Cancer Letters 450 (2019) 98–109

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Original Articles

A novel, liver-specific long noncoding RNA LINC01093 suppresses HCC progression by interaction with IGF2BP1 to facilitate decay of GLI1 mRNA

Jia He[a,1], Qiaozhu Zuo[a,1], Bo Hu[b,1], Haojie Jin[a], Cun Wang[a], Zhuoan Cheng[c], Xuan Deng[d], Chen Yang[d], Haoyu Ruan[d], Chengtao Yu[c], Fangyu Zhao[a], Ming Yao[a], Jingyuan Fang[a], Jianren Gu[a], Jian Zhou[b], Jia Fan[b], Wenxin Qin[a], Xin-Rong Yang[b,**], Hui Wang[a,*]

[a] State Key Laboratory of Oncogenes and Related Genes, Shanghai Cancer Institute, Renji Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, 200032, China

[b] Department of Liver Surgery, Liver Cancer Institute, Zhongshan Hospital and Key Laboratory of Carcinogenesis and Cancer Invasion of Ministry of Education, Fudan University, Shanghai, 200032, China

[c] School of Biomedical Engineering, Shanghai Jiao Tong University, Shanghai, 200030, China

[d] Shanghai Medical College of Fudan University, Shanghai, 200032, China

ARTICLE INFO

Keywords:
lncRNA
Proliferation
Metastasis
mRNA stability
Post-transcriptional regulation

ABSTRACT

Long noncoding RNAs (lncRNAs) are implicated as novel drivers in hepatocellular carcinoma (HCC), but the underlying mechanisms of this relationship with hepatocarcinogenesis are unknown. We report a novel, liver-specific lncRNA LINC01093 that shows significant downregulation in HCC tissues. LINC01093 expression is inversely correlated with cancer embolus and HCC TNM stage and as a prognostic predictor for HCC patients. LINC01093 overexpression significantly suppresses HCC cell proliferation and metastasis *in vitro* and *in vivo*. Conversely, its knockdown promotes HCC progression. Mechanistic analyses indicate that LINC01093 directly binds insulin-like growth factor 2 mRNA–binding protein 1 (IGF2BP1), interfering with interaction between IGF2BP1 and glioma-associated oncogene homolog 1 (GLI1) mRNA. The result is degradation of GLI1 mRNA, further affecting expression of GLI1 downstream molecules involved in HCC progression. The liver-enriched lncRNA LINC01093 is a promising prognostic indicator for HCC patients, and the newly identified LINC01093–IGF2BP1–GLI1 axis shows potential for therapeutic targets in HCC.

1. Introduction

Liver cancer is the sixth most common malignancy and fourth leading cause of cancer death worldwide, and hepatocellular carcinoma (HCC) represents 70%–85% of the total liver cancer burden [1]. Half of the 854,000 incident liver cancer cases and 810,000 deaths in 2015 occurred in China [1,2]. Despite recent advances, most patients with this malignancy are diagnosed at an advanced stage, leading to poor outcomes and high recurrence rate even with curative treatment. Despite previous studies identifying aberrant expression of numerous protein-coding genes in HCC, the molecular events underlying disease progression remain largely unknown. Uncovering these pathways is an urgent need.

Long noncoding RNAs (lncRNAs) are a subclass of transcripts longer than 200 nucleotides with little or no protein coding potential [3]. LncRNAs demonstrate low evolutionary sequence conservation and expression patterns specific to developmental stage, tissue, and disease [4]. Accumulating evidence shows that lncRNAs function in various biological processes by diverse mechanisms [5]. Regarding their role in cancer, lncRNAs have been implicated in cell growth, invasion, differentiation, angiogenesis, apoptosis, metabolism, drug resistance, and immune response [5–7]. Several lncRNAs, such as UFC1, DANCR,

Abbreviations: lncRNA, long noncoding RNA; HCC, hepatocellular carcinoma; GEO, gene expression omnibus; RIP, RNA immunoprecipitation; IGF2BP1, insulin-like growth factor 2 mRNA–binding protein 1; GLI1, glioma-associated oncogene homolog 1; OS, overall survival; TTR, time to recurrence; qPCR, quantitative polymerase chain reaction; RRMs, RNA-recognition motifs; KH, hnRNP-K homology

* Corresponding author. State Key Laboratory of Oncogenes and Related Genes, Shanghai Cancer Institute, Renji Hospital, Shanghai Jiao Tong University School of Medicine, No.25/Ln2200 Xie-Tu Road, Shanghai, 200032, China.

** Corresponding author.

E-mail addresses: yang.xinrong@zs-hospital.sh.cn (X.-R. Yang), hwang@shsci.org (H. Wang).

[1] These authors contribute equally to this work.

https://doi.org/10.1016/j.canlet.2019.02.033
Received 27 August 2018; Received in revised form 25 January 2019; Accepted 14 February 2019

lncTCF7, HULC, miR503HG, HOXD-AS1, TSLNC8, and Dreh, are involved in hepatocarcinogenesis [8–14]. UFC1 increases β-catenin expression by interaction with HuR in HCC cells. DANCR markedly increases HCC cell stemness to enhance tumor progression and intra-/extrahepatic tumor colonization. LncTCF7 recruits the SWI/SNF complex to trigger TCF7 expression, leading to activation of Wnt signaling for priming liver cancer stem cell renewal. The lncRNA HULC can elicit autophagy by stabilizing Sirt1 protein and contribute to HCC cell chemoresistance. Although some lncRNAs have been tied to HCC progression, the role of liver-specific lncRNAs in HCC is not fully understood.

Insulin-like growth factor 2 mRNA–binding protein 1 (IGF2BP1) is a conserved RNA-binding protein acts in post-transcriptional mRNA regulation [15]. It could directly interact with the glioma-associated oncogene homolog 1 (GLI1) mRNA, dampening its degradation [16]. GLI1 contributes to the development of multiple human cancers, including liver [17], lung [18], breast [19], and gastric [20] cancers. Its roles include tumor cell proliferation, invasion, apoptosis, angiogenesis, drug resistance, mismatch repair, self-renewal, and cell fate determination [21–24]. In HCC, increased GLI1 expression proportionally correlates with metastasis and unfavorable overall prognosis [17]. These studies emphasize a central role for GLI1 in cancer biology, but differentially expressed GLI1 mRNA in cancer is not fully understood, suggesting involvement of other regulators.

In the current study, we report a novel, liver-specific lncRNA, LINC01093, which is remarkably downregulated in HCC and could be regarded as an independent predictor for overall survival in HCC patients. Moreover, LINC01093 inhibits HCC cell tumorigenicity and metastasis both *in vitro* and *in vivo*. This lncRNA exerts its tumor-suppressive activity by promoting GLI1 mRNA decay through dissociation of IGF2BP1 from GLI1 mRNA. This pathway involving LINC01093 is highly associated with HCC and may offer therapeutic targets for treatment.

2. Materials and methods

2.1. Patients and specimens

Primary HCC and corresponding adjacent noncancerous liver samples were obtained from patients who underwent hepatectomy in Zhongshan Hospital, Shanghai, China. Normal human liver tissues were obtained from healthy donors for liver transplantation. In light of the American Association for the Study of Liver Diseases guidelines, HCC was diagnosed through imaging technologies or biochemistry like AFP, and confirmed by histopathology. Overall survival was defined as the period between surgery and death or the last follow-up. The time-to-recurrence rate was calculated from the date of surgery to diagnosis of relapse (intrahepatic recurrence and extrahepatic metastasis), death, or last observation. The study was approved by the Zhongshan Hospital Research Ethics Committee, and each patient signed informed consent. Normal human tissue cDNA was purchased from BioChain Institute (Newark, CA, USA).

2.2. Fluorescence in situ hybridization

Fluorescence *in situ* hybridization was performed as described previously [25]. Briefly, cells were plated at a density of 50%–70% per dish and fixed in 4% formaldehyde for 10 min at room temperature. Cells were permeabilized in PBS containing 0.5% Triton X-100 for 5 min at 4 °C, then prehybridized for 30 min at 37 °C. Hybridization using LINC01093, U6, and 18S probes was performed at 37 °C in the dark overnight, and cells were rinsed in 4 × SSC buffer for 15 min, 2 × buffer for 5 min, and 1 × buffer for 5 min at 42 °C, and counterstained with DAPI for 10 min. The images were obtained using a confocal microscope.

2.3. RNA pulldown assay

RNA pulldown assays were performed as described previously [26]. In short, biotin-labeled lncRNA-LINC01093, its antisense RNA, domain truncation fragments, and GLI1 mRNA were *in vitro* transcribed with the Biotin RNA Labeling Mix (Roche Diagnostics, Indianapolis, IN, USA) and SP6/T7 RNA polymerase (Roche). After treatment with RNase-free DNase I (Roche), biotinylated RNAs were purified with the RNeasy Mini Kit (Qiagen, Valencia, CA, USA) and incubated with the indicated cell lysates for 1 h at 4 °C. Streptavidin–agarose beads (Invitrogen, Carlsbad, CA, USA) were added to each tube 1 h at room temperature. Finally, the retrieved proteins were measured on SDS-PAGE gels for mass spectrometry or western blot.

2.4. RNA immunoprecipitation

RNA immunoprecipitation (RIP) experiments were performed with a Magna RIP™ RNA-Binding Protein Immunoprecipitation Kit (Millipore, Billerica, MA, USA) according to the manufacturer's instructions. A total of 5 μg of antibody was used for the IGF2BP1 RIP assays (Proteintech, China), and the dilution of HA antibody (Cell Signaling Technology, USA) was 1:50. Co-precipitated RNAs, total RNAs (input controls), and IgG controls were assayed simultaneously by quantitative polymerase chain reaction (qPCR).

