Annals of Hepatology 29 (2024) 101153

Contents lists available at ScienceDirect

Annals of Hepatology

journal homepage: www.elsevier.es/annalsofhepatology

Concise reviews

Stemness markers in hepatocellular carcinoma of Eastern vs. Western population: Etiology matters?

Caecilia HC Sukowati[a,b,*], Korri El-Khobar[b], Chyntia Olivia Maurine Jasirwan[c], Juferdy Kurniawan[c], Rino Alvani Gani[c]

[a] Liver Cancer Unit, Fondazione Italiana Fegato ONLUS, AREA Science Park campus Basovizza, SS14 km 163.5, Trieste 34149, Italy
[b] Eijkman Research Center for Molecular Biology, National Research and Innovation Agency of Indonesia (BRIN), B.J. Habibie Building, Jl. M.H. Thamrin No. 8, Jakarta Pusat 10340, Indonesia
[c] Hepatobiliary Division, Medical Staff Group of Internal Medicine, Faculty of Medicine, Universitas Indonesia - Dr. Cipto Mangunkusumo General Hospital, Jl. Pangeran Diponegoro No.71, Jakarta 10430, Indonesia

ARTICLE INFO

Article History:
Received 8 August 2023
Accepted 22 August 2023
Available online 19 September 2023

Keywords:
Hepatocellular carcinoma
Stem cells
Etiology
Cancer stem cells
Biomarkers

ABSTRACT

Hepatocellular carcinoma (HCC) is one of the most common cancers with a high mortality rate. HCC development is associated with its underlying etiologies, mostly caused by infection of chronic hepatitis B virus (HBV) and hepatitis C virus (HCV), alcohol, non-alcoholic fatty liver disease, and exposure to aflatoxins. These variables, together with human genetic susceptibility, contribute to HCC molecular heterogeneity, including at the cellular level. HCC initiation, tumor recurrence, and drug resistance rates have been attributed to the presence of liver cancer stem cells (CSC). This review summarizes available data regarding whether various HCC etiologies may be associated to the appearance of CSC biomarkers. It also described the genetic variations of tumoral tissues obtained from Western and Eastern populations, in particular to the oncogenic effect of HBV in the human genome.

Abbreviations: 4-PBA, 4−phenylbutyric acid; ABCB1, ATP-binding cassette superfamily B member 1; ADAMTS7, ADAM metallopeptidase with thrombospondin type 1 motif 7; AFB1, aflatoxin B1; AFP, alpha fetoprotein; ALB, albumin; APC, adenomatous polyposis coli; ARID1A, AT-rich interaction domain 1A; ARID2, AT-rich interaction domain 2; AXIN1, axis inhibition protein 1; BNC1, basonuclin zinc finger protein 1; BPTF, bromodomain PHD finger transcription factor; cccDNA, covalently closed circular DNA; CCNA2, cyclin A2; CDKL2, cyclin dependent kinase like 2; CDH1, cadherin 1; CDKN2A, cyclin-dependent kinase inhibitor 2A; CHB, chronic hepatitis B; CK8, cytokeratin 8; CK19, cytokeratin 19; CSC, cancer stem cell; CTNNB1, catenin beta 1; CYP17A1, cytochrome P450 family 17 subfamily A member 1; DCAMKL1, doublecortin and CaM kinase-like-1; DDX18, DEAD-box helicase 18; DEPDC5, DEP domain containing 5; DKK1, dickkopf WNT signaling pathway inhibitor 1; DLC1, deleted in cancer 1; EMT, epithelial-to-mesenchymal transition; ERCC1, excision repair cross-complementation group 1; FAH, fumarylacetoacetate hydrolase; FN1, fibronectin 1; GATM, glycine amidinotransferase; GWAS, genome-wide association studies; G6PC, glucose-6-phosphatase catalytic; GPI, glycosylphosphatidylinositol; GRIK1, glutamate ionotropic receptor kainate type subunit 1; GSTM1, glutathione S-transferase mu 1; GSTP1, glutathione S-transferase P1; HBV, hepatitis B virus; HBx, hepatitis B virus X protein; HBx-ΔC, truncated HBx protein at the C-terminus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HFE, homeostatic iron regulator; HMBS, hydroxymethylbilane synthase; HNF4α, hepatocyte nuclear factor 4 alpha; HLA-DP, human leukocyte antigen − DP; HLA-DQ, human leukocyte antigen − DQ; HLA-DR, human leukocyte antigen − DR; HSD17B13, hydroxysteroid 17-beta dehydrogenase 13; IGF2, insulin-like growth factor 2; IRF2, interferon regulatory factor 2; IL1B, interleukin 1B; IL10, interleukin 10; IL17, interleukin 17; KIF1B, kinesin family member 1B; KLF4, Kruppler like factor 4; LGR5, leucine-rich repeat-containing G-protein coupled receptor 5; LINE1, long interspersed nuclear element 1; MASLD, metabolic dysfunction-associated steatotic liver disease; MDM2, mouse double minute 2 homolog; MICA, major histocompatibility complex class I polypetide-related sequence A; MLL, lysine methyltransferase 2A; MLL4, myeloid/lymphoid or mixed-lineage leukemia 4; MPO, myeloperoxidase; MTHFR, methylenetetrahydrofolate reductase; NGS, next-generation sequencing; NAFLD, non-alcoholic fatty liver disease; OCT4, octamer binding transcription factor 4; OBI, occult hepatitis B infection; pgRNA, pregenomic RNA; PNPLA, patatin-like phospholipase domain containing protein; PXR, pregnane X receptor; RUNX3, RUNX family transcription factor 3; SAA1, serum amyloid A1; SAGE1, sarcoma antigen 1; SALL4, Sal-like protein 4; SERPINA1, serpin family A member 1; SFRP1, secreted frizzled related protein 1; SHH, sonic hedgehog; siRNA, small interfering RNA; SLC37A4, solute carrier family 37 member 4; SLD, steatotic liver disease; SNP, single nucleotide polymorphism; SOCS1, suppressor of cytokine signaling; SOD2, superoxide dismutase 2; SOX2, SRY-box transcription factor 2; STAT1, signal transducer and activator of transcription 1; STAT3, signal transducer and activator of transcription 3; STAT4, signal transducer and activator of transcription 4; SYT12, synaptotagmin XII; RB1, retinoblastoma transcriptional corepressor 1; TBC1D15, TBC domain family member 15; TCGA, the cancer genome atlas; TERT, telomerase reverse transcriptase; TGFβ, transforming growth factor beta; TISC, tumor-initiating stem-like cell; TLR4, toll-like receptor 4; TM6SF2, transmembrane 6 superfamily member 2; TNFα, tumor necrosis factor alpha; TNFRSF12A, TNF receptor superfamily member 12A; TP53, tumor protein 53; TRPM3, transient receptor potential cation channel subfamily M member 3; UROD, uroporphyrinogen decarboxylase; VEGF, vascular endothelial growth factor; WIF1, WNT inhibitory factor 1; XRCC3, x-ray repair cross complementing 3

* Corresponding author.
E-mail addresses: caecilia.sukowati@fegato.it, caecilia.sukowati@brin.go.id (C.H. Sukowati).

https://doi.org/10.1016/j.aohep.2023.101153

1. Hepatocellular carcinoma: Distribution and etiology

Liver disease is the cause of around 2 million mortality per year, where 1 million death is due to chronic infection of viral hepatitis and liver cancer [1]. Hepatocellular carcinoma (HCC) is the most common histological type, comprising 75 %–85 % of all primary liver cancers. It accounts for the majority of incidence and mortality of all cases [2], with a 5-year overall survival rate of only around 18 % [3]. The main risk factors for HCC are chronic hepatitis C virus (HCV) and hepatitis B virus (HBV), heavy alcohol drinking, aflatoxin-contaminated foods, and recently, the newly named metabolic dysfunction-associated steatotic liver disease (MASLD) [4].

The prevalence of HCC is related to the different underlying etiologies and varies geographically. In Western populations, HCC is predominantly driven by chronic HCV infection and alcohol intake [5]. However, the largest burden is observed in Eastern countries (Asia), predominantly due to the endemic presence of HBV. Although the relevance of nonviral risk factors is increasing, chronic hepatitis B (CHB) is still the leading etiology of HCC worldwide [6]. Chronic HBV infection and HCV infection account for 56 % and 20 % of HCC mortality worldwide, respectively [2]. Global data from the World Health Organization showed that there are 300 million people with chronic hepatitis caused by hepatitis B virus infection [7]. Deaths due to liver cirrhosis in the Asia-Pacific region in 2015 represented 54.3 % of cirrhosis-related deaths globally [8].

