Neoplasma 2021; 68(5): 1052–1062

doi:10.4149/neo_2021_210121N100

An oncogenic lncRNA, GLCC1, promotes tumorigenesis in gastric carcinoma by enhancing the c-Myc/IGF2BP1 interaction

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Received January 21, 2021 / Accepted March 29, 2021

Accumulating evidence has shown that long non-coding RNAs (lncRNAs) are vital regulators of the expression of various genes in multiple human diseases. The aim of this study was to investigate the role of glycolysis-associated lncRNA of colorectal cancer (GLCC1) in the progression of gastric carcinoma as well as the underlying mechanism. The expression levels of GLCC1 and c-Myc were determined in 47 pairs of gastric carcinoma tissues and cell lines using quantitative real-time polymerase chain reaction (qRT-PCR). Next, the functional roles of GLCC1 and c-Myc in the proliferation, apoptosis, migration, and invasion of gastric carcinoma cells (BGC823 and SGC7901 cells) were determined by siRNA-mediated knockdown of these molecules, and the cells were evaluated by Cell Counting Kit-8 (CCK-8), flow cytometry, and Transwell assays. In addition, RIP and RNA pull-down assays were used to examine the interaction between GLCC1 and c-Myc/IGF2BP1. Further mechanistic studies were conducted using western blotting. lncRNA GLCC1 and c-Myc were observed to be significantly increased in both gastric carcinoma tissues and cell lines. Knockdown of GLCC1 or c-Myc suppressed cell proliferation, migration, and invasion but promoted apoptosis in both the BGC823 and SGC7901 cell lines. Mechanistically, c-Myc was identified as a downstream regulator involved in the GLCC1-mediated biological effects in gastric carcinoma. The RNA pull-down and RIP assays further showed that the upregulation of lncRNA GLCC1 enhanced the interaction of the IGF2BP1 protein with c-Myc mRNA, thus promoting the stabilization of c-Myc mRNA. Altogether, we demonstrated that lncRNA GLCC1 modulates gastric cancer cell migration and invasion by enhancing the c-Myc/IGF2BP1 interaction, and lncRNA GLCC1 may serve as a potential therapeutic target for preventing the development and progression of human gastric carcinoma.

Key words: lncRNA GLCC1, IGF2BP1, c-Myc, gastric carcinoma, migration, invasion

Gastric carcinoma is a common malignant tumor of the alimentary system and the second leading cause of tumor-related death worldwide [1, 2]. Due to the absence of obvious symptoms, most patients with gastric carcinoma are not diagnosed until the late stage, and the prognosis is poor [3]. It has been reported that the median survival time of patients suffering from advanced gastric carcinoma is usually less than one year [4]. The occurrence of gastric carcinoma is a complex biological process caused by the imbalance of the expression of various tumor-associated genes. Hence, a better understanding of the pathogenesis of cancer and further exploration of the potential mechanisms underlying gastric carcinoma occurrence and development are crucial for improving the prevention, diagnosis, and treatment of gastric carcinoma.

Long non-coding RNAs (lncRNAs) are non-coding single-stranded RNAs that contain over 200 nucleotides and have the limited protein-coding potential [5]. Recently, accumulating evidence has demonstrated that lncRNAs function as crucial players in regulating gene expression at the epigenetic, transcriptional, and post-transcriptional levels. Additionally, lncRNAs also participate in cell development and differentiation, and many other biological processes [6]. There is increasing evidence that lncRNAs are involved in regulating the pathogenesis of tumor growth, metastasis, and angiogenesis through multiple mechanisms, thus promoting the occurrence and development of malignant tumors [7]. Many lncRNAs, such as HOTAIR, PCAT-1, and MALAT1, have been proven to be key regulators of the development and progression of many cancers. Zhang et al. [8] reported that the upregulation of HOTAIR contributed to the malignant progression of ovarian cancer cells. Zhen and his colleagues [9] found that higher expression of PCAT-1 in esophageal cancer tissues led to increased tumor growth and chemoresistance. In addition, Luan et al. [10] also demonstrated that the aberrant upregulation of MALAT1, which

can act as a competing endogenous RNA, facilitated malignant melanoma growth and metastasis by sponging miR-22. Thus, these findings indicated that lncRNAs might function as vital mediators in the development of various cancers.

lncRNA GLCC1 is significantly upregulated in colorectal cancer cells under glucose starvation conditions, supporting cell survival and proliferation by enhancing glycolysis [11]. GLCC1 exerts its effects on glycolysis and proliferation in a c-Myc-dependent manner. c-Myc has been extensively shown to regulate glucose metabolism as a critical oncogene in metabolic reprogramming; thus, c-Myc is a key switch in regulating the metabolic activity of cancer cells. In addition, many c-Myc target genes are essential for cell growth and cancer progression. However, the biological functions and potential role of GLCC1 in the development of gastric carcinoma remain elusive.

The IGF2BP family proteins (also named IGF-II mRNA-binding proteins [IMPs], coding region instability determinant-binding proteins [CRD-BPs], and zipcode-binding proteins [ZBPs]) include IGF2BP1/2/3 and play important roles in the regulation of translation/turnover of their target transcripts [12]. In addition to regulating the translation of its target mRNAs, IGF2BP1 can also promote mRNA stabilization. Shang et al. demonstrated that lncRNA THOR acted as a retinoblastoma promoter by enhancing the interaction of c-Myc mRNA and the IGF2BP1 protein [13]. The c-Myc gene, discovered as a cellular homolog of the retroviral gene v-Myc, functions as a transcription factor and regulates the expression of genes involved in proliferation, differentiation, metabolism, survival, and apoptosis [14]. c-Myc promotes immortalization by driving unrestricted cell division and proliferation, which is strongly associated with the development of various malignant tumors [15]. Yang et al. proved that c-Myc expression in gastric carcinoma tissues was significantly higher than that in control tissues [16]. However, whether there is a correlation between lncRNA GLCC1 and c-MYC in gastric carcinoma, and the potential molecular mechanism, has not been reported.

Herein, the biological roles of lncRNA GLCC1 and c-Myc/IGF2BP1 in gastric carcinoma were investigated. Our findings showed that lncRNA GLCC1 and c-Myc were significantly upregulated in gastric carcinoma tissues compared with the corresponding non-tumor gastric tissues, knockdown of these molecules inhibited cell proliferation, migration, and invasion *in vitro*. Further studies suggested that lncRNA GLCC1 stimulates gastric cancer metastasis by enhancing the c-Myc/IGF2BP1 interaction, and lncRNA GLCC1 may serve as an oncogene involved in the evolution and aggressive progression of gastric carcinoma.

