ORIGINAL RESEARCH

Mucin 3A's Promotion of the Proliferation and Migration of Gastric Cancer Cells Through Activation of the mTOR Pathway

Jiewei Xu, BM; Jian Qiu, BM

ABSTRACT

Context • Gastric cancer (GC) is a common and life-threatening gastrointestinal malignancy. Although mucin 3A (MUC3A) is an essential oncogenic factor in several cancers, limited information is available on its expression in GC tissues and its impact on prognosis.

Objective • The study aimed to characterize MUC3A in GC and to explore its potential involvement in regulating GC cells' behavior through the mammalian target of rapamycin (mTOR) signaling pathway.

Design • The research team conducted a retrospective genetic analysis.

Setting • The study took place as Huzhou Central Hospital, an Affiliated Central Hospital of Huzhou University in Huzhou, Zhejiang, China.

Participants • Participants were 47 patients with GC who had received treatment at the department of general surgery at the hospital and who gave consent for the use of their tissue samples for the genetic analysis.

Outcome Measures • The research team: (1) performed a differential analysis of MUC3A using GC and normal tissue samples purchased from the American Type Culture Collection; (2) investigated the exposure of cancer tissues to MUC3A and its effects in the tumor, node, metastasis (TNM) stages of GC, using the real-time quantitative polymerase chain reaction (rt-qPCR) method; (3) performed clone formation and conducted transwell assays by knocking down or overexpressing MUC3A to analyze the effects on the behavior of GC cells; and (4) assessed the content of related marker proteins and the phosphoinositide 3-kinase (PI3K)/ protein kinase B (Akt)/ mammalian target of rapamycin (mTOR) pathway proteins, using a Western blot analysis.

Results • A high level of MUC3A existed in GC tissues, and it was associated with TNM staging. Silencing of the MUC3A inhibited GC-cell migration and proliferation, and MUC3A overexpression had the opposite effect. The addition of agonist M05856 restored the inhibitory effect of silencing MUC3A on GC cell proliferation and migration, suggesting that MUC3A regulates GC cells' behavior through the PI3K/Akt/mTOR pathway.

Conclusions • MUC3A plays an oncogenic role in GC and may regulate GC cell behavior through the PI3K/Akt/mTOR pathway. (*Altern Ther Health Med.* [E-pub ahead of print.])

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INTRODUCTION

Gastric cancer (GC) is a common and life-threatening gastrointestinal malignancy, which ranks as the second leading cause of cancer-related deaths worldwide. It seriously threatens the life and health of patients.[1,2] The main clinical treatment for GC is comprehensive surgery.[3]

Early-stage patients have a five-year survival rate of up to 95% but have no characteristic symptoms. Due to the unreliability of early screening mechanisms for GC, most patients already have advanced GC when diagnosed, which increases the difficulty of the surgical clearance of lesions, and the five-year survival rate after surgery is only 30.0-57.1%.[4] Therefore, the search for effective treatments and a prognostic evaluation of GC is now an important topic in medical research.

GC's Pathogenesis

Mucin (MUC) takes part in the occurrence and progression of several diseases. The MUC family comprises glycosylated proteins with relatively large molecular masses. Currently, the family has more than 20 members—MUC1/2-20—which are classified into secretory and trans-model

mucins according to their structures and functions; MUC is widely present on the mucosal surface of the normal organism, playing the role of lubrication and protection.[5]

Recently, Wi et al found that MUC can promote tumor-cell proliferation and metastasis by participating in intercellular signal transduction, immune-antigen presentation, and apoptotic regulation through its special structure under certain environments such as chronic inflammatory environment and immunosuppressive microenvironment.[6]

MUC3A is an isoform of MUC3, a membrane-associated MUC that is closely linked with the development of malignant tumors. [7] Su et al and Sotoudeh et al found that colorectal cancer, extrahepatic bile-duct cancer, and other digestive-tract malignant tumors positively express MUC.[8,9]

Notably, MUC3A is the most common repeated mutation of MUC, especially of those occurring within its sperm protein, enterokinase, and agrin (SEA) domain, and MUC3A is highly likely to induce structural changes in the protein's extracellular region and glycosylation sites. This, in turn, may compromise its effectiveness as a protective barrier against bacterial infections, including those by H. pylori, thereby elevating the risk of infection and potentially contributing to GC's development.

