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# Upregulation of long noncoding RNAs *LINC00941* and *ABHD11-AS1* is associated with intrahepatic cholangiocarcinoma

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## Abstract

**Objective:** Many long noncoding RNAs (lncRNAs) are associated with liver cancers, mainly hepatocellular carcinoma (HCC) and to a smaller extent intrahepatic cholangiocarcinoma (CCA). Most of such lncRNAs show similar dysregulation patterns when the two types of tumors are compared, suggesting that these aberrations are characteristic features of these liver tumor types. In the present study, we aimed to identify some candidate lncRNAs that are associated specifically with CCA.

**Methods:** According to The Cancer Genome Atlas data, we chose *LINC00941*, *ABHD11-AS1*, and *CASC8* as promising biomarkers dysregulated in CCA but unaffected

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in HCC. We first verified their upregulation in an existing transcriptomic dataset for CCA patients. Next, we estimated expression levels of these three lncRNAs by reverse-transcription quantitative PCR in a group of paired (tumorous/adjacent) post-surgery tissue samples from 110 patients with various liver lesions: CCA, HCC, combined HCC-CCA, or benign liver tumors.

**Results:** Significant upregulation of *LINC00941* and *ABHD11-AS1* was noted in most of the investigated CCA samples, whereas in HCC samples, increased expression of these two lncRNAs was observed only in some types of cases (mainly characterized by an advanced tumor stage). In contrast, *CASC8* manifested extremely low expression and no diagnostic potential in all the tested liver samples. Analyzing expression correlations of lncRNAs with candidate genes, we obtained strong evidence for *LINC00941*-mediated upregulation of *CAPRIN2* in CCA.

**Conclusions:** For the first time, we show the upregulation of *LINC00941* and *ABHD11-AS1* in CCA and report their good potential as diagnostic biomarkers for this type of liver tumor.

**Keywords**

Long noncoding RNA, liver cancer, biomarker, cholangiocarcinoma, hepatocellular carcinoma

## Introduction

Long noncoding RNAs (lncRNAs) represent a large class of diverse regulatory molecules longer than 200 nt. They perform various functions in the cell, control gene expression at different levels, and contribute to the development of many diseases including cancer.[1] Consequently, expression levels of lncRNAs are often rather sensitive to any changes in cell metabolism, in particular to those occurring during cell proliferation and carcinogenesis.[2,3] Thus, lncRNAs differentially expressed in tumors compared to normal tissues may be considered promising diagnostic biomarkers.[4,5] Indeed, lncRNA *PCA3* (prostate cancer antigen 3) has been widely used in clinical practice for routine screening of patients with prostate cancer for many years.[6,7] Its unique tissue- and cancer-specificity along with the possibility of its detection in patient urine samples allow for simple non-invasive diagnostics. Nonetheless, at present, *PCA3* is still the only successful lncRNA oncomarker. Accordingly, research efforts are focused on a search for other lncRNAs that may be used as biomarkers for various cancers either alone or as part of a multigene signature/diagnostic panel.

To date, many differentially expressed lncRNAs have already been described for many types of malignant tumors, including primary liver cancer, and at least some of them are widely regarded as promising biomarkers.[8,9] Usually, these lncRNAs are associated with hepatocellular carcinoma (HCC),[10–12] which is the most widespread liver cancer, representing up to 80–90% of all primary malignant tumors of the liver.[13]

Intrahepatic cholangiocarcinoma (CCA) is relatively rare (10–20% of such cases) but still is the second most prevalent type of liver cancer. Distinguishing CCA from HCC can be difficult, especially in a cirrhotic liver,[14] whereas combined HCC-CCA (cHCC-CCA) occurs too.[15,16] CCA originates from bile duct cells[17] and can also be located outside the liver (distal CCA). Notably, in the present study, we analyze only primary liver tumors and intrahepatic CCA.[18]

A number of studies describe lncRNAs associated with CCA [summarized in reviews[19–22]], but most of such known candidate lncRNAs are dysregulated in HCC as well, thereby preventing discrimination between these two types of liver tumors by means of these lncRNA markers. Thus, it is relevant and important to find lncRNAs with expression profiles different between CCA and HCC. In our study, we aimed to identify such CCA-associated lncRNAs differentially expressed between these two cancers. We performed a comprehensive search for potential candidates based on The Cancer Genome Atlas (TCGA) datasets coupled with a literature review focused on lncRNAs not yet analyzed in CCA. Finally, we chose three lncRNAs that possess the above-mentioned features: *LINC00941*, *ABHD11-AS1*, and *CASC8*. None of them has been previously evaluated either as a putative CCA-specific biomarker or as a potential diagnostic or prognostic molecule for a variety of other liver tumors. At the same time, their oncogenic effects have been demonstrated previously in many other malignant tumors and tissues, implying their similar functions in CCA and opening up opportunities to translate the recent research findings into novel cancer treatments. Upregulation of all three lncRNAs in CCA was verified here in existing RNA-Seq data from paired tumorous/adjacent liver CCA tissue samples. We then aimed to validate *LINC00941*, *ABHD11-AS1*, and *CASC8* expression levels by reverse-transcription quantitative PCR (RT-qPCR) in our own group of paired clinical liver samples from patients with primary liver malignant tumors, including CCA, HCC, and cHCC-CCA. The purpose was to determine whether these three lncRNAs can be used for discrimination of these types of liver tumors. We also analyzed a group of control benign liver tumors, namely nonmalignant hepatocellular adenoma (HCA) and focal nodular hyperplasia (FNH).

## Materials and methods

### *Patients' tissue samples*

We analyzed paired tumorous and adjacent liver tissue samples previously collected (January 2016 to May 2023) from patients with a diagnosis of HCC, intrahepatic CCA, cHCC-CCA, FNH, or HCA after tumor resection. The study protocol was approved on 18.01.16 by the Institutional Ethics Committee of Blokhin National Medical Research Center of Oncology affiliated with the Russian Ministry of Health (Ethics Committee Statement No. 18/01/2016). All procedures involving human participants were conducted in accordance with the World Medical Association Declaration of Helsinki of 1975 and its later amendments. Written informed consent for the use of tissue samples for scientific purposes was obtained from all participants of the study before the surgical procedure at Blokhin National Medical Research Center of Oncology (Moscow,

**Table 1.** General characteristics of the patients included in this study.

| Diagnosis | CCA | HCC | cHCC-CCA | Benign tumor[a] |
|---|---|---|---|---|
| Total number | 42 | 48 | 7 | 13 |
| Sex | | | | |
| Male | 18 | 30 | 3 | 6 |
| Female | 24 | 18 | 4 | 7 |
| Age, years | | | | |
| ≤60 | 24 | 31 | 2 | 13 |
| >60 | 18 | 17 | 5 | 0 |
| Median | 60 | 53 | 63 | 37 |
| Stage | | | | n/a |
| I + II | 11 | 24 | 3 | |
| III + IV | 28 | 24 | 4 | |
| ND | 3 | — | — | |

[a]Among them, there were three cases of nonmalignant hepatocellular adenoma (HCA) and 10 cases of focal nodular hyperplasia (FNH).

Russia). All personal data not related to the diagnosis and clinical information were excluded from the analysis, and the tissue samples were anonymized by means of number codes. General demographical characteristics of the subjects are listed in Table 1. Detailed clinical characteristics are listed in the Supplementary Materials (Tables S1–S4). Patient selection criteria were as follows: a primary liver tumor with intrahepatic localization, a certain histological diagnosis (HCC, CCA, cHCC-CCA, or a benign tumor) and availability of paired adjacent tissue samples. The main exclusion criteria were extrahepatic localization of a tumor and bile duct tumor types other than CCA or cHCC-CCA.