2.5. In vivo assays for tumor growth and metastasis

For xenograft experiments, six to eight-week-old BALB/c-nu/nu mice were used. Huh7 cells (2×10^6) that had been stably transfected with overexpression, interfering or control fragment were suspended in 200 μl serum-free DMEM and implanted subcutaneously into the nude mice. Tumors were monitored weekly until mice were sacrificed, and the volume was calculated by length × width^2 × 0.5. Mice were handled and housed according to protocols approved by the Shanghai Medical Experimental Animal Care Commission.

For the lateral tail vein metastasis model, 3×10^6 treated Huh7 cells were suspended in 200 μl serum-free DMEM and injected into the nude mice. After ten weeks, all of mice were sacrificed. The bilateral lung tissues were resected and fixed with 4% phosphate-buffered neutral formalin at room temperature, then analyzed by hematoxylin and eosin staining. Mice were managed and housed according to protocols approved by the Shanghai Medical Experimental Animal Care Commission.

2.6. Statistical analysis

Data are presented as the mean ± standard (s.d.) from one representative experiment of three independent experiments. The Chi-square (χ^2) and Fisher's exact tests were used for nonparametric variables and Student's *t*-test was applied for parametric variables (two-tailed). The paired *t*-test was used to analyze LINC01093 levels in human samples. OS and TTR rate were evaluated using the Kaplan–Meier method and analyzed by the log-rank test. To assess the relative risk for each factor, univariate and multivariate Cox regression analyses were carried out. Pearson correlation analyses were performed to investigate the correlation between LINC01093 and related mRNA expression. A probability value of $p < 0.05$ was considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics V19 package (Armonk, NY, USA).

3. Results

3.1. Identification of LINC01093 as a candidate tumor suppressor in HCC

We conducted transcriptome-wide analyses to determine the transcripts differentially expressed between tumor tissues and adjacent

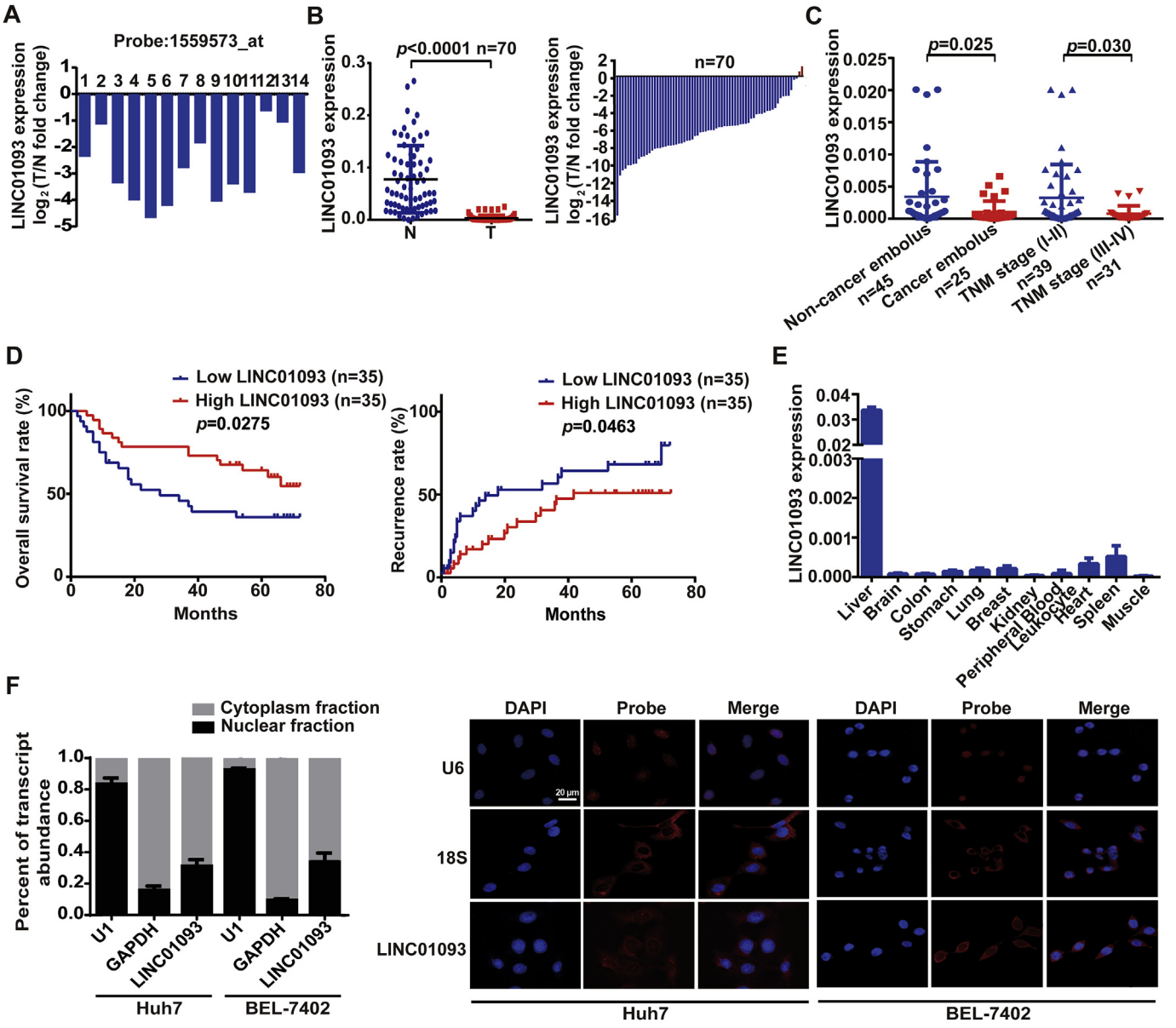

Fig. 1. Identification of LINC01093 as a candidate tumor suppressor in HCC. (A) Fold change of LINC01093 expression in 14 human HCC tissues (T) compared with corresponding noncancerous hepatic tissues (N). (B) Left, LINC01093 expression in 70 matched T/N tissues. Right, fold change of LINC01093 levels in samples. (C) LINC01093 expression in 70 HCC with or without cancer embolus, or with TNM stage (I-II or III-IV). (D) Kaplan-Meier analysis of OS and TTR stratified by LINC01093 levels in 70 HCC patients. (E) RNA levels of LINC01093 in 11 human normal tissues. (F) Left, nuclear and cytoplasmic expression of LINC01093 in HCC cells. Right, RNA FISH analysis of LINC01093 in HCC cells. Values are expressed as mean ± s.d.

noncancerous liver tissues in 14 HCC patients (GSE84402) [27]. Among significantly dysregulated lncRNAs (fold change $> 2^2$ or $< 2^{-2}$, $p < 0.05$), LINC01093 was one of the most downregulated lncRNAs in HCC tissues (Fig. 1A). Next, we verified the expression of LINC01093 in 70 pairs of HCC and corresponding noncancerous liver samples. The data showed that LINC01093 was remarkably downregulated in 97% (68/70) of HCC samples (Fig. 1B). Similar results were obtained from another HCC cohort in the Gene Expression Omnibus (GEO) database (GSE45436) (Fig. S1A). LINC01093 level was also decreased in precancerous disease such as alcohol hepatitis (GSE28619) (Fig. S1B) and hepatitis B virus–associated disease (GSE38941) (Fig. S1C).

We further investigated the clinicopathological and prognostic significance of LINC01093 in 70 HCC tissues. LINC01093 expression was inversely correlated with the existence of cancer embolus and TNM stage of HCC patients (Fig. 1C, Table SI). Of note, HCC patients with lower LINC01093 expression level were associated with poor OS and high rate of TTR (Fig. 1D). Multivariate Cox regression analyses revealed that LINC01093 expression was an independent prognostic indicator for OS (hazard ratio: 2.26; 95% confidence interval: 1.11–4.58; $p = 0.024$) (Table SII).

We next analyzed RNA-Seq Expression Data from GTEx and showed that expression of LINC01093 is liver-specific (Fig. S1D). These results were further confirmed by qPCR in 11 human normal tissues (Fig. 1E). Together, the findings suggest that LINC01093 may be a potential tumor suppressor in hepatic carcinogenesis.

The LINC01093 gene is located on chromosome 4 (Fig. S2A) and exhibits limited protein-coding potential (the ORF finder; Fig. S2B). The LINC01093 transcript was identified by 5′ and 3′ rapid amplification of cDNA ends assays (Fig. S2C). Furthermore, both the subcellular fractionation and *in situ* hybridization analyses showed that LINC01093 was mainly located in the cytoplasm of HCC cells (Fig. 1F).

