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TEAD4-siRNA as an Emerging Drug for the Treatment of Gastric Cancer

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Abstract: As a downstream element that functions as an effector within the Hippo signaling pathway, TEAD4 has been linked to the proliferation, metastasis, and chemoresistance of gastric cancer. Silencing TEAD4 expression may provide a promising treatment approach. This study investigated the effects of TEAD4-targeting siRNA (TEAD4-siRNA) on AGS gastric cancer cells, focusing on cell proliferation, migration, invasion. A siRNA sequence targeting TEAD4 was designed and cloned into a SiRNA vector. AGS cells were transfected with the recombinant plasmid, and functional assays encompassing CCK-8 proliferation, transwell migration/invasion, wound healing, and ROS detection were performed. TEAD4 silencing by siRNA significantly inhibited AGS cell proliferation, migration, and invasion, while reducing colony formation capacity. Our TEAD4-siRNA effectively suppresses malignant phenotypes of gastric cancer cells and represents a potential therapeutic approach for gastric cancer treatment.

1. Introduction

Gastric cancer continues to be among the most widespread and deadly malignancies across the globe, ranking among the primary causes of cancer-related deaths. Its progression involves deregulation of multiple signaling pathways, among which the Hippo pathway has emerged as a crucial regulator of proliferation, differentiation, and stem cell self-renewal [1]. The downstream transcriptional effectors YAP/TAZ interact with TEAD family proteins to regulate gene expression programs linked to tumorigenesis, metastasis, and therapy resistance [2].

TEAD4, one of the major TEAD isoforms, is found to be highly expressed in gastric cancer cells. Previous studies indicate that TEAD4 enhances malignant phenotypes, including epithelial–mesenchymal transition (EMT), stemness maintenance, and chemoresistance [3,4]. Interference with TEAD4 expression or inhibition of its transcriptional activity has been shown to suppress gastric cancer proliferation and invasion [5].

Small interfering RNAs (siRNAs) are synthetic, double-stranded oligonucleotides that mediate sequence-specific degradation of target mRNAs via the RNA interference pathway. They have attracted increasing attention as therapeutic agents due to their high specificity and potential to silence previously 'undruggable' targets [6]. This study aims to evaluate the therapeutic effects of TEAD4-targeted siRNAs on gastric cancer AGS cells and to explore the molecular mechanisms underlying their action. Recent advances highlight siRNA nanocarriers and clinical applications in

solid tumors [7]. Recent studies further confirm TEAD4 as a prognostic marker and therapeutic target in gastric and colorectal cancers [3,8,9].

2. Materials and Methods

2.1 Cell Culture and Transfection

The human gastric adenocarcinoma AGS cell line (ATCC CRL-1739) was cultured in RPMI-1640 medium. This medium was enriched with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin. The cells were housed in a humidified incubator with 5% CO_2 at 37 ℃. For siRNA transfection, Lipofectamine 3000 was used, adhering to the instructions provided by the manufacturer. Control groups included untreated cells, scrambled siRNA controls, and shRNA constructs for comparative analysis.

2.2 Cloning and expression of TEF3-1 and SiRNAs

Using the website http://www.sirnawizard.com/design.php, we designed SiRNAs which were subsequently cloned into a pLKO.1 vector. The following are the sequences of TEF3-1 SiRNAs:

SiTEAD4: GAGAATGGACACTACTCTTAC.

2.3 Cell Transfection

AGS cells were transfected with TEAD4-siRNA plasmids or scrambled controls following the protocol for Lipofectamine 3000 (Invitrogen, USA) supplied by its manufacturer. Transfection efficiency was evaluated by co-transfection with a GFP-reporter plasmid, followed by fluorescence microscopy.

2.4 Western Blot Analysis

Cells were broken down using RIPA buffer containing protease inhibitors (Roche). A matching quantity of protein (30μg) was loaded onto SDS-PAGE gels for separation, after which it was transferred to PVDF membranes (Millipore). After being blocked with 5% non-fat milk, the membranes were incubated overnight at 4 ℃ in the presence of primary antibodies specific to TEAD4 (Cell Signaling Technology, 1:1000) and GAPDH (Abcam, 1:5000). After the membranes were exposed to HRP-conjugated secondary antibodies, ECL substrate (Thermo Fisher Scientific) was used to visualize the resulting signals.

