Discover Oncology

Review

Biological roles and clinical applications of EpCAM in HCC

Peng Liu[1] · **Qun Zhang**[2] · **Fengchao Liu**[2]

Received: 5 December 2024 / Accepted: 7 March 2025
Published online: 14 March 2025

Abstract
Epithelial cell adhesion molecule (EpCAM) is an important biomarker in tumors. In hepatocellular carcinoma (HCC), EpCAM + cells exhibit high invasiveness, tumorigenic ability, therapeutic resistance, and self-renewal ability, often identified as liver cancer stem cells (CSCs). Detecting EpCAM + cells in tumor lesions and circulation is valuable for predicting patient prognosis and monitoring therapeutic outcomes, emphasizing its clinical significance. Given its broad expression in HCC, especially in CSCs and circulating tumor cells (CTCs), EpCAM-targeting agents have garnered substantial research interest. However, the role of EpCAM in HCC progression and its regulatory mechanisms remains poorly understood. Furthermore, clinical applications of EpCAM, such as liquid biopsy and targeted therapies, are still controversial. This review summarizes the biological properties of EpCAM + HCC cells, explores the regulatory mechanisms governing EpCAM expression, and discusses its clinical significance of using EpCAM as a prognostic marker and therapeutic target.

Keywords EpCAM · HCC · Circulating tumour cells · Biomarker · Cancer stem cells

1 Introduction

HCC is a leading cause of cancer-related mortality worldwide. Despite advancements in treatment, including hepatic resection, liver transplantation, chemoembolization and systemic therapies, only surgical options, such as liver transplantation and hepatic resection, are considered potentially curative. However, their efficacy is limited due to high recurrence rates after resection and restricted patient eligibility, as most cases are diagnosed at advanced stages [1, 2]. Early diagnosis significantly improves prognosis, but current screening methods, such as alpha-fetoprotein (AFP) testing, lack sufficient sensitivity for early detection [3, 4].

HCC is marked by a predominant intratumoral heterogeneity, which correlates with worse clinical outcomes [5]. This heterogeneity, compounded by the lack of specific molecular signatures to guide personalized treatment regimens, hinders therapeutic efficacy [6]. Liver CSCs are thought to drive this heterogeneity in HCC [7]. Various biomarkers, including EpCAM, CD133, CD90, CD13, CD44, CD24, CD47, ICAM1, α2δ1, keratin19, OV6, and Lgr5, have been identified to define distinct HCC CSC populations [8, 9]. Each subpopulation may harbor unique oncogenic drivers, complicating efforts to develop effective molecularly targeted therapies. Understanding the cellular functions and regulatory mechanisms of these subpopulations, particularly those at the top of the cellular hierarchy, is critical for improving HCC treatment strategies.

Among these biomarkers, EpCAM stands out as a type I transmembrane glycoprotein expressed in epithelial tissues that was initially identified as a surface antigen in colorectal carcinoma [10]. Subsequent research, EpCAM has revealed

✉ Fengchao Liu, lfchao2012@163.com | [1]Organ Transplantation Center, The Affiliated Hospital of Qingdao University, Qingdao, China. [2]Liver Disease Center, The Affiliated Hospital of Qingdao University, Qingdao, China.

its roles as a prognostic marker, therapeutic target, and anchor molecule for CTCs [11]. EpCAM is a multifunctional transmembrane protein involved in various cancer-related processes, cell proliferation, adhesion, stemness, metabolism, metastasis, chemo- and radio-resistance, angiogenesis, migration, and epithelial-mesenchymal transition (EMT) [11–13]. Its versatility makes it an attractive target for new diagnostic and prognostic approaches in cancer biology.

This review provides a comprehensive overview of EpCAM + cells in HCC, focusing on their properties, interactions with other signaling pathways, and potential clinical applications. Additionally, the progress in EpCAM-based therapies is discussed, shedding light on their implications in HCC management.

2 Biological properties of EpCAM + HCC cells

The expression of EpCAM in HCC is significantly correlated with higher tumor grades and elevated serum AFP levels. Downregulation of EpCAM gene expression significantly decreases the proliferation and invasiveness of HCC cells [14]. Research has shown that EpCAM + cells express hepatic stem cell markers, efficiently form nonadherent spheroids, and demonstrate greater aggressiveness and tumorigenicity compared to EpCAM- cells, demonstrating their stem/progenitor cell-like characteristics [15, 16]. Furthermore, EpCAM-expressing proliferating ductal cells (PDC) in inflamed livers have been identified as potential cellular origins of HCC [17].

A small proportion of EpCAM + cells present in advanced cirrhosis possesses self-renewal capabilities mediated by autocrine Wnt signaling, making them prone to progression into HCC [18]. Follow-up studies of patients with compensated HCV-related cirrhosis showed that EpCAM expression is an independent predictor of HCC occurrence [19]. Additionally, Ogasawara et al. revealed that EpCAM + hepatocellular-cholangiocarcinoma (CHC) cells exhibit stem cell-like features, high tumorigenicity, and the ability to develop tumors with CHC-like tumors, suggesting CHCs may originate from EpCAM(+) cells [20].

EpCAM + HCC CSCs have also been shown to resist natural killer (NK) cell-mediated cytotoxicity by up-regulating carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) expression [21]. Studies found that EpCAM frequently co-expresses with other markers, with these co-expressing cells potentially representing a more precise CSC phenotype. For example, CD133 + EpCAM + cells in Huh7 cells exhibit enhanced self-renewal, differentiation, drug resistance, and tumorigenesis [22]. Liao et al. identified a subset of Wnt-activity^{high}ALDH1 + EpCAM + triple-positive cells as the most tumorigenic, stem cell-like, and phenotypically plastic subpopulation of cells in HCC, referred to as "superpotent CSCs" (spCSCs) [23]. Similarly, EpCAM + AFP + cells, a highly aggressive subgroup associated with metastasis, treatment resistance, and poor prognosis, have been designated hepatic stem cell-like HCC (HPSC HCC) due to their stemness and tumorigenic properties [24].

3 Regulatory mechanisms of EpCAM expression

3.1 Key molecules of EpCAM + HCC cells discovered by Omics techniques

To investigate the regulatory mechanisms of EpCAM + HCC cells, various omics approaches have identified several key molecules. Using GeneNet h50K shRNA library and RNA interference (RNAi) screening, Takai et al. identified PMPCB as a top candidate gene with a synthetic lethal interaction with EpCAM. Blocking PMPCB inhibits EpCAM expression and Wnt/β-catenin signaling by inducing mitochondria-related reactive oxygen species and FOXO activities [25].

Whole exome sequencing of sorted EpCAM + and EpCAM− HCC cells revealed that protocadherin 18 (PCDH18) is functionally suppressed by somatic mutations in a subset of EpCAM + HCC cells, potentially playing an important role in their development [26]. Transcriptomic analysis by Zhao et al. identified YY1-associated protein 1 (YY1AP1) as a critical regulator of EpCAM + AFP + HCC cell survival, with YY1 binding to EpCAM promoter to drive its transcription [27].

Single-cell RNA sequencing (scRNA-seq) by Ho et al. provided detailed transcriptomic landscape of HCC EpCAM + and EpCAM− cells, showing similar mutational profiles between these subtypes. This suggests that mutation acquisition may not be the main factor driving subtype emergence. Notably, scRNA-seq data revealed that EpCAM + cells were enriched in lipid metabolism-related genes, while EpCAM- cells showed higher expression of genes related to the translation and RNA processing [28].

Transcriptomic sequencing also identified epidermal growth factor receptor kinase substrate 8-like protein 3 (EPS8L3) as associated with CD24/CD13/EpCAM-triple positivity in liver CSCs. Akt signaling-driven SP1 upregulated EPS8L3

expression, promoting advanced tumor stages in HCC [29]. Microarray analysis by Zeng et al. found that the Spalt Like Transcription Factor 4 (SALL4) is upregulated in EpCAM + HCC cells, underscoring its important role in maintaining their stemness [30]. These findings collectively provide a deeper understanding of the biological properties and regulatory mechanisms of EpCAM + HCC cells, offering insights into their potential as therapeutic targets.

