Chapter 3

Cancer Stem Cells and Aldehyde Dehydrogenase 1 in Liver Cancers

Hiroyuki Tomita, Tomohiro Kanayama, Ayumi Niwa, Kei Noguchi, Kazuhisa Ishida, Masayuki Niwa and Akira Hara

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/66454

Abstract

The cancer stem cell (CSC) theory posits that a small population of cells with stem cell-like features is responsible for tumor growth, resistance, and recurrence in many malignancies. This theory could be a useful paradigm for designing innovative targeted drug therapies. Liver cancer is the fifth most common cancer worldwide, with hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA) as the predominant forms. Hepatic stem/progenitor cells are believed to be the origin of HCCs and CCAs; however, this remains a controversial topic. Aldehyde dehydrogenase (ALDH) is the main enzymatic system responsible for the clearance of acetaldehyde from the hepatocytes in the liver tissue. Therefore, ALDH1 has been suggested to be a potential, biological and CSC marker in liver cancers. We here provide an overview of the current state of knowledge of CSCs in liver and the role of ALDH1 in the development and progression of liver cancers and discuss its potential value as a prognostic and diagnostic biomarker.

Keywords: aldehyde dehydrogenase, stem cell, cancer stem cell, hepatocellular carcinoma, cholangiocarcinoma, liver cancer

1. Introduction

Liver cancer is the second most common cause of death from cancer and is the fifth most commonly diagnosed cancer worldwide [1]. Given that the incidence of liver cancer has been on the rise globally and its poor prognosis, the overall mortality rate has also been increased [1, 2]. Some hepatic stem/progenitor markers are currently available for identifying a subset of cells with stem cell-like features known as cancer stem cells (CSCs). Identifying CSC-

specific genes and understanding their mechanisms in liver cancers are important issues in the development of cancer therapy. Aldehyde dehydrogenase 1 (ALDH1) has been reported to indicate the therapeutic drug resistance of many malignancies, and shows potential to be widely used as a marker to identify cells with stem cell-like features, including those in primary liver cancers. We describe an overview of CSCs in liver and the role of ALDH1 in liver cancers.

2. The concept of CSCs

The CSC concept derives from the fact that cancer cells are dysregulated clones whose continued propagation occurs in a biologically distinct subset of rare cells. This concept is not novel but has gained prominence in recent years owing to advances in gaining a greater appreciation of the multistep nature of oncogenesis [3]. This concept has important therapeutic implications and may explain why it is possible to treat many malignancies until the tumor can no longer be detected, and yet the cancer returns [4]. Although radiation therapy and chemotherapy have been the mainstay of cancer treatment, these modalities do not show a substantial effect on CSCs [5]. Furthermore, it may be tough to create conditions that assist the production of all the mature cell types of the tissue as well as the survival and self-renewal of the stem cells (SCs) from which the mature cell types derive. Very few phenotypic markers have proven to be reliable surrogates for enumerating SCs, particularly when they have been physiologically or experimentally perturbed [3].

3. SCs in the normal liver

3.1. Liver function and architecture

The liver is the largest parenchymatous organ in the body. It carries out a wide variety of functions for maintaining homeostasis, such as metabolism, glycogen storage, drug detoxification, production of various serum proteins, and bile secretion. Most of the metabolic and synthetic functions of the liver are carried out by hepatocytes. The bile duct is formed by cholangiocytes, a type of epithelial cell. Other cell types that compose the liver are hepatic sinusoidal endothelial cells, Kupffer cells located at the luminal side of the sinusoid, and stellate cells at the space of Disse.

3.2. Liver stem/progenitor cells

In addition to self-renewability, liver stem/progenitor cells have another specific characteristic: the bipotential to differentiate into hepatocytes and cholangiocytes. Liver stem/progenitor cells play important roles in development, homeostasis, and regeneration. Thus, the liver comprises two stem/progenitor cell systems: fetal liver stem/progenitor cells relating to development and adult liver stem/progenitor cells associated with homeostasis and regeneration.

3.3. Fetal liver stem/progenitor cells

The onset of mouse liver development begins at embryonic day (E) 8.5 from the foregut endoderm [6]. The foregut endoderm cells destined for a hepatic fate begin to express the transcription factors HEX and HNF4α as well as the liver-specific genes α-fetoprotein (*Afp*) and albumin (*Alb*) and migrate as cords into the surrounding septum transversum mesenchyme. These cells are common progenitor cells, which give rise to both hepatocytes and cholangiocytes and are called "hepatoblasts" during liver development. Recently, the combination of specific cell-surface markers has been used to isolate fetal liver stem/progenitor cells. The $CD45^-$ $TER119^-$ c-Kit^- $CD29^+$ $CD49f^+$ fraction of the E13.5 mouse liver was shown to include colony-forming cells with the potential to differentiate into hepatocytic and cholangiocytic lineages [7]. Other reported cell-sorting markers that are useful to define fetal liver stem/progenitor cells are c-Kit^{low} [8], c-Kit^- c-Met^+ $CD49f^{+/low}$ [9], $CD13^+$ [10], or $CD13^+$ c-Kit^- $CD49f^{-/low}$ $CD133^+$ [11] in combination with $CD45^-$ and $TER119^-$. Delta-like 1 homolog (DLK1) is expressed in the liver buds as early as E9.0 in the mouse embryo, and $DLK1^+$ cells isolated from E14.5 mouse livers have the capacity to form proliferative colonies *in vitro*, consisting of the hepatocyte and cholangiocyte lineages [12]. E-cadherin and LIV2 are also useful epithelial-specific markers to isolate epithelial cells expressed in the E12.5 mouse liver [13–16]. CD24a and neighbor of Punc E11 (NOPE) were also identified as sorting markers [17]. HNF4α+ liver stem/progenitor cells express epithelial cell adhesion molecule (EpCAM) in mice as early as E9.5. The $EpCAM^+$ $DLK1^+$ cells from the E11.5 mouse liver include cells that form colonies *in vitro* [18]. The $EpCAM^+$ cells isolated from the human fetal liver were shown to contain multipotent precursors of liver stem/progenitor cells [19].

3.4. Adult liver stem/progenitor cells

The liver has a remarkable capacity to regenerate. Liver regeneration depends primarily on the proliferation of adult hepatocytes. In the course of liver generation, hypertrophy of hepatocytes is also observed. In contrast to the regeneration induced by acute liver damage, severe and chronic liver damage induces a defect in the proliferation of mature hepatocytes. Adult liver stem/progenitor cells are thought to be involved in the regeneration induced by such chronic liver damage. During serious liver injury in rodents, the number of characteristic nonparenchymal oval cells increases in the periportal regions. These cells express both cholangiocellular (*Ck7* and *Ck19*) and hepatocellular (*Afp* and *Alb*) marker genes and differentiate into both hepatocytic and cholangiocytic cells, suggesting that oval cells are candidate hepatic progenitors [20–23].

There are several specific markers for sorting cells containing postnatal stem/progenitor cells. Some of them are the same as fetal stem/progenitor cell surface markers such as EpCAM and CD133. Other reported markers are LGR5 [24], $CD13^+$ $CD133^+$ [11], and $CD133^+$ $MIC1\text{-}1C3^+$ [25].

