

Review

An overview of hepatocellular carcinoma study by omics-based methods

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Hepatocellular carcinoma (HCC) is one of the most deadly malignancies worldwide. Scientists have been studying the molecular mechanism of HCC for years, but the understanding of it remains incomplete and scattered across the literature at different molecular levels. Chromosomal aberrations, epigenetic abnormality and changes of gene expression have been reported in HCC. High-throughput omics technologies have been widely applied, aiming at the discovery of candidate biomarkers for cancer staging, prediction of recurrence and prognosis, and treatment selection. Large amounts of data on genetic and epigenetic abnormalities, gene expression profiles, microRNA expression profiles and proteomics have been accumulating, and bioinformatics is playing a more and more important role. In this paper, we review the current omics-based studies on HCC at the levels of genomics, transcriptomics and proteomics. Integrating observations from multiple aspects is an essential step toward the systematic understanding of the disease.

Keywords hepatocellular carcinoma; system biology; omics; bioinformatics

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Introduction

Hepatocellular carcinoma (HCC), the major type of liver cancer, is the fifth most frequent neoplasm and the third most common cause of cancer-related death in the world, especially in Asia and sub-Saharan Africa [1]. In China, HCC ranks the second among all malignancies and its mortality is almost equal to its morbidity [2]. In many cases, HCC arises as a consequence of underlying liver diseases such as viral hepatitis and liver cirrhosis.

Carcinogenesis of HCC is a complex multi-factor, multi-step process, which is associated with many risk factors [3]. Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, and intakes of alcohol and aflatoxin are widely recognized as the four major etiological factors of HCC [4]. Molecular factors including genetic aberrations and cellular changes have been observed in HCC [5]. The mechanisms of HCC differ among etiological factors, and even vary among geographical regions. In China and Africa, HBV is a predominant cause of HCC [6]. But in Japan and Western countries, HCV is the predominant cause [6]. Highly variable clinical phenotypes in HCC patients indicate that HCC comprises several biologically distinctive subgroups. Patients can be categorized in subgroups by different grades of differentiation, proliferation rates, ability to invade vessels, potential for metastasis, sensitivity to chemotherapeutic agents, etc. [7].

Although HCC can be caused by varieties of etiologies, the carcinogenesis of HCC is similar. When the liver gets injured by factors like HBV/HCV, alcohol or aflatoxin B1, necrosis will appear in the liver accompanied by the subsequent hepatocyte proliferation. After continuous cycles of destructive–regenerative process accumulate to some extent, the liver will suffer from cirrhosis. The main characteristic of cirrhosis is that abnormal nodules appear in the liver surrounded by collagens and scarring. Subsequently, the hyperplastic nodules will turn into dysplastic nodules (DNs) inducing a high risk of developing HCC for those patients [4]. DN is classified into low-grade and high-grade according to cytological and architectural atypia on microscopic examination [8]. One-third of high-grade DN will progress to HCC in two years, and the rate increases to 81% in five years [9]. The HCC can be classified into well differentiated, moderately differentiated and poorly differentiated tumors, the last of which is the most severe [4].

Biological research on HCC mainly concentrates on early detection and diagnosis, elucidation of hepatocarcinogenesis by varieties of etiological factors, and prognosis prediction. Investigations have been conducted at different molecular levels including DNA level, RNA level and protein level, with regard to chromosomal imbalance and genetic instability, epigenetic alteration, gene expression, and gene regulation and translation [10–13]. Numbers of omics-based methods have been developed and applied. Large scale profiling technologies, including comparative genomic hybridization (CGH), array-based CGH, microarray and 2D electrophoresis (2DE), mass spectrometry (MS) and other proteomic analysis methods, have been used to detect change of different molecular levels [3,14,15], and computational methods began to play important roles [16–20]. A variety of HCC-associated molecular alterations have been detected. However, because of the lack of good diagnostic markers and treatment strategies and because of clinical heterogeneity, a coherent understanding of the mechanism of HCC development is still limited [21]. The assessment of complex multigenic molecular pathways in HCC remains a difficult challenge.

