CANCER ORIGINS

Functional and genetic deconstruction of the cellular origin in liver cancer

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Abstract | During the past decade, research on primary liver cancers has particularly highlighted the uncommon plasticity of differentiated parenchymal liver cells (that is, hepatocytes and cholangiocytes (also known as biliary epithelial cells)), the role of liver progenitor cells in malignant transformation, the importance of the tumour microenvironment and the molecular complexity of liver tumours. Whereas other reviews have focused on the landscape of genetic alterations that promote development and progression of primary liver cancers and the role of the tumour microenvironment, the crucial importance of the cellular origin of liver cancer has been much less explored. Therefore, in this Review, we emphasize the importance and complexity of the cellular origin in tumour initiation and progression, and attempt to integrate this aspect with recent discoveries in tumour genomics and the contribution of the disrupted hepatic microenvironment to liver carcinogenesis.

Liver cirrhosis
A slow and progressive replacement of healthy liver tissue by fibrotic scar tissue, with subsequent loss of organ function. Cirrhosis occurs as the common end point of the majority of chronic liver diseases.

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doi:10.1038/nrc4017

The two major types of primary liver cancer (PLC) — that is, hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA) — comprise more than 1 million newly diagnosed cases per year, which renders liver cancer an increasing global health care problem[1]. These malignancies are the second most common cause of cancer-related death after lung cancer and are among the few solid tumours (others are melanoma and pancreatic carcinoma) that are increasing in incidence and mortality rates worldwide. The high mortality stems from the lack of suitable biomarkers for early detection, inadequate understanding of the molecular features and genomic traits, and resistance to chemotherapy. Most importantly, aggressive treatment strategies for liver cancer are commonly limited, as most patients have liver cirrhosis and severely compromised liver function. HCC is the most common type of PLC, and is characterized by both phenotypic and molecular heterogeneity[1]. Globally, HCC is the fifth most common cancer in men and the seventh most common in women, and accounts for at least 700,000 deaths worldwide annually[2]. The major aetiological agents responsible for chronic liver disease, cirrhosis and, ultimately, HCC are known and well characterized (for example, infection with hepatitis B virus (HBV) and hepatitis C virus (HCV) as well as ethanol abuse) (BOX 1). Progress in antiviral therapy for chronic HCV infections has led to viral clearance in more than 90% of patients[3]. Despite the substantial impact on global health care, whether this will reduce the incidence of liver cancer, in particular in patients with cirrhosis, is yet unknown and will probably require at least two decades of follow-up to become apparent. Other aetiological factors include non-alcoholic fatty liver disease and other metabolic disorders (for example, diabetes) that are increasing in incidence in Western countries and induce high numbers of cases of HCC without underlying cirrhosis[4]. CCAs are best classified according to their anatomical location as intrahepatic (iCCA), perihilar (pCCA) or distal (dCCA). Incidence rates for CCA show high geographical variation, which is partly related to variations in risk factors. Whereas chronic infection with hepatobiliary flukes (for example, *Opisthorchis viverrini* and *Clonorchis sinensis*) is most frequently observed in Southeast Asia, risk factors for iCCA in Western countries are less well defined. However, an increased risk for patients with chronic viral hepatitis (HBV and HCV) and cirrhosis has recently been noticed[5,6].

Despite differences in aetiological agents, both HCC and CCA are inextricably linked to chronic liver damage. As a result of the constant inflammatory processes, cell damage and high cellular turnover are induced, which results in constant error-prone chronic repair processes as well as selective pressure on the healthy and newly formed hepatocytes. This marked disruption of the hepatic microenvironment creates a pro-oncogenic milieu that promotes malignant transformation (FIG. 1). The well-recognized phenotypic and genetic heterogeneity of PLC is therefore

a reflection of the combined effects of the disrupted liver microenvironment and the cellular origin of the cancer (BOX 1). Despite overlapping risk factors, the histological appearance of and therapeutic strategies for HCC and iCCA are vastly different. Whereas HCC generally displays a solid growth pattern with little or no tumour stroma, the major phenotypic hallmark of iCCA is a marked tumour stroma that comprises up to 60% of the tumour tissue[6,7]. A better understanding of the molecular mechanisms and aetiological factors that drive HCC and iCCA development is imperative for improvement of therapeutic options. Generally, carcinogenesis might be the consequence of the induction of genetic and/or epigenetic alterations in different cell types: that is, stem and progenitor cells or terminally differentiated cells that acquire stemness features[8]. The relative contribution of each cell type to tumorigenesis may depend on diverse factors such as inflammation, structural changes in the hepatic microenvironment and the principal oncogenic agents.

In this Review, we summarize the mutational landscape of the major categories of PLC, highlight the importance of the cellular origin of liver cancer and discuss the outstanding challenges and therapeutic opportunities in the context of precision medicine.

Precision medicine
A form of treatment that focuses on the individual factors of a disease and uses next-generation technologies to improve therapy.

Mutational landscape of PLC

Development of PLC progresses as a branched multistage process via sequential acquisition of genetic[9–12] and morphological[13] traits. The spectrum of liver tumours not only depends on the cellular origin of the malignancy but also involves a broad range of genome aberrations that ultimately drive neoplastic transformation and growth[10,14,15]. Current evidence strongly suggests that PLCs, in particular iCCA and HCC, share several copy number changes and somatic mutations, as well as global epigenetic changes. The identification of core oncogenic signatures[16] further indicates that iCCA and at least a subgroup of HCCs are closely related at the molecular level. Indeed, a close genomic similarity between iCCA and a subset of HCCs with progenitor cell characteristics and poor outcome was shown in several recent studies[17–19]. Moreover, genomic[20,21] and genetic[22,23] analyses of the rare mixed HCC–iCCA lesions showed shared molecular characteristics with, and alterations similar to, both classic iCCA and classic HCC tumours, which suggests that the acquisition of CCA-like transcriptomic traits has a crucial role in the heterogeneous progression of liver tumours. Overall, the genomic landscape of PLCs is complex and is often further complicated by an association with the diverse aetiological features, which precede disease onset by decades. For PLCs, frequent alterations are known to occur in key cancer genes such as *TP53*, *WNT*, *CTNNB1* (which encodes β-catenin), and cell cycle-related genes such as *CCND1* (which encodes cyclin D1) and *CDKN2A* (cyclin-dependent kinase inhibitor 2A). Recently, sequencing approaches have emphasized the importance of early genetic events that affect telomere maintenance (telomerase reverse transcriptase (*TERT*)), epigenetic mechanisms, chromatin modifiers and inflammatory pathways, as well as RNA editing, which present novel therapeutic opportunities for clinical advancement (FIG. 2; TABLE 1).

Sequential evolution of HCC. Clonal branching is probably a key source of genetic heterogeneity in HCC. The full range of lesions found in the liver is complex and remains difficult to study because it requires access to low-grade and high-grade dysplastic nodules, and early and advanced HCCs[7]. Although these lesions are morphologically distinct, it is largely unknown which of the dysplastic and early lesions are persistent and can transform into cancer, and thus require clinical attention, and which alternatively undergo remodelling back into the liver parenchyma. Detailed information about the genetic aberrations that drive this pre-neoplastic conversion is limited.

Several studies have shown that early dysplastic nodules present a relatively uniform genome with little genetic variation (five coding single-nucleotide variants) compared with advanced tumours in which there is extensive molecular heterogeneity or genomic instability (a range of 72–180 mutations)[9,24,25]. The importance of two oncogenic events during hepatocarcinogenesis has emerged: *MYC* activation and *TERT* activation[9,26–28]. Both genes are activated in a range of liver tumours. *MYC* may be of particular importance for the malignant transformation of hepatocytes into HCC (a late event), whereas activation of *TERT* is required for unlimited proliferation. Consistent with an early transforming event, a progressive increase in genetic alterations in the *TERT* locus was

Box 1 | Ethnic, aetiological and geographical diversity of PLCs

Primary liver cancers (PLCs) comprise a heterogeneous group of benign and aggressive solid tumours characterized by ethnic, aetiological and geographical diversity. The two major forms of PLC are hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (iCCA)[142]. Both tumour entities have distinct risk factors but also share common predisposing features. A hallmark of both cancers is chronic inflammation of the liver microenvironment that precedes tumour development[13,54]. Therefore, the aetiological agent and the subsequent type of inflammation as well as the target cell of malignant transformation play an important part in the genesis of the different tumour entities.

HCC is one of the fastest growing causes of cancer-related deaths worldwide, and there is clear male dominance (male/female ratio is 3–5/1)[2]. The highest incidence rates are observed in sub-Saharan Africa and Asia, and are related to chronic infection with hepatitis B virus (HBV). Other major risk factors are chronic hepatitis C virus (HCV) infection, alcoholic liver disease as well as non-alcoholic fatty liver disease and metabolic disorders (for example, haemochromatosis). More than 85% of HCCs develop on the basis of advanced hepatic fibrosis and/or cirrhosis. The cumulative risk of HCC development is significantly influenced by the underlying aetiological condition, in increasing order of incidence: HCV>haemochromatosis>HBV>alcoholic or non-alcoholic fatty liver disease[143].

iCCA is the second most common PLC, and there has been a steady increase in incidence rates and mortality over the past two decades[6]. There is less gender disparity than for HCC (male/female ratio is 1.2–1.5/1), but there are significant racial and ethnic differences, with highest susceptibility noted for Hispanic, followed by Asian, Caucasian and Black ethnicities[144]. Significant differences in incidence rates relate to different risk factors. The highest rates are observed in northeast Thailand, owing to hepatobiliary flukes (for example, *Opisthorchis viverrini* and *Clonorchis sinensis*)[145]. Other risk factors are primary sclerosing cholangitis, biliary tract cysts and chronic biliary inflammation. Recently, factors commonly associated with HCC development — for example, cirrhosis, chronic HBV and HCV infection, obesity, diabetes and alcohol — have been shown to significantly influence iCCA development[144].

