

World Journal of *Gastroenterology*

World J Gastroenterol 2017 June 28; 23(24): 4317-4472



**EDITORIAL**

- 4317 Risk of hepatitis B reactivation in patients treated with direct-acting antivirals for hepatitis C
Aggeletopoulou I, Konstantakis C, Manolakopoulos S, Triantos C
- 4324 Role of new endoscopic techniques in inflammatory bowel disease management: Has the change come?
Goran L, Negreanu L, Negreanu AM

REVIEW

- 4330 Implication of the Hedgehog pathway in hepatocellular carcinoma
Della Corte CM, Viscardi G, Papaccio F, Esposito G, Martini G, Ciardiello D, Martinelli E, Ciardiello F, Morgillo F

ORIGINAL ARTICLE**Basic Study**

- 4341 Indole phytoalexin derivatives induce mitochondrialmediated apoptosis in human colorectal carcinoma cells
Tischlerova V, Kello M, Budovska M, Mojzis J
- 4354 Naringenin prevents experimental liver fibrosis by blocking TGF β -Smad3 and JNK-Smad3 pathways
Hernández-Aquino E, Zarco N, Casas-Grajales S, Ramos-Tovar E, Flores-Beltrán RE, Arauz J, Shibayama M, Favari L, Tsutsumi V, Segovia J, Muriel P
- 4369 Intestinal anti-inflammatory activity of Ground Cherry (*Physalis angulata* L.) standardized CO₂ phytopharmaceutical preparation
Almeida Junior LD, Quaglio AEV, de Almeida Costa CAR, Di Stasi LC
- 4381 *Maytenus erythroxylon* Reissek (Celastraceae) ethanol extract presents antidiarrheal activity *via* antimotility and antisecretory mechanisms
Formiga RO, Quirino ZGM, Diniz MFFM, Marinho AF, Tavares JF, Batista LM
- 4390 Gastric cancer-derived heat shock protein-gp96 peptide complex enhances dendritic cell activation
Lu WW, Zhang H, Li YM, Ji F
- Retrospective Cohort Study**
- 4399 Para-aortic node involvement is not an independent predictor of survival after resection for pancreatic cancer
Sperti C, Gruppo M, Blandamura S, Valmasoni M, Pozza G, Passuello N, Beltrame V, Moletta L

Retrospective Study

- 4407 Risk factors for metachronous gastric carcinoma development after endoscopic resection of gastric dysplasia: Retrospective, single-center study

Moon HS, Yun GY, Kim JS, Eun HS, Kang SH, Sung JK, Jeong HY, Song KS

- 4416 New magnifying endoscopic classification for superficial esophageal squamous cell carcinoma

Kim SJ, Kim GH, Lee MW, Jeon HK, Baek DH, Lee BE, Song GA

- 4422 Procalcitonin as a diagnostic marker to distinguish upper and lower gastrointestinal perforation

Gao Y, Yu KJ, Kang K, Liu HT, Zhang X, Huang R, Qu JD, Wang SC, Liu RJ, Liu YS, Wang HL

Observational Study

- 4428 Healthcare utilization and costs associated with gastroparesis

Wadhwa V, Mehta D, Jobanputra Y, Lopez R, Thota PN, Sanaka MR

- 4437 Variability of anti-human transglutaminase testing in celiac disease across Mediterranean countries

Smarrazzo A, Magazzù G, Ben-Hariz M, Legarda Tamara M, Velmishi V, Roma E, Kansu A, Mičetić-Turk D, Bravi E, Stellato P, Arcidiaco C, Greco L

- 4444 Appropriateness of the study of iron deficiency anemia prior to referral for small bowel evaluation at a tertiary center

Rodrigues JP, Pinho R, Silva J, Ponte A, Sousa M, Silva JC, Carvalho J

Randomized Clinical Trial

- 4454 Comparing reduced-dose sodium phosphate tablets to 2 L of polyethylene glycol: A randomized study

Ako S, Takemoto K, Yasutomi E, Sakaguchi C, Murakami M, Sunami T, Oka S, Kenta H, Okazaki N, Baba Y, Yamasaki Y, Asato T, Kawai D, Takenaka R, Tsugeno H, Hiraoka S, Kato J, Fujiki S

CASE REPORT

- 4462 Case of pediatric traditional serrated adenoma resected *via* endoscopic submucosal dissection

Kondo S, Mori H, Nishiyama N, Kondo T, Shimono R, Okada H, Kusaka T

- 4467 Pancreatic T/histiocyte-rich large B-cell lymphoma: A case report and review of literature

Zheng SM, Zhou DJ, Chen YH, Jiang R, Wang YX, Zhang Y, Xue HL, Wang HQ, Mou D, Zeng WZ

ABOUT COVER

Editorial board member of *World Journal of Gastroenterology*, Christian Martin Grieser, MD, PhD, Associate Professor, Doctor, Center for Modern Diagnostics, Schwachhauser Heerstr. 63a, Bremen 28209, Germany

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 7th, 14th, 21st, and 28th 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 and Directory of Open Access Journals. The 2017 edition of Journal Citation Reports[®] cites the 2016 impact factor for *WJG* as 3.365 (5-year impact factor: 3.176), ranking *WJG* as 29th among 79 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: *Cui-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
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>

PUBLISHER

Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>

<http://www.wjgnet.com>

PUBLICATION DATE
June 28, 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.f6publishing.com>

Implication of the Hedgehog pathway in hepatocellular carcinoma

Carminia Maria Della Corte, Giuseppe Viscardi, Federica Papaccio, Giovanna Esposito, Giulia Martini, Davide Ciardiello, Erika Martinelli, Fortunato Ciardiello, Floriana Morgillo

Carminia Maria Della Corte, Giuseppe Viscardi, Federica Papaccio, Giovanna Esposito, Giulia Martini, Davide Ciardiello, Erika Martinelli, Fortunato Ciardiello, Floriana Morgillo, Dipartimento Medico-Chirurgico di Internistica Clinica e Sperimentale "F. Magrassi e A. Lanzara", Università degli studi della Campania "Luigi Vanvitelli", 80131 Naples, Italy

Author contributions: Della Corte CM conducted data research and wrote the paper; Viscardi G, Papaccio F, Esposito G, Ciardiello D and Martinelli E conducted data research; Ciardiello F and Morgillo F contributed critical revision of the manuscript for important intellectual content.

Conflict-of-interest statement: Authors have no conflict of interest to declare.

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: Floriana Morgillo, MD, PhD, Assistant Professor, Dipartimento Medico-Chirurgico di Internistica Clinica e Sperimentale "F. Magrassi e A. Lanzara", Università degli studi della Campania "Luigi Vanvitelli", Via S. Pansini 5, 80131 Naples, Italy. florianamorgillo@yahoo.com
Telephone: +39-81-5666628
Fax: +39-81-5666785

Received: February 7, 2017

Peer-review started: February 10, 2017

First decision: March 16, 2017

Revised: April 13, 2017

Accepted: May 19, 2017

Article in press: May 19, 2017

Published online: June 28, 2017

Abstract

The prognosis for patients who are diagnosed with advanced stage hepatocellular carcinoma (HCC) is poor because there are few treatment options. Recent research has focused on the identification of novel molecular entities that can be targeted to inhibit oncogenic signals that are involved in the carcinogenesis, proliferation and progression of HCC. Among all of the pathways that are involved in the development of HCC, Hedgehog (HH) signalling has demonstrated a substantial role in hepatocarcinogenesis and HCC progression. HH plays a physiological role in embryogenesis, through the induction of the differentiation of hepatocytes from endodermal progenitors. The re-activation of the HH pathway in chronic damaged liver is a mechanism of fibrotic degeneration and is implicated in various stages of HCC development. HH activation sustains the sub-population of immature liver epithelial cells that are involved in the pathogenesis of cirrhosis and HCC, and HH itself is a mediator of the alcohol-derived malignant transformation of liver cells. High levels of expression of HH protein markers in liver tumour tissues are correlated with aggressive histological and biological features and a poor clinical outcome. *In vitro* and *in vivo* inhibition models of the HH pathway confirm that HH is essential in maintaining tumour growth, metastasis and a mesenchymal phenotype.

Key words: Hedgehog; Epithelial-mesenchymal transition; Hepatocellular carcinoma; Hepatocarcinogenesis; Prognosis

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

Core tip: The hedgehog (HH) pathway is involved in the embryonic development of liver, and its reactivation plays a substantial role in sustaining cancer cell growth and progression in hepatocellular carcinoma (HCC).

In hepatocarcinogenesis, HH signalling is required for differentiation, proliferation and polarity of liver embryonic cells. High levels of expression of HH components in HCC tissues correlate with mesenchymal properties and maintain the proliferation of cancer stem cells, which is a dynamic source of malignant cells in HCC progression. Current data on HH inhibition in preclinical models further confirm the role of HH and deserve future investigations in a clinical setting.

