ISSN 1948-5182 (online)

World Journal of *Hepatology*

World J Hepatol 2017 March 8; 9(7): 349-408

Published by Baishideng Publishing Group Inc

World Journal of Hepatology

A peer-reviewed, online, open-access journal of hepatology

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World Journal of Hepatology

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Three issues per month Volume 9 Number 7 March 8, 2017

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World Journal of Hepatology
Volume 9 Number 7 March 8, 2017

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World Journal of Hepatology (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJH covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

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FLYLEAF

NAME OF JOURNAL
World Journal of Hepatology

ISSN
ISSN 1948-5182 (online)

LAUNCH DATE
October 31, 2009

FREQUENCY
36 Issues/Year (8th, 18th, and 28th of each month)

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PUBLICATION DATE
March 8, 2017

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World J Hepatol 2017 March 8; 9(7): 349-351

DOI: 10.4254/wjh.v9.i7.349

ISSN 1948-5182 (online)

EDITORIAL

Parkin in cancer: Mitophagy-related/unrelated tasks

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Author contributions: Eid N wrote the paper; Kondo Y critically reviewed it.

Conflict-of-interest statement: Eid N declares no conflict of interest related to this publication.

Manuscript source: Invited manuscript

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Received: December 21, 2016
Peer-review started: December 25, 2016
First decision: January 16, 2017
Revised: January 17, 2017
Accepted: February 8, 2017
Article in press: February 13, 2017
Published online: March 8, 2017

Abstract

Dysfunctional mitochondria may produce excessive reactive oxygen species, thus inducing DNA damage, which may be oncogenic if not repaired. As a major role of the PINK1-Parkin pathway involves selective autophagic clearance of damaged mitochondria *via* a process termed mitophagy, Parkin-mediated mitophagy may be a tumor-suppressive mechanism. As an alternative mechanism for tumor inhibition beyond mitophagy, Parkin has been reported to have other oncosuppressive functions such as DNA repair, negative regulation of cell proliferation and stimulation of p53 tumor suppressor function. The authors recently reported that acute ethanol-induced mitophagy in hepatocytes was associated with Parkin mitochondrial translocation and colocalization with accumulated 8-OHdG (a marker of DNA damage and mutagenicity). This finding suggests: (1) the possibility of Parkin-mediated repair of damaged mitochondrial DNA in hepatocytes of ethanol-treated rats (ETRs) as an oncosuppressive mechanism; and (2) potential induction of cytoprotective mitophagy in ETR hepatocytes if mitochondrial damage is too severe to be repaired. Below is a summary of the various roles Parkin plays in tumor suppression, which may or may not be related to mitophagy. A proper understanding of the various tasks performed by Parkin in tumorigenesis may help in cancer therapy by allowing the PINK1-Parkin pathway to be targeted.

Key words: Cancer; Ethanol; Liver; Mitophagy; Parkin; PINK1; 8-OHdG

Core tip: A large number of studies have found that the impaired Parkin function or downregulation of expression may induce cancer initiation and progression *via* mitophagy-related/unrelated mechanisms. Thus, there is a growing belief that Parkin may have tumor suppressor effects. Based on literature and on the authors' recent publications regarding animal models of alcohol abuse, this paper highlights the various roles of Parkin in the suppression of oncogenesis. Proper understanding of Parkin functions may have therapeutic implications in the treatment of various cancers.

Eid N, Kondo Y. Parkin in cancer: Mitophagy-related/unrelated

tasks. *World J Hepatol* 2017; 9(7): 349-351 Available from: URL: http://www.wjgnet.com/1948-5182/full/v9/i7/349.htm DOI: http://dx.doi.org/10.4254/wjh.v9.i7.349

Mutations in the Parkin gene are frequently associated with Parkinson's disease (PD). They lead to defects in autophagic clearance of damaged mitochondria *via* mitophagy, resulting in the characteristic neuronal loss observed in PD[1]. Parkin-mediated mitophagy is characterized by accumulation of PINK1 on the outer mitochondrial membrane (OMM) of damaged mitochondria and subsequent mitochondrial translocation of Parkin and ubiquitination of numerous OMM proteins, followed by clearance of these organelles *via* the microtubule-associated protein light chain 3 (LC3)-mediated autophagic machinery[1,2]. Parkin-mediated ubiquitination of OMM proteins stimulates the recruitment of different LC3 interacting region-containing autophagy receptors which bind ubiquitin-tagged OMM proteins, including p62, optineurin and NBR1[2]. Dysfunctional mitochondria can transform cells and promote tumorigenesis, suggesting that mitophagy may function as a tumor suppressor mechanism[2]. A number of recent studies have investigated the involvement of mitophagy in tumor suppression, with results including the finding that insufficient mitophagy resulted in oncocytic formation in heterogeneous thyroid Hürthle cell tumors[3]. However, a growing body of evidence suggests that Parkin also plays a role in cancer as a putative tumor suppressor. Parkin$^{-/-}$ mice exhibited enhanced hepatocyte proliferation associated with upregulation of endogenous follistatin, resulting in the induction and progression of hepatocellular carcinoma (HCC)[4]. Upon autophagy activation the Atg4 cysteine protease first cleaves pro-LC3 at the C-terminus, thus forming LC3-Ⅰ. Induction of Atg7 conjugates phosphatidylethanolamine (PE) to LC3-Ⅰ, forming LC3-Ⅱ (essential form of LC3 for mitophagosome formation). The Atg5/12/16 complex also acts as an E3 ligase, promoting PE conjugation to LC3[2]. Mice with systemic mosaic deletion of Atg5 and liver-specific Atg7$^{-/-}$ mice develop benign liver adenomas[5]. Parkin deficiency results in overexpression of its substrates, mitotic defects, genomic instability and tumorigenesis[6]. Downregulation of Parkin protein has been observed in HCC, whereas Parkin overexpression inhibits the migration and invasion of multiple cancer cells[7]. Parkin has been reported to contribute to the functions of p53 - another tumor suppressor - *via* regulation of the energy metabolism (especially the Warburg effect) and antioxidant defense[8]. Paradoxically, in some cases Parkin activity may be required for KRAS-driven tumors to maintain mitochondrial quality control and buffer oxidative stress, making it a pro-survival protein[7]. KRAS mutant pancreatic adenocarcinoma has been reported to rely on autophagy and mitophagy to supply bioenergetic intermediates for the TCA cycle. Mitophagy also appears to be a prosurvival mechanism in immortal baby mouse kidney epithelial cells ectopically expressing oncogenic HRAS or KRAS by removing damaged mitochondria[9].

Seitz and Stickel[10] reported that animal models of alcohol abuse have clearly identified ethanol as a hepatic carcinogen *via* mechanisms related to excessive reactive oxygen species and acetaldehyde production, altered methylation and reduction of retinoic acid in hepatocytes. Recently the authors[11,12] and others[13] investigated Parkin-mediated hepatic mitophagy in animal models of acute and chronic alcoholism. The authors found that acute ethanol administration (5 g/kg) to adult rats enhanced hepatocyte mitophagy, which was associated with Parkin mitochondrial translocation and colocalization with accumulated 8-OHdG - a marker of oxidative nuclear and mitochondrial DNA (mtDNA) damage and mutagenicity[11,12,14,15]. Accordingly, Parkin co-localization with accumulated 8-OHdG in hepatocyte mitochondria of acute ETRs may be a signal for mitophagy induction *via* the triggering of Parkin mitochondrial translocation[12,16]. It may also be a stimulus for DNA repair and prevention of oncogenesis, as endogenous Parkin has a reported physical association with mtDNA[12,17] and translocates to nuclei interacting with proliferating cell nuclear antigen in cultured neuronal cells affected by oxidative DNA damage[18]. In addition, Parkin-deficient mice have been reported to show increased 8-oxoguanine in the cerebral cortex. Parkin's promotion of DNA repair may therefore be an important mechanism in the suppression of cancer and neurodegenerative diseases[18,19]. The authors' findings in animal models of ethanol-induced mitophagy may support the above-mentioned literature regarding the tumor suppressor roles of Parkin, which may or may not be mitophagy-related. Parkin has additionally been reported to regulate two additional cytoprotective mechanisms on cellular exposure to oxidative stress: (1) induction of mitochondrial-derived vesicle formation[12,16,20]; and (2) suppression of mitochondrial spheroid formation[11,21,22]. Further studies are needed to determine whether Parkin regulates these two mechanisms in cancer cells and to evaluate the impact of any such regulation on tumorigenesis[23].

The authors believe that their recent publications on animal models of alcoholism and the work of others may provide evidence for Parkin-mediated oncosuppression, which may have implications in cancer therapy.

REFERENCES

1 **Youle RJ**, Narendra DP. Mechanisms of mitophagy. *Nat Rev Mol Cell Biol* 2011; **12**: 9-14 [PMID: 21179058 DOI: 10.1038/nrm3028]

2 **Hamacher-Brady A**, Brady NR. Mitophagy programs: mechanisms and physiological implications of mitochondrial targeting by autophagy. *Cell Mol Life Sci* 2016; **73**: 775-795 [PMID: 26611876 DOI: 10.1007/s00018-015-2087-8]

3 **Lee J**, Ham S, Lee MH, Kim SJ, Park JH, Lee SE, Chang JY, Joung KH, Kim TY, Kim JM, Sul HJ, Kweon GR, Jo YS, Kim KS, Shong YK, Gasparre G, Chung JK, Porcelli AM, Shong M. Dysregulation of Parkin-mediated mitophagy in thyroid Hürthle cell tumors.

Carcinogenesis 2015; **36**: 1407-1418 [PMID: 26354775 DOI: 10.1093/carcin/bgv122]
4 **Fujiwara M**, Marusawa H, Wang HQ, Iwai A, Ikeuchi K, Imai Y, Kataoka A, Nukina N, Takahashi R, Chiba T. Parkin as a tumor suppressor gene for hepatocellular carcinoma. *Oncogene* 2008; **27**: 6002-6011 [PMID: 18574468 DOI: 10.1038/onc.2008.199]
5 **Takamura A**, Komatsu M, Hara T, Sakamoto A, Kishi C, Waguri S, Eishi Y, Hino O, Tanaka K, Mizushima N. Autophagy-deficient mice develop multiple liver tumors. *Genes Dev* 2011; **25**: 795-800 [PMID: 21498569 DOI: 10.1101/gad.2016211]
6 **Lee SB**, Kim JJ, Nam HJ, Gao B, Yin P, Qin B, Yi SY, Ham H, Evans D, Kim SH, Zhang J, Deng M, Liu T, Zhang H, Billadeau DD, Wang L, Giaime E, Shen J, Pang YP, Jen J, van Deursen JM, Lou Z. Parkin Regulates Mitosis and Genomic Stability through Cdc20/Cdh1. *Mol Cell* 2015; **60**: 21-34 [PMID: 26387737 DOI: 10.1016/j.molcel.2015.08.011]
7 **Xu L**, Lin DC, Yin D, Koeffler HP. An emerging role of PARK2 in cancer. *J Mol Med* (Berl) 2014; **92**: 31-42 [PMID: 24297497 DOI: 10.1007/s00109-013-1107-0]
8 **Zhang C**, Lin M, Wu R, Wang X, Yang B, Levine AJ, Hu W, Feng Z. Parkin, a p53 target gene, mediates the role of p53 in glucose metabolism and the Warburg effect. *Proc Natl Acad Sci USA* 2011; **108**: 16259-16264 [PMID: 21930938 DOI: 10.1073/pnas.1113884108]
9 **Bryant KL**, Mancias JD, Kimmelman AC, Der CJ. KRAS: feeding pancreatic cancer proliferation. *Trends Biochem Sci* 2014; **39**: 91-100 [PMID: 24388967 DOI: 10.1016/j.tibs.2013.12.004]
10 **Seitz HK**, Stickel F. Molecular mechanisms of alcohol-mediated carcinogenesis. *Nat Rev Cancer* 2007; **7**: 599-612 [PMID: 17646865 DOI: 10.1038/nrc2191]
11 **Eid N**, Ito Y, Horibe A, Otsuki Y. Ethanol-induced mitophagy in liver is associated with activation of the PINK1-Parkin pathway triggered by oxidative DNA damage. *Histol Histopathol* 2016; **31**: 1143-1159 [PMID: 26935412 DOI: 10.14670/HH-11-747]
12 **Eid N**, Ito Y, Otsuki Y. Triggering of Parkin Mitochondrial Translocation in Mitophagy: Implications for Liver Diseases. *Front Pharmacol* 2016; **7**: 100 [PMID: 27199746 DOI: 10.3389/fphar.2016.00100]
13 **Williams JA**, Ni HM, Ding Y, Ding WX. Parkin regulates mitophagy and mitochondrial function to protect against alcohol-induced liver injury and steatosis in mice. *Am J Physiol Gastrointest Liver Physiol* 2015; **309**: G324-G340 [PMID: 26159696 DOI: 10.1152/ajpgi.00108.2015]
14 **Cederbaum AI**. Effects of alcohol on hepatic mitochondrial function and DNA. *Gastroenterology* 1999; **117**: 265-269 [PMID: 10381938]
15 **Valavanidis A**, Vlachogianni T, Fiotakis C. 8-hydroxy-2' -deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev* 2009; **27**: 120-139 [PMID: 19412858 DOI: 10.1080/10590500902885684]
16 **Roberts RF**, Tang MY, Fon EA, Durcan TM. Defending the mitochondria: The pathways of mitophagy and mitochondrial-derived vesicles. *Int J Biochem Cell Biol* 2016; **79**: 427-436 [PMID: 27443527 DOI: 10.1016/j.biocel.2016.07.020]
17 **Rothfuss O**, Fischer H, Hasegawa T, Maisel M, Leitner P, Miesel F, Sharma M, Bornemann A, Berg D, Gasser T, Patenge N. Parkin protects mitochondrial genome integrity and supports mitochondrial DNA repair. *Hum Mol Genet* 2009; **18**: 3832-3850 [PMID: 19617636 DOI: 10.1093/hmg/ddp327]
18 **Kao SY**. Regulation of DNA repair by parkin. *Biochem Biophys Res Commun* 2009; **382**: 321-325 [PMID: 19285961 DOI: 10.1016/j.bbrc.2009.03.048]
19 **Schüle B**, Byrne C, Rees L, Langston JW. Is PARKIN parkinsonism a cancer predisposition syndrome? *Neurol Genet* 2015; **1**: e31 [PMID: 27066568 DOI: 10.1212/NXG.0000000000000031]
20 **McLelland GL**, Soubannier V, Chen CX, McBride HM, Fon EA. Parkin and PINK1 function in a vesicular trafficking pathway regulating mitochondrial quality control. *EMBO J* 2014; **33**: 282-295 [PMID: 24446486 DOI: 10.1002/embj.201385902]
21 **Manley S**, Ni HM, Williams JA, Kong B, DiTacchio L, Guo G, Ding WX. Farnesoid X receptor regulates forkhead Box O3a activation in ethanol-induced autophagy and hepatotoxicity. *Redox Biol* 2014; **2**: 991-1002 [PMID: 25460735 DOI: 10.1016/j.redox.2014.08.007]
22 **Eid N**, Ito Y, Otsuki Y. Mitophagy in steatotic hepatocytes of ethanol-treated wild-type and Parkin knockout mice. *Am J Physiol Gastrointest Liver Physiol* 2015; **309**: G513-G514 [PMID: 26374875 DOI: 10.1152/ajpgi.00254.2015]
23 **Bernardini JP**, Lazarou M, Dewson G. Parkin and mitophagy in cancer. *Oncogene* 2016; Epub ahead of print [PMID: 27593930 DOI: 10.1038/onc.2016.302]

P- Reviewer: Chen YC, Facciorusso A, Guo JC, Hu XT, Shirai Y
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Li D

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/ *World J Hepatol* 2017 March 8; 9(7): 352-367

DOI: 10.4254/wjh.v9.i7.352 ISSN 1948-5182 (online)

REVIEW

Future of liver disease in the era of direct acting antivirals for the treatment of hepatitis C

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Author contributions: Ponziani FR, Mangiola F and Binda C reviewed the literature, drafted the paper and approved the final version of the paper; Zocco MA, Siciliano M, Grieco A, Rapaccini GL, Pompili M and Gasbarrini A reviewed the literature, revised the paper and approved the final version of the paper.

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

Manuscript source: Invited manuscript

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Received: September 2, 2016
Peer-review started: September 3, 2016
First decision: October 20, 2016
Revised: October 26, 2016
Accepted: December 1, 2016
Article in press: December 2, 2016

Published online: March 8, 2017

Abstract

Hepatitis C virus (HCV) infection has been a global health problem for decades, due to the high number of infected people and to the lack of effective and well-tolerated therapies. In the last 3 years, the approval of new direct acting antivirals characterized by high rates of virological clearance and excellent tolerability has dramatically improved HCV infection curability, especially for patients with advanced liver disease and for liver transplant recipients. Long-term data about the impact of the new direct acting antivirals on liver fibrosis and liver disease-related outcomes are not yet available, due to their recent introduction. However, previously published data deriving from the use of pegylated-interferon and ribavirin lead to hypothesizing that we are going to observe, in the future, a reduction in mortality and in the incidence of hepatocellular carcinoma, as well as a regression of fibrosis for people previously affected by hepatitis C. In the liver transplant setting, clinical improvement has already been described after treatment with the new direct acting antivirals, which has often led to patients delisting. In the future, this may hopefully reduce the gap between liver organ request and availability, probably expanding liver transplant indications to other clinical conditions. Therefore, these new drugs are going to change the natural history of HCV-related liver disease and the epidemiology of HCV infection worldwide. However, the global consequences will depend on treatment accessibility and on the number of countries that could afford the use of the new direct acting antivirals.

Key words: Direct acting antivirals; Hepatitis C; Liver transplantation; Liver fibrosis; Cirrhosis; Hepatocellular carcinoma

Core tip: The approval of new direct acting antivirals with high rates of virological clearance and excellent tolerability has dramatically improved hepatitis C virus (HCV) infection curability, especially for patients with advanced liver disease and for liver transplant recipients. The aim of this review is to draw the possible future scenery in HCV-related liver disease, focusing our attention on the impact of second generation direct acting antivirals on liver fibrosis, hepatocellular carcinoma and liver transplantation.

Ponziani FR, Mangiola F, Binda C, Zocco MA, Siciliano M, Grieco A, Rapaccini GL, Pompili M, Gasbarrini A. Future of liver disease in the era of direct acting antivirals for the treatment of hepatitis C. *World J Hepatol* 2017; 9(7): 352-367 Available from: URL: http://www.wjgnet.com/1948-5182/full/v9/i7/352.htm DOI: http://dx.doi.org/10.4254/wjh.v9.i7.352

INTRODUCTION

Since its discovery, hepatitis C virus (HCV) has been a constant burden for global health, with 3 to 4 million new infections each year and an overall number of 130-170 million infected people in the world[1]. The prevalence of HCV infection has a large geographical variability, ranging from less than 1% to more than 10% in different regions[2,3]. In particular, 2.3 million of the chronically infected subjects have been estimated to reside in the United States, 1.5 in Japan and 11.5-19 in Europe[4].

HCV infection becomes chronic in up to 50%-80% of cases, establishing a damage that may lead to cirrhosis and its complications [*e.g.,* hepatocellular carcinoma (HCC), portal hypertension, liver decompensation and insufficiency] in approximately 10%-20% of patients[5,6]. Nevertheless, chronic HCV infection may be associated with extrahepatic manifestations, such as cryoglobulinemia and non-Hodgkin lymphoma, mainly caused by the continuous stimulation of the immune system[7,8].

Non-pegylated interferon (IFN) or pegylated IFN (PEG-IFN) in combination with ribavirin (RBV) have been the main pharmacological agents for the treatment of HCV infection. However, only 30%-40% of subjects with genotype 1 HCV and 70%-90% of those with genotype 2 and 3 treated with PEG-IFN in association with RBV were able to reach a sustained virological response (SVR), defined as the absence of detectable levels of HCV-RNA 24 wk after the end of treatment[9-14]. In 2011 the association of the first-generation direct acting antivirals (DAAs) boceprevir and telaprevir with PEG-IFN and RBV increased the overall SVR rates to 68%-75% for naive patients and to 59%-88% for treatment-experienced patients, even if these regimens were dedicated just to the treatment of genotype 1 HCV infection[12,14,15]. However, the suboptimal response rates, the long duration of treatment (24-48 wk) and the scarce tolerability of boceprevir and telaprevir, especially by cirrhotic patients, has heavily affected their clinical use and has led to search for new drugs[16].

SECOND-GENERATION DAAs

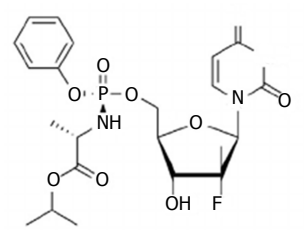
The second-generation DAAs are characterized by elevated SVR rates, good safety profiles, and more comfortable types of administration. They can be used or not in combination with RBV, depending on virological and disease-associated characteristics[17]. Sofosbuvir (SOF) has been the first new agent approved by the Food and Drug Administration (FDA) in December 2013 (Table 1 and Figure 1)[18].

SOF targets HCV-RNA replication with a pangenotypic efficacy since it blocks the nucleotide polymerase NS5B, which is highly preserved among different HCV genotypes[19]. Treatment with SOF, either in combination with PEG-IFN plus RBV or with RBV alone has shown SVR rates above 85% at 12 wk after the end of treatment (SVR12)[20]. Successively, new DAAs for the treatment of HCV infection in association with SOF have been approved: Simeprevir (SMV, a NS3/4A protease inhibitor) and ledipasvir (LDV, a NS5A inhibitor) for genotype 1, and daclatasvir (DCV, a NS5A inhibitor) for genotype 3, reporting SVR12 rates > 90%[21-24]. More recently, the pangenotypic NS5A inhibitor velpatasvir has also been approved for HCV treatment in combination with SOF[25,26].

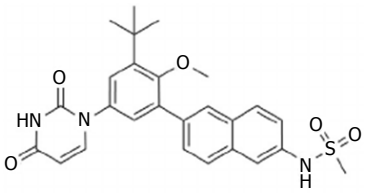
The first antiviral regimen SOF-free was approved in July 2015 and includes paritaprevir (a NS3/4A protease inhibitor), ritonavir (a CYP3A inhibitor, used as a pharmacologic booster) and ombitasvir (a NS5A inhibitor), in association with dasabuvir (a non-nucleoside NS5B polymerase inhibitor), and is indicated for the treatment of genotype 1 (with dasabuvir) and 4 (without dasabuvir) HCV infection[27,28]. Successively, the FDA has approved another SOF-free antiviral regimen including elbasvir and grazoprevir[29], and new drugs with pangenotypic efficacy are in final phase of study and will soon be available[30].

The main advantage of the new DAAs-based antiviral regimens is the achievement of high SVR rates for all HCV genotypes within a short treatment period, together with the infrequent occurrence of side effects, usually of mild grade. Resistance-associated variants (RAVs) of the virus may exist prior to treatment, may persist for years after treatment and affect most frequently the NS3/5A viral protein; RAVs are associated with (but do not inevitably result in) treatment failure, which may occur in about 10%-15% of patients[31,32].

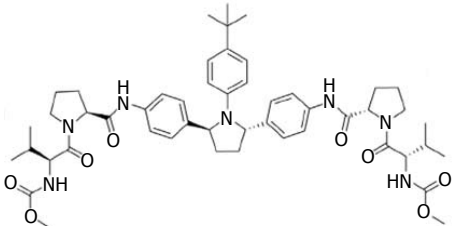
The most ambitious result we might expect from the use of DAAs would be the reduction of liver cirrhosis-related complications, such as HCC development, and in the long-term period a decreased progression towards end-stage liver disease and a decreased need for liver transplant (LT), as well as the prevention of post-LT HCV infection recurrence[33]. Indeed, according to the latest data published by the World Health Organization in 2013, 5%-7% of infected subjects died from a



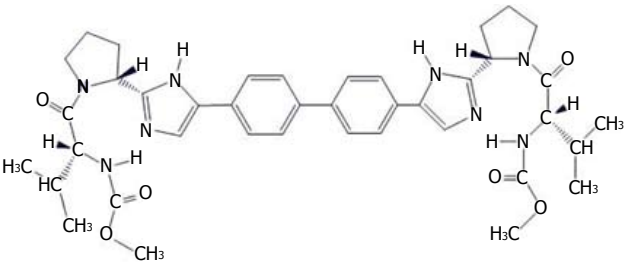
Sofosbuvir



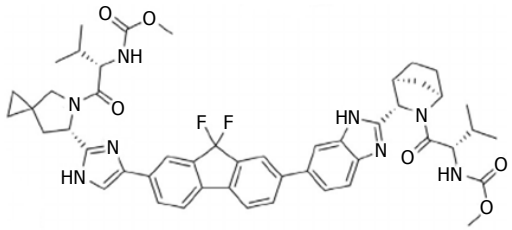
Dasabuvir



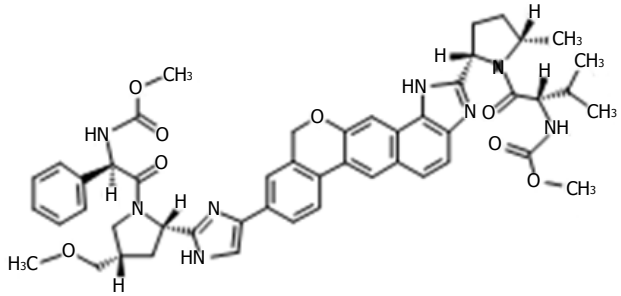
Ombitasvir



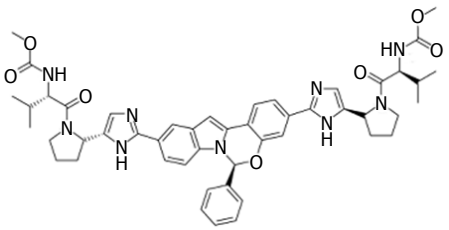
Daclatasvir



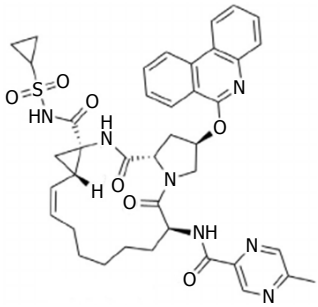
Ledipasvir



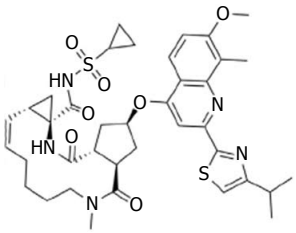
Velpatasvir



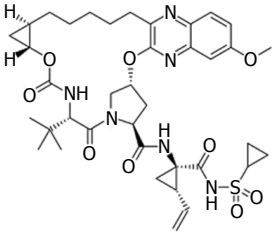
Elbasvir



Paritaprevir



Simeprevir



Grazoprevir

Figure 1 Second-generation direct acting antivirals molecules.

disease related to HCV[34], with an estimated risk of liver failure of 10.4% and 26.5% in patients with F3 and F4 fibrosis, respectively[35]. HCV-associated liver disease represents the most common indication for LT and, in developed countries, is the most common etiological factor of HCC, which is the third leading cause of cancer death worldwide[36-40].

However, due to the relatively recent introduction of these new drugs, data about their impact on liver disease progression, complications and liver-related mortality are

Table 1 Main features of antiviral targets and clinical indications of second-generation direct acting antivirals[17]

Molecule	Class	Target	Genotype	Associations
Sofosbuvir	Nucleotide polymerase inhibitor	NS5B RNA-dependent RNA polymerase	Pangenotypic	Ledipasvir Daclatasvir Simeprevir Velpatasvir
Dasabuvir	Non-nucleoside polymerase inhibitor	NS5B RNA-dependent RNA polymerase	Genotype 1	Ombitasvir + paritaprevir + ritonavir
Ombitasvir		NS5A	Genotype 1, 4	Paritaprevir + ritonavir with or without dasabuvir
Daclatasvir		NS5A	Genotype 1, 2, 3	Sofosbuvir
Ledipasvir		NS5A	Genotype 1, 4	Sofosbuvir
Velpatasvir		NS5A	Pangenotypic	Sofosbuvir
Elbasvir		NS5A	Genotype 1, 4	Grazoprevir
Paritaprevir		NS3/4A protease	Genotype 1, 4	Ombitasvir + ritonavir with or without dasabuvir
Simeprevir		NS3/4A protease	Genotype 1, 4	Sofosbuvir
Grazoprevir		NS3/4A protease	Genotype 1, 4	Elbasvir

scarce. Therefore, previously published data about the impact of SVR achieved with PEG-IFN-based regimens are the only available reference to evaluate the future positive effects that DAAs might produce on liver disease outcomes.

IMPACT OF VIRAL ERADICATION ON LIVER CIRRHOSIS-ASSOCIATED MORBIDITY AND MORTALITY

Published data have demonstrated a correlation between the achievement of SVR and the reduction of HCV-related complications, liver disease severity and mortality (Table 2).

Veldt *et al*[41] reported that among 286 subjects who achieved SVR and were followed-up for 5 years, only 1% experienced liver failure, with a survival similar to that of the general population. Another study including 721 patients with chronic hepatitis C reported a significantly lower annual mortality rate in subjects who had previously achieved SVR after IFN therapy compared to those who had not (0.44%/year, 1.98%/year and 3.19%/year for SVR, non-SVR and untreated patients, respectively; $P < 0.0001$). The study also showed that viral clearance was able to reduce the hazard ratio for total deaths by 0.173[42].

A meta-analysis including 129 trials for a total amount of 15067 patients has demonstrated that SVR achievement reduces the risk of LT requirement by 90%, and the risk of death by 60%-84%[43]. Nevertheless, viral clearance leads to a lower incidence of liver-related morbidity and death (0.62 and 0.61 among SVR patients, respectively, and 4.16 and 3.76 among non-SVR patients, respectively; $P < 0.001$)[44]. Recent data further confirmed that HCV infection resolution allows reduction in the incidence of liver decompensation[45], all-cause mortality[46,47] and annual deaths rate (8.9% in SVR patients *vs* 26.0% in non-SVR patients; $P < 0.001$)[48]. This evidence was confirmed by an extensive review by Szabo *et al*[34]; moreover, survival rates comparable to general population have been reported after the achievement of SVR even in patients with well-compensated cirrhosis[49]. Although useful to figure out the long-term benefits expected from DAAs, the interpretation of data emerging from the use of IFN- or PEG-IFN-based regimens is limited by the selection of patients, since those affected by comorbidities were usually not suitable for treatment and were not included in outcomes analyses; moreover, the lack of homogeneous design and patients' stratification make it difficult to deduce general conclusions.

IMPACT OF VIRAL ERADICATION ON LIVER FIBROSIS

Despite the positive impact of HCV infection eradication on patients' prognosis, few data about liver cirrhosis/fibrosis regression are available.

Regression of liver fibrosis as a result of viral clearance is supported by the reduction of inflammatory mediators that leads to apoptosis of myofibroblasts, and occurs by the inactivation of stellate cells. The down-regulation of inflammation, as well as microvascular remodelling, degradation of extracellular matrix and hepatocyte repopulation leads to the generation of new hepatic tissue[50,51].

Cirrhosis regression has been reported in about 61% of cases after a median time of 3 years from the achievement of SVR (Table 2)[52]. Mallet *et al*[53] observed the evolution of liver fibrosis in 96 patients treated with IFN or PEG-IFN with or without RBV, for a median follow-up of 118 mo. Although statistical significance was not reached, 18 subjects obtained a regression of fibrosis from METAVIR stage 4 to stage $\leqslant$ 2. In another study, among 153 cirrhotic patients treated with IFN or PEG-IFN in combination or not with RBV for 24 or 48 wk, 75 (49%) had a regression of fibrosis after a mean time of 21 ± 4 mo. In addition to SVR, factors independently associated with histology improvement were age < 40 years ($P < 0.001$) and body mass index < 27 kg/m^2 ($P < 0.001$)[54].

Other small studies reported variable rates of fibrosis regression after different time periods from viral

Table 2 Main studies highlighting the effects of hepatitis C virus antiviral therapy on patients' mortality, fibrosis regression and risk of hepatocellular carcinoma

Ref.	HCV genotype	Fibrosis stage	Treatment	SVR rate	Mortality (*n*, pts)	Survival	Other outcomes
Veldt *et al*[47], 2007	G1: 280/474 (59%)	Ishak score 4: 120 (25%) Ishak score 5: 94 (20%) Ishak score 6: 265 (55%)	Duration of treatment, 26 wk (21-48) IFN: 131 (27%) IFN + RBV: 130 (27%) PEG-IFN: 10 (2.1%) PEG-IFN + RBV: 208 (43%)	142/280 (50.7%)	SVR: 2/280 (0.7%) Non-SVR: 24/280 (8.6%)	-	SVR associated with reduction in the hazard of events (adjusted HR = 0.21, 95%CI: 0.07-0.58; $P < 0.003$)
Yoshida *et al*[64], 1999	G1: 1177/2400 (49%) G2: 496/2400 (20.6%)	F0: 45 (1.9%) F1: 665 (27.7%) F2: 896 (37.7%) F3: 564 (23.5%) F4: 230 (9.6%)	IFN-α: 84% IFN-β: 14% Combination of IFN-α and IFN-β: 2%	789/2400 (32.8%)	-	-	Risk of HCC for IFN therapy: Adjusted risk ratio = 0.516, 95%CI: 0.358-0.742 ($P < 0.001$); risk of HCC for SVR pts: risk ratio = 0.197, 95%CI: 0.099-0.392 ($P < 0.002$)
Veldt *et al*[41], 2004	SVR G1: 112/286 (39.2%) Not specified: 174/286 (60.8%) Non-SVR G1: 21/50 (42%) Not specified: 29/50 (58%)	SVR: F4: 15 (5.2%) Non-SVR: F4: 11 (22%)	Recombinant IFN α2a, α2b, or natural IFN monotherapy for 39 wk	286	SVR 6/286 (2.1%) 3/50 (6%)	SVR group: Comparable with the general population	29% regression and 5% progression of fibrosis in SVR group
Maruoka *et al*[42], 2012	Treated (577): G1: 383/577 (66.2%) G2: 144/577 (24.8%) Untreated (144)	Treated: F0: 15 (2.6%) F1: 290 (503%) F2: 132 (22.9%) F3: 82 (12.2%) F4: 58 (10.1%) Untreated: F0: 2 (1.4%) F1: 64 (44.4%) F2: 32 (22.2%) F3: 18 (12.5%)	IFN (not specified)	221/577 (38.3%)	Untreated: 37/144 (25.7%) Non-SVR 74/356 (20.8%) SVR 10/221 (4.5%)	-	Risk ratio of overall death and liver-related death reduced to 0.173 (95%CI: 0.075-0.402)
Bruno *et al*[49], 2016	G1: 88/181 (48.6%)	F4: 100% CPT A5: 154/181 (85.1%) CPT A6: 27/181 (14.9%)	IFN mono-therapy or IFN (pegylated or not) + RBV	181	18/181 (9.9%)	-	-
Cardoso *et al*[44], 2010	G1: 60% G2: 8% G3: 16% G4: 13%	F4: 54%	PEG-IFN + RBV: 252 (82%), PEG-IFN: 22 (7%), IFN ± RBV: 33 (11%)	103/307 (33.5%)	21/307 (6.8%)	-	-
Tada *et al*[46], 2016	G1: 1476/2743 (53.8%) G2: 789/2743 (28.3%) Unknown: 478/2743 (17.4%)	-	IFN (not specified)	587/2267 (25.9%)	137/2267 (6%)	-	-
Van der Meer *et al*[48], 2012	G1: 340/498 (68.3%) G2: 48/498 (9.6%) G3: 88/498 (17.7%) G4: 22/498 (4.4%)	Ishak 4: 143/498 (27%) Ishak 5: 101/498 (19%) Ishak 6: 22/498 (4%)	IFN: 175 (33%) IFN + RBV: 148 (28%) PEG-INF: 176 (33%) PEG-IFN + RBV: 176 (33%)	192/498 (38.5%)	SVR: 13 Non-SVR: 100	-	SVR reduced all-cause mortality (HR = 0.265, 95%CI: 0.14-0.49; $P < 0.001$)
D'Ambrosio *et al*[52], 2012	G1: 11/38 (28.9%) G2: 24/38 (63.2%) G3: 3/38 (7.9%)	Only cirrhotic patients	IFN + RBV: 10/38 (26.3%) PEG-IFN + RBV: 28/38 (73.6%) Duration of treatment 24 mo (24-48)	-	-	-	SVR reduced area of fibrosis by 2.3% ($P < 0.0001$), with a median individual decrease of 71.8%

Mallet *et al*[53], 2008	G1: 51/96 (53.1%)	F4: 100%	IFN or PEG-IFN, with or without RBV	39/96 (40.6%)	SVR: 4 (10.2%) Non-SVR: 17 (29.8%)	-	Regression of fibrosis (according to METAVIR score): Stage 4: 69 (71.9%); stage 3: 9 (9.4%); stage 2: 10 (10.4%); stage 1: 7 (7.3%); stage 0: 1 (1%)
Reichard *et al*[56], 1999	G1: 41/100 (41%) G2: 27/100 (27%) G3: 23/100 (23%) Mixed: 9/100 (9%)	F0-3: 22 F4: 4	IFN alpha2b: 73 Human leucocyte IFN alpha: 42	27/100 (27%)	-	-	Reduction of portal inflammation ($P < 0.0002$), piecemeal necrosis ($P < 0.0004$), lobular necrosis ($P < 0.0005$), fibrosis ($P < 0.0008$) after SVR
Arif *et al*[57], 2003	Naive (52): G1a: 64% G1b: 19% G2: 6% G3: 10% G4: 1% Experienced (79): G1a: 55% G1b: 26% G2: 7% G3: 10% G4: 2%	Naive Fibrosis score: 2.91 ± 1.64 Fibrosis score: 2.83 ± 1.62	IFN alpha2b Duration of treatment: 12-24 wk: 10 24 wk: 56 36 wk: 8 48 wk: 30	Naive 21/52 (40.4%) Experienced 18/79 (22.8%)	-	-	Reduction in fibrosis score in both groups: responders = -0.91 ($P = 0.038$), non-responders = -0.48 ($P = 0.021$)
George *et al*[58], 2009	G1: 75/141 (53%) G2: 49/141 (35%) G3: 14/141 (10%) G4: 3/141 (2%)	Fibrosis stage ⩾ 2: 116 Fibrosis stage = 4: 16 According to Scheuer	IFN alpha2b + RBV: 146 (97%) PEG-IFN alpha2a + RBV: 4 (3%)	100%	-	1	39/49 (79.6%) reduction in fibrosis stage (according to Ishak score) 16/49 (32.6%) pts had 2 point or greater decrease in stage
Poynard *et al*[59], 2002	-	Standard: F0: 12 (15%) F1: 42 (54%) F3: 24 (31%) F4: 0 (0%) Reinforced: F0: 16 (18%) F1: 41 (47%) F3: 30 (35%) F4: 0	Standard: IFN alpha2a 3 MU TIW for 24 wk Reinforced: IFN alpha2a 6 MU daily for 12 d followed by thrice weekly for 22 wk, then 3 MU thrice weekly for 24 wk	Standard: 3/78 (3.8%) Reinforced: 14/87 (16%)	-	-	Decrease in fibrosis index score in SVR group compared with non-responders: From 0.33 ± 0.06 at baseline to 0.18 ± 0.06 at 72 wk *vs* from 0.41 ± 0.03 at baseline to 0.44 ± 0.03 at 72 wk ($P < 0.001$)
Shiratori *et al*[60], 2000	-	SVR: F0: 3 (2%) F1: 42 (23%) F2: 69 (37%) F3: 45 (25%) F4: 24 (13%) Non-SVR: F0: 3 (1%)	IFN alpha2a or IFN alpha2b or Natural IFN alpha weekly for 3 to 6 mo IFN alpha 6-7 times per wk for 8 wk	183/487 (37.6%)	-	-	SVR group: Rate of fibrosis progression -0.28 ± 0.03 unit/year (regression) Non-SVR group: Rate of fibrosis progression: 0.02 ± 0.02 unit/year $P < 0.001$

		F1: 95 (31%) F2: 109 (36%) F3: 67 (22%) F4: 30 (10%)					
Maylin *et al*[62], 2008	G1: 21/210 (39%) G2: 55/210 (18%) G3: 101/210 (32%) G4-5: 33/210 (11%)	F0-1: 121 (38%) F2: 111 (35%) F3: 56 (17%) F4: 31 (10%)	IFN alpha: 3 (1%) IFN-lymphoblastoid: 5 (1%) IFN-hybrid: 9 (3%) IFN alpha2a: 18 (5%) IFN alpha2a + RBV: 5 (2%) PEG-IFN alpha2a + RBV: 27 (8%) IFN alpha2b: 22 (6%) IFN alpha2b + RBV: 41 (12%) PEG-IFN alpha2b + RBV: 214 (62%)	100%	-	-	Fibrosis stage improved in 56%, stable in 32%, deteriorated in 12%; regression of cirrhosis observed in 9 of 14 (64%)

Pts: Patients; IFN: Interferon; PEG: Pegylated; SVR: Sustained virological response; HCC: Hepatocellular carcinoma; RBV: Ribavirin.

clearance[55-62].

