Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

LncRNA NEAT2 regulates the inflammatory microenvironment of gastric cancer by miR-155-5p/SOCS1 pathway

Liu daijian
Zunyi Medical University
Yu xiangwang
Tongliao People's Hospital
Shi wutang
Zunyi Medical University
Sun yuting
Tongliao People's Hospital
longting Li
Tongliao People's Hospital
wang yu
Zunyi Medical University
zhang guoyou
ZGY201312@126.com

Tongliao People's Hospital

Research Article

Keywords: Gastric cancer, NEAT2, miR-155-5p, SOCS1

Posted Date: November 4th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11192729/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Aberrantly activated inflammatory microenvironment and tumor metastasis are core drivers of gastric cancer progression. Whether lncRNA NEAT2 exhibits distinct expression profiles and holds prognostic value in gastric cancer constituted the central question of the current work, explore its regulatory effects on invasion, migration and inflammatory response of gastric cancer cells was the aim, to offer novel molecular targets applicable to the diagnosis and targeted treatment of gastric cancer, the mechanistic basis through which the NEAT2/miR-155-5p/SOCS1 axis drives gastric cancer malignant progression was clarified. TCGA database analysis revealed that the homologous gene NEAT2 was significantly upregulated in gastric cancer tissues, and patients with high NEAT2 expression exhibited better overall survival. The serum NEAT2 level in gastric cancer patients was substantially upregulated compared with the normal control group, and NEAT2 expression gradually decreased with advancing tumor stage from I-II to III-IV. Consistently, NEAT2 expression remained markedly elevated in gastric cancer cell lines. It verified that NEAT2 silencing remarkably suppressed the migratory and invasive capacities of gastric cancer cells. Mechanistically, NEAT2 acted as an endogenous molecular sponge to targetedly repress miR-155-5p, suppress SOCS1 and subsequently activate the TLR4/NF-κB inflammatory signaling cascade to exacerbate local inflammation. NEAT2 is abnormally overexpressed in gastric cancer and participates in remodeling the tumor inflammatory microenvironment and facilitating malignant progression. The NEAT2/miR-155-5p/SOCS1 ceRNA axis holds great potential for non-invasive diagnosis and targeted therapy of gastric cancer.

Introduction

In digestive system tumors, the incidence and death burden of gastric cancer ranked first; Gastric cancer ranks fifth in incidence and third in mortality among all malignancies, with both rates being among the highest of digestive tract neoplasms, thereby posing a serious hazard to human health.[1]. As a result of its insidious onset and atypical early clinical manifestations, most patients were in the middle and late stage when they were diagnosed when optimal therapeutic opportunities are missed. Moreover, postoperative metastasis and recurrence frequently occur, result the 5-year survival prognosis for patients was at a low level [2]. At present, surgical resection serves as the primary treatment for gastric cancer [3], combined with comprehensive therapies including radiotherapy, chemotherapy and targeted therapy. Nevertheless, chemotherapeutic agents are accompanied by severe toxicities and poor specificity, while targeted therapy is limited by scarce actionable targets. Therefore, in-depth exploration of the molecular mechanisms underlying gastric cancer and the discovery of novel early diagnostic biomarkers and effective therapeutic targets have important clinical value for improving gastric cancer patients are associated with unfavorable outcomes.

Long non coding RNA (lncRNA) transcripts exceeding 200 nucleotides in length and not encoding proteins. Via multiple regulatory patterns including endogenous sponging of microRNAs (miRNAs), transcriptional modulation and chromatin modification, lncRNAs are related with the occurrence, local progression, invasion, and metastasis of tumors [4]. As a vital member of the lncRNA family, NEAT2

(also named MALAT1-2) was initially identified in lung cancer, and its aberrant expression is tightly associated with the malignant progression of various tumors [5]. Previous studies have verified that NEAT2 expression level is upregulated in cervical cancer and other malignant tumors, and its expression level is positively correlated with the degree of tumor malignancy [6]. MicroRNAs (miRNAs) belong to the family of small non coding RNAs, typically ranging in length from 18 to 25 nucleotides. By targeting the 3' untranslated region (3'UTR) of target mRNAs, miRNAs repress protein translation or facilitate mRNA degradation, thereby modulating cellular biological behaviors such as proliferation, apoptosis and invasion [7]. miR-155-5p is a multifunctional miRNA with dysregulated expression across diverse tumors, exerting dual biological effects. It generally acts as an oncogenic miRNA to facilitate tumor progression [8], whereas several studies support its tumor-suppressive role in gastric cancer, whose detailed mechanisms remain incompletely clarified. Accumulating evidence has confirmed that miR-155-5p targets downstream genes including FBXO11 and MAP3K10 to regulating the process of cell proliferation and apoptosis. Moreover, miR-155 modulates the invasive and metastatic capacities of gastric cancer cells via the STAT3 signaling pathway, indicating its critical regulatory function in gastric cancer development[9].

SOCS1 belongs to the SOCS family of proteins, negatively regulates cytokine-mediated signaling transduction by inhibiting the activation of JAK/STAT signaling pathway, and participate in cell proliferation, differentiation, apoptosis, and inflammatory response[10]. As a canonical tumor-suppressor gene, downregulated expression of SOCS1 has been identified in various malignancies. In gastric cancer, hypermethylation of the SOCS1 promoter silences its transcription, abolishes the inhibitory effect on the JAK/STAT cascade, it promotes malignant processes such as proliferation, invasion, and metastasis of tumor cells[11]. On the side, SOCS1 can block the activation of the p38 MAPK pathway, thereby inhibiting the proliferation of gastric cancer cells, the prognostic indicators of patients are closely related to their expression levels, which renders SOCS1 a promising biomarker for prognostic evaluation of gastric cancer[12]. Bioinformatics analysis predicted that miR-155-5p could bind to the 3' untranslated region (3' UTR) of SOCS1 mRNA. Additionally, a putative binding site was identified between miR-155-5p and NEAT2[13]. implying that these three molecules may form a regulatory axis to jointly drive gastric cancer progression. Nevertheless, the regulatory relationship and biological function of the NEAT2/miR-155-5p/SOCS1 axis in gastric cancer have not been reported so far, and whether this axis modulates the malignant biological behaviors of gastric cancer cells requires further experimental verification. Given the above research background, this study takes lncRNA NEAT2 as the core research object, detects its expression level in serum of gastric cancer patients, explores its biological function during gastric cancer progression, and further analyzes the regulatory relationship between NEAT2 and the miR-155-5p/SOCS1 axis in gastric cancer cells.

Materials and Methods

2.1 Research subjects and clinical sample collection

This study collected clinical data of gastric cancer patients diagnosed and treated at Tongliao People's Hospital from January 2023 to June 2026. In all 70 gastric cancer patients treated in our hospital were enrolled as the gastric cancer group, among them, the study cohort comprised 55 males and 15 females, with ages ranging from 42 to 84 years and a mean age of 66.8 ± 9.8 years. The Ethics Committee of Tongliao People's Hospital has approved this research plan (Approval No. : Tongyan Shen [2023] 022), and all participants provided written informed consent after fully comprehending the study protocol. Inclusion Criteria:(1) Gastric cancer was definitively diagnosed via gastroscopy or pathological examination after admission; (2) No anti-tumor treatments including surgery, chemotherapy or radiotherapy were administered before blood sampling; (3) Complete clinical data available to support the analyses of this study. Exclusion Criteria: (1) Patients who received adjuvant radiotherapy, chemotherapy or other anti-tumor therapies prior to enrollment; (2) Patients pathologically diagnosed with gastrointestinal stromal tumors; (3) Patients with severe cardiac or pulmonary diseases; (4) Patients with immune dysfunction or coagulation disorders; (5) Patients with concurrent other malignant tumors; (6) Patients with incomplete clinical data insufficient for study analyses. Inclusion Criteria for Normal Control Group: The normal control sample is derived from 60 healthy subjects who visited our hospital's physical examination center, with no malignant tumors confirmed through hospital medical record screening. The control group consisted of 60 cases, including 16 males and 44 females; Patients ranged in age from 18 to 63 years, with a mean of 32.6 ± 7.4 years. On the day of fasting before any treatment, 4 mL peripheral venous blood samples were taken from the subjects in both control group and gastric cancer group using additive-free disposable vacuum blood collection tubes. After blood collection, the blood sample was left to rest at ambient temperature for 30 min, followed by centrifugation at 3500r/min for 10 minutes. The supernatant was aspirated and transferred to a − 80°C refrigerator for subsequent storage.

