

# World Journal of *Gastroenterology*

*World J Gastroenterol* 2017 April 14; 23(14): 2453-2634



### EDITORIAL

- 2453 Noninvasive molecular analysis of *Helicobacter pylori*: Is it time for tailored first-line therapy?  
*Ierardi E, Giorgio F, Iannone A, Losurdo G, Principi M, Barone M, Pisani A, Di Leo A*

### REVIEW

- 2459 Pathogenesis and clinical spectrum of primary sclerosing cholangitis  
*Gidwaney NG, Pawa S, Das KM*
- 2470 Biliary tract cancer stem cells - translational options and challenges  
*Mayr C, Ocker M, Ritter M, Pichler M, Neureiter D, Kiesslich T*

### MINIREVIEWS

- 2483 Potential role of nutraceutical compounds in inflammatory bowel disease  
*Larussa T, Imeneo M, Luzzza F*
- 2493 Unusual gastric tumors and tumor-like lesions: Radiological with pathological correlation and literature review  
*Lin YM, Chiu NC, Li AFY, Liu CA, Chou YH, Chiou YY*
- 2505 New progress in roles of nitric oxide during hepatic ischemia reperfusion injury  
*Zhang YQ, Ding N, Zeng YF, Xiang YY, Yang MW, Hong FF, Yang SL*

### ORIGINAL ARTICLE

#### Basic Study

- 2511 Berberine displays antitumor activity in esophageal cancer cells *in vitro*  
*Jiang SX, Qi B, Yao WJ, Gu CW, Wei XF, Zhao Y, Liu YZ, Zhao BS*

#### Case Control Study

- 2519 Clinical utility of the platelet-lymphocyte ratio as a predictor of postoperative complications after radical gastrectomy for clinical T2-4 gastric cancer  
*Inaoka K, Kanda M, Uda H, Tanaka Y, Tanaka C, Kobayashi D, Takami H, Iwata N, Hayashi M, Niwa Y, Yamada S, Fujii T, Sugimoto H, Murotani K, Fujiwara M, Kodera Y*
- 2527 Colors of vegetables and fruits and the risks of colorectal cancer  
*Lee J, Shin A, Oh JH, Kim J*

**Retrospective Cohort Study**

- 2539 Impact of vitamin D on the hospitalization rate of Crohn's disease patients seen at a tertiary care center  
*Venkata KVR, Arora SS, Xie FL, Malik TA*

- 2545 Barcelona clinic liver cancer nomogram and others staging/scoring systems in a French hepatocellular carcinoma cohort

*Adhoute X, Pénaranda G, Raoul JL, Edeline J, Blanc JF, Pol B, Campanile M, Perrier H, Bayle O, Monnet O, Beaurain P, Muller C, Castellani P, Le Treut YP, Bronowicki JP, Bourlière M*

**Retrospective Study**

- 2556 Laparoscopic approach to suspected T1 and T2 gallbladder carcinoma

*Ome Y, Hashida K, Yokota M, Nagahisa Y, Okabe M, Kawamoto K*

- 2566 Clinical characteristics of peptic ulcer perforation in Korea

*Yang YJ, Bang CS, Shin SP, Park TY, Suk KT, Baik GH, Kim DJ*

- 2575 Effects of omeprazole in improving concurrent chemoradiotherapy efficacy in rectal cancer

*Zhang JL, Liu M, Yang Q, Lin SY, Shan HB, Wang HY, Xu GL*

**Clinical Trials Study**

- 2585 *PIK3CA* gene mutations in Northwest Chinese esophageal squamous cell carcinoma

*Liu SY, Chen W, Chughtai EA, Qiao Z, Jiang JT, Li SM, Zhang W, Zhang J*

- 2592 Endothelial progenitor cells in peripheral blood may serve as a biological marker to predict severe acute pancreatitis

*Ha XQ, Song YJ, Zhao HB, Ta WW, Gao HW, Feng QS, Dong JZ, Deng ZY, Fan HY, Peng JH, Yang ZH, Zhao Y*

- 2601 Comparative study of ROR2 and WNT5a expression in squamous/adenosquamous carcinoma and adenocarcinoma of the gallbladder

*Wu ZC, Xiong L, Wang LX, Miao XY, Liu ZR, Li DQ, Zou Q, Liu KJ, Zhao H, Yang ZL*

**Observational Study**

- 2613 Serum omentin and vaspin levels in cirrhotic patients with and without portal vein thrombosis

*Kukla M, Waluga M, Żorniak M, Berdowska A, Wosiewicz P, Sawczyn T, Buldak RJ, Ochman M, Ziora K, Krzemiński T, Hartleb M*

- 2625 Upper gastrointestinal cancer burden in Hebei Province, China: A population-based study

*Li DJ, Liang D, Song GH, Li YW, Wen DG, Jin J, He YT*

**ABOUT COVER**

Editorial board member of *World Journal of Gastroenterology*, Vicente Lorenzo-Zuniga, MD, PhD, Associate Professor, Chief Doctor, Staff Physician, Endoscopy Unit, Department of Gastroenterology, Hospital Universitari Germans Trias i Pujol/CIBERehd, Badalona 08916, Spain

**AIMS AND SCOPE**

*World Journal of Gastroenterology* (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a peer-reviewed open access journal. *WJG* was established on October 1, 1995. It is published weekly on the 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup>, and 28<sup>th</sup> each month. The *WJG* Editorial Board consists of 1375 experts in gastroenterology and hepatology from 68 countries.

The primary task of *WJG* is to rapidly publish high-quality original articles, reviews, and commentaries in the fields of gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, hepatobiliary surgery, gastrointestinal oncology, gastrointestinal radiation oncology, gastrointestinal imaging, gastrointestinal interventional therapy, gastrointestinal infectious diseases, gastrointestinal pharmacology, gastrointestinal pathophysiology, gastrointestinal pathology, evidence-based medicine in gastroenterology, pancreatology, gastrointestinal laboratory medicine, gastrointestinal molecular biology, gastrointestinal immunology, gastrointestinal microbiology, gastrointestinal genetics, gastrointestinal translational medicine, gastrointestinal diagnostics, and gastrointestinal therapeutics. *WJG* is dedicated to become an influential and prestigious journal in gastroenterology and hepatology, to promote the development of above disciplines, and to improve the diagnostic and therapeutic skill and expertise of clinicians.

**INDEXING/ABSTRACTING**

*World Journal of Gastroenterology* (*WJG*) is now indexed in Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, PubMed Central, Digital Object Identifier, and Directory of Open Access Journals. The 2015 edition of Journal Citation Reports® released by Thomson Reuters (ISI) cites the 2015 impact factor for *WJG* as 2.787 (5-year impact factor: 2.848), ranking *WJG* as 38 among 78 journals in gastroenterology and hepatology (quartile in category Q2).

**FLYLEAF**

I-IX Editorial Board

**EDITORS FOR THIS ISSUE**

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Cai-Hong Wang*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*  
Responsible Science Editor: *Yuan Qi*  
Proofing Editorial Office Director: *Jin-Lei Wang*

**NAME OF JOURNAL**  
*World Journal of Gastroenterology*

**ISSN**  
ISSN 1007-9327 (print)  
ISSN 2219-2840 (online)

**LAUNCH DATE**  
October 1, 1995

**FREQUENCY**  
Weekly

**EDITORS-IN-CHIEF**  
**Damian Garcia-Olmo, MD, PhD, Doctor, Professor, Surgeon**, Department of Surgery, Universidad Autonoma de Madrid; Department of General Surgery, Fundacion Jimenez Diaz University Hospital, Madrid 28040, Spain

**Stephen C Strom, PhD, Professor**, Department of Laboratory Medicine, Division of Pathology, Karolinska Institutet, Stockholm 141-86, Sweden

**Andrzej S Tarnawski, MD, PhD, DSc (Med), Professor of Medicine, Chief Gastroenterology**, VA Long Beach Health Care System, University of California, Irvine, CA, 5901 E. Seventh Str., Long Beach,

CA 90822, United States

**EDITORIAL BOARD MEMBERS**  
All editorial board members resources online at <http://www.wjgnet.com/1007-9327/editorialboard.htm>

**EDITORIAL OFFICE**  
Jin-Lei Wang, Director  
Yuan Qi, Vice Director  
Ze-Mao Gong, Vice Director  
*World Journal of Gastroenterology*  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-2238242  
Fax: +1-925-2238243  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
Help Desk: <http://www.fpublishing.com/helpdesk>  
<http://www.wjgnet.com>

**PUBLISHER**  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-2238242  
Fax: +1-925-2238243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.fpublishing.com/helpdesk>

<http://www.wjgnet.com>

**PUBLICATION DATE**  
April 14, 2017

**COPYRIGHT**  
© 2017 Baishideng Publishing Group Inc. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

**SPECIAL STATEMENT**  
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

**INSTRUCTIONS TO AUTHORS**  
Full instructions are available online at <http://www.wjgnet.com/bpg/gerinfo/204>

**ONLINE SUBMISSION**  
<http://www.fpublishing.com>

## Biliary tract cancer stem cells - translational options and challenges

Christian Mayr, Matthias Ocker, Markus Ritter, Martin Pichler, Daniel Neureiter, Tobias Kiesslich

Christian Mayr, Tobias Kiesslich, Department of Internal Medicine I, Paracelsus Medical University, Salzburger Landeskliniken, 5020 Salzburg, Austria

Christian Mayr, Tobias Kiesslich, Laboratory for Tumour Biology and Experimental Therapies, Institute of Physiology and Pathophysiology, Paracelsus Medical University, 5020 Salzburg, Austria

Matthias Ocker, Translational Medicine Oncology, Bayer AG, 13353 Berlin, Germany

Matthias Ocker, Department of Gastroenterology CBF, Charité University Medicine Berlin, 10117 Berlin, Germany

Markus Ritter, Laboratory for Tumour Biology and Experimental Therapies (TREAT), Laboratory for Functional and Molecular Membrane Physiology, Paracelsus Medical University, 5020 Salzburg, Austria

Markus Ritter, Department for Radon Therapy Research, Ludwig Boltzmann Cluster for Arthritis and Rehabilitation, Institute of Physiology and Pathophysiology, Paracelsus Medical University, 5020 Salzburg, Austria

Martin Pichler, Research Unit for Non-coding RNA and genome editing in cancer, Division of Oncology, Department of Internal Medicine, Medical University of Graz, 8036 Graz, Austria

Daniel Neureiter, Institute of Pathology, Paracelsus Medical University, Salzburger Landeskliniken, 5020 Salzburg, Austria

Daniel Neureiter, Cancer Cluster Salzburg, Salzburg 5020, Austria

**Author contributions:** Mayr C and Kiesslich T wrote the manuscript; Kiesslich T and Neureiter D designed tables and figure; Mayr C, Ocker M, Ritter M, Pichler M, Neureiter D and Kiesslich T equally contributed to literature research and revising the manuscript.

**Supported by** Studies of the authors Mayr C, Pichler M, Neureiter D and Kiesslich T in the research field of this review were supported by research grants of the Jubiläumsfonds der Österreichischen Nationalbank, No. 12677 and No. 14842; and

the research fund of the Paracelsus Medical University Salzburg, No. 08/07/037, No. A-12/02/006-KIE and No. R-16/03/083-MAY.

