the Nikon morphometric system. The mean value, standard error, and statistical differences of data achieved from the Nikon were analyzed by Graph pad statistical software. Unpaired t-test was used to compare controls vs ASH, controls vs NASH, and ASH vs NASH with a p-value < 0.05 considered statistically significant.

Results

The protein expression levels of several candidate molecules in specimens from patients with ASH, NASH, and normal controls were compared. Representative data are shown in (Fig. 1-3). In both NASH and ASH patients, levels of all tested candidate proteins including G9A, LHPP, and ASC (Fig. 1-3) were markedly increased compared with the control group. Although G9A was statistically significant lower in the NASH group specimens compared with the ASH group, LHPP and ASC had no difference (Fig. 1-3).

Discussion and Conclusions

The increasing prevalence of obesity and the metabolic syndrome has

become a major health burden worldwide which is leading to an increased number of patients suffering from NASH to cirrhosis and even hepatocellular carcinoma (HCC). NASH is the most rapid growing risk factor in patients receiving liver transplantation due to HCC [21, 22]. The risk to develop HCC in patients drinking alcohol chronically (longer than 10 years and greater than 80 gram/day) is about 5 fold higher compared with the general population [24]. The most common cause of HCC is ALD which leads to around 1/3 of all HCC cases in US and Italy [2, 4]. Both NASH and ASH could progress to fibrosis, cirrhosis and even HCC, but the mechanisms involved remain unclear [23]. Our published data support that different molecules or pathways may be involved in NASH compared with ASH during the tumorigenesis [12, 13]. We showed that the TLR/NFkB/CXCR4/7, PI3K/AKT/mTORC1, and Tec kinase signaling pathways connected with each other during MDB formation both in ASH and NASH [11-20, 24].

Epigenetic modifications of DNA and histones include methylation, acetylation, and phosphorylation. These processes functionally cooperate modulating tumor development and growth, including that of hepatocellular carcinoma (HCC). SUV39H1, the first SET domain-containing histone lysine methyltransferase (HKMT), was reported in 2000. Since then, research on histone methylation has progressed rapidly. In mammals, Euchromatic histone-lysine N-methyltransferase G9a and GLP are also the primary HKMT enzymes for histone H3 dimethylation at lysine-9 (H3K9me2) and exist predominantly as a G9a-GLP heteromeric complex that appears to be a functional H3K9 methyltransferase in vivo. G9a and GLP play critical roles in various biological processes via epigenetic gene regulation [25]. G9a is upregulated in numerous types of cancer including HCC in at least 5 publically available datasets. G9a inhibition may suppress cell proliferation by arresting cells in G1 phase [26]. G9a regulates liver maturation by silencing neural and proinflammatory genes but maintaining/activating cytoprotective and drug-processing genes, in which the G9a/miR-383/PI3K/Akt/Nrf2 (Chop) pathways may play important roles [27]. Adult G9a liver-specific knockout (Liv-KO) mice show marked loss of H3K9me2 proteins in liver without overt liver injury or infiltration of inflammatory cells; while microRNA-383, a negative regulator of the PI3K/Akt pathway, was strongly induced in G9a Liv-KO mice [28]. Knockdown of G9a reduced H3K9me2 levels and impaired both HCC cell growth and sphere formation. Inhibition of G9a by BIX-01294 resulted in both cell growth inhibition and induction of apoptosis in HCC cells. G9a expression levels were significantly positively correlated with H3K9me2 levels in tumor tissues [29]. Overexpression/upregulation of G9a was significantly associated with HCC progression, aggressive clinicopathological features, and poor prognosis. Inactivation of G9a protein remarkably suppressed HCC cell proliferation and metastasis both in vitro and in vivo [30, 31]. Lysine methylation of histone and non-histone substrates by the methyltransferase G9a is mostly associated with transcriptional repression. However, other studies have highlighted G9a's role as an activator of gene expression through mechanisms that are independent of its methyltransferase activity [32]. Obviously, more detailed studies are needed to clarify the role of G9a in cancers. Our previous work showed that histone methylation factor SUV39H1, acetylation factor HDAC II; DNA methylation factors DNMT1 and DNMT3B were all increased in ASH specimens compared with the control group, and all four factors were much lower in NASH patients when compared with

the ASH group. In the present study, the expression of G9a protein was upregulated both in ASH and NASH specimens. The difference in the

expression of G9a protein between ASH and NASH groups was statistically significant (Fig. 1) [11, 12].

