Cancer Stem Cells in Hepatocellular Carcinoma: Intrinsic and Extrinsic Molecular Mechanisms in Stemness Regulation

Xiaona Fang 1,2,3,†, Qian Yan 4,†, Shan Liu 2,3 and Xin-Yuan Guan 1,2,3,5,6,*

1 Department of Clinical Oncology, The University of Hong Kong-Shenzhen Hospital, Shenzhen 518000, China  
2 Department of Clinical Oncology, The University of Hong Kong, Hong Kong 999077, China  
3 State Key Laboratory for Liver Research, The University of Hong Kong, Hong Kong 999077, China  
4 Guangdong Institute of Gastroenterology, Guangdong Provincial Key Laboratory of Colorectal and Pelvic Floor Diseases, The Sixth Affiliated Hospital, Sun Yat-sen University, Guangzhou 510000, China  
5 MOE Key Laboratory of Tumor Molecular Biology, Jinan University, Guangzhou 510000, China  
6 Advanced Nuclear Energy and Nuclear Technology Research Center, Advanced Energy Science and Technology Guangdong Laboratory, Huizhou 516000, China

* Correspondence: xyguan@hku.hk; Tel.: +852-3917-9782  
† These authors contributed equally to this work.

Abstract: Hepatocellular carcinoma (HCC) remains the most predominant type of liver cancer with an extremely poor prognosis due to its late diagnosis and high recurrence rate. One of the culprits for HCC recurrence and metastasis is the existence of cancer stem cells (CSCs), which are a small subset of cancer cells possessing robust stem cell properties within tumors. CSCs play crucial roles in tumor heterogeneity constitution, tumorigenesis, tumor relapse, metastasis, and resistance to anti-cancer therapies. Elucidation of how these CSCs maintain their stemness features is essential for the development of CSCs-based therapy. In this review, we summarize the present knowledge of intrinsic molecules and signaling pathways involved in hepatic CSCs, especially the CSC surface markers and associated signaling in regulating the stemness characteristics and the heterogeneous subpopulations within the CSC pool. In addition, we recapitulate the effects of crucial extrinsic cellular components in the tumor microenvironment, including stromal cells and immune cells, on the modulation of hepatic CSCs. Finally, we synopsize the currently valuable CSCs-targeted therapy strategies based on intervention in these intrinsic and extrinsic molecular mechanisms, in the hope of shedding light on better clinical management of HCC patients.

Keywords: cancer stem cells; hepatocellular carcinoma; cell surface markers; heterogeneity; stroma cells; immune cells; CSCs-targeting therapies

1. Introduction

According to the Global Cancer Statistics in 2020, hepatocellular carcinoma (HCC) constitutes about 80% of primary liver malignancies, which rank as the sixth most common cancer and the third leading cause of cancer-related death worldwide [1]. The incidence rate and mortality of HCC are continuing to rise, posing a great challenge to global health [2]. Due to the absence of clinical symptoms during its early stages, most patients are in the advanced stage at the first diagnosis and develop liver or distal metastasis. Even for the minority of patients with early-stage HCC who receive surgical resection, the recurrence rate reaches around 70% after surgery [3]. The estimated 5-year survival time in patients with advanced HCC is only 3 to 13 months after systematic therapies [4]. Overall, the therapeutic efficacy and prognosis in HCC patients are extremely poor. It has been documented at single-cell resolution that HCC is a biologically complex malignancy with highly heterogenous genetic and cellular dysregulations and a complicated tumor microenvironment [5–10]. Accumulating evidence has demonstrated that cancer stem cells (CSCs), a small group of cells harboring the capacities to self-renew and initiate tumor, are...
notably responsible for tumor heterogeneity, recurrence, metastasis, as well as resistance to chemotherapy [11]. Therefore, elucidation of how these CSCs maintain their stem cell-like features is essential for the development of CSCs-based therapy and durable treatment of HCC patients. Among the various treatment strategies for HCC patients, CSCs-targeted therapy would be a very promising regimen.

Within the tumor bulk, hepatic CSCs are distinguished by their expression of various stemness-related markers and activation of stemness-associated regulatory signaling pathways that contribute to maintain their stem cell traits. In the surrounding intricate tumor microenvironment (TME), the cellular components, especially stroma cells and immune cells, play critical roles in the stemness regulation of CSCs. Further investigation into the intrinsic and extrinsic molecular mechanisms triggering the biological properties of CSCs could facilitate the advancement of CSCs-directed therapy. In this review, we enumerate the well-recognized and newly discovered CSC surface markers and their representative signaling pathways involved in stemness regulation. We also recapitulate the role of stroma cells and immune cells in driving the tumor-initiating abilities of CSCs, thus providing insights for CSCs-based therapies from these perspectives. The currently valuable CSCs-targeted therapy strategies based on these mechanisms are also summarized.

2. Intrinsic Molecules and Involved Signaling Pathways

2.1. Cell Surface Markers of Hepatic CSCs

The past three decades have witnessed a rich development in excavating important CSCs surface markers. These well-known and newly identified surface markers and associated signaling pathways are summarized in Table 1.

CD133, also known as prominin 1 (PROM1), is considered the most compelling cell surface marker for the identification of hepatic CSCs. Substantial evidence has elucidated the stemness-associated features of CD133+ cells, including self-renewal, chemoradiotherapy resistance, tumorigenicity, and metastasis. The intrinsic activations of multiple signaling pathways were found to be involved in regulating CD133+ CSCs behavior, such as the Wnt/β-catenin signaling [12–15], IL-6/STAT3 signaling [16], TGF-β pathway [17], AKT/PKB pathway [18], IL-8/MAPK signaling [19], TLR4/NANOG and STAT3 signaling [20], STAT3/SOX4 signaling [21], MEK/ERK signaling [22], and ANXA3/JNK signaling [23]. In addition to CD133, CD44 and the epithelial cell adhesion molecule (EpCAM) are also well-characterized CSC surface markers. CD44+ CSCs showed enhanced sphere formation and migration abilities through upregulation of AKT/GSK-3β/β-catenin signaling [24]. In addition, TGF-β, JAK/STAT, and IL-6/STAT3 signaling pathways contributed prominently to the stemness regulation of CD44+ cells, resulting in their vital roles in tumor initiation and growth [25–27]. Regarding EpCAM+ CSCs, various pathways were invariably hijacked to maintain stemness characteristics. For instance, the crosstalk between TNF-α/NF-κB and IL-6/STAT3 signaling was reported to be critical for EpCAM+ CSCs expansion in HCC [28]. Likewise, Wnt/β-catenin signaling was found to be enriched in the EpCAM+ cell population, which showed augmented self-renewal and differentiation abilities [29]. Accumulating studies have revealed that CD90+ HCC cells exhibited self-renewal, pro-tumorigenicity and aggressive phenotypes [30] with the activation of AKT/EphA2 signaling [31], IL-6/JAK2 signaling [32], AKT and mTOR signaling [33] as well as MAP3K8 signaling [34]. Moreover, CD13 is also a well-explored stem cell surface marker in HCC. The molecular mechanisms involved in stemness regulation of CD13+ cells consisted of the activation of Wnt/β-catenin signaling, NF-kB signaling and YAP1 signaling [35–37]. Interestingly, Li Sun et al. reported that aerobic metabolism of tyrosine signaling endowed CD13+ CSCs with increased organoid formation and resistance to chemotherapy [38]. Similar to CD133+ and CD44+ cells, IL-6/STAT3 signaling also contributed largely to the improved stemness properties and tumorigenicity of CD24+ CSCs [28]. Besides, CD24+ cells also employed STAT3-mediated NANOG regulation [39] and iNOS-mediated TACE/ADAM17/NOTCH signaling [40] to fulfill their stem cell traits. Cell surface protein CD47 has been reported to be correlated with self-renewal,
tumor initiation, and metastasis in HCC through upregulating CTSS/PAR2 signaling [41]. Another potential CSC marker, CD54, also known as intercellular adhesion molecule 1 (ICAM1), was demonstrated to confer self-renewal and tumor malignant transformation capacities to HCC cells [42,43]. As the most common and functional stemness-associated pathway, β-catenin signaling was verified to be enriched in leucine rich repeat containing G protein-coupled receptor 5 (LGR5) positive [44–47] and OV6+ liver cancer-initiating cells to sustain their CSCs identities [48,49]. CD34+ HCC cells displayed stem cell features such as self-renewal and tumor initiation via upregulation of various pluripotency drivers such as OCT4, SOX2, NAONG, KLF4 and c-MYC [50–52]. In addition, a recent study found that the calcium channel α2δ1 subunit could be a targetable hepatic tumor-initiating cell marker [53]. The α2δ1+ liver CSCs exhibited enhanced self-renewal and tumorigenicity abilities through ERK1/2 phosphorylation compared to the α2δ1- subset [54,55]. Another cell surface protein delta-like 1 protein (DLK1), endowed HCC cells with stronger ability of self-renewal, chemoresistance and tumorigenicity compared to DLK1− cells [56–58].