However, the neo-formed parenchyma derived from the generation of new liver tissue is different from the healthy one and is characterized by architectural and structural alterations[63]. At present, little is known about its functionality.

IMPACT OF VIRAL ERADICATION ON THE DEVELOPMENT OF HCC

In HCV-infected subjects, the development of liver cirrhosis is the main oncogenic trigger for HCC[5,64,65], though not the only one. Indeed, direct and indirect viral-related mechanisms may contribute to the growth of cancer cells, including the expression of viral proteins with oncogenic effect from infected cells, messy proliferation of non-infected hepatocytes responsive to the apoptotic boost and the oxidative stress caused by inflammation[66].

The first study documenting the importance of viral clearance in reducing the risk of HCC development was published in 1995. Ninety HCV-infected subjects, half of whom had undergone antiviral therapy with IFN, were followed-up for 5 years; seventeen of the untreated subjects developed HCC, compared with only 2 of those treated[67]. A subsequent study also reported a significant difference in HCC occurrence between SVR and non-SVR patients (5.1% and 21.8%, respectively; $P < 0.001$)[48]. A recent meta-analysis including 31528 patients with a median follow-up ranging from 3 years to 8 years demonstrated that SVR is a key factor in reducing the risk of HCC development, since among responders only 1.5% developed tumour lesions compared to 6.2% of non-SVR patients[68]. Moreover, considering only those subjects with advanced fibrosis (F3-F4 according to METAVIR score), the incidence of HCC was 17.9% among non-responders, four times greater than the 4.2% rate reported among responders ($P < 0.001$).

HCV eradication may also reduce the risk of HCC recurrence after surgical treatment. A 63.4% cumulative recurrence rate has been reported in non-treated patients, compared to 63.2% in treated patients who did not achieve SVR and to 41.7% in the SVR group (non-treated *vs* SVR, $P = 0.008$; SVR *vs* treated without SVR, $P = 0.035$)[69]. Mazzaferro *et al*[70] also found SVR as the only factor significantly reducing HCC late recurrence in HCV-pure (hepatitis B anticore antibody negative) patients. A subsequent meta-analysis also reported a reduced rate of early recurrence in 51 patients undergoing surgical resection or percutaneous ablation, reporting a 30% reduction in HCC recurrence rate[71]. In addition, IFN therapy seems to exert beneficial effects, even when started before HCC curative treatments[72].

Although based on heterogeneous studies, these data have raised the issue of the favourable properties of IFN in the prevention of HCC development and recurrence. IFN seems to combine antiviral and antiproliferative effects, such as inhibition of angiogenesis, enhancing of antitumoral immunity and induction of pro-apoptotic boost[70,73]. Recent data have highlighted that IFN may prevent postoperative recurrence of HCC expressing metastatic tumour antigen 1, having 1-year recurrence rate as high as 7% in IFN-treated patients *vs* 24% in the control group ($P < 0.05$)[74].

DAAs may likely modulate the expression of genes involved in the production of endogenous IFN. In patients treated with SOF in association with RBV a reduction in

types Ⅰ and Ⅱ IFN in liver tissue and an increase of IFN-alpha2 have been observed[75]. Conversely, other authors have reported a loss of intrahepatic immune activation by IFN-gamma, associated with normalization of the natural killer cells phenotype and function, consequent to DAAs treatment[76]. How these findings may be associated with DAAs treatment outcome still needs to be further elucidated.

Although the risk of HCC development is significantly reduced by viral clearance it is not completely eliminated, especially in cases of persistence of other cofactors promoting carcinogenesis. Toyoda *et al*[77] reported that 18/522 patients who achieved SVR after IFN treatment developed HCC after a median follow-up of about 7.2 years (1.0-22.9 years), with an incidence of 1.2% and 4.3% at 5 years and 10 years, respectively. In the analysis, the presence of diabetes mellitus and advanced fibrosis (FIB-4 index $\geqslant$ 2) at 24 wk after SVR were correlated to an increased risk of developing HCC. Other data identified type 2 diabetes mellitus and total alcohol intake as independent risk factors for HCC development (HR = 2.77, 95%CI: 2.13-3.60, $P < 0.001$ and HR= 2.13, 95%CI: 1.74-2.61, $P < 0.001$, respectively)[78]. In another study, among 232 SVR patients who underwent liver biopsy between 1992 and 2009, the development of HCC was definitively lower in the group with low-intermediate grade fibrosis (F0-F2 according to Metavir) than in that with F3-F4 grade (1.6% and 8%, respectively)[79].

Data about the impact of DAAs treatment on HCC recurrence in previously treated patients and on the development of new HCC nodules have been recently published. It seems to be clear that these new antivirals are not able to modify the natural history of HCC in cirrhotic patients, and it has also been postulated that they may act as promoters, although other studies have not supported this hypothesis[80-87]. Probably, an investigation focused on the immunologic changes and the microenvironmental hepatic tissue alterations consequent to DAAs treatment may be worthwhile to quell this debate[88].

DAAs AND LT

HCV infection-associated cirrhosis and HCC account for 40% of all cases on the LT waiting list in the United States and for about 1/3 of LTs in cirrhotic patients[39,40]. HCV infection recurrence of the graft is universal and leads to cirrhosis in up to 20%-30% of recipients, being one of the most important causes of death and retransplantation[89,90]. The time course of post-LT HCV reinfection is faster than among immunocompetent individuals; cirrhosis can be histologically documented within 5 years after LT, and from that point on the first episode of decompensation may occur within less than 1 year[91].

After HCV infection eradication, a 62%-84% decrease in 5-year mortality as well as a reduction by 90% of the risk of receiving LT have been reported[43]. This improvement in survival was observed in both sustained virological responders and relapsers[92]. The new available DAAs account for response rates higher than 90% and are better tolerated than either IFN and PEG-IFN, allowing for treatment of patients for whom the previous antivirals were contraindicated and who had low chances of response due to unfavourable virological or clinical conditions[93]. As patients who achieve SVR have a reduced risk of progression to cirrhosis and of developing its complications, the widespread use of the new DAAs will probably change the scenario of LT, potentially reducing the need for liver organs.

DAAs treatment before LT

The aim of antiviral treatment in patients on the waiting list is to prevent the recurrence of HCV infection after LT. To reduce the risk of post-LT recurrence, the achievement of at least 30 d of HCV-RNA negativity before LT has been suggested[94,95]. However, whether it is necessary to continue antiviral therapy after LT in patients who received a very short course of therapy before transplantation is not yet clear[96].

Furthermore, achieving SVR in waiting-list patients may directly impact the severity of liver disease, with possible delisting after treatment. A recent real-life multicentre study[97] including 103 decompensated cirrhotic patients listed for LT and treated with second-generation DAAs reported HCV eradication rates of 16% at 24 wk and of 35% at 48 wk after the beginning of treatment (Table 3). This was associated with delisting of 20% of patients at 48 wk from the end of treatment. The evidence of a significant improvement of liver function also comes from the SOLAR-1 study cohort A[98], including cirrhotic patients with decompensated disease treated with LDV and SOF plus RBV. For this study, similar SVR rates (from 86% to 89%) were reported for Child-Pugh class B and C patients regardless of treatment duration, and this was associated with the improvement of model for end-stage liver disease (MELD) and Child-Pugh scores.

Other studies confirmed liver function amelioration after viral eradication in decompensated cirrhotic patients[26,98-104]; although in cases with more advanced liver impairment (Child-Pugh C, albumin lower than 3.5 g/dL, MELD > 20) and in the elderly worsening of liver function has also been reported[105,106].

Delisting due to clinical improvement may therefore become frequent in the era of DAAs, making it possible to reserve LT only to patients who do not show significant benefit. Munoz[107] estimated that DAAs-induced reduction in MELD score down to the threshold of LT benefit may occur in 592-993 listed patients/year during the first year after treatment, and that approximately 213-515 donated livers/year may become available for redistribution to other patients.

The future impact of DAAs on indications for LT and on organ allocation policy may depend not only on the decreased number of HCV-infected recipients but also on the potential use of anti-HCV positive donors[108]. Indeed, DAAs might introduce a new era, in which anti-

Table 3 Main studies evaluating the effects of direct acting antivirals in patients with advanced cirrhosis and/or listed for liver transplantation

Ref.	HCV genotype	Fibrosis stage	Treatment	SVR rate	Observed improvement
Charlton *et al*[98], 2015	Cohort A G1a: 74/108 (68.5%) G1b: 31/108 (28.7%) G4: 3/108 (2.8%)	Child A: 1/108 (1%) Child B: 65/108 (60.2%) Child C: 42/108 (38.9%)	LDV/SOF + RBV 12 or 24 wk	Child B: -12 wk 26/30 (87%) -24 wk 24/27 (89%) Child C: -12 wk 19/22 (86%) -24 wk 20/23 (87%)	-
Belli *et al*[97], 2016	G1a: 20/103 (19.4%) G1b: 40/103 (38.8%) G2: 3/103 (3%) G3: 20/103 (19.4%) G4: 20/103 (19.4%)	Child A: 0 Child B: 46/103 (44.7%) Child C: 57/103 (55.3%)	SOF/RBV: 52/103 (50.4%) SOF/LDV ± RBV: 9/103 (8.7%) SOF/DCV ± RBV: 35/103 (33.9%) SOF/SMV ± RBV: 7/103 (6.8%)	SOF/RBV (24-48 wk): RVR 61% EVR 98% SOF + 2nd DAA (12-24 wk): RVR 67% EVR 98%	MELD: - 3.4 points Child: -2 points Delisting: 20% Improvement in refractory ascites that became treatable with diuretics
Munoz *et al*[107], 2015	-	Only cirrhosis	SOF/LDV + RBV (12-24 wk): 230 DCV/SOF + RBV (12 wk): 56 GRZ/ELB (12 wk): 27 SOF/LDV/DCV ± RBV (12 wk): 220	SVR 84%	MELD: -2.9 + - 0.1 Child B to Child A: 35% Child C to Child B: 48%
Manns *et al*[101], 2016	G1a: 50/107 (46.7%) G1b: 47/107 (43.9%) G4: 10/107 (9.4%)	Child A: 2/107 (2%) Child B: 60/107 (56%) Child C: 45/107 (42%)	LED/SOF + RBV 12 or 24 wk	genotype 1 Child B: 12 wk 20/23 (87%); 24 wk 22/23 (96%) Child C: 12 wk 17/20 (85%); 24 wk 18/23 (78%), 1/2 (50%) Genotype 4 Child B: 12 wk 2/3 (67%); 24 wk 100% Child C: 12 wk 0% 24 wk	MELD improvement in 72% Child B to Child A: 28% Child C to Child B: 68%
Poordad *et al*[100], 2016	G1a: 34/60 (56.7%) G1b: 11/60 (18.3%) G2: 5/60 (8.3%) G3: 6/60 (10%) G4: 4/60 (6.7%)	Child A: 12/60 (20%) Child B: 32/60 (53.3%) Child C: 16/60 (27.7%)	DCV/SOF + RBV 12 or 24 wk	Child A: 11/12 (92%) Child B: 30/32 (94%) Child C: 9/16 (56%)	MELD improvement in 47% of pts Child improvement in 60% of pts
Jacobson *et al*[99], 2015	Part 1 G1a: 27/30 (90%) G1b: 3/30 (10%)	Only Child B cirrhosis	GRZ/ELB 12 wk	SVR 27/30 (90%)	MELD improvement in 11/30 (36.7%) pts
Curry *et al*[26], 2015	G1a: 159/267 (59.6%) G1b: 48/267 (18%) G2: 12/267 (4.5%) G3: 39/267 (14.6%) G4: 8/267 (3%) G6: 1/267 (0.3%)	Child A: 16/267 (6%) Child B: 240/267 (89.9%) Child C: 11/267 (4.1%)	SOF/VEL 12 or 24 wk SOF/VEL + RBV 12 wk	SOF/VEL 12 wk: 75/90 (83%) SOF/VEL + RBV 12 wk: 82/87 (94%) SOF/VEL 24 wk: 77/90 (86%)	MELD improvement in 51% of pts Child improvement in 47% of pts
Gray *et al*[106], 2016	G1a: 29/101 (28.7%)	Child A: 15/101 (14.8%)	SOF/LDV ± RBV 12 wk	74.3%	No significant differences from baseline

	G1b: 19/101 (18.8%) G1 (no subtype): 27/101 (26.7%) G2: 0 G3: 24/101 (23.8%) G4: 1/101 (1%) Mixed: 1/101 (1%)	Child B: 67/101 (66.3%) Child C: 19/101 (18.8%)			Mortality rate 7.9% (6% Child B, 21% Child C)
Aquel *et al*[103], 2015	G1a: 82/119 (69%) G1b: 24/119 (20%) G1 (no subtype): G13/119 (11%)	Child A: 84/119 (70%) Child B: 34/119 (29%) Child C: 1/119 (1%)	SMV/SOF ± RBV 12 wk	RVR: 82/119 (69%) SVR 12: 92/118 (78%; Child A: 83%, Child B: 68%) (1 pts died after achieving SVR4)	MELD improvement in 61/92 (66.4%) pts that achieved SVR 12
Saxena *et al*[104], 2015	1a: 98/160 (62%) 1b: 62/160 (38%)	Child A: 101/160 (65%) Child B: 49/160 (31%) Child C: 6/160 (4%)	SMV/SOF ± RBV 12 wk	Child A (37% with RBV): 91% Child B/C (35% with RBV): 73%	No significant differences from baseline

Pts: Patients; LDV: Ledipasvir; SOF: Sofosbuvir; RBV: Ribavirin; DCV: Daclatasvir; SMV: Simeprevir; RVR: Rapid virological response (HCV-RNA < 15 UI after 4 wk of treatment); EVR: Early virological response (HCV-RNA < 15 UI after 12 wk of treatment); GRZ: Grazoprevir; ELB: Elbasvir; VEL: Velpatasvir; FCH: Fibrosing cholestatic hepatitis.

HCV positive donors could be reconsidered as a potential source of liver grafts. Moreover, in case of HCV infection transmission from anti-HCV positive donors during LT, it may be easily cured[108]. Recent data suggest that LT outcomes for recipients who accept HCV-positive allografts could be comparable with those of recipients who received HCV-negative allografts[109,110]. Probably, in the future, histological evaluation may become crucial in the choice and the allocation of liver grafts from anti-HCV positive donors, overcoming the issue of previous or active HCV infection. However, these considerations are based on the universal adoption of screening policies for HCV infection, as well as on the widespread use of DAAs for HCV infection treatment, which is still limited by restricted accessibility.

DAAs treatment after LT

DAAs have demonstrated unprecedented results in the treatment of LT recipients (Table 4).

The SOLAR-1 study, cohort B[98], explored the efficacy of LDV and SOF plus RBV in the treatment of LT recipients without cirrhosis (group 3), with compensated cirrhosis (group 4), and with Child-Pugh B (group 5) and C cirrhosis (group 6). In groups 3 and 4 the SVR rates ranged from 96% to 98% independent of treatment duration; in group 5, SVR was achieved by 86% of patients who received 12 wk of treatment and by 88% of those who received 24 wk of treatment, and group 6, instead, had lower rates of SVR, being 60% and 75% in patients receiving 12 wk and 24 wk of treatment, respectively.

Another study with a similar design, the SOLAR-2, also reported excellent SVR rates in LT recipients with decompensated cirrhosis and genotype 1 or 4 HCV infection treated with LDV and SOF plus RBV for 12 wk or 24 wk[101].

The ALLY-1 and the HCV-TARGET study confirmed good outcomes also for the combination regimens including SMV plus SOF with or without RBV and DCV plus SOF with RBV[100,111].

Although these data may highlight that the achievement of SVR is more difficult in LT cirrhotic patients with more advanced liver impairment, the SOLAR-1 and -2 studies also reported an improvement in MELD and Child-Pugh scores in treated patients[98,101]. This was confirmed by a prospective, multicentre study in patients with post-LT hepatitis C recurrence treated with LDV and SOF plus RBV; the response rate was 96% in Child-Pugh A patients compared to 85% and 65% in Child-Pugh class B and C ones, respectively. However, an improvement in Child-Pugh class and MELD scores was observed in patients with decompensated cirrhosis who achieved SVR12[112].

Therefore, liver function improvement consequent to antiviral treatment will hopefully reduce the need for retransplantation and the morbidity and mortality related to liver dysfunction and liver cirrhosis complications.

HCV INFECTION AND DISEASE-RELATED COMPLICATIONS IN THE FUTURE

The future trend of HCV-related morbidity and mortality in the era of IFN-free antiviral regimens is difficult to predict, although encouraging prospects can be inferred by

Table 4 Studies evaluating the effects of direct acting antivirals in liver transplant recipients

Ref.	HCV genotype	Fibrosis stage	Treatment	SVR12 rate	Observed improvement
Charlton *et al*[98], 2015	Cohort B G1a: 164/229 (71.6%) G1b: 63/229 (27.5%) G4: 2/229 (0.9%)	No cirrhosis: 111/229 (48.5%) Child A: 51/229 (22.3%) Child B: 52/229 (22.7%) Child C: 9/229 (3.9%) FCH: 6/229 (2.6%)	LDV/SOF + RBV 12 or 24 wk	No cirrhosis: 12 wk 53/55 (96) 24 wk 55/56 (98) Child A: 12 wk 25/26 (96%) 24 wk 24/25 (96%) Child B: 12 wk 22/26 (85%) 24 wk 23/26 (88%) Child C: 12 wk 3/5 (60%) 24 wk 3/4 (75%) FCH: 12 and 24 wk 100%	-
Manns *et al*[101], 2016	Cohort B G1a: 113/226 (50%) G1b: 86/226 (38%) G4: 27/226 (12%)	No cirrhosis: 101/226 (44.7%) Child A: 71/226 (31.4%) Child B: 40/226 (17.7%) Child C: 9/226 (4%) FCH: 5/226 (2.2%)	LDV/SOF + RBV 12 or 24 wk	Genotype 1: No cirrhosis: 12 wk: 42/45 (93%) 24 wk: 44/44 (100%) Child A: 12 wk: 30/30 (100%) 24 wk: 27/28 (96%) Child B: 12 wk: 19/20 (95%) 24 wk: 20/20 (100%) Child C: 12 wk: 1/2 (50%) 24 wk: 4/5 (80%) FCH: 12 and 24 wk: 100% Genotype 4: No cirrhosis: 12 and 24 wk 100% Child A: 12 wk 3/4 (75%) 24 wk 100% Child B: 12 and 24 wk 100% Child C: 12 wk 0%	MELD improved in 58% Child B to A: 52% Child C to B: 60%
Poordad *et al*[100], 2016	G1a: 31/53 (58.5%) G1b: 10/53 (18.9%) G2: 0 G3: 11/53 (20.7%) G4: 0 G6: 1/53 (1.9%)	F0: 6 F1: 10 F2: 7 F3: 13 F4: 16 ND: 1	DCV/SOF + RBV 12 and 24 wk	50/53 (94%)	-
Brown *et al*[111], 2016	G1a: 87/151 (57.6%) G1b: 42/151 (27.8%) G1 (unspecified): 22/151 (14.6%)	Cirrhosis: 97/151 (64.2%)	SMV/SOF ± RBV	133/151 (88%) SMV/SOF 105/119 (88%) SMV/SOF + RBV 28/32 (88%)	-

LDV: Ledipasvir; SOF: Sofosbuvir; RBV: Ribavirin; DCV: Daclatasvir; SMV: Simeprevir; RVR: Rapid virological response (HCV-RNA < 15 UI after 4 wk of treatment); EVR: Early virological response (HCV-RNA < 15 UI after 12 wk of treatment); GRZ: Grazoprevir; ELB: Elbasvir; VEL: Velpatasvir; FCH: Fibrosing cholestatic hepatitis; HCV: Hepatitis C virus.

recent data and projections.

Sievert *et al*[113] recently analysed the future effects of the increase in SVR rates in the Australian population, taking into account three different models: Without increasing (first scenario) or increasing (second scenario) the number of treated patients and, finally, considering treatment prescription restricted to patients with fibrosis ⩾ F3 only (third scenario).

Applying the model of restricted prescription in the time period between 2015 and 2017, an estimated reduction by 51% of HCC development and by 56% and 54% of compensated and decompensated cirrhosis could be expected in 2030, respectively, as well as a 56% decrease in mortality rates. The cumulative costs of HCV infection were reduced by 26% from the base case. If the time span was extended to all years, a 90% decrease in compensated and decompensated liver cirrhotic patients was expected by 2030, with a reduction of HCC by 84%. In absence of eligibility restriction, chronically infected people were estimated to reduce by 60% in 2030, with a slightly lower decrease of cases of cirrhosis and HCC and comparable

cumulative costs reduction.

A similar study conducted on the French population analysed the reduction in the need for LT associated with HCV infection treatment. Based on two main scenarios constructed by estimating the number of LT candidates between 2013-2022, the authors demonstrated that antiviral treatments will avoid 4425 transplants, reducing by 45% and by 88% the gap between liver organs request and availability for patients with decompensated cirrhosis and HCC, respectively. This will allow for satisfaction of the LT demand for patients affected by HCC within 2022, although (probably) the same results cannot be achieved for decompensated cirrhotic patients[114].

Finally, Kabiri *et al*[115] published a transition model analysis to predict the effect of HCV therapies in the United States. Compared to a scenario including new therapies but with limited treatment capacities and risk-based or birth-cohort screening, a scenario with universal screening and absence of treatment limitations was able to prevent 91000 cases of HCC, 128800 cases of decompensated cirrhosis, 153200 liver-related deaths, and 13400 LT. The authors concluded that HCV might be destined to become a rare disease within 2036.

Although the major limitation of these studies is represented by the correct estimation of treatment response rates, as well as by the quantification of treatment costs, which are in constant evolution, they may provide a useful projection of the evolution of HCV-related health and economic burden in the near future.

CONCLUSION

The discovery of DAAs has radically changed the world scene of hepatitis C infection and its associated morbidity and mortality.

The current evolution and revolution of HCV antiviral treatment has increased the number of patients achieving viral eradication and, therefore, is going to reduce the incidence of cirrhosis, the rate of liver decompensation and HCC development, as well as patients' mortality. This will probably lead to a decrease in the need for LT, providing an adequate supply for nearly all patients with HCC and part of those with decompensated cirrhosis. The future widespread use of these new antivirals might also influence the policy of donor selection, leading to the expansion of the pool of available liver organs, since HCV infection may represent no more a contraindication for the use of liver grafts.

Although DAAs have made it possible to envisage a bright future in the fight against HCV-related liver disease, only long-term follow-up studies will allow for accurate quantification of the benefit obtained. The assessment of less evident effects of the new antivirals, such as microenvironmental and immunologic changes in the liver, is also mandatory to predict and avoid the occurrence of possible unexpected consequences.

Finally, the disparity in the use of DAAs throughout the world caused by the high costs and the restricted availability makes it difficult to draw definitive conclusions about the future epidemiology and evolution of HCV-related liver disease worldwide.

REFERENCES

1 **European Association for Study of Liver.** EASL Recommendations on Treatment of Hepatitis C 2015. *J Hepatol* 2015; **63**: 199-236 [PMID: 25911336 DOI: 10.1016/j.jhep.2015.03.025]

2 **Global Burden Of Hepatitis C Working Group.** Global burden of disease (GBD) for hepatitis C. *J Clin Pharmacol* 2004; **44**: 20-29 [PMID: 14681338 DOI: 10.1177/0091270003258669]

3 **Lavanchy D**. The global burden of hepatitis C. *Liver Int* 2009; **29** Suppl 1: 74-81 [PMID: 19207969 DOI: 10.1111/j.1478-3231.2008.01934.x]

4 **Armstrong GL**, Wasley A, Simard EP, McQuillan GM, Kuhnert WL, Alter MJ. The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. *Ann Intern Med* 2006; **144**: 705-714 [PMID: 16702586]

5 **Hoshida Y**, Fuchs BC, Bardeesy N, Baumert TF, Chung RT. Pathogenesis and prevention of hepatitis C virus-induced hepatocellular carcinoma. *J Hepatol* 2014; **61**: S79-S90 [PMID: 25443348 DOI: 10.1016/j.jhep.2014.07.010]

6 **Westbrook RH**, Dusheiko G. Natural history of hepatitis C. *J Hepatol* 2014; **61**: S58-S68 [PMID: 25443346 DOI: 10.1016/j.jhep.2014.07.012]

7 **Ferri C**, Ramos-Casals M, Zignego AL, Arcaini L, Roccatello D, Antonelli A, Saadoun D, Desbois AC, Sebastiani M, Casato M, Lamprecht P, Mangia A, Tzioufas AG, Younossi ZM, Cacoub P; ISG-EHCV coauthors. International diagnostic guidelines for patients with HCV-related extrahepatic manifestations. A multidisciplinary expert statement. *Autoimmun Rev* 2016; **15**: 1145-1160 [PMID: 27640316 DOI: 10.1016/j.autrev.2016.09.006]

8 **Younossi Z**, Park H, Henry L, Adeyemi A, Stepanova M. Extrahepatic Manifestations of Hepatitis C: A Meta-analysis of Prevalence, Quality of Life, and Economic Burden. *Gastroenterology* 2016; **150**: 1599-1608 [PMID: 26924097 DOI: 10.1053/j.gastro.2016.02.039]

9 **Fried MW**, Shiffman ML, Reddy KR, Smith C, Marinos G, Gonçales FL, Häussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; **347**: 975-982 [PMID: 12324553 DOI: 10.1056/NEJMoa020047]

10 **Manns MP**, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; **358**: 958-965 [PMID: 11583749]

11 **Hadziyannis SJ**, Sette H, Morgan TR, Balan V, Diago M, Marcellin P, Ramadori G, Bodenheimer H, Bernstein D, Rizzetto M, Zeuzem S, Pockros PJ, Lin A, Ackrill AM. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004; **140**: 346-355 [PMID: 14996676]

12 **Bacon BR**, Gordon SC, Lawitz E, Marcellin P, Vierling JM, Zeuzem S, Poordad F, Goodman ZD, Sings HL, Boparai N, Burroughs M, Brass CA, Albrecht JK, Esteban R. Boceprevir for previously treated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1207-1217 [PMID: 21449784 DOI: 10.1056/NEJMoa1009482]

13 **Sherman KE**, Flamm SL, Afdhal NH, Nelson DR, Sulkowski MS, Everson GT, Fried MW, Adler M, Reesink HW, Martin M, Sankoh AJ, Adda N, Kauffman RS, George S, Wright CI, Poordad F. Response-guided telaprevir combination treatment for hepatitis C virus infection. *N Engl J Med* 2011; **365**: 1014-1024 [PMID: 21916639 DOI: 10.1056/NEJMoa1014463]

14 **Jacobson IM**, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, Bzowej NH, Marcellin P, Muir AJ, Ferenci P, Flisiak R, George J, Rizzetto M, Shouval D, Sola R, Terg RA, Yoshida EM,

Adda N, Bengtsson L, Sankoh AJ, Kieffer TL, George S, Kauffman RS, Zeuzem S. Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med* 2011; **364**: 2405-2416 [PMID: 21696307 DOI: 10.1056/NEJMoa1012912]

15 **Poordad F**, McCone J, Bacon BR, Bruno S, Manns MP, Sulkowski MS, Jacobson IM, Reddy KR, Goodman ZD, Boparai N, DiNubile MJ, Sniukiene V, Brass CA, Albrecht JK, Bronowicki JP. Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1195-1206 [PMID: 21449783 DOI: 10.1056/NEJMoa1010494]

16 **Hézode C**, Fontaine H, Dorival C, Zoulim F, Larrey D, Canva V, De Ledinghen V, Poynard T, Samuel D, Bourliere M, Alric L, Raabe JJ, Zarski JP, Marcellin P, Riachi G, Bernard PH, Loustaud-Ratti V, Chazouilleres O, Abergel A, Guyader D, Metivier S, Tran A, Di Martino V, Causse X, Dao T, Lucidarme D, Portal I, Cacoub P, Gournay J, Grando-Lemaire V, Hillon P, Attali P, Fontanges T, Rosa I, Petrov-Sanchez V, Barthe Y, Pawlotsky JM, Pol S, Carrat F, Bronowicki JP. Effectiveness of telaprevir or boceprevir in treatment-experienced patients with HCV genotype 1 infection and cirrhosis. *Gastroenterology* 2014; **147**: 132-142.e4 [PMID: 24704719 DOI: 10.1053/j.gastro.2014.03.051]

17 HCV Guidance: Recommendations for Testing, Managing, and Treating Hepatitis C. Available from: URL: http://www.hcvguidelines.org

18 FDA approves Sovaldi for chronic hepatitis C. FDA news release US food and Drug administration, December 6, 2013

19 **Sofia MJ**, Bao D, Chang W, Du J, Nagarathnam D, Rachakonda S, Reddy PG, Ross BS, Wang P, Zhang HR, Bansal S, Espiritu C, Keilman M, Lam AM, Steuer HM, Niu C, Otto MJ, Furman PA. Discovery of a β-d-2'-deoxy-2'-α-fluoro-2'-β-C-methyluridine nucleotide prodrug (PSI-7977) for the treatment of hepatitis C virus. *J Med Chem* 2010; **53**: 7202-7218 [PMID: 20845908 DOI: 10.1021/jm100863x]

20 **Lawitz E**, Mangia A, Wyles D, Rodriguez-Torres M, Hassanein T, Gordon SC, Schultz M, Davis MN, Kayali Z, Reddy KR, Jacobson IM, Kowdley KV, Nyberg L, Subramanian GM, Hyland RH, Arterburn S, Jiang D, McNally J, Brainard D, Symonds WT, McHutchison JG, Sheikh AM, Younossi Z, Gane EJ. Sofosbuvir for previously untreated chronic hepatitis C infection. *N Engl J Med* 2013; **368**: 1878-1887 [PMID: 23607594 DOI: 10.1056/NEJMoa1214853]

21 **Lawitz E**, Sulkowski MS, Ghalib R, Rodriguez-Torres M, Younossi ZM, Corregidor A, DeJesus E, Pearlman B, Rabinovitz M, Gitlin N, Lim JK, Pockros PJ, Scott JD, Fevery B, Lambrecht T, Ouwerkerk-Mahadevan S, Callewaert K, Symonds WT, Picchio G, Lindsay KL, Beumont M, Jacobson IM. Simeprevir plus sofosbuvir, with or without ribavirin, to treat chronic infection with hepatitis C virus genotype 1 in non-responders to pegylated interferon and ribavirin and treatment-naive patients: the COSMOS randomised study. *Lancet* 2014; **384**: 1756-1765 [PMID: 25078309 DOI: 10.1016/S0140-6736(14)61036-9]

22 **Afdhal N**, Reddy KR, Nelson DR, Lawitz E, Gordon SC, Schiff E, Nahass R, Ghalib R, Gitlin N, Herring R, Lalezari J, Younes ZH, Pockros PJ, Di Bisceglie AM, Arora S, Subramanian GM, Zhu Y, Dvory-Sobol H, Yang JC, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Sulkowski M, Kwo P. Ledipasvir and sofosbuvir for previously treated HCV genotype 1 infection. *N Engl J Med* 2014; **370**: 1483-1493 [PMID: 24725238 DOI: 10.1056/NEJMoa1316366]

23 **Kowdley KV**, Gordon SC, Reddy KR, Rossaro L, Bernstein DE, Lawitz E, Shiffman ML, Schiff E, Ghalib R, Ryan M, Rustgi V, Chojkier M, Herring R, Di Bisceglie AM, Pockros PJ, Subramanian GM, An D, Svarovskaia E, Hyland RH, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Pound D, Fried MW. Ledipasvir and sofosbuvir for 8 or 12 weeks for chronic HCV without cirrhosis. *N Engl J Med* 2014; **370**: 1879-1888 [PMID: 24720702 DOI: 10.1056/NEJMoa1402355]

24 **Sulkowski MS,** Gardiner DF, Rodriguez-Torres M, Reddy KR, Hassanein T, Jacobson I, Lawitz E, Lok AS, Hinestrosa F, Thuluvath PJ, Schwartz H, Nelson DR, Everson GT, Eley T, Wind-Rotolo M, Huang SP, Gao M, Hernandez D, McPhee F, Sherman D, Hindes R, Symonds W, Pasquinelli C, Grasela DM; AI444040 Study Group. Daclatasvir plus sofosbuvir for previously treated or untreated chronic HCV infection. *N Engl J Med* 2014; **370**: 211-221 [PMID: 24428467 DOI: 10.1056/NEJMoa1306218]

25 **Foster GR**, Afdhal N, Roberts SK, Bräu N, Gane EJ, Pianko S, Lawitz E, Thompson A, Shiffman ML, Cooper C, Towner WJ, Conway B, Ruane P, Bourlière M, Asselah T, Berg T, Zeuzem S, Rosenberg W, Agarwal K, Stedman CA, Mo H, Dvory-Sobol H, Han L, Wang J, McNally J, Osinusi A, Brainard DM, McHutchison JG, Mazzotta F, Tran TT, Gordon SC, Patel K, Reau N, Mangia A, Sulkowski M. Sofosbuvir and Velpatasvir for HCV Genotype 2 and 3 Infection. *N Engl J Med* 2015; **373**: 2608-2617 [PMID: 26575258 DOI: 10.1056/NEJMoa1512612]

26 **Curry MP**, O'Leary JG, Bzowej N, Muir AJ, Korenblat KM, Fenkel JM, Reddy KR, Lawitz E, Flamm SL, Schiano T, Teperman L, Fontana R, Schiff E, Fried M, Doehle B, An D, McNally J, Osinusi A, Brainard DM, McHutchison JG, Brown RS, Charlton M. Sofosbuvir and Velpatasvir for HCV in Patients with Decompensated Cirrhosis. *N Engl J Med* 2015; **373**: 2618-2628 [PMID: 26569658 DOI: 10.1056/NEJMoa1512614]

27 **Poordad F**, Hezode C, Trinh R, Kowdley KV, Zeuzem S, Agarwal K, Shiffman ML, Wedemeyer H, Berg T, Yoshida EM, Forns X, Lovell SS, Da Silva-Tillmann B, Collins CA, Campbell AL, Podsadecki T, Bernstein B. ABT-450/r-ombitasvir and dasabuvir with ribavirin for hepatitis C with cirrhosis. *N Engl J Med* 2014; **370**: 1973-1982 [PMID: 24725237 DOI: 10.1056/NEJMoa1402869]

28 **Zeuzem S**, Jacobson IM, Baykal T, Marinho RT, Poordad F, Bourlière M, Sulkowski MS, Wedemeyer H, Tam E, Desmond P, Jensen DM, Di Bisceglie AM, Varunok P, Hassanein T, Xiong J, Pilot-Matias T, DaSilva-Tillmann B, Larsen L, Podsadecki T, Bernstein B. Retreatment of HCV with ABT-450/r-ombitasvir and dasabuvir with ribavirin. *N Engl J Med* 2014; **370**: 1604-1614 [PMID: 24720679 DOI: 10.1056/NEJMoa1401561]

29 **Lawitz E**, Gane E, Pearlman B, Tam E, Ghesquiere W, Guyader D, Alric L, Bronowicki JP, Lester L, Sievert W, Ghalib R, Balart L, Sund F, Lagging M, Dutko F, Shaughnessy M, Hwang P, Howe AY, Wahl J, Robertson M, Barr E, Haber B. Efficacy and safety of 12 weeks versus 18 weeks of treatment with grazoprevir (MK-5172) and elbasvir (MK-8742) with or without ribavirin for hepatitis C virus genotype 1 infection in previously untreated patients with cirrhosis and patients with previous null response with or without cirrhosis (C-WORTHY): a randomised, open-label phase 2 trial. *Lancet* 2015; **385**: 1075-1086 [PMID: 25467591 DOI: 10.1016/S0140-6736(14)61795-5]

30 **Gentile I,** Scotto R, Zappulo E, Buonomo AR, Pinchera B, Borgia G. Investigational direct-acting antivirals in hepatitis C treatment: the latest drugs in clinical development. *Expert Opin Investig Drugs* 2016; **25**: 557-572 [DOI: 10.1517/13543784.2016.1161023]

31 **Forton DM**. How much of a problem is resistance in treating hepatitis C? *Curr Opin Infect Dis* 2016; **29**: 625-631 [PMID: 27673712]

32 **Jiménez-Pérez M,** González-Grande R, España Contreras P, Pinazo Martínez I, de la Cruz Lombardo J, Olmedo Martín R. Treatment of chronic hepatitis C with direct-acting antivirals: The role of resistance. *World J Gastroenterol* 2016; **22**: 6573-6581 [PMID: 27547001 DOI: 10.3748 wjg.v22.i29.6573]

33 **Righi E**, Londero A, Carnelutti A, Baccarani U, Bassetti M. Impact of new treatment options for hepatitis C virus infection in liver transplantation. *World J Gastroenterol* 2015; **21**: 10760-10775 [PMID: 26478668 DOI: 10.3748/wjg.v21.i38.10760]

34 **Szabo SM**, Samp JC, Walker DR, Lane S, Cline SK, Gooch KL, Jimenez-Mendez R, Levy AR. Liver-specific case fatality due to chronic hepatitis C virus infection: a systematic review. *Ann Hepatol* 2015; **14**: 618-630 [PMID: 26256890]

35 **Xu F**, Moorman AC, Tong X, Gordon SC, Rupp LB, Lu M, Teshale EH, Spradling PR, Boscarino JA, Trinacty CM, Schmidt MA, Holmberg SD. All-Cause Mortality and Progression Risks to Hepatic Decompensation and Hepatocellular Carcinoma in Patients Infected With Hepatitis C Virus. *Clin Infect Dis* 2016; **62**: 289-297 [PMID: 26417034 DOI: 10.1093/cid/civ860]

36 **El-Serag HB**, Mason AC. Rising incidence of hepatocellular carcinoma in the United States. *N Engl J Med* 1999; **340**: 745-750 [PMID: 10072408 DOI: 10.1056/NEJM199903113401001]

37 **Kao JH**, Chen DS. Changing disease burden of hepatocellular carcinoma in the Far East and Southeast Asia. *Liver Int* 2005; **25**: 696-703 [PMID: 15998418 DOI: 10.1111/j.1478-3231.2005.01139.x]

38 **Bruix J**, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]

39 **Szabó E**, Lotz G, Páska C, Kiss A, Schaff Z. Viral hepatitis: new data on hepatitis C infection. *Pathol Oncol Res* 2003; **9**: 215-221 [PMID: 14688826 DOI: 10.1007/BF02893380]

40 **Adam R**, Karam V, Delvart V, O'Grady J, Mirza D, Klempnauer J, Castaing D, Neuhaus P, Jamieson N, Salizzoni M, Pollard S, Lerut J, Paul A, Garcia-Valdecasas JC, Rodríguez FS, Burroughs A. Evolution of indications and results of liver transplantation in Europe. A report from the European Liver Transplant Registry (ELTR). *J Hepatol* 2012; **57**: 675-688 [PMID: 22609307 DOI: 10.1016/j.jhep.2012.04.015]

41 **Veldt BJ**, Saracco G, Boyer N, Cammà C, Bellobuono A, Hopf U, Castillo I, Weiland O, Nevens F, Hansen BE, Schalm SW. Long term clinical outcome of chronic hepatitis C patients with sustained virological response to interferon monotherapy. *Gut* 2004; **53**: 1504-1508 [PMID: 15361504 DOI: 10.1136/gut.2003.038257]

42 **Maruoka D**, Imazeki F, Arai M, Kanda T, Fujiwara K, Yokosuka O. Long-term cohort study of chronic hepatitis C according to interferon efficacy. *J Gastroenterol Hepatol* 2012; **27**: 291-299 [PMID: 21793911 DOI: 10.1111/j.1440-1746.2011.06871.x]

43 **Hill A.** Effects of Sustained Virological Response on the risk of liver transplant, hepatocellular carcinoma, death and re-infection: meta-analysis of 129 studies in 34563 patients with Hepatitis C infection. 65th Annual Meeting of the American Association for the Study of Liver Diseases AASLD. Boston, MA, USA, 2014 Nov 7-11