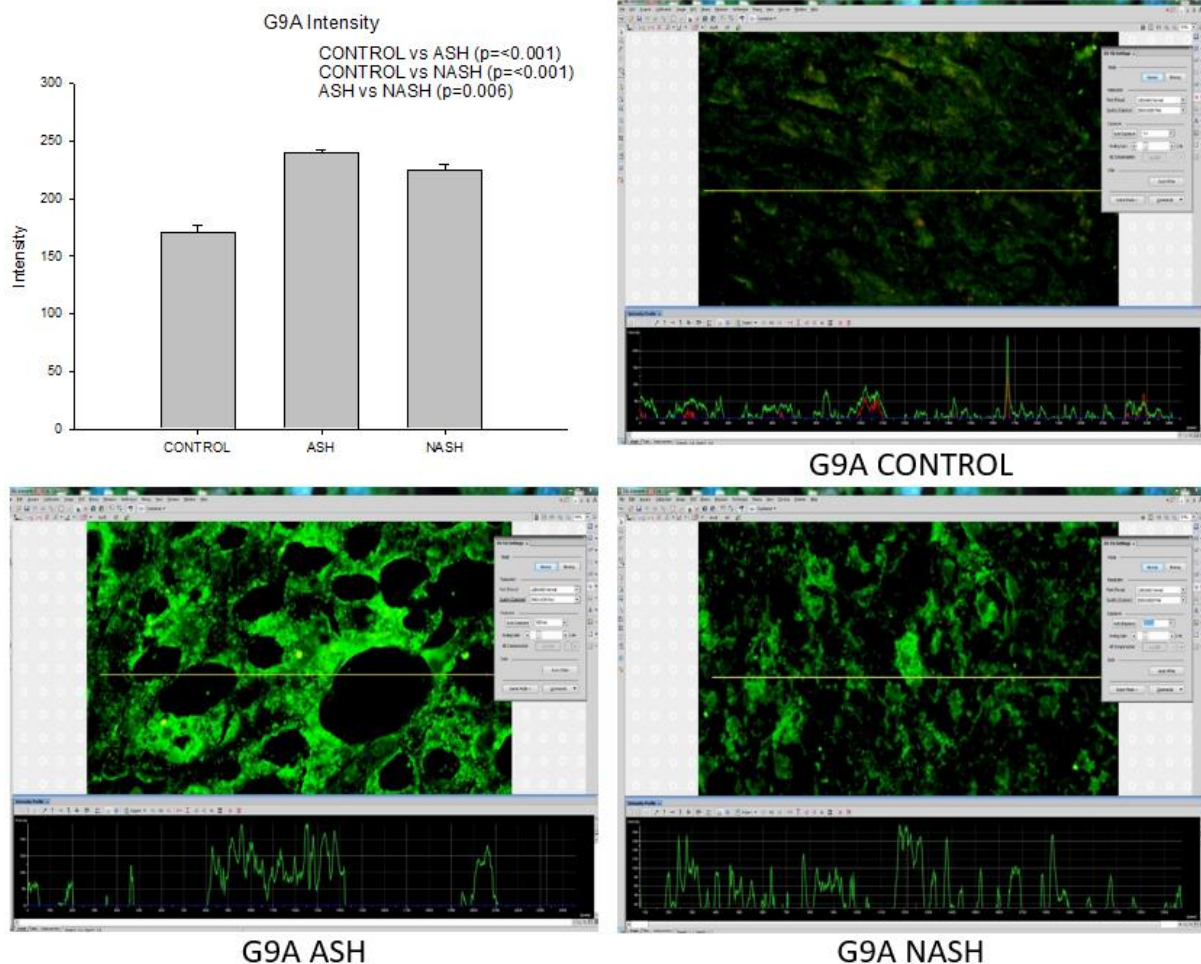


Figure 1: Changes of G9a in ASH and NASH specimens. (A) Level of expression of G9a protein upregulated in ASH and NASH compared with normal controls. Expression is measured as fluorescence intensity and displayed as mean $\pm$ standard deviation. Representative images of fluorescence intensity to measure G9a expression in the normal controls (B), ASH (C) and NASH (D) liver specimens. A line is drawn through the image to yield a fluorescence intensity graph; the intensity of the ten highest peaks are measured, excluding nuclear regions which are highlighted by DAPI (not shown). Three areas per slide are measured in this manner including Figure 2 and 3.