Table 1. qRT-PCR primer sequences.

Genes	Primers	Sequences
GLCC1	Forward	5'-CAGAGAAGGACTGTTTTCCA-3'
	Reverse	5'-CATCCAAGTATCACTATTCCCA-3'
c-Myc	Forward	5'-GTCAAGAGGCGAACACACAAC-3'
	Reverse	5'-TTGGACGGACAGGATGTATGC-3'
GAPDH	Forward	5'-GGAGCGAGATCCCTCCAAAAT-3'
	Reverse	5'-GGCTGTTGTCATACTTCTCATGG-3'

Materials and methods

Patients and specimens. Gastric carcinoma tissues and para-carcinoma tissues (>2 cm from the tumor) were obtained as clinical specimens from 47 gastric cancer patients who underwent surgery in Ganzhou People's Hospital affiliated with Nanchang University from 2012 to 2017. All the patients were diagnosed by pathology, and they did not receive chemotherapy or radiotherapy before the operation. The tissues were stored in liquid nitrogen for subsequent analysis. The research was approved by the Medical Ethics Committee of Ganzhou People's Hospital affiliated with Nanchang University, and all patients provided consent before the research.

Cell culture. Human gastric carcinoma cell lines (BGC-823 and SGC-7901) and a normal gastric epithelium cell line (GES-1) were purchased from the Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences (Shanghai, China). The cells were incubated in RPMI 1640 or DMEM (Gibco-BRL) containing 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 mg/ml streptomycin in humidified air at 37 °C with 5% CO_2. After 2–3 stable passages, cells in the logarithmic phase of growth were used for the subsequent experiments.

Cell transfection. Cells were transfected with specific siRNA oligonucleotides using Lipofectamine® 3000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. siRNAs (including si-GLCC1, si-c-Myc, and the negative control si-NC) were synthesized by Invitrogen to specifically silence the expression of GLCC1 or c-Myc. To construct the c-Myc-overexpressing plasmid, c-Myc cDNA was inserted into p-MSCV-IRES-GFP. For transfection, the cells were seeded in 6-well plates and cultured for 24 h until they reached 70–80% confluence. Then, the cells were transfected with 2 μg of plasmid using Lipofectamine 3000 (Invitrogen, USA) according to the manufacturer's protocol.

RNA extraction and qRT-PCR. Total RNA was extracted from tissues or cultured cells using TRIzol Reagent (Invitrogen, USA) according to the manufacturer's protocol. The First-Strand cDNA Synthesis Kit (Takara, Dalian, China) was used for reverse transcription with random primers. Then, qRT-PCR was performed using SYBR Premix Ex Taq (Takara, USA) according to the manufacturer's instructions. The results were normalized to the expression of GAPDH. An ABI 7500 real-time PCR system (Applied Biosystems, USA) was used for data analysis. Each trial was performed three times (Table 1).

Cell proliferation ability. The cell proliferation capacity was determined using Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Japan). Transfected cells (BGC823

and SGC7901 cells) were cultured in 96-well plates (5×10^3 cells/well). Then, CCK-8 reagents were added to each well at 0, 24, 48, 72, and 96 h, and the cells were further cultured at 37 °C for 2 h. The absorbance at 450 nm was measured by a microplate reader. All the experiments were conducted three times.

Flow cytometry analysis. Transfected cells were harvested by trypsinization and stained with Annexin V-FITC and propidium iodide (PI) according to Annexin V-FITC/PI apoptosis detection kits (BD Biosciences). Next, flow cytometry (FACScan; BD Biosciences) was performed to assess cell apoptosis.

Cell migration and invasion assay. Cell migration analysis was performed using Transwell insert chambers (8 μm pore size, Corning, USA). Approximately 2×10^4 cells were plated into the upper chambers without serum in triplicate. For the invasion assay, 24-well Transwell plates with Matrigel-coated membranes with 8.0 μm pores (BD Bioscience, USA) were used to detect the cell invasion capacity. A total of 2×10^5 cells were seeded into the upper chambers without serum in triplicate. The lower chamber was filled with medium supplemented with 10% FBS as a chemical attractant. After 24 h of cultivation, the cells in the upper chambers that did not migrate or invade were removed with cotton swabs, and the cells that had transferred or invaded onto the lower surface of the membrane were fixed by cold methanol and dyed with 0.1% crystal violet. All the migrated and invaded cells in at least ten random regions of every membrane were observed and counted by light microscopy.

Western blot assay. Total proteins were extracted from cells by using the mammalian protein extraction reagent RIPA (Beyotime, Haimen, China) containing 1% protease inhibitor cocktail (Roche, Diagnostics GmbH, Mannheim, Germany) and PMSF (Roche). Then, the protein extracts were separated by 10% SDS-PAGE and transferred to 0.22 μm nitrocellulose membranes (Sigma-Aldrich). The membranes were blocked with 5% milk in Tris-buffered saline for 1 h at 25 °C, incubated with specific primary antibodies (#8482 anti-IGF2BP1, #13116 anti-N-cadherin, #14472 anti-E-cadherin, #5741 anti-Vimentin, #3871 anti-Snail1, and #9585 anti-Slug, diluted to 1:1000, Cell Signaling Technology) overnight at 4 °C, and then incubated with secondary antibodies (#56970 Goat Anti-Mouse HRP Conjugate; #7074 Anti-rabbit IgG HRP-linked Antibody diluted 1:5000, Santa Cruz Biotechnology) for 2 h at 37 °C. Finally, the protein bands of interest were visualized by the Bio-Rad imaging system.

Subcellular fractionation. Nuclear and cytosolic fractions were separated using the PARIS kit™ (Am1921; Thermo Fisher Scientific, USA) according to the manufacturer's instructions. Quantitative real-time PCR was used to detect the expression levels of GAPDH, U6, and GLCC1.

RNA immunoprecipitation (RIP) assay. RIP assay was performed with the Magna RIP RNA-Binding Protein Immunoprecipitation Kit (Millipore, MA, USA) according to the operation manual. The extracts from BGC823 and SGC7901 cells were incubated with protein-A Sepharose beads that had been preincubated with anti-IGF2BP1 antibodies or IgG at 4 °C for 2 h. Then, the beads were washed with RIP washing buffer 6 times. The purified RNA was used for qRT-PCR analysis.

