Prognostic Value of Long Non-Coding RNA HULC and MALAT1 Following the Curative Resection of Hepatocellular Carcinoma

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Long non-coding RNAs (lncRNAs) were shown to be the crucial regulators of the many diseases. In this study, the expressions of lncRNAs were investigated in resected 158 hepatocellular carcinomas (HCCs) to evaluate the effects of their expression levels on prognosis. The expression levels of HULC and MALAT1 were shown to be significantly higher in the normal background tissue of HCC than those in the normal liver tissue of metastatic liver tumor without hepatitis (HULC: fold change 14.9, \(P = 1.7 \times 10^{-06}\); MALAT1: fold change 17.5, \(P = 1.2 \times 10^{-06}\). The formation of capsule was shown to be correlated with the increased expression of HULC (\(P = 0.041\)), while the size of HCC under 2 cm was correlated with a decrease in MALAT1 expression (\(P = 0.019\). The levels of serum alpha-fetoprotein above 20 ng/mL indicated a decreased expression of both HULC and MALAT1 (HULC: \(P = 0.017\); MALAT1: \(P = 0.0036\). The increase in the expression levels of MALAT1 in HCC tissues was significantly correlated with better overall survival (HULC: \(P = 0.099\), MALAT1: \(P = 0.028\). Thus, the expression of these lncRNAs in HCC potentially correlates with the HCC malignancy and they represent potential prognostic biomarkers of the resected HCC.

Hepatocellular carcinoma (HCC) represents the fifth most common malignancy and the third most common cause of cancer-related death worldwide. Hepatic resection is one of the most effective treatments for non-metastatic HCC. However, after the curative resection, ~80% of patients develop intrahepatic recurrence, and 50% die within 5 years. Therefore, although the surgical resection of early HCC can be curative, the pronounced tendency toward recurrence represents a major concern.

Intrahepatic HCC recurrence is categorized as intrahepatic metastasis (IM) or multicentric occurrence (MO). IM refers to the HCC foci developing from tumor cells that spread into the remnant liver via the portal vein before or during hepatic resection. MO refers to the development of postsurgical HCC foci due to chronic active hepatitis or cirrhosis, caused by viruses, alcohol, toxins, or other HCC-relevant risk factors. The results of previous studies indicated that the clinical progression and outcome of IM and MO differ significantly. Considering these specific characteristics of recurrence, focusing on the analysis of tumor tissue alone may be insufficient. In all HCC cases, the consideration of any correlations between the HCC tissue and the surrounding non-tumor tissue is important. We demonstrated previously that the alterations in the non-malignant liver tissue gene profiles can be used for the prognostic purposes in HCC.

Long non-coding RNAs (lncRNAs) were identified as the key players in tumorigenesis and tumor progression. Many reports showed that lncRNA dysregulation is linked to the development of many diseases, including cancer. Therefore, we hypothesized that the expression of lncRNAs in the background liver tissue of HCC may be important for HCC prognosis as well. We attempted to identify novel lncRNAs related to HCC prognosis by using the microarray analysis of their expression in the background liver tissue of HCC patients and normal liver tissue without HCC and/or hepatitis. We evaluated the differences in lncRNA expression levels in HCC and corresponding non-tumor tissues, and identified the unique prognostic markers for the HCC.

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RT-qPCR. Total cDNA was developed from the RNA extracted from each fresh-frozen tissue with M-MLV Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA). This total cDNA was used as a template for the next step of quantitative PCR. PCR was performed using SYBR Premix Ex Taq II (Takara Clontech, Kyoto, Japan) under the following conditions: denaturing at 95 °C for 10 s, 40 cycles of denaturing at 95 °C for 5 s, and annealing/extension at 60 °C for 30 s. The SYBR Green signal was detected in real-time using StepOne Plus Real-Time PCR System (Life Technologies, Carlsbad, CA, USA). The relative quantification method was used and each gene expression level was determined to be the expression level of the target gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), for each sample. For real-time quantitative reverse transcription PCR (RT-qPCR), HCC and CN tissue samples were collected from 158 consecutive patients who underwent curative resection at Nagoya University Hospital between January 1998 and December 2011. Resection was defined as curative when all gross tumors were removed completely; incidentally found small lesions suspected to be HCC that were treated intraoperatively by radiofrequency therapy or microwave coagulation therapy were also regarded as curative cases. Patient characteristics are summarized in Table 1. After surgery, all patients were monitored by performing blood examinations, ultrasonography, and computed tomography. In the cases of possible recurrence, angiography was performed in order to confirm it. The median follow-up duration of all cases was 48.5 months (range, 0.3 to 193.8 months). All tumor tissue samples were histologically confirmed as HCC samples by pathologists. All surgically obtained tissue samples were immediately frozen in the liquid nitrogen and stored at −80 °C until further analyses. This study and all procedures were approved by the Institutional Review Board at Nagoya University and all patients provided written informed consent. All clinical investigations were conducted in accordance with the principles of the Declaration of Helsinki.

