

Assessment of liver cancer biomarkers

Vahid Mansouri¹, Mohhamadreza Razzaghi², Abdolrahim Nikzamir³, Alireza Ahmadzadeh⁴, Majid Iranshahi⁵, Mehrdad Haghazali⁶, Mostafa Hamdih⁷

¹ Proteomics Research Center, School of Rehabilitation, ShahidBeheshti University of Medical Sciences, Tehran, Iran

² Laser Application in Medical Sciences Research Center, ShahidBeheshti University of Medical Sciences, Tehran, Iran

³ Faculty of Medicine, ShahidBeheshti University of Medical Sciences, Tehran, Iran

⁴ Proteomics Research Center, Faculty of Paramedical Sciences, ShahidBeheshti University of Medical Sciences, Tehran, Iran

⁵ Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran

⁶ Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran

⁷ Department of Psychosomatic, Taleghani Hospital, Faculty of Medicine, ShahidBeheshti University of Medical Sciences, Tehran, Iran

ABSTRACT

Liver cancer is the third cause of cancer-related deaths in the world. It is primarily divided into two main types, namely hepatocellular carcinoma (HC) and cholangiocarcinoma (IC). Due to the increasing number of patients with liver cancer and the high mortality rate, early diagnosis of the disease can be helpful in treatment, but most patients are diagnosed at late stages of HC. The aim of this study is to screen and provide an overview on candidate biomarkers related to primary liver cancer to introduce the critical ones. In this study, various biomarkers related to the diagnosis of primary liver cancer have been studied. Accordingly, biomarkers are divided into different groups as blood biomarkers classified as serum and plasma biomarkers, tissue biomarkers, microRNA biomarkers, proteomic biomarkers and altered genes. Previous researches have focused on liver cells and bile ducts, the surround cellular environment, how cells differentiate, and the types of genes expressed in liver cancer. Some even have focused on the origin of tumor cells and how they differentiate and develop. In all these studies, the expression of specific proteins and genes in liver cancer has been considered. Based on available sources, biomarkers can be considered as candidates to diagnose and prognosis of various types of primary liver cancer, from sources such as blood, tissue, mic-RNA, proteome and genes. However, more investigations are required to introduce a biomarker for precise detection of early liver cancer.

Keywords: Hepatocellular carcinoma, Biomarker, Diagnosis, Prognosis, Proteomics.

(Please cite as: **Mansouri V, Razzaghi MR, Nikzamir A, Ahmadzadeh AR, Iranshahi M, Haghazali M, et al. Assessment of liver cancer biomarkers. Gastroenterol Hepatol Bed Bench 2020;13(Suppl.1):S29-S39.**)

Introduction

Liver cancer with the fourth rank causes death in about 700,000 people annually (1). The most common type of liver cancer is called hepatocellular carcinoma (HC). There are several risk factors for hepatocellular carcinoma, some of which include hepatitis B and C viral infections, alcohol abuse, diabetes, autoimmune hepatitis, obesity and many metabolic diseases (2). Risk

factors that lead to liver damage cause an inflammatory environment and could induce the process of necrosis, tissue repair and chromosomal instability (3). Accumulation of inflammatory cytokines, reactive oxygen species and fibrosis leads to genetic and epigenetic changes resulting in hepatocellular carcinoma (3). Multikinase inhibitors such as Sorafenib and Lenvatinib are currently approved drugs for advanced HC treatment but the survival rate of the patients treated with these drugs is not perfect yet, and new drug discovery is required for HC treatment (4, 5). HC initiation and progression is a multi-step process, but molecular processes leading to HC formation is not completely understood (6). HC is a

Received: 21 March 2020 Accepted: 18 July 2020

Reprint or Correspondence: **Vahid Mansouri**, PhD, Proteomics Research Center, School of Rehabilitation, ShahidBeheshti University of Medical Sciences, Tehran, Iran

E-mail: vm1343@yahoo.com

ORCID ID: 0000-0001-8472-9058

S30 Assessment of liver cancer biomarkers

highly heterogeneous disease, and inter-tumor heterogeneity between different HCs has made effective treatment difficult (7). Target drug treatments are often given without considering the genetic history (8). HC has a stratified basis and unlike target therapies of other cancers with pre-stratified background, we need genetic background history for personalized medication(9). Genomic and transcriptomic studies have their own limitations, and changing them does not necessarily translated to protein levels lead to morphological differences (8). On the other hand, phosphorylation of proteins has not yet been studied for genomic profiling, which can regulate protein activity (8). Advancement in protein profiling technologies as proteomics are useful for identifying the molecular mechanisms of early hepatocellular carcinoma (10). This study investigates and extracts proteomics and genomics researches from valuable data banks to identify recent approaches to introduce new biomarkers related to different stages of HC.

Primary Liver cancer

Primary liver cancer generally include hepatocellular carcinoma(HC) with the incidence of 75-85%, intrahepatic cholangiocarcinoma(IC)with the incidence of 10-15%, and combination of both hepatocellular carcinoma and cholangiocarcinoma (HC-IC)(incidence of 1-4.4%)(11, 12). HC originates from hepatocytes as a malignant tumor and is the fifth common cancer cause(13). The second most common primary liver tumor is IC which originates from biliary epithelium(14) and the rare type of primary hepatic carcinoma originates from both hepatocytes and bile duct cells (15). There are different, sometimes confusing, biological behaviors for these cancers, and laboratory biomarker tests are safe and accurate to early diagnosis. Blood and histochemical biomarkers tracing could manage early monitoring and pathological classification of liver cancers to the prognosis and treatment of liver cancer patients. A-fetoprotein or AFT is the most common biomarker for primary liver cancer yet with low sensitivity (14). Therefore, the use and combination of different tumor biomarkers in order to diagnose primary liver cancer seems necessary to find new functional biomarkers according to prognosis judgments and treatment effect observations.

Serum biomarkers

Biomarkers in the blood are important for the early detection of primary liver cancer and they could be classified as proteins, cytokines, enzymes, and transcripts of dependent genes (16). According to the molecular characteristics of liver cancer biomarkers in the blood, they should not be used separately to diagnose different types of liver cancer and their combined use is necessary to diagnose these types of cancer (Table 1).