**Conflict-of-interest statement:** Ocker M is an employee of Bayer AG, Berlin. Matthias Ocker owns shares of Bayer Pharma AG, Berlin. The other authors have no conflicts of interests.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Matthias Ocker, Professor, Translational Medicine Oncology, Bayer AG, Muellerstrasse 178, 13353 Berlin, Germany. [matthias.ocker@bayer.com](mailto:matthias.ocker@bayer.com)  
**Telephone:** +49-30-468194799

**Received:** January 25, 2017

**Peer-review started:** February 1, 2017

**First decision:** February 10, 2017

**Revised:** February 27, 2017

**Accepted:** March 21, 2017

**Article in press:** March 21, 2017

**Published online:** April 14, 2017

### Abstract

Management of biliary tract cancer remains challenging. Tumors show high recurrence rates and therapeutic resistance, leading to dismal prognosis and short survival. The cancer stem cell model states that a tumor is a heterogeneous conglomerate of cells, in which a certain subpopulation of cells - the cancer stem cells - possesses stem cell properties. Cancer

stem cells have high clinical relevance due to their potential contributions to development, progression and aggressiveness as well as recurrence and metastasis of malignant tumors. Consequently, reliable identification of as well as pharmacological intervention with cancer stem cells is an intensively investigated and promising research field. The involvement of cancer stem cells in biliary tract cancer is likely as a number of studies demonstrated their existence and the obvious clinical relevance of several established cancer stem cell markers in biliary tract cancer models and tissues. In the present article, we review and discuss the currently available literature addressing the role of putative cancer stem cells in biliary tract cancer as well as the connection between known contributors of biliary tract tumorigenesis such as oncogenic signaling pathways, micro-RNAs and the tumor microenvironment with cancer stem cells.

**Key words:** Biliary tract cancer; cancer stem cells; Cancer stem cell markers; Tumor microenvironment; Micro-RNAs

© The Author(s) 2017. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Using a xenograft model, researchers successfully demonstrated that as few as ten of a specific subpopulation of biliary tract cancer cells had the potency to (serially) establish and recapitulate biliary tract cancer in immunodeficient mice. Furthermore, expression of established cancer stem cell markers, cancer stem cell-related signaling pathways and micro-RNAs was reported in biliary specimens and cell lines - in most cases associated with clinical outcome. Based on these results, the existence of cancer stem cells in biliary tract is well-founded and potentially harbors new options for development of therapeutic strategies.

Mayr C, Ocker M, Ritter M, Pichler M, Neureiter D, Kiesslich T. Biliary tract cancer stem cells - translational options and challenges. *World J Gastroenterol* 2017; 23(14): 2470-2482 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v23/i14/2470.htm> DOI: <http://dx.doi.org/10.3748/wjg.v23.i14.2470>

## INTRODUCTION

### General aspects of cancer stem cells

In 1994, Lapidot *et al.*<sup>[1]</sup> identified a subpopulation of cells, characterized by a specific set of surface markers that was able to initiate acute myeloid leukemia in mice. Since then, numerous of such tumor-initiating cells were identified in most solid tumors<sup>[2]</sup>. These tumor-initiating cells, also referred to as cancer stem cells (TICs or CSCs) have the ability to self-renew as well as to differentiate into different lineages - traits that they share with (adult) stem

cells. Further similarities between tumor-initiating cells and normal adult stem cells include the reliance on certain highly conserved embryonic pathways (such as Hedgehog (Hh), Nanog and Wnt), a specialized metabolism (preferential oxidative glycolysis), enhanced protection against DNA damage and oxidative stress, a specific epigenetic profile (*e.g.*, abnormal polycomb repressive complexes activity, see below) as well as the expression of specific surface markers (reviewed in<sup>[2]</sup>). The specific abilities and profiles of CSC may at least in part explain some of the common clinical problems seen when dealing with cancer. CSCs are slow-cycling cells that often are in a quiescent state. Common chemotherapeutics target proliferating, fast cycling cells, thereby erasing the bulk of the tumor while not affecting CSCs - a phenomenon resulting in tumor recurrence. Moreover, CSCs strongly express drug efflux pumps, contributing to the well-known chemoresistance of these cells<sup>[2-5]</sup>.

Currently, two main models are discussed regarding the origin of CSCs<sup>[6]</sup>. In the "stochastic model", each cancer cell is biologically equivalent and unpredictably may acquire a CSC phenotype depending on diverse stochastic events from inside the cells (*via* genetic and epigenetic changes) as well as from the surrounding environment. The second model called "hierarchical model" states that a tumor is, like solid organs, a hierarchically organized heterogeneous cell conglomerate in which only a small subset of cells - the CSC - have the ability to self-renew and to give rise to daughter cells of various differentiation, whereas the majority of cancer cells that form the bulk of the tumor cannot achieve CSC traits. Besides these two main models, a third possible origin of CSC is currently discussed in the literature, namely de-differentiation of already committed cells - a phenomenon that was observed in different tumor entities and that is likely to play a role in biliary tract cancer (BTC) as discussed later in this article<sup>[7-10]</sup>.

## EXPERIMENTAL IDENTIFICATION AND CHARACTERIZATION OF CANCER STEM CELLS

Identification and/or isolation of CSC based on their expression profile (surface markers, signaling pathways) as well as their functional characteristics represent powerful tools in cancer research. The ability of CSCs to form tumors in immunodeficient mice at very low cell numbers surrogates their high tumorigenic potential<sup>[2]</sup>. Besides *in vivo* xenograft experiments, also several *in vitro* techniques are used in CSC research. The clonogenic assay similarly addresses the higher tumorigenic potential of CSC. Here, very few cells (approximate range between 50-200 cells per cm<sup>2</sup> - highly cell line-dependent) are seeded in a cell culture receptacle and the tumorigenic potential is evaluated by counting the number of growing colonies, each of them originating from a single cell clone representing

a potential CSC<sup>[11]</sup>. Anchorage-independent growth, *i.e.*, the formation of tumor spheres in a non-adherent environment, is another well-established experimental approach used in CSC research<sup>[12]</sup>. Likewise, expression of aldehyde-dehydrogenase 1 (ALDH1) is considered as a functional CSC marker<sup>[13]</sup>. ALDH1 is a detoxifying enzyme that was shown to be up-regulated in cancer and associated with various CSC traits such as enhanced tumor growth and the potential of self-renewal and differentiation<sup>[13-16]</sup>. Moreover, the so-called side-population phenotype may signify a CSC population. Side-population cells are defined by their ability to excrete fluorescent dyes such as Hoechst 33342 - this characteristic is based on the enhanced expression of efflux pumps in (cancer) stem cells<sup>[17,18]</sup>. Lastly, potential CSCs are identified and isolated based on their surface markers using fluorescence-activated cell sorting or by analysis of expression of established (cancer) stem cell genes.

---

## BILIARY TRACT CANCER - CLINICAL BACKGROUND

---

BTC is a heterogeneous malignancy that arises from different locations within the biliary tree. It can be categorized into intrahepatic cholangiocarcinoma (IHC), extrahepatic cholangiocarcinoma (EHC), gallbladder cancer (GBC) and mixed hepatocellular-cholangiocarcinoma (HCC-CC). Although BTC is generally a rare disease, it has high clinical significance due to its dismal outcome and limited therapeutic options<sup>[19,20]</sup>. Due to late diagnosis, for most patients only palliative treatment is possible; furthermore, the standard chemotherapeutic approach using a combination of cisplatin and gemcitabine results in median survival of approximately one year only<sup>[21]</sup>. Therefore, advances in understanding the underlying mechanisms of BTC development, progression and aggressiveness are of utmost importance for better management of this disease. BTC is characterized by high recurrence rates, formation of metastasis and high therapeutic resistance towards conventional chemotherapy regimens<sup>[19,20]</sup>. The involvement of CSC subpopulations in BTC is likely, however, the current literature is sparse. In this article, we discuss current studies on the role and impact of CSCs in BTC. Specifically, we focus on potential CSC markers and signaling pathways in BTC and the clinical consequences of their expression as well as on giving an overview of other aspects of BTC tumorigenesis such as miR expression and tumor microenvironment that can be linked to BTC CSCs.

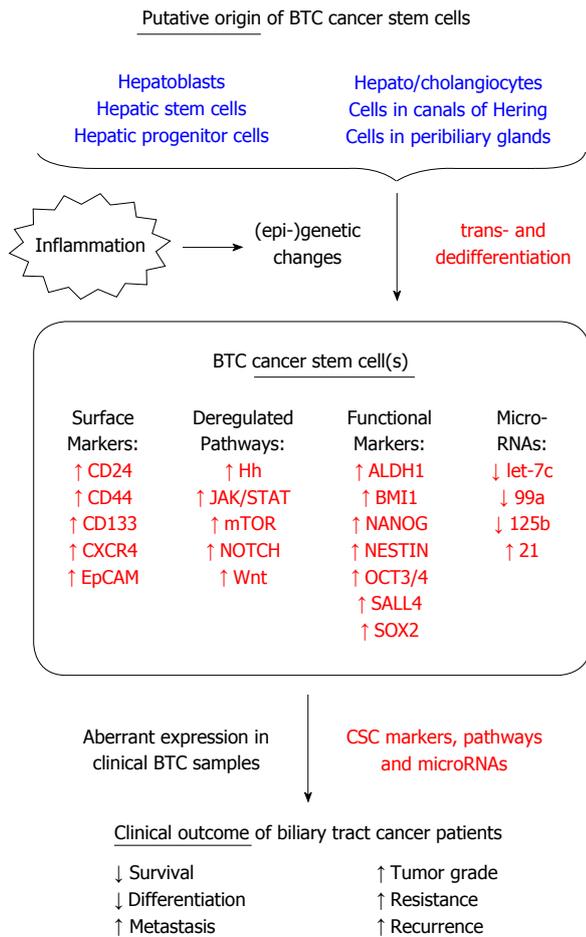
---

## POTENTIAL ORIGINS OF BILIARY TRACT CANCER CELLS WITH STEM CELL-LIKE CHARACTER

---

Several hepatic cell types have been suggested to

represent the origin of BTC CSC (summarized in Figure 1)<sup>[22]</sup>. Mature hepatocytes have the ability to de-differentiate into more pluripotent cells through mechanisms of cell plasticity and reprogramming, thereby acting as a population with stem cell traits<sup>[23]</sup>. IHC is categorized as a primary liver tumor and shows characteristics of both hepatocellular carcinoma and cholangiocarcinoma, suggesting a potential link regarding the cell of origin<sup>[24,25]</sup>. Evidence for mature hepatocytes being the source and quasi-CSC for IHC comes from two studies that showed that mature hepatocytes are able to transdifferentiate and form IHC<sup>[9,10]</sup>. In another study, it was shown that loss of tumor suppressor p53 contributes to dedifferentiation of hepatocytes into progenitor cells that can transform into IHC<sup>[26]</sup>. This is especially interesting, since loss of p53 is a major genetic characteristic of BTC<sup>[27]</sup>. Cells residing in the Canals of Hering, a hepatic stem cell niche, are another possible source of BTC-initiating cells<sup>[28]</sup>. Because the Canals of Hering represent the interface between the liver and the biliary system, residing stem cell populations may be (1) hepatic progenitor cells; (2) biliary progenitor cells; or (3) bi-potential progenitor cells, called hepatoblasts in humans and "oval cells" in rodents which have the ability to differentiate into hepatocytes and cholangiocytes<sup>[25,29-31]</sup>. There is evidence that IHC can originate from hepatic stem cells. Expression of  $\alpha$ -fetoprotein (AFP), a protein expressed by fetal hepatocytes and hepatic progenitor cells, was demonstrated in IHC<sup>[32-34]</sup>. In addition, AFP expression was shown in BTC cell lines and these AFP-expressing BTC cells had characteristics of stem cells<sup>[35]</sup>. Of note, IHC subtypes that were identified to have a hepatic stem-like gene signature had very poor prognosis, underlining not only the connection between hepatic stem cells and IHC, but also the clinical relevance and consequences<sup>[36]</sup>. The peribiliary glands were also described as a source of biliary stem cells as they are involved in normal biliary tissue turnover and repair<sup>[37,38]</sup>. BTC often occurs under (chronic) inflammatory conditions and it was shown that during primary sclerosing cholangitis, a chronic inflammation of the bile ducts, biliary tree stem cells are activated in the peribiliary glands<sup>[39]</sup>. It can be speculated that due to the chronic inflammation and concomitant constant activation of these normally quiescent stem cells, under these tumor-promoting conditions these cells become exposed to an environment that potentially causes malignant transformation - which is especially relevant taking into account the intrinsic longevity of these cells allowing for accumulation of malignant events which eventually may lead to a tumorigenic CSC phenotype. Moreover, p63, which is a homologue of p53 and also a stem cell marker of prostate gland and squamous cells was aberrantly expressed in IHC arisen from cirrhotic liver<sup>[40-42]</sup>, underlining the connection between (chronic) inflammation and CSC in BTC.