Della Corte CM, Viscardi G, Papaccio F, Esposito G, Martini G, Ciardiello D, Martinelli E, Ciardiello F, Morgillo F. Implication of the Hedgehog pathway in hepatocellular carcinoma. *World J Gastroenterol* 2017; 23(24): 4330-4340 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v23/i24/4330.htm> DOI: <http://dx.doi.org/10.3748/wjg.v23.i24.4330>

INTRODUCTION

Liver tumours are the third highest cause of cancer-related deaths and the sixth most frequent type of cancer worldwide^[1]. In the past few years, the incidence of hepatocellular carcinoma (HCC) has been on the rise, especially among males, being correlated with chronic liver disease^[2]. In particular, the majority of patients who develop HCC are infected with viral hepatitis B (HBV) or viral hepatitis C (HCV)^[3,4]. Even though vaccination programmes are commonly used in Western populations, the incidence of HCC remains high because of other risk factors, such as alcoholic and non-alcoholic fatty liver disease and obesity, which are increasingly out of control in Occidental countries^[5].

Screening procedures with periodic liver ultrasonography (every 6 mo), concomitant with the determination of serum alpha-fetoprotein (AFP), are recommended only for cirrhotic patients and HBV- and HCV- serum positive patients^[6].

Treatment options with curative intent, including surgery, local treatment (such as percutaneous thermal ablation, intra-arterial chemotherapy or embolization or radiotherapy) and liver transplantation, are reserved only for select patients with localized tumour extension and/or with good performance status and liver function^[7,8].

Unfortunately, most patients are diagnosed at an advanced stage; the five-year overall survival for unresectable HCC is less than 10% because all medical treatments that were given to these patients exhibited a very poor efficacy^[2]. Actually, according to international oncologic guidelines, sorafenib is the only standard systemic therapy for patients with advanced HCC and adequate liver function^[2]. Sorafenib is a multi-kinase inhibitor with potent inhibitory activity against RAF kinases, including mutant V600E B-RAF, and against various serine/threonine kinases, such as the pro-angiogenic receptor tyrosine kinases vascular endothelial growth factor receptor (VEGFR),

platelet derived growth factor receptor (PDGFR) and fibroblast growth factor receptor (FGFR1), as well as tyrosine kinases such as KIT, Fms-related tyrosine kinase 3 (FLT-3) and RET^[9]. More recent clinical data on new treatment options included regorafenib^[10] and immunotherapy^[11]. Treatment with regorafenib produced a survival benefit in HCC patients on sorafenib treatment (median survival 10.6 mo for regorafenib vs 7.8 mo for the placebo group, with a hazard ratio of 0.63, one-sided $P < 0.0001$)^[10]. At the previous ASCO congress, efficacy data from the CheckMate 040 trial were presented for Nivolumab, an anti-PD1 (Programmed cell Death 1) agent, in advanced HCC patients (70% progressed to sorafenib and 30% were sorafenib naïve). Nivolumab monotherapy provided early, stable and durable responses, irrespective of the hepatitis C or B viral infection status, and of PD-L1 (Programmed cell Death Ligand 1) expression on the tumours^[11]. The overall response rate was 19% in the overall population, and 21.7% in the first-line setting, with an overall 9 month survival rate of 77%^[11].

Recent research has focused on identifying potential new molecular targets that can be “drugable” in order to inhibit the oncogenic signals that drive tumour carcinogenesis, growth and metastasis^[12].

In HCC carcinogenesis and evolution, biological and genetic alterations lead to the activation of oncogenes (V-Myc avian myelocytomatosis viral oncogene homologue, myc, kirsten rat sarcoma viral oncogene homologue, k-ras, raf murine sarcoma viral oncogene homologue B, BRAF), of growth factor-induced signalling (transforming growth factor- α , TGF- α , TGF- β , insulin-like growth factor-2 receptor, IGF-2, epidermal growth factor receptor, EGFR, PDGFR, VEGFR), and/or re-activation of pathways that are implicated in development and embryogenesis (Wingless-Type MMTV integration site family, WNT, and Hedgehog, HH) or disabling of tumour suppressor genes (retinoblastoma, RB, phosphatase and tensin homologues, PTEN, Runt-related transcription factor 3, RUNX3, or p53)^[13,14].

In this review we will focus on current data regarding the role of HH pathway reactivation in the development and progression of HCC. The main role of HH in embryonic hepatogenesis involves the induction of the differentiation of hepatocytes from endodermal progenitors^[15,16]. In hepatocarcinoma cells, the re-induction of expression of HH proteins sustains cancer cell growth and reduces apoptotic stimuli^[17] at various stages of HCC development (Figure 1).

CARCINOGENESIS AND EVOLUTION OF HEPATOCARCINOMA

Carcinogenesis is a multistep process that is characterized by changes at the cellular, genetic and epigenetic levels, and by abnormal cell division that

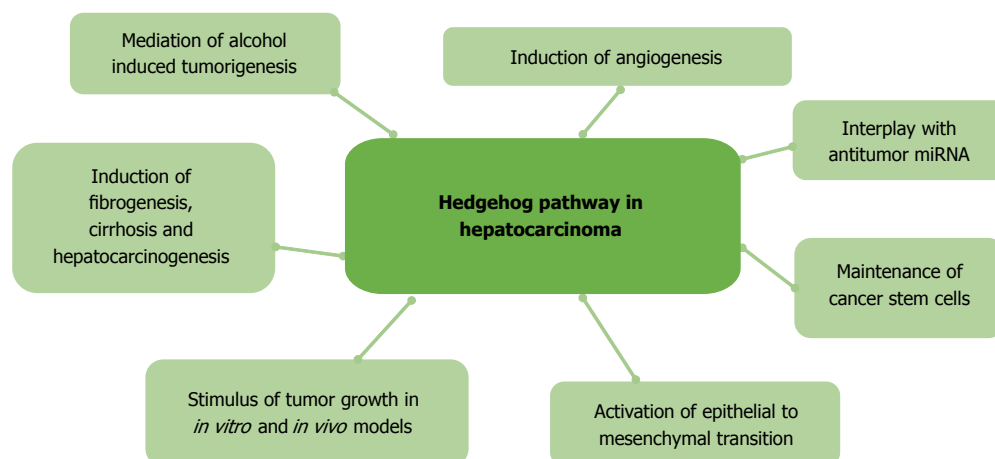


Figure 1 Role of Hedgehog pathway in hepatocarcinoma.

leads to the formation of a malignant mass. There are common mechanisms for carcinogenesis in different human organs and others that are organ-specific^[18].

The principal cause of liver carcinogenesis is an external stimulus: viral infection or alcoholic damage^[5,19-22]. HBV DNA and HCV core proteins are oncogenic, as they induce the formation of pro-proliferative transcription factors and interplay with RAS, WNT or p53 signalling^[19-21]. In fatty liver disease, especially in alcohol related damage, active adipocyte pathways induce chronic inflammation of the microenvironment and liver cell regeneration and proliferation^[5,22].

An important mechanism of oncogenesis and tumour evolution in hepatocarcinoma is the activation of the oncogenes MYC and RAS^[23-26]. MYC gene encodes for a protein that is involved in nucleic acid metabolism and in response to growth factors. MYC amplification has been detected in 12% of single nodular HCC, in 38% of multiple nodular HCC ($P < 0.01$), and with a higher frequency in metastatic and recurrent HCC patients than in primary tumours (45% vs 29%, and 60% vs 38%, respectively), suggesting that MYC alteration can occur in early stages of carcinogenesis rather than in later stages, leading to metastatic processes^[26]. The RAS gene transcript is the RAS protein, a small GTPase protein that represents the main regulator of the mitogen activated protein kinase (MAPK) and of the phosphatidylinositol 3-kinase (PI3K)/AKT pathways that represent the principal signalling pathways for cell survival^[23,24,27].

Among receptors that are involved in HCC carcinogenesis, the MET receptor and its ligand hepatocyte growth factor have been demonstrated to correlate with a poor prognosis in HCC^[28,29], and the selective MET-inhibitor, tivantinib, has shown promising activity in a phase II trial in patients progressing on sorafenib and whose tumours produced high expression levels of MET^[30].

In the evolution and metastasis of HCC, abnormal angiogenesis and epithelial-mesenchymal transition (EMT) are fundamental. VEGFR, FGFR and PDGFR are

the main factors in the uncontrolled neo-angiogenesis of HCC^[31,32]. Sorafenib was originally designed to inhibit VEGFR, PDGFR and KIT; the efficacy of sorafenib confirms the essential role of angiogenesis in the progression of HCC^[10]. TGF- β is a key mediator of EMT and functions as an autocrine or paracrine growth factor for the regulation of inflammatory cells, T-cells and cancer-associated fibroblasts in the microenvironment^[33,34].

A peculiar and interesting aspect of the early phase of hepatocarcinogenesis is the renewal of the developmental pathways of WNT and HH that conduct embryonic growth and the differentiation of normal liver cells in humans^[35-37]. WNT/ β -catenin signalling can be abnormally re-activated in HCC by β -catenin mutations, by nuclear translocations, or by a reduction of the adenomatous polyposis coli gene-related protein, which is a negative regulator of β -catenin^[35,36]. A β -catenin signal induces metastatic behaviour and stem-like features in cancer cells^[35,36].