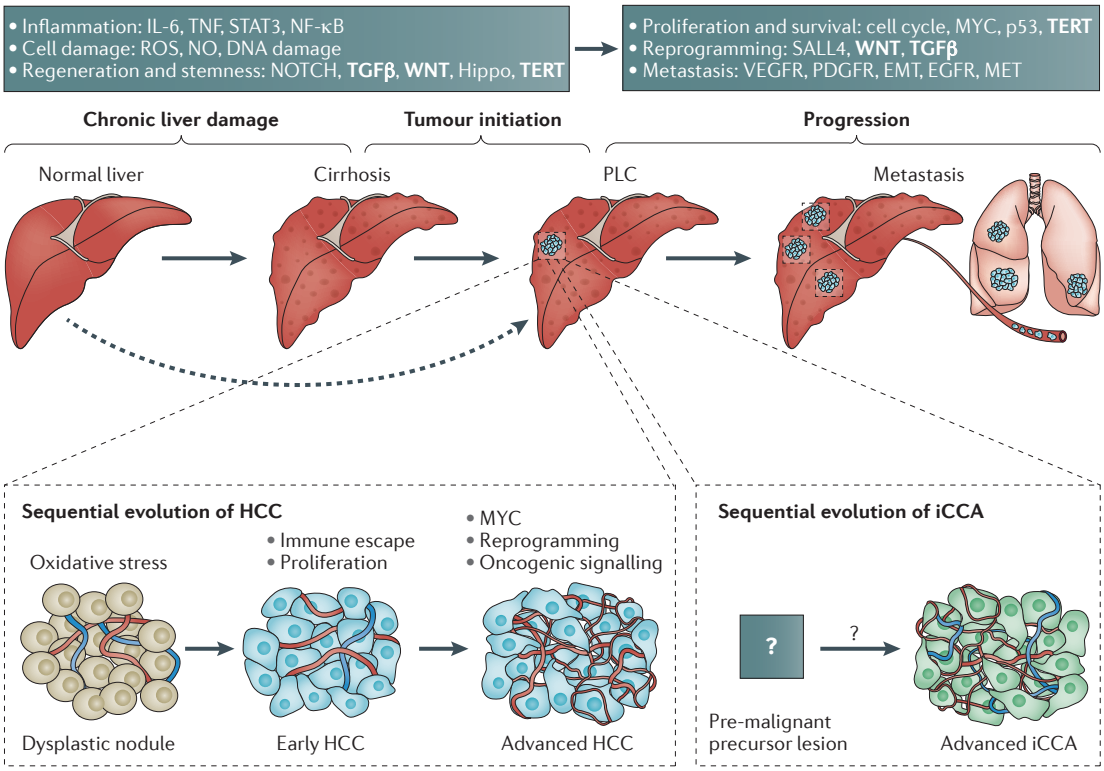

Figure 1 | **Sequential evolution of PLC.** The common end stage of the majority of chronic liver diseases is the development of liver cirrhosis. Therefore, the chronically altered liver microenvironment induced by cirrhosis can be considered a necessary predisposing factor for cancer development in the vast majority of primary liver cancers (PLCs). The dashed arrow indicates that a minority of tumours arise from a normal liver and develop into PLC without cirrhosis. In hepatocellular carcinomas (HCCs), a multistep process is characterized by the occurrence of morphologically distinct stages: dysplastic nodules, early HCC and advanced HCC. Concomitant with disease progression, increased neovascularization is observed and is reflected in the typical hypervascular pattern in imaging (lower panel; veins are depicted in blue, and arteries are depicted in red). For intrahepatic cholangiocarcinoma (iCCA), the sequential evolution and underlying phenotypic features are not well known. Detailed characterization of the molecular events driving chronic liver damage and the development and progression of PLCs, and a thorough molecular risk stratification of pre-malignant lesions to complement the histological classification remain elusive. However, several overlapping molecular pathways that are closely linked to both initiation and progression of liver cancer have been identified (shown in bold in the top panel). These include the transforming growth factor-β (TGFβ), WNT and telomerase reverse transcriptase (TERT) pathways. EGFR, epidermal growth factor receptor; EMT, epithelial–mesenchymal transition; IL-6, interleukin-6; NF-κB, nuclear factor-κB; NO, nitric oxide; PDGFR, platelet-derived growth factor receptor; ROS, reactive oxygen species; SALL4, Sal-like protein 4; STAT3, signal transducer and activator of transcription 3; TNF, tumour necrosis factor; VEGFR, vascular endothelial growth factor receptor.

determined in 6% of 32 cases of low-grade dysplastic nodules, 19% of 16 high-grade dysplastic nodules and 61% of 23 early HCCs[28].

The progressive accrual of genetic alterations at late-stage carcinogenesis causes substantial deregulation of diverse signalling pathways, such as transforming growth factor-β (TGFβ), PI3K and AKT, which affects oncogenic driver genes such as those encoding NOTCH2, Janus kinase 1 (JAK1), insulin-like growth factor 2 (IGF2) and matrix metalloproteinases (MMPs). This supports the notion that cancer onset is a result of multiple genomic alterations[9]. In a study following progression of the disease from background liver cirrhosis to the development of HCC in eight individuals with HBV-associated disease, only one recurrent somatic variant was detected in the gene *IGFALS* (IGF-binding protein, acid-labile subunit)[9]. This mutation causes a gradual loss of function of IGFALS that is predicted to activate IGF signalling and thereby promote HCC progression. Previously, activation of the IGF axis was shown in a subset of patients with HCC whose tumours also demonstrated an association with mTOR signalling[29]. Importantly, an integrative analysis of 12 individual cases of early and advanced HCCs revealed that a core set of 48 genes were mutated in advanced HCCs and that this set was enriched for genes in the WNT–β-catenin pathway[30]. This aberrant regulation further led to enrichment of the epidermal growth factor receptor (EGFR), TGFβ and Hippo pathways. By comparison, early lesions were characterized by activation of genes involved in metabolic functions. Intriguingly, analysis by whole-genome sequencing of two multicentric HCCs showed no common variants, which supports late-stage acquisition of mutations[16]. However, the genome substitution patterns among the tumours were

Multicentric HCCs
(Multicentric hepatocellular carcinomas). More than one independent tumour with different clonal origins detected in the liver.

Genome substitution patterns
Patterns of nucleotide substitution across the whole tumour genome. Characteristic patterns can be detected in different tumours and/or aetiological backgrounds.

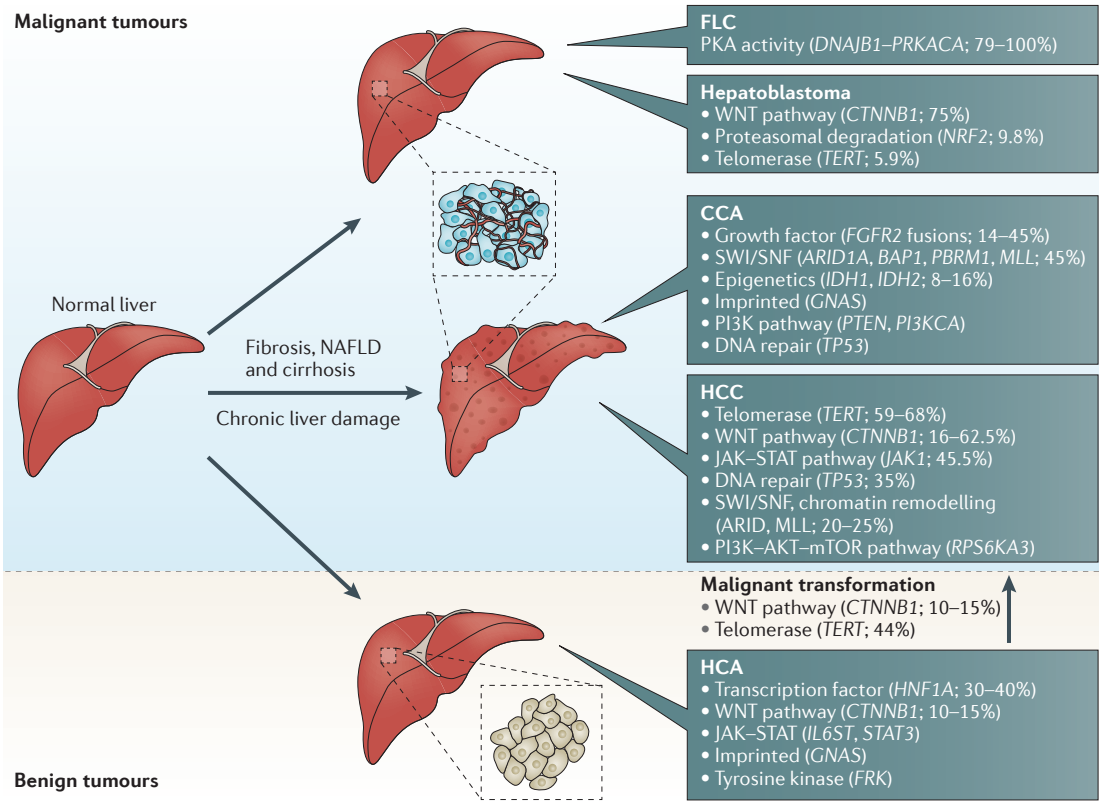

Figure 2 | **The landscape of genetic alterations in PLC.** The schematic illustrates the prevalent mutational landscape and affected pathways in benign and malignant primary liver cancers (PLCs). The majority of malignant hepatic cancers originate on a background of chronic liver damage and fibrosis; non-alcoholic fatty liver disease (NAFLD) and cirrhosis are among the key initiating risk factors for hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA). However, malignant liver tumours that occur in adolescence, such as hepatoblastoma and fibrolamellar hepatocellular carcinoma (FLC), as well as most benign hepatocellular adenomas (HCAs), develop in a non-cirrhotic liver. The earliest genetic variant occurs in the telomerase reverse transcriptase (*TERT*) gene, which has been demonstrated in hepatoblastoma, HCC and during malignant transformation of HCA, which suggests that alterations in *TERT* are common molecular events predisposing to these malignancies in some cases. ARID, AT-rich interactive domain; *BAP1*, BRCA1-associated protein 1; *FGFR2*, fibroblast growth factor receptor 2; *FRK*, FYN-related SRC family tyrosine kinase; *HNF1A*, hepatocyte nuclear factor 1α; IDH, isocitrate dehydrogenase; *IL6ST*, interleukin-6 signal transducer; JAK, Janus kinase; MLL, mixed-lineage leukaemia; *NRF2*, nuclear factor erythroid 2-related factor 2; *PBRM1*, polybromo 1; PKA, protein kinase A; *RPS6KA3*, ribosomal protein S6 kinase α3; STAT, signal transducer and activator of transcription.

similar, which suggests that, although each tumour developed from an independent clone, the mutagenic agents were similar.

The range of genetic alterations in advanced HCCs. In the liver, several mutagens, such as chemical exposure, oxidative stress and variation in the DNA repair and damage response, can cause genome substitutions, resulting in a heterogeneous cancer genome. The first deep-sequencing study in liver cancer, in 2011, was of a Japanese male diagnosed with HCV-positive HCC, and it found a predominance of C>T/G>A and T>C/A>G transitions in the tumour genome[15]. Similar dominant genome substitutions were found in HBV-related cases. Notably, HBV integrations induce chromosomal instability, which subsequently increases during tumour progression[31]. The substitution C>T/G>A is predominant at CpG sites, which may cause the change in the methylation profile that is found in many cancers. In HCCs ($n = 27$ cases) of mixed aetiology, there was a prevalence of T>C/A>G transitions and C>A/G>T transversions, suggesting that this is a unique genetic signature in liver cancer[16]. Furthermore, classification of tumours in six different classes based on their mutational substitution patterns successfully divided the patients according to the major aetiological, demographic and molecular features: for example, aflatoxin B1 exposure, and alcohol and tobacco consumption[24]. Notably, among 30 different cancers, malignancies in the liver show the highest number of distinct mutational signatures[32].

The mutational landscape of HCC was initially defined in 488 tumour samples, which revealed 30 candidate driver genes representing 11 core networks[33]. Besides implicating *TERT* activation (for example, by hotspot mutations and amplifications) as a central factor in at least 68% of cases, the authors also identified alterations that are important for metabolism, chromatin remodelling and mTOR signalling. Several exome

Cellular reprogramming
Conversion of somatic cells to a more stem-like state or to a different developmental lineage.

capture-based studies estimated the genetic variation in advanced HCC from different aetiological backgrounds[10,14,33–37]. A later study[24] used whole-exome sequencing to analyse 243 HCCs and showed that 161 predicted driver genes affect 11 networks. Interestingly, these authors identified mutational signatures unique to the aetiological factors associated with the disease, such as *CTNNB1*, *CDKN2A* and the SWI/SNF chromatin-remodeller genes AT-rich interactive domain 1A (*ARID1A*) and *SMARCA2* (related to alcohol) or *TP53* (related to HBV). Importantly, 28% of the HCC cases investigated harboured genetic variants in genes potentially targetable with known clinically approved drugs.

Several studies have evaluated the importance of HBV integration as an HCC-promoting event[16,38–41]; the frequent and recurrent integration sites at the *TERT* locus support the hypothesis that this may confer a growth advantage in early-stage hepatocarcinogenesis[16]. Sequencing of the *TERT* promoter in 305 cases identified mutations in 25% of early cirrhotic nodules, 44% of adenomas and 59% of HCCs[12]. Interestingly, genetic changes in *TERT* were also associated with activating *CTNNB1* mutations[12], an alteration previously associated with HCV-related cases.

Alterations central to HCC formation include those that occur in genes that are primarily related to four oncogenic networks: metabolic processes, WNT–β-catenin signalling, chromatin modification and PI3K–AKT–mTOR activation[33]. Several studies have emphasized the importance of mutations affecting chromatin-modifying factors, particularly members of the SWI/SNF protein complex[42], including prevalent inactivating mutations as well as homozygous deletions in the ARID family components (*ARID1A*, *ARID1B* and *ARID2*), which suggests that these factors act as tumour suppressor genes[10,14,16,36]. This complex functions as a rheostat that alters the nucleosome organization and is involved in key biological processes such as DNA repair, proliferation, differentiation and cellular reprogramming. In HCV-related tumours, approximately 18% of HCCs from Western countries were found to harbour *ARID2* mutations, including co-occurring genetic variants in *CTNNB1* (REF. 14). This relationship is, however, mutually exclusive with *TP53* mutations, which strongly correlate with HBV infection. A high incidence of mutations in epigenetic regulators such as lysine methyltransferase 'writers' including mixed-lineage leukaemia 1 (*MLL1*; also known as *KMT2A*), *MLL3* (also known as *KMT2C*) and *MLL4* (also known as *KMT2B*), and 'readers' (for example, bromodomain PHD finger transcription factor (*BPTF*) and ring finger protein 20, E3 ubiquitin protein ligase (*RNF20*)) have been proposed to account for up to 50% of all tumours[16]. In a cohort of 87 HCCs, genetic variants in the MLL family were found in 20% of patients (*MLL4* alone was mutated in 7% of cases)[35]. Alterations in chromatin-modifying genes have been observed in 25% of tumours related to alcohol consumption[10].

