Original Article

The miR-155-5p/*FBXO11* axis inhibits the progression of gastric cancer via the mTOR pathway

Tao Yuan[1], Haiyan Liu[2], Fangfang Li[3], Qingyue Meng[4], Yajuan Wang[5], Mei Yuan[6]

[1]Hospital Office, The No. 3 People's Hospital of Qingdao, Qingdao, China; [2]Department of Dermatology, The No. 8 People's Hospital of Qingdao, Qingdao, China; [3]Department of Respiratory Medicine, The No. 8 People's Hospital of Qingdao, Qingdao, China; [4]Department of Health Management Center, The No. 8 People's Hospital of Qingdao, Qingdao, China; [5]Department of Gerontology, The No. 8 People's Hospital of Qingdao, Qingdao, China; [6]Health Management Center, the Affiliated Hospital of Qingdao University, Qingdao, China

Contributions: (I) Conception and design: T Yuan; (II) Administrative support: T Yuan; (III) Provision of study materials or patients: T Yuan; (IV) Collection and assembly of data: F Li; (V) Data analysis and interpretation: F Li; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Mei Yuan, MD. Health Management Center, the Affiliated Hospital of Qingdao University, No. 16 Jiangsu Road, Shinan District, Qingdao 266000, China. Email: qdymyd@163.com.

Background: Gastric cancer (GC) is a leading cause of cancer-related death. MicroRNAs (miRNAs or miRs) play a crucial role in the pathology of GC, including cell proliferation, invasion, and metastasis. In this study, genes targeted by miR-155-5p were predicted using bioinformatic tools. We found that the expression of miR-155-5p in GC cell lines differed relative to the expression of F-box protein 11 (*FBXO11*), which is involved in the regulation of cellular processes. This study sought to examine the function of miR-155-5p and the precise mechanism underlying its regulatory function in modulating proliferation and apoptosis in GC.

Methods: The luciferase reporter assay results showed that miR-155-5p bound directly to the three prime untranslated region (3'-UTR) of *FBXO11*, which further downregulated *FBXO11* expression. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and Western-blot analyses confirmed that miR-155-5p negatively regulated the messenger RNA (mRNA) and protein expression of *FBXO11*. The effects of *FBXO11* on cell proliferation and apoptosis in GC cell lines was further examined using Cell Counting Kit-8 (CCK-8) and flow cytometry.

Results: We found that *FBXO11* promoted proliferation and decreased apoptosis in GC cells. Conversely, rescue experiments showed that the knockdown of *FBXO11* limited the effects of miR-155-5p on the proliferation and apoptosis of GC cells, providing further evidence that *FBXO11* is a functional target of miR-155-5p. Further, the overexpression of miR-155-5p inhibited cell growth via the targeted inhibition of *FBXO11* that regulated mammalian target of rapamycin (mTOR) signaling pathway in the GC cells.

Conclusions: Overall, these results showed that miR-155-5p may serve as a tumor suppressor in GC and that the miR-155-5p/*FBXO11* axis regulates tumor progression via the mTOR signaling pathway. Consequently, our findings may lead to the development a novel treatment strategy for GC.

Keywords: Gastric cancer (GC); *FBXO11*; miR-155-5p; mammalian target of rapamycin pathway (mTOR pathway); proliferation

Submitted Jan 01, 2025. Accepted for publication Feb 20, 2025. Published online Feb 26, 2025.
doi: 10.21037/tcr-2025-8
View this article at: https://dx.doi.org/10.21037/tcr-2025-8

Introduction

Gastric cancer (GC) is one of the most common malignancies worldwide (1). Despite improvements in diagnostics and therapies, poor prognosis and limited treatment options still cause high mortality rates in GC (2). Hence, it is necessary to identify biomarkers with high degrees of sensitivity and specificity to improve the diagnosis and prognosis of GC.

MicroRNAs (miRNAs or miRs) are highly conserved non-coding RNAs that contain 20–22 nucleotides. Research has shown that miRNA dysregulation occurs during the onset and development of GC via diverse molecular mechanisms (3). Each miRNA has a specific seven to eight nucleotide-long seed sequence that directly engages with the three prime untranslated region (3'-UTR) of target genes (4). Some researchers have reported that miRNAs directly regulate 30% of all eukaryotic genes (1). Previous study has revealed the microRNA profiling in human GC (5). Five differentially expressed miRNAs, hsa-miR-132-3p, hsa-miR-155-5p, hsa-miR-19b-3p, hsa-miR-204-5p, and hsa-miR-30a-3p, were significantly down-modulated between the tumoral and peritumoral GC tissues (5). Among them, the function of miR-155-5p was still unclear in GC. Chen *et al.* revealed that miR-155-5p increases oncogenesis by targeting collagen triple helix repeat containing 1 (CTHRC1) and by regulating GSK-3β, which is involved in Wnt/β-catenin signaling in hepatocellular carcinoma (HCC) (6). In addition, it has also been previously reported that the increased upregulation of miR-155 increases proliferation, migration, and invasion in oral squamous cell carcinoma by modulating the BCL6/cyclin D2 axis (7). However, an inverse correlation between miR-155-5p and tumor size and/or pathologic stage was discovered in GC (8). Finally, high expression levels of miR-155-5p are also known to strongly downregulate the activity and propagation of carcinomas (9).

F-box protein 11 (*FBXO11*), an element of the SCF ubiquitin ligase complex, belongs to the F-box protein family (10). *FBXO11* promotes ubiquitination- and proteasome-dependent degradation by recruiting substrates and regulating cellular processes (11). In osteosarcoma, *FBXO11* mediates the role of miR-376a via its tumor suppressive property, and is a possible therapeutic target for osteosarcoma patients (12). *FBXO11* can act either as an oncogene or tumor suppressor in different carcinomas and at different stages of oncogenesis. *FBXO11* promotes the neddylation of p53 in breast cancer, which leads to suppression of the p53 function, and the induction of primary tumor growth and apoptosis suppression. However, at late stages, it prevents metastasis by breaking down Snail in p53-mutated tumor cells (11). However, our current understanding of the expression and function of *FBXO11* in GC is limited.