3.2 miRNAs and EpCAM + HCC cells

Using small RNA deep sequencing, Ji et al. identified miR-155 as overexpressed in EpCAM + HCC cells and demonstrated its critical role in maintaining stemness. They further demonstrated that miR-155 plays an important role in the maintenance of EpCAM + HCC cell stemness [31]. Microarray-based global miRNA profiling methods revealed that members of the miR-181 family are upregulated in EpCAM + HCC cells. Elevated miR-181 expression supports stem cell properties in these cells, while its blockade reduces the proportion of EpCAM + HCC cells and induces their differentiation [32]. The enrichment of miR-429 in EPCAM + HCC cells promotes liver CSC properties by targeting retinoblastoma-binding protein 4(RBBP4) [33]. Additionally, miR-26b-5p has been shown to maintain EpCAM + cells stemness by targeting HSPA8 [34]. miR-30e-3p directly targets EpCAM, contributing to its role in stemness maintenance [35].

3.3 HBx and EpCAM + HCC cells

Chronic viral hepatitis B is a major risk factor for hepatocarcinogenesis, and HBV X protein (HBx) encoded by hepatitis B virus (HBV) is implicated in HCC pathogenesis and HCC CSC maintenance. HBx induces EpCAM expression via RelA-dependent demethylation [36]. Furthermore, HBx activates β-catenin signaling and upregulates miR-181, enhancing EpCAM expression [37]. It also induces EpCAM expression in HCC by activating histone demethylase KDM5B [38]. Wang et al. demonstrated that HBx promotes HCC formation by promoting the expansion and tumorigenicity of EpCAM + hepatic progenitor cells (HPCs) in a DDC-induced mouse model, suggesting a potential origin for HCC arising from chronic hepatitis infection [39].

3.4 Other regulators

Yamashita et al., identified two Tcf binding elements in the EpCAM promoter, showing that EpCAM is a direct transcriptional target of Tcf/β-catenin in HCC cells. They also found that EpCAM + HCC cells are more sensitive to Tcf/β-catenin binding inhibitors compared to EpCAM- HCC cells, further confirming this regulatory relationship [40]. Zinc finger protein X-linked (ZFX) enhances the maintenance of EpCAM + CSCs by promoting nuclear translocation and transactivation of β-catenin [41].

NK cell-derived IFN-γ upregulates EpCAM expression through the STAT1 pathway, inducing epithelial-mesenchymal transition (EMT) and promoting liver inflammation and HCC development [42]. Compared with EpCAM- cells, EpCAM + HCC cells exhibit longer telomeres, higher expression of human telomerase reverse transcriptase (hTERT) and the shelterin complex, as well as increased chromosomal instability [43]. Additionally, NDRG1 stabilizes EpCAM through protein–protein interactions and prevents its ubiquitination [44].

4 EpCAM expression in HCC and prognostic value

EpCAM is overexpressed in various human malignant tumors and is recognized as an important prognostic factor [45, 46]. Its prognostic value in HCC has been extensively studied. Survival analysis has demonstrated that EpCAM overexpression is significantly associated with lower overall survival rates and higher recurrence rates in HCC patients [47–51]. In hepatocellular-cholangiocarcinoma (CHC), EpCAM is highly expressed in 80% of cases and is strongly correlated with poor prognosis [52]. Additionally, overexpression of EpCAM in HCC is associated with other clinicopathological features, including high AFP levels and poorer tumor differentiation [53, 54].

The anatomical distribution of EpCAM expression in tumors also influences postoperative recurrence rates. Patients with peritumoral EpCAM expression exhibit higher recurrence rates and worse prognoses compared to those with pan-tumoral EpCAM expression [21]. Clinical data indicate that recurrent tumors following treatments such as radiofrequency ablation (RFA) or transarterial chemoembolization (TACE) treatment have a higher proportion of EpCAM + cells compared

to primary tumors. This suggests that residual tumors enhanced EpCAM expression may drive aggressive phenotypes and local recurrence [55–59].

However, the spatial heterogeneity of EpCAM expression in HCC adds complexity to its prognostic value. Homogeneous EpCAM expression is significantly associated with early recurrence, while heterogeneous EpCAM expression and EpCAM negativity are linked to similar clinical outcomes [60].

Yamashita et al. hypothesized that EpCAM + HCC can be further subclassified based on AFP expression [24]. EpCAM + AFP + HCC typically occurs in younger patients with advanced TNM stages and portal vein invasion, whereas EpCAM− AFP− HCC is predominately found in older patients with early TNM stages. EpCAM + AFP- HCC also develops in younger early TNM stages and low frequencies of portal vein invasion [24]. Another study showed that EpCAM + AFP + and EpCAM-AFP + HCC are associated with advanced TNM stages and high frequencies of venous invasion, whereas EpCAM + AFP− and EpCAM-AFP− HCC are associated with early TNM stages and low frequencies of venous invasion. Furthermore, EpCAM + AFP + exhibits higher microvessel density (MVD) and vascular endothelial growth factor (VEGF) expression levels of EpCAM + AFP + HCC compared to other subtypes, indicating a more angiogenic tumor phenotype [61]. Thus, the relationship between EpCAM expression profiles and different clinical outcomes is complex, influenced by factors such as tumor subtype, stage, and distribution of EpCAM expression. While EpCAM expression is a significant marker in HCC, Its prognostic value is not fully understood and warrants further investigation.

5 The application of EpCAM in liquid biopsy of HCC

Liver biopsies provide valuable insights into tumor biology but are rarely performed due to their invasiveness and the risk of tumor seeding. Blood-based tests, such as AFP, offer limited sensitivity for the diagnosis or prognosis of HCC. Liquid biopsy, an emerging method for early detection and monitoring of cancer, has gained significant attention in recent years [62]. In HCC, liquid biopsies mainly include circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), and extracellular vesicles (EVs) [63, 64].

5.1 EpCAM-based CTC detection

CTCs are tumor cells derived from the original tumor and extravasate into circulation [65]. Analyzing the characteristics and genomic heterogeneity of CTCs provides critical insights into HCC prognosis and treatment response [66]. Platforms for CTC detection often rely on immunoaffinity methods with the EpCAM being the most commonly used biomarker in HCC and other cancers [66–68].

Studies have reported that EpCAM + CTCs were present in 66.7% of HCC patients, with counts ranging from 1 to 34 per 7.5 mL of blood. A preoperative CTC count of ≥ 2/7.5 mL was identified as an independent prognostic factor for tumor recurrence after surgery [69]. Schulze et al. used the CellSearch™ system to detect ≥ 1 CTC/7.5 mL in 18 of 59 HCC patients but only 1 of 19 patients with cirrhosis or benign hepatic tumors. Compared with CTC-negative patients, CTC-positive patients had a significantly shorter overall Survival (OS) [70]. In patients undergoing chemoembolization, CTC counts were also identified as independent predictors of both OS and progression-free survival (PFS) [71]. HCC patients with ≥ 1 CTC/7.5 mL were more likely to have AFP levels ≥ 400 ng/mL and vascular invasion [70, 72]. Similarly, using fluorescence-activated cell sorting (FACS), Hao et al. demonstrated that HCC patients with ≥ 3.5 CTCs/10 mL experienced higher recurrence rates than those with < 3.5 CTCs/10 mL [73].

The association between EpCAM + CTCs and HCC recurrence after liver transplantation has also been highlighted. Hwang et al. reported that preoperative and postoperative EpCAM + CTC counts were significantly correlated with recurrence [74]. Additionally, Jin et al. enriched CTCs using anti-EpCAM nanoparticles and detected AFP mRNA levels in EpCAM-positive CTCs from HCC patients before hepatectomy by AFP nested RT-PCR. Their findings indicated that positive AFP mRNA expression in EpCAM-positive CTCs could be a pivotal predictor for HCC metastasis before and after hepatectomy [75]. Similarly, Zheng et al. used an integrated immunomagnetic-microfluidic platform (iMAC), which demonstrated significantly higher sensitivity in detecting EpCAM + CTCs in the blood samples from HCC patients compared to the CellSearch system [76].