Integration of whole-exome sequencing with copy number alterations identified 994 somatic mutations with predicted functional consequences in the chromatin-modifier gene *ARID1A*, mTOR signalling (ribosomal protein S6 kinase α3 (*RPS6KA3*)), metabolic pathways (nuclear factor erythroid 2-related factor 2 (*NRF2*; also known as *NFE2L2*)) and interferon regulatory factor 2 (*IRF2*)[10]. Inactivation of IRF2 was found exclusively in HBV-related tumours associated with impaired p53 function. The role of NRF2 is to regulate factors involved in metabolic processes and oxidative stress. Activating mutations in NRF2 or its E3 ubiquitin ligase Kelch-like ECH-associated protein 1 (KEAP1) disrupt this control mechanism, which results in increased NRF2 levels and deregulation of downstream genes that promote metabolic transformation and resistance to stress. In total, the network most affected in HCC (which is found in more than 60% of cases) involves genes associated with WNT–β-catenin signalling[11]. Alterations are most frequently found in *CTNNB1* but also occur in adenomatous polyposis coli (*APC*) and *AXIN1*, two tumour suppressor genes that negatively coordinate β-catenin levels. Deregulation of WNT–β-catenin signalling occurs in a mutually exclusive manner and results in uncontrolled growth by activation of downstream MYC and cyclin D1.

RNA editing is also an important event that influences hepatocarcinogenesis[43–45]. Adenosine-to-inosine editing of *AZIN1* (antizyme inhibitor 1) triggered by ADAR1 (adenosine deaminase acting on RNA 1) in HCC caused conformational changes in the AZIN1 protein that led to increased tumour-initiating potential[44]. This gain-of-function activity, which increases the stability of AZIN1, may promote cell proliferation, probably by targeting cyclin D1 in the G1/S phase of the cell cycle, and thus contributes to increased transcriptome heterogeneity by RNA editing. Patients with cirrhosis who have deregulated levels of ADAR1 and ADAR2 have an increased risk of a poor clinical outcome, including progression to HCCs[43] that have altered gene-specific editing.

Cholangiocarcinoma. CCA is a rare hepatic malignancy in the intrahepatic and extrahepatic biliary ducts with a complex molecular landscape[13] (FIG. 3). Tumours in the interlobular ductules are designated iCCA. Although iCCAs often share aetiological factors with HCC, iCCA presents a distinct molecular pathobiology[46].

Progress in understanding the heterogeneous nature of iCCA genomes has recently been achieved through transcriptome[17,19] and epigenome[18,47] studies. Assessment of somatic mutations in iCCA[48–53] has shown high genetic heterogeneity, with unique prevalence of mutations in key genes that differ from those in HCC; the genetic differences probably depend on aetiology as well as on ethnicity and gender (BOX 1). Within iCCA, 39.8% of tumours associated with *O. viverrini* infection[51] had *TP53* mutations compared with 9.3% of tumours that were not related to liver fluke infection[48]. This difference in the genetic landscape between fluke-related and non-fluke-related iCCA is not exclusive to *TP53* but also applies to *SMAD4*. Conversely, *MLL3*, *GNAS* and *BAP1* (BRCA1-associated protein 1) show prevalent genetic alterations in cases dependent on risk factors other than flukes. Like HCC, iCCA

Table 1 | **Major functional signalling pathways and molecules in PLC**

Functional process	Signalling pathway	Associated molecules	Tumour type	Mode of action in tumours	Phenotypic and tumour features	Refs
Cell cycle	p53 and RB–E2F	p53, CDKN2A, CDKN2B, cyclin D1, cyclin E1, CDKs, ATM and RB1	HCC and iCCA	Inhibition	Loss during tumour progression, aggressive phenotype and loss of DNA damage repair mechanisms	8,10,46
Development and differentiation	WNT–β-catenin	β-catenin, AXIN1, AXIN2 and APC	HCC	Activation	Genomic stability and activation in tumour-initiating cells (early and late stages)	10,46, 119
	TGFβ	SMADs	HCC	• Early stage: inhibition • Late stage: activation	Poor prognosis, metastasis and activation in tumour-initiating cells	30,114
	NOTCH	NOTCH1–3, JAG1, NICD and DLL	iCCA	Activation	Cell fate and stem cell features	88,90, 119
	Hippo–YAP	YAP and MST1 or MST2 (growth control)	HCC	Activation	Stem cell features, tumour initiation and chemoresistance	78–80
	NF2	YAP and EGFR (growth control)	HCC	Inhibition	Stem cell features and tumour initiation	81,82
	SALL4	NuRD complex and PTEN	HCC	Activation	Development and progression, poor prognosis and activation in tumour-initiating cells	103
Proliferation and survival	IGF	IGF1R, IRF2, IRS, SHP and PI3K	HCC	Activation	Pre-neoplastic lesions (early stage)	8,27
	HGF–MET	SH2, MAPK, MEK and ERK	HCC	Activation	Metastatic potential and invasion	97
	EGFR	AKT, STATs and RAS–RAF	HCC and iCCA	Activation	Aggressive phenotype and reprogramming	59,97, 122,123
	PI3K–mTOR	PI3Kα, AKT, RPS6KA3, PTEN, TSC1, RAPTOR and RICTOR	HCC	Activation	Poor prognosis, poorly differentiated tumours and earlier recurrence	10,27, 33
Angiogenesis	VEGF	VEGFR and HIF1α	HCC	Activation	Aggressive phenotype, poor prognosis and metastasis	2,8,28
	FGF	FGF19, FGFRs and SHP2	HCC and iCCA	Activation	Tumour development or progression	56,59
		FGFR2 fusions	iCCA	Activation	Tumour progression and sensitivity to FGF inhibitors	52, 56–59
	PDGF	ROS, PI3K, STAT3 and MMPs	HCC	Activation	Liver cirrhosis and tumour development	114
Immune response	NF-κB	IκB, IKK, NEMO, p65 and IL-20	HCC	Activation	Chronic inflammation and tumour progression	111–116
	TWEAK–FN14	NOTCH and WNT	HCC	Activation	Tumour-initiating cells and cell fate decision	117,118
	IL-6 signalling	STAT3, LIN28, IL-6R, IL-6 and JAK1	HCC	Activation	Progenitor-derived response to adjuvant interferon therapy	106–114
Post-transcriptional modifications	RNA editing	AZIN1, ADARs and ODC	HCC	Alteration	Cirrhosis, tumour development and recurrence, and poor prognosis	44,45

Table 1 (cont.) | **Major functional signalling pathways and molecules in PLC**

Functional process	Signalling pathway	Associated molecules	Tumour type	Mode of action in tumours	Phenotypic and tumour features	Refs
Genome maintenance	Chromatin remodelling	ARID1, ARID2, MLL, BAP1 and EZH2	HCC and iCCA	Activation	Frequently mutated SWI/SNF and poor prognosis	10,14, 28,33–37, 50,54
	Epigenetics	IDH1 and IDH2	iCCA	Abnormal activity	Methylation status and overall survival	50,98
	Telomere stability	TERT	HCC	Activation	Earliest genetic changes, associated with activation of WNT–β-catenin	9,12, 24–26, 33,38
Stress response involving mitochondria	Oxidative response	NRF2, KEAP1 and CUL3	HCC and iCCA	Activation	Oxidative phosphorylation and tumour progression (late stage)	10,15, 33,53

ADAR, adenosine deaminase acting on RNA; APC, adenomatous polyposis coli; ARID, AT-rich interactive domain; ATM, ataxia telangiectasia mutated; AZIN, antizyme inhibitor; BAP1, BRCA1-associated protein 1; CDK, cyclin-dependent kinase; CDKN, CDK inhibitor; CUL3, cullin 3; DLL, delta-like ligand; EGFR, epidermal growth factor receptor; EZH2, enhancer of zeste homologue 2; FGF, fibroblast growth factor; FGFR, FGF receptor; HCC, hepatocellular carcinoma; HGF, hepatocyte growth factor; HIF1α, hypoxia-inducible factor 1α; iCCA, intrahepatic cholangiocarcinoma; IDH, isocitrate dehydrogenase; IκB, inhibitor of nuclear factor-κB; IGF, insulin-like growth factor; IGF1R, IGF1 receptor; IKK, IκB kinase; IL, interleukin; IL-6R, IL-6 receptor; IRF2, interferon regulatory factor 2; IRS, insulin receptor substrate; JAG1, jagged 1; JAK1, Janus kinase 1; KEAP1, Kelch-like ECH-associated protein 1; MLL, mixed-lineage leukaemia; MMP, matrix metalloproteinase; MST, mammalian STE20-like protein kinase; NF2, neurofibromin 2; NF-κB, nuclear factor-κB; NICD, NOTCH intracellular domain; NRF2, nuclear factor erythroid 2-related 2; NuRD, nucleosome remodelling and histone deacetylase (also known as Mi-2); ODC, ornithine decarboxylase; PDGF, platelet-derived growth factor; RAPTOR, regulatory-associated protein of mTOR; RICTOR, rapamycin-insensitive companion of mTOR; ROS, reactive oxygen species; RPS6KA3, ribosomal protein S6 kinase α3; SALL4, Sal-like protein 4; SH2, SRC homology 2; SHP, small heterodimer partner (also known as NR0B2); STAT, signal transducer and activator of transcription; TERT, telomerase reverse transcriptase; TGFβ, transforming growth factor-β; TSC1, tuberous sclerosis 1 protein; TWEAK, tumour necrosis factor-like weak inducer of apoptosis; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; YAP, Yes-associated protein.

has high genomic instability and often presents with prevalent mutations in chromatin-remodelling factors such as the SWI/SNF complex and key epigenetic factors such as isocitrate dehydrogenase 1 (IDH1) and IDH2 (REF. 50). Mutant IDH increases intracellular levels of the metabolite 2-hydroxyglutarate, affecting α-ketoglutarate-dependent enzymes, which regulate diverse cellular processes, including histone and DNA modifications. Approximately 10% of iCCAs contain mutated IDH, leading to increased 5-methylcytosine enrichment on CpGs upstream of transcription start sites, which suggests a global deregulation of promoters[47]. Inactivating recurrent mutations were found in multiple chromatin modifiers, including the most prevalent, such as *BAP1* (in 25% of iCCAs examined; $n = 32$), *ARID1A* (19%) and *PBRM1* (polybromo 1; 17%)[50]. In total, 47% of iCCAs were found to have at least one somatic variant in a chromatin-modifying gene, suggesting that the epigenetic landscape undergoes cumulative changes, which probably affect global gene expression[54]. Recently, 103 tumours obtained from Chinese patients with iCCA were shown to have a preference for C>T/G>A transitions[53]. This substitution is similar to the change observed in the majority of other tumours and commonly affects CpG sites. Besides a few genes in common with HCC (*TP53* and *ARID1A*) and three known iCCA drivers (*KRAS*, *IDH1* and *PTEN*), the study revealed only three novel putative driver genes — epiplakin 1 (*EPPK1*), endothelin-converting enzyme 2 (*ECE2*) and the SRC family tyrosine kinase gene *FYN*. Importantly, several somatic mutations that involve the oxidative phosphorylation pathway were recently observed in mitochondrial genes in iCCA, which suggests a possible contribution of the Warburg effect to iCCA progression[53,55].

