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Genetic and epigenetic analysis of hepatocellular adenomas with atypical morphological features

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List of abbreviation

Hepatocellular adenoma (HCA), Hepatocellular carcinoma (HCC), Formalin-fixed and paraffin-embedded (FFPE), argininosuccinate synthase (ASS1), β-catenin mutated HCA (b-HCA), β-catenin mutated and inflammatory HCA (b-IHCA), C-reactive protein (CRP), glutamine synthetase (GS), hematoxylin and eosin stain (H&E), HNF1A mutated HCA (H-HCA), hepatocyte nuclear factor 1A (HNF1A), inflammatory HCA (IHCA), liver-type fatty acid binding protein (LFABP), serum amyloid A (SAA), sonic hedgehog HCA (shHCA), unclassified HCA (UHCA).

Conflicts of interest

The authors disclose no conflicts of interest.

Author contribution

M.S.M. and S.C. conceived and designed the project. M.S.M., H.S., C.J.O., M.-S.N., S.U., S.J., H.J., W.A., S.F., J.B.A., M.L., T.L. and S.C. analyzed the data. M.S.M., H.S., and S.C. wrote the manuscript. All authors agreed to the content of the manuscript.

Statement of Ethics

The study was approved by the ethic commission of Northern Switzerland (EKNZ; study ID: 2019-00776). Consent was obtained for all patients.

Disclosure Statement

M.S.M. has served as a consultant for ThermoFisher, Merck, GlaxoSmithKline, Roche and Novartis. Otherwise, the authors have no conflicts of interest to declare.

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Data Availability Statement

All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.

Permission to reproduce material from other sources

Nothing to declare

Clinical trial registration

Nothing to declare

Abstract

Background: Hepatocellular adenoma (HCA) is a rare liver tumor, which can have atypical morphological features such as cytological atypia, pseudo-glandular architecture, and altered reticulin framework. Little is known about the genetic and epigenetic alterations of such HCAs and whether they show the alterations classically found in HCC or in HCA without atypical morphology.

Methods: We analyzed five HCAs with atypical morphological features and one HCA with transition to HCC. Every tumor was subtyped by immunohistochemistry, sequenced by a targeted NGS panel and analyzed on a DNA methylation microarray.

Results: Subtyping of the five HCAs with atypical features revealed 2 β-catenin mutated HCA (b-HCA), 2 β-catenin mutated inflammatory HCA (b-IHCA), and 1 sonic hedgehog activated HCA (shHCA). None of them showed mutations typically found in HCC, such as e.g. *TERT* or *TP53* mutations. The epigenomic pattern of HCAs with atypical morphological features clustered with reference data for HCAs without atypical morphological features but not with HCC. Similarly, phylo-epigenetic trees using the DNA methylation data reproducibly showed, that HCAs with morphological atypia are much more similar to non-malignant samples than to malignant samples. Finally, atypical HCAs showed no relevant copy number variations (CNV).

Conclusions: In our series, mutational, DNA methylation, as well as CNV analyses supported a relationship of atypical HCAs with non-atypical HCAs rather than with HCC. Therefore, in cases with difficult differential diagnosis between HCC and HCA, it might be advisable to perform targeted sequencing and/or combined methylation/copy number profiling.

Introduction

Hepatocellular adenomas (HCAs) are rare benign liver tumors with a prevalence of 3 to 4 cases per 100'000 subjects and a predilection for young women.(1) Main risk factors include oral contraceptives and anabolic steroid use.(2, 3) A minority of HCAs, less than 10%, may transform into hepatocellular carcinoma (HCC). (2, 4) Based on genotype-phenotype analyses, various HCA subtypes have been described. (2, 5, 6) *HNF1A* mutated HCA (H-HCA) is defined by inactivating mutations in the *HNF1A* gene, causing the downregulation of liver fatty acid- binding protein (LFABP) demonstrated by immunohistochemistry. β-catenin mutated HCA (b-HCA) shows various mutations in the *CTNNB1* gene. In particular, HCAs with mutations in exon 3 non S45 are associated with an increased risk of malignant transformation into HCC (2, 3). They can be recognized by their specific Glutamine synthetase staining (7, 8). Inflammatory HCA (IHCA) reveals expression of serum amyloid A (SAA) as well as of C-reactive protein (CRP) detectable by immunohistochemistry and is defined by the constitutive activation of the IL6/JAK/STAT pathway due to mutations of *IL6ST*, *STAT3, GNAS*, *FRK*, or *JAK1*. *CTNNB1* mutations can occur as a second event in IHCA leading to a subgroup of b-IHCA. More recent data revealed the existence of an additional subtype with activation of the sonic hedgehog pathway and/or diffuse overexpression of argininosuccinate synthetase-1 (ASS1), by contrast to the periportal pattern of moderate expression in the normal liver.(9) Unclassified hepatocellular adenoma (UHCA), not falling into any of the above-described categories, represents less than 2% of the cases.

