

Original Article

Long non-coding RNA VCAN-AS1 promotes gastric cancer progression via the HuR/F11R pathway

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Abstract: Objectives: To investigate the role of the long non-coding RNA VCAN antisense RNA 1 (VCAN-AS1) in gastric cancer (GC) progression and elucidate its underlying molecular mechanisms, focusing on its interaction with ELAV-like RNA-binding protein 1 (ELAVL1, known as HuR) and its effect on F11 receptor (F11R) expression. Methods: VCAN-AS1 expression levels in GC tissues and cell lines were measured using real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR). In vitro and in vivo experiments, including cell counting kit-8 (CCK-8) assay, colony formation assay, Transwell migration assay, and a xenograft tumor model, were performed to evaluate VCAN-AS1's function in GC progression. RNA immunoprecipitation (RIP) and RNA pull-down assays were used to confirm the interaction between VCAN-AS1 and HuR. Results: VCAN-AS1 expression was significantly elevated in GC tissues and cell lines, with higher expression levels linked to poorer prognosis in GC patients. Functional assays demonstrated that VCAN-AS1 knockdown suppressed GC cell proliferation and migration. RIP and RNA pull-down experiments confirmed a specific interaction between VCAN-AS1 and HuR. Additionally, VCAN-AS1 regulated F11R expression in a HuR-dependent manner, and rescue experiments confirmed that F11R contributed to VCAN-AS1's oncogenic role in GC. Conclusions: These findings suggest that VCAN-AS1 facilitates GC progression through the HuR/F11R pathway, offering new insights into GC pathogenesis and identifying VCAN-AS1 as a potential therapeutic target for GC treatment.

Keywords: VCAN-AS1, gastric cancer, HuR, F11R

Introduction

Gastric cancer (GC) is one of the most prevalent and deadly cancers worldwide, ranking as the fifth most common cancer and the third leading cause of cancer-related deaths [1, 2]. Despite progress in diagnostic and therapeutic strategies, the prognosis for GC remains poor due to late-stage diagnosis, frequent metastasis, and high recurrence rates [3-5]. Understanding the molecular mechanisms underlying GC progression is crucial for developing more effective diagnostic and therapeutic approaches. Recent studies have highlighted the significant roles of non-coding RNAs in various biological processes and their potential as novel biomarkers and therapeutic targets in cancer [6-8].

Long non-coding RNAs (lncRNAs), which are non-coding RNAs longer than 200 nucleotides,

have emerged as key regulators of gene expression at transcriptional, post-transcriptional, and epigenetic levels [9-12]. They are involved in diverse physiological and pathological processes, including cell proliferation, differentiation, and apoptosis [13, 14]. In cancer, lncRNAs can act as oncogenes or tumor suppressors, influencing tumor growth, invasion, metastasis, and resistance to therapy [15-17]. Given their versatile roles, lncRNAs represent a promising research area for understanding cancer biology and developing new therapeutic strategies.

VCAN antisense RNA 1 (VCAN-AS1) is a lncRNA that has recently gained attention for its potential role in cancer progression. Previous studies have reported upregulation of VCAN-AS1 in various cancers, including breast cancer and GC, where it is associated with poor prognosis [18-21]. VCAN-AS1 is believed to regulate cancer progression through different mechanisms, su-

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ch as interacting with proteins or functioning as a competitive endogenous RNA (ceRNA) [18, 19]. For example, it has been shown to promote breast cancer progression by modulating the miR-106a-5p/signal transducer and activator of transcription 3 (STAT3) signaling pathway [19]. Emerging evidence also indicates that VCAN-AS1 is involved in other diseases, such as diabetic kidney disease, where it plays a central role in a lncRNA-associated ceRNA network [22].

However, the role and mechanisms of VCAN-AS1 in GC remain largely unexplored. This study aims to investigate the role of VCAN-AS1 in GC progression and clarify the molecular mechanisms involved. Our findings indicate that VCAN-AS1 promotes GC progression by interacting with Human antigen R (HuR, also known as ELAVL1) and enhancing the stability of F11 receptor (F11R) mRNA. These insights into VCAN-AS1's function in GC may help identify new therapeutic targets for improving the management of this deadly disease.

Materials and methods

Bioinformatic analysis

Expression levels of VCAN-AS1 in gastric cancer (GC) tissues and adjacent normal tissues were obtained from The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) databases. Survival analysis, including overall survival (OS), disease-specific survival (DSS), disease-free interval (DFI), and progression-free interval (PFI), was performed using the Kaplan-Meier method and log-rank test based on data from TCGA database [23, 24]. The interaction between VCAN-AS1 and HuR was predicted using the RNA-Protein Interaction Prediction (RPISeq) tool as previously reported [25].

