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RESEARCH ARTICLE

miR-153-5p Promotes Pancreatic Cancer Progression by Targeting Tumor Suppressor Gene S100A14 Through an Oncogenic Mechanism

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Abstract

Pancreatic cancer is a highly aggressive malignancy with limited treatment options and poor prognosis. MicroRNAs (miRNAs) have emerged as critical regulators of cancer progression. This study investigates the oncogenic role of miRNA-153-5p and its regulatory effects on the tumor suppressor gene S100A14 in pancreatic cancer. The expression levels of miRNA-153-5p and S100A14 were analyzed in 20 pancreatic cancer tissue samples and adjacent normal tissues using quantitative PCR and Western blot techniques. Bioinformatics analysis predicted S100A14 as a direct target of miRNA-153-5p, which was validated via luciferase reporter assays. Functional assays, including migration, invasion, proliferation, cell cycle, and apoptosis analyses, were performed in pancreatic cancer cell lines with miRNA-153-5p overexpression or inhibition. miRNA-153-5p was significantly upregulated in pancreatic cancer tissues and cell lines, while S100A14 was downregulated, demonstrating an inverse correlation. Luciferase assays confirmed S100A14 as a direct target of miRNA-153-5p. Functional assays revealed that miRNA-153-5p overexpression enhanced cancer cell proliferation, migration, and invasion while suppressing apoptosis. Conversely, miRNA-153-5p inhibition reduced tumorigenic properties, an effect reversed by S100A14 knockdown. miRNA-153-5p promotes pancreatic cancer progression by targeting the tumor suppressor S100A14. These findings highlight the potential of miRNA-153-5p as a therapeutic target for pancreatic cancer treatment.

Keywords: Pancreatic cancer, miRNA-153-5p, S100A14, Tumor suppressor, Invasion, Apoptosis, Therapeutic target

Introduction

Pancreatic cancer (PC) is one of the most aggressive and lethal malignancies, ranking as the seventh leading cause of cancer-related deaths worldwide. Its incidence has been steadily rising, and despite advances in early detection and therapeutic strategies, the prognosis remains dismal [1]. The 5-year survival rate for pancreatic cancer is less than 10%, primarily due to late diagnosis, intrinsic resistance to therapy, and rapid disease progression [2]. These challenges highlight the critical need for a deeper understanding of the molecular mechanisms driving pancreatic cancer to identify novel diagnostic markers and therapeutic targets [3].

Among the emerging molecular players in cancer biology are microRNAs (miRNAs), which are small, non-coding RNA molecules typically 18 - 25 nucleotides in length. These molecules regulate gene

expression by binding to the 3′ untranslated region (UTR) of target mRNAs, leading to their degradation or translational repression [4]. miRNAs are involved in numerous cellular processes, including proliferation, differentiation, apoptosis, and stress response. In the context of cancer, miRNAs can function as either oncogenes or tumor suppressors, depending on the genes they regulate and the cellular context [5]. Dysregulation of miRNA expression is a hallmark of cancer and is closely linked to tumor initiation, progression, and metastasis [6].

One such miRNA, miRNA-153-5p, has gained attention in recent years due to its oncogenic potential in various cancers, including renal cell carcinoma Zuo *et al.* [7], breast cancer Chen *et al.* [8], lung cancer [9], and colorectal cancer [10]. In contrast, several other studies have reported its tumor-suppressive function, highlighting its ability to inhibit proliferation, migration, and invasion in certain cancer types [11]. miRNA-153-5p has been implicated in promoting tumor growth and metastasis by targeting key tumor suppressor genes and modulating pathways involved in cell cycle regulation, apoptosis, and angiogenesis [11]. Despite its well-documented roles in other malignancies, the specific function of miRNA-153-5p in pancreatic cancer remains poorly understood, warranting further investigation.