One of the most prevalent genetic alterations in iCCA is the formation of fibroblast growth factor receptor 2 (*FGFR2*) gene fusions, which include *FGFR2–BICC1* (REF. 56), *FGFR2–KIAA1598* (REF. 57), *FGFR2–TACC3* (REF. 57), *FGFR2–AHCYL1* (REF. 58), *FGFR2–MGEA5* (REF. 59) and *FGFR2–PPHLN1* (REF. 52). Several of the fusion products increase cellular transformation, which results in tumour growth *in vivo*. Importantly, *FGFR2* gene fusions represent actionable alterations, which can be inhibited by targeting FGFR2 kinase activity. Moreover, although FGFR2 fusion products are observed at the relatively high incidence of 13.6% (9 of 66) to 44.9% (48 of 107) in patients with iCCA[52,56–59], they have not been detected in HCC (0 of 100)[52] and have been detected only infrequently in mixed HCC–iCCA (1 of 21)[52], suggesting that these fusion products could be used to improve diagnosis[60].

Rare liver tumours. Rare adult and paediatric liver tumours are poorly understood, but evidence suggests that they arise mostly on a background of histologically normal liver[61], which is contrary to classic PLCs, which typically arise on a background of cirrhotic liver disease. Consequently, tumour genomes in these entities are relatively stable and have few genetic alterations (FIG. 2).

Hepatocellular adenoma (HCA) is a rare, usually hormone-induced, monoclonal benign lesion that develops in non-cirrhotic livers and infrequently undergoes neoplastic conversion. HCA occurs mostly in women

of childbearing age (15–45 years of age) and is associated with the use of oestrogen-based oral contraceptives. Other risk factors associated with HCA are anabolic and androgenic steroids, barbiturate and clomiphene use and glycogen storage disease, a disorder that is more frequent in adolescent men than in women (ratio 2/1). In contrast to other hepatic tumours, HCA lesions have been found to harbour a relatively low average rate of 7.5 somatic changes per tumour, including recurrent alterations in only four genes (*CTNNB1*, *IL6ST* (interleukin-6 signal transducer), *HNF1A* (hepatocyte nuclear factor 1α) and *FRK* (FYN-related SRC family tyrosine kinase))[62], which may be related to their more benign nature. HCA can be classified into four morphological and molecular subsets[63]. Inflammatory HCAs (I-HCAs; 40–50%) present with activating mutations in *IL6ST*, *GNAS* and signal transducer and activator of transcription 3 (*STAT3*), which lead to activation of JAK–STAT signalling. HNF1α-mutated HCAs (H-HCAs; 30–40%) are characterized by recurrent somatic loss of heterozygosity at chromosome 12q, which results in a biallelic variant of inactivated *HNF1A* (at locus 12q24.2). β-catenin-mutated HCAs (b-HCAs; 10–15%) frequently include activating mutations in exon 3 of *CTNNB1*. This subset is prone to malignant transformation into HCC in approximately 5% of patients. Interestingly, in addition to *CTNNB1* mutations, which probably occur early, *TERT* promoter alterations as seen in stage-dependent HCC progression are frequently observed in the late-stage adenoma-to-carcinoma conversion[64]. The remaining HCAs — less than 10% — include unclassified cases with an unknown pathobiological cause (or causes). Hepatoblastoma is a malignant tumour type that affects children or infants younger than 4 years of age. Older children and adults (in which this tumour occurs extremely rarely) have the worst prognosis. Hepatoblastoma is the most common type of paediatric liver tumour and has some features reminiscent of adult HCC, indicating a genetic landscape of both common (primarily genotypes that recapitulate a progenitor cell-like phenotype) and distinctive aspects. Recently, the genetic variation of hepatoblastoma was determined to be relatively low, with a range of 1–8 somatic variants per tumour genome[65,66]. The onset of hepatoblastoma has been attributed to activation of the WNT pathway, following the reported finding of *CTNNB1* mutations in ~75% of all patients examined[66]. In total, pathways affected in hepatoblastoma include transcription, chromatin and chromosomal organization as well as the putative involvement of proteasomal degradation triggered by mutations in *NRF2* (9.8%) that result in NRF2–KEAP1 pathway activation, similar to what occurs in HCC[65]. In contrast to HCC and HCA, variants in the *TERT* promoter are observed in only 5.9% of patients with hepatoblastoma[65].

Fibrolamellar hepatocellular carcinoma (FLC) is a rare tumour that is found in less than 1% of PLC cases[67]. Recently, a novel chimeric transcript (*DNAJB1–PRKACA*) was identified in 79–100% of FLCs. This chimeric transcript resulted from a 400 kb deletion in chromosome 19 that led to an in-frame fusion product between exon 1 of the *DNAJB1* gene (a homologue of the molecular chaperone DNAJ) and exons 2–10 of *PRKACA* (which encodes the catalytic subunit of protein kinase A (PKA))[68,69]. The resulting fusion protein has gain-of-function cyclic AMP-dependent PKA activity.

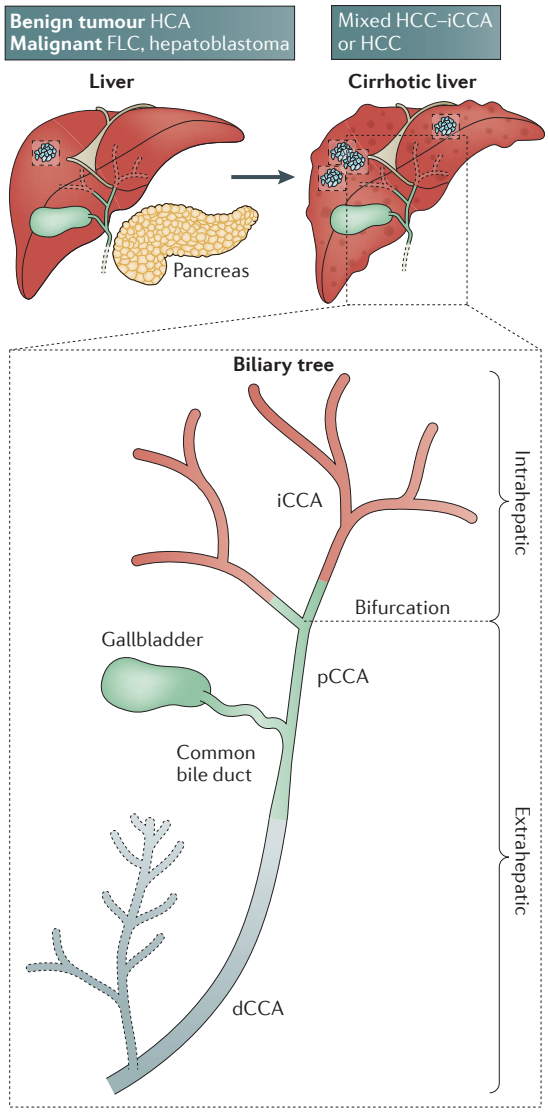

Figure 3 | **Anatomical architecture of the liver and localization of PLC.** The figure gives an overview of the structures in which tumours may occur in the liver and biliary tree. The two most common types of primary liver cancer (PLC) frequently develop in a cirrhotic liver and comprise hepatocellular carcinoma (HCC), accounting for 80% of cases, and cholangiocarcinoma (CCA), accounting for 20% of cases. CCA can be divided according to its anatomical location in the biliary tree into tumours that occur in the intrahepatic ducts (iCCA), and those that are extrahepatic in the bifurcation of the common bile duct (perihilar CCA (pCCA)) and distal to the liver (dCCA). Several rare tumour types arise predominantly in the normal liver of infants or young adults. These include both benign tumours such as hepatocellular adenomas (HCAs) and malignant tumours, including fibrolamellar hepatocellular carcinoma (FLC) and hepatoblastoma tumours. Hepatoblastomas commonly occur in infants or children less than 4 years of age.

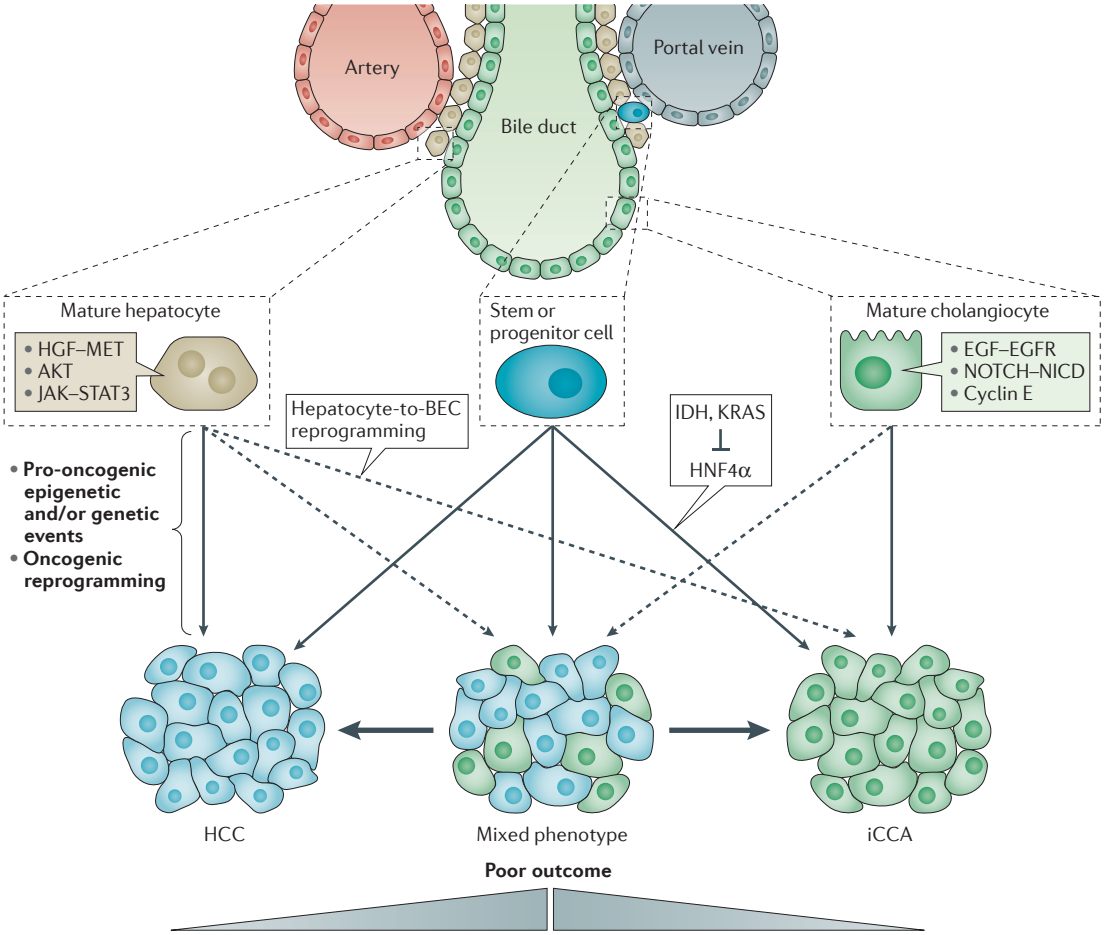

Figure 4 | **Putative cell of origin in PLC.** The scheme illustrates the physiological intrahepatic organization of a portal triad (that is, the portal vein, hepatic artery and bile duct). Hepatic stem or progenitor cells are believed to reside within the most terminal branches of the biliary tree (referred to as Canals of Hering). Various transforming events can induce carcinogenesis in the liver and initiate tumour growth in several cell types: stem or progenitor cells with unlimited self-renewal capacity, mature hepatocytes that harbour excessive proliferation capacity and longevity or mature cholangiocytes exposed to exogenous and endogenous damaging events. Depending on the target cell of the malignant transformation and activation of key signalling pathways, a wide range of different phenotypes including hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma (iCCA) and mixed HCC–iCCA can be induced. However, the differentiation status of the cell of origin might determine the biological traits of a tumour and confer different prognoses. In rare cases, blockade of hepatocytic differentiation might lead to hepatocyte-derived iCCAs (hepatocyte-to-biliary epithelial cell (BEC) trans-differentiation). Genetic alterations in isocitrate dehydrogenase 1 (*IDH1* or *IDH2*) and *KRAS* might promote the development of stem or progenitor cell-derived iCCA by blocking hepatocytic differentiation (for example, by repressing hepatocyte nuclear factor 4α (HNF4α)). Dashed arrows indicate potential phenotypic traits. EGFR, epidermal growth factor receptor; HGF, hepatocyte growth factor; JAK, Janus kinase; NICD, NOTCH intracellular domain; STAT3, signal transducer and activator of transcription 3.