RT-PCR-based platforms have proven to be sensitive, rapid, and cost-effective for identifying CTCs [77–79]. Guo et al. developed a CTC detection platform using negative enrichment and quantitative real-time PCR (qRT-PCR). This platform exhibited high specificity and sensitivity for detecting $EpCAM^{mRNA+}$ CTCs in small blood samples. Their study revealed that pre-treatment $EpCAM^{mRNA+}$ CTCs significantly correlated with higher recurrence rates or worse PFS.

Notably, EpCAM^{mRNA+} CTCs also showed diagnostic value in HCC subgroups with AFP < 20 ng/ml and early-stage HCC [80]. Kocheise et al. also detected CTCs by negative enrichment and qRT-PCR-based CTC detection platform and found that combining EpCAM + CTCs with serum AFP levels improved the identification of HCC patients with poor outcomes after surgical resection [81]. Zhou et al. found that elevated EpCAM^{mRNA+} CTCs and Treg/CD4 + cell levels were associated with postoperative HCC recurrence, suggesting that combined detection of these biomarkers could enhance prognostic accuracy [82].

EpCAM expression is heterogenous and can be absent in certain tumor stages or altered phenotypes, especially during EMT. This limitation can result in undetected CTCs and lead to inaccurate results [65, 83–87]. To address this issue, researchers have investigated combining EpCAM with other biomarkers to improve the sensitivity in HCC. For example, Huang et al. used EpCAM/vimentin/Glypican-3(GPC3) antibody-modified lipid magnetic spheres (LMS) to detect CTCs with epithelial, mesenchymal, and GPC3 phenotypes. This system demonstrated high capture efficiency, low toxicity, high sensitivity and strong specificity [88]. Wu et al. created a dual-targeting functionalized reduced graphene oxide (rGO) film (DTFGF) for HCC CTC detection. By simultaneously targeting EpCAM and HCC cell-specific asialoglycoprotein receptor (ASGPR) to capture and enumerate CTCs in one operation step, the system achieved outstanding selectivity and sensitivity in a single-operation step [89]. An imaging flow cytometry method that incorporates multiple biomarkers, including immunofluorescence of cytokeratin, EpCAM, AFP, glypican-3 and DNA-PK, along with size, morphology, and DNA content analyses, has also enhanced CTC detection sensitivity in HCC [90]. Using the Amnis ImageStreamX Mark II flow cytometer, Debnath et al. employed a biomarker panel (EpCAM, CK, and AFP) that demonstrated strong performance in detecting CTCs in early-stage and AFP-negative HCC patients with high sensitivity and specificity [91]. Xia et al. designed a novel strategy involving Fe3O4 magnetic nanobeads co-assembled with EpCAM antibodies and a tumor cell-specific enzyme, aminopeptidase N (APN). This strategy significantly improved the efficacy of CTC capture while maintaining cell viability [92].

5.2 EpCAM-based EVs detection

Extracellular vesicles (EVs) are cell-derived membranous structures present in biological fluids that facilitate intercellular communication by enabling the exchange of proteins, lipids, and genetic material between cells. This exchange enables EVs to play crucial roles in various physiological and pathological processes [93]. Tumor-secreted EVs are vital mediators of cell-to-cell communications between tumor cells and stromal cells in local and distant microenvironments [94]. EpCAM has been used as a marker of EVs isolation in many tumors [95–98]. However, Similar to tumor cells, tumor-derived EVs are also highly heterogeneous, necessitating the integration of multiple surface markers to enhance the sensitivity and specificity of EVs detection. Julich-Haertel et al. identified AnnexinV + EPCAM + CD147 + tumour-associated microparticles (taMPs), a class of large EVs, as being significantly elevated in HCC. Their study demonstrated that AnnexinV + EpCAM + ASGPR1 + CD133 + taMPs can differentiate HCC from chronic liver disease without liver tumors, suggesting high HCC using this biomarker combination [99]. Similarly, Sun et al. reported that EpCAM + CD63 + HCC EVs are strongly associated with HCC diagnosis. They developed a logistic regression model, named HCC EV ECG score, calculated from the readouts of three HCC EV subpopulations: EpCAM + CD63 +, CD147 + CD63 +, and GPC3 + CD63 + HCC EVs. This model shows significant potential for the early detection of HCC [100].

6 Therapeutic strategies targeting EpCAM in HCC

EpCAM is a key cancer biomarker in primary tumor cells, CTCs, CSCs, and recurrent tumor cells, making it an attractive target for antitumor strategies. Numerous promising anti-tumor strategies targeting EpCAM are widely studied [101–103]. Zhou et al. developed an EpCAM-specific aptamer (EpCAM-apt) for drug delivery in HCC. They conjugated EpCAM-apt with doxorubicin (Dox) to create EpCAM-apt-Dox, which selectively delivered Dox to EpCAM + HCC cells. This approach achieved high drug retention and significant therapeutic effects in HCC organoid xenograft models, extending survival without adverse side effects on major organs [104]. Mitoxantrone (MX), an antitumor drug and photosensitizer, has been integrated into anti-EpCAM antibody-grafted micelle for dual-modality magnetic resonance/upconversion luminescence (MR/UCL)-guided synergetic chemotherapy and photodynamic therapy (PDT). These micelles demonstrated good biocompatibility, high specificity to HCC cells, and effective synergetic antitumor efficacy [105]. Xue et al. developed a nanocomplex comprising 5′-deoxy-5-fluorouridine(5′-DFUR), an EpCAM aptamer, and plasmid DNAs encoding miR-122 through hydrogen bonding. These nanocomplexes specifically targeted EpCAM + HCC cells, releasing 5-FU and

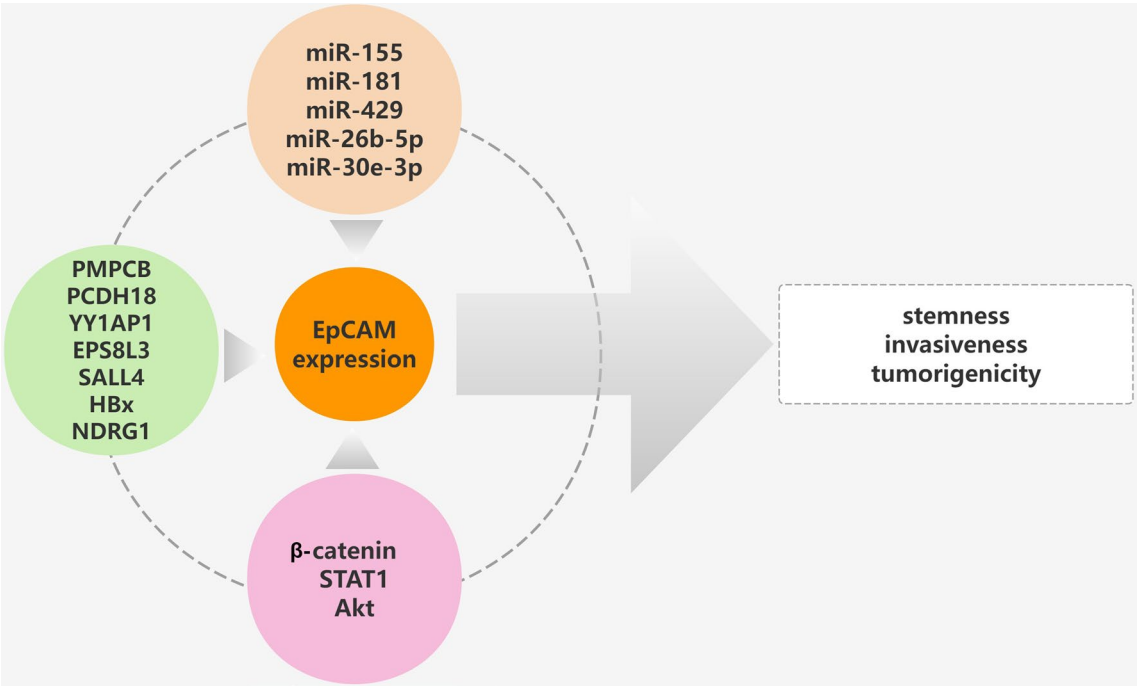

Fig. 1 The molecular mechanism that regulates the expression of EpCAM in HCC

miR-122 into tumor cells and exhibiting potent antitumor effects [106]. Chen et al. synthesized biological porous nanospheres using RNA as a building block and cyclodextrin as an adhesive. These nanospheres delivered EpCAM siRNA and sorafenib to EpCAM + HCC cells, where cytoplasmic degradation by Dicer enzymes released their therapeutic payloads for synergistic therapy [107]. Using RNA nanotechnology, Ishiguro et al. engineered nanoparticles decorated with RNA aptamers against EpCAM. These nanoparticles delivered siRNA targeting β-catenin to EpCAM + liver CSCs, resulting in β-catenin downregulation and effective HCC treatment [108]. Huang et al. developed multicomponent and multifunctional nanoparticles (ICG-CuS-Gd@BSA-EpCAM) for high-sensitivity optical molecular imaging to achieve the purpose of high-sensitivity, noninvasiveness, and high-efficiency photothermal therapy, offering a non-invasive and efficient method for early HCC detection and treatment [109].