A key target of miRNA-153-5p identified through bioinformatics tools such as TargetScan is S100A14, a member of the S100 protein family [12]. The S100 proteins are calcium-binding proteins involved in diverse cellular processes, including cytoskeletal dynamics, cell migration, and intracellular signaling [13]. Aberrant expression of S100 proteins has been implicated in various cancers, where they can act as oncogenes or tumor suppressors depending on the cellular context and specific protein isoform [14]. S100A14, in particular, has been reported to exhibit tumor-suppressive properties in some cancers, such as esophageal squamous cell carcinoma, by regulating cell proliferation, apoptosis, and migration [11]. However, its role in pancreatic cancer remains elusive.

This study aims to investigate the regulatory relationship between miRNA-153-5p and S100A14 in pancreatic cancer, with the objective of elucidating their functional interplay in tumor progression and metastasis. Furthermore, the study explores the potential of this axis as a diagnostic biomarker and therapeutic target, thereby contributing to the understanding of molecular mechanisms underlying pancreatic cancer aggressiveness.

Material and methods

Ethical approval

Consent was obtained or waived by all participants in this study. Kurdistan Regional Government, Iraq, Ministry of Health/Duhok Directorate General of Health/Directorate of Planning Scientific Research Ethics Committee issued approval 13072021-7-17. All individuals signed a written informed consent agreement for the use of their samples for research purposes.

Pancreatic tissue samples

A total of 20 patients with a confirmed diagnosis of PC were included in this study. Following surgical resection, tissue samples from the PC lesions and adjacent normal tissues (used as controls) were collected and preserved at −80 °C for further experiments. The inclusion criteria required patients to have a definitive PC diagnosis confirmed through pathological examination and to have undergone surgical resection. Only patients who provided written informed consent were included. Exclusion criteria encompassed patients who had undergone chemotherapy or radiotherapy before surgery, those with other malignancies or severe comorbid conditions, and individuals who declined participation. Additionally, patients with incomplete medical records or tissue samples deemed unsuitable for analysis were excluded. The study received approval from the hospital's Ethics Committee, and all participants provided written informed consent.

Cell culture

All cell lines used in this study, including the normal pancreatic cell line HPDE6-C7 and pancreatic cancer cell lines ASPC-1, BxPC-3, and MIA PaCA-2, were obtained from the American Type Culture Collection (ATCC). The cells were cultured in either DMEM or RPMI medium, supplemented with 10 % fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin. They were incubated at 37 °C in a humidified atmosphere with 5% CO_2.

Migration and invasion assays

Migration and invasion assays were performed using the Transwell system (Corning, USA). For the migration assay, 2×10^4 cells were suspended in 200 µL of serum-free medium and placed in the upper chamber. Then, 600 µL of complete medium was added to the lower chamber. After 24 h of incubation, the cells that migrated through the membrane were fixed for 15 min with 4% paraformaldehyde and stained with 0.1% crystal violet for 30 min. The invasion assay followed the same procedure, except the membranes were coated with Matrigel (BD Bioscience, USA). The number of cells was counted in 3 representative fields for each independent experiment.

Cell cycle analysis

The cell cycle was assessed using a cell cycle detection kit (Beyotime, China). In brief, fixed cells were stained with propidium iodide and analyzed using a FACSCalibur flow cytometer (BD Biosciences, USA).

RNA isolation and qPCR assay

Total RNA was extracted from tissue or cell samples using TRIzol reagent (Invitrogen, USA) following the manufacturer's standard protocol. Reverse transcription was performed using the GoScript Reverse Transcription System (Promega, USA) after RNA purification. The cDNA first strand was amplified using GoTaq qPCR SYBR GREEN Master Mix (Promega, USA) on 7900 system (Applied Biosystems, USA) using specific primers for miR-153-5p: GUCAUUUUUGUGAUGUUGCAGCU (Qiagen, YP02108124). S100A14: Forward (5′-TGCTCTAGAATGGGACAGTGTCGGTCAGCC-3′) and Reverse (5′-CGCGGATCCTCAGTGCCCCCGGACAGGCCT-3′). U6 was used as a normalization control for miR-153-5p quantification, while GAPDH: Forward (5′-GGAAGGTGAAGGTCGGAGTC-3′) and Reverse (5′-TGAAGGGGTCATTGATGGCA-3′) served as a reference for S100A14 normalization. The 2-ΔΔCt method was applied to calculate the relative gene expression.