**Figure 1 Cancer stem cells in biliary tract cancer.** Biliary tract cancer stem cells are thought to originate from various subpopulations of healthy cells that harbor stem cell or stem cell-like traits. Currently available data on clinical biliary tract cancer (BTC) specimens revealed up-regulation of established cancer stem cells (CSC) cell surface and functional markers as well as aberrant activity of signaling pathways and micro-RNA species. Expression of CSC markers and stemness factors in BTC tissues is associated with diverse unfavorable clinico-pathological features and poor prognosis. See text for details.

In general, these studies suggest that there may not be one BTC CSC population but rather several different CSC populations, which in turn mirrors the heterogeneity of BTC and makes targeted and personalized (CSC-based) therapy very challenging and a demanding aim in the future.

## CANCER STEM CELL MARKERS IN BILIARY TRACT CANCER - AN OVERVIEW

Limiting dilution cell transplantation assay in immunodeficient mice is a method to determine tumorigenic potential of cancer cells. CSCs, by their nature, harbor high tumorigenic potential, meaning that only few of these cells are able to form a heterogeneous tumor. Raggi *et al.*<sup>[43]</sup> isolated BTC cells with sphere formation potential from parental BTC cells and demonstrated that these cells retained

their potential to form spheres over several passages, indicating self-renewal potential. Furthermore, when injected into immunodeficient mice, as few as ten of these cells were able to generate tumors and this high tumorigenic ability was retained when re-transplanted<sup>[43]</sup>. Gene expression analysis of these potential BTC CSCs revealed up-regulation of a number of genes including genes responsible for self-renewal and pluripotency (e.g., SOX2, BMI1, NOTCH1), drug resistance (e.g., ABCG2), surface markers (e.g., CD24, CD44, EpCAM) and metastasis<sup>[43]</sup> that are also expressed in regular stem cell populations. Another study by Wang *et al.*<sup>[44]</sup> further supports the existence of CSCs in BTC: they demonstrated in xenograft experiments that the subpopulation of CD24+/CD44+/EpCAM<sup>high</sup> cells harbor high tumorigenic potential compared to the CD24-/CD44-/EpCAM<sup>low</sup> counterparts. Furthermore, they showed via serial *in vivo* passaging, that the expression profile of CD24/CD44/EpCAM remained stable comparable with the primary tumor. In addition, tumors resulting from injection of CD24+/CD44+/EpCAM<sup>high</sup> cells contained both, CD24+/CD44+/EpCAM<sup>high</sup> as well as phenotypically different cell populations, demonstrating the ability of CD24+/CD44+/EpCAM<sup>high</sup> to self-renew and to produce heterogeneous daughter cell populations<sup>[44]</sup>.

Expression of several of these established surface and general CSC markers was identified in BTC specimens and cells. As shown in Table 1, expression of these markers was generally associated with disadvantageous clinico-pathological characteristics and shorter disease-free and overall survival. In addition, several *in vitro* studies investigated downstream targets and processes that are directly connected with the expression of these CSC markers in BTC. Resistance to anti-tumor treatments is a hallmark of cancer and CSCs and caused by up-regulation of genes responsible for drug efflux and DNA repair<sup>[4]</sup>. Nakashima and colleagues demonstrated an increase of proportion of CD24+/CD44+ cells in gemcitabine-resistant BTC cells and showed that genes of the BRCA/Fanconi repair pathway was over-expressed here, thus connecting the observed chemoresistance in these CSCs with a particular repair pathway<sup>[45]</sup>. Expression of the drug efflux pump ABCG2 is another mechanism of cells to gain therapeutic resistance and also an established CSC marker<sup>[46]</sup>. In BTC, ABCG2 was shown to be over-expressed in BTC tumor spheres and in CD44+/CD133+ cells, making it a candidate for pharmacological intervention in putative BTC CSCs<sup>[43,47,48]</sup>.

Chemokine receptor 4 (CXCR4) plays an important role in repair and regeneration of tissue in adults and was also identified as a surface marker of (cancer) stem cells<sup>[49]</sup>. Using a comprehensive gene analysis array, Leelawat *et al.*<sup>[50]</sup> compared the expression profile of CD24+ and CD24- BTC cells and found enhanced expression of CXCR4 in the CD24+

**Table 1** Surface and functional cancer stem cell markers in biliary tract cancer and their clinical consequences

Marker	Tissue	M	R	S	TS	Comment	Ref
Surface stem cell markers							
CD24	CC, IHC	↑	↑	↓			[68-70]
CD44	CC, EHC, IHC, peri-hilar CC, HCC-CC	↑		↓		↓differentiation, ↑recurrence	[28,71-75]
CD133	CC			↓	↑		[28,63,71]
CXCR4	GBC, IHC	↑		↓	↑	↑vascular invasion	[52,76]
EpCAM	IHC			↓			[28,77]
Functional stem cell markers							
ALDH1	EHC, IHC, perihilar CC		↑	↓			[58,78]
BMI1	EHC, HCC-CC, IHC, perihilar CC					↑in tumor specimens	[71,79,80]
NANOG	EHC, IHC, perihilar CC						
NESTIN	EHC, IHC, perihilar CC						
OCT3/4	GBC, CC	↑		↓	↑	↑tumor size	[63,81]
SALL4	IHC	↑		↓		↑vascular and nerve invasion	[82]
SOX2	EHC, IHC, perihilar CC	↑		↓	↑		[71,74]

CC: Cholangiocarcinoma; EHC: Extrahepatic cholangiocarcinoma; GBC: Gallbladder cancer; HCC-CC: Combined hepatocellular-cholangiocarcinoma; IHC: Intrahepatic cholangiocarcinoma; M: Metastasis; R: Therapeutic resistance; S: Survival; TS: Tumor stage.

subpopulation. Drug-based inhibition of CXCR4 using AMD3100 suppressed migration and invasion of BTC cells, and this effect was only observable in the CD24+ CSC subpopulation<sup>[50]</sup>. Interestingly, AMD3100 treatment also reduced sphere formation potential of BTC cells in another study, further connecting CXCR4 expression with stem cell characteristics<sup>[51]</sup>. RNA interference-mediated knockdown of CXCR4 in an IHC model inhibited proliferation and colony formation *in vitro* as well as tumorigenicity *in vivo*<sup>[52]</sup>.

Cardinale *et al.*<sup>[53]</sup> demonstrated in xenograft experiments that CD13+/CD90+ BTC spheroids were highly tumorigenic. Interestingly, these two surface molecules are established CSC markers that also play a role in liver cancer, further confirming that hepatocellular carcinoma and BTC may share a common origin or CSC subpopulation<sup>[54-57]</sup>.

As mentioned above, CSC can also be identified via functional characteristics such as ALDH1 expression<sup>[13]</sup>. Using a BTC cell model, Shuang *et al.*<sup>[58]</sup> demonstrated that, in contrast to ALDH- cells, ALDH+ cells were able to form tumor spheres. In addition, epithelial markers were reduced in ALDH+ cells, whereas mesenchymal markers were strongly expressed - connecting this cell population to epithelial-to-mesenchymal transition (EMT), a process that is closely related to CSC as discussed later in this article<sup>[58,59]</sup>.

It is well established that deregulated epigenetic events play a huge role in transformation of cells towards a malignant phenotype. The polycomb repressive complexes (PRC) 1 and 2 are multi-protein epigenetic regulators which are known to be aberrantly active in cancer and essential for CSC to maintain their stemness character<sup>[60]</sup>. Several studies indicate a pivotal role of PRC1 and 2 in development and progression of BTC (as reviewed in<sup>[61]</sup>). BMI1, which was shown to be expressed in BTC patient samples (Table 1), is a core component of the PRC1 and recently it was demonstrated in BTC cells, that pharmacological inhibition of BMI1 resulted in

reduction of ALDH+ cells and diminished formation of tumor spheres<sup>[62]</sup>. Moreover, in another study, BMI1 was found to be significantly higher expressed in BTC cells positive for CD133 and OCT3/4, further suggesting BMI1 as a CSC marker in BTC<sup>[63]</sup>.

Regarding the pluripotency markers NANOG, OCT3/4 and SOX2<sup>[64]</sup>, only few studies investigated their expression and associated outcomes in clinical BTC samples. However, several *in vitro* and *in vivo* studies suggest a pivotal role of these pluripotency markers in BTC CSC. In CD133+ spheres derived from GBC cells, OCT4 and NANOG were highly expressed and these cells also showed higher resistance to chemotherapeutics<sup>[65]</sup>. In line with these findings, two other studies found that in tumor spheres derived from BTC cells, stem cell markers such as CD133, NANOG, SOX2, SALL4 and OCT4 were up-regulated<sup>[53,66]</sup>. In addition, these spheres over-expressed ABCG2 and were resistant to cisplatin and additionally displayed high tumorigenic potential when injected into nude mice<sup>[66]</sup>. More evidence for SOX2 being a potentially relevant factor in BTC cells with stem cell character was presented in another study where the authors showed that artificially over-expression of SOX2 enhanced proliferative capacity, apoptosis resistance and migration and invasion potential<sup>[67]</sup>.

## RELEVANCE OF STEMNESS PATHWAYS IN BILIARY TRACT CANCER STEM CELLS

Several signaling pathways are involved in generation and maintenance of CSCs, including the embryonic signaling cascades NOTCH, Wnt and Hh as well as the interleukin-6 (IL-6)-JAK/STAT cascade and the mTOR pathway (for detailed pathway descriptions see<sup>[5,83,84]</sup>). Of note, these embryonic signaling pathways are also involved in basic cholangiocyte differentiation<sup>[85]</sup>.

Several studies found deregulation of these signaling pathways in BTC specimens and corres-

**Table 2** Overly active pathways associated with cancer stem cell-like phenotype in biliary tract cancer specimens

Pathway	Component	Tissue	Outcome	Ref.
Hh	GLI1	GBC	↑ lymph node metastasis	[92,100]
	SHH	CC, GBC	↑ grade (by trend)	[92,100,101]
	SMO	GBC		[92]
JAK / STAT	STAT3	CC, IHC	↑ tumor size, ↑ metastasis, ↑ vascular invasion, ↓ survival, poor histological differentiation	[102,103]
mTOR	mTOR	BTC	↓ survival	[104]
	pmTOR <sup>1</sup>	GBC	↓ survival	[105]
NOTCH	NOTCH 1	EHC	poor histological differentiation, ↓ survival, ↑ tumor grade, ↑ Cyclin E	[86,87]
	NOTCH 2	EHC	↓ survival	[86]
	NOTCH 3	EHC	↓ survival, ↑ tumor grade, ↑ Cyclin E	[86,87]
	NOTCH 4	EHC		[86]
Wnt	HES-1	EHC	↓ survival	[86]
	β-catenin	CC	↑ metastasis	[91] <sup>2</sup>

<sup>1</sup>Phosphorylated mTOR; <sup>2</sup>In this study, no non-tumor control samples were used as control. CC: Cholangiocarcinoma; EHC: Extrahepatic cholangiocarcinoma; GBC: Gallbladder cancer; IHC: Intrahepatic cholangiocarcinoma.

ponding poor clinical outcome parameters (Table 2 and Figure 1). In addition to these findings, in depth experimental approaches as well as experiments on pharmacological intervention of these pathways have shed more light on their roles in putative CSC in BTC. Treatment of cells with the NOTCH pathway inhibitor  $\gamma$ -secretase inhibitor (GSI) IX significantly decreased the CD24+/CD44+ subpopulation in a BTC cell line model<sup>[86]</sup>. Moreover, single treatment with gemcitabine increased the amount of CD24+/CD44+ BTC cells, whereas combined treatment with gemcitabine and GSI IX mitigated this effect<sup>[86]</sup>. In an interesting study by Zender *et al.*<sup>[87]</sup> long-term artificial over-expression of Notch Intracellular Domain (NICD) 1 - an integral factor of the NOTCH signaling cascade - in mouse livers resulted in a cell population that, when injected into immunodeficient mice, was able to form BTC with features of hepatic progenitor cells. This not only demonstrates the involvement of the NOTCH signaling pathway in CSC, but also suggests a role of hepatic progenitor cells in the development of BTC. In the same regard, Ishii and coworkers published that NICD1 was expressed exclusively in BTC cells with CSC characteristics. Furthermore, treatment with GSI DAPT decreased the number of potential BTC CSCs<sup>[35]</sup>. Combined drug-based Hh and mTOR inhibition reduced viability and proliferation of BTC cells *in vitro*<sup>[88]</sup>. Intriguingly, combined treatment also reduced the number of ALDH+ cells as well as the expression of pluripotency factors NANOG and OCT4. Additional *in vivo* experiments also revealed diminished tumorigenic potential of the treated cells, indicating concerted action of the Hh and mTOR pathway in creation and /or maintenance of a CSC phenotype in BTC<sup>[88]</sup>. Constitutively  $\beta$ -catenin expression (mimicking active Wnt pathway) promoted self-renewal of hepatic progenitor cells and injection of these cells generated tumors with characteristics of HCC-CC<sup>[89]</sup>.

