Murine Liver Organoids as a Genetically Flexible System to Study Liver Cancer In Vivo and In Vitro

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The rising incidence of cholangiocarcinoma (CCA) coupled with a low 5-year survival rate that remains below 10% delineates the urgent need for more effective treatment strategies. Although several recent studies provided detailed information on the genetic landscape of this fatal malignancy, versatile model systems to functionally dissect the immediate clinical relevance of the identified genetic alterations are still missing. To enhance our understanding of CCA pathophysiology and facilitate rapid functional annotation of putative CCA driver and tumor maintenance genes, we developed a tractable murine CCA model by combining the cyclization recombination (Cre-lox) system, RNA interference, and clustered regularly interspaced short palindromic repeats/CRISPR associated protein 9 (CRISPR/Cas9) technology with liver organoids, followed by subsequent transplantation into immunocompetent, syngeneic mice. Histologically, resulting tumors displayed cytokeratin 19–positive ductal structures surrounded by a desmoplastic stroma—hallmark features of human CCAs. Despite their initial biliary phenotype in vitro, organoids retained the plasticity to induce a broader differentiation spectrum of primary liver cancers following transplantation into recipient mice, depending on their genetic context. Thus, the organoid system combines the advantage of using nontransformed, premalignant cells to recapitulate liver tumorigenesis as a multistep process, with the advantage of a reproducible and expandable cell culture system that abrogates the need for recurrent isolations of primary cells. Conclusion: Genetically modified liver organoids are able to transform into histologically accurate CCAs. Depending on the oncogenic context, they are also able to give rise to liver cancers that show features of hepatocellular carcinomas. The model can be used to functionally explore candidate cancer genes of primary liver cancers in immunocompetent animals and evaluate novel treatment regimens. (Hepatology Communications 2019;3:423-436).

Three-dimensional (3D) organoid cultures have been derived successfully from a variety of organ systems and cell types, such as intestinal cells, cerebral tissues, and, more recently, liver cells. These culture systems exhibit commitment to the respective tissue of origin and faithfully retain their genetic profiles, even after long-term expansion. Although the exact localization of the liver...
organoid–initiating cell remains elusive, the organoids cultured under conditions described by Huch et al. likely originate from epithelial cell adhesion molecule (EpCAM⁺) biliary epithelial cells and not from hepatocytes, the most abundant cell type in the liver. Consequently, these cells readily express biliary markers in vitro. Seminal work recently demonstrated that, apart from normal liver tissue, organoids can be established from human liver cancer specimens, and organoids derived from hepatocellular carcinoma (HCC), cholangiocarcinoma (CCA), or mixed subtypes are able to maintain the genetic and histological hallmarks of the initial tumor specimen in vitro.

CCA is a dismal malignancy that is highly refractory to current chemotherapeutic regimens and targeted therapies. At the time of diagnosis, most patients present with advanced disease not amenable to surgical resection. Histologically, CCA commonly exhibits a ductal growth pattern with an abundant fibrous stroma.

Despite an increasing wealth of sequencing data from human tumor specimens and the identification of recurrent genetic alterations, as well as key prognostic transcriptome signatures, detailed understanding of actionable molecular alterations remains elusive. Here, we describe how primary murine liver organoids can be used to study CCA in vivo and in vitro and aid in the translational annotation of the CCA mutagenome.

Materials and Methods

Additional information, including antibodies, sample preparations, introduction of short hairpin RNAs (shRNAs) and single-guide RNAs (sgRNAs) into organoids, bioinformatic analyses, and statistical procedures, is provided in the Supporting Information.

ANIMAL EXPERIMENTS

All experiments involving mice were performed according to animal protocols approved by local authorities (the Lower Saxony State Office for Consumer Protection and Food Safety). Animals were maintained under standard housing conditions with a 12-hour day–night cycle and access to food and water ad libitum. All interventions were performed during the day cycle. Recipient mice (C57BL/6) and NSG [NOD.Cg-PrkdcscidIl2rgtm1Wjl/SzJZtm], 5-8 weeks old) were obtained from the local animal facility (Hannover Medical School, Germany). Kirsten rat sarcoma oncogene Kras tm4Tyj (KrasLG12D)(9) mice were a gift from Dieter Saur (Munich, Germany), and Kras tm4Tyj-Trp53 tm1Bmn (KrasLG12D; p53lox/lox) mice were a gift from Florian Kühnel (Hannover, Germany). C57BL/6J mice were used as recipients for organoids derived from C57BL/6J or syngeneic KrasLG12D mice. Organoids derived from KrasLG12D mice. Organoids derived from KrasLG12D; p53lox/lox animals (mixed background) were implanted into NSG mice.
ISOLATION OF MURINE LIVER ORGANOIDs