In the future, however, HCC epidemiology is predicted to change due to increasing alcohol consumption, increasing prevalence of obesity and other metabolic diseases, and advances in the prevention and treatment of HBV and HCV. The prevalence of alcohol as an etiology of HCC varies between countries and regions. The highest is in Europe, especially in Eastern European countries (e.g. up to 63 % in Belarus), 20 % in Southern European countries (e.g. Italy or Spain), and lowest in the Middle East (6 %) [9]. The annual incidence of alcohol-related HCC is reported to be between 0.3 % and 5.6 % in patients with cirrhosis with regard to mortality [10], accounted for 19 % of all liver cancer deaths globally [11].

In parallel, the rise of steatotic liver disease (SLD), an overarching term to encompass the various aetiologies of steatosis, has also become a major liver problem in the world. The new nomenclature of MASLD, replaces the previous term non-alcoholic fatty liver disease (NAFLD) [4,12,13]. In Asia, an endemic region with HBV and/or HCV, an updated population prevalence of MASLD is 34 %, in parallel with a predicted increase of MASLD-associated liver cancer [14,15]. The increasing trends over time are almost identical by region. In both Asia and Europe, the forecast for MASLD prevalence is predicted to reach over 60 % by 2040, with both regions having an average yearly increase of around 2 to 3 % [16].

Aflatoxin B1 (AFB1) is a potent mutagenic toxin that is produced by molds growing in grain, peanuts, or other food. AFB1 is one of the major public health risks in Africa and Asia, partly by synergizing with hepatitis B to dramatically increase the risk of HCC [11].

These various etiologies of HCC result in different mutational landscapes, clinical presentations, and responses to treatment [17]. As previously reviewed by Choo et al., significant differences exist between Eastern and Western populations on many key aspects of HCC. These differences take part in the possible different outcomes upon treatment and can reflect in the future challenges of clinical trial design and data interpretation [18].

2. Biomarkers: Western vs. Eastern populations

Vast and robust technology in -omics profiling and screening, i.e., next-generation sequencing (NGS), had boosted crucial information in cancer biomarkers discovery and molecular pathogenesis of HCC, including its relation to different HCC etiological factors and patients' genetic background.

In May 2012, Guichard and Fujimoto reported in Nature Genetics, the NGS datasets from HCC tumor samples and their respective surrounding tissues as the first representative cohorts of HCC patients, from European (France) and Asian (Japan) cohort, respectively [19,20]. Among a large number of gene alterations, as remarked by Teufel, both studies reported a high number of mutations in tumor protein 53 (*TP53*) and WNT signaling genes, both by number and significance levels in each cohort, indicating NGS as a valuable tool to develop successful targeting strategies [21].

These studies, interestingly, identified a specific association between tumor drivers and HBV-related tumors. In the France cohort, functional analyses showed that only in HBV-related HCC, the inactivation of tumor suppressor properties of interferon regulatory factor 2 (*IRF2*) were noticed. This inactivation led to impaired *TP53* function [20]. In the Japanese study, HBV genome integration in the telomerase reverse transcriptase (*TERT*) locus was frequently observed in a high clonal proportion [19]. More recent analyses, either *via* molecular profiling and/or targeted analysis, accordingly, highlight mutations in the coding regions of *TP53* and catenin beta 1 (*CTNNB1*), together with mutations in the promoter of *TERT* as well-established drivers in HCC development. It is noted, that among different geographic areas, the frequencies of these drivers are variable, again, possibly depending on different HCC etiologies and environmental factors [22].

HCC incidence also varies by demographic factors, including age, sex, and ethnicity. Incidence rates of HCC in most populations are directly correlated with age, with peak incidence at approximately 75 years old [23]. However, the median age at diagnosis is slightly younger in Asia (China and Taiwan) compared to America (USA) [23,24]. HCC incidence and mortality rates are around 2–3 times higher in men compared to women worldwide [2], which may be related to behavioral and endocrine factors. The greatest sex disparity incidence rates can be found in European countries such as France and Malta [23]. However, based on region, the greatest sex disparity incidence rates for HCC were identified in Eastern and Southeastern Asia compared to the other regions [2].

Ethnic disparities have also been observed in HCC, especially in multi-ethic countries like the USA, where the highest rates were found in the American Indians/Alaskan Natives, Hispanics, and Asia/Pacific Islanders populations [23,25]. HCC disparities are linked to certain driver genes dysregulation in the host, in which *TP53* and cyclin-dependent kinase inhibitor 2A (*CDKN2A*) were linked to race (more in Asians than whites); *CTNNB1*, albumin (*ALB*), *TP53*, and axis inhibition protein 1 (*AXIN1*) were significantly linked to patients' gender, and retinoblastoma transcriptional corepressor 1 (*RB1*) was linked to age [26]. In addition, a previous study in the United States showed that among HCV-related HCC samples, altered expression patterns of serum amyloid A1 (*SAA1*) and hepatocyte nuclear factor 4 alpha (*HNF4α*) were more evident in the African American compared to Caucasian American individuals [27].

HCC patients typically have somatic genetic alterations which include somatic mutations, copy number alterations, and viral integration (especially in HBV-related cases). The genes that are frequently mutated in HCCs include *TERT*, *TP53*, *CTNNB1*, *AXIN1*, AT-rich interaction domain 1A (*ARID1A*), AT-rich interaction domain 2 (*ARID2*), and *RB1* [3,24]. However, a recent study had shown that several gene mutations are more common in certain patients populations. Utilizing the whole-exome sequencing data from The Cancer Genome Atlas (TCGA), higher frequencies of *TP53*, *RB1*, and vascular endothelial growth factor (*VEGF*) mutations were observed more in Asian American patients, compared to the European American patients. Further, Asian American patients also have additional transient receptor potential cation channel subfamily M member 3 (*TRPM3*), sarcoma antigen 1 (*SAGE1*), and ADAM metallopeptidase with thrombospondin type 1 motif 7 (*ADAMTS7*) mutations, while interleukin 17 (*IL17*) mutation was only found in European American patients [28].

The variation in host gene polymorphisms had also been associated as predicting factors for HCC development. Single nucleotide polymorphisms (SNPs) variation in the following regulatory genes for iron metabolism (homeostatic iron regulator (*HFE*)), inflammation (tumor necrosis factor alpha (*TNFα*), interleukin 1B (*IL1B*), interleukin 10 (*IL10*), and transforming growth factor beta (*TGFβ*)), oxidative stress (glutathione S-transferase mu 1 (*GSTM1*), superoxide dismutase 2 (*SOD2*), and myeloperoxidase (*MPO*)), cell cycle and DNA repair (mouse double minute 2 homolog (*MDM2*), *TP53*, methylenetetrahydrofolate reductase (*MTHFR*), and x-ray repair cross complementing 3 (*XRCC3*)) had been reported as HCC risk factors [24]. In addition, mutations in hemochromatosis (*HFE*), alpha 1-antitrypsin deficiency (serpin family A member 1 (*SERPINA1*)), glycogen storage diseases (glucose-6-phosphatase catalytic (*G6PC*), solute carrier family 37 member 4 (*SLC37A4*)), porphyrias (hydroxymethylbilane synthase (*HMBS*), uroporphyrinogen decarboxylase (*UROD*)), tyrosinemia (fumarylacetoacetate hydrolase (*FAH*)), and Wilson's disease (ATPase copper transporting beta (*ATP7B*)) had been reported to increase susceptibility to HCC [23]. Gene polymorphisms of DEAD-box helicase 18 (*DDX18*), DEP domain containing 5 (*DEPDC5*), glutamate ionotropic receptor kainate type subunit 1 (*GRIK1*), signal transducer and activator of transcription 4 (*STAT4*), kinesin family member 1B (*KIF1B*), human leukocyte antigen – DP (*HLA-DP*), *HLA-DQ, HLA-DR*, and major histocompatibility complex class I polypetide-related sequence A (*MICA*) had also been correlated with increased risks of HCC in the Asian population with chronic HBV or HCV infections [23,24]. On the other hand, gene polymorphisms of excision repair cross-complementation group 1 (*ERCC1*), glutathione S-transferase P1 (*GSTP1*), cytochrome P450 family 17 subfamily A member 1 (*CYP17A1*), *XRCC3*, and ATP-binding cassette superfamily B member 1 (*ABCB1*) have been identified as predisposition risks for HCC development in Caucasian individuals with HBV or HCV infections [29]. Genome-wide association studies (GWAS) had also identified three SNPs, patatin-like phospholipase domain containing protein (*PNPLA*) (rs738409), transmembrane 6 superfamily member 2 (*TM6SF2*) (rs58542926), and hydroxysteroid 17-beta dehydrogenase 13 (*HSD17B13*) (rs72613567) association with the risk of HCC development in chronic liver disease patients, particularly in alcohol-related and non-alcoholic fatty liver diseases [30].