44 **Cardoso AC**, Moucari R, Figueiredo-Mendes C, Ripault MP, Giuily N, Castelnau C, Boyer N, Asselah T, Martinot-Peignoux M, Maylin S, Carvalho-Filho RJ, Valla D, Bedossa P, Marcellin P. Impact of peginterferon and ribavirin therapy on hepatocellular carcinoma: incidence and survival in hepatitis C patients with advanced fibrosis. *J Hepatol* 2010; **52**: 652-657 [PMID: 20346533 DOI: 10.1016/j.jhep.2009.12.028]

45 **Iacobellis A**, Siciliano M, Perri F, Annicchiarico BE, Leandro G, Caruso N, Accadia L, Bombardieri G, Andriulli A. Peginterferon alfa-2b and ribavirin in patients with hepatitis C virus and decompensated cirrhosis: a controlled study. *J Hepatol* 2007; **46**: 206-212 [PMID: 17125876]

46 **Tada T**, Kumada T, Toyoda H, Kiriyama S, Tanikawa M, Hisanaga Y, Kanamori A, Kitabatake S, Yama T, Tanaka J. Viral eradication reduces all-cause mortality in patients with chronic hepatitis C virus infection: a propensity score analysis. *Liver Int* 2016; **36**: 817-826 [PMID: 26787002 DOI: 10.1111/liv.13071]

47 **Veldt BJ**, Heathcote EJ, Wedemeyer H, Reichen J, Hofmann WP, Zeuzem S, Manns MP, Hansen BE, Schalm SW, Janssen HL. Sustained virologic response and clinical outcomes in patients with chronic hepatitis C and advanced fibrosis. *Ann Intern Med* 2007; **147**: 677-684 [PMID: 18025443]

48 **van der Meer AJ**, Veldt BJ, Feld JJ, Wedemeyer H, Dufour JF, Lammert F, Duarte-Rojo A, Heathcote EJ, Manns MP, Kuske L, Zeuzem S, Hofmann WP, de Knegt RJ, Hansen BE, Janssen HL. Association between sustained virological response and all-cause mortality among patients with chronic hepatitis C and advanced hepatic fibrosis. *JAMA* 2012; **308**: 2584-2593 [PMID: 23268517 DOI: 10.1001/jama.2012.144878]

49 **Bruno S**, Di Marco V, Iavarone M, Roffi L, Crosignani A, Calvaruso V, Aghemo A, Cabibbo G, Viganò M, Boccaccio V, Craxí A, Colombo M, Maisonneuve P. Survival of patients with HCV cirrhosis and sustained virologic response is similar to the general population. *J Hepatol* 2016; **64**: 1217-1223 [PMID: 27059129 DOI: 10.1016/j.jhep.2016.01.034]

50 **Sun M**, Kisseleva T. Reversibility of liver fibrosis. *Clin Res Hepatol Gastroenterol* 2015; **39** Suppl 1: S60-S63 [PMID: 26206574 DOI: 10.1016/j.clinre.2015.06.015]

51 **Iredale JP**. Hepatic stellate cell behavior during resolution of liver injury. *Semin Liver Dis* 2001; **21**: 427-436 [PMID: 11586470]

52 **D'Ambrosio R**, Aghemo A, Rumi MG, Ronchi G, Donato MF, Paradis V, Colombo M, Bedossa P. A morphometric and immunohistochemical study to assess the benefit of a sustained virological response in hepatitis C virus patients with cirrhosis. *Hepatology* 2012; **56**: 532-543 [PMID: 22271347 DOI: 10.1002/hep.25606]

53 **Mallet V**, Gilgenkrantz H, Serpaggi J, Verkarre V, Vallet-Pichard A, Fontaine H, Pol S. Brief communication: the relationship of regression of cirrhosis to outcome in chronic hepatitis C. *Ann Intern Med* 2008; **149**: 399-403 [PMID: 18794559]

54 **Poynard T**, McHutchison J, Manns M, Trepo C, Lindsay K, Goodman Z, Ling MH, Albrecht J. Impact of pegylated interferon alfa-2b and ribavirin on liver fibrosis in patients with chronic hepatitis C. *Gastroenterology* 2002; **122**: 1303-1313 [PMID: 11984517]

55 **Bruno S**, Boccaccio V, Russo ML, Maisonneuve P. Is the benefit of treating patients with cirrhosis proven? *Liver Int* 2016; **36** Suppl 1: 21-27 [PMID: 26725893 DOI: 10.1111/liv.13013]

56 **Reichard O**, Glaumann H, Frydén A, Norkrans G, Wejstål R, Weiland O. Long-term follow-up of chronic hepatitis C patients with sustained virological response to alpha-interferon. *J Hepatol* 1999; **30**: 783-787 [PMID: 10365802]

57 **Arif A**, Levine RA, Sanderson SO, Bank L, Velu RP, Shah A, Mahl TC, Gregory DH. Regression of fibrosis in chronic hepatitis C after therapy with interferon and ribavirin. *Dig Dis Sci* 2003; **48**: 1425-1430 [PMID: 12870807]

58 **George SL**, Bacon BR, Brunt EM, Mihindukulasuriya KL, Hoffmann J, Di Bisceglie AM. Clinical, virologic, histologic, and biochemical outcomes after successful HCV therapy: a 5-year follow-up of 150 patients. *Hepatology* 2009; **49**: 729-738 [PMID: 19072828 DOI: 10.1002/hep.22694]

59 **Poynard T**, Imbert-Bismut F, Ratziu V, Chevret S, Jardel C, Moussalli J, Messous D, Degos F. Biochemical markers of liver fibrosis in patients infected by hepatitis C virus: longitudinal validation in a randomized trial. *J Viral Hepat* 2002; **9**: 128-133 [PMID: 11876795]

60 **Shiratori Y**, Imazeki F, Moriyama M, Yano M, Arakawa Y, Yokosuka O, Kuroki T, Nishiguchi S, Sata M, Yamada G, Fujiyama S, Yoshida H, Omata M. Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy. *Ann Intern Med* 2000; **132**: 517-524 [PMID: 10744587]

61 **Pol S**, Carnot F, Nalpas B, Lagneau JL, Fontaine H, Serpaggi J, Serfaty L, Bedossa P, Bréchot C. Reversibility of hepatitis C virus-related cirrhosis. *Hum Pathol* 2004; **35**: 107-112 [PMID: 14745732]

62 **Maylin S**, Martinot-Peignoux M, Moucari R, Boyer N, Ripault MP, Cazals-Hatem D, Giuily N, Castelnau C, Cardoso AC, Asselah T, Féray C, Nicolas-Chanoine MH, Bedossa P, Marcellin P. Eradication of hepatitis C virus in patients successfully treated for chronic hepatitis C. *Gastroenterology* 2008; **135**: 821-829 [PMID: 18593587 DOI: 10.1053/j.gastro.2008.05.044]

63 **Wanless IR**, Nakashima E, Sherman M. Regression of human cirrhosis. Morphologic features and the genesis of incomplete septal cirrhosis. *Arch Pathol Lab Med* 2000; **124**: 1599-1607 [PMID: 11079009]

64 **Yoshida H**, Shiratori Y, Moriyama M, Arakawa Y, Ide T, Sata M, Inoue O, Yano M, Tanaka M, Fujiyama S, Nishiguchi S, Kuroki T, Imazeki F, Yokosuka O, Kinoyama S, Yamada G, Omata M. Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of Hepatocarcinogenesis by Interferon Therapy. *Ann Intern Med* 1999; **131**: 174-181 [PMID: 10428733]

65 **Yang JD**, Roberts LR. Hepatocellular carcinoma: A global view. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 448-458 [PMID: 20628345 DOI: 10.1038/nrgastro.2010.100]

66 **Lemon SM**, McGivern DR. Is hepatitis C virus carcinogenic? *Gastroenterology* 2012; **142**: 1274-1278 [PMID: 22537433 DOI: 10.1053/j.gastro.2012.01.045]

67 **Nishiguchi S**, Kuroki T, Nakatani S, Morimoto H, Takeda T, Nakajima S, Shiomi S, Seki S, Kobayashi K, Otani S. Randomised

trial of effects of interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. *Lancet* 1995; **346**: 1051-1055 [PMID: 7564784]

68 **Morgan RL**, Baack B, Smith BD, Yartel A, Pitasi M, Falck-Ytter Y. Eradication of hepatitis C virus infection and the development of hepatocellular carcinoma: a meta-analysis of observational studies. *Ann Intern Med* 2013; **158**: 329-337 [PMID: 23460056 DOI: 10.7326/0003-4819-158-5-201303050-00005]

69 **Kanogawa N**, Ogasawara S, Chiba T, Saito T, Motoyama T, Suzuki E, Ooka Y, Tawada A, Kanda T, Mikami S, Azemoto R, Kaiho T, Shinozaki M, Ohtsuka M, Miyazaki M, Yokosuka O. Sustained virologic response achieved after curative treatment of hepatitis C virus-related hepatocellular carcinoma as an independent prognostic factor. *J Gastroenterol Hepatol* 2015; **30**: 1197-1204 [PMID: 25682720 DOI: 10.1111/jgh.12925]

70 **Mazzaferro V**, Romito R, Schiavo M, Mariani L, Camerini T, Bhoori S, Capussotti L, Calise F, Pellicci R, Belli G, Tagger A, Colombo M, Bonino F, Majno P, Llovet JM. Prevention of hepatocellular carcinoma recurrence with alpha-interferon after liver resection in HCV cirrhosis. *Hepatology* 2006; **44**: 1543-1554 [PMID: 17133492 DOI: 10.1002/hep.21415]

71 **Zhang W**, Song TQ, Zhang T, Wu Q, Kong DL, Li Q, Sun HC. Adjuvant interferon for early or late recurrence of hepatocellular carcinoma and mortality from hepatocellular carcinoma following curative treatment: A meta-analysis with comparison of different types of hepatitis. *Mol Clin Oncol* 2014; **2**: 1125-1134 [PMID: 25279210 DOI: 10.3892/mco.2014.386]

72 **Saito T**, Chiba T, Suzuki E, Shinozaki M, Goto N, Kanogawa N, Motoyama T, Ogasawara S, Ooka Y, Tawada A, Kanda T, Miyazaki M, Yokosuka O. Effect of previous interferon-based therapy on recurrence after curative treatment of hepatitis C virus-related hepatocellular carcinoma. *Int J Med Sci* 2014; **11**: 707-712 [PMID: 24843320 DOI: 10.7150/ijms.8764]

73 **George PM**, Badiger R, Alazawi W, Foster GR, Mitchell JA. Pharmacology and therapeutic potential of interferons. *Pharmacol Ther* 2012; **135**: 44-53 [PMID: 22484806 DOI: 10.1016/j.pharmthera.2012.03.006]

74 **Lee D**, Chung YH, Kim JA, Park WH, Jin YJ, Shim JH, Ryu SH, Jang MK, Yu E, Lee YJ. Safety and efficacy of adjuvant pegylated interferon therapy for metastatic tumor antigen 1-positive hepatocellular carcinoma. *Cancer* 2013; **119**: 2239-2246 [PMID: 23564564 DOI: 10.1002/cncr.28082]

75 **Meissner EG**, Wu D, Osinusi A, Bon D, Virtaneva K, Sturdevant D, Porcella S, Wang H, Herrmann E, McHutchison J, Suffredini AF, Polis M, Hewitt S, Prokunina-Olsson L, Masur H, Fauci AS, Kottilil S. Endogenous intrahepatic IFNs and association with IFN-free HCV treatment outcome. *J Clin Invest* 2014; **124**: 3352-3363 [PMID: 24983321 DOI: 10.1172/JCI75938]

76 **Serti E**, Chepa-Lotrea X, Kim YJ, Keane M, Fryzek N, Liang TJ, Ghany M, Rehermann B. Successful Interferon-Free Therapy of Chronic Hepatitis C Virus Infection Normalizes Natural Killer Cell Function. *Gastroenterology* 2015; **149**: 190-200.e2 [PMID: 25754160 DOI: 10.1053/j.gastro.2015.03.004]

77 **Toyoda H**, Kumada T, Tada T, Kiriyama S, Tanikawa M, Hisanaga Y, Kanamori A, Kitabatake S, Ito T. Risk factors of hepatocellular carcinoma development in non-cirrhotic patients with sustained virologic response for chronic hepatitis C virus infection. *J Gastroenterol Hepatol* 2015; **30**: 1183-1189 [PMID: 25678094 DOI: 10.1111/jgh.12915]

78 **Arase Y**, Kobayashi M, Suzuki F, Suzuki Y, Kawamura Y, Akuta N, Kobayashi M, Sezaki H, Saito S, Hosaka T, Ikeda K, Kumada H, Kobayashi T. Effect of type 2 diabetes on risk for malignancies includes hepatocellular carcinoma in chronic hepatitis C. *Hepatology* 2013; **57**: 964-973 [PMID: 22991257 DOI: 10.1002/hep.26087]

79 **Matsumura H**, Nirei K, Nakamura H, Higuchi T, Arakawa Y, Ogawa M, Tanaka N, Moriyama M. Histopathology of type C liver disease for determining hepatocellular carcinoma risk factors. *World J Gastroenterol* 2013; **19**: 4887-4896 [PMID: 23946593 DOI: 10.3748/wjg.v19.i30.4887]

80 **Reig M**, Mariño Z, Perelló C, Iñarrairaegui M, Ribeiro A, Lens S, Díaz A, Vilana R, Darnell A, Varela M, Sangro B, Calleja JL, Forns X, Bruix J. Unexpected high rate of early tumor recurrence in patients with HCV-related HCC undergoing interferon-free therapy. *J Hepatol* 2016; **65**: 719-726 [PMID: 27084592 DOI: 10.1016/j.jhep.2016.04.008]

81 **Conti F**, Buonfiglioli F, Scuteri A, Crespi C, Bolondi L, Caraceni P, Foschi FG, Lenzi M, Mazzella G, Verucchi G, Andreone P, Brillanti S. Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals. *J Hepatol* 2016; **65**: 727-733 [PMID: 27349488 DOI: 10.1016/j.jhep.2016.06.015]

82 **Yang JD**, Aqel BA, Pungpapong S, Gores GJ, Roberts LR, Leise MD. Direct acting antiviral therapy and tumor recurrence after liver transplantation for hepatitis C-associated hepatocellular carcinoma. *J Hepatol* 2016; **65**: 859-860 [PMID: 27392425 DOI: 10.1016/j.jhep.2016.06.023]

83 **ANRS collaborative study group on hepatocellular carcinoma (ANRS CO22 HEPATHER, CO12 CirVir and CO23 CUPILT cohorts).** Lack of evidence of an effect of direct-acting antivirals on the recurrence of hepatocellular carcinoma: Data from three ANRS cohorts. *J Hepatol* 2016; **65**: 734-740 [PMID: 27288051 DOI: 10.1016/j.jhep.2016.05.045]

84 **Cheung MC**, Walker AJ, Hudson BE, Verma S, McLauchlan J, Mutimer DJ, Brown A, Gelson WT, MacDonald DC, Agarwal K, Foster GR, Irving WL. Outcomes after successful direct-acting antiviral therapy for patients with chronic hepatitis C and decompensated cirrhosis. *J Hepatol* 2016; **65**: 741-747 [PMID: 27388925 DOI: 10.1016/j.jhep.2016.06.019]

85 **Yang JD**, Larson JJ, Watt KD, Allen AM, Wiesner RH, Gores GJ, Roberts LR, Heimbach JA, Leise MD. Hepatocellular Carcinoma is the Most Common Indication for Liver Transplantation and Placement on the Waitlist in the United States. *Clin Gastroenterol Hepatol* 2016; Epub ahead of print [PMID: 28013117 DOI: 10.1016/j.cgh.2016.11.034]

86 **Kozbial K**, Moser S, Schwarzer R, Laferl H, Al-Zoairy R, Stauber R, Stättermayer AF, Beinhardt S, Graziadei I, Freissmuth C, Maieron A, Gschwantler M, Strasser M, Peck-Radosalvjevic M, Trauner M, Hofer H, Ferenci P.Unexpected high incidence of hepatocellular carcinoma in cirrhotic patients with sustained virologic response following interferon-free direct-acting antiviral treatment. *J Hepatol* 2016; **65**: 856-858

87 **Cardoso H**, Vale AM, Rodrigues S, Gonçalves R, Albuquerque A, Pereira P, Lopes S, Silva M, Andrade P, Morais R, Coelho R, Macedo G. High incidence of hepatocellular carcinoma following successful interferon-free antiviral therapy for hepatitis C associated cirrhosis. *J Hepatol* 2016; **65**: 1070-1071

88 **Llovet JM**, Villanueva A. Liver cancer: Effect of HCV clearance with direct-acting antiviral agents on HCC. *Nat Rev Gastroenterol Hepatol* 2016; **13**: 561-562 [PMID: 27580683 DOI: 10.1038/nrgastro.2016.140]

89 **Berenguer M**. Natural history of recurrent hepatitis C. *Liver Transpl* 2002; **8**: S14-S18 [PMID: 12362293 DOI: 10.1053/jlts.2002.35781]

90 **Berenguer M**, Prieto M, San Juan F, Rayón JM, Martinez F, Carrasco D, Moya A, Orbis F, Mir J, Berenguer J. Contribution of donor age to the recent decrease in patient survival among HCV-infected liver transplant recipients. *Hepatology* 2002; **36**: 202-210 [PMID: 12085366 DOI: 10.1053/jhep.2002.33993]

91 **Ponziani FR**, Gasbarrini A, Pompili M, Burra P, Fagiuoli S. Management of hepatitis C virus infection recurrence after liver transplantation: an overview. *Transplant Proc* 2011; **43**: 291-295 [PMID: 21335208 DOI: 10.1016/j.transproceed.2010.09.102]

92 **Ponziani FR**, Milani A, Gasbarrini A, Zaccaria R, Viganò R, Iemmolo RM, Donato MF, Rendina M, Toniutto P, Pasulo L, Cescon M, Burra P, Miglioresi L, Merli M, Paolo DD, Fagiuoli S, Pompili M. AISF RECOLT C- Group. Treatment of genotype-1 hepatitis C recurrence after liver transplant improves surivival in both sustained responders and relapsers. *Transplant Int* 2013; **26**: 281-289 [PMID: 23230956 DOI: 10.1111/tri.12027]

93 **Hüsing A**, Kabar I, Schmidt HH, Heinzow HS. Hepatitis C in Special Patient Cohorts: New Opportunities in Decompensated Liver Cirrhosis, End-Stage Renal Disease and Transplant

Medicine. *Int J Mol Sci* 2015; **16**: 18033-18053 [PMID: 26251895 DOI: 10.3390/ijms160818033]

94 **Chen T**, Terrault NA. Perspectives on treating hepatitis C infection in the liver transplantation setting. *Curr Opin Organ Transplant* 2016; **21**: 111-119 [PMID: 26927201 DOI: 10.1097/MOT.0000000000000288]

95 **Curry MP**, Forns X, Chung RT, Terrault NA, Brown R, Fenkel JM, Gordon F, O'Leary J, Kuo A, Schiano T, Everson G, Schiff E, Befeler A, Gane E, Saab S, McHutchison JG, Subramanian GM, Symonds WT, Denning J, McNair L, Arterburn S, Svarovskaia E, Moonka D, Afdhal N. Sofosbuvir and ribavirin prevent recurrence of HCV infection after liver transplantation: an open-label study. *Gastroenterology* 2015; **148**: 100-107.e1 [PMID: 25261839 DOI: 10.1053/j.gastro.2014.09.023]

96 **Donato MF**, Monico S, Malinverno F, Aghemo A, Maggioni M, Reggiani P, Colombo M. Bridging all oral DAA therapy from wait time to post-liver transplant to improve HCV eradication? *Liver Int* 2015; **35**: 1-4 [PMID: 25074044 DOI: 10.1111/liv.12646]

97 **Belli LS**, Berenguer M, Cortesi PA, Strazzabosco M, Rockenschaub SR, Martini S, Morelli C, Donato F, Volpes R, Pageaux GP, Coilly A, Fagiuoli S, Amaddeo G, Perricone G, Vinaixa C, Berlakovich G, Facchetti R, Polak W, Muiesan P, Duvoux C. Delisting of liver transplant candidates with chronic hepatitis C after viral eradication: A European study. *J Hepatol* 2016; **65**: 524-531 [PMID: 27212241 DOI: 10.1016/j.jhep.2016.05.010]

98 **Charlton M**, Everson GT, Flamm SL, Kumar P, Landis C, Brown RS, Fried MW, Terrault NA, O'Leary JG, Vargas HE, Kuo A, Schiff E, Sulkowski MS, Gilroy R, Watt KD, Brown K, Kwo P, Pungpapong S, Korenblat KM, Muir AJ, Teperman L, Fontana RJ, Denning J, Arterburn S, Dvory-Sobol H, Brandt-Sarif T, Pang PS, McHutchison JG, Reddy KR, Afdhal N. Ledipasvir and Sofosbuvir Plus Ribavirin for Treatment of HCV Infection in Patients With Advanced Liver Disease. *Gastroenterology* 2015; **149**: 649-659 [PMID: 25985734 DOI: 10.1053/j.gastro.2015.05.010]

99 **Jacobson I.** Efficacy and safety of grazoprevir and elbasvir in hepatitis C genotype 1-infected patients with Child-Pugh class B cirrhosis (CSALT part A). *J Hepatol* 2015; **62** (Suppl 2): S193-S194

100 **Poordad F**, Schiff ER, Vierling JM, Landis C, Fontana RJ, Yang R, McPhee F, Hughes EA, Noviello S, Swenson ES. Daclatasvir with sofosbuvir and ribavirin for hepatitis C virus infection with advanced cirrhosis or post-liver transplantation recurrence. *Hepatology* 2016; **63**: 1493-1505 [PMID: 26754432 DOI: 10.1002/hep.28446]

101 **Manns M**, Samuel D, Gane EJ, Mutimer D, McCaughan G, Buti M, Prieto M, Calleja JL, Peck-Radosavljevic M, Müllhaupt B, Agarwal K, Angus P, Yoshida EM, Colombo M, Rizzetto M, Dvory-Sobol H, Denning J, Arterburn S, Pang PS, Brainard D, McHutchison JG, Dufour JF, Van Vlierberghe H, van Hoek B, Forns X. Ledipasvir and sofosbuvir plus ribavirin in patients with genotype 1 or 4 hepatitis C virus infection and advanced liver disease: a multicentre, open-label, randomised, phase 2 trial. *Lancet Infect Dis* 2016; **16**: 685-697 [PMID: 26907736 DOI: 10.1016/S1473-3099(16)00052-9]

102 **Lawitz E.** SVR12 results from the Phase II, open-label IMPACT study of simeprevir in combination with daclatasvir and sofosbuvir in treatment- naıve and -experienced patients with chronic HCV genotype 1/4 infection and decompensated liver disease. *J Hepatol* 2015; **62** (Suppl 1): 227A

103 **Aquel BA,** Pungpapong S, Leise M, Werner KT, Chervenak AE, Watt KD, Murphy JL, Ryland K, Keaveny AP, McLemore R, Vargas HE. Multicenter experience using simeprevir and sofosbuvir with or without ribavirin to treat hepatitis C genotype 1 in patients with cirrhosis. *Hepatology* 2015; **62**: 1004-1012 [PMID: 26096332 DOI: 10.1002/hep.27937]

104 **Saxena V,** Nyberg L, Pauly M, Dasgupta A, Nyberg A, Piasecki B, Winston B, Redd J, Ready J, Terrault NA. Safety and efficacy of simeprevir/sofosbuvir in hepatitis C- infected patients with compesansated and decompensated cirrhosis. *Hepatology* 2015; **62**: 715-725 [PMID: 26033798 DOI: 10.1002/hep.27922]

105 **McCaughan G**. The TOSCAR study: sofosbuvir and daclatasvir therapy for decompensated HCV cirrhosis with MELD scores ≥15: what is the point of no return? *J Hepatol* 2015; **62** (Suppl 1): 738A

106 **Gray E**, O'Leary A, Stewart S, Bergin C, Cannon M, Courtney G, Crosbie O, De Gascun CF, Fanning LJ, Feeney E, Houlihan DD, Kelleher B, Lambert JS, Lee J, Mallon P, McConkey S, McCormick A, McKiernan S, McNally C, Murray F, Sheehan G, Norris S. High mortality during direct acting antiviral therapy for hepatitis C patients with Child's C cirrhosis: Results of the Irish Early Access Programme. *J Hepatol* 2016; **65**: 446-448 [PMID: 27130842 DOI: 10.1016/j.jhep.2016.03.022]

107 **Munoz S.** Curing decompensated wait-listed HCV patients with the new DAAs: Potential Significant impact on liver transplant wait list and organ allocation. 66th Annual Meeting of the American Association for the Study of Liver Diseases AASLD. San Francisco, USA, 2015 Nov 13-17

108 **Coilly A**, Samuel D. Pros and Cons: Usage of organs from donors infected with hepatitis C virus - Revision in the direct-acting antiviral era. *J Hepatol* 2016; **64**: 226-231 [PMID: 26375245 DOI: 10.1016/j.jhep.2015.09.002]

109 **Perumpail RB**, Hahambis TA, Aggarwal A, Younossi ZM, Ahmed A. Treatment strategies for chronic hepatitis C prior to and following liver transplantation. *World J Hepatol* 2016; **8**: 69-73 [PMID: 26783422 DOI: 10.4254/wjh.v8.i1.69]

110 **Patwardhan VR**, Curry MP. Reappraisal of the hepatitis C virus-positive donor in solid organ transplantation. *Curr Opin Organ Transplant* 2015; **20**: 267-275 [PMID: 25944236 DOI: 10.1097/MOT.0000000000000191]

111 **Brown RS**, O'Leary JG, Reddy KR, Kuo A, Morelli GJ, Burton JR, Stravitz RT, Durand C, Di Bisceglie AM, Kwo P, Frenette CT, Stewart TG, Nelson DR, Fried MW, Terrault NA. Interferon-free therapy for genotype 1 hepatitis C in liver transplant recipients: Real-world experience from the hepatitis C therapeutic registry and research network. *Liver Transpl* 2016; **22**: 24-33 [PMID: 26519873 DOI: 10.1002/lt.24366]

112 **Reddy KR.** Ledipasvir/Sofosbuvir With Ribavirin for the Treatment of HCV in Patients With Post- Transplant Recurrence: Preliminary Results of a Prospective, Multicenter Study. 65th Annual Meeting of the American Association for the Study of Liver Disease AASLD. Boston, MA, USA, 2014 Nov 7-11

113 **Sievert W**, Razavi H, Estes C, Thompson AJ, Zekry A, Roberts SK, Dore GJ. Enhanced antiviral treatment efficacy and uptake in preventing the rising burden of hepatitis C-related liver disease and costs in Australia. *J Gastroenterol Hepatol* 2014; **29** Suppl 1: 1-9 [PMID: 25055928 DOI: 10.1111/jgh.12677]

114 **Deuffic-Burban S**, Mathurin P, Rosa I, Bouvier AM, Cannesson A, Mourad A, Canva V, Louvet A, Deltenre P, Boleslawski E, Truant S, Pruvot FR, Dharancy S. Impact of emerging hepatitis C virus treatments on future needs for liver transplantation in France: a modelling approach. *Dig Liver Dis* 2014; **46**: 157-163 [PMID: 24119483 DOI: 10.1016/j.dld.2013.08.137]

115 **Kabiri M**, Jazwinski AB, Roberts MS, Schaefer AJ, Chhatwal J. The changing burden of hepatitis C virus infection in the United States: model-based predictions. *Ann Intern Med* 2014; **161**: 170-180 [PMID: 25089861 DOI: 10.7326/M14-0095]

P- Reviewer: Bourgoin SG, Pekgoz M, Wang K, Wang L, Yang SS
S- Editor: Qi Y **L- Editor**: Filipodia **E- Editor**: Li D

World Journal of Hepatology

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World J Hepatol 2017 March 8; 9(7): 368-384

DOI: 10.4254/wjh.v9.i7.368

ORIGINAL ARTICLE

Basic Study

Characterization of a new monoclonal anti-glypican-3 antibody specific to the hepatocellular carcinoma cell line, HepG2

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Supported by National Research Council of Thailand (NRCT), No. 2559A10402115.

Institutional review board statement: Not available.

Institutional animal care and use committee statement: Not available.

Conflict-of-interest statement: The authors declare that there are no conflicts of interest.

Data sharing statement: No additional are available.

Manuscript source: Invited manuscript

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Received: November 21, 2016
Peer-review started: November 23, 2016
First decision: December 15, 2016
Revised: December 19, 2016
Accepted: January 11, 2017
Article in press: January 14, 2017
Published online: March 8, 2017

Abstract

AIM

To characterize the antigen on HepG2 cell that is specifically recognized by a new monoclonal antibody raised against human liver heparan sulfate proteoglycan (HSPG), clone 1E4-1D9.

METHODS

The antigen recognized by mAb 1E4-1D9 was immunoprecipitated and its amino acid sequence was analyzed LC/MS. The transmembrane domain, number of cysteine residues, and glycosylation sites were predicted from these entire sequences. Data from amino acid analysis was aligned with glypican-3 (https://www.ebi.ac.uk/Tools/msa/clustalo/). The competitive reaction of mAb 1E4-1D9 and anti-glypican-3 on HepG2 cells was demonstrated by indirect immunofluorescence and analyzed by flow cytometry. Moreover, co-immunoprecipitation of mAb 1E4-1D9 and anti-glypican-3 was performed in HepG2 cells by Western immunoblotting. The recognition by mAb 1E4-1D9 of a specific epitope on solid tumor and hematopoietic cell lines was studied using indirect immunofluorescence and analyzed by flow cytometry.

RESULTS

Monoclonal antibody 1E4-1D9 reacted with an HSPG isolated from human liver and a band of 67 kD was

detected under both reducing and non-reducing conditions. The specific antigen pulled down by mAb 1E4-1D9, having a MW of 135 kD, was analyzed. The results showed two sequences of interest, gi30722350 (1478 amino acid) and gi60219551 (1378 amino acid). In both sequences no transmembrane regions were observed. Sequence number gi30722350 was 99.7% showed a match to FYCO1, a molecule involved in induction of autophagy. Sequence number gi60219551 contained 15 cysteines and 11 putative glycosylation sites with 6 predicted N-glycosylation sites. It was also matched with all PDZ domain proteins. Moreover, it showed an 85.7% match to glypican-3. Glypican-3 on HepG2 cells competitively reacted with both phycoerythrin-conjugated anti-glypican-3 and mAb 1E4-1C2 and resulted in an increase of double-stained cell population when higher concentration of mAb 1E4-1D9 was used. Moreover, antigens precipitated from HepG2 cell by anti-glypican-3 could be detected by mAb 1E4-1D9 and vice versa. The recognition of antigens, on other solid tumor cell lines, by mAb 1E4-1D9 was studied. The results demonstrated that mAb 1E4-1D9 reacted with Huh7, HepG2, HT29, MCF7, SW620, Caco2, B16F1, U937, K562 and Molt4 cells. It was also found to be weakly positive to SW1353 and HL60 and negative to H460 and Hela cell lines.

CONCLUSION

All findings show that mAb 1E4-1D9 specifically recognizes glypican-3. Moreover, a new partner molecule of glypican-3, FYCO1 is proposed based on the results from co-precipitation studies.

Key words: Monoclonal anti-glypican-3; Hepatocellular carcinoma; HepG2; Heparan sulfate proteoglycan; Co-immunoprecipitation

Core tip: Heparan sulfate proteoglycan (HSPG) was isolated from human liver. Preliminary results showed that it was detected by rabbit anti-glypican. Monoclonal antibody, 1E4-1D9 was raised against human liver HSPG and its specific antigen was characterized. Amino acid sequence analysis revealed that the antigen recognized by mAb 1E4-1D9 specific molecule contained no transmembrane region. It has 15 cysteines and 11 putative glycosylation sites and 6 predicted N-glycosylation sites. The sequence matched to all PDZ domain proteins with an 85.6% match to glypican-3. Studies of co-expression and co-precipitation demonstrated that mAb 1E4-1D9 could compete with anti-glypican-3. It could also react with a various tumor cell lines including solid and hematopoietic cells. The findings suggested that the antigen recognized by 1E4-1D9 was glypican-3. Moreover, findings revealed that FYCO1 co-precipitated with glypican-3 using mAb 1E4-1D9, suggesting that FYCO1 is a partner molecule of glypican-3.

Vongchan P, Linhardt RJ. Characterization of a new monoclonal anti-glypican-3 antibody specific to the hepatocellular carcinoma cell line, HepG2. *World J Hepatol* 2017; 9(7): 368-384 Available from: URL: http://www.wjgnet.com/1948-5182/full/v9/i7/368.htm DOI: http://dx.doi.org/10.4254/wjh.v9.i7.368

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the third most common cause of cancer-related deaths[1,2]. The majority of these cases occur in Asia and Africa. However, the incidence has also been rising in the developed world. Among liver cancer cases, 80% are HCC, which does not respond well to chemotherapy[3]. Early detection is difficult and there are poor outcomes to aggressive therapies[4,5]. Thus, early detection of HCC is a key goal in improving this poor prognosis. In addition, identification of novel molecular targets for development of diagnostic and therapeutic approaches remains of great interest. Glypican-3 is highly expressed in HCC and recently has been suggested as a good diagnostic marker for HCC[6-14]. In addition to effective early diagnosis, drugs targeting different mechanisms of action involving glypican-3 targeted antibody therapy are addressed[15]. To date, several clones of monoclonal antibodies specific to glypican-3 have been described[6,8,16-19]. These have not only been used as research tools and in diagnostic development, but some have been developed for preparing potential agents for HCC immunotherapy[17-21]. Moreover, silencing of glypican-3 was recently reported to induce apoptosis in HCC cell lines[22]. Thus, glypican-3 has great promise as an excellent molecular target for the diagnosis and therapy of HCC.

Glypican is a family of heparan sulfate proteoglycans (HSPGs) that are expressed on the extracellular membrane as a glycosylphosphatidylinositol (GPI)-anchored proteoglycan. These HSPGs regulate cellular signaling during morphogenesis, adult physiology and carcinogenesis by interaction with a multitude of extracellular matrix molecules including chemokines, growth factors or morphogens and their receptors[23-25]. Glypican is expressed in cell-, tissue- and development-specific patterns. Among the six members of the glypican family, glypican-3 has been studied most extensively[23,26,27].

Since glypican-3 is an HSPG, it typically contains a heparan sulfate glycosaminoglycan chain (GAG), but in some instances a chondroitin sulfate (GAG) can also be found on glypican-3[23]. GAG chains carry negative charge, allowing glypican-3 to interact with basic growth factors and morphogens in the extracellular space. Glypican-3 has a 70-kD core protein which can be cleaved by furin generating two fragments of 40-kD N-terminal and 30-kD C-terminal[27]. The GPI anchor linking glypican-3 to the membrane can be cleaved by lipase (notum), releasing glypican-3 to extracellular matrix[28]. The

shedding of glypican-3 plays a role in regulating signaling of Wnts, hedgehogs, fibroblast growth factors, and bone morphogenetic proteins[23,26,29,30]. There has also been a report that soluble glypican-3 can inhibit HCC proliferation both *in vitro* and *in vivo*[31]. Therefore, glypican-3 can play both positive and negative role in cell growth depending on cell type[32,33]. Glypican-3 is expressed in a variety of tissues and acts as oncofetal protein. Among membrane HSPGs, glypican-3 is the only HSPG that is highly expressed on HCC tissue but it is usually not found in normal and in non-tumor liver tissues[34]. Previous findings indicate that glypican-3 stimulates *in vitro* and *in vivo* growth of HCC[26,35-39]. The mechanism in HCC growth promotion of glypican-3 is to regulate Wnt signaling as well as oncogenesis through insulin-like growth factor signaling pathway[40]. It was reported that, in primary HCC, sulfatase-2 (SULF2) enzyme with 6-O-sulfatase activity is up-regulated and associated to poor prognosis[41]. Increasing of SULF2 enhances the expression of glypican-3 *in vitro* and *in vivo*[42].

The liver is a rich source of GAGs and the liver is known to be receptor of many molecules involved in diseases and in pathogen binding[43-46]. Recently, an HSPG was isolated from human liver. The analysis of its GAG component demonstrated that it was heparan sulfate, not heparin[47]. Digestion of liver HSPG with heparin lyase Ⅰ, Ⅱ, Ⅲ yielded a core protein product that could be detected by anti-rat glypican with a band of approximately 61 kD. These results suggested that the HSPG isolated from human liver was a glypican.

Monoclonal antibodies were raised against liver HSPG. Two of the clones obtained are 1E4-1C2 and 1E4-1D9. The clone 1E4-1C2 specifically reacts with membrane molecules of various malignant cell lines, including solid tumor and hematopoietic cells in erythromyeloid series[48]. This antibody can differentiate between acute myeloid leukemia from normal blood cells and normal blast cells in bone marrow. Moreover, mAb 1E4-1C2 strongly reacts with HepG2 cells and inhibits cell proliferation in a dose dependent manner both *in vitro* and in an animal model[49]. Development of HepG2 cell-targeted drug delivery based on mAb 1E4-1C2 has also been studied[50]. Intensive characterization of mAb 1E4-1C2 and its specific antigen is in progress.

Our preliminary results of mAb 1E4-1D9 showed that it could react with HepG2. Together with the previous observations that liver HSPG was a glypican and that glypican-3 is up regulated in HCC, we hypothesized that antigen recognized by mAb 1E4-1D9 was glypican-3. The present study is aimed at characterizing the specific antigen on HepG2 cells recognized by mAb 1E4-1D9.

MATERIALS AND METHODS

Cell lines

HL60 cell line was a kind gift from Associate Professor, Dr. Songyot Anuchpreeda, Department of Medical Technology, Faculty of Associated Medical Sciences, Chiang Mai University. Huh7 was from Professor, Dr. Pa-thai Yenchitsomanus, Faculty of Medicine Siriraj Hospital, Mahidol University. The other cell lines were purchased from ATCC.

Reagents and reagent kits

OPI supplement, fetal bovine serum, 3,3-diamino benzidine (DAB), and SuperSignal™ West Pico Chemiluminescent Substrate were purchased from Sigma-Aldrich (St. Louis, MO, United States). All culture media were from Gibco (Life Technologies, NY, United States). Mouse IgG1 and phycoerythrin (PE) conjugated mouse IgG2a were purchased from Biolegend, CA, United States and anti-glypican-3 [clone 9C2, IgG1, immunogen: Recombinant human glypican-3 (amino acid 1-580)] was from Abcam (United Kingdom). PE conjugated anti-glypican-3 [clone 307801, IgG2a, immunogen: Recombinant human glypican-3 (amino acid 25-558)] was obtained from United States Biological Life Sciences, MA, United States. Fluorescein isothiocyanate (FITC) conjugated anti-mouse Igs and horseradish peroxidase (HRP)-conjugated anti-mouse Igs were purchased from Dako (CA, United States). Protein G agarose was purchased from Pierce (Rockford, IL, United states). IsoStrip was obtained from Roche (IN, United States). Other common reagents used in these studies were purchased from local reputable companies including PCL Holdings (Thailand) and Pacific Sciences (Thailand).

Preparation and purification of mAb 1E4-1D9 antibody

The hybrid clone 1E4-1D9 was grown in OPI containing-Dulbecco's Modified Eagle's medium (DMEM)/high glucose supplemented with 10% fetal bovine serum to exponential phase. Cell culture supernatant was collected and mAb 1E4-1D9 was purified using protein G affinity agarose beads. Briefly, cell culture supernatant was diluted with binding buffer provided (1:1 v/v) before applying and allowed to flow completely into the resin. The column was then washed with binding buffer and eluted with the elution buffer provided. Fractions of 1 mL were collected and neutralized with neutralizing buffer (Tris-base, pH 8.0, 100 μL). Pooled purified mAb was dialyzed against phosphate buffered saline (PBS) pH 7.2, concentrated and aliquots were frozen. Isotype was determined using IsoStrip according to the manufacturer's directions.

Determination of mAb 1E4-1D9 specificity to human liver HSPG

HSPG isolated from human liver[47] was diluted to 5 μg/mL with PBS, pH 7.2. Twenty μL of sample was mixed with 5 μL of 5 × sample buffer (62.5 mmol/L Tris-HCl, pH 6.8, 70 mmol/L sodium dodecylsulfate (SDS), 10% glycerol, 2% bromphenol blue) and non-reducing sample buffer, and boiled for 5 min. Sample was subjected to electrophoresis on 10% SDS-polyacrylamide gel electrophoresis (PAGE) at 200 V for 45 min and blotted onto polyvinyl difluoride (PVDF) membrane. Before probing with mAb 1E4-1D9, non-specific sites were blocked with 5% non-fat dried milk in tris-buffered saline (TBS) pH 7.4

(0.15 mol/L NaCl, 10 mmol/L Tris-base) for 1 h at room temperature on a rocking plate. The membrane was washed 3-times (10 min each) with TBS pH 7.4. Primary antibody (mAb 1E4-1D9, 100 μg/mL in 0.1% Tween-20 in; TBS-Tween) was added onto the membrane. The reaction was performed at room temperature for 1 h on a rocking plate. After completion, membrane was washed with TBS-Tween for 3-times (10 min each) on a rocking plate. The reaction was then detected with HRP-conjugated rabbit anti-mouse Igs for 1 h at room temperature on a rocking plate and washed. Finally, signal was then developed with DAB containing H_2O_2. Molecular weight (kD) was calculated from a plot of log molecular weight standard *vs* migration distance and a $R^2 \geqslant 0.99$ was obtained.