Human tissues

GC tissues and adjacent normal tissues were collected from patients who underwent surgical resection at Suzhou Municipal Hospital between March 2023 and June 2024, with written informed consent from all participants. Tissue samples were immediately snap-frozen in liquid nitrogen and stored in liquid nitrogen until RNA extraction. The study was approved by the Ethics Committee of Suzhou Municipal Hospital.

Cell culture and transfection

Human GC cell lines AGS, HGC27, Hs746T, and NCI-N87, along with the normal gastric epithelial cell line GES-1, were obtained from the Chinese Academy of Cell Collection (Shanghai, China). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37°C in a humidified atmosphere containing 5% CO₂. For transfection, siRNAs targeting VCAN-AS1 and HuR, a negative control (NC) siRNA, and sh-VCAN-AS1 vectors were purchased from GenePharma (Shanghai, China). The pcDNA3.1-F11R overexpression plasmid (OE-F11R) and empty vector were obtained from Sangon (Shanghai, China). Cells were transfected with siRNAs or plasmids using Lipofectamine 2000 (Invitrogen, CA, USA) according to the manufacturer's instructions. Cells were harvested for subsequent experiments 48 hours post-transfection. The siRNA sequences were as follows: si-VCAN-AS1 #1, 5'-AUGUUUUCCUUGGCUUUUGGA-3'; si-VCAN-AS1 #2, 5'-UGGCUUUUGGAUUAAUACA-3'. Sequences targeting HuR and the NC siRNA were used as previously described [25].

RNA extraction and real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR)

Total RNA was extracted from tissue samples and cultured cells using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. The quality and concentration of the RNA were assessed with a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). cDNA synthesis was performed using the PrimeScript RT reagent kit (Vazyme, Nanjing, China) with gDNA Eraser to remove genomic DNA contamination. RT-qPCR was conducted using SYBR Green Master Mix (Vazyme) on an Applied Biosystems 7500 system. The relative expression levels of VCAN-AS1 and F11R were calculated using the 2^{-ΔΔCt} method, with 18s rRNA as the internal control. The primer sequences were listed as follows: VCAN-AS1, forward 5'-AATGCCACATCACAGCTGAC-3' and reverse 5'-AGAGCCACCAACATACTTGACA-3'. The primers for 18s rRNA and F11R amplification were reported previously [25].

Cell counting kit-8 (CCK-8) assay, colony formation assay, and Transwell assay

Cell viability was assessed with the CCK-8 assay (Beyotime, Shanghai, China) as previously

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described [26, 27]. Briefly, transfected cells were seeded in 96-well plates at a density of 2×10^3 cells per well and incubated for 24, 48, 72, and 96 hours. CCK-8 reagent was added, and the absorbance at 450 nm was measured using a microplate reader (Bio-Rad). For the colony formation assay, transfected cells were seeded in 6-well plates at 500 cells per well and cultured for two weeks. Colonies were then fixed with 4% paraformaldehyde, stained with 0.1% crystal violet, and counted under a microscope. Cell migration was evaluated using Transwell chambers (Corning) as previously reported [28, 29]. Cells (1×10^5) were placed in the upper chamber with serum-free medium, while the lower chamber contained medium with 10% FBS. After 48 hours, cells on the lower membrane surface were fixed, stained, and counted under a microscope (Axioskop 2 plus, Zeiss, Germany).

Xenograft model

The tumorigenic potential of VCAN-AS1 knock-down was evaluated in vivo using a xenograft mouse model. Four-week-old female BALB/c nude mice were purchased from Vital River Laboratory (Beijing, China) and housed under specific pathogen-free conditions. The si-VCAN-AS1-1 sequence was cloned into an LV-2N/Puro lentiviral vector (GenePharma). Lentiviral vectors and packaging plasmids were co-transfected into HEK-293T cells (ATCC, USA), and the collected viruses were used to infect AGS cells, which were then selected for puromycin resistance for two weeks. AGS cells stably expressing sh-VCAN-AS1 or control shRNA were subcutaneously injected on both flanks of each mouse (1×10^7 cells per mouse). Tumor growth was measured every three days, and tumor volume was calculated as $V = 0.5 \times \text{length} \times \text{width}^2$. Mice were euthanized 12 days after injection using carbon dioxide exposure followed by cervical dislocation, and tumors were excised and weighed. All animal experiments were approved by the Institutional Animal Care and Use Committee of Nanjing Medical University.

RNA pull-down

Biotin-labeled RNA probes targeting VCAN-AS1 and a control probe were synthesized by Ribobio (Guangzhou, China). RNA pull-down assays were carried out using the Pierce Magnetic RNA-Protein Pull-Down Kit (Thermo Fisher Sci-

entific) as previously described [30]. Briefly, biotinylated RNA probes were incubated with cell lysates from AGS and HGC27 cells at 4°C for 4 hours with gentle rotation. RNA-protein complexes were then captured by adding streptavidin magnetic beads to the mixture, followed by incubation at 4°C for 2 hours with rotation. The bound proteins were eluted, separated by SDS-PAGE, and identified through Western blotting.

