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LncRNA GClnc1 promotes osteosarcoma progression by stabilizing NONO and blocking FBXW7-mediated ubiquitination

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Abstract

Background Long non-coding RNA (lncRNA) plays a vital role in the occurrence and development of varieties of tumors. Previous studies have shown that lncRNA GClnc1 is highly expressed in osteosarcoma (OS). However, the mechanism of lncRNA GClnc1 in osteosarcoma has not been fully elucidated. In this study, we investigated the biological roles of lncRNA GClnc1 in osteosarcoma and unveiled its underlying mechanisms.

Methods The expression of lncRNA GClnc1 in OS cells was detected by real-time quantitative PCR (qRT-PCR). The functional roles of lncRNA GClnc1 were examined by CCK8, trans-well, scratch wound healing assay, colony formation, and apoptosis assays in osteosarcoma cells upon silencing or overexpressing GClnc1. Western blot analysis, qRT-PCR, and RNA co-immunoprecipitation (RIP) assays were used to detect the interaction between lncRNA GClnc1 and NONO.

Results The expression of lncRNA GClnc1 was up-regulated in osteosarcoma cell lines. Knockdown of lncRNA GClnc1 suppressed the cell growth, migration, and invasion of OS cells, whereas the over-expression of GClnc1 improved the proliferation, migration, and invasion of OS cells. Mechanistically, we identified that lncRNA GClnc1 regulates the stability of NONO by blocking FBXW7-mediated ubiquitination degradation. Additionally, overexpression of NONO can reverse GClnc1 silencing exerted suppression of the cell proliferation, migration, and invasion, and vice versa.

Conclusions Our study elucidated that lncRNA GClnc1 participates in the progression of OS by regulating the NONO signal pathway. Targeting GClnc1 provides a potential target for future clinical treatment of OS.

Keywords Osteosarcoma, LncRNA GClnc1, NONO, Migration and invasion, Proliferation

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Introduction

Osteosarcoma (OS) originates from mesenchymal tissue and is highly malignant. Clinically, the distal femur and the proximal tibia are the most common. It is one of the most common malignant tumors in adolescents and children [1, 2]. In addition, OS is highly invasive and metastatic, and the lung is the most common metastatic site [3]. OS is a malignant tumor characterized by rapid progression, early metastasis, high recurrence rate, and poor prognosis. The 5-year survival rate of OS patients is very low, and about 80% of patients relapse within 5 years [4, 5]. Therefore, it's urgent to explore the underline mechanism of the occurrence and progression of OS and identify potential targets for the treatment of OS.

Approximately 80% of the human genome is transcribed into RNA, but only 2% of the genome is translated into protein, indicating that most of the transcriptome consists of non-coding RNA [6]. Long non-coding RNA (lncRNA) is an RNA that is more than 200 nucleotides in length and does not encode a protein [7]. Originally, lncRNA was considered to be non-biologically functional garbage produced by the transcription process [8]. In recent years, studies have shown that lncRNA is involved in the biological processes of varieties of cells. Different lncRNA expression patterns can regulate the cell cycle, proliferation, metastasis, and differentiation [9]. LncRNA performs its functions through multiple mechanisms, such as acting as a scaffold, bait, signal, and guide [10]. At present, growing studies show that lncRNA plays an important role in the occurrence and development of various cancers, including gastric cancer, OS, ovarian cancer, bladder cancer, and colorectal cancer [11–13]. LncRNA GClnc1, also referred to as lncRNA BC041951, is a non-coding RNA molecule exceeding 200 nucleotides in length. Although this molecule does not directly encode proteins, it can finely regulate gene expression at multiple levels, including epigenetic, transcriptional, and post-transcriptional levels [14]. Existing studies have demonstrated that GClnc1 promotes the transcription of the NOTCH1 gene by recruiting the FOXC2 protein, thereby activating the NF-κB/Snail signaling pathway and accelerating the growth and metastasis of epithelial ovarian cancer cells [15]. Concurrently, the expression level of GClnc1 is significantly elevated in gastric cancer tissues and is closely associated with the poor prognosis of gastric cancer patients. As a "scaffolding" molecule, GClnc1 influences the binding and modification of histone modification molecules WDR5 and KAT2A, thereby regulating the activation and function of downstream genes, such as SOD2, which are closely related to tumorigenesis [12]. Furthermore, relevant reports indicate that lncRNA GClnc1 participates in the regulation of signaling pathways in tumors through various mechanisms [16]. However, the mechanism of action of GClnc1 in OS has not been fully elucidated, necessitating further in-depth research to provide new insights for targeted therapy of OS.

NONO, an octameric binding protein lacking a POU domain, is capable of binding to RNA, DNA, and proteins, and plays a significant role in various biological processes, including gene regulation, cell proliferation, migration, and apoptosis, as well as cancer progression [17]. Abnormal expression of the NONO protein is often closely associated with the onset and development of tumors, neurological lesions, vascular calcification, and metabolic diseases. Research indicates that targeting NONO can influence the occurrence and progression of prostate cancer [18]. Wang et al. [19] found that NONO is highly expressed in gliomas and promotes their proliferation and invasion by cleaving specific mRNA precursors, such as GPX1. Additionally, studies by Xie et al. [20] have demonstrated that NONO can inhibit lymphatic metastasis in bladder cancer. While most studies confirm that NONO can directly or indirectly trigger the progression of tumors, research on its upstream signaling pathways remains relatively limited. For instance, FBXW7 and RNF8 have been identified as mediators of NONO's regulatory effects, thereby influencing tumor occurrence and development [21, 22]. FBXW7, a member of the F-box protein family, is recognized as a key tumor suppressor gene and is one of the most frequently dysregulated proteins within the ubiquitin-protease system in human cancers [23]. FBXW7 can inhibit the progression of breast cancer through various complex signaling pathways and targets [24]. Furthermore, some studies have indicated that mutations in FBXW7 may promote the progression of cervical cancer [25].