This review provides a survey on up-to-date omics- and bioinformatics-based studies on HCC from the genomic, transcriptomic and proteomic aspects. These studies include efforts on both the diagnosis and prognosis of the disease, and the mechanisms underlying HCC development with different risk factors. Integrating existing knowledge at multiple molecular levels with systems biology approaches will eventually lead to the understanding of the deadly disease.

Genomic features of HCC

Genomic study on HCC focuses on DNA sequence changes, chromosomal aberrations and epigenetic abnormalities. According to the current understanding, most HCC patients contracted the disease from the accumulation of genetic abnormalities, probably induced by exterior etiological factors especially HBV and HCV infections. These risk factors can induce mutations and damage in DNA sequences, such as p53 mutation induced by aflatoxin and DNA damage induced by the intrusion of the HBV genome [22,23].

Chromosomal aberrations

Chromosomal instability, including gain or loss of the genomic DNA copy number, is commonly seen in liver tumors [24]. Chromosomal amplification regions often

harbor oncogenes, whereas the chromosomal deletion regions often include tumor suppressor genes, both conferring a growth advantage for tumorigenesis in HCC [25]. To identify these crucial regions in hepatocarcinogenesis, a number of approaches have been employed to detect the genomic alterations in HCC samples, including cytogenetics [26], interphase fluorescence *in situ* hybridization (FISH) [27], Southern blot analysis [28], genotyping analyses [29], the conventional metaphase-based CGH [30] and array-based CGH (aCGH). CGH and aCGH are the most widely applied high-throughput methods for detecting copy number variants (CNVs) associated with liver cancer [31]. In the CGH technique, a normal and a pathological DNA sample are differentially labeled and compared by competitive hybridization against a normal metaphase chromosome spread detecting gains and losses based on changes in signal ratios [32]. The aCGH greatly improves the resolution (approximately 1 Mb) of the technique by substituting the hybridization target, the metaphase chromosome spread, with genomic segments spotted in an array format [32].

Several chromosomal loci have been frequently reported in HCC. Some aberrant regions were often correlated with different stages or different prognosis in HCC. For example, gains of chromosome 1q21 were frequently detected in HCC [33], losses of chromosome 5q34 were associated with poor prognosis and losses of 8p23.1-22 were associated with metastasis of HCC [34,35]. Different risk factors of HCC can induce distinct genetic changes. For example, the uptake of aflatoxin can lead to a G to T transversion at codon 249 of tumor protein 53 (TP53), and an ectopic expression of HBx (a core protein of HBV), which can interact with the cellular target HBXIP to dysregulate centrosome dynamics and mitotic spindle formation in HCC [22,23].

The widespread use of aCGH has prompted the development of computational analyses to identify the chromosome aberrations. For example, LS-CAP, an algorithm for identifying cytogenetic aberrations using aCGH data, was built and used on HCC to locate small fragments with cytogenetic aberrations [16]. Wong *et al.* investigated the clonal relationship between tumor nodules in HCC patients to study the recurrence and postoperative treatment by two-way hierarchical clustering [36]. Identifying abnormal chromosomal regions and studying genes located in these regions can help to understand better the hepatocarcinogenesis.

We did a literature mining from PubMed abstracts to collect chromosomal regions reported to be associated

with HCC. We extracted all abstracts with keywords ‘hepatocellular carcinoma’ and ‘chromosome’ from PubMed, and retrieved chromosomal regions reported in these literature. We divided all the extracted HCC-related abstracts into two groups: HBV-related and HCV-related, according to the occurrence of “HBV” or “HCV” in the abstracts. For each frequently reported chromosomal region, we counted its appearance in all the HCC abstracts, and in the HBV- and HCV-related groups, respectively. **Table 1** lists the results. We can see that the appearances in the two groups are uneven for many regions, which could be a sign that some chromosome regions can be associated with a specific virus type.

