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REVIEW

Biomarkers for hepatocellular carcinoma: diagnostic and therapeutic utility

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Abstract: Because of the high prevalence and associated-mortality of hepatocellular carcinoma (HCC), early diagnosis of the disease is vital for patient survival. In this regard, tumor size is one of the two main prognostic factors for surgical resection, which constitutes the only curative treatment for HCC along with liver transplantation. However, techniques for HCC surveillance and diagnosis that are currently used in clinical practice have certain limitations that may be inherent to the tumor development. Thus, it is important to continue efforts in the search for biomarkers that increase diagnostic accuracy for HCC. In this review, we focus on different biological sources of candidate biomarkers for HCC diagnosis. Although those biomarkers identified from biological samples obtained by noninvasive methods have greater diagnostic value, we have also considered those obtained from liver tissue because of their potential therapeutic value. To date, sorafenib is the only US Food and Drug Administration-approved antineoplastic for HCC. However, this therapeutic agent shows very low tumor response rates and frequently causes acquired resistance in HCC patients. We discuss the use of HCC biomarkers as therapeutic targets themselves, or as targets to increase sensitivity to sorafenib treatment.

Keywords: diagnosis, sorafenib, therapy

Introduction

Hepatocellular carcinoma (HCC) represents more than 90% of primary liver cancer, which is the fifth most prevalent cancer in the word and the third leading cause of cancer-related mortality.[1] Unfortunately, HCC development is asymptomatic at early stages of the disease when current curative therapies are available.[2,3] Tests for HCC screening include serological and imaging examinations. Ultrasonography is the most widely used imaging test for screening because of its diagnostic accuracy, noninvasiveness, good acceptance by patients, and moderate cost. However, this technique is highly dependent on the operator's experience. In addition, because HCC develops on a cirrhotic background in up to 90% of cases, fibrosis septa and regenerative nodules present in liver cirrhosis may hinder the identification of small tumors by ultrasonography. Among serological biomarkers, alpha-fetoprotein (AFP) is the most extended serum marker for HCC screening, but it is not routinely used by clinicians due to its insufficient sensitivity and specificity.[4] Thus, biological markers for the early diagnosis of HCC are urgently needed to improve patient survival.

A biomarker is "any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease".[5] It must satisfy the following conditions: achieve high accuracy, be obtained from easily accessible biological samples and have a suitable cost-effectiveness. For these reasons,

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although biomarkers can be obtained from many different sources, those derived from hardly accessible tissue samples are avoided. Furthermore, tissue samples obtained by invasive procedures (biopsy) may result in needle tract seeding. However, biomarkers from tumor tissues can provide direct biological information about the process occurring during tumor development, and may help in identifying potential targets for therapeutic interventions. During the carcinogenesis process, normal cells acquire cancer hallmarks that allow them to become tumorigenic. Linking the hallmarks of HCC with biological alterations has helped to identify new biomarkers of liver cancer and novel targets for HCC treatment. Thus, only biomarkers that directly contribute to tumor growth or invasiveness may be targeted for therapy. Although a cancer biomarker may not be necessarily involved in the development of the disease, it is likely that a biomarker for early diagnosis of cancer is related to the tumor process.

In addition to their diagnostic capacity, biomarkers are commonly used to predict the patient response to a treatment. Thus, patients can be classified as good or poor responders prior to drug administration, which leads to savings in time and side-effects for patients, as well as savings in money for society. This is what the term "personalized medicine" refers to.

In this review, we focused on biomarkers for HCC with diagnostic or therapeutic utility that have been identified at different sources. By its definition, a biomarker can be any biological molecule, so the following can be considered as biomarker sources: genome, epigenome, transcriptome, proteome and metabolome. The development of new "omics" technologies in the last decade has allowed researchers to analyze a large amount of data in search of new reliable biomarkers for HCC.