Although there are data indicating that TGF- β and WNT regulate growth and repair responses in adult livers, the possibility that HH was involved in these processes was not considered until recently^[17,37].

HH pathway activation is required for the differentiation, proliferation and polarity of liver embryonic cells and plays a role in hepatocarcinogenesis^[16,37,38] (Figure 2). It is known that complete silencing of HH signalling in embryos cause a lethal block in the development of nervous and cardiovascular systems and the liver^[38,39]. Partial silencing of HH during liver development is responsible for hepatic defects, along with cardiac, neurological and muscular malformations^[38,39]. In human embryonic development, HH ligands are highly expressed in cells of the ventral endoderm that differentiate in hepatic progenitors, and the principal mediator of HH activity is *foxa2*, a specific transcription factor for hepatic cells^[40]. Inhibition of HH *in vitro* reduced the proliferation of human foetal hepatoblasts^[41], thus confirming that HH is essential in the differentiation process for hepatic and pancreatic

Hedgehog pathway implications in hepatic carcinogenesis

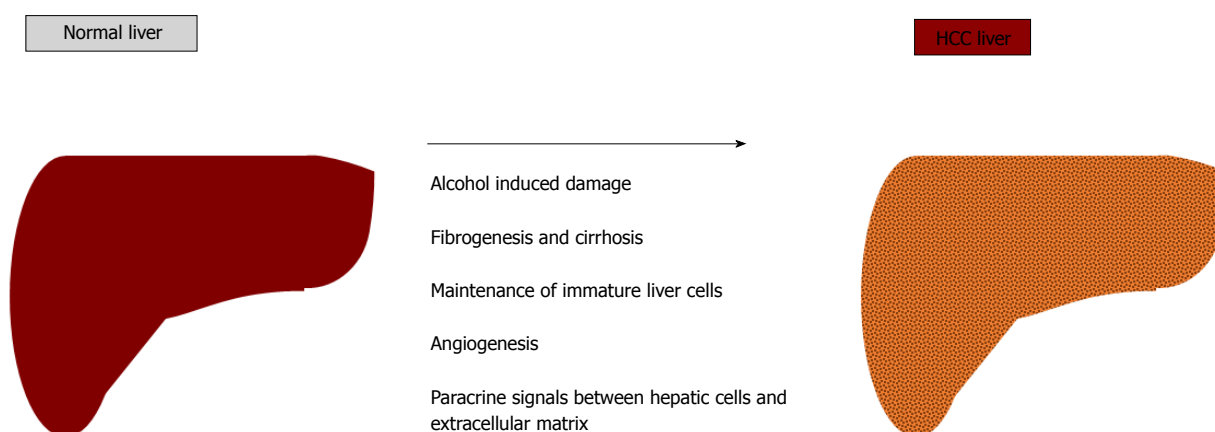


Figure 2 Hedgehog pathway implications in hepatic carcinogenesis. HCC: Hepatocellular carcinoma.

cells from multipotent endodermal progenitor cells. HH activation progressively decreases during liver epithelial cell maturation^[37,41]. HH signalling has no function in the adult liver; indeed, normal hepatocytes display undetectable levels of HH signal proteins with no clear HH pathway activity. The mesenchyme of healthy livers and liver sinusoidal cells, including endothelial and hepatic stellate cells, continues to produce Hhip, a soluble antagonist of HH ligands, which retain soluble HH ligands from the activation of the HH pathway in epithelial cells^[42]. Interestingly, after partial hepatectomy, the hepatic expression of HH ligands re-increases significantly, concomitant with a downregulation of Hhip, and this increment represents a strong stimulus for liver regeneration^[43,44]. Transient activation of the HH pathway is useful for liver regeneration after an acute injury. An alteration of the equilibrium between liver epithelial cells and neighbouring stromal cells is due to persistent re-induction of paracrine HH signalling that sustains the sub-population of immature liver epithelial cells that are involved in the pathogenesis of liver fibrosis, cirrhosis and HCC^[44].

ROLE OF THE HH PATHWAY IN HCC

HH pathway components

The HH pathway is activated by ligands. In humans, three ligands have been identified: Sonic hedgehog (SHH), Indian hedgehog (IHH) and Desert hedgehog, of which SHH is the most studied^[45]. In target cells, SHH binds to its receptor Patched-1 (PTCH1). In the absence of SHH, PTCH1 inhibits Smoothened (SMO), a downstream protein in the HH pathway. Thus, the interaction between SHH and PTCH1 reduces SMO and the subsequent activation of the GLI transcription factors Gli1 and Gli2, which are known activators, as well as Gli3, a repressor^[45]. The effect of this molecular cascade has only partially been studied, and the role of HH is currently the object of research

in various types of human cancers (breast, pancreas, lung, colorectal, gastric and renal cancers and hepatocarcinoma)^[16,17,46-51].

Evidence of HH activation in human liver tumour tissues

Different research groups have demonstrated that mature hepatocytes lack HH activity^[37]. The difference in terms of HH protein marker expression between tumour and normal liver tissues has been investigated to assess the clinical impact of HH activation in HCC patients^[52-54] (Table 1).

The first prospective study was performed in 2006 by Sicklick *et al.*^[52] These authors examined levels of SHH, Gli1 and SMO in tumours and in adjacent normal tissues and confirmed the activation of the HH pathway in cancer cells with overexpression of SMO compared to respective non-tumour tissues. Moreover, elevated SMO levels were significantly associated with the size of the HCC nodules. The ratio of SMO to PTCH1 expression was higher in larger (> 5 cm diameter) than in smaller (< 5 cm diameter) HCC lesions ($r = 1/4$ 0.57, $P < 0.04$)^[52].

Similar results have been presented by Huang *et al.*^[53] who published an interesting analysis of HH markers in HCC tumour tissues and adjacent normal liver tissues. In a case study of 115 patients, the expression of SHH and its target genes were quantified by *in situ* hybridization and polymerase chain reaction (PCR). Expression levels of SHH, Gli1 and PTCH1 in tumour cells were significantly higher than in adjacent normal cells in most cases. HCC cancer cells were positive for SHH in 59% of liver tumours, for Gli1 in 72%, and for PTCH1 in 56%; the majority of HH positive cases were tumours larger than 3 cm, thus underlying the role of this pathway in maintaining tumour growth and progression^[53]. One single case exhibited positive Gli1 and PTCH1 expression both in tumour and in surrounding tissues; deeper analysis also detected positive HH proteins levels in altered pre-cancerous tissues (small cell dysplasia or dysplastic

Table 1 Expression of Hedgehog pathway protein markers in hepatocarcinoma tissues

Ref.	SMO	SHH	PTCH	Gli1	Clinical correlation
Sicklick <i>et al</i> ^[52] , 2006	42.90% (3-fold higher than normal tissues)		50% (3- to 4-fold higher than normal tissues) 21.4% (8- to 9-fold higher than normal tissues)		Tumour size
Huang <i>et al</i> ^[53] , 2006		59% positive tumours	56% positive tumours	72% positive tumours	Tumour size
Che <i>et al</i> ^[59] , 2012	32.61% positive tumours	60.87% positive tumours	50% positive tumours	54.35% positive tumours	Gli1 with disease-free survival (DFS) and overall survival (OS)
Dugum <i>et al</i> ^[54] , 2016		100% tumoural and surrounding tissue	100% tumoural and surrounding tissue, with higher expression in tumour tissue	100% tumoural and surrounding tissue	Tendency to correlation with HCC recurrence after liver transplantation

SMO: Smoothened; SHH: Sonic hedgehog; PTCH1: Patched-1; HCC: Hepatocellular carcinoma.

nodules) and in microscopic HCC, thus reinforcing the substantial role of HH in early stages of carcinogenesis. Another important finding that emerged from this study was the correlation between HH activation and β -catenin positivity, suggesting an interplay between the two developmental pathways (WNT and HH) that are involved in hepatocarcinogenesis^[55].

Furthermore, HH activation appears to be linked to the ingestion of alcohol. A recent study by Chan *et al*^[56] analysed a model of alcohol-derived hepatocarcinogenesis and described a correlation between increased alcohol ingestion and HH signalling activation, with subsequent malignant changes in liver cells. Alcohol exposure induces the over-activation of HH signalling through the elevation of HH ligands and HH target genes in animal models and human tissues. The proliferation of progenitor cells was accompanied by the expression of EMT markers and by an interaction between stroma and hepatic cells. The HH pathway can induce glycolytic activity in myofibroblasts of liver stroma, the so-called "reverse Warburg" effect^[57], and the products of glycolytic stromal cells stimulate the proliferation of malignant liver cells^[58].