HBV DNA integration was considered a strong oncogenic effect in hepatocarcinogenesis. However, HBV genome integration may occur at the early and late onset of HCC [24,25]. HBV preferentially integrated into human repeat regions, where an identified breakpoint in 8q24 was observed more in the early-onset HCCs. The identified recurrent hotspots for the early onset HBV-related HCCs are synaptotagmin XII (*SYT12*), glycine amidinotransferase (*GATM*), and fibronectin 1 (*FN1*), while signal transducer and activator of transcription 1 (*STAT1*), *ALB*, myeloid/lymphoid or mixed-lineage leukemia 4 (*MLL4*), and *TERT* are identified for the late onset HBV-HCCs [31].

In line with the NGS data, the targeted sequencing method showed the effect of HBV DNA integration in the alterations of human genes, especially for *TERT* and lysine methyltransferase 2A (*MLL*), both in the European and Asian cohorts. In another Italian study, HBV DNA integration was noted in around 75 % of samples of HCC patients with occult hepatitis B infection (OBI). The inserted HBV DNA sequences were dominantly X (38 %) and PreS/S HBV sequences (35 %), followed by C (19 %) and P (8 %) sequences. Around 25 % of the inserted HBV sequences were integrated inside the human genome coding regions [32]. This high rate of HBV DNA integrations was also noted in a Chinese study, where the percentage was 69 % among OBI HCC patients. Among these patients, 90 % did not have cirrhosis. In these samples, HBV DNA was found to integrate near genes associated with hepatocarcinogenesis, including *TERT*, *MLL*, and cyclin A2 (*CCNA2*) [33].

Epigenetic dysregulation also plays a crucial role in HCC development. This is achieved by altering gene expression through various epigenetic mechanisms including modifications of DNA methylation, chromatin remodeling, and changes in the levels of noncoding RNAs [30]. Aberrant changes in methylome profiling in multiple regulatory genes have been reported in HCC cases, which have been linked to liver tumorigenesis, including hypermethylation of *CDKN2A* and hypomethylation of insulin-like growth factor 2 (*IGF2*) [30]. A recent study has also highlighted the differences in the DNA methylation rates in HCC cases, stratified by different sex and ethnic backgrounds [34]. Hypermethylation of *CDKN2*, cyclin dependent kinase like 2 (*CDKL2*), and basonuclin zinc finger protein 1 (*BNC1*) and hypomethylation of long interspersed nuclear element 1 (*LINE1*) were observed more in female patients with an Asian background, whereas hypermethylation of adenomatous polyposis coli (*APC*), WNT inhibitory factor 1 (*WIF1*), RUNX family transcription factor 3 (*RUNX3*), deleted in cancer 1 (*DLC1*), secreted frizzled related protein 1 (*SFRP1*), dickkopf WNT signaling pathway inhibitor 1 (*DKK1*), cadherin 1 (*CDH1*), and suppressor of cytokine signaling (*SOCS1*) was observed in Asian male patients. In contrast, hypomethylation of TNF receptor superfamily member 12A (*TNFRSF12A*) was observed in American male patients. Based on the racial background, hypermethylation of *APC*, *GSTP1*, and *SOCS1* was associated with increased in HCC risk in Asian patients compared to non-Asian patients [34].

3. Stemness markers as biomarkers: are they related to HCC etiologies?

3.1. Stem cells markers

Related to its underlying etiologies and host genetic background, HCC has high phenotypic and functional heterogeneity, within the population (inter-patient heterogeneity) and within tumors from the same patient (intra-patient and intra-tumor heterogeneity). The heterogeneity of HCC complicates an efficient and specific targeted therapy for cancer, as different HCC types respond differently to the treatment.

HCC initiation, tumor recurrence, and drug resistance rates have been attributed to the presence of liver cancer stem cells (CSC). Liver CSC are subpopulations of liver cancer cells that have a high capacity for self-renewal, differentiation, and tumorigenesis [35]. The liver CSC has emerged as one of the main players in the initiation of hepatocarcinogenesis and cancer resistance to conventional therapies. The discovery of CSC greatly improved the understanding of HCC development and progression. The origin of CSC remains unclear, although it may have originated either from abnormal differentiation or undifferentiated stem cells or oval cells in the liver, or mutations-induced and dedifferentiation transformation of adult hepatocytes [36,37].

CSC behaves similarly as normal stem cells would, including the capacity for limitless cell division. This ability is achieved by altering the expression of intrinsic cell regulators like cytokines and crucial signaling pathways that maintain the embryonic stem cell self-renewal such as *NANOG*, octamer binding transcription factor 4 (*OCT4*), and SRY-box transcription factor 2 (*SOX2*) pathways [38,39]. CSC divides asymmetrically to generate heterogeneous cell populations, thus enabling CSC to maintain and sustain tumor development [40].

The use of CSC biomarkers, a distinct molecule or protein receptor that mainly coats the surface of cell, is one of the most valid approaches to identify and assess the CSC and their functionalities. As such, the recognition of CSC biomarkers has been widely used in various laboratories. Until now, various proteins had been proposed as CSC markers, including surface proteins CD90/THY-1, CD133/Prom-1, CD326/EpCAM, CD47, CD24, CD13/ANPEP, OV-6, and side population. As we had recently summarized, data showed that various CSC markers can be correlated with patients' clinical features and outcomes of HCC, including prognosis, disease stage, recurrence, and survival [35,41]. Naturally, the oncogenic role of HBV and HCV proteins, for instance, might be related to the acquisition of CSC features [42].

3.2. Stemness markers and HBV

As mentioned above, chronic viral hepatitis infections are still the main etiologies in HCC development. HBV and HCV mainly promote HCC development *via* specific viral proteins, including oncogenic proteins, which disrupt the normal physiological situation of the cells. However, one major difference between HBV and HCV is that as a DNA virus, HBV can also exercise its oncogenicity by integrating its DNA into the human host genome, while HCV cannot. HBV DNA integration into the human genome, in particular HBV S gene and X gene, is a strong direct oncogenic factor for HCC development. HCC is relatively rare in the absence of cirrhosis, except in areas where HBV infection is endemic [43].

Until the writing of this review, only scarce information is available that associates HCC etiologies with a specific CSC marker. For instance, one of the CSC markers that might be related to HCC etiologies is CD90. CD90 (THY-1) is a 25–37 kDa glycosylphosphatidylinositol (GPI)-anchored protein expressed in various cell types, including T cells, thymocytes, neurons, endothelial cells, and fibroblasts. CD90 is involved in multiple pathways, in immunologic and nonimmunologic functions, such as in T cell activation, neurite outgrowth, apoptosis, tumor suppression, wound healing, and fibrosis [44,45]. Due to its pleiotropic roles, CD90 participates in multiple signaling cascades. Several studies had showed that CD90 upregulation was more evident in HBV-related HCC [46,47], compared to other HCC underlying etiologies. Our recent study also showed that CD90 expression was related to HBV infection, as noticeable in the HCC patient's Eastern cohort (from Vietnam) which was mostly related to chronic HBV infection [47].

Regarding the tumorigenicity of the CSC, cellular experimental data was provided by Yamashita et al. [48]. Comparing the tumorigenicity of EpCAM/CD90 sorted cells obtained from xenografts derived from primary HCCs, the tumorigenicity of CD90+ cells was observed only in HBV-related HCCs, whereas EpCAM+ cells were observed in both HCV-related and HBV-related HCCs. However, in agreement with other studies, this study showed that the tumorigenic CD90+ cells may emerge at a later stage of hepatocarcinogenesis, where the majority of isolated CD90+ cells from early HCCs stages may be cancer-associated vascular endothelial cells without tumorigenic capacity [48]. CD90+ CSC have also been associated with increased metastasis risk in HCCs, CD90+ CSCs not only induce metastasis to distant organs for themselves but also for other subsets of CD90- cells, including the EpCAM+ cells that typically have no metastatic capacity [40].

In addition, previously, it has been demonstrated that PreS1 of the HBV S gene activated the expressions of CD90 in normal hepatocytes and HCC cells. However, the expression of other CSC markers, CD133 and CD117, were also upregulated. Nevertheless, these data indicated PreS1 as a new oncoprotein playing a key role in the appearance and self-renewal of CSC during HCC development [49].

