Targeting Snail1 by CRISPR/Cas9 System Inhibits the Proliferation and Migration of Human Gastric Cancer Cells

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Abstract: The zinc-finger transcriptional repressor Snail1 affects cancer progression by controlling the epithelial cell-mesenchymal transition. The RNA-guided clustered regularly interspaced short palindromic (CRISPR) with a CRISPR-associated nuclease 9 (Cas9) nuclease system has been extensively used for gene editing. Here, we used two distinct sgRNAs to successfully target Snail1 in the gastric cancer cell line MGC803 with the CRISPR/Cas9 system. Furthermore, we discovered that Snail1 knockout reduced the proliferation and migration of MGC803 cells.

Keywords: Gastric cancer, Snail1, CRISPR/Cas9, gene editing, cancer proliferation, cancer migration.

1. INTRODUCTION

The zinc-finger transcription factor Snail1, a representative member of the SNAI family (Snail1 /Snail, Snail2/Slug and Snail3/Smuc), was first discovered in Drosophila as involving in embryonic development, and later it was verified that it recognizes and binds to E-BOX (CAGCTG) in the anterior segment of the E-cadherin promoter to inhibit the transcription of E-cadherin [1-3]. Snail1 also regulates the transcription of other genes such as Claudins, Fibronectin, Occludin, etc to control the epithelial cell-mesenchymal transition and affect caner progression [4-7].

The SNAG and zinc finger domains are two main regions of Snail1 protein [8]. The SNAG domain is the evolutionarily highly conserved region, which is located in the region terminal and binds to a variety of co-transcriptional factors and epigenetic recombinant complexes [9]. The zinc-finger domain composes of four highly conserved C2H2 type zinc-fingers to bind to the E-BOX at the front of the promoter of the target gene [10].

Owing to the advantages of being faster and cheaper, the RNA-guided clustered regularly interspaced short palindromic (CRISPR) with a CRISPR-associated nuclease 9 (Cas9) nuclease system has been extensively used for gene editing [11]. In this study, we used CRISPR/Cas9 system targeting Snail1 to verify the role of Snail1 in gastric cancer.

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2. MATERIAL AND METHODS

2.1. Cell Lines and Cell Culture

Human gastric cancer cell line MGC803 and embryonic kidney cell line HEK293T were cultured in DMEM (Gibco) with 10% fetal bovine serum (Gibco), 100 ng/mL streptomycin and 100 U/mL penicillin at 37 °C with 5% CO_2 in a humidified incubator.

2.2. Vector Generation, Lentivirus Production, and Transduction

LentiCRISPRv2 vector (Addgene #52961) was digested with BsmBI (New England Biolabs #R0739S) and ligated with the annealed oligonucleotides (Snail1-sg1F: 5'-CACCGCTTCCAGCAGCCCTACGACC-3', Snail1-sg1R:5'-AAACGGTCGTAGGGCTGCTGGAA GC-3', Snail1-sg2F: 5'- CACCGGCTTCCGATTGGGGT CGGA-3', Snail1-sg2R: 5'- AAACTCCGACCCCAATCG GAAGCC -3'). HEK293T cells were transfected using PEI (Ploysciences #23966-1) at 60%-70% confluency with recombinant CRISPR vectors and packaging vectors pMD2G and psPAX2. The viral supernatant was harvested at 72 hours after transfection and stored at -80°C. MGC803 cells were transducted with the viral supernatant containing HitransGP (from GENECHEM #REVG005), and were selected with puromycin under the concentration of 1ng/mL to establish the monoclonal cell line with stable Snail1 knockout by single-cellculture.

2.3. Genomic PCR and Sequencing Analysis

The genomic DNA of cells was extracted with the QuickExtract DNA extraction kit following the

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manufacturer's protocol and amplified with a pair of primers (Snail1-F: 5'-AGTACTTAAGGGAGTTGGCGG-3'; Snail1-R: 5'-CTCGGCCTCCAAGGAAGAGA-3') designed for the target Snail1 gene region. Then the PCR product was sequenced.