Further evidence for a role of the HH pathway in the carcinogenesis of HCC came from the analysis of HH activation in cirrhotic patients^[52]. Among these patients, levels of PTCH1 were variable, due to the presence of myofibroblastic hepatic stellate cells in cirrhotic non neoplastic tissue that are able to produce PTCH1^[52]. The concomitant detection of SMO mutations and HH activation, demonstrated by high Gli1 levels, suggest that early genetic alteration in HH proteins can promote carcinogenesis and growth of HCC from HH signals^[52].

To define the relationship between the expression of HH pathway components and clinical prognosis, a sample-expanded and prolonged follow-up study was performed on 46 surgically treated HCC patients^[59]. All HH components were present in more than 50% of tumour samples, and in particular, Gli1 expression was significantly correlated with disease-free survival (DFS) and overall survival (OS) ($P < 0.029$ and P

< 0.025 , respectively). The authors concluded that Gli1 expression could become a strong prognostic biomarker for HCC progression. Since Gli1 is a key effector in the HH pathway, and HH components were associated with clinical malignant features (tumour size, capsular invasion and vascular invasion), we speculate that HH signal activation has a clear impact on the clinical progression and development of HCC in patients^[59].

In 2016, Dugum *et al*^[54] assessed HH protein marker expression in HCC and surrounding normal tissue in patients with HCC recurrence after liver transplantation (LT). These data have high clinical relevance, because a complete panel of prognostic biomarkers is required to identify patients who can be selected for LT. SHH, PTCH1 and Gli1 levels were positive in all tumour samples and in cirrhotic tissue, confirming previous data. In particular, PTCH1 expression was higher in HCC than in non-tumour tissue. Although no statistically significant correlation was observed between HH markers and recurrence after LT, the interval time between LT and recurrence indicated a trend towards this correlation. However, the small number of patients limited the value of this study^[54].

HH signal inhibition blocks proliferation of HCC cell lines *in vitro*

In addition to the analysis of tumour tissues from HCC patients, in order to clarify the mechanisms of tumourigenesis and progression that are induced by the HH signalling pathway, Che *et al*^[59] studied the effect of the SMO antagonist cyclopamine on proliferation and apoptosis in HCC cell lines. Cyclopamine is a natural plant corn lily extract^[60] with the ability to downregulate HH signals through direct negative interaction with the SMO receptor. *In vitro* cyclopamine treatment affected cell viability, with a concomitant reduction of mRNA and protein levels of Gli1, demonstrating that HCC cell proliferation was dependent on HH signalling^[59]. Treatment with cyclopamine also induced cell apoptosis through Gli1-

dependent downregulation of Bcl-2 and cleavage of caspase 9 and caspase 3.

Similarly, Huang *et al.*^[53] tested a panel of HCC cells for the expression of HH components, and selected HH positive HCC cells were treated with cyclopamine or anti-SHH-specific antibodies. A cyclopamine-induced reduction of Gli1 mRNA with no substantial change in SHH levels was demonstrated. Each type of HH inhibitor affected the cell proliferation and vitality of HCC cells, supporting the ideas that Gli1 is the best marker of HH activation and that inhibition of Hh can be sufficient to reduce tumour growth in this model^[53]. These results encourage the evaluation of the inhibition of the HH pathway as a promising new therapeutic strategy for treating HCC.

Moreover, the combination of HH inhibitor with radiotherapy enhances the radiosensitivity of HCC cells and orthotopic tumours^[61], and the inhibition of HH induces autophagy and apoptosis in HCC cells^[62], thus suggesting other various potential mechanisms of HH-mediated effects.

In vivo models of HH-induced liver tumourigenesis

Based on these translational evidence, various *in vivo* preclinical models have been created to study the contribution of HH signalling to the pathogenesis of HCC.

Philips *et al.*^[63] focused on the role of the HH pathway in hepatocarcinogenesis and in the development of the pre-cancerous lesion represented by liver fibrosis. To demonstrate this hypothesis, they treated a model of murine HCC represented by Mdr2-deficient mice with the SMO inhibitor, GDC-0449. Mdr2 is the murine homologue protein of human MDR3, and its deletion eliminates P-glycoprotein, which transports phosphatidylcholine (PC) across the canalicular membrane. The absence of functional Mdr2 induces portal inflammation and fibrosis, with ductular proliferation and development of HCC within 60 wk^[64]. Mdr2-deficient mice were the optimal model to study the evolution of HCC *in vivo* in fibrotic livers. Mice showed progressively increasing levels of SHH, IHH, and Gli2, concomitant, in early phases, with an elevation of liver enzymes. Treatment *in vivo* with GDC-0449 decreased the levels of hepatic TGF- β and PDGF- β , of alpha-smooth muscle actin in myofibroblastic hepatic stellate cells, concomitant with a reduction of Gli1 and Gli2, demonstrating that inhibition of HH improved liver fibrosis. Moreover, the expression of markers of liver progenitor cell populations decreased after HH inhibition, suggesting that HH signalling is essential for the regeneration of liver cell carcinogenesis, and for maintenance of progenitor cell populations in advanced stages of disease as a source of malignant cancer cells^[63]. The correlation between HH and PDGF- β in liver fibrosis implied that the fibrogenic repair response favoured HH activation itself. Since stem-like progenitor cells

express CD44, and the receptor for osteopontin and osteopontin itself is controlled by the HH pathway^[65], the authors also investigated the effect of SMO inhibition on osteopontin levels. Of interest, osteopontin expression was significantly reduced after treatment. It is likely that osteopontin is a key point of regulation for cancer stem cell survival by the HH pathway^[63]. Progenitor cells produce fibrogenic factors such as TGF- β and PDGF- β , which activate Gli2 in a non-canonical (SMO-independent) manner. HH is implicated as a principal component of all of these negative stimuli during chronic liver injury that lead to fibrosis and/or cancer^[63].

A prospective *in vivo* animal study confirmed the potency of HH inhibition for reverting HCC growth^[66]. Mice xenografted with mouse hepatoma Mistheton Lectin-1 cells were randomly assigned to receive a placebo or cyclopamine at a dose of 10 or 30 mg/kg per day for 10 d and were monitored for tumour growth. Tumour size, SHH and Gli1 mRNA levels were decreased by cyclopamine treatment, especially with the higher dose, in a statistically significant manner ($P = 0.047$ for tumour size)^[66].

Moreover, Pinter *et al.*^[67] demonstrated that inhibition of HH with the SMO inhibitor GDC-0449 reduces angiogenesis in an orthotopic HCC model by decreasing tumour expression of VEGF, with a subsequent reduction of micro vessel formation and tumour size.

Correlation of HH activation with EMT features and cancer stem cells in HCC

EMT is a complex dynamic process that determines changes in cellular features from an epithelial to a mesenchymal phenotype^[68]. Recent data support the importance of cancer stem cells in inducing EMT because they represent a subpopulation that exhibits a metastatic profile^[68]. Among the heterogeneity of cells that compose a tumour mass, cancer stem cells have been recognized as normal stem cells that are able to generate various types of cells that resemble different clones of tumour evolution and exhibit high invasive potential and drugs resistance^[68]. EMT activation is triggered by cell signalling that represses E-cadherin expression in favour of proteins of the mesenchymal phenotype. In HCC cell lines, SLUG was demonstrated as a principal inducer of EMT and stem-like cancer cell growth, together with high levels of vimentin and over-expression of N-cadherin^[69,70]. Consistent with the phenotype, cancer stem cells display a sphere-forming ability *in vitro*, confirming their invasive behaviour. Of interest, CD133+ cells isolated from a mouse Hepa16-derived tumour revealed over-expression of HH pathway components^[71]. However, cancer stem cells constantly change dynamically in HCC. In particular, cancer stem cells that are characterized by an EMT phenotype, such as expression of vimentin, SNAIL, TGF- β and HH components, are located in

invasive margins of HCC nodules. The location of these components serve as an indicator of their role in the regulation of HCC progression. Their potential role as therapeutic targets in HCC remains unknown^[72,73].

Interestingly, a promising novel target that has been investigated in preclinical studies in HCC cell models is the Bromodomain-containing protein 4 (BRD4), which plays an important role in HCC cancerogenesis by the induction of various oncogenes, including HH signalling-related genes^[74-76]. Wang *et al.*^[75] demonstrated that BRD4 is able to promote HCC cell migration and invasion *in vitro* and that BRD4 activity is mediated by HH signalling through the metalloproteases MMP - 2 and MMP - 9. The activation of metalloprotease by the HH pathway through focal adhesion kinase/AKT signalling has been confirmed also by other studies on liver cancer^[76]. The inhibition of BRD4 by silencing-RNA reduces MMP-2 and MMP-9 levels, blocking cell progression and invasive abilities. All of these metastatic features can be totally restored by stimulating HCC cells with the recombinant ligand SHH, thus confirming that HH is an essential pathway in HCC metastasis^[75].