Immunotherapies targeting EpCAM have emerged in recent years as promising strategies for anticancer treatments, including vaccines against EpCAM [110, 111], EpCAM-targeting CAR-T cell [112, 113] and anti-EpCAM [114, 115]. VB4-845 (Oportuzumab monatox), a conjugated recombinant antibody and immunotoxin targeting EpCAM, has demonstrated antitumor effects [116, 117]. Ogawa et al. showed that VB4-845 suppressed HCC CSC properties and tumor growth when combined with 5-FU in subcutaneous and orthotopic liver xenograft models [118]. Choi et al. showed that activating dendritic cells (DCs) with EpCAM peptides enhanced T cell stimulation, leading to HCC cell cytotoxicity and tumor growth suppression [119]. An anti-EpCAM BiTE (bispecific T cell engager), 1H8/CD3, has been constructed and was shown to eradicate HCC cells as well as CSCs of HCC in vitro and in vivo [120]. These preclinical studies suggest that EpCAM-targeted therapy may offer a promising approach for the treatment of HCC, but there is still a long way to go before it can be clinically applied.

7 Conclusion

EpCAM is overexpressed on the cell surface of many cancers, including HCC, where it plays diverse roles in cancer progression, such as proliferation, CSC stemness, invasiveness, and therapy resistance (Fig. 1). Its expression pattern in HCC lesions holds significant potential for prognostic evaluation. EpCAM has received considerable attention in liquid biopsy applications, including the detection and isolation of CTCs and EVs from the blood of patients with HCC. However, some CTCs and EVs have low or negative EpCAM levels, necessitating the integration of additional markers to improve

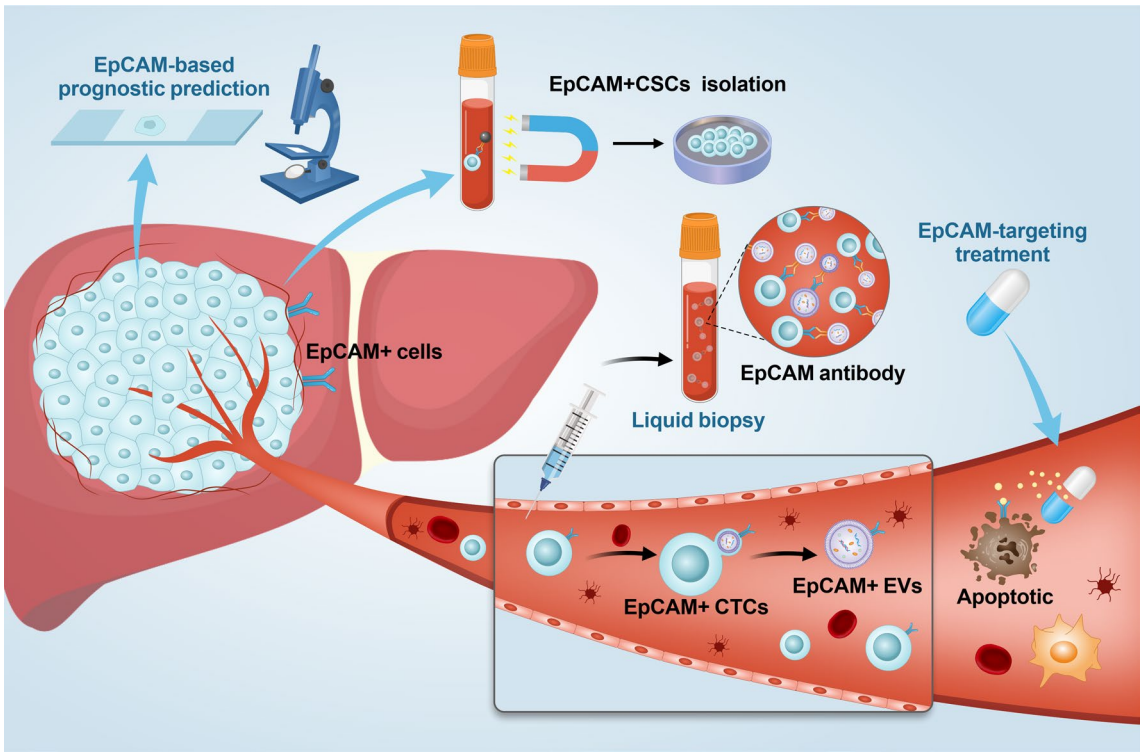

Fig. 2 Applications of EpCAM in HCC. The expression pattern of EpCAM in HCC lesions holds significant potential for prognostic evaluation. EpCAM has received considerable attention in liquid biopsy applications, including the detection and isolation of CTCs and EVs from the blood of patients with HCC. Given its consistent expression in tumor-initiating cells and CTCs, EpCAM remains a promising therapeutic target for HCC

detection efficiency and sensitivity. Given its frequent expression in CSCs and CTCs, EpCAM remains a promising therapeutic target for HCC (Fig. 2). While preclinical studies highlight its potential, clinical application faces challenges due to tumor heterogeneity. Further research and development are needed to realize the full therapeutic potential of EpCAM-targeted strategies.

Acknowledgements Not applicable.

Author contributions L.F.C. and L.P. wrote the main manuscript text; Z.Q. prepared figure. All authors reviewed the manuscript.

Funding This work was supported by the National Natural Science Foundation of China (grant number: 82103662).

Data availability No datasets were generated or analysed during the current study.