Biomarker sources for HCC diagnosis

Genome

It is well known that carcinogenesis, the process by which a normal cell becomes a tumor cell, occurs with the accumulation of genetic alterations in proto-oncogenes and tumor suppressor genes involved in cell growth, metabolism, proliferation, survival, apoptosis and adhesion.[6] In addition to studying somatic mutations accumulated in the genomic DNA, the analysis of copy number alterations[7] and other genetics disturbances, including large chromosomal amplification, translocation, deletion or small fraction loss, is important to identify target genes related to HCC.[8] Some of these HCC genes have shown their usefulness in clinical applications and therapeutic interventions.

In HCC, *TP53* (tumor protein p53) and *CTNNB1* (b-catenin)[9] are the two most-frequently mutated genes. The p53 protein is a crucial regulator of the cell cycle and functions as a tumor suppressor, preventing cancer. The b-catenin is a dual function protein that participates in regulating cell–cell adhesion and acts as an intracellular signal transducer in the Wnt signaling pathway, related to cell proliferation and cell migration. Concerning the use of these two genes as biomarkers of HCC, it has been shown that tumors characterized by chromosomal instability are related to more p53 mutation and less b-catenin mutation.[10,11] While p53 mutation correlates with aggressive HCC and poor prognosis, mutation of b-catenin is associated with less tumor aggression and more favorable prognosis.[12] In addition to these two genes, moderate mutation frequencies have been identified in several genes from multiple HCC cohorts, suggesting that aberrant pathways involved in cell cycle regulation, oxidative stress, chromatin remodeling and oncogenic signaling play critical roles in the process of HCC tumorigenesis.[7] Recently, Zhang et al identified 113 pathways significantly mutated in 207 samples of human HCC by pathway and network analysis.[13] Of them, the five most-frequently mutated pathways were those related to proliferation and apoptosis, tumor microenvironment, neural signaling, metabolism and circadian rhythm. In addition, they identified different key genes and pathways in which the mutations were associated with clinical features such as metastasis or survival.

Apart from genetic mutations, which cause dysfunction of gene products, numerous genetic polymorphisms have been associated with HCC susceptibility, without affecting the protein function.[8] Recently, polymorphisms affecting genes such as insulin-like growth factor-2 (*IGF-2*), insulin-like growth factor-2 receptor (*IGF-2R*), insulin receptor substrate-2 (*IRS-2*)[14] or activating transcription factor-6 (*ATF6*)[15] have been associated with the risk of HCC development. In addition to the genetic alterations described above, mutations and functional polymorphisms in HCC susceptibility genes involved in the chromatin remodeling or the epigenetic process have also been described.[16,17]

Epigenome

Hepatocarcinogenesis is a complex and multistep process involving genetic and epigenetic events.[18] The term epigenetic refers to changes in gene expression and cellular phenotype without affecting DNA sequence, which can be inherited or not.[19] The epigenome consists of the set of such chemical changes, and includes the histone modifications, changes in DNA (CpG) methylation and RNA-mediated gene silencing.

In addition, there is substantial functional crosstalk between these epigenetic elements, which combines to determine cell phenotype.[20] Epigenetic alterations result in changes in chromatin organization and gene expression, and can affect the cellular transcriptome more extensively than genetic alterations. Because the prevalence of HCC varies markedly among different geographic regions, it has been suggested that multiple genetic and environmental factors may interact during disease progression.[8] Thus, the epigenome can be considered as a bridge between the static genome and the highly dynamic environment, which can be altered by different conditions including cancer. In fact, the epigenome is one of the currently active topics in cancer research. Regarding HCC, typical epigenetic alterations include posttranslational modification of histones, changes in DNA methylation and abnormal expression of non-coding RNAs.