Metalloprotease activation is not the only known downstream effector of HH pathway-induced progression in HCC. Another potential "targetable" protein is the kinesin family member 20A that is downstream of Gli2. Gli2 controls the cell cycle in normal and cancerous cells^[77]. Similarly, kinesin family proteins are involved in the regulation of the cell cycle and mitosis^[78]. Shi *et al.*^[77] correlated for the first time Gli2 and KIF20A in HCC cells and tissue samples from patients. They found that aberrant activation of the GLI2-KIF20A axis is crucial for the growth of hepatocarcinoma and that their interplay is mediated by activating Forkhead Box M1 (FoxM1)-MMB complex-mediated transcription. Gli2 or KIF20A knockdown affects the growth of HCC cells *in vitro* and *in vivo*. Analysis of HCC tissues reveals that GLI2, FoxM1 and KIF20A are highly increased in tumour cells and are correlated with HCC recurrence and overall survival of patients. Co-expression of these three markers could be considered a negative predictor for poor prognosis in HCC patients^[77].

Correlation of micro-RNAs and HH signals in HCC

MicroRNAs (miRNAs) are endogenous non-coding RNAs and are characterized by a length of 22 nucleotides. miRNAs suppress the expression of target genes at a post-transcriptional or translational level. In particular, a miRNA seed sequence (6-8 end nucleotides) interacts with a complementary sequence in messenger RNA of target genes. In humans their role is known in normal biological processes including proliferation and differentiation, and in tumours, including HCC^[79,80].

MicroRNAs can regulate EMT in HCC; in particular, miR-338-3p expression is significantly low in HCC

tissues with poor differentiation in advanced stage HCC patients^[81].

MiR- 338-3p reduces HCC cell invasiveness *in vitro* by specifically inhibiting SMO, the receptor of HH signalling, and subsequently reducing MMP-9 activity^[82,83]. In HCC cell lines, miR-338-3p expression levels correlate with the transcriptional activity of E-cadherin and with inhibited N-cadherin^[83]. Transfection of the miR-338-3p mimic in HCC cells induces a regression of Gli1 protein and mRNA levels concomitant with a decrease of SNAIL activity. In contrast, SMO siRNA inhibits the effect of miR-338-3p, confirming that SMO is the mediator of miR-338-3p in HCC cells and that inhibition of miR-338-3p is a mechanism of activation of EMT and HH signalling in HCC development. miR-338-3p and N-cadherin expression levels were assessed in a cohort of 163 HCC tissue specimens by real time PCR, in which HCC tumours were classified as low and high expressers and correlated a low miR-338-3p with a poor clinical DFS. Similarly, high expression of N-cadherin and low expression of miR-338-3p correlated with HCC metastasis^[79,83]. Moreover, real time PCR on HCC tissues for HH components revealed that high levels of miR-338-3p occur together with inhibition of HH signals, and with low expression of SMO, Gli1 and vimentin, thus confirming the complex link between miRNAs, HH and EMT.

In addition, cooperation of HH and miR-378 family members (miR-378a-3p, miR-378b and miR-378d) was demonstrated also in hepatic fibrogenesis. In most chronic liver diseases, fibrogenesis is a base for malignant transformation. The mechanism by which fibrogenesis is controlled by miR-378 is by the inhibition of GLI3 in activated hepatic stellate cells and chronically damaged liver^[80]. Moreover, SMO is an inhibitor of the transcription of miR-378a-3p by nuclear factor-κB. In human tissues, miR-378a-3p levels are inversely correlated with Gli3. In experimental animal models, the introduction of a mimic miR-378a-3p can revert liver fibrotic damage and HH signalling^[80].

In light of these results, miR-378a-3p is considered to be a useful biomarker for hepatic pathogenesis and a potentially new therapeutic agent for treatment of liver tumours.

CONCLUSION

Among all of the pathways that are involved in the development of HCC, HH signalling has a substantial role in hepatocarcinogenesis and HCC progression. The role of HH can have potential therapeutic implications, because chemical inhibitors of the HH pathway are now approved for use in other human tumours, such as in basocellular carcinoma^[84]. All efforts that target the molecular components of the pathways that are activated in the development of HCC have not demonstrated a clinical benefit, and sorafenib remains

the only available therapeutic option for advanced HCC patients. Numerous preclinical data are available that suggest a role of HH in hepatocarcinogenesis and EMT in HCC^[38,75-77]. In HCC, reactivation of the HH pathway is responsible for the maintenance of cancer stem cells that harbour a mesenchymal phenotype, and HH inhibition in preclinical models has a clear anti-tumour effect^[76,77]. Moreover, the correlation between the expression of HH proteins in HCC tumour tissues and a poor clinical outcome demonstrates that HH activation is implicated in HCC invasiveness and progression^[52-54].

Thus, the HH pathway plays a crucial role in the process of carcinogenesis and progression of hepatocarcinoma; however, the prognostic value of the HH pathway protein marker expression in tissues from HCC patients requires further investigation. In the future, targeting the HH pathway in HCC could be an interesting subject for preclinical and clinical studies.

REFERENCES

- 1 **White DL**, Thrift AP, Kanwal F, Davila J, El-Serag HB. Incidence of Hepatocellular Carcinoma in All 50 United States, From 2000 Through 2012. *Gastroenterology* 2017; **152**: 812-820.e5 [PMID: 27889576 DOI: 10.1053/j.gastro.2016.11.020]
- 2 **Verslype C**, Rosmorduc O, Rougier P; ESMO Guidelines Working Group. Hepatocellular carcinoma: ESMO-ESDO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2012; **23** Suppl 7: vii41-vii48 [PMID: 22997453 DOI: 10.1093/annonc/mds225]