Dual-luciferase reporter assays

Dual-luciferase reporter assays were performed by amplifying the 3′-UTR of S100A14, which contains various potential miR-153-5p binding sites, and cloning it into the pmirGLO vector. The miR-153-5p complementary sites, specifically the sequence 5′-GAUUAA-3′ in the S100A14 3′-UTR, were individually mutated to disrupt their complementarity to miR-153-5p. Cells were seeded in 24-well plates and co-transfected with either wild-type or mutated S100A14 3′-UTR constructs along with miR-153-5p mimics or a negative control (NC). After 48 h of transfection, the cells were harvested, and luciferase activity was measured using the Dual-Luciferase® Reporter Assay System.

Western blot

Cells were lysed using Laemmli buffer (Thermo Fisher, USA) and centrifuged at 12,000 g for 10 min. The resulting supernatant, containing the proteins, was collected. Equal amounts of protein from each experimental group were then loaded onto an SDS-PAGE gel. After separation, the proteins were transferred to a PVDF membrane (Millipore, USA) and incubated overnight at 4 °C with primary antibodies specific for S100A14, diluted at 1:500 (Santa Cruz, USA). The membranes were washed with PBS and incubated with a horseradish peroxidase-conjugated secondary antibody (Santa Cruz, USA) for 2 h at room temperature. Protein bands were visualized using an enhanced chemiluminescence kit (Millipore, USA).

MTT assay

MTT colorimetric assay was performed to assess the impact of miR-153-5p expression on the viability of PC cells, 3 days' post-transfection. The cells were plated into 96-well plates, with 2000 cells per well, and incubated at 37 °C for 1 to 3 days. Each day, 20 µl of a 5 mg/mL MTT solution (Thermo Fisher, USA) was added to the wells and incubated for 4 h. After incubation, 100 µL of acidic isopropanol solution (comprising 10% SDS, 5% isopropanol, and 0.01 M HCl) was added to each well, followed by an overnight incubation at 37 °C. The absorbance (optical density, OD) was measured at 570 nm using a Bio-Rad microplate reader (USA).

Caspase activity assay

Caspase 3 and 9 activities were measured using Caspase 3 and 9 Activity Assay Kits (Elabscience

Biotechnology, USA) following the manufacturer's instructions. Briefly, 1×10^5 cells were cultured in 96-well plates and incubated for 24 h. The cells were then detached and centrifuged at 2000 rpm for 5 min. A working solution buffer (60 µL), containing 40 µl of 2× reaction working solution and 5 µL of Ac-DEVD-pNA, was added to the cells, and the plate was placed on an oscillating shaker for 30 min. The optical density at 405 nm was measured using a Bio-Rad microplate reader.

Statistical analysis

Statistical analysis was conducted using GraphPad Prism Software. The differences between 2 groups were evaluated using Student's t-test, while one-way analysis of variance (ANOVA) was used to compare multiple groups. A *p*-value of less than 0.05 was considered statistically significant.

Results

Mir-153-5p expression is inversely correlated with S100A14 protein levels in pancreatic cancer tissues and cells

To investigate the expression patterns of miR-153-5p and S100A14 in pancreatic cancer, we analyzed their levels in both pancreatic cancer cell lines and tumor tissues. The qRT-PCR results demonstrated that miR-153-5p was significantly upregulated in all 3 pancreatic cancer cell lines compared to the control HPDE6-C7 cells. Among the cancer cell lines, MIA PaCa-2 exhibited the highest expression levels of miR-153-5p, while BxPC-3 displayed the lowest expression (**Figure 1(A)**). In contrast, the expression of S100A14 was significantly higher in the control HPDE6-C7 cells compared to MIA PaCa-2 and AsPC-1. Interestingly, no significant difference was observed in S100A14 expression between the control HPDE6-C7 cells and BxPC-3 cells (**Figure 1(B)**). Western blot analysis further validated these findings, showing that S100A14 protein expression was reduced in MIA PaCa-2 and AsPC-1 but remained comparable between BxPC-3 and HPDE6-C7 (**Figure 1(C)**).

