Stemness of liver cancer: From hepatitis B virus to Wnt activation

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Title: Stemness of liver cancer: from hepatitis B virus to Wnt activation

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Hepatocellular carcinoma (HCC) is a heterogeneous disease in terms of etiology, morphology, biological behavior, response to treatment, and clinical outcome. Efforts have been made on classifying HCCs according to the status of gene mutations, chromosomal aberrations, gene/protein expression, and epigenetic modification in order to find hidden molecular features that can explain this heterogeneity [1, 2]. The recent advances in molecular classification and the re-emergence of a cancer stem cell (CSC) hypothesis have highlighted the central role of stemness in HCC pathogenesis [3]. Moreover, sorafenib increased overall survival in a randomized controlled trial against placebo in advanced HCC, and tumor progression invariably occurred. Deciphering the underlying mechanism of stemness acquisition in liver cancer cells is a major issue because stemness contributes to tumor chemo and radio-resistance, two key features of HCC [4].

Epithelial Cell Adhesion Molecule (EpCAM/CD326) is a type 1 transmembrane protein that can antagonize cadherin-mediated cell to cell adhesion [5], and is one of the first tumor associated antigens identified [6]. Although EpCAM is known to be expressed in a variety of normal epithelial cells, it is strongly overexpressed in human adenocarcinomas [7]. In liver cell lineages, EpCAM is absent in mature hepatocytes but detectable in hepatic stem/progenitor cells and cholangiocytes [8]. Numerous studies have suggested the utility of EpCAM as a marker to identify liver cancer stem cells as well as normal hepatic progenitors [3]. In HCC, EpCAM-positivity correlates with a high frequency of vascular invasion, poor prognosis after surgical resection, and high infection rates with hepatitis B

virus (HBV) [9]. Gene signatures of EpCAM-positive HCC have demonstrated an activation of stemness-related genes together with Wnt signaling pathway. EpCAM is reported to be one of the Wnt signaling target genes in HCC [10]. More recently, EpCAM is identified as one of the "cell surface to nuclear missile" that activates Wnt signaling pathway by its intracellular domain (ICD) cleaved by γ-secretase [11]. However, it is unclear why the presence of EpCAM-positive HCC cells is more frequent in HBV-related HCCs than HCV-related or non-B non-C HCCs and if the virus itself could induce an EPCAM-mediated Wnt activation.

In this issue of Journal of Hepatology, Mani and colleagues proposed the potential clues that explain the liaison between EpCAM expression, Wnt signaling activation, and cancer stemness in HCC [12]. First, using two different HBV replication/infection cell lines HepAD38 and HepG2-NTCP, they identified an increase of EpCAM ICD (EpICD) and decrease of full length EpCAM that induces the activation of Wnt signaling pathway in HBV replicating/infected cells. This protein modification was attenuated by γ-secretase inhibitor DAPT, suggesting that EpCAM undergoes regulated intramembrane proteolysis (RIP) by γ-secretase in HBV replicating/infected cells.

Next the authors explored the role of SUZ12, a protein of the Polycomb Repressive Complex 2 (PRC2) that regulates open/close chromatin status. According to their previous findings, HBV X (HBx) protein induced the degradation of SUZ12 by the proteasome [13, 14]. SUZ12 knockdown as well as HBx expression resulted in the induction of EpCAM gene/protein together with the activation of canonical Wnt signaling pathway and stemness-

related genes encoding NANOG, OCT4, and SOX2. As expected, an isolated subset of HBV replicating cells showed an activation of the canonical Wnt signaling and of the stemness-related genes, that promotes chemoresistance to sorafenib and cisplatin. Activation of EpCAM and stemness-related genes with downregulation of SUZ12 in HBV-related HCC was further supported *in vivo* in HBx/c-Myc transgenic mice model. Thus, their data illustrated the role of PRC2 suppression by HBx that induces stemness in cancer cells through chromatin modifications and activation of imprinted genes. Induction of EpCAM together with the RIP by γ-secretase resulted in the activation of EpCAM and Wnt signaling pathways.