Expression of mAb 1E4-1D9 on HepG2 cell lines

HepG2 cells cultured in DMEM high glucose supplemented with 10% fetal bovine serum (FBS) grown to exponential phase. Cells were collected, washed twice with PBS, pH 7.2. Cell viability was checked by trypan blue dye exclusion assay and adjusted to 4 × 10^5 cells/mL with PBS pH 7.2. Heat-inactivated normal human AB serum was added to the final concentration of 10% and incubated on ice for 30 min. An aliquot of cell suspension (50 μL) was added to an equal volume of various final concentrations of mAb 1E4-1D9. Mouse IgG1 and washing buffer [cold 1% bovine serum albumin (BSA)-PBS, 0.02% NaN_3] were used as isotype and conjugated control, respectively. The reactions were incubated on ice for 30 min. After completion, cells were washed 3-times with washing buffer. Fifty microlitre of FITC-conjugated rabbit anti-mouse Igs (1:20 diluted in washing buffer) was added and reaction was incubated for another 30 min on ice. Following with 3-washes, cell pellet was suspended with 300 μL of 0.5% paraformaldehyde in PBS, pH 7.2 and analyzed by flow cytometer (Becton Dickinson, CA, United States).

Immunoprecipitation of mAb 1E4-1D9 specific antigen for amino acid analysis

HepG2 cells grown to exponential phase were harvested, washed 5-times with PBS pH 7.2 (0.137 mol/L NaCl, 2.68 mmol/L KCl, 1.88 mmol/L $NaH_2PO_4.2H_2O$, 8.10 mmol/L Na_2HPO_4) and adjusted to 1 × 10^6 cells/mL. One millilitre of lysis buffer (1% Brij58, 20 mmol/L Tris–HCl pH 7.5, 0.15 mol/L NaCl, 2 mmol/L ethylenediaminetetraacetic acid, 5 mmol/L iodoacetamide, 1 mmol/L phenylmethylsulfonyl fluoride, 2 mmol/L pepstatin A, 10 mg/mL aprotinin) was added. The suspension was mixed thoroughly, centrifuged 12000 rpm at 4 ℃ for 30 min to pellet cell debris. HepG2 cell lysate was used as source of antigen precipitating by mAb immobilized protein G agarose beads.

Prior to immobilization of mAb, 50 μL of protein G was washed 3-times with binding buffer (0.2 mol/L $NaH_2PO_4.H_2O$, 0.15 mol/L NaCl) followed by immunoprecipitation (IP) buffer (25 mmol/L Tris-base, 0.15 mol/L NaCl). One hundred microlitres of mAb 1E4-1D9 (100 μg/mL) was then mixed individually with protein G agarose beads for 30 min at room temperature before washing (3-times) out non-bound mAb with IP buffer. Washing buffer was discarded and 100 μL of HepG2 lysate was added. The reaction was incubated at 4 ℃ overnight. After completion, the reaction was centrifuged, supernatant was discarded and beads were washed 6-times with IP buffer. Fifty microlitre of elution buffer (0.1 mol/L glycine, pH 3.0) was added and mixed for 5 min. Finally beads were pelleted down and eluate, containing specific antigen, was collected. This step was repeated twice. Collected supernatants were pooled and neutralized with 10 μL of neutralizing buffer (1 mol/L Tris-base, pH 8.0).

Twenty microlitres of eluate was mixed with 5 μL of 5 × sample buffer and separated on 10% SDS-PAGE at 200 V for 45 min before blotting onto PVDF membrane. Non-specific binding sites on the membrane were blocked with 5% non-fat dried milk in TBS pH 7.4 (0.15 mol/L NaCl, 10 mmol/L Tris-base) for 1 h at room temperature on a rocking plate. PVDF membrane was then washed, with 0.1% Tween-20 in TBS (TBS-Tween), 3-times for 10 min each on a rocking plate. Primary antibody, mAb 1E4-1D9 (1 mg/mL in 1%BSA TBS-Tween) was added to each membrane. The reaction was performed at 4 ℃ overnight. After completion, membrane was washed (3-times for 10 min each) with 0.1% Tween-20 in TBS (TBS-Tween) on a rocking plate. The reaction was then detected with HRP-conjugated rabbit anti-mouse Igs for 1 h at room temperature on a rocking plate. After washing out the excess antibody (3-times for 10 min each) with 0.1% Tween-20 in TBS (TBS-Tween), signal was visualized by SuperSignal™ West Pico chemiluminescent substrate. Molecular weight was calculated from standard molecular weight graph as previously mentioned.

The eluate was pooled and subject to electrophoresis in 5 × non-reducing sample buffer on 10% SDS-PAGE at 200 V for 45 min. Gel was stained with Coomasie Brilliant Blue to prepare mAb 1E4-1C2 specific antigens for amino acid sequence analysis. The band of interest, selected by as comparison to result on immunoblot, was cut and sent for amino acid analysis by LC-MS (HDMS Synaptat, Waters, MA, United States) at the National Center for Genetic Engineering and Biotechnology, Bangkok, Thailand).

Co-expression of mAb 1E4-1D9 and anti-glypican-3 on HepG2 cells

HepG2 cells in exponential phase were harvested and washed twice with PBS, pH 7.2. Cell viability was determined by trypan blue dye exclusion assay and adjusted to 4 × 10^5 cells/mL with PBS pH 7.2. After blocking with heat-inactivated normal AB serum for 30 min on ice, an aliquot of cell suspension (50 μL) was added to equal volumes of various concentrations of mAb 1E4-1D9. Mouse IgG1 was used as isotype control. The reaction was incubated on ice for 30 min follow by 3-washes with cold washing buffer. Cell pellet was re-

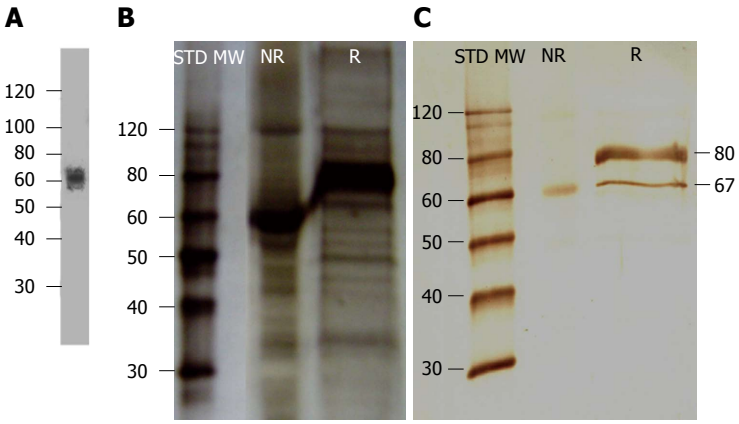


Figure 1 Liver heparan sulfate proteoglycan was detected by anti-rat glypican and mAb 1E4-1D9. A: Liver heparan sulfate proteoglycan (HSPG) was digested with heparin lyase Ⅰ, Ⅱ, Ⅲ and probed with anti-rat glypican[47]; B: Silver stain of liver HSPG; C: Liver HSPG was reacted with mAb 1E4-1D9 and visualized by horseradish peroxidase-conjugated rabbit anti-mouse Igs following with 3,3-diamino benzidine/H_2O_2 substrate.

suspended with 50 μL washing buffer and added with equal volume of FITC-conjugated rabbit anti-mouse Igs (1:20 diluted in washing buffer). After incubating on ice for another 30 min, cells were washed 3-times and PE-conjugated anti-glypican-3 (1:10 diluted with washing buffer). PE conjugated mouse IgG2a was used as isotype control. The reaction was performed on ice for 30 min and washed 3-times. Finally, cells were suspended with 300 μL of 0.5% paraformaldehyde in PBS, pH 7.2 and analyzed by flow cytometer.

Co-immunoprecipitation of mAb 1E4-1C2 and anti-glypican-3 on HepG2 cells

HepG2 cell lysate was prepared as mentioned above and was used as source of antigen. The three different antibody immobilized protein G agarose beads, anti-glypican-3, mAb 1E4-1D9, and mouse IgG1 (isotype control) were immobilized on protein G agarose beads as mentioned.

Twenty microlitres of eluates from mAb 1E4-1D9, anti-glypican-3, or mouse IgG1 immobilized protein G agarose beads was separated on 10% SDS-PAGE at 200 V for 45 min in non-reduced condition and blotted onto PVDF membrane. Three membranes were prepared. Non-specific binding sites on the membrane were blocked with 5% non-fat dried milk in TBS, pH 7.4 for 1 h at room temperature on a rocking plate. PVDF membrane was then washed, 3-times for 10 min, with TBS-Tween each on a rocking plate. Primary antibody [mAb 1E4-1D9, anti-glypican-3, or mouse IgG1 isotype control (100 μg/mL in 1% BSA TBS-Tween)] was added to each individual membrane. The reaction was performed at 4 °C overnight. After completion, membrane was washed, 3-times for 10 min, with TBS-Tween each on a rocking plate. The reaction was then detected with HRP-conjugated rabbit anti-mouse Igs for 1 h at room temperature on a rocking plate. After washing (3-times for 10 min each) out the excess antibody with TBS-Tween, signal was then developed by SuperSignal™ West Pico chemiluminescent substrate and auto-radiographed. The molecular weight was calculated from a standard molecular weight plot as previously described.

Expression of mAb 1E4-1D9 on malignant cell lines

Solid tumor cell lines (Huh7, B16F1, HT29, Caco2, MCF7, SW620, SW1353, H460 and Hela) cultured in DMEM high glucose supplemented with 10% FBS and hematopoietic cell lines (HL60, K562, U937 and Molt4) cultured in RPMI-1640 were grown to exponential phase. Cells were collected, washed twice with PBS, pH 7.2. Cell viability was checked by trypan blue dye exclusion assay and adjusted to 4×10^5 cells/mL with PBS pH 7.2. Heat-inactivated normal human AB serum was added to cell suspension to the final concentration of 10% and incubated on ice for 30 min. Aliquot of cell suspension (50 μL) was added with an equal volume of mAb 1E4-1D9 (20 μg/mL). Mouse IgG1 and washing buffer (cold 1%BSA-PBS, 0.02% NaN_3) were used as isotype control and conjugated control, respectively. The reactions were incubated on ice for 30 min. After completion, cells were washed 3-times with washing buffer. Fifty microlitres of FITC-conjugated rabbit anti-mouse Igs (1:20 diluted in washing buffer) was added and reaction was incubated for another 30 min on ice. Following 3-washes with washing buffer, the cell pellet was suspended with 300 μL of 0.5% paraformaldehyde in PBS, pH 7.2 and was analyzed by flow cytometer (Becton Dickinson, CA, United States).

RESULTS

Before any assay was performed, antibody isotype was determined using a commercial isotyping kit and it was confirmed that mAb 1E4-1D9 was an IgG1. The specificity to the immunogen was also studied by Western immunoblotting of liver HSPG and probed with mAb 1E4-1D9. The results demonstrated a band was detected at approximately 67 kD under both non-reducing and reducing conditions (Figure 1C). The molecular weight

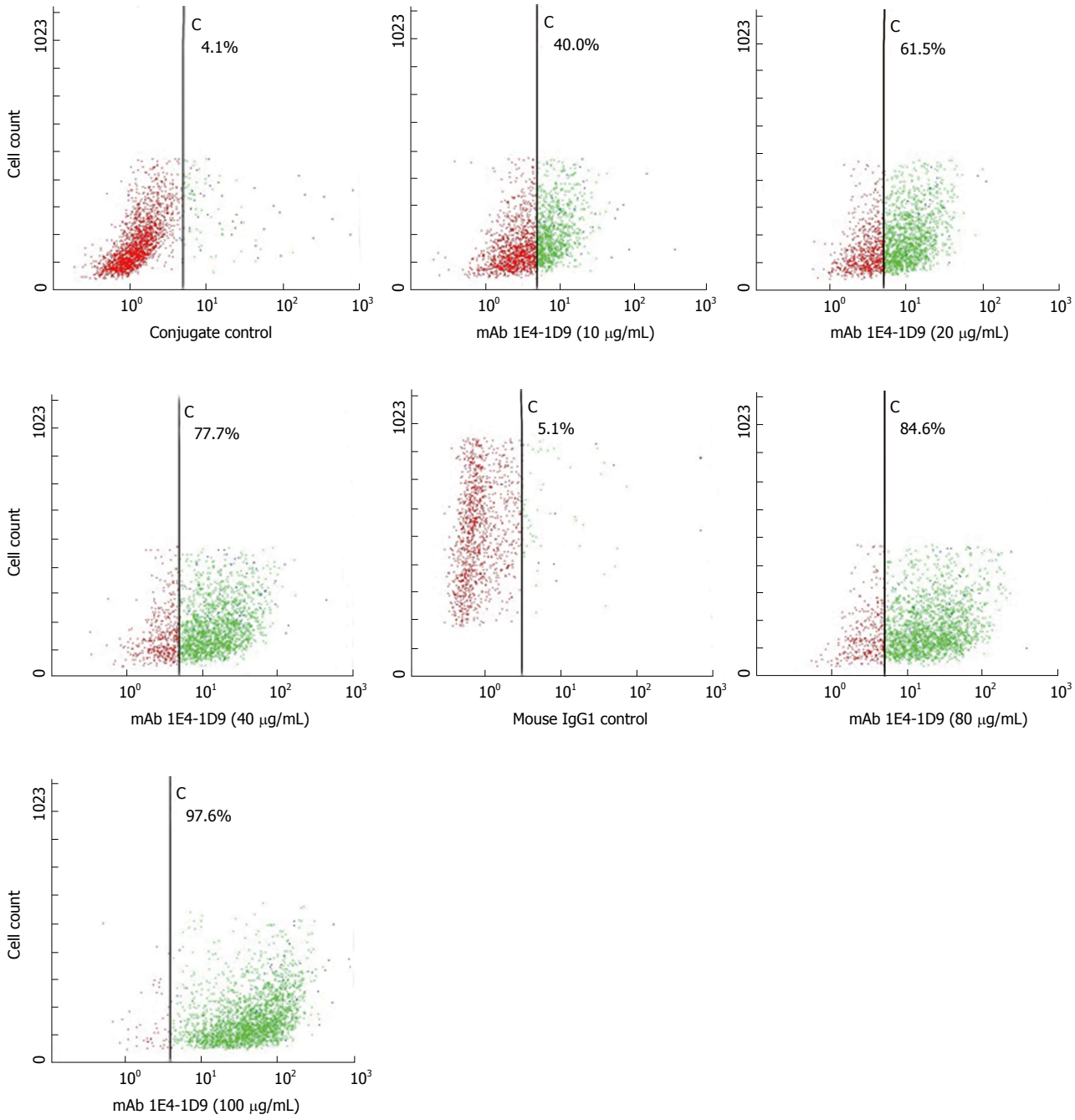


Figure 2 HepG2 cells (4 × 10^5 cells/mL) were reacted with various final concentrations of mAb 1E4-1D9 (0-160 μg/mL) for 30 min on ice. Mouse IgG1 was used as isotype control. After washing, cells were stained with fluorescein isothiocyanate-conjugated rabbit anti-mouse Igs (1:20) for 30 min on ice and washed. Finally, cells were suspended with 300 μL of 0.5% paraformaldehyde in phosphate buffered saline, pH 7.2 and analyzed by flow cytometry.

was close to that reported previously where liver HSPG was probed with rabbit anti-rat glypican[47] (Figure 1A). These results suggest that the epitope of mAb 1E4-1D9 is present in both folded and linear forms.

We next used indirect immunofluorescence to examine the expression of the antigen on HepG2 cells that reacts with mAb 1E4-1D9. mAb 1E4-1D9 reacted specifically to an antigen on HepG2 in concentration dependent manner (Figure 2). Moreover the highest expression of this antigen was observed during incubation (data not shown) while HepG2 was in exponential phase was at day-4 of incubation. The specific antigen was immune-precipitated by mAb 1E4-1D9 immobilized protein G agarose beads. A band at 135 kD was visualized by immunoblotting (Figure 3A) and was cut from the gel (Figure 3B) and sent for analysis.

Amino acid analysis demonstrated the presence of two hypothetical sequences, gi30722350 (1478 amino acid) and gi60219551 (1378 amino acid). Neither sequence had a transmembrane region domain based on analysis

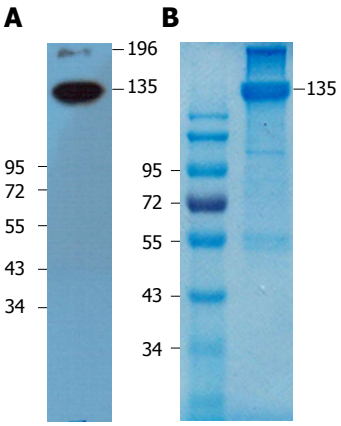


Figure 3 Specific antigen was immunoprecipitated from HepG2 cell lysate by mAb 1E4-1D9-immobilized protein G agarose beads. Eluate was separated under non-reducing conditions in 10% sodium dodecylsulfate-polyacrylamide gel electrophoresis at 200 V for 45 min. One gel was blotted onto polyvinyl difluoride membrane and probed with mAb 1E4-1D9. A: The reaction was detected by HRP-conjugated rabbit anti-mouse Igs and signal was developed by SuperSignal™ West Pico Chemiluminescent Substrate; B: Another gel was stained with Coomasie Brilliant Blue and protein band of 135 kD was cut and sent for amino acid analysis (B).

by TMHMMM software (Figure 4A). Data analysis also demonstrated the number of cysteine residues was 19 and 15 in gi30722350 and gi60219551, respectively. Moreover, gi30722350 contains two putative glycosylation sites while the latter, gi60219551 has 11 putative glycosylation sites at amino acid 139, 227, 377, 393, 577, 721, 891, 911, 1053, 1090 and 1243, respectively with 6 predicted N-glycosylation sites (amino acid 139, 227, 377, 577, 721 and 1053) (Figure 4B). Alignment of gi30722350 (https://blast.ncbi.nlm.nih.gov/Blast.cgi) demonstrated that it was matched to all FYVE containing protein with 99.7% matched to FYCO-1 (data not shown). Interestingly, gi60219551 matched to a PDZ domain protein with 85.7% match to glypican-3 (Figure 4C).

Expression of mAb 1E4-1D9 together in competition with anti-glypican-3 was undertaken to verify that antigen specific to mAb 1E4-1D9 was glypican-3. However, prior to this experiment, the concentration of PE-conjugated anti-glypican-3 was optimized for maximum intensity detection by direct immunofluorescence. The result indicated that PE-conjugated anti-glypican-3 at dilution of 1:10 could specifically react to 97.8% of antigen on HepG2 cells (data not shown). Various final concentrations of mAb 1E4-1D9 were used to react with HepG2 cells followed by fixing with PE-conjugated anti-glypican-3 and analyzed by flow cytometry. The number of cells, in the upper right quadrant (positive both FL1 and FL2), increased in dose dependent manner while FL2 signal of the PE-conjugated anti-glypican-3 decreased (Figure 5). This indicates that mAb 1E4-1D9 is specific to glypican-3 on HepG2 since mAb 1E4-1D9 could compete with PE-conjugated anti-glypican-3 used. Moreover, it suggests that antigenic site of mAb 1E4-1D9 on glypican-3 may be at or close to N-terminal region because immunogen of PE-conjugated anti-glypican-3 used was recombinant human glypican-3 (amino acid 25-558).

Co-immunoprecipitation of a specific antigen on HepG2 cells by mAb 1E4-1D9 and anti-glypican-3 was performed. Mouse IgG1 was used in parallel as an isotype control. Findings from experiments show that mAb 1E4-1D9 precipitated three interesting bands of 69, 115 and 130 kD (Figure 6B), which also reacted with anti-glypican-3 (Figure 6C). A protein band of 130 kD precipitated by anti-glypican-3 was clearly visualized by mAb 1E4-1D9 (Figure 6B). However, anti-glypican-3 itself showed less reaction (Figure 6C). Lysate was probed with anti-glypican-3 to verify that lysate contained glypican-3 and a band was observed at 115 kD (Figure 6D). Taken together this demonstrated that mAb 1E4-1D9 could react with antigen precipitated by anti-glypican-3 and vice versa.

Expression of mAb 1E4-1D9 on other cells was studied by indirect immunofluorescence. We found that the antigen recognized by mAb 1E4-1D9 was expressed on a variety of cell lines tested including B16F1, Caco2, HT29, MCF7, SW620, K562, U937 and Molt4 (Figure 7). Some cells such as SW1353 and HL60 were weakly positive and some were negative (H460 and Hela cells).

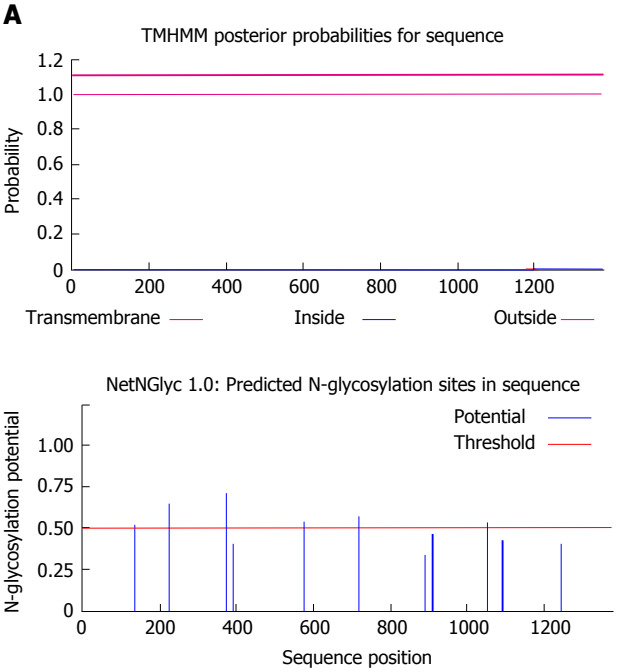
DISCUSSION

HCC is one of the most common cancers worldwide with a poor prognosis and a low 5-year survival rate. Thus, specific biomarkers have become increasingly important to identify HCC. Glypican-3 is upregulated and highly expressed in HCC but not in normal or non-malignant liver tissues. Glypican-3 has important roles in cell growth, differentiation and motility[33]. As a key molecule in relation to signaling with several growth factors and growth factor receptors, glypican-3 can regulate the proliferation of malignant cells both in negative and positive ways[32]. Therefore, antibodies specific to glypican-3 are of interest and many antibody-expressing clones have been developed. Some clones are used to prepare antibodies as tools to study the glypican-3 related cellular activities and some have been applied in tumor investigation and tumor-specific drug development[6,15,20,51].

Our previous report demonstrated that HSPG isolated from human liver contained glypican[47]. Monoclonal antibody raised against human liver HSPG, mAb 1E4-1D9 was, thus, proposed to be specific to glypican-3, which is the only membrane HSPG that highly expressed by HCC[34].

Probing of liver HSPG with mAb 1E4-1D9 resulted a band of 67 kD under both reducing and non-reducing conditions indicate that epitope of mAb 1E4-1D9 can be recognized in either the folded and linear forms. Amino acid analysis of band of 135 kD precipitated from mAb 1E4-1D9 afforded two hypothetical sequences, gi30722350 (1478 amino acid) and gi60219551 (1378

A



B

Name: Sequence gi_60219551 Length: 1378

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VAKPLPLSPEEGYVSAKEDSFLYPPHSCEEAGLADKPLFRADLALVGTNDADLVDESTFESPYSPENDSIYSTQASILSL 240
HGSSCGDGLNYGSSLPSSPPKDVIENSCDPVLDLHMSLEELYTQNLLQRQDENTPSVDISMGPASGFTINDYTPANAIEQ 320
QYECENTIVWTESHLPSEVISSAELPSVLPDSAGKGSEYLLEQSSLACNAECVMLQNVSKESFERTINIAKGNSSLGMTV 400
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QQSGRVMALDIFSSYTGRDIPELPEREEGEGEESELQNTAYSNWNQPRRVELWREPSKSLGISIVGGRGMGSRLSNGEVM 560
RGIFIKHVLEDSPAGKNGTLKPGDRIVEAPSQSESEPEKAPLCSVPPPPPSAFAEMGSDHTQSSASKISQDVDKEDEFGY 640
SWKNIRERYGTLTGELHMIELEKGHSGLGLSLAGNKDRSRMSVFIVGIDPNGAAGKDGRLQIADELLEINGQILYGRSHQ 720
NASSIIKCAPSKVKIIFIRNKDAVNQMAVCPGNAVEPLPSNSENLQNKETEPTVTTSDAAVDLSSFKNVQHLELPKDQGG 800
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GKGLGLSIVGKRNDTGVFVSDIVKGGIADADGRLMQGDQILMVNGEDVRNATQEAVAALLKCSLGTVTLEVGRIKAGPFH 1120
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AVAILKRTKGTVTLMVLS

(Threshold = 0.5)

SeqName	Position	Potential	Jury	N-Glyc agreement result
1	Sequence	139 NSSL	0.5176	(6/9) +
2	Sequence	227 NDSI	0.6440	(8/9) +
3	Sequence	377 NVSK	0.7067	(9/9) ++
4	Sequence	393 NSSL	0.4068	(5/9) -
5	Sequence	577 NGTL	0.5366	(7/9) +
6	Sequence	721 NASS	0.5723	(7/9) +
7	Sequence	891 NSSQ	0.3354	(9/9) --
8	Sequence	911 NTSR	0.4600	(7/9) -
9	Sequence	1053 NDTG	0.5287	(6/9) +
10	Sequence	1090 NATQ	0.4311	(8/9) -
11	Sequence	1243 NASG	0.4047	(9/9) --

C

CLUSTAL O(1.2.3) multiple sequence alignment

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gi|60219551
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NP_001158090.1          ----------MAGTVRTAC--------LVVAMLL-------
NP_001158091.1          ----------MAGTVRTAC--------LVVAMLL-------
NP_001158089.1          ----------MAGTVRTAC--------LVVAMLL-------
AAH35972.1              ----------MAGTVRTAC--------LVVAMLL-------
NP_004475.1             ----------MAGTVRTAC--------LVVAMLL-------
                                  :  *   :           : *:* :

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NP_001158091.1          ----------SLDFPGQAQPPPPPPDATCHQVRSFF-----------
NP_001158089.1          ----------SLDFPGQAQPPPPPPDATCHQVRSFF-----------
AAH35972.1              ----------SLDFPGQAQPPPPPPDATCHQVRSFF-----------
NP_004475.1             ----------SLDFPGQAQPPPPPPDATCHQVRSFF-----------
                                  .*.*          *  *  :*     :* *:.

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NP_001158091.1          QRLQPGLK--WVPETPVP----------
NP_001158089.1          QRLQPGLK--WVPETPVPGSDLQV----------CLP----------
AAH35972.1              QRLQPGLK--WVPETPVPGSDLQV----------CLP----------
NP_004475.1             QRLQPGLK--WVPETPVPGSDLQV----------CLP----------
                         **  ** :  : * :. :

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AAH35972.1              ---KGPTCC-SRKMEEKYQLTARLNM---------EQLLQSASMELKFLIIQNAAVFQEAFEIVV
NP_004475.1             ---KGPTCC-SRKMEEKYQLTARLNM---------EQLLQSASMELKFLIIQNAAVFQEAFEIVV
                                                                                .*:

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NP_001158091.1          RHAKN----------YTNA----------
NP_001158089.1          RHAKN----------YTNA----------
AAH35972.1              RHAKN----------YTNA----------
NP_004475.1             RHAKN----------YTNA----------
                         *...                          **:

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NP_001158091.1          ----------MFKNNYPSLTPQAFEFVGEF
NP_001158089.1          ----------MFKNNYPSLTPQAFEFVGEF
AAH35972.1              ----------MFKNNYPSLTPQAFEFVGEF
NP_004475.1             ----------MFKNNYPSLTPQAFEFVGEF
                                  :  .  :  **: * : :     .*:

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NP_001158089.1          FTDVSLYILG-SDINVDDMVNELFDSLFP----------VIYTQLMNPG--
AAH35972.1              FTDVSLYILG-SDINVDDMVNELFDSLFP----------VIYTQLMNPG--
NP_004475.1             FTDVSLYILG-SDINVDDMVNELFDSLFP----------VIYTQLMNPG--
                        :  : *    . .  : : : :  : * *:   :                    :*   .: : : *

gi|60219551              ISRDGRIAIGDCILSINEESTIS----------VTNAQARAMLRRHSLIGPDIKITYVPAEHL
NP_001158090.1          --LPDSALDINECLRGARRDLKVFGNFPKLIMTQVSKSLQVTRIFLQALNLGIEVINTTD--
NP_001158091.1          --LPDSALDINECLRGARRDLKVFGNFPKLIMTQVSKSLQVTRIFLQALNLGIEVINTTD--
NP_001158089.1          --LPDSALDINECLRGARRDLKVFGNFPKLIMTQVSKSLQVTRIFLQALNLGIEVINTTD--
AAH35972.1              --LPDSALDINECLRGARRDLKVFGNFPKLIMTQVSKSLQVTRIFLQALNLGIEVINTTD--
NP_004475.1             --LPDSALDINECLRGARRDLKVFGNFPKLIMTQVSKSLQVTRIFLQALNLGIEVINTTD--
                           *. : *.:*: .  ..:  .:          :*:..     : *  *  . ::  *  :  : .
```

```
gi|60219551          EEFKISLGQQSGRVMALDIFSSYTGRDIPELPEREEGEGEESELQNTAYSNWNQPRRVEL
NP_001158090.1       ----HLKFSKDCGRMLTRMWYCSYCQGLMMVKPCGG--YCNVVMQGC-M---AGVVEIDKY
NP_001158091.1       ----HLKFSKDCGRMLTRMWYCSYCQGLMMVKPCGG--YCNVVMQGC-M---AGVVEIDKY
NP_001158089.1       ----HLKFSKDCGRMLTRMWYCSYCQGLMMVKPCGG--YCNVVMQGC-M---AGVVEIDKY
AAH35972.1           ----HLKFSKDCGRMLTRMWYCSYCQGLMMVKPCGG--YCNVVMQGC-M---AGVVEIDKY
NP_004475.1          ----HLKFSKDCGRMLTRMWYCSYCQGLMMVKPCGG--YCNVVMQGC-M---AGVVEIDKY
                        : : . :. : : . ** : : :    : . **    :    *       :   : * .       .   .   :

gi|60219551          WREPSKSLG----------------------------------------------------------------------------
NP_001158090.1       WREYILSLEELVNGMYRIYDMENVLLGLFSTIHDSIQYVQKNAGKLTT--------------------
NP_001158091.1       WREYILSLEELVNGMYRIYDMENVLLGLFSTIHDSIQYVQKNAGKLTT--------------------
NP_001158089.1       WREYILSLEELVNGMYRIYDMENVLLGLFSTIHDSIQYVQKNAGKLTTTETEKKIWHFKY
AAH35972.1           WREYILSLEELVNGMYRIYDMENVLLGLFSTIHDSIQYVQKNAGKLTT--------------------
NP_004475.1          WREYILSLEELVNGMYRIYDMENVLLGLFSTIHDSIQYVQKNAGKLTT--------------------
                     ***    **

gi|60219551          ------------ISIVGGRGM----GSRLSNGEVMRGIFIKH-VLEDSPAGKNGTLKPGDRIVE
NP_001158090.1       -----------------TIGKLCAHSQQRQYRSAYYPEDLFIDKKVLKVAHVEHEETLSSRRRELI
NP_001158091.1       -----------------TIGKLCAHSQQRQYRSAYYPEDLFIDKKVLKVAHVEHEETLSSRRRELI
NP_001158089.1       PIFFLCIGLDLQIGKLCAHSQQRQYRSAYYPEDLFIDKKVLKVAHVEHEETLSSRRRELI
AAH35972.1           ----------------TIGKLCAHSQQRQYRFAYYPEDLFIDKKVLKVAHVEHEETLSSRRRELI
NP_004475.1          ----------------TIGKLCAHSQQRQYRSAYYPEDLFIDKKVLKVAHVEHEETLSSRRRELI
                                     *:          :    .    ..:**.:  **:  :  . :: **.    * :

gi|60219551          APSQ-----SESEPEKAPLCSVPPPPPSAFAEMGSDHTQSSASKISQDVDKEDEFGYSWKNI
NP_001158090.1       QKLKSFISFYSALPGYICSHSPV----------------------------------------AENDTLCWNGQEL
NP_001158091.1       QKLKSFISFYSALPGYICSHSPV----------------------------------------AENDTLCWNGQEL
NP_001158089.1       QKLKSFISFYSALPGYICSHSPV----------------------------------------AENDTLCWNGQEL
AAH35972.1           QKLKSFISFYSALPGYICSHSPV----------------------------------------AENDTLCWNGQEL
NP_004475.1          QKLKSFISFYSALPGYICSHSPV----------------------------------------AENDTLCWNGQEL
                        :   *  .   . :**  *                                        : :* :  : . : : :

gi|60219551          RERYGTLTGE----------LHMIELEK-GHSG----------------------------------LGLSLAGNK
NP_001158090.1       VERYSQKAARNGMKNQFNLHELKMKGPEPVVSQIIDKLKHINQLLRTMSMPKGRVLDKNL
NP_001158091.1       VERYSQKAARNGMKNQFNLHELKMKGPEPVVSQIIDKLKHINQLLRTMSMPKGRVLDKNL
NP_001158089.1       VERYSQKAARNGMKNQFNLHELKMKGPEPVVSQIIDKLKHINQLLRTMSMPKGRVLDKNL
AAH35972.1           VERYSQKAARNGMKNQFNLHELKMKGPEPVVSQIIDKLKHINQLLRTMSMPKGRVLDKNL
NP_004475.1          VERYSQKAARNGMKNQFNLHELKMKGPEPVVSQIIDKLKHINQLLRTMSMPKGRVLDKNL
                       ***.  : . .        : : : **:   *  .                              *   *   *

gi|60219551          DRSRMSVFIVGIDPNGAAGKDGRLQIADELLEINGQILYGRSHQNASSIIKCAPSKVKII
NP_001158090.1       DEEGFESGDCGDDEDECIGGSG--------DGMIKVKNQ----------------------------------LR
NP_001158091.1       DEEGFESGDCGDDEDECIGGSG--------DGMIKVKNQ----------------------------------LR
NP_001158089.1       DEEGFESGDCGDDEDECIGGSG--------DGMIKVKNQ----------------------------------LR
AAH35972.1           DEEGFESGDCGDDEDECIGGSG--------DGMIKVKNQ----------------------------------LR
NP_004475.1          DEEGFESGDCGDDEDECIGGSG--------DGMIKVKNQ----------------------------------LR
                     * . . : .     * * :  . * .*      *  : : :: : . *                          :

gi|60219551          FIRNKDAVNQMAVCPGNAVEPLPSNSENLQNKETEPTVTTSDAAVDLSSFKNVQHLELPK
NP_001158090.1       FLAELAYDLDVDDAPGNSQQATPKDN-------------------------------EISTFHNLGNVHSPL
NP_001158091.1       FLAELAYDLDVDDAPGNSQQATPKDN-------------------------------EISTFHNLGNVHSPL
NP_001158089.1       FLAELAYDLDVDDAPGNSQQATPKDN-------------------------------EISTFHNLGNVHSPL
AAH35972.1           FLAELAYDLDVDDAPGNSQQATPKDN-------------------------------EISTFHNLGNVHSPL
NP_004475.1          FLAELAYDLDVDDAPGNSQQATPKDN-------------------------------EISTFHNLGNVHSPL
                     *: :      : :   .* **: :    * . : .                          : :*:* :*:  : : . *

gi|60219551          DQGGLGIAISEEDTLSGVIIKSLTEHGVAATDGRLKVGDQILAVDDEIVVGYPIEKFISL
NP_001158090.1       KL-------------------LTSMA-------------------------------------------------IS----------
NP_001158091.1       KL-------------------LTSMA-------------------------------------------------IS----------
NP_001158089.1       KL-------------------LTSMA-------------------------------------------------IS----------
AAH35972.1           KL-------------------LTSMA-------------------------------------------------IS----------
NP_004475.1          KL-------------------LTSMA-------------------------------------------------IS----------
                     .                  *: . :

gi|60219551          LKTAKMTVKLTIHAENPDSQAVPSAAGAASGEKKNSSQSLMVPQSGSPEPESIRNTSRSS
NP_001158090.1       ------------------------------------------------------------------------------------
NP_001158091.1       ------------------------------------------------------------------------------------
NP_001158089.1       ------------------------------------------------------------------------------------
AAH35972.1           ------------------------------------------------------------------------------------
NP_004475.1          ------------------------------------------------------------------------------------
```

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gi|60219551          TPAIFASDPATCPIIPGCETTIEISKGRTGLGLSIVGGSDTLLGAIIIHEVYEEGAACKD
NP_001158090.1       ------------------------------------------------------------
NP_001158091.1       ------------------------------------------------------------
NP_001158089.1       ------------------------------------------------------------
AAH35972.1           ------------------------------------------------------------
NP_004475.1          ------------------------------------------------------------

gi|60219551          GRLWAGDQILEVNGIDLRKATHDEAINVLRQTPQRVRLTLYRDEAPYKEEEVCDTLTIEL
NP_001158090.1       ------------------------------------------------------------
NP_001158091.1       ------------------------------------------------------------
NP_001158089.1       ------------------------------------------------------------
AAH35972.1           ------------------------------------------------------------
NP_004475.1          ------------------------------------------------------------

gi|60219551          QKKPGKGLGLSIVGKRNDTGVFVSDIVKGGIADADGRLMQGDQILMVNGEDVRNATQEAV
NP_001158090.1       ------------------------------------------------------------
NP_001158091.1       ------------------------------------------------------------
NP_001158089.1       ------------------------------------------------------------
AAH35972.1           ------------------------------------------------------------
NP_004475.1          ------------------------------------------------------------

gi|60219551          AALLKCSLGTVTLEVGRIKAGPFHSERRPSQSSQVSEGSLSSFTFPLSGSSTSESLESSS
NP_001158090.1       ------------------------------------------------------------
NP_001158091.1       ------------------------------------------------------------
NP_001158089.1       ------------------------------------------------------------
AAH35972.1           ------------------------------------------------------------
NP_004475.1          ------------------------------------------------------------

gi|60219551          KKNALASEIQGLRTVEMKKGPTDSLGISIAGGVGSPLGDVPIFIAMMHPTGVAAQTQKLR
NP_001158090.1       ------------------------------------------------------------
NP_001158091.1       ------------------------------------------------------------
NP_001158089.1       ------------------------------------------------------------
AAH35972.1           ------------------------------------------------------------
NP_004475.1          ------------------------------------------------------------

gi|60219551          VGDRIVTICGTSTEGMTHTQAVNLLKNASGSIEMQVVAGGDMSVVTGHQQEPASSSLSFT
NP_001158090.1       ------------------------------------------------------------
NP_001158091.1       ------------------------------------------------------------
NP_001158089.1       ------------------------------------------------------------
AAH35972.1           ------------------------------------------------------------
NP_004475.1          ------------------------------------------------------------

gi|60219551          GLTSSSIFQDDLGPPQCKSITLERGPDGLGFSIVGGYGSPHGDLPIYVKTVFAKGAASED
NP_001158090.1       ------------------------------------------------------------
NP_001158091.1       ------------------------------------------------------------
NP_001158089.1       ------------------------------------------------------------
AAH35972.1           ------------------------------------------------------------
NP_004475.1          ------------------------------------------------------------

gi|60219551          GRLKRGDQIIAVNGQSLEGVTHEEAVAILKRTKGTVTLMVLS
NP_001158090.1       -------------------------------------------
NP_001158091.1       -------------------------------------------
NP_001158089.1       -------------------------------------------
AAH35972.1           -------------------------------------------
NP_004475.1          -------------------------------------------
```

Ref. program: https://www.ebi.ac.uk/Tools/msa/clustalo/

Figure 4 Band of approximately 135 kD precipitated by mAb 1E4-1D9 was analyzed by LC-MS. A: Prediction of glycosylation sites and transmembrane region in hypothetical protein sequence gi60219551 was predicted by TMHMMM software; B: Number of cysteine was determined from all 1378 amino acid sequence, yellow highlight are N-glycosylation sites, green letter are cysteine, blue letter are glycosylation sites; C: Sequence of gi60219551 was aligned with glypican-3 based on the reliable program on website: https://blast.ncbi.nlm.nih.gov/Blast.cgi.