AFP (a-fetoprotein)

AFP is a glycoprotein biomarker derived from AFP is widely used in liver cancer diagnosis. However, it may increase in liver cirrhosis and hepatitis and AFP-L3 may increase in 20-30% of early HC(17). Sensitivity of AFP is 25% less than 3 cm in diameter for HC tumors and combined usage of AFP with other biomarkers can improve early diagnosis of HC(13). As AFP is significantly higher in IC patients compared to HC patients and does not change significantly in IC patients, it could be used as a powerful biomarker in IC diagnosis (15). Significant elevation of AFP in HC-IC patients is reported (18).

AFP-L3 is a fucosylated variant of AFP and derived only from tissues of tumors as a specific biomarker of HC. AFP-L3 specificity for early detection of HC was 90-95% (19). AFP-L3 demonstrates more sensitivity than AFP in early stages of HC, which could also reflect features of tumors such as malignant invasion and little differentiation(20). Combination of AFP and AFP-L3 sensitivity in diagnosis of HC were significant (21), and AFP-L3 could be used as a supplementary test in low-level incidence of AFP in HC patients (16, 22).

Table 1. Blood Biomarkers involved in liver cancer

Biomarker	blood	Cancer type
AFU	Y	HC
AFP/AFP-L3	Y	HC
DCP	Y	HC
DCP & AFT	Y	HC
AFP	Y	HC-IC-HCIC
CA19-9	Y	ICC
AFP & OPN	Y	Small HC with negative AFP
GP73	Y	Early HC

AFP:a-fetoprotein, DCP: des- γ -carboxyprothrombin, AFU: a-L fucosidase, GP73: Golgi protein 73, OPN:Osteopontin, CA18-9: carbohydrate antigen19-9, GPC3: Glypican-3

Des-γ-carboxyprothrombin (DCP)

DCP is a new serological biomarker produced by HC cells. DCP is elevated in HC patients following defects in carboxylation of prothrombin precursor after translation. As reported in a study, DCP was more sensitive than AFP (23). DCP were used as an effective biomarker to distinguish intra-hepatic metastasis and prognosis of HC in east Asian countries (24). Larger tumor size, more tumor numbers and bile duct invasions could be indicated by elevated levels of DCP, resulting in poorer survival of patients (25). DCP could indicate HC in countries with high incidence of hepatitis B virus infections as East Asia and Africa (26). DCP and AFP combination in HC diagnosis with 2-4 cm tumors heightened sensitivity (27).

Golgi protein 73 (GP73)

GP73 are expressed in the epithelial cells of different human tissues as a trans membrane glycoprotein of type II Golgi resident (28). GP73 is highly expressed in HC patients and moderately expressed in viral infections and cirrhosis (29). Expression and sensitivity of GP73 in HC is remarkably higher than AFP (30). GP73 suggested superior than AFP in early detection of HC (16, 30).

α-L-fucosidase or AFU

AFU is an enzyme with the ability of fucoglycoconjugates degradation (31). Serum level of AFU in HC patients was higher than other patients with benign hepatic disorders (32). AFU is introduced as an early detecting biomarker for HC (33). AFU can be combined with AFP because it is positively linked with tumor size for early detection of HC (19).

Carbohydrate antigen 19-9 (CA19-9)

CA19-9 is a general biomarker for the diagnosis of different types of adenocarcinoma as IC (36). CA19-9 is suggested for lymph node metastasis in IC patients (34). CA19-9 levels could be served as an individual prognostic biomarker for IC patients (35).

Osteopontin (OPN)

OPN is a phosphorylated extracellular protein binding to integrin. OPN is expressed in normal cells and tumors (36). Its level in patients diagnosed with hepatitis C viral was associated with HC, and was significantly higher than OPN level in chronic liver disease patients and healthy subjects (37). It seems that it is an early biomarker of HC. OPN elevated one year before HC diagnosis and in HC cases emerging from

liver cirrhosis, OPN were indicated superior to AFP (37). It also induces macrophage activation. Tumor infiltration in OPN knockout HC mouse model through the activation of colony stimulating factor 1 receptor pathway, leading to programmed death with ligand 1 expression in HC (38).

Concurrent detection of AFP, AFP-L3 and DCP in serum is used for early detection of HC at present (39). Elevated 3 biomarkers expression is accompanied with tumors invasiveness (39). AFP is a valuable biomarker for diagnosis and prognosis of HC, and the role of DCP and AFP-L3 in serum is supplement (16).

Histological Biomarkers

Histopathological methods for the diagnosis of liver cancer can be considered as a definitive method that is complemented by immunohistochemistry techniques. In this regard, biomarkers in cancer tissues can indicate the condition and morphology of tumor cells. Therefore, tissue biomarkers play an important role in the diagnosis and treatment of cancers and benign tumors of HC, IC, and HCIC. In the following, we introduce some major tissue biomarkers for the diagnosis of primary liver cancer.

Heat shock protein 70 (HSP70)

HSP70 expression is low in normal cases but in response to hypoxia, heat, genotoxic agents and food starvation, it is expressed significantly high (40). HSP70 gene is highly up-regulated in early HC tissue sections (41). Over expression of HSP70 is accompanied with portal vein invasion (42). Another study revealed that HSP70 expression is associated with vascular invasion, cell proliferation, lymph node metastasis and larger tumor size in HC (43). However, over-expression of HSP70 could not demonstrate survival of patients with HC (42).

Hepatocyte paraffin 1 (Hep-Par1)

Hep-Par1 is a highly sensitive monoclonal antibody for hepatocellular differentiation (44). It is associated with mitochondrial antigens of malignant and nonmalignant hepatic carcinoma cells (45). This biomarker is completely expressed in well differentiated HC but may be negative in poorly differentiated HC (46). Therefore, Hep-Par1 could be used for the diagnosis of poorly differentiated HC and liver cancer metastasis in combination with other biomarkers.