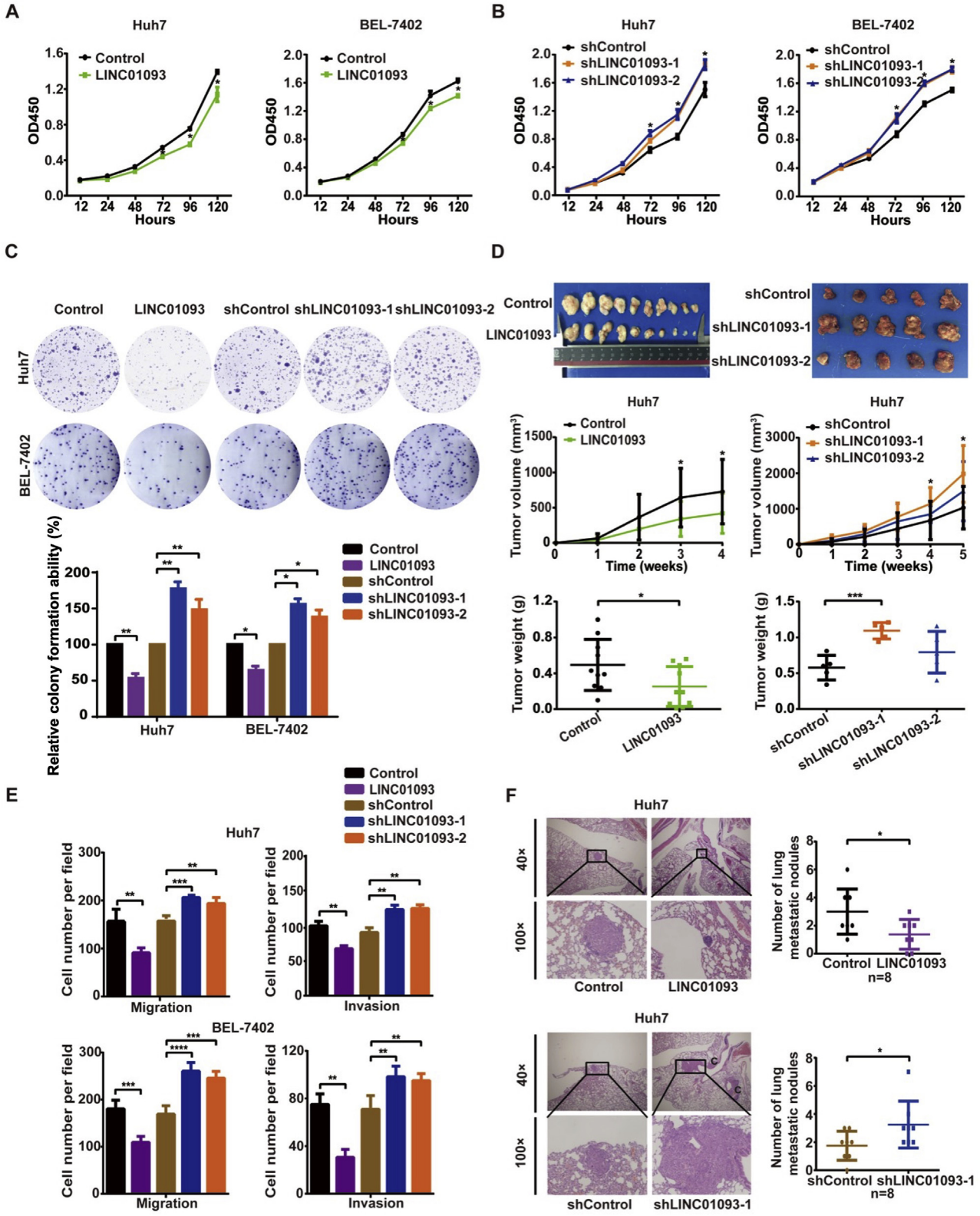

Fig. 2. LINC01093 inhibits HCC cell proliferation and metastasis *in vitro* and *in vivo*. (A) Cell proliferation assays for HCC cells with LINC01093 overexpression or the control lentivirus vector. (B) Cell proliferation assays for HCC cells with LINC01093 knockdown or the control shRNA. (C) Colony formation assays for HCC cells with LINC01093 overexpression or knockdown. Results present colony formation ability relative to the control cells (set to 100%). (D) Tumor growth curve and tumor weight of Huh7 cells with LINC01093 overexpression or knockdown in nude mice. (E) Migration and invasion assays for HCC cells with LINC01093 overexpression or knockdown. (F) Representative pictures of lung metastases by H&E staining in nude mice with Huh7 cells stably transfected with LINC01093 overexpression or knockdown vectors (n = 8). Values are expressed as mean ± s.d. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$.

3.2. LINC01093 inhibits HCC cell proliferation and metastasis in vitro and in vivo

Compared to normal liver tissues, LINC01093 expression was significantly downregulated in HCC cell lines (Fig. S3A). To investigate the biological function of LINC01093 in HCC progression, we established stable models of LINC01093 overexpression or knockdown in Huh7 and BEL-7402 cell lines (Fig. S3B). Cell counting kit-8 analyses showed that overexpression of LINC01093 significantly inhibited the proliferation of HCC cells *in vitro*, whereas knockdown of LINC01093 with short-hairpin RNA significantly promoted proliferation (Fig. 2A and B). Consistent with this finding, LINC01093 overexpression decreased HCC cell colony formation. Conversely, silencing LINC01093 strikingly increased HCC cell colony formation (Fig. 2C). To investigate the role of LINC01093 in tumorigenesis *in vivo*, Huh7 cells with stable overexpression or knockdown were subcutaneously injected into nude mice. We observed that the volume and weight of the tumors were dramatically higher in the LINC01093-shRNA group than in controls, whereas overexpression of LINC01093 inhibited tumor proliferation. These results indicated that LINC01093 remarkably inhibited the tumorigenic ability of HCC cells *in vivo* (Fig. 2D).

The inverse correlation between LINC01093 expression and the existence of cancer embolus in HCC patients prompted us to explore the effect of LINC01093 in cell invasion and metastasis. Transwell assays demonstrated that LINC01093 overexpression remarkably repressed HCC cell migration and invasion. In contrast, LINC01093 knockdown significantly promoted cell migration and invasion (Fig. 2E, Fig. S4). We next explored whether LINC01093 inhibited tumor metastasis *in vivo*. Huh7 cells with LINC01093 overexpression or knockdown were intravenously injected into nude mice, and animals were sacrificed at 10 weeks after injection. Histological analysis confirmed that the numbers of lung metastatic nodules were notably decreased in the LINC01093 overexpression group compared with the control group; in contrast, LINC01093 knockdown strikingly increased the number of lung metastatic nodules compared to the shControl group (Fig. 2F). In short, these findings indicate that LINC01093 can suppress HCC cell invasion and metastasis *in vitro* and *in vivo*.

3.3. LINC01093 physically interacts with IGF2BP1 in HCC cells

To explore interactors with LINC01093, we conducted RNA pulldown assays in whole cell lysates from Huh7 and BEL-7402 cells with biotin-labeled sense and antisense LINC01093 RNA probes. After silver staining, a distinct band approaching 70 kDa was captured by sense LINC01093 and subjected to mass spectrometry analysis (Fig. 3A, left panel). The results identified many proteins in this complex, and the overlap between two groups for the top 10 most abundant protein was with IGF2BP1 (Table SIII). Western blot with anti-IGF2BP1 antibody further confirmed the existence of IGF2BP1 within LINC01093 pulldown samples (Fig. 3A, right panel). In addition, RIP assays showed enrichment of LINC01093 in complex precipitated with IGF2BP1 compared with control IgG (Fig. 3B). These results indicated that the IGF2BP1 protein specifically binds LINC01093.

We then mapped the specific region of IGF2BP1 that is essential for these interactions, using RNA pulldown assays with truncated IGF2BP1 labeled with an HA-tag. These deletion-mapping analyses identified the KH3-KH4 region of IGF2BP1 as responsible for the interaction with LINC01093 (Fig. 3C). In addition, RIP assays with anti-HA antibody showed that this domain was required for the interaction (Fig. 3D). The GxxG loop in the KH domain has been described as crucial for IGF2BP1 to bind RNA, so we constructed a double mutation in the GxxG-GDDG (GKKG-GDDG; GKGG-GDDG) KH3-KH4 region [28]. We silenced endogenous IGF2BP1 in Huh7 cells and expressed wild-type IGF2BP1 (WT-IGF2BP1), mutant-IGF2BP1 (Mut-IGF2BP1), or control, respectively. Through RIP assays, we found Mut-IGF2BP1 in IGF2BP1-silencing cells lacked interaction with LINC01093 (Fig. S5A). Next, in functional assays, we found that suppression of proliferation and migration by LINC01093 was not reversed in IGF2BP1-silencing cells with Mut-IGF2BP1 (Figs. S5B–C).

A series of deletion-mapping analyses of LINC01093 revealed that the 1000–1529 nt fragment was responsible for the interaction of LINC01093 with IGF2BP1 protein (Fig. 3E, Fig. S6A). Furthermore, the LINC01093 fragment with IGF2BP1-binding ability exhibited significant inhibitory effects on HCC cell proliferation and migration, whereas other fragments did not (Fig. 3F, Fig. S6B). Further deletion-mapping analyses of LINC01093-4 (1000–1529 nt) revealed that the fragment 1000-1260 nt was the exact region responsible for the interaction. Based on these results, we constructed mutant LINC01093 (Mut-LINC01093) with deletion of this fragment and found that it could not bind IGF2BP1 (Fig. 3E, Fig. S6A). Restoration of Mut-LINC01093 did not abolish the promotional effects of LINC01093 silencing on cell growth and mobility (Figs. S6C–D). These results indicate that LINC01093 may regulate cell growth and metastasis by interacting with IGF2BP1.

Because lncRNAs could act by regulating expression or cellular localization of binding protein, we performed qPCR and immunoblotting assays for IGF2BP1. The results showed that LINC01093 did not influence IGF2BP1 mRNA level (Fig. S7A, left panel). Moreover, LINC01093 did not affect level of IGF2BP1 total protein level in the cell (Fig. S7A, right panel), or separately in the nucleus or cytoplasm (Fig. S7B).

3.4. LINC01093 facilitates GLI1 mRNA decay via interacting with IGF2BP1 in HCC cells

IGF2BP1 plays a vital role in mRNA regulation, including inhibition/promotion of mRNA decay or inhibition of miR-dependent mRNA decay [15]. To investigate whether LINC01093 participates in regulating mRNA level via IGF2BP1, we examined several selected IGF2BP1 targets by qPCR [15]. With overexpression of LINC01093 in Huh7 and BEL-7402 cells, we found downregulation of GLI1 mRNA and protein levels. Conversely, knockdown of LINC01093 significantly increased GLI1 RNA and protein levels. However, LINC01093 did not modulate levels of BTRC, CD44, MYC, LEF1, MAPK4, MDR1, KRAS, PTEN, β-catenin, and β-actin (Fig. 4A, Figs. S8A–B). It has been reported that IGF2BP1 can bind and stabilize GLI1 mRNA in colorectal cancer cells [16]. We used RIP and RNA pulldown assays to determine the interaction between IGF2BP1 and GLI1 mRNA in HCC cells. The results demonstrated that IGF2BP1 could specifically bind to GLI1 mRNA in HCC cells (Figs. S9A–B). In addition, knockdown of IGF2BP1 reduced GLI1 mRNA and protein levels in HCC cells (Figs. S9C–D).