3.5. Transdifferentiation between hepatocytes and cholangiocytes

Hepatocytes and cholangiocytes are considered to be derived from single stem/progenitor cells, and they show potential to transdifferentiate into other liver epithelial cell types. Tarlow et al. [26] labeled SOX9-positive cells in mice, analyzed the formation of organoids

in culture, monitored the responses of cells in mice on a choline-deficient ethionine diet or diets containing 3,5-diethoxycarbonyl-1,4-dihydrocollidine, and tracked cells transferred into fumarylacetoacetate hydrolase (*Fah*)-deficient mice. Hepatocytes from normal, immune-compatible donors could be transplanted and successfully recolonized the livers of these mice; <1% of the hepatocytes were derived from SOX9-positive precursors [27]. The hepatocyte-derived cholangiocytes continued to express some hepatocyte-specific genes such as *Hnf4* and showed low EpCAM expression [28]. Lu et al. [29] reported the conversion of cholangiocytes to hepatocytes when hepatocyte *Mdm2* (an E3 ubiquitin ligase gene) was deleted. Huch et al. [27] isolated cholangiocytes from the human liver based on the expression of EpCAM. The cells were grown into organoids, induced to transdifferentiate in culture, and expressed hepatocyte-specific genes. Cholangiocytes isolated from liver biopsies of patients with liver diseases also differentiated into hepatocytes in the organoid cultures, but still carried markers of the patients' diseases. However, it is important to note that in these previous studies, the transdifferentiation of cholangiocytes to hepatocytes was observed in culture, and the hepatocyte phenotype detected after transplantation of the cells into mice was observed before the cells were transplanted. It seems therefore fair to conclude that under most conditions of chronic toxic injury or normal liver regeneration, hepatocytes and cholangiocytes proliferate and retain their phenotype. This phenomenon is strongly supported by both rat and mouse studies.

4. CSCs in hepatocellular carcinoma (HCC)

4.1. The characteristics of HCC

HCC represents the major histological subtype of liver cancers, accounting for approximately 85% of primary cancers in the liver [30]. HCC derives from hepatocytes constituting the liver parenchyma, and liver cirrhosis is a precursor in about 80% of all cases. As the precursor lesion of HCC, liver cirrhosis is caused by chronic liver injury, leading to the consecutive liver regeneration and aberrant nodule formation with neighboring fibrosis.

The liver cirrhosis is known to be caused by chronic viral hepatitis B and C infections; metabolic liver diseases, such as nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, hemochromatosis, a1-antitrypsin deficiency, and Wilson's disease; alcoholic liver disease; and autoimmune diseases [2].

4.2. CSC markers in HCC

Recently, cell aggregates with stronger proliferation potency than other tissues comprising HCCs have been discovered. The cell markers of these aggressive tissues have also been identified and classified as SC markers [31]. CD133 (prominin-1), CD90 (THY-1), CD44, CD326 (EpCAM), CD24, and CD13 are the most common cell-surface markers used to detect the CSCs of HCC [32]. Furthermore, several functional markers are available to classify cells according to CSC potency, such as ALDH1, side population, and high green fluorescent molecule fused to the degron of ornithine decarboxylase, associated with low reactive oxygen species (ROS) levels [33].

4.3. Prognosis of HCC

According to the CSC theory, CSCs could influence a patient's prognosis by promoting metastasis and recurrence. Consistent with this hypothesis, recent findings have shown that the presence of CSCs could be associated with patient survival. For example, overexpression of CD90 in HCC is associated with a poor diagnosis. An immunohistochemical study demonstrated the association between CD90 expression and clinical factors, in which CD90 was overexpressed in approximately 70% of the HCC cases. Furthermore, CD90 overexpression was associated with hepatitis B virus infection, age, and histological grade [34]. CD133 overexpression is an independent prognostic factor for survival and tumor recurrence in HCC patients, however, CD133 expression is not shown in normal liver cells. The other report [35] described that the cytoplasmic CD133 expression in HCC patients is associated with high-serum AFP levels, histological high-grade, and invasion. Other studies [36, 37] have demonstrated that CD133 expression is associated with clinical and pathological factors, including poorly differentiated tumors. Furthermore, a significant association was observed between the cytoplasmic expression of CD133 and overall survival of patients with HCC, which was due to multicentric carcinogenicity and hematogenous metastasis to the liver and remote organs. Consequently, positive cytoplasmic expression of CD133 has been proposed to indicate a risk of poor prognosis, especially in patients with HCC at an advanced stage. Chan et al. [36] showed that CD133 is a highly effective prognostic factor for overall survival in patients affected by disease at stage I. In contrast, EpCAM is associated with lower histological differentiation and the invasion of vessel [37]. CK19 expression in HCC is also associated with poor prognosis. Particularly, the increase of CK19-positive cells in HCC was correlated with upregulation of epithelial-mesenchymal transition-related genes. CD44 expression in HCC is related to a higher frequency of extrahepatic metastasis and a shortened survival rate [38] and is correlated with more aggressive tumor behavior and poor clinical outcomes [39].

4.4. Therapy for HCC

Although chemotherapy and ionizing radiation can eliminate tumor cells in proliferating cell cycles, CSCs are intrinsically resistant to these treatments. Therefore, interference with the self-renewal, survival, and niche properties of CSCs is a possible strategy for targeted therapy.

The CSC-specific signal is expected to be a therapeutic target. The self-renewal of CSCs in colorectal cancers is functionally dependent on BMI1, which is one of the polycomb proteins [40]. Furthermore, inhibition of EZH2, a major component of polycomb repressive complex 2 (PRC2), has been demonstrated to dysfunction the self-renewal and tumor-initiating capabilities in some cancers [41], including HCC. Disruption of epigenetic regulations, such as DNA methylation and histone modification, is associated with the initiation and progression of tumors. The efficacy of epigenetic drugs has been proposed to eliminate CSCs in HCC [42]. Zebularine, a DNA methyltransferase (DNMT) inhibitor, declined CSC properties such as self-renewal and tumor-initiating capacities in HCC cells [43]. Histone deacetylase (HDAC) inhibitors such as trichostatin A and vorinostat have been shown to preferentially suppress the cell growth of SALL4-overexpressing HCC cell lines compared with that of $SALL4^-$ HCC cell lines [44, 45]. These findings suggest that epigenetic therapy using DNMT inhibitors and/or HDAC inhibitors may be a promising approach for the eradication of CSCs in HCC.

Another approach for eliminating CSCs has been suggested to be monoclonal antibodies targeting CSC-specific antigens [46], such as CD13, EpCAM, and CD133 antibodies, against hepatic CSCs [47–49]. However, these markers express in not only CSCs but also normal liver cells and tissue SCs. Thus, preclinical experiments and clinical trials will be needed for ensuring safety and efficacy.

On the other hand, hepatocyte nuclear factor-4a (HNF4A), a hepatocyte differentiation factor, decreases the number of CD90+ and CD133+ tumor-initiating cells [50] while simultaneously causing the cells to lose their tumorigenicity by inducing differentiation of the subpopulations. Similarly, oncostatin M (OSM) has been shown to induce the differentiation of EpCAM+ liver CSCs through the OSM receptor signaling pathway [51].