Histone modification and HCC

The posttranslational modification of histones, which package the DNA into chromatin, is an epigenetic change that affects chromatin condensation, DNA accessibility and transcriptional activity. In fact, the transcriptional activity of a gene is determined by the histone "code"; that is, the cumulative influences of multiple histone modifications that are enzymatically regulated.[20] This epigenetic alteration has been related to gene expression silencing occurring in cancer. In particular, histone modifications influence the initiation and progression of liver cancer[21] in a process characterized by dysfunction of histone-modifying enzymes. Thus, histone modifying genes, such as histone methyltransferases *EZH2, G9a* and *SUV39HZ*, and histone phosphorylation proteins, such as Aurora kinases (ARKs) ARK1 and ARK2, are frequently overexpressed in HCC tissue[22,23] and can be associated with poor patient prognosis[24–27] and tumor invasion.[28]

DNA methylation and HCC

The analysis of the methylation profiles has revealed that an aberrant methylation is a frequent event in HCC tissues. It could help researchers to distinguish between tumor and nontumor adjacent tissues, or between cirrhotic liver and HCC.[29] The two most common forms of aberrant methylation are global hypomethylation and site-specific hypermethylation. While the former induces chromosomal and genomic instability, regional hypermethylation is usually related to the silencing of tumor suppressor genes. Epigenetic biomarkers based on promoter hypermethylation that have been developed as potential diagnostic tools for HCC are summarized by Puszyk et al.[30] As potential blood biomarker, DNA methylation has also been associated with HCC. Thus, among many others, the tumor suppressor p16 (*CDKN2A*) is one of the most reported genes whose hypermethylation has been not only described in HCC tissues but also in blood samples from HCC patients.[31]

Non-coding RNAs and HCC

MicroRNAs (miRNAs) are a class of small, non-coding RNAs that act as regulators of gene expression at posttranscriptional level. Thus, a mature miRNA pairs its complementary mRNA and regulates its stability or translation. In HCC, deregulated expression of miRNAs has been suggested to be involved in the development and progression of the disease. MiRNAs are able to regulate cell proliferation and apoptosis and participate in cell migration and invasion by promoting or inhibiting these pathways.[8] As biomarkers of HCC, it has been shown that miRNAs are differentially expressed between tumor and adjacent nontumor liver tissue,[32,33] and they have potential diagnostic value as tissue-based markers.[34] Thus, the combination of multiple miRNAs has been proposed to significantly improve the accuracy in diagnosis and prognosis of HCC. Moreover, the major value of these biomarkers comes from their reported stable expression in serum and urine samples, which makes them potential minimally invasive diagnostic markers for HCC.[33,35] miR-21 is a central oncomiR that is overexpressed in plasma samples from HCC patients when compared with chronic hepatitis patients and healthy individuals. In addition, high levels of miR-21 have been associated with migration and invasion of HCC cells in vitro.[36] Despite its high sensitivity as a biomarker for HCC, miR-21 is overexpressed in many types of cancers and, therefore, increased plasma miR-21 levels should not mean presence of HCC. However, miR-21 shows a diagnostic value higher than AFP, which is significantly stronger when the two plasma markers are combined.[37] In addition to its promising role as a biomarker for HCC, miR-21 has been proposed as a potentially suitable tissue marker for the prediction of the clinical response to therapy in advanced HCC.[38]

Transcriptome

Continuing with the flow of biological information, genetic and epigenetic alterations that occur and accumulate during carcinogenesis involve inevitable changes in the expression profile of transcripts. Use of gene-expression signatures is a powerful tool that has been successfully used for the diagnosis of liver cancer. Kaposi-Novak et al showed that transformation of dysplastic nodules into early HCC was marked by pronounced transcriptomic alterations involving different cluster

genes. Particularly, the activation of the MYC transcription signature was related to the malignant conversion of preneoplastic liver lesions and it was suggested as a useful tool for the early diagnosis of liver cancer.[39] More recently, a Notch gene signature was generated from a murine model where activation of Notch signaling was associated with HCC development. This gene-expression signature predicted activation of Notch in more than 30% of human HCC associated with the hepatitis C virus injection.[40] By integrative transcriptome analysis, Hoshida et al designed a global classification for HCC that defines three major molecular subclasses of HCC. This classification correlates with histologic and clinical features of the tumor, and could be relevant for diagnosis and therapeutic intervention.[41] Furthermore, the combination of the differential gene-expression profile and topological features from the analysis of human protein interaction network has allowed for the enhancement of the diagnostic performance of the HCC classifier.[42]