It has been recognized that morphological analysis of HCAs may reveal atypical features in some cases. They consist in cytological atypia with small cell changes and increased nucleo-cytoplasmic ratio, pseudo-glandular architecture, or even focal reticulin loss, suspicious for malignancy. HCAs with one or more of these features have been called borderline HCAs (10), atypical hepatocellular neoplasm, atypical hepatocellular adenoma, or hepatocellular neoplasm of uncertain malignant potential (HUMP).(11-13) The variety of these names reflects the fact that a precise differential diagnosis between HCA and HCC is difficult in these cases. Indeed, the worrisome features, even if reminiscent for HCC seem to be insufficient for the diagnosis of a carcinoma because being focal. A threshold for atypia such as 5% of the tumor has been proposed for the diagnosis of an atypical hepatocellular neoplasm.(13, 14)

However, this threshold is not generally accepted. Furthermore, a recent study indicated, that strict application of such criteria may lead to many diagnoses of atypical HCAs in cases initially diagnosed as non-atypical HCA, (15) especially if, besides the morphological criteria, an atypical clinical context, or even only the fact that it corresponds to a b-HCA, are also considered to qualify a HCA as being atypical as proposed in some studies (12, 14, 15). The clinical management for HCAs with atypical morphological features needs to be defined. According to the current guidelines by the European Association for the Study of Liver Disease (EASL), HCA with a diameter of more than 5 cm should be resected. (2) If the diameter is < 5 cm, watch and wait after pausing e.g. oral contraceptive is accepted in females, unless the HCA shows a β-catenin mutation in exon 3. (2) However, it is unclear whether HCA with atypical features should be surgically removed, even if the diameter is below 5 cm. One reason for the lack of knowledge on how to treat HCA with atypical morphological features is the fact that they remain poorly defined on a molecular level. Although previous publications have described chromosomal aberrations or mutations to be more frequent in HCA with atypical features, the number of samples investigated still remains low.

We performed an archive search comprising the last 20 years in our institutes of Pathology and collected five formalin-fixed and paraffin-embedded (FFPE) HCAs with atypical features. In addition, we found a HCA with transition to HCC. The goal of our study was to define the molecular landscape of HCAs with carefully defined atypical morphological features. In addition, we aimed to determine whether molecular analyses could be helpful in the differential diagnosis between these HCAs and HCC, and therefore provide a more objective basis for treatment decisions than histomorphology alone.

Methods

Study population

Study samples (n = 6) were selected from the archives of the Service of Clinical Pathology of the Lausanne University Hospital, the Department of Pathology and Molecular Pathology of the University Hospital Zurich, and the Institute of Pathology of Southern Switzerland in Locarno.

Morphological and immunohistochemistry analysis

Immunohistochemistry with respective antibodies (**Supplementary Table 1**) was performed according to standard procedure. In brief, whole FFPE sections were pre-treated with CC1 (Ventana Medical Systems, Tucson, Arizona, USA)(16) and stained for CRP, SAA, β-catenin, glutamine synthetase (GS), LFABP, Ki-67, and argininosuccinate synthase 1 (ASS1). Interpretation followed previously published literature. (1, 5, 8) Discordant cases were jointly reviewed (H.S., M.S.M, S.C.) to reach a consensus.