Niu et al found that tissues of patients with clear-cell renal-cell carcinoma positively expressed MUC3A, which correlated with a high pathological T (pT) stage and tumor recurrence and was a biomarker of a poor prognosis with respect to patient's overall survival.[10]

Sotoudeh et al found that the tissues of GC patients highly express MUC3A and that the GC pathogenic process may involve it.[9] Cui et al found that MUC3A contributed to the development of GC.[11] Jonckheere et al found that the signaling pathway for protein kinase C regulates the expression of MUC3A, which in turn regulates cancer cells' behavior.[12]

These findings underscore the crucial role of MUC3A in GC and emphasize the existing gaps in medical practitioners' understanding of its involvement in the disease.[13] Despite its relevance, limited information is available on the expression of MUC3A in GC tissues and its impact on prognosis.

PI3K/Akt/mTOR Pathway

The phosphoinositide 3-kinase (PI3K)/ protein kinase B (Akt) route represents an intracellular signaling pathway associated with cell growth, viability, metabolism, and inactivity.[14,15] The PI3K/Akt pathway can also promote the progression of GC by participating in the epithelial-mesenchymal transition (EMT).[16] Akt is a key signaling node downstream of PI3K. Abnormalities in Akt activity can lead to the conversion of negative cells to aggressive cells, and its phosphorylation can foster the expansion, invasion, and transposition of tumor cells and arrest their morphology.[17]

Mammalian target of rapamycin (mTOR) is a downstream gene of Akt, which direct or indirect induction of phosphorylation by p-Akt can activate, and p-mTOR is a major regulatory protein of the autophagic process.[18] Pharmacological treatments an induce autophagy-dependent apoptosis in GC cells by modulating the PI3K/Akt/mTOR route.[19] Su et al found that MUC3A can regulate the cellular behavior of colorectal cancer cells through the PI3K/Akt/mTOR molecular route.[8]

Current Study

The current study aimed to characterize MUC3A in GC and to explore its potential involvement in regulating GC cells' behavior through the mTOR signaling pathway.

METHODS

Participants

The research team conducted a retrospective genetic analysis, which took place as Huzhou Central Hospital, an Affiliated Central Hospital of Huzhou University in Huzhou, Zhejiang, China. Potential participants were patients with GC who had received treatment at the department of general surgery at the hospital and who gave consent for the use of their tissue samples for the genetic analysis. As their doctor, we obtained the tissue samples after their consent.

The study included potential participants if they: (1) had received histopathological confirmation of a GC diagnosis; (2) had a history of prior GC-related treatments, including surgery, chemoradiotherapy, or other interventions, before enrollment in the study; and (3) demonstrated a comprehensive understanding of the study's objectives.

The study excluded potential participants if they had multiple tumors.

Participants voluntarily provided informed consent for the use of their tissue samples. The university's Ethics Committee approved the study's protocols. The research team conducted the process of obtaining informed consent and ethical review and approval in accordance with established institutional guidelines, ensuring patients' confidentiality and adherence to ethical standards. Also, the procedures were performed as per the guideline in Helsinki Declaration.

Procedures

Clinical tissue samples. The research team obtained participants' GC tissues and adjacent normal tissues. Participant recruitment: The research team identifies individuals who have been diagnosed with gastric cancer and are willing to participate in the study. The recruitment process involves collaborating with hospitals or medical centers that specialize in the treatment of gastric cancer.

Informed consent: Before any tissue collection takes place, the participants are provided with detailed information about the study, its purpose, potential risks and benefits, and their rights as participants. They are given the opportunity to ask questions and fully understand the implications of their participation. If they agree to take part, they provide written informed consent.

Surgical procedure: In most cases, the collection of gastric cancer tissues and adjacent normal tissues occurs during surgical resection of the tumor. The surgical procedure,

known as gastrectomy, involves removing the affected part of the stomach along with surrounding tissues. This provides access to both the cancerous tissue and the adjacent normal tissue for sampling.

Tissue sampling: Once the surgical resection is performed, the surgeon carefully identifies and separates the cancerous tissue from the adjacent normal tissue. This is done based on visual examination and palpation. The tumor tissue is identified by its distinct appearance and characteristics, such as abnormal growth, discoloration, or structural changes.

Handling and preservation: After the tumor and adjacent normal tissues are separated, they are immediately handled and preserved to maintain their integrity and prevent degradation. The tissues are stored in sterile containers, immersed in a preservative solution.