Apart from early identified CSC markers, other potential surface markers of CSCs in HCC were also discovered and characterized. A recent study revealed that CD73+ HCC cells sustained robust CSC features, including self-renewal ability, expression of stemness-related genes and drug resistance via harnessing AKT signaling and SOX9 expression [59]. CD206, expressed on the cell surface, was also found to be a potential hepatic CSC marker that was co-expressed in spheroids with CD44 and several classic pluripotency transcription factors, including OCT4, SOX2, NANOG and c-MYC [60]. Collectively, Wnt/β-catenin signaling and IL-6/STAT3 signaling are the top two pathways regulating the stem cell features of diverse marker-positive CSCs, including CD133, EpCAM, CD24 and LGR5, indicating that targeting these signaling pathways may be a promising strategy to eliminate CSCs and reverse drug resistance.
| CSC Markers | Source of Identification | Phenotypes | Signaling Involved in CSCs | Clinical Predictive Value | Refs. |
|-------------|-------------------------|------------|---------------------------|--------------------------|-------|
| CD133       | Cell lines, Primary tissues | Self-renewal, Tumorigenicity, Chemoresistance, Invasiveness, Cell proliferation, Radioresistance | Wnt/β-catenin signaling, IL-6/STAT3 signaling, IL-8/MAPK signaling, AKT/PKB pathway, MEK/ERK signaling, STAT3/sox4 signaling, ANXA3/JNK signaling, TGF-β gaining, TLR4/NANOG and STAT3 signaling | Diagnostic, Therapeutic, Prognostic | [12–23,36,61–63] |
| CD44        | Cell lines, Primary tissues | Sphere formation, Tumorigenicity, Targeted drug resistance, TGF-β-mediated mesenchymal phenotype, EMT | TGF-β signaling, JAK/STAT signaling, IL-6/STAT3 signaling, AKT/GSK-3β/β-catenin signaling | Therapeutic, Prognostic | [24–27,64–67] |
| EpCAM       | Cell lines, Primary tissues | Self-renewal, Differentiation, Drug resistance | Wnt/β-catenin signaling, TNF-α/NF-κB, IL-6/STAT3 signaling | Diagnostic, Therapeutic, Prognostic | [28,29,68] |
| CD90        | Cell lines, Primary tissues | Sphere formation, Tumorigenicity, Metastasis, Cell migration and invasion, Cell proliferation, Chemoresistance | AKT/EphA2 signaling, IL-6/JAK2 signaling, AKT and mTOR signaling, MAP3K8 signaling | Diagnostic, Therapeutic, Prognostic | [30–34,69,70] |
| CD13        | Cell lines, Primary tissues | Sphere formation, Tumorigenicity, Chemoresistance, Angiogenesis, ROS-induced DNA damage | Wnt/β-catenin signaling, YAP1 signaling, NF-κB signaling, Aerobic metabolism of tyrosine signaling | Therapeutic, Prognostic | [35–38,71] |
| CD24        | Cell lines, Primary tissues | Sphere formation, Stemness gene expression, Tumorigenicity, Chemoresistance, Cell migration and invasion | IL-6/STAT3 signaling, STAT3-mediated NANOG regulation, iNOS-mediated TACE-ADAM17-NOTCH signaling | Therapeutic, Prognostic | [28,39,40,65,72] |
| CSC Markers | Source of Identification | Phenotypes | Signaling Involved in CSCs | Clinical Predictive Value | Refs. |
|-------------|--------------------------|------------|--------------------------|--------------------------|-------|
| CD47        | Cell lines, Primary tissues | Tumor initiation, Self-renewal, Tumorigenicity, Chemoresistance, Metastasis | CTSS–PAR2 signaling | Therapeutic, Prognostic | [41]  |
| CD54/ICAM1  | Cell lines, Primary tissues | Sphere formation, Tumorigenicity, Metastasis | NANOG dysregulation | Therapeutic, Prognostic | [42,43] |
| LGR5        | Cell lines               | Sphere formation, Tumorigenicity, Organoid initiation, Tumor growth, Drug resistance | Wnt/β-catenin signaling | Therapeutic, Prognostic | [44–47] |
| OV6         | Cell lines, Primary tissues | Tumorigenicity, Chemoresistance, Invasion, Metastasis | β-catenin signaling | Therapeutic, Prognostic | [48,49,73] |
| CD34        | Cell lines               | Self-renewal, Tumorigenicity | OCT4, SOX2, NAONG, KLF4, c-MYC upregulation | Prognostic | [50–52] |
| Calcium channel α2δ1 subunit | Cell lines | Self-renewal, Tumorigenicity | ERK1/2 phosphorylation | Therapeutic | [53–55] |
| DLK1m       | Cell lines               | Chemoresistance, Colony formation, Spheroid formation, Tumorigenicity | Not reported | Therapeutic | [56–58] |
| CD73        | Cell lines               | Sphere formation, Lenvatinib resistance, Stemness gene expression | AKT signaling, SOX9 upregulation | Therapeutic, Prognostic | [59] |
| CD206       | Cell lines               | Cell migration and invasion | Not reported | Prognostic | [60] |
2.2. Heterogeneous Patterns of Hepatic CSC Surface Markers and Phenotypes

Although marker-positive cancer cells were defined as CSCs, these markers are not all concomitantly expressed in the same subpopulation. It is well known that heterogeneity exists not only in tumors but also in hepatic CSCs, which may be organized in a hierarchical relationship [74]. Marker-positive cancer cells are not randomly distributed but spatially heterogeneous, and hepatic CSC subpopulations showed vast heterogeneity biologically and phenotypically [10]. Furthermore, the hepatic CSC subgroup expressing certain CSC markers may not co-occur in all HCC tumors. Different CSC subclusters may exhibit distinct expression patterns of CSC markers and display phenotypically diverse features and functions. Yamashita et al. uncovered that EpCAM+ and CD90+ CSCs represented distinct CSC subpopulations in primary HCC tissues and showed discrete features in terms of cell morphology, tumorigenicity and metastasis propensity [75]. Different CSC markers, including CD133, EpCAM and CD24, defined distinct CSC subclusters, which exhibited different self-renewal potentials [10]. Moreover, the combined expression of certain individual hepatic CSC markers has also fueled CSCs with stronger stemness and tumorigenic characteristics. For example, CD133+CD44+ CSCs showed enhanced abilities of sphere formation, stemness-related gene expression, tumorigenesis and chemoresistance compared with CD133+CD44− cells [76]. CD90+CD44+ CSCs were evidenced with more aggressive and pro-metastatic phenotypes than CD90+CD44− counterparts [77]. Similarly, CD133+EpCAM+ CSCs exhibited remarkable and greater tumor-initiating capacity and high resistance to chemotherapy compared to the CD133+CD13− subset [78] and an enhanced self-renewal and tumorigenicity compared to the CD133-CD13− subset via the IncTCF7-mediated Wnt signaling pathway [15]. Interestingly, it was revealed that the self-renewal ability and radiotherapy-resistant potential of CD13+CD90− cells were significantly superior to CD13-CD90+ cells [78]. Another study uncovered the presence of CD133+CD24+ cells in HCC tumors showing stemness features both in vitro and in vivo, which were not observed in CD133-CD24+ cells [40]. The heterogeneous nature of CSCs in HCC is depicted in Figure 1. These heterogeneous expression patterns also indicate that even in one marker-positive CSC, there may exist distinct subclusters, which were demonstrated by a recent study highlighting the heterogeneity of CD133+ HCC cells [79]. Given the diverse expression of surface markers and distinct stemness traits of CSC subpopulations, the potential hierarchical structure of hepatic CSCs is still far from clear and needs to be further studied. Targeting more than one hepatic CSC marker may be a more effective way to eliminate CSCs and be beneficial for HCC patients.

![Figure 1](image_url)  
**Figure 1.** A cartoon showing the heterogeneous nature of CSCs in HCC. Illustrated are six subsets with individual surface marker expressions harboring distinct stemness features inside the hepatic CSC pool. CSCs with a combined expression of surface markers (shaded) possessed more vital tumor-initiating capacities than unique marker-expressed CSCs.
3. Extrinsic Cellular Components in the Tumor Microenvironment

While intrinsic molecules and signaling pathways in CSCs have been a principal focus of previous studies, how the TME may impact CSC behaviors has been less deciphered. Although little is known about the CSC niche in cancer, it is supposed that CSCs reside in a friendly environment that supports their stemness and maintains the CSC subpopulation [80]. An extensive body of studies has revealed that the various cell types and factors in the tumor ecosystem were indispensable for the formation of the CSC niche, indicating that CSCs-based therapy only targeting CSCs signaling may be insufficient to ablate CSCs [81]. Further study of the CSCs-supportive TME signals may facilitate the development of novel regimens to eradicate CSCs and achieve sustained anti-cancer efficacy. The most critical cellular components essential for the CSC niche are stroma cells such as cancer-associated fibroblasts (CAFs), adipocytes, endothelial cells (ECs) and various kinds of immune cells, including tumor-associated macrophages (TAMs), tumor-associated neutrophils (TANs), T cells, B cells, natural killer (NK), dendritic cells (DCs) and myeloid-derived suppressor cells (MDSCs) [82]. Below, we discuss and summarize how these neighboring cell subsets affect hepatic CSCs and mediate their stemness and malignant properties.

Increasing studies have revealed that CAFs played critical roles in HCC development and aggressiveness. The conditional medium from CAFs could fuel cancer stemness traits, including the self-renewal of CSCs, expression of stemness-associated markers and oncofetal proteins, metastasis, and drug resistance to chemotherapy, suggesting indirect communications between CAFs and cancer cells. It was reported that CAFs mediated the stemness properties of HCC cells through the secretion of the hepatocyte growth factor (HGF), which stimulated MET/FRA1/HEY1 signaling in hepatic CSCs [83]. Similarly, IL-6 produced by CAFs was evidenced to endow tumor-initiating characteristics to HCC cells by the enrichment of STAT3/Notch signaling [84]. Increased excretion of IL-6 and IL-8 from CAFs regulated by HCC-derived miR-1247-3p was also demonstrated to promote stemness and metastasis in HCC [85]. A study by Li et al. further reported that CAFs-derived HGF and IL-6 contributed drastically to the self-renewal, tumorigenicity, metastasis and chemoresistance of CD24+ liver CSCs via the phosphorylation of the STAT3 signaling pathway [86]. Besides, Sun et al. uncovered that CAFs-secreted cartilage oligomeric matrix protein (COMP) could ameliorate the self-renewal and epithelial-mesenchymal transition (EMT) function of HCC cells through the induction of MEK/ERK and PI3K/AKT signaling [87,88]. Surprisingly, autophagy was found to be associated with HCC stemness by CAFs-induced autophagic flux [89]. In addition, the Song group indicated that cardiophrin-like cytokine factor 1 (CLCF1), derived from CAFs, could facilitate stem-like characteristics of HCC cells by STAT3/CXCL6/E2F1 and STAT3/TGF-β/p38 signaling axes [90]. Interestingly, HCC-produced chemokine (C-X-C motif) ligand 6 (CXCL6) and TGF-β could in turn activate ERK1/2 signaling in CAFs and promote CLCF1 expression and secretion and form a positive feedback loop [90]. Furthermore, a recent study revealed that the stemness capacities of HCC cells could be potentiated by CAFs-derived TGF-β1 through P85α/AKT/TBX3 signaling [91]. A newly identified secreted protein by CAFs in HCC, follistatin-like 1 (FSTL1), was also found to have participated in stemness modulation and malignant transformation via activating AKT/mTOR/4EBP1 signaling [92]. The multiple pro-stemness effects of CAFs on hepatic CSCs may provide promising potential targets for CSCs eradication therapy.

Apart from CAFs, ECs and adipocytes are essential elements in the tumor stroma. The vascular niche in TME generates instructive angiocrine factors to nourish CSCs [93]. It was reported that chemokine (C-X-C motif) ligand 9 (CXCL9) in the co-culture system of vascular ECs and CD133+ hepatic CSCs could enhance the migration and invasion of CD133+ cells with the activation of NF-kB signaling [94]. Moreover, lymphatic ECs could produce and secrete IL-17A to potentiate the self-renewal and tumorigenesis properties of hepatic CD133+ stem cells by upregulating programmed cell death-ligand 1 (PD-L1) expression [95]. Besides, adipocytes differentiated from mesenchymal stem cells (MSCs) have been found to secrete factors including IL-6, IL-8 and monocyte chemoattractant
protein 1 (MCP1) to foster the CSC behaviors of CD133+EpCAM+ HCC cells via MET, STAT3 and ERK1/2 signaling [96].