Histidine phosphorylation has broader roles in protein and cellular function including cell cycle regulation, phagocytosis, and tumorigenesis. Two mammalian histidine kinases (NME1 and NME2) and two pHis phosphatases (PHPT1 and LHPP) were identified [33]. Global histidine phosphorylation was significantly upregulated in the liver tumors. Proteomic analysis of 12 tumors from an mTOR-driven HCC mouse model revealed that the expression of the putative histidine phosphatase LHPP was downregulated specifically in the tumors, while the NME1 and NME2 were conversely upregulated [34]. Sustained hepatic expression of LHPP in the HCC mouse model reduced tumor burden and prevented the loss of liver function and low expression of LHPP correlated with more advanced tumor and reduced overall survival in HCC patients [34]. Over-expressed LHPP reduced the tumor cell proliferation, migration and invasion possibly via blocking AKT activation and restraining p53 expression levels in cervical cancer cells [35]. So, LHPP is not only a protein histidine phosphatase but also a

tumor suppressor. In our present data, the expression of LHPP protein was upregulated both in ASH and NASH specimens and there was no significant difference between ASH and NASH groups (Fig. 2).

Chronic inflammatory microenvironment plays a critical role at different stages of cancer development. The most predominant inflammatory pathways studied in hepatocarcinogenesis include NF- κ B and IL-6/STAT3 inflammation signaling pathways [36, 37]. These pathways activate the assembly of the inflammasome and the subsequent secretion of the pro-inflammatory cytokines IL-1 β and IL-18 both of which have been implicated in tumour-genesis/progression [38]. Inflammasomes, the potent inducers of interleukin (IL)-1 β and IL-18 during inflammation, are the central signaling hubs of the inflammatory response. Apoptosis-associated speck-like protein containing a CARD (ASC) is a key adaptor protein in activation of the inflammasome in case of pathogen infection or stressor attack [39, 40]. At the molecular level,

this is accomplished by the sensor-nucleated recruitment and oligomerization of the adapter protein ASC which links activated inflammasome sensors to the effector molecule pro-caspase-1[40]. Inflammasomes are large protein complexes typically consisting of a Nod-like receptor (NLR), the adapter protein ASC, and Caspase-1 [41]. After triggering of inflammasome sensors, ASC assembles into large helical fibrils serving as a supramolecular signaling platform termed the ASC speck. Recruitment of pro-caspase-1 to the inflammasome adapter ASC promotes the autocatalytic activation of caspase-1 and leads to the release of pro-inflammatory cytokines, such as IL-1 β [42, 43]. The various inflammasomes and inflammatory cytokines and chemokines

play contrasting roles in lung, breast, gastric, liver, colon, and prostate cancers and in glioblastomas [44]. Recently, it was reported that Inflammasomes may also exert anticancer effects by specialized programmed cell death called pyroptosis and immune regulatory functions [45]. Our previous work showed that the inflammasome activity was increased in ASH specimens [46]. In the present study, the expression of ASC protein was upregulated both in ASH and NASH specimens and there was no significant difference between ASH and NASH groups (Fig. 3). It suggests that the chronic inflammatory microenvironment and inflammation modulation are important in both ASH and NASH patients.

