SOMATO PUBLICATIONS

Annals of Gastroenterology and Digestive

Review Article

DNA Methylation and Histone Modification in ASH vs NASH before the Development of HCC

Yue Jia*, Ping Ji, Jiajie George Lu, Luan Nguyen and Sameul W French

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

***Address for Correspondence:** Yue Jia, Department of Pathology, Harbor-UCLA Medical Center, Torrance, CA 90502, USA, E-mail: YJia@dhs.lacounty.gov

Received: 01 July 2019; **Accepted:** 16 August 2019; **Published:** 22 August 2019

Citation of this article: Jia, Y., Ji, P., George Lu, J., Nguyen, L., French, SW. (2019) DNA Methylation and Histone Modification in ASH vs NASH before the Development of HCC. Ann Gastroenterol Dig Dis, 2(2): 001-004.

ABSTRACT

Hepatocellular Carcinoma (HCC) is the second leading cause of cancer related deaths worldwide. The main risk factors of HCC include non-alcoholic fatty liver disease, virus infections, alcoholism, aflatoxins exposure, smoking, and others. Epigenetic changes including DNA methylation and histone modification are associated with uncontrolled cell growth and proliferation, and even initiation and progression of HCC from chronic inflammation with or without fibrosis in the liver. Among others, non-alcoholic steatohepatitis (NASH) and alcoholic steatohepatitis (ASH) are two major risk factors as both of them may develop cirrhosis and hepatocellular carcinoma (HCC) if left untreated. As a significantly different percentage of patients may progress to HCC annually between ASH and NASH groups, our group tried to find the different molecular pathways in ASH and NASH patient liver specimens. The present review is to summarize the different epigenetic molecular modulations such as DNA methylation and histone modification between NASH and ASH before HCC development.

Keywords: DNA methylation, Histone modification, ASH, NASH, HCC

Introduction

Hepatocellular Carcinoma (HCC) is the fifth most common cancer and the second leading cause of cancer related deaths in the world, causing up to 1 million deaths annually [1,2]. The main risk factors of HCC include non-alcoholic fatty liver disease (NAFLD), virus infections (HBV and HCV), alcoholic liver disease, aflatoxins exposure, and smoking [3-7]. Epigenetic changes including DNA hypermethylation or hypomethylation, dysregulation of histone modification patterns, chromatin remodeling, and aberrant expression of micro-RNAs (miRNAs) and long noncoding RNAs (lncRNAs) are all associated with initiation and progression of HCC [8,9]. In addition to the abovementioned regulatory mechanisms, transcription factors play critical roles in determining gene expression and cell phenotype.

The liver constantly adapts to circadian rhythm, metabolic processes, changes in the microbiota, and other environmental factors such as viral infections and xenobiotics, which results in the need for its constant repair and regeneration [10]. In the highly variable environment, HCC risk factors including obesity, excessive alcohol consumption, and hepatitis viruses cause a disturbance to the extremely sensitive hepatic epigenome. Alterations of the epigenome such as DNA methylation, chromatin modification, miRNAs, and lncRNAs result in uncontrolled cell growth and proliferation, and even the progression of HCC from chronic inflammation with or without fibrosis in the liver [9-11].

Non-alcoholic steatohepatitis (NASH) and alcoholic steatohepatitis (ASH) are the two major risk factors as both conditions can lead to cirrhosis and hepatocellular carcinoma (HCC) if left untreated [12]. About 3-10% of ASH patients may progress to HCC annually [13], while patients with NASH progress to HCC at a much lower rate around 0.5% annually [14]. Our group reported many molecular findings in both ASH and NASH patient liver specimen [15-18]. The present review is to summarize the different epigenetic molecular modulations including DNA methylation and histone modification between NASH and ASH before HCC development.

DNA Methylation

DNA methylation is a well characterized epigenetic mechanism of gene regulation that occurs in the context of acytosine-phosphate-guanine (CpG, promoter-rich region of gene) dinucleotide. Generally, high density CpG promoters for typical housekeeping genes are rarely methylated, while genes that have intermediate density CpG content are silenced upon methylation. This modification is carried out by three highly conserved enzymes, DNA methyltransferase 1 (DNMT1), the DNMT3A, and DNMT3B enzymes. Approximately 1 to 2% of human genome harbors CpG islands or CpG-rich regions containing hundred to several thousand base pairs and exists in proximity to different gene promoter regions [19].