In the context of the tumor immune environment, the immune cell subsets help to promote the tumor-initiating potential of liver CSCs. As the most studied immune cell type with an ineligible effect on hepatic CSC stemness regulation, M2 TAMs were validated to produce IL-6, which subsequently increased the CD44+ hepatic CSC proportion and tumorigenesis through the STAT3 signaling pathway [27]. TAMs could also augment the stemness characteristics of HCC cells by TGF-β-mediated EMT [97] and CC chemokine ligand 17 (CCL17)-induced EMT, TGF-β1 and Wnt/β-catenin signaling [98]. Besides, TAMs-derived TNF-α played critical roles in inducing the conversion of hepatic CSCs via the TNFR1/STAT3 pathway [99] and boosting EMT and stemness features via the Wnt/β-catenin pathway [100]. Interestingly, a recent study revealed that S100 calcium-binding protein A9 (S100A9) from TAMs improved the stem cell-like properties of HCC cells [101]. In addition, TANs in the TME are also critical inflammatory cells involved in liver cancer initiation, development, and progression. By co-culture with HCC cells, TANs were found to contribute essentially to tumor cell stemness by secretion of TGF-β2 and bone morphogenetic protein 2 (BMP2) to hyperactivate NF-κB signaling [102]. Regulatory T (Treg) cells, which are a subset of immune cells with pro-tumorigenesis capabilities, have also been recognized to exert potent effects on stemness properties of CSCs in HCC [103,104]. Tregs produced high levels of TGF-β1 to promote the generation of liver CSCs by mediating the EMT process [105]. It has been reported that the cancer autocrine molecule C-X-C motif chemokine 11 (CXCL11), accumulated in the TME, could increase the expression of stemness-related genes and maintain the stem cell features of α2δ1+ liver CSCs through chemokine (C-X-C motif) receptor 3 (CXCR3)/ERK1/2 signaling [106]. Simultaneously, CXCL11 could recruit activated T cells to infiltrate into the TME. However, the origins of CXCL11 and whether and how these recruited T cells support the CSC niche are still elusive. For other subtypes of T cells, such as CD8+ cytotoxic T cells, T helper cells Th1 and Th17, several studies have suggested that interferon gamma (IFN-γ) from CD8+ cytotoxic T cells and Th1 could promote the stemness traits in lung cancer [107]. Also, the IL-17 from Th17 was able to augment the self-renewal of CSCs depending on the STAT3 pathway in ovarian cancer [108], pancreatic cancer [109] and gastric cancer [110]. Nevertheless, the potential roles of these T cell subsets in regulating CSC stemness behaviors in HCC have been less explored.

With regard to other neighboring immune cells such as B cells and NK cells, CSCs impose a series of immuno-inhibitory effects on these cells to evade immunosurveillance [111–113]. Nevertheless, whether these cancer cell-recruited B cells and NK cells possess the capability to augment stemness in HCC needs to be further elucidated. The antigen-presenting cells-DCs present tumor antigens to cytotoxic immune cells and instigate an immune response. Cumulative studies have demonstrated that hepatic CSCs could alter the phenotypes of DCs to limit their effects on T cell activation and thus induce immunotolerance [114,115]. However, the reciprocal interactions between liver CSCs and DCs are still far from clear. More explorations are necessary to clarify their interplays and explicate how DCs modulate the stem cell-like characteristics of hepatic CSCs.

Additionally, MDSCs exert immunosuppressive and pro-tumor roles in the TME [116]. It was reported that HCC-derived IL-6 hindered the recruitment of MDSCs and enhanced their immune inhibitory function to hamper anti-tumor immunity [117]. Reciprocally, MDSCs-derived IL-6 conferred stemness properties to breast CSCs via STAT3 and NOTCH signaling pathways [118]. Nevertheless, how MDSCs mediate and maintain the stemness and malignant characteristics of liver CSCs has not come to light yet. Taken together, extrinsic cellular components in the tumor microenvironment play indispensable roles in the stemness modulation of HCC CSCs (Figure 2). Further investigation into the underlying mechanisms will provide a better understanding of the cellular crosstalk between CSCs and the TME and shed light on the development of new therapeutic strategies targeting CSCs.
4. CSCs-Targeted Therapy in HCC

Recent decades have witnessed rapid advancements in CSCs-targeted therapy. In the present review, we have focused on the intrinsic expression and identification of hepatic CSCs surface markers, the heterogeneity of marker-positive subpopulations, and elucidated how the extrinsic cellular elements in the TME colluded with hepatic CSCs to augment their stemness properties. Below, we synopsize the currently valuable hepatic CSCs-targeted therapeutic strategies through interference with these intrinsic and extrinsic molecular mechanisms, including targeting hepatic CSC surface markers and debilitating the cellular support for liver CSCs from the TME.

4.1. Targeting Surface Markers of Hepatic CSCs

Utilizing antibodies, inhibitors, or combinational techniques to target CSC surface markers can powerfully diminish hepatic CSCs. The administration of oncolytic viruses in alleviating tumors has been found as an emerging anti-cancer approach with specific cytotoxicity against cancer cells [119]. It was reported that CD133+ liver cancer cells could be eliminated explicitly by the CD133-targeted oncolytic measles virus termed MV-CD133 [120]. A recent study further revealed that the vesicular stomatitis virus targeting CD133 (VSV-CD133) exerted more powerful oncolytic and anti-tumor activity than MV-CD133 in HCC [121]. Furthermore, several antibody-based strategies can also target surface markers of CSCs, including direct inhibition using monoclonal antibodies and
the indirect effect of antibody-induced cytotoxicity, such as the genetically engineered chimeric antigen receptor (CAR) T cells and dendritic cell vaccines. For example, an anti-CD3/anti-CD133 bispecific antibody binding to cytokine-induced killer cells (BsAb-CIK) was generated and exhibited a significant killing effect on CD133-high CSCs [122]. Recently, a clinical trial (NCT02541370) exploring the activity of CD133-guided CAR T cells in various cancers, including HCC, has been accomplished, showing the safety and efficacy of CD133-targeted CAR T-cells in HCC patients [123,124]. Another study tested the efficacy of the CD133-directed dendritic cell vaccine in HCC and demonstrated that this vaccine could induce CD8+ cytotoxic T cell activity against CD133+ liver CSCs [125]. CD44 and EpCAM peptide-loaded DCs vaccines were also evidenced to endow dramatic anti-tumor immunity to HCC cells [126]. Interestingly, the anti-CD44 antibody was reported to have an inhibitory effect on CD90+ CSCs in HCC cells by inducing apoptosis [77]. Besides, VB4-845, an inhibitor targeting EpCAM+ CSCs in HCC, showed effective anti-tumor cytotoxicity and synergistic effects in combination with 5-FU [127]. Currently, two ongoing clinical trials are evaluating the anti-tumor activity of EpCAM-based CAR T (NCT03013712, NCT02729493) in primary or refractory liver cancer. In addition, novel compounds targeting liver CSCs are also under development. The Kübra group reported that their newly synthesized isoxazole-piperazine analogue compounds could attenuate the proportion of CD133+EpCAM+ cells and downregulate pluripotency marker expression, implying their potential to be developed as CSCs-directed agents [128]. Similarly, the CD133+EpCAM+ cell population was discovered to be reduced by a novel nanoparticle of nitidine chloride named TPGS-FA/NC [129]. Furthermore, a known mTOR pathway inhibitor, OSU-CG5, could impede the maintenance of CD90+ CSCs in liver tumors [130].

Additionally, there are various inhibitors showing inhibitory effects on CD13+ CSCs. Bestatin (ubenimex), identified as a CD13 inhibitor, exerted a potent anti-tumor effect in preclinical mouse models and impeded the stemness features of liver CSCs [131,132]. Likewise, CD13+ CSCs could also be suppressed by a fusion protein, termed NGR-LDP-AE, composed of a CD13-targeted peptide NGR and anti-tumor antibiotic lidamycin, which displayed a prominent anti-tumor effect by killing CD13+ CSCs and suppressing angiogenesis [133]. Another novel conjugate termed poly (ethylene glycol)-poly (lysine) block copolymer-ubenimex conjugate (PEG-b-PLys (Ube)), which used the PEG as a delivery system, showed strong anti-tumor and synergistic effects with combinations of fluorouracil, cisplatin, or doxorubicin targeting CD13+ CSCs [134]. Surprisingly, the Zhou group recently identified a new organic selenium compound termed CU27 using a functional screening and found that CU27 displayed dramatic capacities to destem the CD13+CD133+ population by binding to c-MYC [135].

A BsAb was developed to synchronously recruit NK cells and target CD24+ hepatic CSCs, using the ligand of the NK cell receptor group 2 member D (NKG2D), the MHC class I-related chain A (MICA) to fuse with the flexible pentapeptide cG7 and form BsAb cG7-MICA [136]. It was stated that the hyaluronan synthesis inhibitor 4-methylumbelliferone (4Mu) could induce a potent anti-cancer efficacy in HCC by targeting the CD47+ CSCs [137]. Besides, applying an anti-CD47 antibody to block CD47+ CSCs in HCC significantly decreased stemness and sorafenib resistance [138]. A monoclonal antibody, 1B50-1 showed a therapeutic effect on HCC engraftments to eliminate CSCs by binding to α2δ1 [54]. The current therapeutic strategies targeting hepatic tumor CSC surface markers are shown in Figure 3. Given the complex heterogeneity of distinct marker-positive subpopulations in the CSC niche, novel strategies aiming to target more than one marker may be more successful in CSCs-directed therapy. Although the potential approaches directly targeting surface markers of liver CSCs have been demonstrated to be effective in eliminating CSCs, there is still a long way to go until their application in the clinic.
Figure 3. Current therapeutic strategies targeting hepatic CSC surface markers. The seven surface markers in different colored regions are the essential stemness markers for hepatic CSCs. Distinct approaches, including oncolytic viruses, inhibitors, antibodies, CAR T cells therapy and vaccines, showed potent inhibitory effects on hepatic CSCs. Combining any of these therapeutic strategies may contribute to the better eradication of CSCs.