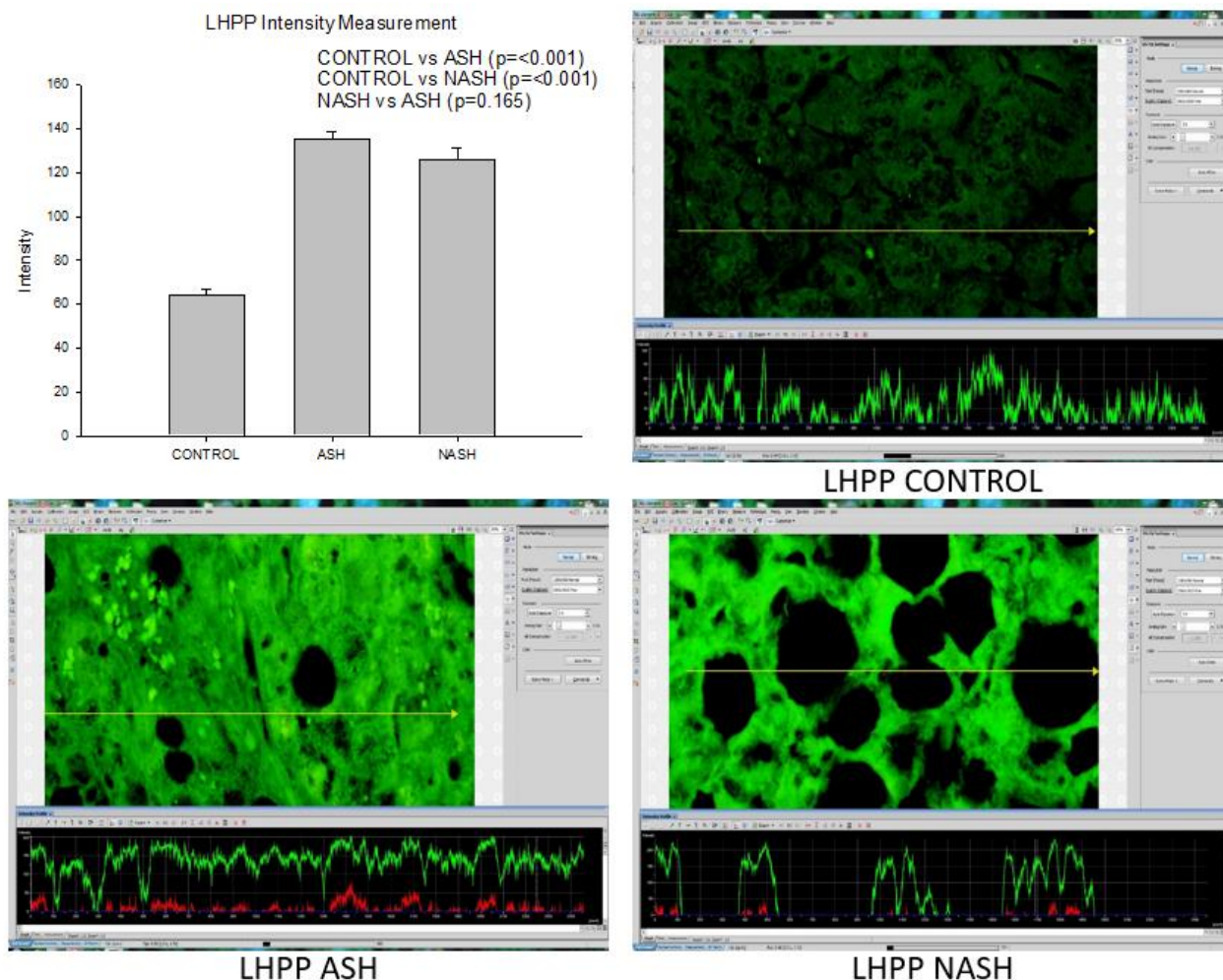


Figure 2: Changes of LHPP in ASH and NASH specimens. (A) Level of expression of LHPP protein upregulated in ASH and NASH compared with normal controls. Expression is measured as fluorescence intensity and displayed as mean $\pm$ standard deviation. Representative images of fluorescence intensity to measure LHPP expression in the normal controls (B), ASH (C) and NASH (D) liver specimens.

In summary, our previous work demonstrated the different expression of proteins which represent different pathways, such as GPCR/PI3K/Akt and TLR4/NF- κ B/STAT3 signaling pathways, and Ras/RASSF/Raf and RUNX3/p53/GSTP1 tumor suppress genes. It is consistent with that the risk rates of those progressing to cirrhosis or HCC annually are much lower in the NASH if compared with those in ASH. In present study, we

showed that epigenetic modulation and chronic inflammation were involved in the HCC tumorigenesis both in ASH and NASH (Fig. 4). These findings may be very helpful to understand the pathogenesis of HCC and to direct possible preventive and therapeutic pathways. To achieve this goal, more detailed experiments and long term studies are needed.

