Key points

- Whole-exome and whole-genome sequencing have provided a comprehensive and high-resolution view of somatic genomic alterations in liver cancer
- Global epigenetic analyses have further identified both unique and complementary molecular alterations in liver cancer
- Somatic mutational signatures of the liver cancer genome are complex and tend to be associated with epidemiological backgrounds
- Integration of genetic and epigenetic alteration profiles has elucidated core oncogenic pathways, potential therapeutic targets and new molecular classifications in liver cancer

Table 1 | Amplified and deleted genes in HCC

Gene name	Locus	Function
Recurrently amplified genes in HCC		
MDM4	1q32.1	p53 pathway
BCL9	1q21.1	WNT pathway
ARNT	1q21.2	Xenobiotics metabolism
ABL2	1q25.2	Proliferation
MET	7q31.2	Proliferation
COPS5	8q13.1	Proteolysis
MTDH	8q22.1	Metastasis
COX6C	8q22.2	Mitochondria
MYC	8q24.21	Proliferation
CCND1	11q13.2	Proliferation
FGF19	11q13.2	WNT pathway
RPS6KB1	17q23.1	Proliferation
EEF1A2	20q13.33	Translation
Recurrently deleted genes in HCC		
TNFRSF14	1p36.33	Immune response
CDKN2C	1p36.11	Cell cycle
ARID1A	1p36.11	Chromatin remodelling
TNFAIP3	6q26	NF-κB pathway
CSMD1	8p23.2	Immune response
DLC1	8p22	Small GTPase
SORBS3	8p21.3	Migration
WRN	8p21.3	DNA repair
SH2D4A	8p21.2	Proliferation
PROSC	8p11.2	Unknown
CDKN2A	9p21.3	Cell cycle
CDKN2B	9p21.3	Cell cycle
PTEN	10q23.31	Proliferation
SPRY2	13q31.1	Proliferation
BRCA2	13q13.1	DNA repair
RB1	13q14.3	Cell cycle
XPO4	13q11	Nuclear export
SMAD4	18q21.31	TGF-β signalling

alteration profiles of 87 HCC tumours, including HBV-associated and HCV-associated cases. Two molecular subgroups were identified that are associated with virus status, the presence of intrahepatic metastasis and patient prognosis. The researchers also reported six distinctive combinations of copy number alterations in HCC.[13] In another study, copy number changes in 63 HCCs of various aetiologies (viral and nonviral) were analysed and 8q24 copy number gains associated with *MYC* overexpression were identified that were unique to viral and alcohol-related HCCs.[15] Amplification of *MDM4* (1q32.1) and copy number gain of *EEF1A2* (20q13.33) were shown to be frequent and aetiology-independent molecular events in HCC.[15] A meta-analysis of four independent microarray comparative genomic hybridization datasets, including 169 samples, identified chromosomal gains in five broad (1q, 6p, 8q, 17q, and 10q) and two narrow (5p15.33 and 9q34.2–34.3) regions, and 88 significant losses frequently present in 4q, 6q, 8p, 9p, 13q, 14q, 16q and 17p.[18] Wang *et al.*[19] reported the results of copy number analysis of 286 HCC tumours by single nucleotide polymorphism array, which identified 29 recurrently amplified and 22 recurrently deleted regions, as well as *BCL9* and *MTDH* as novel amplified oncogenes in HCC.[19]

Whole-exome sequencing

Innovations in sequencing technologies have enabled researchers to explore the liver cancer genome in more depth. The capture or enrichment of DNA fragments containing the exonic region followed by massively parallel sequencing can determine somatic mutations in the whole exon domain (exome).[23,24] This approach enables the comprehensive detection of somatic alterations in the protein-coding region, and has led to the discovery of many novel genes implicated in liver cancer. Exomic sequencing of 10 HCV-positive HCCs and subsequent analysis of an additional tumour cohort of various aetiological backgrounds identified recurrent inactivating mutations of the *ARID2* gene in 18.2% of HCV-associated HCCs.[25] Guichard *et al.*[26] performed copy number analysis of 125 HCC cases and whole-exome sequencing of 24 of these cases and found new recurrent alterations in four genes (*ARID1A*, *RPS6KA3*, *NFE2L2* and *IRF2*). Huang *et al.*[27] performed whole-exome sequencing of nine pairs of HCCs and their intrahepatic metastases. Although most substitutions (94.2%) were common in both primary and metastatic tumours, a fraction of mutations were only detected in primary (1.1%) or metastatic (4.7%) tumours. Among them, *KDM6A*, *CUL9*, *FGD6*, *AKAP4* and *RNF139* were found only in the metastatic tumours of three individuals.