Most of the tissue samples were hepatitis virus (HBV or HCV) negative except for a single cHCC-CCA case (#124) and two CCA cases (#6 and #78) with HBV infection. Among all the subjects (110 patients), there were only eight cases of liver cirrhosis. The exact diagnosis and the origin of all samples studied in this work were confirmed by two independent histopathological analyses of hematoxylin and eosin (H&E)-stained sections. Adjacent normal tissues were also subjected to independent pathology review to confirm that they contained no tumor cells. After tumor resection, all samples were snap-frozen in liquid nitrogen and stored at −80 °C.

## *Study design*

This work is an experimental exploratory study aimed at identifying novel CCA-associated lncRNAs and is based on a preliminary analysis of their expression levels—in liver cancers—retrieved from available high-throughput RNA sequencing (RNA-Seq) datasets, followed by validation by RT-qPCR performed on patients' tissue samples. To identify potential lncRNA candidates, we started from TCGA (www.portal.gdc.cancer.gov/) datasets (detailed description is available at www.docs.gdc.cancer.gov/

Data/Bioinformatics_Pipelines/Expression_mRNA_Pipeline) for tumorous and corresponding normal tissue samples from CCA and HCC patients; these data were downloaded from the UCSC Xena portal (www.xenabrowser.net/datapages), namely GDC TCGA Bile Duct Cancer (CHOL, 51 primary-tumor samples and 21 solid-tissue normal samples) and GDC TCGA Liver Cancer (LIHC, 377 primary-tumor samples and 89 solid-tissue normal samples). Assignment of lncRNA genes was made in accordance with current Ensemble annotation (www.ensembl.org/biomart/martview). According to a phenotypic description of samples (sample type), we focused only on intrahepatic CCA (40 tumorous and 16 normal tissue samples from the TCGA Bile Duct Cancer dataset) and HCC (369 tumorous and 88 normal tissue samples from the GDC TCGA Liver Cancer dataset), excluding cases of a recurrent tumor and mixed diagnoses. To identify potential CCA-associated lncRNA biomarkers, we applied the following criteria: a) average expression in cancer tissues is >0.1 FPKM (fragments per kilobase million); b) the fold change (FC) of differentially expressed lncRNAs in CCA/normal tissues is at least fourfold greater than the FC in HCC/normal tissues [FC(CCA/norm)/FC(HCC/norm) > 4]. We then performed an extensive literature review to select those candidate lncRNAs that had been described previously, were known to be upregulated in some cancers, and were not investigated in liver cancers, especially in CCA. To filter out cancer-associated candidate lncRNAs from nonspecific lncRNAs upregulated in different liver diseases, we used data available in the GepLiver atlas (www.gepliver.org) and in the PCAWG database (www.ebi.ac.uk), which provide brief information about median expression levels of genes of interest in normal liver tissues (on the basis of on the GTEx dataset) and liver diseases of different origin, including datasets for cancer tissue samples and corresponding adjacent liver tissues. To verify candidate lncRNAs for association with CCA, we used existing RNA-Seq data (GSE107943) obtained from 30 pairs of intrahepatic CCA tissues and corresponding adjacent liver tissues.[23] As a result, top three lncRNAs, namely *LINC00941*, *ABHD11-AS1*, and *CASC8*, were selected for subsequent experimental validation in paired tumorous/adjacent liver tissue samples and for a comparison between HCC, CCA, and cHCC-CCA.

## RNA isolation

For RNA isolation, frozen liver tissue samples (~50 mg) from CCA and cHCC-CCA patients were placed in homogenizing tubes (Precellys Lysing Kit CK14) precooled on ice, immediately covered with 0.5 mL of the TRIzol Reagent (Invitrogen), and homogenized by means of Precellys Evolution (Bertin Technologies) according to the standard protocol for soft tissues. The tissue lysate was collected, beads were washed with additional 0.5 mL of the TRIzol Reagent, and finally, 1 mL of the combined solution was used for total-RNA extraction according to the manufacturer's instructions. Obtained RNA pellets were washed three times with precooled (−20 °C) 75% (v/v) ethanol, air-dried, and dissolved in 20 µL of RNase-free water (Sigma). After that, 500 ng of each RNA sample was treated with RNase-free DNase I (Thermo Fisher Scientific) for 1 h at 37 °C, followed by 15 min inactivation at 65 °C in the presence of 5 mM EDTA.

RNA extraction from HCC and liver benign tumor samples (FNH/HCA) was performed by means of the PureLink RNA Mini Kit (Life Technologies) according to a standard manufacturer's protocol including on-column DNase I treatment (PureLink DNase Kit, Life Technologies).

### *Reverse-transcription quantitative PCR (RT-qPCR)*

Reverse transcription was performed with the Maxima H Minus First Strand cDNA Synthesis Kit (Thermo Fisher Scientific) starting with 500 ng of initial RNA in a total volume of 20 µL, including the following steps: 10 min at 25 °C, 40 min at 50 °C, and 5 min at 85 °C (inactivation). Prior to qPCR, cDNA samples were diluted to 100 µL with RNase-free water (Sigma); then, 5 µL of 2× Maxima SYBR Green qPCR Master Mix (Thermo Fisher Scientific) containing 0.6 µM each primer (Table S5) was added to 5 µL of each cDNA sample followed by the addition of 8 µL of mineral oil (Sigma). For qPCR, a three-step cycling protocol was used with annealing temperature 60 °C. All experiments were conducted in a CFX96 Touch Real-Time PCR Detection System (Bio-Rad) on three technical replicates. Relative RNA amounts were calculated by the $2^{-\Delta Ct}$ method and normalized to U6 snRNA.

### *Statistical analysis*

All the data were calculated using GraphPad Prism software v.8.4.3, and the diagrams represent a median of relative RNA expression with a range from a minimal to maximal value. Statistical significance of the observed changes in expression levels between tumorous and adjacent liver tissues was analyzed in two ways (indicated in figures and text): by either unpaired-sample or paired-sample Student's *t* test. Significance of the observed differences in expression levels between multiple groups of samples was evaluated by one-way analysis of variance (ANOVA) with Tukey's *post hoc* test. All presented *p*-values are two-sided, and all values >.05 were assumed to indicate statistical insignificance. Possible associations between lncRNA expression levels in tumor tissues and some clinical parameters were determined by the $\chi^2$ test and Spearman's rank correlation analysis. Correlations between expression levels of lncRNAs and of related candidate genes were estimated by Spearman's and Pearson's rank correlation analyses. To assess the diagnostic performance of the selected lncRNAs, we generated receiver-operating characteristic (ROC) curves and calculated the area under an ROC curve (AUC).

## Results

### *Identification of CCA-associated lncRNAs*

In this study, we aimed to identify lncRNAs with evident upregulation in CCA tissue samples without upregulation in HCC tissue samples. We performed initial screening using TCGA datasets for CCA and HCC followed by thorough analysis of the literature

to select the most promising candidates. As a result, we focused on three lncRNAs—*LINC00941*, *ABHD11-AS1*, and *CASC8*—which had been previously described as biomarkers of several cancers but had not been investigated in primary liver tumors. All three lncRNAs showed remarkable upregulation in CCA tissue samples in comparison to adjacent nontumor liver tissues; this was not the case for HCC samples (Figure 1(a)). According to PCAWG visualization, the lncRNAs of interest have expression levels below the database cutoff (TPM 0.5) in normal liver tissues (GTEx data), in HCC tissues, and in adjacent liver tissues corresponding to CCA or HCC cases. By contrast, these lncRNAs are strongly expressed in CCA (Figure 1(b)). Evaluation of expression levels using the GepLiver atlas (Figure 1(c)) also showed that high expression levels of *LINC00941*, *ABHD11-AS1*, and *CASC8* are distinctive characteristics of CCA (in contrast to other liver malignant tumors) because these lncRNAs are not upregulated in HCC, cHCC-CCA, and hepatoblastoma (HBL) samples. We also examined their expression profiles in nonmalignant liver pathologies, such as cirrhosis, fibrosis, nonalcoholic fatty liver disease, alcoholic liver disease, and viral hepatitis. As shown in the corresponding heatmap (Figure 1(c)), except for slight upregulation in alcoholic liver disease, all three lncRNAs were found to be not overexpressed in other common liver diseases. When compared to a pooled group of normal liver tissues collected from different datasets (including both GTEx data and data on adjacent tissues for various liver conditions/diseases), only *ABHD11-AS1* showed some "nonzero" expression levels (Figure 1(c)), which was still significantly lower (TPM = 1.24) as compared to CCA (TPM = 3.88). Taken together, these data strongly indicated high potential of these three lncRNAs as candidate CCA biomarkers.