In this study, we found that lncRNA GClnc1 improved the cell proliferation, migration, and invasion of OS cell lines and confirmed this in nude mice. Further mechanistic investigations revealed that lncRNA GClnc1 is able to stabilize NONO, while also diminishing the ubiquitin-mediated degradation of NONO facilitated by FBXW7, which in turn contributes to the progression of OS. This study may provide a potential prognostic diagnosis marker and therapeutic target for OS in the future.

Materials and methods

Ethics statement

The research protocol was approved by the Ethics Committee of the Third Affiliated Hospital of Nanchang University. All animal experiments were approved by the Animal Protection and Use Committee, and the experimental procedures were approved by guidelines.

Cell culture

The human OS cell lines (143B, U2OS, U2R, MG63) were obtained from Stem Cell Bank of Chinese Academy of

Sciences. The cells were tested for Mycoplasma before experiments. All the OS cells and 293T cells were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS, Gibco). All cells grew in a 37 °C humidified incubator with 5% CO_2.

Cell transfection

The plasmids delivering GClnc1 and NONO (pcDNA-GClnc1、pcDNA-NONO) were constructed by inserting the full-length sequences into the pcDNA3.1 vector. For different experiments, we used plasmids with different tags, such as HA, Flag, and Myc. To clone shRNAs, the shRNA sequences targeting GClnc1 were cloned into pLKO.1 vector. All siRNAs were purchased from GenePharma Co. Ltd. (Suzhou, China). The transfection was performed using Lipofectamine® RNAiMAX and Lipofecmine 2000 according to the manufacturer's instructions.

Transwell assay

In the invasion experiment, we add 50 μl diluted Matrigel (BD Biosciences, Franklin Lakes, NJ) to the upper chamber. Transwell inserts were applied for detecting cell migratory and invasive potential. Cell suspension (200 μL, 1*10^5 cells) in serum-free DMEM was added to the upper insert, and DMEM (500 μL) containing 10% FBS (Biological Industries, USA) was added to the lower chamber to induce cell migration. MG63 and U2OS cells were allowed to migrate for 22 h and invade for 24 h. And then the cells were fixed, stained, and counted. Images were photographed under a microscope (Olympus, Japan).

Wound healing assay

Pretreated cells were seeded in 6-well plates. After the cells were attached, two lines perpendicular to the well plate were drawn using a pipette tip. The old medium was discarded and the wells were washed three times with PBS before fresh medium was added. Photographs were taken and recorded immediately under microscope. After 24 h of incubation, images were again taken under the microscope. Scratch width was observed at the same position and measured using ImageJ.

MTT assay

MTT assay was used to detect the viabilities of OS cells. Cells (about 1×10^4 cells/well) were placed in 96-well plates and seeded for 24 h, 48 h, and 72 h. Subsequently, the cells were incubated with MTT for 4 h and then dissolved in DMSO. Finally, the optical density (OD) value was detected at a wavelength of 450 nm. Each group was repeated three times to ensure the accuracy of the results.

Flow cytometry

Cell apoptosis was determined using an Annexin V-FITC/PI kit (Cat.no: KGA108, Keygen, China). After 48 h of the transfection, MG63 and U2OS cells were first washed with PBS and resuspended in 500 μl of 1 × binding buffer. 5 μl of Annexin V-FITC and 5 μl of PI were added and then the cells were incubated for 20 min in the dark at room temperature. Flow cytometry (Becton-Dickinson, USA) was used to detect the number of apoptotic cells.

Colony formation

500 OS cells per well were seeded into 6-well plates and cultured for 10 days. Cells were immobilized with paraformaldehyde for 20 min, stained with crystal violet for 30 min, and washed with PBS 3 times. The stained cells were photographed and counted under a microscope (Nikon, Japan).

Real-time quantitative PCR

Total RNA was extracted using TRIzol™ reagent according to the manufactory's instructions (Invitrogen, USA). cDNAs were reversely transcribed using a PrimeScript™ RT reagent kit with gDNA Eraser (Takara, Japan). The qRT-PCR was carried out using SYBR Premix Ex Taq (Takara, Japan) as described in the manufacturer's protocols. The $2-\Delta\Delta Ct$ method, normalized to GAPDH, was utilized to determine the relative expression of genes to be tested. Primer sequences are listed as follows:

GADPH: forward: 5′- GGAGCGAGATCCCTCCAAAAT −3′,

reverse: 5′- GGCTGTTGTCATACTTCTCATGG −3′;

GClnc1: forward: 5′- TGGGGTAACTTAGCAGTTTCAAT-3′,

reverse: 5′- GGCAAGCAGTAATCTTACATGACAC-3′;

si-GClnc1-1: forward: 5′- GUUAGAAAGUAGGCUUUAATT-3′,

reverse: 5′- UUAAAGCCUACUUUCUAACTT-3′;

si-GClnc1-2: forward: 5′- GCAGAUCUGCUGCUUAUATT-3′,

reverse: 5′- UAUAAGUCAGCAGAUCUGCTT-3′;

Western blot analysis

Proteins were extracted using RIPA lysis buffer (50 mMTris-HCl (pH 7.4), 150 mM NaCl, 1% NP-40) supplemented with protease inhibitors cocktail (NCM Biotech, China) on ice for 30 min and then centrifuged at 12,000 g for 20 min at 4 °C. Quantification of proteins was performed using BCA protein quantification kit (Biomega, PW0104, China). Samples, separated by SDS-PAGE gel, transferred to PVDF membrane, were blocked with 5% skimmed milk powder for 1 h and then incubated with the indicated primary antibodies and

secondary antibodies. Antibody detection was performed with a chemiluminescence kit (Beyotime LTD, Shanghai, China). Antibodies against Myc tag (3946 S), HA tag (3724 S), and Flag tag (2368 S) were purchased from Cell Signaling Technology (Boston, USA). Antibodies against NONO (11058-1-AP) and Actin (20536-1-AP) used for western blotting were purchased from Proteintech (Wuhan, China).