To extend our analysis to clinical samples, we measured miR-153-5p and S100A14 expression in tumor tissues compared to control tissues. The results confirmed that miR-153-5p was significantly upregulated in tumor tissues (**Figure 1(D)**). Conversely, S100A14 expression was markedly lower in tumor tissues than in control tissues (**Figure 1(E)**). These findings further support the inverse relationship between miR-153-5p and S100A14 in pancreatic cancer.

Taken together, these results indicate that miR-153-5p is highly expressed in pancreatic cancer cell lines and tumor tissues, whereas S100A14 exhibits reduced expression. This inverse correlation suggests that miR-153-5p may play a regulatory role in suppressing S100A14 expression, potentially contributing to pancreatic cancer progression.

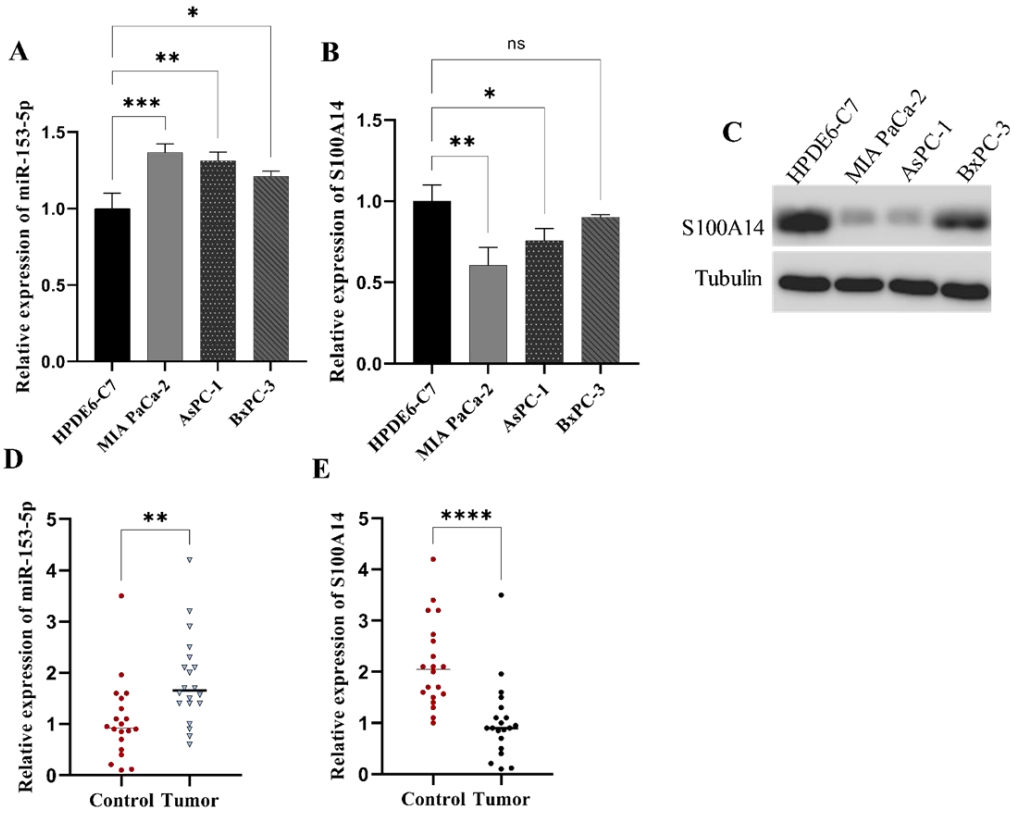

Figure 1 miR-153-5p expression is inversely correlated with S100A14 in pancreatic cancer. (A) qRT-PCR analysis shows increased miR-153-5p expression in pancreatic cancer cell lines (MIA PaCa-2, AsPC-1, BxPC-3) compared to the control HPDE6-C7 cells. (B) S100A14 expression is significantly lower in MIA PaCa-2 and AsPC-1 but shows no significant difference in BxPC-3 compared to HPDE6-C7. (C) Western blot confirms reduced S100A14 protein levels in MIA PaCa-2 and AsPC-1, with similar expression in BxPC-3 and HPDE6-C7. (D) Tumor tissues exhibit significantly higher miR-153-5p expression than control tissues. (E) S100A14 expression is significantly lower in tumor tissues. These results suggest an inverse correlation between miR-153-5p and S100A14 in pancreatic cancer. Statistical significance: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