S32 Assessment of liver cancer biomarkers

Table 2. Histopathological biomarkers for liver cancer

Biomarker	Tissue	Cancer Type
HSP70	Y	EarlyHC
GS	Y	HC
GPC3	Y	HC
Hep Par1	Y	HC
ARG1	Y	HC
CK19	Y	IC
CK7	Y	IC

HSP70:Heat shock protein70. GS: GlutaminsynthetaseGPC3: glypican-3.HepPar1: hepatocyte paraffin 1ARG1: arginase-1. CK19:Cytokeratin19.CK7:Cytokeratin7 Y=Present-N=Absent

Glypican-3 (GPC-3)

GPC-3 is a heparan sulfateproteoglycan, and a member of glypican family (47). Itcan be lysed and released into serum as a soluble form of GPC-3 (48). GP can be considered as a specific biomarker of liver cancer, because its expression in these patients increases up to 80% and it can be used to diagnose HC from IC and other malignant tumors(49). Cell line investigations revealed that GPC-3 can modulate cell cycle progression, promote cell invasion, and migrationinHC cell cultures. It is also regarded asHC proprietary biomarker with high sensitivity of 97% (50). GPC-3 was introduced as a sensitive biomarker candidate for early detection of HC(51). As a strong histological biomarker for HC, it can also be considered as a serological biomarker. Research has shown that the rate of GPC-3 in the early occurrence of HC in serum is higher than AFP and can be used to early detection of HC (33).

Arginase-1 (Arg-1)

Arg-1 is a sensitive biomarker for malignant & benign tumor cells of liver cancer and acts as an enzyme for arginine to ornithine hydrolysis (52). It is a suitable biomarker for hepatocellular differentiation too (53). Compared to Hep Par1, this enzyme is more sensitive in early HC differentiation, although Hep Par1 expression in non-hepatic tumors is also reported(54). Microarray analysis revealed that Arg-1 can be considered as a specific biomarker of HC (55). Therefore, itmay introduced as a specific candidate biomarker for HC (16).

Cytokeratin or CK7

CK7 is a member of cytokeratin family as intermediate filament. Itis expressed in epithelial cells and is mainly presented in gland ducts and absent in hepatocytes (56). The presence of CK7(similar to CK19) in gland ducts as bile ducts can justify its usage as a

immunohistochemical biomarker to distinguish between IC and HC cells (57). Expression of CK7 and CK19 in IC patients is associated with aggressive tumor phenotypes and co-expression of CK7 & CK19 is considered as a top factor for independent prognosis of IC (58). The high- and low-level expressions of combined CK7 & CK19were respectively associated with low survival rate of patients (58). Therefore, CK7 & CK19 canbe assumed as a powerful biomarker for prognosis of IC but furtherresearch needs to confirm it.

Glutamine synthetize (GS)

GS catalyzes glutamate and ammonia to glutamine in the liver. It isexpressed in the hepatic veins beside hepatocytes in normal cases, but spreads on hepatocellular tumors (59). Glutamine provides energy for tumor cells and it is therefore diffused in HC cells and its expression is dependent on the WNT signal pathway. Itspositivity in liver cancer can also be associated with symptoms such as tumor size, cellular swelling, fatty liver and fibrosis (59). GC and HSP70 combined positive expression revealed well differentiated HC; therefore, two biomarkers expression may be a more efficienttool to distinguish between atypical neoplasms and well differentiated HC (60). Another study reported that immunoreactivity of GS and HSP70 could not identify IC cells origin properly (52). On the other hand, another study revealed that the specificity and sensitivity of GS in cirrhotic livers was significantly higher than non-cirrhotic livers (65). However, HSP70 and GS could not be identified in ICC origin tumors immunologically (66). Apparently, GC is an efficient tool for differentiating HC tracing(16).

Biomarkers of hepatocytes commonly use GS, Arg-1,Hep-Par1and GPC-3. Bile duct cells biomarkers are CK7 and CK19. Biomarkers requiredistinguishing HC from ICC and separate different classes of HC as primary to metastatic.

HCC involved genes

Whole genome sequencing analysis of anHC related to hepatitis C viral infection were reported in 2011 for the first time to enter next-generation sequencing era (61). Sequencing researcheson HCC samples with different backgrounds have offered us novel insights. They have introducedviral host genomic interactions, gene mutations,epigenetic modifications and transcriptomic changes of HC (62-64). Frequently mutated genes as

CTNNB1(B-catenin),TERT promoter and Tp53 have been explored recently as HC genomic alterations (65). Wheeler et al. suggested multiple genomic platforms and they believed in the central role of Sonic hedgehog signaling in HC (66). HC cells utilize dysregulation of phosphorylation mechanism to acquire cancer properties, but classical genomic and proteomic analysis could not reflect these changes (8). HCC patients with Tuberous Sclerosis Complex (TSC) mutation have a significant increase in S6 protein phosphorylation due to the activation of mTOR kinase (67). Jiang et al. published a large-scale phosphoproteomics profiling study on early stages of HCC associated with hepatitis B virus. They stratified cohorts into three subtypes SI, SII and SIII tumors. Results demonstrated that TGF- β , HIF-1, Integrin and Rho GTPase pathways were up-regulated in SIII tumors with poor outcome after surgery and increased both AFP protein level and sterol O-acyltransferase 1 (SOAT1) expression (10). They concluded that SOAT1 is critical for maintaining cholesterol level to localize the trans-membrane receptors of HCC cells to growth and metastasis. Other researches in cholesterol maintenance revealed that loss of tumor suppressor factor p53 activates master transcription regulator of cholesterol synthesis pathway, and promotes maturation of sterol regulatory element binding protein 2 (SREBP2) for HCC development (68). However, liver cells with the lack of fatty acid synthase (FASN) utilize the cholesterol synthesis pathway, supporting c-MET oncogene mediated liver tumor formation through the regulation of SREBP2 (69). It seems that Cholesterol biosynthesis pathway is required for HCC development. In a proteomics study on hepatitis B leading to hepatocellular carcinoma, Gao et al. introduced the biomarkers of PYCR2 and ADH1A for metabolic programming in proteomic subgroups. They revealed CTNNB1 and TP53 mutation associated signaling and metabolic profiles with HC (70). PYCR2 is up-regulated in various types of cancer (71). ADH1A is involved in various xenobiotic substrates (72). Bioinformatics analysis of hepatocellular carcinoma biomarkers in patients revealed that FOXM1 was the most strongly connected gene among up-regulated genes in PPI network (73). Other researchers suggested FOMX1 elevation in many tumors such as intrahepatic cholangiocarcinoma, esophageal

adenocarcinoma, gastric cancer and HC (74-77). KIF4A gene expression couples with FOMX1 leads to excessive cell proliferation and promotes tumor development (77). Another study introduced FTCD as a core gene to distinguish early HC from benign tumors as a potential marker for early diagnosis of HC (78). Auto-immune hepatitis probability is 6 to 7 percent to induce HC (79). EPH2A mutations are frequent in IC and lymph node metastasis and angiogenesis was associated with the IC patients (80).