| Characteristics                  | Value                                           |
|----------------------------------|-------------------------------------------------|
| Age (years)                      | Median (range) 65 (37–84)                      |
| Sex                              | Male: female 132 (84):26 (16)                   |
| Viral infection                  | HBV/HCV:non-HBV/HCV 41 (26):92 (58):28 (18)    |
| Child-Pugh classification        | A:B 148 (94):9 (6)                             |
| Liver damage classification      | A:B:C 126 (83):25 (16):1 (1)                   |
| Albumin (g/dL)                   | Median (range) 3.9 (2.3–4.9)                   |
| Total bilirubin (mg/dL)          | Median (range) 0.7 (0.2–7.3)                   |
| PT (%)                           | Median (range) 89.7 (46.9–138)                 |
| AFP (ng/mL)                      | Median (range) 17 (0.8–119923)                 |
| Tumor size (cm)                  | Median (range) 3.5 (0.15–15)                   |
| Tumor multiplicity               | Solitary: multiple 124 (78):34 (22)            |
| ICG-R15 (%)                      | Median (range) 11.5 (1.6–35.2)                 |
| Stage                            | Child-Pugh classification A:B 148 (94):9 (6)    |
|                                  | Liver damage classification A:B:C 126 (83):25 (16):1 (1) |
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Table 1. HCC patient characteristics (n = 158). Abbreviations: HCC, hepatocellular carcinoma; n, number; HBV, hepatitis B virus; HCV, hepatitis C virus; PT, prothrombin time; AFP, alpha-fetoprotein; ICG-R15, retention rate of indocyanine green 15 minutes after administration.

Methods
Patients and Samples. For microarray analysis, the representative non-tumor liver tissue of a HCC patient (CN, for the corresponding normal) was obtained from a typical HCC patient, a 58-year-old man during hepatectomy. This HCC was caused by the chronic hepatitis C infection, and it recurred three years after the resection. Pathology confirmed the absence of cancerous regions from the CN sample. As controls, non-cancerous liver tissue without hepatitis (SN, for super normal) was obtained from 11 patients with liver metastases who underwent hepatectomy at Nagoya University Hospital, Japan. Their primary diseases were colorectal cancer (n = 5), gastrointestinal stromal tumor (n = 2), or gastric cancer, esophageal cancer, cervical cancer, or tongue cancer (n = 1, each).

For real-time quantitative reverse transcription PCR (RT-qPCR), HCC and CN tissue samples were collected from 158 consecutive patients who underwent curative resection at Nagoya University Hospital between January 1998 and December 2011. Resection was defined as curative when all gross tumors were removed completely; incidentally found small lesions suspected to be HCC that were treated intraoperatively by radiofrequency therapy or microwave coagulation therapy were also regarded as curative cases. Patient characteristics are summarized in Table 1. After surgery, all patients were monitored by performing blood examinations, ultrasonography, and computed tomography. In the cases of possible recurrence, angiography was performed in order to confirm it. The median follow-up duration of all cases was 48.5 months (range, 0.3 to 193.8 months). All tumor tissue samples were histologically confirmed as HCC samples by pathologists. All surgically obtained tissue samples were immediately frozen in the liquid nitrogen and stored at −80 °C until further analyses. This study and all procedures were approved by the Institutional Review Board at Nagoya University and all patients provided written informed consent. All clinical investigations were conducted in accordance with the principles of the Declaration of Helsinki.