4.2. Hampering Cellular Supports for Hepatic CSCs in TME

As stroma cells and immune cells could confer pro-stemness capacities to hepatic CSCs through the secretion of various cytokines, chemokines, and secreted proteins (Figure 2), CSCs-based approaches impeding the supportive signals from TME would be promising to wipe out CSCs. Given that IL6 can be derived from various cell types, targeting IL-6 may exert potent CSCs-inhibitory effects. A previous study has illustrated that the blockade of IL-6 with tocilizumab, approved by the Food and Drug Administration for treatment of rheumatoid arthritis, could attenuate the self-renewal of CD44+ liver CSCs and decrease tumorigenesis, suggesting its potential to target liver CSCs and application in the clinical management of HCC patients [27]. A phase I clinical trial (NCT02536469) was completed, which tested the anti-tumor efficacy of humanized anti-IL-8 monoclonal antibodies in solid tumors, providing possibilities to utilize the IL-8 antibody to abrogate CSCs in HCC [139]. Besides, the production of CAFs-derived COMP could be diminished by a potential agent named resolvin D1 and thus obstruct the stemness properties of HCC CSCs [87]. In addition, the FSTL1 neutralizing antibody exerted significant effects on the eradication of the hepatic CSC subset and the reversal of sorafenib resistance [92]. Overall, the inhibitors or antibodies directly targeting the secreted proteins in the TME that contribute to stemness maintenance of liver CSCs are being discovered continuously. Further development of these agents may open a new era for CSCs-based therapy. The multiple hepatic CSCs-targeted therapeutic strategies are recapitulated in Table 2.

Figure 3. Current therapeutic strategies targeting hepatic CSC surface markers. The seven surface markers in different colored regions are the essential stemness markers for hepatic CSCs. Distinct approaches, including oncolytic viruses, inhibitors, antibodies, CAR T cells therapy and vaccines, showed potent inhibitory effects on hepatic CSCs. Combining any of these therapeutic strategies may contribute to the better eradication of CSCs.

4.2. Hampering Cellular Supports for Hepatic CSCs in TME

As stroma cells and immune cells could confer pro-stemness capacities to hepatic CSCs through the secretion of various cytokines, chemokines, and secreted proteins (Figure 2), CSCs-based approaches impeding the supportive signals from TME would be promising to wipe out CSCs. Given that IL6 can be derived from various cell types, targeting IL-6 may exert potent CSCs-inhibitory effects. A previous study has illustrated that the blockade of IL-6 with tocilizumab, approved by the Food and Drug Administration for treatment of rheumatoid arthritis, could attenuate the self-renewal of CD44+ liver CSCs and decrease tumorigenesis, suggesting its potential to target liver CSCs and application in the clinical management of HCC patients [27]. A phase I clinical trial (NCT02536469) was completed, which tested the anti-tumor efficacy of humanized anti-IL-8 monoclonal antibodies in solid tumors, providing possibilities to utilize the IL-8 antibody to abrogate CSCs in HCC [139]. Besides, the production of CAFs-derived COMP could be diminished by a potential agent named resolvin D1 and thus obstruct the stemness properties of HCC CSCs [87]. In addition, the FSTL1 neutralizing antibody exerted significant effects on the eradication of the hepatic CSC subset and the reversal of sorafenib resistance [92]. Overall, the inhibitors or antibodies directly targeting the secreted proteins in the TME that contribute to stemness maintenance of liver CSCs are being discovered continuously. Further development of these agents may open a new era for CSCs-based therapy. The multiple hepatic CSCs-targeted therapeutic strategies are recapitulated in Table 2.
### Table 2. The multiple hepatic CSCs-targeted therapeutic strategies.

| Approach               | Agent or Inhibitor                      | Target     | Applied Model                        | Refs.        |
|------------------------|-----------------------------------------|------------|--------------------------------------|--------------|
| Oncolytic viruses      | MV-CD133                                | CD133      | Cell lines                           | [119–121]    |
|                        | VSV-CD133                               | CD133      | Mouse Models                         |              |
| Antibody and cell-based| BsAb-CIK                                | CD133      | Cell lines                           | [122]        |
| CAR T                  | CAR-CD133                               | CD133      | Clinical Trials                      | [123,124]    |
|                        | CAR-EpCAM                               | EpCAM      | Clinical Trials                      | /            |
| Vaccines               | DC vaccines                             | CD133, CD44| Cell lines                           | [125,126]    |
|                        |                                        | EpCAM      | Mouse Models                         |              |
| Compounds              | VB4-845                                 | EpCAM      | Cell lines                           | [127]        |
|                        | isoazole-piperazine analogue            | CD133, EpCAM| Cell lines                           | [128]        |
|                        | TPGS-FA/NC                              | CD133      | Cell lines                           | [129]        |
|                        | OSU-CG5                                 | CD90       | Cell lines                           | [130]        |
|                        | Bestatin                                | CD13       | Cell lines                           | [131,132]    |
|                        | NGR-LDP-AE                              | CD13       | Cell lines                           | [133]        |
|                        | PEG-b-PLys (Ube)                        | CD13       | Cell lines                           | [134]        |
|                        | CU27                                    | CD133, CD13| Cell lines                           | [135]        |
|                        |                                        | CD47       | Mouse Models                         | [137]        |
|                        | Resolvin D1                             | CAFs-derivedCOMP| Cell lines                           | [87]         |
| Antibodies             | anti-CD44 antibody                      | CD44, CD90 | Cell lines                           | [77]         |
|                        | BsAb cG7-MICA                           | CD24       | Cell lines                           | [136]        |
|                        | anti-CD47 antibody                      | CD47       | Cell lines                           | [138]        |
|                        | 1B50-1                                  | α2δ1       | Cell lines                           | [54]         |
|                        | Tocilizumab                             | CD44       | Cell lines                           | [27]         |
|                        | FSTL1 neutralizing antibody             | CAFs-derivedFSTL1| Cell lines                           | [92]         |

### 5. Conclusions

Hepatic CSCs remain the major culprit for intratumor heterogeneity, tumor aggressiveness, relapse, metastasis and resistance to chemotherapy and targeted therapy in HCC. The existence of CSCs poses great obstacles to the durative anti-cancer effect of multiple therapies. A profound understanding of the CSCs’ behaviors and their regulatory network will pave the way for developing novel and effective anti-CSCs therapeutic strategies. Numerous studies have demonstrated that the elements in the TME function as a supportive
backup force to form a friendly niche for the maintenance of CSCs features. Our review
gives an overview of the common and newly identified surface markers for the identification
of hepatic CSCs as well as the impact of cellular components in the TME on liver CSCs.
The heterogeneous expression patterns of distinct liver CSCs are also illustrated, which may
provide novel directions for the development of CSCs-based therapy. Finally, we underline
the currently practical CSCs-targeted therapeutic approaches based on the two avenues
we summarized, in the hope of accelerating the development of CSCs-based therapy and
ultimately improving HCC patient outcomes. The advancement in single-cell RNA-seq
technology and 3D organoid culture of patient-derived tumors enables researchers to gain
a comprehensive knowledge of hepatic CSCs in HCC tumors and investigate the bidirectional
communications between CSCs and TME, facilitating our understanding of the CSC niche
and further promoting translational medical research.

Author Contributions: Conceptualization: X.-Y.G., X.F., Q.Y.; table and figures: X.F., Q.Y. and S.L.;
writing—original draft preparation: X.F.; writing—review and editing: X.-Y.G. and Q.Y. All authors
have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Shenzhen Fundamental Research Program (Grant No.
JCYJ20180508153249223), Shenzhen Science and Technology Program (Grant No. KQTD2018041118-
5028798), National Natural Science Foundation of China (Grant No. 81903049), Hong Kong Research
Grant Council (RGC) including Collaborative Research Funds (C7065-18GF, C7026-18GF and C4039-
19GF). XY Guan is Sophie YM Chan Professor in Cancer Research.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

HCC, hepatocellular carcinoma; CSCs, cancer stem cells; TME, tumor microenvironment;
PROM1, prominin 1; EpCAM, epithelial cell adhesion molecule; ICAM1, intercellular
cell adhesion molecule 1; LGR5, leucine rich repeat containing G protein-coupled receptor 5;
DLK1, delta-like 1 protein; CAFs, cancer-associated fibroblasts; ECs, endothelial cells; TAMs,
tumor-associated macrophages; TANs, tumor-associated neutrophils; NK, natural killer;
DCs, dendritic cells; MDSCs, myeloid-derived suppressor cells; HGF, hepatocyte growth
factor; COMP, cartilage oligomeric matrix protein; EMT, epithelial–mesenchymal transition;
CLCF1, cardiotoxin-like cytokine factor 1; CXCL6, chemokine (C-X-C motif) ligand 6;
FSTL1, follistatin-like 1; CXCL9, chemokine (C-X-C motif) ligand 9; PD-L1: programmed
cell death ligand 1; MSCs, mesenchymal stem cells; MCP1, monocyte chemoattractant
protein 1; CCL17, CC chemokine ligand 17; S100A9, S100 calcium-binding protein A9;
BMP2, bone morphogenetic protein 2; Regulatory T, Treg; CXCL11, C-X-C motif chemokine
11; CXCR3, chemokine (C-X-C motif) receptor 3; IFN-γ, interferon gamma; VSV-CD133,
vesicular stomatitis virus targeting CD133; CAR, chimeric antigen receptor; BsAb, bispecific
antibody; BsAb-CIK, bispecific antibody binding to cytokine-induced killer cells; PEG-
b-PLys (Ube), poly (ethylene glycol)-poly (lysine) block copolymer-ubenimex conjugate;
NKG2D, NK cell receptor group 2 member D; MICA, MHC class I-related chain A; 4Mu,
4-methylumbelliferone.
23. Tong, M.; Fung, T.M.; Luk, S.T.; Ng, K.Y.; Lee, T.K.; Lin, C.H.; Yam, J.W.; Chan, K.W.; Ng, F.; Zheng, B.J.; et al. ANXA3/JNK Signaling Promotes Self-Renewal and Tumor Growth, and its Blockade Provides a Therapeutic Target for Hepatocellular Carcinoma. Stem Cell Rep. 2015, 5, 45–59. [CrossRef] [PubMed]

24. Park, N.R.; Cha, J.H.; Jang, J.W.; Bae, S.H.; Jang, B.; Kim, J.H.; Hur, W.; Choi, J.Y.; Yoon, S.K. Synergistic Effects of CD44 and TGF-β1 through AKT/GSK-3β-β-catenin Signaling During Epithelial-Mesenchymal Transition in Liver Cancer Cells. Biochem. Biophys. Res. Comm. 2016, 477, 568–574. [CrossRef] [PubMed]

25. Toh, T.B.; Lim, J.J.; Hooi, I.; Rashid, M.; Chow, E.K. Targeting Jak/Stat Pathway as a Therapeutic Strategy Against SP/CD44+ Tumorigenic Cells in Akt/β-catenin-driven Hepatocellular Carcinoma. J. Hepatol. 2020, 72, 104–118. [CrossRef] [PubMed]

26. Zhang, J.; Han, C.; Ungerleider, N.; Chen, W.; Song, K.; Wang, Y.; Kwon, H.; Ma, W.; Wu, T. A Transforming Growth Factor-Beta and H19 Signaling Axis in Tumor-Initiating Hepatocytes that Regulates Hepatic Carcinogenesis. Hepatology 2019, 69, 1549–1563. [CrossRef]

