Cell of origin in biliary tract cancers and clinical implications

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Summary

Biliary tract cancers (BTCs) are aggressive epithelial malignancies that can arise at any point of the biliary tree. Albeit rare, their incidence and mortality rates have been rising steadily over the past 40 years, highlighting the need to improve current diagnostic and therapeutic strategies. BTCs show high inter- and intra-tumour heterogeneity both at the morphological and molecular level. Such complex heterogeneity poses a substantial obstacle to effective interventions. It is widely accepted that the observed heterogeneity may be the result of a complex interplay of different elements, including risk factors, distinct molecular alterations and multiple potential cells of origin. The use of genetic lineage tracing systems in experimental models has identified cholangiocytes, hepatocytes and/or progenitor-like cells as the cells of origin of BTCs. Genomic evidence in support of the distinct cell of origin hypotheses is growing. In this review, we focus on recent advances in the histopathological subtyping of BTCs, discuss current genomic evidence and outline lineage tracing studies that have contributed to the current knowledge surrounding the cell of origin of these tumours.

Keywords: Biliary tract cancers; Cholangiocarcinoma; Cell of origin; Genomics; Lineage tracing; Personalized therapy

Received 2 November 2020; received in revised form 14 December 2020; accepted 16 December 2020; available online 19 January 2021

Introduction

Biliary tract cancers (BTCs) are a highly heterogeneous group of malignancies that affect both the small intrahepatic and large extrahepatic bile ducts (cholangiocarcinoma) or the gallbladder (gallbladder cancer).[1,2] While dismal clinical prognosis is a common underlying trait, histological and epidemiological features significantly differ across the BTC subtypes.[1–6] Furthermore, the recent application of next-generation sequencing (NGS) technologies in large international BTC cohorts suggests a pronounced intra- and inter-tumoural molecular heterogeneity.[7–16] Understanding the origins of such heterogeneity is paramount to improve diagnosis and treatment strategies for this disease.

Both cell-autonomous (*i.e.* genetics) and non-cell autonomous elements (*i.e.* microenvironment) have been proposed as sources of tumour heterogeneity across several cancer types.[17–21] An increasing body of evidence also suggests that the different tumour profiles may be significantly influenced by the existence of multiple cells of origin.[22–24] For example, early progenitor cells or stem cells have emerged as the potential cell of origin in several solid cancers,[22,25] including colon,[26,27] prostate[28,29] and glioblastoma;[30,31] at the same time, in other malignancies such as breast cancer,[32] different mature cell types may be the target for oncogenesis, ultimately leading to tumours with different morphology and metastatic behaviour. The possibility of multiple cells of origin is particularly relevant in BTCs which may exhibit distinct phenotypical traits of cholangiocytes and undifferentiated cells. In addition, the remarkable plasticity of cholangiocytes and hepatocytes in response to various injuries has fuelled the intriguing, albeit controversial, hypothesis that both cell types may represent the cell of origin of some BTCs.

Herein, we provide an overview of the current classification system of BTCs and review the evidence supporting hypotheses regarding the cell of origin of the distinct subtypes. In the foreseeable future, integration of this information into current histopathological and molecular stratification systems may have important implications in the design of tailored therapeutic strategies.

Clinicopathological insights into the origin of BTCs

Overall, BTCs are relatively rare malignancies accounting for ~3% of all gastrointestinal tumours. Tumours of the biliary tract can be classified as cholangiocarcinoma (CCA) and gallbladder cancer (GBC) (Fig. 1).[1,2] According to the anatomical location, CCA can be further sub-classified into intrahepatic (iCCA), perihilar (pCCA) and distal (dCCA). iCCA accounts for 20–30% of all CCAs and refers to those neoplastic lesions forming in the bile ductules and segmental ducts located within the liver (Fig. 1). pCCA (50% of all CCAs) and dCCA (20-30% of CCAs) arise outside of the liver, with pCCA developing on the large bile ducts in the hepatic hilum

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https://doi.org/10.1016/j.jhepr.2021.100226

and above the insertion of the cystic duct, while distal dCCA arises below the insertion of the cystic duct.[33,34] pCCA and dCCA have been referred to collectively as extrahepatic cholangiocarcinoma (eCCA), although increasing evidence suggests that these subtypes may represent distinct molecular entities.[2]

Over the past 40 years, distinct epidemiological trends have been reported for the BTC subtypes.[5,35] Multiple studies have shown rising incidence and mortality rates for iCCA, while rates for eCCA have been relatively steady or even decreasing in some European countries.[36–40] Collectively, the widely reported rising incidences in iCCA need to be interpreted with caution since the accelerated trends may reflect, in part, the establishment of better classification systems. The epidemiological patterns of CCA also show great geographical variability, broadly reflecting differences in risk factors as well as genetic determinants (Table 1). In the Western world the annual incidence of CCA is between 0.3 to 6 cases per 100,000 people, whereas in South-East Asia it reaches up to 85 cases per 100,000.[5] While recognised risk factors account for approximately half of CCA cases, these are more prevalent in South-East Asia (*i.e.* liver fluke infections and biliary malformations), whereas most cases occur sporadically in the West.

GBC is the most prevalent type of BTC, occurring at an incidence of 1.6 cases per 100,000 people, and it is the only digestive cancer that is more common in women than men.[35,41] According to a recent analysis of the World Health Organization (WHO)'s Cancer Mortality database, rates for GBC are decreasing in most countries but increasing in several high-income countries due to emerging trends in lifestyle changes, such as increase in excess body weight.[35] Like CCA, a disparity in the global disease burden has been observed, with the highest incidence rates of GBC among indigenous populations in South America and Northern India.[42]

Key points

- Biliary tract cancers are clinically and molecularly heterogeneous.
- Histopathological diversity and emerging integrative genomic analyses support the hypothesis of multiple cells of origin of BTCs.
- The existence of hepatic progenitor cells in adult liver remains controversial, largely due to mixed results obtained from lineage tracing studies.
- Depending on the oncogenic insult and presence of liver injury, lineage tracing systems identify both mature hepatocytes and cholangiocytes as potential sources of liver regeneration and iCCA development.
- Understanding the nature of the cell of origin of BTCs holds the potential to guide a more accurate diagnosis and personalised treatment decision-making.

The distinct BTC subtypes also differ regarding specific risk factors, clinical presentation and management. Below, we review the current staging system for BTCs, focusing on the histopathological features that, over the years, have fuelled the hypothesis of multiple cells of origin.