Epigenetic alterations

Epigenetic modifications refer to changes in DNA/chromatin that do not involve changes in the DNA sequence [37]. DNA methylation and histone modifications are two major aspects of epigenetics. The epigenetic

regulation is a key mechanism for cellular differentiation and cell fate decisions and is known to play a major role in ageing and cancer [38]. Some genes have been reported to have abnormal epigenetic alterations in HCC. The most standard technology for detecting DNA methylation is bisulphite genomic sequencing, which maps sites with the resolution of single base-pair. The method utilizes the selective deamination of cytosine to uracil by incubation with sodium bisulphite and the sequencing subsequently generated PCR products. In contrast to cytosine, the 5-methylcytosine can be distinguished because it does not react with bisulphite [39,40]. The most widely used technique for mapping histone modification is chromatin immunoprecipitation assay [41]. Chromatin fragments are isolated using specific antibodies of histones and amplified DNA are used for PCR or microarray [42].

DNA methylation abnormalities of CpG islands have been observed in a group of malignancies [43].

Table 1 The frequently reported aberrated regions in HCC

Chromosome region	Type of aberrations ^a	Numbers of appearance in HCC-abstracts	Numbers of appearance in HBV-related HCC-abstracts	Numbers of appearance in HCV-related HCC-abstracts
1q	Gains	131	38	16
1p	Losses	130	33	16
17p	Losses	120	42	16
6q	Losses	117	37	16
4q	Losses	116	39	15
8p	Losses	109	35	15
13q	Losses	96	34	14
16q	Losses	95	34	15
8q	Gains	84	31	9
7q	Gains	72	20	8
6p	Gains	65	24	7
17q	Gains	52	17	8
20q	Gains	24	6	3
19p	Losses	18	4	0
16q22	Losses	16	3	1
1q21	Gains	11	2	1
11q12	Gains	4	2	1
14q12	Gains	3	2	1
12p11	Gains	2	1	0
5q34	Losses	2	1	0
4q28	Losses	3	0	0
13q21	Losses	2	0	0
19q13.1	Gains	1	1	0

^a The major types of aberration reported in HCC. “Gains” indicates that the region was reported to be amplified in most literature, and “Losses” indicates that the region was reported to be deleted in most literature.

A number of studies have indicated that promoter hypermethylation may be a key mechanism involved in the inactivation of some tumor suppressor genes in HCC. Promoter methylation and subsequent loss of protein expression have been demonstrated in many oncogenes and tumor suppressor genes in HCC. **Table 2** lists genes we collected that have been frequently reported to involve abnormal methylation in HCC.

DNA methylation status of some genes can be used as potential biomarkers. For example, p15, p16 and RASSF1A was suggested as potential diagnosis markers because their methylated DNA sequences can be detected in the serum of HCC patients [44]. The T-cadherin down-regulation by its promoter methylation is associated with the development and progression of HCC [45]. Some methylated genes can also be markers of prognosis. For example, the frequent promoter methylation of M-cadherin is associated with poor prognosis in HCC [46]. Different etiological factors such as HBV and HCV infections can induce different methylation statuses of variety of genes: HBx may play an important role in the early stage of HBV-associated hepatocarcinogenesis via induction of hypermethylation of p16INK4A promoter [47,48]; methylation of SOCS-1, APC, and p15 was more frequently seen in HCV-positive HCC than HCV/HBV-negative HCC [49]. Many studies indicated that the abnormal gene promoter methylation was a common event in HCC [50,51]. Besides DNA methylation, some aberrant histone modifications were also identified in HCC. Kondo *et al.* have demonstrated that histone modifications at H3K4, H3K9 and H3K27 regulate the expression of tumor suppressors genes in HCC [40].