Proteome

HCC development and progression are associated with proteomic changes as a consequence of protein secretion by the tumor cells or by other organs in response to the presence of cancer. Therefore, the blood composition of patients may reflect such changes when it is analysed by proteomics. Serum or plasma protein markers are the most applicable for routine clinical analysis because the tests are normally noninvasive, require a very low quantity of sample, have low dependence on the operator expertise, are low cost, have high reproducibility and the samples need no pretreatment. In addition, as occurs with the epigenome, the proteome defines what is happening at the moment. For these reasons, serum/plasma proteome has been and continues to be extensively investigated as a source of cancer biomarkers.

AFP is a glycoprotein produced by the fetal liver and yolk sac during pregnancy. Although serum AFP levels may be elevated initially in the early stages of HCC and it has been used as a tumor marker of HCC for a long time, an AFP increase has also been recognized in the presence of acute and chronic hepatitis or cirrhosis related to hepatitis C virus infection.[43] Thus, an AFP elevation could be more indicative of HCC in noninfected patients. Different studies have shown that the fucosylated fraction of AFP (AFP-L3) may be a more useful marker than total AFP.[44,45] Furthermore, the highly sensitive AFP-L3 has been proposed as a candidate tumor marker for HCC in patients with chronic liver disease and low serum AFP concentration.[46] However, because few studies have validated these results in independent cohorts of human samples, AFP remains the most widely used serum biomarker for HCC. Despite this, the suboptimal diagnosis accuracy of AFP in detecting early HCC[47] led to the American Association for the Study of Liver Diseases (AASLD) Practice Guidelines Committee to recommend that ultrasound alone (without AFP) be used for HCC surveillance.[1] Therefore, reliable biomarkers to complement ultrasound are needed.

Des-gamma-carboxy prothrombin (DCP), or protein induced by vitamin K absence or antagonist II (PIVKA-II), is an abnormal molecule that is generated as a result of an acquired defect in the posttranslational carboxylation of the prothrombin precursor in malignant cells. DCP is a promising serum marker that has been extensively studied and used in the diagnosis and surveillance of HCC in Japan. However, it does not seem to offer substantial advantages with respect to AFP in the early detection of HCC in patients with advanced hepatitis C, even when combined.[4] By contrast, DCP was the most useful predisposing clinical parameter for the development of portal venous invasion (an important survival prognostic factor for HCC and the largest independent risk factor for cancer recurrence) in a study that included 227 patients with HCC.[48]

Dickkopf-1 (DKK1) is a secretory antagonist of the canonical Wnt signaling pathway, which has also been suggested as a potential diagnostic and prognostic biomarker for HCC[49] in combination with AFP.[50] Thus, DKK1 is overexpressed in HCC tissue and in serum of hepatitis B virus (HBV)-infected HCC patients, where it shows high sensitivity and specificity for HCC. In addition, serum level decrease of DKK1 after surgery indicates that this protein may be a useful surveillance marker to assess the therapeutic response of HCC patients. Although the expression pattern of DKK1 in serum seems to be not specific for HCC,[51,52] its predictive ability for the diagnosis of HCC needs to be further investigated.

Osteopontin (OPN) is a glycol-phosphoprotein cytokine physiologically expressed by different cells and tissues, which binds alpha V integrins and CD44 families of receptors, and participates in multiple processes such as cell adhesion, chemotaxis regulation of immune functions, or vascular remodeling. In cancer, overexpression of OPN has been widely documented.[53] High expression of OPN in tumor tissue or plasma samples from HCC patients has been associated with metastasis of HBV-positive patients[54] or HCC recurrence after surgery,[55] respectively. Similarly, increased plasma level of OPN has been suggested as a promising biomarker[56] at early stages of the liver disease.[57] All these data are consistent with the critical role of the protein for tumor growth of

human HCC.[58] Although OPN shows better sensitivity than AFP in comparing cirrhosis and HCC, the best performance has been obtained by combining OPN and AFP.