To validate these findings, we searched for additional available RNA-Seq data from paired tumorous/adjacent liver tissues of patients with CCA. Among such data, we focused on studies dealing with only intrahepatic CCA of nonviral origin with relatively large sample size. Dataset GSE107943 presented by Ahn et al.[23] met our requirements. All three lncRNAs were found to be significantly overexpressed in 30 CCA tissue samples in comparison to adjacent surrounding liver tissues (Figure 1(d) and (e)). In line with the information obtained from databases (Figure 1(a)–(c)), among the three lncRNAs in question, the highest expression levels in CCA samples were noted for *ABHD11-AS1* (Figure 1(d) and (e)), whereas this lncRNA was not expressed at all in most of the adjacent normal liver tissues. *CASC8* was not detectable in the majority of adjacent normal tissue samples. Nevertheless, upregulation of *CASC8* was observed in all but three CCA samples. Respective ROC curves also indicated high diagnostic value of all three candidate lncRNA biomarkers (Figure 1(f)).

## *Experimental validation of expression of the three lncRNAs in CCA tissue samples*

To validate the RNA-Seq data, we analyzed expression levels of lncRNAs *LINC00941*, *ABHD11-AS1*, and *CASC8* by RT-qPCR in our own patient cohort represented by 42 pairs of postsurgery tissue samples (CCA in comparison to corresponding adjacent

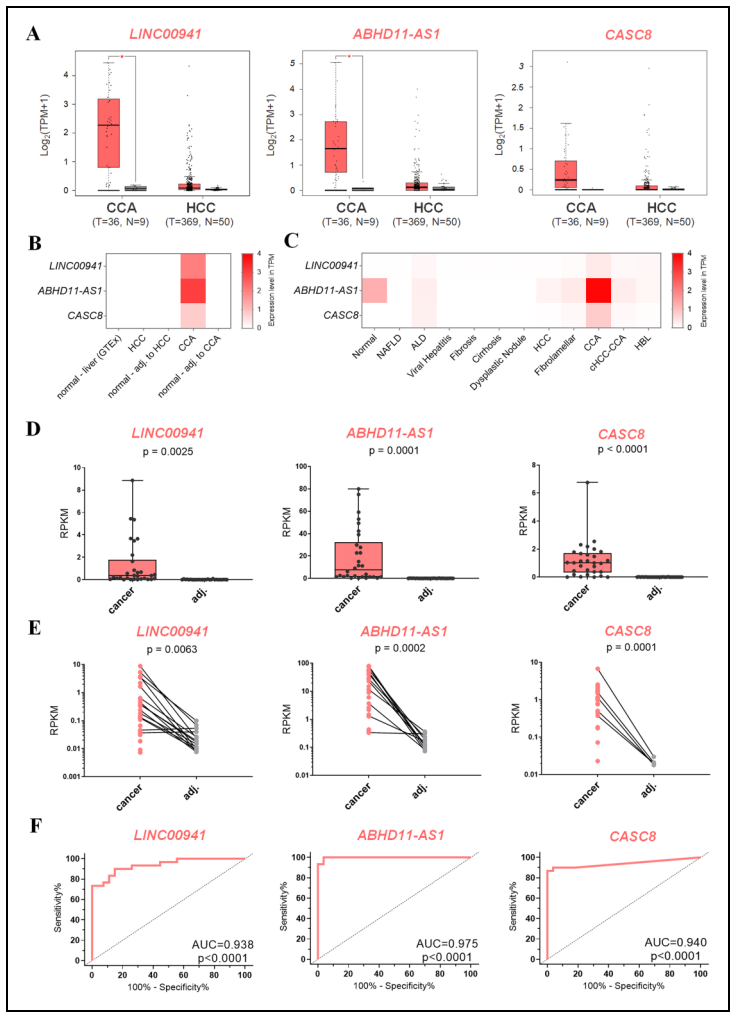


**Figure 1.** *LINC00941*, *ABHD11-AS1*, and *CASC8* as candidate biomarkers of CCA. (a) Expression levels of the three lncRNAs in tumorous (red) and adjacent (gray) liver tissues from patients with CCA or HCC according to TCGA datasets visualized by GEPIA (www.gepia.cancer-pku.cn). (b) Relative expression of the three lncRNAs in the datasets from normal liver tissues (GTEx), CCA and HCC cancerous tissues, and corresponding adjacent liver tissues according to the PCAWG database (www.ebi.ac.uk). (c) Relative expression of the three lncRNAs in the datasets from different liver diseases versus normal healthy tissues according to the GepLiver database (www.gepliver.org). NAFLD: nonalcoholic fatty liver disease, ALD: alcoholic liver disease. (d, e) Relative expression of lncRNAs *LINC00941* (left panels), *ABHD11-AS1* (central panels), and *CASC8* (right panels) in paired tumorous/adjacent liver tissue samples from patients with CCA according to transcriptomic data extracted from dataset GSE107943. *P*-values were calculated either by unpaired-sample (d) or paired-sample (e) Student's *t* test. (f) Corresponding ROC curves for *LINC00941*, *ABHD11-AS1*, and *CASC8*.

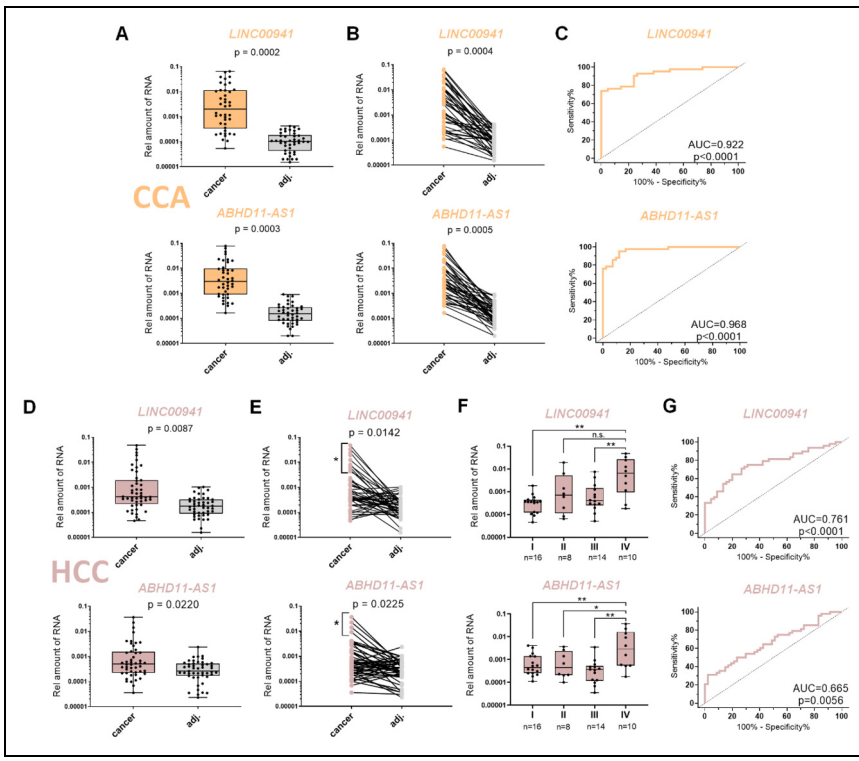


**Figure 2.** Validation (RT-qPCR) of *LINC00941* and *ABHD11-AS1* as biomarkers of CCA (a–c) and HCC (d–f) in paired (tumorous/adjacent) liver tissue samples from the patients. *P*-values were calculated either by unpaired-sample (a, d) or paired-sample (b, e) Student's *t* test. Samples with the highest expression of lncRNAs mainly representing stage IV of HCC. (c, g) Corresponding ROC curves for *LINC00941* and *ABHD11-AS1*. (f) Correlation of expression levels of *LINC00941* and *ABHD11-AS1* with the tumor stage. Statistical significance of the results according to analysis of variance (ANOVA): $*p < .05$, $**p < .01$, n.s.: not significant.