As epigenetic factors are gaining more attention in cancer study, several databases about abnormal epigenetic modifications in cancers have been developed. PubMeth (<http://www.pubmeth.org/>) is a cancer methylation database curated by text-mining and expert annotation and it includes genes that have been reported to be methylated in various cancers [52]. Some computational methods have been developed to identify novel targets of methylation in specific cancers. Perry *et al.* predicted 338 potential novel targets of methylation by screening CpG islands in 631 genes that were down-regulated in prostate cancer [53]. Similar work has not been reported on HCC yet, and there is still no specific database of epigenetic alterations in HCC.

Transcriptomics studies on HCC

Transcriptomics analyzes all RNA molecules transcribed from DNA in a cell, tissue or organ. Several high-throughput profiling technologies have been developed to detect thousands of transcripts simultaneously, such as DNA microarray, SAGE (serial analysis of gene expression) and MPSS (massively parallel signature sequencing). Microarrays have been the most popular profiling method in cancer study.

Microarray and gene expression profiles in HCC

Microarray is a technique that can measure the mRNA expression of thousands of genes on a single chip by detecting their hybridization to the array of DNA probes on the chip. Many researchers applied this technology to explore the molecular mechanisms of HCC and to find markers or patterns for early detection and diagnosis,

Table 2 A list of abnormally methylated genes frequently reported in HCC

Gene	Type of aberrant methylation	Numbers of appearance in HCC abstracts	Known functions
CDKN2A	hypermethylation	141	Tumor suppressor gene
GSTP1	methylation	123	Susceptibility to cancer
CDH1	hypermethylation	109	Mutations are correlated with cancer
APC	hypermethylation	60	Tumor suppressor gene
SOCS1	hypermethylation	28	Suppressor of cytokine signaling
RASSF1A	hypermethylation	25	Tumor suppressor gene
ARHGAP24	hypermethylation	25	Encodes negative regulators of Rho GTPases
MRPL28	hypermethylation	24	Helps protein synthesis in the mitochondrion
RARB	hypermethylation	24	Flanks a hepatitis B virus integration site
SPEN	hypermethylation	11	Encodes a hormone inducible transcriptional repressor
PRDM2	demethylation	10	Tumor suppressor gene

Table 3 Downloadable microarray data sets for HCC

Dataset ID and Web link	Sample description	Number of genes ^a	Microarray platform	Data format ^b	Reference
GSE3500 http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE3500	102 primary HCC, 74 non-tumor liver tissues, 7 benign liver tumor samples, 10 metastatic cancers, and 10 HCC cell lines	About 20,000	cDNA	Soft	[51,59]
GDS274(GSE364) http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE364	67 primary and metastatic HCC	9984	cDNA	Soft	[60]
GDS2239 http://www.ncbi.nlm.nih.gov/projects/geo/gds/gds_browse.cgi?gds=2239	6 HepG2 human hepatocyte cell line with inducible expression of HCV Core protein (HCV-1b)	22,283	Affymetrix HG-U133A	Soft	[61]
GSE5975 http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE5975	238 HBV-positive HCC	22,297	cDNA	Soft	[62]
GSE6764 http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE6764	75 samples covering 8 stages of HCV induced HCC	54,675	Affymetrix HG-U133 Plus 2.0	Cel	[63]
GSE5093 http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE5093	115 non-cancerous surrounding hepatic tissues from two HCC patient groups, those with primary HCC with a metastasis-inclined microenvironment (MIM) and a metastasis-averse microenvironment (MAM)	9,128	cDNA	Soft	[64]
GSE4024 http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE4024	49 HCC	21,794	cDNA	Soft	[7]
GSE1898 http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE1898	91 HCC	21,794	cDNA	Soft	[7,65]
GSE7474 http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE7474	19 HCC	About 40,000	cDNA	Soft	[66]
E-TABM-36.processed http://www.ebi.ac.uk/ebisearch/search.ebi?db=geneExpression&t=E-TABM-36	57HCC, 3 hepatocellular adenoma, 5 non-tumoral pools	22,283	Affymetrix HG-U133	Txt	[67]

^a For the microarrays developed on more than one platform, the order of magnitude rather than the exact gene number is shown.