RNA pull-down assay. RNA pull-down assays were used to verify the interaction of c-Myc RNA with the IGF2BP1 protein with the Pierce™ Magnetic RNA-Protein Pull-Down Kit (Thermo Fisher Scientific, MA, USA). Accordingly, the 3'-UTR RNA of c-Myc and 50 pmol of poly(A) RNA were labeled with desthiobiotinylated cytidine bisphosphate and T4 RNA ligase. Subsequently, before first washing the beads using 20 mM Tris (pH 7.5) and then with Protein-RNA Binding Buffer, the labeled RNA was incubated with 50 μl of streptavidin magnetic beads. The beads were incubated with A431 extract (40 μg) for 45 min at 4 °C, incubated with biotin elution buffer for 15 min at 37 °C, and eluted. An antibody against IGF2BP1 (No. ab82968, Abcam, MA, USA) was utilized to determine the specificity of the RNA pull-down assay by western blotting.

Co-immunoprecipitation (Co-IP) assay. The RIP assay was carried out as described in a previous study [17]. In brief, cells were lysed with 25 mM Tris-HCl buffer (pH 7.5) containing 1 mM EDTA, 5% (v/v) glycerol, 1% (v/v) Nonidet P-40, 150 mM NaCl, and 100 U/ml RNase inhibitor (Promega; MA, USA). Then, the extracts were incubated with protein-A sepharose beads that had been preincubated with 3 μg of anti-IGF2BP1 antibody/IgG at 4 °C for 2 h. Subsequently, the beads were incubated with 20 U of RNase-free DNase I (Invitrogen) for 20 min at 37 °C and further incubated in NT2 buffer containing 0.1% SDS (v/v) and 0.5 g/l proteinase K for 25 min at 55 °C. Finally, the RNA in the IP materials was assessed by RT-PCR analysis.

Statistical analysis. The SPSS 17.0 software package (IBM, Chicago, IL) was used for statistical analysis. The results are shown as the mean ± standard deviation (SD). When comparing two groups, Student's t-test was performed to calculate differences; when comparing multiple groups, one-way ANOVA was performed. Differences were significant when the p-value <0.05.

Results

The expression levels of GLCC1 and c-Myc were upregulated in gastric carcinoma tissues and positively correlated. The expression levels of GLCC1 and c-Myc in 47 paired gastric carcinoma tissues and para-carcinoma tissues were detected by qRT-PCR and western blot assays, respectively. As shown in Figures 1A and 1B, the levels of GLCC1 and c-Myc were significantly upregulated in the gastric carcinoma tissues compared with the control tissues. Consistently, the protein level of c-Myc was also increased in the gastric carcinoma tissues (Figure 1C). Furthermore, the Pearson correlation analysis data revealed that there was a positive

correlation between the GLCC1 and c-Myc expression levels in the gastric cancer tissues.

Knockdown of GLCC1 inhibited the proliferation, migration, and invasion of gastric carcinoma cells. To explore the potential biological processes in which GLCC1 is involved, the expression pattern of GLCC1 was detected in a normal gastric epithelial cell line (GES-1 cells) and in gastric carcinoma cell lines (BGC823 and SGC7901 cells). First, qRT-PCR assays showed that GLCC1 was more highly expressed in the BGC823 and SGC7901 cells than in the GES-1 cells (Figure 2A). Next, to further ascertain the function of high GLCC1 expression in the development of gastric carcinoma, two effective siRNA sequences targeting GLCC1 (si-GLCC1 #1 and si-GLCC1 #2) were used to assess the biological effects. The qRT-PCR analysis demonstrated that the expression of GLCC1 was significantly decreased in the si-GLCC1 groups compared to the si-NC group (Figure 2B). Moreover, the CCK-8 analysis revealed that knockdown of GLCC1 significantly inhibited cell viability in both the BGC823 and SGC7901 cell lines (Figure 2C). Moreover, flow cytometry analysis indicated that downregulation of GLCC1 promoted apoptosis in both the BGC823 and SGC7901 cell lines (Figure 2D). In addition, the effects of GLCC1 on the migration and invasion of BGC823 and SGC7901 cells were also examined using Transwell assays. The results showed that the number of migratory and invasive cells was markedly decreased after transfection with si-GLCC1 #1 or si-GLCC1 #2 compared to si-NC (Figures 2E, 2F). These findings suggested that lncRNA GLCC1 might function as an oncogene in the development of gastric carcinoma.

Knockdown of c-Myc inhibited the proliferation, migration, and invasion of gastric carcinoma cells. To determine the function of high expression of c-Myc in the development of gastric carcinoma, we applied siRNA to silence the c-Myc expression for *in vitro* investigation. As expected, c-Myc expression was also increased in the gastric carcinoma cell lines (BGC823 and SGC7901 cells) compared with the gastric epithelial cell line (GES-1 cells) (Figure 3A). The qRT-PCR analysis of cells transfected with c-Myc siRNA confirmed that the c-Myc expression was downregulated (Figure 3B). Similarly, mechanistic experiments also showed that knockdown of c-Myc inhibited viability in both the BGC823 and SGC7901 cell lines (Figure 3C). Next, flow cytometry analysis revealed that the number of apoptotic cells was notably increased in the si-c-Myc group (Figure 3D). Furthermore, the cell migration and invasion capacities were also determined. We demonstrated that the number of migratory and invasive cells was significantly reduced in the cells transfected with si-c-Myc compared to the cells transfected with si-NC (Figures 3E, 3F), which was similar to GLCC1 knockdown results.

c-Myc participates in lncRNA GLCC1-mediated biological effects. To reveal the underlying correlation between GLCC1 and c-Myc in gastric carcinoma, further experiments were carried out. As shown in Figure 4A, the mRNA expression of c-Myc was downregulated with GLCC1 knockdown and significantly upregulated with GLCC1 overexpression. In addition, western blot analysis of the c-Myc protein levels showed similar results (Figure 4B). To further verify whether c-Myc is involved in GLCC1-mediated biological effects,