2.4. Western Blot Analysis

Cells were first harvested then lysed in RIPA buffer (1% NP-40, 0.5% sodium deoxycholate, 0.1%SDS, 10 ng/ml PMSF, 0.03% aprotinin, 1μM sodium orthovanadate) at 4°C for 30 minutes. Lysates were centrifuged for 15 min at 12,000×g. Protein concentration was quantified using with Bradford assay. Proteins were separated on 10% SDS-PAGE gels and transferred to polyvinylidene difluoride membranes. Membranes were blocked with 5% skimmed milk powder and incubated with the primary antibodies including anti-β-tubulin antibody (Yeasen #30302ES60) and anti-Snail1 antibody (Proteintech #30302ES60). The corresponding horseradish peroxidase-conjugated secondary antibodies were used against each primary antibody. Signals were detected with the ChemiDoc XRS chemiluminescent gel imaging system (Analytik Jena).

2.5. Cell Proliferation Analysis

Cell proliferation was monitored by the CCK-8 and clone formation assays. For the CCK-8 assay, cells were cultured in 96-well plates as 8×10^2 cells/well for 1, 2, 3, 4 and 5 days, incubated 10 μL CCK-8 for another 1 hour and measured at 450 nm absorbance on a multifunctional microplate reader. For the colony formation assay, cells were cultured in 12-well plates as 4×10^2 cells/well for 15 days, fixed with 4% paraformaldehyde and stained with 0.1% crystal violet for 10 minutes. Cell colonies were imaged and counted.

2.6. Cell Migration Analysis

Cell migration was detected by the transwell and scratch assays. For the transwell assay, the upper chamber of the transwell chamber was loaded with 150 μl serum-free medium and cells at the density of 2.0×10^4. The lower chamber was filled with 600 μl of medium containing 10% FBS. After incubation for 24 hours at 37 ˚C, the cells were washed with PBS and fixed with 4% paraformaldehyde for 10 minutes. Non-migrating cells in the upper chamber were wiped with a cotton swab, and the membrane with migrated cells were stained with 0.1% crystal violet stain for 10 minutes. After washing, cells were photographed with microscope. For the scratch assay, cells were planted in 6-well plates at a density of 5×10^7 cells/well for 12 hours and then scratched with a 200 μl pipet tip. Cell migration distances were recorded by microscope at 0 and 24 hours.

2.7. Statistical Analysis

The experimental data are the results of three independent repetitions and presented as the average and standard deviation. Statistical analysis of data differences using T test method. A P-value of <0.05 was set as the criterion for statistical significance.

3. RESULTS

3.1. Knockout of Snail1 by CRISPR/Cas9 System

To target Snail1 with CRISPR/Cas9 system, we established lentiCRISPRv2 vector which contains a targeting sequences from exon 1 or 2 of human Snail1 gene (Figure **1A**) [12]. MGC803 cells were infected with LentiCRISPRv2 viral supernatant, and cultured as monoclonal cell. After detected by Western blot, two monoclonal cells with undetectable Snail1 protein were generated (Figure **1B**). MGC803-sg1 was deleted one base, and MGC803-sg2 was deleted 56 bases, according to genome sequencing (Figure **1C**).

3.2. Knockout of Snail1 Inhibits Cell Proliferation

To explore the effect of Snail1 knockout on the proliferation of MGC803 cells, the CCK-8 and clone formation experiments were performed. As shown in Figure **2A**, knockout of Snail1 blocked the growth of MGC803 cells. Further clony formation experiments showed that knockout of Snail1 attenuated the clone size and number of MGC803 cells (Figures **2B-D**). These data suggest that knockout of Snail1 inhibits cell proliferation.