Another marker, the transcription factor Sal-like protein 4 (SALL4), a novel oncofetal protein has been related to CSC in HCC [50]. SALL4, known to regulate stemness in embryonic and hematopoietic stem cells, was found to be activated in an HCC subtype with stem cell features [51]. SALL4 expression is associated with EpCAM-positivity and a poor prognosis [52]. SALL4 is often associated with chronic HBV infection, where HBV infection induces DNA demethylation of specific SALL4 CpG sites [53]. SALL4 was found expressed in almost half (47.7 %) of Chinese HCC individuals [54] and 20.5 % in Korean HCC individuals [52]. On the contrary, it was a rare event in the Western HCC cohort (majorly with HCV), and noticeable only in 1.3 % of cases, although its immunoreactivity was also correlated with higher grade and poor prognosis [55]. A positive association between SALL4 and EpCAM and NANOG in HBV-related HCC had also been demonstrated [50,52,53,56].

Taking advantage of animal models, the effect of HBV DNA sequence insertion into the host genome can be straightforwardly analyzed and quantified. By using a transgenic mouse model of HBV S gene insertion into liver cells genome [57], we had shown previously that in a time-course study, there was a progressive increase of the expression of CSC markers *CD133*, *EpCAM*, and cytokeratin 19 (*CK19*) genes along with progressing liver injury and hepatocarcinogenesis, Further, a significant correlation between CSC markers and diagnosis were also observed [58].

Even though the integrated fragments of HBV DNA sequences in the cells cannot support HBV replication and transcription, the presence of HBV genomic templates such as covalently closed circular DNA (cccDNA) and pregenomic RNA (pgRNA) may still be present in HCC cells [59]. A recent study showed the importance of OCT4 expression in HBV-related HCC, where HBV pgRNA was found positively correlated with CSC markers EpCAM and CD133 expressions in adjacent non-tumor tissues of HCC, where HBV DNA level was also found correlated with OCT4 expression. By contrast, the levels of HBV pgRNA was only positively correlated to CD133 and OCT4 expressions, and total RNA levels to CD44 and OCT4 in the distal non-tumor tissue [60].

In another study, in a HBV X transgenic mice (HBx) transgenic mice fed with 3,5-diethoxycarbonyl-1,4-dihydrocollidine, an elevated number of EpCAM+ CSC cells with characteristics of human progenitor cells was observed [61]. This *in vivo* study confirmed a previous *in vitro* study where HBx-expressing cells had upregulation of EpCAM and β-catenin, together with pluripotent transcription factors OCT4, NANOG, and Kruppler like factor 4 (KLF4) [62].

Another CSC marker that have been associated with HBV-related HCC is OV-6. Clinically, OV-6-positive cells have been shown to relate to biological invasion and poor prognosis in HCC patients [63]. The stem-like properties of OV-6-positive cells were found induced by the expression of HBV X protein (HBx) through the dysregulation of the β-catenin signaling pathway [61].

Further, random HBV genome integration can also lead to the truncation of the HBx protein at the C-terminus (HBx-ΔC). This HBx-ΔC insertion had been shown to promote hepatocarcinogenesis by conferring enhanced invasiveness and diminished apoptotic response. An *in vitro* study using stable overexpressed HBx-ΔC resulted in the induction of CD133, mediated through signal transducer and activator of transcription 3 (*STAT3*) activation [64]. Data from another study also confirmed the effect of HBx-ΔC, although this study identified NANOG and SOX2 as the stemness markers [65], perhaps due to the difference in the site of integration.

3.3. Stemness markers and HCV

Regarding HCV as HCC etiology, in contrast to the clear direct oncogenicity of HBV to stemness markers, the association between CSC markers in HCV-related HCC is still very limited [42]. It must be noted that in general, the oncogenicity of HCV is generally indirect, triggered *via* chronic inflammation, fibrosis, which led to liver cirrhosis [66].

Previously, primary human hepatocytes infected with cell culture-grown HCV display epithelial to mesenchymal transition (EMT) characteristics *via* the activation of the AKT/β-catenin signaling pathway [67]. The sphere-forming hepatocytes express many stem cell markers, including high levels of the stem cell factor receptor c-Kit, pluripotency factors SOX2, and NANOG [68].

In an *in vitro* study, the insertion of an HCV subgenomic replicon resulted in the acquisition of CSC traits, including an enhanced expression of putative stem cell marker doublecortin and CaM kinase-like-1 (*DCAMKL1*) together with leucine-rich repeat-containing G-protein coupled receptor 5 (LGR5), CD133, alpha fetoprotein (AFP), CK19, Lin28, and c-Myc. DCAMKL1 is also elevated in response to the overexpression of a cassette of pluripotency factors. Curing the replicon of these cells results in a diminished expression of these factors, and significant reduction of HCV RNA abundance and NS5B

levels using the small interfering RNA (siRNA), leading to the depletion of *DCAMKL1* [69].

Further, not only (sub)genomic replicon, it seemed that certain HCV proteins might affect the molecular pathways of the cells, inducing the transformation of the cells. Previously, it was demonstrated that in HCC cell line Huh7, the transfection of NS5A induced the expression of the toll-like receptor 4 (*TLR4*) [70]. In a more complex *in vivo* transgenic mouse model, even though NS5A transgenic mouse alone resulted in hepatoma, the prolonged alcohol feeding resulted in the development of HCC, with the presence of CD133/NANOG-positive cells in the tumor [71].

This study was then followed with the isolation of tumor-initiating stem-like cells (TISCs) with the phenotype of CD133+/CD49f+ in NS5A mice fed with high-fat diet [72,73]. In more recent data, the molecular mechanism of this was explored where the role of cell-fate-determinant molecule NUMB-interacting protein (*TBC1D15*) contribution was demonstrated in an alcohol Western diet-fed HCV NS5A mice model. TBC1D15 was found to be overexpressed and contributes to p53 degradation in TISCs. Liver-specific deletion of TBC1D15 also attenuated p53 loss. Further, TBC1D15 activated three novel oncogenic pathways to promote self-renewal, p53 loss, and NANOG transcription in TISCs [74].

3.4. Stemness markers, fatty liver, and aflatoxin

As compared to viral etiologies, metabolic HCC etiologies such as alcohol and MASLD and toxin etiology of AFB1 to the appearance of CSC cells, have not been extensively explored. As previously mentioned, in tumorigenic primary cells obtained from fifteen fresh HCC samples with different etiologies (five HBV-related, four HCV-related, three non-B, non-C hepatitis-related, and three alcohol-related), the tumorigenic CSCs were only obtained from the HBV or HCV-related cases [48].

In cell line and animal studies, however, it was shown that prolonged alcohol feeding resulted in the development of HCC in NS5A transgenic mice, with the presence of CD133/NANOG-positive cells in the tumor [71] and the appearance of TISCs with CD133+/CD49f+ phenotype in NS5A mice fed with high-fat diet [72,73]. This mechanism is associated with the Toll-like receptor (TLR) signaling pathway and is upregulated in chronic liver diseases. It is known that alcoholism is associated with endotoxemia that stimulates the expression of proinflammatory cytokine expression and inflammation in the liver and fat tissues [75]. More recently, it was also demonstrated in both *in vitro* and *in vivo* studies, that a long exposure, up to 21 days, to ethanol increased the CSC population of CD133+ and EpCAM+. By mechanic analysis, this was due to the induction of EMT through activation of the WNT /β-catenin signaling pathway in HCC cells [76]. As known, cancer cells undergoing EMT will acquire stemness characteristics [77].

For MASLD, it was previously demonstrated in an *in vitro* study that saturated fatty acid palmitic acid significantly enhanced the sphere-forming ability of HepG2 cells, and increased stemness gene expressions of SOX2 and OCT4, and production of sonic hedgehog (SHH) [78]. More recent data was demonstrated using 4-phenylbutyric acid (4-PBA), a small molecular weight fatty acid. 4-PBA has been used in clinical practice to treat inherited urea cycle disorders and 4-PBA alone did not induce liver tumors in the long term. However, 4-PBA might significantly increase liver tumor burden when administered at an early stage in fibrotic-induced HCC animal models, even though liver inflammation and fibrosis seemed lessened [79]. 4-PBA promoted liver tumorigenesis in HCC mice models *via* initiation of hepatic CSC through the WNT5b/FZD5 mediating β-catenin signaling. In this study, higher CD133 protein expression was detected in fresh frozen sections along with the elevation of other CSC-related genes, including *Epcam, Cd90, Bmi1, Oct4, Sox2, Cd133*, and *Stat3* [79]. In a subsequent study, two palmitoylation inhibitors, tunicamycin and 2-bromohexadecanoic acid significantly decreased CSC sphere formation without affecting the cell viability [80].