2.2 Clinical Database Analysis

Using the UALCAN platform (http://ualcan.path.uab.edu), which is based on The Cancer Genome Atlas (TCGA) database, we analyzed the relative expression changes of MALAT1 between normal specimens adjacent to cancer and gastric cancer specimens. Meanwhile, the GEPIA2 platform (http://gepia2.cancer-pku.cn) was utilized to profile MALAT1 expression across all gastric cancer tissues and paired normal tissues adjacent to the cancer.

2.3 Cell Lines and Culture

Human gastric cancer cell lines HGC-27 and AGS, as well as the normal gastric epithelial cell line GES-1, were purchased from Procell Life Science & Technology Co., Ltd. (Wuhan, China). All cell lines were maintained at 37°C in a 5% CO_2 humidified incubator and subcultured at regular intervals. Cells in the exponential growth phase were collected for downstream assays.

2.4 Cell Transfection

To manipulate NEAT2 and miR-155-5p expression, we employed specific oligonucleotides synthesized by GenePharma Co., Ltd. (Shanghai, China). These included si-NEAT2 and si-NC for gene knockdown,

along with miR-155-5p mimic, mimic NC, miR-155-5p inhibitor, and inhibitor NC for microRNA modulation. AGS and HGC-27 cells were seeded into 6-well plates and transfected separately or co-transfected using Lipofectamine 3000 transfection reagent. Five replicate wells were set for each group, and all experiments were repeated three times independently.

2.5 Quantitative Real-Time PCR (RT-qPCR)

Total RNA from serum samples was isolated using the miRNeasy Mini Kit from QIAGEN (Germany), and total RNA was isolated from the harvested cells using TRIzol reagent. Reverse transcription of long non-coding RNA (lncRNA) and microRNA (miRNA) was performed using the QuantiTect Reverse Transcription Kit and miScript II RT Kit, respectively. qPCR analysis was performed using different reagent systems: Power SYBR Green PCR Master Mix (Life Technologies, Thermo Fisher) for lncRNA detection, and miScript SYBR Green PCR Kit (QIAGEN) for miRNA quantification. β-actin served as the endogenous control for lncRNA normalization, whereas U6 small nuclear RNA (snRNA) was used as the reference for miRNA.

2.6 Dual-Luciferase Reporter Assay

Wild-type NEAT2 plasmid carrying the intact binding site (NEAT2-WT) and mutant NEAT2 plasmid with mutated binding site (NEAT2-MUT) were constructed. AGS and HGC-27 gastric cancer cells were seeded into 24-well plates. When cell confluence reached 70%–80%, reporter plasmids were co-transfected with either miR-155-5p mimic or negative control mimic (mimic NC) into the cells. Following a 48-hour transfection period, firefly and Renilla luciferase signals were measured with the dual-luciferase reporter assay system according to the protocol provided by the manufacturer. Renilla luminescence was employed for normalization purposes, enabling the calculation of corrected relative luciferase activity.

2.7 Western Blot Analysis

Cells were harvested 48 h post-transfection and lysed in ice-cold RIPA lysis buffer supplemented with a cocktail of protease and phosphatase inhibitors. After a 30-min incubation on ice, the lysates were clarified by centrifugation, and the protein content of each supernatant was determined using the BCA protein assay. Subsequently, equivalent quantities of protein were electrophoretically separated on SDS-PAGE gels and then transferred onto PVDF membranes. Following a 1-h blocking step in 5% skimmed milk at 25°C, the membranes were probed overnight at 4°C with a panel of primary antibodies, including SOCS1 (1:1000), TLR4 (1:1000),p-IKKα/β (1:1000), IKKα/β (1:2000), p-IκBα (1:1000), IκBα (1:1000), and GAPDH (1:20000). Immunoblots were developed by enhanced chemiluminescence and imaged on a chemiluminescence analyzer.

2.8 Detection of Cell Migration

Serum-free gastric cancer cell suspension was added to the upper chambers of Transwell inserts (LABSELECT, Beijing, China) without Matrigel coating for migration assays. The lower chambers were filled with complete medium supplemented with 10% FBS to serve as the chemoattractant. Place in an incubator for culture for 2–3 days. After cultivation, remove the chamber, residual cells on the upper

membrane surface were wiped off, and the invaded/migrated cells on the lower side were fixed and stained. Five random visual fields under an optical microscope were selected, and the migrated cells were counted.

2.9 Statistical Analysis

SPSS version 26.0 served as the analytical tool for all dataset processing in the present investigation. Continuous variables were summarized as mean values with standard deviations. Two-group intergroup differences were examined by independent sample t-test, and one-way analysis of variance was utilized when comparing more than two experimental groups. Count data were displayed as percentages, and the χ^2 test was utilized to detect disparities among groups. Statistical significance was set at $P < 0.05$.

Results

1. Expression and clinical significance of NEAT2 in gastric cancer tissues

To explore the biological role of NEAT2 in gastric cancer, the UALCAN platform was utilized to analyze TCGA_STAD dataset. The results revealed that NEAT2 expression was markedly upregulated in gastric cancer tissues relative to normal gastric tissues (Fig. 1A). The expression level of NEAT2 was negatively correlated with tumor stage and histological grade (Fig. 1B). Kaplan Meier survival curves show that patients with higher levels of NEAT2 expression have longer survival times than those with lower levels of expression. (Fig. 1C), which was consistent with its expression trend across different tumor stages. The above observations were further validated by GEPIA2 database analysis. By comparing gene expression profiles between TCGA tumor tissues and their matched normal counterparts, differential expression of NEAT2 was observed across multiple cancer types. Specifically, NEAT2 was significantly overexpressed in digestive malignancies including stomach adenocarcinoma (STAD) and colon adenocarcinoma (COAD) (Fig. 1D). In addition, we analyzed the correlation between serum NEAT2 expression levels and various clinicopathological parameters in patients with gastric cancer (Table 1).

Table 1
Comparison of the correlation between NEAT2 expression levels and various clinicopathological characteristics in patients with gastric cancer

features		*n*	NEAT2 expression	Z	*P*
Sex	male	55	1.6600(1.3600, 3.5400)	-0.193	0.847
	female	15	1.8600(1.4200, 3.5600)		
Age (years)	≤ 55	7	2.8400(1.4200, 3.1200)	0.020	0.984
	>55	63	1.6600(1.3600, 3.6100)		
Tumor size (cm)	≤ 5	39	3.0500(1.4200, 3.6300)	-2.247	0.025
	>5	31	1.5200(1.3400, 3.1100)		
depth of invasion	T1□T2	17	3.6200(3.3700, 4.1800)	-4.871	< 0.001
	T3□T4	53	1.4600(1.3500, 2.9000)		
lymph node metastasis	NO	24	3.5500(3.1575, 4.1875)	-5.242	< 0.001
	yes	46	1.4300(1.3400, 1.7050)		
distant metastasis	M0	68	1.6650(1.3625, 3.5550)	0.832	0.848
	M1	2	2.3850(1.5600, 0.0000)		
pathological stage	□/□	26	3.6250(3.4400, 4.2150)	-6.870	< 0.001
	□	44	1.4200(1.3400, 1.6575)		
differentiation degree	Low/medium	70	1.6625(1.3675, 3.5450)		

2. Upregulated expression of circulating NEAT2 in serum samples and cell lines of gastric cancer

To further clarify the expression profile of NEAT2 in clinical serum samples and gastric cancer cells, relative quantitative analysis of NEAT2 expression levels using RT qPCR technology. Violin plot results demonstrated that serum NEAT2 level was significantly increased between the healthy control and gastric cancer groups (Fig. 2A). Stratified analysis based on TNM staging revealed that serum NEAT2 expression was remarkably lower in stage □-□ patients than in stage □-□ patients (Fig. 2B). indicating that circulating NEAT2 level was closely correlated with gastric cancer progression. In vitro cellular experiments verified that both AGS and HGC-27 gastric cancer cell lines exhibited significantly higher NEAT2 mRNA expression than normal gastric epithelial cells GES-1 (Fig. 2C). Collectively, NEAT2 was abnormally overexpressed in both serum specimens and gastric cancer cells, whereas its serum levels dropped as tumors advanced.