To explore the specific region of IGF2BP1 required for interaction with GLI1 mRNA, we first conducted deletion-mapping assays with truncated IGF2BP1. GLI1 mRNA primarily bound the KH3-KH4 domain of IGF2BP1, where the LINC01093-binding site is located (Figs. S9E–F). We thus hypothesized that LINC01093 might be implicated in GLI1 mRNA regulation through competitive binding to IGF2BP1. To test this hypothesis, we performed RIP analysis in HCC cells with LINC01093 knockdown or overexpression. Knockdown of LINC01093 led to an increased interaction between IGF2BP1 and GLI1 mRNA. WT-LINC01093, but not Mut-LINC01093, prevented interaction of IGF2BP1 with GLI1 mRNA (Fig. 4B). Subsequent RNA pulldown assays showed that LINC01093 overexpression impaired interaction between IGF2BP1 with GLI1 mRNA and that LINC01093 knockdown strengthened the assembly of GLI1 mRNA with IGF2BP1 (Fig. 4C). However, these results clearly demonstrate that LINC01093 mediates the association between IGF2BP1 and GLI1 mRNA, in a lncRNA–protein–mRNA pattern.

To further verify whether LINC01093 negatively regulates the level of GLI1 via IGF2BP1, we conducted transient interference and overexpression of IGF2BP1 conducted in Huh7 and BEL-7402 cells. Knockdown of IGF2BP1 significantly attenuated induction of GLI1 by LINC01093 depletion. We also observed increased expression of GLI1 in LINC01093-overexpressing cells with IGF2BP1 (Fig. 4D). These results

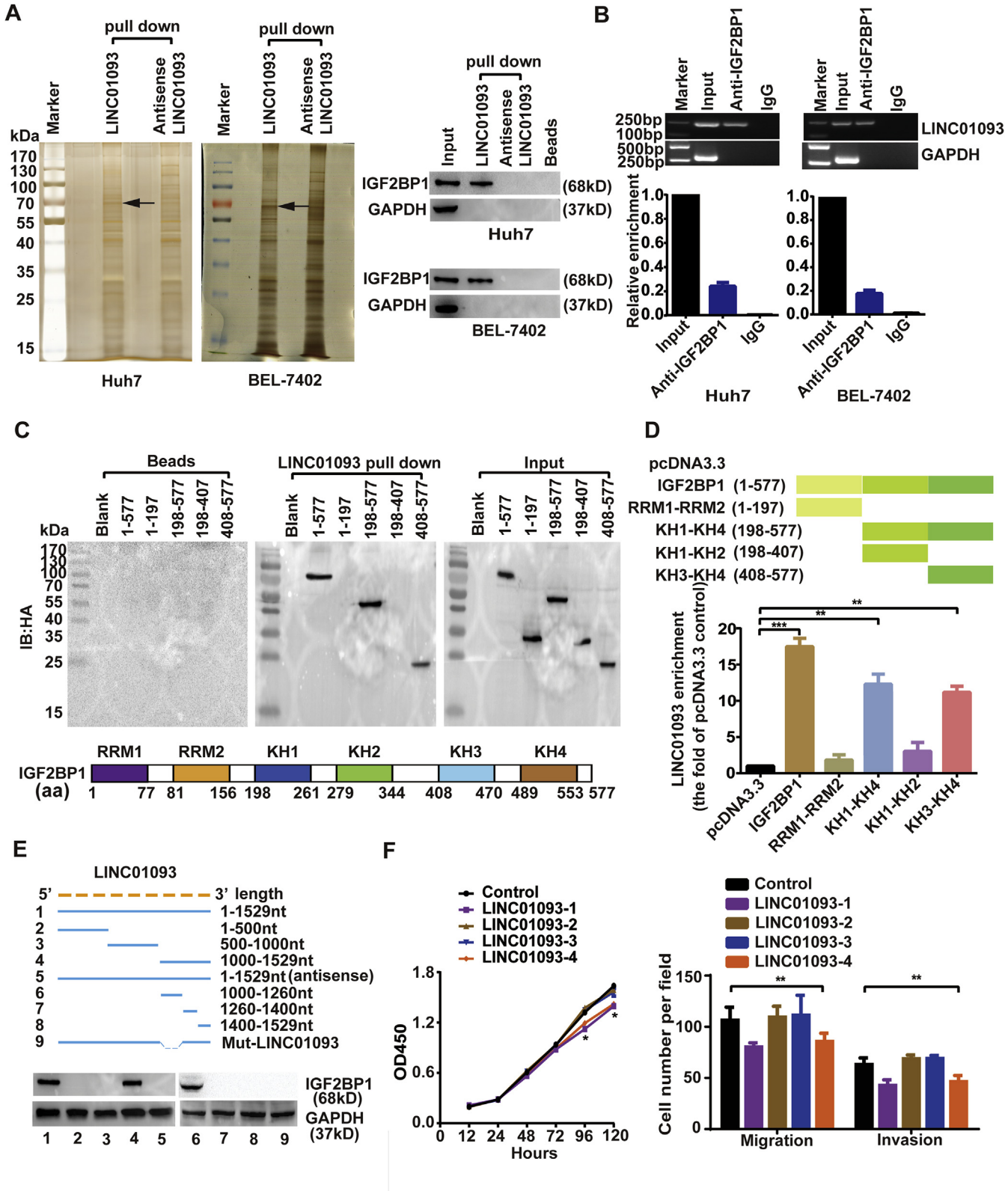

Fig. 3. LINC01093 physically interacts with IGF2BP1 in HCC cells. (A) Left: identification of the LINC01093-protein complex by incubation of biotinylated LINC01093 with protein extracts. Right: immunoblot analysis showed the specific association of IGF2BP1 with LINC01093. (B) RIP assays showed association of IGF2BP1 with LINC01093. (C) Immunoblot analysis of HA-tagged IGF2BP1 (full-length or domain truncation fragments) retrieved by LINC01093. RRM, RNA-recognition motif; KH, hnRNP-K homology. (D) Deletion mapping of the LINC01093-binding domain in IGF2BP1. Top, diagrams of full-length IGF2BP1 and the deletion fragments. Bottom, qPCR detection of LINC01093 retrieved by indicated vectors using a HA antibody. (E) Deletion mapping of the IGF2BP1-binding domain in LINC01093. Top, diagrams of full-length LINC01093 and the deletion fragments. Bottom, immunoblot analysis for IGF2BP1 in protein samples pulled down by the different LINC01093 constructs. (F) Cell proliferation, migration and invasion assays for Huh7 cells infected with the lentivirus expressing full-length LINC01093, deletion fragments, or the control. Values are expressed as mean ± s.d. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