The tissue specimens were fixed in 4% formalin immediately after removal and were embedded in paraffin for immunohistochemical staining. Each sample was frozen and stored at -80°C.

Cell culture and transfection. The research team: (1) purchased normal human gastric mucosal epithelium GES-1 cells and the GC cell lines N87 and AGS from the American Type Culture Collection (Manassas, VA, USA); (2) cultured them using Roswell Park Memorial Institute (RPMI)-1640 medium (Biosun, Shanghai, China) containing 10% fetal bovine serum (FBS) from Genetimes (Shanghai, China); and (3) inoculated the cells into six-well plates with 1x105 cells per well and transfected the sh-NC, sh-MUC3A-1, sh-MUC3A-2, pcDNA3.1, and overexpressed MUC3A into the N87 and AGS cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). sh-NC: This refers to the control group in which a non-targeting or scrambled short hairpin RNA (shRNA) was transfected into the N87 and AGS cells. It serves as a control to compare against the MUC3A knockdown groups.

s**h-MUC3A-1 and sh-MUC3A-2**: These are experimental groups where specific shRNAs targeting the MUC3A gene were transfected into the N87 and AGS cells. The purpose is to knock down or reduce the expression of the MUC3A gene and assess its effects on gastric cancer-related processes.

pcDNA3.1: This group represents the control group for the overexpression of MUC3A. pcDNA3.1 is a plasmid vector commonly used for gene overexpression studies. In this group, the empty pcDNA3.1 vector was transfected into the N87 and AGS cells as a control comparison for the MUC3A overexpression group.

Overexpressed MUC3A: This experimental group involves the transfection of a plasmid carrying the MUC3A gene into the N87 and AGS cells. This results in an increased expression of the MUC3A gene in the cells, allowing the researchers to study the effects of MUC3A overexpression in gastric cancer cells.

The source of the sh-NC, sh-MUC3A-1, sh-MUC3A-2, pcDNA3.1, and overexpressed MUC3A was GenePharma (Shanghai, China). At 6 hours posttransfection, the research team replaced the culture medium and collected cells for subsequent experiments after a 24-hour incubation period.

Real-time quantitative polymerase chain reaction (RT-qPCR). The research team: (1) extracted total RNA from the collected cells using TRIzol Reagent (Invitrogen) and performed reverse transcription into complementary DNA (cDNA) using avian myeloblastosis virus (AMV) Reverse Transcriptase (Solarbio, Beijing, China); and (2) performed RT-qPCR amplification using SYBR Premix Ex Taq II (TaKaRa, Dalian, China), with the following cycling conditions: initial denaturation at 94°C for 3 min; 40 PCR cycles at 94°C for 30 s, 55°C for 30s, 72°C for 30s; and 72°C for 10 min. The team used the reference gene β-actin for normalization. The primer sequence was: MUC3A: F: 5'-TCTTGGTCCTGAAGCCTGTTC-3', R: 5'-TGGATGTGGCCGTGGATAAA-3'; β-actin: F: 5'-TGGATCAGCAAGCAGGAGTA-3', R: 5'-TCGGCCACATTGTGAACTTT-3'.

Western blot analysis. The research team: (1) extracted the total protein in the GC cells using radio-immunoprecipitation assay (RIPA) reagent (Thermo Fisher Scientific, Waltham, MA, USA) and determined the protein content using a bicinchoninic acid (BCA) kit (Beyotime, Shanghai, China); (2) determined the total protein content using sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) at 30 μg/well; (3) after electrophoresis, wet-transferred and sealed the polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA) for one hour in a 5% skim-milk powder solution; and (4) then added primary antibodies and incubated the cells at 4°C overnight.

The team purchased all primary antibodies from Abcam (Cambridge, MA, USA) and diluted them at 1:1000. The primary antibodies were: (1) β-actin—ab8224, (2) MUC3A—ab270247, (3) cyclin dependent kinase 6 (CDK6)—ab124821, (4) matrix metallopeptidase 9 (MMP9)—ab76003, (5) phosphoinositide 3-kinase (PI3K)—ab131067, (6) protein kinase B (Akt)—ab8805, and (7) mTOR—ab134903.

The team: (1) added goat anti-rabbit immunoglobulin G (IgG) H&L—ab205718—before incubation for one hour; (2) added a chemiluminescent reagent, eyo/ enhanced chemiluminescence (ECL) Plus Kit (Beyotime), to the cells and developed them away from light and exposed them and took pictures.