Intrahepatic cholangiocarcinoma and other rare primary liver cancers

Among BTCs, iCCA stands out due to a highly heterogeneous macro- and microscopic appearance. This is reflected in its

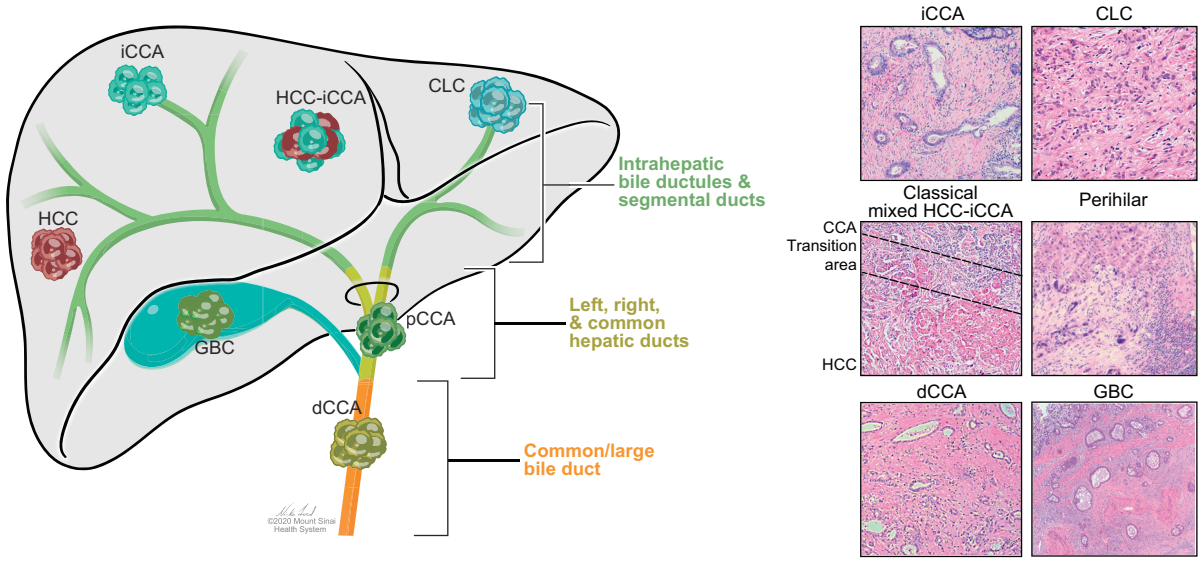

Fig. 1. Biliary tract cancer classification, anatomical location and histopathological traits. Based on the anatomical site of origin, BTCs are classified into iCCA, pCCA, dCCA, and GBC. iCCA is defined as tumours located in the periphery of intrahepatic bile ductules and segmental ducts. iCCA together with HCC and mixed HCC-CCA represent the main types of primary liver cancers arising in the liver parenchyma. Mixed HCC-CCAs are a group of histologically heterogeneous tumours sharing unequivocal phenotypical characteristics of both HCC and iCCA. Multiple subtypes of mixed HCC-CCA have been identified, including classical HCC-CCA and CLC among others. Classical mixed HCC-CCA presents hepatocytic and cholangiocytic components, either admixed or as separate areas within the same tumour (displayed in the fig.); CLC presents malignant ductular-like structures embedded in a dense stroma (displayed in the figure). Among the extrahepatic CCA subtypes, pCCA arises in the right and/or left hepatic duct and/or at their junction, and dCCA involves the common bile duct. Representative histopathological images of different BTCs subtypes are included to ultimately highlight the heterogeneity in cellular phenotypes. BTCs, biliary tract cancers; CCA, cholangiocarcinoma; CLC, cholangiolocarcinoma; dCCA, distal cholangiocarcinoma; eCCA, extrahepatic cholangiocarcinoma; GBC, gallbladder cancer; HCC, hepatocellular carcinoma; iCCA, intrahepatic cholangiocarcinoma; pCCA, perihilar cholangiocarcinoma.

Table 1. Main clinico-pathological features and potential cell of origin.

		eCCA				
Tumour type	**GBC**	**pCCA**	**dCCA**	**iCCA**	**Mixed HCC-CCA**	**HCC**
Annual incidence*	1.6/100,000	1.12/100,000		0.92/100,000	<0.1/100,000	9.5/100,000
Male to female ratio	<1:2	1.3:1	1.5:1	1.4:1	1.9:1	3:1
High incidence regions	Chile/Northern India	South-East Asia	South-East Asia	South-East Asia	Unknown	East Asia and Sub-Saharan Africa
Underlying disease	Cholecystolithiasis Gallbladder polyps Obesity Chronic cholecystitis	PSC Liver flukes Biliary cysts	PSC Liver flukes Choledocholithiasis Biliary cysts	PSC Viral hepatitis Cirrhosis Liver flukes	Viral hepatitis Cirrhosis	Viral hepatitis Cirrhosis NAFLD/NASH
Expected 5 year OS**	~20%	10%	11%	8%	<10%	~19%
Potential cell of origin	Mature cholangiocyte, Gallbladder epithelial cell	Biliary progenitor cell, mature cholangiocyte		HPCs, mature hepatocyte and cholangiocyte		HPCs and mature hepatocyte

dCCA, distal cholangiocarcinoma; eCCA, extrahepatic cholangiocarcinoma; GBC, gallbladder cancer; HCC, hepatocellular carcinoma; HPCs, hepatic progenitor cells; iCCA, intrahepatic cholangiocarcinoma; pCCA, perihilar cholangiocarcinoma; PSC, primary sclerosing cholangitis; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; OS, overall survival.
* Age-adjusted annual incidence in USA.
** Including all stages.

histological classification which defines conventional tumours as well as rare variants.[43,44] Based on the size of the affected duct, conventional iCCA tumours are sub-stratified into small and large bile duct iCCAs. Small bile duct iCCA mostly derives from interlobular and septal bile ducts and displays a mass-forming growth pattern.[45] On the other hand, large bile duct iCCA arises in large intrahepatic ducts, presents increased mucin production and is more frequently preceded by precancerous lesions

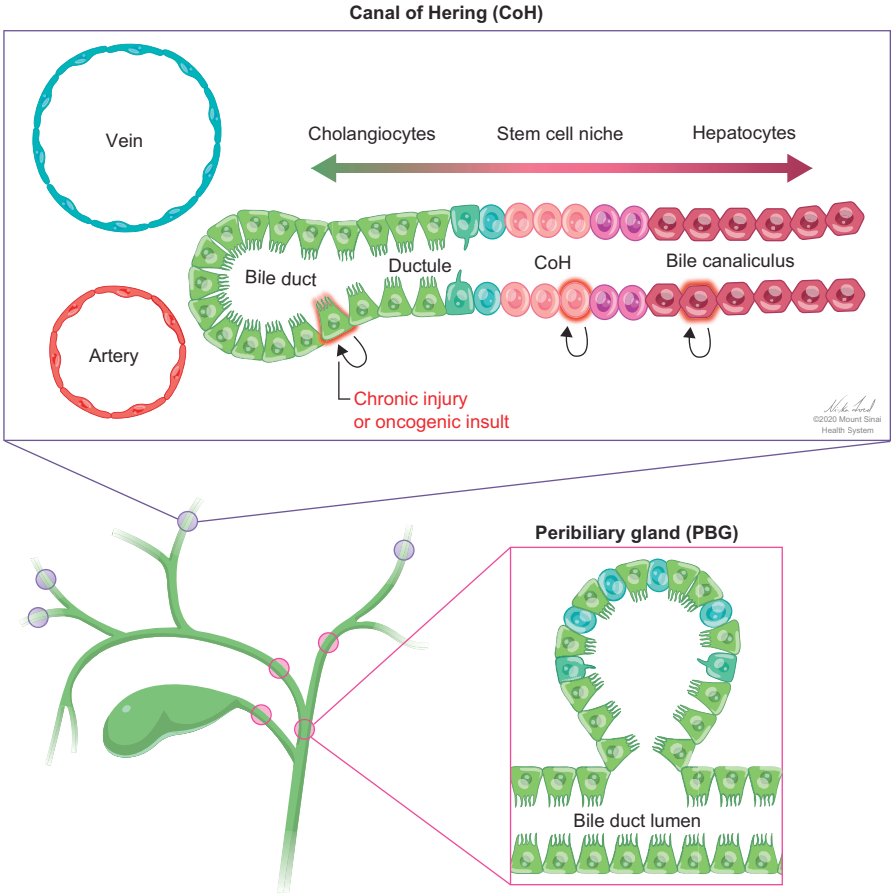

Fig. 2. Hepatobiliary stem cell niches. Schematic representation of the biliary system anatomy with emphasis on the location and structure of stem cell niches. In the adult liver, HPCs are postulated to be located within the CoH (purple circles) near the portal triads. HPCs are thought to have the potential to differentiate into hepatocytes and cholangiocytes. Mature hepatocytes and cholangiocytes have high self-renewal capability and are responsible for normal tissue turnover upon injury or oncogenic insult (represented as highlighted cells with red hallow). Biliary tree stem cell niches containing biliary-committed progenitor cells are located in the PBGs (pink circles). PBGs are occasionally observed as small evaginations of the bile duct epithelium at the level of the septal intrahepatic bile ducts and along the extrahepatic duct, containing less differentiated (in blue) to fully differentiated biliary cells (in green). CoH, canal of Hering; HPCs, hepatic progenitor cells; PBGs, peribiliary glands.