RNA immunoprecipitation (RIP)

RIP assays were performed using the Magna RIP RNA-Binding Protein Immunoprecipitation Kit (Millipore) as previously described [31, 32]. AGS and HGC27 cell lysates were incubated with HuR antibodies (Proteintech, Chicago, IL, USA) or control IgG at 4°C overnight with rotation. RNA-protein complexes were captured using Protein A/G beads, followed by 2 hours of incubation at 4°C with rotation. Co-precipitated RNAs were extracted with TRIzol reagent, and the presence of VCAN-AS1 and F11R was detected by RT-qPCR.

Western blotting

Total protein was extracted from cells using RIPA lysis buffer (Beyotime) supplemented with protease inhibitors, as described previously [33, 34]. Protein concentrations were measured with the BCA Protein Assay Kit (Beyotime). Equal amounts of protein were separated via SDS-PAGE and transferred onto PVDF membranes (Millipore). Membranes were blocked with 5% non-fat milk in TBST and incubated overnight at 4°C with primary antibodies against HuR (1:1000, Proteintech) and Tubulin (1:5000, Beyotime). After washing, membranes were incubated with HRP-conjugated secondary antibodies (1:1000, Thermo Fisher Scientific), and the immunoreactive bands were visualized using the ECL detection system (Tanon, Shanghai, China).

Statistical analysis

Data were analyzed with GraphPad Prism software (version 8.0). Quantitative results are presented as mean \pm standard deviation (SD) from at least three independent experiments. Differences between groups were assessed using Student's t-test or one-way ANOVA followed by Tukey's post-hoc test. A p -value < 0.05 was considered statistically significant.

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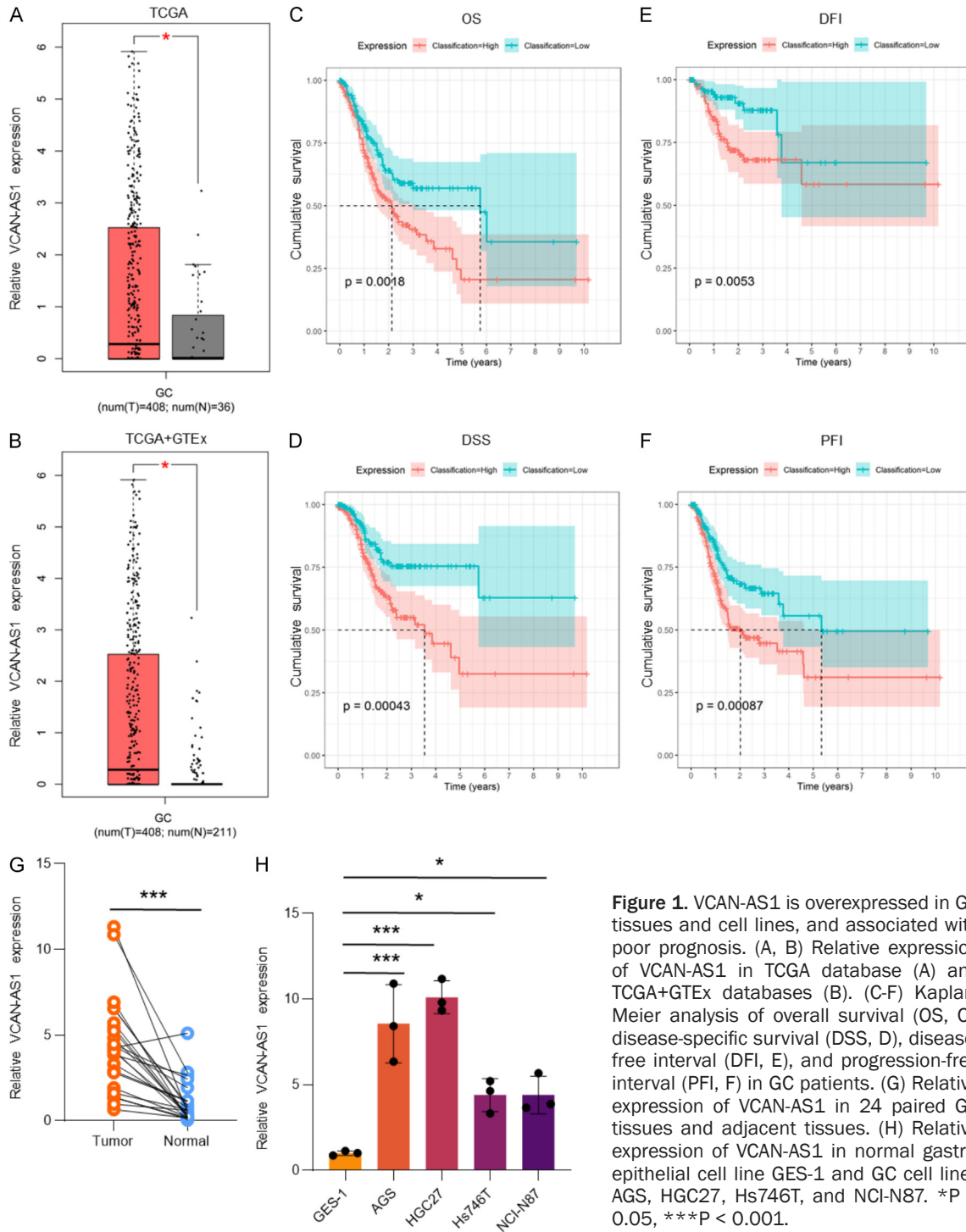