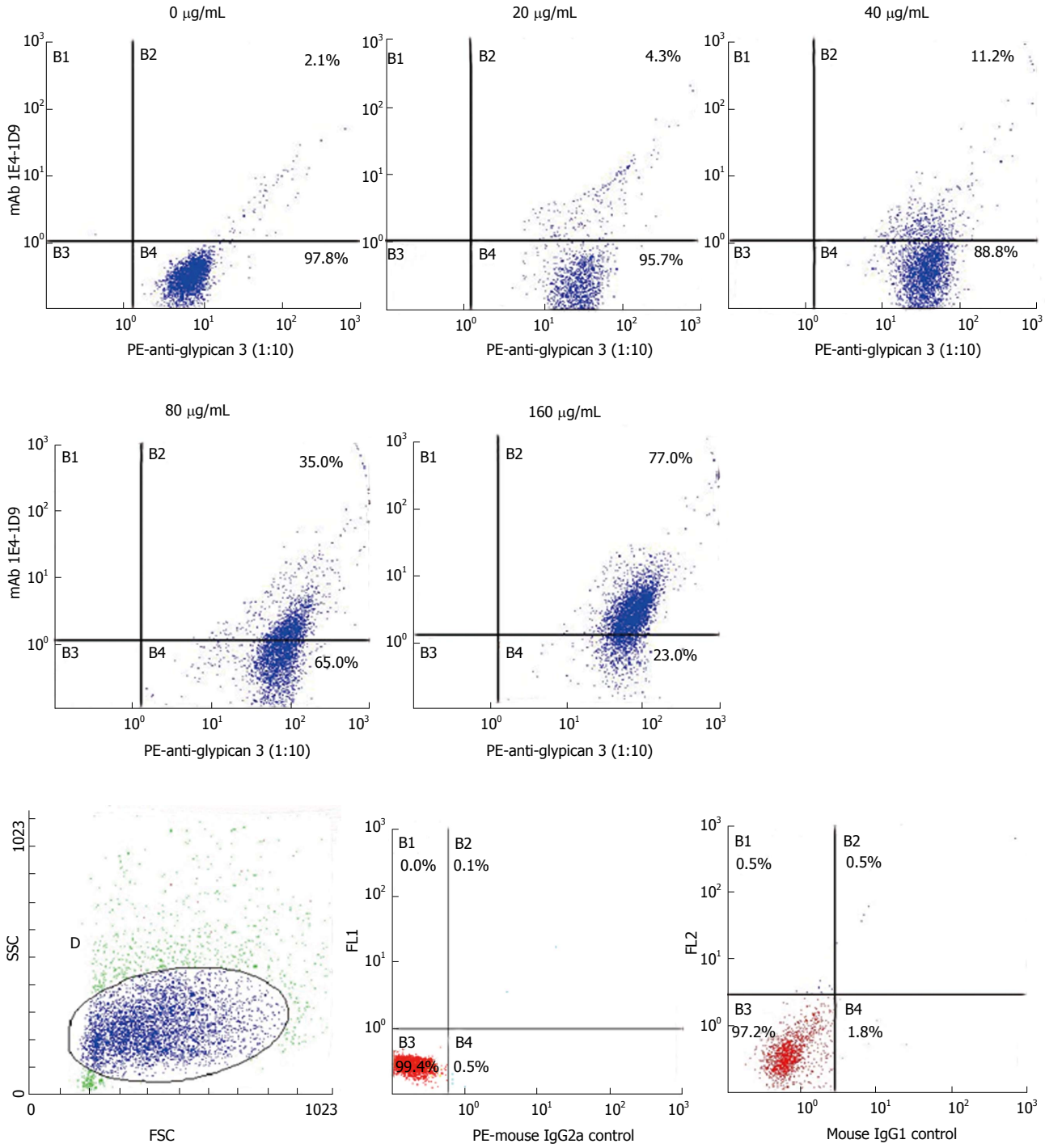

Figure 5 HepG2 cells (4 × 10^5 cells/mL) were reacted with various final concentrations of mAb 1E4-1D9 (0-160 μg/mL) for 30 min on ice. Mouse IgG1 was used as isotype control. After washing, cells were stained with fluorescein isothiocyanate-conjugated rabbit anti-mouse Igs (1:20) for 30 min on ice and washed. PE-conjugated anti-glypican-3 (1:10) was added and reaction was incubated for another 30 min on ice. Phycoerythrin-conjugated mouse IgG2a was used as isotype control in this step. After washing, cells were suspended with 300 μL of 0.5%paraformaldehyde in phosphate buffered saline, pH 7.2 and analyzed by flow cytometry.

amino acid). Both sequences had no transmembrane domain indicating that they might be either intracellular or external proteins. The first sequence with 19 cysteines, gi30722350 was FYVE containing protein and found 99.7% matched to FYCO1. This is very surprising since there have been no reports of a relationship between FYCO1 and glypican-3. FYCO1 is FYVE (Fab1, YOYB, Vac1, EEA1) and coiled-coil domain containing[52] FYCO1, an endogenous protein with MW of 150 kD resides on perinuclear cytosolic vesicles. However, during a starvation period, FYCO1 redistributes to the cell periphery in microtubule-dependent manner[53]. It functions as an adapter mediating autophagosome to microtubule plus-end-directed molecular motors[54]. FYCO1 can be dimerized

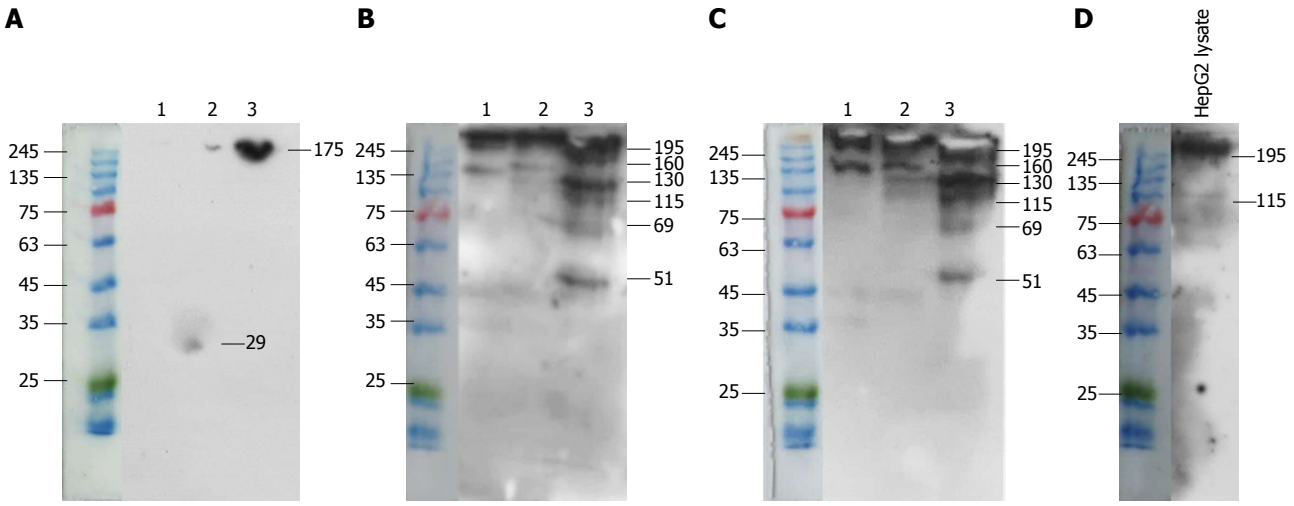

Figure 6 Specific antigen was precipitated from HepG2 lysate by mouse IgG1 (1), or anti-glypican-3 (2), or mAb 1E4-1D9 (3). The antigen was electrophoresed in 10% sodium dodecylsulfate-polyacrylamide gel electrophoresis at 200V for 45 min in non-reduced condition and blotted onto PVDF membrane. The antigen was probed with mouse IgG1, isotype control (A), or anti-glypican-3 (B), or mAb 1E4-1D9 (C), compared to HepG2 lysate probed with anti-glypican-3 (D). The reaction was detected by horseradish peroxidase-conjugated rabbit anti-mouse Igs and visualized by SuperSignal™ West Pico Chemiluminescent Substrate. PVDF: Polyvinyl difluoride.

and recruited to the phosphatidylinositol-3-phosphate, PtdIns(3)P. Findings in the study demonstrate a protein band of 160 kD co-precipitated with a band of 69 kD by anti-glypican-3 itself or mAb 1E4-1D9. This band of 160 kD was identified FYCO1. Additional studies, are required to better understand the biological function of this relationship.

Amino acid sequence analysis revealed that the second sequence, gi60219551 with 1378 amino acid was a PDZ containing protein and 85.7% matched to glypican-3. More information confirmed the structure since there are 15 cysteines and 11 putative glycosylation sites with 6 predicted N-glycosylation sites. A band of 69 kD was precipitated with mAb 1E4-1D9 as was with anti-glypican-3. However, there were two bands of 115 and 130 kD with higher MW observed which might correspond to GAG-remaining attached protein. Indirect immunofluorescence staining of various concentrations of mAb 1E4-1D9 on HepG2 cells following with the PE-conjugated anti-glypican-3 was performed to confirm the glypican-3 specificity of mAb 1E4-1D9. It was revealed that increasing amount of mAb 1E4-1D9 showed the higher number cells in upper right quadrant. This demonstrates that HepG2 can react with both antibodies through the same antigen. PE-conjugated anti-glypican-3 used in the experiment was raised against recombinant human glypican-3 at amino acid 25-558 (available information from datasheet). According to the competition experiments, we suggest that antigenic sites of mAb 1E4-1D9 are at or closed to N-terminus. This hypothesis was confirmed by co-precipitation of specific antigen from HepG2 lysate that the same protein bands were precipitated and visualized by cross-reaction between two antibodies. The protein band precipitated by mAb 1E4-1D9 was also detected by anti-glypican-3.

Moreover, since glypican-3 is expressed on a variety of malignant cell lines, indirect immunofluorescence technique was performed. We found that mAb 1E4-1D9 strongly reacted with an antigen on malignant cell lines including B16F1, Caco2, HT29, MCF7 and SW620. A strongly positive signal was observed when staining hematopoietic cell lines including K562, U937 and Molt4. In some cell lines, mAb 1E4-1D9 was weakly reacted (SW1353 and HL60) and in some no reaction was observed (H460 and Hela). These results are consistent with previous reports[55-59].

Glypican-3 is highly expressed on HCC and plays roles in cellular bioactivities, thus, it is the attractive molecule for developing a therapeutic antibody for HCC treatment. In addition, effect of anti-glypican-3 on proliferation inhibition is dependent on functional epitope of antibody[15]. Taken together, these findings support that mAb 1E4-1D9 raised against human liver HSPG is specific for glypican-3. This antibody is specific to HepG2 and glypican-3 expressing malignant cells. The effect of antibody on cell proliferation needs to be studied to understand whether it can be used as a tool for anti-cancer drug development. However, based on its specificity, it should be an excellent candidate monoclonal antibody for applications in tumor investigation as well as for tumor-targeted immunotherapy. Interestingly, the present study also discovers FYCO1 as a possible partner molecule of glypican-3. The findings merit further investigation, which may be applicable and beneficial for immuno- or gene-therapy in clinical settings for the treatment of HCC.

ACKNOWLEDGMENTS

The authors are gratefully acknowledged Dr. Sitthiruk Roytrakul, National Center for Genetic Engineering and Biotechnology, Bangkok, for his kind analysis of amino acid sequence. Many thanks were sent to Ms. Yupanun Wutti-In for her excellent technical assistance.

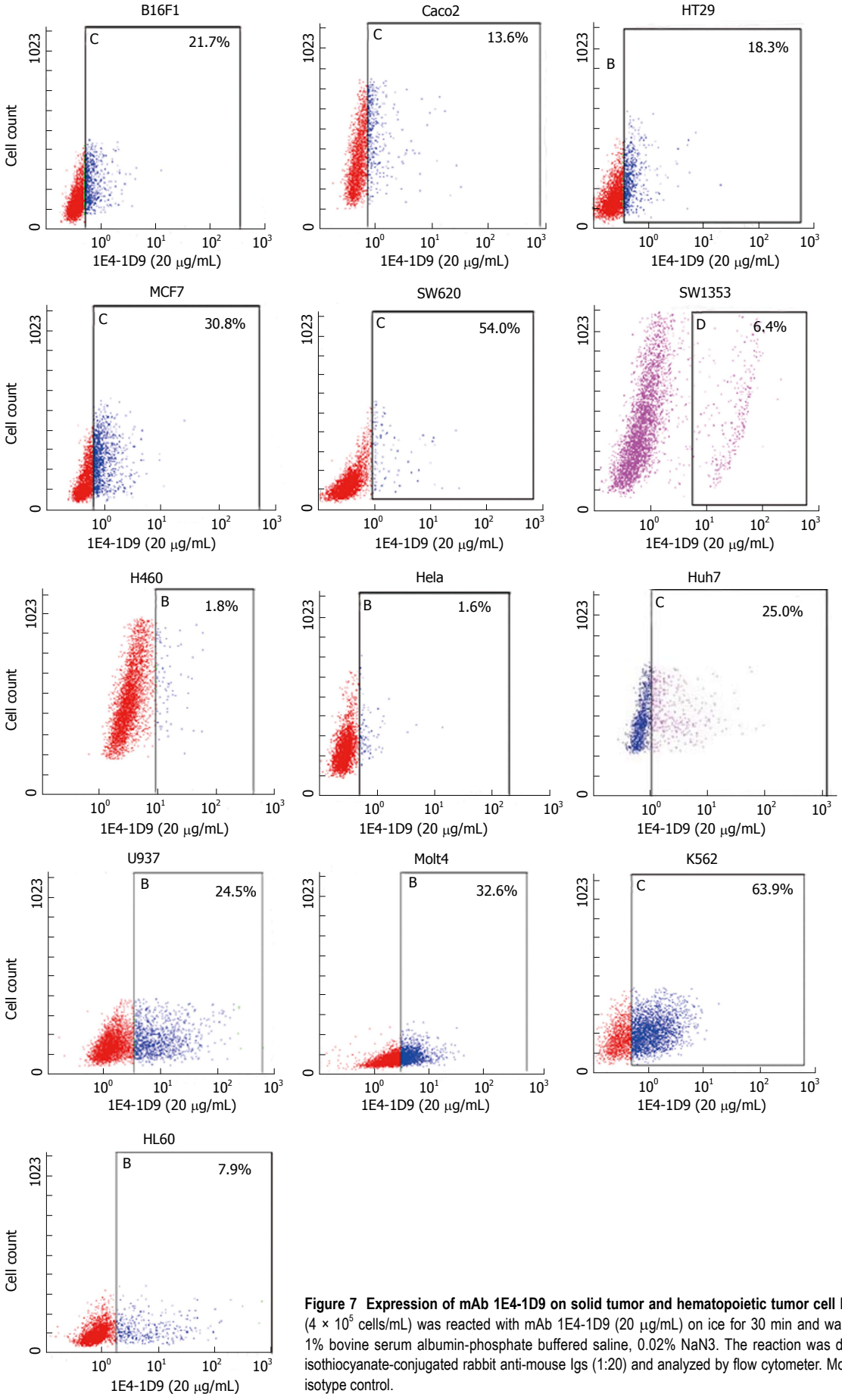

Figure 7 Expression of mAb 1E4-1D9 on solid tumor and hematopoietic tumor cell lines. Various cell lines (4×10^5 cells/mL) was reacted with mAb 1E4-1D9 (20 μg/mL) on ice for 30 min and washed 3 times with cold 1% bovine serum albumin-phosphate buffered saline, 0.02% NaN3. The reaction was detected by fluorescein isothiocyanate-conjugated rabbit anti-mouse Igs (1:20) and analyzed by flow cytometer. Mouse IgG1 was used as isotype control.

COMMENTS

Background

Among most malignant tumors worldwide hepatocellular carcinoma (HCC) is ranked in the fifth most common malignancy and the third leading cause of death. Patients with HCC have a very poor prognosis and the 5-year survival rate of less than 5%-10%. The reasons are that clinical diagnosis usually occurs at a late stage and there are limitations in drug- and surgery-based treatment. Therefore, new strategies and effective treatment as well as early detection using tumor specific monoclonal antibodies are needed. Glypican-3, a glycosylphosphatidylinositol-linked cell surface heparan sulfate proteoglycan (HSPG) is highly expressed in HCC. In some particular conditions, glypican-3 can be cleaved and released into serum and used as a biomarker for HCC. Glypican-3 is involved in growth signalling through Wnts, hedhogs, fibroblast growth factor, and bone morphogenetic proteins. Based on its function in tumor growth regulation, an antibody specific to glypican-3, would be important for the development of tumor-targeted drug delivery and immunotherapy. Previously, HSPG was isolated from human liver. Biochemical characterization revealed that liver HSPG consisted of heparan sulfate chain with a high level of sulfation. Preliminary result showed that liver HSPG could reacted with anti-rat glypican. A monoclonal antibody against liver HSPG was raised and mAb 1E4-1D9 obtained was studied to determine whether it recognized glypican-3.

Research frontiers

Important fields related to this study using mAb 1E4-1D9 as a tool include: (1) tumor detection and investigation such as developing of serological detection system and other clinical applications; (2) tumor-targeted drug delivery and drug design both in immunotherapy and gene therapy; and (3) understanding the role of glypican-3 in regulation of intracellular signalling in many cell types.

Innovations and breakthroughs

Glypican-3 is upregulated in HCC and many tumor cell types where it enhances cell growth in particular growth-signalling pathways. Research focusing on the production of monoclonal antibodies specific to glypican-3 are important to explore new diagnostic and therapeutic candidates. Findings from present study based on HepG2 cells indicates that specific antigen of a new monoclonal antibody, 1E4-1D9 is glypican-3. In addition, this is the first report showing FYCO1 as a potential partner molecule for glypican-3. The findings merit further investigation, which may be applicable and beneficial for immune- or gene therapy in clinical setting for the treatment of HCC.

Applications

Glypican-3 specific monoclonal antibody, 1E4-1D9, can be a tool for development of laboratory investigation for HCC and other glypican-3 expressed tumors. In addition, it will be a good candidate for tumor-targeted drug development, immunotherapy and gene therapy.

Peer-review

Early detection of HCC is very important to study, the glypican-3 is a good point to research, so topic of paper is novel and design of experiment is precise.

REFERENCES

1 **Dhanasekaran R**, Limaye A, Cabrera R. Hepatocellular carcinoma: current trends in worldwide epidemiology, risk factors, diagnosis, and therapeutics. *Hepat Med* 2012; **4**: 19-37 [PMID: 24367230]

2 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]

3 **Cao H**, Phan H, Yang LX. Improved chemotherapy for hepatocellular carcinoma. *Anticancer Res* 2012; **32**: 1379-1386 [PMID: 22493374]

4 **Calvisi DF**, Ladu S, Gorden A, Farina M, Lee JS, Conner EA, Schroeder I, Factor VM, Thorgeirsson SS. Mechanistic and prognostic significance of aberrant methylation in the molecular pathogenesis of human hepatocellular carcinoma. *J Clin Invest* 2007; **117**: 2713-2722 [PMID: 17717605 DOI: 10.1172/JCI31457]

5 **Kern MA**, Breuhahn K, Schirmacher P. Molecular pathogenesis of human hepatocellular carcinoma. *Adv Cancer Res* 2002; **86**: 67-112 [PMID: 12374281 DOI: 10.1016/S0065-230X(02)86003-1]

6 **Capurro M**, Wanless IR, Sherman M, Deboer G, Shi W, Miyoshi E, Filmus J. Glypican-3: a novel serum and histochemical marker for hepatocellular carcinoma. *Gastroenterology* 2003; **125**: 89-97 [PMID: 12851874 DOI: 10.1016/S0016-5085(03)00689-9]

7 **Kandil DH**, Cooper K. Glypican-3: a novel diagnostic marker for hepatocellular carcinoma and more. *Adv Anat Pathol* 2009; **16**: 125-129 [PMID: 19550373 DOI: 10.1097/PAP.0b013e3181992455]

8 **Yamauchi N**, Watanabe A, Hishinuma M, Ohashi K, Midorikawa Y, Morishita Y, Niki T, Shibahara J, Mori M, Makuuchi M, Hippo Y, Kodama T, Iwanari H, Aburatani H, Fukayama M. The glypican 3 oncofetal protein is a promising diagnostic marker for hepatocellular carcinoma. *Mod Pathol* 2005; **18**: 1591-1598 [PMID: 15920546 DOI: 10.1038/modpathol.3800436]

9 **Wang XY**, Degos F, Dubois S, Tessiore S, Allegretta M, Guttmann RD, Jothy S, Belghiti J, Bedossa P, Paradis V. Glypican-3 expression in hepatocellular tumors: diagnostic value for preneoplastic lesions and hepatocellular carcinomas. *Hum Pathol* 2006; **37**: 1435-1441 [PMID: 16949914 DOI: 10.1016/j.humpath.2006.05.016]

10 **Coston WM**, Loera S, Lau SK, Ishizawa S, Jiang Z, Wu CL, Yen Y, Weiss LM, Chu PG. Distinction of hepatocellular carcinoma from benign hepatic mimickers using Glypican-3 and CD34 immunohistochemistry. *Am J Surg Pathol* 2008; **32**: 433-444 [PMID: 18300806 DOI: 10.1097/PAS.0b013e318158142f]

11 **Wang HL**, Anatelli F, Zhai QJ, Adley B, Chuang ST, Yang XJ. Glypican-3 as a useful diagnostic marker that distinguishes hepatocellular carcinoma from benign hepatocellular mass lesions. *Arch Pathol Lab Med* 2008; **132**: 1723-1728 [PMID: 18976006]

12 **Wang F**, Jing X, Wang T, Li G, Li T, Zhang Q, Huang Y, Li J, Wang Y, Gao Y, Han T, Du Z. Differential diagnostic value of GPC3-CD34 combined staining in small liver nodules with diameter less than 3 cm. *Am J Clin Pathol* 2012; **137**: 937-945 [PMID: 22586053 DOI: 10.1309/ajcp0kzz5dsigmny]

13 **Wang FH**, Yip YC, Zhang M, Vong HT, Chan KI, Wai KC, Wen JM. Diagnostic utility of glypican-3 for hepatocellular carcinoma on liver needle biopsy. *J Clin Pathol* 2010; **63**: 599-603 [PMID: 20501450 DOI: 10.1136/jcp.2010.075374]

14 **Zhang L**, Liu H, Sun L, Li N, Ding H, Zheng J. Glypican-3 as a potential differential diagnosis marker for hepatocellular carcinoma: a tissue microarray-based study. *Acta Histochem* 2012; **114**: 547-552 [PMID: 22119409 DOI: 10.1016/j.acthis.2011.10.003]

15 **Feng M**, Ho M. Glypican-3 antibodies: a new therapeutic target for liver cancer. *FEBS Lett* 2014; **588**: 377-382 [PMID: 24140348 DOI: 10.1016/j.febslet.2013.10.002]

16 **Midorikawa Y**, Ishikawa S, Iwanari H, Imamura T, Sakamoto H, Miyazono K, Kodama T, Makuuchi M, Aburatani H. Glypican-3, overexpressed in hepatocellular carcinoma, modulates FGF2 and BMP-7 signaling. *Int J Cancer* 2003; **103**: 455-465 [PMID: 12478660 DOI: 10.1002/ijc.10856]

17 **Nakano K**, Orita T, Nezu J, Yoshino T, Ohizumi I, Sugimoto M, Furugaki K, Kinoshita Y, Ishiguro T, Hamakubo T, Kodama T, Aburatani H, Yamada-Okabe H, Tsuchiya M. Anti-glypican 3 antibodies cause ADCC against human hepatocellular carcinoma cells. *Biochem Biophys Res Commun* 2009; **378**: 279-284 [PMID: 19022220 DOI: 10.1016/j.bbrc.2008.11.033]

18 **Ishiguro T**, Sugimoto M, Kinoshita Y, Miyazaki Y, Nakano K, Tsunoda H, Sugo I, Ohizumi I, Aburatani H, Hamakubo T, Kodama T, Tsuchiya M, Yamada-Okabe H. Anti-glypican 3 antibody as a potential antitumor agent for human liver cancer. *Cancer Res* 2008; **68**: 9832-9838 [PMID: 19047163 DOI: 10.1158/0008-5472.CAN-08-1973]

19 **Nakano K**, Ishiguro T, Konishi H, Tanaka M, Sugimoto M, Sugo I, Igawa T, Tsunoda H, Kinoshita Y, Habu K, Orita T, Tsuchiya M, Hattori K, Yamada-Okabe H. Generation of a humanized anti-glypican 3 antibody by CDR grafting and stability optimization. *Anticancer Drugs* 2010; **21**: 907-916 [PMID: 20847643 DOI: 10.1097/CAD.0b013e32833f5d68]

20 **Feng M**, Gao W, Wang R, Chen W, Man YG, Figg WD, Wang XW, Dimitrov DS, Ho M. Therapeutically targeting glypican-3 via

a conformation-specific single-domain antibody in hepatocellular carcinoma. *Proc Natl Acad Sci USA* 2013; **110**: E1083-E1091 [PMID: 23471984 DOI: 10.1073/pnas.1217868110]
21 **Zhu AX**, Gold PJ, El-Khoueiry AB, Abrams TA, Morikawa H, Ohishi N, Ohtomo T, Philip PA. First-in-man phase I study of GC33, a novel recombinant humanized antibody against glypican-3, in patients with advanced hepatocellular carcinoma. *Clin Cancer Res* 2013; **19**: 920-928 [PMID: 23362325 DOI: 10.1158/1078-0432.CCR-12-2616]
22 **Liu S**, Li Y, Chen W, Zheng P, Liu T, He W, Zhang J, Zeng X. Silencing glypican-3 expression induces apoptosis in human hepatocellular carcinoma cells. *Biochem Biophys Res Commun* 2012; **419**: 656-661 [PMID: 22382024 DOI: 10.1016/j.bbrc.2012.02.069]
23 **Filmus J**, Capurro M, Rast J. Glypicans. *Genome Biol* 2008; **9**: 224 [PMID: 18505598 DOI: 10.1186/gb-2008-9-5-224]
24 **Häcker U**, Nybakken K, Perrimon N. Heparan sulphate proteoglycans: the sweet side of development. *Nat Rev Mol Cell Biol* 2005; **6**: 530-541 [PMID: 16072037 DOI: 10.1038/nrm1681]
25 **Lin X**. Functions of heparan sulfate proteoglycans in cell signaling during development. *Development* 2004; **131**: 6009-6021 [PMID: 15563523 DOI: 10.1242/dev.01522]
26 **Capurro MI**, Xiang YY, Lobe C, Filmus J. Glypican-3 promotes the growth of hepatocellular carcinoma by stimulating canonical Wnt signaling. *Cancer Res* 2005; **65**: 6245-6254 [PMID: 16024626 DOI: 10.1158/0008-5472.CAN-04-4244]
27 **Filmus J**, Selleck SB. Glypicans: proteoglycans with a surprise. *J Clin Invest* 2001; **108**: 497-501 [PMID: 11518720 DOI: 10.1172/JCI200113712]
28 **Traister A**, Shi W, Filmus J. Mammalian Notum induces the release of glypicans and other GPI-anchored proteins from the cell surface. *Biochem J* 2008; **410**: 503-511 [PMID: 17967162 DOI: 10.1042/BJ20070511]
29 **De Cat B**, Muyldermans SY, Coomans C, Degeest G, Vanderschueren B, Creemers J, Biemar F, Peers B, David G. Processing by proprotein convertases is required for glypican-3 modulation of cell survival, Wnt signaling, and gastrulation movements. *J Cell Biol* 2003; **163**: 625-635 [PMID: 14610063 DOI: 10.1083/jcb.200302152]
30 **Capurro MI**, Shi W, Sandal S, Filmus J. Processing by convertases is not required for glypican-3-induced stimulation of hepatocellular carcinoma growth. *J Biol Chem* 2005; **280**: 41201-41206 [PMID: 16227623 DOI: 10.1074/jbc.M507004200]
31 **Zittermann SI**, Capurro MI, Shi W, Filmus J. Soluble glypican 3 inhibits the growth of hepatocellular carcinoma in vitro and in vivo. *Int J Cancer* 2010; **126**: 1291-1301 [PMID: 19816934]
32 **Filmus J**, Capurro M. The role of glypican-3 in the regulation of body size and cancer. *Cell Cycle* 2008; **7**: 2787-2790 [PMID: 18787398 DOI: 10.4161/cc.7.18.6672]
33 **Stigliano I**, Puricelli L, Filmus J, Sogayar MC, Bal de Kier Joffé E, Peters MG. Glypican-3 regulates migration, adhesion and actin cytoskeleton organization in mammary tumor cells through Wnt signaling modulation. *Breast Cancer Res Treat* 2009; **114**: 251-262 [PMID: 18404367 DOI: 10.1007/s10549-008-0009-2]
34 **Suzuki M**, Sugimoto K, Tanaka J, Tameda M, Inagaki Y, Kusagawa S, Nojiri K, Beppu T, Yoneda K, Yamamoto N, Ito M, Yoneda M, Uchida K, Takase K, Shiraki K. Up-regulation of glypican-3 in human hepatocellular carcinoma. *Anticancer Res* 2010; **30**: 5055-5061 [PMID: 21187490]
35 **Zhu ZW**, Friess H, Wang L, Abou-Shady M, Zimmermann A, Lander AD, Korc M, Kleeff J, Büchler MW. Enhanced glypican-3 expression differentiates the majority of hepatocellular carcinomas from benign hepatic disorders. *Gut* 2001; **48**: 558-564 [PMID: 11247902 DOI: 10.1136/gut.48.4.558]
36 **Nakatsura T**, Yoshitake Y, Senju S, Monji M, Komori H, Motomura Y, Hosaka S, Beppu T, Ishiko T, Kamohara H, Ashihara H, Katagiri T, Furukawa Y, Fujiyama S, Ogawa M, Nakamura Y, Nishimura Y. Glypican-3, overexpressed specifically in human hepatocellular carcinoma, is a novel tumor marker. *Biochem Biophys Res Commun* 2003; **306**: 16-25 [PMID: 12788060 DOI: 10.1016/S0006-291X(03)00908-2]
37 **Hippo Y**, Watanabe K, Watanabe A, Midorikawa Y, Yamamoto S, Ihara S, Tokita S, Iwanari H, Ito Y, Nakano K, Nezu J, Tsunoda H, Yoshino T, Ohizumi I, Tsuchiya M, Ohnishi S, Makuuchi M, Hamakubo T, Kodama T, Aburatani H. Identification of soluble NH2-terminal fragment of glypican-3 as a serological marker for early-stage hepatocellular carcinoma. *Cancer Res* 2004; **64**: 2418-2423 [PMID: 15059894 DOI: 10.1158/0008-5472.CAN-03-2191]
38 **Baumhoer D**, Tornillo L, Stadlmann S, Roncalli M, Diamantis EK, Terracciano LM. Glypican 3 expression in human nonneoplastic, preneoplastic, and neoplastic tissues: a tissue microarray analysis of 4,387 tissue samples. *Am J Clin Pathol* 2008; **129**: 899-906 [PMID: 18480006 DOI: 10.1309/HCQWPWD50XHD2DW6]
39 **Llovet JM**, Chen Y, Wurmbach E, Roayaie S, Fiel MI, Schwartz M, Thung SN, Khitrov G, Zhang W, Villanueva A, Battiston C, Mazzaferro V, Bruix J, Waxman S, Friedman SL. A molecular signature to discriminate dysplastic nodules from early hepatocellular carcinoma in HCV cirrhosis. *Gastroenterology* 2006; **131**: 1758-1767 [PMID: 17087938 DOI: 10.1053/j.gastro.2006.09.014]
40 **Cheng W**, Tseng CJ, Lin TT, Cheng I, Pan HW, Hsu HC, Lee YM. Glypican-3-mediated oncogenesis involves the Insulin-like growth factor-signaling pathway. *Carcinogenesis* 2008; **29**: 1319-1326 [PMID: 18413366 DOI: 10.1093/carcin/bgn091]
41 **Lai JP**, Oseini AM, Moser CD, Yu C, Elsawa SF, Hu C, Nakamura I, Han T, Aderca I, Isomoto H, Garrity-Park MM, Shire AM, Li J, Sanderson SO, Adjei AA, Fernandez-Zapico ME, Roberts LR. The oncogenic effect of sulfatase 2 in human hepatocellular carcinoma is mediated in part by glypican 3-dependent Wnt activation. *Hepatology* 2010; **52**: 1680-1689 [PMID: 20725905 DOI: 10.1002/hep.23848]
42 **Chen M**, Li G, Yan J, Lu X, Cui J, Ni Z, Cheng W, Qian G, Zhang J, Tu H. Reevaluation of glypican-3 as a serological marker for hepatocellular carcinoma. *Clin Chim Acta* 2013; **423**: 105-111 [PMID: 23643963 DOI: 10.1016/j.cca.2013.04.026]
43 **Bazin HG**, Marques MA, Owens AP, Linhardt RJ, Crutcher KA. Inhibition of apolipoprotein E-related neurotoxicity by glycosaminoglycans and their oligosaccharides. *Biochemistry* 2002; **41**: 8203-8211 [PMID: 12069613 DOI: 10.1021/bi025817e]
44 **Lin YL**, Lei HY, Lin YS, Yeh TM, Chen SH, Liu HS. Heparin inhibits dengue-2 virus infection of five human liver cell lines. *Antiviral Res* 2002; **56**: 93-96 [PMID: 12323403 DOI: 10.1016/S0166-3542(02)00095-5]
45 **Pradel G**, Garapaty S, Frevert U. Proteoglycans mediate malaria sporozoite targeting to the liver. *Mol Microbiol* 2002; **45**: 637-651 [PMID: 12139612 DOI: 10.1046/j.1365-2958.2002.03057.x]
46 **Barth H**, Schafer C, Adah MI, Zhang F, Linhardt RJ, Toyoda H, Kinoshita-Toyoda A, Toida T, Van Kuppevelt TH, Depla E, Von Weizsacker F, Blum HE, Baumert TF. Cellular binding of hepatitis C virus envelope glycoprotein E2 requires cell surface heparan sulfate. *J Biol Chem* 2003; **278**: 41003-41012 [PMID: 12867431 DOI: 10.1074/jbc.M302267200]
47 **Vongchan P**, Warda M, Toyoda H, Toida T, Marks RM, Linhardt RJ. Structural characterization of human liver heparan sulfate. *Biochim Biophys Acta* 2005; **1721**: 1-8 [PMID: 15652173 DOI: 10.1016/j.bbagen.2004.09.007]
48 **Vongchan P**, Linhardt RJ. Expression of human liver HSPGs on acute myeloid leukemia. *Clin Immunol* 2007; **122**: 194-206 [PMID: 17035092 DOI: 10.1016/j.clim.2006.08.017]
49 **Vongchan P**, Kothan S, Wutti-In Y, Linhardt RJ. Inhibition of human tumor xenograft growth in nude mice by a novel monoclonal anti-HSPG isolated from human liver. *Anticancer Res* 2011; **31**: 4067-4074 [PMID: 22199263]
50 **Vongchan P**, Wutti-In Y, Sajomsang W, Gonil P, Kothan S, Linhardt RJ. N,N,N-Trimethyl chitosan nanoparticles for the delivery of monoclonal antibodies against hepatocellular carcinoma cells. *Carbohydr Polym* 2011; **85**: 215-220 [PMID: 21552341 DOI: 10.1016/j.carbpol.2011.02.018]
51 **Zaghloul RA**, Al-Gayyar MM, El-Shishtawy MM, Ebrahim MA. Cytotoxic effects of antiglypican-3 against HepG2 cell line. *J App Pharm Sci* 2013; **3**: 5 [DOI: 10.7324/JAPS.2013.31206]

52 **Stenmark H**, Aasland R, Toh BH, D'Arrigo A. Endosomal localization of the autoantigen EEA1 is mediated by a zinc-binding FYVE finger. *J Biol Chem* 1996; **271**: 24048-24054 [PMID: 8798641 DOI: 10.1074/jbc.271.39.24048]
53 **Pankiv S**, Alemu EA, Brech A, Bruun JA, Lamark T, Overvatn A, Bjørkøy G, Johansen T. FYCO1 is a Rab7 effector that binds to LC3 and PI3P to mediate microtubule plus end-directed vesicle transport. *J Cell Biol* 2010; **188**: 253-269 [PMID: 20100911 DOI: 10.1083/jcb.200907015]
54 **Pankiv S**, Johansen T. FYCO1: linking autophagosomes to microtubule plus end-directing molecular motors. *Autophagy* 2010; **6**: 550-552 [PMID: 20364109 DOI: 10.4161/auto.6.4.11670]
55 **Motomura Y**, Senju S, Nakatsura T, Matsuyoshi H, Hirata S, Monji M, Komori H, Fukuma D, Baba H, Nishimura Y. Embryonic stem cell-derived dendritic cells expressing glypican-3, a recently identified oncofetal antigen, induce protective immunity against highly metastatic mouse melanoma, B16-F10. *Cancer Res* 2006; **66**: 2414-2422 [PMID: 16489048 DOI: 10.1158/0008-5472.CAN-05-2090]
56 **Gonzalez AD**, Kaya M, Shi W, Song H, Testa JR, Penn LZ, Filmus J. OCI-5/GPC3, a glypican encoded by a gene that is mutated in the Simpson-Golabi-Behmel overgrowth syndrome, induces apoptosis in a cell line-specific manner. *J Cell Biol* 1998; **141**: 1407-1414 [PMID: 9628896 DOI: 10.1083/jcb.141.6.1407]
57 **Huber R**, Hansen RS, Strazzullo M, Pengue G, Mazzarella R, D'Urso M, Schlessinger D, Pilia G, Gartler SM, D'Esposito M. DNA methylation in transcriptional repression of two differentially expressed X-linked genes, GPC3 and SYBL1. *Proc Natl Acad Sci USA* 1999; **96**: 616-621 [PMID: 9892682 DOI: 10.1073/pnas.96.2.616]
58 **Marquez BV**, Zheleznyak A, Lapi SE. Glypican-3-targeted 89Zr PET imaging of hepatocellular carcinoma: where antibody imaging dares to tread. *J Nucl Med* 2014; **55**: 708-709 [PMID: 24665087 DOI: 10.2967/jnumed.113.136234]
59 **Khan S**, Blackburn M, Mao DL, Huber R, Schlessinger D, Fant M. Glypican-3 (GPC3) expression in human placenta: localization to the differentiated syncytiotrophoblast. *Histol Histopathol* 2001; **16**: 71-78 [PMID: 11193214]

P- Reviewer: Jin B, Shen F, Yao DF **S- Editor**: Ji FF **L- Editor**: A
E- Editor: Li D

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/

World J Hepatol 2017 March 8; 9(7): 385-390

DOI: 10.4254/wjh.v9.i7.385

ORIGINAL ARTICLE

Case Control Study

Risk factors for hepatocellular carcinoma in cirrhosis due to nonalcoholic fatty liver disease: A multicenter, case-control study

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Institutional review board statement: This study was approved by the Institutional Review Boards at the respective institutions.

Informed consent statement: Informed consent was waived by all Institutional Review Boards as the data collected was retrospective in nature and risk was considered minimal.

Conflict-of-interest statement: No potential conflicts of interest relevant to this article were reported.

Data sharing statement: Statistical code and dataset are available from the corresponding author at kcorey@partners.org. Consent for data sharing was not obtained but the presented data are anonymized and risk of identification is low.

Manuscript source: Invited manuscript

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Received: July 12, 2016
Peer-review started: July 13, 2016
First decision: December 13, 2016
Revised: December 20, 2016
Accepted: February 8, 2017
Article in press: February 13, 2017
Published online: March 8, 2017

Abstract

AIM

To identify risk factors associated with hepatocellular

carcinoma (HCC), describe tumor characteristics and treatments pursed for a cohort of individuals with nonalcoholic steatohepatitis (NASH) cirrhosis.

METHODS

We conducted a retrospective case-control study of a well-characterized cohort of patients among five liver transplant centers with NASH cirrhosis with (cases) and without HCC (controls).