In the Western blot section, the team used positive control samples for each protein marker to validate the assay's accuracy and specificity. Positive control samples are crucial for confirming the reliability of a Western blot's results. For each protein under analysis, including β-actin, MUC3A, CDK6, MMP9, PI3K, Akt, and mTOR, the team used established positive control lysates or protein extracts known to express the respective protein of interest. The team ran these positive control samples alongside the experimental samples, and they served as a reference to validate the specificity of the antibody probes and the assay's overall integrity.

To further investigate the regulatory role of the PI3K/Akt/mTOR pathway by MUC3A, the team employed specific pharmacological agents: 10 μmol/L of the PI3K/Akt inhibitor LY294002[20] and agonist M05856. The team based the selection

of these agents on their established roles in modulating the PI3K/Akt/mTOR pathway. M05856 is known for its ability to activate the PI3K/Akt pathway, while LY294002 is a well-recognized inhibitor of this pathway.

The team chose these agents to provide insights into how MUC3A can influence the PI3K/Akt/mTOR pathway, and subsequently, the behavior of GC cells. This approach allowed the team to explore the potential interactions and regulatory mechanisms involved in the study. The team categorized the GC cells into three groups: (1) sh-NC, (2) sh-MUC3A-2, and (3) sh-MUC3A-2+M05856. Second, the team classified the GC cells into the following three groups: (1) pcDNA3.1, (2) MUC3A, and (3) MUC3A+LY294002.

Colony formation assay. The research team: (1) inoculated logarithmic growth-phase cells into a 24-well plates at 50 cells/well and cultured them for 2 weeks, and (2) observed clones larger than 50 cells and counted them under a microscope (Leica (Germany)).

Transwell assay. The research team: (1) adjusted the cells to a cell suspension with a density of 5×10^4 cells/mL; (2) then added 100 μL of cell suspension to the upper chamber of the Transwell and 500 μL of conventional medium to the lower chamber; (3) after 24 h of culture, discarded the medium; (4) fixed the cells in paraformaldehyde (Santa Cruz Biotechnology, United States)) for 30 min and stained the cells with crystal violet for 15 min; and (5) took photographs and counted the migrated cells.

Outcome measures. The research team: (1) performed a differential analysis of MUC3A using GC and normal tissue samples; (2) investigated the exposure of cancer tissues to MUC3A and its effects in the tumor, node, metastasis (TNM) stages of GC, using the real-time quantitative polymerase chain reaction (rt-qPCR) method; (3) performed clone formation and conducted transwell assays by knocking down or overexpressing MUC3A to analyze the effects on the behavior of GC cells; and (4) assessed the content of related marker proteins and the phosphoinositide 3-kinase (PI3K)/ protein kinase B (Akt)/ mammalian target of rapamycin (mTOR) pathway proteins, using a Western blot analysis.

Outcome Measures

Mucin 3A in gastric cancer. The research team identified the MUC3A level in gastric adenocarcinoma. The team performed further analysis: (1) to determine MUC3A's expression in the TNM stages, (2) to evaluate its effects using the receiver operating characteristic curve, and (3) to determine MUC3A's effects on patients' five-year survival rates.

Knockdown of Mucin3A. The research team: (1) compared the levels of MUC3A mRNA and protein in N87, AGS, and GES-1 cells; (2) transfected knockdown MUC3A into N87 and AGS cells; (3) determined the silencing rate of MUC3A-1 for the N87 and AGS cells; (4) compared the sh-MUC3A-2 group's clone cell numbers with those of the sh-NC group; and (5) compared the sh-MUC3A-2 group's inhibition of the levels of CDK6 and MMP9 protein those of the sh-NC group.

Overexpressed Mucin3A. The research team further constructed an overexpression MUC3A vector and transfected it into GC cells and verified its high expression and transfection efficiency using a Western blot assay. The team: (1) compared the expression of MUC3A protein in the MUC3A and pcDNA3.1 groups; (2) determined the clone cell numbers and migration rates of N87 and AGS in the MUC3A and pcDNA3.1 groups; and (3) compared the expression of the proliferation marker protein CDK6 and migration marker protein MMP9 in GC cells in the MUC3A and pcDNA3.1 groups.