Table 2. Main genetic alterations and potential targeted therapies in BTCs.

Genes	Alteration type	Frequencies*			Targetable alteration	Drug	Clinical outcome		Therapy line
		iCCA	eCCA	GBC			ORR % (DCR)	Median PFS, months	
TP53	Mutation	30%	40%	53%	No	n.a.	n.a.	n.a.	n.a.
KRAS	Mutation	15%	30%	10%	Yes	AMG 510	NR	NR	NR
IDH1/2	Mutation	20%	3%	2%	Yes	Ivosidenib[122]	2 (51)	2.7	2nd line
						Enasidenib	NR	NR	NR
						Dasatinib	NR	NR	NR
FGFR1-3	Fusion, mutation	20%	1%	3%	Yes	Pemigatinib[121]	35.5 (82)	6.9	2nd line
						Infigratinib[134]	14.8 (75.4)	5.8	2nd line
						Derazantinib[135]	20 (76.7)	NR	2nd line
						Fusibatinib[136]	25 (79)	NR	2nd line
ARID1A	Mutation	15%	12%	13%	No	n.a.	n.a.	n.a.	n.a.
CDKN2A/B	Loss	15%	17%	10%	No	n.a.	n.a.	n.a.	n.a.
BAP1	Mutation	13%	0%	1%	No	n.a.	n.a.	n.a.	n.a.
RNF43	Mutation	9%	0%	4%	Yes	RXC004	NR	NR	NR
ERBB2/3	Mutation, amplification	7%	15%	20%	Yes	Lapatinib[130] #	0 (26)	1.8	1st & 2nd line
						Erlotinib[131] ##	6 (35)	2.0	1st line
						Neratinib[141]	10.5 (31.6)	1.8	2nd line
PIK3CA	Mutation	6%	7%	10%	Yes	Alpelisib, copanlisib	NR	NR	NR
BRAF	Mutation	3%	3%	4%	Yes	Dabrafenib[137] ###	51 (91)	9 mo	2nd line
MET	Amplification	5%	3%	0%	Yes	Tivantinib	NR	NR	NR
NTRK1-3	Fusion	4%	4%	4%	Yes	Entrectinib Larotectinib	NR	NR	NR
SMAD4	Mutation	10%	21%	4%	No	n.a.	n.a.	n.a.	n.a.
PRKACA/B	Fusion	0%	2%	0%	Yes	DNAJB1-PRKACA vaccine	n.a.	n.a.	n.a.

ARID1A, AT-rich interactive domain-containing protein 1A; BAP1, BRCA1-associated protein 1; BRAF, v-Raf murine sarcoma viral oncogene homolog B; CDKN2A/B, cyclin-dependent kinase inhibitor 2A/B; DCR, disease control rate; FGFR2, fibroblast growth factor receptor 2; GBC, gallbladder cancer; iCCA, intrahepatic cholangiocarcinoma; IDH1/2, isocitrate dehydrogenases 1/2; KRAS, Kirsten Rat Sarcoma Viral Oncogene Homolog; MET, Hepatocyte Growth Factor Receptor; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PRKACA/B, Protein Kinase CAMP-Activated Catalytic Subunit Alpha/Beta; NR, not reported; n.a., not applicable; NTRK, neurotrophic receptor tyrosine kinase 1; ORR, objective response rate; PFS, progression-free survival; RNF43, ring finger protein 43; SMAD4, SMAD Family Member 4.

* Frequencies adopted from Lamarca *et al.*[138]

\# Drug tested in non-enriched population.

\## Tested in combination with sorafenib in a non-enriched population.

\### Drug tested in combination with the MEK inhibitor trametinib.

(*i.e.* biliary intraepithelial neoplasm or intraductal papillary neoplasm).[46,47] It has been speculated that the different histopathology of these subtypes may be due to a different cell of origin and pathogenesis. For example, large bile duct iCCA shares phenotypic traits with pCCA and pancreatic cancer, perhaps suggesting a common origin.[48,49] Similarly, given the association of small bile duct iCCA with chronic liver disease, it has been proposed that its onset may be linked to activation of hepatic stem cells and senescence of mature hepatocytes in chronic liver diseases. Nonetheless, conclusive data validating such hypotheses are lacking.[50]

iCCA is the second most common primary liver cancer (PLC) after hepatocellular carcinoma (HCC).[2] Even though the vast majority of iCCAs are sporadic, in recent years risk factors for chronic liver disease, which are conventionally associated with HCC (*i.e.* viral hepatitis, alcohol consumption, non-alcoholic steatohepatitis, *etc.*), have been shown to concomitantly increase the risk of iCCA (Table 1).[51,52] The association between iCCA and chronic liver disease, as well as the the existence of mixed HCC-CCA tumours, has fuelled the hypotheses of a common cell of origin for these PLCs. Mixed HCC-CCA encompasses a group of rare (<1% of all PLCs) and histologically diverse tumours that, based on histopathological features, do not fit into either typical HCC or iCCA subtypes (Fig. 1).[53] These tumours have attracted great attention due to their possible genesis from stem cells, although it should be noted that a stem/cell progenitor phenotype is not solid proof of their *bona fide* cell of origin. According to the latest World Health Organization classification, the term mixed HCC-CCA refers to tumours containing unequivocal intimately mixed components of both HCC and iCCA and/or bi-phenotypic stem-like cells.[54] Cholangiolocarcinoma (CLC) is also grouped with mixed HCC-CCA, although it may represent a unique biliary subtype.[55,56] CLC arises in small intrahepatic ductules and is characterised by low grade cytologic atypia, and anastomosing cords and glands resembling cholangioles or canals of Hering.[54] From a histopathological point of view, CLCs are negative for hepatocyte markers such as HepPar1, but positive for biliary lineage markers including cytokeratin (CK)7, CK19 and the progenitor-like marker NCAM.[55] Nonetheless, recent studies have reported that CLC and CK19+ HCC share clinico-pathological features, and thus may originate from a single hepatic progenitor cell (HPC).[57,58]

Perihilar and distal cholangiocarcinoma CCA

The other CCA tumour types, pCCA and dCCA, are conventional mucin-producing adenocarcinomas, although several rare histological variants have been described. These tumours may display 4 main patterns of growth including:[59] i) polypoid papillary tumours, ii) nodular tumours, iii) scirrhous constricting, the most common type, and (iv) diffusely infiltrating tumours. Predisposing factors for pCCA and dCCA revolve around chronic inflammation within the large bile ducts.[51,60] This is frequently precipitated by primary sclerosing cholangitis (PSC) in Western countries and liver fluke infestation in Eastern countries.[5]