RESULTS

Ninety-four cases and 150 controls were included. Cases were significantly more likely to be male than controls (67% *vs* 45%, $P < 0.001$) and of older age (61.9 years *vs* 58 years, $P = 0.002$). In addition, cases were more likely to have had complications of end stage liver disease (83% *vs* 71%, $P = 0.032$). On multivariate analysis, the strongest association with the presence of HCC were male gender (OR 4.3, 95%CI: 1.83-10.3, $P = 0.001$) and age (OR = 1.082, 95%CI: 1.03-1.13, $P = 0.001$). Hispanic ethnicity was associated with a decreased prevalence of HCC (OR = 0.3, 95%CI: 0.09-0.994, $P = 0.048$). HCC was predominantly in the form of a single lesion with regional lymph node(s) and distant metastasis in only 2.6% and 6.3%, respectively. Fifty-nine point three percent of individuals with HCC underwent locoregional therapy and 61.5% underwent liver transplantation for HCC.

CONCLUSION

Male gender, increased age and non-Hispanic ethnicity are associated with HCC in NASH cirrhosis. NASH cirrhosis associated HCC in this cohort was characterized by early stage disease at diagnosis and treatment with locoregional therapy and transplant.

Key words: Hepatocellular carcinoma; Nonalcoholic fatty liver disease; Cirrhosis, Gender; Ethnicity

Core tip: The present paper identifies male gender, increased age and non-Hispanic ethnicity as factors associated with hepatocellular carcinoma (HCC) in nonalcoholic steatohepatitis cirrhosis. In this series, HCC in nonalcoholic fatty liver disease cirrhosis was diagnosed at an early stage and treated predominantly with locoregional therapy and liver transplantation.

Corey KE, Gawrieh S, deLemos AS, Zheng H, Scanga AE, Haglund JW, Sanchez J, Danford CJ, Comerford M, Bossi K, Munir S, Chalasani N, Wattacheril J. Risk factors for hepatocellular carcinoma in cirrhosis due to nonalcoholic fatty liver disease: A multicenter, case-control study. *World J Hepatol* 2017; 9(7): 385-390 Available from: URL: http://www.wjgnet.com/1948-5182/full/v9/i7/385.htm DOI: http://dx.doi.org/10.4254/wjh.v9.i7.385

INTRODUCTION

The burden of nonalcoholic fatty liver disease (NAFLD) is substantial. Estimates suggest 75-100 million people in the United States have NAFLD, and alarmingly, many of these patients are not aware of or evaluated for this condition[1,2]. A subset of individuals with NAFLD will develop nonalcoholic steatohepatitis (NASH), the inflammatory phenotype of NAFLD. Hepatic fibrosis and eventual cirrhosis is a consequence of NASH progression, particularly in genetically predisposed individuals. NASH cirrhosis is projected to be the leading indication for liver transplantation in the next 10-20 years[3].

Hepatocellular carcinoma (HCC), like NAFLD, is also underrecognized. In fact, a recent retrospective study suggested that only 20% of patients received appropriate surveillance before their HCC diagnosis[4]. Inadequate screening is a serious concern for patients with cirrhosis of any type. However, recent data suggests that a deficiency in screening may be particularly problematic for patients with NAFLD HCC who present at a later tumor stage, have shorter survival times, and lower rates of liver transplantation[5].

Thus, the convergence of NAFLD and HCC uniquely focuses the narrative for providers caring for these patients to enhance the screening and diagnosis of both diseases. Simultaneously, identifying risk factors for HCC development in patients with underlying NASH cirrhosis is critically important to improve screening and treatment. We have conducted a retrospective case-control study of a well-characterized cohort of patients with NASH cirrhosis with and without HCC in order to identify risk factors associated with HCC. We also provide tumor characteristics and survival data for this cohort. Our data, derived from five academic liver transplant centers, highlights patient characteristics associated with HCC and enhances the growing body of evidence on HCC in the setting NAFLD.

MATERIALS AND METHODS

We conducted a case-control study of individuals with NAFLD cirrhosis with and without HCC from five academic medical centers in the United States. NAFLD was diagnosed between 1991-2015 and all HCC cases were diagnosed between 2004-2015. This study was approved by the Institutional Review Boards at the respective institutions.

A diagnosis of NAFLD cirrhosis was made either (1) by histology; or (2) clinically. Clinical NAFLD was defined by the exclusion of other causes of chronic liver disease and the presence of one or more risk factors for NAFLD including diabetes, obesity or $\geqslant$ 1 component of the metabolic syndrome. The diagnosis of cirrhosis was made either by histology or by imaging suggestive of cirrhosis (nodular liver, splenomegaly, ascites or varices) in combination with laboratory values suggesting portal hypertension or impaired synthetic function (platelet count

< 150000/μL, albumin < 3.5 g/dL) or complications of end-stage liver disease. Characteristic liver histology for NASH served as one diagnostic modality; NAFLD Activity Score values were not available for all subjects.

Definition of cases

Cases were individuals with NAFLD cirrhosis and well-characterized HCC. HCC was defined by histology or imaging consistent with Organ Procurement and Transplantation Network criteria[6].

Definition of controls

Controls were defined as individuals meeting criteria for NASH cirrhosis but without evidence of HCC on imaging within one year following the diagnosis of cirrhosis. For each case, depending on the availability, we enrolled 1-3 controls from the same institution. Cases and controls were matched for the year of enrollment, *i.e.,* ascertainment of absence of HCC by imaging in the controls was in the same year as the HCC diagnosis in the cases.

Data collection

Charts were reviewed for weight, height, body mass index (BMI) and co-morbid disease including diabetes mellitus, hypertension, dyslipidemia, cardiovascular disease, obstructive sleep apnea, polycystic ovary syndrome and obesity. Complications of liver disease were also recorded including the presence of ascites, spontaneous bacterial peritonitis, hepatorenal syndrome, hepatic encephalopathy and gastroesophageal varices. These complications were combined in to a composite cirrhosis complication variable. Use of medications including metformin, pioglitazone, vitamin E, HMG-CoA reductase inhibitors ("statins") was also collected. Laboratory values for platelet count, INR, fasting insulin, fasting glucose, creatinine, alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase, total bilirubin, albumin, total cholesterol, low-density lipoprotein level, high-density lipoprotein level, triglycerides, glycosylated hemoglobin (A1C), ferritin, alpha-fetoprotein, and model for end-stage liver disease (MELD) score.MELD score was calculated according to the published formula[7].

Pathology reports were reviewed for the presence of HCC as well as TMN classification of malignant tumor status, differentiation status, vascular and/or perineural invasion and lymph node involvement. Imaging including ultrasound, computerized tomography scan or magnetic resonance imaging was reviewed for tumor number, size and location.

Statistical analysis

All statistical analyses were performed using SAS software, version V.9.2 (SAS Institute, Cary, NC). Continuous variables were analyzed using a Student's *t*-test for normally distributed variables and a Wilcoxon rank sum test for variables that were not normally distributed. Categorical variables were analyzed using a χ^2 test or Fisher's exact test as appropriate. Nominal, two-sided *P* values were used and were considered statistically significant if $P < 0.05$. The final model was selected by combining clinical judgment and statistical assessment. We included variables with $P < 0.1$ in univariate analysis and variables that are considered as known confounders. All analyses were carried out using SAS 9.4 (SAS Institute, Cary, NC) and Stata 13.1 (Stata Corp., College Station, TX).

RESULTS

Baseline characteristics

Two hundred and forty-four individuals (94 cases and 150 controls) were included. Individuals were predominantly male (54.7%), and Caucasian (81.8%) with a mean age of 59 years. Diabetes (69.5%), dyslipidemia (47.9%) and hypertension (60.1%) were frequent. Mean BMI was 33.5 kg/m^2 and mean MELD score was 12.

Seventy-five point four percent had a complication of cirrhosis with the most frequent being gastroesophageal varices (58.0%), ascites (48.6%) and encephalopathy (39.6%). Hepatorenal syndrome and spontaneous bacterial peritonitis were infrequent (3.3% and 4.2%, respectively).

Characteristics of cases and controls: Univariate analysis

Ninety-four cases and 150 controls were included in the present study. Cases were significantly more likely to be male than controls (67% *vs* 45%, $P < 0.001$) and be of older age (mean, 61.9 ± 9.4 *vs* 58.0 ± 9.9, $P = 0.002$). In addition, cases were more likely to have had complications of end-stage liver disease including ascites, SBP, HRS, gastroesophageal varices or encephalopathy (composite 83% *vs* 71%, $P = 0.032$). There was no difference between cases and controls by comorbidities, medication use including statins or vitamin E, or biochemical markers such as ALT or MELD score (Table 1).

Characteristics of cases and controls: Multivariate analysis

On multivariate analysis, after adjustment for the relevant confounders, the strongest association with the presence of HCC among those with NASH cirrhosis was male gender (OR = 4.3, 95%CI: 1.83-10.3, $P = 0.001$). In addition, age (OR = 1.082, 95%CI: 1.03-1.13, $P = 0.001$) was associated with HCC. Hispanic ethnicity was associated with a decreased prevalence of HCC (OR = 0.3, 95%CI: 0.09-0.994, $P = 0.048$) (Table 2).

Characteristics of HCC in NAFLD cirrhosis

HCC diagnosed in this cohort of individuals with NASH cirrhosis was predominantly in the form of a single lesion (median 1.0, IQR 1.0) with a median size of 2.7 cm (IQR 2.5) (Table 3). Regional lymph node and distant metastasis were recorded in only 2.6% and 6.3%, respectively. Vascular or perineural invasion was documented in

Table 1 Characteristics of cases and controls

Characteristic		Case (n = 94)	Control (n = 150)	P value
Age, yr (mean ± SD)		61.9 ± 9.4	58.0 ± 9.9	0.002
Gender	Female	33%	55%	< 0.001
	Male	67%	45%	
Race	White	85%	80.0%	0.605
	Black	1%	17%	
	Other	14%	3%	
Ethnicity	Not Hispanic	82%	90%	0.149
	Hispanic	18%	10%	
Diabetes mellitus	Yes	74%	67%	0.237
	No	26%	33%	
Hypertension	Yes	61%	60%	0.888
	No	39%	40%	
Dyslipidemia	Yes	50%	47%	0.609
	No	50%	53%	
Cardiovascular disease	Yes	28%	19%	0.093
	No	72%	81%	
Metformin use	Yes	40%	37%	0.660
	No	60%	63%	
Statin use	Yes	25%	21%	0.401
	No	75%	79%	
Vitamin E use	Yes	11%	9%	0.620
	No	89%	91%	
Ascites	Yes	51%	47%	0.628
	No	49%	53%	
Gastroesophageal varices	Yes	66%	53%	0.072
	No	34%	47%	
Hepatic encephalopathy	Yes	40%	40%	0.995
	No	60%	60%	
Complications of cirrhosis	Yes	83%	71%	0.032
	No	17%	29%	
BMI (kg/m^2)		32.8 ± 5.8	33.9 ± 7.3	0.222
ALT (IU/L) (mean ± SD)		43.3 ± 25.4	42.18 ± 37.3	0.819
MELD score (mean ± SD)		11.6 ± 4.4	12.39 ± 4.8	0.382

BMI: Body mass index; ALT: Alanine aminotransferase; MELD: Model for end-stage liver disease.

13.9% and 1.3%, respectively. Fifty-five prcent of tumors involved a single lobe of the liver, 25.6% were bilobar, while the lobar distribution was unknown in 32.4%.

Treatment for HCC

In this cohort, 59.3% of individuals with HCC underwent either locoregional therapy with radiofrequency ablation, transarterial chemoembolization or radiation. In addition, 61.5% of the entire cohort underwent liver transplantation for HCC. Resection was infrequent and took place in only 10% of the HCC cohort. Sorafenib and/or palliative care was administered in 10% of patients.

DISCUSSION

NASH cirrhosis is projected to become the leading indication for liver transplantation by 2020, surpassing alcohol and chronic hepatitis C infection[3]. Despite its public health impact, however, relatively little is known about the risk factors for HCC development in NASH cirrhosis. The present case-control study sought to address this gap by evaluating individuals with NASH cirrhosis with and without HCC.

Table 2 Variables associated with presence of hepatocellular carcinoma on multivariate analysis[1]

Variable	Univariate P value	Multivariate OR 95%CI	Multivariate P value
Age	0.002	1.08 (1.032-1.13)	0.001
Gender	< 0.001	4.34 (1.83-10.31)	< 0.001
BMI	0.22	0.96 (0.90-1.02)	0.20
Ethnicity	0.15	0.300 (0.090-0.994)	0.045
Platelet count	0.14	1.004 (1.00-1.01)	0.14
CVD	0.09	1.21 (0.61-2.41)	0.58
Gastroesophageal varices	0.07	1.43 (0.63-3.21)	0.39
Complications of cirrhosis	0.03	1.15 (0.43-3.02)	0.78

[1]The final model was selected by combining clinical judgment and statistical assessment. We included variables with P < 0.1 in univariate analysis and variables that are considered as known confounders. CVD: Cardiovascular disease; BMI: Body mass index.

We found that HCC was associated with male gender and older age. There was no difference between cases and controls with regards to comorbidities, prescription medications, vitamin E use, or biochemical markers such as ALT or MELD score. Surprisingly, the Hispanic ethnicity conferred a decreased risk of HCC.

The observed differences in sex and age are consistent with prior studies. Ascha *et al*[8] compared patients with HCC secondary to NASH cirrhosis to those with HCV cirrhosis and HCC. Compared to those with HCV, individuals with NASH and HCC were significantly older and had a trend toward an increased risk of HCC in men. Bugianesi *et al*[9] also evaluated risk factors for HCC in a cohort of 641 individuals with chronic liver disease of varying etiologies. Six point nine percent of the cohort had cryptogenic cirrhosis largely attributed to NASH. HCC in cryptogenic cirrhosis was associated with older age although no difference in gender was seen. These studies also found that HCC in NASH cirrhosis/cryptogenic cirrhosis was associated with BMI, obesity and diabetes mellitus. The present study did not find associations between diabetes, obesity, BMI or insulin resistance. Our use of NASH cirrhosis controls with high prevalence of diabetes and obesity may account for this difference as prior studies have compared NASH patients who are often characterized by diabetes and obesity to those with other forms of chronic liver disease among whom these comorbidities are less frequent.

Metabolic stress including development of the metabolic syndrome is not only associated with increased risk of cancer in general, but with risk for HCC. Presumably, most NAFLD patients meet criteria for diagnosis of the metabolic syndrome, yet a great proportion of these patients do not develop HCC. The present study did not find a significant difference in comorbidities between cases and controls. Just as only a subset of NAFLD patients progress to NASH, this lends further support to a genetic determinant for development of HCC within NAFLD. Investigation of genetic alterations in insulin signaling including the PI3K-AKT-PTEN pathway and other factors

Table 3 Tumor characteristics of hepatocellular carcinoma

Characteristics of HCC	*n* (%)
Primary tumor (T)	
1	27 (42.86)
2	28 (44.44)
3	8 (12.70)
Regional lymph nodes (N)	
Yes	2 (2.63)
No	43 (56.58)
Unknown	31 (40.79)
Distant metastasis (M)	
Yes	5 (6.33)
No	45 (56.96)
Unknown	29 (36.71)
Tumor size, median (IQR)	2.7 (2.5)
Number of lesions, median (IQR)	1.0 (1.0)
Vascular invasion	
Yes	11 (13.92)
No	56 (70.89)
Unknown	12 (15.19)
Perineural invasion	
Yes	1 (1.30)
No	51 (66.23)
Unknown	25 (32.47)
Bilobar involvement of tumor	
Yes	21 (25.61)
No	45 (54.88)
Unknown	16 (19.51)

HCC: Hepatocellular carcinoma.

in inflammatory pathways including NF-KB may be promising[10-12] and possible with a prospective study in a similar cohort of subjects.

Genetic variation may explain reported racial/ethnic disparities. Racial/ethnic disparities have been reported both in NAFLD and HCC: Hispanics tend to have a more progressive course in NAFLD; and have lower rates of curative therapies for HCC[13]. Our finding that Hispanic ethnicity was associated with a decreased risk of development of HCC within NAFLD is surprising and needs confirmation with a larger cohort of individuals with NASH and other etiologies of chronic liver disease. Indeed among other causes of chronic liver disease, specifically hepatitis C, Hispanics are more likely to progress to cirrhosis and HCC[14]. The present study is limited by a small number of Hispanic patients among both cases and controls and further evaluation of this relationship between ethnicity and HCC among those with NASH cirrhosis is needed.

The tumors observed in our study were typically a single lesion, confined to a single lobe and without any invasion to adjacent structures. This is in contrast with a recent study by Piscaglia *et al*[15] who found that NAFLD-HCC tended to be more advanced when compared to HCC in the background of HCV cirrhosis (HCV-HCC). The authors concluded that this was a result of delayed diagnosis of NAFLD and subsequent lack of screening in advanced fibrosis. There was no significant difference in mortality when propensity score analysis was performed. Certainly, detection of early stage HCC centers around appropriate screening. Our patients were established in our respective clinics and routinely followed. Resection was infrequent and the majority of our patients (61.5%) underwent orthotopic liver transplantation. The earlier stage observed in our study may be a product of referral and/or selection bias, as this cohort was selected from tertiary care and transplant medical center populations.

The limitations of our study include its retrospective nature; only cirrhotic patients were included in the study by design, thus limiting our ability to add to the body of data of HCC in the absence of advanced fibrosis. Similarly, we did not include HCC arising within other etiologies of cirrhosis, and therefore, cannot report that our findings are unique to NAFLD but that these characteristics play a role in the development of HCC in the context of NAFLD cirrhosis. The duration of cirrhosis is not known in this cohort given the case- control design and absence of longitudinal data.

In conclusion, the present study found that male gender and advanced age were associated with increased risk for the development of HCC among individuals with NASH cirrhosis whereas Hispanic ethnicity was associated with lower risk. Larger cohorts of individuals with HCC, from NASH and other etiologies are needed to further explore these associations. Additionally, prospective studies will help address these factors as predictors of HCC development and to risk stratify patients with NAFLD at increased risk for HCC who may benefit from more intense surveillance for HCC.

COMMENTS

Background

Both nonalcoholic steatohepatitis (NASH) and hepatocellular carcinoma (HCC) are rising in prevalence worldwide. Recent data suggest that HCC surveillance rates are poor in those with nonalcoholic fatty liver disease (NAFLD) cirrhosis.

Research frontiers

The authors sought to identify risk factors for HCC in NAFLD cirrhosis to identify individuals at highest risk for HCC.

Innovations and breakthroughs

Male gender, increased age and non-Hispanic ethnicity are associated with HCC in NASH cirrhosis. NASH cirrhosis associated HCC in this cohort was characterized by early stage disease at diagnosis and treatment with locoregional therapy and transplant.

Applications

The present study suggests that among those with NAFLD cirrhosis, men with increased age and of non-Hispanic ethnicity are at highest risk of HCC and should be targeted for screening.

Terminology

NAFLD is a chronic liver disease characterized by hepatic steatosis and can lead to the development of cirrhosis in a subset of patients.

Peer-review

Kathleen *et al* found male gender, increased age, and non-Hispanic ethnicity are associated with HCC in NASH cirrhosis, and suggested that these parameters may be useful for diagnosis and treatment of NASH cirrhosis associated HCC.

REFERENCES

1 **Rinella ME**. Nonalcoholic fatty liver disease: a systematic review. *JAMA* 2015; **313**: 2263-2273 [PMID: 26057287 DOI: 10.1001/jama.2015.5370]

2 **Blais P**, Husain N, Kramer JR, Kowalkowski M, El-Serag H, Kanwal F. Nonalcoholic fatty liver disease is underrecognized in the primary care setting. *Am J Gastroenterol* 2015; **110**: 10-14 [PMID: 24890441 DOI: 10.1038/ajg.2014.134]

3 **Charlton MR**, Burns JM, Pedersen RA, Watt KD, Heimbach JK, Dierkhising RA. Frequency and outcomes of liver transplantation for nonalcoholic steatohepatitis in the United States. *Gastroenterology* 2011; **141**: 1249-1253 [PMID: 21726509 DOI: 10.1053/j.gastro.2011.06.061]

4 **Singal AG**, Yopp AC, Gupta S, Skinner CS, Halm EA, Okolo E, Nehra M, Lee WM, Marrero JA, Tiro JA. Failure rates in the hepatocellular carcinoma surveillance process. *Cancer Prev Res* (Phila) 2012; **5**: 1124-1130 [PMID: 22846843 DOI: 10.1158/1940-6207.CAPR-12-0046]

5 **Younossi ZM**, Otgonsuren M, Henry L, Venkatesan C, Mishra A, Erario M, Hunt S. Association of nonalcoholic fatty liver disease (NAFLD) with hepatocellular carcinoma (HCC) in the United States from 2004 to 2009. *Hepatology* 2015; **62**: 1723-1730 [PMID: 26274335 DOI: 10.1002/hep.28123]

6 Use of ethyl esters of tryptophan to bypass the absorption defect in Hartnup disease. *Nutr Rev* 1990; **48**: 22-24 [PMID: 2336209 DOI: 10.1148/radiol.12121698]

7 **Kamath PS**, Kim WR. The model for end-stage liver disease (MELD). *Hepatology* 2007; **45**: 797-805 [PMID: 17326206 DOI: 10.1002/hep.21563]

8 **Ascha MS**, Hanouneh IA, Lopez R, Tamimi TA, Feldstein AF, Zein NN. The incidence and risk factors of hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. *Hepatology* 2010; **51**: 1972-1978 [PMID: 20209604 DOI: 10.1002/hep.3527]

9 **Bugianesi E**, Leone N, Vanni E, Marchesini G, Brunello F, Carucci P, Musso A, De Paolis P, Capussotti L, Salizzoni M, Rizzetto M. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. *Gastroenterology* 2002; **123**: 134-140 [PMID: 12105842]

10 **Michelotti GA**, Machado MV, Diehl AM. NAFLD, NASH and liver cancer. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 656-665 [PMID: 24080776 DOI: 10.1038/nrgastro.2013.183]

11 **Zoller H**, Tilg H. Nonalcoholic fatty liver disease and hepatocellular carcinoma. *Metabolism* 2016; **65**: 1151-1160 [PMID: 26907206 DOI: 10.1016/j.metabol.2016.01.010]

12 **Rinella M**, Charlton M. The globalization of nonalcoholic fatty liver disease: Prevalence and impact on world health. *Hepatology* 2016; **64**: 19-22 [PMID: 26926530 DOI: 10.1002/hep.28524]

13 **Ha J**, Yan M, Aguilar M, Tana M, Liu B, Frenette CT, Bhuket T, Wong RJ. Race/Ethnicity-specific Disparities in Hepatocellular Carcinoma Stage at Diagnosis and its Impact on Receipt of Curative Therapies. *J Clin Gastroenterol* 2016; **50**: 423-430 [PMID: 26583267 DOI: 10.1097/MCG.0000000000000448]

14 **El-Serag HB**, Kramer J, Duan Z, Kanwal F. Racial differences in the progression to cirrhosis and hepatocellular carcinoma in HCV-infected veterans. *Am J Gastroenterol* 2014; **109**: 1427-1435 [PMID: 25070058 DOI: 10.1038/ajg.2014.214]

15 **Piscaglia F**, Svegliati-Baroni G, Barchetti A, Pecorelli A, Marinelli S, Tiribelli C, Bellentani S. Clinical patterns of hepatocellular carcinoma in nonalcoholic fatty liver disease: A multicenter prospective study. *Hepatology* 2016; **63**: 827-838 [PMID: 26599351 DOI: 10.1002/hep.28368]

P- Reviewer: de la Monte SM, Murotomi K **S- Editor**: Qi Y
L- Editor: A **E- Editor**: Li D

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/ *World J Hepatol* 2017 March 8; 9(7): 391-400

DOI: 10.4254/wjh.v9.i7.391 ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Retrospective Study

Features of hepatocellular carcinoma in Hispanics differ from African Americans and non-Hispanic Whites

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Institutional review board statement: This was a retrospective study which was approved by the University of Illinois IRB as an expedited review, under expedited category 5 (Protocol 2005-0283). As such, it was granted a waiver of informed consent and HIPAA authorization.

Informed consent statement: This study was approved under expedited category 5 (Protocol 2005-0283). As such, it was granted a waiver of informed consent and HIPAA authorization.

Conflict-of-interest statement: We have no financial relationships to disclose.

Data sharing statement: No additional data are available.

Manuscript source: Unsolicited manuscript

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Received: September 24, 2016
Peer-review started: September 28, 2016
First decision: October 20, 2016
Revised: December 29, 2016
Accepted: January 16, 2017
Article in press: January 18, 2017
Published online: March 8, 2017

Abstract

AIM

To compare features of hepatocellular carcinoma (HCC) in Hispanics to those of African Americans and Whites.

METHODS

Patients treated for HCC at an urban tertiary medical center from 2005 to 2011 were identified from a tumor registry. Data were collected retrospectively, including demographics, comorbidities, liver disease characteristics, tumor parameters, treatment, and survival (OS) outcomes. OS analyses were performed using Kaplan-Meier

method.

RESULTS

One hundred and ninety-five patients with HCC were identified: 80.5% were male, and 22% were age 65 or older. Mean age at HCC diagnosis was 59.7 ± 9.8 years. Sixty-one point five percent of patients had Medicare or Medicaid; 4.1% were uninsured. Compared to African American (31.2%) and White (46.2%) patients, Hispanic patients (22.6%) were more likely to have diabetes (P = 0.0019), hyperlipidemia (P = 0.0001), nonalcoholic steatohepatitis (NASH) (P = 0.0021), end stage renal disease (P = 0.0057), and less likely to have hepatitis C virus (P < 0.0001) or a smoking history (P < 0.0001). Compared to African Americans, Hispanics were more likely to meet criteria for metabolic syndrome (P = 0.0491), had higher median MELD scores (P = 0.0159), ascites (P = 0.008), and encephalopathy (P = 0.0087). Hispanic patients with HCC had shorter OS than the other racial groups (P = 0.020), despite similarities in HCC parameters and treatment.

CONCLUSION

In conclusion, Hispanic patients with HCC have higher incidence of modifiable metabolic risk factors including NASH, and shorter OS than African American and White patients.

Key words: Hepatocellular carcinoma; Epidemiology; Treatment pattern; Survival; Hispanics

Core tip: This is a retrospective study evaluating features of hepatocellular carcinoma (HCC) in Hispanics compared to those of African Americans and Whites. This large single institution study found that Hispanic patients with HCC presented with more modifiable risk factors, more advanced liver disease, and shorter survival compared to African American and White patients with HCC. Early identification and intervention upon modifiable risk factors may ameliorate HCC development and HCC morbidity in Hispanic patients.

Venepalli NK, Modayil MV, Berg SA, Nair TD, Parepally M, Rajaram P, Gaba RC, Bui JT, Huang Y, Cotler SJ. Features of hepatocellular carcinoma in Hispanics differ from African Americans and non-Hispanic Whites. *World J Hepatol* 2017; 9(7): 391-400 Available from: URL: http://www.wjgnet.com/1948-5182/full/v9/i7/391.htm DOI: http://dx.doi.org/10.4254/wjh.v9.i7.391

INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common malignancy worldwide and the third leading cause of cancer related mortality[1,2]. While the highest prevalence rates of HCC are in Asia and Africa accounting for 85% of cases in 2008, the incidence of HCC has increased steadily in the United States among most racial and ethnic groups with a greater rate of growth observed in non-White populations[3,4]. Recent SEER analyses reported higher incidence rates for Hispanics (2.5 times) compared to non-Hispanics, and Asian-Pacific Islanders (4 times) and African Americans (1.7 times) compared to Whites[3].

HCC in Hispanics Americans will continue to increase as Hispanics are the most rapidly growing immigrant population in the United States, and projected to comprise 30% of the total population in 2050[5]. Recent studies report differences in HCC presentation in Hispanics compared to non-Hispanics, including younger age at diagnosis and greater prevalence of metabolic risk factors for Hispanic patients compared to non-Hispanic Whites[6,7], and higher incidence of HCC in Hispanic women compared to Hispanic men[6]. Notably, the incidence of liver cancer in Hispanic men has doubled between 1992 and 2012, and is double that of non-Hispanic men[8]. While national HCC incidence is highest in Asians[3,6], Hispanic patients continue to have poorer 5 year survival in comparison to their White and Asian counterparts (respectively; 15% *vs* 18% *vs* 23%), and higher overall mortality rates[9,10]. Age adjusted HCC-related mortality rates were reported as more than double in native Hispanic men *vs* immigrant Hispanic men, suggesting that synergy between biologic, environmental, and acquired risk factors contributes to HCC development in Hispanics in the United States[6]. Despite disproportionately higher incidence and mortality rates of HCC in Hispanics, there is a paucity of information about HCC presentation and features in Hispanics compared to non-Hispanics.

Identifying the role of modifiable risk factors associated with HCC in Hispanics will be critical to begin to address racial disparities in HCC incidence rates and outcomes. The aim of the current study was to evaluate HCC risk factors with specific emphasis on modifiable risk factors, disease characteristics, and treatment outcomes in Hispanic patients seen in an academic tertiary medical center in Chicago, Illinois and to compare HCC presentation and outcomes in Hispanics to African American and White patients.

MATERIALS AND METHODS

Patient populations

All adult patients ⩾ 18 years of age with HCC treated at the University of Illinois at Chicago (UIC) from January 2005 to December 2011 were identified from the UIC tumor registry. HCC diagnosis was confirmed by histopathology or according to the American Association of the Study of Liver Diseases non-invasive diagnostic criteria[11]. Hispanic, African American, and White patients were included in the study population; other racial groups were excluded. Patient charts were reviewed for relevant demographic data including comorbidities, liver disease etiology and characteristics, tumor parameters, treatment patterns, and length of survival from presentation. The protocol for this study was approved by

the Institutional Review Board at UIC as an expedited review under expedited category 5 (Protocol 2005-0283), and was granted a waiver of informed consent and HIPAA authorization.

Variable selection

The primary category of interest was patient identified race/ethnicity. The primary outcome of interest was patient survival. Demographic factors included race, age at diagnosis, gender, insurance status, body mass index (BMI), and metabolic syndrome criteria per the adult treatment panel Ⅲ guidelines[12]. Comorbidities included diabetes, hyperlipidemia, end stage renal disease requiring dialysis (ESRD), and history of smoking and alcohol use. Assessment of smoking and alcohol consumption was based on patient self-reporting per chart review. Cirrhosis was confirmed by liver biopsy or *via* characteristic clinical and radiologic features. Liver disease etiology was characterized as hepatitis B virus, hepatitis C virus (HCV), alcoholic liver disease, nonalcoholic steatohepatitis (NASH), and other non-viral, non-NASH etiologies including autoimmune, hemochromatosis, and cryptogenic (other). Liver disease characteristics included MELD score calculated based on baseline laboratory values rather than tumor exception points, baseline AFP level, presence of hepatic encephalopathy, and presence of ascites.

Tumor parameters were categorized by size of the largest tumor, stage at diagnosis, portal vein involvement, tumor grade (when tissue was available), and whether HCC was within Milan criteria. Stage at diagnosis was defined as unifocal, multifocal, or metastatic. Assessment was performed regarding whether patients were diagnosed during active HCC surveillance.

Type of treatment was recorded including loco regional therapy, resection, transplantation, chemotherapy, or observation. Cause of death was categorized as attributable to HCC (evidence of radiographic progression in the last 3 mo of life), decompensated cirrhosis (evidence of liver failure or complications of portal hypertension), other, or unknown based on review of outpatient notes within 1 mo of death, discharge summary, and hospice documentation. Two physicians independently reviewed cause of death categorization to ensure criteria standardization.

Statistical analysis

Patient characteristics were first summarized using mean ± SD for normally distributed continuous variables, median and interquartile range for non-normally distributed continuous variables, and percentages for categorical variables. Analysis of variance was used to examine mean differences by race for continuous variables with regards to demographics, comorbidities, liver disease etiology and characteristics, tumor parameters, and treatment patterns. Two-sided χ^2 tests or Fishers' exact test ($\leqslant$ 5 patients) were conducted to assess specific pairwise differences by race (between Hispanics, African Americans, and Whites) for variables that showed significant overall differences by race ($P < 0.05$). Further analysis was not performed for groups including $\leqslant$ 5 patients.

A Cox proportional hazard regression model was developed to evaluate survival adjusted by demographic and clinical factors, and a stepwise model was used for variable selection. Variables approaching statistical significance in univariate analysis ($P = 0.10$) and clinically meaningful variables were included in a forward stepwise selection. Potential confounders examined included gender, race, insurance, stage at diagnosis, MELD at diagnosis, Milan Criteria, receipt of locoregional therapy, HCV, hepatic encephalopathy, metabolic syndrome, diabetes, ascites, NASH, smoking history, and AFP level. Only variables reaching statistical significance at 0.05 α level were retained in the final multivariable model. Multivariable analysis rather than multivariate analysis was conducted to best assess for multiple independent variables and relationships while adjusting for potential confounders[13,14].

The Kaplan-Meier method was utilized to estimate survival distribution for two overall survival analyses, first with inclusion of all patients, and second with exclusion of liver transplant recipients. Overall survival was defined as the interval between date of HCC diagnosis and date of death due to any cause, or date of data censorship (June 6, 2013) for patients still alive.

All tests were two sided. Analysis was performed *via* SAS 9.3 (SAS Institute, Cary, NC).

RESULTS

Patient characteristics

One hundred and ninety-five patients with HCC were identified for analysis, including 44 Hispanics, 61 African Americans, and 90 Whites. Patient characteristics and selected pairwise comparisons between races are summarized in Table 1. Patients were predominantly male (80.5%), White (46.1%), and had a median age of 59.7 years (range, 50.0-69.5) with 22% of patients $\geqslant$ 65 years old. The majority of patients had Medicare or Medicaid insurance (61.5%) with a small group of uninsured patients (4.1%).

The observed female to male ratio was 1:2.8 in the Hispanic group, 4:5 in the African American group, and 1:2.5 in the White group, showing a higher proportion of women in the Hispanic and African American groups ($P = 0.022$). Among Hispanic patients, women presented at an older age in comparison to men (respectively: 71.7 ± 6.5 years *vs* 59.4 ± 12.6 years; $P = 0.0037$).

Comorbidities and modifiable risk factors

Hispanic patients demonstrated a higher prevalence of modifiable metabolic risk factors and comorbidities than African Americans or Whites. In comparison to African American and Whites, Hispanic patients had more frequent diagnoses of diabetes ($P = 0.0007$; $P =$

Table 1 Demographics, comorbid conditions and disease characteristics of hepatocellular carcinoma patients, by race

Patient characteristics	Total (n = 195)	Hispanic (n = 44)	African-American (n = 61)	White (n = 90)	P[1]
Demographics					
Age (yr, mean ± SD)	59.7 ± 9.8	62.5 ± 12.5	58.7 ± 10.2	58.9 ± 7.7	
Female (n, %)	38, 19.5	11, 25.0	17, 27.9	10, 11.1	[a]HW, AW
Insurance (n, %)					
Medicare/medicaid	120, 61.5	31, 70.5	36, 59.0	53, 58.9	
Private	67, 34.4	12, 27.3	22, 36.1	33, 36.7	
None	8, 4.1	1, 2.3	3, 4.9	4, 4.4	
BMI > 24.9 (n, %)	137, 70.3	31, 70.5	44, 72.1	62, 68.9	
Metabolic syndrome[3] (n, %)	27, 14.1	8, 18.2	3, 4.9	16, 18.4	[a]HA, AW
Comorbid conditions					
Hyperlipidemia (n, %)	31, 25.4	16, 55.2	5, 13.2	10, 18.2	[b]HW, HA
On dialysis (n, %)	7, 3.6	5, 11.4	0, 0	2, 2.3	[b]HNH[2]
Diabetes mellitus 2 (n, %)	91, 46.7	29, 65.9	19, 31.1	43, 47.8	[b]HA, [a]AW
History smoking (n, %)	126, 65	16, 36.3	43, 70.5	67, 75.2	[b]HW, HA; [a]AW
Current smoker (n, %)	57, 29.4	2, 4.5	28, 45.9	27, 30.3	[b]HW, HA; [a]AW
Triglycerides (median ± SD)	99.5 ± 66.5	101.0 ± 70.2	111.0 ± 64.7	80.5 ± 65.8	
History alcohol use (n, %)	163, 83.6	39, 88.6	50, 82	74, 82.2	
Cirrhosis characteristics					
Etiology[4]					
HCV (n, %)	132, 67.7	18, 40.9	49, 80.3	65, 72.2	[b]HW, HA
HBV (n, %)	14, 8.1	2, 5.0	8, 14.3	4, 5.2	
ETOH (n, %)	55, 28.2	11, 25.0	15, 24.6	29, 32.2	
NASH (n, %)	35, 18.0	15, 34.9	5, 8.2	15, 16.7	[b]HA, [a]HW
Other[5] (n, %)	22, 11.3	11, 25	2, 3.3	9, 11.3	0.056
MELD (median ± SD)	11.0 ± 4.6	11.5 ± 4.4	9.0 ± 3.1	12.0 ± 5.1	[a]HA, [b]AW
AFP					
Median (IQR)	22.4 (6.1-217.2)	19 (5.9-434.85)	82 (11.9-434.8)	12 (5.0-53.2)	
AFP > 200	49, 25.1	12, 27.3	22, 36.1	15, 16.7	[a]HA, [b]AW
Hepatic encephalopathy (n, %)	65, 33.3	16, 36.4	8, 13.1	41, 45.6	[a]HA, [b]AW
Ascites (n, %)	80, 44.5	23, 54.8	14, 26	43, 51.2	[a]HW, HA, AW
HCC surveillance performed (n, %)	113, 61.1	27, 65.9	31, 51.7	55, 65.5	
Tumor parameters					
Tumor size (cm)					
Median (± SD) (IQ range)	3.0 ± 3.7 (2.0-6.0)	3.0 ± 3.9 (2.0-5.0)	3.0 ± 3.6 (2.0-6.0)	3.0 ± 3.7 (2.0-6.0)	
> 5 cm (n, %)	43, 26.9	8, 22.2	14, 28.6	21, 28	
Median > 5 cm (± SD) (IQ range)	8.0 ± 4.0 (6.0-12.0)	13.0 ± 3.4 (7.5-13.0)	9.5 ± 3.1 (6.0-11.0)	7.0 ± 4.7 (6.0-8.0)	
Stage at diagnosis (n, %)	99, 52.1	20, 45.5	30, 50.8	49, 56.3	
Unifocal	77, 40.5	21, 47.7	25, 42.4	31, 35.6	
Multifocal	14, 7.4	3, 6.8	4, 6.8	7, 8.0	
Metastatic	29, 24.2	9, 33.3	9, 23.1	11, 20.4	
Portal vein involvement (n, %)	35, 47.9	6, 54.5	13, 54.2	16, 42.1	
Poorly differentiated within milan criteria (n, %)	121, 62.1	28, 63.6	36, 59.0	57, 63.3	

[1]P values from χ^2 tests (two-sided) and fischer for overall race effect followed by pairwise comparisons, for $P < 0.05$. A significance level of 0.05 was used for the overall race comparisons. [a]$P < 0.05$, [b]$P < 0.001$, P values were not calculated for $n < 5$; [2]Given $n < 5$ for AA ($n = 0$) and W ($n = 2$), limited statistical tests for Hispanic to non-Hispanic with $P = 0.0074$; [3]Metabolic syndrome: Three of the following five traits per adult treatment panel Ⅲ guidelines. Abdominal obesity, defined as a waist circumference in men ⩾ 102 cm (40 in) and in women ⩾ 88 cm (35 in): (1) serum triglycerides ⩾ 150 mg/dL (1.7 mmol/L) or drug treatment for elevated triglycerides; (2) serum HDL cholesterol < 40 mg/dL (1 mmol/L) in men and < 50 mg/dL (1.3 mmol/L) in women or drug treatment for low HDL-C; (3) blood pressure ⩾ 130/85 mmHg or drug treatment for elevated blood pressure; (4) fasting plasma glucose (FPG) ⩾ 100 mg/dL (5.6 mmol/L) or drug treatment for elevated blood glucose; [4]Some patients with more than one listed etiology of cirrhosis; [5]Other: Cryptogenic, hemochromatosis, autoimmune, other not specified. H: Hispanics; A: African Americans; W: Non-Hispanic Caucasians; SD: Standard deviation; IQ: Percentile interquartile range (25%, 75%); HW: Hispanics compared to Whites; HA: Hispanics compared to African Americans; AW: African Americans compared to Whites; HNH: Hispanics compared to non-Hispanics; BMI: Body mass index; HBV: Hepatitis B virus; HCV: Hepatitis C virus; NASH: Nonalcoholic steatohepatitis; HCC: Hepatocellular carcinoma.