Figure 1. VCAN-AS1 is overexpressed in GC tissues and cell lines, and associated with poor prognosis. (A, B) Relative expression of VCAN-AS1 in TCGA database (A) and TCGA+GTEx databases (B). (C-F) Kaplan-Meier analysis of overall survival (OS, C), disease-specific survival (DSS, D), disease-free interval (DFI, E), and progression-free interval (PFI, F) in GC patients. (G) Relative expression of VCAN-AS1 in 24 paired GC tissues and adjacent tissues. (H) Relative expression of VCAN-AS1 in normal gastric epithelial cell line GES-1 and GC cell lines AGS, HGC27, Hs746T, and NCI-N87. * $P < 0.05$, *** $P < 0.001$.

Results

VCAN-AS1 is upregulated in GC tissues and cells, and associated with poor prognosis

Analysis of the TCGA and GTEx databases showed that VCAN-AS1 was significantly upregulated in GC tissues compared to normal tis-

sues (Figure 1A and 1B). Higher expression levels of VCAN-AS1 in GC tissues were correlated with worse patient outcomes, including OS (Figure 1C), DSS (Figure 1D), DFI (Figure 1E), and PFI (Figure 1F). Further validation by RT-qPCR confirmed elevated VCAN-AS1 expression in both GC tissues (Figure 1G) and cell lines (Figure 1H). Among the cell lines tested,

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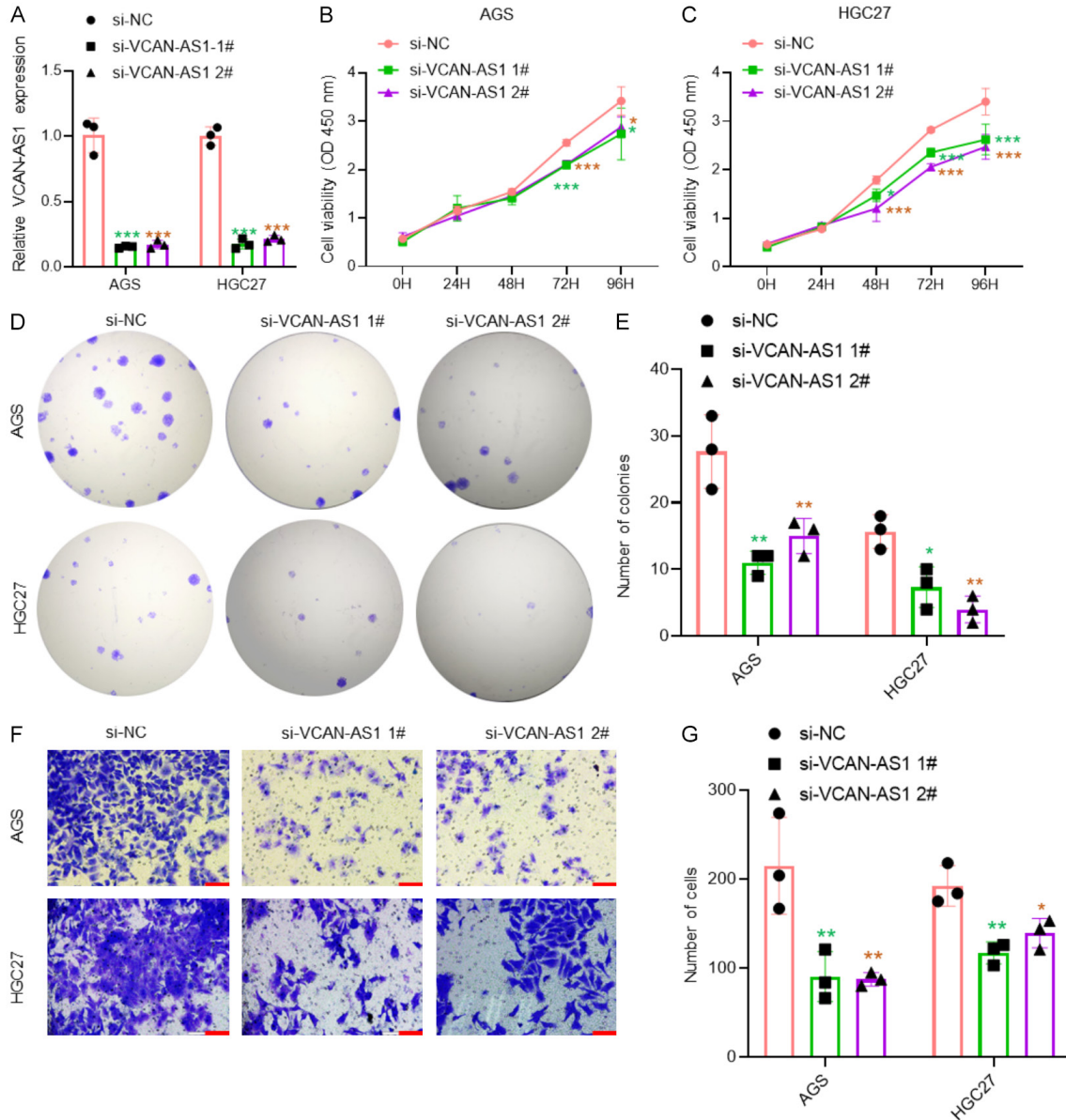