Cellular plasticity of differentiation is discussed as a characteristic of CSC in BTC<sup>[90]</sup>. A phenomenon tightly connected to cellular plasticity and therefore

to a CSC phenotype is the ability of cells to detach from the primary tumor and to gain access to the lymphatic and/or vascular system, *i.e.*, the cells become invasive. In order to do so, these invasive and potentially stem cell-like cancer cells have to carve through the surrounding extracellular matrix. Matrix metalloproteinases (MMP) are enzymes that are centrally involved in the breakdown of the extracellular matrix. Active Wnt pathway was shown to directly up-regulate MMP expression in BTC cells, giving BTC cells the ability to gain invasive capabilities<sup>[91]</sup>. More evidence that the Wnt pathway influences the CSC phenotype in BTC comes from Zhao and coworkers: knockdown of the BTC CSC marker CXCR4 (Table 1) also caused inhibition of the Wnt pathway<sup>[52]</sup>. Direct causality of these events was proven by providing the ligand CXCL12, which resulted in activation the Wnt pathway and the respective downstream targets including CD44<sup>[52]</sup>.

Artificial activation of Hh pathway (*via* up-regulation of the ligand SHH) enhanced invasiveness of GBC cells and this effect was reversible via shRNA-mediated or drug-based (cyclopamine) intervention<sup>[92]</sup>. Further experiments showed that regulation of MMP expression via the Hh pathway was a potential underlying molecular mechanism of this observation<sup>[92]</sup>. In the same study, Hh pathway activation was associated with enhanced colony formation and this effect was reversed by RNA interference-mediated Hh pathway blockage. Finally, GBC cells with active Hh signaling harbored greater tumor-generating capability *in vivo*, underlining the importance of this pathway in BTC for several aspects connected with CSC characteristics<sup>[92]</sup>.

BTC often develops under inflammatory conditions and in this regard, diverse cytokines such as IL-6 are involved. It was shown that under chronic inflammatory conditions, neoplastic cholangiocytes are able to produce and secrete IL-6 in an autocrine loop, resulting in proliferation and induction of DNA damaging molecules such as reactive oxygen species and nitric oxide<sup>[24]</sup>. The JAK/STAT signaling

cascade is one of the downstream pathways that is activated via inflammation-related cytokines in BTC development<sup>[93,94]</sup>. In an interesting study using GBC cells, Kong *et al.*<sup>[95]</sup> isolated side-population cells and compared the functional and molecular characteristics of these potential CSCs with the non-side-population cells. They found enhanced expression of IL-6 and activated (*i.e.*, phosphorylated) STAT3 in the side-population cells. Intriguingly, these cells harbored multiple CSC traits: enhanced tumor sphere and colony formation potential, chemoresistance, the ability to generate both, side-population and non-side-population cells and, finally, high tumorigenic potential *in vivo*<sup>[95]</sup>. Drug-based inhibition of STAT3 reduced growth and migration/wound healing potential of BTC cells<sup>[94]</sup>. Moreover, treatment of cells with the natural compound luteolin suppressed the activation of the IL-6-induced JAK/STAT3 cascade in BTC cells which resulted in diminished migration, wound healing and colony formation potential<sup>[96]</sup>.

mTOR inhibition was directly connected to loss of CSC characteristics in BTC cell lines: treatment with rapamycin decreased migration, invasion as well as sphere formation potential<sup>[97]</sup>. Similar results were presented by two other studies in GBC cells<sup>[98,99]</sup>. Of note, activated mTOR pathway was found in highly proliferative and metastatic GBC cells<sup>[99]</sup>.

## MIRNAS AND THEIR ROLE IN REGULATION OF BILIARY TRACT CANCER STEM CELL CHARACTERISTICS

Micro-RNAs (miRs) are non-coding RNAs that regulate gene expression by binding and degradation of target mRNAs<sup>[106]</sup>. As recently reviewed, aberrant miR expression is involved in different aspects of BTC development and progression<sup>[107]</sup>. However, up to now, only few studies associated deregulated miR expression with CSC characteristics in BTC. The miRs let-7c/99a and 125b originate from the same gene cluster and were shown to be down-regulated in CC patient material, *de-facto* being tumor suppressor miRs<sup>[108]</sup>. Interestingly, expression of these miRs was reduced in tumor spheres derived from BTC cells and in addition, enforced expression of let-7c/99a and 125b reduced the expression of CD133 and CD44 in BTC cells as well as the potential to form tumor spheres<sup>[108]</sup>. Of note, in the same study, the Interleukin 6 (IL-6) pathway, including IL-6 itself, its receptor IL-6R and the downstream transcription factor STAT3 were identified as targets of this particular miR cluster.

MiR21 is a potent oncogenic miR in BTC with several established targets that contributes to disadvantageous clinical outcome<sup>[107]</sup>. Zhang *et al.*<sup>[109]</sup> published that miR21 is necessary for survival of CD24+ cells in primary liver cancer, indicating a role of miR21 in the CSC phenotype in BTC.

Although the number of studies regarding miRs

and CSC in BTC is limited as of today, the overlap between described deregulated miRs in BTC and the role of these miRs in CSC of other tumor types strongly suggests a role of these miRs also in CSC of BTC. For example, miR200b is down-regulated in BTC samples, resulting in shorter survival<sup>[110]</sup>. In lung adenocarcinoma, reduced miR200b expression was shown to be a marker of CSC and, interestingly, restoration of miR200b expression resulted in loss of CSC maintenance and chemoresistance<sup>[111]</sup>. MiR145 is another miR species that was found to be down-regulated in BTC specimens and associated with poor survival<sup>[112]</sup>. Likewise, in lung adenocarcinoma tissues, miR145 was found to be down-regulated and negatively correlated with expression of OCT4<sup>[113]</sup>. Moreover, forced expression of miR145 in lung cancer initiating cells markedly reduced CSC features *in vitro* and *in vivo*<sup>[113]</sup>. In prostate cancer, miR34a was down-regulated in the CD44+ CSC subpopulation and the down-regulation of this miR species was also measured in BTC samples and correlated with advanced clinical stage, lymph node metastasis and poor survival<sup>[114,115]</sup>.

## BRIEF OUTLOOK ON BILIARY TRACT CANCER STEM CELLS AND TUMOR MICROENVIRONMENT

CSCs are thought to reside in specific environments called stem cell niches. The CSC niche consists of various cell types and structures including immune cells, mesenchymal (stem) cells, fibroblasts, vascular network, soluble factors and extracellular matrix components and has the function to preserve the exclusive features of CSC as well as to protect them from therapeutic intervention<sup>[116]</sup>. By creating a suitable tumor microenvironment (TME), the CSC niche plays an outstanding role in development and progression of cancer, essentially supporting tumor growth in multiple aspects. On the other hand, CSCs also support their TME for example by inducing the expression of survival genes<sup>[116]</sup>. Data regarding CSC niches and TME in BTC are very limited, however, the TME likely contributes to angiogenesis, invasion, metastasis, therapeutic resistance, maintenance of CSC niche and survival of CSC also in BTC<sup>[117]</sup>. In an interesting study, Raggi *et al.*<sup>[43]</sup> demonstrated the importance of the interaction between BTC cells and macrophages for tumor development. Medium gathered from BTC cells with CSC characteristics (spheres) activated CD14+ macrophages and shifted their phenotype towards CD163+ so-called tumor-associated macrophages (TAM) which harbored high invasive capacity accompanied by expression of the matrix-remodeling gene MMP2. In the same study, CD163+ macrophages were found at the tumor front in BTC samples, suggesting the importance of these immune cells in progression of BTC. Moreover, the levels of TAMs were associated with poor prognosis of

BTC patients<sup>[118-120]</sup>. Evidence that BTC CSCs not only are able to activate TAMs but also to chemo-attract them was shown *via* chemotaxis experiments, in which CD14+ strongly migrated towards medium derived from BTC spheres<sup>[43]</sup>.

Mesenchymal stem cells (MSCs) are multipotent adult stromal stem cells that have the ability to generate diverse types of connective tissue<sup>[121]</sup>. They produce a wide range of cytokines and chemokines, thereby strongly communicating with their near and far environment. Several studies demonstrated that MSCs not only support tumor growth and CSC as a part of the TME, but also that MSC actively migrate towards tumor sites<sup>[122]</sup>. Regarding BTC, media from MSCs increased migration, invasion, proliferation, chemoresistance and colony formation potential of BTC cells *in vitro*<sup>[91]</sup>. Moreover, media from MSCs activated Wnt/ $\beta$ -catenin signaling as well as MMP expression. In a xenograft model, the authors also demonstrated that injection of BTC cells together with MSCs resulted in significantly larger tumors<sup>[91]</sup>. Recently, it was shown that media derived from BTC cells enhanced the migratory potential of MSC as well as the release of IL-6<sup>[123]</sup>. On the other hand, culture media derived from these "activated" MSCs significantly increased proliferation of BTC cells as well as the amount of pSTAT3. This effect was completely blocked by the addition of an anti-IL-6 antibody. This is especially interesting because of the well-known role of the IL-6/JAK/STAT cascade in CSCs (see above), thus indicating that MSC can directly support CSCs in BTC. Further evidence that MSCs may directly interact with CSC comes from the fact that MSCs secrete CXCL12, the ligand of the putative BTC stem cell surface marker CXCR-4 (Table 1)<sup>[121]</sup>.

Epithelial-to-mesenchymal transition (EMT) is a process in which cells lose their epithelial traits and gain mesenchymal character, allowing them to detach from the primary tumor and subsequently to form secondary tumors. On molecular level, loss of epithelial markers such as E-Cadherin and enhanced expression of mesenchymal markers such as Vimentin can be observed during EMT<sup>[124,125]</sup>. Key factors that can initiate EMT include Slug, Snail and Twist, which are repressors of E-Cadherin<sup>[125]</sup>. The process of EMT is closely related to CSC phenotype. For example, several established CSC pathways such as Wnt, NOTCH and mTOR are involved in EMT and forced expression of EMT resulted in enrichment of CSC subpopulation (as reviewed in<sup>[126]</sup>). For BTC, Shuang *et al.*<sup>[58]</sup> connected an EMT phenotype with CSC characteristics. They demonstrated that the ALDH+ subpopulation expressed low levels of E-Cadherin and high levels of the mesenchymal markers Vimentin and N-Cadherin. In another study, the authors recognized reduced expression of the ubiquitin ligase FBXW7 in BTC samples and found a correlation with the metastasis status<sup>[97]</sup>. Interestingly, the authors demonstrated *in vitro* and *in vivo* experiments in

BTC cells, that silencing of FBXW7 resulted in both, an EMT and a CSC phenotype: the epithelial marker E-Cadherin was found to be down-regulated, whereas Vimentin was up-regulated. Regarding CSC markers, silencing of FBXW7 increased the expression of OCT4 and NANOG and enhanced tumor sphere formation capability of the tested BTC cells. Conversely, forced expression of FBXW7 reversed both, the EMT and the CSC phenotype<sup>[97]</sup>. Results from a study conducted by Matsushita and colleagues also suggests a role of the Hh pathway in EMT regulation in BTC: knockdown of the Hh pathway component SMO caused up-regulation of E-Cadherin and down-regulation of Vimentin, accompanied by decreased invasive potential of these cells<sup>[92]</sup>. Lastly, Kong *et al.*<sup>[95]</sup> demonstrated an EMT phenotype (high Vimentin and low E-Cadherin protein expression) in side-population CSCs in GBC, further connecting the EMT process with the CSC phenotype.

