Perspective

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Tumorigenesis from non-alcoholic steatohepatitis to hepatocellular carcinoma

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Abstract: Non-alcoholic steatohepatitis (NASH) with metabolic syndrome is increasing to be a main cause of hepatocellular carcinoma (HCC). However, the mechanism of tumorigenesis in NASH induced HCC is still not clear. In this perspective, we will discuss the recent progress that has been made to understand the genetic change and the immune microenvironment of HCC, and the remaining questions. Based on the current study, NASH-HCC is likely to have novel mechanism, which needs more investigation in future.

Keywords: hepatocellular carcinoma; non-alcoholic steatohepatitis; tumorigenesis.

Liver cancer is one of the most common causes of cancer deaths. The predominant type of liver cancer is hepatocellular carcinoma (HCC), which accounts for approximately 90% of liver cancer cases [1]. The five-year survival rate of HCC is less than 20%, and the best treatment is still liver transplantation. HCC is tightly associated with chronic liver disease, which can be caused by hepatitis C virus (HCV) infection, hepatitis B virus (HBV) infection, alcoholic consumption, and non-alcoholic steatohepatitis (NASH) with metabolic syndrome. Given increased availability of vaccines to prevent HBV, and effective anti-viral treatment for HCV, the risk of viral hepatitis to cause HCC is declining over time. NASH with metabolic syndrome, however, is increasing in prevalence and will soon be the main cause of HCC.

NASH features hepatocyte steatosis and injury, which provokes inflammation, and is closely related to obesity and type 2 diabetes (T2D). Liver inflammation in turn provokes hepatic stellate cell (HSC) activation, leading to liver fibrosis and cirrhosis, a major risk factor of HCC. How cirrhosis facilitates tumorigenesis and whether or not NASH induced HCC has different mechanisms is still under investigation. However, with recent advances in sequencing methodologies, a great deal of progress has been made in the past few years to understand specific gene mutations, overall transcriptomic changes and the immune microenvironment of HCC [2]. As in other cancers, tumor initiation in HCC is hypothesized to begin with a driver mutation. The top three mutated genes in HCC patients are telomerase reverse transcriptase ([TERT], 40%–60% incidence), catenin beta 1 ([CTNNB1], 15%–30% incidence), and tumor protein 53 ([TP53], 15%–30% incidence) [3, 4]. These three genes are the most frequently mutated in NASH-induced HCC, similar to HBV/HCV/alcoholic-induced HCC, suggesting that these mutations may be the final determinants of tumorigenesis, independent of HCC etiology. However, there are some notable differences between HCC etiologies. For example, activin A receptor type 2A (ACVR2A), a receptor for transforming growth factor β (TGF-β), has a higher mutation rate in NASH-HCC than HCC of other causes (10% vs. 3%) (3), leading to an enriched molecular signature of TGF-β signaling in NASH-HCC.

After mutation of driver genes, the expansion of the tumor initiation cell is considered an evolutionary process associated with additional mutations to confer faster growth. This further adds to the complexity of tumorigenesis in HCC, leading to both inter-tumor and intra-tumor heterogeneity. This iterative process also emphasizes the importance of looking for convergent mutation among different tumor clones. For example, a recent study showed forkhead box O1 (FOXO1) S22W gain-of-function mutation in about 20% of patients with HCC, mostly in independent hepatocyte clones indicating a convergent evolution [5]. FOXO1 is a key transcriptional factor that regulates insulin/Akt-control of hepatic gluconeogenesis. Disrupted expression or modification of liver FOXO1 is tightly correlated with T2D and NASH. Whether or not $FOXO1^{S22W}$ contributes to the development of HCC still needs to be verified *in vivo*, but this study may pave the road for discovery of mutation of other metabolism related genes [6].