^b Format of the data provided on the web. Soft, the SOFT (Simple Omnibus Format in Text) format is a simple line-based, plain text format designed for rapid batch submission and download of microarray data; Cel, The cel file is a data format that contains fluorescence intensities for each probe on the Affymetrix GeneChips. Txt, plain text file of the expression matrix.

Table 4 Genes that have been highlighted in HCC microarray studies

Gene	Numbers of appearance in HCC abstracts	Known functions
AFP	3421	A serum marker of hepatoma and teratoma
RB1	245	Tumor suppressor gene
MAPK1	206	Integration point for biochemical signals
TCEAL1	128	Encoding a nuclear phosphoprotein
VIM	74	Well-characterized cytoskeletal elements
POSTN	69	Osteoblast specific factor
AKT1	55	Mediator of growth factor-induced neuronal survival
GPC3	41	Deletion mutations are associated with Simpson-Golabi-Behmel syndrome
CD44	34	Related to tumor metastasis
IGFBP-3	25	Prolongs the half-life of IGF and alters their interaction with cell surface receptors
MDK	18	Neurite growth-promoting factor 2
PEG10	10	Overexpression is associated with HCC
CSTB	8	Mutations are responsible for the primary defects in patients with progressive myoclonic epilepsy
ILK	7	Associated with integrin-mediated signaling
SPP1	6	Secreted phosphoprotein 1
TACSTD1	6	Encoding an antigen for immunotherapy treatment of cancer
ANXA2	4	Autocrine factor heightening osteoclast formation and bone resorption
FGL1	4	Involved in development of HCC

PTEN [78]. Wang *et al.* found miR-224 increases apoptotic cell death as well as proliferation and targets apoptosis inhibitor-5 (API-5) to inhibit API-5 transcript expression [79]. Budhu *et al.* used a supervised algorithm to predict metastasis characterization through examining the miRNA expression profiles of 482 cancerous and non-cancerous specimens from radical resection of 241 patients with HCC [80]. **Table 5** lists a summary of these studies. Most of the original data of these studies are not publicly available yet. In **Table 6**, we collected the miRNAs that were reported to be abnormally expressed in HCC.

Biomarkers and proteomics studies on HCC

A biomarker is a biological molecule found in blood or other body fluids or tissues that can be a sign of a disease. Most proteomics studies on HCC are aimed at the discovery of new biomarkers. Proteomics is the large-scale study of total proteins, particularly their structures and functions at a given time and conditions in biological system [83,84]. The 2DE and mass spectrometry (MS) are the two major methods in proteomics.

The 2DE methods can simultaneously separate large numbers of proteins by isoelectric focusing and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. It was demonstrated that 2DE can detect the differences between proteins from normal and diseased tissues. Mass spectrometry is an analytical technique that identifies the chemical composition of a compound or sample on the basis of the mass-to-charge ratio of charged particles [85]. In proteomics, MS deduces the composition of molecules by determining the particular peptide mass that results from certain combinations of amino acids. There are several different MS methods, such as MALDI-TOF-MS and SELDI-TOF-MS. Bioinformatics analysis is adopted for identifying features from mass spectrometry data, similar to methods used in microarray study [19,20]. By searching databases of known proteins or by further *de novo* protein identification, these features can be identified as potential biomarkers.

Biomarkers can be used routinely for population screening, prognosis, monitoring of therapy, and prediction of therapeutic response in HCC. The most widely used biomarkers for diagnosis of HCC are AFP, AFP-L3 and des-gamma-carboxy prothrombin (DCP) that are all present in the serum. A number of protein markers for