Figure 2. Silencing VCAN-AS1 inhibits proliferation and migration in GC cells. A. RT-qPCR analysis of VCAN-AS1 expression in AGS and HGC27 cells transfected with si-NC and si-VCAN-AS1 for 48 hours. B, C. CCK-8 assays in AGS and HGC27 cells transfected with si-NC and si-VCAN-AS1 for 48 hours. n=6 per group. D, E. Colony formation assays in AGS and HGC27 cells transfected with si-NC and si-VCAN-AS1 for 48 hours. n=3 per group. F, G. Transwell assays in AGS and HGC27 cells transfected with si-NC and si-VCAN-AS1 for 48 hours. n=3 per group. Scale bar: 100 μ m. *P < 0.05, **P < 0.01, ***P < 0.001.

AGS and HGC27 showed the highest VCAN-AS1 expression and were thus selected for subsequent experiments.

Knockdown of VCAN-AS1 inhibits GC cell proliferation and migration in vitro

To investigate the role of VCAN-AS1 in GC, two specific siRNAs were designed to knock down VCAN-AS1 expression in AGS and HGC27 cells, with RT-qPCR confirming effective knockdown

(**Figure 2A**). Functional assays, including CCK-8, colony formation, and Transwell assays, demonstrated that silencing VCAN-AS1 significantly reduced the proliferation (**Figure 2B-E**) and migration (**Figure 2F, 2G**) of GC cells.

VCAN-AS1 knockdown suppresses tumor growth in vivo

The effect of VCAN-AS1 knockdown on tumor growth was further evaluated in a subcutane-

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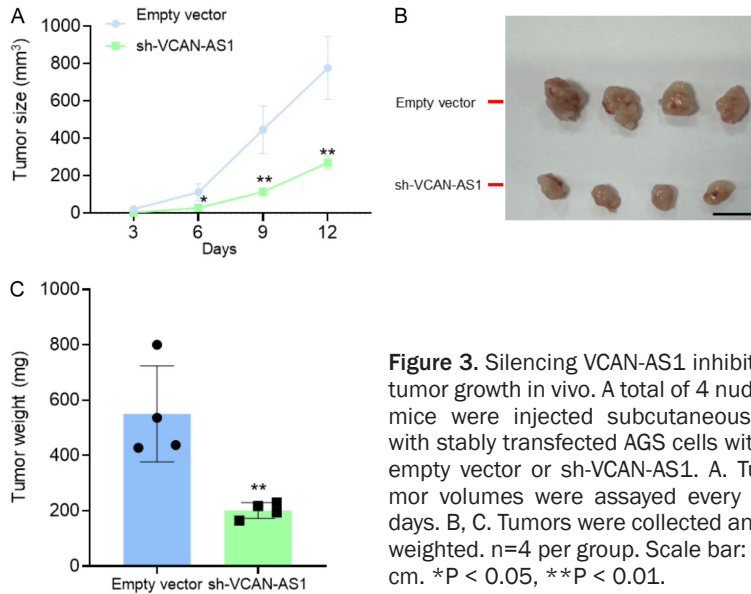


Figure 3. Silencing VCAN-AS1 inhibits tumor growth in vivo. A total of 4 nude mice were injected subcutaneously with stably transfected AGS cells with empty vector or sh-VCAN-AS1. A. Tumor volumes were assayed every 3 days. B, C. Tumors were collected and weighted. n=4 per group. Scale bar: 1 cm. *P < 0.05, **P < 0.01.

ous xenograft model using nude mice. AGS cells stably transfected with sh-VCAN-AS1 or an empty vector were injected into the mice. Tumors derived from VCAN-AS1-knockdown cells exhibited significantly slower growth and lower tumor weight compared to the control group (Figure 3A-C). These findings indicate that silencing VCAN-AS1 inhibits GC cell growth in vivo.