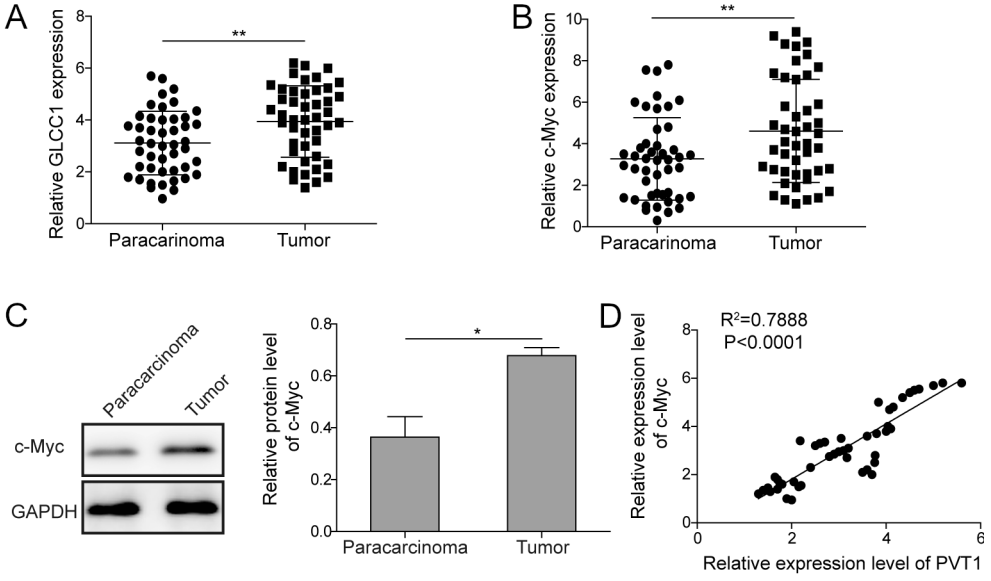

Figure 1. Expression of GLCC1 and c-Myc in gastric carcinoma tissues. A, B) Detection of the relative expression of GLCC1 and c-Myc in gastric carcinoma tissues and paracarcinoma tissues (N=47, respectively) via qRT-PCR assay. C) Detection of the relative expression of c-MYC in gastric carcinoma tissues and paracarcinoma tissues via western blot analysis. D) Pearson's correlation analysis of GLCC1 and c-Myc expression in gastric carcinoma tissues was performed. *p<0.05, **p<0.01

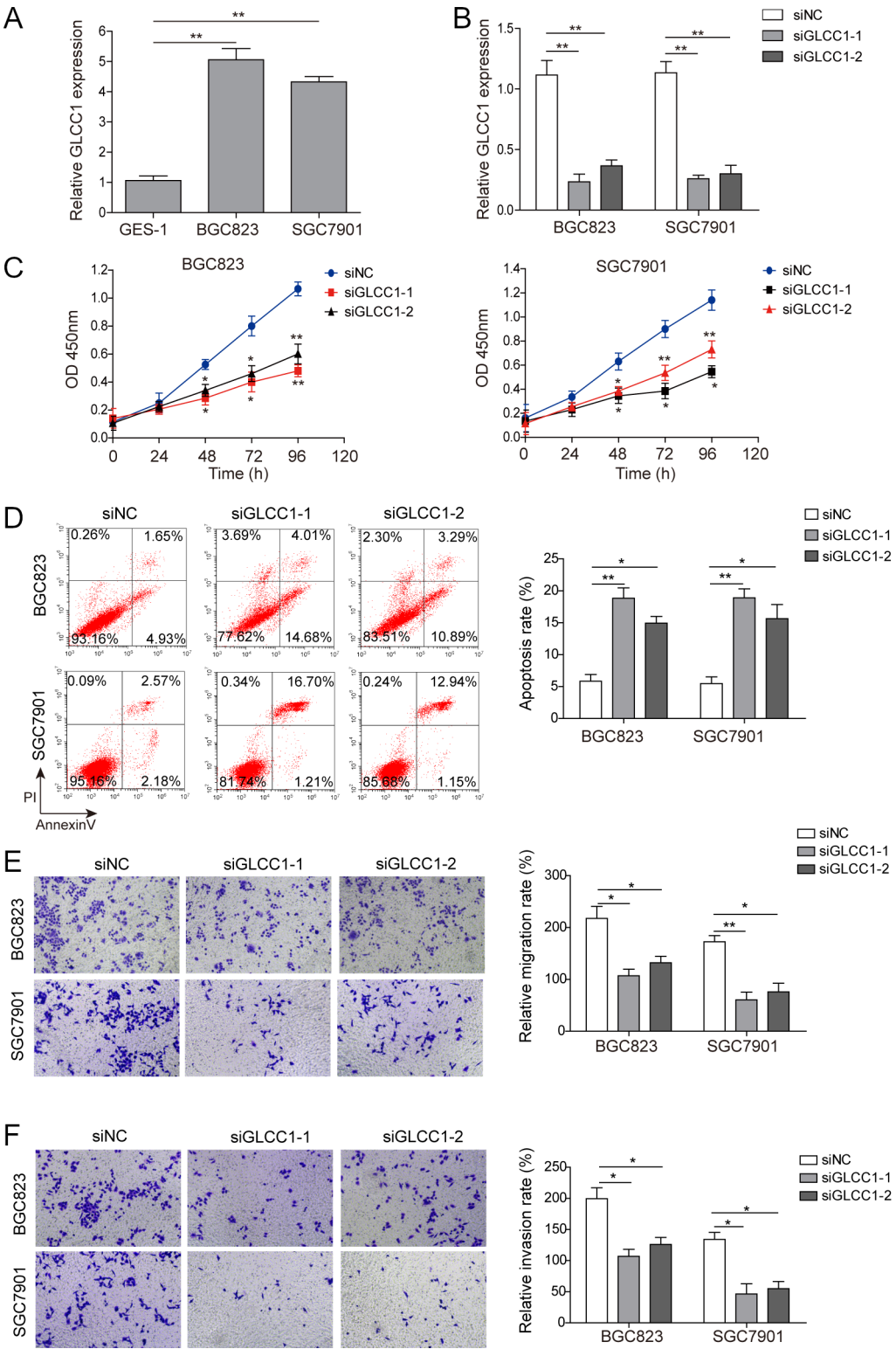

Figure 2. Effects of GLCC1 on the proliferation, apoptosis, migration, and invasion of gastric carcinoma cells. A) GLCC1 expression in gastric carcinoma cell lines (BGC823 and SGC7901 cells) compared to that in a gastric epithelial cell line (GES-1 cells) was measured using the qRT-PCR assay. B) qRT-PCR was performed to measure the relative expression of GLCC1 in BGC823 and SGC7901 cells after transfection with si-GLCC1 (si-GLCC1# and si-GLCC1#) or si-NC. C) A CCK-8 assay was performed to assess the viability of BGC823 and SGC7901 cells after transfection with si-GLCC1 or si-NC. D) Cell apoptosis was assessed by flow cytometry. E, F) Cell migration and invasion were assessed by Transwell analysis. *p<0.05, **p<0.01

we transfected GLCC1-overexpressing gastric carcinoma cells with si-c-Myc. The results of Transwell analysis demonstrated that overexpression of GLCC1 promoted cell migration and invasion, but this effect was blocked by silencing c-Myc (Figures 4C, 4D). Taken together, these data further suggested that c-Myc might be a downstream target of GLCC1 in regulating the development of gastric carcinoma.