Murine organoids were isolated from adult C57BL/6J mice, Kras<sup>lslG12D</sup> mice, or Kras<sup>lslG12D/wt, p53lox/lox</sup> mice according to published protocols. (11) Briefly, murine livers were minced and enzymatically digested in Earle’s Balanced Salt Solution (EBSS; Thermo Fisher, Waltham, MA) containing 2.5 mg/mL Collagenase Type IV (Sigma-Aldrich, St. Louis, MO) and 0.1 mg/mL DNase I (Sigma-Aldrich) for 20 to 40 minutes with repeated pipetting and passed through a 70-µm cell strainer. After additional washes, cells were spun at 300g, resuspended in 100% Growth Factor Reduced Matrigel (Corning, NY), and plated (two 50-µL droplets per 24-well). After solidification, Matrigel droplets were overlaid with 500-µL murine liver organoid media according to published protocols. (11) Alternatively, residual fragments retained in the cell strainer after collagenase digest were plated in Matrigel. For passaging, organoids were mechanically disrupted by repeated pipetting using a P200 pipette tip, followed by a 5-minute to 8-minute enzymatic digestion in TrypLE Express solution (Thermo Fisher).

TUMOR CELL ISOLATION

Tumors were minced and enzymatically digested in a shaking incubator with Collagenase IV 1 mg/mL (Sigma-Aldrich) in EBSS (Thermo Fisher) for 30 minutes at 37°C. Cells were washed, spun at 300g, and resuspended either in Matrigel and overlaid with organoid expansion medium for organoid culture or resuspended and plated on tissue culture dishes in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin for 2-dimensional (2D) culture.

CELL VIABILITY ASSAY

Kras<sup>lslG12D/wt, p53lox/lox</sup> and Kras<sup>G12D/wt, p53Δ/Δ</sup> organoids were seeded in 100-µL 10% Matrigel per 96-well. Two, 24, and 48 hours after seeding, the organoids were lysed and CellTiter-Glo 3D Luminescent Cell Viability Assay (Promega, Madison, WI) was performed according to the manufacturer’s protocol.

For inhibitor treatment, Kras<sup>G12D/wt, p53Δ/Δ, LMP_shRenilla.713</sup> tumor cell lines established as 2D or 3D cultures from primary, organoid-derived tumors were plated at 1,000 cells (2D cell line) and 10,000 cells (3D cell line) per 96-well and treated with selumetinib (3.36 µM; MedChem Express, Monmouth, NJ), NVP-BKM120 (0.25 µM; MedChem Express), or a combination of both inhibitors for 24 hours and 48 hours. At the indicated time points, cells were lysed using CellTiter-Glo Luminescent Cell Viability Assay or CellTiter-Glo 3D Luminescent Cell Viability Assay, and luminescence was acquired on a Glomax Multi Detection System (Promega, Madison, WI).

FLOW CYTOMETRY

Single cell suspensions from murine livers or organoids were prepared and incubated with primary antibody (1:100 dilution) for 30 minutes at 4°C (Allophycocyanin-EpCAM; Thermo Fisher; Cat. #17-5791-80). Flow cytometry was performed on a FacsCanto (BD Biosciences, San Jose, CA).

Results

EXPRESSION OF ONCOGENIC KRAS IN COMBINATION WITH P53 LOSS IN TRANSPLANTED LIVER ORGANOIDs GIVES RISE TO CCA IN RECIPIENT MICE

We previously developed a murine model for CCA based on the transplantation of fetal liver cells. (12) Because fetal liver cells cannot be propagated long term in vitro, the model required the continuous maintenance of multi-allelic colonies, timed matings, and isolation of primary cells. We aimed to evaluate the use of liver organoids to create a CCA model that eliminates the need for repetitive fetal liver cell isolations while maintaining the advantage of using nontransformed, premalignant cells to recapitulate tumorigenesis as a multistep process.