- 3 **Chen JD**, Yang HI, Iloeje UH, You SL, Lu SN, Wang LY, Su J, Sun CA, Liaw YF, Chen CJ; Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer in HBV (REVEAL-HBV) Study Group. Carriers of inactive hepatitis B virus are still at risk for hepatocellular carcinoma and liver-related death. *Gastroenterology* 2010; **138**: 1747-1754 [PMID: 20114048 DOI: 10.1053/j.gastro.2010.01.042]
- 4 **Mahale P**, Torres HA, Kramer JR, Hwang LY, Li R, Brown EL, Engels EA. Hepatitis C virus infection and the risk of cancer among elderly US adults: A registry-based case-control study. *Cancer* 2017; **123**: 1202-1211 [PMID: 28117886 DOI: 10.1002/CNCR.30559]
- 5 **Marrero JA**, Fontana RJ, Fu S, Conjeevaram HS, Su GL, Lok AS. Alcohol, tobacco and obesity are synergistic risk factors for hepatocellular carcinoma. *J Hepatol* 2005; **42**: 218-224 [PMID: 15664247 DOI: 10.1016/j.jhep.2004.10.005]
- 6 **Dulku G**, Dhillon R, Goodwin M, Cheng W, Kontorinis N, Mendelson R. The role of imaging in the surveillance and diagnosis of hepatocellular cancer. *J Med Imaging Radiat Oncol* 2017; **61**: 171-179 [PMID: 27981791 DOI: 10.1111/1754-9485.12568]
- 7 **Poon RT**, Fan ST, Tsang FH, Wong J. Locoregional therapies for hepatocellular carcinoma: a critical review from the surgeon's perspective. *Ann Surg* 2002; **235**: 466-486 [PMID: 11923602]
- 8 **Llovet JM**, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: Chemoembolization improves survival. *Hepatology* 2003; **37**: 429-442 [PMID: 12540794 DOI: 10.1053/jhep.2003.50047]
- 9 **Josephs DH**, Ross PJ. Sorafenib in hepatocellular carcinoma. *Br J Hosp Med (Lond)* 2010; **71**: 451-456 [PMID: 20852487 DOI: 10.12968/hmed.2010.71.8.77669]
- 10 **Bruix J**, Qin S, Merle P, Granito A, Huang YH, Bodoky G, Pracht M, Yokosuka O, Rosmorduc O, Breder V, Gerolami R, Masi G, Ross PJ, Song T, Bronowicki JP, Ollivier-Hourmand I, Kudo M, Cheng AL, Llovet JM, Finn RS, LeBerre MA, Baumhauer A, Meinhardt G, Han G. Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 2017; **389**: 56-66 [PMID: 27932229 DOI: 10.1016/S0140-6736(16)32453-9]
- 11 **Melero I**, Sangro B, Yau TC. Nivolumab dose escalation and expansion in patients with advanced hepatocellular carcinoma: The CheckMate 040 study. Presented January 20, 2017. Gastrointestinal Cancers Symposium ASCO, 2017: Abstract 226
- 12 **Fornaro L**, Vivaldi C, Lorenzoni G, Masi G, Bargellini I. Moving beyond sorafenib alone in advanced hepatocellular carcinoma: is hepatic arterial infusion chemotherapy the best option? *Ann Oncol* 2017; **28**: 667 [PMID: 27993799 DOI: 10.1093/annonc/mdw664]
- 13 **Kgatlhe MM**, Setshedi M, Hairwadzi HN. Hepatoepigenetic Alterations in Viral and Nonviral-Induced Hepatocellular Carcinoma. *Biomed Res Int* 2016; **2016**: 3956485 [PMID: 28105421 DOI: 10.1155/2016/3956485]
- 14 **Yoo JE**, Kim YJ, Rhee H, Kim H, Ahn EY, Choi JS, Roncalli M, Park YN. Progressive Enrichment of Stemness Features and Tumor Stromal Alterations in Multistep Hepatocarcinogenesis. *PLoS One* 2017; **12**: e0170465 [PMID: 28114366 DOI: 10.1371/journal.pone.0170465]
- 15 **Ramallo-Santos M**, Melton DA, McMahon AP. Hedgehog signals regulate multiple aspects of gastrointestinal development. *Development* 2000; **127**: 2763-2772 [PMID: 10821773]
- 16 **Parkin CA**, Ingham PW. The adventures of Sonic Hedgehog in development and repair. I. Hedgehog signaling in gastrointestinal development and disease. *Am J Physiol Gastrointest Liver Physiol* 2008; **294**: G363-G367 [PMID: 18063705 DOI: 10.1152/ajpgi.00457.2007]
- 17 **Zheng X**, Zeng W, Gai X, Xu Q, Li C, Liang Z, Tuo H, Liu Q. Role of the Hedgehog pathway in hepatocellular carcinoma (review). *Oncol Rep* 2013; **30**: 2020-2026 [PMID: 23970376 DOI: 10.3892/or.2013.2690]
- 18 **Vassilev A**, DePamphilis ML. Links between DNA Replication, Stem Cells and Cancer. *Genes (Basel)* 2017; **8**: [PMID: 28125050 DOI: 10.3390/genes8020045]
- 19 **Minami M**, Daimon Y, Mori K, Takashima H, Nakajima T, Itoh Y, Okanoue T. Hepatitis B virus-related insertional mutagenesis in chronic hepatitis B patients as an early drastic genetic change leading to hepatocarcinogenesis. *Oncogene* 2005; **24**: 4340-4348 [PMID: 15806150 DOI: 10.1038/sj.onc.1208628]
- 20 **Yamanaka T**, Kodama T, Doi T. Subcellular localization of HCV core protein regulates its ability for p53 activation and p21 suppression. *Biochem Biophys Res Commun* 2002; **294**: 528-534 [PMID: 12056798 DOI: 10.1016/S0006-291X(02)00508-9]
- 21 **Fukutomi T**, Zhou Y, Kawai S, Eguchi H, Wands JR, Li J. Hepatitis C virus core protein stimulates hepatocyte growth: correlation with upregulation of wnt-1 expression. *Hepatology* 2005; **41**: 1096-1105 [PMID: 15841445 DOI: 10.1002/hep.20668]
- 22 **McKillop IH**, Schrum LW. Role of alcohol in liver carcinogenesis. *Semin Liver Dis* 2009; **29**: 222-232 [PMID: 19387921 DOI: 10.1055/s-0029-1214377]
- 23 **Zakaria MK**, Khan I, Mani P, Chattopadhyay P, Sarkar DP, Sinha S. Combination of hepatocyte specific delivery and transformation dependent expression of shRNA inducing transcriptional gene silencing of c-Myc promoter in hepatocellular carcinoma cells. *BMC Cancer* 2014; **14**: 582 [PMID: 25108398 DOI: 10.1186/1471-2407-14-582]
- 24 **Kolch W**. Meaningful relationships: the regulation of the Ras/Raf/MEK/ERK pathway by protein interactions. *Biochem J* 2000; **351** Pt 2: 289-305 [PMID: 11023813 DOI: 10.1042/0264-6021]
- 25 **Huynh H**, Nguyen TT, Chow KH, Tan PH, Soo KC, Tran E. Over-expression of the mitogen-activated protein kinase (MAPK) kinase (MEK)-MAPK in hepatocellular carcinoma: its role in tumor progression and apoptosis. *BMC Gastroenterol* 2003; **3**: 19 [PMID: 12906713 DOI: 10.1186/1471-230X-3-19]
- 26 **Wang Y**, Wu MC, Sham JS, Zhang W, Wu WQ, Guan XY. Prognostic significance of c-myc and AIB1 amplification in hepatocellular carcinoma. A broad survey using high-throughput tissue microarray. *Cancer* 2002; **95**: 2346-2352 [PMID: 12436441]