normal liver tissues). The obtained data confirmed significant upregulation of *LINC00941* and *ABHD11-AS1* in CCA (Figure 2(a)). Of note, this was true in every pair of samples for *ABHD11-AS1*, and in all but two pairs for *LINC00941* (Figure 2(b)). Notably, we registered approximately 15-fold (median) overexpression of both lncRNAs in CCA samples in comparison to adjacent tissues. By contrast, relative amounts of *CASC8* appeared to be extremely low in both cancerous and adjacent liver tissues without any appreciable patterns (Figure S1(a)). In fact, we were able to detect *CASC8* only in 14 pairs of samples and additionally in 12 cancer cases (with no expression in corresponding adjacent normal tissues). We attempted to quantify *CASC8* with an alternative qPCR primer pair (Table S5), but this approach resulted in even worse detection of this lncRNA (only in 36% of tissue samples regardless their tumor/adjacent origin, Figure S1), consistently

with other studies, showing that *CASC8* transcripts harboring this region are even less abundant.[24,25] Our statistical analysis revealed no significant correlations between expression levels of *LINC00941* or *ABHD11-AS1* and available clinical characteristics of each case, suggesting that their upregulation in CCA is likely to be universal and independent of the cancer stage or other clinical parameters of the tumor. Meanwhile, ROC analysis pointed to high diagnostic potential of both lncRNAs *LINC00941* (AUC = 0.922, $p < .0001$) and *ABHD11-AS1* (AUC = 0.968, $p < .0001$) (Figure 2(c)).

## *Expression of the three lncRNAs in HCC samples*

Next, we assessed expression levels of the investigated lncRNAs in 48 pairs of samples classified as HCC cases. In cancer tissues, *LINC00941* showed moderate overall upregulation, which was lower by at least an order of a magnitude (median) as compared to CCA (Figure 2(d) and (e)). Nonetheless, average *LINC00941* overexpression mostly resulted from extremely high expression in 10 distinct HCC samples, mainly with tumor necrosis. Among them, six cases were classified as stage IV HCC (#2, #18, #23, #62, #67, and #72), and two cases as stage III (#7 and #8); two cases of stage II (#71 and #86) had some specific features. Sample #86 had mixed etiology that likely derived from HCA. Notably, the patient in question died 1 month after the surgical operation. Case #71 featured tumor invasion into blood vessels, liver cirrhosis, recurrence of the disease, and elevated alpha-fetoprotein (AFP) serum levels (Table S2). We grouped HCC patients to analyze the disease stage and indeed revealed a moderately significant correlation of *LINC00941* levels with stage IV HCC (Figure 2(f)). Nevertheless, diagnostic value of *LINC00941* for HCC was not as pronounced as that for CCA (Figure 2(g)).

Similar results were obtained for the *ABHD11-AS1* lncRNA: 10-fold lower expression (median) in comparison to CCA samples and lower significance of its upregulation in cancer tissue samples (Figure 2(d) and (e)). The highest levels of *ABHD11-AS1* were also detected in four distinct samples with stage IV HCC (#2, #23, #62, and #72), and further analysis additionally revealed a correlation with tumor stage IV (Figure 2(f)). On the other hand, overall diagnostic value of *ABHD11-AS1* for HCC tumors proved to be rather low (Figure 2(g), AUC = 0.665, $p < .0001$), especially in comparison with CCA samples.

*CASC8* was detectable only in 11 out of 24 paired HCC samples, but its expression levels did not differ significantly between tumorous and adjacent tissues (Figure S1(b)).

We examined a probable association of *LINC00941* and *ABHD11-AS1* expression levels with clinical parameters by the $\chi^2$ test and confirmed a significant correlation with the tumor stage for both lncRNAs ($p^{\text{LINC00941}} = .0204$; $p^{\text{ABHD11-AS1}} = .0095$; Table S6). Besides, we observed some marginally significant correlations with elevated serum AFP levels and invasion into blood vessels in cases with high *LINC00941* expression and an association with the differentiation grade and tumor size in cases of *ABHD11-AS1* overexpression (Table S6). A marginally significant ($p = .0408$) negative correlation ($r = -.4393$) between *ABHD11-AS1* expression and HCC tumor size was confirmed by Spearman's rank test too (Figure S2).

## Expression of the lncRNAs in cHCC-CCA samples

Because cHCC-CCA cases are extremely rare, we had only seven tissue samples of these tumors in our collection. *CASC8* expression levels appeared to be below the detection limit in all these samples. *LINC00941* and *ABHD11-AS1* turned out to be upregulated in most of these samples (Figure S3(a)–(c)), but the expression levels varied rather widely among the tissue samples, resulting in insignificance of these differences. We compared expression levels of each lncRNA in cHCC-CCA with corresponding values in HCC and CCA; the observed differences were not significant (Figure S3(d)). *LINC00941* expression levels in cHCC-CCA tended to be similar to those in HCC, whereas the levels of *ABHD11-AS1* in cHCC-CCA were somewhere between HCC and CCA. This discrepancy might be explained by the complex origin of this specific liver tumor type, which is not associated with even well-known biomarkers investigated in the literature.[16,26]

## Expression of the lncRNAs in benign tumors of the liver

To examine the expression of the investigated lncRNAs in nonmalignant liver tumors, we analyzed paired (tumorous/adjacent) liver tissue samples from 10 FNH cases and three HCA cases. *CASC8* levels were mostly similar between adjacent and tumorous tissues (Figure S1(c)). Although amounts of *LINC00941* and *ABHD11-AS1* were extremely low in these tumor samples, the obtained results were significant, thereby confirming upregulation of both lncRNAs in these tumors (Figure 3(a) and (b)). We next compared the expression of *LINC00941* and *ABHD11-AS1* in various liver tumors (CCA, HCC, and benign tumors) with their amounts detected in all adjacent tissues representing a nontumor group of tissue samples (Figure 3(c) and (d)). Median expression levels in the CCA cohort differed by 1–2 orders of magnitude from the values observed in HCC and benign tumor samples. Statistical analysis confirmed that among these groups, only CCA samples significantly differ from all the other types of liver samples. Thus, *LINC00941* and *ABHD11-AS1* have promising potential as cancer biomarkers mainly specific to CCA, not other types of liver tumors.