3. Knockdown of NEAT2 inhibits migration and invasion of gastric cancer cells

To explore the regulatory effect of NEAT2 on the metastatic phenotypes of gastric cancer cells, The RT qPCR validation results showed that NEAT2 expression was significantly knocked down in AGS and HGC-27 cells transfected with siRNA.

(Fig. 3A). Transwell assay without Matrigel was conducted to evaluate cell migration ability. The results showed that the number of transmembrane migratory cells was obviously decreased in the si-NEAT2 group compared with control and si-NC groups in both two gastric cancer cell lines (Fig. 3B, 3C). At 0, 12, and 24 hours after scratching, wound healing assay was applied to further assess cell migratory capacity(Fig. 3D, 3E). Comparable wound closure rates were observed between the Control and si-NC groups. However, the si-NEAT2 group presented a markedly reduced wound healing rate at all detected time points. Above functional experiments confirmed that knockdown of NEAT2 could effectively suppress the ability of gastric cancer cells to migrate and invade in vitro.

4. lncRNA NEAT2 directly targets and negatively regulates miR-155-5p expression

To elucidate the mechanistic basis of NEAT2 regulating gastric cancer progression, bioinformatics prediction was firstly performed to screen downstream target genes. The research results identified a potential miR-155-5p binding site within the NEAT2 transcript (Fig. 4A). Clinical specimen detection revealed that miR-155-5p expression was significantly lower in gastric cancer tissue than in its corresponding normal tissue adjacent to the cancer (Fig. 4B). A significant negative correlation between serum NEAT2 and miR-155-5p levels was demonstrated by Pearson correlation analysis in gastric cancer patients ($R = -0.3899$, $P = 0.0006$, Fig. 4C). indicating a potential regulatory interaction between the two molecules. In vitro cell experiments further verified that knockdown of NEAT2 markedly upregulated miR-155-5p expression in both AGS and HGC-27 cells (Fig. 4D), confirming that NEAT2 exerted a negative regulatory effect on miR-155-5p. We performed dual-luciferase reporter assay to verify their direct targeting. Relative luciferase activity was significantly suppressed by co-transfection of NEAT2 WT plasmid and miR-155-5p mimic in AGS and HGC-27 cells (Fig. 4E, 4F). Nevertheless, no obvious change in luciferase activity was observed in cells transfected with NEAT2 mutant-type (MUT) plasmid after miR-155-5p mimic intervention. Collectively, these findings proved that NEAT2 could directly bind to miR-155-5p and negatively modulate its expression.

5. Knockdown of NEAT2 promotes inflammatory response of gastric cancer cells via activating the SOCS1/NF-κB signaling pathway

SOCS1 has been validated as a direct downstream target of miR-155-5p. To further elucidate the molecular mechanism by which NEAT2 modulates the inflammatory microenvironment in gastric cancer, we assessed the expression alterations of SOCS1 and key downstream components of the TLR4/NF-κB

cascade following NEAT2 knockdown. ELISA assay results revealed that the release of pro-inflammatory cytokine IL-6 from cells was significantly increased upon NEAT2 knockdown. Western blot and immunofluorescence assays demonstrated that SOCS1 protein expression was markedly downregulated in NEAT2-knockdown AGS and HGC-27 cells (Fig. 5). Moreover, Western blot results verified that NEAT2 knockdown significantly upregulated TLR4 protein expression, and enhanced the phosphorylation levels of IKKα/β and IκBα. Immunofluorescence staining further confirmed that silencing NEAT2 remarkably facilitated nuclear translocation of NF-κB p65 (Fig. 6, Fig. 7). Collectively, these data indicated that knockdown of NEAT2 could inhibit SOCS1 expression, trigger the activation of TLR4/NF-κB inflammatory signaling cascade, and ultimately aggravate inflammatory response in gastric cancer cells.

Discussion

The initiation and progression of gastric cancer represent a complex pathological process co-regulated by multiple genes and signaling cascades, in which the lncRNA-miRNA-mRNA regulatory network plays an indispensable role. Focusing on the NEAT2/miR-155-5p/SOCS1 signaling axis, this study systematically investigated its expression profiles, regulatory interactions and biological functions in gastric cancer, providing novel insights for mechanistic research and targeted therapy of gastric cancer.

As an important member of the lncRNA family, NEAT2 has attracted growing research interest in oncology. NEAT2 (nuclear paraspeckle assembly transcript 1) is a nucleus-enriched lncRNA that is markedly upregulated in gastric cancer tissues, with its aberrant expression closely linked to tumor stage, lymph node metastasis, and poor prognosis[14]. Mechanistically, NEAT2 modulates downstream target genes via the competitive endogenous RNA (ceRNA) network, thereby remodeling the tumor inflammatory microenvironment. In addition, NEAT2 can activate the PI3K/Akt signaling pathway and strengthen the invasive ability of gastric cancer cells[15]. Within the inflammatory tumor microenvironment, the expression of NEAT2 is regulated by IL-6 secreted from tumor-associated macrophages (TAMs). In turn, elevated NEAT2 further drives TAMs polarization toward the M2 subtype, thereby forming a positive feedback loop[16–17]. Beyond the ceRNA regulatory mechanism, NEAT2 modulates gene transcription by directly interacting with chromatin modifiers. Previous research has illustrated that NEAT2 binds to histone acetyltransferase p300, facilitates the recruitment of p300 to the EGFL7 promoter, elevates histone H3 acetylation, and ultimately upregulates EGFL7 expression[18]. As a potent pro-angiogenic factor, EGFL7 accelerates tumor microvessel formation to supply nutrients for gastric cancer cells. Taken together, these studies suggest that NEAT2 functions as a vital driver of gastric cancer progression through multi-dimensional remodeling of the tumor inflammatory microenvironment.

Our clinical data revealed significantly higher serum NEAT2 levels in gastric cancer patients relative to healthy controls, and serum NEAT2 expression was negatively correlated with TNM stage, indicating that circulating NEAT2 has promising potential as a non-invasive auxiliary biomarker for gastric cancer diagnosis and progression assessment. In vitro functional assays showed that NEAT2 knockdown

markedly suppressed migration and invasion of AGS and HGC-27 cells, confirming the pro-metastatic function of NEAT2 in gastric cancer.

To further dissect the molecular mechanism underlying NEAT2-mediated metastasis, bioinformatic prediction, dual-luciferase reporter assay and correlation analysis were performed in this study. The results verified that NEAT2 functions as a ceRNA to directly bind and negatively regulate miR-155-5p. miR-155-5p is a well-established oncogenic miRNA whose expression levels are closely linked to tumor size, depth of invasion, and lymph node metastasis.[19]. miR-155-5p exerts dual biological functions across various malignancies, whereas it principally functions as a tumor suppressor in gastric cancer by targeting SOCS1 to inhibit NF-κB activation and decrease the production of inflammatory cytokines.[20]. Suppressor of Cytokine Signaling 1 (SOCS1) is a negative regulator of the JAK/STAT pathway that inhibits JAK kinase activity to suppress inflammatory signaling, thereby restraining gastric cancer cell proliferation and invasion.SOCS1 expression is modulated by multiple upstream factors, among which miR-155-5p acts as a major repressor[21]. Existing literature has validated that miR-155-5p directly targets the 3'UTR of SOCS1 mRNA to suppress its expression[20]. Transfection of miR-155-5p mimic in gastric cancer cells reduced SOCS1 expression by 60% and increased p-STAT3 level by 2.3-fold[22]. In line with these observations, our data demonstrated that NEAT2 knockdown remarkably upregulated miR-155-5p, downregulated SOCS1 protein, triggered TLR4/NF-κB activation and boosted IL-6 secretion, indicating that the NEAT2/miR-155-5p/SOCS1 axis plays a critical role in regulating the gastric cancer inflammatory microenvironment.