Mani and colleagues then took advantage of gene sets analysis (hCSC-like gene signature) derived from their EpCAM RIP analysis in HBV-related HCC. They sub-classified HCCs according to this gene signature in two independent cohorts, and confirmed the prognostic values of the hCSC-like gene signature in HCC. Importantly, *CTNNB1* mutations, which resulted in the activation of canonical Wnt signaling, were not detected in hCSC-like HCCs. Intriguingly, these data were consistent with previous genomic studies suggesting the existence of two different types of Wnt signaling activation in HCC according to the presence of *CTNNB1* mutation or not (Figure 1) [1]. Somatic *CTNNB1* (coding for β-catenin) mutations have been described in 20 to 40% of HCC [1]. These mutations impair the degradation of β-catenin by the proteasome and promote its translocation in the nucleus and the expression of hepatocyte-related Wnt-target-genes such as *GLUL* and *LGR5*. Interestingly, these tumors belong to a homogeneous molecular subtype associated with histological features (well differentiated tumor with cholestasis) and etiology (hepatitis C

virus infection) [1] Furthermore, this HCC subtype frequently exhibited the uptake of the contrast reagent gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid (Gd-EOB-DTPA), known to be metabolized in hepatocytes and secreted into bile, in the hepatobiliary phase of magnetic resonance imaging (MRI), consistent with their cholestatic feature [15, 16]. In contrast, a second subgroup of HCC without *CTNNB1* mutations harbored Wnt activation with induction of other Wnt target genes (such as *MYC* and *CCND1*) together with stem cells features and TGF beta pathway upregulation [17]. This subclass of tumors is associated with a poor prognosis and a high rate of HBV related HCC.

This study has several implications in terms of mechanistic insights about the development of HCC with stem cell features and its treatment strategy. First, this study suggests that HBx protein may activate stemness-related genes through chromatin modifications by PRC2 repression, EpCAM RIP activation, and Wnt signaling activation. Because the simple canonical Wnt signaling activation by *CTNNB1* mutations did not enhance the stemness-related genes, one can speculate that HBV infection together with HBx and EpCAM protein expression may somehow erase the epigenetic memories to reprogram the cell fate of infected hepatocytes (Figure 1). It is unclear if complete eradication of HBV including HBx, cccDNA, and minichromosome can restore the epigenetic memories of infected hepatocytes or HCC cells.

Because γ-secretase activity may regulate Notch signaling as well as EpCAM RIP and cadherin, it is possible that other signaling pathways such as Notch signaling pathway may also play a role in the induction of stemness in HBV-related HCC. Exploration of γ-

secretase activation mechanism may provide important clues to understand the “cell surface to nuclear missile” signaling in HCC in the future.

Second, this study identifies potential therapeutic targets such as Wnt, γ–secretase, and PRC2 in HBV-related HCC with stem cell features. Unfortunately, no US Food and Drug Administration (FDA)-approved drugs are available that can target these pathways [18], and further efforts are required to provide new treatment for patients with HCC harboring stem cell features.

A potential limitation of this study is that the main conclusion was drawn from the experimental data of HCC cell lines and not of untransformed hepatocytes. It is still unclear if epigenetic reprogramming occurs in HBV-infected normal hepatocytes and stem/progenitor cells, when epigenetic reprogramming starts after HBV infection, and how frequently such epigenetic reprogramming can affect the cellular phenotypes in normal liver lineages. Further studies are required.