It has been reported that DNA methylation is increased in livers of alcoholic hepatitis patients [20]. Loss of DNMT1 in murine hepatic progenitor cells leads to severe DNA damage, cell cycle arrest, senescence and death [21]. DNMT3B loss inhibits proliferation, migration, and invasion in hepatocellular carcinoma cell lines [22]. We reported that in both ASH and NASH patients, levels of DNMT1 and DNMT3B proteins were higher than those in control groups. However the DNMT3B protein level was higher in AH compared to NASH [16].

In HCC and many other tumors, specific promoter hypermethylation are often associated with inactivation of tumor-suppressor genes (TSGs). Silencing of tumor-suppressor genes such as RASSF1A downregulates mRNA transcript expression [9]. A significant level of DNA methylation of GSTP1 was reported in HCC tissues [23]. In our study, the protein levels of three TSGs such as RASSF1A and GSTP1 were both increased in ASH, but not in NASH, if compared with control groups. Another TSG, RUNX3, although increased in both ASH and NASH compared with controls, the RUNX3 showed much lower expression in NASH compared to ASH [18]. These findings are different from the loss of the TSGs in HCC which may suggest that the suppressing/silencing of TSGs expression in hepatocytes from ASH and NASH patients could be one of the mechanisms for HCC development.

Histone Modification

Histone modifications have a direct impact on chromatin structure and gene expression and play important roles in gene silencing during tumorigenesis. N-terminal tails of nucleosomal histones can be modulated via methylation, acetylation, phosphorylation, and ubiquitination to regulate gene activities and cellular processes, including DNA repair, DNA replication, and gene transcription.

DNA methylation is closely associated with histone modification because inhibitors reverse the histone modification changes on H3-K4 and H3-K9 codes [24]. H3K27 trimethylation is another distinct histone modification and is one of the candidates for a silencing mechanism for tumor-suppressor genes [9]. Histone methyltransferases (HMTs), SUV39H1 and G9a, mediate the histone H3-K9 trimethylation and dimethylation (H3-K9 diMe), respectively [25]. H3K9 methylation is associated with silencing of several tumor-suppressor genes. In our study, SUV39H1 protein level increased dramatically in ASH but not in NASH compared with control [15]. In contrast to SUV39H1, G9a protein level was increased in both ASH and NASH, although the G9a level was higher in ASH compared to NASH [Jia Y., et al.unpublished data, reported in EB2019 annual meeting].

Histone acetylation is associated with active transcription and is under the control of HATs and HDACs. Histone deacetylases (HDACs) are the enzymes that regulate gene expression by removing acetyl group from histones that make the DNA more compact leading to gene silencing. HDACs may act as tumor suppressors and therapeutic targets [26]. Accumulating evidence suggests a correlation of individual HDAC overexpression with poor prognosis in different types of cancer including HCC [27]. Overexpression of HDAC3 was correlated with early recurrence of HCC after surgery and advance tumor stage. A tumor-suppressor role of HDACs has also been noticed, as overt HCC occurred as a result of liver-specific knockdown of HDAC3 [28]. Suppressing/silencing of HDAC3 may lead to cancer development and DNA damage by increasing the histone acetylation during S phase [29]. HDAC2 removes acetyl groups from histones resulting in further winding of DNA around histones and transcriptional suppression [30]. Overexpressed HDAC2 in hepatocellular carcinoma patients is associated with poor survival in low-grade and early-grade tumors [31]. We found that in ASH and NASH patients, the level of HDAC2 was elevated compared to controls, but there was no difference between ASH and NASH groups [16]. HDACs also remove acetyl-lysine on diverse nonhistone proteins such as NFkB, transcription factors p53, and many others [32]. HDAC2 knockdown studies revealed that HDAC2 dysregulation contributes to HCC pathogenesis by modulating expression of genes involved in apoptosis, cell cycle and lipid metabolism [33].