Using whole-exome sequencing of 87 HCC cases, Cleary *et al.*[28] identified recurrent alterations in the *NFE2L2–KEAP1* and *KMT2A* (also known as *MLL*) pathways, and other genes (*C16orf62* and *RAC2*) with lower mutation frequencies. Eight fluke-associated cholangiocarcinomas (the predominant type of liver cancer in northern Thailand and neighbouring countries) were analysed, and showed that the number of coding mutations per tumour ranged from 19 to 34, with an average of 26 mutations per sample.[29] In addition to *TP53* and *KRAS*, recurrent inactivating mutations in the *MLL3*, *ROBO2*, *RNF43* and *PEG3* genes were identified, and activating mutations were found in the *GNAS* gene.

Whole-genome sequencing

Several research groups have sequenced the full liver cancer genome in further attempts to identify all somatic driver events related to hepatocarcinogenesis, including substitutions in noncoding regions, structural rearrangements, and viral genome integration. Totoki *et al.*[30] first performed whole-genome sequencing of one HCV-associated HCC case (tumour genome and corresponding normal genome) and identified >16,000 somatic mutations and 26 intra-chromosomal and inter-chromosomal rearrangements generating four fusion transcripts. Among them, one in-frame fusion transcript (*BCORL1-ELF4*), generated by a small inversion on the X chromosome, showed reduced transcriptional repression activity compared to wild-type *BCORL1*, which encodes a tumour suppressor gene.

Fujimoto *et al.*[31] reported the results of whole-genome sequencing of 27 HCCs and matched normal genomes, 25 of which were associated with HBV or HCV infection. The average number of somatic point mutations at the whole-genome level was 4.2 per Mb. One tumour that contained an exceptionally large number of somatic mutations (24,147 substitutions) showed a DNA mismatch-repair defect caused by a somatic nonsense mutation in the *MLH1* gene. Furthermore, mutations in several chromatin regulators, including *ARID1A*, *ARID1B*, *ARID2*, *KMT2A* and *MLL3*, were detected in ~50% of the tumours.

Whole-genome sequencing of 88 HCC tumour and normal tissue pairs, including 81 HBV-positive and no HCV-positive cases showed an average somatic mutation rate of 3.69 per Mb and a mean protein-altering mutation rate of 1.8 per Mb, which are mid-range among different cancer types.[32] In this study, the WNT/CTNNB1 and JAK/STAT pathways were shown to be major oncogenic drivers in HCC and activating *JAK1* mutations were identified in 9.1% of total cases, suggesting that these pathways could be novel therapeutic targets in HCC.

HBV genome integrations in the host genome

HBV is a DNA virus whose genome is integrated into the host genome. The integration of the viral genome affects host gene expression near the integration site and its effect on the integrity of the host genome is associated with virus-mediated hapatocarcinogenesis.[33] In the past, Southern blot analysis or inverse PCR was applied to identify viral genome integration sites. However, current genome sequencing technology can detect virus integration events more comprehensively and at higher resolution than previously.

Jiang *et al.*[34] performed high-depth (>80× and 240× coverage of the genome, two or three times more than that used for conventional whole-genome sequencing) whole-genome and transcriptome sequencing of four pairs of HBV-positive HCCs and identified 225 HBV genome integration sites by taking advantage of paired reads mapping to both human and viral genomes. A variety of genomic aberrations near viral integration sites were found, including direct gene disruption, viral promoter-driven gene transcription, viral–human transcript fusion, and DNA copy number alterations. Frequent HBV integration in *TERT* and *MLL4* loci has also been reported.[31] Sung *et al.*[35] conducted whole-genome sequencing, at >30× coverage on average, of 81 HBV-positive and seven HBV-negative HCC samples. Analysis of HBV integration sites identified 399 integration breakpoints (4.9 per case). Frequent HBV integration breakpoints were observed in the *TERT*, *KMT2D* (also known as *MLL4*), *CCNE1* and *FN1* genes.

Somatic change of retrotransposons in HCC

The human genome contains a variety of repetitive sequences, including tandem repeats (such as satellite DNA and microsatellite DNA) and retrotransposons, such as short interspersed nuclear elements (SINEs) and long interspersed nuclear elements (LINEs). In the human genome, Alu and LINE-1 are major forms of SINEs and LINEs, respectively. Given that current massive parallel sequencing technologies can produce only short reads (~200 bp), repetitive sequences, which constitute ~20% of the human genome, remain to be explored in genome sequencing.[36]

Retrotransposon capture sequencing applied to HCC samples revealed two LINE-1-mediated somatic changes associated with liver tumorigenesis.[37] One was a germline retrotransposon insertion in the *MCC* gene, a tumour suppressor gene that is known to be mutated in colorectal cancers. This retrotransposon insertion was found to downregulate *MCC* expression and activate the WNT/CTNNB1 pathway. The other event, a tumour-specific LINE-1 insertion, activates a potential oncogene, *ST18*, in liver tumours.