## CONCLUSION

Although several studies suggest the existence of BTC-specific CSC, additional independent studies should verify these results in functional BTC models, especially in xenograft experiments. Furthermore, due to the heterogenic character of BTC, it should be taken into account that multiple CSC subpopulations with potentially different genetic background and surface marker profiles may exist. Involvement of classical stemness pathways and complexes such as Hh, Wnt, NOTCH and the PRCs as well as of diverse miR species in generation and maintenance of CSC in BTC is very likely. However, up to now, only few studies directly associated the ascertained role of these factors for BTC development with CSC characteristics (Table 2). Moreover, future studies should also concentrate on the role of the TME in the creation and maintenance of potential CSC niches in BTC. The CSC status is strongly dependent on the microenvironment, meaning that CSC traits may very well be not a static, but rather a dynamic phenomenon in which the TME and CSC niche play an absolutely pivotal role<sup>[127]</sup>. In this light, limitations of current *in vitro* and *in vivo* models should always be kept in mind, as (artificial) imitation of an environment as complex and as dynamic as the TME seems very challenging. Current clinical trials involving the CSC concept mainly include immunotherapies, use of CSCs as biomarkers, several CSC-targeted therapies (*e.g.*, metformin, Hh or Notch inhibitors with/without concomitant chemotherapy). However, currently no studies are registered at clinicaltrials.gov specifically enrolling patients with BTC. In addition, future studies should also concentrate on the investigation of the role crosstalk between stemness pathways regarding BTC CSC as well as on the role of (chronic) inflammation in the formation and maintenance of a CSC phenotype in BTC, especially due to the fact that in BTC cells positive for CD133 or OCT3/4, enhanced levels of

inflammation-related DNA damage was observed<sup>[63]</sup>.

Taken together, CSCs are promising and attractive targets for pharmacological intervention. Therefore, regarding BTC, after identification and validation of putative CSC populations, screening and testing of anti-CSC-targeting compounds in BTC models will be of great importance in order to develop new therapeutic strategies and approaches.

## REFERENCES

- Lapidot T**, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, Minden M, Paterson B, Caligiuri MA, Dick JE. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature* 1994; **367**: 645-648 [PMID: 7509044 DOI: 10.1038/367645a0]
- S Franco S**, Szczesna K, Iliou MS, Al-Qahtani M, Mobasher A, Kobolák J, Dinnyés A. In vitro models of cancer stem cells and clinical applications. *BMC Cancer* 2016; **16**: 738 [PMID: 27766946 DOI: 10.1186/s12885-016-2774-3]
- Kreso A**, Dick JE. Evolution of the cancer stem cell model. *Cell Stem Cell* 2014; **14**: 275-291 [PMID: 24607403 DOI: 10.1016/j.stem.2014.02.006]
- Deshmukh A**, Deshpande K, Arfuso F, Newsholme P, Dharmarajan A. Cancer stem cell metabolism: a potential target for cancer therapy. *Mol Cancer* 2016; **15**: 69 [PMID: 27825361 DOI: 10.1186/s12943-016-0555-x]
- Oren O**, Smith BD. Eliminating Cancer Stem Cells by Targeting Embryonic Signaling Pathways. *Stem Cell Rev* 2017; **13**: 17-23 [PMID: 27730468 DOI: 10.1007/s12015-016-9691-3]
- Dick JE**. Looking ahead in cancer stem cell research. *Nat Biotechnol* 2009; **27**: 44-46 [PMID: 19131997 DOI: 10.1038/nbt0109-44]
- Muñoz P**, Iliou MS, Esteller M. Epigenetic alterations involved in cancer stem cell reprogramming. *Mol Oncol* 2012; **6**: 620-636 [PMID: 23141800 DOI: 10.1016/j.molonc.2012.10.006]
- Krivtsov AV**, Twomey D, Feng Z, Stubbs MC, Wang Y, Faber J, Levine JE, Wang J, Hahn WC, Gilliland DG, Golub TR, Armstrong SA. Transformation from committed progenitor to leukaemia stem cell initiated by MLL-AF9. *Nature* 2006; **442**: 818-822 [PMID: 16862118 DOI: 10.1038/nature04980]
- Fan B**, Malato Y, Calvisi DF, Naqvi S, Razumilava N, Ribback S, Gores GJ, Dombrowski F, Evert M, Chen X, Willenbring H. Cholangiocarcinomas can originate from hepatocytes in mice. *J Clin Invest* 2012; **122**: 2911-2915 [PMID: 22797301 DOI: 10.1172/JCI63212]
- Sekiya S**, Suzuki A. Intrahepatic cholangiocarcinoma can arise from Notch-mediated conversion of hepatocytes. *J Clin Invest* 2012; **122**: 3914-3918 [PMID: 23023701 DOI: 10.1172/JCI63065]
- Rafehi H**, Orłowski C, Georgiadis GT, Ververis K, El-Osta A, Karagiannis TC. Clonogenic assay: adherent cells. *J Vis Exp* 2011; (49): pii: 2573 [PMID: 21445039 DOI: 10.3791/2573]
- Schatton T**, Frank NY, Frank MH. Identification and targeting of cancer stem cells. *Bioessays* 2009; **31**: 1038-1049 [PMID: 19708024 DOI: 10.1002/bies.200900058]
- Douville J**, Beaulieu R, Balicki D. ALDH1 as a functional marker of cancer stem and progenitor cells. *Stem Cells Dev* 2009; **18**: 17-25 [PMID: 18573038 DOI: 10.1089/scd.2008.0055]
- Charafe-Jauffret E**, Ginestier C, Bertucci F, Cabaud O, Wicinski J, Finetti P, Josselin E, Adelaide J, Nguyen TT, Monville F, Jacquemier J, Thomassin-Piana J, Pinna G, Jalaguier A, Lambaudie E, Houvenaeghel G, Xerri L, Harel-Bellan A, Chaffanet M, Viens P, Birnbaum D. ALDH1-positive cancer stem cells predict engraftment of primary breast tumors and are governed by a common stem cell program. *Cancer Res* 2013; **73**: 7290-7300 [PMID: 24142344 DOI: 10.1158/0008-5472.CAN-12-4704]
- Ginestier C**, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, Jacquemier J, Viens P, Kleer CG, Liu S, Schott A, Hayes D, Birnbaum D, Wicha MS, Dontu G. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. *Cell Stem Cell* 2007; **1**: 555-567 [PMID: 18371393 DOI: 10.1016/j.stem.2007.08.014]
- Wu A**, Luo W, Zhang Q, Yang Z, Zhang G, Li S, Yao K. Aldehyde dehydrogenase 1, a functional marker for identifying cancer stem cells in human nasopharyngeal carcinoma. *Cancer Lett* 2013; **330**: 181-189 [PMID: 23220285 DOI: 10.1016/j.canlet.2012.11.046]
- Hadnagy A**, Gaboury L, Beaulieu R, Balicki D. SP analysis may be used to identify cancer stem cell populations. *Exp Cell Res* 2006; **312**: 3701-3710 [PMID: 17046749 DOI: 10.1016/j.yexcr.2006.08.030]
- Wu C**, Alman BA. Side population cells in human cancers. *Cancer Lett* 2008; **268**: 1-9 [PMID: 18487012 DOI: 10.1016/j.canlet.2008.03.048]
- Razumilava N**, Gores GJ. Cholangiocarcinoma. *Lancet* 2014; **383**: 2168-2179 [PMID: 24581682 DOI: 10.1016/S0140-6736(13)61903-0]
- Patel T**. Cholangiocarcinoma--controversies and challenges. *Nat Rev Gastroenterol Hepatol* 2011; **8**: 189-200 [PMID: 21460876 DOI: 10.1038/nrgastro.2011.20]
- Valle JW**, Furuse J, Jitlal M, Beare S, Mizuno N, Wasan H, Bridgewater J, Okusaka T. Cisplatin and gemcitabine for advanced biliary tract cancer: a meta-analysis of two randomised trials. *Ann Oncol* 2014; **25**: 391-398 [PMID: 24351397 DOI: 10.1093/annonc/mdt540]
- Kokuryo T**, Yokoyama Y, Nagino M. Recent advances in cancer stem cell research for cholangiocarcinoma. *J Hepatobiliary Pancreat Sci* 2012; **19**: 606-613 [PMID: 22907641 DOI: 10.1007/s00534-012-0542-6]
- Tarlow BD**, Pelz C, Naugler WE, Wakefield L, Wilson EM, Finegold MJ, Grompe M. Bipotential adult liver progenitors are derived from chronically injured mature hepatocytes. *Cell Stem Cell* 2014; **15**: 605-618 [PMID: 25312494 DOI: 10.1016/j.stem.2014.09.008]
- Wei M**, Lü L, Lin P, Chen Z, Quan Z, Tang Z. Multiple cellular origins and molecular evolution of intrahepatic cholangiocarcinoma. *Cancer Lett* 2016; **379**: 253-261 [PMID: 26940139 DOI: 10.1016/j.canlet.2016.02.038]
- Joo I**, Kim H, Lee JM. Cancer stem cells in primary liver cancers: pathological concepts and imaging findings. *Korean J Radiol* 2015; **16**: 50-68 [PMID: 25598674 DOI: 10.3348/kjr.2015.16.1.50]
- Tschaharganeh DF**, Xue W, Calvisi DF, Evert M, Michurina TV, Dow LE, Banito A, Katz SF, Kastenhuber ER, Weissmueller S, Huang CH, Lechel A, Andersen JB, Capper D, Zender L, Longerich T, Enikolopov G, Lowe SW. p53-Dependent Nestin Regulation Links Tumor Suppression to Cellular Plasticity in Liver Cancer. *Cell* 2016; **165**: 1546-1547 [PMID: 27259155 DOI: 10.1016/j.cell.2016.05.058]
- Guest RV**, Boulter L, Kendall TJ, Minnis-Lyons SE, Walker R, Wigmore SJ, Sansom OJ, Forbes SJ. Cell lineage tracing reveals a biliary origin of intrahepatic cholangiocarcinoma. *Cancer Res* 2014; **74**: 1005-1010 [PMID: 24310400 DOI: 10.1158/0008-5472.CAN-13-1911]
- Iwahashi S**, Utsunomiya T, Shimada M, Saito Y, Morine Y, Imura S, Ikemoto T, Mori H, Hanaoka J, Bando Y. High expression of cancer stem cell markers in cholangiolocellular carcinoma. *Surg Today* 2013; **43**: 654-660 [PMID: 23192764 DOI: 10.1007/s00595-012-0437-9]
- Saxena R**, Theise N. Canals of Hering: recent insights and current knowledge. *Semin Liver Dis* 2004; **24**: 43-48 [PMID: 15085485 DOI: 10.1055/s-2004-823100]
- Fausto N**. Liver regeneration and repair: hepatocytes, progenitor cells, and stem cells. *Hepatology* 2004; **39**: 1477-1487 [PMID: 15185286 DOI: 10.1002/hep.20214]
- Roskams T**. Liver stem cells and their implication in hepatocellular and cholangiocarcinoma. *Oncogene* 2006; **25**: 3818-3822 [PMID: 16799623 DOI: 10.1038/sj.onc.1209558]
- Gitlin D**, Perricelli A, Gitlin GM. Synthesis of -fetoprotein by liver, yolk sac, and gastrointestinal tract of the human conceptus.