Glypican-3 (GPC3) is a cell-surface heparan sulfate proteoglycan that is secreted into the plasma. GPC3 is overexpressed in tissue[59] and serum samples from HCC patients, while it remains undetectable or barely detectable in healthy individuals and in patients with hepatitis or hepatitis plus cirrhosis.[60] These data, along with the finding that serum GPC3 levels are more frequently elevated in small HCCs than those of AFP, indicate that GPC3 could be a highly specific marker for early diagnosis of HCC.[60,61] Despite this, the simultaneous use of GPC3 and AFP has shown to increase the sensitivity of the serum test for HCC detection without compromising specificity. As a tissue marker, GPC3 expression is also associated with poor prognosis of primary HCC.[62,63] In addition, its combination with two other candidate tissue markers for HCC such as heat shock protein 70 (Hsp70) and glutamine synthetase (GS) has been proven to be useful in the differential diagnosis of dysplastic precancerous nodules and HCC.[64] However, this combination only slightly increases the diagnostic accuracy in an expert setting.[65]

The serine protease inhibitor squamous cell carcinoma antigen (SCCA) is physiologically found in the spinous and granular layers of normal squamous epithelium, but is typically overexpressed in neoplastic cells including liver cancer cells.[66,67] In this regard, circulating SCCA–IgM immune complexes have been described as novel biomarkers for HCC.[68] Moreover, progressive increase of SCCA–IgM in cirrhotic patients has been associated with development of HCC and has been suggested as a surveillance test.[69]

Although there are many other proteins that have been proposed as candidate biomarkers of HCC, only a very small number of them have been clinically validated. Again, the simultaneous measurement of several candidate biomarkers has been proven to offer advantages over detection of a unique biomarker. Serum proteomic signature has been pointed out to be an interesting tool for HCC diagnosis in high-risk cirrhotic patients, with better diagnostic accuracy than AFP.[70,71]

Metabolome

The metabolome is defined as the metabolic status of a biological system, and metabolomics is the tool to study it. As has occurred with other omics, metabolomics has been applied to discover biomarkers for disease diagnosis.

HCC development from cirrhosis induces a number of cellular metabolic alterations that can be detected as changes in serum concentration of metabolites mainly involved in the biosynthesis of bile acids and the metabolism of long-chain carnitines, small peptides, phospholipids or sphingolipids.[72–75] In addition, metabolomic profiling has been suggested not only as a promising tool to identify biomarkers for early diagnosis of HCC in high-risk patients, but also to monitor the progression of HCC.[73]

Gut homeostasis

As occurs with epigenome, intestinal microflora can be altered in response to a highly variable environment. Thus, disruption of gut homeostasis has been associated with different human diseases. Regarding the liver, it has been shown that obesity induction[76] and exposure to a liver carcinogen[77] are associated with the imbalance of the gut microflora and the development of HCC. Thus, the analysis of the intestinal microflora might be interesting for HCC diagnosis in high-risk patients.

Circulating tumor cells

The clinical application of circulating tumor cells (CTCs) in peripheral blood has been suggested as a viable alternative to liver cancer diagnosis and early metastasis prediction. Thus, liver cancer cells enter the circulation at an early tumor stage and CTC level in the blood is directly correlated to tumor size, stage, and metastasis after cancer surgery.[78] In addition, CTCs may serve as a real-time parameter for monitoring treatment response for HCC.[79] A recently established platform for CTC detection solves questions about sensitivity, specificity and reproducibility, and could be clinically useful in HCC diagnosis and monitoring.[80]

Biomarkers with therapeutic utility in HCC

As has been shown, the etiology of HCC is very complex and involves a large number of genetic and epigenetic alterations as well as the consequent disruption of numerous signaling pathways, which can be candidate therapeutic targets for HCC. Thus, although tumor tissue-derived biomarkers are not practical for HCC screening tasks, they have helped in the identification of new cellular targets in liver cancer by using different technical approaches. Disturbed signaling pathways related to HCC include the Wnt/b-catenin, Ras/Raf/MAPK, PI3K/AKT/mTOR, HGF/c-MET, IGF, VEGF and PDGF pathways. Currently, there are chemical agents targeting all these pathways, which are available or under investigation. Among them, points of therapeutic intervention along the Wnt/b-catenin pathway have been identified.[41,81,82]