The Kras oncogene and the tumor suppressor Tp53 are among the most frequently mutated genes in CCA. (13,14) Hence, we isolated organoids from adult Kras<sup>lslG12D/wt, p53lox/lox</sup> mice and cultured them according to published protocols. (11) As anticipated, we could detect expression of cytokeratin 19 (CK19), cytokeratin 7 (CK7), sex determining region Y (SRY)-box 9, and EpCAM, in line with a biliary phenotype of liver organoids in vitro (Fig. 1 A,B; Supporting Fig. S1).
Interestingly, when we slightly modified the isolation procedure by plating the fragments that remained after collagenase digestion instead of plating liver cell suspensions, organoids budded abundantly from the fragments, leading to an accelerated establishment of stable organoid cultures (Supporting Fig. S2).

To activate the latent mutant Kras allele and excise the p53 allele, we transduced the organoids with a retroviral vector containing a tamoxifen regulatable Cre-recombinase and a neomycin resistance cassette (Fig. 1D), and confirmed excision of the respective lox sites by polymerase chain reaction (PCR).

**FIG. 1.** Murine liver organoids express markers of biliary differentiation. (A) Immunofluorescence staining of organoids reveals expression of biliary markers such as CK7, CK19, and SOX9. Scale bars correspond to 50 μm. (B) The adult murine liver contains only a minor fraction of EpCAM-positive cells as determined by flow cytometry (left histogram), whereas nearly all liver-derived organoids are EpCAM-positive (right histogram). (C) Activation of a latent Kras<sup>G12D</sup> and loss of p53 lead to increased proliferation as assessed by cell viability assay. (D) Schematic of the retroviral vector pSIN-PGK-CreERT-IRES-NeomycinResistance that was used to activate the latent Kras allele and delete p53 following tamoxifen treatment. Abbreviations: DAPI, 4′,6-diamidino-2-phenylindole; RLU, relative light units; and SOX9, sex determining region Y (SRY)-box 9.
Following activation of the latent alleles, proliferation of the organoids increased without any overt morphological alterations (Fig. 1C and Supporting Fig. S3B).

Next, we aimed to determine whether transplantation of the genetically modified organoids leads to tumor development in recipient mice (Fig. 2A). Following subcutaneous (sq) injection, tumors developed with 100% penetrance and were harvested after 8 weeks. Consistent with activation of mutant Kras and loss of p53, Cre-recombined organoids before transplantation as well as organoids derived from resulting tumors (tumoroids) exhibited increased phosphorylated p44/42 mitogen-activated protein kinase (p-p44/42 MAPK) levels and loss of p21 (cyclin-dependent kinase inhibitor 1A) expression (Fig. 2C). Histological examination uniformly revealed gland-forming adenocarcinomas with CK19-positive tumor cells surrounded by an abundant stromal reaction, compatible with moderately differentiated CCA (Fig. 2B).

**LIVER ORGANOIDS FOR PRECLINICAL DRUG TESTING IN VIVO AND IN VITRO**

Therapeutic approaches are limited in patients with CCA, and first-line chemotherapy includes gemcitabine-based treatment protocols. Therefore, we tested the effect of gemcitabine on tumors arising in our murine CCA model. Similar to human patients, gemcitabine treatment led to a moderate survival benefit (median overall survival gemcitabine versus vehicle: 42 days versus 32 days; Supporting Fig. S5), but not to complete tumor regression, suggesting that the model is suitable for preclinical drug testing in an immunocompetent in vivo situation.

Apart from more complex in vivo model systems, tumor cell lines are an indispensable tool to study the molecular pharmacology and explore the efficacy of anticancer drugs in a preclinical setting.

To assess the scalability of the system for drug testing, we first tested whether the system is suitable to generate cancer-derived cell lines that are serially transplantable, while maintaining the histological features of the primary malignancy. Tumors derived from sq-injected Kras\(^{G12D/wt}\), p53\(^{Δ/Δ}\) organoids were collagenase-digested, and cells were cultured and passaged either under standard 2D cell culture conditions in serum-containing media or kept as 3D tumoroid cultures over 14 days (Fig. 3A). Both 2D and 3D cell lines gave rise to predominantly moderately differentiated CCAs with similar relative stromal content (CK19-negative area) following sq transplantation (Fig. 3A-C). Thus, the organoid model can be used to generate well-expandable, genetically defined 2D and 3D tumor cell lines that maintain the histology of the primary tumor following retransplantation. Next,
we interrogated whether in vitro susceptibility toward targeted therapies depends on the culture condition. Combinatorial treatment with mitogen-activated protein kinase kinase (MAPKK) and phosphatidylinositol 3-kinase (PI3K) inhibitors has been suggested for Kras mutant cancers \(^{(15,16)}\) and CCA \(^{(17)}\). Genetically engineered tumor-derived cell lines established in parallel as 2D versus 3D cultures from the same Kras...
FIG. 2. Genetically modified liver organoids give rise to CCA in vivo. (A) Technical outline: Murine organoids lead to tumor formation in recipient mice following Cre-mediated activation of mutant Kras and loss of the tumor suppressor p53. (B) Tumors exhibit glandular differentiation and stromal desmoplasis, resembling human CCA. CK7 and CK19 expression in tumor cells confirm ductal differentiation. Alcian blue staining indicates elevated production of mucins. Cluster of differentiation 31 stains blood vessels, and sirius red/fast green confirms the presence of collagen fibers. Scale bars correspond to 200 µm. (C) Immunoblotting shows a comparable growth inhibition (Supporting Fig. S8C). Tumors derived from non-Kras mutant tumors did not exhibit S-phase reduction (Fig. 3D, lower panel). Cell lines derived from these tumors are a viable tool for in vitro drug studies. Cell cycle analysis revealed that, although organoids can be conveniently used to engineer genetically defined primary tumors, 2D cell lines derived from non-Kras mutant tumors did not exhibit comparable growth inhibition (Supporting Fig. S8C).