2.5 Cell Proliferation Assay (CCK-8)

To evaluate cell proliferation, we utilized the Cell Counting Kit-8 (CCK-8, Dojindo Laboratories, Japan)—a reagent that identifies dehydrogenase activity in viable living cells. A density of 3×10^3 AGS gastric cancer cells per well was used for seeding in 96-well plates, with overnight adherence allowed. Following transfection with TEAD4-siRNA#1 or control siRNA, 10μL of CCK-8 reagent was introduced into each well at the 24-hour and 48-hour time points after transfection. The cells were placed in an incubator at 37 ℃ for 2 hours, after which a microplate reader (BioTek, USA) was used to measure the absorbance at 450 nm. Triplicate runs were conducted for each experiment, and proliferation rates were expressed as relative optical density compared with negative controls.

2.6 Wound-healing assays

AGS cells (or the target cells) were seeded into 6-well culture plates and cultured until they reached roughly 90% confluence. At this point, a sterile 200 µL pipette tip was used to create a scratch through the layer of confluent cells. After rinsing the cells with PBS, they were maintained in medium without serum. Images were captured at 0, 24, and 48 hours using an inverted microscope (Olympus, Japan). The wound closure rate was computed with the following formula: [(initial wound width – wound width at each time point) / initial wound width] × 100%.

2.7 Migration and Invasion Assays

Transwell chambers fitted with 8 µm pore filters (Corning, USA) were used to assess cell migration. In the migration experiments, 5×10^4 transfected AGS cells suspended in serum-free medium were plated into the upper compartment, while the lower compartment was loaded with medium supplemented with 10% fetal bovine serum (FBS) to act as a chemoattractant. After being incubated for 24 hours, any cells that failed to migrate were removed by wiping. On the other hand, cells that had migrated to the membrane's lower side were fixed in 4% paraformaldehyde, stained with 0.1% crystal violet, and then counted across five randomly selected microscopic fields. As for the invasion assays, the protocol was identical, with the exception that the upper chamber was pre-treated with Matrigel (BD Biosciences, USA)—a step designed to replicate the extracellular matrix-derived barriers.

2.8 Statistical Analysis

In the current study, each experiment was carried out in triplicate on separate occasions. Data are expressed as the mean ± standard deviation (SD). Statistical analyses were performed using Student's t-test or one-way ANOVA, with a P-value below 0.05 regarded as signifying a statistically significant difference. Graphs were created using GraphPad Prism 9 software.

3. Results

3.1 Construction of the siRNA Targeted to TEAD4

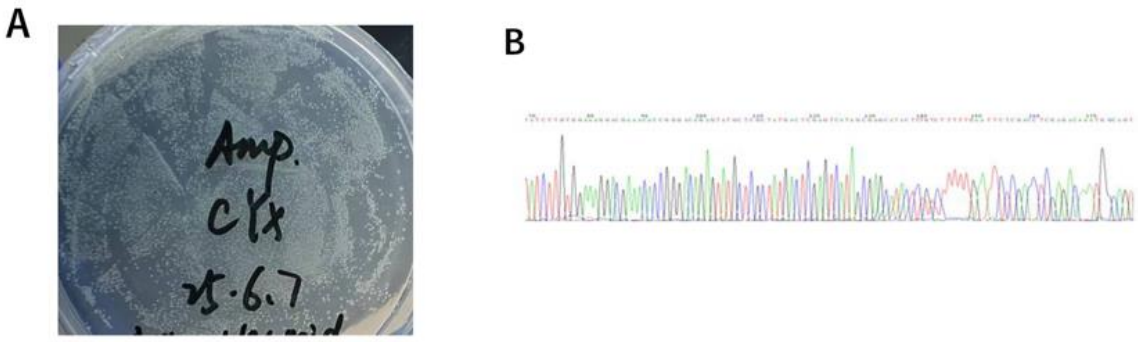


Figure 1 Construction of the TEAD4-siRNA expression plasmids: (A) Single colonies selected from LB agar plates following bacterial transformation; (B) Representative sequencing chromatogram confirming the insertion of TEAD4-siRNA#1 into a pLKO.1 vector.

RNA interference (RNAi) using small interfering RNA (siRNA) offers a powerful approach for transient gene silencing in mammalian cells. In this study, siRNA constructs specifically targeting

the coding sequence (CDS) of the human TEAD4 gene were designed based on established siRNA design guidelines. The siRNA sequence (TEAD4-siRNA) was selected to minimize off-target effects and enhance silencing efficiency.