- Cancer Res* 1972; **32**: 979-982 [PMID: 4111729]
- 33 **Shiojiri N**, Lemire JM, Fausto N. Cell lineages and oval cell progenitors in rat liver development. *Cancer Res* 1991; **51**: 2611-2620 [PMID: 1708696]
- 34 **Ishikawa K**, Sasaki A, Haraguchi N, Yoshikawa Y, Mori M. A case of an alpha-fetoprotein-producing intrahepatic cholangiocarcinoma suggests probable cancer stem cell origin. *Oncologist* 2007; **12**: 320-324 [PMID: 17405896 DOI: 10.1634/theoncologist.12-3-320]
- 35 **Ishii T**, Yasuchika K, Suemori H, Nakatsuji N, Ikai I, Uemoto S. Alpha-fetoprotein producing cells act as cancer progenitor cells in human cholangiocarcinoma. *Cancer Lett* 2010; **294**: 25-34 [PMID: 20149523 DOI: 10.1016/j.canlet.2010.01.019]
- 36 **Oishi N**, Kumar MR, Roessler S, Ji J, Forgues M, Budhu A, Zhao X, Andersen JB, Ye QH, Jia HL, Qin LX, Yamashita T, Woo HG, Kim YJ, Kaneko S, Tang ZY, Thorgeirsson SS, Wang XW. Transcriptomic profiling reveals hepatic stem-like gene signatures and interplay of miR-200c and epithelial-mesenchymal transition in intrahepatic cholangiocarcinoma. *Hepatology* 2012; **56**: 1792-1803 [PMID: 22707408 DOI: 10.1002/hep.25890]
- 37 **Carpino G**, Cardinale V, Onori P, Franchitto A, Berloco PB, Rossi M, Wang Y, Semeraro R, Anceschi M, Brunelli R, Alvaro D, Reid LM, Gaudio E. Biliary tree stem/progenitor cells in glands of extrahepatic and intrahepatic bile ducts: an anatomical in situ study yielding evidence of maturational lineages. *J Anat* 2012; **220**: 186-199 [PMID: 22136171 DOI: 10.1111/j.1469-7580.2011.01462.x]
- 38 **Cardinale V**, Wang Y, Carpino G, Cui CB, Gatto M, Rossi M, Berloco PB, Cantafora A, Wauthier E, Furth ME, Inverardi L, Dominguez-Bendala J, Ricordi C, Gerber D, Gaudio E, Alvaro D, Reid L. Multipotent stem/progenitor cells in human biliary tree give rise to hepatocytes, cholangiocytes, and pancreatic islets. *Hepatology* 2011; **54**: 2159-2172 [PMID: 21809358 DOI: 10.1002/hep.24590]
- 39 **Carpino G**, Cardinale V, Renzi A, Hov JR, Berloco PB, Rossi M, Karlsen TH, Alvaro D, Gaudio E. Activation of biliary tree stem cells within peribiliary glands in primary sclerosing cholangitis. *J Hepatol* 2015; **63**: 1220-1228 [PMID: 26119688 DOI: 10.1016/j.jhep.2015.06.018]
- 40 **Pellegrini G**, Dellambra E, Golisano O, Martinelli E, Fantozzi I, Bondanza S, Ponzin D, McKeon F, De Luca M. p63 identifies keratinocyte stem cells. *Proc Natl Acad Sci USA* 2001; **98**: 3156-3161 [PMID: 11248048 DOI: 10.1073/pnas.061032098]
- 41 **Signoretti S**, Waltregny D, Dilks J, Isaac B, Lin D, Garraway L, Yang A, Montironi R, McKeon F, Loda M. p63 is a prostate basal cell marker and is required for prostate development. *Am J Pathol* 2000; **157**: 1769-1775 [PMID: 11106548 DOI: 10.1016/S0002-9440(10)64814-6]
- 42 **Nomoto K**, Tsuneyama K, Cheng C, Takahashi H, Hori R, Murai Y, Takano Y. Intrahepatic cholangiocarcinoma arising in cirrhotic liver frequently expressed p63-positive basal/stem-cell phenotype. *Pathol Res Pract* 2006; **202**: 71-76 [PMID: 16377099 DOI: 10.1016/j.prp.2005.10.011]
- 43 **Raggi C**, Correnti M, Sica A, Andersen JB, Cardinale V, Alvaro D, Chiorino G, Forti E, Glaser S, Alpini G, Destro A, Sozio F, Di Tommaso L, Roncalli M, Banales JM, Coulouarn C, Bujanda L, Torzilli G, Invernizzi P. Cholangiocarcinoma stem-like subset shapes tumor-initiating niche by educating associated macrophages. *J Hepatol* 2017; **66**: 102-115 [PMID: 27593106 DOI: 10.1016/j.jhep.2016.08.012]
- 44 **Wang M**, Xiao J, Shen M, Yahong Y, Tian R, Zhu F, Jiang J, Du Z, Hu J, Liu W, Qin R. Isolation and characterization of tumorigenic extrahepatic cholangiocarcinoma cells with stem cell-like properties. *Int J Cancer* 2011; **128**: 72-81 [PMID: 20232394 DOI: 10.1002/ijc.25317]
- 45 **Nakashima S**, Kobayashi S, Nagano H, Tomokuni A, Tomimaru Y, Asaoka T, Hama N, Wada H, Kawamoto K, Marubashi S, Eguchi H, Doki Y, Mori M. BRCA/Fanconi anemia pathway implicates chemoresistance to gemcitabine in biliary tract cancer. *Cancer Sci* 2015; **106**: 584-591 [PMID: 25736055 DOI: 10.1111/cas.12652]
- 46 **Nakanishi T**, Ross DD. Breast cancer resistance protein (BCRP/ABC2): its role in multidrug resistance and regulation of its gene expression. *Chin J Cancer* 2012; **31**: 73-99 [PMID: 22098950 DOI: 10.5732/cjc.011.10320]
- 47 **Mayr C**, Wagner A, Stoecklinger A, Jakab M, Illig R, Berr F, Pichler M, Di Fazio P, Ocker M, Neureiter D, Kiesslich T. 3-Deazaneplanocin A May Directly Target Putative Cancer Stem Cells in Biliary Tract Cancer. *Anticancer Res* 2015; **35**: 4697-4705 [PMID: 26254359]
- 48 **Shi C**, Tian R, Wang M, Wang X, Jiang J, Zhang Z, Li X, He Z, Gong W, Qin R. CD44+ CD133+ population exhibits cancer stem cell-like characteristics in human gallbladder carcinoma. *Cancer Biol Ther* 2010; **10**: 1182-1190 [PMID: 20948317]
- 49 **Furusato B**, Mohamed A, Uhlén M, Rhim JS. CXCR4 and cancer. *Pathol Int* 2010; **60**: 497-505 [PMID: 20594270 DOI: 10.1111/j.1440-1827.2010.02548.x]
- 50 **Leelawat K**, Keeratichamroen S, Leelawat S, Tohtong R. CD24 induces the invasion of cholangiocarcinoma cells by upregulating CXCR4 and increasing the phosphorylation of ERK1/2. *Oncol Lett* 2013; **6**: 1439-1446 [PMID: 24179538 DOI: 10.3892/ol.2013.1587]
- 51 **Mayr C**, Neureiter D, Pichler M, Berr F, Wagner A, Kiesslich T, Namberger K. Cytotoxic effects of chemokine receptor 4 inhibition by AMD3100 in biliary tract cancer cells: Potential drug synergism with gemcitabine. *Mol Med Rep* 2015; **12**: 2247-2252 [PMID: 25846744 DOI: 10.3892/mmr.2015.3589]
- 52 **Zhao S**, Wang J, Qin C. Blockade of CXCL12/CXCR4 signaling inhibits intrahepatic cholangiocarcinoma progression and metastasis via inactivation of canonical Wnt pathway. *J Exp Clin Cancer Res* 2014; **33**: 103 [PMID: 25471741 DOI: 10.1186/s13046-014-0103-8]
- 53 **Cardinale V**, Renzi A, Carpino G, Torrice A, Bragazzi MC, Giuliani F, DeRose AM, Fraveto A, Onori P, Napoletano C, Franchitto A, Cantafora A, Grazi G, Caporaso N, D'Argenio G, Alpini G, Reid LM, Gaudio E, Alvaro D. Profiles of cancer stem cell subpopulations in cholangiocarcinomas. *Am J Pathol* 2015; **185**: 1724-1739 [PMID: 25892683 DOI: 10.1016/j.ajpath.2015.02.010]
- 54 **Sukowati CH**, Anfusio B, Torre G, Francalanci P, Crocè LS, Tiribelli C. The expression of CD90/Thy-1 in hepatocellular carcinoma: an in vivo and in vitro study. *PLoS One* 2013; **8**: e76830 [PMID: 24116172 DOI: 10.1371/journal.pone.0076830]
- 55 **Röcken C**, Licht J, Roessner A, Carl-McGrath S. Canalicular immunostaining of aminopeptidase N (CD13) as a diagnostic marker for hepatocellular carcinoma. *J Clin Pathol* 2005; **58**: 1069-1075 [PMID: 16189153 DOI: 10.1136/jcp.2005.026328]
- 56 **Nagano H**, Ishii H, Marubashi S, Haraguchi N, Eguchi H, Doki Y, Mori M. Novel therapeutic target for cancer stem cells in hepatocellular carcinoma. *J Hepatobiliary Pancreat Sci* 2012; **19**: 600-605 [PMID: 22892595 DOI: 10.1007/s00534-012-0543-5]
- 57 **Shaikh MV**, Kala M, Nivsarkar M. CD90 a potential cancer stem cell marker and a therapeutic target. *Cancer Biomark* 2016; **16**: 301-307 [PMID: 27062695 DOI: 10.3233/CBM-160590]
- 58 **Shuang ZY**, Wu WC, Xu J, Lin G, Liu YC, Lao XM, Zheng L, Li S. Transforming growth factor- $\beta$ 1-induced epithelial-mesenchymal transition generates ALDH-positive cells with stem cell properties in cholangiocarcinoma. *Cancer Lett* 2014; **354**: 320-328 [PMID: 25194504 DOI: 10.1016/j.canlet.2014.08.030]
- 59 **Ishiwata T**. Cancer stem cells and epithelial-mesenchymal transition: Novel therapeutic targets for cancer. *Pathol Int* 2016; **66**: 601-608 [PMID: 27510923 DOI: 10.1111/pin.12447]
- 60 **Sauvageau M**, Sauvageau G. Polycomb group proteins: multifaceted regulators of somatic stem cells and cancer. *Cell Stem Cell* 2010; **7**: 299-313 [PMID: 20804967 DOI: 10.1016/j.stem.2010.08.002]
- 61 **Mayr C**, Neureiter D, Wagner A, Pichler M, Kiesslich T. The role of polycomb repressive complexes in biliary tract cancer. *Expert Opin Ther Targets* 2015; **19**: 363-375 [PMID: 25424424 DOI: 10.1517/14728222.2014.986460]
- 62 **Mayr C**, Wagner A, Loeffelberger M, Bruckner D, Jakab M, Berr F, Di Fazio P, Ocker M, Neureiter D, Pichler M, Kiesslich T. The