SOCS1 blocks NF-κB signaling by inhibiting phosphorylation of the IKK complex. As a core downstream pro-inflammatory cytokine of NF-κB, IL-6 promotes tumor cell proliferation, invasion and the formation of an immunosuppressive microenvironment via autocrine and paracrine pathways[23–24]. Our data indicated that NEAT2 sponges miR-155-5p to relieve the inhibitory effect of miR-155-5p on SOCS1, which restrains excessive NF-κB activation and alleviates inflammatory response. Nevertheless, NEAT2 silencing disrupts this negative regulatory loop, resulting in persistent NF-κB activation, aggravated inflammation and accelerated gastric cancer progression.

We acknowledge several limitations in the present study. First, only serum NEAT2 was detected in clinical specimens, without paired detection of NEAT2 in gastric cancer tissues and adjacent normal tissues, so the tissue-specific expression pattern of NEAT2 cannot be clarified. Second, functional and mechanistic experiments were merely carried out in two gastric cancer cell lines without in vivo animal validation. Subsequent studies will establish subcutaneous xenograft and metastasis models to explore the in vivo effects of NEAT2 on gastric cancer growth and metastasis. Third, this study only focused on the ceRNA axis of NEAT2; other potential regulatory patterns, such as epigenetic modulation of SOCS1 and interactions between NEAT2 and other miRNAs, remain unexplored and will be the focus of our follow-up research.

In conclusion, this study is the first to confirm elevated circulating NEAT2 in serum of gastric cancer patients. NEAT2 modulates NF-κB signaling activation, inflammatory cytokine release and malignant

behaviors of gastric cancer cells through the miR-155-5p/SOCS1 ceRNA axis. Our findings uncover the essential role of NEAT2 in gastric cancer progression and provide a novel target for gastric cancer targeted therapy. Further investigations will concentrate on in vivo functional validation and clinical transformation, so as to provide sufficient experimental evidence for developing NEAT2-based diagnostic biomarkers and targeted therapeutic strategies for gastric cancer.

Declarations

Research Project Funding：Inner Mongolia Medical Doctor Association Clinical Research and New Technology Promotion Project (YSXH2024KYF106), Science and Technology Program of the Joint Fund of Scientific Research for the Public Hospitals of Inner Mongolia Academy of Medical Sciences (2023GLLH0388、2024GLLH0926、2023GLLH0390), Tongliao City Science and Technology Planning Project (TL2024YF001)

Conflicts of Interest: The authors declare no conflicts of interest.

Availability of Data and Materials：The original data supporting the conclusions of this study are available upon reasonable request from the corresponding author.

UALCAN---(https://ualcan.path.uab.edu/cgi-bin/TCGA-lncRNA-Result.pl?genenam=MALAT1&ctype=STAD)

GEPIA2---(http://gepia2.cancer-pku.cn/#general)

Acknowledgments

We would like to express our sincere gratitude to the Gastrointestinal Hernia Surgery Department, Tongliao People's Hospital, Tongliao, Inner Mongolia 028000, China, for providing [clinical data / patient samples / surgical specimens / research.

We also thank the Key Laboratory of Basic Pharmacology of Ministry of Education and Joint International Research Laboratory of Ethnomedicine of Ministry of Education, Zunyi Medical University, Zunyi, Guizhou 563099, China, for providing Experimental Equipment / Experimental Platform / Technical Support.

Finally, we thank the anonymous reviewers for their insightful comments, which have greatly improved the quality of this manuscript.

Ethical approval and informed consent：

The Ethics Committee of Tongliao People's Hospital has approved this research plan (Approval No.: Tongyan Shen [2023] 022), and all participants provided written informed consent after fully comprehending the study protocol.

Clinical trial designation: Not applicable

Research involving humans and/or human data complies with the provisions of the Declaration of Helsinki.

Statement:

The patient consents to publication.

Agreement to Publication Statement

Author Contributions

Yu xiangwang Methodology, Resources, Writing – original draft

Daijian Liu Methodology, Resources, Writing – original draft

Shi wutang:Data curation , Formal analysis

Sun yuting Data curation , Formal analysis

longting Li Data curation, Resources

wang yu Formal analysis, Writing – review & editing

zhang guoyou Conceptualization, Writing – review & editing, Funding acquisition

References

1. Pradhan, Smruti Priyambada et al. “Epidemiology and prevention of gastric cancer: A comprehensive review.” Seminars in oncology vol. 52,3 (2025): 152341. doi:10.1016/j.seminoncol.2025.152341
2. Ramachandran, Rajarajeshwari et al. “Gastric Cancer: Clinical Features, Screening, Diagnosis, Treatment, and Prevention.” Journal of community hospital internal medicine perspectives vol. 14,2 49-57. 4 Mar. 2024, doi:10.55729/2000-9666.1304
3. Ramos, Marcus Fernando Kodama Pertille et al. “Surgical treatment of gastric cancer: A single center's 15-year experience.” Clinics (Sao Paulo, Brazil) vol. 80 (2025): 100828. doi:10.1016/j.clinsp.2025.100828
4. Tam, Chit et al. “LncRNAs with miRNAs in regulation of gastric, liver, and colorectal cancers: updates in recent years.” Applied microbiology and biotechnology vol. 103,12 (2019): 4649-4677. doi:10.1007/s00253-019-09837-5
5. Gutschner, Tony et al. “The noncoding RNA MALAT1 is a critical regulator of the metastasis phenotype of lung cancer cells.” Cancer research vol. 73,3 (2013): 1180-9. doi:10.1158/0008-5472.CAN-12-2850

6. Yang, L et al. “High MALAT1 expression predicts a poor prognosis of cervical cancer and promotes cancer cell growth and invasion.” European review for medical and pharmacological sciences vol. 19,17 (2015): 3187-93.
7. Catalanotto, Caterina et al. “MicroRNA in Control of Gene Expression: An Overview of Nuclear Functions.” International journal of molecular sciences vol. 17,10 1712. 13 Oct. 2016, doi:10.3390/ijms17101712
8. Lu, Jian et al. “miR-155-5p promotes osteosarcoma progression and cisplatin resistance by targeting the SOCS1/STAT1/c-Fos signaling axis.” Discover oncology vol. 17,1 723. 1 Apr. 2026, doi:10.1007/s12672-026-04913-5
9. Li, Shiqing et al. “The tumor suppressor role of miR-155-5p in gastric cancer.” Oncology letters vol. 16,2 (2018): 2709-2714. doi:10.3892/ol.2018.8932
10. Yoshikawa, H et al. “SOCS-1, a negative regulator of the JAK/STAT pathway, is silenced by methylation in human hepatocellular carcinoma and shows growth-suppression activity.” Nature genetics vol. 28,1 (2001): 29-35. doi:10.1038/ng0501-29
11. To, K F et al. “Constitutional activation of IL-6-mediated JAK/STAT pathway through hypermethylation of SOCS-1 in human gastric cancer cell line.” British journal of cancer vol. 91,7 (2004): 1335-41. doi:10.1038/sj.bjc.6602133
12. Souma, Yoshihito et al. “Antiproliferative effect of SOCS-1 through the suppression of STAT3 and p38 MAPK activation in gastric cancer cells.” International journal of cancer vol. 131,6 (2012): 1287-96. doi:10.1002/ijc.27350
13. Montagne, Kevin et al. “Modulation of the long non-coding RNA Mir155hg by high, but not moderate, hydrostatic pressure in cartilage precursor cells.” PloS one vol. 17,12 e0275682. 20 Dec. 2022, doi:10.1371/journal.pone.0275682
14. Xia, Hongwei et al. “The lncRNA MALAT1 is a novel biomarker for gastric cancer metastasis.” Oncotarget vol. 7,35 (2016): 56209-56218. doi:10.18632/oncotarget.10941
15. Elimam, Hanan et al. “Unraveling the influence of LncRNA in gastric cancer pathogenesis: a comprehensive review focus on signaling pathways interplay.” Medical oncology (Northwood, London, England) vol. 41,9 218. 5 Aug. 2024, doi:10.1007/s12032-024-02455-w
16. Wang, Zhenqiang et al. “LncRNA MALAT1 promotes gastric cancer progression via inhibiting autophagic flux and inducing fibroblast activation.” Cell death & disease vol. 12,4 368. 6 Apr. 2021, doi:10.1038/s41419-021-03645-4
17. Wang, Xuedong et al. “Effect of tumor-derived extracellular vesicle-shuttled lncRNA MALAT1 on proliferation, invasion and metastasis of triple-negative breast cancer by regulating macrophage M2 polarization via the POSTN/Hippo/YAP axis.” Translational oncology vol. 49 (2024): 102076. doi:10.1016/j.tranon.2024.102076
18. Deng, Quan-Jun et al. “Overexpressed MALAT1 promotes invasion and metastasis of gastric cancer cells via increasing EGFL7 expression.” Life sciences vol. 157 (2016): 38-44. doi:10.1016/j.lfs.2016.05.041