Mutation signatures and aetiological factors

There are six patterns of somatic substitution (C>A/G>T, C>G/G>C, C>T/G>A, T>A/A>T, T>C/A>G and T>G/A>C) in the cancer genome and they are affected by exogenous or endogenous mutagens, such as oxidative stress, exposure to chemicals or UV, and defects in the DNA repair machinery.[38] Whole-genome sequencing in cancer can identify large numbers of neutral mutations and is more appropriate for the analysis of mutation signatures in an unbiased manner than is whole-exome sequencing.

The first whole-genome sequencing study of a Japanese HCV-positive HCC case showed a distinct mutation signature (dominance in C>T/G>A and T>C/A>G) in the liver cancer genome.[30] A similar substitution pattern was also reported in Asian HBV-positive HCC cases.[32,33] Guichard *et al.*[26] reported the over-representation of C>A/G>T substitutions in HCC in a Western population with multiple aetiological backgrounds, although their data was obtained using whole-exome sequencing.[26] Using whole-genome sequencing, a study of 27 HCC cases of different aetiological backgrounds demonstrated a dominance of T>C/A>G transitions as well as C>A/G>T transversions and C>T/G>A transitions, particularly at CpG sites.[31] As C>T/G>A transitions are commonly found in other cancers, T>C/A>G transitions and C>A/G>T transversions could be characteristic mutational signatures

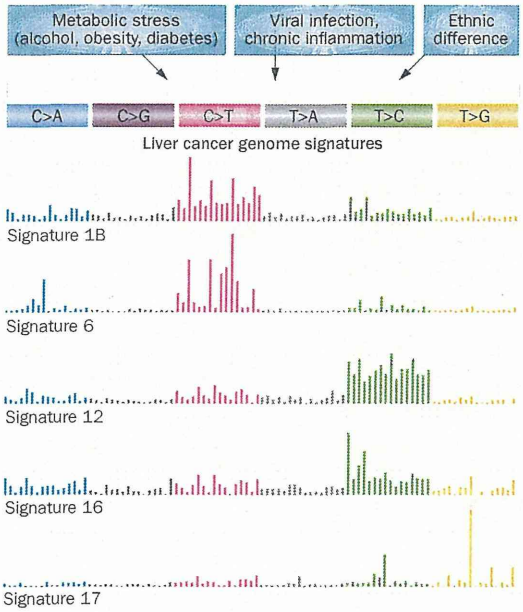

Figure 1 | Multiple aetiological factors and ethnic differences affect somatic mutation signatures in liver cancer. Five characteristic mutation signatures identified in the liver cancer genome are shown.[39] Permission obtained from Nature Publishing Group © Alexandrov, L. B. *Nature* **500**, 415–421 (2013).

of HCC genomes. Habitual alcohol drinking and the occurrence of synchronous or metachronous multiple liver nodules were significantly associated with the principal components of the somatic substitution patterns.[31] Somatic substitutions in IHCC associated with liver fluke are predominantly C>T/G>A transitions, the majority of which are identified in the context of a CpG-to-TpG change as the result of 5-methylcytosine deamination.[29]

In addition to six different substitutions, information on the bases immediately 5′ and 3′ to each mutation has been used to identify context-dependent mutation patterns in a wide range of cancers. Among 22 mutational signatures identified by this cross-tumour analysis, HCC contained five distinct signatures, which was the highest number among the 30 tumour types and indicates that a complex mutagenesis process operates in this tumour (Figure 1).[39]

Epigenetic alterations in HCC

HCC is a heterogeneous disease in terms of aetiology and cell of origin.[40] Various environmental agents and lifestyles known to be risk factors for HCC are suspected to promote its development by eliciting epigenetic changes, which have a key role in a wide range of human malignancies.[41]

DNA methylation in HCC

Altered DNA methylation is an early event in HCC development. Global hypomethylation mainly affects intergenic regions of the genome and has a critical role in increasing chromosomal instability.[42] DNA methylation of gene promoters, which is important in transcriptional regulation and the cellular differentiation process,[43] is a common mechanism of gene silencing in cancer cells. Furthermore, CpG island hypermethylation phenotypes have been reported in various types of cancers, such as colorectal,[44] uterine,[45] glioma,[46] and renal[47] cancers. However, the presence of such phenotypes is still controversial in HCC.[48,49]