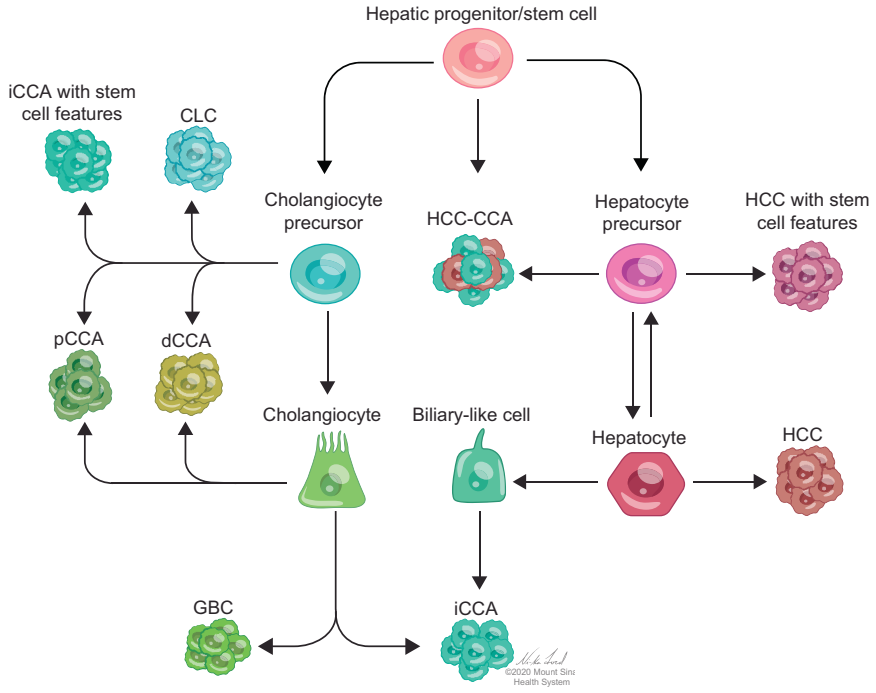

Fig. 3. Potential cells of origin of hepatobiliary cancers. Current evidence from histopathological, genomic and preclinical models suggest multiple potential cells of origin. HPCs or dedifferentiated hepatocytes can potentially generate liver tumours with biliary features. In addition, intermediate states of HPCs, such as biliary-committed precursors, may represent the cell of origin of CLC or iCCA with stem cell features. Furthermore, recent evidence supports the hypothesis that mature hepatocytes can transdifferentiate into biliary-like cells, leading to the development of iCCA. Finally, cholangiocyte lineage cells (precursor and/or mature cholangiocyte) are considered to be the common cell of origin of all anatomical subtypes of BTCs. BTCs, biliary tract cancers; CLC, cholangiolocarcinoma; dCCA, distal cholangiocarcinoma; HPCs, hepatic progenitor cells; iCCA, intrahepatic cholangiocarcinoma; GBC, gallbladder cancer; HCC, hepatocellular carcinoma; iCCA, intrahepatic cholangiocarcinoma; pCCA, perihilar cholangiocarcinoma.

Histological analysis of samples containing extrahepatic or large intrahepatic bile duct tissue obtained from patients with PSC has revealed a key role of peribiliary glands (PBGs, Fig. 2) in the progression of bile duct lesions.[61] PBGs are clusters of epithelial cells residing in the sub-mucosal compartment of large intrahepatic and extrahepatic bile ducts. Although the majority of cells contained in PBGs are mature epithelial cells, few populations expressing stem/progenitor cell markers and immature phenotypes have been identified.[62,63] Interestingly, these cells proliferate in response to bile duct injury as observed in patients with PSC[61] and liver fluke infection,[64] thus functioning as a biliary stem cell niche. Accordingly, PBGs may represent the potential cell of origin of eCCA.

Gallbladder cancer

Unlike other BTCs, GBC is more common in women and often grows within the fundus of the gallbladder.[60] At the histological level, adenocarcinomas constitute the most frequent phenotype of GBC; nonetheless, less frequent variants (for example, intestinal type, clear cell carcinoma and signet ring cell carcinoma) have recently been recognised by the WHO.[65] Similar to eCCA, GBC is associated with underlying chronic inflammation of the large bile ducts.[51,60] Overall, the gallbladder shares a common embryological origin with the liver and ventral pancreas.[66] In a recent study, it has been demonstrated that while gallbladders do not have PBGs, pluripotent EpCAM+ endodermal stem/progenitors are present in the mucosal crypt of the human gallbladder.[67] However, whether such cells could be a potential cell of origin of GBC remains to be explored.

Genomic data supporting the cell of origin of BTCs

In the past decade, significant efforts have been conducted to elucidate the molecular pathogenesis of BTCs, particularly iCCA, through the application of multi-omics approaches, including genomic, epigenomic, transcriptomic, and metabolomic analyses.[9,13,68–71] Integration of these findings into the clinical staging system is key for the development of novel drugs and to improve current histology-based diagnosis of specific subtypes.

Overall, the molecular evidence suggests that BTC tumours arising from distinct anatomical sites represent different molecular entities. For example, while *TP53* and *KRAS* mutations are relatively common to all BTCs, the occurrence of isocitrate dehydrogenase (*IDH)1/2, EPHA2* and BRCA1-associated protein 1 (*BAP1)* mutations and fibroblast growth factor receptor (*FGFR)2* fusions is significantly higher in iCCA; on the other hand, *PRKACA* and *PRKACB* fusions, *ELF3* and *ARID1B* mutations are detected almost exclusively in eCCA subsets (Table 2). Notably, iCCA and eCCA subtypes share some predisposing risk factors, such as PSC and liver fluke infections (Table 1). In this regard, genome-wide association studies[13–15] have shown the occurrence of a higher level of non-synonymous mutations and genome-wide epigenetic derangements in fluke-positive CCA, regardless of the subtype. Among the most commonly mutated genes in CCA, mutations in *SMAD4* and *TP53* as well as *ERBB2* (also called HER2) amplification were more frequent in fluke-associated CCA, while fluke-negative CCAs were enriched in mutations in *IDH1/2* and *BAP1* (Table 2).[13,14,72] Whether these molecular features reflect either specific carcinogenic processes or differential vulnerabilities in distinct cells of origin remains to be clarified.

Table 3. Genetically engineered mouse models evaluating the potential cells of origin of BTCs.