Next-generation sequencing, library preparation and data analysis

NGS was conducted using a customized panel covering 40 genes involved in HCA and HCC or a panel covering 161 relevant cancer genes (see complete list of genes in the Supplementary Information). Library preparation and sequencing was performed according to the manufacturer's recommendation (ThermoFisher Scientific) and is described in more detail in the supplementary information. Variants were evaluated for their pathogenicity based on previous literature, databases (COSMIC, ClinVar, OncoKB, Varsome), and by using the open-access version from the Cancer Core Europe online portal.(12) Mutations were classified as pathogenic, likely pathogenic, variant of unknown significance (VUS), likely benign, and benign. Mutations classified as benign or likely benign were not reported.

Copy number variation and methylation profiling analysis

DNA was processed and hybridized on a beadchip microarray (Infinium human methylation EPIC, Illumina) covering approx. 850'000 CpG islands distributed across the entire genome. Top differentially methylated probes were determined by calculating standard deviations across the entire dataset comprising >18'000 cases obtained from public resources including TCGA and Gene Expression Omnibus (GEO), as well as from in-house reference collections (n=18537, case Sentrix IDs and coordinates in Supplementary File 1). Filtered set of methylation beta values were compared by uniform manifold approximation projection (UMAP) for dimension reduction as previously described.(17, 18) Copy number plots were generated with conumee (in R). All methylation data and annotations were curated in our public methylation analysis platform EpiDiP (www.epidip.org).

Further information is provided in the Supplementary Information.

Results

Clinical Characteristics

Our study cohort included six female patients with an average age of 31.8 years (±8.75, range 23 – 42 years). Liver tumors had a mean diameter of 12 cm (± 5.9; range 6 - 24 cm). None of the patients had chronic hepatitis or a biliary disease, and neither bridging fibrosis or cirrhosis. Patients also did not suffer from hepatic adenomatosis, glycogen storage diseases, or Fanconi anemia.

Morphological and immunohistochemical features

The five HCAs with atypia showed atypical features in at least 5% of the sectional tumor area (**Fig. 1, Table 1**). However, the changes were not regarded sufficient for the definitive diagnosis of HCC. All HCA exhibited nuclear atypia in 5 - 10% of the tumor area, two showed small cell changes in up to 30% of the tumor area, four revealed focal areas of pseudo-glandular formation in up to 10% of the tumor area, and four showed areas with loss of reticulin fibers in up to 30% of the tumor area. It is important to note that the HCA with 30% reticulin fiber loss also revealed hemorrhages, which might have contributed to the loss of reticulin fibers. However, foci of reticulin loss were present even at distance of the hemorrhages. At least two of the four aforementioned atypical morphological features were present in each HCA included in the series. All 5 HCAs were subtyped by immunohistochemistry following current guidelines (**Table 1**). Two were b-HCA and showed immunohistochemical positivity for glutamine synthetase (GS) at the border of the tumor (**Fig. 1**), and within the rest of the HCA a focal patchy GS positivity (case #1) or a diffuse heterogeneous GS staining (case #2). Both showed negative nuclear immunohistochemistry for β-catenin. One HCA was classified as b-lHCA with immunohistochemical positivity for CRP, SAA, diffuse homogenous positivity for GS and nuclear β-catenin (case #3). One HCA (case #4) showed immunohistochemical positivity for CRP and SAA but was negative for nuclear β-catenin and GS was negative to questionably weakly positive. Targeted sequencing (see below) of this HCA showed a *CTNNB1* missense mutation (p.K335I), which typically leads to an immunohistochemically negative nuclear β-catenin staining and a weak GS staining.(8, 19) Based on the sequencing results in combination with the immunohistochemical results, this HCA (case #4) was classified as a b-lHCA. One

HCA showed a strong overexpression for ASS1 with negative CRP, SAA, β-catenin and GS staining, and retained expression of LFABP and was classified as shHCA (case #5). The case with HCC arising in HCA (case #6), revealed classical adenoma portions but also an area in which the criteria for a HCC were fulfilled with increased atypia, few pseudo-glandular formations, and an extensive reticulin fiber loss (**Fig. 1**). In contrast to the HCAs described above, this lesion further presented with a clearly demarcated pushing border and could easily be separated from the classical adenoma parts (**Fig. 1**). Immunohistochemical analysis revealed diffuse homogenous positivity for GS within the HCC and HCA portion, but negativity for SAA, CRP and nuclear β-catenin. Furthermore, we found a decreased expression of LFABP within both the HCC and the HCA portion, whereas it was weakly retained in the non-tumoral liver.