lncRNA GLCC1 enhanced the c-Myc/IGF2BP1 interaction. Localization of lncRNAs within the cell is the primary determinant of their molecular functions. The subcellular localization of GLCC1 in BGC823 and SGC7901 cells was detected. GLCC1 was distributed in both the cytoplasm and nucleus in the BGC823 and SGC7901 cells and was mainly present in the cytoplasm (Figure 5A). Furthermore, we performed RNA pull-down and RIP assays to evaluate whether lncRNA GLCC1 enhanced the interaction between the IGF2BP1 protein and c-Myc mRNA. As shown in Figure 5B, the upregulation of lncRNA GLCC1 significantly enhanced the interaction between the IGF2BP1 protein and c-Myc mRNA (Figure 5B), which also enhanced the recruitment of c-Myc mRNA to the IGF2BP1 protein (Figure 5C). In addition, the qRT-PCR assays verified that IGF2BP1 overexpression inhibited the degradation of c-Myc mRNA (Figure 5D). Affinity pull-down assays showed that IGF2BP1 can physically bind to c-Myc mRNA (Figure 5E). These findings suggested that lncRNA GLCC1 induces the upregulation of c-Myc by enhancing the c-Myc/IGF2BP1 interaction.

lncRNA GLCC1 enhances the stability of c-Myc mRNA via IGF2BP1 in GC. To further investigate the mechanism by which lncRNA GLCC1/c-Myc/IGF2BP1 regulates gastric carcinoma progression, western blot analysis was performed to examine the expression of IGF2PB1 in cells with GLCC1 overexpression or knockdown. The data indicated the positive regulation of the IGF2BP1 protein levels by GLCC1 (Figure 6A). Furthermore, the qRT-PCR assays also demonstrated that the upreg-

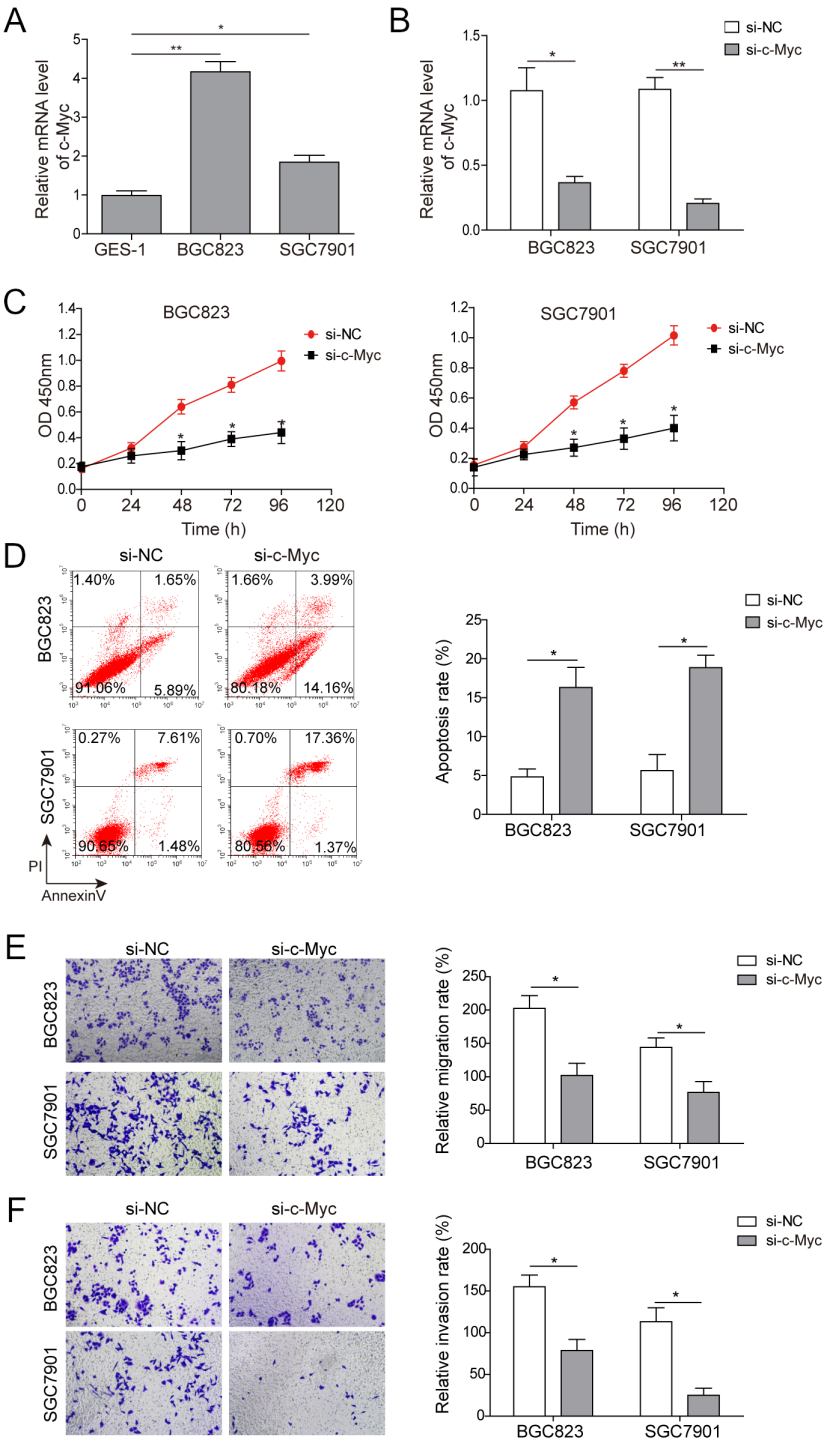

Figure 3. Effects of c-Myc on the proliferation, apoptosis, migration, and invasion of gastric carcinoma cells. A) c-Myc expression in gastric carcinoma cell lines (BGC823 and SGC7901 cells) was compared to that in a gastric epithelial cell line (GES-1 cells) via qRT-PCR. B) The qRT-PCR assays were performed to measure the relative expression of c-Myc in BGC823 and SGC7901 cells after transfection with si-c-Myc or si-NC. C) A CCK-8 assay was used to measure the viability of BGC823 and SGC7901 cells after transfection with si-c-Myc or si-NC. D) Cell apoptosis was assessed by flow cytometry. E, F) Cell migration and invasion were assessed by Transwell assays. *p<0.05, **p<0.01