Both CSCs and normal tissue SCs are thought to reside in specialized microenvironments called niches. Brain tumor CSCs have been reported to exist in vascular niches where they are maintained in an undifferentiated state by endothelial cells [52]. An oral multikinase inhibitor, sorafenib, is the sole molecular target drug clinically approved to treat advanced HCC. This drug blocks tumor cell proliferation by targeting Raf/mitogen-activated protein kinase/extracellular signal-regulated kinase signaling and exerts an antiangiogenic effect by targeting tyrosine kinase receptors such as vascular endothelial growth factor receptor and platelet-derived growth factor receptor [53]. Although its role in the CSC niche in HCC has not been investigated, sorafenib may contribute to the eradication of CSCs in HCC.

5. CSCs in cholangiocarcinoma (CCA)

5.1. The characteristics of CCA

CCA is an epithelial cell malignancy arising from varying locations within the biliary tree showing markers of cholangiocyte differentiation. CCA is classified by the anatomical location, including intrahepatic, perihilar, and distal CCA. Intrahepatic CCA is defined by the location from proximally to the second-degree bile ducts in the liver. Perihilar CCA is defined by the location from the second-degree bile ducts to the insertion of the cystic duct into the common bile duct. Distal CCA is defined by the location from the origin of the cystic duct to ampulla of Vater.

Perihilar, distal, and intrahepatic disease represent about 50%, 40%, and <10% of CCA cases, respectively [54]. Mixed hepatocellular CCA was only recently acknowledged and accounts for about 1% of CCA cases. The incidence of intrahepatic CCA increases in western countries [55, 56]. The age-matched rate of CCA has been reported to be the highest in Hispanic and Asian populations (approximately 3 per 100,000) and the lowest in non-Hispanic white and black populations [57–59].

The mortality rate in intrahepatic CCA is largest in American Indian, Alaska Native groups, and Asian populations and is lowest in white and black populations [56]. Increases in both the recognition and incidence have contributed to the rising interest in this type of cancer. Most cases of CCA arise *de novo*, and no risk factors have yet been identified.

Cirrhosis and hepatitis C and B virus infections have been implicated as risk factors for CCA, in particular intrahepatic CCA. In the USA and European studies, viral hepatitis C was shown to be a risk factor for CCA with the strongest association observed for intrahepatic CCA [60], and a Japanese study subsequently confirmed these findings [61]. However, studies from South Korea and China have shown that hepatitis B is a more consistent risk factor for intrahepatic CCA [62–64]. A meta-analysis of several case-control studies on risk factors for intrahepatic CCA showed that the combined odds ratios (ORs) (95% confidence interval [CI]) of cirrhosis, hepatitis C, and hepatitis B were 22.92 (18.24–28.79), 4.84 (2.41–9.71), and 5.10 (2.91–8.95), respectively [65].

Southeast Asia has a very high incidence of CCA due to the high prevalence of the hepatobiliary flukes *Opisthorchis viverrini* and *Clonorchissinensis*, which are risk factors for CCA [65]. This risk is probably increased by environmental and genetic factors. Several genetic polymorphisms have been reported to increase the risk of CCA. The genes have been indicated as risk factors associated with DNA repair, cellular protection against toxins, or immunological surveillance [57].

Hepatolithiasis and biliary enteric drainage, predisposing patients to enteric bacteria bile duct colonization and infections, are additional risk factors for CCA [66]. The results from the studies on the role of alcohol and smoking exposure have been inconsistent [57]. Furthermore, metabolic syndrome was associated with an increased risk of intrahepatic CCA in the Surveillance and Epidemiology Results database analysis (OR: 1.6, 95% CI: 1.32–1.83, $p < 0.0001$). Consistent with these observations, a meta-analysis of the US and Danish studies identified an association of intrahepatic CCA with diabetes (OR: 1.89, 95% CI: 1.74–2.07) and obesity (OR: 1.56, 95% CI: 1.26–1.94). Although obesity is a biologically plausible risk factor for CCA development, the data are too scarce to definitively establish an association at this time.

5.2. The molecular pathway in CCA

The genetic pathways contributing to the selective growth advantage of cancer cells can be organized into those governing cell fate and differentiation, proliferation, cell survival, and maintenance of genome integrity. Several studies identifying genetic changes in CCA have been published, but most of the data generated from these single studies need further validation.

The Ras/mitogen-activated protein kinase pathway is one of the main signaling networks in CCA biology and was reported in several studies. Sia et al. described two distinct gene signature classes: a proliferation class and an inflammatory class. The proliferation class (62% of cases) was associated with copy number variations in several oncogenes, whereas the inflammatory class showed activation of inflammatory pathways causing overexpression of cytokines and the transcriptional factor STAT3, which modulates cell growth and survival and has been implicated in carcinogenesis [67, 68]. The Hedgehog survival signaling pathway in CCA has been identified to have tumor-suppressive activity in several studies [69, 70]. Hotspot mutations of genes encoding IDH1 and IDH2 were recently reported by several groups to be fairly specific to intrahepatic CCA among various gastrointestinal and biliary cancers (10–23%) [71, 72].

5.3. CSC markers in CCA

In CCA, chemotherapy adding surgery is usually needed for improving patient survival. The CSCs in CCA involves cell-surface markers, such as CD24, CD133, CD44, and EpCAM. CD133, known as prominin-1, is an important CSC marker, and has been also found in normal epithelial SCs [73]. CD133 also is an important CSC marker in CCA [74]. CD133-positive cells showed higher invasiveness compared with CD133-negative cells. Shimada et al. [75] analyzed CD133 expression in 29 patients with intrahepatic CCA and found that the 5-year survival rate in the CD133-positive group (8%) was worse than that in the CD133-negative group [76]. However, Fan et al. [77] reported contrasting results, in which CD133 expression was correlated with a higher tumor differentiation status in 54 consecutively analyzed CCA specimens. Moreover, positive CD133 expression significantly correlated with a better prognosis.

CD24 is expressed in cellular adhesion processes, cell motility, and invasive cell growth in cancers [78]. The median survival for patients with high CD24 expression was shorter than that for patients with low expression [79]. CD24 expression is also associated with a poor response to chemotherapy and radiation therapy [80]. However, CD24 is not detected in either the normal or inflamed epithelium, indicating that it may be a useful marker for early CCA carcinogenesis.

EpCAM is a hemophilic, Ca^{2+}-independent cell-cell adhesion molecule that is expressed in many human epithelial tissues while the expression in CCA remains unclear. There is just one report that EpCAM is much expressed in CCA cells compared with HCCs cells [81].

CD44 glycoprotein is expressed on epithelial cells and cancer cells. Wang et al. demonstrated that $CD24^{+}$ $CD44^{+}$ $EpCAM^{high}$ cells isolated from CCA xenografts had high tumorigenic potential compared with $CD24^{-}$ $CD44^{-}$ $EpCAM^{low/-}$ cells. Cells with high EpCAM expression exhibited the characteristic SC properties of self-renewal and heterogenous progeny [82]. The other markers, CD49f, CD117, and SCA-1, have been only scarcely investigated.

5.4. Therapy for CCA

Surgical treatment is the main therapy for improving patient survival in CCA [83]. The 5-year survival rate after radical surgical resection is approximately 35% in intrahepatic CCA and about 40% in perihilar CCA [83–85]. Regarding liver transplantation, the experience of liver transplantation for CCA is still limited, having performed in only a few selective centers, and it is mainly limited to early-stage perihilar CCA [86]. The first line of the chemotherapy in advanced and metastatic CCAs has been proposed to use the gemcitabine with cisplatin [87, 88]. The role of radiation or chemoradiation in CCA remains to be defined. The patterns of recurrence following resection of hilar or distal CCA play an important role in defining the appropriate strategy for adjuvant therapy [89].

