Genetics and epigenetics of liver cancer

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Hepatocellular carcinoma (HCC) represents a major form of primary liver cancer in adults. Chronic infections with hepatitis B (HBV) and C (HCV) viruses and alcohol abuse are the major factors leading to HCC. This deadly cancer affects more than 500,000 people worldwide and it is quite resistant to conventional chemo- and radiotherapy. Genetic and epigenetic studies on HCC may help to understand better its mechanisms and provide new tools for early diagnosis and therapy. Recent literature on whole genome analysis of HCC indicated a high number of mutated genes in addition to well-known genes such as TP53, CTNNB1, AXIN1 and CDKN2A, but their frequencies are much lower. Apart from CTNNB1 mutations, most of the other mutations appear to result in loss-of-function. Thus, HCC-associated mutations cannot be easily targeted for therapy. Epigenetic aberrations that appear to occur quite frequently may serve as new targets. Global DNA hypomethylation, promoter methylation, aberrant expression of non-coding RNAs and dysregulated expression of other epigenetic regulatory genes such as EZH2 are the best-known epigenetic abnormalities. Future research in this direction may help to identify novel biomarkers and therapeutic targets for HCC.

Introduction
The most frequent primary liver cancers are hepatocellular carcinoma (HCC) and cholangiocarcinoma in adults, and hepatoblastoma in children. More than 80% of liver tumours are HCCs [1]. This review will focus primarily on HCC, one of the most frequent cancers worldwide with more than 500,000 new cases observed each year. Almost the same number of deaths is observed because of this cancer could not be easily treated. The most efficient treatment for HCC is liver transplantation, provided that it is detected early enough. Surgical removal and chemo-embolisation of tumour nodules are other alternatives. These tumours are usually resistant to chemo- or radiotherapy [1–3]. Targeted therapy of HCC is in its infancy. The only clinically relevant drug is a kinase inhibitor, Sorafenib, has only a modest effect on patient survival [4].

The aetiology of HCC is well known. Chronic liver injury associated primarily with hepatitis B (HBV) and C (HCV) virus infection constitutes the most important cause of HCC. Other factors, such as alcohol abuse and dietary exposure to aflatoxins, are also established causes, but their contribution to the disease aetiology is much less than the contributions of viral agents. The unprecedented increase in obesity rates in both developed and developing countries is a rising concern for HCC risk that may account for the unexpected increase in HCC incidence in the Western world [1].

Molecular mechanisms of hepatocellular carcinogenesis remain ill-defined, mainly due to disease heterogeneity. The heterogeneity of agents that cause chronic liver injury (HBV, HCV, aflatoxins and alcohol) and the ways they interact with the host DNA and epigenetic players are the most probable parameters contributing to HCC heterogeneity.

Chromosomal aberrations and hepatitis B virus integration into the host genome
Chromosomal aberrations such as deletions and copy number gains are frequent in HCC. Initial studies identified that HCC...
Since the discovery of TP53 as the first mutated gene in HCC over 20 years ago [10] and until very recently, only four genes were known to display frequent alterations in liver cancers. While TP53, CTNNB1 (encoding β-catenin) and AXIN1 genes usually display point mutations and small deletions, CDKN2A (encoding p16INK4a) undergoes homozygous deletions and epigenetic silencing [11,12].

During the past two years, the first reports of whole-genome or exome sequencing data for HCC have appeared [6,9,13]. This is the beginning of a new era of HCC genetics, because of the fact that these new techniques will allow the visualisation of the mutational landscape of HCC. Figure 1 shows a summary of primary findings gathered by ourselves from two recently published reports [6,9]. Each study first analysed a small set of tumours (n = 20–25) for a genome-wide search of somatically mutated genes; significantly mutated genes were then further tested for mutations using a larger set of tumours (n > 100).