- DOI: 10.1002/cncr.10963]
- 27 **Chung TW**, Lee YC, Kim CH. Hepatitis B viral HBx induces matrix metalloproteinase-9 gene expression through activation of ERK and PI-3K/AKT pathways: involvement of invasive potential. *FASEB J* 2004; **18**: 1123-1125 [PMID: 15132991]
 - 28 **Boix L**, Rosa JL, Ventura F, Castells A, Bruix J, Rodés J, Bartrons R. c-met mRNA overexpression in human hepatocellular carcinoma. *Hepatology* 1994; **19**: 88-91 [PMID: 8276372 DOI: 10.1002/hep.1840190115]
 - 29 **Ueki T**, Fujimoto J, Suzuki T, Yamamoto H, Okamoto E. Expression of hepatocyte growth factor and its receptor, the c-met proto-oncogene, in hepatocellular carcinoma. *Hepatology* 1997; **25**: 619-623 [PMID: 9049208 DOI: 10.1002/hep.510250321]
 - 30 **Santoro A**, Rimassa L, Borbath I, Daniele B, Salvagni S, Van Laethem JL, Van Vlierberghe H, Trojan J, Kolligs FT, Weiss A, Miles S, Gasbarrini A, Lencioni M, Cicalese L, Sherman M, Gridelli C, Buggisch P, Gerken G, Schmid RM, Boni C, Personeni N, Hassoun Z, Abbadessa G, Schwartz B, Von Roemeling R, Lamar ME, Chen Y, Porta C. Tivantinib for second-line treatment of advanced hepatocellular carcinoma: a randomised, placebo-controlled phase 2 study. *Lancet Oncol* 2013; **14**: 55-63 [PMID: 23182627 DOI: 10.1016/S1470-2045(12)70490-4]
 - 31 **Semela D**, Dufour JF. Angiogenesis and hepatocellular carcinoma. *J Hepatol* 2004; **41**: 864-880 [PMID: 15519663 DOI: 10.1016/j.jhep.2004.09.006]
 - 32 **El-Assal ON**, Yamanoi A, Soda Y, Yamaguchi M, Igarashi M, Yamamoto A, Nabika T, Nagasue N. Clinical significance of microvessel density and vascular endothelial growth factor expression in hepatocellular carcinoma and surrounding liver: possible involvement of vascular endothelial growth factor in the angiogenesis of cirrhotic liver. *Hepatology* 1998; **27**: 1554-1562 [PMID: 9620326 DOI: 10.1002/hep.510270613]
 - 33 **Lu WQ**, Qiu JL, Huang ZL, Liu HY. Enhanced circulating transforming growth factor beta 1 is causally associated with an increased risk of hepatocellular carcinoma: a mendelian randomization meta-analysis. *Oncotarget* 2016; **7**: 84695-84704 [PMID: 27835897 DOI: 10.18632/ONCOTARGET.13218]
 - 34 **Mitra A**, Yan J, Xia X, Zhou S, Chen J, Mishra L, Li S. IL6-mediated inflammatory loop reprograms normal to epithelial-mesenchymal transition(+) metastatic cancer stem cells in preneoplastic liver of transforming growth factor beta-deficient $\beta 2$ -spectrin(+/-) mice. *Hepatology* 2017; **65**: 1222-1236 [PMID: 27863449 DOI: 10.1002/hep.28951]
 - 35 **Zhang Y**, Wei W, Cheng N, Wang K, Li B, Jiang X, Sun S. Hepatitis C virus-induced up-regulation of microRNA-155 promotes hepatocarcinogenesis by activating Wnt signaling. *Hepatology* 2012; **56**: 1631-1640 [PMID: 22610915 DOI: 10.1002/hep.25849]
 - 36 **Srisuttee R**, Koh SS, Kim SJ, Malilas W, Boonying W, Cho IR, Jhun BH, Ito M, Horio Y, Seto E, Oh S, Chung YH. Hepatitis B virus X (HBX) protein upregulates β -catenin in a human hepatic cell line by sequestering SIRT1 deacetylase. *Oncol Rep* 2012; **28**: 276-282 [PMID: 22562294]
 - 37 **McMahon AP**, Ingham PW, Tabin CJ. Developmental roles and clinical significance of hedgehog signaling. *Curr Top Dev Biol* 2003; **53**: 1-114 [PMID: 12509125]
 - 38 **Omenetti A**, Diehl AM. The adventures of sonic hedgehog in development and repair. II. Sonic hedgehog and liver development, inflammation, and cancer. *Am J Physiol Gastrointest Liver Physiol* 2008; **294**: G595-G598 [PMID: 18218671 DOI: 10.1152/ajpgi.00543.2007]
 - 39 **Ingham PW**, McMahon AP. Hedgehog signaling in animal development: paradigms and principles. *Genes Dev* 2001; **15**: 3059-3087 [PMID: 11731473 DOI: 10.1101/gad.938601]
 - 40 **Lee CS**, Friedman JR, Fulmer JT, Kaestner KH. The initiation of liver development is dependent on Foxa transcription factors. *Nature* 2005; **435**: 944-947 [PMID: 15959514 DOI: 10.1038/nature03649]
 - 41 **Sicklick JK**, Li YX, Melhem A, Schmelzer E, Zdanowicz M, Huang J, Caballero M, Fair JH, Ludlow JW, McClelland RE, Reid LM, Diehl AM. Hedgehog signaling maintains resident hepatic progenitors throughout life. *Am J Physiol Gastrointest Liver Physiol* 2006; **290**: G859-G870 [PMID: 16322088 DOI: 10.1152/ajpgi.00456.2005]
 - 42 **Choi SS**, Omenetti A, Witek RP, Moylan CA, Syn WK, Jung Y, Yang L, Sudan DL, Sicklick JK, Michelotti GA, Rojkind M, Diehl AM. Hedgehog pathway activation and epithelial-to-mesenchymal transitions during myofibroblastic transformation of rat hepatic cells in culture and cirrhosis. *Am J Physiol Gastrointest Liver Physiol* 2009; **297**: G1093-G1106 [PMID: 19815628 DOI: 10.1152/ajpgi.00292.2009]
 - 43 **Ochoa B**, Syn WK, Delgado I, Karaca GF, Jung Y, Wang J, Zubiaga AM, Fresnedo O, Omenetti A, Zdanowicz M, Choi SS, Diehl AM. Hedgehog signaling is critical for normal liver regeneration after partial hepatectomy in mice. *Hepatology* 2010; **51**: 1712-1723 [PMID: 20432255 DOI: 10.1002/hep.23525]
 - 44 **Schmelzer E**, Wauthier E, Reid LM. The phenotypes of pluripotent human hepatic progenitors. *Stem Cells* 2006; **24**: 1852-1858 [PMID: 16627685 DOI: 10.1634/stemcells.2006-0036]
 - 45 **Nybakken K**, Perrimon N. Hedgehog signal transduction: recent findings. *Curr Opin Genet Dev* 2002; **12**: 503-511 [PMID: 12200154]
 - 46 **Habib JG**, O'Shaughnessy JA. The hedgehog pathway in triple-negative breast cancer. *Cancer Med* 2016; **5**: 2989-3006 [PMID: 27539549 DOI: 10.1002/cam4.833]
 - 47 **Della Corte CM**, Bellevecine C, Vicidomini G, Vitagliano D, Malapelle U, Accardo M, Fabozzi A, Fiorelli A, Fasano M, Papaccio F, Martinelli E, Troiani T, Troncone G, Santini M, Bianco R, Ciardiello F, Morgillo F. SMO Gene Amplification and Activation of the Hedgehog Pathway as Novel Mechanisms of Resistance to Anti-Epidermal Growth Factor Receptor Drugs in Human Lung Cancer. *Clin Cancer Res* 2015; **21**: 4686-4697 [PMID: 26124204 DOI: 10.1158/1078-0432.CCR-14-3319]
 - 48 **Klieser E**, Swierczynski S, Mayr C, Jäger T, Schmidt J, Neureiter D, Kiesslich T, Illig R. Differential role of Hedgehog signaling in human pancreatic (patho-) physiology: An up to date review. *World J Gastrointest Pathophysiol* 2016; **7**: 199-210 [PMID: 27190692 DOI: 10.4291/wjgp.v7.i2.199]
 - 49 **Papadopoulos V**, Tsapakidis K, Riobo Del Galdo NA, Papandreou CN, Del Galdo F, Anthoney A, Sakellaris N, Dimas K, Kamposioras K. The Prognostic Significance of the Hedgehog Signaling Pathway in Colorectal Cancer. *Clin Colorectal Cancer* 2016; **15**: 116-127 [PMID: 27032873 DOI: 10.1016/J.CLCC.2016.02.010]
 - 50 **Lu L**, Wu M, Zhao F, Fu W, Li W, Li X, Liu T. Prognostic and clinicopathological value of Gli-1 expression in gastric cancer: A meta-analysis. *Oncotarget* 2016; **7**: 69087-69096 [PMID: 27634907 DOI: 10.18632/ONCOTARGET.12011]
 - 51 **Shigemura K**, Fujisawa M. Hedgehog signaling and urological cancers. *Curr Drug Targets* 2015; **16**: 258-271 [PMID: 25429711]
 - 52 **Sicklick JK**, Li YX, Jayaraman A, Kannangai R, Qi Y, Vivekanandan P, Ludlow JW, Owzar K, Chen W, Torbenson MS, Diehl AM. Dysregulation of the Hedgehog pathway in human hepatocarcinogenesis. *Carcinogenesis* 2006; **27**: 748-757 [PMID: 16339184 DOI: 10.1093/carcin/bgi292]
 - 53 **Huang S**, He J, Zhang X, Bian Y, Yang L, Xie G, Zhang K, Tang W, Stelter AA, Wang Q, Zhang H, Xie J. Activation of the hedgehog pathway in human hepatocellular carcinomas. *Carcinogenesis* 2006; **27**: 1334-1340 [PMID: 16501253 DOI: 10.1093/carcin/bgi378]
 - 54 **Dugum M**, Hanouneh I, McIntyre T, Pai R, Aucejo F, Eghtesad B, Zein N. Sonic hedgehog signaling in hepatocellular carcinoma: A pilot study. *Mol Clin Oncol* 2016; **4**: 369-374 [PMID: 26998285 DOI: 10.3892/mco.2016.728]
 - 55 **Giakoustidis A**, Giakoustidis D, Mudan S, Sklavos A, Williams R. Molecular signalling in hepatocellular carcinoma: Role of and crosstalk among WNT/ β -catenin, Sonic Hedgehog, Notch and Dickkopf-1. *Can J Gastroenterol Hepatol* 2015; **29**: 209-217 [PMID: 25965442]
 - 56 **Chan IS**, Guy CD, Machado MV, Wank A, Kadiyala V, Michelotti