A molecular mechanism of active DNA demethylation has been identified and shown to be involved in tumorigenesis,[50] particularly in glioma and haematological malignancies. Hydroxymethylcytosine is present at a considerable level in normal adult liver tissues and is often decreased in tumour tissues;[51] however, its role in liver carcinogenesis remains unknown. *IDH1* and *IDH2* mutations are frequent in IHCCs and have been detected in 34 of 326 cases (10%).[52] Tumours containing mutations in *IDH1* or *IDH2* had lower 5-hydroxymethylcytosine and higher 5-methylcytosine levels compared with those without mutations, and 50% of hypermethylated genes overlapped with DNA hypermethylation in *IDH1*-mutant glioblastomas.[51]

To investigate DNA methylation patterns comprehensively, aberrantly methylated genes are identified by methylated DNA immunoprecipitation (meDIP) followed by tiling array[53] or next-generation sequencing. Deng *et al.*[54] applied the meDIP-chip method to identify 15 genes preferentially methylated in HCV-related HCCs. Alternatively, a genome-wide DNA methylation assay that was developed on Beadchip™ (Illumina Inc., San Diego, CA) technology[55] can measure methylation levels quantitatively at single CpG sites, and yield largely comparable results to meDIP sequencing[56] and whole-genome bisulphite sequencing. This assay has been applied to methylation profiling in various cancers and in the cancer genome atlas project.[57] A few distinct epigenetic subtypes identified on the basis of the methylation pattern have been detected in HCCs and will be integrated with genetic alteration data.

Shen *et al.*[58] used a 27K Infinium™ array (Illumina) to analyse 62 HCC cases and identified 2,324 differentially methylated CpG sites, of which 684 hypermethylation markers could be utilized for plasma DNA diagnostics. They also analysed 66 HCC cases using a 450K array in which the top 500 significant CpG sites that were differentially methylated were able to distinguish HCC from adjacent tissues.[59] Meanwhile, Tao *et al.*[60] analysed non-cancerous tissues of HBV-associated HCC on a 27K array and identified hypermethylated genes. Accumulation of such methylations would form "an epigenetic field for cancerization".[61]

An early study[62] showed that extensive methylation is associated with *CTNNB1* mutations, while HCC with a *TP53* mutation is often characterized by chromosomal instability. Given that *CTNNB1* and *TP53* mutations are mutually exclusive in HCCs, such distinct methylation patterns could be associated with particular genetic alterations.

Promoter CpG islands of the *CDKN2A* and *CDKN2B* tumour suppressor genes are frequently hypermethylated, leading to inactivation of the *RB* pathway.[63] Methylation of the *CDKN2A* gene promoter occurs in 73% of HCC

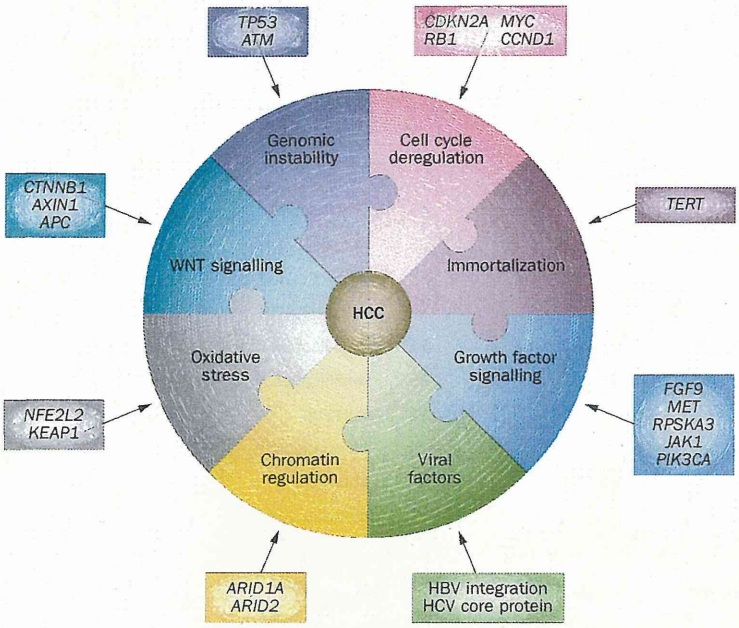

Figure 2 | Core oncogenic pathways in hepatocarcinogenesis. Representative genes involved in each pathway are indicated.

tissues,[64] 56% of HBV-related HCC, and 84% of HCV-related HCC.[65] *RASSF1A* is methylated in up to 85% of HCCs,[66] *GSTP1* in 50–90%,[67,68] and *MGMT* in 40%.[69]

Transcriptome analysis and beyond

RNA sequencing technology has enabled not only transcriptomic profiling, but also the identification of rearranged transcripts, such as translocations and inversions, and tumour-specific expression of noncoding RNAs, although the latter analysis requires deep coverage of sequencing reads. No recurrent fusion genes have been reported in HCC to date.