PI3K/Akt/mTOR pathway. For the N87 and AGS cells, the research team: (1) compared the protein levels of p-PI3K, p-Akt, and p-mTOR for the sh-NC, sh-MUC3A-2, and sh-MUC3A-2+M05856 groups and for the pcDNA3.1, MUC3A, and MUC3A+LY294002 groups.

Cell migration through PI3K/Akt/mTOR pathway. The research team: (1) to verify that MUC3A regulated GC cell behavior through the PI3K/Akt/mTOR pathway, sliced MUC3A and added M05856 and (2) compared the clone cell numbers and migration rates of the sh-NC, sh-MUC3A-2, and MUC3A+M05856 groups.

Statistical Analysis

The research team analyzed the data using GraphPad Prism 7 software (GraphPad Software, Inc., La Jolla, CA). The team repeated each experimental design three times and expressed the results as means ± standard deviations (SDs), and (2) used Student's t test for pairwise comparisons between groups and one-way analysis of variance (ANOVA) followed by post-hoc tests for comparisons between multiple groups. $P < .05$ indicated statistically significant differences.

RESULTS

Mucin 3A in Gastric Cancer

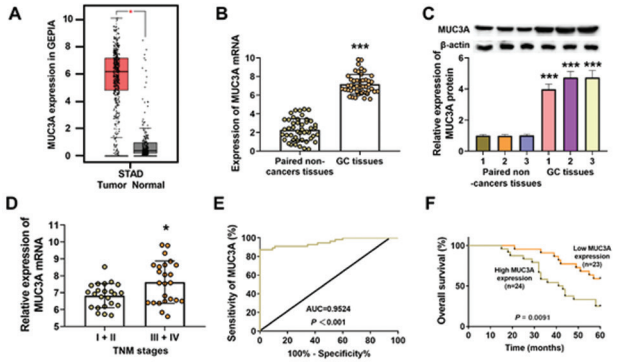
Figure 1A shows that the MUC3A level was significantly elevated in gastric adenocarcinoma ($P < .05$). In addition, Figures 1B and 1C show that participants' clinical GC tissues had a significantly higher level of MUC3A compared to their normal tissues ($P < .001$). For MUC3A's expression as related to TNM stage, Figure 1D shows that the MUC3A expression was significantly higher in stages III and IV than in stages I and II ($P < .05$).

Figure 1E shows the receiver operating characteristic curve had area under the curve of 0.9524, which was statistically significant ($P < .001$), indicating the significance of MUC3A for diagnosing GC. In addition, Figure 1F shows that GC patients with high MUC3A levels had a significantly lower five-year survival rate than patients with low MUC3A expression ($P = .0091$). These data suggest a high level of MUC3A in GC patients, correlated with TNM staging and prognosis.

Knockdown of Mucin3A

The levels of MUC3A mRNA (Figure 2A) and protein (Figure 2B) were significantly higher in the N87 and AGS cells than in GES-1 cells (both $P < .001$).

Figure 1. High Expression of Mucin 3A (MUC3A) in Gastric Cancer (GC). Figure 1A shows the MUC3A in gastric adenocarcinoma, using GEPIA; Figure 1B shows the MUC3A in GC tissues (n=47), using RT-qPCR; Figure 1C shows the MUC3A in GC tissues as tested three times (n=47), using a Western blot; Figure 1D shows the MUC3A in GC TNM stages, using qRT-PCR; Figure 1E shows the receiver operating characteristic curve; and Figure 1F shows the five-year survival rate of GC patients.

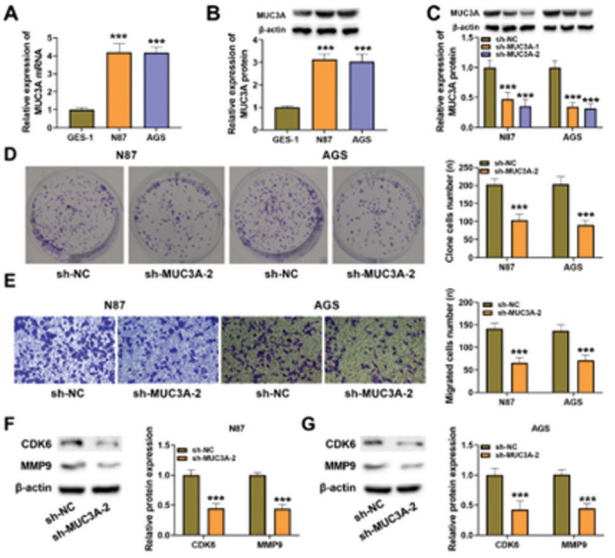


*P < .05, indicating that the MUC3A level was significantly elevated in gastric adenocarcinoma (Figure 1A) and that the MUC3A expression was significantly higher in stages III and IV than in stages I and II of GC (Figure 1D)