Histidine phosphorylation has important roles in protein and cellular function including cell cycle regulation, phagocytosis, and tumorigenesis. Two mammalian histidine kinases and two pHis phosphatases (PHPT1 and LHPP) were identified [34]. Histidine phosphorylation increases significantly in liver tumors. Proteomic analysis of 12 tumors from an mTOR-driven HCC mouse model revealed that expression of the putative histidine phosphatase LHPP was downregulated specifically in the tumors [35]. Sustained expression of LHPP in the HCC mouse model liver suppresses tumor and preserves the liver function, while decreased LHPP level correlates with more advanced tumor and reduces overall survival in HCC patients [35]. Over-expressed LHPP reduced tumor cell proliferation, migration and invasion, possibly via blocking AKT activation and restraining p53 expression levels in cervical cancer cells [36]. LHPP is not only a protein histidine phosphatase but also a tumor suppressor. In our study, the expression of LHPP protein was upregulated both in ASH and NASH specimens and there was no significant difference between ASH and NASH groups [Jia, unpublished data, reported in EB2019 annual meeting].

A significant decrease in histone H2A ubiquitination was reported in HCC [37]. It has been reported that the functionally important histone H2A PTM H2A119ub (H2Aub) markedly decreases in hepatocellular carcinoma [37]. The H2A deubiquitinase, Usp21, is probably responsible for decrease of H2Aub. In addition, H2Aub levels were inversely correlated with H3S10 phosphorylation (H3S10p) and the proliferative state of the cells.

Summary

The hepatic epigenome modulation is extremely important to keep the liver's adaptability to daily challenges including dietary, metabolic, xenobiotic and microbial factors. However, if this capacity is compromised, epigenetic adaptions can become dysfunctional and lead to disorder, even tumorogenesis. We summarized the key

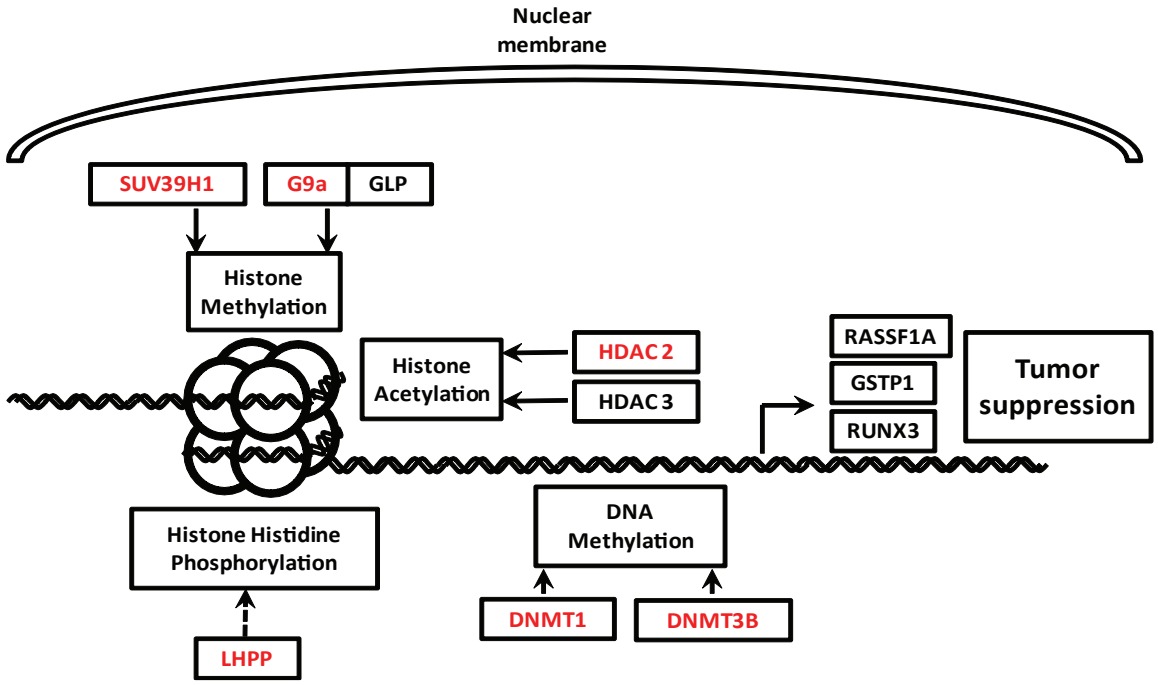

Figure 1: Putative pathway for Epigenetic Modulation in the HCC tumorigenesis. The similar expression pattern of G9a and LHPP proteins suggests similar pathways been involved in ASH and NASH, including certain epigenetic modulators in HCC tumorigenesis. But other epigenetic modulators such as DNMTs, SUV39H1, and HDAC 2 have different expression pattern in ASH and NASH specimens which indicates the complexity of HCC tumorigenesis from different stressors.

components of the epigenetic machinery such as DNA methylation and histone modification in the context of ASH and NASH patients before the HCC development. These studies may be very helpful to understand the pathogenesis of HCC in ASH and NASH patients and to direct possible diagnosis, prevention and treatment choices.