Targeted sequencing

Next, we wanted to determine if mutational changes characteristic for HCA and/or HCC were present in the HCA with atypia. Therefore, we isolated DNA from the areas with atypical features and performed targeted sequencing (**Table 2**) for the most commonly mutated genes in HCA (e.g. *CTNNB1*, *IL-6ST/JAK/STAT*) and HCC (e.g. *TERT* promoter, *TP53*, or *ARID1A*). As expected from the subtyping by immunohistochemistry, both b-HCA showed mutations in *CTNNB1* gene: one in exon 7 (p.K335I, case #1) and one in exon 3 (p.S45F, case #2). No additional variants were detected in these two b-HCA. The two b-IHCA also showed mutations in *CTNNB1*: one again in exon 7 (p.K335I, case #4) and one in exon 3 (p.V22_D32del, case #3). In addition, the latter revealed a typical mutation for IHCA in *IL6ST* at exon 6 (p.P216H). No mutations typically present in inflammatory adenomas, like *IL6ST, STAT3, GNAS, FRK or JAK1* could be detected for the other b-IHCA. Finally, the shHCA revealed a missense mutation in *LRP1B* and a frameshift deletion in *APOB* (p.G3793fs) of unknown significance (case #5). The mutation found in *LRP1B* has already been described in an adenocarcinoma of the gastroesophageal junction, but neither in HCC nor HCA.(20) The HCC arising in an HCA showed a pathogenic nonsense mutation in *BRD7* in exon 12 in the HCC part. However, no *BRD7* or other mutations, including *HNF1A*, were found in the classical adenoma area of that tumor (case #6), even though the tissue derived from close to the HCC. In addition, we

performed sequencing for 161 relevant cancer genes, which confirmed the already found mutation, yet did not reveal any additional mutation.

Methylation signature and copy number variations

For epigenetic analysis, we first established a reference cohort. From the Cancer Genome Atlas (TCGA and GEO) databases and own samples, we obtained reference cases with the epigenetic signatures of HCA (n=48), HCC (n=381), normal liver tissue (n=74) and "degraded DNA" (n=26). In a uniform manifold approximation projection (UMAP) analysis (https://arxiv.org/abs/1802.03426), HCA, HCC, and normal liver tissue could be separated (**Fig. 2 A-C**).

Next, we performed epigenetic analysis by performing a whole-genome methylation array of all liver tumors analyzed for this study. In accordance with the mutational analysis, HCA with atypia clustered with classical HCAs but not with normal liver tissue or HCC (**Fig. 2D**). Interestingly, the HCC arising within an adenoma clustered with HCCs for the HCC portion, whereas the adenoma portion clustered with HCAs (**Fig. 2D**). Note that the HCA samples typically exhibit methylome profiles most similar to normal liver tissue but yet differ from this signature. HCC samples close to the HCA/LIV cluster typically have low amplitude CNVs (not shown).

To confirm our data in a larger cohort, we re-analyzed public data corresponding to surrounding liver (SL), dysplastic nodules (DN), HCA, early HCC (eHCC), and HCC. We then randomized these samples to a discovery cohort and a replication cohort. In each of these cohorts, we compared the DNA methylation profile of each of these entities to our HCA with atypia. By constructing phylo-epigenetic trees using the DNA methylation data, we were able to reproducibly show that HCA with morphological atypia are much more similar to the non-malignant samples (SL, DN, HCA) than to the malignant samples (early HCC, HCC)(**Fig. 2E**).

In addition, in CNV analysis none of the cases showed copy number changes including the HCC arising in an adenoma (**Supplementary Figure 1**). In particular, no alterations typical for HCC such as gains at chromosomal regions 1q, 7q, 8p, 8q and X as well as losses at 4p, 11q and 16q (11, 21) were observed in any of the six liver tumors.