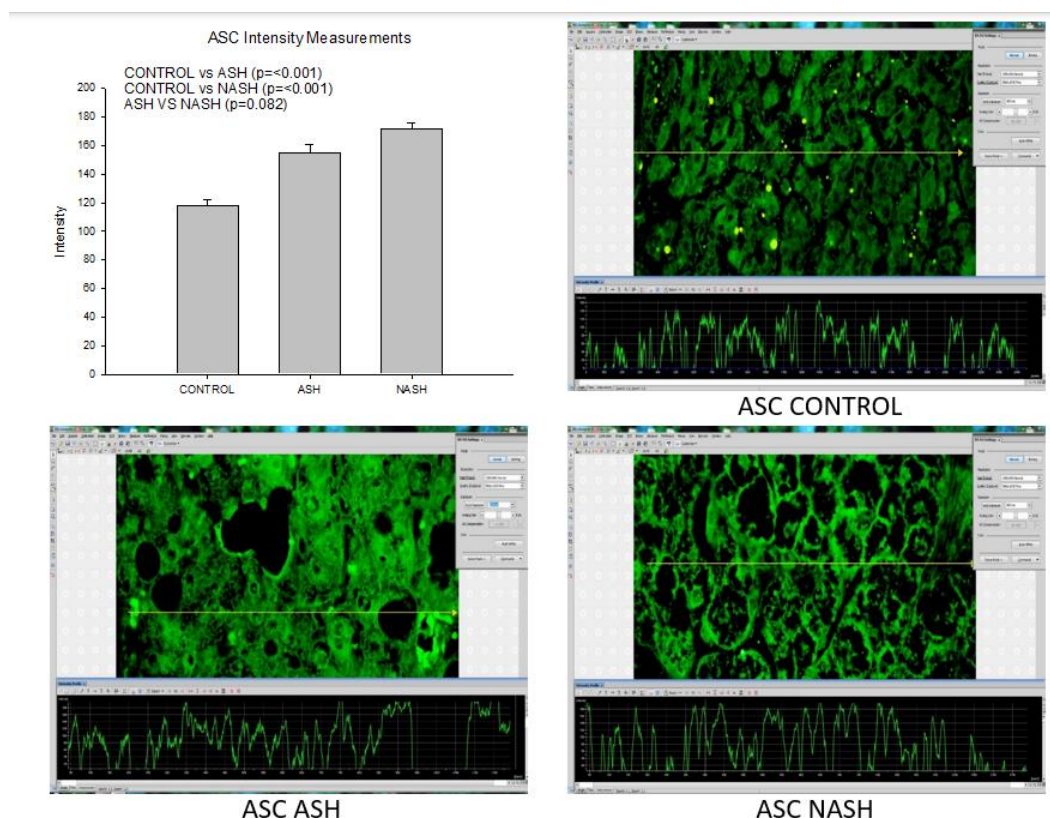


Figure 3: Changes of ASC in ASH and NASH specimens. (A) Level of expression of ASC protein upregulated in ASH and NASH compared with normal controls. Expression is measured as fluorescence intensity and displayed as mean $\pm$ standard deviation. Representative images of fluorescence intensity to measure ASC expression in the normal controls (B), ASH (C) and NASH (D) liver specimens.

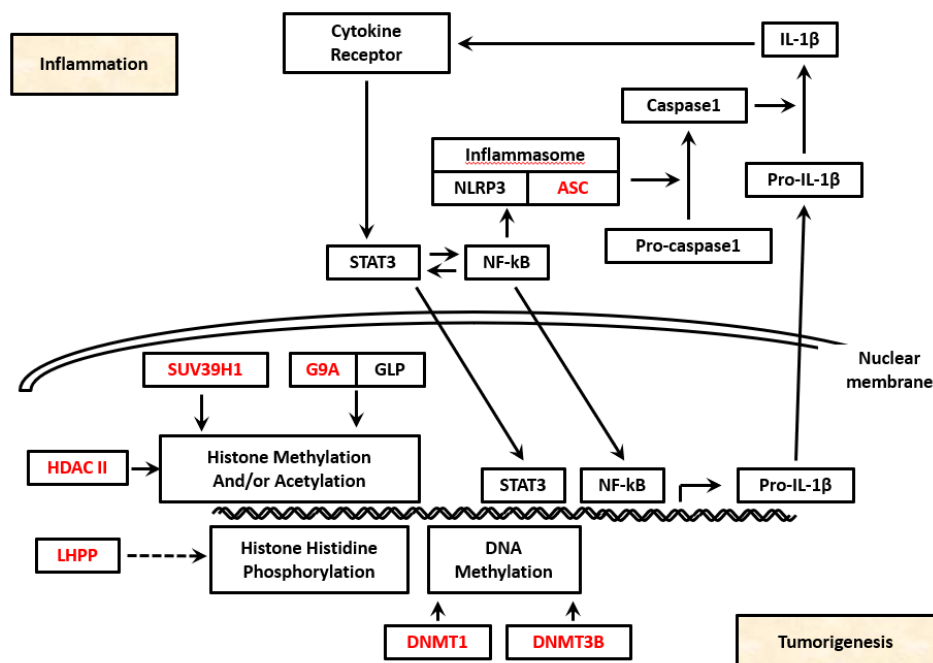


Figure 4: Putative pathway for DNMTs, SUV39H1, HDAC II, G9a, LHPP, and ASC in the HCC tumorigenesis. The similar expression pattern of G9a, LHPP, and ASC proteins suggest similar pathways been involved in ASH and NASH, including certain epigenetic modulators and inflammasome component in HCC tumorigenesis. But other epigenetic modulators such as DNMTs, SUV39H1, HDAC II still have different expression pattern in ASH and NASH specimens which shows the complexity of HCC tumorigenesis from different stressors

Acknowledgements

This study was funded by NIH/AAA grant # UO-21898-05.