Complementary oligonucleotides corresponding to the selected siRNA sequences were synthesized, annealed to form double-stranded inserts, and subsequently cloned into the vector using BamHI and EcoRI restriction sites. The ligation products were transformed into E. coli DH5α competent cells and plated on LB agar containing ampicillin for positive clone selection (Figure 1A). Single colonies were picked and expanded for plasmid extraction. The correct insertion of the siRNA sequences into the vector backbone was confirmed by Sanger sequencing (Figure 1B), ensuring the fidelity of the constructs. As a result, plasmids expressing TEAD4-siRNA were successfully generated and validated for downstream applications.

3.2 siRNA Plasmid Expression Efficiency in AGS Gastric Cancer Cells

To evaluate the transfection efficiency of the TEAD4-siRNA plasmids, AGS gastric cancer cells were transfected with a pLKO.1 vector harboring the siRNA constructs using Lipofectamine 2000. At 24–48 hours post-transfection, cells were observed under both bright-field and fluorescence microscopy. The bright-field images revealed normal cellular morphology and adherence, while the corresponding fluorescence images showed robust green fluorescent protein (GFP) expression in a majority of the cells, indicating high transfection efficiency.

Images confirmed that the green fluorescence signal co-localized with viable cell boundaries, validating successful plasmid uptake and expression in AGS cells. These results confirmed the suitability of the system for functional knockdown studies of TEAD4 in gastric cancer cells (Figures 2A and 2B).

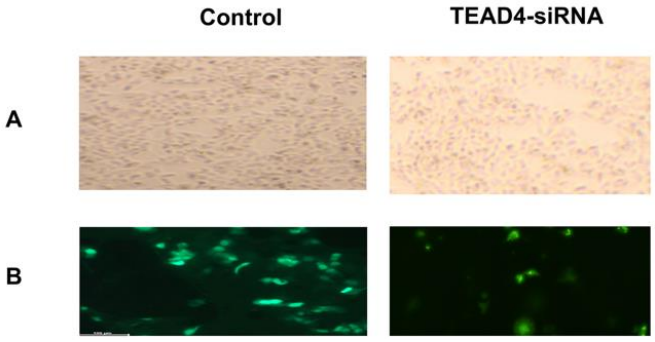


Figure 2 Validation of TEAD4-siRNA plasmid transfection efficiency in AGS cells: (A) Bright-field microscopy image showing normal morphology and cell distribution following plasmid transfection; (B) Corresponding fluorescence image displaying strong GFP expression in transfected AGS cells. Scale bar: 100 μm.

3.3 Inhibition of TEAD4 expression

In Figure 3, western blot analysis was performed to assess the protein-level knockdown efficiency of TEAD4 following siRNA transfection in AGS gastric cancer cells. Total protein was extracted 48 hours after transfection with RIPA lysis buffer that contained protease inhibitors. An equal quantity of protein (usually 20–30 μg) was separated on SDS-PAGE gels and then transferred to PVDF membranes. Following blocking in 5% non-fat milk, the membranes underwent overnight incubation at 4 ℃ with a primary antibody that specifically recognizes TEAD4. After being washed, membranes were placed in incubation with secondary antibodies linked to HRP and developed using enhanced chemiluminescence (ECL) reagents. A robust reduction in TEAD4 protein levels

was observed in cells transfected with TEAD4-siRNA compared to the negative control siRNA group. β-actin (or GAPDH) functioned as a loading control to ensure proteins were loaded in equal amounts. Densitometric analysis further confirmed a marked decrease in TEAD4 expression, validating the knockdown efficiency at the protein level.

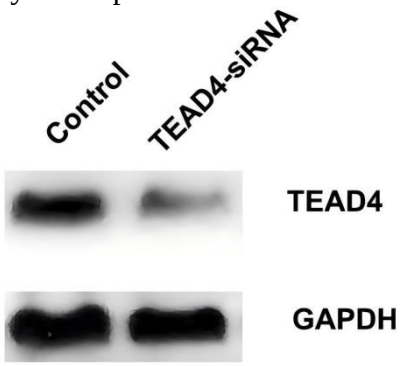


Figure 3 TEAD4 knockdown efficiency in AGS gastric cancer cells: Western blot analysis showing TEAD4 protein expression in AGS cells transfected with TEAD4-siRNA compared with scrambled siRNA control. GAPDH was used as a loading control.

3.4 Inhibition of Cell Proliferation

In Figure 4, we examined how silencing TEAD4 affects the growth of gastric cancer cells using the Cell Counting Kit-8 (CCK-8) method. We seeded AGS cells into 96-well plates at 3×10^3 cells per well, then transfected them with TEAD4-siRNA#1 or negative control siRNA. Two time points—24 and 48 hours after transfection—were chosen to assess cell viability: 10 μL of CCK-8 reagent was added to each well, and the plates were incubated at 37 ℃ for 2 hours to complete the measurement. Absorbance at 450 nm was quantified with the use of a microplate reader.