Discussion

In this study we assessed the immunohistochemical, genomic, and epigenomic features of 6 HCAs with atypical morphological features. Although several criteria have been proposed for the diagnosis of HCA with atypia, the distinction between HCC and HCA with atypia remains difficult. Therefore, we wanted to analyze in more detail molecular changes in HCA with morphological atypia, which might aid in easier differential diagnosis with HCC. Moreover, we wanted to know whether HCAs with morphological atypia more closely resembled HCA without morphological atypia or HCC on a molecular level.

Our cohort of cases comprised two b-HCA, two b-IHCA, and one sh-HCA, all showing at least 2 atypical morphological features, even 3 in the majority of them. Sequencing showed that while two cases had a *CTNNB1* mutation in exon 3, namely, p.S45F and p.V22_D32del, in two other, *CTNNB1* was mutated in exon 7, namely p.K335I. Interestingly, we found only mutations known to occur in HCAs and we did not detect any mutation in *TERT*, *TP53*, or *ARID1A*, which are frequently affected in HCC.(22) This is consistent with previous studies in which *TERT* promoter mutations were only sometimes found in HCAs with morphological atypia and *TP53* mutations were very rarely found.(23, 24) Differences might reflect the variation in the criteria used to define HCAs as being atypical in other studies.

DNA methylation signatures can be used to characterize cancers of unknown origin, brain tumors, sarcomas, and metastases from squamous cell carcinomas.(25-27) In analogy, after obtaining methylation data from the TCGA databases, we observed that methylation signatures separated HCA from HCC and normal liver tissue. In accordance with the sequencing analysis, our methylation signature analysis of the HCAs with morphological atypia revealed that they all clustered within HCA devoid of morphological atypia. Moreover, phylogenetic trees confirmed that these atypical HCAs were much more similar to the non-malignant samples than to malignant samples, such as eHCC and HCC. Therefore, HCA with morphological atypia are molecularly closely related to HCA without atypia, and it can be presumed that they also share similar biological behavior in most cases. Interestingly, the HCA showing transition to HCC clustered with HCCs for the HCC part, but with HCAs for the HCA part. In addition, in the HCC part, we found a non-sense mutation in *BRD7*, which has been observed in various cancers, including HCC. (28, 29). In contrast, this

mutation was not detected in the HCA part, even close to the HCC, further indicating that an abrupt transition from HCA to HCC occurred. However, this is only one case and needs confirmation in further studies.

The five morphologically atypical HCAs of our cohort did not show any copy number variations. In particular, gains of 1q and 8q, which occur early in hepatocarcinogenesis and have also been found in in up to 50% of atypical HCAs, were not identified (11, 21, 24, 30), which may be explained by the small size of our cohort, but also by a different selection of the cases. Indeed, even if small, it was built on strict morphological criteria to define the cases as atypical.

In conclusion, DNA sequencing, CNV analysis and methylome profiling of HCAs with morphological atypia reflected their molecular relationship with HCAs not showing morphological atypia rather than with HCC. In addition, sequencing and methylation signature analyses suggest that HCC foci within HCA already harbor molecular changes separating them from the HCA part. Consequently, when it is difficult to distinguish between an HCA with atypical morphological features and a well-differentiated HCC, DNA methylation profiling and, potentially, targeted sequencing might offer valuable diagnostic tools.

Figure and Table legend

Table 1

Morphological and immunohistochemical characteristics of the 5 HCAs with atypia.

Table 2

Mutational analysis by NGS using a panel covering the most relevant genes affected in HCA and HCC and an additional panel comprising 161 relevant cancer genes.

Figure 1

Morphological and immunohistochemical analyses of HCAs with atypia (A-E) and HCC arising within HCA (F-H). Representative areas with cytonuclear atypia (**A,** H&E, bar = 10 μm), small cell changes (**B**; H&E, bar = 100 μm), pseudo-glandular architecture (**C**; H&E, arrow in yellow, bar = 100 μm), and reticulin fiber loss (**D,** Novotny, bar = 100 μm). Immunohistochemical positivity for GS at the border of the HCA with atypia with diffuse heterogeneous positivity inside the lesion (case #2) (**E**; bar = 200 μm). Representative area of the HCC arising within HCA; note pushing border (**F**; bar = 200 μm) and sharp demarcation (**G**; bar = 100 μm) with reticulin loss (**H**; bar = 100 μm).