RNA binding protein immunoprecipitation (RIP)

Cell extracts were prepared on ice using RIP buffer (150 mM KCl, 25 mM Tris (pH 7.4), 5 mM EDTA, 0.5 mM dithiothreitol, 0.5% NP40, 1× protease inhibitor). Anti-NONO or anti-HA antibodies, or a corresponding control IgG antibody was incubated with protein A/G agarose beads (Thermo Scientific, USA) for 4 h at 4 °C, followed by incubation with precleared nuclear extracts in RIP buffer supplemented with RNase inhibitor at 4 °C overnight. The co-IPed complexes were digested with DNase at 37 °C for 15 min and with proteinase K at 37 °C for 15 min. Input and co-IPed RNAs were extracted by TRIzol RNA extraction reagent. Analysis was performed by reverse transcription qPCR.

Xenograft experiments

All nude mice (3 to 4 weeks old) purchased from the Laboratory Animal (Shanghai SLAC Laboratory Animal Co, Ltd) were randomly divided into three groups: the control group, the shGClnc1-1 group, and the shGClnc1-2 group. There were six mice in each group. Each nude mouse was injected subcutaneously with about 200ul of cells containing 1×10^6 corresponding stable lines. The tumor volume was measured every 5 days for a total of 30 days. The calculation method was as follows: length (L) and width (W) with a caliper and calculated with the formula $V = (L \times W^2) \times 0.5$. Rats were sacrificed and weighed after 30 days. The experiment was approved by the Animal Experiment Animal Use Committee of Jiangxi University of Traditional Chinese Medicine.

Statistical analyses

All of the experimental data were repeated at least three times and expressed as the mean±SD. Student's t-test was used to compare differences between the two groups. SPSS 22.0 (SPSS Inc., Chicago, IL, USA), R (http://www.r-project.org) 4.2.1, and ImageJ (https://imagej.nih.gov/ij) software for Windows were used for statistical analysis. A *p value* of less than 0.05 was considered to be statistically significant.

Results

GClnc1 improves the proliferating and colony formatting ability of OS cells

To explore the expression of GClnc1 in OS cell lines, we detected the expression of GClnc1 in four OS cells (MG63, 143B, U2OS, U2R). Our analysis revealed a statistically significant difference in GClnc1 expression among these OS cell lines, with the highest levels observed in MG63 cells and the lowest in U2OS cells (Fig. 1A). We knocked down GClnc1 in MG63 cells using siRNAs and the silencing efficiency was evaluated by real-time quantitative PCR (qRT-PCR) (Fig. 1B). The knockdown of GClnc1 effectively reduced the proliferation and colony formation of MG63 cells (Fig. 1C and D). On the contrary, overexpression of GClnc1 promoted the cell viability and colony formation of U2OS (Fig. 1E, F and G).

GClnc1 increases the migratory and invasive ability and repressed the apoptosis of OS cells

We then examined the roles of GClnc1 in regulating the malignant features of OS cells. Transwell assay results showed that silence of GClnc1 significantly reduces the migration, and invasion in MG63 cells (Fig. 2A), whereas overexpression of GClnc1 promotes the migration and invasion in U2OS cells (Fig. 2B). In addition, scratch assays supported these results (Fig. 2C and D). Besides, annex V-FITC staining results showed that knockdown of GClnc1 promote the apoptosis of OS (Fig. 2E), while overexpression of GClnc1 effectively represses apoptosis (Fig. 2F). Altogether, these results indicated that GClnc1 plays an important role in the progress of the OS.

GClnc1 regulates the protein stability of NONO and blocks the ubiquitin degradation of NONO by FBXW7

The lncRNA Gclncl-RBP (RNA Binding Protein) pathway is an important mode of lncRNA function. To explore the specific mechanism of GClnc1 regulating OS progression, we used the online database RBPDB (The database of RNA binding protein) to predict its interacting protein. Among them, NONO, an RNA-binding protein with a crucial role in tumorigenesis provoked our interest. We firstly confirmed the interaction between GClnc1 and NONO by RNA co-Immunoprecipitation (RIP) (Fig. 3A). To explore the functional role of this interaction, we examined whether GClnc1 regulated the expression of NONO. Firstly, over-expression of GClnc1 increased the expression of NONO protein, while knocking down GClnc1 decreased the protein level of NONO OS cells (Fig. 3B). Half-life experiments showed that GClnc1 silence of GClnc1 reduced but overexpression of GClnc1 increased the stability of NONO (Fig. 3C and D). To map the domains of NONO that mediate the interaction of NONO with GClnc1, we constructed a series of plasmids