MicroRNA biomarkers(miR)

miRs are small RNA molecules usually containing 21-23 nucleotides which regulate gene expression through miR degradation or translational repression (81). They are considered as a useful biomarkers for the diagnosis of HC and IC (82). Investigation of miRs in one study revealed that miR-25, miR-375 and Let7f could be significantly expressed in HC cells compared to the control group. Also, miR-122, miR-21, miR-192, miR223, miR27a, miR26a and miR-801 are introduced as specific biomarkers of liver cancer in comparison with healthy samples of cirrhosis and hepatitis B (83). Another study revealed that miR-122 and miR-21 are better for distinguishing HC (84). Between the two miRs, the miR122 had better performance because research in mice, lacking the miR122 gene, has prone to HC along with expression of AFP and IGF2 (85). In another study, miR-122 is significantly low in HC patients compared to Hepatitis C and control patients while the expression of miR-224 was significantly higher (86). It should be noted that the presence of miR-122 and miR-224 is associated with AFP, alkaline phosphatase level and tumor size, and they can be considered as biomarkers for early detection of liver cancer (86). Moshiri et al. used plasma RNA sequencing and found as HC biomarkers miR-101, miR-1246 and miR-106b-3p in combination or individually (87). Hypoxia induced factors (HIF-1a and HIF-2a), miR-21 and miR-10b can be activated in acidic environment of HC to stimulate HC cells proliferation and migration (88).

Proteomic study in HC

Proteomic analysis as a powerful tool can uncover unidentified biomarkers in liver cancer with therapeutic potentials. Jiang et al. performed a large-scale proteomic profiling of early hepatocellular carcinoma associated with hepatitis B virus (8). They found that

S34 Assessment of liver cancer biomarkers

sterol O-acyltransferase 1 (SOAT1) expression is significantly high in liver cancer tumors the same as protein expression related to oncogenic pathways as integrin, Rho-GTBase, TGF- β and hIF-1. Moon et al. revealed that loss of tumor suppressor p53 could promote maturation of sterol regulating binding protein 2 (SRBP-2) in liver cancer cells development (68). Another study mentioned that absence of fatty acid synthase in liver cells alternatively activate cholesterol synthesis pathways and up-regulation of SREBP2 (69). Therefore, it seems that cholesterol synthesis pathways lead to liver cancer. Nevertheless, more proteomic investigations will introduce suitable biomarkers for HC and IC according to metabolic pathways of cancer cells proliferation and migration.

Signaling pathway biomarkers

These biomarkers pathways contribute to the appearance and development of liver cancer. There are several signaling pathways associated with liver cancer formation and progression such as wnt, p53 signaling pathway, and c-Met. Canonical Wnt/ β -catenin signaling pathway occurs in hepatocellular carcinoma through mutations in N terminal part of B-Catenin (89). Wnt signaling is active in most HCs (90). Combination of GPC-3 with Wnt leads to Wnt signaling pathway stimulation and localization of B-Catenin and liver carcinoma activation processes (48). Wnt presentation in tissues with hepatocellular carcinoma are reported in two classes named CTNNB1 and Wnt-TGF- β with different characteristics in liver carcinoma (91). Mutations in p53 signaling pathways may lead to HC. MDM2 is a transcriptional target and negative regulator of p53, and homeostasis between p53 and MDM2 as a feedback loop can control HC initiation and progression. With silencing p53 and over-expression of MDM2, hepatitis viruses can lost defense mechanisms of hepatocytes survival (92). c-Met signaling pathway is known with hepatocyte growth factor (HGF) and its receptor named mesenchymalepithelial transitional factor (c-Met). The pivot role of HGF-c-Met is in liver growth, regeneration and degeneration; however, c-Met inconsistent function may lead to the onset, proliferation and migration of HC. HGF-c-Met axis is a prognostic biomarker of HC (93). GPC-3 can control migration of HC cells via HGF-c-Met pathway and heparin sulfate chain mediated growthfactor (94).

Discussion

Biomarkers can be used for early detection of liver cancer. In this respect, following the occurrence of various complications of the disease, and according to the expression of different biomarkers, appropriate diagnostic and therapeutic methods can be applied. On the other hand, it should be noted that many of the introduced biomarkers are not efficient and cancer patients should not be tested, because of subsequent complications (95). Nevertheless, biomarkers still play an important role in the diagnosis and prognosis of liver cancer. Early diagnosis of liver cancer depends on biomarker sensitivity and specificity. Serum biomarkers such as AFP are used to diagnose liver cancer in high-risk patients with minimal invasiveness and rapid response. Combined use of biomarkers for early detection of liver cancer is prevalent. AFP, DPC and AFP-L3 biomarkers are used in combination every six months for liver cancer detection (96). Recent studies introduced novel biomarkers for accurate diagnosis and early treatment of liver cancer such as AFU, GP73 and OPN. Biomarkers such as GP73, GPC3, AKR1B10 seem to be promising but require more validation (97). They have no more privilege over than AFP as demonstrates for osteopontin biomarker (98). Heterogeneity in meta-analysis approaches to HCC management with ultrasonography and AFP in patients of 14 countries with different and distinct outcomes justifies early cancer detection requirements (99). Mic-RNA can be evaluated as a diagnostic or prognostic tool or therapeutic target for liver cancer. However, inconsistency of assessed molecules measured in plasma and serum revealed discrepancy observed in researches, but mir-21 and mic-122 are promising as they were not differentially expressed in utilizing RNA sequencing (84). Moshiri et al. introduced some additional mic-RNAs, perhaps with more potential accuracy (87), but those observations remain preliminary and more investigation is required because of the lack of reproducibility of the findings. The use of immunohistochemical methods and H&E staining confirms the diagnosis of liver cancer, with routine histochemical biomarkers such as CPC-3, HSP-70, Hep Par1, CK7 and Arg-1 (16).

Gene target therapies also indicated good curative influence. Genomics studies indicated positive CTNNB-1 & IDH potentials in HC & IC target therapies (100, 101) and gene target therapies can improve prognosis of liver cancer.