27. Wan, S.; Zhao, E.; Kryczek, I.; Vatana, L.; Sadowskaya, A.; Ludema, G.; Simeone, D.M.; Zou, W.; Welling, T.H. Tumor-Associated Macrophages Produce Interleukin 6 and Signal Via STAT3 to Promote Expansion of Human Hepatocellular Carcinoma Stem Cells. Gastroenterology 2014, 147, 1393–1404. [CrossRef]

28. Wang, X.; Sun, W.; Shen, W.; Xia, M.; Chen, C.; Xiang, D.; Ning, B.; Cui, X.; Li, H.; Li, X.; et al. Long Non-Coding RNA DILC Regulates Liver Cancer Stem Cells Via IL-6/STAT3 Axis. J. Hepatol. 2016, 64, 1283–1294. [CrossRef]

29. Yamashita, T.; Ji, J.; Budhu, A.; Forgues, M.; Yang, W.; Wang, H.Y.; Jia, H.; Ye, Q.; Qin, L.X.; Waithier, E.; et al. EpCAM-positive Hepatocellular Carcinoma Cells are Tumor-Initiating Cells with Stem/Progenitor Cell Features. Gastroenterology 2009, 136, 1012–1024. [CrossRef]

30. Zhang, K.; Che, S.; Su, Z.; Zheng, S.; Zhang, H.; Yang, S.; Li, W.; Liu, J. CD90 Promotes Cell Migration, Viability and Sphere Forming Ability of Hepatocellular Carcinoma Cells. Int. J. Mol. Med. 2018, 41, 944–954. [CrossRef]

31. Asakura, N.; Nakamura, N.; Muroi, A.; Nojima, Y.; Yamashita, T.; Kaneko, S.; Ikeda, K.; Koshikawa, N.; Suzuki, T. Expression of Cancer Stem Cell Markers EpCAM and CD90 is Correlated with Anti- and Pro-Oncogenic EphA2 Signaling in Hepatocellular Carcinoma. Int. J. Mol. Sci. 2021, 22, 8652. [CrossRef]

32. Zhang, K.; Che, S.; Pan, C.; Su, Z.; Zheng, S.; Yang, S.; Zhang, H.; Li, W.; Wang, W.; Liu, J. The SHH/Gli Axis Regulates CD90-mediated Liver Cancer Stem Cell Function by Activating the IL6/JAK2 Pathway. J. Cell. Mol. Med. 2018, 22, 3679–3690. [CrossRef]

33. Moustafa, M.; Dähling, K.K.; Günther, A.; Riebandt, L.; Smit, D.J.; Riecken, K.; Schröder, C.; Zhuang, R.; Krech, T.; Kriegs, M.; et al. Combined Targeting of AKT and mTOR Inhibits Tumor Formation of EpCAM(+) and CD90(+) Human Hepatocellular Carcinoma Cells in an Orthotopic Mouse Model. Cancers 2022, 14, 1882. [CrossRef]

34. Zhang, X.; Jiang, P.; Shuai, L.; Chen, K.; Li, Z.; Zhang, Y.; Jiang, Y.; Li, X. MiR-589-5p Inhibits MAP3K8 and Suppresses CD90(+) Cancer Stem Cells in Hepatocellular Carcinoma. J. Exp. Clin. Cancer Res. 2016, 35, 176. [CrossRef]

35. Hu, B.; Xu, Y.; Li, Y.C.; Huang, J.F.; Cheng, J.W.; Guo, W.; Yin, Y.; Gao, Y.; Wang, P.; Wu, S.Y.; et al. CD13 Promotes Hepatocellular Carcinogenesis and Sorafenib Resistance by Activating HDAC5-LSD1-NF-kappaB Oncogenic Signaling. Clin. Transl. Med. 2020, 10, e233. [CrossRef]

36. Zhu, P.; Wang, Y.; Wu, J.; Huang, G.; Liu, B.; Ye, B.; Du, Y.; Gao, G.; Tian, Y.; He, L.; et al. LncBRM Initiates YAP1 Signalling Activation to Drive Self-Renewal of Liver Cancer Stem Cells. Nat. Commun. 2016, 7, 13608. [CrossRef]

37. Zhu, P.; Wang, Y.; Huang, G.; Ye, B.; Liu, B.; Du, Y.; He, L.; Fan, Z. Lnc-beta-Catm Elicits EZH2-dependent Beta-Catenin Stabilization and Sustains Liver CSC Self-Renewal. Nat. Struct. Mol. Biol. 2016, 23, 631–639. [CrossRef]

38. Sun, L.; Zhang, L.; Chen, J.; Li, C.; Sun, H.; Wang, J.; Xiao, H. Activation of Tyrosine Metabolism in CD13+ Cancer Stem Cells Drives Relapse in Hepatocellular Carcinoma. Cancer Res. Treat. 2020, 52, 604–621. [CrossRef]

39. Lee, T.K.; Castillo, A.; Cheung, V.C.; Tang, K.H.; Ma, S.; Ng, I.O. CD24(+) Liver Tumor-Initiating Cells Drive Self-Renewal and Tumor Initiation through STAT3-mediated NANOG Regulation. Cell Stem Cell 2011, 9, 50–63. [CrossRef]

40. Wang, R.; Li, Y.; Tsung, A.; Huang, H.; Du, Q.; Yang, M.; Deng, M.; Xiong, S.; Wang, X.; Zhang, L.; et al. INOS Promotes CD24(+) CD133(+) Liver Cancer Stem Cell Phenotype through a TACE/ADAM17-dependent Notch Signaling Pathway. Proc. Natl. Acad. Sci. USA 2018, 115, E10127–E10136. [CrossRef]

41. Lee, T.K.; Cheung, V.C.; Lu, P.; Lau, E.Y.; Ma, S.; Tang, K.H.; Tong, M.; Lo, J.; Ng, I.O. Blockade of CD47-mediated Cathepsin S/protease-activated Receptor 2 Signaling Provides a Therapeutic Target for Hepatocellular Carcinoma. Hepatology 2014, 60, 179–191. [CrossRef]

42. Guo, W.; Liu, S.; Cheng, Y.; Lu, L.; Shi, J.; Xu, G.; Li, N.; Cheng, K.; Wu, M.; Cheng, S.; et al. ICAM-1-Related Noncoding RNA in Cancer Stem Cells Maintains ICAM-1 Expression in Hepatocellular Carcinoma. Clin. Cancer Res. 2016, 22, 2041–2050. [CrossRef]

43. Liu, S.; Li, N.; Yu, X.; Xiao, X.; Cheng, K.; Hu, J.; Wang, J.; Zhang, D.; Cheng, S.; Liu, S. Expression of Intercellular Adhesion Molecule 1 by Hepatocellular Carcinoma Stem Cells and Circulating Tumor Cells. Gastroenterology 2013, 144, 1031–1041. [CrossRef]

44. Lei, Z.J.; Wang, J.; Xiao, H.L.; Guo, Y.; Wang, T.; Li, Q.; Liu, L.; Luo, X.; Fan, L.L.; Lin, L.; et al. Lysine-Specific Demethylase 1 Promotes the Stemness and Chemoresistance of Lgr5(+) Liver Cancer Initiating Cells by Suppressing Negative Regulators of Beta-Catenin Signaling. Oncogene 2015, 34, 3188–3198. [CrossRef]