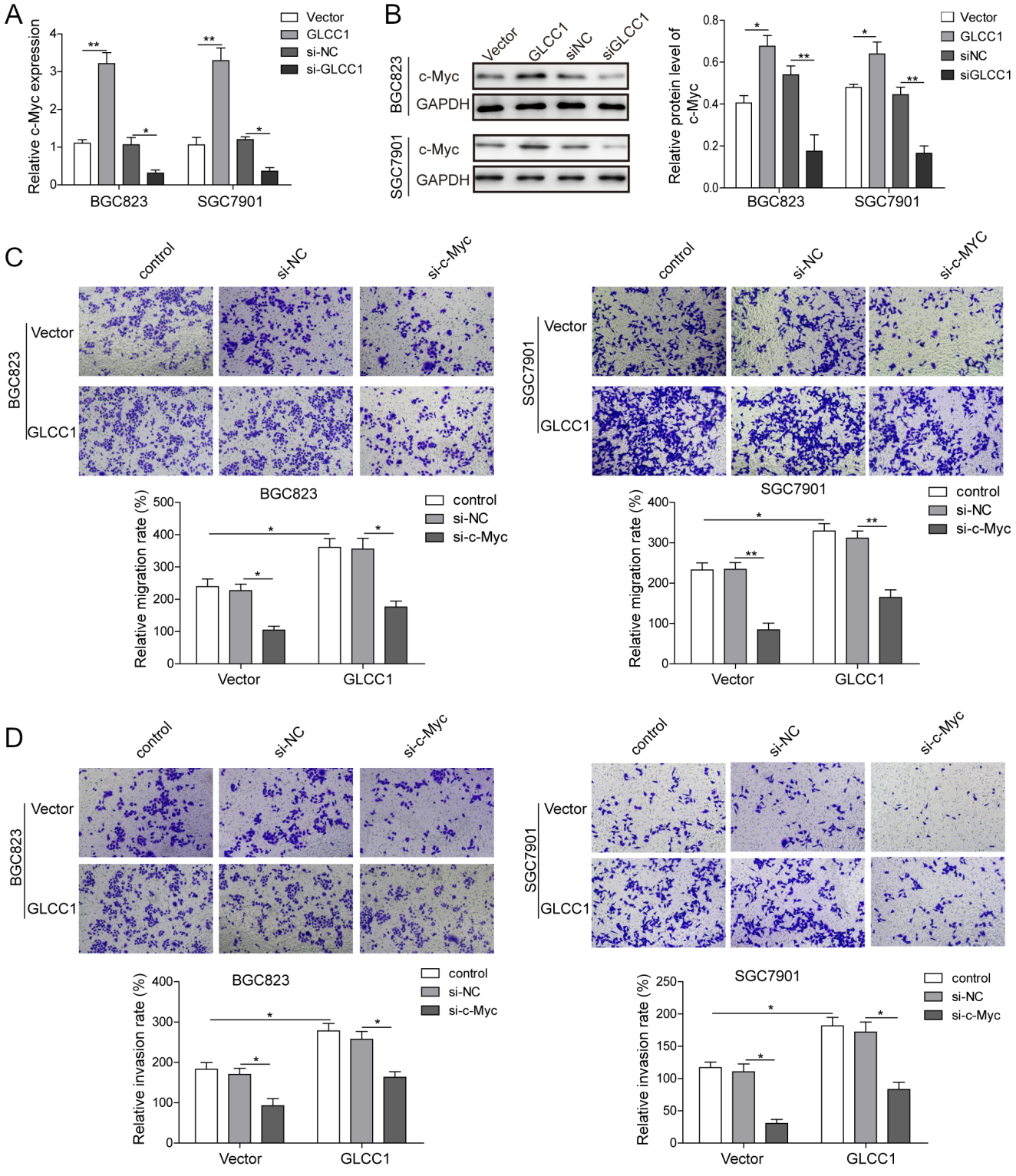

Figure 4. Verification of the functions of GLCC1 and c-Myc in gastric carcinoma cells. A, B) qRT-PCR assay and western blot analysis were performed to detect the mRNA and protein levels of c-Myc, respectively, in gastric carcinoma cell lines (BGC823 and SGC7901) with GLCC1 knockdown and overexpression. C, D) The cell migration and invasion capacities of si-c-Myc-transfected cells overexpressing GLCC1 were determined using Transwell assays. *p<0.05, **p<0.01