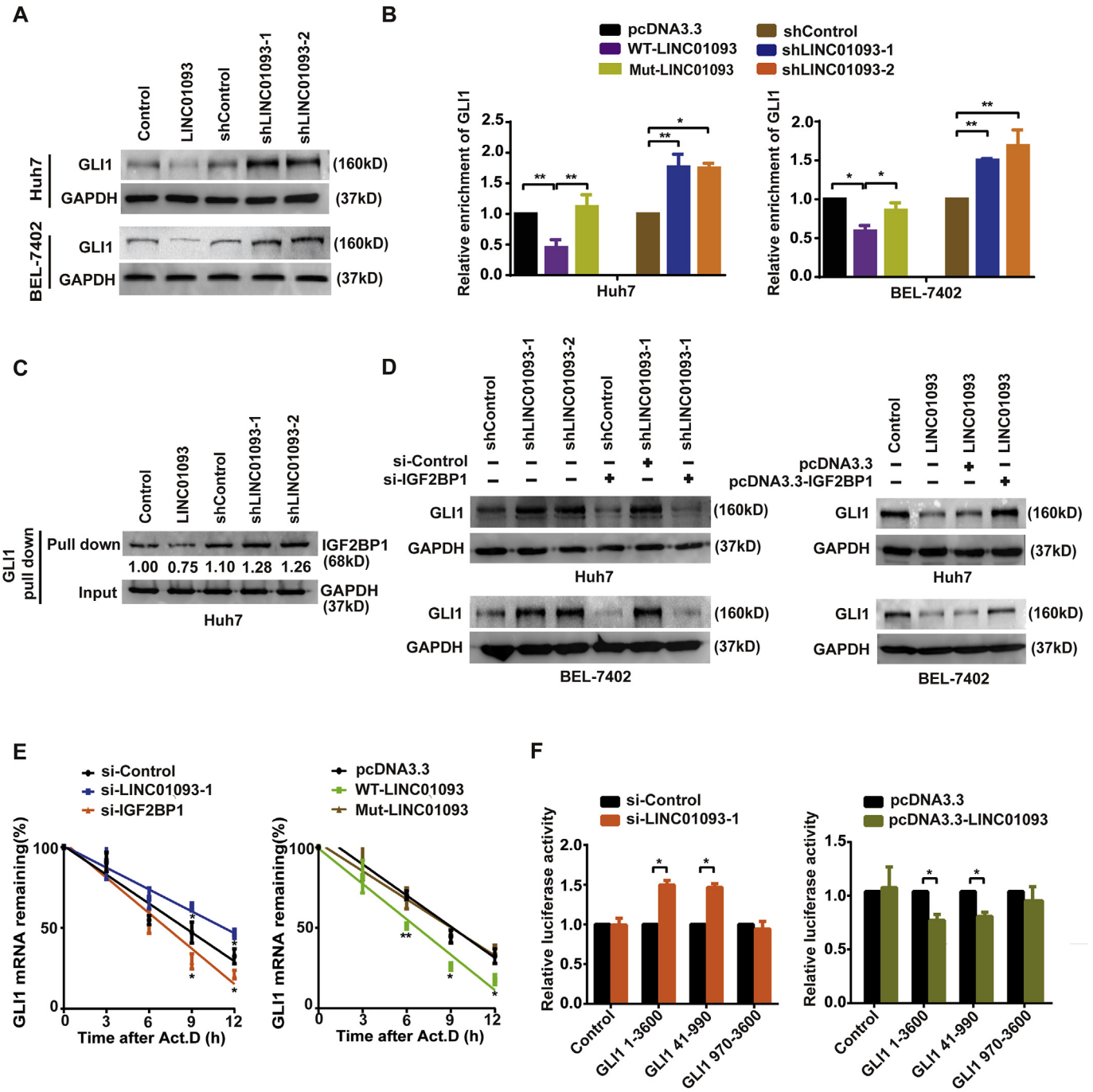

Fig. 4. LINC01093 facilitates GLI1 mRNA decay via interacting with IGF2BP1 in HCC cells. (A) LINC01093 regulated GLI1 protein level. (B) RIP assays for the interaction between IGF2BP1 and GLI1 mRNA in HCC cells with LINC01093 overexpression or knockdown. (C) Immunoblot analysis showed the specific association of IGF2BP1 with GLI1 mRNA in Huh7 cells with LINC01093 overexpression or knockdown. (D) Immunoblot analysis of GLI1. (E) mRNA decay of GLI1 was affected by interaction of LINC01093 with IGF2BP1. The remaining transcript of GLI1 was measured in Huh7 cells after treatment of actinomycin D (Act.D). (F) Luciferase assays of the reporter containing the full length or truncated fragments of GLI1 for Huh7 cells with LINC01093 knockdown or overexpression. Relative luciferase activity is the ratio of firefly luciferase to Renilla luciferase. Data are represented as mean ± s.d. $*p < 0.05$, $**p < 0.01$.

suggest that LINC01093 antagonizes IGF2BP1 binding to the GLI1 mRNA.

We next examined whether LINC01093 regulated the stability of GLI1 mRNA. To block new RNA synthesis, we treated different cell lines with actinomycin D and then measured GLI1 mRNA levels every 3 h IGF2BP1 knockdown accelerated GLI1 mRNA degradation on actinomycin D treatment, whereas LINC01093 downregulation yielded a pattern of greater GLI1 mRNA stability (Fig. 4E, left panel). However, neither LINC01093 nor IGF2BP1 regulated degradation of GAPDH (Fig. S10A). Wild-type LINC01093 significantly decreased the half-life of GLI1 mRNA, but the mutant LINC01093 did not (Fig. 4E, right panel; Fig. S10B). Altogether, these results demonstrated that LINC01093 helps regulate GLI1 mRNA decay.

To specifically address the effect of LINC01093 in the GLI1 mRNA stability, we conducted luciferase reporter assays. IGF2BP1 can directly bind GLI1 mRNA, with the strongest interaction within the 41–990 bases of the coding region [16]. Therefore, we cloned full-length and truncated fragments of GLI1 into the region downstream of the firefly

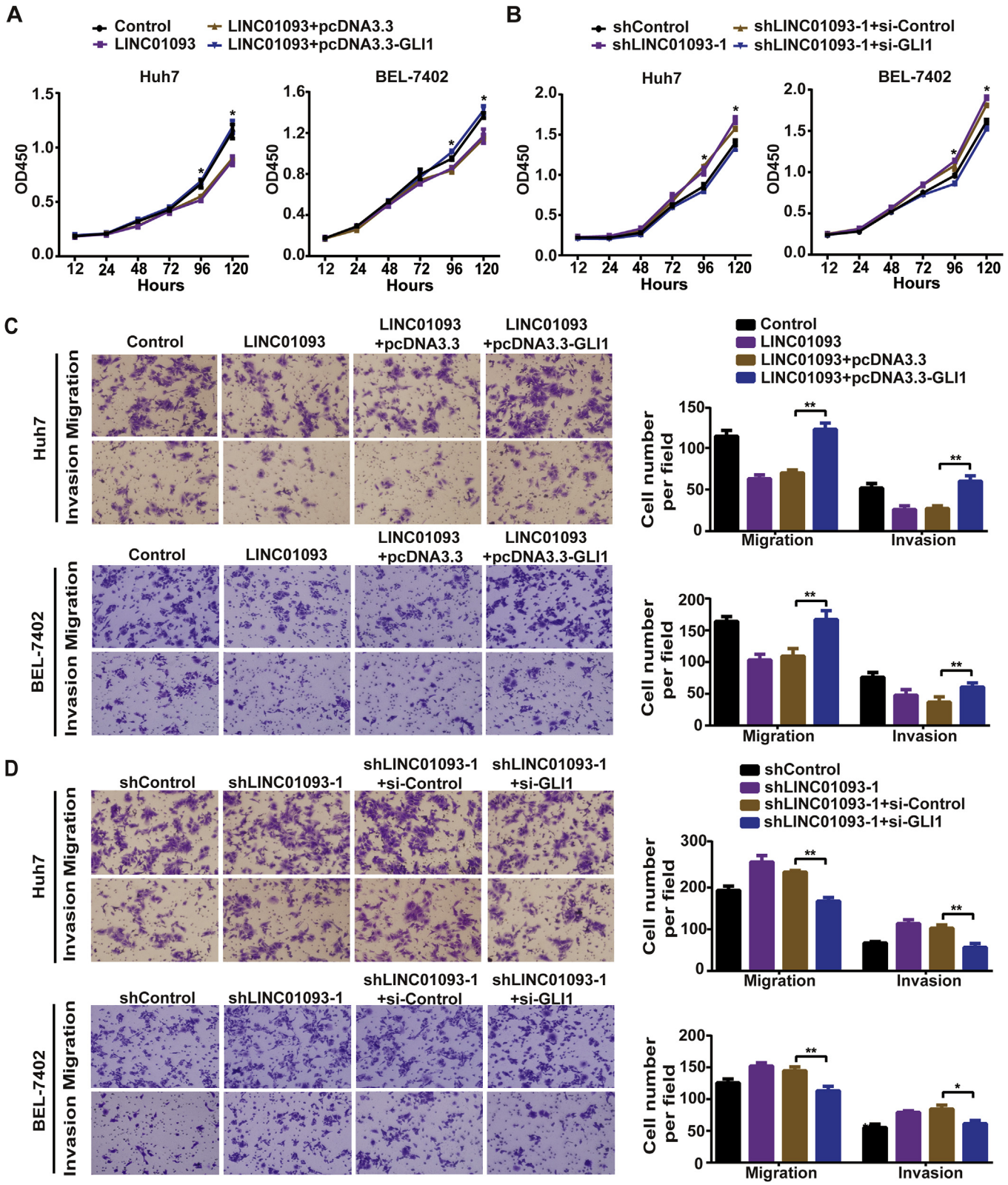

Fig. 5. GLI1 is required for effect of LINC01093 on regulation of HCC cell proliferation and metastasis. (A) Proliferation profiles of LINC01093 overexpression cells transiently transfected with pcDNA3.3-GLI1 or the control. *$p < 0.05$, LINC01093 overexpression cells compared with the cells overexpressing LINC01093 and transfected with GLI1. (B) Proliferation profiles of LINC01093 interference cells transiently transfected with si-GLI1 or the control. *$p < 0.05$, LINC01093 interference cells compared with the cells expressing LINC01093 shRNA and transfected with GLI1 siRNA. (C) Representative images and cell counts of migration and invasion assays for LINC01093 overexpression cells transiently transfected with pcDNA3.3-GLI1 or the control. (D) Representative images and cell counts of migration and invasion assays for LINC01093 interference cells transiently transfected with si-GLI1 or the control. Values are expressed as mean ± s.d.*$p < 0.05$, **$p < 0.01$.

luciferase gene of the pGL3-Control vector. Each vector was respectively transfected into Huh7 cells along with LINC01093 transient overexpression or knockdown vector. The results showed that LINC01093 knockdown led to an augmentation of luciferase activity in both full-length GLI1 mRNA and the first 990 bases (41–990) of GLI1 compared to empty vector as control. The activity of the reporter was reduced, however, after LINC01093 overexpression (Fig. 4F). These data suggest that LINC01093 plays an important role in GLI1 mRNA stability.

3.5. GLI1 is required for LINC01093 regulation of HCC cell proliferation and metastasis

We further investigated whether GLI1 is required for LINC01093 to act in tumor suppression. We transfected HCC cells with si-GLI1 or pcDNA3.3-GLI1, respectively. Cell proliferation assays showed that increased expression of GLI1 specifically abrogated inhibition of HCC cell growth arising from LINC01093 overexpression (Fig. 5A). In contrast, decreased GLI1 could readily rescue the effects of LINC01093 silencing (Fig. 5B). Moreover, the restoration of GLI1 remarkably abolished the inhibitory effect of LINC01093 on cell migration and invasion (Fig. 5C), and GLI1 siRNA erased the promoting effect of LINC01093 knockdown (Fig. 5D). These results demonstrate that GLI1 is required for LINC01093 regulation of HCC cell proliferation and metastasis.