Regarding AFB1, only a little information is available on whether this toxin might independently induce the appearance of hepatic CSC. It was previously shown that long exposure of AFB1 in WB-F344 cells, a rat non-tumorigenic epithelial cell line, transformed the cells ability to form colonies in soft agar, a characteristic of stem cells [81]. In combination with other HCC etiologies, in a study using a partial transformation of rat oval cells with HBV X gene and the exposure of AFB1, rat oval cells can generate HCC through the combined effects of the HBx and AFB1 in the liver microenvironment. The intrahepatic HCC cells were immunopositive for HepPar1, ALB, cytokeratin 8 (CK8), and AFP [82]. One of the explanations of this mechanism was HBx sustained activation of pregnane X receptor (PXR) that might aggravate the hepatotoxicity or genotoxicity of AFB1 [83]. PXR is a xenobiotic receptor that is responsible for the metabolic activation or detoxification of several carcinogens and may play various roles in hepatocellular carcinogenesis.

4. Problem and perspective

HCC cellular and molecular heterogeneity is vast. Although it is accepted that chronic infection of HBV and HCV are the main underlying etiologies for HCC, in the real-world setting, the outcome of these infections are diverse. HBV genetics varies, consisting of at least nine genotypes (A to I) and one putative genotype (J), based on genome-wide divergence of more than 7.5 % [84,85]. These differences are associated with HBV replicative capacity, infective capability, gene expressions, and different clinical manifestations, including the development of HCC, even though its mechanisms are still unknown [86]. As for HBV, HCV is also highly heterogenous, with at least 7 genotypes and 67 based on the differences of the whole viral genome. A complex of genetic variants within individual isolates is defined as quasispecies [87]. Even though HCV genotype is still considered an epidemiological marker, such as HBV, many studies have shown that different hepatitis C genotypes have different responses to treatment [88].

Moreover, as described in details above, cancer biomarkers are related not only to different HCC etiological factors but also to patient's genetic backgrounds. Abundant NGS data dan GWAS studies from HCC vs. healthy subjects had demonstrated the susceptibility of human genetic predisposition to viral infection and metabolic diseases [89]. For example, mutations in the promoter of *TERT* is a well-established driver in HCC development [90]. *TERT* locus is a recognized hot site for HBV genome integration and it is frequently observed in a high clonal proportion [19]. At the same time, in Asian population, the *TERT* genetic variants of rs2736098 and rs2853669 alleles were associated with a significantly increased risk of HCC [91,92]. In HCC, *TERT* is considered as a critical factor that promotes cell immortalization [93]. *hTERT* increased expression and telomere length were significantly higher in HCC tissues expressing stemness markers of CK19, EpCAM, and CD133 [94]. In a recent study, *hTERT* was reported to be regulated by the bromodomain PHD finger transcription factor (*BPTF*), where BPTF knockdown inhibited cell proliferation, colony formation, and CSC traits in both HCC cell lines and in animal models [93]. Further, HCC etiologies are also related to sex, age, and other factors (e.g. smoking) [6], not to mention the high prevalence of co-infection between HBV and HCV, where both viruses must share or compete for these host proteins [95]. Hence, the inter-variabilities to assess the effect of a certain etiological factor on a certain stemness marker is enormous.

In line with this, in most cases, the expression of a CSC marker itself in a single tumor may vary. For instance, the most studied CSC markers above, EpCAM and CD90. A recent study using 314 tumor spots from 69 HCC patients showed around 48 % of contained spots with and without EpCAM expression with diverse quality and

intensity. It confirmed the presence of spatial heterogeneity (intra-heterogeneity) of CSC features in nearly half of the patients with HCC [96]. CD90 itself is a pleiotropic molecule that can be found in many cell types, not only progenitor cells [45]. Several studies had also indicated a contradictory role for CD90, where it acts as a tumor suppressor instead of a tumor promoter [97,98].

Therefore, as a perspective, we emphasize that, regardless of the etiologies and the genetic background of the HCC patient, the focus must placed on the CSC population itself. It is important to understand the oncogenic mechanism of the CSC, to avoid the initiation of the CSC, and to inhibit HCC development and metastasis by eliminating and/or eliminating the CSC. Until now, various studies, using small molecule inhibitors, oncolytic measles virus (e.g. for CD133), and anti-surface CSC marker antibodies had demonstrated efficient and selective (reviewed in Ref. [99]. In phase II/III clinical trial, catumaxomab (anti-EpCAM and anti-CD3) was shown to improve patient's survival with ovarian and gastric cancer [100,101], however its efficacy in HCC patients is still unproven.

Regarding HCC molecular and cellular heterogeneity, it is also necessary to search for the most commonly expressed molecular target. Since the liver stemness marker is a surface protein whose expression might be altered in regards to stimuli and tumor microenvironment, targeting dysregulated molecular pathways involved in the CSC initiation and hepatocarcinogenesis might be more efficient. The inhibition of these pathways may lead to the inhibition of CSC survival.

Availability of data and materials

Not applicable.

Funding

CHCS is supported by 2022-2023 grant of the Fondazione Umberto Veronesi, Milan, Italy. KEEK received a fellowship of the Ministero degli Affari Esteri e della Cooperazione Internazionale (MAECI) of Italy for the Progetto Co-Tutela at the FIF.

Declaration of interests

None.

Author contributions

Caecilia HC Sukowati: Conceptualization, Writing – review & editing, Supervision. **Korri El-Khobar:** Writing – review & editing, Supervision. **Chyntia Olivia Maurine Jasirwan:** Writing – review & editing, Supervision. **Juferdy Kurniawan:** Writing – review & editing, Supervision. **Rino Alvani Gani:** Writing – review & editing, Supervision.