The CSC-target therapy has been challenged *in vivo* experiments. CD133 inhibits cell growth of Hep3B human hepatoma cell line and abrogated tumor growth *in vivo* [49]. The EpCAM inhibition by small-interfering RNA (siRNA) in hepatic progenitor cells decreased tumorigenicity [90]. Further, CCA cell lines were inhibited by CD44 siRNA on invasiveness and

migration [91]. CD24 suppression decreased the invasive ability of CCA cells [79]. These data suggest that the therapy associated with the surface markers is a new candidate for a CSC-target therapy for CCA.

6. ALDH1 in liver cancers

The *ALDH* gene superfamily contains 19 putatively human functional genes, which encode enzymes that are critical for detoxification through the $NAD(P)^+$-dependent oxidation of aldehyde substrates. Among the 19 genes, *ALDH1* has been reported to encode the key ALDH isozyme linked to SC and CSC populations. In the liver SCs and CSCs, retinoic acid (RA), ROS, and aldehyde metabolism are likely to be deeply associated with the functional roles of ALDH1 (**Figure 1**).

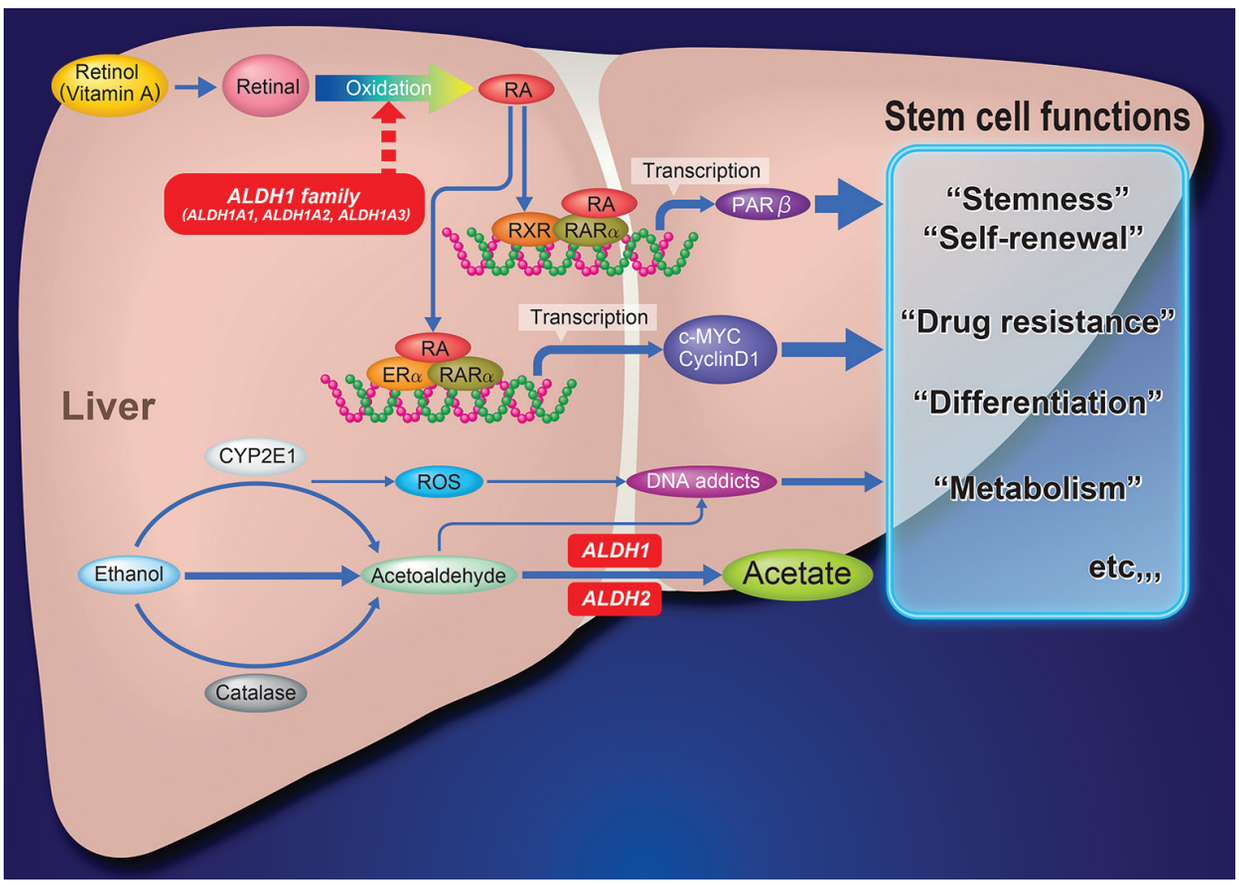

Figure 1. Regulation and function of ALDH1 in normal SCs and CSCs in the liver. Members of the ALDH1 family metabolize RA, regulating the self-renewal, differentiation, and drug resistance of SCs and CSCs. Retinol absorbed by cells is oxidized to retinal, which in turn is oxidized to RA by ALDH1 enzymes. RA binds to RARα and RXRs to induce the transcription of downstream target genes. RA can bind to dimers of RXRs and ERα and induces the expression c-MYC and cyclin D1. Furthermore, ALDH1 and ALDH2 reduce the levels of ROS and reactive aldehydes, thereby promoting tumor growth and initiating carcinogenesis in CSCs. SC, stem cell; CSC, cancer stem cell; RA, retinoic acid; RAR, retinoic acid receptor; RXR, retinoid X receptors; ER, estrogen receptor; and ROS, reactive oxygen species.

6.1. ALDH1 in retinoid signaling

Retinoid signaling has important roles in SCs and CSCs [92]. In retinoid signaling, retinol dehydrogenases oxidize the retinol absorbed by cells to retinal [93]. Retinal is then oxidized to RA in a reaction catalyzed by ALDH1 family members such as ALDH1A1, ALDH1A2, and ALDH1A3. The metabolized product RA includes all-*trans* RA, 9-*cis* RA, and 13-*cis* RA. RA enters the nucleus and induces the transcription of downstream genes through the activation of retinoic acid receptors (RARs) and retinoid X receptors (RXRs). Finally, increased ALDH1 contributes to not only RA synthesis but also cellular protection against cytotoxic drugs.

ALDH1 has been reported to regulate CSCs in breast cancer by promoting the metabolism of retinoid [94]. RA binds to RARs and RXRs and activates the expression of genes associated with differentiation, cell cycle arrest, and morphological variation [95]. Increasing RAR and RXR levels creates a positive feedback loop for retinoid signaling. RA formation by the oxidation of all-*trans*-retinal and 9-*cis*-retinal in retinoid signaling is closely associated with the function of SCs and CSCs [96].

6.2. ALDH1 in acetaldehyde metabolism

Alcohol dehydrogenase catalase and cytochrome P4502E1 metabolize ethanol to acetaldehyde. Acetaldehyde produces ROS, which suppress DNA repair and methylation and form DNA and protein adducts, thereby promoting carcinogenesis and tumor growth [97, 98]. ALDH1A1 and ALDH2 primarily metabolize acetaldehyde to acetate. ALDH activity maintains a low ROS level and inhibits CSC apoptosis [99]. Reactive aldehydes' metabolism and the ROS level are closely related to the characteristics of CSCs and cancer development. However, the relationship between ALDH and ROS in the functions of SCs and CSCs is still unclear.