## The search for genes associated with LINC00941 and ABHD11-AS1 upregulation in CCA

On the basis of the validation of *LINC00941* and *ABHD11-AS1* as lncRNAs upregulated in CCA samples, we decided to predict possible mechanisms of their association with the pathogenesis of this cancer and analyzed sets of the most relevant genes (Tables S7 and S8) coexpressed in the corresponding TCGA dataset (GDC TCGA Bile Duct Cancer, CHOL) with the help of the GEPIA genomic browser (www.gepia.cancer-pku.cn). We also compared these data to an HCC TCGA dataset (GDC TCGA Liver Cancer, LIHC). Notably, we did not find any overlap (common genes) between two gene sets (top 100 genes coexpressed with *ABHD11-AS1* in CCA and top 100 genes coexpressed with *ABHD11-AS1* in HCC), whereas in a similar analysis of *LINC00941*, the only one such common gene was *TNFAIP6* (tumor necrosis factor alpha induced protein 6).

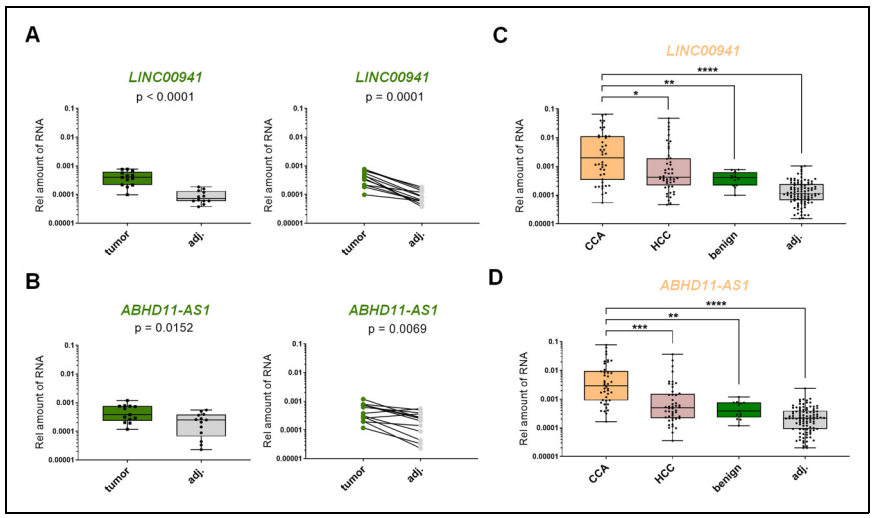


**Figure 3.** A comparison of *LINC00941* and *ABHD11-AS1* expression profiles between malignant and benign liver tumors. (a, b) Relative expression of lncRNAs *LINC00941* (a) and *ABHD11-AS1* (b) in paired (tumorous/adjacent) liver tissue samples from patients with a diagnosis of a benign liver tumor (FNH or HCA). *P*-values were calculated either by unpaired-sample (left panels) or paired-sample (right panels) Student's *t* test. (c, d) The comparison of *LINC00941* (c) and *ABHD11-AS1* (d) expression levels among different groups of samples: CCA, HCC, benign tumors, and total adjacent liver tissues. Statistical significance of the results according to analysis of variance (ANOVA): **p* < .05, ***p* < .01, ****p* < .001, *****p* < .0001.

Therefore, we supposed that *LINC00941* and *ABHD11-AS1* each plays different roles when CCA is compared with HCC. A further comparison revealed minor expression of *TNFAIP6* in HCC without statistical significance (Figure S4(a)–(c)), which should not be considered evidence for a probable correlation between *TNFAIP6* and *LINC00941* expression levels in HCC. In contrast, we confirmed significant upregulation of *TNFAIP6* in CCA samples in comparison to adjacent liver tissues in validation dataset GSE107943 as well as its correlation with *LINC00941* expression (Figure S4(d)–(f)). TNFAIP6 (also known as TSG-6, aka tumor necrosis factor alpha stimulated gene 6) is an inflammation-associated protein widely expressed by cancer-associated fibroblasts and plays an important part in extracellular-matrix formation and cell migration. It is upregulated in a number of cancers, including CCA, and is associated with poor prognosis.[27,28] Considering TNFAIP6-mediated activation of β-catenin (CTNNB1) and yes1 associated transcriptional regulator (YAP1) in the liver,[29] this gene may be a promising target for future analyses regarding its correlation with *LINC00941* in terms of expression.

Although previously, *LINC00941* and *ABHD11-AS1* have not been investigated in terms of CCA, there are numerous experimental data—obtained in other types of cancers—indicating their oncogenic roles and involvement in cancer progression pathways (for details see the Discussion). Accordingly, we hypothesized that at least some such

processes may take place in CCA too and searched for corresponding genes among those listed as coexpressed with *LINC00941* and *ABHD11-AS1* (Tables S7 and S8). Of note, the top candidate (with the highest correlation rank coefficient) among *LINC00941*-associated genes in CCA was *CAPRIN2* (Figure 4(a) and (b)) encoding cytoplasmic activation/proliferation-associated protein 2, which has previously been shown to be directly upregulated by *LINC00941* in nasopharyngeal carcinoma[30] and in oral squamous cell carcinoma.[31] We observed the strong positive correlation between *CAPRIN2* and *LINC00941* expression levels as well as overall upregulation of *CAPRIN2* in CCA tissues in validation dataset GSE107943 (Figure 4(c)–(e)). We further analyzed *CAPRIN2* mRNA expression levels by RT-qPCR in our own cohort of CCA patients (Figure 4(f) and (g)) and confirmed its correlation with *LINC00941* in cancerous tissues (Figure 4(h)). *CAPRIN2* is known as an activator of the WNT/β-catenin signaling pathway (for details see the Discussion). Therefore, our data are suggestive of a similar function of *LINC00941* in CCA (Figure 4(i)). We also observed some weak correlations between *LINC00941* and genes *PMEPA1* (prostate transmembrane protein, androgen induced 1), *LAMB3* (laminin subunit beta 3) and *NCOR2* (nuclear receptor corepressor 2) in terms of expression judging by TCGA data, but these were not confirmed in the validation dataset owing to low significance (Figure S4).

Among the genes predicted to have expression profiles similar to the profile of *ABHD11-AS1* (Table S8), in validation dataset GSE107943 we observed expression correlations of *NBL1* (neuroblastoma suppressor of tumorigenicity 1) and *TSPAN3* (tetraspanin 3) with *ABHD11-AS1* (Figure S5); however, in the literature, we found no experimental evidence for the expression and functions of NBL1 and TSPAN3 in the liver. Recently, lncRNA *ABHD11-AS1* was reported to regulate in cervical cancer the expression of its neighboring gene *ABHD11*,[34] encoding abhydrolase domain containing 11 protein – a mitochondrial hydrolase highly expressed in gastrointestinal tissues. We checked expression levels of *ABHD11* in TCGA and GSE107943 datasets and observed some marginally significant correlations with *ABHD11-AS1* expression (Figure S6). We also analyzed *ABHD11* mRNA expression levels by RT-qPCR in our own cohort of CCA patients and verified the correlation with *ABHD11-AS1* expression (Figure S6(e) and (f)); the correlation was found to be very similar to that in RNA-Seq data, in TCGA data for CCA and in TCGA data for cervical cancer (Figure S6(a)). The *ABHD11-AS1* lncRNA is reported to directly bind to RNA-binding protein FUS in a cervical-cancer cell line, resulting in inhibition of *ABHD11* mRNA degradation and an increase in the ABHD11 amount[34]; we believe that a similar mechanism exists in CCA (Figure S6(g)). We expect that further experimental verification of the identified associations both in clinical samples and in model *in vitro* systems will allow to determine more exactly the mechanisms underlying possible involvement of the lncRNAs under study in the pathogenesis of CCA.