S100A14 is a functional target of miR-153-5p

Our results demonstrate that the 3′-UTR of S100A14 contains multiple potential binding sites for miR-153-5p, as identified through sequence analysis and subsequent cloning into the pmirGLO luciferase reporter vector (**Figure 2(A)**). Luciferase reporter assays revealed that, in wild-type constructs, cells transfected with NC-mimics exhibited significantly higher luciferase activity compared to those transfected with miR-153-5p mimics, suggesting that miR-153-5p directly targets S100A14 (**Figure 2(B)**). In contrast, no significant difference in luciferase activity was observed in the mutant constructs, indicating that the predicted binding sites are essential for this interaction (**Figure 2(B)**). Furthermore, qRT-PCR analysis confirmed that miR-153-5p expression was significantly higher in mimics-transfected cells compared to NC-mimic controls (**Figure 2(C)**). Conversely, the expression of S100A14 mRNA was significantly downregulated in miR-153-5p mimic-transfected cells relative to NC-mimics (**Figure 2(D)**). To validate these findings at the protein level, Western blot analysis was performed, revealing a corresponding decrease in S100A14 protein expression upon miR-153-5p overexpression (**Figure 2(E)**). These findings collectively suggest that miR-153-5p negatively regulates S100A14 expression by directly binding to its 3′-UTR.

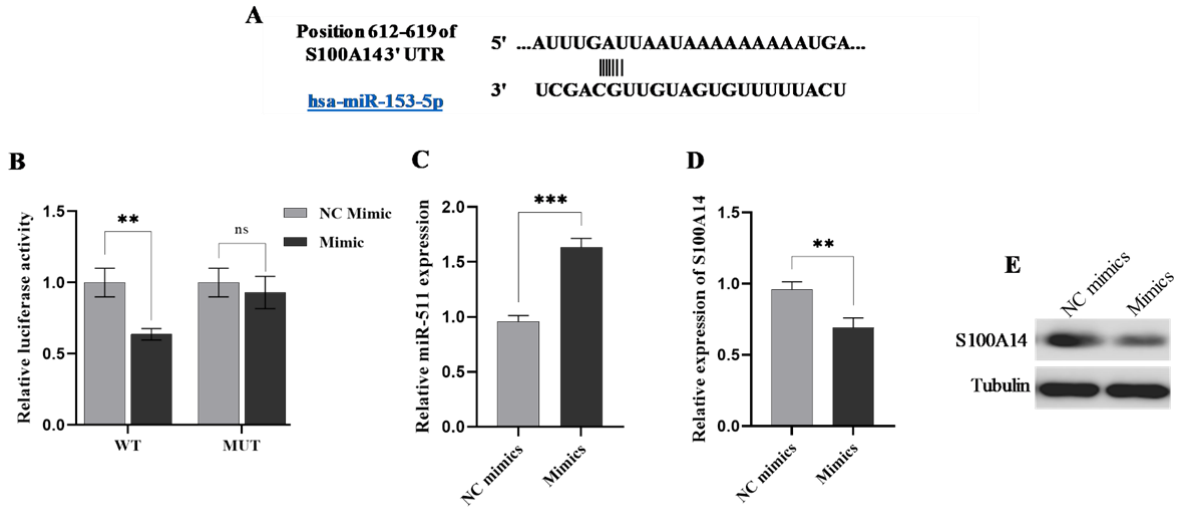

Figure 2 miR-153-5p directly targets S100A14. (A) Predicted binding sites of miR-153-5p in the 3′-UTR of S100A14 cloned into the pmirGLO vector. (B) Luciferase activity assay showing reduced luciferase expression in wild-type but not mutant constructs upon miR-153-5p overexpression. (C) qRT-PCR analysis confirming increased miR-153-5p expression in mimic-transfected cells. (D) qRT-PCR analysis showing a significant decrease in S100A14 mRNA levels in miR-153-5p mimics. (E) Western blot analysis demonstrating reduced S100A14 protein expression in miR-153-5p mimics compared to NC-mimics. Data are presented as mean ± SD; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

miR-153-5p Inhibition suppresses pancreatic cancer cell migration and invasion via S100A14 Regulation