3.6. LINC01093 inhibits expression of GLI1 downstream genes via binding with IGF2BP1

Several molecules downstream of GLI1 signaling (Bcl-xl, CD24, CXCR4, EGFR, FOXM1, MMP9, Snai1, TGFβ1, and VEGF) are reported to be involved in tumor growth or metastasis [29–35]. In agreement with the changes in GLI1 by LINC01093, expression levels of these GLI1 downstream molecules altered notably in the respective cells. Silencing of LINC01093 increased transcript and protein levels of GLI1 downstream genes, but LINC01093 overexpression suppressed GLI1 downstream signaling activation (Fig. 6A and B, Figs. S11A–B). Additionally, we performed rescue experiments to detect the effects of IGF2BP1 on LINC01093-induced genes. With suppressed IGF2BP1 in HCC cells via LINC01093 knockdown, we found that inhibition of IGF2BP1 could rescue the increased GLI1 targeting molecules (Fig. 6A). We also transiently upregulated IGF2BP1 in HCC cells with LINC01093 overexpression and observed that ectopic expression of IGF2BP1 could abrogate the decrease in GLI1 downstream molecules (Fig. 6B). Taken together, these results suggest that LINC01093 is involved in HCC progression by acting as an endogenous competitor of GLI1 mRNA through IGF2BP1.

To investigate the clinical correlations between LINC01093 and GLI1 as well as its downstream genes, we conducted qPCR in 70 HCC tissues. LINC01093 was negatively correlated with GLI1, CD24, CXCR4, FOXM1, Snai1, and TGFβ1 in 70 HCC (Fig. 6C). However, LINC01093 and Bcl-xl, EGFR, MMP9, and VEGF showed no association (data not shown). Furthermore, we detected the transcriptional level of LINC01093 and the protein level of GLI1 in the same group of HCC tissues. The level of LINC01093 transcript also negatively correlated with level of GLI1 protein in 26 HCC tissues (Fig. 6D). Altogether, these results demonstrated that LINC01093 was negatively correlated with GLI1 in HCC samples.

4. Discussion

LncRNAs are emerging as crucial regulators in the pathogenesis of human cancers and can be used as novel biomarkers for diagnosis, prognosis, and prediction of response to therapy [36]. Here, we identified LINC01093 as a novel liver-specific lncRNA that is downregulated in HCC tissues. Furthermore, patients with lower LINC01093 levels appeared to have more cancer embolus risk, higher TNM stage, and shorter OS compared to those with higher levels. The GEO database also showed that LINC01093 is downregulated in chronic inflammation, as occurs with alcohol abuse or viral infections, both critical factors triggering liver carcinogenesis. All of this evidence points to a suppressive function of LINC01093 in HCC progression.

Our findings indicated that overexpression of LINC01093 results in inhibition of cell growth and metastasis of HCC, with opposite effects from LINC01093 knockdown. The *in vivo* results also indicated that LINC01093 might be an endogenous tumor suppressor of HCC. Of note, we identified LINC01093 as a liver-enriched lncRNA according to our results in 11 human tissues and RNA-Seq expression data in 53 human normal tissues. LncRNAs are more tissue- and cell-type-–specific in comparison with protein-coding genes [37] and appear to be more promising prognostic indicators. In clinical trials, for example, PCA3, a prostate-specific lncRNA highly overexpressed in prostate cancer, has proved a superior biomarker compared to prostate-specific antigen in terms of specificity and predictive value [38]. Among liver-specific lncRNAs, recently, a novel liver-enriched lnc18q22.2 was identified as being involved in cell viability in the liver of nonalcoholic steatohepatitis patients [39]. LncLSTR is a liver-specific lncRNA that regulates systemic lipid metabolism in mice [40]. A fetal liver-specific lncRNA PVT1 promotes proliferation and stem cell–like properties of HCC by stabilizing NOP2 [41]. However, the molecular mechanisms underlying the action of HCC-specific lncRNAs in the development and progression of HCC are still poorly understood.

LncRNAs often interact with specific proteins to exert their effects, especially in specific subcellular locations. We found that LINC01093 was mainly located in cytoplasm in HCC cells, suggesting a role in posttranscriptional gene regulation. To detect if LINC01093 in the nucleus has a potential function in mediating gene expression by influencing the epigenetic status of target genes, we performed RNA pulldown assays. Our preliminary results showed that LINC01093 did not bind with EZH2 (the catalytic subunit of polycomb repressive complex 2) in HCC cell lines (Fig. S12). Further study is needed to explore whether LINC01903 acts as a scaffold for chromatin remodeling complexes.

Through RNA pulldown and RIP assays, IGF2BP1, a predominantly cytoplasmic RNA-binding protein, was found to interact with LINC01093. IGF2BP1 carries two RNA-recognition motifs in the *N*-terminal part and four KH domains in the *C*-terminal region [15]. The RNA-recognition motif domain could stabilize the protein–RNA complex, and RNA-binding is mainly facilitated by the KH domain [15]. Recent reports have indicated that the KH3 and KH4 domains are structurally linked to act as a di-domain unit, with the two RNA-binding grooves on opposite sides. The target RNA molecule can loop around the protein to bind to both domains [42]. In our study, we found that the KH3-KH4 region of IGF2BP1 is responsible for the interaction with LINC01093. Moreover, according to the fragment truncated assays, LINC01093 did not interact with a separate domain of KH3 or KH4 (data not shown). As Chao et al. reported, the target sequences of KH3 and KH4 are not continuous. They are separated by a spacer, and the length of this spacer (between 10 and 23 nucleotides) is important for interaction with the pseudo-domain [43,44]. The conserved UGGAC motif is reported as the putative binding element for the IGF2BP1–RNA association [45]. In this study, we observed two repeats of this motif in the 1000–1260 bp of LINC01093, which may represent a portion of the integrating site for IGF2BP1 to form homo- or heterodimers.

IGF2BP1 is a conserved oncofetal protein that is overexpressed in several human cancers [15]. To date, it has been described as having an oncogenic role because of its influence on transcript targets and the fate of classical oncogenes including GLI1, CD44, MYC, MAPK4, MDR1, KRAS, and β-catenin [15]. Research on multiple mRNA targets has verified that IGF2BP1 physically interacts with the corresponding mRNAs, resulting in their stabilization, increased expression, and ultimately the presentation of the cancerous phenotypes [15,16]. In our work, we found that GLI1 is a target of IGF2BP1 in HCC cells [16]. GLI1

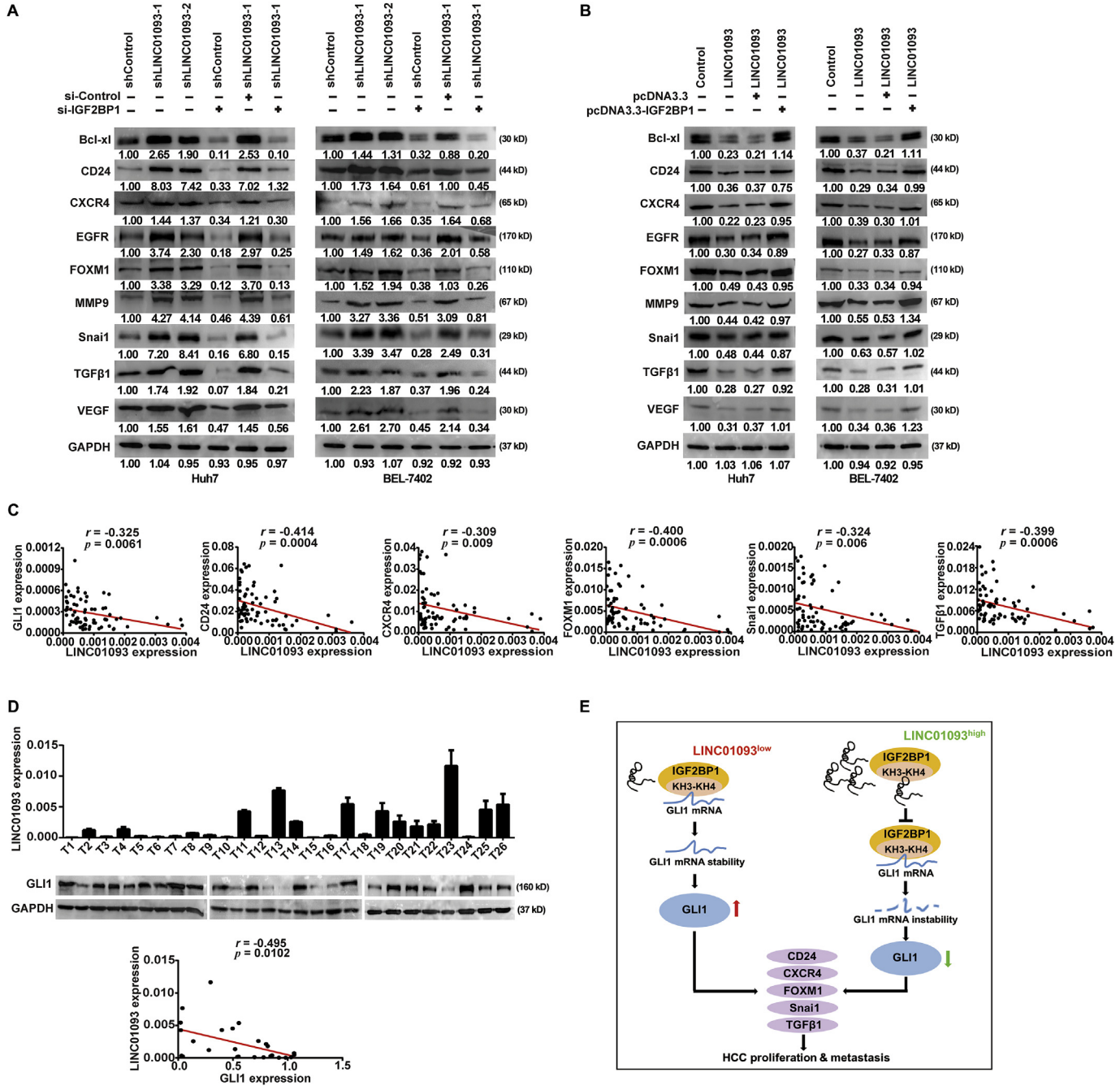

Fig. 6. LINC01093 inhibits gene expression of GLI1 downstream by binding with IGF2BP1. (A) Immunoblot analysis of the GLI1 downstream genes related to cancer proliferation and metastasis (Bcl-xl, CD24, CXCR4, EGFR, FOXM1, MMP9, Snai1, TGFβ1, and VEGF) in HCC cells expressing LINC01093 shRNAs or control cells with or without transient transfection with IGF2BP1 siRNA. (B) Immunoblot analysis of the GLI1 downstream genes in HCC cells expressing LINC01093 or control cells with or without transient transfection with pcDNA3.3-IGF2BP1. (C) Negative correlations between LINC01093 levels and the mRNA levels of GLI1, CD24, CXCR4, FOXM1, Snai1, and TGFβ1 in HCC samples (n = 70). (D) Protein level of GLI1 was negatively related to LINC01093 RNA levels in HCC samples (n = 26). (E) Proposed model for the function of LINC01093 in regulating stability of GLI1 mRNA. LINC01093 binds to IGF2BP1, inhibits IGF2BP1 occupancy to GLI1 mRNA, then leading to the instability of GLI1 mRNA and suppression of the GLI1-related oncogenic pathway, and exerts its tumor suppressive role in HCC.

mRNA shares the same region that IGF2BP1 binds to with LINC01093. LINC01093 depletion influences GLI1 mRNA stability and the effects of binding to IGF2BP1. As shown above, we found that LINC01093 competitively binds to IGF2BP1, preventing GLI1 binding to IGF2BP1 and promoting its degradation.