LIVER ORGANOIDNS CAN BE USED TO PROBE CANDIDATE CANCER GENES IN VIVO

The advent of next-generation sequencing technologies led to the identification of the mutational landscape of human CCA. However, the relevance of individual genetic alterations for initiation and maintenance of the tumors is still unknown. To probe whether our model is suitable for the functional characterization of candidate tumor suppressor genes, we retrovirally introduced an shRNA directed against the tumor suppressor phosphatase and tensin homolog (shPten) or a nontargeting control (shRenilla) into KrasG12D/WT; p53+/- recombinant organoids. Pten knockdown activated thymoma viral proto-oncogene (Akt) signaling (Fig. 4C) and markedly accelerated tumor growth compared with control-transduced cells (Fig. 4A). Histologically, tumors from both groups were indistinguishable and not altered by loss of Pten (Fig. 4B,D). This experiment highlights the ease with which candidate cancer genes can be probed by RNA interference–mediated knockdown. Of note, a comparable transgenic approach would have required breeding six different transgenic alleles (e.g., Albinum-Cre; KrasG12D/WT; p53+/− organoids; Pten+/- organoids (pre-injection)). Abbreviations: aCGH, array comparative genomic hybridization; CD31, cluster of differentiation 31; Chr, chromosome; CNAs, copy number alterations; H&E, hematoxylin and eosin; Isl, lox-stop-lox; p21, cyclin-dependent kinase inhibitor 1A; and p-p44/42 MAPK, phosphorylated p44/42 mitogen-activated protein kinase.

mutant murine CCA were treated with a MAPKK inhibitor (selumetinib) and a PI3K inhibitor (BKM-120), alone and in combination. Independent of the culture conditions, both 2D and 3D cultures exhibited a comparable response to targeted therapy in vitro, as assessed by luminescence assay (Fig. 3D), indicating that, although organoids can be conveniently used to engineer genetically defined primary tumors, 2D cell lines derived from these tumors are a viable tool for in vitro drug studies. Cell cycle analysis revealed that 24-hour treatment of the 2D cell lines with the respective drugs caused an accumulation of cells in the gap 0 phase/gap 1 phase (G0/G1 phase) and a concomitant S-phase reduction (Fig. 3D, lower panel). Cell lines derived from non-Kras mutant tumors did not exhibit comparable growth inhibition (Supporting Fig. S8C).

The shRNAs were expressed from a retroviral backbone (LMP) co-encoding for a fluorescent reporter gene (green fluorescent protein [GFP]) that, in addition to testing candidate cancer drivers, allows tracing of cells derived from the transplanted organoids in the recipient animal. The stroma cells were GFP-negative, indicating that stroma cells were exclusively derived from the recipient host and not from epitheli-to-mesenchymal transition of tumor cells (Fig. 4D). Stromal content did not differ significantly in tumors with and without loss of PTEN (Supporting Fig. S6).