To investigate the role of miR-153-5p in pancreatic cancer cell migration and invasion, BxPC-3 cells were transfected with either a miR-153-5p inhibitor or a NC-inhibitor (negative control inhibitor). qRT-PCR analysis confirmed that miR-153-5p expression was significantly reduced in cells transfected with the miR-153-5p inhibitor compared to the NC-inhibitor group (**Figure 3(A)**).

To further assess the functional impact of miR-153-5p inhibition, cells were co-transfected with miR-153-5p inhibitor and either siRNA-S100A14 (to silence S100A14) or siRNA-NC (negative control siRNA). Transwell migration and invasion assays revealed that knockdown of miR-153-5p led to a significant reduction in both migration and invasion of BxPC-3 cells compared to the NC-inhibitor group (**Figures 3(B)** - **3(C)**).

Interestingly, co-transfection with miR-153-5p inhibitor and siRNA-S100A14 rescued the inhibitory effects on cell migration and invasion, indicating that S100A14 is a critical downstream target of miR-153-5p in regulating these processes. In contrast, cells transfected with miR-153-5p inhibitor and siRNA-NC continued to show significantly decreased migration and invasion, further supporting the role of miR-153-5p in promoting pancreatic cancer cell motility (**Figures 3(B)** - **3(C)**). These findings suggest that miR-153-5p knockdown suppresses pancreatic cancer cell migration and invasion, and this effect is mediated through the regulation of S100A14.