Notably, aside from this deletion, recurrent genetic and structural variations are limited, indicating that, similar to the hepatoblastoma genome, a relatively stable genomic landscape is characteristic of the FLC genome[69,70].

Cellular origin of liver cancer

Genomic analyses suggest that PLCs may be considered a continuum of overlapping neoplasms rather than entirely distinct entities. The extent of this spectrum depends largely on the underlying aetiological background, the composition of the pre-neoplastic microenvironment and, importantly, the target cell of malignant transformation: that is, the cell of origin. The human liver contains three distinct types of resident cell: hepatocytes, biliary epithelial cells (BECs; also known as cholangiocytes) and adult stem and progenitor cells (often referred to as oval cells), all of which are potential cells of origin in liver cancer. Depending on the individual target of malignant transformation, hepatic oncogenesis is characterized by a large and heterogeneous spectrum of histological patterns that ranges from classical HCC and iCCA to mixed HCC–iCCA[71] (FIG. 4).

Despite the above-mentioned similarities of certain molecules and pathways that are commonly associated with an increased overall risk of PLC, a detailed understanding of the epigenetic and/or genetic factors

Hydrodynamic gene delivery
A highly efficient method for delivery of membrane-impermeable genetic information by physical force. A solution is rapidly injected at high volume to enable the delivery of genetic information to the target cell.

that induce malignant transformation in the putative cells of origin remains to be formally established[4]. Although many common oncogenic pathways are activated, the different putative cells of origin in human PLC are reflected in the observed molecular heterogeneity within different tumours. This heterogeneity hampers effective therapeutic targeting and necessitates precision and individualized approaches to treatment[72,73] (TABLE 1).

Hepatocytes as the origin of PLCs. Mature hepatocytes are characterized by longevity and remarkable regenerative potential without loss of functional properties[74,75]. This strongly supports the susceptibility of mature hepatocytes to malignant transformation in HCC under selective pressure induced by chronic inflammatory cell death. This concept is well supported by numerous mouse models of hepatocarcinogenesis and, more recently, by hydrodynamic gene delivery, which predominantly induces genetic alterations in hepatocytes (reviewed in REFS 76–78). Among the most prominent pathways commonly dysregulated in hepatocyte-derived HCC are known tumorigenic pathways such as p53, WNT–β-catenin, TGFβ, hepatocyte growth factor (HGF)–MET, IGF and EGFR (TABLE 1). Other important pathways that antagonize organ size control in the liver, differentiation status and plasticity of hepatocytes are the Hippo–YAP (Yes-associated protein) and the neurofibromin 2 (NF2; also known as merlin) pathways[79]. Activation of the Hippo–YAP pathway promotes tissue overgrowth and HCC development by YAP-mediated regulation of key transcriptional programmes, including HNF4α, jagged 1 (JAG1), NOTCH, β-catenin and E2F[80,81]; loss of the tumour suppressor NF2 has a similar effect[82]. These studies emphasize that disruption of signalling pathways with a regulatory role in organ homeostasis and tissue integrity is a crucial step in hepatocarcinogenesis.

Several lines of evidence indicate that, in response to acute and chronic biliary injury, hepatocytes undergo hepatocyte-to-BEC reprogramming (although stem and progenitor cells might also be a source of new BECs[83]) in a NOTCH-dependent manner[84–87]. Accordingly, constitutive activation of NOTCH signalling in hepatocytes as well as forced co-activation of NOTCH and AKT signalling by hydrodynamic gene delivery not only induced biliary lineage cells but also led to the development of biliary tract cancers, which supports the concept that hepatocyte-to-BEC reprogramming can lead to iCCA development[88–90]. Interestingly, both human and mouse iCCAs express the cyclin E protein, which is a direct transcriptional target of the NOTCH signalling pathway. Conversely, blockade of NOTCH signalling by inhibition of γ-secretase activity in human iCCA xenotransplants inhibited cyclin E expression and attenuated tumorigenicity[90]. However, a recent study demonstrated that, in response to excessive damage, hepatocyte-derived newly formed biliary cells retained a memory of their hepatocytic origin and differentiated back into the hepatocytic lineage upon clearance of the liver injury[91]. This implies that, although hepatocytes could be a potential source of iCCA, extensive and ongoing liver damage seems to be a prerequisite condition, and that the transforming event (or events) probably occurs in the trans-differentiated cells.

BECs as the origin of PLCs. Despite the compelling evidence for hepatocyte-derived iCCA development via hepatocyte-to-BEC reprogramming, a rigorous cholangiocyte-lineage tracing system applied to an inflammatory liver injury model in combination with p53 loss formally addressed the contribution of BECs to iCCA development[92]. Upregulation of endogenous NOTCH signalling could be detected in both BECs and hepatocytes, and was subsequently also found in mouse and human iCCAs. However, BEC lineage labelling of tumours provided strong experimental evidence that at least in the context of chronic biliary inflammation and p53 loss — two major determinants of iCCA development — both BECs and hepatocytes can be the cell of origin for iCCAs. Notably, the frequency of hepatocyte-derived and BEC-derived iCCAs is still unclear.

Stem and progenitor cells as the origin of PLCs. The notion that hepatic stem and progenitor cells can drive hepatobiliary carcinogenesis and be a source of tumour initiation is historically well documented. Experimental induction of liver stem and/or progenitor cells in rodents has been extensively studied in liver injury models as well as in liver carcinogenesis. These stem or progenitor cells are thought to reside within the most terminal branches of the bile duct (so-called Canals of Hering) and were first characterized as bi-potential cells (that is, they can give rise to both BECs and hepatocytes) by Opie in 1944, and later by Farber[93–95]. In agreement with a stem or progenitor cell-derived origin in PLC, various experimental protocols in rodents resulted in activation and proliferation of putative adult liver stem and progenitor cells, and also frequently caused carcinogenesis. These models commonly combined hepatic injury with impaired regenerative potential of hepatocytes, thereby mimicking the excessive liver injury present in many human diseases[74].

A recent study used a robust culture system to dissect the multilineage differentiation capacity of hepatic stem and progenitor cells and to further examine the individual roles of HGF–MET and EGF–EGFR in stem and progenitor cell self-renewal as well as in the binary cell fate decision[96]. Both MET and EGFR collaboratively increased self-renewal in an ERK-dependent manner. Whereas MET predominantly induced hepatocyte differentiation via AKT and STAT3, EGFR selectively induced NOTCH1 to promote cholangiocyte specification and branching morphogenesis. Interestingly, EGFR-dependent NOTCH1 activation concomitantly suppressed hepatocyte differentiation. Consistently, suppression of EGFR in hepatocytes improved stem or progenitor cell-mediated liver regeneration by promoting hepatocytic lineage differentiation. Both MET and EGFR pathways are frequently disrupted in various subtypes of PLC, corroborating the importance of these molecular alterations for liver carcinogenesis. A recent study showed that aberrant EGFR signalling in stem or

progenitor cell-derived HCCs might be directly activated by NF2, which implicates another important regulatory function of this pathway beyond YAP signalling[97].

Tschaharganeh *et al.*[98] recently provided evidence to support the crucial role of p53 in restricting cellular plasticity and tumorigenesis in the liver. This study demonstrates that p53 can indirectly repress the hepatic progenitor cell marker Nestin in an SP1- or SP3-dependent manner. As a consequence and depending on the lineage of the target cell, loss of p53 was also associated with mutations in WNT and NOTCH signalling pathways, and thereby promoted the development of either HCCs or iCCAs. These results again confirm the importance of cellular reprogramming of differentiated cells into a more pluripotent state during the generation of PLCs.

IDHs might also be crucial in determining the cell fate of progenitor cells during malignant transformation. IDH mutations are frequently observed in iCCA, and these can suppress differentiation of liver progenitor cells into hepatocytes via inhibition of the key differentiation factor HNF4α[99]. Furthermore, coexisting mutations in *IDH1* or *IDH2* and *KRAS* cooperatively promoted the development of pre-malignant biliary lesions and progression to metastatic iCCA[99]. Additional evidence to support a progenitor cell origin of liver cancer is based on the observation that activation and proliferation of hepatic progenitor cells are observed in many precancerous conditions in humans, such as chronic inflammation (caused by HBV, HCV, alcoholic hepatitis and steatohepatitis)[100,101].

A recent study assessed the relative capacity of distinct hepatic lineage cells (mouse primary hepatic progenitor cells, lineage-committed hepatoblasts and differentiated adult hepatocytes) to induce liver cancer by forced expression of oncogenic HRAS and simian virus 40 (SV40) large T antigen[27]. This investigation provided formal evidence that any cell type in the mouse hepatic lineage can undergo oncogenic reprogramming into a tumour-initiating cell of PLC, by activating different cell type-specific pathways. Integrated gene expression analyses further demonstrated that transformation of the cells into tumour-initiating cells required the activation of MYC and its target genes. In another study, the potential causative role of MYC during malignant transformation in human HCC and the effect of distinct levels of MYC overexpression on the biology of putative hepatic cancer stem cells in different hepatoma cell lines were tested[102]. The results of that study demonstrated that different levels of MYC expression have a differential impact on stemness characteristics. At low levels, MYC activation led to increased proliferation and enhanced stemness properties. However, when its expression exceeded a threshold level, MYC induced a pro-apoptotic programme and loss of stemness potential both *in vitro* and *in vivo*. Mechanistically, the MYC-induced self-renewal capacity of liver cancer cells was exerted in a p53-dependent manner.

Overall, the studies discussed in this section emphasize that many different cell types may be the potential targets of a transforming event (or events) and thereby contribute to hepatocarcinogenesis. Regardless of the primary cellular source of PLCs, the cellular origin seems to be retained and reflected in the biological traits of the tumour. In this context, it seems abundantly clear that liver cancers with stem or progenitor cell features show activation of oncogenic signalling pathways that are associated with a poor outcome[4]. The observation that acquisition of stemness traits is crucial not only for tumour initiation but also for the generation of distant metastasis, and relapse after therapy therefore has broad clinical implications[73,103,104]. Although successful early diagnosis would require biomarkers that specifically detect stemness and early signs of malignant transformation, identification of the cellular origin (the 'cell-at-risk') would be necessary to reduce the number of tumour-initiating cells effectively, for example, using cancer stem cell-directed therapies.

Importance of the hepatic microenvironment

More than 80% of liver cancers develop on the basis of a chronic liver disease, most commonly liver cirrhosis (FIG. 1). As such, the disruption of the microenvironment seems to be the most important determinant of malignant progression and requires particular attention[105]. Considerable research efforts have focused on the identification of predetermining factors that contribute to the disruption of the liver microenvironment and thereby lead to the generation of a pre-malignant niche (or niches) that promotes liver cancer development. In support of this, a recent study elegantly demonstrated that transplantation of hepatic progenitor cells gave rise to cancer only when they were introduced into livers with pre-existing chronic damage and compensatory hepatocyte proliferation[106]. Interestingly, similar to observations made in human hepatocarcinogenesis, cells with progenitor cell features quiescently resided within dysplastic lesions for several months before the appearance of HCC. Malignant transformation of the progenitor cells and stimulation of their growth were subsequently attained by acquisition of autocrine IL-6 signalling, which might be a general mechanism of progenitor cell-induced HCC[107,108].

Notably, IL-6 is a highly abundant cytokine in the liver and actively regulates defence mechanisms of immune cells as well as growth and differentiation of epithelial tumour cells via paracrine and autocrine regulatory loops[107,108]. IL-6 is further associated with the treatment response to targeted therapies and progression in various cancers by, for example, promoting immune evasion of cancer cells[109,110]. The dual role of IL-6 signalling in both cancer cells and non-parenchymal cells (for example, immune cells) therefore contributes substantially to crosstalk between the tumour and the microenvironment[111]. In support of this finding, genomic analyses provided evidence that gene sets associated with a poor prognosis in liver cancer contained several downstream targets of IL-6 (REF. 112). Importantly, this prognostic gene expression signature was generated from the surrounding non-tumour liver tissue, whereas gene expression profiles of tumour tissue failed to yield significant association with survival. Pro-inflammatory and pro-oncogenic effects of IL-6 in the liver are frequently controlled by nuclear factor-κB

(NF-κB) signalling, which is one of the most dominant signalling pathways involved in the so-called inflammation–fibrosis–cancer axis[113,114]. Consistent with this, both IL-6 and NF-κB signalling pathways are commonly disrupted in most inflammatory chronic liver diseases. Although the prominent role of NF-κB activation during multistage hepatocarcinogenesis is well documented, the absence of NF-κB as a result of the genetic loss of the regulatory NF-κB essential modulator subunit *NEMO* in hepatocytes also promoted liver cancer development[115]. However, we recently demonstrated that inhibition of NF-κB effectively diminished stemness features in a subgroup of liver cancer cells by NF-κB-mediated inhibition of histone deacetylases (HDACs). Specific blockade of the NF-κB pathway might therefore be a promising new therapeutic strategy for liver cancers that share progenitor cell features[116]. In summary, the above-mentioned conflicting context and cell type dependency of pathways involved in inflammation and cancer indicate the necessity of considering molecular traits that are crucial not only for tumour development but also in the pre-malignant stages of liver cancer[117].