6.3. ALDH1 in HCC

ALDH1 expression evaluated by immunohistochemistry is heterogenous and is present in the normal liver tissue, especially in hepatocytes [100]. However, ALDH bright cells, including ALDH1 isoforms, evaluated using the Aldefluor assay, have been reported to be a marker of liver progenitor cells in the normal liver tissue [101] and of CSCs in HCC [102]. Interestingly, ALDH bright cells are attributed to ALDH1 activity. Thus, ALDH1 expression in immunohistochemistry is considered to be slightly different from ALDH bright cells in HCC [93].

ALDH1 expression is associated with a favorable outcome for HCC patients [100, 103]. Furthermore, putative CSC markers such as CD24, CD13, CD90, EpCAM, BMI1, and CD133 were not colocalized with ALDH1-expressing cells in HCC [100]. Consequently, immunohistochemistry with an ALDH1 antibody shows differentiated cells that look like mature hepatocytes but not CSCs.

Taken together, these findings suggest that increased ALDH1 expression is associated with a factor indicative of a well-differentiated morphology and favorable prognosis in HCC. Furthermore, ALDH1-expressing cells may serve as a useful differentiation biological marker for HCC rather than as a CSC marker.

6.4. ALDH1 in CCA

Shuang et al. [104] demonstrated that ALDH1 is a valuable marker of CSCs in CCA. Further, patients with high ALDH1 expression had a poor prognosis in the cases of both intrahepatic and extrahepatic CCA. ALDH1 and CD133 are two other molecular markers of putative CSCs in extrahepatic CCA [105]. ALDH1 has been reported to play a crucial role in the identification of CSCs and/or tumor-initiating cells in various types of cancers [106]. In breast cancer, ALDH1+ seems to be a more significant predictive marker than other markers for the identification of breast CSCs. However, the identification of putative CSCs using a single marker such as ALDH1 is controversial. Nevertheless, ALDH1 has been shown to be a very important molecular marker for CSCs. To clarify the correlation among ALDH1 and other putative CSC markers, i.e., CD133, CD24, CD44, and EpCAM, and to identify cells with multiple CSC phenotypes might improve the selection of CSCs, and further studies are needed in this regard.

Recently, HCC and CCA have been shown to share the same origin. Hepatic progenitor cells can differentiate into hepatocytes and cholangiocytes and give rise to HCC as well as CCA [107]. ALDH1 expression has been reported to be specific to the liver CSCs' population [102] and can be assessed to reliably identify CCA cells with stem-like properties. With respect to other ALDH isoforms, only one study has described that ALDH1A3 was a poor prognostic factor and a good biomarker of gemcitabine resistance in intrahepatic CCA [108].

7. Conclusions

CSCs represent key cell populations among the heterogenous malignant cells of liver cancers, and their biological characteristics highlight them as a major target for cancer research. In particular, they provide reliable biomarkers for prognosis, such as ALDH1. Discovery of the mechanisms and molecules associated with CSCs offers great potential to accelerate the development of novel therapeutic options and improve the treatment outcome and quality of life of patients with liver cancers.

Conflicting interest

The authors declare no conflicts of interest.

Acknowledgements

We thank all the members of our laboratory and our collaborators for their research work and helpful discussions. This work was partly supported by grants from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (#15K11289 and #26430111).

Author details

Hiroyuki Tomita[1,*], Tomohiro Kanayama[1], Ayumi Niwa[1], Kei Noguchi[1], Kazuhisa Ishida[1], Masayuki Niwa[2] and Akira Hara[1]

*Address all correspondence to: h_tomita@gifu-u.ac.jp

1 Department of Tumor Pathology, Gifu University Graduate School of Medicine, Gifu, Japan

2 Medical Science Division, United Graduate School of Drug Discovery and Medical Information Sciences, Gifu, Japan

References

[1] Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):E359–E386.

[2] Grandhi MS, Kim AK, Ronnekleiv-Kelly SM, Kamel IR, Ghasebeh MA, Pawlik TM. Hepatocellular carcinoma: from diagnosis to treatment. Surg Oncol. 2016;25(2):74–85.

[3] Nguyen LV, Vanner R, Dirks P, Eaves CJ. Cancer stem cells: an evolving concept. Nat Rev Cancer. 2012;12(2):133–143.

[4] Vescovi AL, Galli R, Reynolds BA. Brain tumour stem cells. Nat Rev Cancer. 2006;6(6):425–436.

[5] Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature. 2006;444(7120):756–760.

[6] Tremblay KD, Zaret KS. Distinct populations of endoderm cells converge to generate the embryonic liver bud and ventral foregut tissues. Dev Biol. 2005;280(1):87–99.

[7] Suzuki A, Zheng Y, Kondo R, Kusakabe M, Takada Y, Fukao K, et al. Flow-cytometric separation and enrichment of hepatic progenitor cells in the developing mouse liver. Hepatology. 2000;32(6):1230–1239.

[8] Minguet S, Cortegano I, Gonzalo P, Martinez-Marin JA, de Andres B, Salas C, et al. A population of c-Kit(low)(CD45/TER119)- hepatic cell progenitors of 11-day post-coitus mouse embryo liver reconstitutes cell-depleted liver organoids. J Clin Invest. 2003;112(8):1152–1163.

[9] Suzuki A, Iwama A, Miyashita H, Nakauchi H, Taniguchi H. Role for growth factors and extracellular matrix in controlling differentiation of prospectively isolated hepatic stem cells. Development. 2003;130(11):2513–2524.

[10] Kakinuma S, Ohta H, Kamiya A, Yamazaki Y, Oikawa T, Okada K, et al. Analyses of cell surface molecules on hepatic stem/progenitor cells in mouse fetal liver. J Hepatol. 2009;51(1):127–138.

[11] Kamiya A, Kakinuma S, Yamazaki Y, Nakauchi H. Enrichment and clonal culture of progenitor cells during mouse postnatal liver development in mice. Gastroenterology. 2009;137(3):1114–1126, 1126e1–14.

[12] Tanimizu N, Nishikawa M, Saito H, Tsujimura T, Miyajima A. Isolation of hepatoblasts based on the expression of Dlk/Pref-1. J Cell Sci. 2003;116(Pt 9):1775–1786.

[13] Miyajima A, Tanaka M, Itoh T. Stem/progenitor cells in liver development, homeostasis, regeneration, and reprogramming. Cell Stem Cell. 2014;14(5):561–574.

[14] Nierhoff D, Ogawa A, Oertel M, Chen YQ, Shafritz DA. Purification and characterization of mouse fetal liver epithelial cells with high in vivo repopulation capacity. Hepatology. 2005;42(1):130–139.

[15] Watanabe T, Nakagawa K, Ohata S, Kitagawa D, Nishitai G, Seo J, et al. SEK1/MKK4-mediated SAPK/JNK signaling participates in embryonic hepatoblast proliferation via a pathway different from NF-kappaB-induced anti-apoptosis. Dev Biol. 2002;250(2):332–347.