Disclosure

All authors have no financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

Abbreviations

ASH: alcoholic steatohepatitis
 ALD: alcoholic liver disease
 NAFLD: non-alcoholic fatty liver disease
 NASH: non-alcoholic steatohepatitis
 HCC: hepatocellular carcinoma
 G9a: histone-lysine N-methyltransferase G9a
 NME1: Nucleoside diphosphate kinase A, also known as nm23-H1, nonmetastatic gene 23-H1
 NME2: Nucleoside diphosphate kinase B, also known as nm23-H2, nonmetastatic gene 23-H2
 PHPT1: Phosphohistidine Phosphatase 1
 LHPP: Phospholysine Phosphohistidine Inorganic Pyrophosphate Phosphatase
 ASC: Apoptosis-associated speck-like protein containing a CARD
 SUV39H1: Suppressor Of Variegation 3-9 Homolog 1
 RASSF1A: Ras association domain family protein 1A
 RUNX3: Runt-related transcription factor
 GSTP1: glutathione S-transferase protein 1
 MEK: Mitogen-activated protein kinase kinase
 GPCR: G protein-coupled receptor
 PI3K: phosphatidylinositol 3-kinase
 Akt: also known as PKB, Protein Kinase B
 TLR4: Toll-like receptor 4
 NF- κ B: nuclear factor kappa-light-chain-enhancer of activated B cells
 STAT3: signal transducer and activator of transcription 3
 FAT10: HLA-F-adjacent transcript 10; FOXO1: forkhead box protein O1
 FOXO1: forkhead box protein O1