Classification based on gene expression, copy number and DNA methylation profiling data would help elucidate the correlation between mutation profiles and molecular subclasses.[70,71] Gene expression profiles in cancer are the result of genetic and epigenetic alterations. Therefore, an integrated genomic analysis is necessary to determine how these genetic and epigenetic alterations affect cancer phenotypes, because the combination of somatic mutations, promoter methylation, and chromosomal loss might lead to gene inactivation.[72]

Vetter *et al.*[73] reported on the association between the increase of splicing variants of the *KLF6* gene and increased hepatocarcinogenesis. Splicing variants in HCCs have been reported in several genes,[74] including *LLGL1* (also known as *HUGL1*),[75] *TCF4*,[76] and *p73*.[77] Transcriptome sequencing of HCC samples combined with genotyping validation identified a frequent adenosine-to-inosine RNA editing event in the *AZIN1* gene in HCC.[78,79] This editing induces a serine-to-glycine amino acid change that confers gain-of-function activity and a stronger affinity of the edited protein to antizyme. Increased AZIN1 (antizyme inhibitor 1) protein stability could promote cell proliferation, presumably through the neutralization of ornithine decarboylase (ODC) and G1/S-specific cyclin-D1 (CCND1) degradation mediated by antizyme. Adenosine-to-inosine RNA editing will contribute to more transcriptome diversity and liver carcinogenesis.

Core liver cancer genes and pathways

Comprehensive analyses of the liver cancer genome have demonstrated that multiple cancer genes and molecular pathways are recurrently altered and have pivotal roles in hepatocarcinogenesis (Figure 2). Table 2 summarizes important mutated genes in liver cancer.

TP53 pathway

TP53 is the top gene among recurrently mutated genes in HCC, and its mutation frequency varies between 18% and 35.2% (25.9% on average) of HCCs.[80] Alterations of other genes located upstream and downstream on the TP53 pathway, such as recurrent mutations of the *ATM* (an upstream regulator of TP53 activation[81]) and *CDKN1A* (a target of TP53[82]) genes, have also been reported. Moreover, mutations of the *IRF2* gene, which encodes a positive regulator of TP53 protein expression, are mutually exclusive to the TP53 mutation in a cohort of patients with HCC.[26]

Cell cycle regulation pathway

The G1/S cell cycle checkpoint and cell senescence are regulated by *RB* and *CDKN2A*. Inactivation of the *RB* and *CDKN2A* genes by homozygous deletion and promoter CpG hypermethylation or point mutations has been reported in HCC.[83,84] The tumour suppressing activity of *RB* in the liver was evaluated in a mouse model, and RB inactivation was found to be associated with both increased cell proliferation and chromosomal instability.[85]

TERT pathway

Activation of telomerase (encoded by the *TERT* gene), which is physiologically silenced in most normal cells, is required for infinite replication in cancer cells.[86] Somatic mutations in the *TERT* gene promoter have been shown to promote *TERT* gene expression in melanoma.[87,88] Killela *et al.*[89] screened these mutations in >1,000 tumours of various organs and reported that 27% of HCC cases harboured these alterations. Nault *et al.* reported *TERT* promoter mutations in 54% of human HCCs and 25% of cirrhotic preneoplastic nodules, suggesting that this alteration could be the earliest recurrent genetic event in hepatocarcinogenesis.[90]

WNT pathway

Aberrant activation of WNT signalling is a driving molecular event in a wide range of tumours, including liver cancers.[91] Somatically acquired missense mutations in exon 3 of the *CTNNB1* gene are frequently reported in HCC (10.0–32.8% in genome-wide sequencing studies).[92] In addition to *CTNNB1*, alterations of *APC* and *AXIN1*, which are tumour suppressor genes that negatively regulate catenin β-1 (CTNNB1) protein levels in a post-transcriptional manner, have been recurrently reported

Table 2 | Candidate driver genes in hepatocellular carcinoma with recurrent genetic alterations