45. Akbari, S.; Hunter, I.; Azbazdar, Y.; Ozhan, G.; Atabay, N.; Firtina, K.Z.; Erdal, E. LGR5/R-Spo1/Wnt3a Axis Promotes Stemness and Aggressive Phenotype in Hepatoblast-Like Hepatocellular Carcinoma Cell Lines. Cell. Signal. 2021, 82, 109972. [CrossRef]
63. Ma, S.; Chan, K.W.; Hu, L.; Lee, T.K.; Wo, J.Y.; Ng, I.O.; Zheng, B.J.; Guan, X.Y. Identification and Characterization of Tumorigenic
60. Fan, W.; Yang, X.; Huang, F.; Tong, X.; Zhu, L.; Wang, S. Identification of CD206 as a Potential Biomarker of Cancer Stem-Like
69. Jia, Q.; Zhang, X.; Deng, T.; Gao, J. Positive Correlation of Oct4 and ABCG2 to Chemotherapeutic Resistance in CD90(+)/CD133(+)
68. Zhou, K.; Nguyen, R.; Qiao, L.; George, J. Single Cell RNA-seq Analysis Identifies a Noncoding RNA Mediating Resistance to
65. Ho, D.W.; Tsui, Y.M.; Sze, K.M.; Chan, L.K.; Cheung, T.T.; Lee, E.; Sham, P.C.; Tsui, S.K.; Lee, T.K.; Ng, I.O. Single-Cell Transcriptionomics Reveals the Landscape of Intra-Tumoral Heterogeneity and Stemness-Related Subpopulations in Liver Cancer.
62. Piao, L.S.; Hur, W.; Kim, T.K.; Hong, S.W.; Kim, S.W.; Choi, J.E.; Sung, P.S.; Song, M.J.; Lee, B.C.; Hwang, D.; et al. CD133+ Liver Tumor-Initiating Cells Modulate Radiosensitivity in Human Hepatocellular Carcinoma. Cancer Lett. 2020, 459, 176–185. [CrossRef]
61. Jun, S.Y.; Jeon, S.J.; Yoon, J.Y.; Lee, J.J.; Yoon, H.R.; Choi, M.H.; Halder, D.; Lee, K.; Kim, N.S. The Positive Correlation of TIPRL with LC3 and CD133 Contributes to Cancer Aggressiveness: Potential Biomarkers for Early Liver Cancer. Sci. Rep. 2019, 9, 16802. [CrossRef]
60. Fan, W.; Yang, X.; Huang, F.; Tong, X.; Zhu, L.; Wang, S. Identification of CD206 as a Potential Biomarker of Cancer Stem-Like Cells and Therapeutic Agent in Liver Cancer. Oncol. Lett. 2019, 18, 3218–3226. [CrossRef]
59. Ma, X.; Hu, B.; Tang, W.; Xie, S.; Ren, N.; Guo, L.; Lu, R. CD73 Sustained Cancer-Stem-Cell Traits by Promoting SOX9 Expression and Stability in Hepatocellular Carcinoma. J. Hepatol. Oncol. 2020, 13, 11. [CrossRef]
58. Xu, X.; Liu, R.F.; Zhang, X.; Huang, L.Y.; Chen, F.; Fei, Q.L.; Han, Z.G. DLK1 as a Potential Target Against Cancer Stem/Progenitor Cells of Hepatocellular Carcinoma. Mol. Cancer Ther. 2012, 11, 629–638. [CrossRef]
57. Yanai, H.; Nakamura, K.; Hijioka, S.; Kamei, A.; Ikari, T.; Ishikawa, Y.; Shinozaki, E.; Mizunuma, N.; Hatake, K.; Miyajima, A. Dlk-1, a Cell Surface Antigen On Foetal Hepatic Stem/Progenitor Cells, is Expressed in Hepatocellular, Colon, Pancreas and Breast Carcinomas at a High Frequency. J. Biochem. 2010, 148, 85–92. [CrossRef]
56. Zhao, W.; Wang, L.; Han, H.; Jin, K.; Lin, N.; Guo, T.; Chen, Y.; Cheng, H.; Lu, F.; Fang, W.; et al. 1B50-1, a mAb Raised Against Recurrent Tumor Cells, Targets Liver Tumor-Initiating Cells by Binding to the Calcium Channel Alpha2delta1 Subunit. Cancer Cell 2013, 23, 541–556. [CrossRef]
55. Zhao, W.; Lv, M.; Yang, X.; Zhou, J.; Xing, B.; Zhang, Z. Liver Tumor-Initiating Cells Initiate the Formation of a Stiff Cancer Stem Cell Microenvironment Niche by Secreting LOX. Carcinogenesis 2022, 43, 766–778. [CrossRef]
54. Zhang, Y.; Zhao, W.; Han, H.; Li, S.; Chen, D.; Zhang, Z. MicroRNA-31 Suppresses the Self-Renewal Capability of Alpha2delta1(+) Liver Tumor-Initiating Cells by Targeting ISL1. Oncotarget 2017, 8, 87647–87657. [CrossRef]
53. Zhang, Y.; Zhao, W.; Han, H.; Jin, K.; Lin, N.; Guo, T.; Chen, Y.; Cheng, H.; Lu, F.; Fang, W.; et al. 1B50-1, a mAb Raised Against Recurrent Tumor Cells, Targets Liver Tumor-Initiating Cells by Binding to the Calcium Channel Alpha2delta1 Subunit. Cancer Cell 2013, 23, 541–556. [CrossRef]
52. Park, S.C.; Nguyen, N.T.; Eun, J.R.; Zhang, Y.; Tsudchy-Seney, B.; Jung, Y.J.; Theise, N.D.; Zern, M.A.; Duan, Y. CD34(+) Cells and Therapeutic Agent in Liver Cancer. Oncol. Lett. 2019, 18, 3218–3226. [CrossRef]
51. Zeng, C.; Zhang, Y.; Park, S.C.; Eun, J.R.; Nguyen, N.T.; Tsudchy-Seney, B.; Jung, Y.J.; Theise, N.D.; Zern, M.A.; Duan, Y. CD34(+) Liver Cancer Stem Cells were Formed by Fusion of Hepatobiliary Stem/Progenitor Cells with Hematopoietic Precursor-Derived Myeloid Intermediates. Stem Cells Dev. 2015, 24, 2467–2476. [CrossRef]
50. Park, S.C.; Nguyen, N.T.; Eun, J.R.; Zhang, Y.; Jung, Y.J.; Tsudchy-Seney, B.; Trotsyuk, A.; Lam, A.; Ramsamooj, R.; Zhang, Y.; et al. Identification of Cancer Stem Cell Subpopulations of CD34(+/)PLC/PRF/5 that Result in Three Types of Human Liver Carcinomas. Stem Cells Dev. 2015, 24, 1008–1021. [CrossRef]
49. Yang, W.; Wang, C.; Lin, Y.; Liu, Q.; Yu, L.X.; Tang, L.; Yan, H.X.; Fu, J.; Chen, Y.; Zhang, H.L.; et al. OV6 Protein Promotes the Stem-Like Properties of OV6(+) Cancer Cells in Hepatocellular Carcinoma. Cell Death Dis. 2017, 8, e2560. [CrossRef]
48. Wang, C.; Wang, C.; Lin, Y.; Liu, Q.; Yu, L.X.; Tang, L.; Yan, H.X.; Fu, J.; Chen, Y.; Zhang, H.L.; et al. OV6 Protein Promotes the Stem-Like Properties of OV6(+) Cancer Cells in Hepatocellular Carcinoma. Cell Death Dis. 2017, 8, e2560. [CrossRef]
47. Cao, W.; Li, M.; Liu, J.; Zhang, M.; Noordam, L.; Verstegen, M.; Wang, L.; Ma, B.; Li, S.; Wang, W.; et al. LGR5 Marks Targetable Tumor-Initiating Cells in Mouse Liver Cancer. Nat. Commun. 2020, 11, 1961. [CrossRef]
46. Ang, C.H.; Hsu, S.H.; Guo, F.; Tan, C.T.; Yu, V.C.; Vissader, J.E.; Chow, P.; Fu, N.Y. Lgr5(+) Pericentral Hepatocytes are Self-Maintained in Normal Liver Regeneration and Susceptible to Hepatocarcinogenesis. Proc. Natl. Acad. Sci. USA 2019, 116, 19530–19540. [CrossRef]
70. Yang, Z.F.; Ngai, P.; Ho, D.W.; Yu, W.C.; Ng, M.N.; Lau, C.K.; Li, M.L.; Tam, K.H.; Lam, C.T.; Poon, R.T.; et al. Identification of Local and Circulating Cancer Stem Cells in Human Liver Cancer. *Hepatology* 2008, 47, 919–928. [CrossRef] PubMed

71. Yamanaka, C.; Wada, H.; Eguchi, H.; Hatano, H.; Gotoh, K.; Noda, T.; Yamada, D.; Asaoka, T.; Kawamoto, K.; Nagano, H.; et al. Clinical Significance of CD13 and Epithelial Mesenchymal Transition (EMT) Markers in Hepatocellular Carcinoma. *Jpn. J. Clin. Oncol.* 2018, 48, 52–60. [CrossRef] [PubMed]

72. Yang, Y.; Hou, J.; Lin, Z.; Zhuo, H.; Chen, D.; Zhang, X.; Chen, Y.; Sun, B. Attenuated Listeria Monocytogenes as a Cancer Vaccine Vector for the Delivery of CD24, a Biomarker for Hepatic Cancer Stem Cells. *Cell. Mol. Immunol.* 2014, 11, 184–196. [CrossRef] [PubMed]

73. Zhu, J.; Yu, H.; Chen, S.; Yang, P.; Dong, Z.; Ling, Y.; Tang, H.; Bai, S.; Yang, W.; Tang, L.; et al. Prognostic Significance of Combining High Mobility Group Box-1 and OV-6 Expression in Hepatocellular Carcinoma. *Sci. China Life Sci.* 2018, 61, 912–923. [CrossRef] [PubMed]

74. Gu, Y.; Zheng, X.; Ji, J. Liver Cancer Stem Cells as a Hierarchical Society: Yes Or No? *Acta Biochim. Biophys. Sin.* 2020, 52, 723–735. [CrossRef]

75. Yamashita, T.; Honda, M.; Nakamoto, Y.; Baba, M.; Nio, K.; Haru, Y.; Zeng, S.S.; Hayashi, T.; Kondo, M.; Takatori, H.; et al. Combining High Mobility Group Box-1 and OV-6 Expression in Hepatocellular Carcinoma. *Cancer Cell* 2018, 38, 302–316. [CrossRef] [PubMed]

76. Lau, E.Y.; Lo, J.; Cheng, B.Y.; Ma, M.K.; Lee, J.M.; Ng, J.K.; Chai, S.; Lin, C.H.; Tsang, S.Y.; Ma, S.; et al. Cancer-Associated Fibroblasts Promotes Hepatocellular Carcinoma Metastasis and Stemness. *Cancer Manag. Res.* 2015, 7, 140. [CrossRef] [PubMed]

77. Muller, L.; Tungger, A.; Plesca, I.; Wehner, R.; Temme, A.; Westphal, D.; Meier, F.; Bachmann, M.; Schmitz, M. Bidirectional Crosstalk Between Cancer Stem Cells and Immune Cell Subsets. *Front. Immunol.* 2020, 11, 140. [CrossRef]

78. Sun, L.; Wang, Y.; Wang, L.; Yao, B.; Chen, T.; Li, Q.; Liu, Z.; Liu, R.; Niu, Y.; Song, T.; et al. Lineage Tracing and Single-Cell Analysis Reveal Proliferative From1 + Tumour-Propagating Cells and their Dynamic Cellular Transition During Liver Cancer Progression. *Gut* 2022, 71, 1656–1666. [CrossRef]

79. Fang, T.; Lv, H.; Lv, G.; Li, T.; Wang, C.; Han, Q.; Yu, L.; Su, B.; Guo, L.; Huang, S.; et al. Tumor-Derived Exosomal miR-1247-3p Induces Cancer-Associated Fibroblast Activation to Foster Lung Metastasis of Liver Cancer. *Nat. Commun.* 2019, 10, 191. [CrossRef] PubMed

80. Oshimori, N.; Guo, Y.; Taniguchi, S. An Emerging Role for Cellular Crosstalk in the Cancer Stem Cell Niche. *J. Pathol.* 2021, 254, 384–394. [CrossRef]

81. Zheng, X.; Yu, C.; Xu, M. Linking Tumor Microenvironment to Plasticity of Cancer Stem Cells: Mechanisms and Application in Cancer Therapy. *Front. Oncol.* 2021, 11, 678333. [CrossRef] PubMed

82. Muller, L.; Tungger, A.; Plesca, I.; Wehner, R.; Temme, A.; Westphal, D.; Meier, F.; Bachmann, M.; Schmitz, M. Bidirectional Crosstalk Between Cancer Stem Cells and Immune Cell Subsets. *Front. Immunol.* 2020, 11, 140. [CrossRef]

83. Lau, E.Y.; Lo, J.; Cheng, B.Y.; Ma, M.K.; Lee, J.M.; Ng, J.K.; Chai, S.; Lin, C.H.; Tsang, S.Y.; Ma, S.; et al. Cancer-Associated Fibroblasts Regulate Tumor-Initiating Cell Plasticity in Hepatocellular Carcinoma through c-Met/FRα1/HEY1 Signaling. *Cell Rep.* 2016, 15, 1175–1189. [CrossRef] PubMed

84. Xiong, S.; Wang, R.; Chen, Q.; Luo, J.; Wang, J.; Zhao, Z.; Li, Y.; Wang, Y.; Wang, X.; Cheng, B. Cancer-Associated Fibroblasts Promote Stem Cell-Like Properties of Hepatocellular Carcinoma Cells through IL-6/STAT3/Notch Signaling. *Am. J. Cancer Res.* 2018, 8, 302–316. [CrossRef]

85. Fang, T.; Lv, H.; Lv, G.; Li, T.; Wang, C.; Han, Q.; Yu, L.; Su, B.; Guo, L.; Huang, S.; et al. Tumor-Derived Exosomal miR-1247-3p Induces Cancer-Associated Fibroblast Activation to Foster Lung Metastasis of Liver Cancer. *Nat. Commun.* 2019, 10, 191. [CrossRef] PubMed