REFERENCES

1. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S et al. (2012) Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11. Lyon. *Internat Agency Res Cancer*.
2. Morgan TR, Mandayam S, Jamal MM (2004) Alcohol and hepatocellular carcinoma. *Gastroenterology* 127: S87-S96. [[Crossref](#)]
3. Ramadori P, Cubero FJ, Liedtke C, Trautwein C, Nevzorova YA (2017) Alcohol and Hepatocellular Carcinoma: Adding Fuel to the Flame. *Cancers (Basel)* 9. [[Crossref](#)]
4. Testino G, Leone S, Borro P (2014) Alcohol and hepatocellular carcinoma: a review and a point of view. *World J Gastroenterol* 20: 15943-15954. [[Crossref](#)]
5. Scaglioni F, Ciccia S, Marino M, Bedogni G, Bellantani S (2011) ASH and NASH. *Dig Dis* 29: 202-210. [[Crossref](#)]
6. Lindenmeyer CC, McCullough AJ (2018) The natural history of nonalcoholic fatty liver disease – an evolving view. *Clin Liver Dis* 22: 11-21. [[Crossref](#)]
7. Schwartz JM, Reinus JF (2012) Prevalence and natural history of alcoholic liver disease. *Clin Liver Dis* 16: 659-666. [[Crossref](#)]
8. Dam-Larsen S, Franzmann M, Andersen IB, Christoffersen P, Jensen LB et al. (2004) Long term prognosis of fatty liver: risk of chronic liver disease and death. *Gut* 53: 750-755. [[Crossref](#)]
9. Stickel F, Schuppan D, Hahn EG, Seitz HK (2002) Cocarcinogenic effects of alcohol in hepatocarcinogenesis. *Gut* 51: 132-139. [[Crossref](#)]
10. Stickel F (2015) Alcoholic cirrhosis and hepatocellular carcinoma. *Adv Exp Med Biol* 815: 113-130. [[Crossref](#)]
11. Nguyen L, Masouminia M, Mendoza A, Samadzadeh S, Tillman B et al. (2018) Alcoholic hepatitis versus non-alcoholic steatohepatitis: Levels of expression of some proteins involved in tumorigenesis. *Exp Mol Pathol* 104: 45-49. [[Crossref](#)]
12. Lu JG, Nguyen L, Samadzadeh S, Masouminia M, Mendoza A et al. (2018) Expression of proteins upregulated in hepatocellular carcinoma in patients with alcoholic hepatitis (AH) compared to non-alcoholic steatohepatitis (NASH): An immunohistochemical analysis of candidate proteins. *Exp Mol Pathol* 104: 125-129. [[Crossref](#)]
13. Jia Y, French B, Tillman B, French S (2018) Different roles of FAT10, FOXO1, and ADRA2A in hepatocellular carcinoma tumorigenesis in patients with alcoholic steatohepatitis (ASH) vs non-alcoholic steatohepatitis (NASH). *Exp Mol Pathol* 105: 144-149. [[Crossref](#)]
14. Liu H, Li J, Tillman B, Morgan TR, French BA et al. (2014) TLR3/4 signaling is mediated via the NF κ B-CXCR4/7 pathway in human alcoholic hepatitis and non-alcoholic steatohepatitis which formed Mallory-Denk bodies. *Exp Mol Pathol* 97: 234-240. [[Crossref](#)]
15. French SW, Bardag-Gorce F, Li J, French BA, Oliva J (2010) Mallory-Denk body pathogenesis revisited. *World J Hepatol* 2: 295-301. [[Crossref](#)]
16. French SW, French BA, Oliva J, Li J, Bardag-Gorce F et al. (2012) FAT10 knock out mice livers fail to develop Mallory-Denk bodies in the DDC mouse model. *Exp Mol Pathol* 93: 309-314. [[Crossref](#)]
17. French SW, Lee J, Zhong J, Morgan TR, Buslon V et al. (2012) Alcoholic liver disease - Hepatocellular carcinoma transformation. *J Gastrointest Oncol* 3: 174-181. [[Crossref](#)]
18. Nan L, Wu Y, Bardag-Gorce F, Li J, French BA et al. (2005) The p105/50 NF-kappaB pathway is essential for Mallory body formation. *Exp Mol Pathol* 78: 198-206. [[Crossref](#)]
19. Afifiyan N, Tillman B, French BA, Masouminia M, Samadzadeh S et al. (2017) Over expression of proteins that alter the intracellular signaling pathways in the cytoplasm of the liver cells forming Mallory-Denk bodies. *Exp Mol Pathol* 102: 106-114. [[Crossref](#)]
20. Afifiyan N, Tillman B, French BA, Sweeny O, Masouminia M et al. (2017) The role of Tec kinase signaling pathways in the development of Mallory Denk Bodies in balloon cells in alcoholic hepatitis. *Exp Mol Pathol* 103: 191-199. [[Crossref](#)]
21. Wong RJ, Cheung R, Ahmed A (2014) Nonalcoholic steatohepatitis is the most rapidly growing indication for liver transplantation in patients with hepatocellular carcinoma in the U.S. *Hepatology* 59: 2188-2195. [[Crossref](#)]
22. Wong RJ, Aguilar M, Cheung R, Perumpail RB, Harrison SA et al. (2015) Nonalcoholic steatohepatitis is the second leading etiology of liver disease among adults awaiting liver transplantation in the United States. *Gastroenterology* 148: 547-555. [[Crossref](#)]