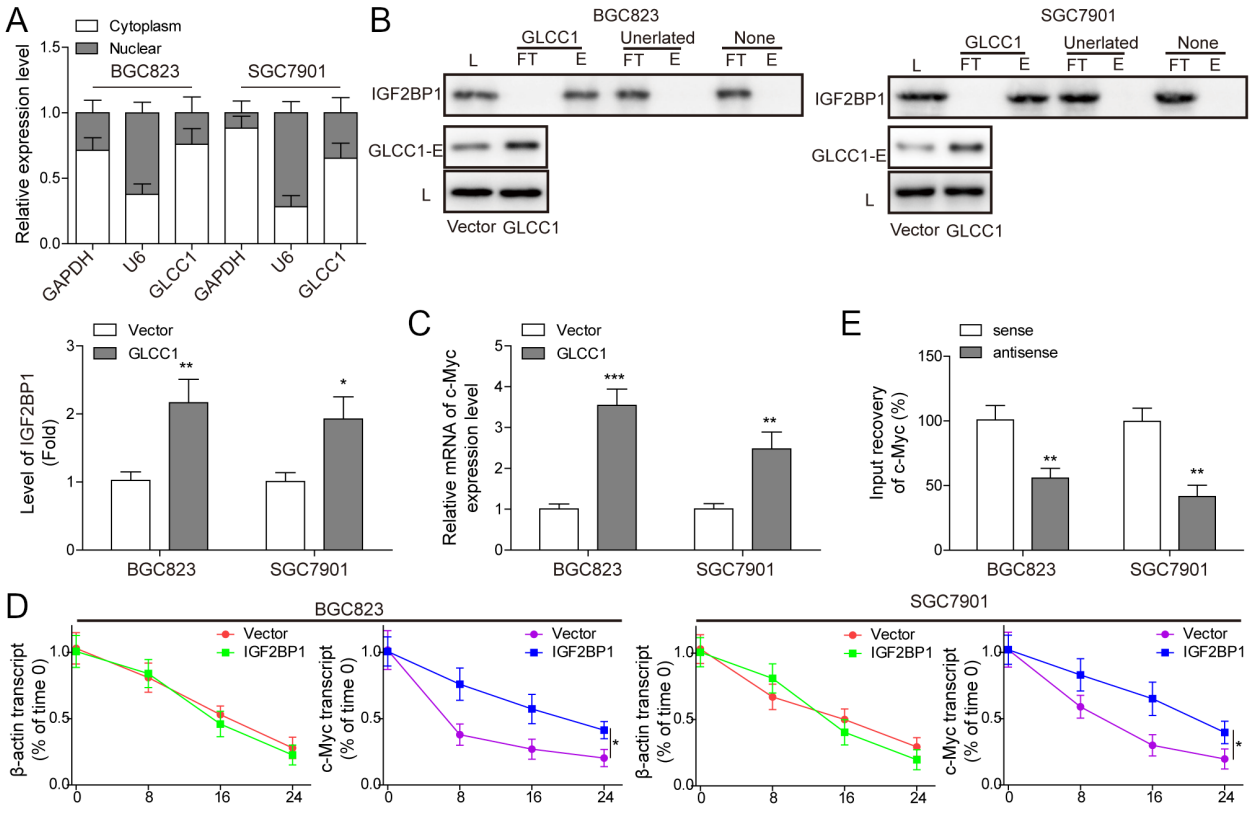

Figure 5. lncRNA GLCC1 enhanced the c-Myc/IGF2BP1 interaction. A) The subcellular localization of GLCC1 in BGC823 and SGC7901 cells was detected. B) RNA pull-down assay was performed to verify the regulatory mechanism of lncRNA GLCC1/c-Myc/IGF2BP2, and the data showed that the upregulation of lncRNA GLCC1 markedly strengthened the level of the IGF2BP1 protein bound to c-myc mRNA. C) RIP assay was used to validate the interaction between c-Myc mRNA and the IGF2BP1 protein. D) qRT-PCR assay was conducted to determine the regulatory effect of IGF2BP1 on c-Myc mRNA, and the results showed that IGF2BP1 overexpression inhibited the degradation of c-Myc mRNA. E) Affinity pull-down assays showed that IGF2BP1 can physically bind to c-Myc mRNA. *p<0.05, **p<0.01, ***p<0.001

ulation of lncRNA GLCC significantly promoted the mRNA expression of c-Myc, but this effect was abolished by silencing IGF2BP1 (Figure 6B). In addition, epithelial-mesenchymal transition (EMT) is an important mechanism associated with human cancer metastasis [18]. To further confirm whether GLCC1 could affect gastric carcinoma metastasis via EMT, western blot analysis was also used to assess EMT-related signaling molecules in gastric carcinoma cell lines (BGC823 and SGC7901 cells). The results showed that the upregulation of GLCC1 increased the expression of N-cadherin, Vimentin, Snail1, and Slug but reduced the expression of E-cadherin (Figure 6C). However, these positive effects of GLCC1 overexpression were reversed by silencing IGF2BP1 (Figure 6C). Thus, these results further supported the role of GLCC1 in gastric carcinoma metastasis.

Discussion

Gastric carcinoma is the fifth most frequently diagnosed cancer in the world [19]. Despite improvements in surgical techniques and chemotherapy, cancer is still highly lethal, and little progress has been made in improving overall survival [4]. With the development of biotechnology, bio-targeted therapies have become important methods for the treatment of gastric carcinoma. Currently, identifying new targets is critical for the treatment of gastric carcinoma to improve the survival rate of patients.

Recently, increasing evidence has shown that several lncRNAs are involved in the tumorigenesis, metastasis, prognosis, and drug resistance of gastric carcinoma [16, 20, 21]. GLCC1 is an oncogenic lncRNA in colorectal cancer. GSEAs have demonstrated that cell proliferation and glycolytic pathways in cancer are significantly enriched in response to changes in GLCC1 in colorectal cancer patient datasets. In cultured colorectal cancer cells and xenograft mouse models, the downregulation of GLCC1 markedly suppresses cell growth and inhibits glycolysis progression in colorectal cancer. The data consistently suggest that high GLCC1 expression is a decisive factor that controls human colorectal cancer aggressiveness. Our results were consistent