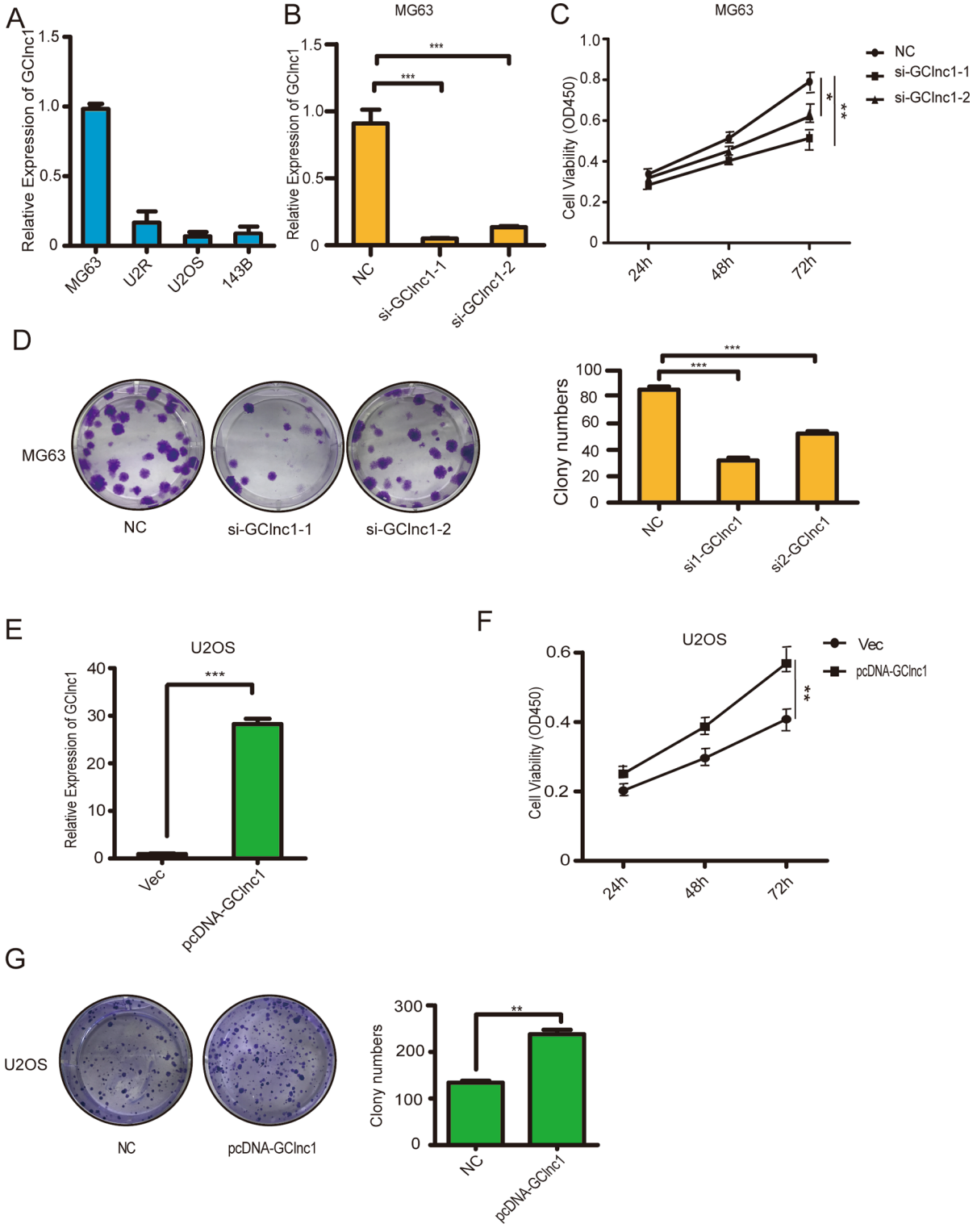

Fig. 1 LncRNA GClnc1 regulates the proliferation and colony formation of osteosarcoma (**A**) RT-qPCR examined the expression of GClnc1 in OS cell lines (MG63, U2R, U2OS, 143B) (**B**-**C**) RT-qPCR detected the expression of GClnc1 and proliferation ability was determined by CCK8 assay in MG63 cells after transfection with GClnc1 siRNA (si-GClnc1) (**D**) The colony numbers were reduced in the si-GClnc1 group (**E**-**F**) RT-qPCR detected the expression of GClnc1 and proliferation ability was determined by CCK8 assay in U2OS cells after transfection with pcDNA-GClnc1 (**G**) The colony numbers increased in GClnc1 over-expression group. ($n = 3$ per group; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$, t-test)