[16] Nitou M, Sugiyama Y, Ishikawa K, Shiojiri N. Purification of fetal mouse hepatoblasts by magnetic beads coated with monoclonal anti-e-cadherin antibodies and their in vitro culture. Exp Cell Res. 2002;279(2):330–343.

[17] Nierhoff D, Levoci L, Schulte S, Goeser T, Rogler LE, Shafritz DA. New cell surface markers for murine fetal hepatic stem cells identified through high density complementary DNA microarrays. Hepatology. 2007;46(2):535–547.

[18] Tanaka M, Okabe M, Suzuki K, Kamiya Y, Tsukahara Y, Saito S, et al. Mouse hepatoblasts at distinct developmental stages are characterized by expression of EpCAM and DLK1: drastic change of EpCAM expression during liver development. Mech Dev. 2009;126(8–9):665–676.

[19] Schmelzer E, Zhang L, Bruce A, Wauthier E, Ludlow J, Yao HL, et al. Human hepatic stem cells from fetal and postnatal donors. J Exp Med. 2007;204(8):1973–1987.

[20] Okabe M, Tsukahara Y, Tanaka M, Suzuki K, Saito S, Kamiya Y, et al. Potential hepatic stem cells reside in EpCAM+ cells of normal and injured mouse liver. Development. 2009;136(11):1951–1960.

[21] Yovchev MI, Grozdanov PN, Zhou H, Racherla H, Guha C, Dabeva MD. Identification of adult hepatic progenitor cells capable of repopulating injured rat liver. Hepatology. 2008;47(2):636–647.

[22] Suzuki A, Sekiya S, Onishi M, Oshima N, Kiyonari H, Nakauchi H, et al. Flow cytometric isolation and clonal identification of self-renewing bipotent hepatic progenitor cells in adult mouse liver. Hepatology. 2008;48(6):1964–1978.

[23] Rountree CB, Barsky L, Ge S, Zhu J, Senadheera S, Crooks GM. A CD133-expressing murine liver oval cell population with bilineage potential. Stem Cells. 2007;25(10):2419–2429.

[24] Huch M, Dorrell C, Boj SF, van Es JH, Li VS, van de Wetering M, et al. In vitro expansion of single Lgr5+ liver stem cells induced by Wnt-driven regeneration. Nature. 2013;494(7436):247–250.

[25] Dorrell C, Erker L, Schug J, Kopp JL, Canaday PS, Fox AJ, et al. Prospective isolation of a bipotential clonogenic liver progenitor cell in adult mice. Genes Dev. 2011;25(11):1193–1203.

[26] Tarlow BD, Finegold MJ, Grompe M. Clonal tracing of Sox9+ liver progenitors in mouse oval cell injury. Hepatology. 2014;60(1):278–289.

[27] Huch M, Gehart H, van Boxtel R, Hamer K, Blokzijl F, Verstegen MM, et al. Long-term culture of genome-stable bipotent stem cells from adult human liver. Cell. 2015;160(1–2):299–312.

[28] Tarlow BD, Pelz C, Naugler WE, Wakefield L, Wilson EM, Finegold MJ, et al. Bipotential adult liver progenitors are derived from chronically injured mature hepatocytes. Cell Stem Cell. 2014;15(5):605–618.

[29] Lu WY, Bird TG, Boulter L, Tsuchiya A, Cole AM, Hay T, et al. Hepatic progenitor cells of biliary origin with liver repopulation capacity. Nat Cell Biol. 2015;17(8):971–983.

[30] Janevska D, Chaloska-Ivanova V, Janevski V. Hepatocellular carcinoma: risk factors, diagnosis and treatment. Open Access Maced J Med Sci. 2015;3(4):732–736.

[31] Romano M, De Francesco F, Pirozzi G, Gringeri E, Boetto R, Di Domenico M, et al. Expression of cancer stem cell biomarkers as a tool for a correct therapeutic approach to hepatocellular carcinoma. Oncoscience. 2015;2(5):443–456.

[32] Anfuso B, Tiribelli C, Sukowati CH. Recent insights into hepatic cancer stem cells. Hepatol Int 2014;8 Suppl 2:458–463.

[33] Chiba T, Iwama A, Yokosuka O. Cancer stem cells in hepatocellular carcinoma: therapeutic implications based on stem cell biology. Hepatol Res. 2016;46(1):50–57.

[34] Lu JW, Chang JG, Yeh KT, Chen RM, Tsai JJ, Hu RM. Overexpression of Thy1/CD90 in human hepatocellular carcinoma is associated with HBV infection and poor prognosis. Acta Histochem. 2011;113(8):833–838.

[35] Sasaki A, Kamiyama T, Yokoo H, Nakanishi K, Kubota K, Haga H, et al. Cytoplasmic expression of CD133 is an important risk factor for overall survival in hepatocellular carcinoma. Oncol Rep. 2010;24(2):537–546.

[36] 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):935–950.

[37] Song W, Li H, Tao K, Li R, Song Z, Zhao Q, et al. Expression and clinical significance of the stem cell marker CD133 in hepatocellular carcinoma. Int J Clin Pract. 2008;62(8):1212–1218.

[38] Hirohashi K, Tanaka H, Kanazawa A, Kubo S, Ohno K, Tsukamoto T, et al. Living-related liver transplantation in a patient with end-stage hepatolithiasis and a biliary-bronchial fistula. Hepatogastroenterology. 2004;51(57):822–824.

[39] Yang XR, Xu Y, Yu B, Zhou J, Li JC, Qiu SJ, et al. CD24 is a novel predictor for poor prognosis of hepatocellular carcinoma after surgery. Clin Cancer Res. 2009;15(17):5518–5527.

[40] Kreso A, van Galen P, Pedley NM, Lima-Fernandes E, Frelin C, Davis T, et al. Self-renewal as a therapeutic target in human colorectal cancer. Nat Med. 2014;20(1):29–36.

[41] Suva ML, Riggi N, Janiszewska M, Radovanovic I, Provero P, Stehle JC, et al. EZH2 is essential for glioblastoma cancer stem cell maintenance. Cancer Res. 2009;69(24):9211–9218.

[42] Marquardt JU, Thorgeirsson SS. SnapShot: hepatocellular carcinoma. Cancer Cell. 2014;25(4):550e1.

[43] Raggi C, Factor VM, Seo D, Holczbauer A, Gillen MC, Marquardt JU, et al. Epigenetic reprogramming modulates malignant properties of human liver cancer. Hepatology. 2014;59(6):2251–2262.

[44] Zeng SS, Yamashita T, Kondo M, Nio K, Hayashi T, Hara Y, et al. The transcription factor SALL4 regulates stemness of EpCAM-positive hepatocellular carcinoma. J Hepatol. 2014;60(1):127–134.

[45] Yong KJ, Gao C, Lim JS, Yan B, Yang H, Dimitrov T, et al. Oncofetal gene SALL4 in aggressive hepatocellular carcinoma. N Engl J Med. 2013;368(24):2266–2276.

[46] Deonarain MP, Kousparou CA, Epenetos AA. Antibodies targeting cancer stem cells: a new paradigm in immunotherapy? MAbs. 2009;1(1):12–25.

[47] Ogawa K, Tanaka S, Matsumura S, Murakata A, Ban D, Ochiai T, et al. EpCAM-targeted therapy for human hepatocellular carcinoma. Ann Surg Oncol. 2014;21(4):1314–1322.