19. Wang, Ziwei et al. “Relationship between the degree of Helicobacter pylori infection and miR-155 and miR-543 expression levels in gastric cancer.” BMC gastroenterology vol. 25,1 669. 29 Sep. 2025, doi:10.1186/s12876-025-04220-3
20. Wang, Dong et al. “Exosomal miR-146a-5p and miR-155-5p promote CXCL12/CXCR7-induced metastasis of colorectal cancer by crosstalk with cancer-associated fibroblasts.” Cell death & disease vol. 13,4 380. 20 Apr. 2022, doi:10.1038/s41419-022-04825-6
21. Nuovo, Gerard J et al. “MiR-155-driven loss of ICOSL and SOCS1 in EBV+ gastric cancers renders abundant cytotoxic T cells ineffective, enabling immune evasion.” Proceedings of the National Academy of Sciences of the United States of America vol. 123,19 (2026): e2536375123. doi:10.1073/pnas.2536375123
22. Yin, Libin et al. “The MRTF-A/miR-155/SOX1 pathway mediates gastric cancer migration and invasion.” Cancer cell international vol. 20 303. 11 Jul. 2020, doi:10.1186/s12935-020-01395-5
23. Shaibu, Zakari et al. “Role of mesenchymal stem cells in modulating cytokine networks and immune checkpoints in gastric cancer therapy.” Biochimica et biophysica acta. Reviews on cancer vol. 1880,5 (2025): 189433. doi:10.1016/j.bbcan.2025.189433
24. Yu, Bingting et al. “IL-6 facilitates cross-talk between epithelial cells and tumor- associated macrophages in Helicobacter pylori-linked gastric carcinogenesis.” Neoplasia (New York, N.Y.) vol. 50 (2024): 100981. doi:10.1016/j.neo.2024.100981

Figures

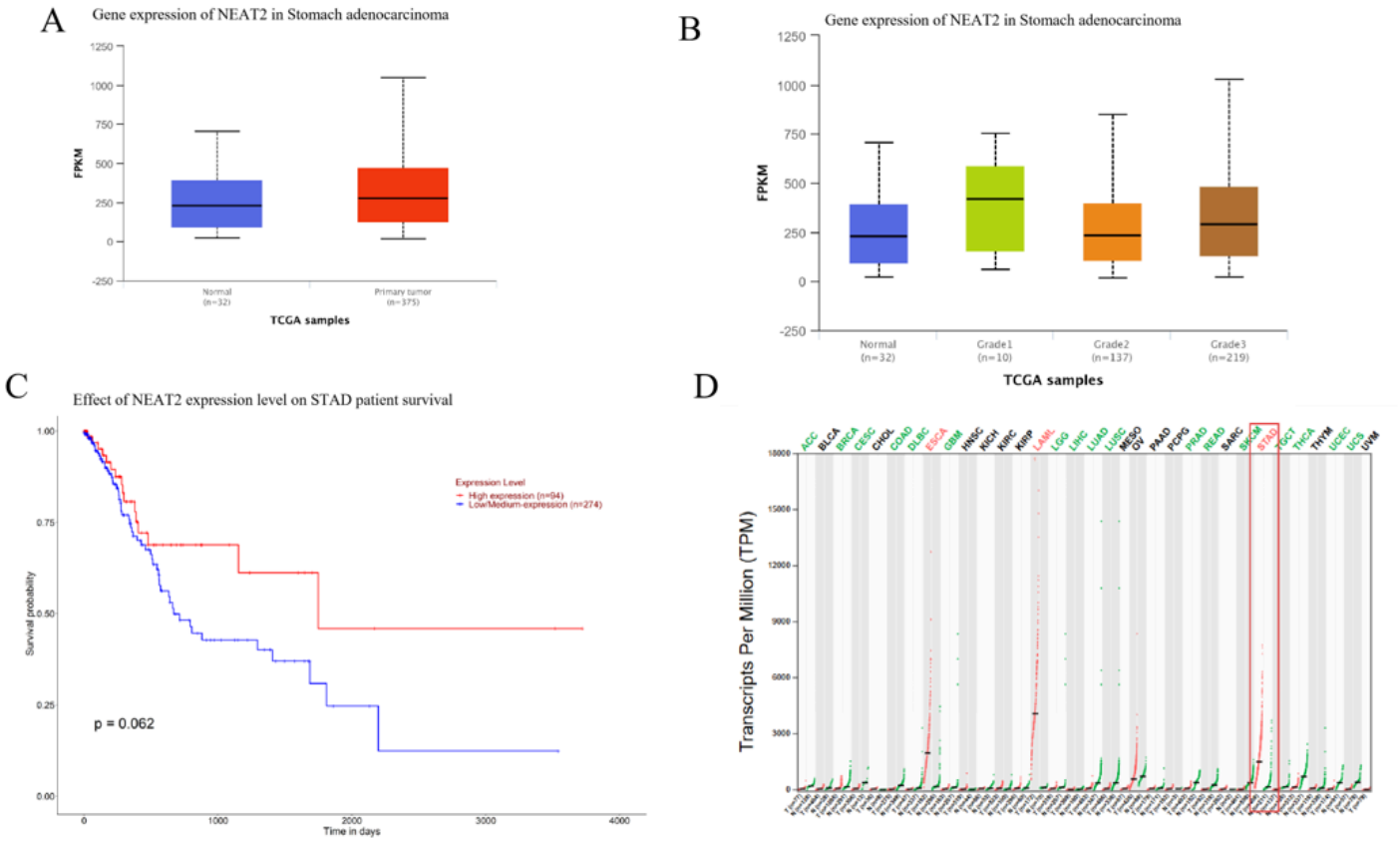


Figure 1

Expression and clinical significance of NEAT2 in gastric cancer tissues

(A) Gene expression of NEAT2 in stomach adenocarcinoma stratified by sample types.

(B) Gene expression of NEAT2 in stomach adenocarcinoma stratified by tumor grade.

(C) Effects of NEAT2 expression level on the survival rate of STAD patients.

(D) Gene expression profiles of NEAT2 across all tumor samples and matched normal tissues.