Table 5 Recent microarray-based miRNA studies on HCC

Sample	Numbers of miRNAs on microarray	Major observation	Reference
Ten pairs of HCC and non-tumor (NT) samples from 10 non-viral hepatitis patients	331 human miRNAs, 236 rat miRNAs, 189 mouse miRNAs and 142 human predicted miRNAs	18 miRNAs▼, 6 miRNAs▲. For others, 15 miRNAs ↑ , 1miRNA ↓	[77]
Malignant hepatocyte cell lines and normal human hepatocytes	328 human miRNAs, 266 mouse miRNAs, 238 rat miRNAs, and 152 ambi-miRNA	Inhibition of miR-21 increased expression of PTEN	[78]
17 HCC and 21 liver cirrhosis	381 probes for 238 mature and 143 precursor human miRNAs	miR-122a ↓ . Cyclin G1 is it's target	[81]
96 pairs of HCC and NT tissues; Cell lines: Hep3B, HepG2, Huh7, SK-HEP-1, QGY7703, BEL7402, BEL7404, MHCC97H	59 miRNAs	Only four variations in miR-106b, miR-192 and let-7a-2. Mutation of miRNA is a rare event in HCC	[82]
25 pairs of HCC and NT and nine chronic hepatitis specimens	180 mature human miRNAs and 206 precursor human miRNAs	↑: miR-18, precursor miR-18, and miR-224; ↓ : miR-199a*, miR-195, miR-199a, miR-200a, and miR-125a	[76]
19 paired tumorous and adjacent nontumorous liver tissues from hepatocellular carcinoma patients	157 miRNAs	miR-224 ↑ increases apoptotic cell death and proliferation and targets API-5 to inhibit API-5 transcript expression	[79]
241 paired HCC and NT tissues	OSU-CCC MicroRNA Microarray Version 2.0, include hundreds of human and mouse	A 20-miRNA signature was found, which may help predict the metastases or recurrence in HCC	[80]

▲, miRNA only expresses in HCC samples; ▼, miRNA only expresses in NT samples; ↑ , miRNA expresses higher in HCC vs non-tumor (NT) samples; ↓, miRNA expresses lower in HCC vs NT samples.

Table 6 Differentially expressed miRNAs between HCC and non-tumor

High expression in HCC ^a	Low expression in HCC ^b
miR-18, miR-224, miR-21, miR-182, miR-182*, miR-183, miR-222, miR-96, miR-9*, miR-216, miR-155, miR-301, miR-221, miR-324-5p, miR-186, miR-151, miR-374	miR-199a*, miR-125a, miR-195, miR-199a, miR-200a, miR-122a, miR-139, miR-214

^a MicroRNAs highly expressed in HCC vs non-tumor; ^b microRNAs lowly expressed in HCC vs non-tumor.

diagnosis and prognosis of HCC have been reported. These biomarkers include metabolic enzymes, proteins involved in calcium homeostasis, cytoskeleton and tumor suppression factors, and proteins that can increase cell's resistance to apoptosis.

New potential biomarkers were continuously discovered from HCC cell lines, tissues and serum. Ding *et al.* found that CK19 was significantly decreased in the MHCC97L cell line (low metastasis potential) compared with the MHCC97H cell line (high metastasis potential). It indicated that CK19 was associated with metastasis [86]. Tong *et al.* used 2-DE and MS/MS analysis to compare and identify differentially expressed proteins between an HBV-producing cell line HepG2.2.15 and its parental cell line HepG2. The proteins identified would be useful in revealing the mechanisms underlying HBV–host cell interactions and the development of HCC [87]. Sun *et al.* used two-dimensional fluorescence DIGE to study the differentially expressed proteins in tumor and adjacent non-tumor tissue samples. They identified that Hcp70/Hsp90-organizing protein and heterogeneous nuclear ribonucleoproteins C1/C2 can be potential biomarkers in HCC [88]. Zinkin *et al.* used the serum of patients with HCC and HCV liver cirrhosis by SELDI-TOF-MS to distinguish patients accurately [89]. Lee *et al.* performed SELDI-TOF-MS to identify differentially expressed proteins in HCC serum. Complement C3a was identified as a candidate biomarker in human chronic hepatitis C and HCV-related HCC [90]. **Table 7** lists a representative collection of reported biomarkers in HCC including some markers that are widely used and other potential markers that are still in test.