References

[1] Asrani SK, Devarbhavi H, Eaton J, Kamath PS. Burden of liver diseases in the world. J Hepatol 2019;70:151–71. https://doi.org/10.1016/j.jhep.2018.09.014.
[2] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71:209–49. https://doi.org/10.3322/caac.21660.
[3] Vogel A, Meyer T, Sapisochin G, Salem R, Saborowski A. Hepatocellular carcinoma. Lancet 2022;400:1345–62. https://doi.org/10.1016/S0140-6736(22)01200-4.
[4] Rinella ME, Lazarus JV, Ratziu V, Francque SM, Sanyal AJ, Kanwal F, et al. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. J Hepatol 2023. https://doi.org/10.1016/j.jhep.2023.06.003.
[5] El-Serag HB. Epidemiology of viral hepatitis and hepatocellular carcinoma. Gastroenterology 2012;142:1264–73. https://doi.org/10.1053/j.gastro.2011.12.061.
[6] Kulik L, El-Serag HB. Epidemiology and management of hepatocellular carcinoma. Gastroenterology 2019;156:477–91. https://doi.org/10.1053/j.gastro.2018.08.065.
[7] World Health Organization. Global hepatitis report, 2017. https://www.who.int/publications-detail-redirect/9789241565455; 2023 [accessed April 20, 2023].
[8] Sarin SK, Kumar M, Eslam M, George J, Al Mahtab M, Akbar SMF, et al. Liver diseases in the Asia-Pacific region: a lancet gastroenterology & hepatology commission. Lancet Gastroenterol Hepatol 2020;5:167–228. https://doi.org/10.1016/S2468-1253(19)30342-5.
[9] Ganne-Carrié N, Nahon P. Hepatocellular carcinoma in the setting of alcohol-related liver disease. J Hepatol 2019;70:284–93. https://doi.org/10.1016/j.jhep.2018.10.008.
[10] Jacob R, Prince DS, Kench C, Liu K. Alcohol and its associated liver carcinogenesis. J Gastroenterol Hepatol 2023. https://doi.org/10.1111/jgh.16248.
[11] Huang MN, Yu W, Teoh WW, Ardin M, Jusakul A, Ng AWT, et al. Genome-scale mutational signatures of aflatoxin in cells, mice, and human tumors. Genome Res 2017;27:1475–86. https://doi.org/10.1016/S2468-1253(19)30342-5.
[12] Latin American Association for the Study of the Liver. A call for unity: the path towards a more precise and patient-centric nomenclature for NAFLD. Ann Hepatol 2023;28:101115. https://doi.org/10.1016/j.aohep.2023.101115.
[13] American Association for the Study of Liver Diseases. A call for unity: the path towards a more precise and patient-centric nomenclature for NAFLD. Hepatology 2023;78:3. https://doi.org/10.1097/HEP.0000000000000412.
[14] Li J, Zou B, Yeo YH, Feng Y, Xie X, Lee DH, et al. Prevalence, incidence, and outcome of non-alcoholic fatty liver disease in Asia, 1999–2019: a systematic review and meta-analysis. Lancet Gastroenterol Hepatol 2019;4:389–98. https://doi.org/10.1016/S2468-1253(19)30039-1.
[15] Yip TC-F, Lee HW, Chan WK, Wong GL-H, Wong VW-S. Asian perspective on NAFLD-associated HCC. J Hepatol 2022;76:726–34. https://doi.org/10.1016/j.jhep.2021.09.024.
[16] Le MH, Yeo YH, Zou B, Barnet S, Henry L, Cheung R, et al. Forecasted 2040 global prevalence of nonalcoholic fatty liver disease using hierarchical Bayesian approach. Clin Mol Hepatol 2022;28:841–50. https://doi.org/10.3350/cmh.2022.0239.
[17] Fenton SE, Burns MC, Kalyan A. Epidemiology, mutational landscape and staging of hepatocellular carcinoma. Chin Clin Oncol 2021;10:2. https://doi.org/10.21037/cco-20-162.
[18] Choo SP, Tan WL, Goh BKP, Tai WM, Zhu AX. Comparison of hepatocellular carcinoma in Eastern versus Western populations. Cancer 2016;122:3430–46. https://doi.org/10.1002/cncr.30237.
[19] Fujimoto A, Totoki Y, Abe T, Boroevich KA, Hosoda F, Nguyen HH, et al. Whole-genome sequencing of liver cancers identifies etiological influences on mutation patterns and recurrent mutations in chromatin regulators. Nat Genet 2012;44:760–4. https://doi.org/10.1038/ng.2291.
[20] Guichard C, Amaddeo G, Imbeaud S, Ladeiro Y, Pelletier L, Maad IB, et al. Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. Nat Genet 2012;44:694–8. https://doi.org/10.1038/ng.2256.
[21] Teufel A, Marquardt JU, Galle PR. Next generation sequencing of HCC from European and Asian HCC cohorts. Back to p53 and Wnt/β-catenin. J Hepatol 2013;58:622–4. https://doi.org/10.1016/j.jhep.2012.10.006.
[22] Lombardo D, Saitta C, Giosa D, Di Tocco FC, Musolino C, Caminiti G, et al. Frequency of somatic mutations in TERT promoter, TP53 and CTNNB1 genes in patients with hepatocellular carcinoma from Southern Italy. Oncol Lett 2020;19:2368–74. https://doi.org/10.3892/ol.2020.11332.
[23] McGlynn KA, Petrick JL, El-Serag HB. Epidemiology of hepatocellular carcinoma. Hepatology 2021;73(Suppl 1):4–13. https://doi.org/10.1002/hep.31288.
[24] Zhang C-H, Cheng Y, Zhang S, Fan J, Gao Q. Changing epidemiology of hepatocellular carcinoma in Asia. Liver Int 2022;42:2029–41. https://doi.org/10.1111/liv.15251.
[25] Liu Y, Liu L. Changes in the epidemiology of hepatocellular carcinoma in Asia. Cancers 2022;14:4473. https://doi.org/10.3390/cancers14184473.
[26] Chaudhary K, Poirion OB, Lu L, Huang S, Ching T, Garmire LX. Multimodal meta-analysis of 1,494 hepatocellular carcinoma samples reveals significant impact of consensus driver genes on phenotypes. Clin Cancer Res 2019;25:463–72. https://doi.org/10.1158/1078-0432.CCR-18-0088.
[27] Yeh MM, Boukhar S, Roberts B, Dasgupta N, Daoud SS. Genomic variants link to hepatitis C racial disparities. Oncotarget 2017;8:59455–75. https://doi.org/10.18632/oncotarget.19755.
[28] Yao S, Johnson C, Hu Q, Yan L, Liu B, Ambrosone CB, et al. Differences in somatic mutation landscape of hepatocellular carcinoma in Asian American and European American populations. Oncotarget 2016;7:40491–9. https://doi.org/10.18632/oncotarget.9636.
[29] De Mattia E, Cecchin E, Polesel J, Bignucolo A, Roncato R, Lupo F, et al. Genetic biomarkers for hepatocellular cancer risk in a Caucasian population. World J Gastroenterol 2017;23:6674–84. https://doi.org/10.3748/wjg.v23.i36.6674.
[30] Rebouissou S, Nault J-C. Advances in molecular classification and precision oncology in hepatocellular carcinoma. J Hepatol 2020;72:215–29. https://doi.org/10.1016/j.jhep.2019.08.017.
[31] Yan H, Yang Y, Zhang L, Tang G, Wang YZ, Xue G, et al. Characterization of the genotype and integration patterns of hepatitis B virus in early- and late-onset hepatocellular carcinoma. Hepatology 2015;61:1821–31. https://doi.org/10.1002/hep.27722.
[32] Saitta C, Tripodi G, Barbera A, Bertuccio A, Smedile A, Ciancio A, et al. Hepatitis B virus (HBV) DNA integration in patients with occult HBV infection and hepatocellular carcinoma. Liver Int 2015;35:2311–7. https://doi.org/10.1111/liv.12807.
[33] Wong N. Frequent integration of HBV DNA in genomes of hepatocellular carcinoma cells from patients with occult infections. Clin Gastroenterol Hepatol 2020;18:302–3. https://doi.org/10.1016/j.cgh.2019.08.045.
[34] Ye W, Siwko S, Tsai RYL. Sex and race-related DNA methylation changes in hepatocellular carcinoma. Int J Mol Sci 2021;22:3820. https://doi.org/10.3390/ijms22083820.

[35] Wang N, Wang S, Li M-Y, Hu B-G, Liu L-P, Yang S-L, et al. Cancer stem cells in hepatocellular carcinoma: an overview and promising therapeutic strategies. Ther Adv Med Oncol 2018;10:1758835918816287. https://doi.org/10.1177/1758835918816287.

[36] Holczbauer A, Factor VM, Andersen JB, Marquardt JU, Kleinere DE, Raggi C, et al. Modeling pathogenesis of primary liver cancer in lineage-specific mouse cell types. Gastroenterology 2013;145:221–31. https://doi.org/10.1053/j.gastro.2013.03.013.

[37] Marquardt JU, Andersen JB, Thorgeirsson SS. Functional and genetic deconstruction of the cellular origin in liver cancer. Nat Rev Cancer 2015;15:653–67. https://doi.org/10.1038/nrc4017.

[38] Lv D, Chen L, Du L, Zhou L, Tang H. Emerging regulatory mechanisms involved in liver cancer stem cell properties in hepatocellular carcinoma. Front Cell Dev Biol 2021;9:691410. https://doi.org/10.3389/fcell.2021.691410.

[39] Lee TKW, Guan X-Y, Ma S. Cancer stem cells in hepatocellular carcinoma - from origin to clinical implications. Nat Rev Gastroenterol Hepatol 2022;19:26–44. https://doi.org/10.1038/s41575-021-00508-3.

[40] Yamashita T, Kaneko S. Liver cancer stem cells: recent progress in basic and clinical research. Regen Ther 2021;17:34–7. https://doi.org/10.1016/j.reth.2021.03.002.

[41] Sukowati CHC. Heterogeneity of hepatic cancer stem cells. Adv Exp Med Biol 2019;1139:59–81. https://doi.org/10.1007/978-3-030-14366-4_4.

[42] Sukowati CHC, Reyes PAC, Tell G, Tiribelli C. Oncogenicity of viral hepatitis B and C in the initiation of hepatic cancer stem cells. Hepatoma Res 2019;5:2. https://doi.org/10.20517/2394-5079.2018.106.

[43] Tang A, Hallouch O, Chernyak V, Kamaya A, Sirlin CB. Epidemiology of hepatocellular carcinoma: target population for surveillance and diagnosis. Abdom Radiol 2018;43:13–25. https://doi.org/10.1007/s00261-017-1209-1.

[44] Rege TA, Hagood JS. Thy-1 as a regulator of cell-cell and cell-matrix interactions in axon regeneration, apoptosis, adhesion, migration, cancer, and fibrosis. FASEB J 2006;20:1045–54. https://doi.org/10.1096/fj.05-5460rev.

[45] Rege TA, Hagood JS. Thy-1, a versatile modulator of signaling affecting cellular adhesion, proliferation, survival, and cytokine/growth factor responses. Biochim Biophys Acta 2006;1763:991–9. https://doi.org/10.1016/j.bbamcr.2006.08.008.