To address whether the prominent stromal reaction is a direct consequence of injecting the Kras mutant organoids into the subcutaneous microenvironment or whether recruitment of stromal cells is rather an intrinsic feature of the transplanted organoids, we orthotopically injected organoids into the liver. Despite the profoundly different injection sites, tumors developing within the liver were histologically comparable to tumors that formed from sq-injected organoids. In both settings, the organoid-derived, Kras mutant tumor cells were able to recruit stromal cells and induce a desmoplastic reaction (Supporting Fig. S7A,B). In contrast to RNA interference, CRISPR/Cas9 technology facilitates genome editing and allows for
complete loss of function or the generation of genomic deletions. To introduce genetic perturbations into liver organoids using CRISPR/Cas9, we transfected a plasmid co-encoding for a Cre-recombinase, Cas9 protein, and sgRNAs directed against the tumor suppressors Pten and p53 into \( \text{Kras}^{12D/\text{wt}} \) organoids (sgp53/sgPten-CC)\(^{(19)} \) (Fig. 4E, top panel). Cre-mediated recombination was confirmed by PCR and efficient target cleavage by T7 endonuclease assays (Fig. 4E, left panel). Similar to their shRNA-transgenic counterparts, these organoids gave rise to \( \text{CK}19^{+} \)-positive, moderately differentiated murine CCAs (Fig. 4F). Isolated tumoroids showed complete loss of p53 and Pten expression (Fig. 4E, right panel). In summary, liver organoids can be genetically modified with either RNA interference or CRISPR/Cas9 technology to interrogate gene function in vivo, and fluorescent marker proteins facilitate the tracing of transplanted cells in the recipient animal.

ORGANOIDs EXPRESSING A BILIARY MARKER PHENOTYPE ARE ABLE TO GIVE RISE TO TUMORS RESEMBLING HCC FOLLOWING MALIGNANT TRANSFORMATION

Next, we assessed whether the intrinsic biliary marker constellation expressed by the organoids in vitro translates into the development of tumors of biliary differentiation or whether the organoids retain the plasticity to give rise to a broader differentiation spectrum. We transduced organoids derived from WT C57/Bl6 livers with retroviruses encoding for murine myelocytomatosis oncogene (Myc) and co-expressing a red fluorescent reporter (mCherry), an shRNA directed against p53 coupled to a GFP reporter, and transfected an sgRNA targeting \( Apc \) (adenomatous polyposis coli) (Fig. 5A,B and Supporting Fig. S8A). Intriguingly, substituting Kras with Myc as an oncogenic driver resulted in tumors of a different and distinct differentiation: Unlike the duct-forming, stroma-rich adenocarcinomas that arise in the context of mutant Kras, Myc-overexpressing tumors display a compact, solid growth pattern of tumor cell nests lacking prominent desmoplasia. The tumor cells stained negative for CK19 and exhibited more prominent nucleoli, overall more resembling HCC than CCA (Fig. 5C).

Comparative analyses of the CCA tumoroids and the HCC-like tumoroids by RNA sequencing identified 4,723 differentially expressed genes. Unsupervised clustering confirmed the distinct gene expression profile of Myc overexpressing and Kras mutant tumoroids (Fig. 5D). Consistently, GSEA (Fig. 5E) confirmed that Kras-mutated tumoroids were predominantly driven by activation of Kras-dependent gene sets, whereas HCC-like tumoroids showed an activation of MYC target genes, overall matching the differences in histology and thus validating the approach presented here. Similar to what we observed in CCA organoids (Fig. 2D), Myc-overexpressing pre-injection organoids and WT C57Bl/6j organoids, but not tumoroids, clustered together (Supporting Fig. S8B). Moreover, comparative analyses and cross-species integration of the tumoroid expression signatures with a previously published data set of 70 human HCCs, 13 CCAs, and 7 liver cancers of mixed HCC/CCA histology\(^{(20)} \) confirmed that both the HCC-like and CCA-like tumoroids closely reflected the transcriptome profile of respective authentic human cancers (Fig. 5F).

These results indicate that, despite their initial biliary phenotype, the genetic profile of the liver organoids significantly contributes to the morphology of resulting tumors in vivo.

Discussion

CCA is the second-most common primary liver cancer. A rising incidence and pronounced resistance
to current treatment regimens emphasize the need for a better pathophysiological understanding, which is pivotal for the development of novel treatment approaches. Multiple studies have investigated the mutational landscape of CCAs.\(^{13,14}\) To functionally address the role of potential cancer drivers during tumor development and maintenance, as well as to identify effective drug targets, histologically accurate and genetically flexible \textit{in vivo} systems are needed. Organoid cultures offer unique opportunities to model CCA. Unlike other primary culture systems, such as fetal liver cells, organoids can be serially passaged in an untransformed state, cryoconserved, infinitely expanded, and genetically modified according to specific objectives of individual investigations. Importantly, organoid cultures offer the opportunity to minimize cost and time required for continuous animal husbandry and/or repeated isolations of primary cells, thus significantly accelerating the translation of scientific hypotheses into experimental approaches.