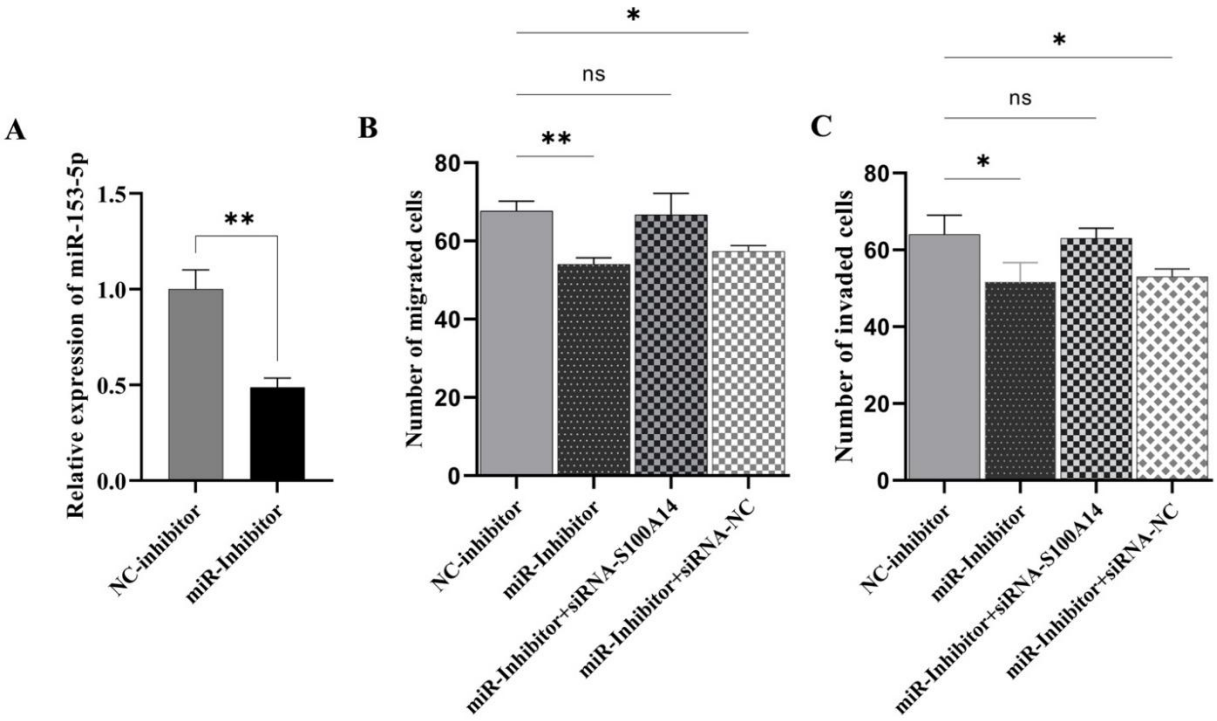

Figure 3 Inhibition of miR-153-5p reduces pancreatic cancer cell migration and invasion. (A) Migration assay showing a significant reduction in migrated cell numbers upon miR-153-5p inhibition, with rescue observed in the miR-153-5p inhibitor + siRNA-S100A14 group. (B) Invasion assay demonstrating decreased invaded cell numbers after miR-153-5p inhibition, which was reversed by siRNA-S100A14. (C) qRT-PCR analysis confirming the successful knockdown of miR-153-5p after transfection with its inhibitor. Data are presented as mean ± SD; *$p < 0.05$, **$p < 0.01$ from 3 independent biological replicates (n = 3).

MiR-153-5p inhibition reduces cell cycle progression and cell proliferation while inducing apoptosis by targeting S100A14

To further investigate the role of miR-153-5p in pancreatic cancer progression, BxPC-3 cells were transfected with either miR-153-5p inhibitor, NC-inhibitor (negative control inhibitor), miR-153-5p inhibitor + siRNA-S100A14, or miR-153-5p inhibitor + siRNA-co.

Cell cycle analysis revealed that inhibition of miR-153-5p significantly reduced cell cycle progression, as observed in cells treated with miR-153-5p inhibitor and miR-153-5p inhibitor + siRNA-co. In contrast, no significant promotion of the cell cycle was detected in NC-inhibitor-treated cells or in cells co-transfected with miR-153-5p inhibitor and siRNA-S100A14 (**Figure 4(A)**).

To further evaluate the effects of miR-153-5p on cell proliferation, an MTT assay was performed at different time points (0, 24, 48 and 72 h). The results showed a decrease in proliferation in miR-153-5p inhibitor-treated cells at 24 h, as well as in cells co-transfected with miR-153-5p inhibitor + siRNA-co. However, no significant changes were observed in cells treated with miR-153-5p inhibitor + siRNA-S100A14. Interestingly, at 48 and 72 h, a highly significant decrease in proliferation was observed in cells treated with miR-153-5p inhibitor and miR-153-5p inhibitor + siRNA-co, suggesting that miR-153-5p inhibition reduce cell proliferation unless S100A14 is also silenced (**Figure 4(B)**).

To examine the apoptotic effects of miR-153-5p inhibition, caspase 3 and caspase 9 activity were measured. The results revealed an inverse correlation between miR-153-5p inhibition and caspase activity, with a significant increase in caspase 3 and caspase 9 activity in cells treated with miR-153-5p inhibitor and miR-153-5p inhibitor + siRNA-co. However, no significant changes in caspase 3 and caspase 9 activity were detected in NC-inhibitor-treated cells or in cells

transfected with miR-153-5p inhibitor + siRNA-S100A14, further supporting the role of S100A14 in apoptosis regulation (**Figure 4(C)**). These findings suggest that miR-153-5p inhibition promotes cell cycle arrest and apoptosis while reducing proliferation, effects that are mediated through its regulation of S100A14.