[46] Lu J-W, Chang J-G, Yeh K-T, Chen R-M, Tsai JJP, Hu R-M. Overexpression of Thy1/CD90 in human hepatocellular carcinoma is associated with HBV infection and poor prognosis. Acta Histochem 2011;113:833–8. https://doi.org/10.1016/j.acthis.2011.01.001.

[47] Luong AB, Do HQ, Tarchi P, Bonazza D, Bottin C, Cabral LKD, et al. The mRNA distribution of cancer stem cell marker CD90/Thy-1 is comparable in hepatocellular carcinoma of eastern and western populations. Cells 2020;9:2672. https://doi.org/10.3390/cells9122672.

[48] Yamashita T, Honda M, Nakamoto Y, Baba M, Nio K, Hara Y, et al. Discrete nature of EpCAM+ and CD90+ cancer stem cells in human hepatocellular carcinoma. Hepatology 2013;57:1484–97. https://doi.org/10.1002/hep.26168.

[49] Liu Z, Dai X, Wang T, Zhang C, Zhang W, Zhang W, et al. Hepatitis B virus PreS1 facilitates hepatocellular carcinoma development by promoting appearance and self-renewal of liver cancer stem cells. Cancer Lett 2017;400:149–60. https://doi.org/10.1016/j.canlet.2017.04.017.

[50] Zeng SS, Yamashita T, Kondo M, Nio K, Hayashi T, Hara Y, et al. The transcription factor SALL4 regulates stemness of EpCAM-positive hepatocellular carcinoma. J Hepatol 2014;60:127–34. https://doi.org/10.1016/j.jhep.2013.08.024.

[51] Yong KJ, Gao C, Lim JSJ, Yan B, Yang H, Dimitrov T, et al. Oncofetal gene SALL4 in aggressive hepatocellular carcinoma. N Engl J Med 2013;368:2266–76. https://doi.org/10.1056/NEJMoa1300297.

[52] Park H, Lee H, Seo AN, Cho JY, Choi YR, Yoon YS, et al. SALL4 expression in hepatocellular carcinomas as associated with EpCAM-positivity and a poor prognosis. J Pathol Transl Med 2015;49:373–81. https://doi.org/10.4132/jptm.2015.07.09.

[53] Fan H, Cui Z, Zhang H, Mani SK, Diab A, Lefrancois L, et al. DNA demethylation induces SALL4 gene re-expression in subgroups of hepatocellular carcinoma associated with Hepatitis B or C virus infection. Oncogene 2017;36:2435–45. https://doi.org/10.1038/onc.2016.399.

[54] Han S, Wang J, Guo X, He CC, Ying X, Ma JL, et al. Serum SALL4 is a novel prognosis biomarker with tumor recurrence and poor survival of patients in hepatocellular carcinoma. J Immunol Res 2014;2014:e262385. https://doi.org/10.1155/2014/262385.

[55] Liu T-C, Vachharajani N, Chapman WC, Brunt EM. SALL4 immunoreactivity predicts prognosis in Western hepatocellular carcinoma patients but is a rare event: a study of 236 cases. Am J Surg Pathol 2014;38:966–72. https://doi.org/10.1097/PAS.0000000000000218.

[56] Kwon J, Liu YV, Gao C, Bassal MA, Jones AI, Yang J, et al. Pseudogene-mediated DNA demethylation leads to oncogene activation. Sci Adv 2021;7 eabg1695. https://doi.org/10.1126/sciadv.abg1695.

[57] Chisari FV, Klopchin K, Moriyama T, Pasquinelli C, Dunsford HA, Sell S, et al. Molecular pathogenesis of hepatocellular carcinoma in hepatitis B virus transgenic mice. Cell 1989;59:1145–56. https://doi.org/10.1016/0092-8674(89)90770-8.

[58] Anfuso B, El-Khobar KE, Ie SI, Avellini C, Radillo O, Raseni A, et al. Activation of hepatic stem cells compartment during hepatocarcinogenesis in a HBsAg HBV-transgenic mouse model. Sci Rep 2018;8:13168. https://doi.org/10.1038/s41598-018-31406-5.

[59] Halgand B, Desterke C, Rivière L, Fallot G, Sebagh M, Calderaro J, et al. Hepatitis B virus pregenomic RNA in hepatocellular carcinoma: A nosological and prognostic determinant. Hepatology 2018;67:86–96. https://doi.org/10.1002/hep.29463.

[60] Xiao Y, Cao J, Zhang Z, Zeng C, Ou G, Shi J, et al. Hepatitis B virus pregenomic RNA reflecting viral replication in distal non-tumor tissues as a determinant of the stemness and recurrence of hepatocellular carcinoma. Front Microbiol 2022;13:830741. https://doi.org/10.3389/fmicb.2022.830741.

[61] Wang C, Yang W, Yan H-X, Luo T, Zhang J, Tang L, et al. Hepatitis B virus X (HBx) induces tumorigenicity of hepatic progenitor cells in 3,5-diethoxycarbonyl-1,4-dihydrocollidine-treated HBx transgenic mice. Hepatology 2012;55:108–20. https://doi.org/10.1002/hep.24675.

[62] Arzumanyan A, Friedman T, Ng IOL, Clayton MM, Lian Z, Feitelson MA. Does the hepatitis B antigen HBx promote the appearance of liver cancer stem cells? Cancer Res 2011;71:3701–8. https://doi.org/10.1158/0008-5472.CAN-10-3951.

[63] Zhu J, Yu H, Chen S, Yang P, Dong Z, Ling Y, et al. Prognostic significance of combining high mobility group Box-1 and OV-6 expression in hepatocellular carcinoma. Sci China Life Sci 2018;61:912–23. https://doi.org/10.1007/s11427-017-9188-x.

[64] Ng K-Y, Chai S, Tong M, Guan X-Y, Lin C-H, Chinh YP, et al. C-terminal truncated hepatitis B virus X protein promotes hepatocellular carcinogenesis through induction of cancer and stem cell-like properties. Oncotarget 2016;7:24005–17. https://doi.org/10.18632/oncotarget.8209.

[65] Ching RHH, Sze KMF, Lau EYT, Chiu Y-T, Lee JMF, Ng IOL, et al. C-terminal truncated hepatitis B virus X protein regulates tumorigenicity, self-renewal and drug resistance *via* STAT3/Nanog signaling pathway. Oncotarget 2017;8:23507–16. https://doi.org/10.18632/oncotarget.15183.

[66] Castello G, Scala S, Palmieri G, Curley SA, Izzo F. HCV-related hepatocellular carcinoma: from chronic inflammation to cancer. Clin Immunol 2010;134:237–50. https://doi.org/10.1016/j.clim.2009.10.007.

[67] Bose SK, Meyer K, Di Bisceglie AM, Ray RB, Ray R. Hepatitis C virus induces epithelial-mesenchymal transition in primary human hepatocytes. J Virol 2012;86:13621–8. https://doi.org/10.1128/JVI.02016-12.

[68] Kwon Y-C, Bose SK, Steele R, Meyer K, Di Bisceglie AM, Ray RB, et al. Promotion of cancer stem-like cell properties in Hepatitis C virus iInfected hepatocytes. J Virol 2015;89:11549–56. https://doi.org/10.1128/JVI.01946-15.

[69] Ali N, Allam H, May R, Sureban SM, Bronze MS, Bader T, et al. Hepatitis C virus-induced cancer stem cell-like signatures in cell culture and murine tumor xenografts. J Virol 2011;85:12292–303. https://doi.org/10.1128/JVI.05920-11.

[70] Machida K, Cheng KTH, Sung VM-H, Levine AM, Foung S, Lai MMC. Hepatitis C virus induces toll-like receptor 4 expression, leading to enhanced production of beta interferon and interleukin-6. J Virol 2006;80:866–74. https://doi.org/10.1128/JVI.80.2.866-874.2006.

[71] Machida K, Tsukamoto H, Mkrtchyan H, Duan L, Dynnyk A, Liu HM, et al. Toll-like receptor 4 mediates synergism between alcohol and HCV in hepatic oncogenesis involving stem cell marker Nanog. Proc Natl Acad Sci U S A 2009;106:1548–53. https://doi.org/10.1073/pnas.0807390106.

[72] Machida K. NANOG-dependent metabolic reprogramming and symmetric division in tumor-initiating stem-like cells. Adv Exp Med Biol 2018;1032:105–13. https://doi.org/10.1007/978-3-319-98788-0_8.