Declarations

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

References

1. Bruix J, Sherman M. American Association for the Study of Liver D: management of hepatocellular carcinoma: an update. Hepatology. 2011;53(3):1020–2.
2. Llovet JM, Kelley RK, Villanueva A, Singal AG, Pikarsky E, Roayaie S, Lencioni R, Koike K, Zucman-Rossi J, Finn RS. Hepatocellular carcinoma. Nat Rev Dis Primers. 2021;7(1):6.
3. Xu RH, Wei W, Krawczyk M, Wang W, Luo H, Flagg K, Yi S, Shi W, Quan Q, Li K, et al. Circulating tumour DNA methylation markers for diagnosis and prognosis of hepatocellular carcinoma. Nat Mater. 2017;16(11):1155–61.
4. Lee YT, Fujiwara N, Yang JD, Hoshida Y. Risk stratification and early detection biomarkers for precision HCC screening. Hepatology. 2023;78(1):319–62.
5. Craig AJ, von Felden J, Garcia-Lezana T, Sarcognato S, Villanueva A. Tumour evolution in hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol. 2020;17(3):139–52.
6. Chan LK, Tsui YM, Ho DW, Ng IO. Cellular heterogeneity and plasticity in liver cancer. Semin Cancer Biol. 2022;82:134–49.
7. Prasetyanti PR, Medema JP. Intra-tumor heterogeneity from a cancer stem cell perspective. Mol Cancer. 2017;16(1):41.
8. Gu Y, Zheng X, Ji J. Liver cancer stem cells as a hierarchical society: yes or no? Acta Biochim Biophys Sin (Shanghai). 2020;52(7):723–35.
9. Nio K, Yamashita T, Kaneko S. The evolving concept of liver cancer stem cells. Mol Cancer. 2017;16(1):4.
10. Herlyn M, Steplewski Z, Herlyn D, Koprowski H. Colorectal carcinoma-specific antigen: detection by means of monoclonal antibodies. Proc Natl Acad Sci U S A. 1979;76(3):1438–42.
11. Gires O, Pan M, Schinke H, Canis M, Baeuerle PA. Expression and function of epithelial cell adhesion molecule EpCAM: where are we after 40 years? Cancer Metastasis Rev. 2020;39(3):969–87.
12. Gaber A, Lenarcic B, Pavsic M. Current view on EpCAM structural biology. Cells. 2020. https://doi.org/10.3390/cells9061361.
13. Liu Y, Wang Y, Sun S, Chen Z, Xiang S, Ding Z, Huang Z, Zhang B. Understanding the versatile roles and applications of EpCAM in cancers: from bench to bedside. Exp Hematol Oncol. 2022;11(1):97.
14. Bae JS, Noh SJ, Jang KY, Park HS, Chung MJ, Park CK, Moon WS. Expression and role of epithelial cell adhesion molecule in dysplastic nodule and hepatocellular carcinoma. Int J Oncol. 2012;41(6):2150–8.
15. Yamashita T, Ji J, Budhu A, Forgues M, Yang W, Wang HY, Jia H, Ye Q, Qin LX, Wauthier E, et al. EpCAM-positive hepatocellular carcinoma cells are tumor-initiating cells with stem/progenitor cell features. Gastroenterology. 2009;136(3):1012–24.
16. Kimura O, Takahashi T, Ishii N, Inoue Y, Ueno Y, Kogure T, Fukushima K, Shiina M, Yamagiwa Y, Kondo Y, et al. Characterization of the epithelial cell adhesion molecule (EpCAM)+ cell population in hepatocellular carcinoma cell lines. Cancer Sci. 2010;101(10):2145–55.
17. Matsumoto T, Takai A, Eso Y, Kinoshita K, Manabe T, Seno H, Chiba T, Marusawa H. Proliferating EpCAM-positive ductal cells in the inflamed liver give rise to hepatocellular carcinoma. Cancer Res. 2017;77(22):6131–43.
18. Khosla R, Rastogi A, Ramakrishna G, Pamecha V, Mukhopadhyay A, Vasudevan M, Sarin SK, Trehanpati N. EpCAM+ liver cancer stem-like cells exhibiting autocrine wnt signaling potentially originate in cirrhotic patients. Stem Cells Transl Med. 2017;6(3):807–18.
19. Wendum D, Layese R, Ganne-Carrie N, Bourcier V, Merabtene F, Cagnot C, Sauce E, Barget N, Bedossa P, Terris B, et al. Influence of progenitor-derived regeneration markers on hepatitis C virus-related cirrhosis outcome (ANRS CO12 CirVir Cohort). Hepatology. 2018;68(4):1534–48.
20. Ogasawara S, Akiba J, Nakayama M, Nakashima O, Torimura T, Yano H. Epithelial cell adhesion molecule-positive human hepatic neoplastic cells: development of combined hepatocellular-cholangiocarcinoma in mice. J Gastroenterol Hepatol. 2015;30(2):413–20.
21. Park DJ, Sung PS, Kim JH, Lee GW, Jang JW, Jung ES, Bae SH, Choi JY, Yoon SK. EpCAM-high liver cancer stem cells resist natural killer cell-mediated cytotoxicity by upregulating CEACAM1. J Immunother Cancer. 2020. https://doi.org/10.1136/jitc-2019-000301.
22. Chen Y, Yu D, Zhang H, He H, Zhang C, Zhao W, Shao RG. CD133(+)EpCAM(+) phenotype possesses more characteristics of tumor initiating cells in hepatocellular carcinoma Huh7 cells. Int J Biol Sci. 2012;8(7):992–1004.
23. Liao WY, Hsu CC, Chan TS, Yen CJ, Chen WY, Pan HW, Tsai KK. Dishevelled 1-regulated superpotent cancer stem cells mediate Wnt heterogeneity and tumor progression in hepatocellular carcinoma. Stem Cell Rep. 2020;14(3):462–77.
24. Yamashita T, Forgues M, Wang W, Kim JW, Ye Q, Jia H, Budhu A, Zanetti KA, Chen Y, Qin LX, et al. EpCAM and alpha-fetoprotein expression defines novel prognostic subtypes of hepatocellular carcinoma. Cancer Res. 2008;68(5):1451–61.
25. Takai A, Dang H, Oishi N, Khatib S, Martin SP, Dominguez DA, Luo J, Bagni R, Wu X, Powell K, et al. Genome-wide RNAi screen identifies PMPCB as a therapeutic vulnerability in EpCAM(+) hepatocellular carcinoma. Cancer Res. 2019;79(9):2379–91.
26. Hayashi T, Yamashita T, Okada H, Nio K, Hara Y, Nomura Y, Hayashi T, Asahina Y, Yoshida M, Oishi N, et al. Sporadic PCDH18 somatic mutations in EpCAM-positive hepatocellular carcinoma. Cancer Cell Int. 2017;17:94.
27. Zhao X, Parpart S, Takai A, Roessler S, Budhu A, Yu Z, Blank M, Zhang YE, Jia HL, Ye QH, et al. Integrative genomics identifies YY1AP1 as an oncogenic driver in EpCAM(+) AFP(+) hepatocellular carcinoma. Oncogene. 2015;34(39):5095–104.
28. Ho DW, Tsui YM, Sze KM, Chan LK, Cheung TT, Lee E, Sham PC, Tsui SK, Lee TK, Ng IO. Single-cell transcriptomics reveals the landscape of intra-tumoral heterogeneity and stemness-related subpopulations in liver cancer. Cancer Lett. 2019;459:176–85.
29. Tsui YM, Ho DW, Sze KM, Lee JM, Lee E, Zhang Q, Cheung GC, Tang CN, Tang VW, Cheung ET, et al. Sorted-cell sequencing on HCC specimens reveals EPS8L3 as a key player in CD24/CD13/EpCAM-triple positive, stemness-related HCC cells. Cell Mol Gastroenterol Hepatol. 2024;18(3): 101358.
30. Zeng SS, Yamashita T, Kondo M, Nio K, Hayashi T, Hara Y, Nomura Y, Yoshida M, Hayashi T, Oishi N, et al. The transcription factor SALL4 regulates stemness of EpCAM-positive hepatocellular carcinoma. J Hepatol. 2014;60(1):127–34.
31. Ji J, Zheng X, Forgues M, Yamashita T, Wauthier EL, Reid LM, Wen X, Song Y, Wei JS, Khan J, et al. Identification of microRNAs specific for epithelial cell adhesion molecule-positive tumor cells in hepatocellular carcinoma. Hepatology. 2015;62(3):829–40.
32. Ji J, Yamashita T, Budhu A, Forgues M, Jia HL, Li C, Deng C, Wauthier E, Reid LM, Ye QH, et al. Identification of microRNA-181 by genome-wide screening as a critical player in EpCAM-positive hepatic cancer stem cells. Hepatology. 2009;50(2):472–80.
33. Li L, Tang J, Zhang B, Yang W, LiuGao M, Wang R, Tan Y, Fan J, Chang Y, Fu J, et al. Epigenetic modification of MiR-429 promotes liver tumour-initiating cell properties by targeting Rb binding protein 4. Gut. 2015;64(1):156–67.