[48] Haraguchi N, Ishii H, Mimori K, Tanaka F, Ohkuma M, Kim HM, et al. CD13 is a therapeutic target in human liver cancer stem cells. J Clin Invest. 2010;120(9):3326–3339.

[49] Smith LM, Nesterova A, Ryan MC, Duniho S, Jonas M, Anderson M, et al. CD133/prominin-1 is a potential therapeutic target for antibody-drug conjugates in hepatocellular and gastric cancers. Br J Cancer. 2008;99(1):100–109.

[50] Yin C, Lin Y, Zhang X, Chen YX, Zeng X, Yue HY, et al. Differentiation therapy of hepatocellular carcinoma in mice with recombinant adenovirus carrying hepatocyte nuclear factor-4alpha gene. Hepatology. 2008;48(5):1528–1539.

[51] Yamashita T, Honda M, Nio K, Nakamoto Y, Yamashita T, Takamura H, et al. Oncostatin M renders epithelial cell adhesion molecule-positive liver cancer stem cells sensitive to 5-fluorouracil by inducing hepatocytic differentiation. Cancer Res. 2010;70(11):4687–4697.

[52] Gilbertson RJ, Rich JN. Making a tumour's bed: glioblastoma stem cells and the vascular niche. Nat Rev Cancer. 2007;7(10):733–736.

[53] Wilhelm SM, Carter C, Tang L, Wilkie D, McNabola A, Rong H, et al. BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Res. 2004;64(19):7099–7109.

[54] DeOliveira ML, Cunningham SC, Cameron JL, Kamangar F, Winter JM, Lillemoe KD, et al. Cholangiocarcinoma: thirty-one-year experience with 564 patients at a single institution. Ann Surg. 2007;245(5):755–762.

[55] Khan SA, Emadossadaty S, Ladep NG, Thomas HC, Elliott P, Taylor-Robinson SD, et al. Rising trends in cholangiocarcinoma: is the ICD classification system misleading us? J Hepatol. 2012;56(4):848–854.

[56] McLean L, Patel T. Racial and ethnic variations in the epidemiology of intrahepatic cholangiocarcinoma in the United States. Liver Int. 2006;26(9):1047–1053.

[57] Tyson GL, El-Serag HB. Risk factors for cholangiocarcinoma. Hepatology. 2011;54(1):173–184.

[58] Everhart JE, Ruhl CE. Burden of digestive diseases in the United States part I: overall and upper gastrointestinal diseases. Gastroenterology. 2009;136(2):376–386.

[59] Shaib Y, El-Serag HB. The epidemiology of cholangiocarcinoma. Semin Liver Dis. 2004;24(2):115–125.

[60] Welzel TM, Mellemkjaer L, Gloria G, Sakoda LC, Hsing AW, El Ghormli L, et al. Risk factors for intrahepatic cholangiocarcinoma in a low-risk population: a nationwide case–control study. Int J Cancer. 2007;120(3):638–641.

[61] Yamamoto S, Kubo S, Hai S, Uenishi T, Yamamoto T, Shuto T, et al. Hepatitis C virus infection as a likely etiology of intrahepatic cholangiocarcinoma. Cancer Sci. 2004;95(7):592–595.

[62] Sekiya S, Suzuki A. Intrahepatic cholangiocarcinoma can arise from Notch-mediated conversion of hepatocytes. J Clin Invest. 2012;122(11):3914–3918.

[63] Zhou HB, Chen JM, Cai JT, Du Q, Wu CN. Anticancer activity of genistein on implanted tumor of human SG7901 cells in nude mice. World J Gastroenterol. 2008; 14(4):627–631.

[64] Lee TY, Lee SS, Jung SW, Jeon SH, Yun SC, Oh HC, et al. Hepatitis B virus infection and intrahepatic cholangiocarcinoma in Korea: a case–control study. Am J Gastroenterol. 2008;103(7):1716–1720.

[65] Palmer WC, Patel T. Are common factors involved in the pathogenesis of primary liver cancers? A meta-analysis of risk factors for intrahepatic cholangiocarcinoma. J Hepatol. 2012;57(1):69–76.

[66] Tocchi A, Mazzoni G, Liotta G, Lepre L, Cassini D, Miccini M. Late development of bile duct cancer in patients who had biliary-enteric drainage for benign disease: a follow-up study of more than 1,000 patients. Ann Surg. 2001;234(2):210–214.

[67] Sia D, Hoshida Y, Villanueva A, Roayaie S, Ferrer J, Tabak B, et al. Integrative molecular analysis of intrahepatic cholangiocarcinoma reveals 2 classes that have different outcomes. Gastroenterology. 2013;144(4):829–840.

[68] Sansone P, Bromberg J. Targeting the interleukin-6/Jak/stat pathway in human malignancies. J Clin Oncol. 2012;30(9):1005–1014.

[69] Jinawath A, Akiyama Y, Sripa B, Yuasa Y. Dual blockade of the Hedgehog and ERK1/2 pathways coordinately decreases proliferation and survival of cholangiocarcinoma cells. J Cancer Res Clin Oncol. 2007;133(4):271–278.

[70] Berman DM, Karhadkar SS, Maitra A, Montes De Oca R, Gerstenblith MR, Briggs K, et al. Widespread requirement for Hedgehog ligand stimulation in growth of digestive tract tumours. Nature. 2003;425(6960):846–851.

[71] Wang P, Dong Q, Zhang C, Kuan PF, Liu Y, Jeck WR, et al. Mutations in isocitrate dehydrogenase 1 and 2 occur frequently in intrahepatic cholangiocarcinomas and share hypermethylation targets with glioblastomas. Oncogene. 2013;32(25):3091–3100.

[72] Borger DR, Tanabe KK, Fan KC, Lopez HU, Fantin VR, Straley KS, et al. Frequent mutation of isocitrate dehydrogenase (IDH)1 and IDH2 in cholangiocarcinoma identified through broad-based tumor genotyping. Oncologist. 2012;17(1):72–79.

[73] Mizrak D, Brittan M, Alison M. CD133: molecule of the moment. J Pathol. 2008;214(1):3–9.

[74] Wang M, Xiao J, Shen M, Yahong Y, Tian R, Zhu F, et al. Isolation and characterization of tumorigenic extrahepatic cholangiocarcinoma cells with stem cell-like properties. Int J Cancer. 2011;128(1):72–81.

[75] Shimada M, Sugimoto K, Iwahashi S, Utsunomiya T, Morine Y, Imura S, et al. CD133 expression is a potential prognostic indicator in intrahepatic cholangiocarcinoma. J Gastroenterol. 2010;45(8):896–902.

[76] Leelawat K, Thongtawee T, Narong S, Subwongcharoen S, Treepongkaruna SA. Strong expression of CD133 is associated with increased cholangiocarcinoma progression. World J Gastroenterol. 2011;17(9):1192–1198.

[77] Fan L, He F, Liu H, Zhu J, Liu Y, Yin Z, et al. CD133: a potential indicator for differentiation and prognosis of human cholangiocarcinoma. BMC Cancer. 2011;11:320.

[78] Riener MO, Vogetseder A, Pestalozzi BC, Clavien PA, Probst-Hensch N, Kristiansen G, et al. Cell adhesion molecules P-cadherin and CD24 are markers for carcinoma and dysplasia in the biliary tract. Hum Pathol. 2010;41(11):1558–1565.