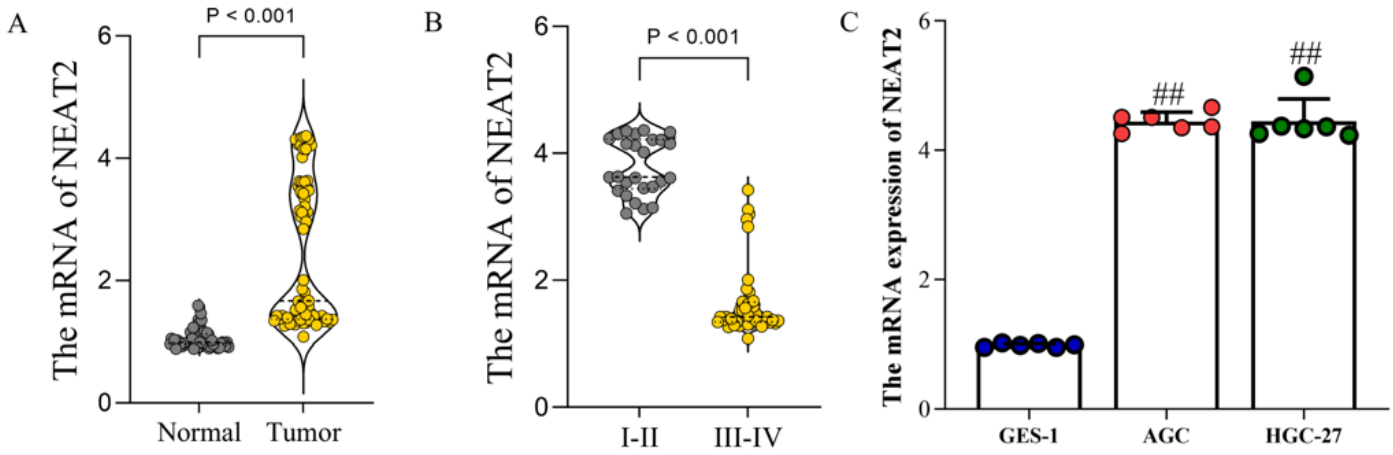


Figure 2

Expression of lncRNA NEAT2 in serum and cell lines

(A) Serum NEAT2 expression was markedly higher in gastric cancer patients than healthy controls (Normal vs. Tumor, P < 0.001).

(B) Differential serum NEAT2 expression among gastric cancer patients with different TNM stages: serum NEAT2 level was significantly higher in stage Ⅰ–Ⅱ early-stage patients than stage Ⅲ–Ⅳ advanced patients (P < 0.001).

(C) RT-qPCR detection of NEAT2 mRNA expression in normal gastric epithelial cell line GES-1 and gastric cancer cell lines AGS, HGC-27. NEAT2 was significantly upregulated in both gastric cancer cell lines compared with GES-1 (##P < 0.01 vs. GES-1).

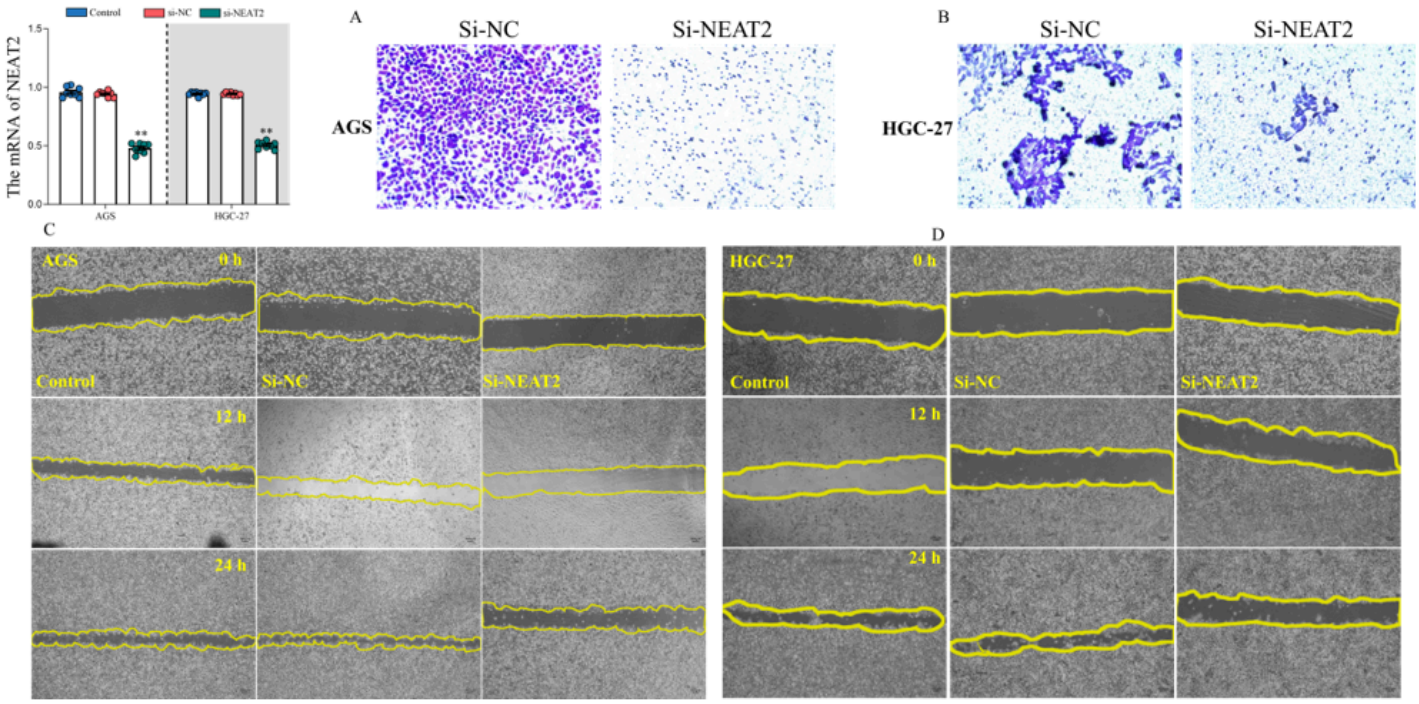


Figure 3

Effects of NEAT2 knockdown on migration and invasion of gastric cancer cells

(C) RT-qPCR verified the knockdown efficiency of NEAT2 siRNA ($P < 0.01$ vs. si-NC).

(A/B) Transwell migration assay showed that NEAT2 knockdown significantly suppressed the migratory capacity of AGS and HGC-27 cells (crystal violet staining, magnification ×200).

(D) Wound healing assay demonstrated that NEAT2 knockdown markedly reduced the wound closure rate of AGS and HGC-27 cells at 0, 12 and 24 h.