34. Khosla R, Hemati H, Rastogi A, Ramakrishna G, Sarin SK, Trehanpati N. miR-26b-5p helps in EpCAM+cancer stem cells maintenance via HSC71/HSPA8 and augments malignant features in HCC. Liver Int. 2019;39(9):1692–703.
35. Gramantieri L, Pollutri D, Gagliardi M, Giovannini C, Quarta S, Ferracin M, Casadei-Gardini A, Callegari E, De Carolis S, Marinelli S, et al. MiR-30e-3p influences tumor phenotype through MDM2/TP53 axis and predicts sorafenib resistance in hepatocellular carcinoma. Cancer Res. 2020;80(8):1720–34.
36. Fan H, Zhang H, Pascuzzi PE, Andrisani O. Hepatitis B virus X protein induces EpCAM expression via active DNA demethylation directed by RelA in complex with EZH2 and TET2. Oncogene. 2016;35(6):715–26.
37. Arzumanyan A, Friedman T, Ng IO, Clayton MM, Lian Z, Feitelson MA. Does the hepatitis B antigen HBx promote the appearance of liver cancer stem cells? Cancer Res. 2011;71(10):3701–8.
38. Wang X, Oishi N, Shimakami T, Yamashita T, Honda M, Murakami S, Kaneko S. Hepatitis B virus X protein induces hepatic stem cell-like features in hepatocellular carcinoma by activating KDM5B. World J Gastroenterol. 2017;23(18):3252–61.
39. Wang C, Yang W, Yan HX, Luo T, Zhang J, Tang L, Wu FQ, Zhang HL, Yu LX, Zheng LY, 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(1):108–20.
40. Yamashita T, Budhu A, Forgues M, Wang XW. Activation of hepatic stem cell marker EpCAM by Wnt-β-catenin signaling in hepatocellular carcinoma. Cancer Res. 2007;67(22):10831–9.
41. Wang C, Fu SY, Wang MD, Yu WB, Cui QS, Wang HR, Huang H, Dong W, Zhang WW, Li PP, et al. Zinc finger protein X-linked promotes expansion of EpCAM(+) cancer stem-like cells in hepatocellular carcinoma. Mol Oncol. 2017;11(5):455–69.
42. Chen Y, Hao X, Sun R, Wei H, Tian Z. Natural killer cell-derived interferon-gamma promotes hepatocellular carcinoma through the epithelial cell adhesion molecule-epithelial-to-mesenchymal transition axis in hepatitis B virus transgenic mice. Hepatology. 2019;69(4):1735–50.
43. Kim H, Yoo JE, Cho JY, Oh BK, Yoon YS, Han HS, Lee HS, Jang JJ, Jeong SH, Kim JW, et al. Telomere length, TERT and shelterin complex proteins in hepatocellular carcinomas expressing "stemness"-related markers. J Hepatol. 2013;59(4):746–52.
44. Cheng Q, Ning S, Zhu L, Zhang C, Jiang S, Hao Y, Zhu J. NDRG1 facilitates self-renewal of liver cancer stem cells by preventing EpCAM ubiquitination. Br J Cancer. 2023;129(2):237–48.
45. Mohtar MA, Syafruddin SE, Nasir SN, Low TY. Revisiting the roles of pro-metastatic EpCAM in cancer. Biomolecules 2020;10(2):255.
46. Keller L, Werner S, Pantel K. Biology and clinical relevance of EpCAM. Cell Stress. 2019;3(6):165–80.
47. El-Kholy MA, Abu-Seadah SS, Hasan A, Elhussiny MEA, Abdelwahed MS, Hanbazazh M, Samman A, Alrashdi SA, Rashed ZF, Ashmawy D, et al. The role of epithelial cell adhesion molecule cancer stem cell marker in evaluation of hepatocellular carcinoma. Medicina (Kaunas). 2024. https://doi.org/10.3390/medicina60060915.
48. Noh CK, Wang HJ, Kim CM, Kim J, Yoon SY, Lee GH, Cho HJ, Yang MJ, Kim SS, Hwang JC, et al. EpCAM as a predictive marker of tumor recurrence and survival in patients who underwent surgical resection for hepatocellular carcinoma. Anticancer Res. 2018;38(7):4101–9.
49. Murakata A, Tanaka S, Mogushi K, Yasen M, Noguchi N, Irie T, Kudo A, Nakamura N, Tanaka H, Arii S. Gene expression signature of the gross morphology in hepatocellular carcinoma. Ann Surg. 2011;253(1):94–100.
50. Guo Z, Li LQ, Jiang JH, Ou C, Zeng LX, Xiang BD. Cancer stem cell markers correlate with early recurrence and survival in hepatocellular carcinoma. World J Gastroenterol. 2014;20(8):2098–106.
51. Chan AW, Tong JH, Chan SL, Lai PB, To KF. Expression of stemness markers (CD133 and EpCAM) in prognostication of hepatocellular carcinoma. Histopathology. 2014;64(7):935–50.
52. Wang A, Wu L, Lin J, Han L, Bian J, Wu Y, Robson SC, Xue L, Ge Y, Sang X, et al. Whole-exome sequencing reveals the origin and evolution of hepato-cholangiocarcinoma. Nat Commun. 2018;9(1):894.
53. Zhou L, Zhu Y. The EpCAM overexpression is associated with clinicopathological significance and prognosis in hepatocellular carcinoma patients: a systematic review and meta-analysis. Int J Surg. 2018;56:274–80.
54. Yamashita T, Honda M, Nakamoto Y, Baba M, Nio K, Hara Y, Zeng SS, Hayashi T, Kondo M, Takatori H, et al. Discrete nature of EpCAM+ and CD90+ cancer stem cells in human hepatocellular carcinoma. Hepatology. 2013;57(4):1484–97.
55. Wang S, Liu J, Wu H, Jiang A, Zhao K, Yan K, Wu W, Han H, Zhang Y, Yang W. All-trans retinoic acid (ATRA) inhibits insufficient radiofrequency ablation (IRFA)-induced enrichment of tumor-initiating cells in hepatocellular carcinoma. Chin J Cancer Res. 2021;33(6):694–707.
56. Yamada S, Utsunomiya T, Morine Y, Imura S, Ikemoto T, Arakawa Y, Kanamoto M, Iwahashi S, Saito Y, Takasu C, et al. Expressions of hypoxia-inducible factor-1 and epithelial cell adhesion molecule are linked with aggressive local recurrence of hepatocellular carcinoma after radiofrequency ablation therapy. Ann Surg Oncol. 2014;21(Suppl 3):S436-442.
57. Kim M, Hui KM, Shi M, Reau N, Aloman C. Differential expression of hepatic cancer stemness and hypoxia markers in residual cancer after locoregional therapies for hepatocellular carcinoma. Hepatol Commun. 2022;6(11):3247–59.
58. Zeng Z, Ren J, O'Neil M, Zhao J, Bridges B, Cox J, Abdulkarim B, Schmitt TM, Kumer SC, Weinman SA. Impact of stem cell marker expression on recurrence of TACE-treated hepatocellular carcinoma post liver transplantation. BMC Cancer. 2012;12:584.
59. Wu JY, Bai XM, Wang H, Xu Q, Wang S, Wu W, Yan K, Yang W. The perfusion features of recurrent hepatocellular carcinoma after radiofrequency ablation using contrast-enhanced ultrasound and pathological stemness evaluation: compared to initial tumors. Front Oncol. 2020;10:1464.
60. Krause J, von Felden J, Casar C, Frundt TW, Galaski J, Schmidt C, Jung C, Ittrich H, Weidemann SA, Krech T, et al. Hepatocellular carcinoma: intratumoral EpCAM-positive cancer stem cell heterogeneity identifies high-risk tumor subtype. BMC Cancer. 2020;20(1):1130.
61. Shan YF, Huang YL, Xie YK, Tan YH, Chen BC, Zhou MT, Shi HQ, Yu ZP, Song QT, Zhang QY. Angiogenesis and clinicopathologic characteristics in different hepatocellular carcinoma subtypes defined by EpCAM and alpha-fetoprotein expression status. Med Oncol. 2011;28(4):1012–6.
62. Nikanjam M, Kato S, Kurzrock R. Liquid biopsy: current technology and clinical applications. J Hematol Oncol. 2022;15(1):131.
63. Ye Q, Ling S, Zheng S, Xu X. Liquid biopsy in hepatocellular carcinoma: circulating tumor cells and circulating tumor DNA. Mol Cancer. 2019;18(1):114.
64. Lehrich BM, Zhang J, Monga SP, Dhanasekaran R. Battle of the biopsies: role of tissue and liquid biopsy in hepatocellular carcinoma. J Hepatol. 2024;80(3):515–30.
65. Lin D, Shen L, Luo M, Zhang K, Li J, Yang Q, Zhu F, Zhou D, Zheng S, Chen Y, et al. Circulating tumor cells: biology and clinical significance. Signal Transduct Target Ther. 2021;6(1):404.