[73] Uthaya Kumar DB, Chen C-L, Liu J-C, Feldman DE, Sher LS, French S, et al. TLR4 signaling *via* NANOG cooperates with STAT3 to activate Twist1 and promote formation of tumor-initiating stem-like cells in livers of mice. Gastroenterology 2016;150:707–19. https://doi.org/10.1053/j.gastro.2015.11.002.

[74] Choi HY, Siddique HR, Zheng M, Kou Y, Yeh D-W, Machida T, et al. p53 destabilizing protein skews asymmetric division and enhances NOTCH activation to direct self-renewal of TICs. Nat Commun 2020;11:3084. https://doi.org/10.1038/s41467-020-16616-8.

[75] Machida K. TLRs, Alcohol, HCV, and tumorigenesis. Gastroenterol Res Pract 2010;2010:518674. https://doi.org/10.1155/2010/518674.

[76] Chen D, Yu D, Wang X, Liu Y, He Y, Deng R, et al. Epithelial to mesenchymal transition is involved in ethanol promoted hepatocellular carcinoma cells metastasis and stemness. Mol Carcinog 2018;57:1358–70. https://doi.org/10.1002/mc.22850.

[77] Chen Y, Wen H, Zhou C, Su Q, Lin Y, Xie Y, et al. TNF-α derived from M2 tumor-associated macrophages promotes epithelial-mesenchymal transition and cancer stemness through the Wnt/β-catenin pathway in SMMC-7721 hepatocellular carcinoma cells. Exp Cell Res 2019;378:41–50. https://doi.org/10.1016/j.yexcr.2019.03.005.

[78] Chong L-W, Chou R-H, Liao C-C, Lee T-F, Lin Y, Yang K-C, et al. Saturated fatty acid induces cancer stem cell-like properties in human hepatoma cells. Cell Mol Biol 2015;61:85–91 (Noisy-le-grand). https://doi.org/10.14715/cmb/2015.61.6.11.

[79] Chen S-Z, Ling Y, Yu L-X, Song Y-T, Chen X-F, Cao Q-Q, et al. 4-phenylbutyric acid promotes hepatocellular carcinoma *via* initiating cancer stem cells through activation of PPAR-α. Clin Transl Med 2021;11:e379. https://doi.org/10.1002/ctm2.379.

[80] Chong L-W, Tsai C-L, Yang K-C, Liao C-C, Hsu Y-C. Targeting protein palmitoylation decreases palmitate-induced sphere formation of human liver cancer cells. Mol Med Rep 2020;22:939–47. https://doi.org/10.3892/mmr.2020.11172.

[81] Yang L, Ji J, Chen Z, Wang H, Li J. Transcriptome profiling of malignant transformed rat hepatic stem-like cells by aflatoxin B1. Neoplasma 2014;61:193–204. https://doi.org/10.4149/neo_2014_025.

[82] Li C-H, Wang Y-J, Dong W, Xiang S, Liang H-F, Wang H-Y, et al. Hepatic oval cell lines generate hepatocellular carcinoma following transfection with HBx gene and treatment with aflatoxin B1 *in vivo*. Cancer Lett 2011;311:1–10. https://doi.org/10.1016/j.canlet.2011.05.035.

[83] Niu Y, Fan S, Luo Q, Chen L, Huang D, Chang W, et al. Interaction of hepatitis B virus X protein with the pregnane X receptor enhances the synergistic effects of aflatoxin B1 and hepatitis B virus on promoting hepatocarcinogenesis. J Clin Transl Hepatol 2021;9:466–76. https://doi.org/10.14218/JCTH.2021.00036.

[84] Ingasia LAO, Kinge CW, Kramvis A. Genotype E: the neglected genotype of hepatitis B virus. World J Hepatol 2021;13:1875–91. https://doi.org/10.4254/wjh.v13.i12.1875.

[85] Kramvis A. Genotypes and genetic variability of hepatitis B virus. Intervirology 2014;57:141–50. https://doi.org/10.1159/000360947.
[86] Liu Y, Park D, Cafiero TR, Bram Y, Chandar V, Tseng A, et al. Molecular clones of genetically distinct hepatitis B virus genotypes reveal distinct host and drug treatment responses. JHEP Rep 2022;4:100535. https://doi.org/10.1016/j.jhepr.2022.100535.
[87] Smith DB, Bukh J, Kuiken C, Muerhoff AS, Rice CM, Stapleton JT, et al. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment web resource. Hepatology 2014;59:318–27. https://doi.org/10.1002/hep.26744.
[88] Keikha M, Eslami M, Yousefi B, Ali-Hassanzadeh M, Kamali A, Yousefi M, et al. HCV genotypes and their determinative role in hepatitis C treatment. Virus Disease 2020;31:235–40. https://doi.org/10.1007/s13337-020-00592-0.
[89] Yip TC-F, Vilar-Gomez E, Petta S, Yilmaz Y, Wong GL-H, Adams LA, et al. Geographical similarity and differences in the burden and genetic predisposition of NAFLD. Hepatology 2023;77:1404–27. https://doi.org/10.1002/hep.32774.
[90] Nault JC, Calderaro J, Di Tommaso L, Balabaid C, Zafrani ES, Bioulac-Sage P, et al. Telomerase reverse transcriptase promoter mutation is an early somatic genetic alteration in the transformation of premalignant nodules in hepatocellular carcinoma on cirrhosis. Hepatology 2014;60:1983–92. https://doi.org/10.1002/hep.27372.
[91] Su L-Y, Li X-L, Shen L, Zhang Y, Zhao M-M, Yin Z-H, et al. Polymorphisms of TERT and CLPTM1L and the risk of hepatocellular carcinoma in Chinese males. Asian Pac J Cancer Prev 2014;15:8197–201. https://doi.org/10.7314/apjcp.2014.15.19.8197.
[92] Ko E, Seo H-W, Jung ES, Kim B, Jung G. The TERT promoter SNP rs2853669 decreases E2F1 transcription factor binding and increases mortality and recurrence risks in liver cancer. Oncotarget 2016;7:684–99. https://doi.org/10.18632/oncotarget.6331.
[93] Zhao X, Zheng F, Li Y, Hao J, Tang Z, Tian C, et al. BPTF promotes hepatocellular carcinoma growth by modulating hTERT signaling and cancer stem cell traits. Redox Biol 2019;20:427–41. https://doi.org/10.1016/j.redox.2018.10.018.
[94] Kim H, Yoo JE, Cho JY, Oh B-K, Yoon Y-S, Han H-S, et al. Telomere length, TERT and shelterin complex proteins in hepatocellular carcinomas expressing "stemness"-related markers. J Hepatol 2013;59:746–52. https://doi.org/10.1016/j.jhep.2013.05.011.
[95] Airewele NE, Shiffman ML. Chronic hepatitis B virus in patients with chronic hepatitis C virus. Clin Liver Dis 2021;25:817–29. https://doi.org/10.1016/j.cld.2021.06.008.
[96] Krause J, von Felden J, Casar C, Frundt TW, Galaski J, Schmidt C, et al. Hepatocellular carcinoma: intratumoral EpCAM-positive cancer stem cell heterogeneity identifies high-risk tumor subtype. BMC Cancer 2020;20:1130. https://doi.org/10.1186/s12885-020-07580-z.
[97] Kumar A, Bhanja A, Bhattacharyya J, Jaganathan BG. Multiple roles of CD90 in cancer. Tumour Biol 2016;37:11611–22. https://doi.org/10.1007/s13277-016-5112-0.
[98] Sauzay C, Voutetakis K, Chatziioannou A, Chevet E, Avril T. CD90/Thy-1, a cancer-associated cell surface signaling molecule. Front Cell Dev Biol 2019;7:66. https://doi.org/10.3389/fcell.2019.00066.
[99] Liu Y-C, Yeh C-T, Lin K-H. Cancer stem sell functions in hepatocellular carcinoma and comprehensive therapeutic strategies. Cells 2020;9:1331. https://doi.org/10.3390/cells9061331.
[100] Jäger M, Schoberth A, Ruf P, Hess J, Hennig M, Schmalfeldt B, et al. Immunomonitoring results of a phase II/III study of malignant ascites patients treated with the trifunctional antibody catumaxomab (anti-EpCAM x anti-CD3). Cancer Res 2012;72:24–32. https://doi.org/10.1158/0008-5472.CAN-11-2235.
[101] Knödler M, Körfer J, Kunzmann V, Trojan J, Daum S, Schenk M, et al. Randomised phase II trial to investigate catumaxomab (anti-EpCAM × anti-CD3) for treatment of peritoneal carcinomatosis in patients with gastric cancer. Br J Cancer 2018;119:296–302. https://doi.org/10.1038/s41416-018-0150-6.