0.0648; $P = 0.0019$ on initial χ^2 analysis, see appendix A), hyperlipidemia ($P = 0.0004$; $P = 0.001$), and end stage renal disease requiring dialysis ($P < 0.0001$), and were less likely to have a smoking history ($P < 0.0001$). In comparison to African Americans, Hispanic patients were more likely to meet criteria for metabolic syndrome ($P = 0.0491$). The three racial groups were similar with regards to age at presentation, insurance status, other comorbidities including BMI > 24.9 and history of alcohol use.

Among Hispanics, women trended towards a higher frequency of metabolic syndrome compared to men (36.4% *vs* 12.1%, $P = 0.09$).

Liver disease characteristics

Etiology and liver disease features in Hispanic patients differed from African Americans and Whites. NASH cirrhosis was significantly more common in Hispanics compared to

Table 2 Treatment patterns for non-metastatic patients at diagnosis: First line treatment patterns for non-metastatic patients by race

First line treatment characteristics	Total (n = 176)[1]	Hispanic (n = 41) (n, %)	African American (n = 55) (n, %)	White (n = 80)[1] (n, %)	P[2]
Surgery	2	0, 0	2, 3.6	0, 0	
Liver directed	154	38, 92.7	48, 87.2	68, 85	
Chemotherapy	6	1, 2.4	2, 3.6	3, 3.8	
Observation	6	0, 0	1, 1.8	4, 5	
Lost to follow-up	7	2, 4.9	2, 3.6	4, 5	

[1]One patient missing information; [2]P values from χ^2 tests (two-sided) and fischer for overall race effect followed by pairwise comparisons, for $P < 0.05$. A significance level of 0.05 was used for the overall race comparisons.

Table 3 Treatment patterns for non-metastatic patients at diagnosis: Transplantation patterns by race

Transplantation patterns	Total listed	Hispanic	African American	White	P[2]
Met milan criteria	121	28	36	57	
Transplanted	34[1], 1.4%	10, 35.7%	6, 16.7%	18[1], 31.6%	
Listed	68	20	17	31	
Tumor exception points	33, 48.5%	10, 50%	6, 35.3%	17, 48.4%	
Transplanted	38[1], 55.9%	10, 50%	6, 35.3%	22[1], 71%[1]	[a]AW
Removed from list	28, 41.1%	9, 45%	11, 64.7%	8, 25.8%	[b]AW
Death	7	3	0	4	
Progression	10	3	5	2	
Transfer of care	5	2	2	1	
Other	5	1	4	0	

[1]Four patients initially outside of Milan criteria, subsequently listed and transplanted after reassessment and locoregional treatment; [2]P values from χ^2 tests (two-sided) and fischer for overall race effect followed by pairwise comparisons, for $P < 0.05$. A significance level of 0.05 was used for the overall race comparisons. [a]$P < 0.05$; [b]$P < 0.001$. P values were not calculated for $n < 5$. AW: African American compared to Whites.

African Americans and Whites ($P < 0.0001$; $P = 0.026$) while HCV cirrhosis was less common in Hispanics ($P < 0.0001$). There was a trend towards more non-viral, non-NASH cirrhosis etiologies in the Hispanic patients compared to other groups ($P = 0.056$).

Hispanic patients with HCC showed more evidence of advanced liver disease. In comparison to African Americans and White, ascites was more common in Hispanics ($P = 0.006$; $P = 0.042$). Hispanic patients presented with higher median MELD scores ($P = 0.0159$) and more hepatic encephalopathy ($P = 0.0087$) than African Americans. Median AFP levels were similar among groups, although Hispanic and African Americans demonstrated more variability in AFP based on inter-quartile range, and Hispanics were more likely to have AFP > 200 IU/mL in comparison to Whites ($P = 0.035$).

Among Hispanics, women had a lower prevalence of alcoholic cirrhosis compared to men (0% *vs* 37.93%, $P = 0.0186$), while the prevalence of HCV cirrhosis was similar by gender.

Tumor parameters

The three groups demonstrated similar frequency of HCC diagnosis made during active surveillance, and similar tumor parameters at presentation including stage at diagnosis, tumor size, tumor differentiation, presence of portal vein invasion, and transplant eligibility *via* Milan criteria at diagnosis (Table 1).

HCC treatment patterns

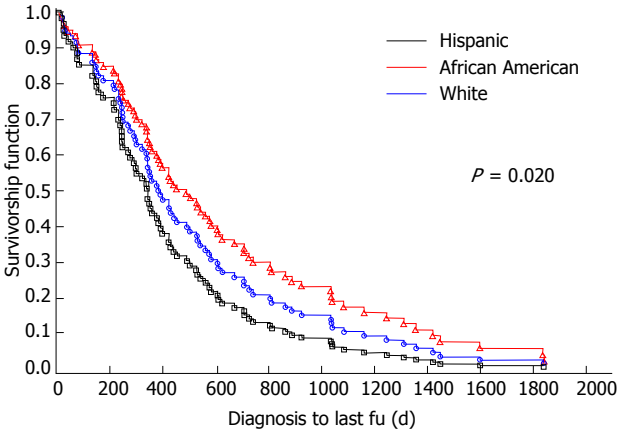
While median time from HCC diagnosis to time of last follow-up was similar among groups, median time from HCC diagnosis to time of first treatment was longer for African Americans in comparison to both Hispanics and Whites (median time to first line treatment; Hispanics 25.0 d (IQR 7.0-34.0 d); African Americans 39.0 d (IQR 17.0-70.0 d), Whites 32.5 d (IQR 13.0-64.0 d, $P = 0.0373$).

As shown in Table 2, the use of loco regional therapy (chemoembolization and radiofrequency ablation) for non-metastatic HCC was similar among racial groups ($P = 0.1168$). The vast majority of patients (87.5%) received loco regional therapy as their initial treatment, while the remaining 12.5% of patients received other initial treatments including chemotherapy, resection, or observation. P values are not reported for the remaining 12.5% due to small numbers of patients per individual group, by race.

There was no difference in HCC presentation within Milan Criteria, listing for transplant, receipt of tumor exception points, or liver transplantation for patients meeting Milan Criteria among the three ethnic/racial groups (Table 3). However, once listed, African Americans were more frequently removed from the transplant list due to HCC progression and death (64.7% *vs* 25.8%, $P = 0.0084$) and were less likely to receive liver transplantation (35.3% *vs* 71%, $P = 0.0165$) compared to Whites. Hispanics did not differ significantly from Whites or African Americans with regard to being transplanted once listed, or removed from the list (Table 3).

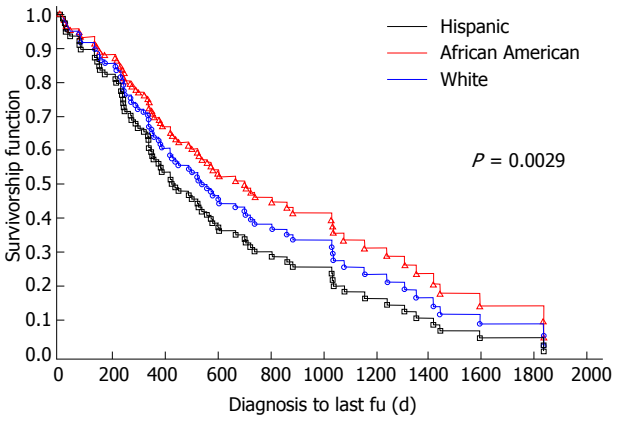
Overall survival

Forty-nine of the 195 patients died from all causes during



	Day 0	Day 500	Day 1000	Day 1500	Day 2000
Hispanic	44	16	8	2	0
African American	61	29	12	4	0
White	90	20	7	1	0

Figure 1 Unadjusted survival curve stratified in patients with hepato–cellular carcinoma by race from time of presentation to time of death or censorship (with numbers of subject at risk). Hispanic (n = 44), African American (n = 61), Whites (n = 90). P-value was obtained by the log-rank test.



	Day 0	Day 500	Day 1000	Day 1500	Day 2000
Hispanic	34	10	3	1	0
African American	55	27	11	4	0
White	69	13	4	0	

Figure 2 Overall survival curves by race after exclusion of patients who underwent orthotopic liver transplantation (with numbers of subjects at risk). Hispanic (n = 34), African American (n = 55), Whites (n = 69). P-value was obtained with the use of the log-rank test.

the study period (Hispanic n = 9; African American n =15; Whites n = 25). The median follow-up for the entire cohort was 563 d and was similar among racial groups. In a multivariable analysis examining possible confounders, three variables were identified as independently related to survival including HCV, metabolic syndrome, and race. However, when all three variables were entered in a stepwise fashion for model building, only race was found to be predictive of survival.

Hispanic patients appeared to have poorer survival compared to both African American and Whites (log-rank test for overall differences by race: P = 0.0220) (Figure 1). The hazard ratio for death was 1.52 (95%CI: 0.354, 1.223), for Hispanics in comparison to African Americans and 1.36 (95%CI: 0.739-2.511), for Hispanic in comparison to Whites. After excluding patients who underwent liver transplantation, a second multivariable model adjusting for the factors mentioned above confirmed that Hispanics with HCC had the highest mortality rate (log-rank test for overall differences by race: P = 0.0029) (Figure 2). Cause of death was similar for all groups for cases in which the cause of death could be discerned (Figure 3), with similar frequency of death due to HCC (n = 11) *vs* liver cirrhosis (n = 19) *vs* other (n = 11) in Hispanics, African Americans, and Whites.

DISCUSSION

Hispanics with HCC had significantly shorter survival in comparison to both African American and Whites, with race as the only independent predictor of survival in multivariable analysis. This observation is consistent with previous studies showing that Hispanic ethnicity was an independent risk factor for HCC-related mortality, with shorter 5 year survival[9,10] in Hispanic patients with HCC compared to White and Asian counterparts, and higher mortality rates in Hispanics aged 50-64[15].

A substantive body of prior research has shown that health disparities, barriers to care, socioeconomic characteristics, and later diagnosis of more advanced malignancy impact on survival in minority groups[16-18]. An important contribution of the current study was that we found no evidence that reduced survival in Hispanics with HCC was related to differences in access to care; groups were similar with regard to insurance status, age at diagnosis, HCC diagnoses made during active surveillance, and tumor parameters at presentation including stage and tumor grade at diagnosis.

Little is known about features of patients with HCC that might contribute to disparate outcomes by race. Data from the current study shows important and intriguing differences in HCC presentation and disease characteristics for Hispanics. Characteristics that distinguished Hispanic patients included significantly higher rates of comorbidities and modifiable risk factors for liver disease such as diabetes, hyperlipidemia, metabolic syndrome, as well as a greater prevalence of NASH and ESRD. Hispanics also had evidence of more advanced liver disease with higher rates of ascites than African Americans and Whites and higher MELD scores and more hepatic encephalopathy than African Americans.

The clinical correlates of HCC in Hispanics provide a context to consider potential causes for the shorter overall survival in Hispanics. Patients with HCC are at risk for complications and mortality from cirrhosis, HCC, and other comorbidities. Consistent with prior studies, we found that Hispanic patients had higher rates of comorbidities including metabolic syndrome[19,20] and ESRD[21-23]. Our data builds on existing literature by

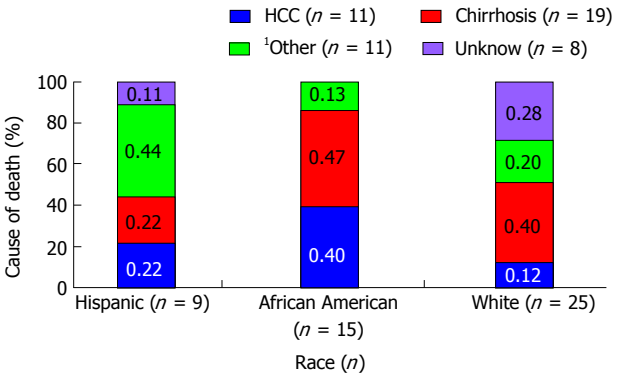


Figure 3 Distribution of cause of death in patients with hepatocellular carcinoma by race. There was no difference in HCC (P = 0.1051), cirrhosis (P = 0.6162), or other (P = 0.0581) as cause of death between Hispanics, African Americans, and White. [1]Cause of death other includes: Immediate complications post liver transplant (n = 3), sepsis (n = 3), complications from second malignancy (n = 2), cardiogenic shock (n = 1), PEA (n = 1), intracerebral hemorrhage (n = 1). Of Hispanic patient (n = 4), immediate complications post liver transplant (n = 2), cardiogenic shock (n = 1), complications from a second malignancy (n = 1). Fischers pairwise comparison not performed due to n < 5 per group. HCC: Hepatocellular carcinoma.

showing that these differences persist in patients with HCC. Moreover, metabolic disease might contribute to the development of HCC and to poorer outcomes in Hispanics. There is increasing evidence that diabetes and obesity are individually associated with significant risk of HCC development[24-26], and Hispanics appear to demonstrate a stronger association between diabetes and HCC compared to non-Hispanics[27]. A longitudinal study reported that diabetic Hispanics had a 3.3 × higher risk of HCC development compared to non-diabetic Hispanics, and that there was a 2.17 × higher risk of HCC for diabetic non-Hispanics compared to non-diabetic counterparts[28].

In addition to higher rate of comorbidities and modifiable risk factors, Hispanics had more complications of portal hypertension and compared to African Americans had higher MELD scores at presentation, indicating more advanced liver disease. This is consistent with national data reporting a higher prevalence of chronic liver disease, more advanced disease features at presentation, and higher liver disease related mortality in Hispanics[29-31]. Although chronic liver disease is the 6th most common cause of death in Hispanic populations in 2010 per the United States National Center for Health Statistics data, it is not within the top ten causes of death for African American or White populations. Mortality rates from chronic liver disease are almost 50% higher in Hispanics than non-Hispanics[32]. One potential explanation may be that increased comorbidities in Hispanics could contribute to higher chronic liver disease mortality. Recent SEER data found parallel mortality trends for diabetes, chronic liver disease, and HCC by state; states with high HCC mortality also demonstrated elevated mortality rates for diabetes and liver disease, including cirrhosis[15]. Racial/ethnic biologic differences in cirrhosis pathogenesis might also contribute; Hispanics with HCV appear at significantly higher risk for cirrhosis and HCC development compared to non-Hispanic Whites and African Americas, independently of BMI, diabetes, HCV treatment and genotype[33]. Additionally, Hispanics with HCV cirrhosis showed lower median time to cirrhosis at a younger age[34], and higher rates of cirrhosis mortality for Hispanics in both the United States and Mexico[29-31]. The finding of higher rates of ESRD in Hispanics in the current study is consistent with prior literature reporting higher incidence of ESRD in Hispanics than non-Hispanics, and a higher risk of kidney failure despite similar prevalence of stage 3 and 4 chronic kidney disease[22,23,35,36]. Renal failure is associated with increased risk of mortality in patients with cirrhosis[37]. It is intriguing that Hispanics carry a disproportionate burden of ESRD and cirrhosis severity and incidence, although ESRD did not independently predict shorter survival in our study.

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in the United States[38-40] and is increasingly being recognized as an important cause of cirrhosis and HCC[41] with higher prevalence in Hispanics compared to non-Hispanic[42]. Recent data also suggest NAFLD's role in hepatocarcinogenesis in the absence of cirrhosis[40]. NASH comprises a subgroup of NAFLD with hepatocyte injury and inflammation, and is considered to be the hepatic manifestation of the metabolic syndrome. In the current series, NASH was the second leading cause of liver disease in Hispanics with HCC, accounting for 34% of cases. Consistent with prior studies, we found Hispanics demonstrated notable differences in cirrhosis etiologies compared to non-Hispanics, including higher rates of NASH[43,44] and autoimmune cirrhosis[45], and lower incidence of HCV cirrhosis[46]. Additionally, we observed that NASH was particularly prevalent in Hispanic women compared to Hispanic men (72.7% *vs* 21.9%). Although the risk of HCC development in NAFLD is lower than with HCV[47], NASH is poised to become the primary etiology for cirrhosis and HCC in developed countries over the next decade. One new observation from our study is that Hispanics and Whites with HCC had similar rates of diabetes and metabolic syndrome, although Hispanics had more NASH cirrhosis and hypertriglyceridemia. This suggests that Hispanics may have differences in NAFLD progression, NASH pathogenesis and a greater susceptibility towards cirrhosis. A role for biologic differences in cirrhosis pathogenesis and hepatocarcinogenesis unique to Hispanics has been suggested by prior studies demonstrating that Hispanic patients with NASH, NAFLD, and hepatitis C[48] demonstrate more fibrosis and higher rate of aminotransferase abnormalities in comparison to other ethnic groups[49,50]. The high prevalence of metabolic disease and NASH in Hispanics with HCC has a critically important implication. Early identification of Hispanics with risk factors for NASH and intervention to modify metabolic risk factors could have a major impact on reducing the development of HCC in Hispanics. Specifically, elimination of diabetes and metabolic syndrome could significantly

decrease HCC incidence across all ethnic groups, but with largest reduction in Hispanics. Additionally, targeted HCC screening for Hispanics with metabolic syndrome, diabetes, and NASH risk factors for NASH could also improve diagnosis, timely treatment, and survival for Hispanics with HCC.

The retrospective design of the current study made it difficult to assess whether reduced survival in Hispanics with HCC was related to increased mortality from complications of cirrhosis, HCC, or comorbid conditions. It is likely that synergy between biologic, genetic, and environmental factors may contribute to racial differences in cirrhosis pathogenesis, HCC development, and survival. Recent proteomic and tissue microarray studies have demonstrated racial and regional differences in molecular pathogenesis of cirrhosis and HCC, including variations of molecular signatures for HCV induced HCC[51] unique to African Americans compared to Whites, down-regulation of p53 and MDM2 in Americans compared to South Koreans[52], higher prevalence of PNPLA3 polymorphisms associated with high NAFLD susceptibility and worse survival in Hispanics[53] and greater expression of genetic polymorphisms predisposing towards higher NASH severity in Hispanics compared to non-Hispanics[8]. Genetic and biologic differences are associated with susceptibility to increased fibrosis and inflammation in NAFLD, NASH and HCV, influencing more aggressive cirrhosis progression and hepatocarcinogenesis[48-50,54-56]. Racial and ethnic differences modulating insulin resistance have also been identified; compared to non-Hispanics, Hispanics express a higher frequency of an insulin receptor gene regulator (high-mobility group AT-hook, or HMGA1) associated with higher BMI, lower HDL, and type 2 diabetes[57]. While our study did not include biologic correlates, given the paucity of Hispanic specific information, studies comparing Hispanic tumor and cirrhosis samples to other multiethnic HCC and cirrhotic cohorts are necessary to better understand ongoing racial disparities in HCC and cirrhosis mortality and progression.

Despite being one of the largest single institution studies of HCC in Hispanics, African Americans and Whites, the major limitation of the present study was the retrospective design. The study identified important clinical factors associated with HCC in Hispanics. However, it was unable to discern the cause of reduced overall survival in Hispanics with HCC. Moreover, single center data might not be applicable to all Hispanic populations. Prospective studies with molecular analyses are needed to determine the relative contributions of co-morbidities, cirrhosis, HCC and biologic correlative information to the reduced overall survival in Hispanics.

In conclusion, the current study provides important new insights into clinical factors distinguishing Hispanics with HCC. Hispanics with HCC present with a higher prevalence of modifiable metabolic risk factors, more advanced liver disease, and shorter survival compared to African Americans and Whites. Increased mortality in Hispanics with HCC may be explained by compounding risk from metabolic comorbidities, NASH cirrhosis, and unique biologic gene-environment interactions influencing higher susceptibility towards NAFLD development, and more aggressive cirrhosis progression and hepatocarcinogenesis. Further clinical, epidemiologic, and molecular data are necessary to determine the relative contributions of modifiable comorbidities such as diabetes, hyperlipidemia, metabolic syndrome, and NASH to HCC pathogenesis in Hispanics. Development of prospective multi-institutional HCC databases with specimen sharing is essential. There is an additional need for prospective case controlled studies, and therapeutic clinical trials with proportional representation of Hispanics to assess the impact of modifying comorbidities such as metabolic syndrome, hyperlipidemia, ESRD, diabetes, and NASH through lifestyle and medical management upon cirrhosis and HCC progression in Hispanic and non-Hispanics. Identification of clinical factors associated with HCC in Hispanics provides direction for public health efforts at HCC prevention through intervening on modifiable risk factors, targeted HCC screening for high risk ethnic populations, and more timely HCC treatment and management in this population.

COMMENTS

Background

There is a dearth of information about hepatocellular carcinoma (HCC) race specific risk factors and disease characteristics in Hispanic patients, compared to African American and White patients, despite higher incidence and mortality rates. This is one of the largest published single institution retrospective studies of Hispanic, African American, and White patients treated for HCC at an urban tertiary academic medical center.

Research frontiers

The results of this study contribute to new insights and a deeper understanding of racial disparities in HCC incidence, cirrhosis progression and mortality in Hispanic patients, compared to African American and White patients.

Innovations and breakthroughs

The results of this study demonstrate significant differences in survival and modifiable risk factors for Hispanic patients compared to other racial groups, with Hispanic patients showing lower survival, more advanced liver disease, and higher incidence of modifiable risk factors including metabolic syndrome, nonalcoholic steatohepatitis (NASH), and end stage renal disease. This is consistent with prior data suggesting compounding risks unique to Hispanic patients, including modifiable risk factors, biologic differences in cirrhosis and NASH pathogenesis, and gene-environmental interactions influencing a higher susceptibility towards hepatocarcinogenesis and more aggressive cirrhosis progression.

Applications

Identification of clinical factors associated with HCC in Hispanics provides direction for public health efforts at HCC prevention through intervening on modifiable risk factors, targeted HCC screening for high risk ethnic populations, and more timely HCC treatment and management in this population.

Terminology

HCC: Hepatocellular carcinoma; OS: Overall survival; UIC: University of Illinois, Chicago; AASLD: American Association for the Study of Liver Diseases; BMI: Body mass index; ATP: Adult treatment panel; ESRD: End stage renal disease; HBV: Hepatitis B virus; HCV: Hepatitis C virus; NASH: Nonalcoholic steatohepatitis; NAFLD: Nonalcoholic fatty liver disease.

Peer-review

An interesting observation study for the clinical outcome between HCC in

Hispanics to those of African Americans and Whites. A clearly data presentation and manuscript written.

REFERENCES

1 **Ferlay J**, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010; **127**: 2893-2917 [PMID: 21351269 DOI: 10.1002/ijc.25516]

2 GLOBOCAN 2008. Available from: URL: http://globocan.iarc.fr/factsheets/populations/factsheet.asp?uno=900

3 **Ahmed F**, Perz JF, Kwong S, Jamison PM, Friedman C, Bell BP. National trends and disparities in the incidence of hepatocellular carcinoma, 1998-2003. *Prev Chronic Dis* 2008; **5**: A74 [PMID: 18558024]

4 **Nguyen MH**, Whittemore AS, Garcia RT, Tawfeek SA, Ning J, Lam S, Wright TL, Keeffe EB. Role of ethnicity in risk for hepatocellular carcinoma in patients with chronic hepatitis C and cirrhosis. *Clin Gastroenterol Hepatol* 2004; **2**: 820-824 [PMID: 15354283]

5 Census Brief 2010: The Hispanic Population. 2010. Available from: URL: http://www.census.gov/prod/cen2010/briefs/c2010br-04.pdf

6 **El-Serag HB**, Lau M, Eschbach K, Davila J, Goodwin J. Epidemiology of hepatocellular carcinoma in Hispanics in the United States. *Arch Intern Med* 2007; **167**: 1983-1989 [PMID: 17923599 DOI: 10.1001/archinte.167.18.1983]

7 **Ramirez AG**, Weiss NS, Holden AE, Suarez L, Cooper SP, Munoz E, Naylor SL. Incidence and risk factors for hepatocellular carcinoma in Texas Latinos: implications for prevention research. *PLoS One* 2012; **7**: e35573 [PMID: 22530052 DOI: 10.1371/journal.pone.0035573]

8 **Siegel RL**, Fedewa SA, Miller KD, Goding-Sauer A, Pinheiro PS, Martinez-Tyson D, Jemal A. Cancer statistics for Hispanics/Latinos, 2015. *CA Cancer J Clin* 2015; **65**: 457-480 [PMID: 26375877 DOI: 10.3322/caac.21314]

9 **Altekruse SF**, McGlynn KA, Dickie LA, Kleiner DE. Hepatocellular carcinoma confirmation, treatment, and survival in surveillance, epidemiology, and end results registries, 1992-2008. *Hepatology* 2012; **55**: 476-482 [PMID: 21953588 DOI: 10.1002/hep.24710]

10 **Younossi ZM**, Stepanova M. Hepatitis C virus infection, age, and Hispanic ethnicity increase mortality from liver cancer in the United States. *Clin Gastroenterol Hepatol* 2010; **8**: 718-723 [PMID: 20435163 DOI: 10.1016/j.cgh.2010.04.017]

11 **Bruix J**, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]

12 **Grundy SM**, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC, Spertus JA, Costa F. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 2005; **112**: 2735-2752 [PMID: 16157765 DOI: 10.1161/CIRCULATIONAHA.105.169404]

13 **Hidalgo B**, Goodman M. Multivariate or multivariable regression? *Am J Public Health* 2013; **103**: 39-40 [PMID: 23153131 DOI: 10.2105/AJPH.2012.300897]

14 **Katz MH**. Multivariable analysis: a primer for readers of medical research. *Ann Intern Med* 2003; **138**: 644-650 [PMID: 12693887 DOI: 10.7326/0003-4819-138-8-200304150-00012]

15 **Altekruse SF**, Henley SJ, Cucinelli JE, McGlynn KA. Changing hepatocellular carcinoma incidence and liver cancer mortality rates in the United States. *Am J Gastroenterol* 2014; **109**: 542-553 [PMID: 24513805 DOI: 10.1038/ajg.2014.11]

16 **Mathur AK**, Osborne NH, Lynch RJ, Ghaferi AA, Dimick JB, Sonnenday CJ. Racial/ethnic disparities in access to care and survival for patients with early-stage hepatocellular carcinoma. *Arch Surg* 2010; **145**: 1158-1163 [PMID: 21173289 DOI: 10.1001/archsurg.2010.272]

17 **Artinyan A**, Mailey B, Sanchez-Luege N, Khalili J, Sun CL, Bhatia S, Wagman LD, Nissen N, Colquhoun SD, Kim J. Race, ethnicity, and socioeconomic status influence the survival of patients with hepatocellular carcinoma in the United States. *Cancer* 2010; **116**: 1367-1377 [PMID: 20101732 DOI: 10.1002/cncr.24817]

18 **Wong LL**, Hernandez BY, Albright CL. Socioeconomic factors affect disparities in access to liver transplant for hepatocellular cancer. *J Transplant* 2012; **2012**: 870659 [PMID: 23304446 DOI: 10.1155/2012/870659]

19 **Boden-Albala B**, Cammack S, Chong J, Wang C, Wright C, Rundek T, Elkind MS, Paik MC, Sacco RL. Diabetes, fasting glucose levels, and risk of ischemic stroke and vascular events: findings from the Northern Manhattan Study (NOMAS). *Diabetes Care* 2008; **31**: 1132-1137 [PMID: 18339972 DOI: 10.2337/dc07-0797]

20 **Rodriguez F**, Naderi S, Wang Y, Johnson CE, Foody JM. High prevalence of metabolic syndrome in young Hispanic women: findings from the national Sister to Sister campaign. *Metab Syndr Relat Disord* 2013; **11**: 81-86 [PMID: 23259587 DOI: 10.1089/met.2012.0109]

21 **Benabe JE**, Rios EV. Kidney disease in the Hispanic population: facing the growing challenge. *J Natl Med Assoc* 2004; **96**: 789-798 [PMID: 15233489]

22 **Peralta CA**, Shlipak MG, Fan D, Ordoñez J, Lash JP, Chertow GM, Go AS. Risks for end-stage renal disease, cardiovascular events, and death in Hispanic versus non-Hispanic white adults with chronic kidney disease. *J Am Soc Nephrol* 2006; **17**: 2892-2899 [PMID: 16959827 DOI: 10.1681/ASN.2005101122]

23 **Coresh J**, Astor BC, Greene T, Eknoyan G, Levey AS. Prevalence of chronic kidney disease and decreased kidney function in the adult US population: Third National Health and Nutrition Examination Survey. *Am J Kidney Dis* 2003; **41**: 1-12 [PMID: 12500213 DOI: 10.1053/ajkd.2003.50007]

24 **Calle EE**, Teras LR, Thun MJ. Obesity and mortality. *N Engl J Med* 2005; **353**: 2197-2199 [PMID: 16291995 DOI: 10.1056/NEJM200511173532020]

25 **El-Serag HB**, Hampel H, Javadi F. The association between diabetes and hepatocellular carcinoma: a systematic review of epidemiologic evidence. *Clin Gastroenterol Hepatol* 2006; **4**: 369-380 [PMID: 16527702 DOI: 10.1016/j.cgh.2005.12.007]

26 **Wang C**, Wang X, Gong G, Ben Q, Qiu W, Chen Y, Li G, Wang L. Increased risk of hepatocellular carcinoma in patients with diabetes mellitus: a systematic review and meta-analysis of cohort studies. *Int J Cancer* 2012; **130**: 1639-1648 [PMID: 21544812 DOI: 10.1002/ijc.26165]

27 **Setiawan VW**, Hernandez BY, Lu SC, Stram DO, Wilkens LR, Le Marchand L, Henderson BE. Diabetes and racial/ethnic differences in hepatocellular carcinoma risk: the multiethnic cohort. *J Natl Cancer Inst* 2014; **106**: pii: dju326 [PMID: 25326644 DOI: 10.1093/jnci/dju326]

28 **Wendy Setiawan PD**. Multiethnic Cohort Study Analysis: Diabetes Identified as Risk Factor for Liver Cancer Across Ethnic Groups. Paper presented at: AACRSixth AACR Conference on the Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved, held December 6-9, 2013. Available from: URL: https://www.sciencedaily.com/releases/2013/12/131209084147.htm

29 **Flores YN**, Yee HF, Leng M, Escarce JJ, Bastani R, Salmerón J, Morales LS. Risk factors for chronic liver disease in Blacks, Mexican Americans, and Whites in the United States: results from NHANES IV, 1999-2004. *Am J Gastroenterol* 2008; **103**: 2231-2238 [PMID: 18671818 DOI: 10.1111/j.1572-0241.2008.02022.x]

30 **Stewart SH**. Racial and ethnic differences in alcohol-associated aspartate aminotransferase and gamma-glutamyltransferase elevation. *Arch Intern Med* 2002; **162**: 2236-2239 [PMID: 12390068 DOI: 10.1001/archinte.162.19.2236]

31 **Stinson FS**, Grant BF, Dufour MC. The critical dimension of ethnicity in liver cirrhosis mortality statistics. *Alcohol Clin Exp Res* 2001; **25**: 1181-1187 [PMID: 11505049 DOI: 10.1111/j.1530-0277.2001.tb02333.x]

32 **Asrani SK**, Larson JJ, Yawn B, Therneau TM, Kim WR. Underestimation of liver-related mortality in the United States. *Gastroenterology* 2013; **145**: 375-382.e1-2 [PMID: 23583430 DOI:

10.1053/j.gastro.2013.04.005]
33 **El-Serag HB**, Kramer J, Duan Z, Kanwal F. Racial differences in the progression to cirrhosis and hepatocellular carcinoma in HCV-infected veterans. *Am J Gastroenterol* 2014; **109**: 1427-1435 [PMID: 25070058 DOI: 10.1038/ajg.2014.214]
34 **Rodríguez-Torres M**, Ríos-Bedoya CF, Rodríguez-Orengo J, Fernández-Carbia A, Marxuach-Cuétara AM, López-Torres A, Salgado-Mercado R, Bräu N. Progression to cirrhosis in Latinos with chronic hepatitis C: differences in Puerto Ricans with and without human immunodeficiency virus coinfection and along gender. *J Clin Gastroenterol* 2006; **40**: 358-366 [PMID: 16633110 DOI: 10.1097/01.mcg.0000210105.66994.dc]
35 **Lora CM**, Daviglus ML, Kusek JW, Porter A, Ricardo AC, Go AS, Lash JP. Chronic kidney disease in United States Hispanics: a growing public health problem. *Ethn Dis* 2009; **19**: 466-472 [PMID: 20073150]
36 **Fischer MJ**, Go AS, Lora CM, Ackerson L, Cohan J, Kusek JW, Mercado A, Ojo A, Ricardo AC, Rosen LK, Tao K, Xie D, Feldman HI, Lash JP. CKD in Hispanics: Baseline characteristics from the CRIC (Chronic Renal Insufficiency Cohort) and Hispanic-CRIC Studies. *Am J Kidney Dis* 2011; **58**: 214-227 [PMID: 21705121 DOI: 10.1053/j.ajkd.2011.05.010]
37 **Belcher JM**, Garcia-Tsao G, Sanyal AJ, Bhogal H, Lim JK, Ansari N, Coca SG, Parikh CR. Association of AKI with mortality and complications in hospitalized patients with cirrhosis. *Hepatology* 2013; **57**: 753-762 [PMID: 22454364 DOI: 10.1002/hep.25735]
38 **Ratziu V**, Bellentani S, Cortez-Pinto H, Day C, Marchesini G. A position statement on NAFLD/NASH based on the EASL 2009 special conference. *J Hepatol* 2010; **53**: 372-384 [PMID: 20494470 DOI: 10.1016/j.jhep.2010.04.008]
39 **Starley BQ**, Calcagno CJ, Harrison SA. Nonalcoholic fatty liver disease and hepatocellular carcinoma: a weighty connection. *Hepatology* 2010; **51**: 1820-1832 [PMID: 20432259 DOI: 10.1002/hep.23594]
40 **Torres DM**, Harrison SA. Nonalcoholic steatohepatitis and noncirrhotic hepatocellular carcinoma: fertile soil. *Semin Liver Dis* 2012; **32**: 30-38 [PMID: 22418886 DOI: 10.1055/s-0032-1306424]
41 **White DL**, Kanwal F, El-Serag HB. Association between non-alcoholic fatty liver disease and risk for hepatocellular cancer, based on systematic review. *Clin Gastroenterol Hepatol* 2012; **10**: 1342-1359.e2 [PMID: 23041539 DOI: 10.1016/j.cgh.2012.10.001]
42 **Ruhl CE**, Everhart JE. Determinants of the association of overweight with elevated serum alanine aminotransferase activity in the United States. *Gastroenterology* 2003; **124**: 71-79 [PMID: 12512031 DOI: 10.1053/gast.2003.50004]
43 **Younossi ZM**, Stepanova M, Negro F, Hallaji S, Younossi Y, Lam B, Srishord M. Nonalcoholic fatty liver disease in lean individuals in the United States. *Medicine* (Baltimore) 2012; **91**: 319-327 [PMID: 23117851 DOI: 10.1097/MD.0b013e3182779d49]
44 **Browning JD**. Statins and hepatic steatosis: perspectives from the Dallas Heart Study. *Hepatology* 2006; **44**: 466-471 [PMID: 16871575 DOI: 10.1002/hep.21248]
45 **Wong RJ**, Gish R, Frederick T, Bzowej N, Frenette C. The impact of race/ethnicity on the clinical epidemiology of autoimmune hepatitis. *J Clin Gastroenterol* 2012; **46**: 155-161 [PMID: 21814143 DOI: 10.1097/MCG.0b013e318228b781]
46 **Ditah I**, Ditah F, Devaki P, Ewelukwa O, Ditah C, Njei B, Luma HN, Charlton M. The changing epidemiology of hepatitis C virus infection in the United States: National Health and Nutrition Examination Survey 2001 through 2010. *J Hepatol* 2014; **60**: 691-698 [PMID: 24291324 DOI: 10.1016/j.jhep.2013.11.014]
47 **Ascha MS**, Hanouneh IA, Lopez R, Tamimi TA, Feldstein AF, Zein NN. The incidence and risk factors of hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. *Hepatology* 2010; **51**: 1972-1978 [PMID: 20209604 DOI: 10.1002/hep.23527]
48 **Kallwitz ER**, Layden-Almer J, Dhamija M, Berkes J, Guzman G, Lepe R, Cotler SJ, Layden TJ. Ethnicity and body mass index are associated with hepatitis C presentation and progression. *Clin Gastroenterol Hepatol* 2010; **8**: 72-78 [PMID: 19686868 DOI: 10.1016/j.cgh.2009.08.009]
49 **Bambha K**, Belt P, Abraham M, Wilson LA, Pabst M, Ferrell L, Unalp-Arida A, Bass N. Ethnicity and nonalcoholic fatty liver disease. *Hepatology* 2012; **55**: 769-780 [PMID: 21987488 DOI: 10.1002/hep.24726]
50 **Kallwitz ER**, Kumar M, Aggarwal R, Berger R, Layden-Almer J, Gupta N, Cotler SJ. Ethnicity and nonalcoholic fatty liver disease in an obesity clinic: the impact of triglycerides. *Dig Dis Sci* 2008; **53**: 1358-1363 [PMID: 18347982 DOI: 10.1007/s10620-008-0234-x]
51 **Dillon ST**, Bhasin MK, Feng X, Koh DW, Daoud SS. Quantitative proteomic analysis in HCV-induced HCC reveals sets of proteins with potential significance for racial disparity. *J Transl Med* 2013; **11**: 239 [PMID: 24283668 DOI: 10.1186/1479-5876-11-239]
52 **Song TJ**, Fong Y, Cho SJ, Gönen M, Hezel M, Tuorto S, Choi SY, Kim YC, Suh SO, Koo BH, Chae YS, Jarnagin WR, Klimstra DS. Comparison of hepatocellular carcinoma in American and Asian patients by tissue array analysis. *J Surg Oncol* 2012; **106**: 84-88 [PMID: 22234941 DOI: 10.1002/jso.23036]
53 **Hassan MM**, Kaseb A, Etzel CJ, El-Serag H, Spitz MR, Chang P, Hale KS, Liu M, Rashid A, Shama M, Abbruzzese JL, Loyer EM, Kaur H, Hassabo HM, Vauthey JN, Wray CJ, Hassan BS, Patt YZ, Hawk E, Soliman KM, Li D. Genetic variation in the PNPLA3 gene and hepatocellular carcinoma in USA: risk and prognosis prediction. *Mol Carcinog* 2013; **52** Suppl 1: E139-E147 [PMID: 23776098 DOI: 10.1002/mc.22057]
54 **Lade A**, Noon LA, Friedman SL. Contributions of metabolic dysregulation and inflammation to nonalcoholic steatohepatitis, hepatic fibrosis, and cancer. *Curr Opin Oncol* 2014; **26**: 100-107 [PMID: 24275855 DOI: 10.1097/CCO.0000000000000042]
55 **Guzman G**, Brunt EM, Petrovic LM, Chejfec G, Layden TJ, Cotler SJ. Does nonalcoholic fatty liver disease predispose patients to hepatocellular carcinoma in the absence of cirrhosis? *Arch Pathol Lab Med* 2008; **132**: 1761-1766 [PMID: 18976012 DOI: 10.1043/1543-2165-132.11.1761]
56 **Liu Y**, El-Serag HB, Jiao L, Lee J, Moore D, Franco LM, Tavakoli-Tabasi S, Tsavachidis S, Kuzniarek J, Ramsey DJ, White DL. WNT signaling pathway gene polymorphisms and risk of hepatic fibrosis and inflammation in HCV-infected patients. *PLoS One* 2013; **8**: e84407 [PMID: 24386373 DOI: 10.1371/journal.pone.0084407]
57 **Pullinger CR**, Goldfine ID, Tanyolaç S, Movsesyan I, Faynboym M, Durlach V, Chiefari E, Foti DP, Frost PH, Malloy MJ, Brunetti A, Kane JP. Evidence that an HMGA1 gene variant associates with type 2 diabetes, body mass index, and high-density lipoprotein cholesterol in a Hispanic-American population. *Metab Syndr Relat Disord* 2014; **12**: 25-30 [PMID: 24148075 DOI: 10.1089/met.2013.0086]

P- Reviewer: Chiu KW, Ma L, Wong GLH **S- Editor**: Kong JX
L- Editor: A **E- Editor**: Li D

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/

World J Hepatol 2017 March 8; 9(7): 401-408

DOI: 10.4254/wjh.v9.i7.401

ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Prospective Study

Phase angle obtained by bioelectrical impedance analysis independently predicts mortality in patients with cirrhosis

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Author contributions: Belarmino G, Gonzalez MC, Waitzberg DL, Heymsfield SB, Pereira RMR and D'Albuquerque LAC contributed to the conception and design and/or coordination of the study; Belarmino G, Gonzalez MC, Torrinhas RS, Waitzberg DL, Sala P, Andraus W, Heymsfield SB and Caparbo VF were responsible for the acquisition, analysis, and/or interpretation and discussion of data; Belarmino G, Torrinhas RS, Gonzalez MC and Ravacci GR drafted the manuscript; Damiani L performed statistical analyses; all authors read and approved the final manuscript.