66. Ahn JC, Teng PC, Chen PJ, Posadas E, Tseng HR, Lu SC, Yang JD. Detection of circulating tumor cells and their implications as a biomarker for diagnosis, prognostication, and therapeutic monitoring in hepatocellular carcinoma. Hepatology. 2021;73(1):422–36.
67. Zhang Q, Rong Y, Yi K, Huang L, Chen M, Wang F. Circulating tumor cells in hepatocellular carcinoma: single-cell based analysis, preclinical models, and clinical applications. Theranostics. 2020;10(26):12060–71.
68. Prasoppokakorn T, Buntho A, Ingrungruanglert P, Tiyarattanachai T, Jaihan T, Kulkraisri K, Ariyaskul D, Phathong C, Israsena N, Rerknimitr R, et al. Circulating tumor cells as a prognostic biomarker in patients with hepatocellular carcinoma. Sci Rep. 2022;12(1):18686.
69. Sun YF, Xu Y, Yang XR, Guo W, Zhang X, Qiu SJ, Shi RY, Hu B, Zhou J, Fan J. Circulating stem cell-like epithelial cell adhesion molecule-positive tumor cells indicate poor prognosis of hepatocellular carcinoma after curative resection. Hepatology. 2013;57(4):1458–68.
70. Schulze K, Gasch C, Staufer K, Nashan B, Lohse AW, Pantel K, Riethdorf S, Wege H. Presence of EpCAM-positive circulating tumor cells as biomarker for systemic disease strongly correlates to survival in patients with hepatocellular carcinoma. Int J Cancer. 2013;133(9):2165–71.
71. Shen J, Wang WS, Zhu XL, Ni CF. High epithelial cell adhesion molecule-positive circulating tumor cell count predicts poor survival of patients with unresectable hepatocellular carcinoma treated with transcatheter arterial chemoembolization. J Vasc Interv Radiol. 2018;29(12):1678–84.
72. Kelley RK, Magbanua MJ, Butler TM, Collisson EA, Hwang J, Sidiropoulos N, Evason K, McWhirter RM, Hameed B, Wayne EM, et al. Circulating tumor cells in hepatocellular carcinoma: a pilot study of detection, enumeration, and next-generation sequencing in cases and controls. BMC Cancer. 2015;15:206.
73. Hao S, Chen S, Tu C, Huang T. Anterior approach to improve the prognosis in HCC patients via decreasing dissemination of EpCAM(+) circulating tumor cells. J Gastrointest Surg. 2017;21(7):1112–20.
74. Hwang HS, Yoo JE, Han DH, Choi JS, Lee JG, Joo DJ, Kim MS, Kim SI, Choi GH, Park YN. Circulating cancer stem cells expressing EpCAM/CD90 in hepatocellular carcinoma: a pilot study for predicting tumor recurrence after living donor liver transplantation. Gut Liver. 2022;16(3):443–55.
75. Jin J, Niu X, Zou L, Li L, Li S, Han J, Zhang P, Song J, Xiao F. AFP mRNA level in enriched circulating tumor cells from hepatocellular carcinoma patient blood samples is a pivotal predictive marker for metastasis. Cancer Lett. 2016;378(1):33–7.
76. Zheng WJ, Wang PX, Sun YF, Cheng JW, Zhong YC, Xu Y, Guo W, Hu B, Zhou J, Fan J, et al. Uncovering the heterogeneity and clinical relevance of circulating tumor-initiating cells in hepatocellular carcinoma using an integrated immunomagnetic-microfluidic platform. ACS Appl Mater Interfaces. 2022;14(32):36425–37.
77. Sergeant G, Roskams T, van Pelt J, Houtmeyers F, Aerts R, Topal B. Perioperative cancer cell dissemination detected with a real-time RT-PCR assay for EpCAM is not associated with worse prognosis in pancreatic ductal adenocarcinoma. BMC Cancer. 2011;11:47.
78. Masuda T, Hayashi N, Iguchi T, Ito S, Eguchi H, Mimori K. Clinical and biological significance of circulating tumor cells in cancer. Mol Oncol. 2016;10(3):408–17.
79. Khoja L, Lorigan P, Dive C, Keilholz U, Fusi A. Circulating tumour cells as tumour biomarkers in melanoma: detection methods and clinical relevance. Ann Oncol. 2015;26(1):33–9.
80. Guo W, Yang XR, Sun YF, Shen MN, Ma XL, Wu J, Zhang CY, Zhou Y, Xu Y, Hu B, et al. Clinical significance of EpCAM mRNA-positive circulating tumor cells in hepatocellular carcinoma by an optimized negative enrichment and qRT-PCR-based platform. Clin Cancer Res. 2014;20(18):4794–805.
81. Kocheise L, Schoenlein M, Behrends B, Joerg V, Casar C, Fruendt TW, Renne T, Heumann A, Li J, Huber S, et al. EpCAM-positive circulating tumor cells and serum AFP levels predict outcome after curative resection of hepatocellular carcinoma. Sci Rep. 2023;13(1):20827.
82. Zhou Y, Wang B, Wu J, Zhang C, Zhou Y, Yang X, Zhou J, Guo W, Fan J. Association of preoperative EpCAM circulating tumor cells and peripheral Treg cell levels with early recurrence of hepatocellular carcinoma following radical hepatic resection. BMC Cancer. 2016;16:506.
83. Bailey PC, Martin SS. Insights on CTC biology and clinical impact emerging from advances in capture technology. Cells. 2019. https://doi.org/10.3390/cells8060553.
84. Gabriel MT, Calleja LR, Chalopin A, Ory B, Heymann D. Circulating tumor cells: a review of non-EpCAM-based approaches for cell enrichment and isolation. Clin Chem. 2016;62(4):571–81.
85. Austin RG, Huang TJ, Wu M, Armstrong AJ, Zhang T. Clinical utility of non-EpCAM based circulating tumor cell assays. Adv Drug Deliv Rev. 2018;125:132–42.
86. Chen L, Wu LL, Zhang ZL, Hu J, Tang M, Qi CB, Li N, Pang DW. Biofunctionalized magnetic nanospheres-based cell sorting strategy for efficient isolation, detection and subtype analyses of heterogeneous circulating hepatocellular carcinoma cells. Biosens Bioelectron. 2016;85:633–40.
87. Wang L, Li Y, Xu J, Zhang A, Wang X, Tang R, Zhang X, Yin H, Liu M, Wang DD, et al. Quantified postsurgical small cell size CTCs and EpCAM(+) circulating tumor stem cells with cytogenetic abnormalities in hepatocellular carcinoma patients determine cancer relapse. Cancer Lett. 2018;412:99–107.
88. Huang XY, Li F, Li TT, Zhang JT, Shi XJ, Huang XY, Zhou J, Tang ZY, Huang ZL. A clinically feasible circulating tumor cell sorting system for monitoring the progression of advanced hepatocellular carcinoma. J Nanobiotechnol. 2023;21(1):25.
89. Wu C, Li P, Fan N, Han J, Zhang W, Zhang W, Tang B. A dual-targeting functionalized graphene film for rapid and highly sensitive fluorescence imaging detection of hepatocellular carcinoma circulating tumor cells. ACS Appl Mater Interfaces. 2019;11(48):44999–5006.
90. Ogle LF, Orr JG, Willoughby CE, Hutton C, McPherson S, Plummer R, Boddy AV, Curtin NJ, Jamieson D, Reeves HL. Imagestream detection and characterisation of circulating tumour cells—a liquid biopsy for hepatocellular carcinoma? J Hepatol. 2016;65(2):305–13.
91. Debnath P, Dalal K, Dalal B, Athalye S, Chandnani S, Jain S, Shukla A, Rathi P, Shankarkumar A. Characterization of circulating tumor cells using imaging flow cytometry in liver disease patients. J Clin Exp Hepatol. 2023;13(4):608–17.
92. Xia W, Li H, Li Y, Li M, Fan J, Sun W, Li N, Li R, Shao K, Peng X. In vivo coinstantaneous identification of hepatocellular carcinoma circulating tumor cells by dual-targeting magnetic-fluorescent nanobeads. Nano Lett. 2021;21(1):634–41.
93. van Niel G, D'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. Nat Rev Mol Cell Biol. 2018;19(4):213–28.
94. Becker A, Thakur BK, Weiss JM, Kim HS, Peinado H, Lyden D. Extracellular vesicles in cancer: cell-to-cell mediators of metastasis. Cancer Cell. 2016;30(6):836–48.
95. Deng J, Zhao S, Li J, Cheng Y, Liu C, Liu Z, Li L, Tian F, Dai B, Sun J. One-step thermophoretic AND gate operation on extracellular vesicles improves diagnosis of prostate cancer. Angew Chem Int Ed Engl. 2022;61(33): e202207037.
96. Ostenfeld MS, Jensen SG, Jeppesen DK, Christensen LL, Thorsen SB, Stenvang J, Hvam ML, Thomsen A, Mouritzen P, Rasmussen MH, et al. miRNA profiling of circulating EpCAM(+) extracellular vesicles: promising biomarkers of colorectal cancer. J Extracell Vesicles. 2016;5:31488.