23. Nault JC (2014) Pathogenesis of hepatocellular carcinoma according to aetiology. *Best Pract Res Clin Gastroenterol* 28: 937-947. [[Crossref](#)]
24. Samuel W French (2013) Epigenetic events in liver cancer resulting from alcoholic liver disease. *Alcohol Res* 35: 57-67. [[Crossref](#)]
25. Shinkai Y, Tachibana M (2011) H3K9 methyltransferase G9a and the related molecule GLP. *Genes Dev* 25: 781-788. [[Crossref](#)]
26. Qin J, Li Q, Zeng Z, Wu P, Jiang Y et al. (2018) Increased expression of G9a contributes to carcinogenesis and indicates poor prognosis in hepatocellular carcinoma. *Oncol Lett* 15: 9757-9765. [[Crossref](#)]
27. Chen WL, Sun HP, Li DD, Wang ZH, You QD et al. (2017) G9a - An Appealing Antineoplastic Target. *Curr Cancer Drug Targets* 17: 555-568. [[Crossref](#)]
28. Lu H, Lei X, Zhang Q (2018) Liver-specific knockout of histone methyltransferase G9a impairs liver maturation and dysregulates inflammatory, cytoprotective, and drug-processing genes. *Xenobiotica* 23: 1-13.
29. Yokoyama M, Chiba T, Zen Y, Oshima M, Kusakabe Y et al. (2017) Histone lysine methyltransferase G9a is a novel epigenetic target for the treatment of hepatocellular carcinoma. *Oncotarget* 8: 21315-21326. [[Crossref](#)]
30. Wei L, Chiu DK, Tsang FH, Law CT, Cheng CL et al. (2017) Histone methyltransferase G9a promotes liver cancer development by epigenetic silencing of tumor suppressor gene RARRES3. *J Hepatol* 67: 758-769. [[Crossref](#)]
31. Bárcena-Varela M, Caruso S, Llerena S, Álvarez-Sola G, Uriarte I et al. (2018) Dual targeting of histone methyltransferase G9a and DNA-methyltransferase 1 for the treatment of experimental hepatocellular carcinoma. *Hepatology* 69: 587-603. [[Crossref](#)]
32. Shankar SR, Bahirvani AG, Rao VK, Bharathy N, Ow JR (2013) G9a, a multipotent regulator of gene expression. *Epigenetics* 8: 16-22. [[Crossref](#)]
33. Fuhs SR, Hunter T (2017) pHisphorylation: the emergence of histidine phosphorylation as a reversible regulatory modification. *Curr Opin Cell Biol* 45: 8-16. [[Crossref](#)]
34. Hindupur SK, Colombi M, Fuhs SR, Matter MS, Guri Y et al (2018) The protein histidine phosphatase LHPP is a tumour suppressor. *Nature* 555: 678-682. [[Crossref](#)]
35. Zheng J, Dai X, Chen H, Fang C, Chen J (2018) Down-regulation of LHPP in cervical cancer influences cell proliferation, metastasis and apoptosis by modulating AKT. *Biochem Biophys Res Commun* 503: 1108-1114. [[Crossref](#)]
36. Jin K, Li T, Sánchez-Duffhues G, Zhou F, Zhang L (2017) Involvement of inflammation and its related microRNAs in hepatocellular carcinoma. *Oncotarget* 8: 22145-22165. [[Crossref](#)]
37. Greten TF, Wang XW, Korangy F (2015) Current concepts of immune based treatments for patients with HCC: from basic science to novel treatment approaches. *Gut* 64: 842-848. [[Crossref](#)]
38. Yazdi AS, Drexler SK (2015) The two faces of the inflammasome adaptor ASC in epithelial skin carcinogenesis. *Clin Exp Rheumatol* 33: S94-S96. [[Crossref](#)]
39. Ramakrishna G, Rastogi A, Trehanpati N, Sen B, Khosla R (2013) From cirrhosis to hepatocellular carcinoma: new molecular insights on inflammation and cellular senescence. *Liver Cancer* 2: 367-383. [[Crossref](#)]
40. Franklin BS, Latz E, Schmidt FI (2018) The intra- and extracellular functions of ASC specks. *Immunol Rev* 281: 74-87. [[Crossref](#)]
41. Kolb R, Liu GH, Janowski AM, Sutterwala FS, Zhang W (2014). Inflammasomes in cancer: a double-edged sword. *Protein Cell* 5: 12-20. [[Crossref](#)]
42. Hoss F, Rodriguez-Alcazar JF, Latz E (2017) Assembly and regulation of ASC specks. *Cell Mol Life Sci* 74: 1211-1229. [[Crossref](#)]
43. Place DE, Kanneganti TD (2017) Recent advances in inflammasome biology. *Curr Opin Immunol* 50: 32-38. [[Crossref](#)]
44. He Q, Fu Y, Tian D, Yan W (2018) The contrasting roles of inflammasomes in cancer. *Am J Cancer Res* 8: 566-583. [[Crossref](#)]
45. Lin C, Zhang J (2017) Inflammasomes in Inflammation-Induced Cancer. *Front Immunol* 8: 271. [[Crossref](#)]
46. Peng Y, French BA, Tillman B, Morgan TR, French SW (2014) The inflammasome in alcoholic hepatitis: Its relationship with Mallory-Denk body formation. *Exp Mol Pathol* 97: 305-313. [[Crossref](#)]
47. French SW, French BA, Oliva J, Li J, Bardag-Gorce F et al. (2014) FAT10 knock out mice livers fail to develop Mallory-Denk bodies in the DDC mouse model. *Exp Mol Pathol* 93: 309-314.