***P < .001, indicating that participants' clinical GC tissues had a significantly higher level of MUC3A compared to their normal tissues (Figures 1A and 1B), that the receiver operating characteristic curve had an area under the curve of 0.9524, which was statistically significant (Figure 1E), and that GC patients with high MUC3A levels had a significantly lower five-year survival rate than patients with a low MUC3A expression (Figure 1F)

Abbreviations: GEPIA, Gene Expression Profiling Interactive Analysis; RT-qPCR, real-time quantitative polymerase chain reaction; STAD, stomach adenocarcinoma; TNM, tumor, node, metastasis

Figure 2. Inhibition of Gastric Cancer (GC) Cell Proliferation and Migration From Knockdown of Mucin 3A (MUC3A). Figure 2A shows the level of MUC3A mRNA in GC cells, using RT-qPCR; Figure 2B shows the content of MUC3A protein in GC cells, using a Western blot analysis; Figure 2C shows the expression of MUC3A, using a Western blot analysis; Figure 2D shows the clone cell number, using a colony formation assay; Figure 2E shows the migration rate of GC cells, using a transwell assay; and Figures 2F and 2G show the levels of CDK6 and MMP9 proteins, using a Western blot analysis.



***P < .001, indicating for the N87 and AGS cells that the levels of MUC3A mRNA (Figure 2A) and protein (Figure 2B) were significantly higher than those in the GES-1 cells, that the MUC3A-1 group's expressions of MUC3A protein were significantly lower than that of the sh-NC group and that the sh-MUC3A-2 group's expressions of MUC3A protein were significantly lower than that of the sh-MUC3A-1 group (Figure 2C), that the sh-MUC3A-2 group's clone cell number was significantly lower than that of the sh-NC group (Figure 2D), that the sh-MUC3A-2 group's migration rate was significantly lower than that of the sh-NC group (Figure 2E), and that the MUC3A-2 group's levels of CDK6 and MMP9 protein were significantly lower than those for the sh-NC group (Figures 2F and 2G)

Abbreviations: CDK6, cyclin dependent kinase 6; MMP9, matrix metallopeptidase 9; RT-qPCR, real-time quantitative polymerase chain reaction

Figure 2C shows that for the N87 and AGS cells, the MUC3A-1 group's expressions of MUC3A protein were significantly lower than that for the sh-NC group and that the sh-MUC3A-2 group's expressions of MUC3A were significantly lower than that of the sh-MUC3A-1 group (both P < .001). Therefore, the research team selected sh-MUC3A-2 for the follow-up cell experiments.

For the N87 and AGS cells: (1) Figure 2D shows that the sh-MUC3A-2 group's clone cell number was significantly lower than those of the sh-NC group (both P < .001); (2) Figure 2E shows that the sh-MUC3A-2 group's migration rate was significantly lower than that of the sh-NC group (both P < .001); and (3) Figures 2F and 2G show that the MUC3A-2 group's levels of CDK6 and MMP9 protein were significantly lower than those of the sh-NC group (all P < .001).

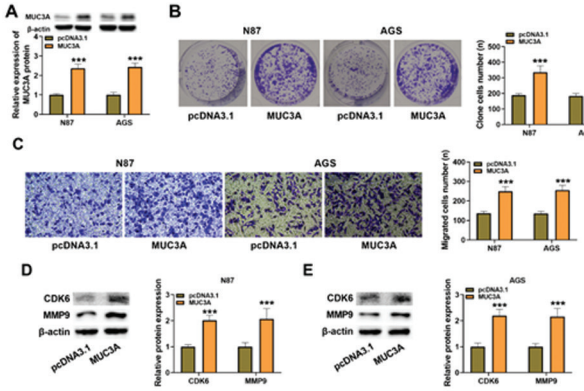
Overexpressed Mucin3A

For the N87 and AGS cells: (1) Figure 3A shows that the overexpressed MUC3A group's expression of MUC3A protein was significantly higher than of the pcDNA3.1 group (both P < .001); (2) Figure 3B shows that the overexpressed MUC3A group's clone cell number was significantly higher than that of the pcDNA3.1 group (both P < .001); (3) Figure 3C shows that the overexpressed MUC3A group's migration rate was significantly higher than that of the pcDNA3.1 group (both P < .001); and (4) Figures 3D and 3E show that the overexpressed MUC3A group's levels of the proliferation marker protein CDK6 and migration marker protein MMP9 were significantly higher than the levels of the pcDNA3.1 group (both P < .001).