Figure 2

Methylation signature (A-D). (**A**) UMAP dimension reduction plot based on top 25'000 differentially methylated CpG sites from 18'537 datasets of the EpiDiP data lake at the time of figure production. (**B**) Overview including all relevant samples, whereof samples clustering in "Liver" includes *bona fide* HCC, HCA and normal liver (see in C). Annotation of HCC cell lines (CL), samples with artifacts and insufficient amounts of intact DNA (DEG), and N_HCC, which are primarily TCGA samples that cluster with other non-HCC neoplasms and inflammatory changes. (**C**) Higher magnification of samples clustered in B as "Liver" with annotation for HCA (blue), HCC (red) and normal liver (NLIV; yellow). (**D**) Higher magnification of samples in C. HCA with atypia from our cohort (2 - 5) marked with black dots. Sample (1) from our cohort had insufficient intact DNA. HCA with transition to HCC with HCA portion (6A) and HCC portion (6b). (**E**) Phylo-epigenetic trees using the DNA methylation profile of HCAs with morphological atypia in comparison to publicly available data from surrounding liver

(SL; n=85), dysplastic nodules (DN; n=30), HCA (n=50), early HCC (eHCC; n=9), and HCC (n=589). Samples were randomized in a discovery cohort and a replication cohort. Abbreviations: CL: HCC cell lines, DEG: degenerated samples, N_HCC: non-HCC neoplasms and inflammatory changes, NLIV: normal liver, HCA: hepatocellular adenma, HCC: hepatocellular carcinoma, AHCA: atypical hepatocellular adenoma from cohort. SL: surrounding liver, DN: dysplastic nodules, eHCC: early HCC.

Supplementary Figure 1

Copy number profiles of liver tumor of our cohort generated from methylation microarray data. Case #1 - # 6.

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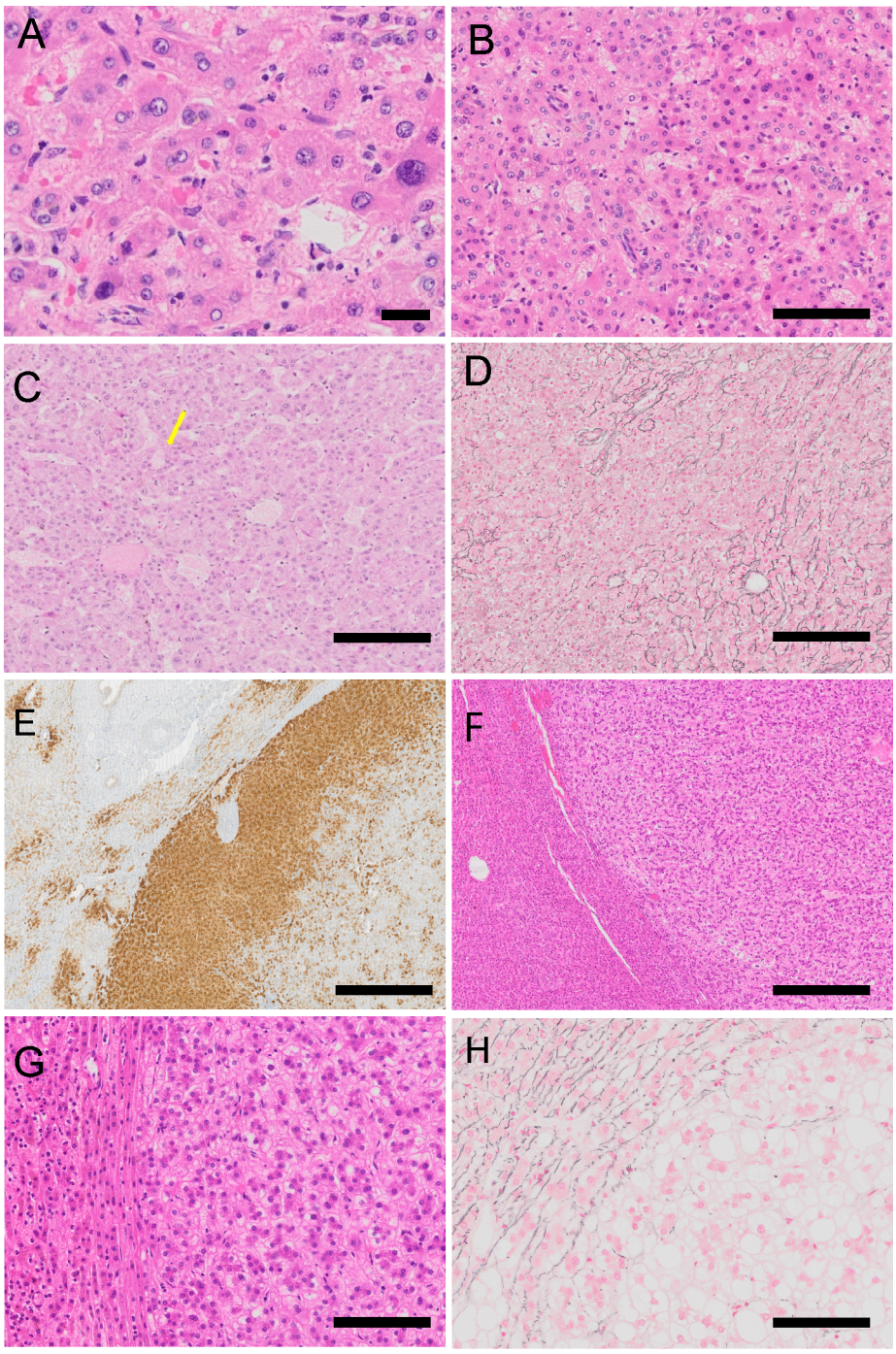