This study sought to examine the function of miR-155-5p and the precise mechanism underlying its regulatory function in modulating proliferation and apoptosis in GC. It has been observed that *FBXO11* is a particular target of miR-155-5p and is involved in regulating cell progression by modulating the mammalian target of rapamycin (mTOR) signaling pathway. Thus, the miR-155-5p/*FBXO11*/mTOR pathway might affect GC cell progression. Our findings might lead to the development of novel diagnostic and therapeutic strategies for GC. We present this article in accordance with the MDAR reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-2025-8/rc).

Highlight box

Key findings

- We found that miR-155-5p may serve as a tumor suppressor in gastric cancer (GC).
- The miR-155-5p/F-box protein 11 (*FBXO11*) axis regulates tumor progression via the mammalian target of rapamycin (mTOR) signaling pathway.

What is known, and what is new?

- GC is a main cause of death related to cancer. MicroRNAs (miRNAs or miRs) play an essential part in the pathology of GC.
- The expression of miR-155-5p in GC cell lines showed different patterns relative to the expression of *FBXO11*, which is involved in the regulation of cellular processes.

What is the implication, and what should change now?

- *FBXO11* may regulate GC cell progression via the PI3K/AKT pathway and via TGF-β1-induced activation of the mammalian target of rapamycin complex 1 (mTORC1) pathway.
- The miR-155-5p/*FBXO11*/mTOR pathway might affect GC cell progression. Our findings may lead to the development of new diagnostic and therapeutic strategies for GC.

Methods

Cell culture

Human GC cell lines (i.e., AGS, SGC7901, BGC803, and HGC27) were obtained from the Beijing Institute for Cancer Research (Beijing, China). The gastric epithelial cell

line gastric epithelial cells (GES)-1 used in the present study was purchased from the Cell Bank of the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Roswell Park Memorial Institute Medium-1640 (RPMI-1640) supplemented with 10% fetal bovine serum (FBS) was used for culturing all cell lines, which were cultivated in 5% carbon dioxide (CO_2) at 37 ℃.

Extraction of RNA and quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

In accordance with the instructions supplied by the manufacturer, total RNA was extracted from the gastric cell lines using TRIzol® reagent (Invitrogen, USA). The RevertAid RT kit (Thermo Fisher Scientific, USA) was used to reverse transcribe samples of RNA, and the miRNA RT kit (Takara, Japan) was used to reverse transcribe miRNA. In both cases, the end product was complementary DNA. SYBR Premix ExTaq TM II (Takara, Japan) was used to perform the qRT-PCR on an ABI 7900 qRT-PCR System (Applied Biosystems, USA). U6 and GAPDH were normalized as internal controls to measure the expression of miR-155-5p and *FBXO11*. The following primers were used for qRT-PCR: *FBXO11*, forward: 5'-TGGCAAGCAGGTTGGTGTTT-3'; reverse: 5'-ACCATTCTGTCCTCCCCAGA-3'; GAPDH, forward: 5'-GCACCGTCAAGGCTGAGAAC-3', reverse: 5'-TGGTGAAGACGCCAGTGGA-3'; and U6, forward: 5'-CTCGCTTCGGCAGCACA3' and reverse, 5'-AACGCTTCACGAATTTGCGT-3'. The miR-155-5p, 5'-ACGCTTAATGCTAATCGTGATAGGGGTT-3' quantification of the messenger RNA (mRNA) levels was performed using the $2^{-\Delta\Delta Cq}$ method. All samples were analyzed in triplicate.

Prediction of the target genes of miR-155-5p

Four prediction online databases, for example, DIANA-microT (http://diana.imis.athena-innovation.gr/), TarBase (http://microrna.gr/tarbase/), mirTarbase (https://miRTarBase.cuhk.edu.cn), and Miranda (http://www.mirdb.org/) were used to successfully identify the predicted targets of miR-155-5p. These targets were merged by an R package and exported to VennPainter. Overlapping genes were then identified as possible targets of miR-155-5p. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses

The ClusterProfiler R package was used to perform the GO annotation and KEGG pathway analyses of the identified genes. A P value <0.05 was set as the statistically significance threshold for pathway selection. Based on the P value, the top 10 items (in ascending order) were selected for visualization.

Cell transfection

The cells were added to 6-well plates, after which RPMI-1640 was added, and the cells were then cultivated at 37 ℃ in 5% CO_2. Once 70–80% confluence was reached, the transfection process began. Specifically, the miR-155-5p mimic, miR-155-5p inhibitor, synthetic small-interfering RNAs (siRNAs) against *FBXO11* (si-*FBXO11*), and a corresponding negative control (NC) were synthesized by GenePharma (Shanghai, China). The transfection of these sequences was performed as per the instructions provided in the Lipofectamine 2000 Kit (Invitrogen). After 6 h, the cells were then cultivated at 37 ℃ for 48 h in RPMI-1640 consisting of 10% FBS with 5% CO_2. The resulting samples were used for experiments 48 h after transfection.

Western-blot analysis and reagents

RIPA buffer (Beyotime, Shanghai, China) was used for the cell lysis experiments. Protein concentrations were assessed using a BCA kit (Beyotime). The samples were segregated by 10% SDS-PAGE before being transferred to PVDF membranes. The proteins were blocked with 5% skimmed milk for 2 h. The membranes were then cultured with the primary antibodies of *FBXO11* (1:1,000, Abcam) and GAPDH (1:1,000, CST) overnight at 4 ℃. They were then washed three times in TBST before being incubated with secondary antibodies (1:5,000, Bioss) for 2 h at room temperature. GAPDH was used as the control protein. The visualization of the samples was performed using an ECL kit. The band quantification analysis was carried out using ImageJ. MHY1485 was purchased from Beyotime (China).

Dual-luciferase reporter assay

The potential binding regions of miR-155-5p and the 3'UTR of *FBXO11* were examined using DIANA Tools.