VCAN-AS1 interacts with HuR and regulates F11R expression

Using RPISeq software, we predicted a potential interaction between VCAN-AS1 and the RNA-binding protein HuR (Figure 4A). This interaction was confirmed by RNA pull-down (Figure 4B) and RNA immunoprecipitation (RIP) assays (Figure 4C and 4D). Previous studies have shown that HuR binds to the 3'-UTR of F11R mRNA, stabilizing it and promoting its oncogenic role in GC [25, 35]. Our results revealed that knockdown of either VCAN-AS1 or HuR significantly reduced F11R mRNA expression in GC cells (Figure 4E and 4F). Notably, silencing VCAN-AS1 did not alter HuR protein levels but impaired HuR's ability to bind F11R mRNA (Figure 4G-I). These findings suggest that VCAN-AS1 participates in the HuR-F11R regulatory axis involved in GC progression.

F11R overexpression rescues the effects of VCAN-AS1 knockdown on GC cell proliferation and migration

To further clarify the mechanism by which VCAN-AS1 promotes GC progression, we per-

formed rescue experiments by overexpressing F11R in VCAN-AS1-knockdown cells. The results showed that F11R overexpression effectively restored the reduced proliferation (Figure 5A-D) and migration (Figure 5E and 5F) caused by VCAN-AS1 knockdown, indicating that VCAN-AS1 promotes GC cell proliferation and migration through F11R regulation.

Discussion

This study investigated the role of the long non-coding RNA (lncRNA) VCAN-AS1 in GC and uncovered its involvement in GC progression. Our results demonstrate that VCAN-AS1 is

significantly upregulated in GC tissues and cell lines, correlating with poor prognosis. Both in vitro and in vivo knockdown of VCAN-AS1 suppressed tumor growth. Mechanistically, we identified that VCAN-AS1 interacts with HuR, which in turn enhances the stability of F11R mRNA, promoting GC progression. These findings provide new insights into the molecular pathways of GC involving lncRNAs, suggesting the VCAN-AS1-HuR-F11R axis as a potential therapeutic target for GC.

Furthermore, identifying VCAN-AS1 as a critical regulator of GC progression may offer a novel biomarker for predicting GC prognosis. Unlike previous studies focusing primarily on protein-coding genes in GC biology [36, 37], our work expands the understanding of GC carcinogenesis, emphasizing the significant role of lncRNAs in cancer regulation [38-40]. A common mechanism through which lncRNAs function as competing endogenous RNAs (ceRNAs), binding to microRNAs (miRNAs) and preventing them from interacting with their target mRNAs. For example, lncRNA PCED1B-AS1 has been shown to promote GC metastasis and epithelial-mesenchymal transition by sponging miR-3681-3p, leading to the upregulation of MAP2K7 [41]. Similarly, LINC01140, in conjunction with miR-140-5p and FGF9, forms a lncRNA-miRNA-mRNA axis that promotes GC aggressiveness [42].

In addition to acting as ceRNAs, accumulating evidence indicates that many lncRNAs exert their oncogenic roles by directly binding to pro-