In a recent study, Zhou *et al.* studied protein–protein interaction networks in liver cancer from glycoprotein expression profiles of human liver cancer cell lines with diverse metastasis potential [112]. They found many interactions among proliferation and apoptosis-related proteins and differential glycoproteins, and concluded that instead of analyzing a single, or a few, proteins, a “‘molecule groups’ concept should be introduced in the diagnosis and metastasis prediction of HCC” [112].

Perspectives

Liver cancer has been extensively studied with various high-throughput omics technologies. The observed genetic aberrations associated with HCC include the amplification or deletion of chromosomal regions, copy number changes of genes and abnormal epigenetic alterations. These aberrations can be caused by different environmental factors like virus infection and alcohol and/or aflatoxin consumption. Changes in the expression of many genes are also evident at both mRNA and protein levels. Such changes can be the consequences of the genetic aberrations and environmental interactions. Noncoding miRNAs are also observed to play roles in the disease. As a complex disease, the genesis and development of HCC could not be decided by a single factor or a simple collection of single factors, but rather by interactions of multiple proteins, genes and miRNAs in biological pathways. **Fig. 1** illustrates a diagram of the putative relations of multiple types of factors at different molecular levels in HCC. Examples of some of the relations (illustrated in solid links) have been already reported and some still lack observations (illustrated in dash links).

The accumulation of omics data at multiple levels provides an opportunity to comprehend the mechanisms of HCC. Integrating different omics data is being increasingly regarded as a key approach in cancer biology [113,114]. Zender *et al.* combined genomics and transcriptomics to identify and validate Yap and cIAP1 as oncogenes in liver cancer [115]. In fact this omics combination is beginning to be widely applied [116,117]. Hou *et al.* used aCGH in conjunction with gene expression profiling and found RAB23 as an invasion mediator gene in diffuse-type gastric cancer [118]. Onken *et al.* utilized gene expression profiling, aCGH, array-based global DNA methylation profiling, and single nucleotide polymorphism-based detection of loss of heterozygosity to identify modifiers of metastatic risk and ascertained LZTS1 to be a good candidate in primary uveal melanomas [119]. More recently, a large

Table 7 Biomarkers of HCC reported by proteomics studies

Protein	Potential biomarker type	Status	Reference
AFP	Diagnosis marker and prognosis marker	Currently used (FDA approved)	[91]
AFP-L3	Diagnosis marker	Currently used (FDA approved)	[92]
DCP	Diagnosis marker	Currently used (Available in Asia)	[93]
Hypercholesterolemia	Diagnosis marker	Research stage	[94]
α -L-fucosidase	Diagnosis marker	Research stage	[95]
Squamous cell carcinoma antigen (SCCA)	Diagnosis marker	In test	[96]
Glypican-3	Diagnosis marker	In test	[97]
TGF β -1	Diagnosis marker	Research stage	[98]
IGF-II	Diagnosis marker	Research stage	[99]
IGFBP-2	Diagnosis marker	Research stage	[100]
HCCR	Diagnosis marker	Research stage	[101]
GP73	Diagnosis marker	Research stage	[102]
HGF	Diagnosis marker	Research stage	[103]
KL-6	Diagnosis marker	Research stage	[104]
RASSF1A	Diagnosis marker	In test	[105]
C-terminal fragment of complement C3 protein Isoform of ApoA1 Autoantibodies against HSP70, GA3PDH, peroxyredoxin, and Mn SOD	Diagnosis marker	Research stage	[106]
EpCAM and α -fetoprotein HSPA1B P2	Prognosis marker	Research stage	[107,108]
Cytokeratin 19, CYFRA21-1	Metastasis marker	Research stage	[109]
Haptoglobin, α 1 antitrypsin, transthyretin, ApoAIV, isoforms of ApoAI, and Topoisomerase IIB	HBV-HCC marker	Research stage	[110]
Autoantibodies against calreticulin, cytokeratin 8, nucleoside diphosphate kinase A, and ATP synthase b chain	HCV-HCC marker	Research stage	[111]

cohort study of about 50,000 women has been initiated to study breast cancer using the omics technologies [120]. There are other successful examples of integrating omics data to obtain potential biomarkers and interesting findings [121,122].