PI3K/Akt/mTOR Pathway

For the N87 and AGS cells, Figures 4A and 4B, respectively, show that the sh-MUC3A-2 group had significantly lower protein levels of p-PI3K, p-Akt, and p-mTOR than those of the sh-NC group (all P < .001) and the sh-MUC3A-2+M05856 group (all P < .01). For the N87 and AGS cells, Figures 4C and 4D, respectively, show that the MUC3A group had significantly higher protein levels of p-PI3K, p-Akt, and p-mTOR than

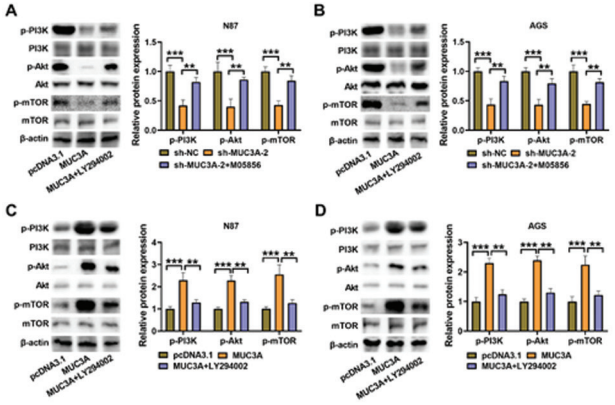
Figure 3. Promotion of Gastric Cancer (GC) Cell Proliferation and Migration From Overexpressed Mucin 3A (MUC3A). Figure 3A shows the expression of MUC3A, using a Western blot analysis; Figure 3B shows the clone cell number, using a colony formation assay; Figure 3C shows the migration rate of GC cells, using a transwell assay; and Figure 3D and 3E show the levels of CDK6 and MMP9 proteins, using a Western blot analysis.



***P < .001, indicating that the overexpressed MUC3A group's expression of MUC3A protein was significantly higher than of the pcDNA3.1 group (Figure 3A), that the overexpressed MUC3A group's clone cell number was significantly higher than that of the pcDNA3.1 group (Figure 3B), that the overexpressed MUC3A group's migration rate was significantly higher than that of the pcDNA3.1 group (Figure 3C), and that the overexpressed MUC3A group's levels of the proliferation marker protein CDK6 and migration marker protein MMP9 were significantly higher than the levels of the pcDNA3.1 group

Abbreviations: CDK6, cyclin dependent kinase 6; MMP9, matrix metallopeptidase 9

Figure 4. Effect of Mucin 3A (MUC3A) on the PI3K/AKT/mTOR Pathway in Gastric Cancer (GC) CELLS. Figures 4A-4D show the levels of p-PI3K, p-Akt, and p-mTOR protein in GC cells, using a Western blot.

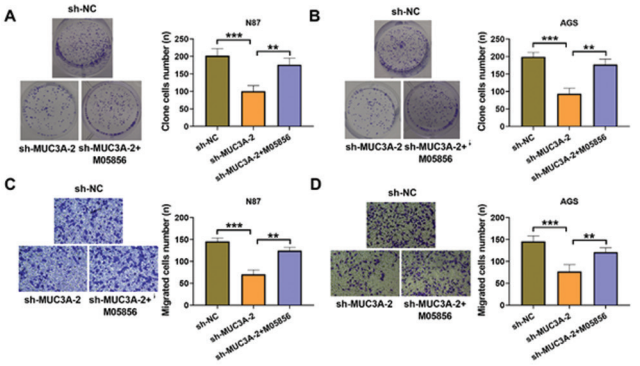


**P < .01, indicating that the sh-MUC3A-2 group had significantly lower protein levels of p-PI3K, p-Akt, and p-mTOR than those of the sh-MUC3A-2+M05856 group and that the MUC3A group had significantly higher protein levels of p-PI3K, p-Akt, and p-mTOR than those of the MUC3A+LY294002 group

***P < .001, indicating that the sh-MUC3A-2 group had significantly lower protein levels of p-PI3K, p-Akt, and p-mTOR than those of the sh-NC group and that the MUC3A group had significantly higher protein levels of p-PI3K, p-Akt, and p-mTOR than those of the pcDNA3.1 group

Abbreviations: AKT, protein kinase B; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide 3-kinase

Figure 5. M05856's Restoration of the Cell Behavior That sh-MUC3A Inhibited. Figures 5A and 5B show the colony formation assay; Figures 5C and 5D show the Transwell assay.