Cell of origin	Tumour type	Signalling pathway	Method/system	Model	Ref.
HPCs, Hepatocytes or cholangiocytes	iCCA	NOTCH	GEMM	Alb-Cre;NotchIC	97
	iCCA	TP53 and NOTCH	GEMM	Alb-Cre;Tp53$^{f/f}$;NotchICD	101
	iCCA, HCC, mixed HCC-CCA	RAS and TP53	GEMM	Alb-Cre;Kras$^{LSLG12D/+}$;Tp53$^{f/f}$	104
	iCCA	RAS and PTEN	GEMM with liver injury	Alb-Cre:Kras$^{LSLG12D/+}$, Pten$^{f/f}$	107
	iCCA	RAS and TGF-beta	GEMM	Alb-Cre:Smad4$^{f/f}$, Pten$^{f/f}$	94
HPCs/hepatoblast	HCC and iCCA	Hippo/YAP	GEMM	Alb-Cre; sav1$^{fl/fl}$ or mst1$^{fl/fl}$and mst2$^{fl/fl}$	110
	iCCA and HCC	TP53	GEMM	Alfp-Cre;Tp53$^{f/f}$	139
	iCCA	IDH and RAS	GEMM	Alb-Cre;IDH2$^{LSL-R172}$;Kras$^{LSL-G12D}$	70
Mature hepatocytes	iCCA, HCC, mixed HCC-CCA	YAP and AKT	Transposon-based model	Overexpression of PIK3CA and Yap	113
	iCCA	NOTCH and AKT	Hepatocyte fate-tracing, Transposon-based model	Overexpression of NICD1 and AKT	98
	iCCA	YAP and AKT	Transposon-based model	Overexpression of myrAKT and YAPS127A	111
	iCCA	NOTCH and RAS	Transposon-based model	Overexpression of NICD in Kras^{LSLG12D} mice	100
	iCCA	NOTCH and AKT	Transposon-based model	Overexpression of AKT and Jag1	99
	iCCA	NOTCH	Fate-tracing, GEMM with liver injury	Administration of TAA Alb-Cre^{ERT2};R26$^{RlacZ/+}$; Ck19-Cre^{ERT2};R26$^{RlacZ/+}$; *Alb-CreER^{T2};R26R$^{Notch/+}$*	96
	iCCA	MYC and RAS or MYC and AKT	Transposon-based model	Overexpression of mouse Myc and *Nras^{G12V}* or human *AKT1* in ROSA$^{mT/mG}$ × Alb-cre × p19Arf$^{-/-}$ mice	102
	iCCA	PTEN and TGFβ	GEMM	AAV8-TBG-Cre: Pten$^{f/f}$; Tgfbr2$^{f/f}$	105
	iCCA, HCC, mixed HCC-CCA	RAS and TP53	GEMM with liver injury	Administration of DDC diet AAV8-TBG-Cre:Kras$^{LSLG12D/+}$;Tp53$^{f/f}$	106
Mature cholangiocyte	iCCA	NOTCH and TP53	GEMM with liver injury	Administration of TAA Ck19-Cre$^{ERT/eYFP}$;Tp53$^{f/f}$,Notch3	103
	iCCA	RAS and PTEN	GEMM	Tamoxifen-inducible Alb-Cre^{ERT2+};Kras$^{LSL-G12D/+}$;Pten$^{flox/flox}$, or Ck19Cre$^{ERT/+}$;Kras$^{LSL-G12D/+}$;Pten$^{flox/flox}$	108
	iCCA, pCCA/dCCA	RAS and PTEN	GEMM	Ah-Cre^{ERT}:Kras$^{V12/+}$, Pten$^{f/f}$	140
	iCCA	RAS and TP53	GEMM	Sox9-Cre^{ERT2};Kras$^{LSLG12D/+}$, Tp53$^{f/f}$	106
	pCCA/dCCA	RAS and TGFβ	GEMM	Ck19-Cre^{ERT}:Kras^{LSLG12D};Tgfbr2$^{f/f}$;Cdh1$^{f/f}$	118
	GBC, pCCA/dCCA	EGFR	GEMM	Bk5-Erbb2	117
	iCCA	PTEN and TGFβ	GEMM with liver injury	Administration of DDC diet Ck19-Cre^{ERT} or Prom1-Cre^{ERT2}; Pten$^{f/f}$;Tgfbr2$^{f/f}$	105
	iCCA	AKT and YAP	Transposon-based model	Intrabiliary transduction of active AKT (myr-AKT) and human YAP (YAPS127A)	109
Gallbladder epithelium	GBC	RAS and NOTCH	GEMM	Pdx1-Cre: Kras$^{LSL-G12D/+}$	119
	GBC	Estrogen and TGFβ	GEMM	LXRbeta(-/-)	120

CCA, cholangiocarcinoma; dCCA, distal cholangiocarcinoma; DDC, 3,5-diethoxycarbonyl-1,4-dihydrocollidine; eCCA, extrahepatic cholangiocarcinoma; EGFR, epidermal growth factor receptor; GBC, gallbladder cancer; GEMM, genetically engineered mouse model; HCC, hepatocellular carcinoma; iCCA, intrahepatic cholangiocarcinoma; IDH, isocitrate dehydrogenase; pCCA, perihilar cholangiocarcinoma; TAA, thioacetamide; TBG, thyroid-binding globulin; TGFβ, transforming growth factor-β; TP53, Tumor Protein P53.

Independent studies incorporating transcriptomic and mutational analysis have identified 2 main subtypes of iCCA:[68,73] (i) the inflammation class, characterised by inflammatory signalling and STAT3 activation; and (ii) the proliferation class which features the upregulation of classical oncogenic pathways (KRAS, epidermal growth factor receptor [EGFR] and NOTCH). Tumours belonging to the proliferation class present with an adverse outcome and a more aggressive phenotype. Of note, a subset of tumours belonging to the iCCA proliferation class are enriched with liver-specific stem cell gene signatures[73,74] and molecular signatures of HCC with poor prognosis,[75,76] thus suggesting that iCCA and HCC may share a common ancestor.[68,73]

The hypothesis that HPCs may represent the cell of origin of iCCA and other PLCs is further supported by the molecular features of mixed HCC-CCA subtypes. In an attempt to clarify the clonality of these neoplasms, recent integrative molecular analyses of the micro-dissected HCC and iCCA foci within these tumours have shown similar allelic imbalances in most cases, suggesting a potential single clonal derivation in some subtypes of PLCs.[55,77] Similarly, the subtype of mixed HCC-CCA with stem cell features shows a molecular profile characteristic of undifferentiated and CK19+ HCCs, hinting at a single bi-potential cell of origin (Fig. 3).

Among mixed HCC-CCA, CLC seem to represents a distinct biliary-derived entity.[55,58,78] Whole-genome transcriptome-based analyses of CLCs have suggested a molecular profile more similar to iCCA than conventional HCCs.[55,79] In addition, CLC presents mutations in *IDH1/2*, a common feature in a subset of iCCA tumours which display stem cell-like features and higher chromosomal instability.[68,70,73] Overall, these findings suggest that a biliary-committed precursor may represent the potential cell of origin for CLC and a subset of iCCA with stem cell features (Fig. 3).

Due to the low number of eCCA samples analysed in large international initiatives, no molecular classification had been proposed for eCCA until recently. In an analysis of 189 clinically annotated eCCAs (76% pCCA and 24% dCCA) from Western countries, 4 distinct eCCA molecular classes were identified: i) metabolic (19%); ii) proliferation (22%); iii) mesenchymal (47%); and iv) immune (12%). Direct comparison of eCCA with