The HEK293 cells were planted at a density of 1×10^5 cells per well into 24-well plates. The 3'-UTR of *FBXO11* was also cloned into a pGL3-basic vector. The pGL3-*FBXO11*-3'-UTR-WT or pGL3-*FBXO11*-3'-UTR-mut vectors were co-transfected with the miR-155-5p-mimic or matched controls into the HEK293T cells using Lipofectamine 2000 (Invitrogen); all the procedures were performed in accordance with the guidelines of the manufacturer. A Dual-Luciferase® Reporter assay system (Promega Corporation) was used to detect luciferase activity after 48 h; all procedures were performed in accordance with the manufacturer's guidelines. Each experiment was carried out at least three times.

Cell proliferation assay

A Cell Counting Kit-8 (CCK-8; KeyGentech, China) was used for the cell proliferation assay; all the procedures were performed in accordance with the manufacturer's protocol. The GC cell lines (i.e., AGC and MGC803) and GES-1 were planted into 96-well plates at a density of 5×10^3/well, and incubated for 24 h. The cells were collected after reaching the logarithmic growth state, and they were then transfected with 50 nM of the miR-155-5p mimic, the inhibitor, or the NC, respectively. After treatment for 0, 24, 48, or 72 hours, 90 µL of serum-free RPMI-1640 and 10 µL of CCK-8 reagent were applied for staining for 1 h. The optical density was then measured using the Soft-Max apparatus (Bio-Tek ELx808, Winooski, USA) at a wavelength of 450 nm.

Apoptosis assay

After transfection for 48 h, the cells were collected and washed in ice-cold phosphate-buffered saline, and 500 µL of binding buffer was then added for suspension. Next, 10 µL of propidium-iodide and 10 µL of Annexin V-FITC were used for staining. The cells were then incubated for 15 min without light at room temperature. Next, 300 µL of 1× binding buffer was added to all the cell samples. Apoptotic activity was identified using the FACSCalibur apparatus (BD Biosciences, Franklin Lakes, USA).

Statistical analysis

GraphPad Prism 5.0 (GraphPad Software) was used for all the statistical analyses. All the outcomes are presented as the mean ± standard deviation of three independent experiments for three technical or biological replicates. P value <0.05 was set as the threshold of statistical significance for all results.

Results

The expression of FBXO11 differed to that of miR-155-5p in the GC cell lines

Four GC cell lines (i.e., AGS, SGC7901, BGC803, and HGC27) were chosen and used to examine the expression of miR-155-5p and *FBXO11*. The qRT-PCR results showed that relative to the immortalized GES-1 line, miR-155-5p expression was significantly downregulated in all the GC cell lines (P<0.05, *Figure 1A*). Conversely, as *Figure 1B,1C* show, the qRT-PCR and Western-blot analyses showed that both the *FBXO11* mRNA and protein were significantly upregulated in the GC cell lines relative to the control cell lines. Thus, the mode of miR-155-5p expression in the GC cell lines differed from that of *FBXO11*.

miR-155-5p target genes identified by GO and pathway enrichment analyses

Previous research has shown that miR-155-5p affects cellular growth and apoptosis by amending the expression of target genes (13). Four prediction databases (i.e., DIANA-microT, TarBase, mirTarbase, and miranda—respectively identified 941, 4,315, 2,468, and 608 target genes of human miR-155-5p. The intersection of these results was then determined using VennPainter. In total, 89 target genes were identified (*Figure 2A*).

To further analyze these 89 genes on a functional level, these genes were analyzed for KEGG/GO pathway enrichment as implemented by the ClusterProfiler R package (*Figure 2B-2D*). The results of KEGG pathway analysis showed significant enrichment in the following pathways: osteoclast differentiation, lipid and atherosclerosis, MAPK signaling pathway, Rap1 signaling pathway, Toll-like receptor signaling pathway, Yersinia infection, Measles, Ubiquitin mediated proteolysis, Signaling pathways regulating pluripotency of stem cells, and Hepatitis B.

Furthermore, GO analysis results showed that for the biological process group, the target genes were mostly enriched in the following processes: regulation of hemopoiesis, myeloid cell differentiation, mononuclear cell differentiation, regulation of myeloid cell differentiation, miRNA metabolic process, osteoclast differentiation,