Another important microenvironmental signalling pathway associated with selective mitogenic effects on stem or progenitor cell-derived PLC development during liver injury is the TWEAK–FN14 (tumour necrosis factor (TNF)-like weak inducer of apoptosis–FN14 (also known as TNFRSF12A)) pathway[118]. Interestingly, infiltrating macrophages could be identified as an important source of TWEAK–FN14-mediated stem or progenitor cell activation during liver injury[119]. Furthermore, the cell fate decision of stem or progenitor cells and the specific activation of NOTCH or WNT signalling were shown to be dependent on the type of liver injury (that is, biliary or hepatocyte damage) as well as on the interaction of stem or progenitor cells with activated myofibroblasts or macrophages[120].

Several other inflammatory mediators and pro-oncogenic molecules revealed unexpected tumour-suppressing effects when activated in diverse parenchymal and non-parenchymal cell types (for example, immune cells versus hepatocytes) and different aetiological contexts (for example, inflammation, fibrosis or cirrhosis), which underlines the crucial importance of the interaction of signals from the microenvironment and the tumour cells for tumour initiation and progression[121,122]. In the case of EGFR, a key molecule in PLCs, overexpression significantly promotes liver cancer development and is associated with poor outcome only when it is present in macrophages; however, EGFR showed no prognostic implications when upregulated in hepatocytes[123,124]. Furthermore, loss of JUN activity in hepatocytes during the course of chronic liver inflammation precedes malignant transformation, thereby inducing a pro-tumorigenic switch in liver macrophages. As a result of this immunosuppressive switch, several pro-oncogenic chemokines are released that subsequently lead to the recruitment of other pro-tumorigenic immune cells such as regulatory T cells, again confirming that the crosstalk between parenchymal and non-parenchymal liver cells is important during sequential hepatocarcinogenesis[125]. Another study showed that activated hepatic stellate cells, the major source of extracellular matrix production during liver fibrogenesis, also promote the pro-tumorigenic switch of macrophages in the diseased liver microenvironment, thereby accelerating HCC progression[126,127]. Hepatic stellate cells also contribute to the direct crosstalk between stromal and tumour cells in PLC[128]. Consistently, activation of hepatic stellate cells leads to subsequent upregulation of signalling pathways that are important for liver fibrogenesis, such as TGFβ, stromal cell-derived factor 1 (SDF1)–CXCR4 (chemokine (C-X-C motif) receptor 4), platelet-derived growth factor (PDGF), Hedgehog and NOTCH[115]. Notably, many of these pathways have been shown to be prognostic in PLC[129–131]. Results of these studies clearly show that the pro-oncogenic changes in the hepatic microenvironment during chronic liver inflammation are orchestrated by the interaction of parenchymal cells with diverse types of non-parenchymal cells[105], and provide a rationale for immunotherapy or other treatment approaches that involve interference with the tumour microenvironment in PLC[132].

Challenges and outlook

Despite the complexity of the cellular origin and molecular mechanisms that influence the initiation, cell fate decisions and progression into PLC, much has been achieved to clarify the genomic alterations and crosstalk between immune and cancer cells in the hepatic microenvironment. However, treatment of patients with PLC still constitutes a major clinical challenge[133].

In contrast to most cancers, a large majority of HCCs arise in livers that have chronic fibrotic damage (that is, cirrhosis), which severely compromises liver functions (for example, xenobiotic metabolism and regenerative capacity) and substantially limits therapeutic options[2]. However, extensive and detailed knowledge at both the cellular and molecular levels of all stages of HCC and, to a lesser degree, of iCCA has provided new and exciting therapeutic opportunities. This is predominantly the case in the context of precision medicine, in which the effect of the treatment is determined by matching detailed information about patients at the molecular level with a unique targeted therapy guided by genomics[134].

In this context, the microenvironment-dependent effect of deregulated signalling pathways during PLC initiation and progression will demand increased attention. Importantly, the tumour–stroma–immune cell interaction adds another layer of complexity to hepatocarcinogenesis that has considerable mechanistic implications[135]. In light of the importance of inflammation in liver cancer, the rationale for targeting immune cell–cancer cell interactions by innovative immunotherapeutic approaches is evident, and these approaches are currently being pursued in several preclinical and early-stage clinical trials[132,136]. The success of recent checkpoint inhibitors and oncolytic virotherapies in several solid tumours, including HCC, provides grounds for optimism as regards to the application of immunotherapy in PLC[137–141].

In summary, next-generation genomic technologies have provided a detailed understanding of general mechanisms of hepatocarcinogenesis. Similarly, several functional studies have substantially improved our understanding of PLC development by identifying key cellular targets during malignant transformation in the liver. The cellular and molecular heterogeneity outlined in this Review clearly indicates that improved patient classification and response prediction will be crucial steps in overcoming the present unmet therapeutic need in liver cancer. Therefore, the future goal will be the successful implementation of molecular-guided patient care in daily clinical routine. Definition of the hallmarks of PLC at the cellular level (that is, resident parenchymal and non-parenchymal cells) and dissection of the contributions of each cell type to hepatocarcinogenesis in the context of therapeutic opportunities are major challenges. The formation of multicentre academic consortia to enable integrative and dynamic clinical trial designs using well-stratified patients as well as the broad application of genomic technologies might be the first essential step in this endeavour.

1. Lozano, R. *et al.* Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* **380**, 2095–2128 (2012).
2. El-Serag, H. B. Hepatocellular carcinoma. *N. Engl. J. Med.* **365**, 1118–1127 (2011).
3. Pawlotsky, J. M., Feld, J. J., Zeuzem, S. & Hoofnagle, J. H. From non-A, non-B hepatitis to hepatitis C virus cure. *J. Hepatol.* **62**, S87–S99 (2015).
4. Marquardt, J. U., Galle, P. R. & Teufel, A. Molecular diagnosis and therapy of hepatocellular carcinoma (HCC): an emerging field for advanced technologies. *J. Hepatol.* **56**, 267–275 (2012).
5. Razumilava, N. & Gores, G. J. Classification, diagnosis, and management of cholangiocarcinoma. *Clin. Gastroenterol. Hepatol.* **11**, 13–21 (2013).
6. Bridgewater, J. *et al.* Guidelines for the diagnosis and management of intrahepatic cholangiocarcinoma. *J. Hepatol.* **60**, 1268–1289 (2014).
These are the first clinical practice guidelines for iCCA, summarizing epidemiological data and the current state of the art in diagnosis and therapy.
7. The International Consensus Group for Hepatocellular Neoplasia. Pathologic diagnosis of early hepatocellular carcinoma: a report of the international consensus group for hepatocellular neoplasia. *Hepatology* **49**, 658–664 (2009); erratum **49**,1058 (2009)
8. Farazi, P. A. & DePinho, R. A. Hepatocellular carcinoma pathogenesis: from genes to environment. *Nat. Rev. Cancer* **6**, 674–687 (2006).
This is a landmark review summarizing the molecular pathomechansims of HCC.
9. Marquardt, J. U. *et al.* Sequential transcriptome analysis of human liver cancer indicates late stage acquisition of malignant traits. *J. Hepatol.* **60**, 346–353 (2014).
10. Guichard, C. *et al.* Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. *Nat. Genet.* **44**, 694–698 (2012).
11. Kan, Z. *et al.* Whole-genome sequencing identifies recurrent mutations in hepatocellular carcinoma. *Genome Res.* **23**, 1422–1433 (2013).
12. Nault, J. C. *et al.* High frequency of telomerase reverse-transcriptase promoter somatic mutations in hepatocellular carcinoma and preneoplastic lesions. *Nat. Commun.* **4**, 2218 (2013).
13. Raggi, C., Invernizzi, P. & Andersen, J. B. Impact of microenvironment and stem-like plasticity in cholangiocarcinoma: molecular networks and biological concepts. *J. Hepatol.* **62**, 198–207 (2015).
14. Li, M. *et al.* Inactivating mutations of the chromatin remodeling gene *ARID2* in hepatocellular carcinoma. *Nat. Genet.* **43**, 828–829 (2011).
15. Totoki, Y. *et al.* High-resolution characterization of a hepatocellular carcinoma genome. *Nat. Genet.* **43**, 464–469 (2011).
This is the first landmark study to use next-generation sequencing in HCC on 12 individual Japanese tumours.
16. Fujimoto, A. *et al.* Whole-genome sequencing of liver cancers identifies etiological influences on mutation patterns and recurrent mutations in chromatin regulators. *Nat. Genet.* **44**, 760–764 (2012).
17. Andersen, J. B. *et al.* Genomic and genetic characterization of cholangiocarcinoma identifies therapeutic targets for tyrosine kinase inhibitors. *Gastroenterology* **142**, 1021–1031.e15 (2012).
This is a seminal study on the CCA transcriptome. The study revealed different prognostic subtypes in CCA with distinct genetic alterations that could be used for therapeutic interventions.
18. Oishi, N. *et al.* Transcriptomic profiling reveals hepatic stem-like gene signatures and interplay of miR-200c and epithelial-mesenchymal transition in intrahepatic cholangiocarcinoma. *Hepatology* **56**, 1792–1803 (2012).
19. Sia, D. *et al.* Integrative molecular analysis of intrahepatic cholangiocarcinoma reveals 2 classes that have different outcomes. *Gastroenterology* **144**, 829–840 (2013).
20. Seok, J. Y. *et al.* A fibrous stromal component in hepatocellular carcinoma reveals a cholangiocarcinoma-like gene expression trait and epithelial-mesenchymal transition. *Hepatology* **55**, 1776–1786 (2012).
21. Woo, H. G. *et al.* Identification of a cholangiocarcinoma-like gene expression trait in hepatocellular carcinoma. *Cancer Res.* **70**, 3034–3041 (2010).
22. Cazals-Hatem, D. *et al.* Clinical and molecular analysis of combined hepatocellular-cholangiocarcinomas. *J. Hepatol.* **41**, 292–298 (2004).
23. Fujii, H. *et al.* Genetic classification of combined hepatocellular-cholangiocarcinoma. *Hum. Pathol.* **31**, 1011–1017 (2000).
24. Schulze, K. *et al.* Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. *Nat. Genet.* **47**, 505–511 (2015).
25. Jiang, J. H. *et al.* Clinical significance of the ubiquitin ligase UBE3C in hepatocellular carcinoma revealed by exome sequencing. *Hepatology* **59**, 2216–2227 (2014).
26. Kaposi-Novak, P. *et al.* Central role of c-Myc during malignant conversion in human hepatocarcinogenesis. *Cancer Res.* **69**, 2775–2782 (2009).
27. Holczbauer, A. *et al.* Modeling pathogenesis of primary liver cancer in lineage-specific mouse cell types. *Gastroenterology* **145**, 221–231 (2013).
This study demonstrates that induction of malignant transformation by selected oncogenes can induce liver cancer development regardless of the putative cell of origin in the hepatic lineage.
28. Nault, J. C. *et al.* Telomerase reverse transcriptase promoter mutation is an early somatic genetic alteration in the transformation of premalignant nodules in hepatocellular carcinoma on cirrhosis. *Hepatology* **60**, 1983–1992 (2014).
29. Tovar, V. *et al.* IGF activation in a molecular subclass of hepatocellular carcinoma and pre-clinical efficacy of IGF-1R blockage. *J. Hepatol.* **52**, 550–559 (2010).
30. Woo, H. G. *et al.* Profiling of exome mutations associated with progression of HBV-related hepatocellular carcinoma. *PLoS ONE* **9**, e115152 (2014).
31. Sung, W. K. *et al.* Genome-wide survey of recurrent HBV integration in hepatocellular carcinoma. *Nat. Genet.* **44**, 765–769 (2012).
32. Alexandrov, L. B. *et al.* Signatures of mutational processes in human cancer. *Nature* **500**, 415–421 (2013).
33. Totoki, Y. *et al.* Trans-ancestry mutational landscape of hepatocellular carcinoma genomes. *Nat. Genet.* **46**, 1267–1273 (2014).
This is a large next-generation sequencing study on PLC performed on data from 488 HCCs from different populations. The study revealed 30 candidate driver genes and 11 core pathway modules.
34. Chen, Y., Wang, L., Xu, H., Liu, X. & Zhao, Y. Exome capture sequencing reveals new insights into hepatitis B virus-induced hepatocellular carcinoma at the early stage of tumorigenesis. *Oncol. Rep.* **30**, 1906–1912 (2013).
35. Cleary, S. P. *et al.* Identification of driver genes in hepatocellular carcinoma by exome sequencing. *Hepatology* **58**, 1693–1702 (2013).
36. Huang, J. *et al.* Exome sequencing of hepatitis B virus-associated hepatocellular carcinoma. *Nat. Genet.* **44**, 1117–1121 (2012).
37. Liu, Y. X. *et al.* Whole-exome sequencing identifies mutated PCK2 and HUWE1 associated with carcinoma cell proliferation in a hepatocellular carcinoma patient. *Oncol. Lett.* **4**, 847–851 (2012).
38. Chen, Y. L. *et al.* TERT promoter mutation in resectable hepatocellular carcinomas: a strong association with hepatitis C infection and absence of hepatitis B infection. *Int. J. Surg.* **12**, 659–665 (2014).
39. Ding, D. *et al.* Recurrent targeted genes of hepatitis B virus in the liver cancer genomes identified by a next-generation sequencing-based approach. *PLoS Genet.* **8**, e1003065 (2012).
40. Jhunjhunwala, S. *et al.* Diverse modes of genomic alteration in hepatocellular carcinoma. *Genome Biol.* **15**, 436 (2014).
41. Shiraishi, Y. *et al.* Integrated analysis of whole genome and transcriptome sequencing reveals diverse transcriptomic aberrations driven by somatic genomic changes in liver cancers. *PLoS ONE* **9**, e114263 (2014).
42. Zhong, R. *et al.* Genetic variant in SWI/SNF complexes influences hepatocellular carcinoma risk: a new clue for the contribution of chromatin remodeling in carcinogenesis. *Sci. Rep.* **4**, 4147 (2014).
43. Chan, T. H. *et al.* A disrupted RNA editing balance mediated by ADARs (Adenosine DeAminases that act on RNA) in human hepatocellular carcinoma. *Gut* **63**, 832–843 (2014).
44. Chen, L. *et al.* Recoding RNA editing of *AZIN1* predisposes to hepatocellular carcinoma. *Nat. Med.* **19**, 209–216 (2013).
45. Kang, L. *et al.* Genome-wide identification of RNA editing in hepatocellular carcinoma. *Genomics* **105**, 76–82 (2015).
46. Andersen, J. B. Molecular pathogenesis of intrahepatic cholangiocarcinoma. *J. Hepatobiliary Pancreat. Sci.* **22**, 101–113 (2015).
47. Wang, P. *et al.* Mutations in isocitrate dehydrogenase 1 and 2 occur frequently in intrahepatic cholangiocarcinomas and share hypermethylation targets with glioblastomas. *Oncogene* **32**, 3091–3100 (2013).
48. Chan-On, W. *et al.* Exome sequencing identifies distinct mutational patterns in liver fluke-related and non-infection-related bile duct cancers. *Nat. Genet.* **45**, 1474–1478 (2013).
49. Gao, Q. *et al.* Activating mutations in *PTPN3* promote cholangiocarcinoma cell proliferation and migration and are associated with tumor recurrence in patients. *Gastroenterology* **146**, 1397–1407 (2014).