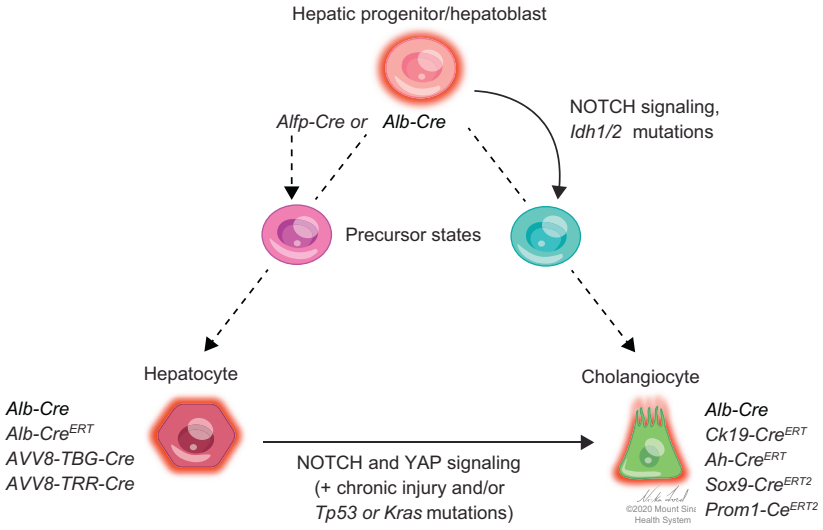

Fig. 4. Lineage tracing promoter systems and related pathways involved in the onset of BTCs. Depending on the experimental setting and developmental stage, the Cre system may recombine floxed alleles in different cell types. The Cre^{ERT} system, a fusion of *Cre* and the tamoxifen-inducible domain of the estrogen receptor, enables spatiotemporal control, which has been crucial for manipulating genes in the adult liver. Induction of alterations in signalling pathways (black continuous arrows), for example activation of NOTCH and YAP signalling, together with oncogenic insults (*i.e. Tp53 or Kras* mutations) or liver injury, result in the malignant transformation of potential cells of origin (highlighted with red hallow). KRAS, Kirsten Rat Sarcoma Viral Oncogene Homolog; TP53, Tumor Protein P53.

previously described iCCA molecular classes only identified significant similarities in the *Proliferation* classes.[7] However, it remains unclear if the shared molecular features may reflect a common origin.

GBC remains relatively underexplored at the genomic level compared to other BTCs. Reports are widely confined to mutational analysis and a deeper understanding of its molecular pathogenesis is still lacking. Of note, frequently mutated genes include *KRAS*, *TP53* as well as mutations in the ErbB pathway,[11] which occur in all BTC subtypes (Table 2).

In a recent NGS-based international study, Wardell *et al.* analysed the genomic landscape of 412 BTC samples from Japanese and Italian populations, including 136 iCCAs, 101 dCCAs, 109 pCCAs and 66 GBCs.[16] The authors predicted the cell of origin of a subset of the BTC samples and 3 additional HCC series by combining somatic mutation patterns and epigenetic features. Interestingly, the majority of HCCs (~90%) were classified as originating from hepatocytes. Conversely, BTCs were classified as originating from the liver or epithelial cells in 33% and 36% of cases, respectively. Overall, iCCA samples were more commonly classified as originating from hepatocytes than epithelial cells (43.5% *vs.* 17%), ultimately supporting results of lineage tracing studies (discussed in the next paragraphs). The opposite was true for dCCAs and GBCs whose origin was more commonly assigned to a non-liver source. Interestingly, iCCAs on a background of hepatitis were more likely to be predicted to derive from liver sources. Unfortunately, in this study only 39 BTC samples were used to infer the cell of origin. Further studies are awaited to further corroborate these results in larger cohorts and further explore the genomic landscape of these tumours, particularly eCCAs and GBCs.

Evidence from mouse models

In several instances, the tumour phenotype may not directly reflect the tumour histology and the underlying process of tumorigenesis.[22] Therefore, the use of genetically engineered mouse models (GEMM) and lineage tracing systems has proven indispensable to elucidate the cell of origin of cancer, including BTCs.

A unique feature of biliary cancers is that they manifest in the hepatic parenchyma or large intrahepatic and extrahepatic bile ducts, which are furnished by 2 distinct stem cell niches: the canals of Hering and PBGs, respectively (Fig. 2).[80] Despite recent advances, the existence of compartmentalised *bona fide* stem cell populations within the liver and their role as a potential cell of origin of BTCs remains debatable. This is largely due to the mixed results obtained from different lineage tracing studies as well as accumulating evidence demonstrating the high cellular plasticity of the mature liver epithelium. In the adult liver, both mature hepatocytes and cholangiocytes exhibit high self-renewal capacity and are responsible for normal tissue turnover. In addition, under certain circumstances, this cellular plasticity seems to be bidirectional, with hepatocytes able to transdifferentiate into biliary-like cells and cholangiocytes able to act as facultative stem cells. Below, we summarise the experimental evidence supporting these highly controversial claims (Fig. 3 and Table 3).

The cellular source of liver regeneration following chronic injury

The liver is a unique organ with an extraordinary capacity to self-repair and regenerate upon various injuries.[81] In homeostasis, adult hepatocytes are mostly quiescent with slow cell turnover; however, upon hepatic injury or partial hepatectomy, hepatocyte turnover accelerates to restore hepatic mass and function.[82] Several studies using multiple lineage tracing of different hepatic cell lines have demonstrated that, after partial hepatectomy or in the context of acute liver injuries, regeneration predominantly relies on the self-renewal of hepatocytes rather than stem cell differentiation.[83–87] Conversely, when hepatocyte proliferation is impaired, bipotential HPCs or oval cells, postulated to be localised in the canals of Hering (Fig. 2), are the cellular source of

hepatocyte turnover.[81,88,89] To further complicate the scenario, 2 recent studies have demonstrated that cholangiocytes can also act as facultative liver stem cells and contribute to hepatocyte regeneration in the context of severe and prolonged liver damage.[90,91] Indeed, Raven *et al.* demonstrated that the combination of liver injury (*i.e.* loss of β1-Integrin) with inhibition of hepatocyte proliferation (*i.e.* p21 overexpression) causes regeneration of functional hepatocytes from biliary cells.[90] These findings have been further validated by Deng *et al.* who showed that biliary-derived hepatocytes replenish a large fraction of the liver parenchyma following severe chronic injuries induced by long-term thioacetamide or 3,5-diethoxycarbonyl-1,4-dihydrocollidine treatment.[91] All together these data suggest that: i) the nature of the injury can determine the cellular source of epithelial regeneration; and ii) cholangiocytes represent a potential cellular source of hepatic regeneration.[92] Although significant gaps exist between the animal models of liver regeneration and the complex clinical scenarios of chronic liver injuries, these studies provide insights into the potential mechanisms driving liver regeneration. Whether the injury-induced plasticity of hepatocytes and cholangiocytes could also occur in the setting of BTC tumorigenesis remains to be clarified.

Hepatocytes or cholangiocytes: who should we blame for iCCA?

Based on histological observations, the mature cholangiocyte within the intrahepatic small bile ducts has traditionally been considered the cell of origin of iCCA. Nonetheless, during the past decade, the use of sophisticated cell lineage tracing systems and GEMMs has provided compelling evidence supporting the hypothesis that iCCA may also originate from the malignant transformation of hepatocytes (Fig. 3). Most of these models are based on the use of hepatocyte-targeted Cre or overepression systems (Table 3). Among them, it should be noted that the activity of constitutive albumin (Alb)-Cre system in some instances is not restricted to fully differentiated hepatocytes (Fig. 4). In fact, depending on the developmental stage, the *Alb-Cre* allele may recombine floxed alleles in both hepatocytes, cholangiocytes and hepatoblasts.[93,94] Alternatively, other investigators have adopted the hydrodynamic delivery of transposon-based system that is known to preferentially transduce 5–40% of hepatocytes in the adult liver.[95]