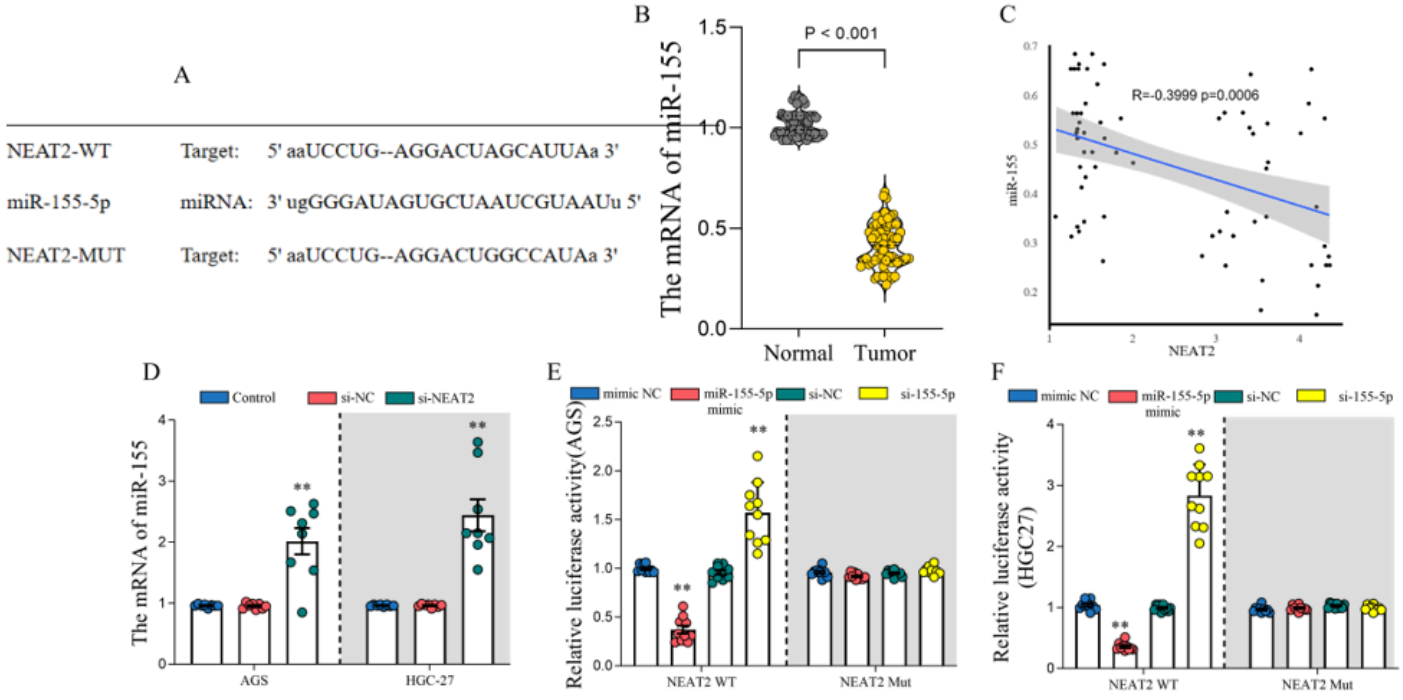


Figure 4

lncRNA NEAT2 directly targets and negatively regulates miR-155 expression

(A) Bioinformatic prediction of potential binding sites between NEAT2 and miR-155.

(B) miR-155 expression was significantly lower in gastric cancer tumor tissues than paired adjacent normal tissues ($P < 0.001$).

(C) Serum NEAT2 level was significantly negatively correlated with miR-155 level in gastric cancer patients ($R=-0.3899$, $P=0.0006$).

(D) RT-qPCR revealed that NEAT2 knockdown significantly upregulated miR-155 expression in AGS and HGC-27 cells ($P < 0.01$ vs. si-NC).

(E/F) Dual-luciferase reporter assay validated the direct targeting interaction between NEAT2 and miR-155. Co-transfection of NEAT2-WT and miR-155 mimic dramatically reduced luciferase activity in AGS (E) and HGC-27 (F) cells compared with miR-NC co-transfection ($P < 0.01$ vs. miR-NC); no obvious change was observed in cells co-transfected with NEAT2-MUT and miR-155 mimic.

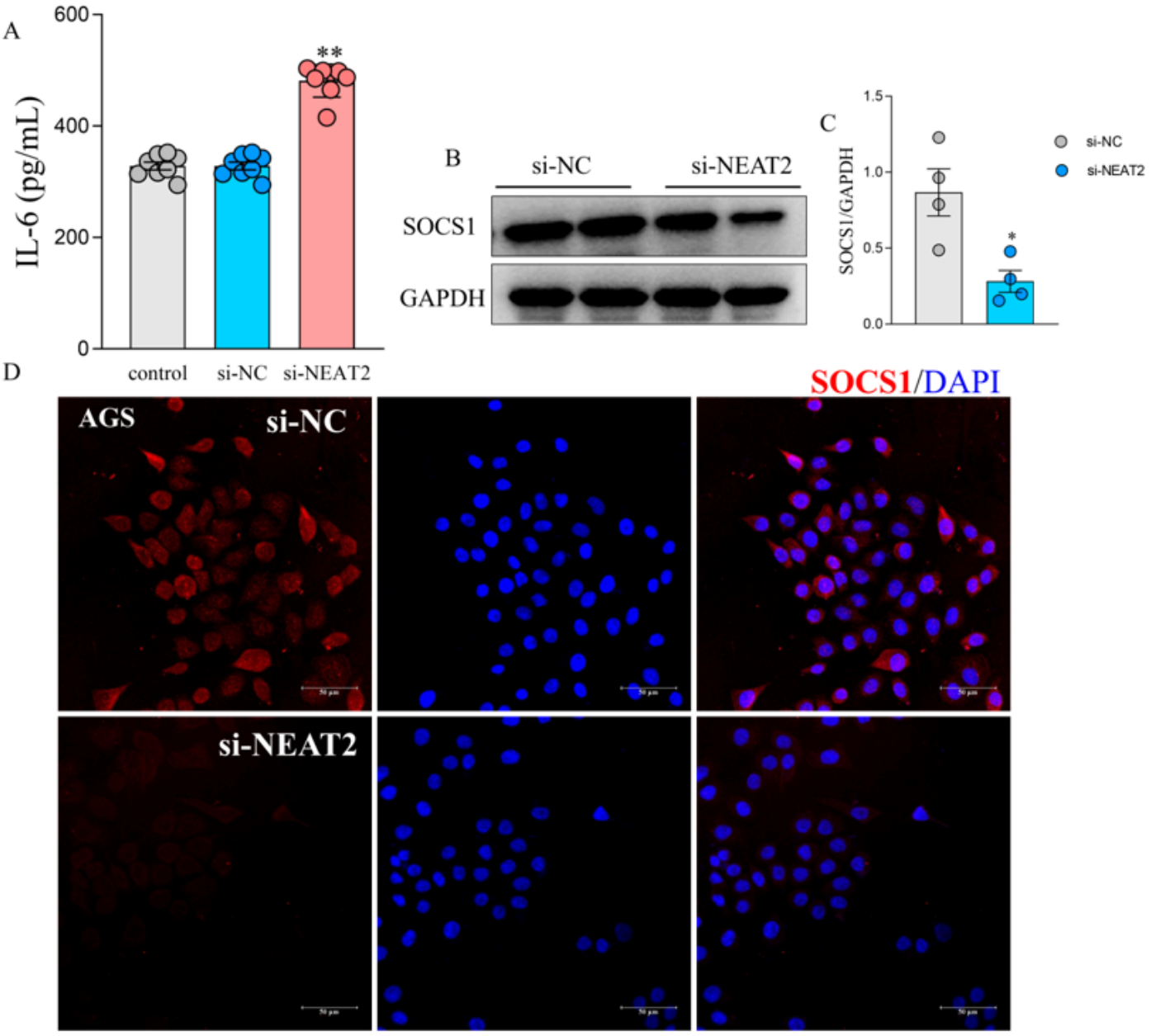

A
600
400
200
0
IL-6 (pg/mL)
**
control
si-NC
si-NEAT2
B
si-NC
si-NEAT2
SOCS1
GAPDH
C
1.5
1.0
0.5
0.0
SOCS1/GAPDH
*
si-NC
si-NEAT2
D
SOCS1/DAPI
AGS
si-NC
si-NEAT2