Gene Expression Analysis. Microarray. Total RNA was extracted from the fresh-frozen CN and SN tissue samples using a Qiagen miRNAeasy mini-kit (Qiagen, Hilden, Germany). Eleven SN samples were pooled so that the individual differences are eliminated. RNA quality was confirmed based on the RNA integrity number ≥ 8 as measured using an Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA). RNA was labeled with cyanine-3 dye using a Quick Amp labeling kit (Agilent, Santa Clara, CA, USA) and hybridized to Agilent whole human genome (4 × 44 K) microarrays for 17 h in a rotating SciGene model 700 oven (SciGene, Sunnyvale, CA, USA). The arrays were scanned (Agilent DNA microarray scanner), and the data were feature-extracted using Feature Extraction software 10.5.1.1 and statistically analyzed using the default settings for GeneSpring GX 11.0.1 software (Agilent, Santa Clara, CA, USA)17.

RT-qPCR. Total cDNA was developed from the RNA extracted from each fresh-frozen tissue with M-MLV Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA). This total cDNA was used as a template for the next step of quantitative PCR. PCR was performed using SYBR Premix Ex Taq II (Takara Clontech, Kyoto, Japan) under the following conditions: denaturing at 95 °C for 10 s, 40 cycles of denaturing at 95 °C for 5 s, and annealing/extension at 60 °C for 30 s. The SYBR Green signal was detected in real-time using StepOne Plus Real-Time PCR System (Life Technologies, Carlsbad, CA, USA). The relative quantification method was used and each gene expression level was determined to be the expression level of the target gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), for each sample.

The PCR primers used in current study were for the 84 base pairs fragment of Hepatocellular Carcinoma Up-Regulated Long Non-Coding RNA (HULC) (sense, 5′-ACTCTGAATGAAAGGCCGGAA-3′; antisense 5′-GCCAGGAAACTTTCTTTGCTTA-3′) and for the 14 base pairs fragment of Metastasis Associated Lung Adenocarcinoma Transcript 1 (MALAT1) (sense, 5′-CCAGTTGAATTCACCGTGAC-3′; antisense 5′-AGTTGTCATCGCCAGTTAC-3′). GAPDH (sense, 5′-GAAGGTGAAGGTCGGAGTC-3′; antisense 5′-TGAAGACGCCAGAGGCTTC-3′).
Expression Profiling. To identify novel tumor-related IncRNA in HCC, we compared the non-coding RNA expression profiles of a CN sample and the pooled SN samples. Microarray analysis revealed that IncRNAs upregulated in the CN sample (fold change higher than 1.2) were Urothelial Cancer Associated 1 (UCA1; fold change 7.98), HULC (fold change 3.49), MALAT1 (fold change 1.85), Growth Arrest Specific 5 (GASS; fold change 1.28), and Taurine Up-Regulated 1 (TUG1; fold change 1.27) (Supplementary Table S1).

Figure 1. HULC and MALAT1 expression levels in SN, CN, and HCC samples. (a) HULC expression levels in SN, CN, and HCC samples. (b) MALAT1 expression in SN, CN, and HCC samples. (c) Correlation between HULC expression and MALAT1 expression in HCC. CN, corresponding normal; HCC, hepatocellular carcinoma; HULC, Hepatocellular Carcinoma Up-Regulated Long Non-Coding RNA; MALAT1, Metastasis Associated Lung Adenocarcinoma Transcript 1.

5′-GAAGATGGTGATGGGATTTC-3′; probe 5′-[Fl] CAAGCTTCCCGTTCTCAGCC [Fl-Q]-3′ expression was quantified in each sample in order to perform the normalization. All RT-qPCR experiments were performed in triplicate, including the template-omitted negative controls.

Public Available Dataset. Normalized TCGA RNA-sequencing data for hepatocellular carcinoma was downloaded from the Broad GDAC Firehose (http://gdac.broadinstitute.org/, accessed on November 1st, 2017). This dataset includes 360 HCC cases including seven HCCs mixed with hepatocholangiocarcinoma and two cases with fibrolamellar carcinoma. There are 266 cases with information of recurrence-free survival (RFS) and 336 cases with information of overall survival (OS) out of the 360 cases.