## Discussion

HCC is the most widespread type of primary malignant tumor of the liver and is linked with a number of risk factors such as HBV and HCV infections,[35,36] liver cirrhosis,

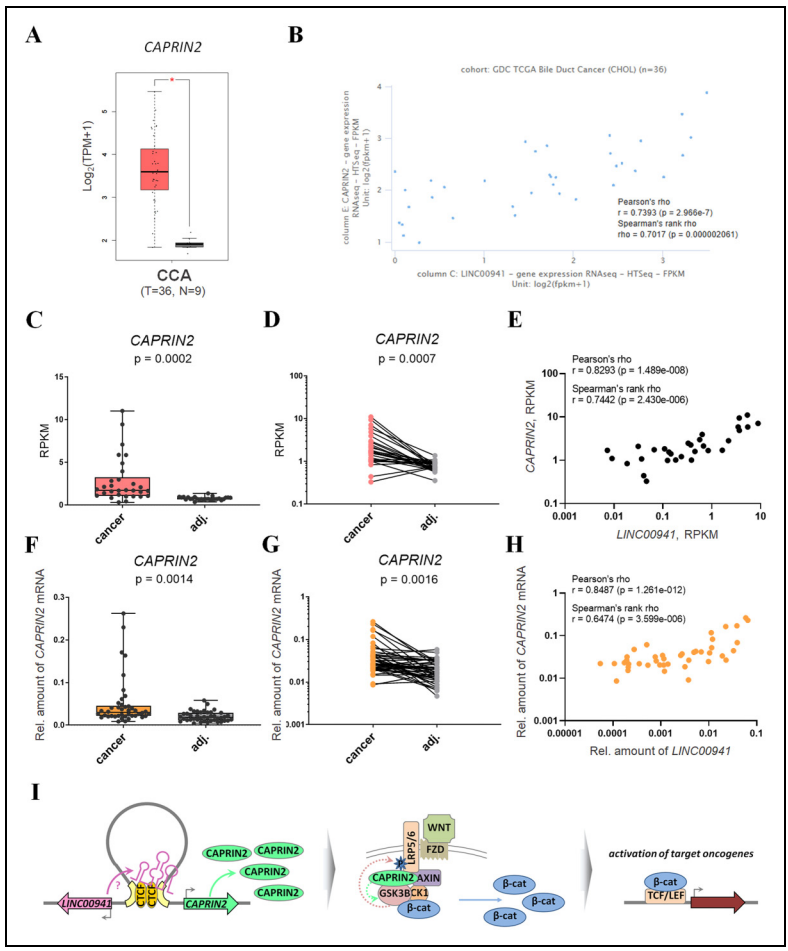


**Figure 4.** *CAPRIN2* is a candidate gene upregulated by *LINC00941* in CCA. (a) Expression levels of *CAPRIN2* mRNA in tumorous (red) and adjacent (gray) liver tissues from patients with CCA according to TCGA datasets visualized by GEPIA (www.gepia.cancer-pku.cn). (b) Correlation between *CAPRIN2* mRNA and *LINC00941* expression levels in the CCA TCGA dataset visualized by UCSC Xena (www.xenabrowser.net). (c, d) Relative expression of *CAPRIN2* mRNA in paired tumorous/adjacent liver tissue samples from patients with CCA according to transcriptomic data extracted from dataset GSE107943. *P*-values were calculated either by unpaired-sample (c) or paired-sample (d) Student's *t* test. (e) The correlation between *CAPRIN2* mRNA and *LINC00941* expression levels in CCA tissue samples from dataset GSE107943. (f, g) Relative expression (RT-qPCR) of *CAPRIN2* mRNA in paired tumorous/adjacent liver tissue samples in our own cohort of CCA patients. *P*-values were calculated either by unpaired-sample (f) or paired-sample (g) Student's *t* test. (h) Correlation between *CAPRIN2* mRNA and *LINC00941* expression levels in CCA tissue samples of our own cohort of patients. (i) Schematic representation of *LINC00941*'s involvement in activation of the WNT/β-catenin pathway via the regulation of *CAPRIN2* expression [according to mechanisms described in Ref.[32,33]; β-cat: β-catenin (CTNNB1)].

nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, regular alcohol consumption, and environmental factors, such as aflatoxins.[13,37] In general, most of liver pathologies can increase the risk of HCC or other types of liver cancers, including intrahepatic CCA.

CCA is the second most prevalent primary malignant tumor of the liver and features high aggressiveness and lethality. It strongly differs from HCC by a number of morphological and functional features but is also very difficult to diagnose at early stages.[38] To date, the only diagnostic CCA biomarker used in clinical practice is the serum level of carbohydrate antigen 19-9 (CA 19-9), which has rather limited practical applicability owing to its low specificity and frequent overexpression in other gastrointestinal diseases, including pancreatic ductal adenocarcinoma.[39,40] Identification of novel cancer biomarkers may substantially facilitate early diagnosis of CCA as well as its differential diagnosis vis-a-vis other types of liver tumors. Circulating biomarkers are the most appealing in terms of noninvasive detection of liver cancers[41,42]; however, initial candidates are usually identified directly by RNA-Seq profiling of postoperative tumor samples or biopsies.[43] Moreover, transcriptome data have revealed that noncoding transcripts have much higher cell/tissue specificity as compared to protein-coding genes.[44] Consequently, lncRNAs show promise as valuable cancer biomarkers.

Regarding liver malignant tumors, there are several examples of tissue-specific RNAs, for example, *HULC* (aka highly upregulated in liver cancer), *LINC01093*, *HELIS* (aka healthy liver specific), and *FAM99A* (family with sequence similarity 99 member A). *HULC* is the most widely known liver-specific lncRNA that is highly upregulated in HCC,[45] but it is downregulated in CCA despite having some prognostic value.[46] *LINC01093* and *HELIS* have been reported to be underexpressed in HCC and to be absent in CCA samples.[27,47] There are no experimental data on *FAM99A* expression in CCA, but according to a recent *in silico* analysis, it is downregulated in CCA,[48] consistently with results of such expression profiling in HCC.[49] Therefore, none of the above-mentioned liver-specific lncRNAs can be regarded as prospective CCA biomarkers. On the other hand, there are dozens of known non-tissue-specific lncRNAs, such as *DANCR* (differentiation antagonizing non-protein coding RNA), *HOTAIR* (HOX transcript antisense RNA), *NEAT1* (nuclear paraspeckle assembly transcript 1) and *H19*, that are dysregulated in many types of malignant tumors thus being universal cancer biomarkers. Some of them are dysregulated in CCA as well, but their diagnostic value and prognostic value seem to be disputable.[22]

To find some candidate lncRNAs that are more specific to CCA, we performed a large-scale literature search and revealed that most of lncRNAs reported as CCA-associated are also dysregulated in HCC. For example, *HCG18* is a promising CCA oncomarker that can be found even in exosomes secreted by cancer cells[50]; however, its upregulation is a characteristic of HCC too.[51] *ST8SIA6-AS1* has been shown to be overexpressed in CCA compared to adjacent liver tissues and to be associated with worse survival of patients[52]; similar trends have been observed in HCC.[53] The lncRNAs recently implicated in CCA—*LOXL1-AS1*, *LINC00313*, and *HARN* (aka HCC associated lncRNA)—also correlate with HCC.[54–56] The latest meta-analysis revealed dozens of lncRNAs that can be considered successful diagnostic and/or

prognostic biomarkers of CCA, but their applicability to HCC was not addressed.[57] To further explore this field, we analyzed TCGA database and searched for lncRNAs featuring distinct overexpression in CCA datasets but unaffected in HCC. We focused on those genes that have been previously validated in other research articles as upregulated in other types of cancers. We then verified the upregulation of those lncRNAs using available RNA-Seq data[23] for intrahepatic CCA samples paired with corresponding adjacent liver tissues. This pipeline resulted in a final list of three promising candidate lncRNAs—*LINC00941*, *ABHD11-AS1*, and *CASC8*—that had not been previously studied as CCA biomarkers. None of them appeared to be liver- or other tissue-specific, but overexpression of all three lncRNAs has been associated with a number of other types of cancer, implying their universal oncogenic activities (Figure S7).