- G, Choi S, Swiderska-Syn M, Karaca G, Pereira TA, Yip-Schneider MT, Max Schmidt C, Diehl AM. Alcohol activates the hedgehog pathway and induces related procarcinogenic processes in the alcohol-preferring rat model of hepatocarcinogenesis. *Alcohol Clin Exp Res* 2014; **38**: 787-800 [PMID: 24164383 DOI: 10.1111/ACER.12279]
- 57 **Pavlidis S**, Whitaker-Menezes D, Castello-Cros R, Flomenberg N, Witkiewicz AK, Frank PG, Casimiro MC, Wang C, Fortina P, Addya S, Pestell RG, Martinez-Outschoorn UE, Sotgia F, Lisanti MP. The reverse Warburg effect: aerobic glycolysis in cancer associated fibroblasts and the tumor stroma. *Cell Cycle* 2009; **8**: 3984-4001 [PMID: 19923890]
- 58 **Chan IS**, Guy CD, Chen Y, Lu J, Swiderska-Syn M, Michelotti GA, Karaca G, Xie G, Krüger L, Syn WK, Anderson BR, Pereira TA, Choi SS, Baldwin AS, Diehl AM. Paracrine Hedgehog signaling drives metabolic changes in hepatocellular carcinoma. *Cancer Res* 2012; **72**: 6344-6350 [PMID: 23066040 DOI: 10.1158/0008-5472.CAN-12-1068]
- 59 **Che L**, Yuan YH, Jia J, Ren J. Activation of sonic hedgehog signaling pathway is an independent potential prognosis predictor in human hepatocellular carcinoma patients. *Chin J Cancer Res* 2012; **24**: 323-331 [PMID: 23359030 DOI: 10.3978/J.ISSN.1000-9604.2012.10.10]
- 60 **Chen JK**, Taipale J, Cooper MK, Beachy PA. Inhibition of Hedgehog signaling by direct binding of cyclopamine to Smoothened. *Genes Dev* 2002; **16**: 2743-2748 [PMID: 12414725 DOI: 10.1101/gad.1025302]
- 61 **Tsai CL**, Hsu FM, Tzen KY, Liu WL, Cheng AL, Cheng JC. Sonic Hedgehog inhibition as a strategy to augment radiosensitivity of hepatocellular carcinoma. *J Gastroenterol Hepatol* 2015; **30**: 1317-1324 [PMID: 25682950 DOI: 10.1111/JGH.12931]
- 62 **Wang Y**, Han C, Lu L, Magliato S, Wu T. Hedgehog signaling pathway regulates autophagy in human hepatocellular carcinoma cells. *Hepatology* 2013; **58**: 995-1010 [PMID: 23504944 DOI: 10.1002/HEP.26394]
- 63 **Philips GM**, Chan IS, Swiderska M, Schroder VT, Guy C, Karaca GF, Moylan C, Venkatraman T, Feuerlein S, Syn WK, Jung Y, Witek RP, Choi S, Michelotti GA, Rangwala F, Merkle E, Lascola C, Diehl AM. Hedgehog signaling antagonist promotes regression of both liver fibrosis and hepatocellular carcinoma in a murine model of primary liver cancer. *PLoS One* 2011; **6**: e23943 [PMID: 21912653 DOI: 10.1371/JOURNAL.PONE.0023943]
- 64 **Van Nieuwkerk CM**, Elferink RP, Groen AK, Ottenhoff R, Tytgat GN, Dingemans KP, Van Den Bergh Weerman MA, Offerhaus GJ. Effects of Ursodeoxycholate and cholate feeding on liver disease in FVB mice with a disrupted mdr2 P-glycoprotein gene. *Gastroenterology* 1996; **111**: 165-171 [PMID: 8698195]
- 65 **Syn WK**, Choi SS, Liaskou E, Karaca GF, Agboola KM, Oo YH, Mi Z, Pereira TA, Zdanowicz M, Malladi P, Chen Y, Moylan C, Jung Y, Bhattacharya SD, Teaberry V, Omenetti A, Abdelmalek MF, Guy CD, Adams DH, Kuo PC, Michelotti GA, Whittington PF, Diehl AM. Osteopontin is induced by hedgehog pathway activation and promotes fibrosis progression in nonalcoholic steatohepatitis. *Hepatology* 2011; **53**: 106-115 [PMID: 20967826 DOI: 10.1002/hep.23998]
- 66 **Jeng KS**, Sheen IS, Jeng WJ, Yu MC, Tsai HH, Chang FY, Su JC. Blockade of the sonic hedgehog pathway effectively inhibits the growth of hepatoma in mice: An in vivo study. *Oncol Lett* 2012; **4**: 1158-1162 [PMID: 23205113 DOI: 10.3892/ol.2012.935]
- 67 **Pinter M**, Sieghart W, Schmid M, Dauser B, Prager G, Dienes HP, Trauner M, Peck-Radosavljevic M. Hedgehog inhibition reduces angiogenesis by downregulation of tumoral VEGF-A expression in hepatocellular carcinoma. *United European Gastroenterol J* 2013; **1**: 265-275 [PMID: 24917971 DOI: 10.1177/2050640613496605]
- 68 **Jordan CT**, Guzman ML, Noble M. Cancer stem cells. *N Engl J Med* 2006; **355**: 1253-1261 [PMID: 16990388 DOI: 10.1056/NEJMr061808]
- 69 **Sun Y**, Song GD, Sun N, Chen JQ, Yang SS. Slug overexpression induces stemness and promotes hepatocellular carcinoma cell invasion and metastasis. *Oncol Lett* 2014; **7**: 1936-1940 [PMID: 24932263 DOI: 10.3892/ol.2014.2037]
- 70 **Mitra A**, Satelli A, Xia X, Cutrera J, Mishra L, Li S. Cell-surface Vimentin: A mislocalized protein for isolating cVimentin(+) CD133(-) novel stem-like hepatocellular carcinoma cells expressing EMT markers. *Int J Cancer* 2015; **137**: 491-496 [PMID: 25487874 DOI: 10.1002/ijc.29382]
- 71 **Jeng KS**, Sheen IS, Jeng WJ, Yu MC, Hsiao HI, Chang FY, Tsai HH. Activation of the sonic hedgehog signaling pathway occurs in the CD133 positive cells of mouse liver cancer Hepa 1-6 cells. *Onco Targets Ther* 2013; **6**: 1047-1055 [PMID: 23950652 DOI: 10.2147/OTT.S44828]
- 72 **Fan QM**, Jing YY, Yu GF, Kou XR, Ye F, Gao L, Li R, Zhao QD, Yang Y, Lu ZH, Wei LX. Tumor-associated macrophages promote cancer stem cell-like properties via transforming growth factor-beta1-induced epithelial-mesenchymal transition in hepatocellular carcinoma. *Cancer Lett* 2014; **352**: 160-168 [PMID: 24892648 DOI: 10.1016/j.canlet.2014.05.008]
- 73 **Plaks V**, Kong N, Werb Z. The cancer stem cell niche: how essential is the niche in regulating stemness of tumor cells? *Cell Stem Cell* 2015; **16**: 225-238 [PMID: 25748930 DOI: 10.1016/j.stem.2015.02.015]
- 74 **Tang Y**, Gholamin S, Schubert S, Willardson MI, Lee A, Bandopadhyay P, Berghthold G, Masoud S, Nguyen B, Vue N, Balansay B, Yu F, Oh S, Woo P, Chen S, Ponnuswami A, Monje M, Atwood SX, Whitson RJ, Mitra S, Cheshier SH, Qi J, Beroukhir R, Tang JY, Wechsler-Reya R, Oro AE, Link BA, Bradner JE, Cho YJ. Epigenetic targeting of Hedgehog pathway transcriptional output through BET bromodomain inhibition. *Nat Med* 2014; **20**: 732-740 [PMID: 24973920 DOI: 10.1038/NM.3613]
- 75 **Wang YH**, Sui XM, Sui YN, Zhu QW, Yan K, Wang LS, Wang F, Zhou JH. BRD4 induces cell migration and invasion in HCC cells through MMP-2 and MMP-9 activation mediated by the Sonic hedgehog signaling pathway. *Oncol Lett* 2015; **10**: 2227-2232 [PMID: 26622824]
- 76 **Chen JS**, Huang XH, Wang Q, Huang JQ, Zhang LJ, Chen XL, Lei J, Cheng ZX. Sonic hedgehog signaling pathway induces cell migration and invasion through focal adhesion kinase/AKT signaling-mediated activation of matrix metalloproteinase (MMP)-2 and MMP-9 in liver cancer. *Carcinogenesis* 2013; **34**: 10-19 [PMID: 22948179 DOI: 10.1093/carcin/bgs274]
- 77 **Shi C**, Huang D, Lu N, Chen D, Zhang M, Yan Y, Deng L, Lu Q, Lu H, Luo S. Aberrantly activated Gli2-KIF20A axis is crucial for growth of hepatocellular carcinoma and predicts poor prognosis. *Oncotarget* 2016; **7**: 26206-26219 [PMID: 27036048 DOI: 10.18632/ONCOTARGET.8441]
- 78 **Vale RD**, Reese TS, Sheetz MP. Identification of a novel force-generating protein, kinesin, involved in microtubule-based motility. *Cell* 1985; **42**: 39-50 [PMID: 3926325]
- 79 **Chen JS**, Liang LL, Xu HX, Chen F, Shen SL, Chen W, Chen LZ, Su Q, Zhang LJ, Bi J, Zeng WT, Li W, Ma N, Huang XH. miR-338-3p inhibits epithelial-mesenchymal transition and metastasis in hepatocellular carcinoma cells. *Oncotarget* 2016; Epub ahead of print [PMID: 27331410 DOI: 10.18632/ONCOTARGET.10138]
- 80 **Hyun J**, Wang S, Kim J, Rao KM, Park SY, Chung I, Ha CS, Kim SW, Yun YH, Jung Y. MicroRNA-378 limits activation of hepatic stellate cells and liver fibrosis by suppressing Gli3 expression. *Nat Commun* 2016; **7**: 10993 [PMID: 27001906 DOI: 10.1038/NCOMMS10993]
- 81 **Zhang LY**, Liu M, Li X, Tang H. miR-490-3p modulates cell growth and epithelial to mesenchymal transition of hepatocellular carcinoma cells by targeting endoplasmic reticulum-Golgi intermediate compartment protein 3 (ERGIC3). *J Biol Chem* 2013; **288**: 4035-4047 [PMID: 23212913 DOI: 10.1074/jbc.M112.410506]
- 82 **Huang XH**, Chen JS, Wang Q, Chen XL, Wen L, Chen LZ, Bi J, Zhang LJ, Su Q, Zeng WT. miR-338-3p suppresses invasion of liver cancer cell by targeting smoothened. *J Pathol* 2011; **225**: 463-472 [PMID: 21671467 DOI: 10.1002/path.2877]
- 83 **Huang JY**, Zhang K, Chen DQ, Chen J, Feng B, Song H, Chen Y,

Zhu Z, Lu L, De W, Wang R, Chen LB. MicroRNA-451: epithelial-mesenchymal transition inhibitor and prognostic biomarker of hepatocellular carcinoma. *Oncotarget* 2015; **6**: 18613-18630 [PMID: 26164082 DOI: 10.18632/oncotarget.4317]

84 **Becker LR**, Aakhus AE, Reich HC, Lee PK. A Novel Alternate Dosing of Vismodegib for Treatment of Patients With Advanced Basal Cell Carcinomas. *JAMA Dermatol* 2017; **153**: 321-322 [PMID: 28114606 DOI: 10.1001/JAMADERMATOL.2016.5058]

P- Reviewer: Meng ZQ, Şendur MAN, Zhao HT **S- Editor:** Ma YJ
L- Editor: A **E- Editor:** Wang CH





Published by **Baishideng Publishing Group Inc**
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>



ISSN 1007-9327