3.3. Knockout of Snail1 Inhibits Cell Migration

To explore the effect of Snail1 knockout on the migration of MGC803 cells, the scratch and transwell experiments were performed. As shown in Figure **3A-B**, knockout of Snail1 suppressed the migration rate of MGC803 cells. Further transwell experiments showed that knockout of Snail1 attenuated the migration distance of MGC803 cells (Figures **3C-D**). These results indicate that knockout of Snail1 inhibits cell proliferation.

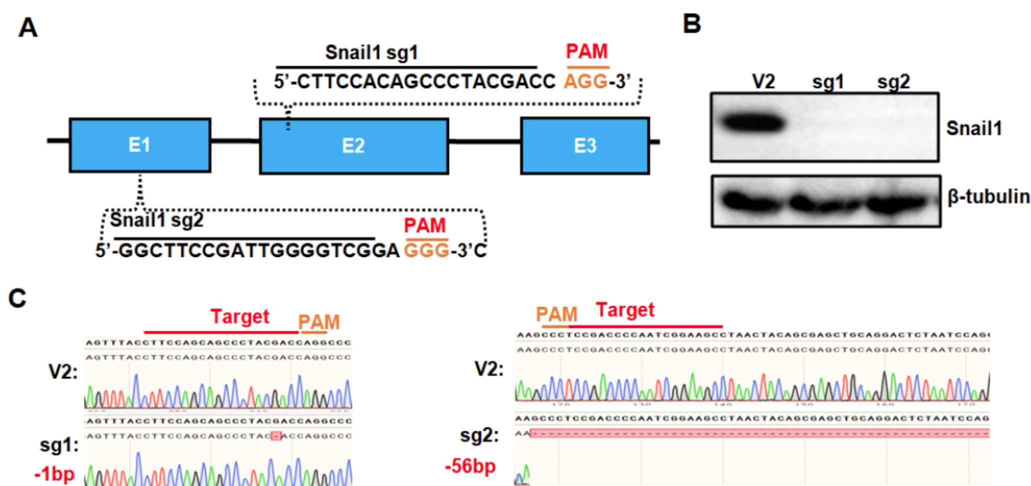

Figure 1: Knockout of Snail1 by CRISPR/Cas9 system. (A) The locations and sequences of two sgRNAs of Snail1. **(B)** Snail1 protein expression of cells were examined by Western blot. **(C)** The Snail1 genomic sequences of cells were shown.

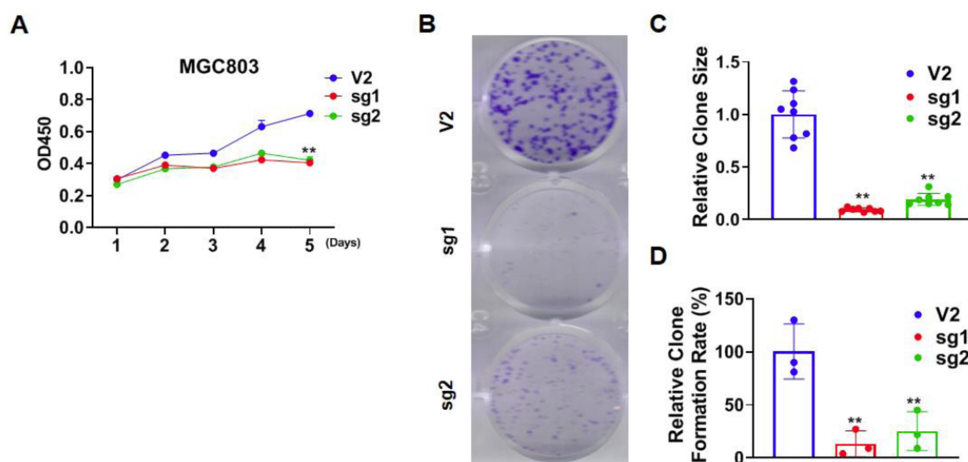

Figure 2: Knockout of Snail1 affects cell proliferation. (A) Cell proliferation was evaluated by CCK-8 experiments. **(B-D)** Cell proliferation was examined with clony formation experiments. The representative images and quantitative results were shown. **P < 0.01 against. corresponding control.