86. Li, Y.; Wang, R.; Xiong, S.; Wang, X.; Zhao, Z.; Bai, S.; Wang, Y.; Zhao, Y.; Cheng, B. Cancer-Associated Fibroblasts Promote the Stemness of CD24(+) Liver Cells Via Paracrine Signaling. *J. Mol. Med.* 2019, 97, 243–255. [CrossRef]

87. Sun, L.; Wang, Y.; Wang, L.; Yao, B.; Chen, T.; Li, Q.; Liu, Z.; Liu, R.; Niu, Y.; Song, T.; et al. Resolvin D1 Prevents Epithelial-Mesenchymal Transition and Reduces the Stemness Features of Hepatocellular Carcinoma by Inhibiting Paracrine of Cancer-Associated Fibroblast-Derived COMP. *J. Exp. Clin. Cancer Res.* 2019, 38, 170. [CrossRef]

88. Li, Q.; Wang, C.; Wang, Y.; Sun, L.; Liu, Z.; Wang, L.; Song, T.; Yao, Y.; Liu, Q.; Tu, K. HSCs-derived COMP Drives Hepatocellular Carcinoma Progression by Activating MEK/ERK and PI3K/AKT Signaling Pathways. *J. Exp. Clin. Cancer Res.* 2018, 37, 231. [CrossRef] PubMed

89. Zhao, Z.; Bai, S.; Wang, R.; Xiong, S.; Li, Y.; Wang, X.; Chen, W.; Cheng, B. Cancer-Associated Fibroblasts Endow Stem-Like Qualities to Liver Cancer Cells by Modulating Autophagy. *Cancer Manag. Res.* 2019, 11, 5737–5744. [CrossRef]

90. Song, M.; He, J.; Pan, Q.Z.; Yang, J.; Zhao, J.; Zhang, Y.J.; Huang, Y.; Tang, Y.; Wang, Q.; He, J.; et al. Cancer-Associated Fibroblast-Mediated Cellular Crosstalk Supports Hepatocellular Carcinoma Progression. *Hepatology* 2021, 73, 1717–1735. [CrossRef]

91. Liu, B.; Fang, X.; Kwong, D.L.; Zhang, Y.; Verhoeft, K.; Gong, L.; Zhang, B.; Chen, J.; Yu, Q.; Luo, J.; et al. Targeting TROY-mediated PS8A/AKT/TBX3 Signaling Attenuates Tumor Stemness and Elevates Treatment Response in Hepatocellular Carcinoma. *J. Exp. Clin. Cancer Res.* 2022, 41, 182. [CrossRef] PubMed

92. Loh, J.J.; Li, T.W.; Zhou, L.; Wong, T.L.; Liu, X.; Ma, V.; Lo, C.M.; Man, K.; Lee, T.K.; Ning, W.; et al. FSTL1 Secreted by Activated Fibroblasts Promotes Hepatocellular Carcinoma Metastasis and Stemness. *Cancer Res.* 2021, 81, 5692–5705. [CrossRef] PubMed

93. Yao, H.; Liu, N.; Lin, M.C.; Zheng, J. Positive Feedback Loop Between Cancer Stem Cells and Angiogenesis in Hepatocellular Carcinoma. *Cancer Lett.* 2016, 379, 213–219. [CrossRef] PubMed
94. Ding, Q.; Xia, Y.; Ding, S.; Lu, P.; Sun, L.; Fan, Y.; Li, X.; Wang, Y.; Tian, D.A.; Liu, M. Potential Role of CXCL9 Induced by Endothelial cells/CD133+ Liver Cancer Cells Co-Culture System in Tumor Transendothelial Migration. *Genes Cancer* 2016, 7, 254–259. [CrossRef] [PubMed]

95. Wei, R.; Shi, D.; Liang, Z.; Liu, Y.; Li, Y.; Xing, Y.; Liu, W.; Ai, Z.; Zhuang, J.; Chen, X.; et al. IL-17A Secreted From Lymphatic Endothelial Cells Promotes Tumorigenesis by Upregulation of PD-L1 in Hepatoma Stem Cells. *J. Hepatol.* 2019, 71, 1206–1215. [CrossRef] [PubMed]

96. Firtina, K.Z.; Koc, D.; Sahin, E.; Avci, S.T.; Yilmaz, M.; Atabey, N.; Erdal, E. Effect of Adipocyte-Secreted Factors On EpCAM+ /CD133+ Hepatic Stem Cell Population. *Biochem. Biophys. Res. Commun.* 2016, 474, 482–490. [CrossRef] [PubMed]

97. Fan, Q.M.; Jing, Y.Y.; Yu, G.F.; Kou, X.R.; Ye, F.; Gao, L.; Li, R.; Zhao, Q.B.; Yang, Y.; Lu, Z.H.; et al. Tumor-Associated Macrophages Promote Cancer Stem-Cell-Like Properties Via Transforming Growth Factor-Beta1-Induced Epithelial-Mesenchymal Transition in Hepatocellular Carcinoma. *Cancer Lett.* 2014, 352, 160–168. [CrossRef] [PubMed]

98. Zhu, F.; Li, X.; Chen, S.; Zeng, Q.; Zhao, Y.; Luo, F. Tumor-Associated Macrophage Or Chemokine Ligand CCL17 Positively Regulates the Tumorigenesis of Hepatocellular Carcinoma. *Med. Oncol.* 2016, 33, 17. [CrossRef]

99. Li, X.F.; Chen, C.; Xiang, D.M.; Qu, L.; Sun, W.; Lu, X.Y.; Zhou, T.F.; Chen, S.Z.; Ning, B.F.; Cheng, Z.; et al. Chronic Inflammation-Elicted Liver Progenitor Cell Conversion to Liver Cancer Stem Cell with Clinical Significance. *Hepatology* 2017, 66, 1934–1951. [CrossRef]

100. Wei, R.; Zhu, W.W.; Yu, G.Y.; Wang, X.; Gao, C.; Zhou, X.; Lin, Z.F.; Shao, W.Q.; Wang, S.H.; Lu, M.; et al. S100 Calcium-Binding Protein A9 From Tumor-Associated Macrophage Enhances Cancer Stem Cell-Like Properties of Hepatocellular Carcinoma. *Int. J. Cancer* 2021, 148, 1233–1244. [CrossRef] [PubMed]

101. Zhong, M.; Zhong, C.; Cui, W.; Wang, Y.; Tian, D.A.; Liu, M. Potential Role of CXCL9 Induced by Endothelial cells/CD133+ Liver Cancer Cells Co-Culture System in Tumor Transendothelial Migration. *Genes Cancer* 2016, 7, 254–259. [CrossRef] [PubMed]

102. Zhou, S.L.; Yin, D.; Hu, Z.Q.; Luo, C.B.; Zhou, Z.J.; Xin, H.Y.; Yang, X.R.; Shi, Y.H.; Wang, Z.; Huang, X.W.; et al. A Positive Feedback Loop Between Cancer Stem-Like Cells and Tumor-Associated Neutrophils Controls Hepatocellular Carcinoma Progression. *Hepatology* 2019, 70, 1214–1230. [CrossRef] [PubMed]

103. Tsuchiya, H.; Shiota, G. Immune Evasion by Cancer Stem Cells. *Regen. Ther.* 2021, 17, 20–33. [CrossRef]

104. Yu, X.; Li, H.; Ren, X. Interaction Between Regulatory T Cells and Cancer Stem Cells. *Int. J. Cancer* 2012, 131, 1491–1498. [CrossRef]

105. Shi, C.; Chen, Y.; Chen, Y.; Yang, Y.; Binging, W.; Qi, J. CD4(+) CD25(+) Regulatory T Cells Promote Hepatocellular Carcinoma Invasion Via TGF-beta1-induced Epithelial-Mesenchymal Transition. *Onco Targets Ther.* 2019, 12, 279–289. [CrossRef]

106. Zhang, Y.; Zhao, W.; Li, S.; Lv, M.; Yang, X.; Li, M.; Zhang, Z. CXCL11 Promotes Self-Renewal and Tumorigenicity of Alphaf2delta1(+) Liver Tumor-Initiating Cells through CXCR3/ERK1/2 Signaling. *Cancer Lett.* 2019, 449, 163–171. [CrossRef]

107. Song, M.; Ping, Y.; Zhang, K.; Yang, L.; Li, F.; Zhang, C.; Cheng, S.; Yue, D.; Mainema, N.R.; Qu, J.; et al. Low-Dose IFN gamma Induces Tumor Cell Stemness in Tumor Microenvironment of Non-Small Cell Lung Cancer. *Cancer Res.* 2019, 79, 3737–3748. [CrossRef]

108. Xiang, T.; Long, H.; He, L.; Han, X.; Lin, K.; Liang, Z.; Zhuo, W.; Xie, R.; Zhu, B. Interleukin-17 Produced by Tumor Microenvironment Promotes Self-Renewal of CD133+ Cancer Stem-Like Cells in Ovarian Cancer. *Oncogene* 2015, 34, 165–176. [CrossRef]

109. Zhang, Y.; Zoltan, M.; Riquelme, E.; Xu, H.; Sahin, I.; Castro-Pando, S.; Montiel, M.F.; Chang, K.; Jiang, Z.; Ling, J.; et al. Immune Cell Production of Interleukin 17 Induces Stem Cell Features of Pancreatic Intraepithelial Neoplasia Cells. *Gastroenterology* 2018, 155, 210–223. [CrossRef]

110. Jiang, Y.X.; Yang, S.W.; Li, P.A.; Luo, X.; Li, Z.Y.; Hao, Y.X.; Yu, P.W. The Promotion of the Transformation of Quiescent Gastric Cancer Stem Cells by IL-17 and the Underlying Mechanisms. *Oncogene* 2017, 36, 1256–1264. [CrossRef]

111. Shokouhifar, A.; Firouzi, J.; Nouri, M.; Sarab, G.A.; Ebrahimi, M. NK Cell Upraise in the Dark World of Cancer Stem Cells. *Cancer Lett.* 2019, 417, 1–9. [CrossRef] [PubMed]

112. Schwartz, M.; Zhang, Y.; Rosenblatt, J.D. B Cell Regulation of the Anti-Tumor Response and Role in Carcinogenesis. *J. Immunother. Cancer* 2016, 4, 40. [CrossRef] [PubMed]

113. Kimura, Y.; Tsumedomi, R.; Yoshimura, K.; Matsukuma, S.; Shindo, Y.; Matsui, H.; Tokumitsu, Y.; Yoshida, S.; Iida, M.; Suzuki, N.; et al. Immune Evasion of Hepatoma Cancer Stem-Like Cells from Natural Killer Cells. *Ann. Surg. Oncol.* 2022. [CrossRef]