The mechanism underlying the effects of LINC01093 on the interaction between IGF2BP1 and its RNA targets is direct binding to and sequestering of IGF2BP1 from GLI1 mRNA. However, other indirect mechanisms are possible. IGF2BP1 could interact with several RNA-binding proteins [46,47]. The altered level of these RNA-binding proteins or interplay with IGF2BP1 upon LINC01093 depletion could influence how IGF2BP1 interacts with its targets. A recent report showed that lncRNA-AB074169 destabilized *KHSRP* mRNA which might be regulated by STAU1-mediated mRNA decay pathway [48], so LINC01093 may act as a protein scaffold to recruit not only IGF2BP1 but also other molecules to regulate GLI1 mRNA. Whether LINC01093 can bind with other RNA binding proteins to regulate stability of GLI1 mRNA needs further investigation.

GLI1, as a transcriptional activator, is crucial to the development and progression of multiple malignancies. GLI1 downstream genes have been identified in cell growth and invasion, which include EGFR, FOXM1, VEGF, and PDGFR for proliferation [49–52]; Bcl-xl, Bcl-2 for anti-apoptosis [29,53]; and CD24, CXCR4, MMP9, Snail1, TGFβ1, and Vimentin for invasion [54–57]. These molecules may be potential targets for LINC01093 in exerting its tumor-suppressive function in HCC cells. In this study, knockdown of LINC01093 increased GLI1 levels and its downstream target genes. The result was elevated EGFR, FOXM1, VEGF, and Bcl-xl, which allow tumor cells to proliferate. In addition, increased expression of CD24, CXCR4, MMP9, Snai1, and TGFβ1 led to a high-metastasis characteristic of HCC cells. However, several molecules like Vimentin, PDGFR, and Bcl-2 were not significantly changed (data not shown).

We observed negative correlations between LINC01093 and mRNA levels of GLI1, CD24, CXCR4, FOXM1, Snai1, and TGFβ1 in 70 HCC samples. These results confirmed the inhibitory effect of LINC01093 on GLI1 downstream signaling, showing that LINC01093 plays a pivotal role in suppressing neoplasm proliferation and metastasis. Future studies should involve even larger sample sizes. In addition, the tissue specimens in our study were acquired from Chinese patients, whose HCC was mainly caused by hepatitis B infection; thus, LINC01093 in HCC of other etiologies needs investigation.

In summary, we show that LINC01093, as a liver-specific lncRNA, is implicated in HCC growth and metastasis. We propose that LINC01093 binds to IGF2BP1 and disrupts the interplay of IGF2BP1 with GLI1 mRNA, leading to degradation of GLI1 mRNA (Fig. 6E). Our results indicate that LINC01093 may act as a regulator by dissociating the protein–RNA interaction, affecting GLI1 mRNA stability at the post-transcriptional level.

Conflicts of interest

The other authors declare no potential conflicts of interest.

Acknowledgements

This work was supported by the National Key Basic Research Program of China (grant number 2015CB553905), the National Key Research and Development Program (grant numbers 2016YFC0902400 and 2016YFF0101405), the State Key Program of National Natural Science of China (grant number 81530077), the National Natural Science Foundation of China (grant numbers 81201626, 81421001, 81572311, 81772461, 81702838, 81672933, 81472676, 81772551, 81772578, 81672839), the Strategic Priority Research Program of the Chinese Academy of Sciences (grant numbers XDA12020105, XDA12020103), the Shanghai Natural Science Foundation of China (grant number 16ZR1434700), the Shanghai Municipal Commission of Health and Family Planning (grant numbers 201640055, 201640007), the State Key Laboratory of Oncogenes and Related Gene (grant number 91-17-06), and the Doctoral Innovation Fund of Shanghai Jiao Tong University School of Medicine (grant number BXJ201717).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.canlet.2019.02.033.

References

[1] T. Akinyemiju, S. Abera, M. Ahmed, N. Alam, M.A. Alemayohu, C. Allen, et al., The burden of primary liver cancer and underlying etiologies from 1990 to 2015 at the global, regional, and national level results from the global burden of disease study 2015, JAMA Oncol 3 (2017) 1683–1691.
[2] L.A. Torre, F. Bray, R.L. Siegel, J. Ferlay, J. Lortet-Tieulent, A. Jemal, Global cancer statistics, 2012, Ca-Cancer. J. Clin. 65 (2015) 87–108.
[3] J.L. Rinn, H.Y. Chang, Genome regulation by long noncoding RNAs, Annu. Rev. Biochem. 81 (2012) 145–166.
[4] M. Cesana, D. Cacchiarelli, I. Legnini, T. Santini, O. Sthandier, M. Chinappi, et al., A long noncoding RNA controls muscle differentiation by functioning as a competing endogenous RNA, Cell 147 (2011) 358–369.
[5] J.R. Prensner, A.M. Chinnaiyan, The emergence of lncRNAs in cancer biology, Cancer Discov. 1 (2011) 391–407.
[6] M. Huarte, The emerging role of lncRNAs in cancer, Nat. Med. 21 (2015) 1253–1261.
[7] A.M. Schmitt, H.Y. Chang, Long noncoding RNAs in cancer pathways, Cancer Cell 29 (2016) 452–463.
[8] C. Cao, J. Sun, D. Zhang, X. Guo, L. Xie, X. Li, et al., The long intergenic noncoding RNA UFC1, a target of microRNA 34a, interacts with the mRNA stabilizing protein HuR to increase levels of beta-catenin in HCC cells, Gastroenterology 148 (2015) 415–426.
[9] Y. Wang, L. He, Y. Du, P. Zhu, G. Huang, J. Luo, et al., The long noncoding RNA lncTCF7 promotes self-renewal of human liver cancer stem cells through activation of Wnt signaling, Cell Stem Cell 16 (2015) 413–425.
[10] J.H. Yuan, F. Yang, F. Wang, J.Z. Ma, Y.J. Guo, Q.-f. Tao, et al., A long noncoding RNA activated by TGF-beta promotes the invasion-metastasis cascade in hepatocellular carcinoma, Cancer Cell 25 (2014) 666–681.
[11] H. Wang, L. Liang, Q. Dong, L. Huan, J. He, B. Li, et al., Long noncoding RNA miR503HG, a prognostic indicator, inhibits tumor metastasis by regulating the HNRNPA2B1/NF-kappaB pathway in hepatocellular carcinoma, Theranostics 8 (2018) 2814–2829.
[12] H. Wang, X. Huo, X.-R. Yang, J. He, L. Cheng, N. Wang, et al., STAT3-mediated upregulation of lncRNA HOXD-AS1 as a ceRNA facilitates liver cancer metastasis by regulating SOX4, Mol. Canc. 16 (2017) 136.
[13] S.X. Yuan, J. Wang, F. Yang, Q.F. Tao, J. Zhang, L.L. Wang, et al., Long noncoding RNA DANCR increases stemness features of hepatocellular carcinoma by derepression of CTNNB1, Hepatology 63 (2016) 499–511.
[14] H. Xiong, Z. Ni, J. He, S. Jiang, X. Li, J. He, et al., LncRNA HULC triggers autophagy via stabilizing Sirt1 and attenuates the chemosensitivity of HCC cells, Oncogene 36 (2017) 3528–3540.
[15] J.L. Bell, K. Waechter, B. Muehleck, N. Pazaitis, M. Koehn, M. Lederer, et al., Insulin-like growth factor 2 mRNA-binding proteins (IGF2BPs): post-transcriptional drivers of cancer progression, Cell. Mol. Life Sci. 70 (2013) 2657–2675.
[16] F.K. Noubissi, S. Goswami, N.A. Sanek, K. Kawakami, T. Minamoto, A. Moser, et al., Wnt signaling stimulates transcriptional outcome of the hedgehog pathway by stabilizing GLI1 mRNA, Cancer Res. 69 (2009) 8572–8578.
[17] L. Che, Y.-H. Yuan, J. Jia, J. Ren, Activation of sonic hedgehog signaling pathway is an independent potential prognosis predictor in human hepatocellular carcinoma patients, Chin. J. Canc. Res. 24 (2012) 323–331.
[18] Z. Hong, A. Bi, D. Chen, L. Gao, Z. Yin, L. Luo, Activation of Hedgehog signaling pathway in human non-small cell lung cancers, Pathol. Oncol. Res. 20 (2014) 917–922.
[19] S. Mukherjee, N. Frolova, A. Sadlonova, Z. Novak, A. Steg, G.P. Page, et al., Hedgehog signaling and response to cyclopamine differ in epithelial and stromal cells in benign breast and breast cancer, Cancer Biol. Ther. 5 (2006) 674–683.
[20] A. Yoshizaki, T. Nakayama, S. Naito, C.-Y. Wen, I. Sekine, Expressions of sonic hedgehog, patched, smoothened and Gli-1 in human intestinal stromal tumors and their correlation with prognosis, World J. Gastroenterol. 12 (2006) 5687–5691.
[21] P. Infante, R. Alfonsi, B. Botta, M. Mori, L. Di Marcotullio, Targeting GLI factors to inhibit the Hedgehog pathway, Trends Pharmacol. Sci. 36 (2015) 547–558.
[22] A. Po, E. Ferretti, E. Miele, E. De Smaele, A. Paganelli, G. Canettieri, et al., Hedgehog controls neural stem cells through p53-independent regulation of Nanog, EMBO J. 29 (2010) 2646–2658.
[23] B. Stecca, A. Ruiz i Altaba, Context-dependent regulation of the GLI code in cancer by HEDGEHOG and con-HEDGEHOG Signals, J. Mol. Cell Biol. 2 (2010) 84–95.
[24] K. Palle, C. Mani, K. Tripathi, M. Athar, Aberrant GLI1 activation in DNA damage response, carcinogenesis and chemoresistance, Cancers 7 (2015) 2330–2351.
[25] P. Wang, Y. Xue, Y. Han, L. Lin, C. Wu, S. Xu, et al., The STAT3-binding long noncoding RNA lnc-DC controls human dendritic cell differentiation, Science 344 (2014) 310–313.
[26] M.-C. Tsai, O. Manor, Y. Wan, N. Mosammaparast, J.K. Wang, F. Lan, et al., Long noncoding RNA as modular scaffold of histone modification complexes, Science 329 (2010) 689–693.
[27] H. Jin, C. Wang, G. Jin, H. Ruan, D. Gu, L. Wei, et al., Regulator of calcineurin 1 gene isoform 4, down-regulated in hepatocellular carcinoma, prevents proliferation, migration, and invasive activity of cancer cells and metastasis of orthotopic tumors by inhibiting nuclear translocation of NFAT1, Gastroenterology 153 (2017) 799–811.
[28] D. Hollingworth, A.M. Candel, G. Nicastro, S.R. Martin, P. Briata, R. Gherzi, et al., KH domains with impaired nucleic acid binding as a tool for functional analysis, Nucleic Acids Res. 40 (2012) 6873–6886.
[29] Y. Sun, W. Guo, T. Ren, W. Liang, W. Zhou, Q. Lu, et al., Gli1 inhibition suppressed cell growth and cell cycle progression and induced apoptosis as well as autophagy depending on ERK1/2 activity in human chondrosarcoma cells, Cell Death Dis. 5 (2014) e979.
[30] X. Cao, J. Geradts, M.W. Dewhirst, H.W. Lo, Upregulation of VEGF-A and CD24 gene expression by the tGLI1 transcription factor contributes to the aggressive behavior of breast cancer cells, Oncogene 31 (2012) 104–115.
[31] N. Bora-Singhal, D. Perumal, J. Nguyen, S. Chellappan, Gli1-mediated regulation of Sox2 facilitates self-renewal of stem-like cells and confers resistance to EGFR inhibitors in non-small cell lung cancer, Neoplasia 17 (2015) 538–551.
[32] Q. Liu, W. Sheng, M. Dong, X. Dong, Q. Dong, F. Li, Glil promotes transforming growth factor-betal- and epidermal growth factor-induced epithelial to