References

1. Jemal, A., Bray, F., Center, MM., Ferlay, J., Ward, E., Forman, D. (2011) Global cancer statistics.CA Cancer J Clin, 61(2): 69-90.
2. Ferlay, J., Soerjomataram, I., Ervik, M., Dikshit, R., Eser, S., et al. (2012) Cancer Incidence and Mortality Worldwide: IARC Cancer Base No. 11. Lyon: International Agency for Research on Cancer.
3. Toh, TB., Lim, JJ., Chow, EK. (2019) Epigenetics of hepatocellular carcinoma. ClinTransl Med, 8(1): 13.
4. Herceg, Z., Paliwal, A. (2011) Epigenetic mechanisms in hepatocellular carcinoma: how environmental factors influence the epigenome. Mutat Res, 727(3): 55-61.
5. Morgan, TR., Mandayam, S., Jamal, MM. (2004) Alcohol and hepatocellular carcinoma. Gastroenterology, 127(5 Suppl 1): S87-96.
6. Ramadori, P., Cubero, FJ., Liedtke, C., Trautwein, C., Nevzorova, YA. (2017) Alcohol and Hepatocellular Carcinoma: Adding Fuel to the Flame. Cancers (Basel), 9(10): 130.
7. Testino, G., Leone, S., Borro, P. (2014) Alcohol and hepatocellular carcinoma: a review and a point of view. World J Gastroenterol, 20(43): 15943-15954.
8. Ma, L., Chua, MS., Andrisani, O., So, S. (2014) Epigenetics in hepatocellular carcinoma: an update and future therapy perspectives. World J Gastroenterol, 20(2): 333-345.
9. Wahid, B., Ali, A., Rafique, S., Idrees, M. (2017) New Insights into the Epigenetics of Hepatocellular Carcinoma. Biomed Res Int, 2017: 1609575
10. Wilson, CL., Mann, DA., Borthwick, LA. (2017) Epigenetic reprogramming inliver fibrosis and cancer. Adv Drug Deliv Rev, 121: 124–132.
11. Bhat, V., Srinathan, S., Pasini, E., Angeli, M., Chen, E., Baciu, C., et al. (2018) Epigenetic basis of hepatocellular carcinoma: A network-based integrativemeta-analysis. World J Hepatol,10(1): 155–165
12. Neuman, MG., French, SW., French, BA., Seitz, HK., Cohen, LB., Mueller, S., et al. (2014) Alcoholic and non-alcoholic steatohepatitis. Exp Mol Pathol, 97(3): 492-510.
13. Schwartz, JM., Reinus, JF. (2012) Prevalence and natural history of alcoholic liver disease. Clin Liver Dis, 16(4): 659-666.
14. Lindenmeyer, C., McCullough, A. (2018) The natural history of nonalcoholic fatty liver disease – an evolving view. Clin Liver Dis. 22(1): 11–21.
15. Nguyen, L., Masouminia, M., Mendoza, A., Samadzadeh, S., Tillman, B., Morgan, T., et al. (2018) Alcoholic hepatitis versus non-alcoholic steatohepatitis: Levels of expression of some proteins involved in tumorigenesis. Exp Mol Pathol, 104(1): 45-49
16. Lu, JG., Nguyen, L., Samadzadeh, S., Masouminia, M., Mendoza, A., Sweeney, O., 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(2): 125-129.

17. 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(1): 144-149.

18. Jia, Y., Ji, P., French, B., Tillman, B., French, SW. (2019) The different expression of tumor suppressors, RASSF1A, RUNX3, and GSTP1, in patients with alcoholic steatohepatitis (ASH) vs non-alcoholic steatohepatitis (NASH). Exp Mol Pathol, 108: 156-163.

19. Deaton, AM., Bird, A. (2011) CpG islands and the regulation of transcription. Genes Dev, 25(10): 1010–1022.

20. Shen, H., French, BA., Tillman, BC., Li, J., French, SW. (2015) Increased DNA methylation in the livers of patients with alcoholic hepatitis. ExpMolPathol, 99(2): 326–329.