To date, several studies have consistently shown that hepatocyte-specific activation of NOTCH, either alone[96,97] or in cooperation with AKT activation,[98,99] gain-of-function *Kras*[100] or loss-of-function *Tp53* mutations,[101] results in the conversion of mature hepatocytes into malignant biliary cells. In order to provide the direct evidence that fully differentiated hepatocytes can give rise to iCCA, Fan *et al.* used a hepatocyte fate-tracing model and elegantly showed that NOTCH and AKT signalling cooperate to convert normal hepatocytes into biliary cells that act as precursors in iCCA development.[98] Using both hepatic and biliary lineage tracing systems, Sekiya *et al.* have further demonstrated that, upon chronic liver injury, iCCA arises from biliary lineage cells derived from hepatocytes and not cholangiocytes, with NOTCH signalling being the key mediator of biliary differentiation.[96] The tumour microenvironment may also play a role in determining iCCA growth from oncogenically transformed hepatocytes. Indeed, it has recently been demonstrated that in the presence of an apoptotic microenvironment, hepatocytes with aberrantly activated oncogenes (loss of *p19Arf* in combination with gain-of-function alterations including *Myc* and *NrasG12V* or *Myc* and AKT1) give rise to HCC whereas a necroptotic-associated cytokine microenvironment will lead to iCCA.[102]

Hepatocytes are not always the cell undergoing the neoplastic transformation. Indeed, Guest *et al.* have demonstrated that in the context of chronic inflammation and biliary-specific (*Ck19-Cre*) activation of *NOTCH* and mutant *TP53,* cholangiocytes lead to iCCA formation.[103] In addition, murine models based on the combination of *Pten* and *Tgfbr2* deletions or *Tp53* loss and *Kras* activation suggest that iCCA can derive from either biliary (*Ck19-Cre* or *Sox9-Cre*, respectively) or hepatic (*Alb-Cre* or hepatic-specific thyroid-binding globulin promoter [*TBG*]-*Cre*) compartments (Table 3).[104–106] Interestingly, the use of an adeno-associated vector to express Cre recombinase only in adult hepatocytes under the *TGB* promoter (AAV8-TBG-Cre) suggested that *Tp53* and *Kras* mutated adult hepatocytes are refractory to malignant transformation in the absence of liver injury.[106]

The application of sophisticated approaches further supports the role of cholangiocytes in iCCA formation. For example, the use of a cell lineage visualisation system using tamoxifen-inducible Cre-*loxP* has recently demonstrated that *Kras* activation and *Pten* deletion in both adult hepatocytes or cholangiocytes leads to iCCA tumours from cholangiocytes rather than hepatocytes.[107,108] Finally, using a technically challenging approach for specific intrabiliary instillation of a transposon system expressing activated forms of YAP and AKT coupled with IL-33 administration, Yamada *et al.* have further demonstrated the role of cholangiocytes in the onset of iCCA.[109]

Overall, this evidence suggests that depending on the oncogenic insults, adult hepatocytes can undergo a phenotypic switch to induce iCCA development, while adult cholangiocytes can readily go through malignant transformation. Further research is needed to understand in which circumstances and under which oncogenic insults one cell type rather than the other will ultimately give rise to iCCA.

Lesson learnt from rare bi-phenotypic primary liver cancers

As previously mentioned, the existence of mixed HCC-CCA tumours suggests the possibility that a progenitor-like cell may represent the cell of origin of iCCA and other PLCs. Experimental models based on the activation of the HIPPO-YAP pathway via Alb-Cre derived knock-out of the neurofibromatosis type 2 (*Nf2*) gene, inactivation of *Mst1/2 or Sav1,* and ectopic expression of *Yap,* have demonstrated the expansion of atypical ductal cells or progenitor-like cells and the subsequent development of iCCA, HCC and mixed HCC-iCCA tumours.[110–113] Similarly, viral-related specific transduction of mouse HPCs/hepatoblasts with transgenes encoding oncogenic H-Ras and SV40LT or *Bmi1* and mutated β-catenin results in the formation of a broad spectrum of liver tumours with iCCA and/or HCC features.[114,115] However, due to the limitations of the *Alb-Cre* promoter, whether the HPCs are the actual cell giving rise to these tumours remains debatable. To date, the only study specifically pointing to the expansion of progenitor-like cells as a key mechanism contributing to iCCA development is based on the expression of gain-of-function *Idh* mutations.[70] In this study, Saha *et al.* demonstrated that *Idh* mutations block hepatocyte lineage progression and enhance the expansion of HPCs which, upon acquisition of an oncogenic insult (*i.e. Kras* mutations), lead to the development of premalignant biliary lesions and progression to iCCA.[70]

Peribiliary glands as potential niche for the cell of origin of eCCA

During embryogenesis, intrahepatic and extrahepatic bile ducts originate from the intrahepatic ductal plate and hepatic diverticulum,[116] respectively, thus suggesting different tumorigenic processes for iCCA and eCCA subtypes. However, due to the lack of specific models of eCCA development, our understanding of the mechanisms underlying the onset of pCCA/dCCA remains scarce. Interestingly, well-differentiated eCCA tumours have been detected in the extrahepatic bile duct of a mouse model of iCCA generated by liver-specific *Kras* activation and *Pten* deletion[107] and in transgenic mice over-expressing *Erbb2* in the basal layer of the biliary tract epithelium[117] (Table 3). Of note, in the latter study, mice also developed GBC suggesting that mature cholangiocytes may represent a common cell of origin for all BTC subtypes.

Recent efforts have been focused on generating a clinically relevant model of eCCA development by incorporating the most frequent genetic alterations identified in NGS-based studies.[7,9,13] In this regard, Nakagawa *et al.* developed a mouse model of injury-related eCCA through biliary-cell-specific *Kras* activation and deletion of TGFβ receptor type 2 (*Tgfbr2*) and E-cadherin (*Cdh1*).[118] Remarkably, these mice fully recapitulate the characteristic features of the human disease, including thickening of the extrahepatic bile duct wall accompanied by a swollen gallbladder, and moderately-differentiated adenocarcinoma cells with periductal infiltrating growth patterns of pCCA/dCCA. Cell lineage tracing in these mice suggested biliary precursors of the PGB as the cell of origin of eCCA (Figs. 2 and 3).[118]

Cell of origin of GBC

Similar to eCCA, only few murine models of GBC are currently available (Table 3). As discussed, overexpression of *Erbb2* in the basal layer of the biliary tract epithelium under the bovine keratin 5 (BK5) promoter leads to tumour formation at various sites along the biliary tract.[117] Interestingly, GBC develops in 90% of these transgenic mice[117] suggesting a common cell of origin for GBC and CCA (Fig. 2). Data obtained in an independent investigation showed that targeted transduction of the gallbladder epithelium via *Pdx1-Cre* driven expression of oncogenic *Kras* leads to upregulated NOTCH signalling and gallbladder tumourigenesis, thus suggesting gallbladder epithelial cells, rather than cholangiocytes, are the direct target of carcinogenesis.[119] Similarly, malignant transformation of the gallbladder epithelium was observed in a knock-out model of the oxysterol receptor liver X receptor–β (LXRβ), which is involved in the control of lipid homeostasis and glucose metabolism.[120] Carcinogenesis in this model was estrogen dependent, consistent with a higher incidence of GBC in women (Table 1).

Potential therapeutic impact of the cell of origin

Outcomes of patients with BTC remain poor, particularly at advanced stages when most cases are diagnosed. Until recently, available therapeutic strategies relied almost exclusively on systemic chemotherapy. However, thanks to the discovery of targetable molecular alterations, the treatment landscape of BTCs has been rapidly evolving, and it is becoming evident that tailored approaches elicit superior outcomes (Table 2). In this regard, the recent accelerated FDA-approval of the FGFR inhibitor pemigatinib in patients with CCA harbouring *FGFR2* fusion genes is exemplary.[121] While these recent advances are encouraging, the magnitude of the benefit conveyed by these therapies is limited and there remains an unmet need to improve outcomes for all patients.