mesenchymal transition in pancreatic cancer cells, Surgery 158 (2015) 211–224.
[33] L. Yang, J. Huang, X. Ren, A.E. Gorska, A. Chytil, M. Aakre, et al., Abrogation of TGF beta signaling in mammary carcinomas recruits Gr-1 +CD11b+ myeloid cells that promote metastasis, Cancer Cell 13 (2008) 23–35.
[34] J.A. Burger, T.J. Kipps, CXCR4: a key receptor in the crosstalk between tumor cells and their microenvironment, Blood 107 (2006) 1761–1767.
[35] P. Raychaudhuri, H.J. Park, FoxM1: a master regulator of tumor metastasis, Cancer Res. 71 (2011) 4329–4333.
[36] R. Maruyama, H. Suzuki, Long noncoding RNA involvement in cancer, BMB Rep 45 (2012) 604–611.
[37] T. Gutschner, S. Diederichs, The hallmarks of cancer A long non-coding RNA point of view, RNA Biol. 9 (2012) 703–719.
[38] G.L. Lee, A. Dobi, S. Srivastava, Diagnostic performance of the PCA3 urine test, Nat. Rev. Urol. 8 (2011) 123–124.
[39] B. Atanasovska, S.S. Rensen, M.R. van der Sijde, G. Marsman, V. Kumar, I. Jonkers, et al., A liver-specific long noncoding RNA with a role in cell viability is elevated in human nonalcoholic steatohepatitis, Hepatology 66 (2017) 794–808.
[40] P. Li, X. Ruan, L. Yang, K. Kiesewetter, Y. Zhao, H. Luo, et al., A liver-enriched long non-coding RNA, lncLSTR, regulates systemic lipid metabolism in mice, Cell Metabol. 21 (2015) 455–467.
[41] F. Wang, J.H. Yuan, S.B. Wang, F. Yang, S.X. Yuan, C. Ye, et al., Oncofetal long noncoding RNA PVT1 promotes proliferation and stem cell-like property of hepatocellular carcinoma cells by stabilizing NOP2, Hepatology 60 (2014) 1278–1290.
[42] G. Nicastro, A.M. Candel, M. Uhl, A. Oregioni, D. Hollingworth, R. Backofen, et al., Mechanism of beta-actin mRNA recognition by ZBP1, Cell Rep. 18 (2017) 1187–1199.
[43] V.L. Patel, S. Mitra, R. Harris, A.R. Buxbaum, T. Lionnet, M. Brenowitz, et al., Spatial arrangement of an RNA zipcode identifies mRNAs under post-transcriptional control, Genes Dev. 26 (2012) 43–53.
[44] J.A. Chao, Y. Patskovsky, V. Patel, M. Levy, S.C. Almo, R.H. Singer, ZBP1 recognition of beta-actin zipcode induces RNA looping, Genes Dev. 24 (2010) 148–158.
[45] H. Huang, H. Weng, W. Sun, X. Qin, H. Shi, H. Wu, et al., Recognition of RNA N-6-methyladenosine by IGF2BP proteins enhances mRNA stability and translation, Nat. Cell Biol. 20 (2018) 285–295.
[46] M. Haemmerle, T. Gutschner, H. Uckelmann, S. Ozgur, E. Fiskin, M. Gross, et al., Posttranscriptional destabilization of the liver-specific long noncoding RNA HULC by the IGF2 mRNA-binding protein 1 (IGF2BP1), Hepatology 58 (2013) 1703–1712.
[47] L. Jonson, J. Vikesaa, A. Krogh, L.K. Nielsen, T.v. Hansen, R. Borup, et al., Molecular composition of IMP1 ribonucleoprotein granules, Mol. Cell. Proteomics 6 (2007) 798–811.
[48] Q. Gou, L. Gao, X. Nie, W. Pu, J. Zhu, Y. Wang, et al., Long noncoding RNA AB074169 inhibits cell proliferation via modulation of KHSRP-mediated CDKN1a expression in papillary thyroid carcinoma, Cancer Res. 78 (2018) 4163–4174.
[49] F. Goetschel, D. Berg, W. Gruber, C. Bender, M. Eberl, M. Friedel, et al., Synergism between hedgehog-GLI and EGFR signaling in hedgehog-responsive human medulloblastoma cells induces downregulation of canonical hedgehog-target genes and stabilized expression of GLI1, PLoS One 8 (2013) e65403.
[50] M.T. Teh, S.T. Wong, G.W. Neill, L.R. Ghali, M.P. Philpott, A.G. Quinn, FOXM1 is a downstream target of Gli1 in basal cell carcinomas, Cancer Res. 62 (2002) 4773–4780.
[51] M.H. Shahi, M. Afzal, S. Sinha, C.G. Eberhart, J.A. Rey, X. Fan, et al., Regulation of sonic hedgehog-GLI1 downstream target genes PTCH1, Cyclin D2, Plakoglobin, PAX6 and NKX2.2 and their epigenetic status in medulloblastoma and astrocytoma, BMC Canc. 10 (2010) 614.
[52] J.W. Xie, M. Aszterbaum, X.L. Zhang, J.M. Bonifas, C. Zachary, E. Epstein, et al., A role of PDGFR alpha in basal cell carcinoma proliferation, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 9255–9259.
[53] K. Wang, L. Pan, X. Che, D. Cui, C. Li, Gli1 inhibition induces cell-cycle arrest and enhanced apoptosis in brain glioma cell lines, J. Neuro Oncol. 98 (2010) 319–327.
[54] Q.S. Zhu, K. Rosenblatt, K.L. Huang, G. Lahat, R. Brobey, S. Bolshakov, et al., Vimentin is a novel AKT1 target mediating motility and invasion, Oncogene 30 (2011) 457–470.
[55] S. Inaguma, K. Kasai, H. Ikeda, GLI1 facilitates the migration and invasion of pancreatic cancer cells through MUC5AC-mediated attenuation of E-cadherin, Oncogene 30 (2011) 714–723.
[56] K. Wang, L. Pan, X. Che, D. Cui, C. Li, Sonic Hedgehog/GLI1 signaling pathway inhibition restricts cell migration and invasion in human gliomas, Neurol. Res. 32 (2010) 975–980.
[57] H.W. Lo, H. Zhu, X. Cao, A. Aldrich, F. Ali-Osman, A novel splice variant of GLI1 that promotes glioblastoma cell migration and invasion, Cancer Res. 69 (2009) 6790–6798.