Cancer phenomics and metabolomics are emerging omics and will play an increasingly important role in the future. Cancer phenomics can be defined as the systematic acquisition and objective documentation of host and/or somatic cancer phenotypic data at many levels [123]. Metabolomics is the global analysis of metabolites, small molecules generated in the process of metabolism at given times in a cell, tissue, organ, and so on, potentially providing information about the functional state [124]. To facilitate the integration of those diverse data, some computer systems have also been developed: Shannon *et al.* created the Gaggle, which aims at

helping system biologists by integrating different data types and sources as well as different bioinformatics platforms. Shah *et al.* built a platform enabling users to manage, integrate and retrieve high-throughput data [125]. Agrawal *et al.* developed a sophisticated database containing a large collection and variety of omics data for type 2 diabetes [126].

Similar to the concept of systems biology, Vivekanandan *et al.* proposed the term 'high-dimensional biology' (HDB), which is the integration of genomics, transcriptomics, proteomics and metabolomics for studying complex diseases [127]. There have been two databases OncoDB.HCC [70] and ECHO, [71] which collected data from HCC-related reports. Efforts on integrating multiple data sources at different levels, especially with regard to biological pathways and interaction networks, are still lacking on HCC. It is time now

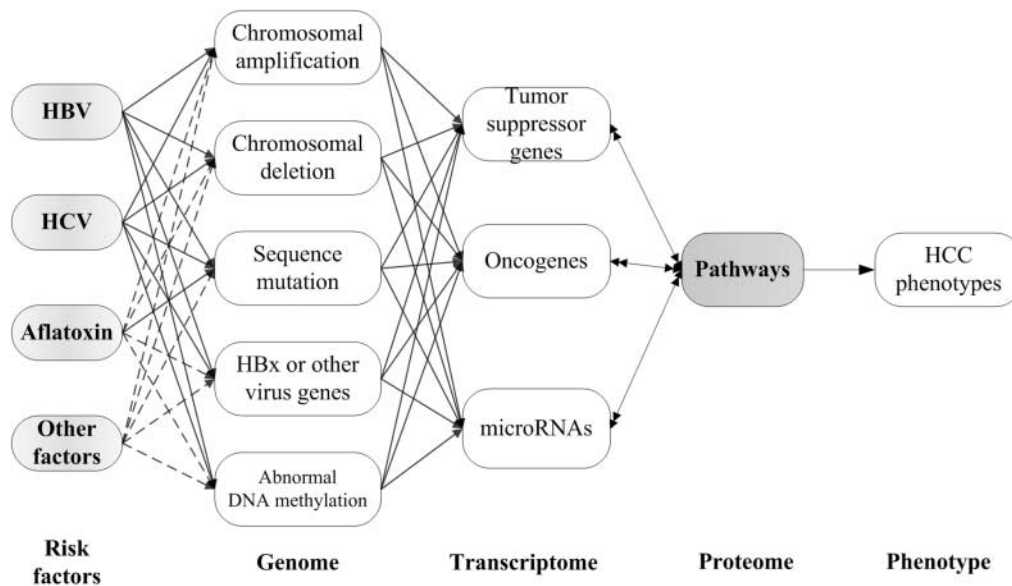


Fig. 1 The putative relations between multiple factors behind HCC A diagram showing the putative conceptual relation between multiple factors that play important roles in the genesis and development of HCC. Solid lines indicate relations that have reported instances; dash lines show links that could exist but have not been reported in the literature.

that computational and experimental scientists come together to develop systems biology strategies toward the comprehension of the deadly malignance.

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