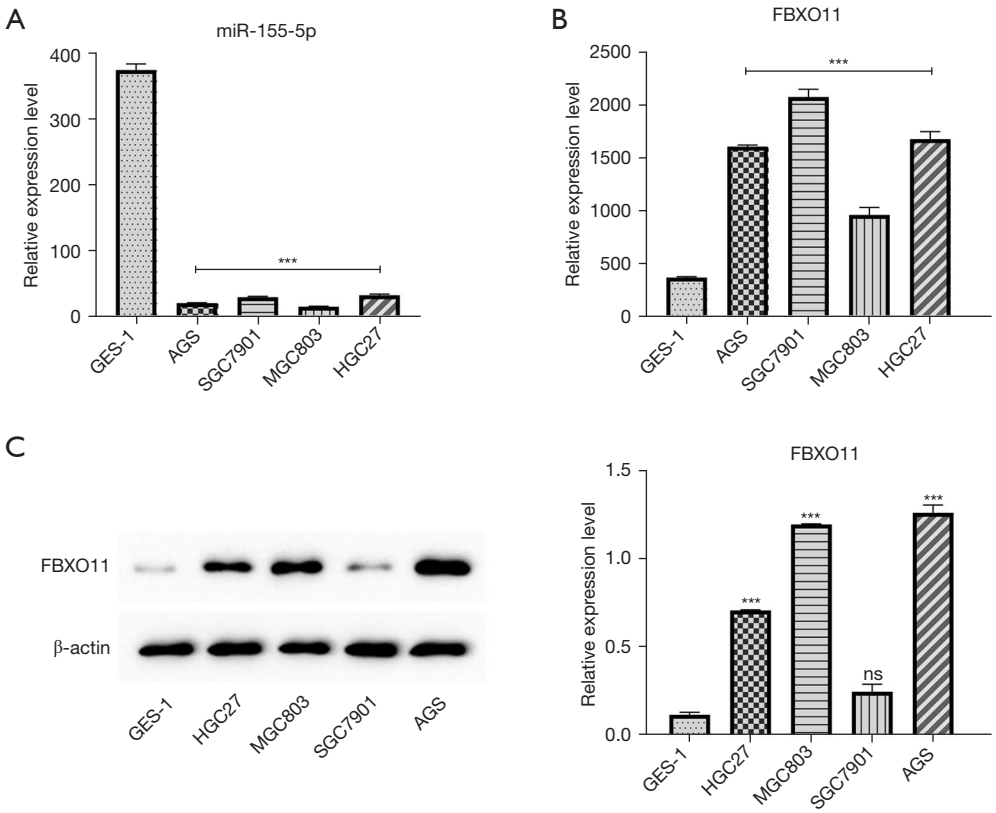

Figure 1 MiR-155-5p and *FBXO11* expression in GC cells. (A) qRT-PCR showed miR-155-5p mRNA expression in GES-1 and GC. (B) qRT-PCR showed *FBXO11* mRNA expression in GES-1 and GC. (C) Western blot showed the level of expression of *FBXO11* in GC (HGC27, SGC7901, AGS, and MGC803) and GES-1. ***, P<0.001; ns, not significant. Every experiment was conducted at least three times. GC, gastric cancer; GES, gastric epithelial cells; qRT-PCR, quantitative reverse transcription-polymerase chain reaction.

negative regulation of fibroblast proliferation, negative regulation of receptor-mediated endocytosis, forebrain neuron differentiation, and interleukin-17-mediated signaling pathway. Similarly, for the cellular component group, the target genes were mostly enriched in extrinsic component of membrane, cell leading edge, nuclear matrix, nuclear periphery, RNA polymerase II transcription regulator complex, condensed chromosome, extrinsic component of plasma membrane, remodel the structure of chromatin (RSC)-type complex, phosphatidylinositol 3-kinase complex, and SWI/SNF complex. Finally, for the molecular function group, the target genes were mostly enriched in DNA-binding transcription activator activity, RNA polymerase II-specific, DNA-binding transcription activator activity, modification-dependent protein binding, transcription coactivator activity, ubiquitin binding, ubiquitin-like protein binding, core promoter sequence-specific DNA binding, polyubiquitin modification-dependent protein binding, Tat protein binding, RNA polymerase II core promoter sequence-specific DNA binding.

FBXO11 is a target of miR-155-5p in GC cell lines

According to the bioinformatic analysis of miR-155-5p, the 3'-UTR of *FBXO11* includes a sequence that is complementary to the seed sequence of miR-155-5p (*Figure 3A*). We therefore produced luciferase reporters of both the WT and MUT, which contained the seeding sequence and a mutant sequence from the 3'-UTR of *FBXO11*. These vectors were then co-transfected with miR-155-5p or miR-NC into HEK293T.

As *Figure 3B* shows, a considerable decrease was observed in WT luminescence intensity following transfection with miR-155-5p mimics. However, the overexpression of miR-155-5p did not affect the MUT reporter. Further, the qRT-PCR and Western-blot analyses showed that the