[79] Keeratichamroen S, Leelawat K, Thongtawee T, Narong S, Aegem U, Tujinda S, et al. Expression of CD24 in cholangiocarcinoma cells is associated with disease progression and reduced patient survival. Int J Oncol. 2011;39(4):873–881.

[80] Agrawal S, Kuvshinoff BW, Khoury T, Yu J, Javle MM, LeVea C, et al. CD24 expression is an independent prognostic marker in cholangiocarcinoma. J Gastrointest Surg. 2007;11(4):445–451.

[81] de Boer CJ, van Krieken JH, Janssen-van Rhijn CM, Litvinov SV. Expression of Ep-CAM in normal, regenerating, metaplastic, and neoplastic liver. J Pathol. 1999;188(2):201–206.

[82] Zhu Z, Hao X, Yan M, Yao M, Ge C, Gu J, et al. Cancer stem/progenitor cells are highly enriched in CD133+CD44+ population in hepatocellular carcinoma. Int J Cancer. 2010;126(9):2067–2078.

[83] de Jong MC, Nathan H, Sotiropoulos GC, Paul A, Alexandrescu S, Marques H, et al. Intrahepatic cholangiocarcinoma: an international multi-institutional analysis of prognostic factors and lymph node assessment. J Clin Oncol. 2011;29(23):3140–3145.

[84] Zaydfudim VM, Rosen CB, Nagorney DM. Hilar cholangiocarcinoma. Surg Oncol Clin N Am. 2014;23(2):247–263.

[85] Fendrich V, Langer P, Celik I, Bartsch DK, Zielke A, Ramaswamy A, et al. An aggressive surgical approach leads to long-term survival in patients with pancreatic endocrine tumors. Ann Surg. 2006;244(6):845–851; discussion 52–53.

[86] Darwish Murad S, Kim WR, Harnois DM, Douglas DD, Burton J, Kulik LM, et al. Efficacy of neoadjuvant chemoradiation, followed by liver transplantation, for perihilar cholangiocarcinoma at 12 US centers. Gastroenterology. 2012;143(1):88–98 e3; quiz e14.

[87] Valle J, Wasan H, Palmer DH, Cunningham D, Anthoney A, Maraveyas A, et al. Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. N Engl J Med. 2010;362(14):1273–1281.

[88] Okusaka T, Nakachi K, Fukutomi A, Mizuno N, Ohkawa S, Funakoshi A, et al. Gemcitabine alone or in combination with cisplatin in patients with biliary tract cancer: a comparative multicentre study in Japan. Br J Cancer. 2010;103(4):469–474.

[89] Jarnagin WR, Ruo L, Little SA, Klimstra D, D'Angelica M, DeMatteo RP, et al. Patterns of initial disease recurrence after resection of gallbladder carcinoma and hilar cholangiocarcinoma: implications for adjuvant therapeutic strategies. Cancer. 2003;98(8):1689–1700.

[90] Yamashita T, Ji J, Budhu A, Forgues M, Yang W, Wang HY, et al. EpCAM-positive hepatocellular carcinoma cells are tumor-initiating cells with stem/progenitor cell features. Gastroenterology. 2009;136(3):1012–1024.

[91] Nathan H, Pawlik TM, Wolfgang CL, Choti MA, Cameron JL, Schulick RD. Trends in survival after surgery for cholangiocarcinoma: a 30-year population-based SEER database analysis. J Gastrointest Surg. 2007;11(11):1488–1496; discussion 96–97.

[92] Chanda B, Ditadi A, Iscove NN, Keller G. Retinoic acid signaling is essential for embryonic hematopoietic stem cell development. Cell. 2013;155(1):215–227.

[93] Tomita H, Tanaka K, Tanaka T, Hara A. Aldehyde dehydrogenase 1A1 in stem cells and cancer. Oncotarget. 2016;7(10):11018–11032.

[94] Ginestier C, Wicinski J, Cervera N, Monville F, Finetti P, Bertucci F, et al. Retinoid signaling regulates breast cancer stem cell differentiation. Cell Cycle. 2009;8(20):3297–3302.

[95] Ying M, Wang S, Sang Y, Sun P, Lal B, Goodwin CR, et al. Regulation of glioblastoma stem cells by retinoic acid: role for Notch pathway inhibition. Oncogene. 2011;30(31):3454–3467.

[96] Marcato P, Dean CA, Giacomantonio CA, Lee PW. Aldehyde dehydrogenase: its role as a cancer stem cell marker comes down to the specific isoform. Cell Cycle. 2011;10(9):1378–1384.

[97] Seitz HK, Stickel F. Molecular mechanisms of alcohol-mediated carcinogenesis. Nat Rev Cancer. 2007;7(8):599–612.

[98] Brennan P, Boffetta P. Mechanistic considerations in the molecular epidemiology of head and neck cancer. IARC Sci Publ. 2004(157):393–414.

[99] Xu X, Chai S, Wang P, Zhang C, Yang Y, Yang Y, et al. Aldehyde dehydrogenases and cancer stem cells. Cancer Lett. 2015;369(1):50–57.

[100] Tanaka K, Tomita H, Hisamatsu K, Nakashima T, Hatano Y, Sasaki Y, et al. ALDH1A1-overexpressing cells are differentiated cells but not cancer stem or progenitor cells in human hepatocellular carcinoma. Oncotarget. 2015;6(28):24722–24732.

[101] Dolle L, Best J, Empsen C, Mei J, Van Rossen E, Roelandt P, et al. Successful isolation of liver progenitor cells by aldehyde dehydrogenase activity in naive mice. Hepatology. 2012;55(2):540–552.

[102] Ma S, Chan KW, Lee TK, Tang KH, Wo JY, Zheng BJ, et al. Aldehyde dehydrogenase discriminates the CD133 liver cancer stem cell populations. Mol Cancer Res. 2008;6(7):1146–1153.

[103] Suzuki E, Chiba T, Zen Y, Miyagi S, Tada M, Kanai F, et al. Aldehyde dehydrogenase 1 is associated with recurrence-free survival but not stem cell-like properties in hepatocellular carcinoma. Hepatol Res. 2012;42(11):1100–1111.

[104] Shuang ZY, Wu WC, Xu J, Lin G, Liu YC, Lao XM, et al. Transforming growth factor-beta1-induced epithelial-mesenchymal transition generates ALDH-positive cells with stem cell properties in cholangiocarcinoma. Cancer Lett. 2014;354(2):320–328.

[105] Wang M, Xiao J, Jiang J, Qin R. CD133 and ALDH may be the molecular markers of cholangiocarcinoma stem cells. Int J Cancer. 2011;128(8):1996–1997.

[106] Moreb JS. Aldehyde dehydrogenase as a marker for stem cells. Curr Stem Cell Res Ther. 2008;3(4):237–246.

[107] Roskams T. Liver stem cells and their implication in hepatocellular and cholangiocarcinoma. Oncogene. 2006;25(27):3818–3822.

[108] Chen MH, Weng JJ, Cheng CT, Wu RC, Huang SC, Wu CE, et al. ALDH1A3, the major aldehyde dehydrogenase isoform in human cholangiocarcinoma cells, affects prognosis and gemcitabine resistance in cholangiocarcinoma patients. Clin Cancer Res. 2016;22(16)4225–4235.

IntechOpen