Gene	Frequency (%)	Total number of cases analysed	Number of mutation-positive cases	Genetic alteration	Pathway
TP53	31	2,720	844	Mutation, LOH	TP53
ARID1A	28.2	85	24	Mutation, LOH	Chromatin modifying
CTNNB1	18.8	3,238	609	Mutation	WNT
MTDH	14.7	286*	42	Amplification	Cell adhesion
AXIN1	14.2	466	66	Mutation, LOH	WNT
CDKN2A	11.7	686	80	Mutation, LOH	Cell cycle
ARID2	10.9	202	22	Mutation, LOH	Chromatin modifying
CHD1L	10.7	286*	31	Amplification	Chromatin modifying
BCL9	8.7	286*	25	Amplification	Chromatin modifying
NFE2L2	7.4	162	12	Mutation	Oxidative stress
ATM	6.9	72	5	Mutation, LOH	TP53
PIK3CA	6.3	631	40	Mutation	Growth factor signalling
SMARCA4	6.2	129	8	Mutation, LOH	Chromatin modifying
TSC2	5.2	77	4	Mutation, LOH	Growth factor signalling
CCND1	4.7	286*	14	Amplification	Cell cycle
APC	4.7	107	5	Mutation, LOH	WNT
JAK2	4.7	85	4	Mutation	Growth factor signalling
PTEN	4.4	451	20	Mutation, LOH	Growth factor signalling
BRAF	4.4	360	16	Mutation	Growth factor signalling
FGF19	4.3	286*	13	Amplification	Growth factor signalling
RB1	4.3	94	4	Mutation, LOH	Cell cycle
COL1A1	4.2	71	3	Mutation	Cell adhesion
HNF1A	3.9	233	9	Mutation	Chromatin modifying
KRAS	2.7	672	18	Mutation	Growth factor signalling
NRAS	1.6	426	7	Mutation	Growth factor signalling

*Copy number change. Abbreviation: LOH, loss of heterozygosity.

in HCC and hepatoblastoma.[93–95] Frequent epigenetic inactivation of SFRPs and SOX1, both of which are negative regulators of WNT signalling, has also been detected.[96,97] Alterations in the *CTNNB1, APC* and *AXIN1* genes occur in a mutually exclusive way and activate downstream signals, including transcriptional activation of the *MYC* and *CCND1* genes, which are also amplified in HCC.[98–100] *CTNNB1* mutation is reported to be associated with HCV-related HCC.[28]

Chromatin modifying factors

DNA is tightly associated with proteins, mainly various types of histones, and compactly packed in the nucleus. This DNA-protein complex is called chromatin, and its structure (open or closed) or position is dynamically regulated by histone modifications or ATP-dependent mobilization, which affect gene expression and convey epigenetic information beyond DNA replication. The SWI/SNF (switch/sucrose non-fermentable) protein complex regulates chromatin structure by altering the position of the nucleosome, the basic unit of the DNA-histone complex, and participates in a wide range of biological phenomena, such as differentiation, growth, DNA repair, and reprogramming.[101,102] *ARID1A, ARID1B* and *ARID2* encode core proteins of SWI/SNF complexes and are frequently altered in HCC.[26,31] Alterations of these ARID family members have been reported in other tumour types, including ovarian cancer, renal cell cancer and gastric cancer.[103] In addition, the presence of frameshift mutations, copy number loss and homozygous deletions observed in *in vitro* studies demonstrated that members of the ARID family function as tumour suppressor genes.

Alterations of other epigenetic regulators have also been reported in HCC. As an epigenetic writer (functioning in histone modification), mutations in the gene encoding histone-lysine N-methyltransferase 2A (*KMT2A*; also known as *MLL*)[104,105] and its family members (*MLL3* and *MLL4*) are frequent.[28] A group of genes encoding epigenetic readers (specifically recognizing histone modification) including *BPTF*[106] and other histone binding proteins (*RNF20* [also known as *BRE1A*] and BRDT) are also altered in certain HCCs.[31] Alterations in these epigenetic regulators account for >50% of HCC cases.[31]

Growth factor signalling pathway

Copy number analyses of HCC identified focal gene amplification of the genes encoding the receptor tyrosine

kinase MET, FGF19 (which is a ligand for FGFR4), and downstream signalling components (MYC and RPS6KB1). Furthermore, HCC genome sequencing studies have revealed recurrent somatic mutations in genes encoding other kinases (*RPS6KA3* and *JAK1*). Epigenetic silencing of SOCS-1, a negative regulator of the JAK/STAT pathway, occurs frequently in HCC.[107] Compared to other epithelial cancers, such as lung or colorectal cancer, activating mutations in the RAS (*KRAS, NRAS* and *HRAS*) and *PIK3CA* genes are rarely reported in HCC, but occur more frequently in IHCC.[108–110] Activation of other growth factors including TGF-β,[111] IGF[112] and VEGF[113] are also involved in hepatocarcinogenesis. These genomic alterations, especially *JAK1*/*PIK3CA* mutations,[32] are potential therapeutic targets in liver cancer.