50. Jiao, Y. *et al.* Exome sequencing identifies frequent inactivating mutations in *BAP1*, *ARID1A* and *PBRM1* in intrahepatic cholangiocarcinomas. *Nat. Genet.* **45**, 1470–1473 (2013).
51. Ong, C. K. *et al.* Exome sequencing of liver fluke-associated cholangiocarcinoma. *Nat. Genet.* **44**, 690–693 (2012).
52. Sia, D. *et al.* Massive parallel sequencing uncovers actionable *FGFR2–PPHLN1* fusion and *ARAF* mutations in intrahepatic cholangiocarcinoma. *Nat. Commun.* **6**, 6087 (2015).
53. Zou, S. *et al.* Mutational landscape of intrahepatic cholangiocarcinoma. *Nat. Commun.* **5**, 5696 (2014).
This is currently the largest next-generation sequencing study on iCCA. The study identified mutational signatures associated with liver inflammation, fibrosis and cirrhosis. Twenty-five genes were substantially mutated, including eight potential driver genes.
54. Marquardt, J. U. & Andersen, J. B. Liver cancer oncogenomics: opportunities and dilemmas for clinical applications. *Hepat. Oncol.* **2**, 79–93 (2015).
55. Muisuk, K. *et al.* Novel mutations in cholangiocarcinoma with low frequencies revealed by whole mitochondrial genome sequencing. *Asian Pac. J. Cancer Prev.* **16**, 1737–1742 (2015).
56. Wu, Y. M. *et al.* Identification of targetable FGFR gene fusions in diverse cancers. *Cancer Discov.* **3**, 636–647 (2013).
57. Ross, J. S. *et al.* New routes to targeted therapy of intrahepatic cholangiocarcinomas revealed by next-generation sequencing. *Oncologist* **19**, 235–242 (2014).
58. Arai, Y. *et al.* Fibroblast growth factor receptor 2 tyrosine kinase fusions define a unique molecular subtype of cholangiocarcinoma. *Hepatology* **59**, 1427–1434 (2014).
59. Borad, M. J. *et al.* Integrated genomic characterization reveals novel, therapeutically relevant drug targets in FGFR and EGFR pathways in sporadic intrahepatic cholangiocarcinoma. *PLoS Genet.* **10**, e1004135 (2014).
60. Graham, R. P. *et al.* Fibroblast growth factor receptor 2 translocations in intrahepatic cholangiocarcinoma. *Hum. Pathol.* **45**, 1630–1638 (2014).
61. Klein, W. M. *et al.* Primary liver carcinoma arising in people younger than 30 years. *Am. J. Clin. Pathol.* **124**, 512–518 (2005).
62. Pilati, C. *et al.* Genomic profiling of hepatocellular adenomas reveals recurrent FRK-activating mutations and the mechanisms of malignant transformation. *Cancer Cell* **25**, 428–441 (2014).
This is the first study to perform large-scale analyses on HPAs. The study identified recurrent *FRK*-activating mutations and other genetic predictors of malignant transformation, including *TERT* alterations.
63. Nault, J. C., Bioulac-Sage, P. & Zucman-Rossi, J. Hepatocellular benign tumors — from molecular classification to personalized clinical care. *Gastroenterology* **144**, 888–902 (2013).
64. Marquardt, J. U. & Thorgeirsson, S. S. Next-generation genomic profiling of hepatocellular adenomas: a new era of individualized patient care. *Cancer Cell* **25**, 409–411 (2014).
65. Eichenmuller, M. *et al.* The genomic landscape of hepatoblastoma and their progenies with HCC-like features. *J. Hepatol.* **61**, 1312–1320 (2014).
66. Jia, D. *et al.* Exome sequencing of hepatoblastoma reveals novel mutations and cancer genes in the Wnt pathway and ubiquitin ligase complex. *Hepatology* **60**, 1686–1696 (2014).
This is the first study to perform whole-exome sequencing of six paired hepatoblastoma tumours. It provides a detailed description of the landscape of genetic alterations in hepatoblastoma, highlighting the importance of WNT and ubiquitin pathways.
67. Eggert, T. *et al.* Fibrolamellar hepatocellular carcinoma in the USA, 2000–2010: a detailed report on frequency, treatment and outcome based on the Surveillance, Epidemiology, and End Results database. *United European Gastroenterol. J.* **1**, 351–357 (2013).
68. Honeyman, J. N. *et al.* Detection of a recurrent *DNAJB1–PRKACA* chimeric transcript in fibrolamellar hepatocellular carcinoma. *Science* **343**, 1010–1014 (2014).
69. Cornella, H. *et al.* Unique genomic profile of fibrolamellar hepatocellular carcinoma. *Gastroenterology* **148**, 806–818 (2014).
This study provides integrative analyses of 78 FLCs. The study identified three subtypes with distinct genetic alterations and survival rates.
70. Darcy, D. G. *et al.* The genomic landscape of fibrolamellar hepatocellular carcinoma: whole genome sequencing of ten patients. *Oncotarget* **6**, 755–770 (2014).
71. Yamashita, T. & Wang, X. W. Cancer stem cells in the development of liver cancer. *J. Clin. Invest.* **123**, 1911–1918 (2013).
72. Llovet, J. M. & Bruix, J. Molecular targeted therapies in hepatocellular carcinoma. *Hepatology* **48**, 1312–1327 (2008).
73. Marquardt, J. U. & Thorgeirsson, S. S. Stem cells in hepatocarcinogenesis: evidence from genomic data. *Semin. Liver Dis.* **30**, 26–34 (2010).
74. Duncan, A. W., Dorrell, C. & Grompe, M. Stem cells and liver regeneration. *Gastroenterology* **137**, 466–481 (2009).
75. Overturf, K., al-Dhalimy, M., Ou, C. N., Finegold, M. & Grompe, M. Serial transplantation reveals the stem-cell-like regenerative potential of adult mouse hepatocytes. *Am. J. Pathol.* **151**, 1273–1280 (1997).
In this study, serial transplantations of hepatocytes confirmed that hepatocytes possess high proliferative potential.
76. Chen, X. & Calvisi, D. F. Hydrodynamic transfection for generation of novel mouse models for liver cancer research. *Am. J. Pathol.* **184**, 912–923 (2014).
77. Fausto, N. & Campbell, J. S. Mouse models of hepatocellular carcinoma. *Semin. Liver Dis.* **30**, 87–98 (2010).
78. Zender, L. *et al.* Cancer gene discovery in hepatocellular carcinoma. *J. Hepatol.* **52**, 921–929 (2010).
79. Yimlamai, D. *et al.* Hippo pathway activity influences liver cell fate. *Cell* **157**, 1324–1338 (2014).
80. Tschaharganeh, D. F. *et al.* Yes-associated protein up-regulates Jagged-1 and activates the Notch pathway in human hepatocellular carcinoma. *Gastroenterology* **144**, 1530–1542.e12 (2013).
81. Fitamant, J. *et al.* YAP inhibition restores hepatocyte differentiation in advanced HCC, leading to tumor regression. *Cell Rep.* **10**, 1692–1707 (2015).
82. Zhang, N. *et al.* The Merlin/NF2 tumor suppressor functions through the YAP oncoprotein to regulate tissue homeostasis in mammals. *Dev. Cell* **19**, 27–38 (2010).
83. Dorrell, C. *et al.* Prospective isolation of a bipotential clonogenic liver progenitor cell in adult mice. *Genes Dev.* **25**, 1193–1203 (2011).
84. Lemaigre, F. P. Hepatocytes as a source of cholangiocytes in injured liver. *Hepatology* **59**, 726–728 (2014).
85. Malato, Y. *et al.* Fate tracing of mature hepatocytes in mouse liver homeostasis and regeneration. *J. Clin. Invest.* **121**, 4850–4860 (2011).
86. Sekiya, S. & Suzuki, A. Hepatocytes, rather than cholangiocytes, can be the major source of primitive ductules in the chronically injured mouse liver. *Am. J. Pathol.* **184**, 1468–1478 (2014).
87. Yanger, K. *et al.* Robust cellular reprogramming occurs spontaneously during liver regeneration. *Genes Dev.* **27**, 719–724 (2013).
88. Sekiya, S. & Suzuki, A. Intrahepatic cholangiocarcinoma can arise from Notch-mediated conversion of hepatocytes. *J. Clin. Invest.* **122**, 3914–3918 (2012).
89. Fan, B. *et al.* Cholangiocarcinomas can originate from hepatocytes in mice. *J. Clin. Invest.* **122**, 2911–2915 (2012).
90. Zender, S. *et al.* A critical role for notch signaling in the formation of cholangiocellular carcinomas. *Cancer Cell* **23**, 784–795 (2013).
This study induced CCA by forced expression of the NOTCH1 intracellular domain in mouse livers, confirming the importance of the NOTCH pathway for iCCA development.
91. Tarlow, B. D. *et al.* Bipotential adult liver progenitors are derived from chronically injured mature hepatocytes. *Cell Stem Cell* **15**, 605–618 (2014).
92. Guest, R. V. *et al.* Cell lineage tracing reveals a biliary origin of intrahepatic cholangiocarcinoma. *Cancer Res.* **74**, 1005–1010 (2014).
93. Farber, E. Carcinoma of the liver in rats fed ethionine. *AMA Arch. Pathol.* **62**, 445–453 (1956).
94. Opie, E. L. The pathogenesis of tumors of the liver produced by butter yellow. *J. Exp. Med.* **80**, 231–246 (1944).
95. Evarts, R. P., Nagy, P., Marsden, E. & Thorgeirsson, S. S. A precursor-product relationship exists between oval cells and hepatocytes in rat liver. *Carcinogenesis* **8**, 1737–1740 (1987).
96. Kitade, M. *et al.* Specific fate decisions in adult hepatic progenitor cells driven by MET and EGFR signaling. *Genes Dev.* **27**, 1706–1717 (2013).
97. Benhamouche, S. *et al.* Nf2/Merlin controls progenitor homeostasis and tumorigenesis in the liver. *Genes Dev.* **24**, 1718–1730 (2010).
98. Tschaharganeh, D. F. *et al.* p53-dependent Nestin regulation links tumor suppression to cellular plasticity in liver cancer. *Cell* **158**, 579–592 (2014).
99. Saha, S. K. *et al.* Mutant IDH inhibits HNF-4α to block hepatocyte differentiation and promote biliary cancer. *Nature* **513**, 110–114 (2014).
100. Hsia, C. C., Evarts, R. P., Nakatsukasa, H., Marsden, E. R. & Thorgeirsson, S. S. Occurrence of oval-type cells in hepatitis B virus-associated human hepatocarcinogenesis. *Hepatology* **16**, 1327–1333 (1992).
101. Roskams, T. & Desmet, V. Ductular reaction and its diagnostic significance. *Semin. Diagn. Pathol.* **15**, 259–269 (1998).
102. Akita, H. *et al.* MYC activates stem-like cell potential in hepatocarcinoma by a p53-dependent mechanism. *Cancer Res.* **74**, 5903–5913 (2014).
103. Kakarala, M. & Wicha, M. S. Implications of the cancer stem-cell hypothesis for breast cancer prevention and therapy. *J. Clin. Oncol.* **26**, 2813–2820 (2008).
104. Yong, K. J. *et al.* Oncofetal gene *SALL4* in aggressive hepatocellular carcinoma. *N. Engl. J. Med.* **368**, 2266–2276 (2013).
105. Hernandez-Gea, V., Toffanin, S., Friedman, S. L. & Llovet, J. M. Role of the microenvironment in the pathogenesis and treatment of hepatocellular carcinoma. *Gastroenterology* **144**, 512–527 (2013).
106. He, G. *et al.* Identification of liver cancer progenitors whose malignant progression depends on autocrine IL-6 signaling. *Cell* **155**, 384–396 (2013).
This study demonstrates that cancer progenitor cells can only induce tumours when introduced into a liver with chronic damage, underlining the importance of the tumour microenvironment for HCC development.
107. Naugler, W. E. *et al.* Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. *Science* **317**, 121–124 (2007).
108. Park, E. J. *et al.* Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression. *Cell* **140**, 197–208 (2010).
109. Guo, Y., Xu, F., Lu, T., Duan, Z. & Zhang, Z. Interleukin-6 signaling pathway in targeted therapy for cancer. *Cancer Treat. Rev.* **38**, 904–910 (2012).
110. Makarova-Rusher, O. V., Medina-Echeverz, J., Duffy, A. G. & Greten, T. F. The yin and yang of evasion and immune activation in HCC. *J. Hepatol.* **62**, 1420–1429 (2015).
111. Wan, S. *et al.* Tumor-associated macrophages produce interleukin 6 and signal via STAT3 to promote expansion of human hepatocellular carcinoma stem cells. *Gastroenterology* **147**, 1393–1404 (2014).
112. Hoshida, Y. *et al.* Gene expression in fixed tissues and outcome in hepatocellular carcinoma. *N. Engl. J. Med.* **359**, 1995–2004 (2008).
113. Maeda, S. *et al.* IkB kinaseβ/nuclear factor-κB activation controls the development of liver metastasis by way of interleukin-6 expression. *Hepatology* **50**, 1851–1860 (2009).
114. Elsharkawy, A. M. & Mann, D. A. Nuclear factor-κB and the hepatic inflammation–fibrosis–cancer axis. *Hepatology* **46**, 590–597 (2007).
115. Luedde, T. & Schwabe, R. F. NF-κB in the liver — linking injury, fibrosis and hepatocellular carcinoma. *Nat. Rev. Gastroenterol. Hepatol.* **8**, 108–118 (2011).
116. Marquardt, J. U. *et al.* Curcumin effectively inhibits oncogenic NF-kB signaling and restrains stemness features in liver cancer. *J. Hepatol.* **63**, 661–669 (2015).
117. Karin, M. Nuclear factor-κB in cancer development and progression. *Nature* **441**, 431–436 (2006).
118. Jakubowski, A. *et al.* TWEAK induces liver progenitor cell proliferation. *J. Clin. Invest.* **115**, 2330–2340 (2005).
119. Bird, T. G. *et al.* Bone marrow injection stimulates hepatic ductular reactions in the absence of injury via macrophage-mediated TWEAK signaling. *Proc. Natl Acad. Sci. USA* **110**, 6542–6547 (2013).
120. Boulter, L. *et al.* Macrophage-derived Wnt opposes Notch signaling to specify hepatic progenitor cell fate in chronic liver disease. *Nat. Med.* **18**, 572–579 (2012).