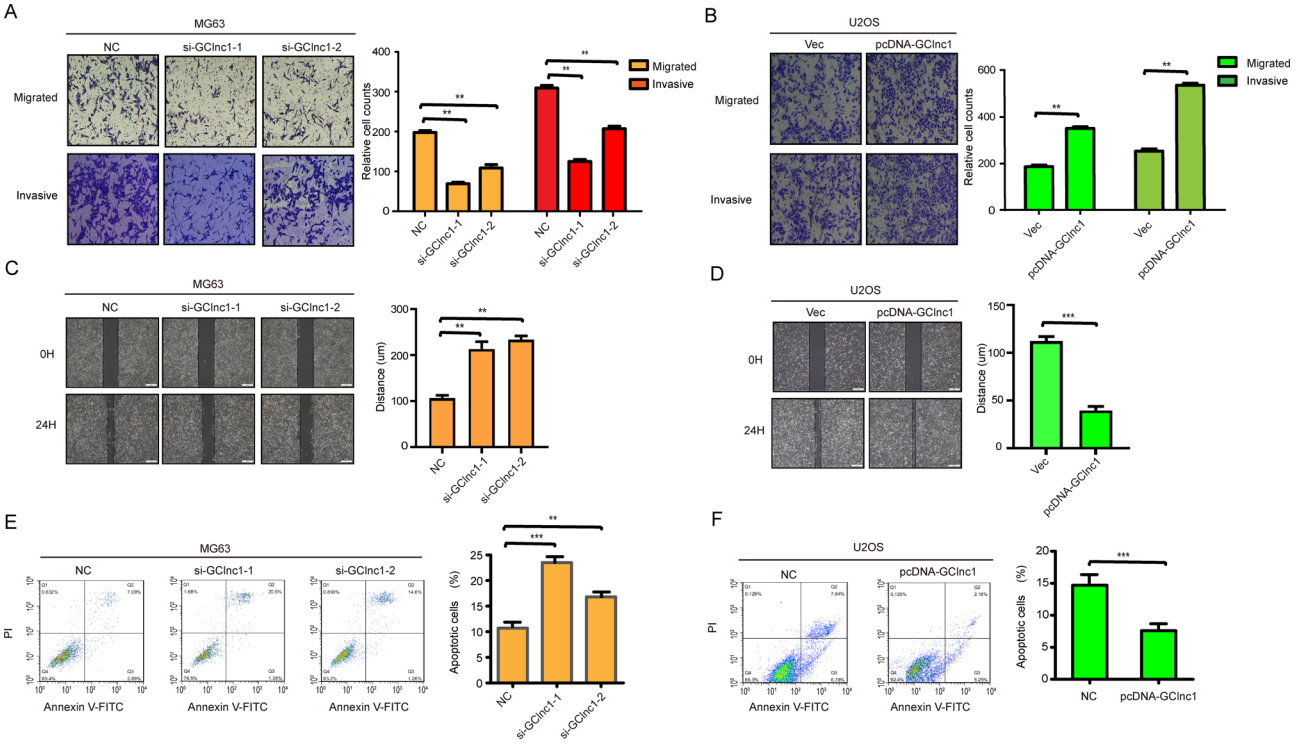

Fig. 2 LncRNA GClnc1 regulates the apoptosis, migration, and invasion of osteosarcoma cells. MG63 and U2OS cells were transfected with si-NC, si-GClnc1-1, si-GClnc1-2, empty vector, and pcDNA-GClnc1 for 48 h, respectively (**A**-**B**) Cell migration and invasion assay (**C**-**D**) Scratch wound healing assay of cells (**E**-**F**) Cell apoptosis assay. ($n = 3$ per group; ***, $P < 0.001$; **, $P < 0.01$, t-test)

delivering truncated NONO mutants. RIP assay showed that deletion of the two RRM domains of NONO abolished the interaction of NONO with GClnc1, indicating that the RRM domain of NONO mediates the interaction of NONO with GClnc1 (Fig. 3E). It was reported that the ubiquitin ligase FBXW7 could mediate the ubiquitination degradation of NONO [26]. We sought to determine whether GClnc1 stabilized NONO by blocking FBXW7 mediated-ubiquitin degradation. Indeed, co-IP assays showed that overexpression of GClnc1 inhibited the binding of NONO to FBXW7 (Fig. 3F). Consistently, overexpression of GClnc1 reduced the ubiquitination level of NONO mediated by FBXW7, thereby stabilizing the expression of NONO (Fig. 3G). Taken together, these results indicated that GClnc1 reduced the ubiquitin degradation of NONO mediated by FBXW7.

"Rescue" experiments indicate that the GClnc1-NONO pathway regulates OS metastasis and proliferation

To determine whether NONO mediates the tumor-promoting roles of GClnc1 in OS, we performed "rescue" assays in OS cells. While the silence of GClnc1 repressed the cell growth, migration, and invasion, overexpression of NONO in GClnc1-silenced MG63 cells significantly reversed the repression (Fig. 4A, B and C). In addition, the silence of NONO could reverse the increased proliferation, migration, and invasion by GClnc1 transfection in U2OS cells (Fig. 4D, E and F). Collectively, these results indicate that GClnc1 modulates the malignant features of OS cells through NONO.

GClnc1 improves the cell growth of OS in the animal model

To further confirm the roles of GClnc1 in OS, we performed an in vivo assay using a xenograft model. MG63 cells with or without stably silencing GClnc1 were injected subcutaneously into nude mice. The volume and weight of xenograft tumors in the GClnc1-silencing group were significantly smaller than the control group (Fig. 5A and C).

Discussion

OS is a primary bone sarcoma that originates from mesenchymal cells and often occurs in children and young adults [27, 28]. A large number of studies have shown that lncRNA plays a key role in the progression of tumors [29, 30]. Therefore, revealing the pathogenesis of OS from the point of view of lncRNA molecular biology may bring a new dawn for the treatment of OS.

Previous studies have confirmed that GClnc1 plays an important regulator in a variety of human tumor diseases, and it also reflects the clinical value potential in the treatment of tumor diseases [13, 31]. For example, GClnc1 may contribute to the progression of ovarian cancer by regulating the p53 signaling pathway [32].