This study showed a variety of biomarkers related to different types of liver cancer. The new biomarkers will be put to clinical trials in the near future and open windows of hope for early detection and definitive treatment of liver cancer. Target drugs for some biomarkers may improve the survival rate of the liver cancer patients. Further studies on the signaling pathway in which biomarkers are involved will increase our knowledge of the molecular mechanism of the progression of liver cancer. Significant advances in technology are encouraging more researchers to use those advances to better identify cancer biomarkers to find a definitive and early treatment for the liver cancer.

Despite numerous studies and the introduction of numerous biomarkers, it seems that a specific biomarker that has the ability to detect liver cancer early along with AFP has not been introduced yet. Further clinical trials in different centers are required to specify the risk of liver cancer in various populations. Comprehensive data are needed to decide and select the appropriate biomarker, and future technological advances will help achieve this goal.

Conflict of interests

The authors declare that they have no conflict of interest.

References

- Villanueva A, Schwartz ME, Llovet JM. Liver cancer. In: Oh W, Chari A, Editors. Mount Sinai Expert Guides: Oncology. New York: John Wiley & Sons Ltd; 2019. P.89-100.
- Yang JD, Roberts LR. Hepatocellular carcinoma: a global view. *Nat Rev Gastroenterol Hepatol* 2010;7:448.
- Karagozian R, Derdák Z, Baffy G. Obesity-associated mechanisms of hepatocarcinogenesis. *Metabolism* 2014;63:607-17.
- Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008;359:378-90.
- Llovet JM, Hernandez-Gea V. Hepatocellular carcinoma: reasons for phase III failure and novel perspectives on trial design. *Clin Cancer Res* 2014;20:2072-9.
- Zucman-Rossi J, Villanueva A, Nault JC, Llovet JM. Genetic landscape and biomarkers of hepatocellular carcinoma. *Gastroenterology* 2015;149:1226-39.
- Torrecilla S, Sia D, Harrington AN, Zhang Z, Cabellos L, Cornella H, et al. Trunk mutational events present minimal intra-and inter-tumoral heterogeneity in hepatocellular carcinoma. *J Hepatol* 2017;67:1222-31.
- Chan LK, Ng IOL. Proteomic profiling in liver cancer: another new page. *Trans Gastroenterol Hepatol* 2019;4.
- Lee SC, Tan HT, Chung MCM. Prognostic biomarkers for prediction of recurrence of hepatocellular carcinoma: current status and future prospects. *World J Gastroenterol* 2014;20:3112.
- Jiang Y, Sun A, Zhao Y, Ying W, Sun H, Yang X, et al. Proteomics identifies new therapeutic targets of early-stage hepatocellular carcinoma. *Nature* 2019;567:257-61.
- Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018;68:394-424.
- Gera S, Ettl M, Acosta-Gonzalez G, Xu R. Clinical features, histology, and histogenesis of combined hepatocellular-cholangiocarcinoma. *World J Hepatol* 2017;9:300.
- Saffroy R, Pham P, Reffas M, Takka M, Lemoine A, Debuire B. New perspectives and strategy research biomarkers for hepatocellular carcinoma. *Clin Chem Lab Med* 2007;45:1169-79.
- Zhou YM, Yang JM, Li B, Yin ZF, Xu F, Wang B, et al. Clinicopathologic characteristics of intrahepatic cholangiocarcinoma in patients with positive serum α -fetoprotein. *World J Gastroenterol* 2008;14:2251.
- Yin X, Zhang BH, Qiu SJ, Ren ZG, Zhou J, Chen XH, et al. Combined hepatocellular carcinoma and cholangiocarcinoma: clinical features, treatment modalities, and prognosis. *Ann Surg Oncol* 2012;19:2869-76.
- Yamashita T, Forgues M, Wang W, Kim JW, Ye Q, Jia H, et al. EpCAM and α -fetoprotein expression defines novel prognostic subtypes of hepatocellular carcinoma. *Cancer Res* 2008;68:1451-61.
- Li R, Yang D, Tang CL, Cai P, Ma KS, Ding SY, et al. Combined hepatocellular carcinoma and cholangiocarcinoma (biphenotypic) tumors: clinical characteristics, imaging features of contrast-enhanced ultrasound and computed tomography. *BMC Cancer* 2016;16:158.
- Bertino G, Ardiri A, Malaguarnera M, Malaguarnera G, Bertino N, Calvagno GS, et al. Hepatocellular carcinoma serum markers. *Semin Oncol* 2012;39:410-33.