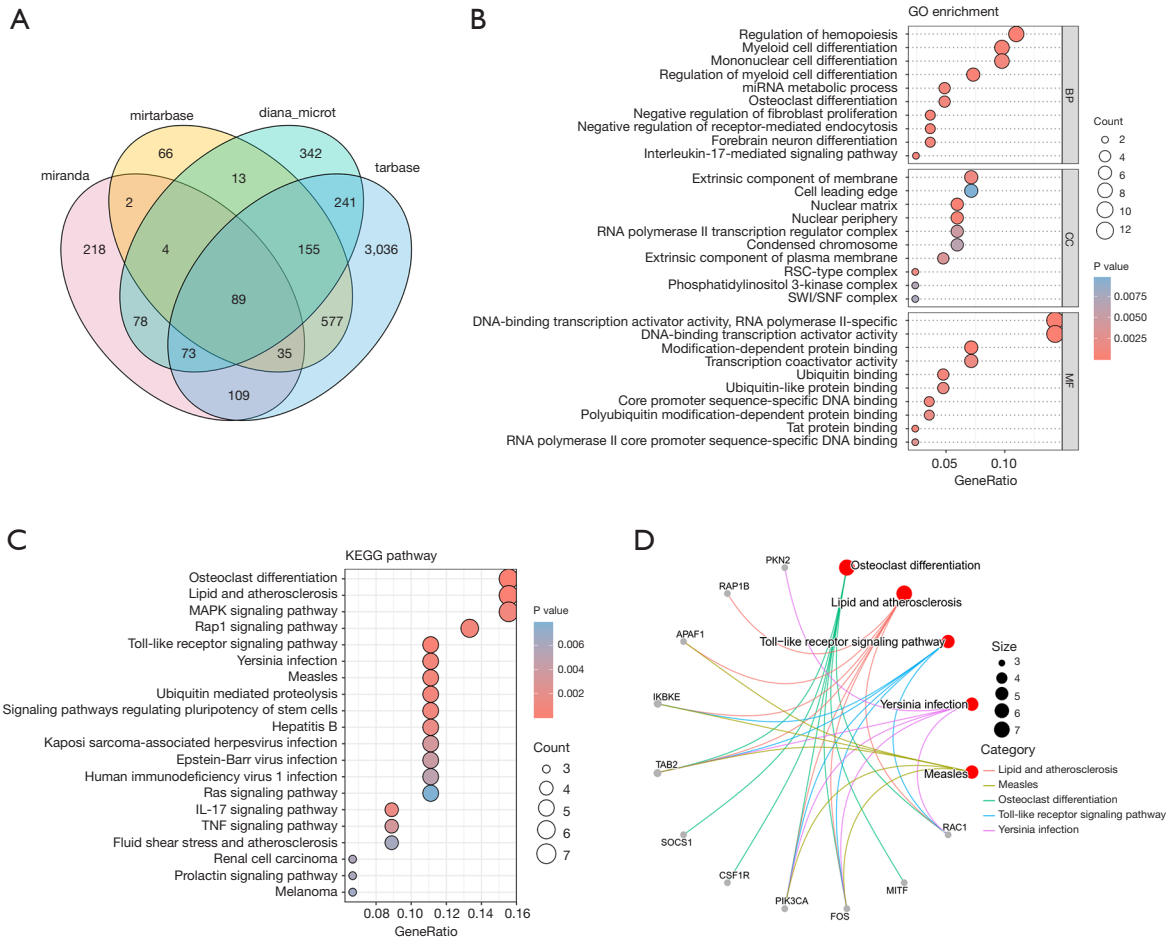

Figure 2 Target genes of miR-155-5p GO and pathway enrichment analysis. (A) VennPainter intersected the target genes of miR-155-5p from four prediction databases (i.e., diana-microt, mirtarbase, mianda, and miranda). (B) R package cluster Profiler showed the top 10 GO terms enriched in this category based on the P values, Count: represents how many genes are enriched in each term. (C) R package cluster Profiler showed the top 10 KEGG terms enriched in this category based on the P values; Count: represents the quantity of enriched genes in each term. (D) String diagram for the KEGG analysis: KEGG pathway enrichment analysis of miR-155-5p target genes. BP, biological process; CC, cell component; MF, molecular function; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

overexpression of miR-155-5p led to the suppression of *FBXO11* at both the protein and mRNA levels in the AGS and MGC803 cell lines relative to the NC cell lines (P<0.05, *Figure 3C,3D*). However, the expression of *FBXO11* was increased in GES-1 transfected with the miR-155-5p inhibitor relative to the NC cells (P<0.05, *Figure 3E,3F*). Hence, it is reasonable to conclude that miR-155-5p directly targets *FBXO11* in GC cells.

Downregulation of FBXO11 abolishes the low expression effect of miR-155-5p in GC cell lines

To conduct a more in-depth investigation of the role of *FBXO11* in the proliferation and apoptosis of GC cells, *FBXO11* was silenced by introducing siRNA into MGC803. Western blotting was then used to measure the transfection efficiency and verify the optimum conditions. We observed that the protein levels of *FBXO11* were considerably increased in the miR-155-5p inhibitor and si-*FBXO11*-co-transfected MGC803 cell lines relative to the si-*FBXO11*-transfected cell lines (P<0.05, *Figure 4A*). The CCK-8 assays showed that the proliferative capacity of the MGC803 cells was decreased relative to the NC cells after treatment with si-*FBXO11*. Conversely, the knockdown of miR-155-5p by an inhibitor somewhat recovered the low degree of proliferation induced by silencing *FBXO11* (P>0.05,