**P < .01, indicating that the sh-MUC3A-2 group's clone cell numbers and migration rates were significantly lower than those of the MUC3A-2+M05856 group

***P < .001, indicating that the sh-MUC3A-2 group's clone cell numbers and migration rates were significantly lower than those of the sh-NC group

those of the pcDNA3.1 group (all P < .001) and the MUC3A+LY294002 group (all P < .01).

MUC3A overexpression clearly promoted the protein profiling of p-PI3K, p-Akt, and p-mTOR, while the addition of the inhibitor LY294002 suppressed this effect.

Cell Migration Through PI3K/Akt/mTOR Pathway

For the N87 (Figure 5A) and AGS (Figure 5B) cells, the sh-MUC3A-2 group's clone cell numbers were significantly lower than those of the sh-NC group (both P < .001) and the sh-MUC3A-2+M05856 group (both P < .01). For the N87 (Figure 5C) and AGS (Figure 5D) cells, the sh-MUC3A-2 group's migration rates were significantly lower than those of the sh-NC group (both P < .001) and the sh-MUC3A-2+M05856 group (both P < .01).

The results demonstrated that slicing MUC3A significantly could repress cell proliferation while adding M05856 significantly diminished that effect and that the M05856 had significantly facilitated the effect of the migrated cells that sh-MUC3A-2 suppressed.

DISCUSSION

The present study found that the positive rate of MUC3A in the tissues of GC patients was clearly higher than that in normal tissues, which was in line with Masoud Sotoudeh et al's findings.[9] In addition, the MUC3A expression was significantly higher in TMN stages III and IV than in stages I and II, which is similar to Cui et al's findings.[11]

The current study observed further that patients with elevated expressions of MUC3A in GC tissues had lower five-year survival rates than those with a low expression, suggesting that patients with high MUC3A expression in GC have shorter survival times, higher morbidity and mortality rates, and poorer prognoses. In addition, the current study's cellular assays showed that MUC3A overexpression contributed to the proliferation and migration of GC cells,

demonstrating that high MUC3A expression may be linked to the development of GC.

To further elucidate the mechanisms by which MUC3A may activate the PI3K/Akt/mTOR pathway in GC cells, the current study found that silencing MUC3A with sh-MUC3A-2 led to a significant reduction in the levels of phosphorylated PI3K (pPI3K), phosphorylated Akt (p-Akt), and phosphorylated mTOR (p-mTOR) in GC cells when compared to the sh-NC group.

Notably, the use of the PI3K/Akt agonist M05856 was able to reverse the impaired effect of silencing MUC3A on GC cell behavior. These findings provide compelling evidence that support the idea that MUC3A promotes GC's development by enhancing the expression and activation of the PI3K/Akt/mTOR pathway. However, the field needs further studies to unravel the precise molecular interactions and downstream targets involved in this process.

The current study had some limitations, including a relatively small sample size, which may have impacted the findings' generalizability. The field needs further research with larger and more diverse cohorts to validate these observations and to provide a more comprehensive understanding of MUC3A's role in GC.

In summary, the positive correlation between MUC3A expression and TNM stage in GC patients indicates its potential role in disease progression. The observed association between high MUC3A expression and poor prognoses suggests that MUC3A could serve as a valuable prognostic indicator for GC. These findings may have important clinical implications for the management of GC patients. Identifying MUC3A's expression levels in patients could aid in risk stratification and treatment planning. Additionally, future research could explore the development of targeted therapies that modulate the PI3K/Akt/mTOR signaling pathway, potentially providing more effective treatment options for GC.

CONCLUSIONS

MUC3A plays an oncogenic role in GC and may regulate GC cell behavior through the PI3K/Akt/mTOR pathway.

AUTHORS' DISCLOSURE STATEMENT

The authors declare that they have no financial conflicts of interest related to the study.

ACKNOWLEDGMENTS

The authors thank the Huzhou Central Hospital at Huzhou Normal University for their support of the study.

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