LncRNA *LINC00941* (also known as *MUF* aka mesenchymal stem cell upregulated factor) is an lncRNA mostly investigated in pancreatic ductal adenocarcinoma.[58] It is a well-known cancer-associated lncRNA also correlating with other gastrointestinal cancers.[59] Among the lncRNAs that we examined here, *LINC00941* is the only one that has previously been reported to be upregulated in HCC. On the other hand, that was a single study involving only five primary HCC tissue samples and three samples of recurrent HCC; among the eight cases, 50% were HBV-infected and 50% had liver cirrhosis. Thus, one cannot definitely state that *LINC00941* upregulation is associated with cancer progression but not with these concomitant liver diseases.[60] Nevertheless, according to TCGA data, among the three lncRNAs under study, only *LINC00941* has some prognostic value for liver cancer because an elevated level has been associated with slightly worse overall survival in patients with HCC (Figure S8). *In vitro* experiments on several HCC cell lines and HCC-associated mesenchymal stem cells indicate *LINC00941*-mediated upregulation of WNT/β-catenin signaling and epithelial–mesenchymal transition (EMT) via sponging of miR-34a and through direct binding to annexin A2 (ANXA2), with consequent disruption of complex formation between glycogen synthase kinase-3 beta (GSK3β) and β-catenin.[61] In bladder cancer cell lines, *LINC00941* has been found to interact with the IMP2 protein (mitochondrial inner membrane protease subunit 2, associated with tumor chemoresistance) and to maintain its stability under hypoxic stress conditions.[62] In pleural mesothelioma, *LINC00941* has been shown to bind translation initiation factor eIF4G, promoting selective synthesis of the cMYC transcription factor and subsequent activation of expression of downstream oncogenes.[63] *LINC00941* is also reported to contribute tumorigenesis through induction of cell autophagy in non–small cell lung cancer.[64] Thus, despite different mechanisms of action in different cancers, *LINC00941* is very likely an oncogenic lncRNA with good potential as a diagnostic or prognostic biomarker or even as a therapeutic target. Nevertheless, its involvement in CCA has not been previously investigated and requires further research. The putative overexpression of *LINC00941* in CCA was only mentioned in a recent review,[65] in line with the fact that it has not yet been experimentally confirmed.

*ABHD11-AS1* (also known as *LINC00035* and *BICDL3P*) appears to be upregulated in a number of malignant tumors, including colorectal, pancreatic, and gastric cancers, but has never been studied in liver cancers.[66] A recent meta-analysis provides comprehensive data on *ABHD11-AS1* upregulation in eight types of cancer, including CCA.[67]

*ABHD11-AS1* acts as an oncogenic lncRNA mainly by sponging numerous microRNAs, such as miR-199a, miR-133a, miR-1231, and miR-1301, activating PI3K–AKT, EGFR, and RhoC signaling pathways, and by affecting the cell cycle [reviewed in detail in ref.[66]]. *ABHD11-AS1* has also been found to serve as a "classic" antisense lncRNA in cervical cancer, by promoting *ABHD11* mRNA expression through prevention of its degradation, which is mediated by direct binding of *ABHD11-AS1* to the FUS protein.[34]

The *CASC8* (aka cancer susceptibility 8) lncRNA is best known for the fact that its gene is located in a so-called gene desert upstream of the *MYC* oncogene; this "desert" is enriched with numerous genetic variants associated with elevated risk of various cancers,[68,69] in particular pancreatic cancer.[70] Recently, the gene of *CASC8* was also mentioned as one of the m6A/m5C/m1A-regulated genes that correlate with unfavorable prognosis of lung adenocarcinoma.[71] Functions of *CASC8* itself are poorly investigated, but some authors have reported its oncogenic role based on sponging of miR-34a and miR-129-5p.[72,73]

Overall, these three lncRNAs—*LINC00941*, *ABHD11-AS1*, and *CASC8*—represented a set of cancer-related biomolecules with promising diagnostic value in CCA, as predicted from TCGA data (Figure 1(a)). Our initial validation using an existing RNA-Seq dataset from CCA patients (Figure 1(d)–(f)) confirmed expression and upregulation of all three lncRNAs in CCA tissue samples.

We next analyzed the expression levels of lncRNAs by RT-qPCR in our own collection of 110 paired (tumorous/adjacent) clinical tissue samples. We used U6 snRNA as a reference RNA for normalization because it has been successfully applied in previous studies dealing with liver cancer tissues including CCA[74,75] and has stabler expression in liver cancer and nonmalignant tissues in contrast to conventional *GAPDH* and *ACTB* mRNAs.[76] Giving the design of our study (see the Methods section), we did not estimate sample size and statistical power for the study, thus creating certain limitations. Nonetheless, we obtained significant results that are supported by a confidence interval, and we provide *p*-values with all the findings.

We first showed significant upregulation of *LINC00941* and *ABHD11-AS1* in CCA tissue compared to adjacent normal liver tissues (Figure 2(a)). Elevated levels of *ABHD11-AS1* in cancer tissue samples were found in every tested pair of samples here. The same trend was documented for *LINC00941*, except for only two cases (Figure 2(b)). ROC analysis (Figure 2(c)) additionally confirmed the high potential of both lncRNAs as promising CCA diagnostic biomarkers. On the contrary, diagnostic value of *LINC00941* and *ABHD11-AS1* for HCC seemed to be lower than that for CCA (Figure 2(d)). In fact, the significance of the obtained data for HCC resulted from strongest upregulation in a few samples mainly from patients with a stage IV tumor (Figure 2(e) and (f)). Appropriate assessment of the observed trend needs additional research with larger sample size. Nevertheless, the overall diagnostic value of both lncRNAs appears to be less promising for HCC than for CCA (as also evident in a comparison of the respective ROC curves, Figure 2(g)), especially considering the fact that candidate biomarkers are more valuable for early detection of liver cancer.

Of note, we registered very low but significant upregulation of both *LINC00941* and *ABHD11-AS1* in a group of benign liver tumors (Figure 3(a) and (b)). These results may

indicate extremely high sensitivity of these two potential lncRNA biomarkers to initial proliferative (tumorigenic) processes within liver tissues. These lncRNAs can also be tested as a possible diagnostic panel for HCA prognosis because this type of benign liver tumors is at a high risk of malignant transformation into HCC.[77] We compared median expression levels of both lncRNAs among all analyzed groups of liver tissue samples and revealed the highest and most significant upregulation only in CCA; these data may help to distinguish CCA from HCC and benign lesions (Figure 3(c) and (d)).

For the *CASC8* lncRNA, the diagnostic potential was not confirmed for either CCA or HCC (Figure S1). We believe that this is a vivid example pointing to the extreme need for validation of RNA-Seq data by alternative methods such as RT-qPCR, including other sets of patients' tissue samples. Notably, among the three candidate lncRNAs under study, *CASC8* has the lowest expression according to TCGA data (Figure 1(a)). This pattern is even more evident when the same TCGA dataset is visualized using the UCSC Xena browser, which shows elevated expression of *CASC8* in only a small proportion of CCA samples (Figure S9), in contrast to *LINC00941* and *ABHD11-AS1*. Indeed, the low expression levels of the majority of lncRNAs, in particular of tissue-specific or cancer-specific candidates, is a challenging problem for current research focusing on both bioinformatic and biomedical studies.[78,79] Thus, we can assume that overall expression of *CASC8* in liver tissues is very low, and even in case of its upregulation in some cancer, this aberration is not so significant and informative. We also noticed that *CASC8* expression has previously been experimentally analyzed in patients' tissue samples only in pancreatic cancer.[80] Indeed, *CASC8* expression is highest in this type of tumor, according to pan-cancer TCGA data (Figure S7). Researchers in reference[80] also reported approximately a ninefold increase in expression within pancreatic-adenocarcinoma samples compared to normal pancreas tissues according to RNA-Seq data; however, subsequent RT-qPCR analysis of their clinical samples (13 pairs of tumorous/adjacent tissues) showed no significant *CASC8* upregulation.