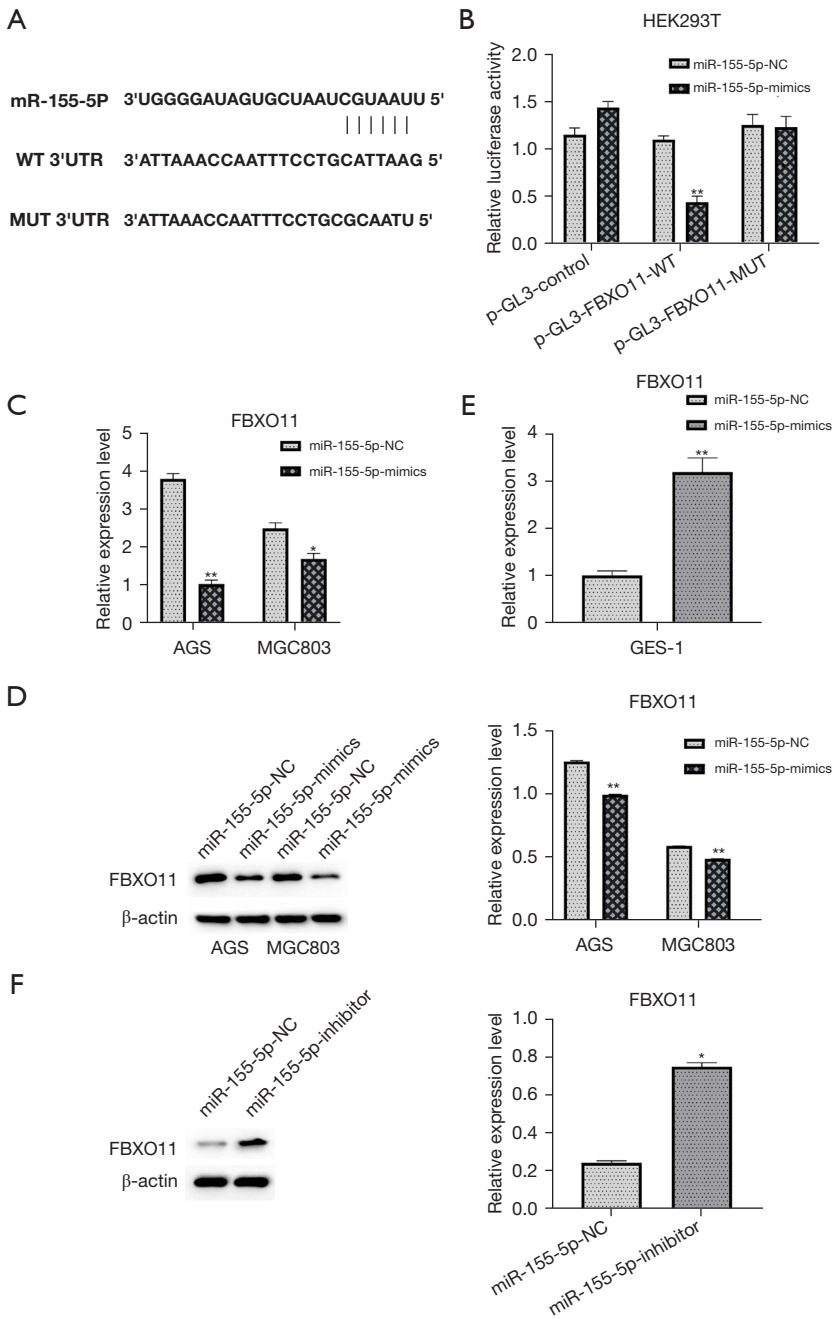

Figure 3 MiR-155-5p directly targets *FBXO11* in GC cells. (A) Seed matched outcomes of the 3'UTR of *FBXO11* mRNA and miR-155-5p. *FBXO11* mRNA (MUT-*FBXO11*) showed a 3'UTR mutation in the seed-matching sequence. (B) In the luciferase assay experiments, the HEK293T cells were co-transfected with 1.5 μg of recombinant plasmid (mutant or wild *FBXO11*-3'UTR) and 50 nM of miRNA mimics (miR-155-5p or NC) in each well. Luciferase activity was recorded 48 h post-transfection. (C) qRT-PCR showed the mRNA expression of *FBXO11* after transfecting miR-155-5p mimics in the AGS and MGC803 cells. (D) Western blot was used to detect the protein expression of *FBXO11* after the transfection of miR-155-5p mimics in the AGS and MGC803 cells. (E) qRT-PCR was performed to examine the mRNA expression of *FBXO11* after the miR-155-5p inhibitor was transfected in the GES-1 cells. (F) Western blot was used to detect the expression of *FBXO11* after the miR-155-5p inhibitor was transfected in the GES-1 cells. *, P<0.05; **, P<0.01. Every experiment was conducted at least three times. GC, gastric cancer; GES, gastric epithelial cells; MUT, mutant; NC, negative control; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; WT, wild type.

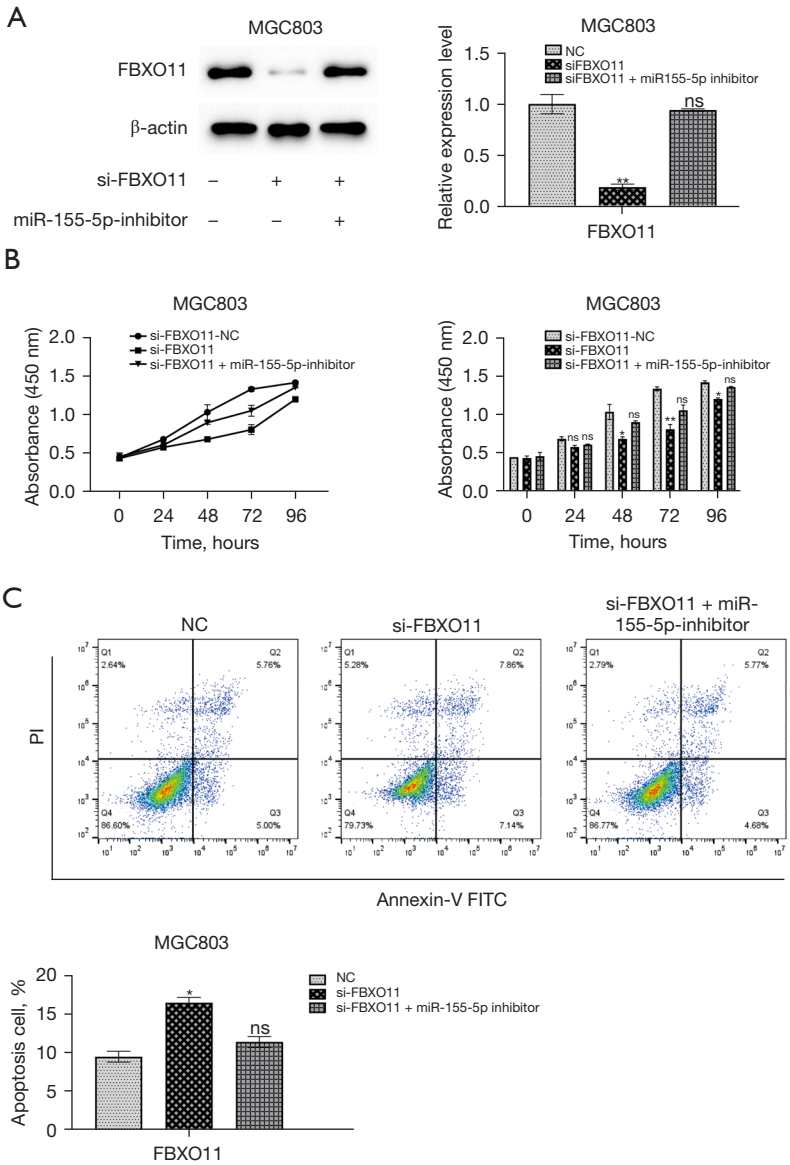

Figure 4 The inhibitory effect of miR-155-5p on the proliferation and apoptosis of GC cells was reversed by *FBXO11*. (A) The protein expression of *FBXO11* was decreased in the MGC803 cells after transfection with the miR-155-5p inhibitor. Conversely, an increase in the expression was observed after transfection with the si-*FBXO11* and miR-155-5p inhibitor. (B) CCK-8 demonstrated that the cellular proliferation rate was decreased in the MGC803 cells after miR-155-5p inhibitor transfection, but increased after the transfection with the miR-155-5p inhibitor + si-*FBXO11*. (C) Flow cytometry analysis showed that transfection with the miR-155-5p inhibitor increased the rate of apoptosis in MGC803 cells. Conversely, transfection with si-*FBXO11* and miR-155-5p inhibitor magnified this rate. *, P<0.05; **, P<0.01; ns, not significant. CCK-8, Cell Counting Kit-8; FITC, Fluorescein Isothiocyanate; GC, gastric cancer; NC, negative control; PI, Propidium Iodide.