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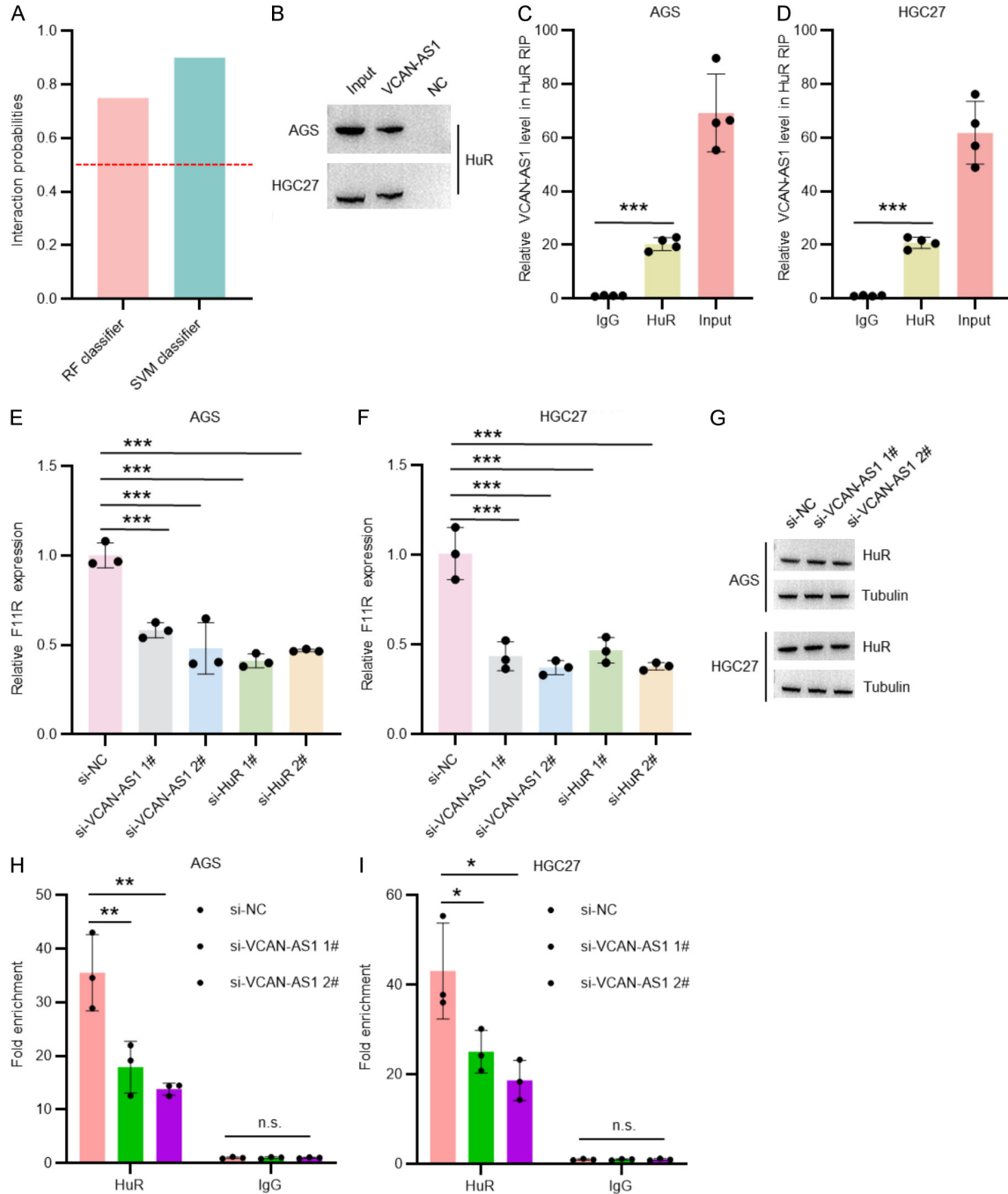


Figure 4. VCAN-AS1 interacts with HuR and affects F11R mRNA expression. A. VCAN-AS1-HuR interactions were predicted by RPISeq. B. Western blot analysis of RNA pull-down products based on an anti-HuR antibody in AGS and HGC27 cells. C, D. RIP assays of VCAN-AS1 binding to HuR in AGS and HGC27 cells. n=3 per group. E, F. RT-qPCR analysis of F11R expression in AGS and HGC27 cells transfected with si-NC, si-VCAN-AS1, and si-HuR for 48 hours. n=3 per group. G. Western blot analysis of HuR expression in AGS and HGC27 cells transfected with si-NC and si-VCAN-AS1 for 48 hours. H, I. RIP assays of F11R binding to HuR in AGS and HGC27 cells transfected with si-NC and si-VCAN-AS1 for 48 hours. n=3 per group. *P < 0.05, **P < 0.01, ***P < 0.001.

teins. For instance, lncRNA HOXA11-AS has been shown to facilitate GC progression by scaffolding the epigenetic factors EZH2, LSD1, and DNMT1 [43]. Similarly, lncRNA NRSN2-AS1

plays a crucial role in ovarian cancer malignancy through interaction with PTK2, thereby activating β -catenin signaling [30]. In this study, we found that VCAN-AS1 directly interacts with the