Figure 1

HIS_14858_Figure 1.tif

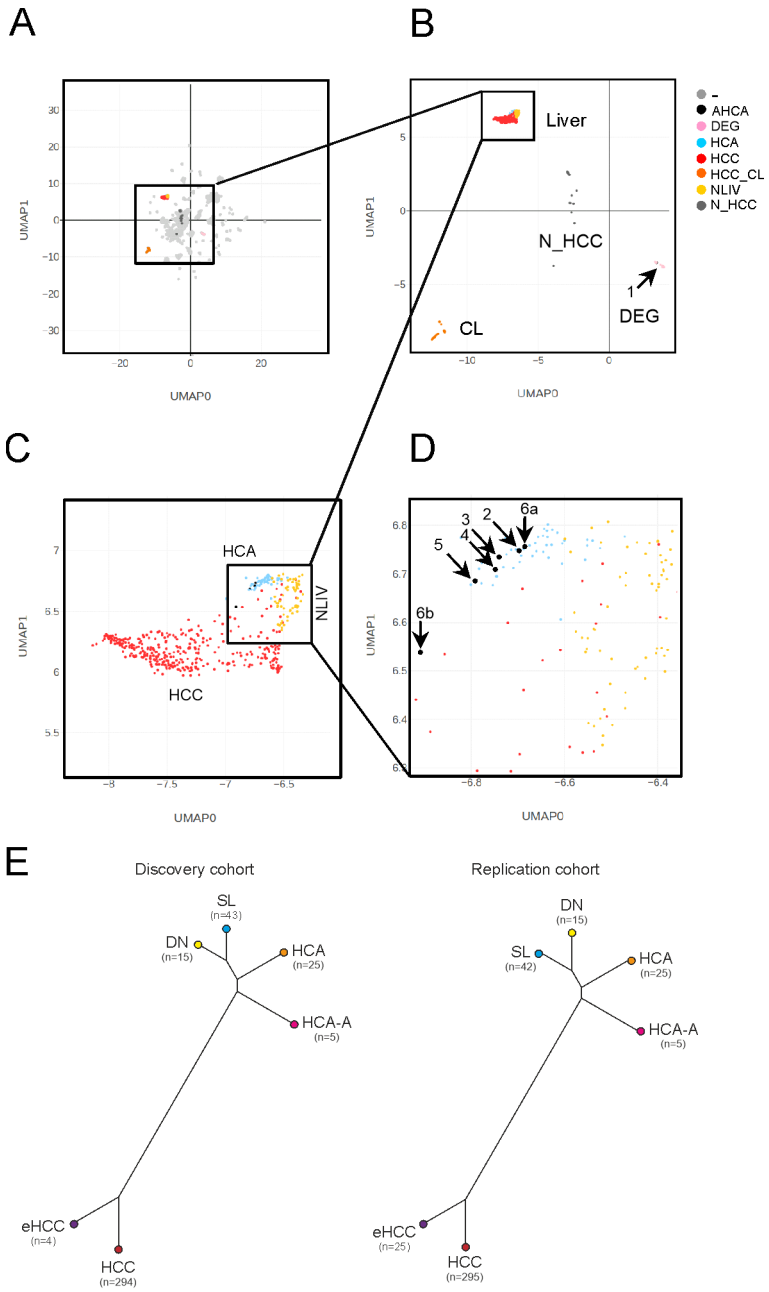

Figure 2

HIS_14858_Figure 2.tif

Patient ID	Adenoma subtype	Nuclear atypia	Small cell change	Pseudoglandular formation	Reticulin loss	Glutaminsynthetase	β-catenin	CRP	SAA	L-FABP	ASS1
1	b-HCA	10%	15%	5%	No	Border Pos.	Neg.	Neg.	Neg.	Ret.	Neg.
2	b-HCA	10%	30%	No	10%	Border Pos.	Neg.	Neg.	Neg.	Ret.	Neg.
3	b-IHCA	5%	No	5%	10%	Positive	Pos.	Pos.	Pos.	Ret.	Neg.
4	b-IHCA	10%	No	10%	10%	Neg./weakly Pos.	Neg.	Pos.	Pos.	Ret.	Neg.
5	sh-HCA	5%	No	No	30%	Neg.	Neg.	Neg.	Neg.	Ret.	Pos.

Table 1: Morphological and immunohistochemical characteristics of the 5 HCA with atypia

Abbreviations: Neg: Negative, Pos: Positive, Ret: Retained,CRP: C reactive protein, SAA: serum amyloid A, L-FABP: liver fatty acid- binding protein

ASS1: argininosuccinate synthetase-1, b-HCA: β-catenin mutated HCA, b-IHCA: β-catenin mutated and inflammatory HCA, sh-HCA: sonic hedgehog HCA

Patient ID	Adenoma Subtype	Gene altered	Coding	AA variation	Variant interpretation	Allelic frequency (%)