We demonstrate that genetic modifications can be rapidly introduced into liver organoid cultures by RNA interference or CRISPR/Cas9 technology. Co-introduction of antibiotic resistance genes facilitates the \textit{in vitro} selection of efficiently targeted cell populations before transplantation. This feature is especially convenient when studying genetic alterations that do not convey a selective advantage to the emerging tumor and might otherwise be outgrown by nonmodified clones. Furthermore, compared with traditional transgenic models, this model can incorporate genetic alterations in multiple genes simultaneously, offering the genetic flexibility to model highly complex genetic interactions across diverse genomic loci.

Our work demonstrates that, in the context of mutant Kras, liver organoids derived from adult murine livers can give rise to tumors histologically matching CCAs with 100% penetrance when injected orthotopically or sq in syngeneic animals. Unlike in transgenic approaches, in which poorly differentiated sarcomatoid tumors or overlaps to tumors of hepatocellular differentiation are frequently observed,\(^{21}\) more than 90% of CCAs are moderately differentiated CCAs (G2). Given the decisive importance of the tumor stroma in tumor development and therapy response, the observed distinct stromal reaction in the genetically modified organoid model closely recapitulates this hallmark feature of human CCAs. Therefore, the model can be highly instrumental to study tumor–stroma interactions in the emerging field of stroma biology.

Liver tumorigenesis is considered a multistep process. Using expression profiling and array comparative genomic hybridization, we provide initial evidence for how the molecular profile of organoids undergoes gradual genetic changes, reflecting the key transforming events from cells that are WT or harbor latent cancer alleles, toward a “premalignant” \textit{in vitro} activated phenotype following introduction of defined genetic alterations, until reaching the fully transformed state of malignant tumoroids.

Indeed, transcriptome alterations are most pronounced in tumoroids, likely due to the outgrowth of highly malignant clones under selective pressure, in combination with the acquisition of additional lesions during \textit{in vivo} tumorigenesis. Similarly, the acquisition of pro-oncogenic copy number alterations can be detected during the progression from \textit{in vitro}
modified organoids to tumoroids. Our study highlights that the de novo introduction of driver oncogenes in combination with tumor suppressor gene loss is capable of introducing specific genomic changes, thus significantly altering the genetic landscape of liver organoids.

The murine liver organoids complement the human counterpart by enabling scientific work in genetically induced tumors that allow, among others, the study of vulnerabilities in the presence of defined combinations of genetic alterations that occur in large fractions of patients with CCA. In addition, considering the significant advances made in the field of tumor immunology, transplantable murine organoids allow us to study CCA in an immunocompetent and syngeneic environment. An additional benefit of our model is the simplicity with which the transition between in vitro and in vivo work can be accomplished. Retransplantation of both 2D tumor-derived cell lines and tumoroids gives rise to cancers that recapitulate the histology of the primary tumors, which indicates that the tumor cell lines retain crucial features of corresponding parental tumors. In addition, 2D and 3D tumor-derived cell lines exhibit comparable susceptibility to targeted therapies. These results suggest that these cell lines are a competent and viable tool for functional in vitro studies, such as drug screening.

The cellular origin of liver cancer remains unresolved. Indeed, in experimental animal models, hepatocytes, as well as bipotent progenitor cells, can give rise to CCA and HCC in a context-dependent and genotype-dependent fashion. Liver organoid cultures are most likely initiated from cells that reside within the biliary epithelial compartment, as suggested by the expression of biliary markers and the inability of mature hepatocytes to contribute to organoid cultures under the described conditions. Despite their biliary phenotype, we were able to show that, in direct dependence on the oncogenic driver, organoids retain their plasticity and can be transformed into tumors that show a markedly different histology than CCA and more closely resemble HCC. Importantly, these tumors also exhibit expression profiles that cluster with human HCCs. Therefore, our genetically modified liver organoids can recapitulate the full morphological and molecular spectrum of primary human liver cancer.

In summary, we have shown that, depending on the genetic context, organoids can give rise to aggressive liver cancers that exhibit characteristics of human CCA as well as HCCs in immunocompetent mice. These tumors not only histologically resemble their human counterparts but also show similar expression profiles. Thus, organoid-based mouse models can serve as a scalable system to facilitate the rapid interrogation of putative cancer genes and vulnerabilities using RNA interference and CRISPR/Cas9 technology.

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Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep4.1312/supplinfo.