Statistical Analyses. Continuous variables were expressed as median (range) and expression of each target gene was compared by a Wilcoxon signed-rank test. Categorical variables were compared using the χ² or Fisher’s exact tests, as appropriate. Correlation between each gene expression was analyzed with Spearman’s rank correlation coefficient. OS and RFS rate at each point of the follow-up time were estimated using the Kaplan-Meier method and compared using a log-rank test. The Cox proportional hazard regression model was used to perform univariate analysis for OS. All statistical analyses were performed using R (version 3.4.1; http://www.r-project.org/) and statistical significance was set at P < 0.05, which was obtained using two-tailed tests.

Results
SN, CN, and HCC Tissue Sample Analysis. As determined by analyzing 11 SN and 48 paired CN and HCC, HULC and MALAT1 expression levels were significantly higher in the CN tissue samples (HULC: median, 0.019 [range, 0.0008 to 0.068]; MALAT1: 0.71 [0.12 to 6.8]; n = 48) than in the SN tissue samples (HULC: 0.0013 [0.0002 to 0.0076], P = 1.7e-06; MALAT1: 0.041 [0.019 to 0.45], P = 1.2e-06; n = 11) (Fig. 1a and b). However, no significant difference in HULC expression between CN and HCC was observed (0.012 [range, 0.0001 to 0.29], P = 0.12, n = 158) (Fig. 1a).

MALAT1 expression levels in the HCC samples (0.19 [range, 0.011 to 3.4], n = 158) were shown to be significantly lower than those in the CN samples (P = 1.7e-10) (Fig. 1b). There was a weak positive correlation between HULC and MALAT1 expression in HCC tissue (correlation coefficient r = 0.46, P = 1.1e-06) (Fig. 1c).

When 158 curably resected HCC tissue samples were analyzed, HULC expression levels were shown to be higher in the HCC samples (0.047 [range, 0.00007 to 4.1], n = 158) in comparison with those in the CN tissues (0.036 [0.0005 to 1.9], P = 0.063; n = 58) (Fig. 2a). However, the expression of MALAT1 did not differ between

Figure 2. HULC and MALAT1 expression levels in 158 HCC samples. (a) HULC expression in CN and HCC samples. (b) MALAT1 expression levels in CN and HCC tissue samples. (c) HULC expression in the HCC cases accompanied by the formation of capsule (Fc) or without Fc. (d) MALAT1 expression in HCC samples larger or smaller than 2 cm. (e) HULC expression level in the HCC cases with higher serum AFP levels. (f) MALAT1 expression level in the HCC cases with higher serum AFP levels. CN, corresponding normal; HCC, hepatocellular carcinoma; Fc, formation of capsule; AFP, alpha-fetoprotein; HULC, Hepatocellular Carcinoma Up-Regulated Long Non-Coding RNA; MALAT1, Metastasis Associated Lung Adenocarcinoma Transcript 1.
CN (0.69 [0.026 to 19.1], n = 158) and HCC tissues (0.57 [0.032 to 23.1], P = 0.11; n = 158) (Fig. 2b). In the CN samples, no significant difference was found between other clinicopathological factors and HULC or MALAT1 expression. However, HULC expression levels in HCC cases where the capsular formation can be observed (0.059 [0.0003 to 4.0], n = 101) was significantly higher than in the HCC samples without capsular formation (0.039 [0.00007 to 4.1], P = 0.041, n = 45) (Fig. 2c). MALAT1 expression levels in HCC samples larger than 2 cm (0.51 [0.031 to 21], n = 116) were significantly lower than those in the HCC under 2 cm (0.94 [0.23 to 2.9], P = 0.019, n = 23) (Fig. 2d). The expression of HULC and MALAT1 in HCC cases with higher serum alpha-fetoprotein (AFP) levels (>20 ng/mL) (HULC: 0.03 [0.0007 to 1.7], n = 64; MALAT1: 0.36 [0.031 to 11.1, n = 64) were significantly lower than those in the HCC samples with lower serum AFP values (<20 ng/mL) (HULC: 0.061 [0.0007 to 4.1], P = 0.019, n = 80; MALAT1: 0.88 [0.058 to 23.1], P = 0.0036, n = 81) (Fig. 2e and f).