Compared to the control groups, cells transfected with TEAD4-siRNA exhibited a significant decrease in absorbance values at both time points, indicating impaired proliferative capacity. The decrease in cell viability indicates that TEAD4 serves a key function in facilitating the proliferation of AGS gastric cancer cells. These findings support the hypothesis that TEAD4 is essential for maintaining the proliferative phenotype in this cancer cell model.

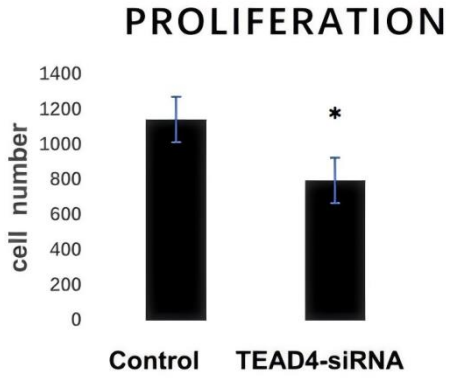
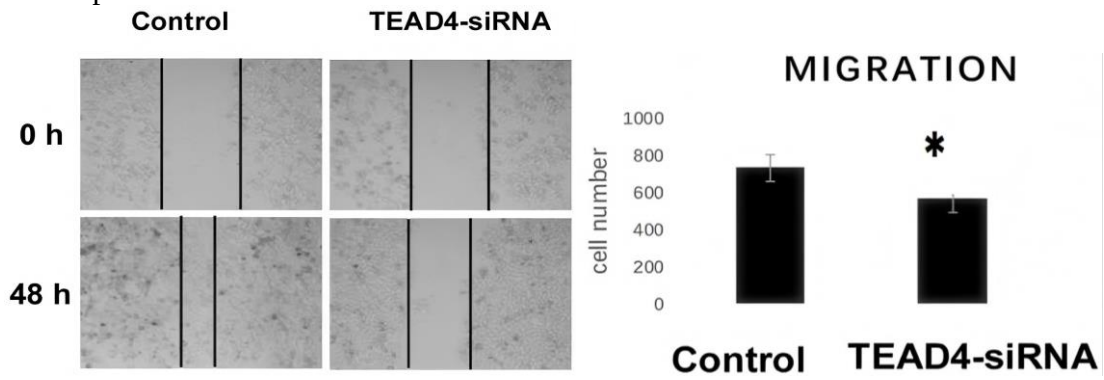


Figure 4 Knocking down TEAD4 suppresses the proliferation of AGS gastric cancer cells: (A) The Cell Counting Kit-8 (CCK-8) test revealed cell viability 48 hours following transfection with TEAD4-siRNA or scrambled siRNA as a control. Readings of absorbance were taken at a wavelength of 450nm; (B) Relative cell proliferation rates were quantified and normalized to the control group (mean±SD, n=3). Student's t-test was applied for statistical analysis, with *$p<0.05$ indicating a significant difference compared to the control.

3.5 Wound-Healing Assay to Evaluate Cell Migration Following TEAD4 Knockdown

We examined how TEAD4 silencing affects the migration ability of gastric cancer cells using a wound-healing experiment. After AGS cells were plated in 6-well plates, they were cultured continuously until the cell layer reached nearly full confluence. Using a sterile 200 μL pipette tip, a steady scratch was made in the confluent cell layer after the cells were transfected with TEAD4-siRNA or negative control siRNA. Cells that had detached were softly rinsed away with PBS, and the original medium was substituted with RPMI-1640 medium containing 1% FBS (serum-reduced) to reduce cell proliferation to a minimum.



(A) (B)

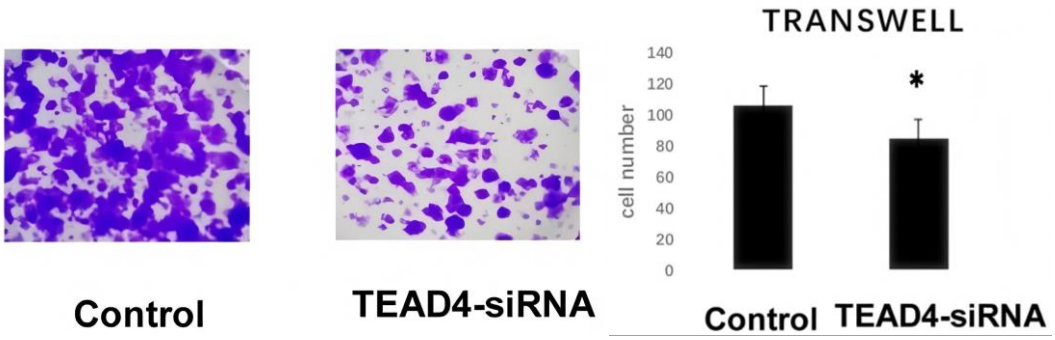
Figure 5 TEAD4 knockdown impairs migration of AGS gastric cancer cells: (A) Representative phase-contrast images of wound-healing assays at 0 h and 48 h after scratching in AGS cells transfected with TEAD4-siRNA or scrambled siRNA control; (B) Quantification of wound closure percentage at 48 h (mean ±SD, n=3). Statistical analysis was performed using Student's t-test; *$p<0.05$ vs. control.