- BMI1 inhibitor PTC-209 is a potential compound to halt cellular growth in biliary tract cancer cells. *Oncotarget* 2016; **7**: 745-758 [PMID: 26623561 DOI: 10.18632/oncotarget.6378]
- 63 **Thanan R**, Pairojkul C, Pinlaor S, Khuntikeo N, Wongkham C, Sripa B, Ma N, Vaeteewoottacharn K, Furukawa A, Kobayashi H, Hiraku Y, Oikawa S, Kawanishi S, Yongvanit P, Murata M. Inflammation-related DNA damage and expression of CD133 and Oct3/4 in cholangiocarcinoma patients with poor prognosis. *Free Radic Biol Med* 2013; **65**: 1464-1472 [PMID: 23917144 DOI: 10.1016/j.freeradbiomed.2013.07.034]
- 64 **Ruiz-Vela A**, Aguilar-Gallardo C, Simón C. Building a framework for embryonic microenvironments and cancer stem cells. *Stem Cell Rev* 2009; **5**: 319-327 [PMID: 20058196 DOI: 10.1007/s12015-009-9096-7]
- 65 **Shi CJ**, Gao J, Wang M, Wang X, Tian R, Zhu F, Shen M, Qin RY. CD133(+) gallbladder carcinoma cells exhibit self-renewal ability and tumorigenicity. *World J Gastroenterol* 2011; **17**: 2965-2971 [PMID: 21734809 DOI: 10.3748/wjg.v17.i24.2965]
- 66 **Yin BB**, Wu SJ, Zong HJ, Ma BJ, Cai D. Preliminary screening and identification of stem cell-like sphere clones in a gallbladder cancer cell line GBC-SD. *J Zhejiang Univ Sci B* 2011; **12**: 256-263 [PMID: 21462380 DOI: 10.1631/jzus.B1000303]
- 67 **Sun Q**, Li J, Wang G, Xie Y. Role of the embryonic protein SOX2 in cholangiocarcinoma. *Cell Biochem Biophys* 2014; **70**: 1311-1316 [PMID: 24906237 DOI: 10.1007/s12013-014-0056-8]
- 68 **Su MC**, Hsu C, Kao HL, Jeng YM. CD24 expression is a prognostic factor in intrahepatic cholangiocarcinoma. *Cancer Lett* 2006; **235**: 34-39 [PMID: 16125303 DOI: 10.1016/j.canlet.2005.03.059]
- 69 **Agrawal S**, Kuvshinoff BW, Khoury T, Yu J, Javle MM, LeVea C, Groth J, Coignet LJ, Gibbs JF. CD24 expression is an independent prognostic marker in cholangiocarcinoma. *J Gastrointest Surg* 2007; **11**: 445-451 [PMID: 17436128 DOI: 10.1007/s11605-007-0091-5]
- 70 **Keerattichamroen S**, Leelawat K, Thongtawe T, Narong S, Aegem U, Tujinda S, Praditphol N, Tohtong R. Expression of CD24 in cholangiocarcinoma cells is associated with disease progression and reduced patient survival. *Int J Oncol* 2011; **39**: 873-881 [PMID: 21687942 DOI: 10.3892/ijo.2011.1088]
- 71 **Kemmerling R**, Alinger B, Dietze O, Bösmüller HC, Ocker M, Wolkersdörfer GW, Berr F, Neureiter D, Kiesslich T. Association of stem cell marker expression pattern and survival in human biliary tract cancer. *Int J Oncol* 2012; **41**: 511-522 [PMID: 22614781 DOI: 10.3892/ijo.2012.1477]
- 72 **Thanee M**, Loilome W, Techasen A, Sugihara E, Okazaki S, Abe S, Ueda S, Masuko T, Namwat N, Khuntikeo N, Titapun A, Pairojkul C, Saya H, Yongvanit P. CD44 variant-dependent redox status regulation in liver fluke-associated cholangiocarcinoma: A target for cholangiocarcinoma treatment. *Cancer Sci* 2016; **107**: 991-1000 [PMID: 27176078 DOI: 10.1111/cas.12967]
- 73 **Kunlabut K**, Vaeteewoottacharn K, Wongkham C, Khuntikeo N, Warasawapati S, Pairojkul C, Wongkham S. Aberrant expression of CD44 in bile duct cancer correlates with poor prognosis. *Asian Pac J Cancer Prev* 2012; **13** Suppl: 95-99 [PMID: 23480770]
- 74 **Gu MJ**, Jang BI. Clinicopathologic significance of Sox2, CD44 and CD44v6 expression in intrahepatic cholangiocarcinoma. *Pathol Oncol Res* 2014; **20**: 655-660 [PMID: 24482053 DOI: 10.1007/s12253-014-9745-2]
- 75 **Kim R**, Kim SB, Cho EH, Park SH, Park SB, Hong SK, Chae G. CD44 expression in patients with combined hepatocellular cholangiocarcinoma. *Ann Surg Treat Res* 2015; **89**: 9-16 [PMID: 26131439 DOI: 10.4174/astr.2015.89.1.9]
- 76 **Yao X**, Zhou L, Han S, Chen Y. High expression of CXCR4 and CXCR7 predicts poor survival in gallbladder cancer. *J Int Med Res* 2011; **39**: 1253-1264 [PMID: 21986127]
- 77 **Sulpice L**, Rayar M, Turlin B, Boucher E, Bellaud P, Desille M, Meunier B, Clément B, Boudjema K, Coulouarn C. Epithelial cell adhesion molecule is a prognosis marker for intrahepatic cholangiocarcinoma. *J Surg Res* 2014; **192**: 117-123 [PMID: 24909871 DOI: 10.1016/j.jss.2014.05.017]
- 78 **Chen MH**, Weng JJ, Cheng CT, Wu RC, Huang SC, Wu CE, Chung YH, Liu CY, Chang MH, Chen MH, Chiang KC, Yeh TS, Su Y, Yeh CN. ALDH1A3, the Major Aldehyde Dehydrogenase Isoform in Human Cholangiocarcinoma Cells, Affects Prognosis and Gemcitabine Resistance in Cholangiocarcinoma Patients. *Clin Cancer Res* 2016; **22**: 4225-4235 [PMID: 27076629 DOI: 10.1158/1078-0432.CCR-15-1800]
- 79 **Sasaki M**, Ikeda H, Itatsu K, Yamaguchi J, Sawada S, Minato H, Ohta T, Nakanuma Y. The overexpression of polycomb group proteins Bmi1 and EZH2 is associated with the progression and aggressive biological behavior of hepatocellular carcinoma. *Lab Invest* 2008; **88**: 873-882 [PMID: 18591938 DOI: 10.1038/labinvest.2008.52]
- 80 **Sasaki M**, Yamaguchi J, Ikeda H, Itatsu K, Nakanuma Y. Polycomb group protein Bmi1 is overexpressed and essential in anchorage-independent colony formation, cell proliferation and repression of cellular senescence in cholangiocarcinoma: tissue and culture studies. *Hum Pathol* 2009; **40**: 1723-1730 [PMID: 19695678 DOI: 10.1016/j.humpath.2009.01.027]
- 81 **Zou Q**, Yang L, Yang Z, Huang J, Fu X. PSCA and Oct-4 expression in the benign and malignant lesions of gallbladder: implication for carcinogenesis, progression, and prognosis of gallbladder adenocarcinoma. *Biomed Res Int* 2013; **2013**: 648420 [PMID: 23984394 DOI: 10.1155/2013/648420]
- 82 **Deng G**, Zhu L, Huang F, Nie W, Huang W, Xu H, Zheng S, Yi Z, Wan T. SALL4 is a novel therapeutic target in intrahepatic cholangiocarcinoma. *Oncotarget* 2015; **6**: 27416-27426 [PMID: 26317546 DOI: 10.18632/oncotarget.4862]
- 83 **Heinrich PC**, Behrmann I, Haan S, Hermanns HM, Müller-Newen G, Schaper F. Principles of interleukin (IL)-6-type cytokine signalling and its regulation. *Biochem J* 2003; **374**: 1-20 [PMID: 12773095 DOI: 10.1042/BJ20030407]
- 84 **Pires BR**, DE Amorim ÍS, Souza LD, Rodrigues JA, Mencilha AL. Targeting Cellular Signaling Pathways in Breast Cancer Stem Cells and its Implication for Cancer Treatment. *Anticancer Res* 2016; **36**: 5681-5691 [PMID: 27793889 DOI: 10.21873/anticancer.11151]
- 85 **Liu WH**, Ren LN, Chen T, Liu LY, Tang LJ. Stages based molecular mechanisms for generating cholangiocytes from liver stem/progenitor cells. *World J Gastroenterol* 2013; **19**: 7032-7041 [PMID: 24222945 DOI: 10.3748/wjg.v19.i41.7032]
- 86 **Aoki S**, Mizuma M, Takahashi Y, Haji Y, Okada R, Abe T, Karasawa H, Tamai K, Okada T, Morikawa T, Hayashi H, Nakagawa K, Motoi F, Naitoh T, Katayose Y, Unno M. Aberrant activation of Notch signaling in extrahepatic cholangiocarcinoma: clinicopathological features and therapeutic potential for cancer stem cell-like properties. *BMC Cancer* 2016; **16**: 854 [PMID: 27821106 DOI: 10.1186/s12885-016-2919-4]
- 87 **Zender S**, Nিকেleit I, Wuestefeld T, Sörensen I, Dauch D, Bozko P, El-Khatib M, Geffers R, Bektas H, Manns MP, Gossler A, Wilkens L, Plentz R, Zender L, Malek NP. A Critical Role for Notch Signaling in the Formation of Cholangiocellular Carcinomas. *Cancer Cell* 2016; **30**: 353-356 [PMID: 27505676 DOI: 10.1016/j.ccell.2016.07.005]
- 88 **Zuo M**, Rashid A, Churi C, Vauthey JN, Chang P, Li Y, Hung MC, Li D, Javle M. Novel therapeutic strategy targeting the Hedgehog signalling and mTOR pathways in biliary tract cancer. *Br J Cancer* 2015; **112**: 1042-1051 [PMID: 25742482 DOI: 10.1038/bjc.2014.625]
- 89 **Chiba T**, Zheng YW, Kita K, Yokosuka O, Saisho H, Onodera M, Miyoshi H, Nakano M, Zen Y, Nakanuma Y, Nakauchi H, Iwama A, Taniguchi H. Enhanced self-renewal capability in hepatic stem/progenitor cells drives cancer initiation. *Gastroenterology* 2007; **133**: 937-950 [PMID: 17673212 DOI: 10.1053/j.gastro.2007.06.016]
- 90 **Oikawa T**. Cancer Stem cells and their cellular origins in primary liver and biliary tract cancers. *Hepatology* 2016; **64**: 645-651 [PMID: 26849406 DOI: 10.1002/hep.28485]
- 91 **Wang W**, Zhong W, Yuan J, Yan C, Hu S, Tong Y, Mao Y, Hu T, Zhang B, Song G. Involvement of Wnt/ $\beta$ -catenin signaling in the mesenchymal stem cells promote metastatic growth and