19. Tamura Y, Igarashi M, Kawai H, Suda T, Satomura S, Aoyagi Y. Clinical advantage of highly sensitive on-chip immunoassay for fucosylated fraction of alpha-fetoprotein in patients with hepatocellular carcinoma. *Dig Dis Sci* 2010;55:3576-83.
20. Choi J, Kim GA, Han S, Lee W, Chun S, Lim YS. Longitudinal assessment of three serum biomarkers to detect very early-stage hepatocellular carcinoma. *Hepatology* 2019;69:1983-94.
21. Hagiwara S, Kudo M, Kawasaki T, Nagashima M, Minami Y, Chung H, et al. Prognostic factors for portal venous invasion in patients with hepatocellular carcinoma. *J Gastroenterol* 2006;41:1214-9.
22. Hu B, Tian X, Sun J, Meng X. Evaluation of individual and combined applications of serum biomarkers for diagnosis of hepatocellular carcinoma: a meta-analysis. *Int J Mol Sci* 2013;14:23559-80.
23. Song P, Tobe RG, Inagaki Y, Kokudo N, Hasegawa K, Sugawara Y, et al. The management of hepatocellular carcinoma around the world: a comparison of guidelines from 2001 to 2011. *Liver Int* 2012;32:1053-63.
24. Zhang YS, Chu JH, Cui SX, Song ZY, Qu XJ. Des- γ -carboxy prothrombin (DCP) as a potential autologous growth factor for the development of hepatocellular carcinoma. *Cell Physiol Biochem* 2014;34:903-15.
25. Chen J, Wu G, Li Y. Evaluation of serum des-gamma-carboxy prothrombin for the diagnosis of hepatitis B virus-related hepatocellular carcinoma: a meta-analysis. *Dis Markers* 2018;2018: 8906023.
26. Masuzaki R, Karp SJ, Omata M. New serum markers of hepatocellular carcinoma. *Semin Oncol* 2012;39(4):434-9.
27. Wei C, Yang X, Liu N, Geng J, Tai Y, Sun Z, et al. Tumor microenvironment regulation by the endoplasmic reticulum stress transmission mediator Golgi protein 73 in mice. *Hepatology* 2019;70:851-70.
28. Liu Y, Zhang X, Zhou S, Shi J, Xu Y, He J, et al. Knockdown of Golgi phosphoprotein 73 blocks the trafficking of matrix metalloproteinase-2 in hepatocellular carcinoma cells and inhibits cell invasion. *J Cell Mol Med* 2019;23:2399-409.
29. Mao Y, Yang H, Xu H, Lu X, Sang X, Du S, et al. Golgi protein 73 (GOLPH2) is a valuable serum marker for hepatocellular carcinoma. *Gut* 2010;59:1687-93.
30. Mintz K, Waidely E, Zhou Y, Peng Z, Al-Youbi AO, Bashammakh AS, et al. Carbon dots and gold nanoparticles based immunoassay for detection of alpha-L-fucosidase. *Anal Chim Acta* 2018;1041:114-21.
31. El-Tayeh SF, Hussein TD, El-Houseini ME, Amer MA, El-Sherbini M, Elshemey WM. Serological biomarkers of hepatocellular carcinoma in Egyptian patients. *Dis Markers* 2012;32:255-63.
32. Waidely E, Al-Youbi AO, Bashammakh AS, El-Shahawi MS, Leblanc RM. Alpha-L-fucosidase immunoassay for early detection of hepatocellular carcinoma. *Anal Chem* 2017;89:9459-66.
33. Yamada T, Nakanishi Y, Okamura K, Tsuchikawa T, Nakamura T, Noji T, et al. Impact of serum carbohydrate antigen 19-9 level on prognosis and prediction of lymph node metastasis in patients with intrahepatic cholangiocarcinoma. *J Gastroenterol Hepatol* 2018;33:1626-33.
34. Wang Y, Li J, Xia Y, Gong R, Wang K, Yan Z, et al. Prognostic nomogram for intrahepatic cholangiocarcinoma after partial hepatectomy. *J Clin Oncol* 2013;31:1188-95.
35. Ying X, Zhao Y, Wang JL, Zhou X, Zhao J, He CC, et al. Serum anti-osteopontin autoantibody as a novel diagnostic and prognostic biomarker in patients with hepatocellular carcinoma. *Oncol Rep* 2014;32:1550-6.
36. Shang S, Plymoth A, Ge S, Feng Z, Rosen HR, Sangrajang S, et al. Identification of osteopontin as a novel marker for early hepatocellular carcinoma. *Hepatology* 2012;55:483-90.
37. Zhu Y, Yang J, Xu D, Gao XM, Zhang Z, Hsu JL, et al. Disruption of tumour-associated macrophage trafficking by the osteopontin-induced colony-stimulating factor-1 signalling sensitises hepatocellular carcinoma to anti-PD-L1 blockade. *Gut* 2019;68:1653-66.
38. Carr BI, Kanke F, Wise M, Satomura S. Clinical evaluation of Lens culinaris agglutinin-reactive α -fetoprotein and des- γ -carboxy prothrombin in histologically proven hepatocellular carcinoma in the United States. *Dig Dis Sci* 2007;52:776-82.
39. Wang C, Zhang Y, Guo K, Wang N, Jin H, Liu Y, et al. Heat shock proteins in hepatocellular carcinoma: Molecular mechanism and therapeutic potential. *Int J Cancer* 2016;138:1824-34.
40. Chuma M, Sakamoto M, Yamazaki K, Ohta T, Ohki M, Asaka M, et al. Expression profiling in multistage hepatocarcinogenesis: identification of HSP70 as a molecular marker of early hepatocellular carcinoma. *Hepatology* 2003;37:198-207.
41. Kang GH, Lee BS, Lee ES, Kim SH, Lee HY, Kang DY. Prognostic significance of p53, mTOR, c-Met, IGF-1R, and HSP70 overexpression after the resection of hepatocellular carcinoma. *Gut liver* 2014;8:79.
42. Shin E, Ryu HS, Kim SH, Jung H, Jang JJ, Lee K. The clinicopathological significance of heat shock protein 70 and glutamine synthetase expression in hepatocellular carcinoma. *J Hepato-biliary-pancreatic Sci* 2011;18:544-50.
43. Ibrahim TR, Abdel-Raouf SM. Immunohistochemical study of Glypican-3 and HepPar-1 in differentiating hepatocellular carcinoma from metastatic carcinomas in FNA of the liver. *Pathol Oncol Res* 2015;21:379-87.
44. SY Leong A, Sormunen RT, Tsui W, Liew C. Hep Par 1 and selected antibodies in the immunohistological distinction of hepatocellular carcinoma from