21. Kaji, K., Factor, VM., Andersen, JB., Durkin, ME., Tomokuni, A., Marquardt, JU., et al. (2016) DNMT1 is a required genomic regulator for murine liver histogenesis and regeneration. Hepatology, 64(2): 582–598.

22. Wang, JC., Wang, Z., Fan, YX., Si, YQ., Wang, JX. (2015) DNA methyltransferase3b silencing affects locus-specific DNA methylation and inhibits proliferation, migration and invasion in human hepatocellular carcinoma SMMC-7721 and BEL-7402 cells. Oncol Lett, 9(6): 2499–4506.

23. Song, MA., Tiirikainen, M., Kwee, S., Okimoto, G., Yu, H., Wong, LL. (2013) Elucidating the landscape of aberrant DNA methylation in hepatocellular carcinoma.PLoS One, 8(2): e55761.

24. Chen, H., Zhang, T., Sheng, Y., Zhang, C., Peng, Y., Wang, X., et al. (2015) Methylation Profiling of Multiple Tumor Suppressor Genes in Hepatocellular Carcinoma and the Epigenetic Mechanism of 3OST2 Regulation. J Cancer, 6(8): 740-749.

25. Lachner, M., O'Sullivan, RJ., Jenuwein, T. (2003) An epigenetic road map for histone lysine methylation.J Cell Sci, 116(Pt 11): 2117-2124.

26. Ceccacci, E., Minucci, S. (2016) Inhibition of histone deacetylases in cancer therapy: lessons from leukaemia. Br J Cancer, 114(6): 605-611.

27. Rikimaru, T., Taketomi, A., Yamashita, Y., Shirabe, K., Hamatsu, T., Shimada, M., et al. (2007) Clinical significance of histone deacetylase 1 expression in patients with hepatocellular carcinoma. Oncology, 72(1-2): 69-74.

28. Liu, C., Liu, L., Shan, J., Shen, J., Xu, Y., Zhang, Q., et al. (2013) Histone deacetylase 3 participates in self-renewal of liver cancer stem cells through histone modification. Cancer Lett, 339(1): 60-69.

29. Bhaskara, S., Knutson, SK., Jiang, G., Chandrasekharan, MB., Wilson, AJ., Zheng, S., et al. (2010) Hdac3 is essential for the maintenance of chromatin structure and genome stability. Cancer Cell, 18(5): 436-447.

30. Chen, HP., Zhao, YT., Zhao, TC. (2015) Histone deacetylases and mechanisms of regulation of gene expression. Crit Rev Oncog, 20(1-2): 35–47.

31. Quint, K., Agaimy, A., Di Fazio, P., Montalbano, R., Steindorf, C., Jung, R., et al. (2011) Clinical Significance of Histone Deacetylases 1, 2, 3, and 7: HDAC2 Is an Independent Predictor of Survival in HCC. VirchowsArchiv, 459(2): 129–139.

32. Falkenberg, KJ., Johnstone, RW. (2014) Histone deacetylases and their inhibitors in cancer, neurological diseases and immune disorders. Nat Rev Drug Discov, 13(9): 673-691.

33. Lee, YH., Seo, D., Choi, KJ., Andersen, JB., Won, MA., Kitade, M., et al. (2014) Antitumor effects in hepatocarcinoma of isoformselectiveinhibition of HDAC2. Cancer Res, 74(17): 4752–4761.

34. Fuhs, SR., Hunter, T. (2017) pHisphorylation: the emergence of histidine phosphorylation as a reversible regulatory modification. CurrOpin Cell Biol, 45: 8-16.

35. Hindupur, SK., Colombi, M., Fuhs, SR., Matter, MS., Guri, Y., Adam, K., et al. (2018) The protein histidine phosphatase LHPP is a tumour suppressor. Nature, 555(7698): 678-682.

36. Zheng, J., Dai, X., Chen, H., Fang, C., Chen, J., Sun, L. (2018) Down-regulation of LHPP in cervical cancer influences cell proliferation, metastasis and apoptosis by modulating AKT. BiochemBiophys Res Commun, 503(2): 1108-1114.

37. Bhattacharya, S., Reddy, D., Ingle, A., Khade, B., Gupta, S. (2016) Brief Communication: Featured Article: Histone H2A mono-ubiquitination and cellular transformation are inversely related in N-nitrosodiethylamine-induced hepatocellular carcinoma. ExpBiol Med (Maywood), 241(16): 1739-1744.