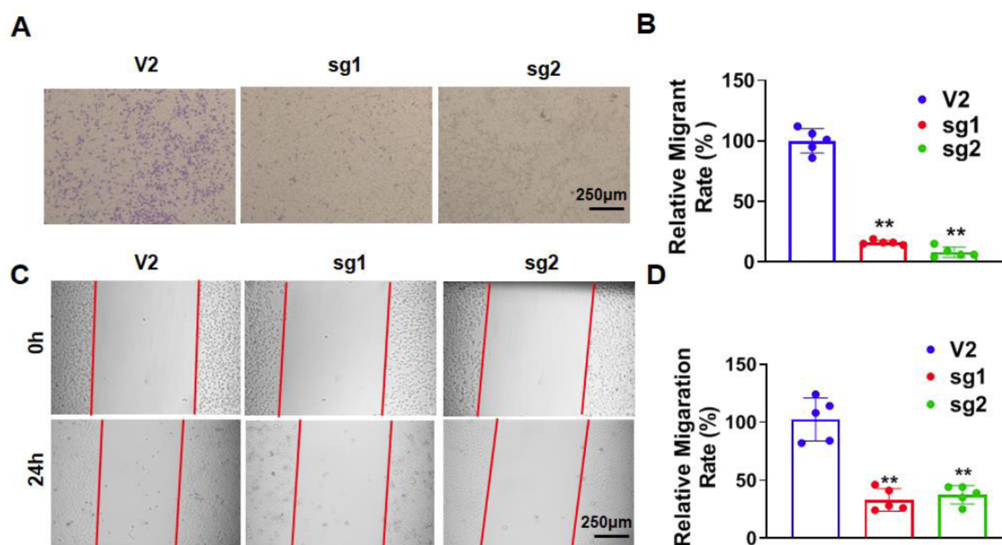

Figure 3: Knockout of Snail1 inhibits cell migration. (A, B) Cell migration was detected with transwell experiments. The representative images and quantitative results were shown. **(C, D)** Cell migration was investigated by scratch experiments. The representative images and quantitative results were shown. **P < 0.01 vs. corresponding control.

4. DISCUSSION

Snail1 was reported as possible prognostic markers and therapeutic targets for gastric cancer [4, 13]. Snail upregulation, which connects the dedifferentiation process to cancer stem cell features in gastric cancer cells, is a promising marker for predicting patient prognosis and a possible biological target for gastric carcinoma therapy [14-16]. Pantoprazole reduced gastric cancer cell lines AGS and MKN-28 cell migration and invasion by dramatically downregulating Snail1 [17]. By implementing shRNA to suppress Snail1 in gastric cancer cell lines SNU216 and SNU484, Snail is demonstrated to relate with lymph node metastasis [18]. Recent study shows that miRNA-200a reduces Snail1 expression, resulting in epithelial cell-mesenchymal transition inhibition in gastric cancer cells [19].

In this study, we used two distinct sgRNAs to successfully target Snail1 in the gastric cancer cell line MGC803 with the CRISPR/Cas9 system. Furthermore, we discovered that Snail1 knockout reduced the proliferation and migration of MGC803 cells. Delivering the Cas9 protein and a synthetic guide RNA into the cell and then combining the two parts to cut the target genome location, enables effective gene editing [11]. Snail1 also affects the emergence and progression of ovarian cancer, according to an earlier research that used CRISPR/ Cas9 to eradicate the gene in ovarian cancer RMG-1 cells [20].

5. CONCLUSION

In summary, our findings show that targeting Snail1 by CRISPR/Cas9 System inhibits the proliferation and migration of human gastric cancer cells, providing the important evidence for the role of Snail1 in gastric cancer.

AUTHORS' CONTRIBUTION

Peng-Wei Zhang performed the experiments, analyzed the data, and wrote the paper.

Zhe-Sheng Chen designed the experiments and wrote the paper.

Zhi Shi designed the experiments, analyzed the data, and wrote the paper.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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