Figure 5

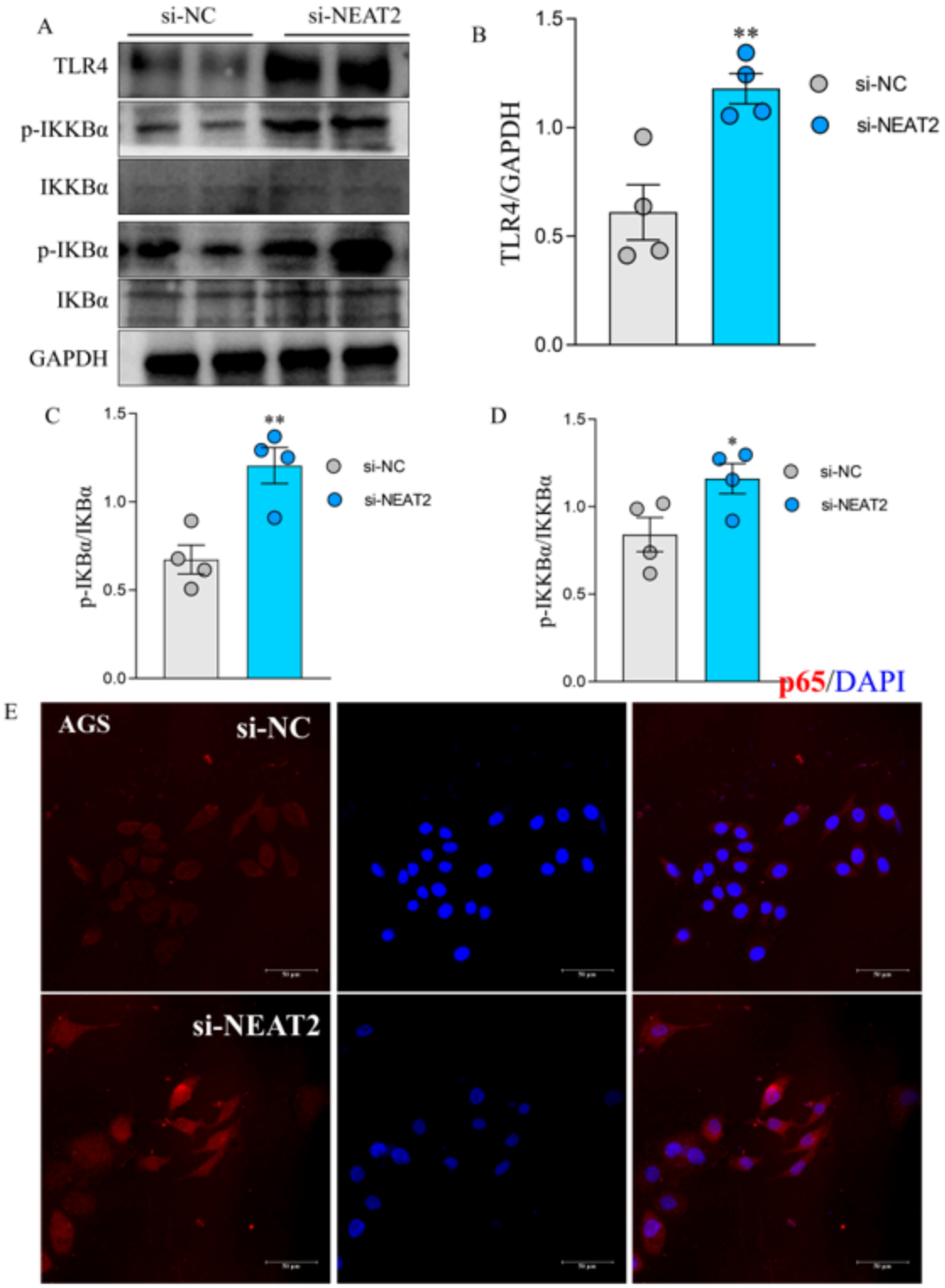


Figure 6

Effects of NEAT2 knockdown on NF-κB signaling pathway in AGS cells

(A-D) Western blot results showed that NEAT2 knockdown significantly upregulated TLR4 protein expression and enhanced phosphorylation of IKKα/β and IκBα ($P < 0.01$ vs. si-NC); GAPDH served as internal control.

(E) Immunofluorescence staining demonstrated that NEAT2 knockdown reduced SOCS1 fluorescence intensity and facilitated nuclear translocation of NF-κB p65 in AGS cells (SOCS1/p65: red; DAPI: blue; scale bar = 50 μm).

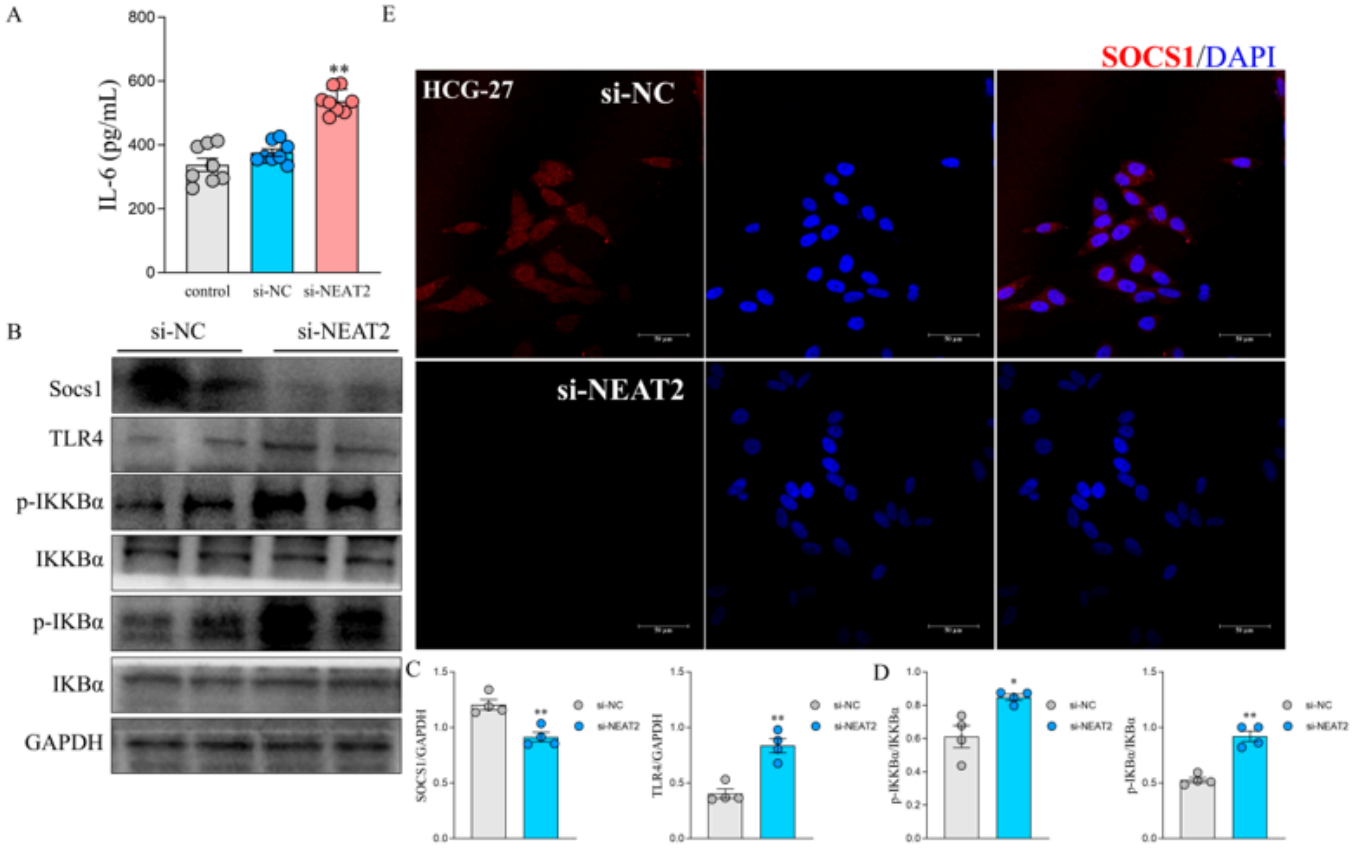


Figure 7

NEAT2 knockdown facilitates inflammatory response via modulating the SOCS1/NF-κB pathway in HGC-27 cells

(A) ELISA assay showed that NEAT2 knockdown markedly elevated IL-6 secretion ($P < 0.01$ vs. si-NC).

(B–D) Western blot detection revealed that NEAT2 knockdown significantly decreased SOCS1 protein expression ($P < 0.05$ vs. si-NC), upregulated TLR4 expression, and increased phosphorylation levels of IKKα/β and IκBα ($P < 0.01$ vs. si-NC); GAPDH was used as internal reference.

(E) Immunofluorescence staining exhibited decreased SOCS1 fluorescence intensity upon NEAT2 knockdown (SOCS1/p65: red; DAPI: blue; scale bar = 50 μm).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- file.pdf