- cholangiocarcinoma, combined tumours and metastatic carcinoma. *Histopathology* 1998;33:318-24.
45. Kakar S, Gown AM, Goodman ZD, Ferrell LD. Best practices in diagnostic immunohistochemistry: hepatocellular carcinoma versus metastatic neoplasms. *Arch Pathol Lab Med* 2007;131:1648-54.
 46. Nishida T, Kataoka H. Glypican 3-targeted therapy in hepatocellular carcinoma. *Cancers* 2019;11:1339.
 47. Chen C, Huang X, Ying Z, Wu D, Yu Y, Wang X, et al. Can glypican-3 be a disease-specific biomarker? *Clin Trans Med* 2017;6:18.
 48. Shirakawa H, Kuronuma T, Nishimura Y, Hasebe T, Nakano M, Gotohda N, et al. Glypican-3 is a useful diagnostic marker for a component of hepatocellular carcinoma in human liver cancer. *Int J Oncol* 2009;34:649-56.
 49. Kolluri A, Ho M. The role of glypican-3 in regulating Wnt, YAP and hedgehog in liver cancer. *Front Oncol* 2019;9:708.
 50. Liu H, Li P, Zhai Y, Qu CF, Zhang LJ, Tan YF, et al. Diagnostic value of glypican-3 in serum and liver for primary hepatocellular carcinoma. *World J Gastroenterol* 2010;16:4410.
 51. Lagana SM, Moreira RK, Remotti HE, Bao F. Glutamine synthetase, heat shock protein-70, and glypican-3 in intrahepatic cholangiocarcinoma and tumors metastatic to liver. *Appl Immunohistochem Mol Morphol* 2013;21:254-7.
 52. Yan BC, Gong C, Song J, Krausz T, Tretiakova M, Hyjek E, et al. Arginase-1: a new immunohistochemical marker of hepatocytes and hepatocellular neoplasms. *Am J Surg Pathol* 2010;34:1147.
 53. Fujiwara M, Kwok S, Yano H, Pai RK. Arginase-1 is a more sensitive marker of hepatic differentiation than HepPar-1 and glypican-3 in fine-needle aspiration biopsies. *Cancer Cytopathol* 2012;120:230-7.
 54. Timek DT, Shi J, Liu H, Lin F. Arginase-1, HepPar-1, and Glypican-3 are the most effective panel of markers in distinguishing hepatocellular carcinoma from metastatic tumor on fine-needle aspiration specimens. *Am J Clin Pathol* 2012;138:203-10.
 55. Moll R, Divo M, Langbein L. The human keratins: biology and pathology. *Histochem Cell Biol* 2008;129:705.
 56. Ryu HS, Lee K, Shin E, Kim SH, Jing J, Jung HY, et al. Comparative analysis of immunohistochemical markers for differential diagnosis of hepatocellular carcinoma and cholangiocarcinoma. *Tumori Journal* 2012;98:478-84.
 57. Liu LZ, Yang LX, Zheng BH, Dong PP, Liu XY, Wang ZC, et al. CK7/CK19 index: a potential prognostic factor for postoperative intrahepatic cholangiocarcinoma patients. *J Surg Oncol* 2018;117:1531-9.
 58. Dal Bello B, Rosa L, Campanini N, Tinelli C, Viera FT, D'Ambrosio G, et al. Glutamine synthetase immunostaining correlates with pathologic features of hepatocellular carcinoma and better survival after radiofrequency thermal ablation. *Clin Cancer Res* 2010;16:2157-66.
 59. Nguyen TB, Roncalli M, Di Tommaso L, Kakar S. Combined use of heat-shock protein 70 and glutamine synthetase is useful in the distinction of typical hepatocellular adenoma from atypical hepatocellular neoplasms and well-differentiated hepatocellular carcinoma. *Mod Pathol* 2016;29:283-92.
 60. Totoki Y, Tatsuno K, Yamamoto S, Arai Y, Hosoda F, Ishikawa S, et al. High-resolution characterization of a hepatocellular carcinoma genome. *Nat Genet* 2011;43:464-9.
 61. Sung W-K, Zheng H, Li S, Chen R, Liu X, Li Y, et al. Genome-wide survey of recurrent HBV integration in hepatocellular carcinoma. *Nat Genet* 2012;44:765-9.
 62. Kan Z, Zheng H, Liu X, Li S, Barber TD, Gong Z, et al. Whole-genome sequencing identifies recurrent mutations in hepatocellular carcinoma. *Genome Res* 2013;23:1422-33.
 63. Cancer Genome Atlas Research Network. Electronic address: wheeler@bcm.edu; Cancer Genome Atlas Research Network. Comprehensive and Integrative Genomic Characterization of Hepatocellular Carcinoma. *Cell* 2017;169:1327-41.
 64. Schulze K, Imbeaud S, Letouzé E, Alexandrov LB, Calderaro J, Rebouissou S, et al. Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. *Nat Gen* 2015;47:505-11.
 65. Wheeler DA, Roberts LR, Network CGAR. Comprehensive and integrative genomic characterization of hepatocellular carcinoma. *Cell* 2017;169:1327.
 66. Ho DW, Chan LK, Chiu YT, Xu IM, Poon RT, Cheung TT, et al. TSC1/2 mutations define a molecular subset of HCC with aggressive behaviour and treatment implication. *Gut* 2017;66:1496-506.
 67. Moon S-H, Huang C-H, Houlihan SL, Regunath K, Freed-Pastor WA, Morris IV JP, et al. p53 represses the mevalonate pathway to mediate tumor suppression. *Cell* 2019;176:564-80.
 68. Che L, Chi W, Qiao Y, Zhang J, Song X, Liu Y, et al. Cholesterol biosynthesis supports the growth of hepatocarcinoma lesions depleted of fatty acid synthase in mice and humans. *Gut* 2020;69:177-86.
 69. Gao Q, Zhu H, Dong L, Shi W, Chen R, Song Z, et al. Integrated proteogenomic characterization of HBV-related hepatocellular carcinoma. *Cell* 2019;179:561-77.
 70. Ding J, Kuo ML, Su L, Xue L, Luh F, Zhang H, et al. Human mitochondrial pyrroline-5-carboxylate reductase 1 promotes invasiveness and impacts survival in breast cancers. *Carcinogenesis* 2017;38:519-31.
 71. Molotkov A, Deltour L, Foglio MH, Cuenca AE, Duester G. Distinct retinoid metabolic functions for

alcohol dehydrogenase genes Adh1 and Adh4 in protection against vitamin A toxicity or deficiency revealed in double null mutant mice. *J Biol Chem* 2002;277:13804-11.

72. Teng L, Wang K, Liu Y, Ma Y, Chen W, Bi L. Based on integrated bioinformatics analysis identification of biomarkers in hepatocellular carcinoma patients from different regions. *BioMed Res Int* 2019;2019.

73. Liu L, Wu J, Guo Y, Xie W, Chen B, Zhang Y, et al. Overexpression of FoxM1 predicts poor prognosis of intrahepatic cholangiocarcinoma. *Aging* 2018;10:4120.

74. Dibb M, Han N, Choudhury J, Hayes S, Valentine H, West C, et al. The FOXM1-PLK1 axis is commonly upregulated in oesophageal adenocarcinoma. *Br J Cancer* 2012;107:1766-75.

75. Zeng J, Wang L, Li Q, Li W, Björkholm M, Jia J, et al. FoxM1 is up-regulated in gastric cancer and its inhibition leads to cellular senescence, partially dependent on p27kip1. *J Pathol* 2009;218:419-27.