KEAP1–NFE2L2 pathway

The *NFE2L2* gene encodes a sequence-specific transcriptional factor that upregulates genes associated with oxidative stress and other metabolic pathways.[114] The level of the NFE2L2 protein is regulated by the ubiquitin-proteasome pathway, and KEAP1 functions as an E3 ubiquitin ligase. Activating missense mutations in the *NFE2L2* gene,[115] which disrupt direct NFE2L2–KEAP1 interaction, or inactivating mutations of the *KEAP1* gene are recurrently reported in HCC.[26,28] These alterations result in the accumulation of the NFE2L2 protein and promote aberrant activation of downstream genes that confer resistance to oxidative stress and induce metabolic transformation in cancer cells.[114,116]

NOTCH pathway

The role of the NOTCH cascade in solid tumours is controversial. Comparative functional genomics integrating transcriptome data from mice and human HCC samples indicate that NOTCH is activated in this cancer,[117,118] whereas other reports identified activation of NOTCH signalling as a suppressor feedback mechanism during HCC progression.[119,120] These contradictions suggest that biological activities of NOTCH signalling during hepatocarcinogenesis largely depend on the cellular contexts, as reported in other tumour types.[121]

Genomic changes during tumour progression

Midorikawa *et al.*[72] analysed copy number changes during multistep hepatocarcinogenesis and found that 1q21.3–44 gain and loss of heterozygosity on 1p36.21–36.32 and 17p13.1–13.3 were frequently observed in the early stage of HCC, whereas the combination of chromosomal gains on 5q11.1–35.3 and 8q11.1–24.3 and loss of heterozygosity on 4q11–34.3 and 8p11.21–23.3 are late molecular events in advanced HCC.

Roessler *et al.*[20] combined array comparative genomic hybridization and gene expression data in 76 HBV-positive HCCs and attempted to elucidate genomic signatures associated with tumour progression and the prognosis of patients. These authors found a substantial correlation between copy number aberration and gene expression. In particular, a cluster of six genes located on chromosome 8p were deleted in tumours from patients with a poor prognosis; these genes included *PROSC, SH2D4A* and *SORBS3*, which showed tumour suppressive activities, along with *DLEC1* (also known as *DLC1*), a known tumour suppressor gene.

Classification and prognosis prediction

In clinical settings, prognosis assessment and decisions regarding treatment are made on the basis of various tumour staging systems. The Edmondson–Steiner grading system has been applied to assess tumour aggressiveness in HCC, but data supporting its independent prognostic impact are quite weak.[122] Therefore, new approaches and methodologies are needed to develop independent prognostic and predictive tools that might finally assist the clinical decision-making process to further improve curative strategies in HCC.

Genomic profiling, such as gene expression profiling, has been applied to classify HCCs.[123,124] Copy number alterations have also been integrated for classification and therapeutic target identification.[125] In prognosis prediction, the expression pattern from the adjacent non-tumour tissue, which reflects "carcinogenic field effect",[126] was previously reported to correlate with patient survival.[127] A large collection of human HCC samples from patients undergoing curative resection was analysed by microarray profiling. A panel of five genes, including *TAF9, RAMP3, HN1, KRT19* and *RAN*, showed the strongest prognostic relevance and was selected for further analysis.[128] The five-genes score was further validated in an independent, large cohort and also increased its prognostic accuracy when combined with the expression pattern in non-tumour tissues as described above.[127]

Integrative genomic analysis with gene mutation profiles will enable us to elucidate the genetic and epigenetic mechanism of HCC for better classification and to construct a better scoring system for prognosis prediction and treatment selection.

Conclusions

As summarized in this Review, advances in sequencing technologies have enabled the examination of liver cancer genomes at high resolution. In addition to copy number changes and mutations, analyses have identified additional genome alterations, including structural alterations, HBV integration, and retrotransposon changes. Integrated analyses of trans-omics data (genome, transcriptome and methylome data) have identified multiple critical genes and pathways implicated in hepatocarcinogenesis.

These comprehensive genomic analyses have already identified many potential therapeutic targets in liver cancer, including growth factor signalling/kinases (MET, FGF9/FGFR, PIK3CA/AKT/mTOR and JAK/STAT), the NFE2L2-mediated oxidative pathway and chromatin modifying factors. Functional analysis of these targets and the identification of novel potential driver mutations, and the construction of *in vitro* and *in vivo* therapeutic models to evaluate new molecular-targeting compounds are necessary for effective translational research connecting basic molecular science to the clinic.