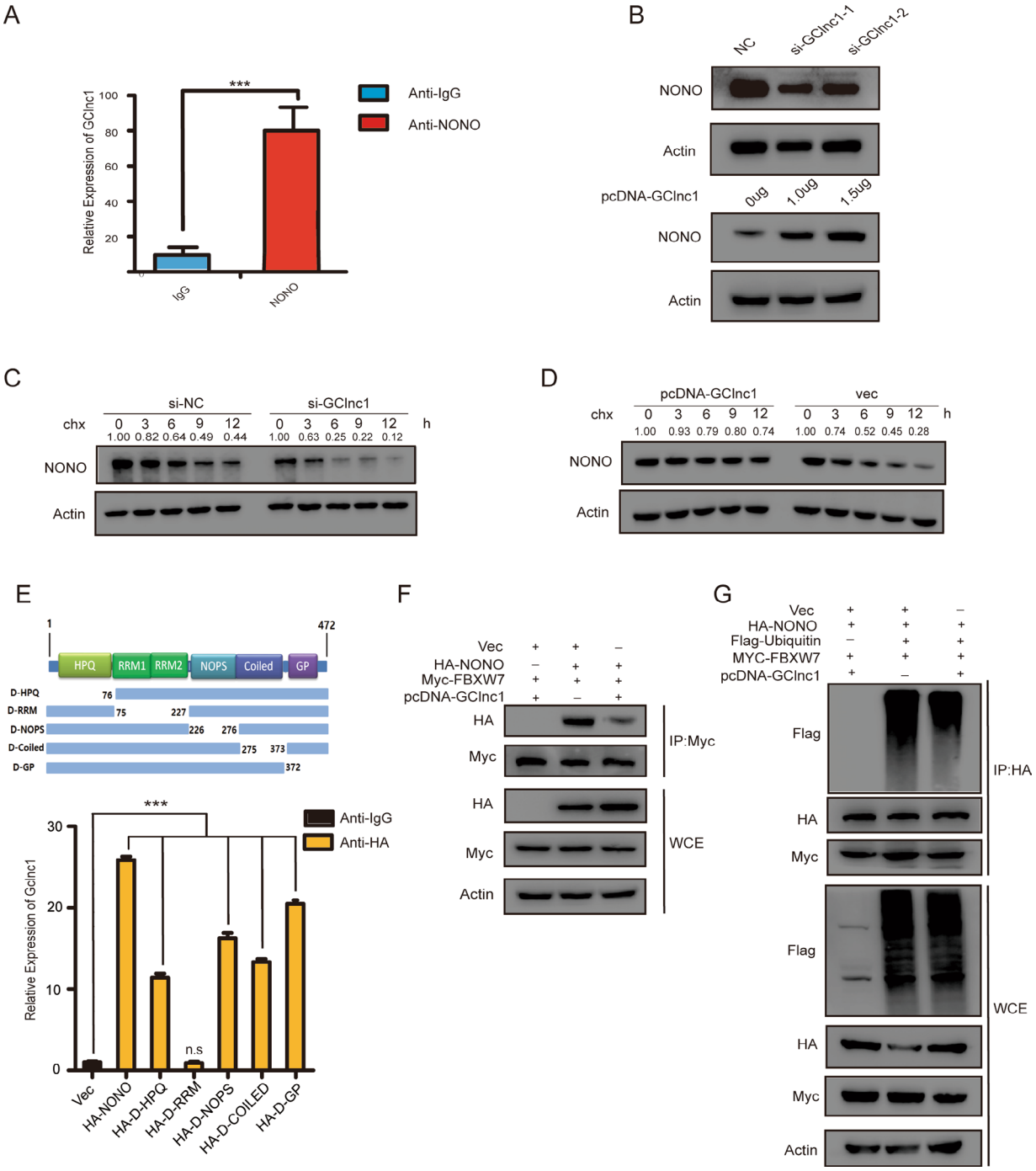

Fig. 3 GClnc1 blocks ubiquitin degradation of NONO regulated by FBXW7 (**A**) In HEK293T cells, the RNA expression of GClnc1 was analyzed by RIP and RT-qPCR with IgG and NONO antibodies (**B**) In MG63 cells, si-GClnc1 reduced the expression of NONO protein. In U2OS cells, overexpression of GClnc1 increased the expression of NONO protein (**C**-**D**) GClnc1 increased the stability of the NONO protein. 293T cells were transfected with si-GClnc1, empty vector, and pcDNA-GClnc1, respectively, and then treated with CHX for the indicated times before harvesting. NONO protein levels were measured by Western blot, and then gray values were calculated by ImageJ software (**E**) Schematic representation of the deletion of different domains in NONO. In 293T cells, HA-tagged NONO and empty plasmid were transfected, and then the RNA expression of GClnc1 was analyzed by RIP and RT-qPCR with IgG and HA-tagged antibodies (**F**) Over-expression of GClnc1 inhibits FBXW7 binding to NONO. HA-tagged NONO, Myc-tagged FBXW7, and pcDNA-GClnc1 were co-transfected into 293T cells. The effect of GClnc1 on the binding of FBXW7 to NONO was verified by coIP and Western blotting. (**G**) Overexpression of GClnc1 hindered the ubiquitination degradation of NONO by FBXW7. HA-tagged NONO, Flag-tagged ubiquitin, Myc-tagged FBXW7, and GClnc1 were co-transfected into 293T cells. Western blotting was used to detect the ubiquitination level of NONO. ($n = 3$ per group; ***, $P < 0.001$; *, $P < 0.05$, t-test). The full-length blots are presented in Supplementary Fig. 1