Sorafenib is a multikinase inhibitor with reported activity against tyrosine protein kinases, such as VEGFR, PDGFR and c-Kit receptors, and serine/threonine kinases, such as C-Raf and B-Raf. Sorafenib is the first and only drug approved to treat advanced HCC, which has shown a statistically significant increase in median overall survival compared to placebo.[83,84] However, the tumor response rates are very low, and patients frequently develop acquired resistance to the therapeutic agent. For this reason, the identification of biomarkers for sorafenib sensitivity is essential to identify candidate patients with greater options to benefit from therapy. In addition, it will allow that resistant patients may benefit from sorafenib treatment. The largest study on this topic to date was based on the pivotal Phase III study of sorafenib (the SHARP study) and did not find any plasma marker that could predict the efficacy of sorafenib. However, the angiogenesis biomarkers Ang2 and VEGF were independent predictors of survival in patients with advanced HCC.[85] Other studies have described potential biomarkers for response to sorafenib such as early AFP response and serum IGF-1 level at baseline.[86] Activation of PI3K/Akt signaling[87] or epithelial–mesenchymal transition,[88] and overexpression of EGFR and HER3,[89] hypoxia-inducible factor[90] or nucleophosmin,[91] may confer HCC cell resistance to sorafenib. In addition, a number of histone methyltransferases (HMTs) have been involved in histone methylation and the epigenetic regulation of the expression of genes related to sorafenib resistance of HCC cells. Thus, whereas the expression of HMTs such as EZH2[92] or ASH1L, C17ORF49 and SETD4[93] is associated with sorafenib resistance, its inhibition promotes sorafenib-induced HCC cell growth arrest and apoptosis, and it has been suggested as potential therapeutic combination for sorafenib treatment in HCC.

In a similar way, the inhibition of protein activities related to the epigenetic modulation of histones and the expression regulation of miRNAs have been reported as useful therapeutic strategies against HCC. Thus, the use of the pan-ARK inhibitor VE-465 or the ARK2 selective inhibitor AZD1152-HQPA shows anticancer effects in preclinical models of human HCC[94] and in HCC cells,[28] respectively. Using histone deacetylase inhibitors suppresses self-renewal and induced differentiation of liver cancer stem cells, and has been suggested as a candidate strategy in liver cancer therapy.[27] On the other hand, downregulation of miR-21 has been noted as a potential target for HCC therapy since antisense miR-21 inhibitor increases the sensitivity of HCC cell lines to antitumor treatments.[38] In addition, in vitro silencing of miR-21 has been related to reduction in the migration and invasion of HCC cells with stem cell-like properties.[36]