A close examination of the data of Fig. 1 indicates that TP53 and CTNNB1 represent the two most frequently mutated genes. A second group of genes (AXIN1 and ARID1A) was found to present less frequent mutations, but still present in more than 10% of HCC samples studied. The third group is the largest with 22 genes displaying recurrent mutations in less than 10% of tumours. Guichard et al. [6] reported that Wnt/β-catenin, p53, P13K/Ras signalling, oxidative, endoplasmic reticulum stress pathways and chromatin remodelling were frequently affected by these mutations.

Whole genome sequencing allowed the detection of recurrent somatic mutations in several genes annotated as associated with chromatin regulation, such as ARID1A, ARID1B, ARID2, MLL, MLL3, BAZ2B, BRD8, BPTF, BRET and HIST1H4B. Notably, 14 out of the 27 tumours (52%) had either somatic point mutations or indels in at least one of these chromatin regulators. In both sets of experiments (whole genome sequencing and the validation sets), the number of indels in chromatin regulator genes was significantly higher than those in genes belonging to the other categories. This suggests that loss-of-function mutations are enriched in these chromatin-regulator genes in HCC genomes [9].

As shown in Table 1, the frequent mutations that identified so far in HCC are likely to result in loss of function with the notable exception of CTNNB1 mutations. It will be interesting to study why loss of function rather than gain of function of crucial genes is associated with HCC. By contrast, this pattern of mutation does not offer a broad spectrum of therapeutic intervention applications. Cancer cells can easily be targeted by blocking genes that are aberrantly overactive in these cells. The restoration of a lost gene activity to achieve a therapeutic intervention is difficult to achieve. Thus, although the genome-wide analyses have been very helpful in establishing the list of a large set of mutated genes in HCC, this will most probably serve diagnostic needs while the chance of their therapeutic use is more limited.

**Epigenetic deregulation**

Epigenetic regulation of gene expression involves DNA methylation, post-translational histone modifications, chromatin changes and non-coding RNAs that are often affected in cancer cells [14,15]. The role of epigenetic deregulation in HCC is being increasingly recognised [16]. In addition to changes in DNA methylation, microRNA expression, mutations affecting epigenetic regulatory genes have recently been discovered in HCC [6,9,13].

HCC cells display global hypomethylation as well as promoter hypermethylation of a large set of genes [17]. Promoter hypermethylation appears to affect mainly tumour suppressor and anti-proliferative genes resulting in downregulation of gene expression (Fig. 2). Aberrations in microRNA expression have also been observed with several of them being linked to metabolic and phenotypic changes in HCC cells [14,18–20].
Several genes encoding epigenetic regulatory proteins are involved in hepatocellular malignancy. The EZH2 (KMT6) encodes the catalytic component of the Polycomb Repressive Complex 2 (PRC2), creating the transcriptionally repressive H2K27Me3 histone mark which results in transcriptional silencing [21]. EZH2 is over-expressed in HCC and mostly associated with the progression and aggressive biological behaviour of HCC [22,23]. EZH2 protein silences Wnt pathway antagonists and constitutively activates Wnt/β-catenin signalling causing cell proliferation in HCC cells [24]. EZH2 also exerts a prometastatic function through epigenetic silencing of multiple tumour suppressor miRNAs including miR-139-5p, miR-125b, miR-101, let-7c and miR-200b [25]. Yang et al. identified an IncRNA called IncRNA-HEIH (High Expression in HCC) that associates with EZH2 to repress EZH2 target genes such as p16\textsuperscript{ink4a} and p21\textsuperscript{Cip1} in HBV-related HCC [26]. BMI1 is another PRC2 member overexpressed in HCC. Effendi et al. determined that BMI1 is upregulated in early and well-differentiated HCC and this expression correlates with ABCB1 expression [27].