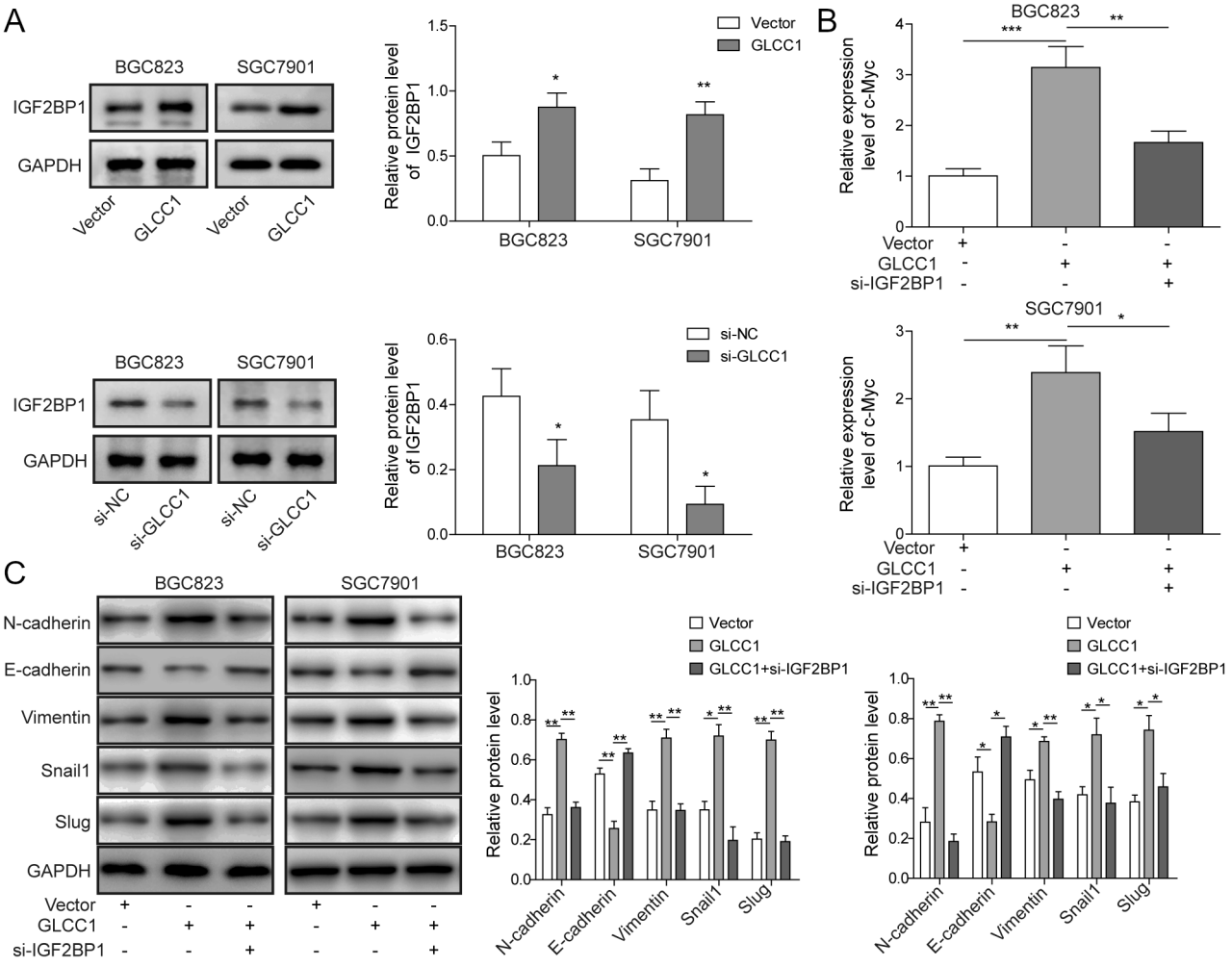

Figure 6. lncRNA GLCC1 enhances the stability of c-Myc mRNA via IGF2BP1 in gastric carcinoma. A) Western blot assays were performed to determine the protein levels of IGF2BP1 in BGC823 and SGC7901 gastric cancer cells with GLCC1 knockdown. B) qRT-PCR was carried out to measure the mRNA levels of c-Myc. C) Expression of EMT-related signaling molecules in cells transfected with the GLCC1-overexpressing plasmid with or without si-IGF2BP1 was determined using western blot analysis. *p<0.05, **p<0.01, ***p<0.001

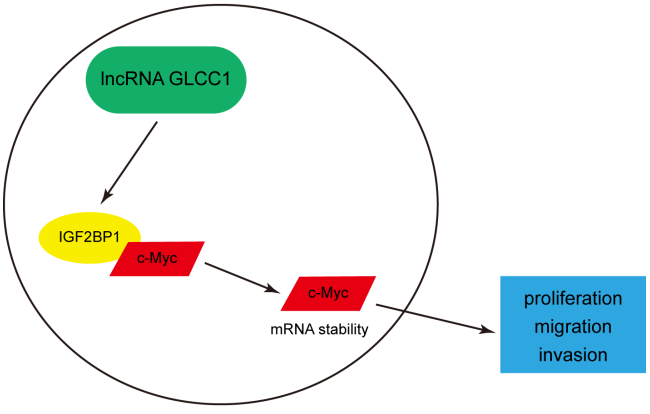

Figure 7. Schematic diagram of the role of lncRNA GLCC1 in the progression of gastric carcinoma. lncRNA GLCC1 promotes the progression of gastric carcinoma by enhancing the c-Myc/IGF2BP1 interaction.

with previous results, which suggest that GLCC1 is highly expressed in gastric carcinoma cells and promotes cell proliferation, metastasis, and invasion. The expression of GLCC1 may be a crucial prognostic indicator of gastric cancer with poor survival.

Studies have confirmed that IGF2BP1 stabilizes c-Myc mRNA by binding to a sequence located in the coding region determinant (CRD) (open reading frame) and stabilizes CD44 mRNA by binding to the 3'-untranslated regions of the transcript with IGF2BP1 [22–24]. Weidensdorfer et al. [25] found that IGF2BP1 enhanced the mRNA stability of c-Myc through IGF2BP1-related cytoplasmic ribonucleoproteins. In this study, we observed that the level of the IGF2BP1 protein bound to c-Myc mRNA and the level of the c-Myc mRNA bound to the IGF2BP1 protein, as well as both the mRNA and protein expression levels of c-Myc, were obviously increased

when BGC823 and SGC7901 cells expressed upregulated levels of lncRNA GLCC1. These results illustrate that lncRNA GLCC1 enhances c-Myc mRNA stability by enhancing its interaction with the IGF2BP1 protein.

c-Myc has been proven to promote tumorigenesis in many malignant tumors [26]. High expression of c-Myc in gastric adenocarcinoma, esophageal squamous cell carcinoma, and soft tissue leiomyosarcoma is associated with a low survival rate [27, 28]. Ninomiya et al. reported that patients with c-Myc protein-positive gastric cancer had a significantly poorer prognosis than those with c-Myc protein-negative gastric cancer, and the level of c-Myc was closely related to the recurrence of cancer [29]. Teixeira De Souza et al. demonstrated that the increase in c-Myc immunoreactivity and mRNA expression is closely related to the deep invasion and metastasis of gastric carcinoma [30]. In the present study, our data showed that c-Myc was highly expressed in gastric cancerous tissues and gastric cancerous cells. However, we found that the overexpression or knockdown of GLCC1 could cause the up- or downregulation of c-Myc, respectively. Mechanistic experiments verified that silencing c-Myc could significantly reverse GLCC1-mediated cell biological effects, indicating a positive correlation between GLCC1 and c-Myc in the development of gastric carcinoma. Moreover, we demonstrated that c-Myc expression was positively modulated by lncRNA GLCC1 during gastric carcinoma progression, illustrating that the lncRNA GLCC1/c-Myc molecular cascade might be a potent target for gastric carcinoma treatment.

In conclusion, the data presented in this research show that lncRNA GLCC1 modulates gastric cancer cell migration and invasion by enhancing the c-Myc/IGF2BP1 interaction, which may provide new evidence for developing novel therapeutic strategies to prevent the development and progression of human gastric carcinoma (Figure 7).

Acknowledgments: We would like to give our sincere gratitude to the reviewers for their constructive comments.

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