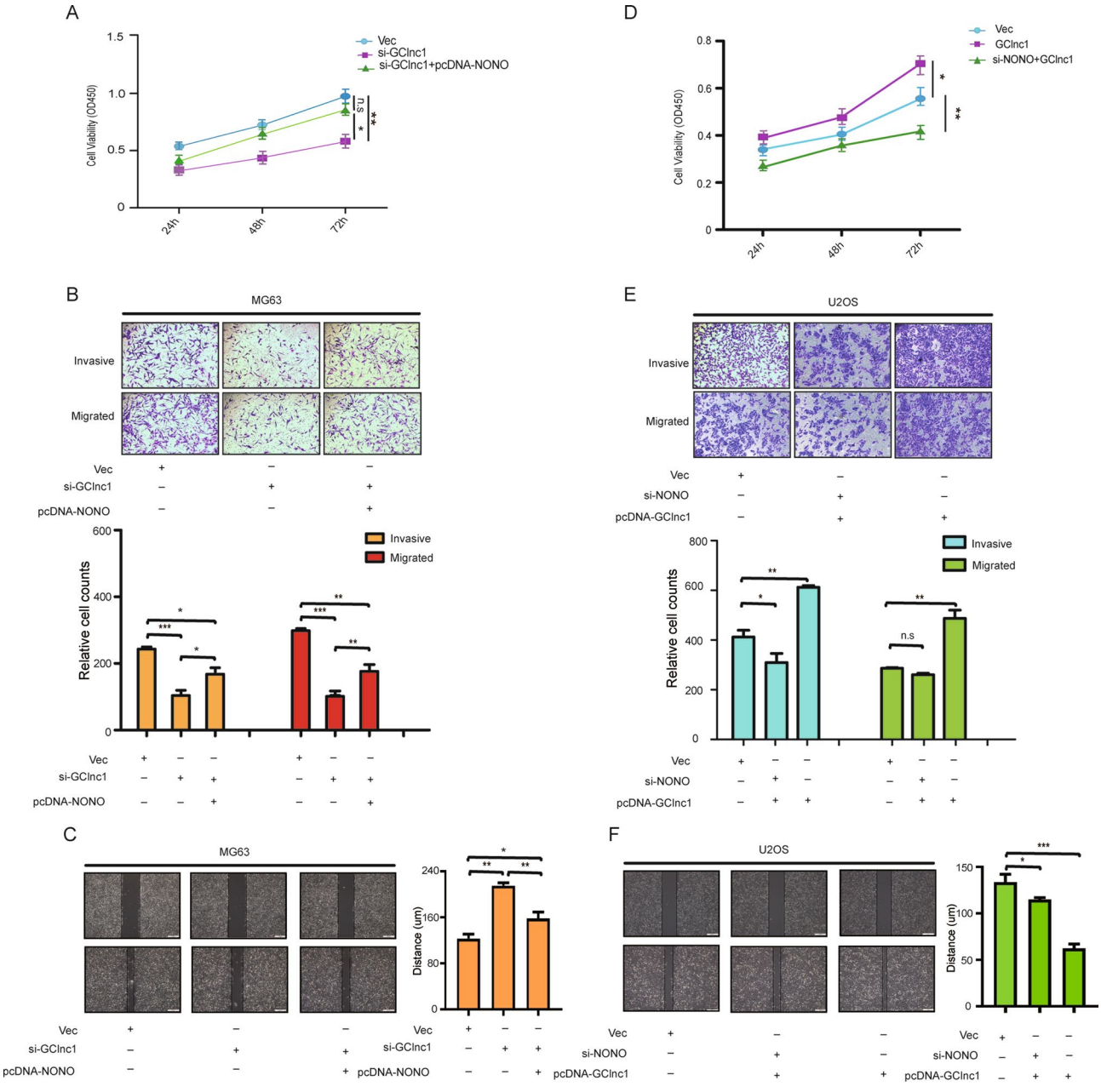

Fig. 4 The GClnc1-NONO pathway regulates OS metastasis and proliferation (**A**-**B**) Investigate the effects of GClnc1 knockdown and NONO overexpression on the proliferation, migration, and invasion of MG63 cells (**C**) Investigate the effects of GClnc1 knockdown and NONO overexpression on the scratch healing of MG63 cells (**D**-**E**) Investigate the effects of NONO knockdown and GClnc1 overexpression on the proliferation, migration, and invasion of U2OS cells (**F**) Investigate the effects of NONO knockdown and GClnc1 overexpression on the scratch healing of U2OS cells. ($n = 3$ per group; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$, t-test)

GClnc1 was also highly expressed in bladder cancer tissue and promotes proliferation and invasion through the activation of MYC [31]. Moreover, GClnc1 could increase the proliferation of colorectal cancer cells by reducing the expression of p21 as well as BAX via the P53 signal pathway [33]. These studies show that GClnc1 is involved in the occurrence and development of human cancer and plays an important role in some biological processes. Nonetheless, the specific way in which GClnc1 operates in OS remains unclear. In this study, we first confirmed the over-expression of GClnc1 promoted the proliferation, migration, and invasion of OS cells and inhibited their apoptosis through a series of functions in vitro. Meanwhile, knocking down GClnc1 got the opposite results.

Related studies have confirmed that NONO plays an essential role in the occurrence and development of malignant tumors. For example, studies have shown that