114. Zhong, M.; Zhong, C.; Cui, W.; Wang, G.; Zheng, G.; Li, L.; Zhang, J.; Ren, R.; Gao, H.; Wang, T.; et al. Induction of Tolerogenic Dendritic Cells by Activated TGF-beta1/Akt/Smad2 Signaling in RIG-I-deficient Stemness-High Human Liver Cancer Cells. *BMC Cancer* 2019, 19, 439. [CrossRef] [PubMed]

115. Ruiz, D.G.M.; Bresnahan, E.; Molina-Sanchez, P.; Lindblad, K.E.; Maier, B.; Sia, D.; Puigvehi, M.; Miguela, V.; Casanova-Acebes, M.; Dhaanaut, M.; et al. Beta-Catenin Activation Promotes Immune Escape and Resistance to Anti-PD-1 Therapy in Hepatocellular Carcinoma. *Cancer Discov.* 2019, 9, 1124–1141. [CrossRef] [PubMed]

116. Ma, C.; Zhang, Q.; Greten, T.F. MDSCs in Liver Cancer: A Critical Tumor-Promoting Player and a Potential Therapeutic Target. *Cell. Immunol.* 2021, 361, 104295. [CrossRef]

117. Xu, M.; Zhao, Z.; Song, J.; Lan, X.; Lu, S.; Chen, M.; Wang, Z.; Chen, W.; Fan, X.; Wu, F.; et al. Interactions Between Interleukin-6 and Myeloid-Derived Suppressor Cells Drive the Chemoresistant Phenotype of Hepatocellular Cancer. *Exp. Cell Res.* 2017, 351, 142–149. [CrossRef]
118. Peng, D.; Tanikawa, T.; Li, W.; Zhao, L.; Vatan, L.; Szelig, W.; Wan, S.; Wei, S.; Wang, Y.; Liu, Y.; et al. Myeloid-Derived Suppressor Cells Endow Stem-Like Qualities to Breast Cancer Cells through IL6/STAT3 and NO/NOTCH Cross-Talk Signaling. *Cancer Res.* 2016, 76, 3156–3165. [CrossRef]

119. Cao, G.D.; He, X.B.; Sun, Q.; Chen, S.; Wan, K.; Xu, F.; Feng, X.; Li, P.P.; Chen, B.; Xiong, M.M. The Oncolytic Virus in Cancer Diagnosis and Treatment. *Front. Oncol.* 2020, 10, 1786. [CrossRef]

120. Bach, P.; Abel, T.; Hoffmann, C.; Gal, Z.; Braun, G.; Voelker, J.; Ball, C.R.; Johnston, I.C.; Lauer, U.M.; Herold-Mende, C.; et al. Specific Elimination of CD133+ Tumor Cells with Targeted Oncolytic Measles Viruses. *Cancer Res.* 2013, 73, 865–874. [CrossRef]

121. Kleinlutzum, D.; Hanauer, J.; Muik, A.; Henschmann, K.M.; Kays, S.K.; Ayala-Breton, C.; Peng, K.W.; Muhlebach, M.D.; Abel, T.; Buchholz, C.J. Enhancing the Oncolytic Activity of CD133-Targeted Measles Virus: Receptor Extension or Chimerism with Vesicular Stomatitis Virus are Most Effective. *Front. Oncol.* 2017, 7, 127. [CrossRef] [PubMed]

122. Huang, J.; Li, C.; Wang, Y.; Lv, H.; Guo, Y.; Dai, H.; Wicha, M.S.; Chang, A.E.; Li, Q. Cytokine-Induced Killer (CIK) Cells Bound with anti-CD3/anti-CD133 Bispecific Antibodies Target CD133(high) Cancer Stem Cells in Vitro and in Vivo. *Clin. Immunol.* 2013, 149, 156–168. [CrossRef] [PubMed]

123. Dai, H.; Tong, C.; Shi, D.; Chen, M.; Guo, Y.; Chen, D.; Han, X.; Wang, H.; Wang, Y.; Shen, P. Efficacy and Biomarker Analysis of CD133-directed CAR T Cells in Advanced Hepatocellular Carcinoma: A Single-Arm, Open-Label, Phase II Trial. *Oncoimmunology* 2020, 9, 1846926. [CrossRef] [PubMed]

124. Wang, Y.; Chen, M.; Wu, Z.; Tong, C.; Dai, H.; Guo, Y.; Liu, Y.; Huang, J.; Lv, H.; Luo, C.; et al. CD133-directed CAR T Cells for Advanced Malignancies: A Phase I Trial. *Oncoimmunology* 2018, 7, e140169. [CrossRef] [PubMed]

125. Sun, J.C.; Pan, K.; Chen, M.S.; Wang, Q.J.; Wang, H.; Ma, H.Q.; Li, Y.Q.; Liang, X.T.; Li, J.J.; Zhao, J.J.; et al. Specific Elimination of CD133+ Tumor Cells with Targeted Oncolytic Measles Viruses. *Cancer Res.* 2013, 73, 865–874. [CrossRef]

126. Dai, H.; Tong, C.; Shi, D.; Chen, M.; Guo, Y.; Chen, D.; Han, X.; Wang, H.; Wang, Y.; Shen, P. Efficacy and Biomarker Analysis of CD133-directed CAR T Cells in Advanced Hepatocellular Carcinoma: A Single-Arm, Open-Label, Phase II Trial. *Oncoimmunology* 2020, 9, 1846926. [CrossRef] [PubMed]

127. Ogawa, K.; Tanaka, S.; Matsumura, S.; Murakata, A.; Ban, D.; Ochiai, T.; Irie, T.; Kudo, A.; Nakamura, N.; Tanabe, M.; et al. Efficacy and Biomarker Analysis of CD133-directed CAR T Cells in Advanced Hepatocellular Carcinoma: A Single-Arm, Open-Label, Phase II Trial. *Oncoimmunology* 2020, 9, 1846926. [CrossRef] [PubMed]

128. Choi, Y.J.; Park, S.J.; Park, Y.S.; Park, H.S.; Yang, K.M.; Heo, K. EpCAM Peptide-Primed Dendritic Cell Vaccination Confers Significant Anti-Tumor Immunity in Hepatocellular Carcinoma Cells. *PloS ONE* 2018, 13, e0190638. [CrossRef]

129. Li, D.; Zhang, Q.; Zhou, Y.; Zhu, H.; Li, T.; Du, F. A Novel Nitidine Chloride Nanoparticle Overcomes the Stemness of CD133(+)EPCAM(+) Huh7 Hepatocellular Carcinoma Cells for Liver Cancer Therapy. *BMC Pharmacol. Toxicol.* 2022, 23, 48. [CrossRef]

130. Chen, W.C.; Chang, Y.S.; Hsu, H.P.; Yen, M.C.; Huang, H.L.; Cho, C.Y.; Wang, C.Y.; Weng, T.Y.; Lai, P.T.; Chen, C.S.; et al. Therapeutics Targeting CD90-integrin-AMPK-CD133 Signal Axis in Liver Cancer. *Oncotarget* 2015, 6, 42923–42937. [CrossRef]

131. Dou, C.; Fang, C.; Zhao, Y.; Fu, X.; Zhang, Y.; Zhu, D.; Wu, H.; Liu, H.; Zhang, J.; Xu, W.; et al. BC-02 Eradicates Liver Cancer Stem Cells by Upregulating the ROS-dependent DNA Damage. *Int. J. Oncol.* 2017, 51, 1775–1784. [CrossRef] [PubMed]

132. Toshiyama, R.; Konno, M.; Eguchi, H.; Takemoto, H.; Noda, T.; Asai, A.; Koseki, J.; Haraguchi, N.; Ueda, Y.; Matsushita, K.; et al. Poly(Ethylene Glycol)-Poly(Lysine) Block Copolymer-Ubenimex Conjugate Targets Aminopeptidase N and Exerts an Antitumor Effect in Hepatocellular Carcinoma Stem Cells. *Mol. Carcinog.* 2016, 55, 244–260. [CrossRef] [PubMed]

133. Yamashita, M.; Hara, T.; Hara, H.; Tsukamoto, H.; Nakamura, Y.; Nakamura, N.; Tanabe, M.; et al. Functional Screening Identifies a New Organic Selenium Compound Targeting Cancer Stem Cells: Role of c-Myc Transcription Activity Inhibition in Hepatocellular Carcinoma Stem Cells. *Oncogene* 2019, 38, 244–260. [CrossRef] [PubMed]

134. Zhou, J.N.; Zhang, B.; Wang, H.Y.; Wang, D.X.; Zhang, M.M.; Zhang, M.; Wang, X.K.; Fan, S.Y.; Xu, Y.C.; Zeng, Q.; et al. A Functional Screening Identifies a New Organic Selenium Compound Targeting Cancer Stem Cells: Role of c-Myc Transcription Activity Inhibition in Hepatocellular Carcinoma. *Adv. Sci.* 2022, 9, e2201166. [CrossRef] [PubMed]

135. Han, Y.; Sun, F.; Zhang, X.; Wang, T.; Jiang, J.; Cai, J.; Gao, Q.; Hezam, K.; Liu, Y.; Xie, J.; et al. CD24 Targeting Bi-Specific Antibody that Simultaneously Stabilizes NKG2D Enhances the Efficacy of Cancer Immunotherapy. *J. Cancer Res. Clin. Oncol.* 2019, 145, 1179–1190. [CrossRef]

136. Rodriguez, M.M.; Fiore, E.; Bayo, J.; Atorrasagasti, C.; Garcia, M.; Onorato, A.; Dominguez, L.; Malvincini, M.; Mazzolini, G. 4Mu Decreases CD47 Expression on Hepatic Cancer Stem Cells and Primes a Potent Antitumor T Cell Response Induced by Interleukin-12. *Mol. Ther.* 2018, 26, 2738–2750. [CrossRef]

137. Lo, J.; Lau, E.Y.; Ching, R.H.; Cheng, B.Y.; Ma, M.K.; Ng, I.O.; Lee, T.K. Nuclear Factor Kappa B-mediated CD47 Up-Regulation Promotes Sorafenib Resistance and its Blockade Synergizes the Effect of Sorafenib in Hepatocellular Carcinoma in Mice. *Hepatology* 2015, 62, 534–545. [CrossRef]

138. Bilusic, M.; Heery, C.R.; Collins, J.M.; Donahue, R.N.; Palena, C.; Madan, R.A.; Karzai, F.; Marte, J.L.; Strauss, J.; Gatti-Mays, M.E.; et al. Phase I Trial of HuMax-IL8 (BMS-986253), an anti-IL-8 Monoclonal Antibody, in Patients with Metastatic Or Unresectable Solid Tumors. *J. Immunother. Cancer* 2019, 7, 240. [CrossRef]