The aetiological factors associated with liver cancer (for example hepatitis infection, alcohol and obesity) are well known, and ethnic differences in the prevalence of this disease are prominent. However, the effect of these factors on the accumulation of somatic changes in the liver and the influence of ethnic variation in risk factors on the susceptibility to this tumour remain unknown. In this sense, the international collaboration of cancer genome sequencing projects, such as the International Cancer Genome Consortium (ICGC), will contribute to an improved understanding of this tumour.

Review criteria

We initially selected the articles by searching PubMed, COSMIC and OMIM with the following keywords: "HCC", "sequencing", "exome", "mutation", "CGH", "copy number", "methylation", and "HBV integration". We then searched the reference lists of the identified papers or the above databases by using specific keywords, including "TP53", "CTNNB1" and "RB" among others. Selected papers included mainly full-text papers, and abstracts if we could not access the full text. Only papers published in English were selected, with no publication date restrictions.

1. Jemal, A. *et al.* Global cancer statistics. *CA Cancer J. Clin.* **61**, 69–90 (2011).
2. Forner, A., Llovet, J. M. & Bruix, J. Hepatocellular carcinoma. *Lancet* **379**, 1245–1255 (2012).
3. El-Serag, H. B. Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology* **142**, 1264–1273 (2012).
4. Yu, J., Shen, J., Sun, T. T., Zhang, X. & Wong, N. Obesity, insulin resistance, NASH and hepatocellular carcinoma. *Semin. Cancer Biol.* **23**, 483–491 (2013).
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Fibroblast Growth Factor Receptor 2 Tyrosine Kinase Fusions Define a Unique Molecular Subtype of Cholangiocarcinoma

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Cholangiocarcinoma is an intractable cancer, with limited therapeutic options, in which the molecular mechanisms underlying tumor development remain poorly understood. Identification of a novel driver oncogene and applying it to targeted therapies for molecularly defined cancers might lead to improvements in the outcome of patients. We performed massively parallel whole transcriptome sequencing in eight specimens from cholangiocarcinoma patients without *KRAS/BRAF/ROS1* alterations and identified two fusion kinase genes, *FGFR2-AHCYL1* and *FGFR2-BICC1*. In reverse-transcriptase polymerase chain reaction (RT-PCR) screening, the *FGFR2* fusion was detected in nine patients with cholangiocarcinoma (9/102), exclusively in the intrahepatic subtype (9/66, 13.6%), rarely in colorectal (1/149) and hepatocellular carcinoma (1/96), and none in gastric cancer (0/212). The rearrangements were mutually exclusive with *KRAS/BRAF* mutations. Expression of the fusion kinases in NIH3T3 cells activated MAPK and conferred anchorage-independent growth and *in vivo* tumorigenesis of subcutaneous transplanted cells in immune-compromised mice. This transforming ability was attributable to its kinase activity. Treatment with the fibroblast growth factor receptor (FGFR) kinase inhibitors BGJ398 and PD173074 effectively suppressed transformation. *Conclusion: FGFR2* fusions occur in 13.6% of intrahepatic cholangiocarcinoma. The expression pattern of these fusions in association with sensitivity to FGFR inhibitors warrant a new molecular classification of cholangiocarcinoma and suggest a new therapeutic approach to the disease. (HEPATOLOGY 2014;59:1427-1434)

Cholangiocarcinoma (CC) is a highly malignant invasive carcinoma that arises through malignant transformation of cholangiocytes.[1] It is an intractable tumor with poor prognosis, whose incidence and mortality rates are high in East Asia and have been rapidly increasing worldwide.[1,2] CC can be subdivided into intrahepatic (ICC) and extrahepatic (ECC) types, which show distinct etiological and clinical features.[2] ICC is the second most common primary hepatic malignancy after hepatocellular carcinoma, and is associated with hepatitis virus infection. Somatic mutations of *KRAS* and *BRAF* are the most common genetic alterations in CC.[3,4] Surgical resection is the only curative treatment for CC, and no standard chemotherapy regimens have been established for inoperative cases or those showing recurrence after surgical resection.[5,6]

Abbreviations: CC, cholangiocarcinoma; ECC, extrahepatic cholangiocarcinoma; FGFR, fibroblast growth factor receptor; FISH, fluorescent in situ hybridization; ICC, intrahepatic cholangiocarcinoma; TKI, tyrosine kinase inhibitor.

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