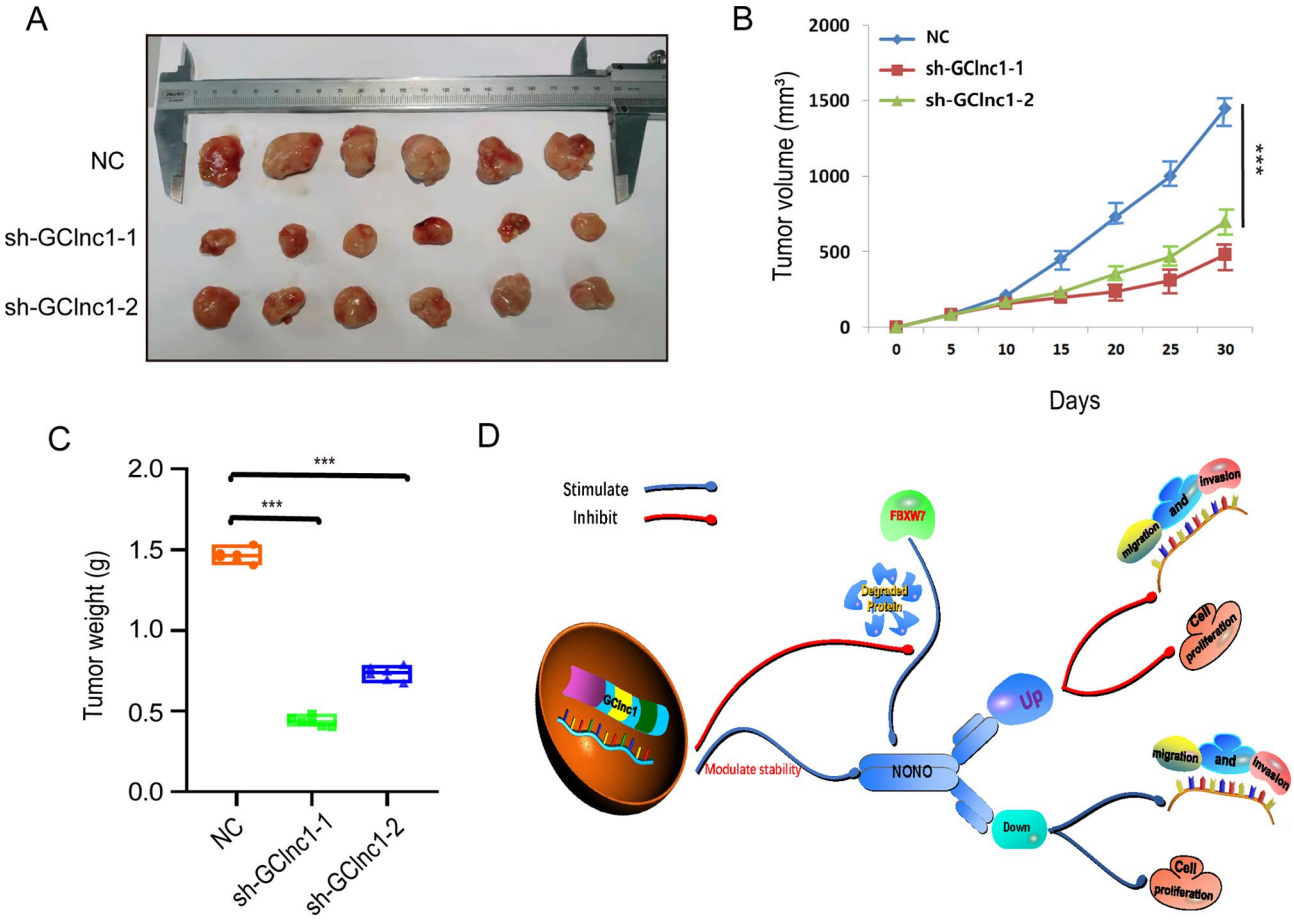

Fig. 5 GClnc1 is essential for tumorigenesis of OS in vivo (**A**) Representative tumors were excised from mice at the end of the experiment on day 30 (**B**) Tumor growth kinetics were measured every 5 days (**C**) Endpoints of tumor weight were measured (**D**) Schematic diagram of lncRNA GClnc1/NONO/FBXW7 axis in OS progression. ($n=6$ per group; ***, $P<0.001$; **, $P<0.01$; *, $P<0.05$, t-test)

NONO is highly expressed in malignant melanoma cell lines and tissues, and participates in early tumor formation, which plays a role in promoting tumor cell proliferation and metastasis [34, 35]. In breast cancer, ZHU et al. found that the expression of NONO in breast cancer tissues was higher than in paracancerous tissues, which was positively correlated with the level of SREBP-1a protein [36]. In addition, NONO regulates the proliferation and metastasis of esophageal squamous cell carcinoma by activating Akt and Erk1/2 signaling pathways [37]. Single-cell transcriptome analysis has revealed that NONO is highly expressed in OS tissues [38]. In brief, these results show that NONO is closely related to tumors and plays a carcinogenic role. In this study, our analysis of the database indicates that GClnc1 can bind to the NONO protein, a finding that we have confirmed through RNA immunoprecipitation (RIP) experiments. Furthermore, we confirmed the interaction between GClnc1 and NONO domain, and GClnc1 can affect the stability of NONO. This shows that the unstable expression of NONO may be an important reason for the proliferation and apoptosis of OS. Of course, we also learned that the ubiquitin degradation pathway of NONO plays an important role in the proliferation and metastasis of OS [39]. Next, we tried to explore the specific mechanism by which GClnc1 regulates the stability of NONO protein, and affects the progression of OS. Through a series of experiments such as RIP, deletion mutation, protein half-life method, etc. we found that NONO, an RNA binding protein, significantly interacts with GClnc1. Meanwhile, GClnc1 also regulates the stability of NONO.

Existing research shows that FBXW7, also known as Fbw7, CDC4, Sel10, or Ago, is a highly conserved F-box family protein from yeast to mammals [40, 41]. FBXW7, together with SKP1, CUL1, and RBX1, forms SCF^{FBXW7} E3 ubiquitin ligase, which specifically binds and degrades substrate proteins by ubiquitin [42]. In OS, researchers found that FBXW7 was down-regulated in OS, and its low expression was associated with malignant clinicopathological features. And the expression of FBXW7 can predict the 5-year survival prognosis of patients with OS [43]. However, the specific mechanism of FBXW7 in

OS is still unclear. Previous studies have reported that NONO can bind to ubiquitin ligase FBXW7 [26]. We speculated that there is a potential regulatory relationship between GClnc1, NONO, and FBXW7. Then, we adopted experimental methods such as RIP and ubiquitin analysis in vitro. Our results show that GClnc1 hinders the binding of NONO and FBXW7, and reduces the FBXW7-mediated degradation of NONO ubiquitin modification, resulting in increased NONO expression, which promotes proliferation, migration, and invasion (Fig. 5D). However, our study also has limitations. While we experimentally demonstrated that GClnc1 plays a role in OS, this finding requires further validation in clinical specimens from patients diagnosed with OS.

In summary, we have clarified the function of GClnc1 in the progression of OS and it regulates the malignant progression of OS through the NONO/FBXW7 pathway. These results show that GClnc1 plays a key role as a cancer-promoting molecule in OS. In the future treatment of OS, it is expected to bring potential treatment hope for clinical work as a new target.

Abbreviations

OS	Osteosarcoma
lncRNA	Long non-coding RNA
qRT-PCR	Real-time quantitative PCR
RIP	RNA co-Immunoprecipitation

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-024-13138-0.

Supplementary Materials 1

Acknowledgements

None.

Author contributions

JFZ, XBL and ZPZ designed the experiments and wrote the paper. JFZ, CG and XHL performed the western blotting assays, and co-IP, and interpreted the data. JFZ, ZZD, XFT, FFZ and QHJ performed in vitro cell line studies and the acquisition of data. JFZ, XHL, CG, SFL and LZ performed a Xenograph assay, and assist with data analysis. All authors reviewed the manuscript.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on request.

Declarations

Ethics approval and consent to participate

Ethics committee belonging to the Third Affiliated Hospital of Nanchang University ratified the current study (grant number: KY2023009). The experiment was approved by the Animal Experiment Animal Use Committee of Jiangxi University of Traditional Chinese Medicine. All animal experiments were approved by the Animal Protection and Use Committee, and the experimental procedures were approved by guidelines. All methods were carried out in accordance with relevant guidelines and regulations. All methods were performed in accordance with the ARRIVE guidelines for reporting animal experiments.

Competing interests

The authors declare no competing interests.

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