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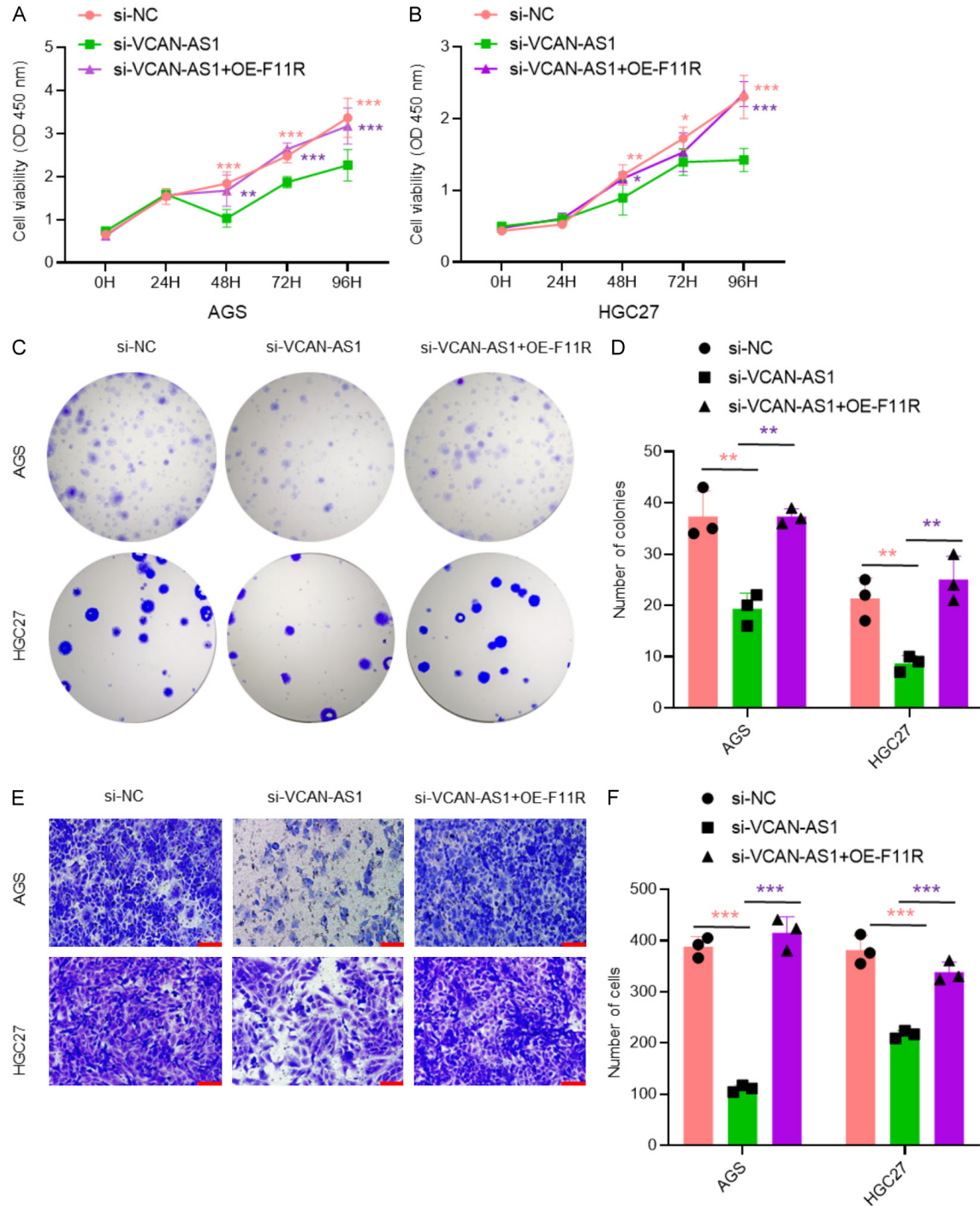


Figure 5. F11R is a major downstream of VCAN-AS1/HuR axis. A, B. CCK-8 assays in AGS and HGC27 cells transfected with si-NC, si-VCAN-AS1, and si-VCAN-AS1+OE-F11R for 48 hours. n=6 per group. C, D. Colony formation assays in AGS and HGC27 cells transfected with si-NC, si-VCAN-AS1, and si-VCAN-AS1+OE-F11R for 48 hours. n=3 per group. E, F. Transwell assays in AGS and HGC27 cells transfected with si-NC, si-VCAN-AS1, and si-VCAN-AS1+OE-F11R for 48 hours. n=3 per group. Scale bar: 100 μ m. *P < 0.05, **P < 0.01, ***P < 0.001.

RNA-binding protein HuR, which enhances the stability of F11R mRNA, thereby promoting its oncogenic function in GC. This novel mecha-

nism illustrates the diverse functions of lncRNAs and their complex roles in cancer biology.

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HuR is a well-established RNA-binding protein known for its function in stabilizing target mRNAs by binding to AU-rich elements in their 3'-untranslated regions (3'-UTRs) [44, 45]. Previous research has shown that HuR stabilizes TIMM44 mRNA, promoting ovarian cancer proliferation [46], and enhances the stability of CDK3 mRNA in breast cancer, contributing to cancer cell growth [47]. In GC, HuR has been reported to interact with lincRNAs such as LINC00707 and DEPDC1-AS1, leading to the stabilization of F11R mRNA and driving GC progression [25, 35]. Our findings demonstrate that HuR interacts with VCAN-AS1, which in turn stabilizes F11R mRNA, uncovering a new layer of regulatory control in GC progression.

In conclusion, our findings emphasize the significance of VCAN-AS1 in GC progression and suggest new avenues for developing targeted therapies aimed at the VCAN-AS1-HuR-F11R axis.

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Disclosure of conflict of interest

None.

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