**HULC and MALAT1 Correlation with HCC Prognosis.** Based on the results obtained by the real time RT-qPCR, 158 HCC cases were divided into two groups, according to the HULC and MALAT1 expression levels in HCC tissues (HULC: < median and ≥ median) and the effects of the expression levels on RFS and OS were evaluated. The distribution of each clinicopathological features stratified by HULC or MALAT1 expression. However, HULC expression levels in HCC cases where the capsular formation can be observed (0.059 [0.0003 to 4.0], n = 101) was significantly higher than in the HCC samples without capsular formation (0.039 [0.00007 to 4.1], P = 0.041, n = 45) (Fig. 2c). MALAT1 expression levels in HCC samples larger than 2 cm (0.51 [0.031 to 21], n = 116) were significantly lower than those in the HCC under 2 cm (0.94 [0.23 to 2.9], P = 0.019, n = 23) (Fig. 2d). The expression of HULC and MALAT1 in HCC cases with higher serum alpha-fetoprotein (AFP) levels (>20 ng/mL) (HULC: 0.03 [0.0007 to 1.7], n = 64; MALAT1: 0.36 [0.031 to 11.1, n = 64) were significantly lower than those in the HCC samples with lower serum AFP values (<20 ng/mL) (HULC: 0.061 [0.0007 to 4.1], P = 0.019, n = 80; MALAT1: 0.88 [0.058 to 23.1], P = 0.0036, n = 81) (Fig. 2e and f).

**Figure 3.** Survival analysis stratified by HULC and MALAT1 expression levels in HCC tissues. (a) RFS analysis stratified by HULC expression. (b) OS analysis stratified by HULC expression. (c) RFS analysis stratified by MALAT1 expression. (d) OS analysis stratified by MALAT1 expression. HULC, Hepatocellular Carcinoma Up-Regulated Long Non-Coding RNA; MALAT1, Metastasis Associated Lung Adenocarcinoma Transcript 1; OS, overall survival; RFS, recurrence-free survival.
prothrombin time < 70% (HR, 2.03; 95% CI, 1.11 to 3.72; \( P = 0.02 \)), B or C liver damage (HR, 2.09; 95% CI, 1.21 to 3.62; \( P = 0.01 \)), multiple tumor (HR, 1.68; 95% CI, 1.00 to 2.81; \( P = 0.049 \)), AFP \( \geq 20 \) ng/mL (HR, 2.15; 95% CI, 1.35 to 3.45; \( P = 0.001 \)), poorly differentiated (HR, 2.29; 95% CI, 1.14 to 4.62; \( P = 0.02 \)), serosal invasion (HR, 2.43; 95% CI, 1.44 to 4.11; \( P = 0.001 \)), vascular invasion (HR, 2.39; 95% CI, 1.47 to 3.87; \( P = 0.004 \)), and positive surgical margin (HR, 1.94; 95% CI, 1.09 to 3.45; \( P = 0.02 \)) (Table 2).

The cohort used in this study is composed of cases with HBV 38 (24%), HCV 89 (56%), HBV + HCV 3 (2%), and None-virus 28 (18%). This proportion is similar to those in the EU, US, and Japan, as reported previously\(^{18}\). Then, using only HCV cases (81 informative cases with RFS and 82 informative cases with OS), a prognostic analysis was performed. Interestingly, the result revealed that the RFS and OS value, as stratified by the expression level of HULC was significantly different (RFS: HR, 0.30; 95% CI, 0.17 to 0.55; \( P < 0.0001 \); OS: HR, 0.32; 95% CI, 0.17 to 0.63; \( P = 0.0005 \), Fig. 4a and b). However the RFS and OS value, as stratified by the expression level of MALAT1 had the same tendency as seen in entire cohort without any significant difference (RFS: HR, 0.78; 95% CI, 0.48 to 1.3; \( P = 0.33 \); OS: HR, 0.62; 95% CI, 0.34 to 1.1; \( P = 0.12 \), Fig. 4c and d).

**HULC and MALAT1 Correlation with HCC Prognosis in a Public Available Dataset.** Finally, we confirmed the impact of HULC and MALAT1 expression on HCC prognosis using TCGA dataset. Based on the normalized RNA-sequencing data, HCC cases were divided into two groups, according to the HULC and MALAT1 expression levels in HCC tissues (HULC: \( < 0.45 \) and \( \geq 0.45 \); MALAT1: \( < 9.35 \) and \( \geq 9.35 \)). As a result, the prognosis of HCC cases from TCGA stratified by HULC and MALAT1 expression have the same tendency for HCC prognosis as seen in our collected 158 cases (Supplementary Figure S1).