In Figure 5, at 0 hours and 48 hours following the scratch, images of the wounded region were obtained with a phase-contrast microscope. To analyze wound closure quantitatively, the remaining width of the wound at each time point was measured using ImageJ software. Compared to control cells, TEAD4-silenced AGS cells displayed a markedly reduced rate of wound closure, indicating impaired migratory ability. These findings suggest that TEAD4 is required for efficient gastric cancer cell motility. TEAD4 knockdown significantly reduced the number of migrated and invaded cells. These findings collectively demonstrate that TEAD4 is essential for maintaining the migratory and invasive capacity of gastric cancer cells.

3.6 Transwell Assay to Evaluate Cell Invasion Following TEAD4 Knockdown

The influence of TEAD4 silencing on the invasive potential of AGS gastric cancer cells was analyzed with the help of Matrigel-coated transwell chambers. Following transfection of AGS cells with TEAD4-siRNA or scrambled siRNA, a 24-hour period elapsed. At this point, non-invading cells on the upper membrane surface were discarded, and invading cells that had traversed the Matrigel to the lower surface were subjected to fixation with 4% paraformaldehyde, staining with 0.1% crystal violet, and counting in five random microscopic fields.

As illustrated in Figure 6, silencing TEAD4 resulted in a notable decline in the count of invading AGS cells when compared to the scrambled siRNA control group ($p<0.01$). Quantitative assessments verified a considerable reduction in the number of invasive cells per microscopic field, which suggests that TEAD4 knockdown significantly weakens the invasive capability of AGS gastric cancer cells.



(A) (B)

Figure 6 TEAD4-siRNA suppresses migration and invasion in AGS gastric cancer cells: (A) Representative micrographs of transwell migration and Matrigel invasion experiments, carried out 24 h following transfection with TEAD4-siRNA or scrambled siRNA. Cells were stained with 0.1% crystal violet; (B) Quantification of migrated and invaded cells per field (mean ±SD, n=3). Student's t-test was used for statistical analysis; *p<0.05 relative to the control.

4. Discussion

Our findings firmly place TEAD4 at the centre of the malignant programme of AGS gastric cancer cells. Silencing TEAD4 with siRNAs dismantled several hallmarks of aggressiveness—cell proliferation, migration, invasion and clonogenic growth—revealing TEAD4 as more than just a downstream effector of the Hippo pathway but as a critical oncogenic driver in this context. These observations echo and extend previous reports of Hippo pathway dysregulation and TEAD4 activation as a unifying theme in gastric and other epithelial cancers [10-12].

Unlike more conventional shRNA approaches, siRNAs offer a non-integrative, transient, and therefore safer route to target TEAD4, which is particularly attractive from a translational standpoint. This is timely: siRNA-based drugs are no longer theoretical, with several already approved by the FDA—most notably patisiran—and lipid nanoparticle technologies have revolutionised their delivery to solid tumours [4,7,13,14]. These developments create a favourable backdrop for translating our findings into therapeutic strategies.

Taken together, our work frames TEAD4 as an actionable vulnerability in gastric cancer and positions TEAD4-targeting siRNAs as a promising therapeutic avenue. Future investigations in animal models and refinements in delivery platforms, including next-generation lipid nanoparticles [15-17], will be key to bringing this concept to the clinic. Combination approaches that simultaneously blunt multiple oncogenic pathways are increasingly supported by preclinical data and could further enhance efficacy[18,19].

5. Conclusion

Silencing TEAD4 with siRNAs powerfully suppresses proliferation, migration and invasion of AGS gastric cancer cells. TEAD4 is elevated from a prognostic marker to a tangible therapeutic target by these results, and a foundation is laid for the advancement of siRNA-based strategies that aim to suppress primary tumor growth as well as metastatic spread in gastric cancer.

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