A better understanding of the cells of origin of BTCs could facilitate the identification of specific molecular mechanisms of carcinogenesis amenable to therapeutic targeting. Unfortunately, thus far, no biomarker able to trace back each patient's tumour to its originating cell has been identified; in particular, we still do not know if the cell of origin may determine the genetic mutation landscape or vulnerability to specific therapeutic strategies. Similarly, it is important to keep in mind that the previously described molecular classes, in particular the progenitor-like cluster of iCCA, as well as aberrations in specific pathways may just reflect a unique transcriptional profile and do not necessarily mirror the cell of origin of that tumour.

In this scenario, iCCA with stem cell features and CLC harbour frequent mutations in *IDH1/2* genes. These gain-of-function mutations result in the accumulation of oncometabolites and promote tumour progression. Several IDH-inhibitors have been developed and recently the IDH1 inhibitor ivosidenib has been proven superior to placebo in terms of progression-free survival in an enriched population in the ClarIDHy phase III trial.[122] Aside from targeting IDH1/2, signalling pathways involved in trans-differentiation of hepatocytes and tumorigenesis (*i.e.* NOTCH,[123] WNT/beta-catenin[124] and Hippo/Yap[125]) represent attractive targets. However, since these pathways play a key role in normal physiology, toxicity remains a concern. Serious adverse effects are an overarching theme, particularly in the exploration of NOTCH inhibitors.[126] Likewise, targeting YAP in tumours with a stem cell phenotype may be an intriguing therapeutic approach but concerns regarding toxicity outweigh potential benefits. Finally, WNT-pathway inhibition is an enticing option currently under evaluation in several phase I clinical trials.[127] The role of the TGFβ signalling pathway in tumour progression has prompted several preclinical studies investigating the efficacy of selective inhibitors targeting this pathway. A phase I study of bintrafusp alfa, a bifunctional fusion protein[128] targeting both TGFβRII and the immune checkpoint PD-L1 (programmed cell death 1 ligand 1), was able to elicit promising objective response rates of 20% in a non-enriched population of BTCs.[129]

While the therapeutic repertoire for iCCA is expanding, drug development for pCCA, dCCA and GBC has failed to keep pace. The main potentially targetable alterations detected in these tumours relate to EGFR and HER2-4, but trials investigating the HER-pathway inhibitors, lapatinib and erlotinib, have failed to show a meaningful benefit.[130,131]

Immunotherapy with checkpoint inhibitors has elicited impressive survival benefit in many solid cancers. Several clinical trials testing these agents are currently underway in patients with BTC. Unfortunately, initial results have been disappointing, ultimately suggesting that these tumours may be mostly resistant.[2] Considering the potential role of the microenvironment in dictating the onset of different types of liver cancer,[102] a more comprehensive understanding of the complexity and diversity of the immune microenvironment of BTCs and its relationship with tumour genotypes and cellular hierarchies may provide sound rationale for the use of immunotherapy in combination with targeted therapies in selected populations. This is of high relevance since tumours displaying stem cell features correlate with more aggressive tumour behaviour and poor clinical outcome.

Concluding remarks

BTCs represent an extremely heterogenous group of cancers with different anatomic location, risk factors, molecular features and potentially distinct cells of origin. Although several genomic advances have helped elucidate the molecular pathogenesis of BTCs and improve their histopathological classifications, the critical question of which cell is responsible for cancer initiation remains unanswered. Experimental studies applying sophisticated lineage tracing systems have indicated that HPCs, mature hepatocytes and cholangiocytes may give rise to iCCA. Albeit less explored, emerging data suggest that while eCCAs may derive from either biliary progenitor cells or mature cholangiocytes, GBCs may arise from mature cholangiocytes and/or gallbladder epithelial cells. In addition, the phenotypic complexity and presence of progenitor/stem cell features in BTC could also be explained by the so-called *cancer stem cell model*, which suggests that tumour cells dedifferentiate to acquire progenitor cell features and become cancer stem cells (extensively reviewed in[132,133]). However, controversy exists. Future research will need to clarify the similarities between experimental models and the human disease and the exact relationship between the cell of origin, tumour genotype and immune microenvironment. Incorporating this information into current staging systems could provide the rationale for combining targeted therapies with immune checkpoint inhibitors and ultimately identify potential biomarkers of response and resistance. This is particularly relevant given that only a small fraction of patients seems to benefit from currently available immunotherapies and the understanding of the immune landscape of BTCs remains limited. In this regard, the analysis of the tumour, immune and stroma compartment at the single cell level via single-cell RNA sequencing could provide critical information to clarify the role of the microenvironment in shaping distinct tumour phenotypes.

Abbreviations

ARID1A, AT-rich interactive domain-containing protein 1A; BAP1, BRCA1-associated protein 1; BTC, biliary tract cancer; BRAF, v-Raf murine sarcoma viral oncogene homolog B; CCA, cholangiocarcinoma; CDKN2A/B, cyclin-dependent kinase inhibitor 2A/B; CK, cytokeratin; CLC, cholangiolocarcinoma; CoH, Canal of Hering; dCCA, distal cholangiocarcinoma; DCR, disease control rate; eCCA, extrahepatic cholangiocarcinoma; ER, estrogen receptor; ERBB2/3, Erb-B2 Receptor Tyrosine Kinase 2/3; FGFR, fibroblast growth factor receptor; FGFR2, Fibroblast Growth Factor Receptor 2; GBC, gallbladder cancer; GEMM, genetically engineered mouse models; HCC, hepatocellular carcinoma; HPCs, hepatic progenitor cells; iCCA, intrahepatic cholangiocarcinoma; IDH, isocitrate dehydrogenase; KRAS, Kirsten Rat Sarcoma Viral Oncogene Homolog; MET, Hepatocyte Growth Factor Receptor; mo, months; MST1, Macrophage Stimulating 1; NA, not applicable; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NGS, next-generation sequencing; NR, not reported; NTRK, Neurotrophic Receptor Tyrosine Kinase 1; ORR, objective response rate; OS, overall survival; PBG, peribiliary gland; pCCA, perihilar cholangiocarcinoma; PFS, progression-free survival; PIK3CA, Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha; PROM1, Prominin 1; PLC, primary liver cancer; PRKACA/B, Protein Kinase CAMP-Activated Catalytic Subunit Alpha/Beta; PSC, primary sclerosing cholangitis; RNF43, Ring Finger Protein 43; SMAD4, SMAD Family Member 4; TBG, thyroid binding globulin; TP53, Tumor Protein P53; WHO, World Health Organization.

Financial support

P.K.H. is supported by the fellowship grant of the German Research Foundation (DFG: HA 8754/1-1). The work of D.S. is supported by the Tisch Cancer Institute, the Dr. Franklin M. Klion Young Scientist Research Award and the PhD Scientist Innovative Research Award.

Conflicts of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Conception and design [AM, DS]. Literature review and drafting of the manuscript [AM, PKH, DS]. Critical revision and editing [DS]. All the authors approved the final manuscript.

Acknowledgements

We would like to thank Prof. Swan N. Thung for providing the histological images and Ni-ka Ford for her contribution in the design work of the figures.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2021.100226.

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Author names in bold designate shared co-first authorship

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