- chemoresistance of cholangiocarcinoma. *Oncotarget* 2015; **6**: 42276-42289 [PMID: 26474277 DOI: 10.18632/oncotarget.5514]
- 92 **Matsushita S**, Onishi H, Nakano K, Nagamatsu I, Imaizumi A, Hattori M, Oda Y, Tanaka M, Katano M. Hedgehog signaling pathway is a potential therapeutic target for gallbladder cancer. *Cancer Sci* 2014; **105**: 272-280 [PMID: 24438533 DOI: 10.1111/cas.12354]
- 93 **Maemura K**, Natsugoe S, Takao S. Molecular mechanism of cholangiocarcinoma carcinogenesis. *J Hepatobiliary Pancreat Sci* 2014; **21**: 754-760 [PMID: 24895231 DOI: 10.1002/jhbp.126]
- 94 **Senggunprai L**, Kukongviriyapan V, Prawan A, Kukongviriyapan U. Quercetin and EGCG exhibit chemopreventive effects in cholangiocarcinoma cells via suppression of JAK/STAT signaling pathway. *Phytother Res* 2014; **28**: 841-848 [PMID: 24038588 DOI: 10.1002/ptr.5061]
- 95 **Kong X**, Ma MZ, Zhang Y, Weng MZ, Gong W, Guo LQ, Zhang JX, Wang GD, Su Q, Quan ZW, Yang JR. Differentiation therapy: sesamin as an effective agent in targeting cancer stem-like side population cells of human gallbladder carcinoma. *BMC Complement Altern Med* 2014; **14**: 254 [PMID: 25038821 DOI: 10.1186/1472-6882-14-254]
- 96 **Aneknan P**, Kukongviriyapan V, Prawan A, Kongpetch S, Sripan B, Senggunprai L. Luteolin arrests cell cycling, induces apoptosis and inhibits the JAK/STAT3 pathway in human cholangiocarcinoma cells. *Asian Pac J Cancer Prev* 2014; **15**: 5071-5076 [PMID: 24998588]
- 97 **Yang H**, Lu X, Liu Z, Chen L, Xu Y, Wang Y, Wei G, Chen Y. FBXW7 suppresses epithelial-mesenchymal transition, stemness and metastatic potential of cholangiocarcinoma cells. *Oncotarget* 2015; **6**: 6310-6325 [PMID: 25749036 DOI: 10.18632/oncotarget.3355]
- 98 **Zong H**, Yin B, Zhou H, Cai D, Ma B, Xiang Y. Inhibition of mTOR pathway attenuates migration and invasion of gallbladder cancer via EMT inhibition. *Mol Biol Rep* 2014; **41**: 4507-4512 [PMID: 24623408 DOI: 10.1007/s11033-014-3321-4]
- 99 **Cao Y**, Liu X, Lu W, Chen Y, Wu X, Li M, Wang XA, Zhang F, Jiang L, Zhang Y, Hu Y, Xiang S, Shu Y, Bao R, Li H, Wu W, Weng H, Yen Y, Liu Y. Fibronectin promotes cell proliferation and invasion through mTOR signaling pathway activation in gallbladder cancer. *Cancer Lett* 2015; **360**: 141-150 [PMID: 25657110 DOI: 10.1016/j.canlet.2015.01.041]
- 100 **Kiesslich T**, Mayr C, Wachter J, Bach D, Fuehrer J, Wagner A, Alinger B, Pichler M, Di Fazio P, Ocker M, Berr F, Neureiter D. Activated hedgehog pathway is a potential target for pharmacological intervention in biliary tract cancer. *Mol Cell Biochem* 2014; **396**: 257-268 [PMID: 25064451 DOI: 10.1007/s11010-014-2161-9]
- 101 **Al-Bahrani R**, Nagamori S, Leng R, Petryk A, Sergi C. Differential Expression of Sonic Hedgehog Protein in Human Hepatocellular Carcinoma and Intrahepatic Cholangiocarcinoma. *Pathol Oncol Res* 2015; **21**: 901-908 [PMID: 25740074 DOI: 10.1007/s12253-015-9918-7]
- 102 **Yang XW**, Li L, Hou GJ, Yan XZ, Xu QG, Chen L, Zhang BH, Shen F. STAT3 overexpression promotes metastasis in intrahepatic cholangiocarcinoma and correlates negatively with surgical outcome. *Oncotarget* 2017; **8**: 7710-7721 [PMID: 28032598 DOI: 10.18632/oncotarget.13846]
- 103 **Dokduang H**, Techasen A, Namwat N, Khuntikeo N, Pairojkul C, Murakami Y, Loilome W, Yongvanit P. STATs profiling reveals predominantly-activated STAT3 in cholangiocarcinoma genesis and progression. *J Hepatobiliary Pancreat Sci* 2014; **21**: 767-776 [PMID: 25044480 DOI: 10.1002/jhbp.131]
- 104 **Herberger B**, Puhalla H, Lehnert M, Wrba F, Novak S, Brandstetter A, Gruenberger B, Gruenberger T, Pirker R, Filipits M. Activated mammalian target of rapamycin is an adverse prognostic factor in patients with biliary tract adenocarcinoma. *Clin Cancer Res* 2007; **13**: 4795-4799 [PMID: 17699857 DOI: 10.1158/1078-0432.CCR-07-0738]
- 105 **Leal P**, Garcia P, Sandoval A, Letelier P, Brebi P, Ili C, Álvarez H, Tapia O, Roa JC. Immunohistochemical expression of phospho-mTOR is associated with poor prognosis in patients with gallbladder adenocarcinoma. *Arch Pathol Lab Med* 2013; **137**: 552-557 [PMID: 23544944 DOI: 10.5858/arpa.2012-0032-OA]
- 106 **Lee Y**, Jeon K, Lee JT, Kim S, Kim VN. MicroRNA maturation: stepwise processing and subcellular localization. *EMBO J* 2002; **21**: 4663-4670 [PMID: 12198168]
- 107 **Mayr C**, Beyreis M, Wagner A, Pichler M, Neureiter D, Kiesslich T. Deregulated MicroRNAs in Biliary Tract Cancer: Functional Targets and Potential Biomarkers. *Biomed Res Int* 2016; **2016**: 4805270 [PMID: 27957497 DOI: 10.1155/2016/4805270]
- 108 **Lin KY**, Ye H, Han BW, Wang WT, Wei PP, He B, Li XJ, Chen YQ. Genome-wide screen identified let-7c/miR-99a/miR-125b regulating tumor progression and stem-like properties in cholangiocarcinoma. *Oncogene* 2016; **35**: 3376-3386 [PMID: 26455324 DOI: 10.1038/ncr.2015.396]
- 109 **Zhang J**, Jiao J, Cermelli S, Muir K, Jung KH, Zou R, Rashid A, Gagea M, Zabludoff S, Kalluri R, Beretta L. miR-21 Inhibition Reduces Liver Fibrosis and Prevents Tumor Development by Inducing Apoptosis of CD24+ Progenitor Cells. *Cancer Res* 2015; **75**: 1859-1867 [PMID: 25769721 DOI: 10.1158/0008-5472.CAN-14-1254]
- 110 **Urbas R**, Mayr C, Klieser E, Fuehrer J, Bach D, Stättner S, Primavesi F, Jaeger T, Stanzer S, Röss AL, Löffelberger M, Wagner A, Berr F, Ritter M, Pichler M, Neureiter D, Kiesslich T. Relevance of MicroRNA200 Family and MicroRNA205 for Epithelial to Mesenchymal Transition and Clinical Outcome in Biliary Tract Cancer Patients. *Int J Mol Sci* 2016; **17**: [PMID: 27941621 DOI: 10.3390/ijms17122053]
- 111 **Chen DQ**, Huang JY, Feng B, Pan BZ, De W, Wang R, Chen LB. Histone deacetylase 1/Sp1/microRNA-200b signaling accounts for maintenance of cancer stem-like cells in human lung adenocarcinoma. *PLoS One* 2014; **9**: e109578 [PMID: 25279705 DOI: 10.1371/journal.pone.0109578]
- 112 **Zhan M**, Zhao X, Wang H, Chen W, Xu S, Wang W, Shen H, Huang S, Wang J. miR-145 sensitizes gallbladder cancer to cisplatin by regulating multidrug resistance associated protein 1. *Tumour Biol* 2016; **37**: 10553-10562 [PMID: 26852750 DOI: 10.1007/s13277-016-4957-6]
- 113 **Hu J**, Qiu M, Jiang F, Zhang S, Yang X, Wang J, Xu L, Yin R. MiR-145 regulates cancer stem-like properties and epithelial-to-mesenchymal transition in lung adenocarcinoma-initiating cells. *Tumour Biol* 2014; **35**: 8953-8961 [PMID: 24903381 DOI: 10.1007/s13277-014-2158-8]
- 114 **Jin K**, Xiang Y, Tang J, Wu G, Li J, Xiao H, Li C, Chen Y, Zhao J. miR-34 is associated with poor prognosis of patients with gallbladder cancer through regulating telomere length in tumor stem cells. *Tumour Biol* 2014; **35**: 1503-1510 [PMID: 24078448 DOI: 10.1007/s13277-013-1207-z]
- 115 **Qiao P**, Li G, Bi W, Yang L, Yao L, Wu D. microRNA-34a inhibits epithelial mesenchymal transition in human cholangiocarcinoma by targeting Smad4 through transforming growth factor-beta/Smad pathway. *BMC Cancer* 2015; **15**: 469 [PMID: 26077733 DOI: 10.1186/s12885-015-1359-x]
- 116 **Borovski T**, De Sousa E Melo F, Vermeulen L, Medema JP. Cancer stem cell niche: the place to be. *Cancer Res* 2011; **71**: 634-639 [PMID: 21266356 DOI: 10.1158/0008-5472.CAN-10-3220]
- 117 **Romano M**, De Francesco F, Gringeri E, Giordano A, Ferraro GA, Di Domenico M, Cillo U. Tumor Microenvironment Versus Cancer Stem Cells in Cholangiocarcinoma: Synergistic Effects? *J Cell Physiol* 2016; **231**: 768-776 [PMID: 26357947 DOI: 10.1002/jcp.25190]
- 118 **Subimerb C**, Pinlaor S, Lulitanond V, Khuntikeo N, Okada S, McGrath MS, Wongkham S. Circulating CD14(+) CD116(+) monocyte levels predict tissue invasive character of cholangiocarcinoma. *Clin Exp Immunol* 2010; **161**: 471-479 [PMID: 20636398 DOI: 10.1111/j.1365-2249.2010.04200.x]
- 119 **Subimerb C**, Pinlaor S, Khuntikeo N, Leelayuwat C, Morris A, McGrath MS, Wongkham S. Tissue invasive macrophage density is correlated with prognosis in cholangiocarcinoma. *Mol Med Rep* 2010; **3**: 597-605 [PMID: 21472285 DOI: 10.3892/

mmr\_00000303]

- 120 **Banales JM**, Cardinale V, Carpino G, Marzioni M, Andersen JB, Invernizzi P, Lind GE, Folseraas T, Forbes SJ, Fouassier L, Geier A, Calvisi DF, Mertens JC, Trauner M, Benedetti A, Maroni L, Vaquero J, Macias RI, Raggi C, Perugorria MJ, Gaudio E, Boberg KM, Marin JJ, Alvaro D. Expert consensus document: Cholangiocarcinoma: current knowledge and future perspectives consensus statement from the European Network for the Study of Cholangiocarcinoma (ENS-CCA). *Nat Rev Gastroenterol Hepatol* 2016; **13**: 261-280 [PMID: 27095655 DOI: 10.1038/nrgastro.2016.51]
- 121 **Sherman LS**, Shaker M, Mariotti V, Rameshwar P. Mesenchymal stromal/stem cells in drug therapy: New perspective. *Cytotherapy* 2017; **19**: 19-27 [PMID: 27765601 DOI: 10.1016/j.jcyt.2016.09.007]
- 122 **Norozi F**, Ahmadzadeh A, Shahrabi S, Vosoughi T, Saki N. Mesenchymal stem cells as a double-edged sword in suppression or progression of solid tumor cells. *Tumour Biol* 2016; **37**: 11679-11689 [PMID: 27440203 DOI: 10.1007/s13277-016-5187-7]
- 123 **Haga H**, Yan IK, Takahashi K, Wood J, Zubair A, Patel T. Tumour cell-derived extracellular vesicles interact with mesenchymal stem cells to modulate the microenvironment and enhance cholangiocarcinoma growth. *J Extracell Vesicles* 2015; **4**: 24900 [PMID: 25557794 DOI: 10.3402/jev.v4.24900]
- 124 **Kiesslich T**, Pichler M, Neureiter D. Epigenetic control of epithelial-mesenchymal-transition in human cancer. *Mol Clin Oncol* 2013; **1**: 3-11 [PMID: 24649114 DOI: 10.3892/mco.2012.28]
- 125 **Kalluri R**, Weinberg RA. The basics of epithelial-mesenchymal transition. *J Clin Invest* 2009; **119**: 1420-1428 [PMID: 19487818 DOI: 10.1172/JCI39104]
- 126 **Mladinich M**, Ruan D, Chan CH. Tackling Cancer Stem Cells via Inhibition of EMT Transcription Factors. *Stem Cells Int* 2016; **2016**: 5285892 [PMID: 27840647 DOI: 10.1155/2016/5285892]
- 127 **Kuhn NZ**, Tuan RS. Regulation of stemness and stem cell niche of mesenchymal stem cells: implications in tumorigenesis and metastasis. *J Cell Physiol* 2010; **222**: 268-277 [PMID: 19847802 DOI: 10.1002/jcp.21940]

**P- Reviewer:** Ishikawa T, Vaccarezza M, Saeki K, Tanabe S

**S- Editor:** Qi Y **L- Editor:** A **E- Editor:** Wang CH





Published by **Baishideng Publishing Group Inc**  
8226 Regency Drive, Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.f6publishing.com/helpdesk>  
<http://www.wjgnet.com>



ISSN 1007-9327