The inhibition of expression or activity of key proteins that have a role in the carcinogenesis process is another therapeutic strategy that is being developed to prevent and suppress HCC. Among the different ways to inhibit protein expression, immunotherapy by using monoclonal antibodies has been identified as a very interesting tool. Anti-DKK1 antibody has shown its anticancer activity by inhibiting the invasive activity and the growth of cancer cell lines with high expression of DKK1, and by suppressing the growth of engrafted tumors in vivo.[52] In addition to being a highly specific marker of HCC that is not expressed by normal or cirrhotic liver, GPC3 promotes the in vitro and in vivo growth of HCC by stimulating the Wnt signaling pathway.[95] Because of this, GPC3 has been chosen as a promising target for HCC immunotherapy.[61] Anti-GPC3 antibodies and inoculation of GPC3 peptide-reactive cytotoxic T-lymphocytes are currently under investigation in different stages of preclinical or clinical development as novel targeted treatments for advanced HCC.[61,96] TM4SF5 is a member of the tetraspanins or transmembrane 4 superfamily that is highly expressed in HCC and induces uncontrolled growth of tumor cells via the loss of contact inhibition.[97] As a therapeutic target, the inhibition of TM4SF5 by the synthetic inhibitor 4′-(p-toluenesulfonyl-amido)-4-hydroxychalcone (TSAHC) suppresses HCC growth and metastasis in vitro and in nude mice.[98] Furthermore, the injection of monoclonal antibodies specific to an epitope of the human TM4SF5 (hTM4SF5R2-3) suppresses the growth of HCC cell lines in vitro[99,100] and in vivo.[101] In addition, the immunization with the hTM4SF5R2-3 epitope has proven to be a useful peptide vaccine to prevent tumor formation and tumor growth in vivo.[100] The accumulation of immune suppressive cells is a key mechanism for tumor progression that has been associated with HCC. The combining of intratumoral injection of secondary lymphoid-tissue chemokine (SLC) and depletion of CD25+ regulatory T-cells by anti-CD25 monoclonal antibodies has also emerged as an effective treatment for HCC in animal models.[102] OPN is another candidate biomarker that has been proposed as a promising target for therapy against HCC. Because OPN is an extracellular cytokine ligand, its interaction with receptors is more readily accessible to pharmaceuticals than intracellular targets. Gene or protein expression of OPN can be suppressed by different modes.[103,104] Among them, the expression induction of the 30 kDa Tat-interacting protein (TIP30)[105] and the use of short hairpin RNA,[106] antisense oligonucleotides[107] or specific antibodies targeting OPN[54] have been reported to effectively block HCC cell invasion in vitro and inhibit metastasis of HCC cells in nude mice. In addition to the direct

action on OPN, the suppression of the main receptors for the cytokine on tumors cells by cytotoxic and immunotherapeutic approaches has also been identified as a potential target for therapeutic intervention.[103,104]

Due to the relationship between the number of preoperative HCC CTCs and the risk for postoperative recurrence and metastasis, the use of preoperative neoadjuvant therapy to eliminate CTCs has been suggested as another hopeful approach against HCC.[79] In this regard, clinical studies evaluating the use of sorafenib and other preoperative neoadjuvant therapies targeting HCC CTCs have been required.[108]

Finally, the modulation of gut microflora by probiotics in an animal model of chemically induced HCC has been proposed as a plausible therapeutic alternative to treat or prevent hepatocarcinogenesis. This is consistent with the view that HCC development is closely related to the status of gut homeostasis. Thus, high-doses of probiotics could restore or supply essential bacterial strains that have been disrupted during the liver cancer development.[77]

Conclusion

The search for HCC biomarkers is imperative because curative options for the disease depend highly on early diagnosis. The development of new-generation tools applied to molecular biology has allowed for the identification of HCC markers that are able to overcome the current predictive capability of AFP. However, most of them need to be validated. Among others, nucleic acids, protein or metabolites have been proposed. In many cases, the combination between them appears to have a better diagnosis/prognosis value. Thus, obtaining specific signatures or expression profiles, developed from the analysis of hundreds or thousands of data, seems to offer great advantages over the use of a single biomarker. This is logical since the molecular basis of HCC is highly heterogeneous and involves a large number of gene products that participate in different signaling pathways and processes. However, the numerous research studies in this regard are based on the combination of biological molecules of the same chemical nature. Maybe, the simultaneous detection of biomarkers of different chemical nature, which were previously identified and validated, could add value in the diagnosis of HCC. After all, HCC mainly arises from exposure to external environmental factors that are associated with gut homeostasis and epigenetic changes, which in turn result in changes in gene expression, protein expression and metabolism.

Although the use of a newly proposed marker for HCC has not been extended in clinical practice to date, the identification of candidate biomarkers has proven valuable in the development of new therapeutic strategies. Currently, there are a number of drugs in development that target different molecules that are deregulated in HCC and participate in hepatocarcinogenesis.

In conclusion, the massive search for biomarkers or molecular signatures related to HCC development has led to a greater and better knowledge of the disease, making it possible to increase the options for therapeutic intervention and therefore success in the fight against cancer.

Disclosure

The authors report no conflicts of interest in this work.

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