Supported by The Fundação de Amparo à Pesquisa do Estado de São Paulo, Nos. 2011/13243-3, 2012/15677-3 [GB].

Institutional review board statement: The study was reviewed and approved by the Institutional Ethics Review Board (0646/11) of the Hospital das Clínicas (São Paulo, SP, Brazil).

Clinical trial registration statement: This clinical trial was registered at ClinicalTrials.gov with the identifier NCT02421848. Details can be found at https://clinicaltrials.gov/ct2/show/NCT02421848.

Informed consent statement: All study participants, or their legal guardians, provided written consent prior to study enrollment.

Conflict-of-interest statement: The authors of this manuscript have no conflict of interest to disclose.

Data sharing statement: No additional data are available.

Manuscript source: Invited manuscript

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Received: July 30, 2016
Peer-review started: August 2, 2016
First decision: September 8, 2016
Revised: December 22, 2016
Accepted: January 11, 2017
Article in press: January 14, 2017
Published online: March 8, 2017

Abstract

AIM

To evaluate the prognostic value of the phase angle (PA)

obtained from bioelectrical impedance analysis (BIA) for mortality prediction in patients with cirrhosis.

METHODS

In total, 134 male cirrhotic patients prospectively completed clinical evaluations and nutritional assessment by BIA to obtain PAs during a 36-mo follow-up period. Mortality risk was analyzed by applying the PA cutoff point recently proposed as a malnutrition marker (PA $\leqslant$ 4.9°) in Kaplan-Meier curves and multivariate Cox regression models.

RESULTS

The patients were divided into two groups according to the PA cutoff value (PA > 4.9°, n = 73; PA $\leqslant$ 4.9°, n = 61). Weight, height, and body mass index were similar in both groups, but patients with PAs > 4.9° were younger and had higher mid-arm muscle circumference, albumin, and handgrip-strength values and lower severe ascites and encephalopathy incidences, interleukin (IL)-6/IL-10 ratios and C-reactive protein levels than did patients with PAs $\leqslant$ 4.9° ($P \leqslant 0.05$). Forty-eight (35.80%) patients died due to cirrhosis, with a median of 18 mo (interquartile range, 3.3-25.6 mo) follow-up until death. Thirty-one (64.60%) of these patients were from the PA $\leqslant$ 4.9° group. PA $\leqslant$ 4.9° significantly and independently affected the mortality model adjusted for Model for End-Stage Liver Disease score and age (hazard ratio = 2.05, 95%CI: 1.11-3.77, P = 0.021). In addition, Kaplan-Meier curves showed that patients with PAs $\leqslant$ 4.9° were significantly more likely to die.

CONCLUSION

In male patients with cirrhosis, the PA $\leqslant$ 4.9° cutoff was associated independently with mortality and identified patients with worse metabolic, nutritional, and disease progression profiles. The PA may be a useful and reliable bedside tool to evaluate prognosis in cirrhosis.

Key words: Bioelectrical impedance analysis; Body composition; Phase angle; Nutritional assessment; Liver disease; Cirrhosis; Mortality

Core tip: This article provides original data displaying the good performance of the phase angle (PA) obtained by bioelectrical impedance analysis in the evaluation of mortality prognosis in patients with cirrhosis. The findings suggest that the PA is a safe, practical, and inexpensive tool for the prediction of mortality potentially associated with malnutrition.

Belarmino G, Gonzalez MC, Torrinhas RS, Sala P, Andraus W, D'Albuquerque LAC, Pereira RMR, Caparbo VF, Ravacci GR, Damiani L, Heymsfield SB, Waitzberg DL. Phase angle obtained by bioelectrical impedance analysis independently predicts mortality in patients with cirrhosis. *World J Hepatol* 2017; 9(7): 401-408 Available from: URL: http://www.wjgnet.com/1948-5182/full/v9/i7/401.htm DOI: http://dx.doi.org/10.4254/wjh.v9.i7.401

INTRODUCTION

Liver transplantation (LT) is the best option for patients with advanced cirrhosis, but its clinical application is often limited by the low availability of organ donors, risk of organ rejection, and implied high cost[1,2]. Consequently, the control and treatment of cirrhosis-associated complications remains the mainstay for this population. Malnutrition is a major complication often observed in patients with cirrhosis, and it has been associated with more severe disease, the manifestation of other cirrhosis-associated complications, and mortality[3]. Early diagnosis of malnutrition in patients with cirrhosis is important for prompt management and to improve quality of life[4-7].

In general, ascites, edema, and other chronic liver disease-associated complications (*i.e.,* altered immunocompetence, decreased protein synthesis, and renal failure) can impair the performance of traditionally applied criteria for nutritional assessment (NA)[8]. Consequently, weight loss, anthropometric measurements, the creatinine-height index, nitrogen balance, lymphocyte count, and serum albumin, transferrin, prealbumin, and retinol-bound protein levels should be interpreted with restrictions when assessing the nutritional status of cirrhotic patients[9]. In this scenario, a gold standard NA method is required for the proper diagnosis of malnutrition in this patient population[10-15].

The phase angle (PA) obtained from bioimpedance analysis (BIA) has been proposed as a nutritional status marker, with low values associated with malnutrition and nutritional risk at the time of hospital admission[16]. The PA reflects the relationship between the resistance component (R), meaning tissue opposition to the passage of electric current, and reactance (Xc), meaning the resistance effect produced by the interface of tissues and cell membranes[17]. A main advantage of the use of PA is that it can be applied even under unstable tissue hydration conditions, such as edema and ascites[18].

By potentially reflecting malnutrition, the PA can be a useful prognostic marker in several clinical settings[16,18-29]. As with any biological marker, the PA is influenced by the specific characteristics of each clinical population and may vary according to sex and age. Thus, specific PA reference and cutoff values have been proposed to establish prognoses for different diseases[16,18-26,30-34]. Recently, the 4.9° PA value was identified as the best cut-off for malnutrition associated to disease severity of patients with liver cirrhosis and shown to have important prognostic value for malnutrition-associated mortality in this patient population[35].

In this study, we aimed to test whether this PA cutoff ($\leqslant$ 4.9°) had prognostic value for mortality in a population of patients with cirrhosis of different ethnicity than used for its initial identification.

MATERIALS AND METHODS

Patients

This study included 134 male patients with biopsy-proven cirrhosis who were recruited prospectively from the Digestive Tract Surgery Service at the Hospital das Clínicas of the University of São Paulo Medical School between January 2012 and December 2014. Exclusion criteria were alcohol abuse; human immunodeficiency virus positivity; cancer diagnosis, acute liver failure, or chronic or acute disease of the lung, kidney, or heart; previous LT; orthopedic prosthesis use; and dementia. All patients provided written informed consent before trial participation.

Protocol design

Our protocol was designed to determine whether the PA has prognostic value for mortality in male patients with cirrhosis, by considering the PA cutoff point proposed by Ruiz-Margáina *et al*[35] (PA $\leqslant$ 4.9°) as a malnutrition marker. All recruited subjects were instructed to refrain from excessive physical activity, diuretic use, and alcohol consumption for 24 h before the assessment, which was performed in a 4-h fasting state[36]. Demographic data were recorded for all subjects. Death events were recorded for all patients with cirrhosis during the 36-mo follow-up period. A single trained technician performed all study procedures according to the ethical standards of the Declaration of Helsinki of the World Medical Association. All procedures were approved by the Institutional Ethics Review Board (0646/11) and registered at www.clinicalTrials.gov (NCT02421848).

Demographic and clinical data collection

The following demographic, clinical, inflammatory, and anthropometric data were collected: Age, liver cirrhosis etiology, Child-Pugh and Model for End-Stage Liver Disease (MELD) scores, presence of severe ascites, presence of encephalopathy, interleukin (IL)-6/IL-10 ratio, C-reactive protein (CRP) level, body weight and height, body mass index (BMI), non-dominant handgrip-strength (ND-HGS), and mid-arm muscle circumference (MAMC). Body weight was measured with the participant standing in the center of a single electronic scale platform (ADP; BOD POD™ BC system device; Life Measurement Instruments, Concord, CA, United States) while barefoot and wearing only light clothes[37]. Height was measured with a single stadiometer (Sanny, São Paulo, SP, Brazil) with the individual standing barefoot with the heels together, back upright, and arms extended next to the body[38]. BMI was calculated as weight divided by height squared (kg/m^2). ND-HGS was measured using a digital dynamometer (Charder Co. Ltd., Taichung City, Taiwan), as described previously[39]. Arm circumference (AC) was measured around the mid-upper arm, between the shoulder and elbow, using a flexible tape. Triceps skinfold thickness (TST) was assessed and MAMC was calculated using the formula: MAMC = AC (cm) = π × [TST (mm)/10].

Phase angle estimation

The PA was assessed by whole-body BIA[40] at 50 kHz (Bodystat 4000 model; Bodystat Ltd., Douglas, Isle of Man, British Isles) with APEX software (version 4.02; Hologic Inc., Bedford, MA, United States). Participants removed all metal objects and other items that might interfere with the scan and were instructed to empty the bladder. Each participant was positioned supine in the center of the scanning table with the palms down and the arms beside the body. His age, height, weight, sex, and ethnicity were entered into the computer. The PA value was calculated as PA = arc tangent Xc/R × 180/π. Patients were grouped according to PA value (PA > 4.9°, PA $\leqslant$ 4.9°)[35].

Survival

Death events were assessed by telephone calls at the end of the study period. Only deaths related directly to cirrhosis complications were counted. The prognostic value of the PA for mortality prediction was evaluated in mortality models adjusted for variables potentially impacting nutritional status and/or cirrhosis severity (age, Child-Pugh and MELD score)[35,41,42]. A longitudinal analysis of mortality was used to assess the prognostic value of malnutrition.

Sample size

The sample size required to analyze the prognostic value of the PA for mortality was calculated using the G Power software package (version 3.1.9.2; Heinrich Heine University, Dusseldorf, Germany). A sample size of 134 patients was obtained from a Cox proportional-hazards regression model, considering a significance level of 5% and rate of 36% at 36 mo of follow-up, with 80% power to detect a hazard ratio (HR) of 2.50 for mortality prediction.

Statistical analysis

Survival probabilities were estimated by the Kaplan-Meier method, compared using the log-rank test, and estimated in terms of the failure rate according to independent and multiple models of Cox proportional hazards. The mortality models included PA values and were adjusted for MELD score and age. Data were expressed as means ± SDs, medians, interquartile ranges (IQRs; 25th-75th percentile), or percentages, depending on the normality of distribution and type of variable. Data were analyzed using the R software package (version 3.1.3, 2015; R Core Team, Vienna, Austria) and a significance level of 5%.

RESULTS

Patient characteristics

A total of 134 patients (mean age, 54.30 ± 10.10 years) with cirrhosis of different etiologies (59.80% alcoholic,

Table 1 Baseline characteristics and body composition of patients with cirrhosis

Variable	PA > 4.9° (*n* = 73)	PA ⩽ 4.9° (*n* = 61)	Total (*n* = 134)	*P* value[a]
Age (yr)	52.10 ± 9.80	56.90 ± 9.80	54.30 ± 10.10	0.005[1]
Weight (kg)	76.60 ± 13.10	76.40 ± 15.30	76.50 ± 14.10	0.919[1]
Height (m)	1.70 ± 0.10	1.70 ± 0.10	1.70 ± 0.10	0.536[1]
Child Pugh A (%)	25	10	18	
Child Pugh B (%)	45	65	55	
Child Pugh C (%)	30	25	27	0.031[3]
Model for end-stage liver disease score	13.41 ± 5.11	14.95 ± 4.65	14.11 ± 4.95	0.073[3]
Severe ascites (%)	10.00	29.00	18.20	0.016[3]
Encephalopathy (%)	40.00	60.00	50.00	0.044[3]
Body mass index (kg/m^2)	26.70 ± 4.10	26.40 ± 5.00	26.60 ± 4.50	0.683[1]
Mid-arm muscle circumference (cm)	25.80 ± 3.20	23.20 ± 3.10	24.70 ± 3.40	< 0.001[1]
Handgrip strength (kg)	31.80 ± 7.00	24.40 ± 8.90	28.60 ± 8.70	< 0.001[1]
IL-6/IL-10 ratio (pg/mL)	1.10 (0.51; 2.35)	1.29 (0.71; 4.68)	1.17 (0.58; 2.68)	0.086[2]
C-reactive protein (mg/dL)	0.88 (0.42; 1.96)	1.20 (0.60; 4.72)	1.09 (0.54; 2.62)	0.030[2]
Albumin (g/dL)	3.90 (3.40; 4.30)	3.50 (2.90; 3.80)	3.60 (3.20; 4.20)	0.002[2]

[a]PA > 4.9° *vs* PA ⩽ 4.9°; [1]Student's *t* test; [2]Mann-Whitney test; [3]χ^2 test. Data are presented as mean ± SD (confidence interval), or percentage. PA: Phase angle; IL: Interleukin.

Table 2 Mortality estimates for patients with cirrhosis from a multiple Cox regression model

Variable	HR (95%CI)	*P* value
Age (yr)	1.03 (1.00, 1.06)	0.042
MELD score	1.10 (1.05, 1.16)	0.001
Phase angle 50 kHz (< 4.9°)	2.05 (1.11, 3.77)	0.021

P values for independent Cox regression models refer to three models explained by age, MELD score, and phase angle. HR: Hazard ratio; MELD: Model for end-stage liver disease.

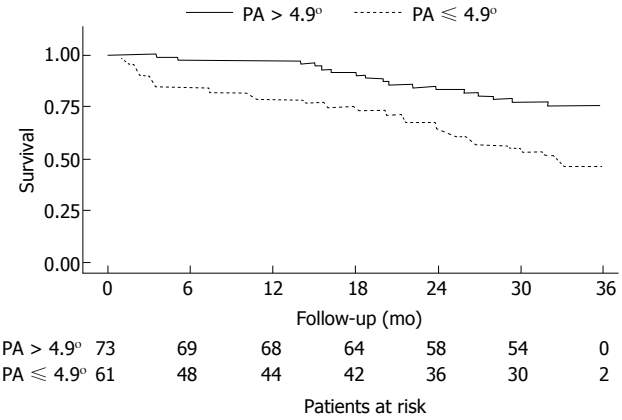

Figure 1 Kaplan-Meier survival curves for 134 patients with cirrhosis, obtained using cutoff scores based on phase angle obtained by bioelectrical impedance analysis (PA < 4.9°, *n* = 61; PA > 4.9°, *n* = 73). PA: Phase angle.

20.10% viral, 10.40% cryptogenic, and 9.70% other), presenting as 17.90% Child A, 54.50% Child B, and 27.60% Child C and with a mean MELD score of 14.11 ± 4.95, were enrolled in the study. Of these patients, 73 (54.48%) were assigned to the PA > 4.9° group and 61 (45.52%) were assigned to the PA ⩽ 4.9° group. Weight, height, and BMI were similar in both groups, but patients from the PA > 4.9° group were younger and had higher MAMC, albumin, and ND-HGS values and lower severe ascites and encephalopathy incidences, IL-6/IL-10 ratios, and CRP levels than did patients from the PA ⩽ 4.9° group (Table 1).

Prognostic value of malnutrition, identified by the phase angle

The mean follow-up duration was 25 mo (median, 32.1 mo). Of the 134 patients included in the mortality prediction analysis, 48 (35.80%) died due to cirrhosis, with a median of 18 mo (IQR, 3.3; 25.6 mo) of follow-up until death. Thirty-one (64.60%) patients who died were from the PA ⩽ 4.9° group.

The Child-Pugh score had no significant effect in the initial mortality model and was not included in the final model (Table 2). PA values ⩽ 4.9° significantly affected the mortality model adjusted for MELD score and age (HR = 2.05, 95%CI: 1.11-3.77, *P* = 0.021). In addition, the mortality prediction was not influenced by MELD or age. Patients from the PA ⩽ 4.9° group were significantly more likely to die, as demonstrated by Kaplan-Meier curves (Figure 1). In the median follow-up period of 18 mo, the incidence ratios of death were 27.10% for patients from the PA ⩽ 4.9° group and 9.90% for those from the PA > 4.9° group.

DISCUSSION

Although malnutrition implies a poor prognosis for patients with cirrhosis, its diagnosis has been masked in this population due to the unavailability of a clinically accessible method that is not affected by edema and/or ascites[18]. The PA is not affected by hydric changes and was recently proposed as a good tool for malnutrition diagnosis in patients with cirrhosis, with a cutoff value of ⩽ 4.9°[35]. Here, we showed that PA ⩽ 4.9° predicted mortality in male cirrhotic patients, in a model adjusted for age and MELD score.

We identified four studies evaluating the prognostic value of the PA in Brazilian (n = 2), German, and, more recently, Mexican patients with cirrhosis. These studies showed that PA cutoff values of 5.18°, 5.44°, 5.4° and 4.9°, respectively, were related to disease severity and even mortality, when controlling for age and other nutritional variables[14,18,35,43]. Here, we applied the PA cutoff value proposed recently by Ruiz-Margáin *et al*[35] ($\leqslant$ 4.9°), which was further used to establish malnutrition with good prognostic value for mortality in a cohort of Mexican cirrhotic patients.

In our study, the prognostic value of this PA cutoff was tested in mortality models adjusted for age and MELD score, as the main markers of PA performance and disease severity, respectively. Age has been proposed as the main indicator for PA determination in women and men, and the MELD score has been considered a good predictor of short-term mortality in patients with cirrhosis[35,41,42].

The Child-Pugh score was added to our initial mortality model because it may reflect the progression of liver damage and indirectly detect metabolic changes that may influence the prognosis of the disease[42]. However, it had no significant effect on mortality prediction. Notably, the MELD score has been validated as a good predictor of the survival of adult patients on the LT list, and has been found to better predict short-term results than does the CP score[44]. This difference in performance may explain the significant value of the MELD score, and not the CP score, for mortality prediction in our initial model. Data from the final mortality model support the prognostic value of PA $\leqslant$ 4.9°, as it was associated independently with mortality. Furthermore, our HR for mortality was similar to that reported by Ruiz-Margáin *et al*[35].

Results from some studies suggest that malnutrition is related strongly to mortality and cirrhosis-related complications[14,18,27,35,43,44]. Despite evidence suggesting the utility of the PA as a nutritional marker, its validity has been questioned. According to our data, the PA $\leqslant$ 4.9° cutoff was able to identify patients with significant changes in inflammatory and nutritional markers highly indicative of catabolism and malnutrition (*i.e.,* increased IL-6/IL-10 ratio and CRP level and decreased albumin level and HGS, a relevant marker of muscle loss associated largely with poor prognosis in cirrhosis). The notably increased mortality rate observed in our patients with PAs $\leqslant$ 4.9° may be associated closely with the deleterious effects of malnutrition.

PA values change in response to nutritional interventions, with greater sensitivity than achieved with other nutritional markers[45]. Thus, even if the PA cannot actually represent the nutritional status of a patient, it seems to adequately reflect minimal changes in this clinical variable. In this scenario, the PA could be applied for nutritional monitoring of patients for whom the risk of malnutrition could significantly influence clinical outcomes. For instance, the incidences of severe ascites and encephalopathy complications were significantly higher among patients with PAs $\leqslant$ 4.9° than among those with PAs above this cutoff in our study, in response to the metabolic consequences of the disease.

Patients with cirrhosis often display circulatory dysfunction with portal hypertension, leading to vasodilatation of splanchnic vessels and favoring decreased peripheral resistance and effective central blood volume, with consequent arterial hypotension and hyperdynamic circulation. These abnormalities result in the activation of vasoconstrictor systems through the renin-angiotensin-aldosterone system and of the sympathetic nervous system, with increased levels of antidiuretic hormone and renal vasoconstriction that culminate in ascites and/or edema[46]. These altered physiological states limit the application of available methods to evaluate nutritional status[47].

Indeed, as a result of ascites and/or edema, anthropometric measures such as BMI usually overestimate lean mass in patients with end-stage liver disease who require LT[3]. Consequently, although easier, traditional NA may underestimate the prevalence and severity of malnutrition in patients with cirrhosis[13]. Moreover, the presence of body fluid changes, mainly ascites, may explain the marked discrepancies in malnutrition frequencies (ranging from 5.4% to 68.2%) among NA methods in patients with cirrhosis[12,47-54]. As PA values are not influenced by unstable hydration, we suggest that this tool is useful for nutritional monitoring of patients with cirrhosis, and that the PA cutoff value proposed by Ruiz-Margáina *et al*[35] can identify those at high risk of death if not nutritionally treated.

One limitation of our study was the inclusion of solely male patients. We assessed only male patients to make our sample as uniform as possible, as liver cirrhosis *per se* is a progressive disease and hepatic damage may differ, even slightly, among patients. In addition, cirrhosis is more common in men and malnutrition seems to have greater prognostic value for disease progression in men than in women. The prognostic ability of the studied cutoff value for phase angle is associated directly with malnutrition. Thus, by evaluating only men, we were able to access not only a more uniform sample, but also the population most susceptible to the studied disease and its associated nutritional complications. Ruiz-Margáin *et al*[35] did not specify the sex of the cirrhotic patients with which the studied PA cutoff value was developed. Thus, we cannot confirm whether this value performs similarly in the prediction of malnutrition-associated mortality in women. We can conclude that the PA $\leqslant$ 4.9° cutoff was associated independently with mortality in male patients with cirrhosis, potentially associated to malnutrition. The PA may be a useful and reliable bedside tool to evaluate prognosis in cirrhosis.

ACKNOWLEDGMENTS

The authors thank the patients and nurses who par-

ticipated in the study.

COMMENTS

Background

Liver transplantation is the best option for patients with advanced cirrhosis, but its clinical application is often limited. Malnutrition is a major complication often observed in patients with cirrhosis. Early diagnosis of malnutrition in patients with cirrhosis is important. In general, ascites, edema, and other chronic liver disease-associated complications can impair the performance of traditionally applied criteria for nutritional assessment (NA). Consequently, weight loss, anthropometric measurements, the creatinine-height index, nitrogen balance, lymphocyte count, and serum albumin, transferrin, prealbumin, and retinol-bound protein levels should be interpreted with restrictions when assessing the nutritional status of cirrhotic patients. In this scenario, a gold standard NA method is required for the proper diagnosis of malnutrition in this patient population.

Research frontiers

The phase angle (PA) obtained from bioimpedance analysis has been proposed as a nutritional status marker, with low values associated with malnutrition and nutritional risk at the time of hospital admission. The PA reflects the relationship between the resistance component, meaning tissue opposition to the passage of electric current, and reactance, meaning the resistance effect produced by the interface of tissues and cell membranes. A main advantage of the use of PA is that it can be applied even under unstable tissue hydration conditions, such as edema and ascites.

Innovations and breakthroughs

This article provides original data displaying the good performance of the PA obtained by bioelectrical impedance analysis in the evaluation of mortality prognosis in patients with cirrhosis.

Applications

The findings suggest that the PA is a safe, practical, and inexpensive tool for the prediction of mortality potentially associated with malnutrition.

Peer-review

The authors aim to explore the potential value of PA in cirrhosis. In general, the topic is interesting, and the design is sound.

REFERENCES

1 **Habka D**, Mann D, Landes R, Soto-Gutierrez A. Future Economics of Liver Transplantation: A 20-Year Cost Modeling Forecast and the Prospect of Bioengineering Autologous Liver Grafts. *PLoS One* 2015; **10**: e0131764 [PMID: 26177505 DOI: 10.1371/journal.pone.0131764]

2 **Axelrod DA**, Dzebisashvili N, Lentine K, Segev DL, Dickson R, Tuttle-Newhall E, Freeman R, Schnitzler M. Assessing variation in the costs of care among patients awaiting liver transplantation. *Am J Transplant* 2014; **14**: 70-78 [PMID: 24165015 DOI: 10.1111/ajt.12494]

3 **Ritter L**, Gazzola J. [Nutritional evaluation of the cirrhotic patient: an objective, subjective or multicompartmental approach?]. *Arq Gastroenterol* 2006; **43**: 66-70 [PMID: 16699622 DOI: 10.1590/S0004-28032006000100016]

4 **Montano-Loza AJ**, Meza-Junco J, Prado CM, Lieffers JR, Baracos VE, Bain VG, Sawyer MB. Muscle wasting is associated with mortality in patients with cirrhosis. *Clin Gastroenterol Hepatol* 2012; **10**: 166-173, 173.e1 [PMID: 21893129 DOI: 10.1016/j.cgh.2011.08.028]

5 **Kalafateli M**, Mantzoukis K, Choi Yau Y, Mohammad AO, Arora S, Rodrigues S, de Vos M, Papadimitriou K, Thorburn D, O'Beirne J, Patch D, Pinzani M, Morgan MY, Agarwal B, Yu D, Burroughs AK, Tsochatzis EA. Malnutrition and sarcopenia predict post-liver transplantation outcomes independently of the Model for End-stage Liver Disease score. *J Cachexia Sarcopenia Muscle* 2016 [PMID: 27239424 DOI: 10.1002/jcsm.12095]

6 **Masuda T**, Shirabe K, Ikegami T, Harimoto N, Yoshizumi T, Soejima Y, Uchiyama H, Ikeda T, Baba H, Maehara Y. Sarcopenia is a prognostic factor in living donor liver transplantation. *Liver Transpl* 2014; **20**: 401-407 [PMID: 24357065 DOI: 10.1002/lt.23811]

7 **Pinzani M**, Rosselli M, Zuckermann M. Liver cirrhosis. *Best Pract Res Clin Gastroenterol* 2011; **25**: 281-290 [PMID: 21497745 DOI: 10.1016/j.bpg.2011.02.009]

8 **Alvares-da-Silva MR**, Reverbel da Silveira T. Comparison between handgrip strength, subjective global assessment, and prognostic nutritional index in assessing malnutrition and predicting clinical outcome in cirrhotic outpatients. *Nutrition* 2005; **21**: 113-117 [PMID: 15723736 DOI: 10.1016/j.nut.2004.02.002]

9 **Barbosa-Silva MC**, de Barros AJ. [Subjective global assessment: Part 2. Review of its adaptations and utilization in different clinical specialties]. *Arq Gastroenterol* 2002; **39**: 248-252 [PMID: 12870085]

10 **Hanai T**, Shiraki M, Nishimura K, Ohnishi S, Imai K, Suetsugu A, Takai K, Shimizu M, Moriwaki H. Sarcopenia impairs prognosis of patients with liver cirrhosis. *Nutrition* 2015; **31**: 193-199 [PMID: 25441595 DOI: 10.1016/j.nut.2014.07.005]

11 **Johnson TM**, Overgard EB, Cohen AE, DiBaise JK. Nutrition assessment and management in advanced liver disease. *Nutr Clin Pract* 2013; **28**: 15-29 [PMID: 23319353 DOI: 10.1177/0884533612469027]

12 **Figueiredo FA**, De Mello Perez R, Kondo M. Effect of liver cirrhosis on body composition: evidence of significant depletion even in mild disease. *J Gastroenterol Hepatol* 2005; **20**: 209-216 [PMID: 15683423 DOI: 10.1111/j.1440-1746.2004.03544.x]

13 **Figueiredo FA**, Perez RM, Freitas MM, Kondo M. Comparison of three methods of nutritional assessment in liver cirrhosis: subjective global assessment, traditional nutritional parameters, and body composition analysis. *J Gastroenterol* 2006; **41**: 476-482 [PMID: 16799890 DOI: 10.1007/s00535-006-1794-1]

14 **Fernandes SA**, Bassani L, Nunes FF, Aydos ME, Alves AV, Marroni CA. Nutritional assessment in patients with cirrhosis. *Arq Gastroenterol* 2012; **49**: 19-27 [PMID: 22481682]

15 **Roberts HC**, Denison HJ, Martin HJ, Patel HP, Syddall H, Cooper C, Sayer AA. A review of the measurement of grip strength in clinical and epidemiological studies: towards a standardised approach. *Age Ageing* 2011; **40**: 423-429 [PMID: 21624928 DOI: 10.1093/ageing/afr051]

16 **Kyle UG**, Genton L, Pichard C. Low phase angle determined by bioelectrical impedance analysis is associated with malnutrition and nutritional risk at hospital admission. *Clin Nutr* 2013; **32**: 294-299 [PMID: 22921419 DOI: 10.1016/j.clnu.2012.08.001]

17 **Baumgartner RN**, Chumlea WC, Roche AF. Bioelectric impedance phase angle and body composition. *Am J Clin Nutr* 1988; **48**: 16-23 [PMID: 3389323]

18 **Selberg O**, Selberg D. Norms and correlates of bioimpedance phase angle in healthy human subjects, hospitalized patients, and patients with liver cirrhosis. *Eur J Appl Physiol* 2002; **86**: 509-516 [PMID: 11944099 DOI: 10.1007/s00421-001-0570-4]

19 **Krause L**, Becker MO, Brueckner CS, Bellinghausen CJ, Becker C, Schneider U, Haeupl T, Hanke K, Hensel-Wiegel K, Ebert H, Ziemer S, Ladner UM, Pirlich M, Burmester GR, Riemekasten G. Nutritional status as marker for disease activity and severity predicting mortality in patients with systemic sclerosis. *Ann Rheum Dis* 2010; **69**: 1951-1957 [PMID: 20511612 DOI: 10.1136/ard.2009.123273]

20 **Faisy C**, Rabbat A, Kouchakji B, Laaban JP. Bioelectrical impedance analysis in estimating nutritional status and outcome of patients with chronic obstructive pulmonary disease and acute respiratory failure. *Intensive Care Med* 2000; **26**: 518-525 [PMID: 10923724]

21 **Maggiore Q**, Nigrelli S, Ciccarelli C, Grimaldi C, Rossi GA, Michelassi C. Nutritional and prognostic correlates of bioimpedance indexes in hemodialysis patients. *Kidney Int* 1996; **50**: 2103-2108 [PMID: 8943496]

22 **Ott M**, Fischer H, Polat H, Helm EB, Frenz M, Caspary WF, Lembcke B. Bioelectrical impedance analysis as a predictor of survival in patients with human immunodeficiency virus infection. *J Acquir Immune Defic Syndr Hum Retrovirol* 1995; **9**: 20-25 [PMID: 7712230]

23 **Schwenk A**, Ward LC, Elia M, Scott GM. Bioelectrical impedance analysis predicts outcome in patients with suspected bacteremia. *Infection* 1998; **26**: 277-282 [PMID: 9795784]

24 **Schwenk A**, Beisenherz A, Römer K, Kremer G, Salzberger B, Elia M. Phase angle from bioelectrical impedance analysis remains an independent predictive marker in HIV-infected patients in the era of highly active antiretroviral treatment. *Am J Clin Nutr* 2000; **72**: 496-501 [PMID: 10919947]

25 **Gupta D**, Lammersfeld CA, Burrows JL, Dahlk SL, Vashi PG, Grutsch JF, Hoffman S, Lis CG. Bioelectrical impedance phase angle in clinical practice: implications for prognosis in advanced colorectal cancer. *Am J Clin Nutr* 2004; **80**: 1634-1638 [PMID: 15585779]

26 **Gupta D**, Lammersfeld CA, Vashi PG, King J, Dahlk SL, Grutsch JF, Lis CG. Bioelectrical impedance phase angle as a prognostic indicator in breast cancer. *BMC Cancer* 2008; **8**: 249 [PMID: 18727837 DOI: 10.1186/1471-2407-8-249]

27 **Alberino F**, Gatta A, Amodio P, Merkel C, Di Pascoli L, Boffo G, Caregaro L. Nutrition and survival in patients with liver cirrhosis. *Nutrition* 2001; **17**: 445-450 [PMID: 11399401]

28 **Bosy-Westphal A**, Danielzik S, Dörhöfer RP, Piccoli A, Müller MJ. Patterns of bioelectrical impedance vector distribution by body mass index and age: implications for body-composition analysis. *Am J Clin Nutr* 2005; **82**: 60-68 [PMID: 16002801]

29 **Roman M**, Torres S, Casanova M. Bases físicas del análisis de la impedância bioeléctrica. Universidad de Cádiz, 1999: 39-143

30 **Llames L**, Baldomero V, Iglesias ML, Rodota LP. [Values of the phase angle by bioelectrical impedance; nutritional status and prognostic value]. *Nutr Hosp* 2013; **28**: 286-295 [PMID: 23822677 DOI: 10.3305/nh.2013.28.2.6306]

31 **Norman K**, Stobäus N, Zocher D, Bosy-Westphal A, Szramek A, Scheufele R, Smoliner C, Pirlich M. Cutoff percentiles of bioelectrical phase angle predict functionality, quality of life, and mortality in patients with cancer. *Am J Clin Nutr* 2010; **92**: 612-619 [PMID: 20631202 DOI: 10.3945/ajcn.2010.29215]

32 **Souza Thompson Motta R**, Alves Castanho I, Guillermo Coca Velarde L. CUTOFF POINT OF THE PHASE ANGLE IN PRE-RADIOTHERAPY CANCER PATIENTS. *Nutr Hosp* 2015; **32**: 2253-2260 [PMID: 26545685 DOI: 10.3305/nh.2015.32.5.9626]

33 **Kyle UG**, Soundar EP, Genton L, Pichard C. Can phase angle determined by bioelectrical impedance analysis assess nutritional risk? A comparison between healthy and hospitalized subjects. *Clin Nutr* 2012; **31**: 875-881 [PMID: 22560739 DOI: 10.1016/j.clnu.2012.04.002]

34 **da Silva TK**, Berbigier MC, Rubin Bde A, Moraes RB, Corrêa Souza G, Schweigert Perry ID. Phase angle as a prognostic marker in patients with critical illness. *Nutr Clin Pract* 2015; **30**: 261-265 [PMID: 25829343 DOI: 10.1177/0884533615572150]

35 **Ruiz-Margáin A**, Macías-Rodríguez RU, Duarte-Rojo A, Ríos-Torres SL, Espinosa-Cuevas Á, Torre A. Malnutrition assessed through phase angle and its relation to prognosis in patients with compensated liver cirrhosis: a prospective cohort study. *Dig Liver Dis* 2015; **47**: 309-314 [PMID: 25618555 DOI: 10.1016/j.dld.2014.12.015]

36 **Kyle UG**, Bosaeus I, De Lorenzo AD, Deurenberg P, Elia M, Manuel Gómez J, Lilienthal Heitmann B, Kent-Smith L, Melchior JC, Pirlich M, Scharfetter H, M W J Schols A, Pichard C. Bioelectrical impedance analysis-part II: utilization in clinical practice. *Clin Nutr* 2004; **23**: 1430-1453 [PMID: 15556267 DOI: 10.1016/j.clnu.2004.09.012]

37 **Ginde SR**, Geliebter A, Rubiano F, Silva AM, Wang J, Heshka S, Heymsfield SB. Air displacement plethysmography: validation in overweight and obese subjects. *Obes Res* 2005; **13**: 1232-1237 [PMID: 16076993 DOI: 10.1038/oby.2005.146]

38 **McDowell MA**, Fryar CD, Ogden CL, Flegal KM. Anthropometric reference data for children and adults: United States, 2003-2006. National Health Statistics Report; no 10. Hyattsville, MD: National Center for Health Statistics; 2008. Available from: URL: https://www.cdc.gov/nchs/data/nhsr/nhsr010.pdf

39 **Gottschall CA**, Álvares-da-Silva MR, Camargo AC, Burtett RM, Silveira TR. Avaliação nutricional de pacientes com cirrose pelo vírus da hepatite C: a aplicação da calorimetria indireta. *Arq Gastroenterol* 2004; **41**: 220-224 [PMID: 15806264]

40 **Barbosa-Silva MCG**, Barros AJD. Bioelectrical impedance analysis in clinical practice: a new perspective on its use beyond body composition equations. *Curr Opin Clin Nutr Metab Care* 2005; **8**: 311-317 [PMID: 15809535]

41 **Gonzalez MC**, Barbosa-Silva TG, Bielemann RM, Gallagher D, Heymsfield SB. Phase angle and its determinants in healthy subjects: influence of body composition. *Am J Clin Nutr* 2016; **103**: 712-716 [PMID: 26843156 DOI: 10.3945/ajcn.115.116772]

42 **Kamath PS**, Wiesner RH, Malinchoc M, Kremers W, Therneau TM, Kosberg CL, D'Amico G, Dickson ER, Kim WR. A model to predict survival in patients with end-stage liver disease. *Hepatology* 2001; **33**: 464-470 [PMID: 11172350 DOI: 10.1053/jhep.2001.22172]

43 **Peres WA**, Lento DF, Baluz K, Ramalho A. Phase angle as a nutritional evaluation tool in all stages of chronic liver disease. *Nutr Hosp* 2012; **27**: 2072-2078 [PMID: 23588459 DOI: 10.3305/nh.2012.27.6.6015]

44 **Sam J**, Nguyen GC. Protein-calorie malnutrition as a prognostic indicator of mortality among patients hospitalized with cirrhosis and portal hypertension. *Liver Int* 2009; **29**: 1396-1402 [PMID: 19602136 DOI: 10.1111/j.1478-3231.2009.02077.x]

45 **Norman K**, Stübler D, Baier P, Schütz T, Ocran K, Holm E, Lochs H, Pirlich M. Effects of creatine supplementation on nutritional status, muscle function and quality of life in patients with colorectal cancer--a double blind randomised controlled trial. *Clin Nutr* 2006; **25**: 596-605 [PMID: 16701923 DOI: 10.1016/j.clnu.2006.01.014]

46 **Urrunaga NH**, Magder LS, Weir MR, Rockey DC, Mindikoglu AL. Prevalence, Severity, and Impact of Renal Dysfunction in Acute Liver Failure on the US Liver Transplant Waiting List. *Dig Dis Sci* 2016; **61**: 309-316 [PMID: 26386861 DOI: 10.1007/s10620-015-3870-y]

47 **Morgan MY**, Madden AM, Soulsby CT, Morris RW. Derivation and validation of a new global method for assessing nutritional status in patients with cirrhosis. *Hepatology* 2006; **44**: 823-835 [PMID: 17006918 DOI: 10.1002/hep.21358]

48 **Shawcross DL**, Shabbir SS, Taylor NJ, Hughes RD. Ammonia and the neutrophil in the pathogenesis of hepatic encephalopathy in cirrhosis. *Hepatology* 2010; **51**: 1062-1069 [PMID: 19890967 DOI: 10.1002/hep.23367]

49 **Putadechakum S**, Klangjareonchai T, Soponsaritsuk A, Roongpisuthipong C. Nutritional status assessment in cirrhotic patients after protein supplementation. *ISRN Gastroenterol* 2012; **2012**: 690402 [PMID: 23304537 DOI: 10.5402/2012/690402]

50 **Pirlich M**, Schütz T, Spachos T, Ertl S, Weiss ML, Lochs H, Plauth M. Bioelectrical impedance analysis is a useful bedside technique to assess malnutrition in cirrhotic patients with and without ascites. *Hepatology* 2000; **32**: 1208-1215 [PMID: 11093726 DOI: 10.1053/jhep.2000.20524]

51 **Chang WT**, Ker CG, Hung HC, Lee KT, Chen LS, Chiang HC, Huang MC. Albumin and prealbumin may predict retinol status in patients with liver cirrhosis. *Hepatogastroenterology* 2008; **55**: 1681-1685 [PMID: 19102369]

52 **Masuda T**, Shirabe K, Yoshiya S, Matono R, Morita K, Hashimoto N, Ikegami T, Yoshizumi T, Baba H, Maehara Y. Nutrition support and infections associated with hepatic resection and liver transplantation in patients with chronic liver disease. *JPEN J Parenter Enteral Nutr* 2013; **37**: 318-326 [PMID: 22898793 DOI: 10.1177/0148607112456041]

53 **Stephenson GR**, Moretti EW, El-Moalem H, Clavien PA, Tuttle-Newhall JE. Malnutrition in liver transplant patients: preoperative subjective global assessment is predictive of outcome after liver transplantation. *Transplantation* 2001; **72**: 666-670 [PMID:

11544428]
54 **Merli M**, Giusto M, Gentili F, Novelli G, Ferretti G, Riggio O, Corradini SG, Siciliano M, Farcomeni A, Attili AF, Berloco P, Rossi M. Nutritional status: its influence on the outcome of patients undergoing liver transplantation. *Liver Int* 2010; **30**: 208-214 [PMID: 19840246 DOI: 10.1111/j.1478-3231.2009.02135.x]

P- Reviewer: Ikura Y, Zheng MH **S- Editor**: Kong JX **L- Editor**: A
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