121. Feng, G. S. Conflicting roles of molecules in hepatocarcinogenesis: paradigm or paradox. *Cancer Cell* **21**, 150–154 (2012).
This review critically discusses the context and cell type dependency of key oncogenic pathways for liver cancer development and progression.
122. Li, S. C. *et al.* Cancer genomic research at the crossroads: realizing the changing genetic landscape as intratumoral spatial and temporal heterogeneity becomes a confounding factor. *Cancer Cell. Int.* **14**, 115 (2014).
123. Lanaya, H. *et al.* EGFR has a tumour-promoting role in liver macrophages during hepatocellular carcinoma formation. *Nat. Cell Biol.* **16**, 972–981 (2014).
124. Timchenko, N. A. Cell-type specific functions of EGFR are involved in development of hepatocellular carcinoma. *Hepatology* **62**, 314–316 (2015).
125. Hefetz-Sela, S. *et al.* Acquisition of an immunosuppressive protumorigenic macrophage phenotype depending on c-Jun phosphorylation. *Proc. Natl Acad. Sci. USA* **111**, 17582–17587 (2014).
126. Ji, J. *et al.* Hepatic stellate cell and monocyte interaction contributes to poor prognosis in hepatocellular carcinoma. *Hepatology* **62**, 481–495 (2015).
127. Trautwein, C., Friedman, S. L., Schuppan, D. & Pinzani, M. Hepatic fibrosis: concept to treatment. *J. Hepatol.* **62**, S15–S24 (2015).
128. Coulouarn, C. & Clement, B. Stellate cells and the development of liver cancer: therapeutic potential of targeting the stroma. *J. Hepatol.* **60**, 1306–1309 (2014).
129. Coulouarn, C. *et al.* Hepatocyte–stellate cell cross-talk in the liver engenders a permissive inflammatory microenvironment that drives progression in hepatocellular carcinoma. *Cancer Res.* **72**, 2533–2542 (2012).
130. Kang, N., Gores, G. J. & Shah, V. H. Hepatic stellate cells: partners in crime for liver metastases? *Hepatology* **54**, 707–713 (2011).
131. Gentilini, A. *et al.* Role of the stromal-derived factor-1 (SDF-1)–CXCR4 axis in the interaction between hepatic stellate cells and cholangiocarcinoma. *J. Hepatol.* **57**, 813–820 (2012).
132. Greten, T. F., Wang, X. W. & Korangy, F. Current concepts of immune based treatments for patients with HCC: from basic science to novel treatment approaches. *Gut* **64**, 842–848 (2015).
133. Bruix, J., Gores, G. J. & Mazzaferro, V. Hepatocellular carcinoma: clinical frontiers and perspectives. *Gut* **63**, 844–855 (2014).
134. Quetglas, I. M., Moeini, A., Pinyol, R. & Llovet, J. M. Integration of genomic information in the clinical management of HCC. *Best Pract. Res. Clin. Gastroenterol.* **28**, 831–842 (2014).
135. Sprinzl, M. F. & Galle, P. R. Immune control in hepatocellular carcinoma development and progression: role of stromal cells. *Semin. Liver Dis.* **34**, 376–388 (2014).
136. Sprinzl, M. F. *et al.* Sorafenib inhibits macrophage-induced growth of hepatoma cells by interference with insulin-like growth factor-1 secretion. *J. Hepatol.* **62**, 863–870 (2015).
137. Sangro, B. *et al.* A clinical trial of CTLA-4 blockade with tremelimumab in patients with hepatocellular carcinoma and chronic hepatitis C. *J. Hepatol.* **59**, 81–88 (2013).
This is the first clinical trial to evaluate the safety and efficacy of checkpoint blockade in HCC. The results underline the potential of immunotherapy as a novel therapeutic approach for liver cancer.
138. Snyder, A. *et al.* Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N. Engl. J. Med.* **371**, 2189–2199 (2014).
139. Heo, J. *et al.* Randomized dose-finding clinical trial of oncolytic immunotherapeutic vaccinia JX-594 in liver cancer. *Nat. Med.* **19**, 329–336 (2013).
140. Le, D. T. *et al.* Safety and survival with GVAX pancreas prime and *Listeria Monocytogenes*-expressing mesothelin (CRS-207) boost vaccines for metastatic pancreatic cancer. *J. Clin. Oncol.* **33**, 1325–1333 (2015).
141. Wang, J., Reiss Binder, K., Khatri, R., Jaffee, E. & Laheru, D. Immune therapy in GI malignancies: a review. *J. Clin. Oncol.* **33**, 1745–1753 (2015).
142. Bruix, J., Han, K. H., Gores, G., Llovet, J. M. & Mazzaferro, V. Liver cancer: approaching a personalized care. *J. Hepatol.* **62**, S144–S156 (2015).
143. Forner, A., Llovet, J. M. & Bruix, J. Hepatocellular carcinoma. *Lancet* **379**, 1245–1255 (2012).
144. Rizvi, S. & Gores, G. J. Pathogenesis, diagnosis, and management of cholangiocarcinoma. *Gastroenterology* **145**, 1215–1229 (2013).
145. Qian, M. B., Utzinger, J., Keiser, J. & Zhou, X. N. Clonorchiasis. *Lancet* http://dx.doi.org/10.1016/S0140-6736(15)60313-0 (2015).

Acknowledgements
This project was supported by the Intramural Research Program of the Center for Cancer Research, National Cancer Institute, Bethesda, Maryland, USA (to S.S.T.). J.U.M. is supported by grants from the German Research Foundation (MA 4443/2-1), German Cancer Aid (DKH 110989) and the Volkswagen Foundation (Lichtenberg program). J.B.A. is supported by grants from the Danish Cancer Society (Knæk cancer program), the Novo Nordisk Foundation (Hallas-Møller fellowship), the Danish Medical Research Council (Sapere Aude program) and the A.P. Møller Foundation.

Competing interests statement
The authors declare no competing interests.