Figure 4B). Moreover, the flow cytometry results showed that relative to control cells, *FBXO11* silencing promoted apoptosis in the MGC803 cells. In addition, the expression of *FBXO11* in the MGC803 and AGS cells weakened the action of miR-155-5p in relation to cell apoptosis (P>0.05, *Figure 4C*). However, a contrasting trend was observed in relation to the function of *FBXO11* and miR-155-5p on both the proliferation and apoptosis in the GC cell lines.

The miR-155-5p/FBXO11 axis modulates cell proliferation and apoptosis via mTORC1 signaling pathways in GC cells

Next, the role of the miR-155-5p/*FBXO11* axis in the mammalian target of rapamycin complex 1 (mTORC1) pathway was examined using the MGC803 cells. The mTOR, p-mTOR, p-p70s6k, p-4EBP1, and *FBXO11* protein levels in the MGC803 cells were quantified by Western blot following transfection with either si-*FBXO11* only or si-*FBXO11* co-transfected with miR-155-5p inhibitor treatments. As *Figure 5A* shows, the knockdown of *FBXO11* lowered p-mTOR, p-p70s6k, and p-4EBP1 expression, but did not affect mTOR expression. Further, treatment with MHY1485, an mTOR pathway activator, partially reversed the *FBXO11*-mediated effect on the relative protein expression of the mTORC1 signaling pathway. In addition, the proliferation and apoptosis rates of this group were decreased and increased, respectively, relative to the control group. Once again, MHY1485 treatment partly recovered the *FBXO11*-mediated inhibitory effect on cell proliferation and apoptosis (*Figure 5B*,*5C*), which suggests that miR-155-5p regulates cell growth and the mTORC1 signaling pathway by targeting *FBXO11* in GC cells.

Discussion

At present, GC is a leading cause of cancer-related death worldwide (14). Due to the rapid progression of GC cells, the 5-year survival rate of GC patients remains poor (15). Hence, it is essential to identify new markers to diagnose patients at an early stage. MiRNAs are small non-coding RNAs implicated in cancer development and progression. A study has demonstrated that many miRNAs play important roles in GC. MiRNA-766-3p inhibited GC via targeting COL1A1 and regulating PI3K/AKT signaling pathway (16). miR-148-3p inhibits GC cell malignant phenotypes and chemotherapy resistance by targeting Bcl2 (17). Meanwhile, circulating miRNAs were verified as biomarkers of GC (18). In our study, It has been speculated that miR-155-5p has a tumor suppressive role in the progression of GC, but has different roles in colorectal cancer and breast cancer (19,20). For example, cyclin D1, a cell cycle regulator, was found to be targeted and hindered by the overexpression of miR-155-5p. This affected the regulation of the progression of the G1 to the S phase in GC; thus, the inhibition of cyclin D1 by miR-155-5p could reduce cell proliferation, promote G1 phase arrest, and subsequently promote apoptosis (21). In addition, miR-155-5p was also found to target Smad2, thereby blocking the transforming growth factor beta (TGF-β) pathway, which effectively promotes tumor cell invasion in GC (22). However, the exact way in which miR-155-5p affects cell function in GC remains unexplained. Based on bioinformatics analyses and dual-luciferase reporter assays, we established that *FBXO11* is a target of miR-155-5p. Western-blot analyses and qRT-PCR further verified this relationship. Subsequent functional analyses revealed that relative to the control cells, the knockdown of *FBXO11* reduced propagation and caused apoptosis in the GC cells. Rescue experiments then showed that *FBXO11* knockdown significantly terminated the miR-155-5p inhibitor-mediated promotion of cell tumor growth in GC cells. The outcomes of this study are consistent with those of previous studies that reported that miR-155-5p expression inhibits GC cell progression.

FBXO11 shows E3 ubiquitin ligase activity and methyltransferase activity via its F-box domains (23). However, to date, the regulatory mechanism of *FBXO11* and its role in GC remained largely unexplored. In this study, the KEGG pathway analysis showed that miR-155-5p may regulate the MAPK, PI3K/Akt, and mTOR pathways to induce the biological function of cells. Previous research has shown that the PI3K/AKT pathway is vital for many pathophysiological processes, including the growth, apoptosis, migration, and autophagy of carcinomas (24-26). Sun *et al.* reported that *FBXO11* overexpression negatively modulated phosphatase and tensin homolog (PTEN) to regulate the PI3K/AKT pathway, and to subsequently promote proliferation in GC cells (27). PTEN is a negative regulator of the PI3K/AKT cascade, which itself regulates multiple cell activities, including growth, differentiation, proliferation, invasion, and intracellular trafficking. By inhibiting the tumor suppressor PTEN, the PI3K/AKT/mTOR axis can be activated to regulate a critical node of oncogenic transformation (28). Conversely, the overexpression of *FBXO11* promotes HCC development by inducing epithelial–mesenchymal transition through the PI3K/AKT/GSK-3β/Snail signaling pathway. Thus, *FBXO11* may regulate proliferative patterns via the PTEN/PI3K/AKT axis, which mediates the cellular function of miR-155-5p. A recent study identified *FBXO11* as a new avenue of TGF-β signaling by controlling CDT2 activity; it could thus become both a therapeutic target and a biomarker of the early stages of GC (29), in which epithelial growth is restricted by the tumor suppressive effects of