**Discussion**

With the development of whole genome and transcriptome sequencing, many lncRNAs were shown to be highly expressed in tumor tissue\(^{19}\). Gene expression profile of the surrounding normal liver tissue is important for HCC prognosis and the potential for recurrence\(^{12}\). We attempted to identify lncRNAs that may be used as prognostic biomarkers for HCC, by comparing their expression levels in the non-tumorous background liver tissue of HCC patients and non-tumorous and non-hepatitis liver tissue from the metastatic hepatic cancer patient\(^{12}\). In this way, we found five lncRNAs, shown to be highly expressed in CN, and the expression profiles obtained using a small-sized cohort confirmed that HULC and MALAT1 show higher expression in the CN than SN samples.

According to the cancer genome atlas (TCGA) dataset, HULC and MALAT1 are highly expressed in the HCC tissues (HULC: 371 tumor vs. 50 normal, fold change 2.04; MALAT1: 373 tumor vs. 50 normal, fold change 1.72)\(^{20}\).
HULC was first described as a lncRNA with induced expression in HCC compared with the non-neoplastic tissue. In osteosarcoma and pancreatic cancer, an increased expression of HULC in tumor tissue was shown to indicate poor prognosis. MALAT1 is highly conserved and it was originally reported as a biomarker of metastasis in the early lung adenocarcinoma. Functionally, HULC has not been completely elucidated, and a previous report showed that HULC can act as an oncogene in HCC through the deregulation of lipid metabolism through a signaling pathway involving miR-9, PPARα, and ACSL1. MALAT1 acts as a proto-oncogene through the Wnt pathway activation and induction of the oncogenic splicing factor SRSF1.

In this study, we showed that the expression of HULC was significantly higher in HCC samples, but the expression of MALAT1 was not altered between CN and HCC. This may be caused by the presence of hepatitis in these cases. The underlying cause of hepatitis differed between the samples and in each study, the distribution of causes for hepatitis may vary. Furthermore, in breast cancer, the expression pattern of MALAT1 differs between transcript variants, indicating that the transcript variant should be considered when determining the expression level of these lncRNAs in HCC.

Unexpectedly, we did not observe the difference in lncRNA expression profiles between the background liver tissue and HCC tissue, and no correlation between HULC and MALAT1 levels in CN and other background factors was determined. However, we demonstrated a significant difference between the expression of these two lncRNAs in HCC and other clinicopathological tumor-related factors, indicating that the decreased aggressiveness of HCC may be associated with the expression level of both HULC and MALAT1. Additionally, we demonstrated that the induced HULC and MALAT1 expression in HCC correlates with a better prognosis. Few reports demonstrated that these two genes contribute to tumor development and recurrence and they are tumor indicators. The preliminary HULC analysis with Gene Expression Omnibus (GEO) and in silico analysis showed that the higher expression of HULC is correlated with the better prognosis in HCC. To the best of our knowledge, the relationship between these two lncRNAs and HCC prognosis, using clinical samples, has not been established.

Figure 4. Survival analysis stratified by HULC and MALAT1 expression levels in HCC tissues from HCV patients. (a) RFS analysis stratified by HULC expression. (b) OS analysis stratified by HULC expression. (c) RFS analysis stratified by MALAT1 expression. (d) OS analysis stratified by MALAT1 expression. HULC, Hepatocellular Carcinoma Up-Regulated Long Non-Coding RNA; MALAT1, Metastasis Associated Lung Adenocarcinoma Transcript 1; OS, overall survival; RFS, recurrence-free survival.
previously. Our results indicate that these two IncRNAs may act as oncogenes, but they do not accelerate the progression of HCC.

This study is limited due to the use of a single-center cohort, without the possibility of the analysis of an independent cohort. In order to confirm these results further, they should be validated using an independent HCC cohort.

In conclusion, our findings suggest that the increased expression of HULC and MALAT1 in HCC tissue may represent a good prognostic biomarker for curatively resected HCC. Therefore, in combination with other tumor prognostic factors, the determination of the expression levels of these IncRNAs may lead to a more accurate prediction of HCC prognosis.

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Additional Information
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