1	b-HCA	CTNNB1, Exon 7	c.1004A>T	p.Lys335Ile	missense	29.7
2	b-HCA	CTNNB1, Exon 3	c.134C>T	p.Ser45Phe	missense	30.16
3*	b-IHCA	CTNNB1, Exon 3	c.63_95del	p.Val22_Asp32del	non-frameshift deletion	60.82
		IL6ST, Exon 6	c.647C>A	p.Pro216His	missense	24.95
4	b-IHCA	CTNNB1, Exon 7	c.1004A>T	p.Lys335Ile	missense	17.46
5	sh-HCA	APOB	c.11377delG	p.Glu3793fs	frameshift deletion	32.55
		LRP1B, Exon 75	c.11447C>T	p.Ala3816Val	missense	40.91
6	HCC in HCA	6a (HCA): -	6a (HCA): -	6a (HCA): -	6a (HCA): -	6a (HCA): -
		6b (HCC): BRD7, Exon 12	6b (HCC): c.1365T>G	6b(HCC): p.Tyr455Ter	6b(HCC): nonsense	6b(HCC): 49.56

Table 2: Mutational analysis by NGS using a panel covering the most relevant genes affected in HCA and HCC and a panel comprising 161 relevant cancer genes.

Abbreviations: b-HCA: β-catenin mutated HCA, b-IHCA: β-catenin mutated and inflammatory HCA, sh-HCA: sonic hedgehog HCA, u-HCA: unclassified HCA, AA: amino acide; *only analyzed with panel for relevant genes affected in HCA and HCC