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Besides looking for genomic mutations in HCC, parallel efforts have been made to understand the tumorigenesis from NASH to HCC through changes of hepatic transcription factor network. As NASH progresses to advanced fibrosis, hepatocytes may lose identity by dysregulated expression of transcription factors, including but not limited to higher expression of E74 like ETS transcription factor 3 (ELF3) and GLIS family zinc finger 2 (GLIS2) [7]. Reduced hepatocyte expression of either ELF3 or GLIS2 ameliorates liver fibrosis in mice fed NASH-inducing diet, perhaps through reduction of secreted phosphoprotein 1 (SPP1) and connective tissue growth factor (CTGF) [7]. Whether or not these transcription factor network alterations facilitate tumor initiation in HCC needs further investigation.

Other data have highlighted the re-awakening of developmental pathways in NASH and fibrosis, including activation of the Notch signaling pathway, which may contribute to HCC tumorigenesis [8]. Notch activity is important for intrahepatic bile duct formation during liver development, but it is absent in normal post-development hepatocytes. Recent studies have shown, however, that expression of hepatic Notch targets is increased in patients with NASH, and forced hepatocyte Notch activity in mice is sufficient to activate HSCs and induce fibrosis [9, 10]. When combined with a NASH inducing diet, hepatocyte Notch activation will cause HCC in mice. Interestingly, 30% of HCC patients in various database show a Notch-activity signature, and Notch-high tumors shows decreased CTNNB1 mutations [11]. How Notch drives NASH to HCC is still unknown, and exploration of downstream targets will lead to a greater understanding of HCC heterogeneity and may be critical to develop personalized HCC treatment.

The progression of HCC growth is also closely related to compromised immunity, wherein programmed death-ligand 1 (PD-L1) from tumor cells or cells in the tumor microenvironment bind to programmed death-1 (PD-1) receptors on T-cell, leading to T-cell apoptosis and tumor immune evasion [12]. Immunotherapy targeting PD-1/PD-L1 did not change overall survival rate in HCC patients [13, 14]. Combining PD-1/PD-L1 inhibitors with blockade of other activated pathways in HCC, for example, vascular endothelial growth factor (VEGF), does lead to improved survival [14], albeit with significant heterogeneity. This heterogeneity may be explained by PD-L1 expression in neoplastic cells of only 17% of HCC patients [15]; intriguingly, these tumors showed higher aggressiveness. PD-1 expression is similarly found in intratumor lymphocytes in a minority (about 27%) of patients [15]. These data suggest PD-1/PD-L1 inhibitor efficacy may be restricted to specific HCC variants. In fact, single cell sequencing (scRNA-seq) of lymphocytes isolated from a NASH-HCC mouse model and NAFLD patients showed an enrichment of $CD8^+PD1^+$ T cells [16], but surprisingly, anti-PD1 treatment leads to a paradoxical increase of tumor number and size in NASH-HCC mice. Consistently, treatment with anti-PD-1 or anti-PD-L1 showed relatively decreased efficacy in NASH-HCC patients as compared to other etiologies of HCC. These data suggest other immune evasion mechanisms may exist in NASH-HCC. To comprehensively characterize the landscape of immune cell in HCC, efforts have been made to sequence $CD45^+$ cells from HCC patients [17, 18], which revealed that $LAMP3^+$ dendritic cells (DCs) are enriched in tumor as compared to surrounding tissue. DCs may produce relative T cell dysfunction by decreasing T-regulatory cells (Tregs) and/or increasing tumoral $CD8^+$ $CD161^+$ T cells, significantly associated with worse prognosis [18]. Other immune cells, including tumor-associated macrophages (TAMs), were also found to be associated with poor prognosis [17]. How these specific immune cell populations regulate tumorigenesis in HCC needs more study, which will be helpful to develop new immunotherapy for HCC treatment. Also important would be to determine if NASH-HCC have specific alterations in immune cell population.

In summary, animal modeling and single-cell level molecular studies from human HCC have elucidated novel mechanisms of NASH-HCC, which is likely differentially regulated based on alterations in genetic signature and differential response to immunotherapy targeting the PD-L1/PD1 axis. As more and more studies demonstrate HCC heterogeneity, it will be helpful to classify NASH-HCC variants both to understand the underlying mechanism and develop effective biomarker and therapeutics for NASH-HCC.

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