76. Hu G, Yan Z, Zhang C, Cheng M, Yan Y, Wang Y, et al. FOXM1 promotes hepatocellular carcinoma progression by regulating KIF4A expression. *J Exp Clin Cancer Res* 2019;38:188.

77. Seimiya M, Tomonaga T, Matsushita K, Sunaga M, Oh-ishi M, Kodera Y, et al. Identification of novel immunohistochemical tumor markers for primary hepatocellular carcinoma; clathrin heavy chain and formiminotransferase cyclodeaminase. *Hepatology* 2008;48:519-30.

78. Yeoman AD, Al-Chalabi T, Karani JB, Quaglia A, Devlin J, Mieli-Vergani G, et al. Evaluation of risk factors in the development of hepatocellular carcinoma in autoimmune hepatitis: implications for follow-up and screening. *Hepatology* 2008;48:863-70.

79. Sheng Y, Wei J, Zhang Y, Gao X, Wang Z, Yang J, et al. Mutated EPHA2 is a target for combating lymphatic metastasis in intrahepatic cholangiocarcinoma. *Int J Cancer* 2019;144:2440-52.

80. Singh G, Yoshida EM, Rathi S, Marquez V, Kim P, Erb SR, et al. Biomarkers for hepatocellular cancer. *World J Hepatol* 2020;12:558.

81. Huang W. MicroRNAs: biomarkers, diagnostics, and therapeutics. In: Huang J, Borchert GM, Dou D, Huan J, Lan W, Tan M, et al, Editors. *Bioinformatics in MicroRNA Research*. Totowa, New Jersey, United States: Humana Press; 2017.

82. Zhou J, Yu L, Gao X, Hu J, Wang J, Dai Z, et al. Plasma microRNA panel to diagnose hepatitis B virus-related hepatocellular carcinoma. *J Clin Oncol* 2011;29:4781-8.

83. Huang JT, Liu SM, Ma H, Yang Y, Zhang X, Sun H, et al. Systematic review and meta-analysis: Circulating miRNAs for diagnosis of hepatocellular carcinoma. *J Cell Physiol* 2016;231:328-35.

84. Tsai WC, Hsu SD, Hsu CS, Lai TC, Chen SJ, Shen R, et al. MicroRNA-122 plays a critical role in liver

homeostasis and hepatocarcinogenesis. *J Clin Invest* 2012;122:2884-97.

85. Amr KS, Atia HAE, Elbnhawy RAE, Ezzat WM. Early diagnostic evaluation of miR-122 and miR-224 as biomarkers for hepatocellular carcinoma. *Gen Dis* 2017;4:215-21.

86. Moshiri F, Salvi A, Gramantieri L, Sangiovanni A, Guerriero P, De Petro G, et al. Circulating miR-106b-3p, miR-101-3p and miR-1246 as diagnostic biomarkers of hepatocellular carcinoma. *Oncotarget* 2018;9:15350.

87. Tian XP, Wang CY, Jin XH, Li M, Wang FW, Huang WJ, et al. Acidic microenvironment up-regulates exosomal miR-21 and miR-10b in early-stage hepatocellular carcinoma to promote cancer cell proliferation and metastasis. *Theranostics* 2019;9:1965.

88. Takigawa Y, Brown A. Wnt signaling in liver cancer. *Curr Drug Targ* 2008;9:1013-24.

89. Armengol C, Cairo S, Fabre M, Buendia M. Wnt signaling and hepatocarcinogenesis: the hepatoblastoma model. *Int J Biochem Cell Biol* 2011;43:265-70.

90. Austinat M, Dunsch R, Wittekind C, Tannapfel A, Gebhardt R, Gaunitz F. Correlation between β -catenin mutations and expression of Wnt-signaling target genes in hepatocellular carcinoma. *Mol Cancer* 2008;7:1-9.

91. Cao H, Chen X, Wang Z, Wang L, Xia Q, Zhang W. The role of MDM2-p53 axis dysfunction in the hepatocellular carcinoma transformation. *Cell Death Discov* 2020;6:1-14.

92. García-Vilas JA, Medina MÁ. Updates on the hepatocyte growth factor/c-Met axis in hepatocellular carcinoma and its therapeutic implications. *World J Gastroenterol* 2018;24:3695.

93. Gao W, Kim H, Ho M. Human monoclonal antibody targeting the heparan sulfate chains of glypican-3 inhibits HGF-mediated migration and motility of hepatocellular carcinoma cells. *PloS one* 2015;10:e0137664.

94. Sherman M. How to improve HCC surveillance outcomes. *JHEP Rep* 2019;1:460-7.

95. Tzartzeva K, Singal AG. Testing for AFP in combination with ultrasound improves early liver cancer detection. *Expert Rev Gastroenterol Hepatol* 2018;12:947-49.

96. Han C, Gao L, Zhao L, Sheng Q, Zhang C, An Z, et al. Immunohistochemistry detects increased expression of aldo-keto reductase family 1 member b10 (AKR1B10) in early-stage hepatocellular carcinoma. *Int Med J Exp Clin Res* 2018;24:7414.

97. Wan HG, Xu H, Gu YM, Wang H, Xu W, Zu MH. Comparison osteopontin vs AFP for the diagnosis of HCC: a meta-analysis. *Clin Res Hepatol Gastroenterol* 2014;38:706-14.

98. Park JW, Chen M, Colombo M, Roberts LR, Schwartz M, Chen PJ, et al. Global patterns of

hepatocellular carcinoma management from diagnosis to death: the BRIDGE Study. *Liver Int* 2015;35:2155-66.

99. Gao YX, Yang TW, Yin JM, Yang PX, Kou BX, Chai MY, et al. Progress and prospects of biomarkers in primary liver cancer. *Int J Oncol* 2020;57:54-66.

100. Calderaro J, Couchy G, Imbeaud S, Amaddeo G, Letouzé E, Blanc JF, et al. Histological subtypes of

hepatocellular carcinoma are related to gene mutations and molecular tumour classification. *J Hepatol* 2017;67:727-38.

101. Wang P, Dong Q, Zhang C, Kuan PF, Liu Y, Jeck WR, et al. Mutations in isocitrate dehydrogenase 1 and 2 occur frequently in intrahepatic cholangiocarcinomas and share hypermethylation targets with glioblastomas. *Oncogene* 2013;32:3091-100.