We also examined seven rare cases of cHCC-CCA to determine whether *LINC00941*, *ABHD11-AS1*, and *CASC8* have some distinct expression patterns in this type of tumor. These tissue samples manifested rather varied patterns, in agreement with previous studies.[27] This finding indicates that the identification of biomarkers for cHCC-CCA diagnostics may be very challenging.

The present study is focused on postsurgery liver tissues and is not aimed at evaluating the secretion of the lncRNAs in question into biological fluids of patients with liver tumors. Nevertheless, we view the detectability of *LINC00941* and *ABHD11-AS1* as a subject of our next studies. Not so long ago, *LINC00941* serum levels were shown to be elevated in patients with HCC in comparison to healthy patients.[81] On the other hand, they proved to be upregulated to an even larger extent in HBV-associated liver cirrhosis and in chronic HBV infection (all the analyzed tissue samples including HCC tissues were found to be HBV-infected).[81] These data partially support our hypothesis that *LINC00941* expression is somehow activated in response to liver injury caused by factors other than cancer. Nevertheless, in our comparison of existing transcriptomic datasets from various liver diseases, CCA appeared to be a more prevalent correlate of *LINC00941* upregulation (Figure 1(c)) in comparison to viral infections. Consequently,

we expect that if *LINC00941* is detectable in blood serum in HBV-associated HCC cases, then this pattern may also be applicable to CCA, especially because of the higher expression of *LINC00941* in this type of liver cancer. In light of all these findings, *LINC00941* may be regarded as a potential noninvasive biomarker of CCA.

*ABHD11-AS1* has not been reported to be dysregulated either in CCA or in HCC; therefore, we showed its diagnostic potential for CCA for the first time. However, this lncRNA has been detected by RT-qPCR in the plasma of patients with pancreas cancer.[82] We cannot rule out the possibility of different functions of *ABHD11-AS1* in these two types of cancer and possible absence of its secretion into the bloodstream in case of CCA for some specific reason. Nevertheless, we believe that this possibility indeed needs to be tested in an extended experiment.

Possible molecular mechanisms underlying the upregulation of *LINC00941* and *ABHD11-AS1* during CCA progression are still unknown, and neither are subsequent consequences for regulation of intracellular processes. We can assume that general *LINC00941*- and *ABHD11-AS1*-mediated pathways found in other types of cancers are also relevant for CCA. *ABHD11-AS1* has been found to affect the activity of PI3K–AKT, EGFR, and RhoC signaling pathways in various cancers.[66] We can hypothesize that it possibly plays a similar role in CCA, because activation of the same regulatory cascades is known to stimulate CCA progression too.[83–85] In the present study, we uncovered a positive correlation of *ABHD11-AS1* expression levels with *ABHD11* mRNA expression in CCA samples (Figure S6), similarly to a trend previously demonstrated in cervical cancer.[34] Thus, we suppose that at least one function of *ABHD11-AS1* in CCA is likely realized by a similar mechanism, through direct FUS binding and prevention of *ABHD11* mRNA degradation, although further *in vitro* studies on CCA cell lines are needed to test this hypothesis.

*LINC00941* has been shown *in vitro* to sponge miR-34a and activate EMT in HCC cell lines.[61] Decreased miR-34a levels have been reported to correlate with a worse prognosis for CCA patients.[86] Thus, one can suppose that at least one of the molecular consequences of *LINC00941* upregulation in CCA is implemented via sponging of miR-34a. Similar mechanisms have been documented for circular RNA circEIF3C, which sponges miR-34a thereby promoting intrahepatic CCA progression.[87] Another mechanism could be mediated by direct binding of *LINC00941* to the ANXA2,[61] which is considered one of important factors of CCA tumorigenesis as well.[88] In the present study focusing specifically on CCA, we were able to identify at least one potential direct target of *LINC00941: CAPRIN2* mRNA. Of note, *CAPRIN2* proved to be the top CCA gene from the TCGA dataset that has the highest correlation with the *LINC00941* expression level (Table S7). It was subsequently confirmed by means of independent RNA-Seq data and was experimentally validated by RT-qPCR on our own cohort of CCA patients (Figure 4). *LINC00941* has been previously found to loop the promoter of its neighboring gene *CAPRIN2* with the help of CTCF transcription factor and to activate its expression. This mechanism was demonstrated in two independent studies—on nasopharyngeal carcinoma[30] and on oral squamous cell carcinoma[31]—and is probably similar in CCA. It was also recently reported that *CAPRIN2* is one of the six mostly upregulated genes in CCA cell lines generated from xenograft models derived from specimens associated with a

patient's unfavorable prognosis.[89] CAPRIN2 directly binds low-density lipoprotein-related receptor LRP5/6 and promotes its phosphorylation by GSK3β. Subsequent stabilization of free cytosolic β-catenin leads to its nuclear translocation and results in upregulation of the canonical WNT/β-catenin signaling pathway (Figure 4(f)). Due to the critical impact of this pathway on CCA progression,[90] *LINC00941* can potentially be considered a therapeutic target in this type of liver cancer. Furthermore, our study provides the first evidence of possible involvement of *LINC00941* in CCA pathogenesis.

## Conclusions

Our paper provides the first evidence for significant upregulation of *LINC00941* and *ABHD11-AS1* in tumor tissue samples from patients with intrahepatic CCA, thereby indicating good prospects for the use of these lncRNAs as CCA oncomarkers. This study has certain limitations, mainly related to modest sample sizes, especially in case of cHCC-CCA and benign tumors, although this is consistent with the total prevalence of such tumors among liver lesions. We also did not analyze widespread liver diseases, for example, hepatitis or liver cirrhosis; however, the main purpose of our work was to screen different types of liver tumors unrelated to chronic infection or other concomitant diseases, in order to identify a possible association of lncRNA upregulation with cancer only and to initially validate candidate lncRNAs as biomarkers of CCA. Further verification of our data on independent patient cohorts by other research groups is expected to accelerate progress in this area of research.

## Abbreviations

| | |
|---|---|
| CCA | cholangiocarcinoma |
| cHCC-CCA | combined HCC and CCA |
| EMT | epithelial–mesenchymal transition |
| FNH | focal nodular hyperplasia |
| HBL | pediatric hepatoblastoma |
| HBV | hepatitis B virus |
| HCA | hepatocellular adenoma |
| HCC | hepatocellular carcinoma |
| HCV | hepatitis C virus |
| lncRNA | long noncoding RNA. |

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## Statements and declarations

### Ethical considerations

The study protocol was approved on 18 January 2016 by the Institutional Ethics Committee of Blokhin National Medical Research Center of Oncology affiliated with the Russian Ministry of Health (Ethics Committee Statement No. 18/01/2016). All procedures involving human participants were conducted in accordance with the Declaration of Helsinki of 1975 and its later amendments (World Medical Association, 2013).

### Consent to participate

Written informed consent for the use of tissue samples for scientific purposes was obtained from all participants of the study.

### Author contributions/CRediT

Study concept and design (OYB, NLL); resources (DAS, EAM, NEK, YIP); experiments (OYB); analysis and interpretation of the data (OYB, NLL, IFK); manuscript writing (OYB); review and editing (NLL, MPR); supervision (MPR, OAD); and funding acquisition (OYB, OAD). All authors contributed to the article and approved the submitted version.

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### Conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

### Data availability

The data necessary to reproduce our results are included in the article; raw data are available from the corresponding author.

## Supplemental material

Supplemental material for this article is available online.

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