97. Jiang L, Shen Y, Guo D, Yang D, Liu J, Fei X, Yang Y, Zhang B, Lin Z, Yang F, et al. EpCAM-dependent extracellular vesicles from intestinal epithelial cells maintain intestinal tract immune balance. Nat Commun. 2016;7:13045.
98. Yildizhan Y, Driessens K, Tsao HSK, Boiy R, Thomas D, Geukens N, Hendrix A, Lammertyn J, Spasic D. Detection of breast cancer-specific extracellular vesicles with fiber-optic SPR biosensor. Int J Mol Sci 2023;24(4):3764.
99. Julich-Haertel H, Urban SK, Krawczyk M, Willms A, Jankowski K, Patkowski W, Kruk B, Krasnodebski M, Ligocka J, Schwab R, et al. Cancer-associated circulating large extracellular vesicles in cholangiocarcinoma and hepatocellular carcinoma. J Hepatol. 2017;67(2):282–92.
100. Sun N, Zhang C, Lee YT, Tran BV, Wang J, Kim H, Lee J, Zhang RY, Wang JJ, Hu J, et al. HCC EV ECG score: an extracellular vesicle-based protein assay for detection of early-stage hepatocellular carcinoma. Hepatology. 2023;77(3):774–88.
101. Ni J, Cozzi PJ, Duan W, Shigdar S, Graham PH, John KH, Li Y. Role of the EpCAM (CD326) in prostate cancer metastasis and progression. Cancer Metastasis Rev. 2012;31(3–4):779–91.
102. Xu T, Karschnia P, Cadilha BL, Dede S, Lorenz M, Seewaldt N, Nikolaishvili E, Muller K, Blobner J, Teske N, et al. In vivo dynamics and anti-tumor effects of EpCAM-directed CAR T-cells against brain metastases from lung cancer. Oncoimmunology. 2023;12(1):2163781.
103. Dutta D, Al Hoque A, Paul B, Park JH, Chowdhury C, Quadir M, Banerjee S, Choudhury A, Laha S, Sepay N, et al. EpCAM-targeted betulinic acid analogue nanotherapy improves therapeutic efficacy and induces anti-tumorigenic immune response in colorectal cancer tumor microenvironment. J Biomed Sci. 2024;31(1):81.
104. Zhou K, Huo X, Nguyen R, Bae SDW, Han S, Zhang Z, Duan W, Yuen L, Lam V, George J, et al. Aptamer-mediated doxorubicin delivery reduces HCC burden in 3D organoids model. J Control Release. 2022;341:341–50.
105. Han Y, An Y, Jia G, Wang X, He C, Ding Y, Tang Q. Theranostic micelles based on upconversion nanoparticles for dual-modality imaging and photodynamic therapy in hepatocellular carcinoma. Nanoscale. 2018;10(14):6511–23.
106. Xue F, Lin X, Cai Z, Liu X, Ma Y, Wu M. Doxifluridine-based pharmacosomes delivering miR-122 as tumor microenvironments-activated nanoplatforms for synergistic treatment of hepatocellular carcinoma. Colloids Surf B Biointerfaces. 2021;197: 111367.
107. Chen X, Chen T, Zhang L, Wang Z, Zhou Q, Huang T, Ge C, Xu H, Zhu M, Zhao F, et al. Cyclodextrin-mediated formation of porous RNA nanospheres and their application in synergistic targeted therapeutics of hepatocellular carcinoma. Biomaterials. 2020;261: 120304.
108. Ishiguro K, Yan IK, Lewis-Tuffin L, Patel T. Targeting liver cancer stem cells using engineered biological nanoparticles for the treatment of hepatocellular cancer. Hepatol Commun. 2020;4(2):298–313.
109. Huang M, Qi M, Yang H, Peng Z, Chen S, Liang M, Hu Y, Deng L, Hu M. Noninvasive strategies for the treatment of tiny liver cancer: integrating photothermal therapy and multimodality imaging EpCAM-guided nanoparticles. ACS Appl Mater Interfaces. 2023;15(18):21843–53.
110. Elia L, Mennuni C, Storto M, Podda S, Calvaruso F, Salucci V, Aurisicchio L, Scarito A, Ciliberto G, La Monica N, et al. Genetic vaccines against Ep-CAM break tolerance to self in a limited subset of subjects: initial identification of predictive biomarkers. Eur J Immunol. 2006;36(5):1337–49.
111. Mosolits S, Markovic K, Fagerberg J, Frodin JE, Rezvany MR, Kiaii S, Mellstedt H, Jeddi-Tehrani M. T-cell receptor BV gene usage in colorectal carcinoma patients immunised with recombinant Ep-CAM protein or anti-idiotypic antibody. Cancer Immunol Immunother. 2005;54(6):557–70.
112. Yang Y, Yang H, Alcaina Y, Puc J, Birt A, Vedvyas Y, Gallagher M, Alla S, Riascos MC, McCloskey JE, et al. Inducible expression of interleukin-12 augments the efficacy of affinity-tuned chimeric antigen receptors in murine solid tumor models. Nat Commun. 2023;14(1):2068.
113. Li D, Guo X, Yang K, Yang Y, Zhou W, Huang Y, Liang X, Su J, Jiang L, Li J, et al. EpCAM-targeting CAR-T cell immunotherapy is safe and efficacious for epithelial tumors. Sci Adv. 2023;9(48): eadg9721.
114. Wang L, Qiao Y, Zong H, Han L, Ke Y, Pan Z, Chen J, Lu J, Li J, Ying T, et al. IgG-like bispecific antibody CD3xEpCAM generated by split intein against colorectal cancer. Front Pharmacol. 2022;13: 803059.
115. Wang Z, Wang M, Li Q, Wu Y, Ying T. High-affinity fully human anti-EpCAM antibody with biased IL-2 exhibits potent antitumor activity. Biomolecules 2024;14(11):1399.
116. MacDonald GC, Rasamoelisolo M, Entwistle J, Cizeau J, Bosc D, Cuthbert W, Kowalski M, Spearman M, Glover N. A phase I clinical study of VB4-845: weekly intratumoral administration of an anti-EpCAM recombinant fusion protein in patients with squamous cell carcinoma of the head and neck. Drug Des Devel Ther. 2009;2:105–14.
117. Kowalski M, Entwistle J, Cizeau J, Niforos D, Loewen S, Chapman W, MacDonald GC. A Phase I study of an intravesically administered immunotoxin targeting EpCAM for the treatment of nonmuscle-invasive bladder cancer in BCGrefractory and BCG-intolerant patients. Drug Des Devel Ther. 2010;4:313–20.
118. Ogawa K, Tanaka S, Matsumura S, Murakata A, Ban D, Ochiai T, Irie T, Kudo A, Nakamura N, Tanabe M, et al. EpCAM-targeted therapy for human hepatocellular carcinoma. Ann Surg Oncol. 2014;21(4):1314–22.
119. Choi YJ, Park SJ, Park YS, Park HS, Yang KM, Heo K. EpCAM peptide-primed dendritic cell vaccination confers significant anti-tumor immunity in hepatocellular carcinoma cells. PLoS ONE. 2018;13(1): e0190638.
120. Zhang P, Shi B, Gao H, Jiang H, Kong J, Yan J, Pan X, Li K, Zhang P, Yao M, et al. An EpCAM/CD3 bispecific antibody efficiently eliminates hepatocellular carcinoma cells with limited galectin-1 expression. Cancer Immunol Immunother. 2014;63(2):121–32.