Expression of histone deacetylases (HDACs) is deregulated in different cancers [28], and some of them are also deregulated in HCC. HDACs-1, -2 and -3 are over-expressed in HCC [29,30]. LC3B-II-induced inactivation of HDAC1 caused regression of HCC cell proliferation and triggered caspase independent autophagy. p21\textsuperscript{Cip1} and p21\textsuperscript{WAF1/Cip1} were selectively induced while cyclin D1 and CDK2 were suppressed by inactivation of HDAC1. As a result, HDAC1 inactivation resulted in hypophosphorylation of pRb in the G1/S checkpoint to inactivate E2F/DP1 transcriptional activity. Also, p21\textsuperscript{WAF1/Cip1} transcriptional activity was suppressed by

\begin{table}[h]
\centering
\caption{Most frequent gene mutations in hepatocellular carcinoma are predicted to lead to a loss-of-function.}
\begin{tabular}{|l|l|l|l|}
\hline
Genes & % mutation rates & Protein function & Known/expected outcome \\
\hline
TP53 & 35 & DNA damage response, other & Loss-of-function \\
CTNNB1 & 19 & Positive regulator of Wnt signalling & Gain-of-function \\
AXIN1 & 13 & Negative regulator of Wnt signalling & Loss-of-function \\
ARID1A & 12 & Chromatin remodelling & Loss-of-function \\
WWP1 & 9 & E3 ubiquitin ligase & Loss-of-function? \\
RPS6KA3 & 8 & Ribosomal protein S6 kinase & ? \\
ATM & 8 & DNA damage response & Loss-of-function? \\
ARID1B & 7 & Chromatin remodelling & Loss-of-function? \\
CDKN2A & 6 & Positive regulator of senescence & Loss-of-function \\
NFE2L2 & 5 & Redox homeostasis? & ? \\
IGSF10 & 5 & EGFR/ERB2 kinase inhibitor & Loss-of-function \\
ERRFI1 & 5 & ? & Loss-of-function \\
ARID2 & 5 & Chromatin remodelling & Loss-of-function? \\
\hline
\end{tabular}
\end{table}

**FIGURE 2**
The frequency of promoter methylation in hepatocellular carcinoma.
HDAC1 by interaction with an Sp1-binding site in the p21(WAF1/Cip1) promoter [31]. HDAC4 also suppresses the promoter activity of miR-200a and its expression and interacts with Sp1 in the miR-200a promoter to attenuate histone H3 acetylation levels. miR-200a represses HDAC4 expression through targeting the 3′-untranslated region of messenger RNA of HDAC4. In this respect, miR-200a has an ability to induce its own transcription and increase the levels of histone H3 acetylation at its promoter. Furthermore, miR-200a induces up-regulation of the levels of total acetyl-histone H3 and histone H3 acetylation in the p21(CIP1) promoter [32].

DNA methylating enzymes DNMT1, DNMT3A and DNMT3B are over-expressed in HCC compared to noncancerous liver samples [33,34]. Finally, CENPA expression was found to be significantly elevated in HCC tissues, and a positive correlation exists between CENPA expression and HBx COOH mutations in HCC tissues. HBx mutant increases the expression of CENPA mRNA [35].

**Future perspectives**

Recent advances in genome sequencing technologies will change radically our capabilities for fine mapping of hepatocellular cancer genomes. It is expected that patient tumours will be fully analysed in a short time at a moderate cost. Therefore, the genomic and epigenomic status of the patient’s own tumour will be a crucial element for decision making in terms of disease prognosis, therapeutic choices and prediction of patient survival. However, most of the known mutations observed in HCC are associated with a loss of function. Apparently, targetable genes found in other cancers such as growth factor receptors and intracellular protein kinases are not mutated at significant levels in HCC. Therefore, we need to find other targets for the treatment of liver cancers. Epigenetic characterisation of HCC has allowed the discovery of many epigenetic players in this disease. However, these studies are far from being complete. The rarity of targetable mutations in HCC justifies a systematic study of epigenetic changes to identify new targets for the therapy of this disease.

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