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Conflicts of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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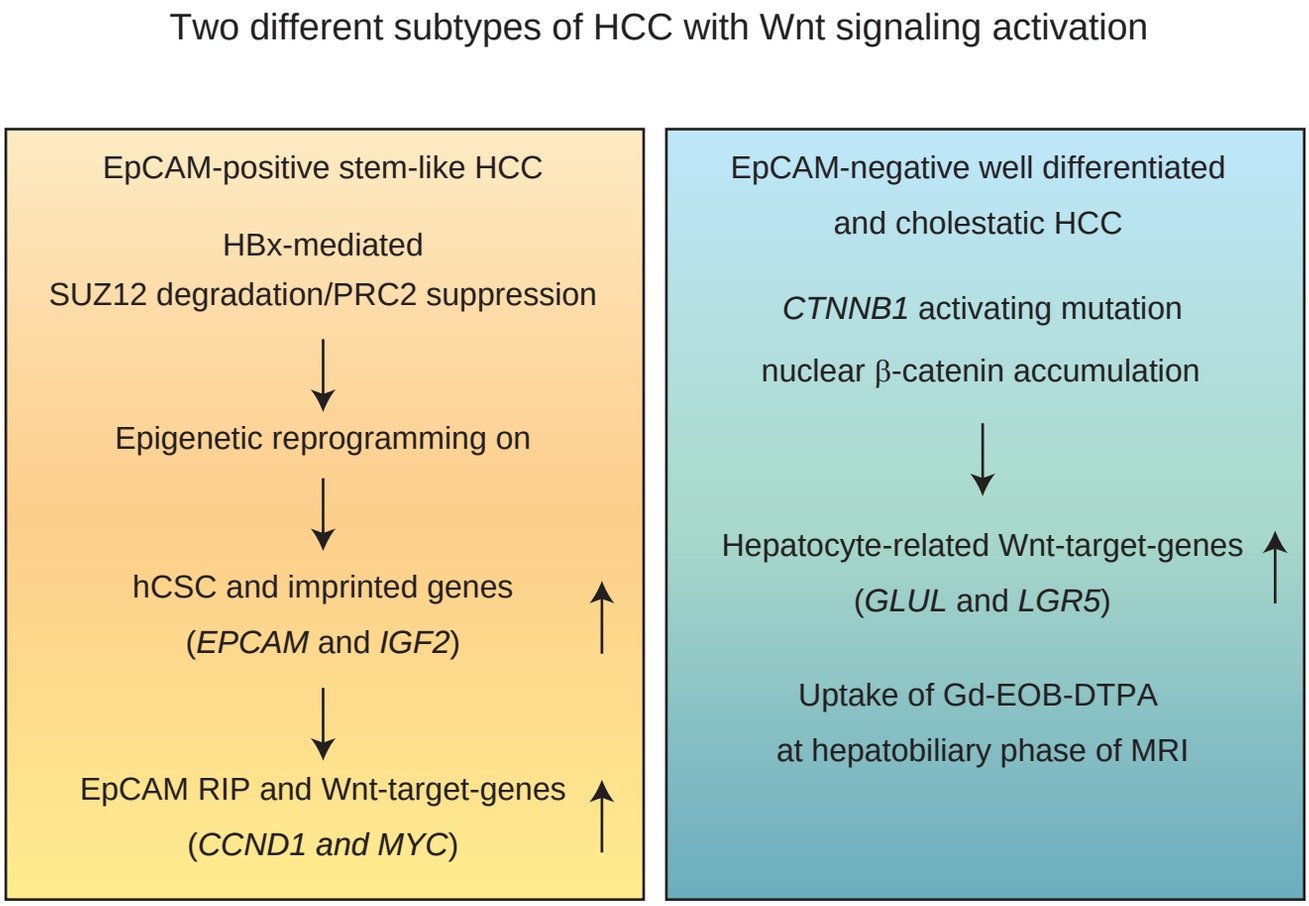
Figure Legend

Fig. 1. HBx and induction of HCC stemness.

Accumulation of HBx results in the degradation of SUZ12, suppression of the PRC2 function and leads to epigenetic reprogramming and activation of hCSC genes and imprinted genes including *EPCAM*. EpCAM then undergoes RIP by γ-secretase and induces Wnt-target-genes such as *CCND1* and *MYC*. In contrast, *CTNNB1* mutations result in the nuclear accumulation of β-catenin without affecting epigenetic reprograming, and activate hepatocyte-related Wnt-target-genes such as *GLUL* and *LGR5*.

Fig. 1


Two different subtypes of HCC with Wnt signaling activation
EpCAM-positive stem-like HCC
HBx-mediated
SUZ12 degradation/PRC2 suppression
Epigenetic reprogramming on
hCSC and imprinted genes
(EPCAM and IGF2)
EpCAM RIP and Wnt-target-genes
(CCND1 and MYC)
EpCAM-negative well differentiated
and cholestatic HCC
CTNNB1 activating mutation
nuclear β-catenin accumulation
Hepatocyte-related Wnt-target-genes
(GLUL and LGR5)
Uptake of Gd-EOB-DTPA
at hepatobiliary phase of MRI