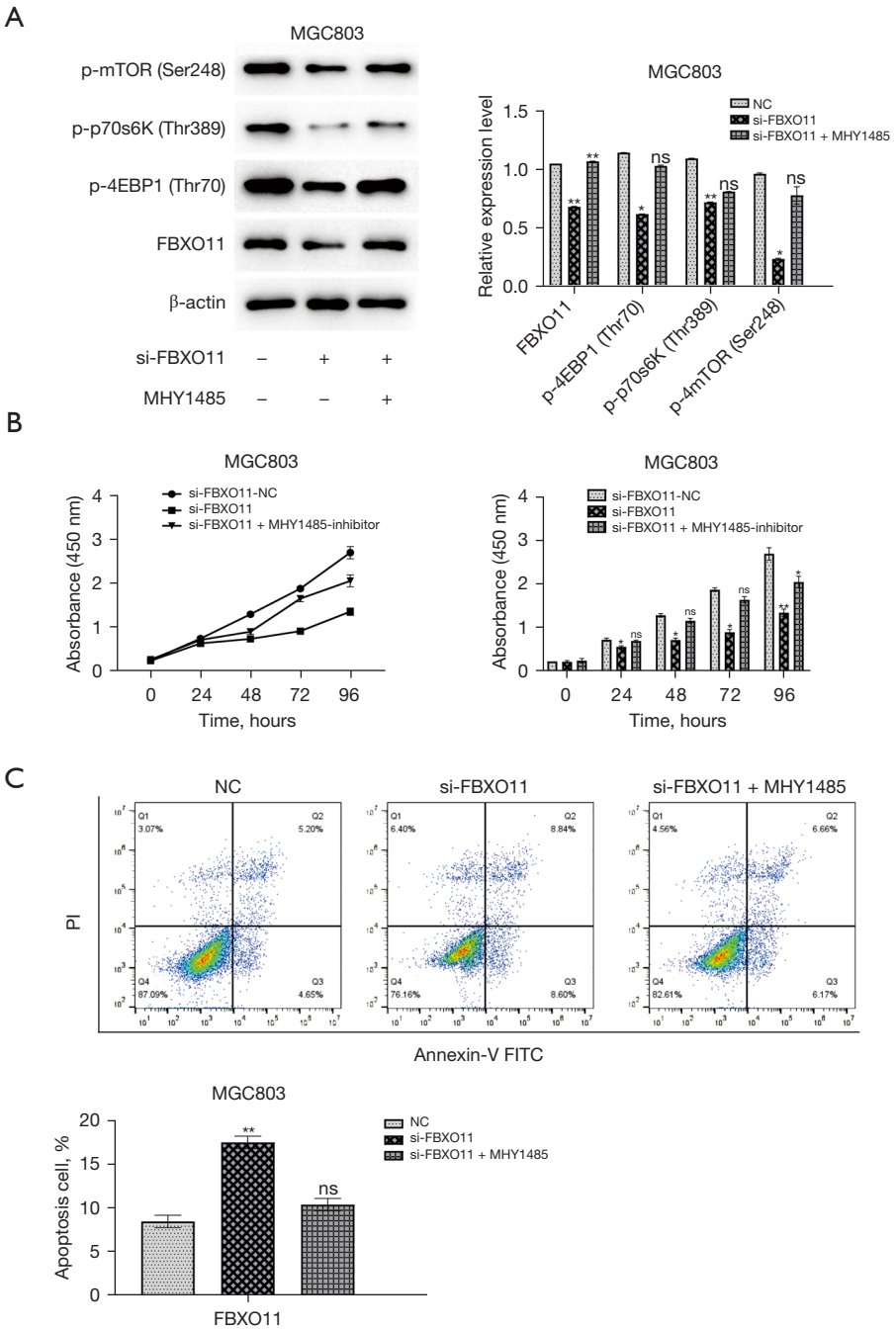

Figure 5 miR-155-5p/*FBXO11* axis affected proliferation and apoptosis via mTORC1 signaling pathways in GC cells. (A) Western blot was used to detect the related protein expression of the mTORC1 pathway after (respectively or common) transfected si-*FBXO11* and MHY1485 in MGC803 cells. (B) CCK-8 assay revealed that the rate of cell proliferation after (respectively or common transfected) si-*FBXO11* and MHY1485 in MGC803 cells. (C) Flow cytometry analysis revealed cell apoptosis after (respectively or common) transfected si-*FBXO11* and MHY1485 in MGC803 cells. *, P<0.05; **, P<0.01; ns, not significant. Every experiment was conducted at least three times. CCK-8, Cell Counting Kit-8; FITC, Fluorescein Isothiocyanate; GC, gastric cancer; mTORC1, mammalian target of rapamycin complex 1; NC, negative control; PI, Propidium Iodide.

TGF-β (30). Based on these results, it is likely that miR-155-5p also controls propagation and apoptosis through *FBXO11* to regulate the TGF-β pathway.

One of the most vital regulators of the G1 cell cycle is the mTORC1. Its downstream target genes are the eukaryotic initiation factor 4E binding protein-1 and the ribosomal subunit S6 kinase (S6K) (31). The present study showed that the knockdown of *FBXO11* in MGC803 cells inhibited the mTORC1 signaling pathway, and the inhibitory force of *FBXO11* on the mTORC1 signaling pathway may be reversed by MHY1485. We speculate that the miR-155-5p/*FBXO11* axis may regulate cell proliferation and apoptosis via the mTORC1 pathway, as previous studies have reported that p-AKT, which is activated downstream of mTORC1, is vital for many types of tumorigenesis and metastases (32-33). In addition, TGF-β can activate the PI3K/Akt/mTOR pathway in human lens epithelial cells (34). Thus, *FBXO11* may also regulate GC cell progression via the PI3K/AKT pathway and by TGF-β1–induced activation of the mTORC1 pathway.

Limitations

The scope of this study was limited, as it only explored whether the mTORC1 signaling pathway affected *FBXO11*-regulated progression.

Conclusions

Overall, this study showed that miR-155-5p targets *FBXO11* to suppress GC cell progression via the regulation of mTORC1 signaling. Future research should examine whether *FBXO11* could serve as a possible target in novel GC treatments.

Acknowledgments

None.

Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-2025-8/rc

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-2025-8/rc

Peer Review File: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-2025-8/prf

Funding: None.

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-2025-8/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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(English Language Editor: L. Huleatt)

Cite this article as: Yuan T, Liu H, Li F, Meng Q, Wang Y, Yuan M. The miR-155-5p/*FBXO11* axis inhibits the progression of gastric cancer via the mTOR pathway. Transl Cancer Res 2025;14(2):1375-1387. doi: 10.21037/tcr-2025-8