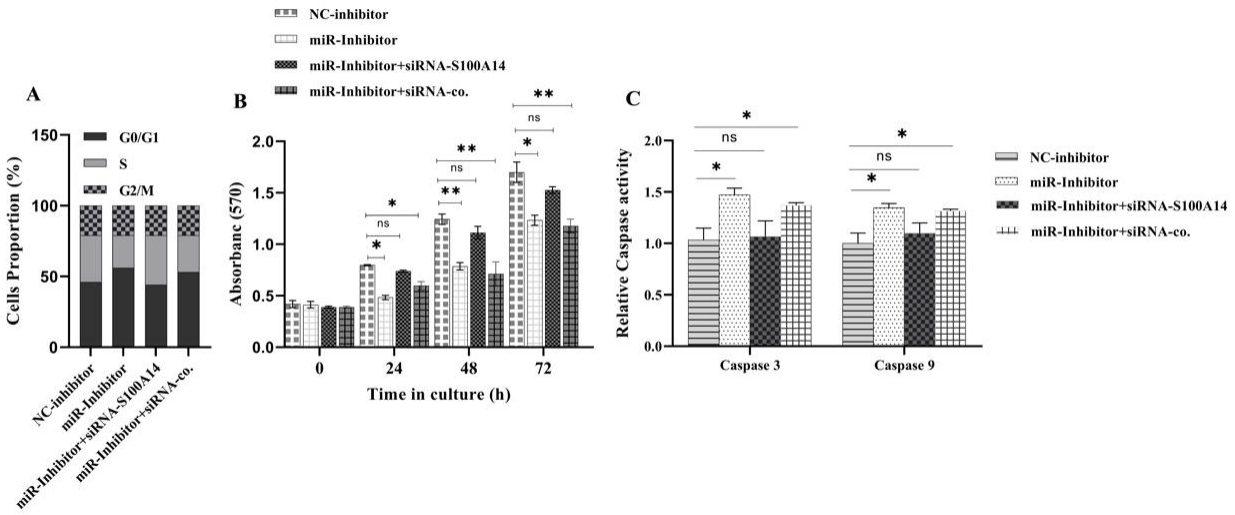

Figure 4 miR-153-5p inhibition reduced cell cycle progression and cell proliferation while inducing apoptosis by targeting S100A14. (A) Cell cycle analysis showing decreased cell cycle progression in miR-153-5p inhibitor and miR-153-5p inhibitor + siRNA-co groups, with no significant changes in NC-inhibitor or miR-153-5p inhibitor + siRNA-S100A14 groups. (B) Cell proliferation assay (MTT) showing a decrease in proliferation at 24h in miR-153-5p inhibitor and miR-153-5p inhibitor + siRNA-co groups, while proliferation significantly decreased at 48h and 72h in miR-153-5p inhibitor + siRNA-co cells. (C) Caspase 3 and caspase 9 activity showing increased expression in miR-153-5p inhibitor and miR-153-5p inhibitor + siRNA-co groups, with no significant changes in NC-inhibitor or miR-153-5p inhibitor + siRNA-S100A14 groups. Data are presented as mean ± SD; *$p < 0.05$, **$p < 0.01$ from 3 independent biological replicates (n = 3).

Discussion

Pancreatic cancer remains one of the most challenging malignancies to treat due to its aggressive nature and poor prognosis [15]. The findings of this study provide significant insights into the molecular mechanisms underlying pancreatic cancer progression, particularly the roles of miRNA-153-5p and its target, S100A14. By elucidating the interplay between these 2 molecular markers, this study highlights potential avenues for targeted therapeutic strategies.

The data presented in this study underscore the oncogenic role of miRNA-153-5p in pancreatic cancer. Elevated expression of miRNA-153-5p in pancreatic cancer tissues compared to adjacent normal tissues aligns with findings in other malignancies such as advanced stages of colorectal cancer Shi *et al*. [16] and breast cancers [17]. Functional assays further confirmed that miRNA-153-5p promotes key hallmarks of cancer, including increased migration, invasion, and proliferation of pancreatic cancer cells, while suppressing apoptosis. These findings support previous reports suggesting that miRNA-153-5p plays a pivotal role in tumor progression by modulating critical cellular pathways [18].

The therapeutic potential of miRNA inhibition was also demonstrated in this study. Use of a miRNA-153-5p inhibitor significantly reduced the aggressive phenotype of pancreatic cancer cells, restoring apoptotic mechanisms and diminishing their migratory and invasive capabilities. These findings are consistent with the growing body of evidence that miRNA inhibition could serve as a viable therapeutic strategy in cancer treatment [19].

S100A14 has been implicated as a tumor suppressor in various cancers, including esophageal squamous cell carcinoma Sapkota *et al.* [20] and oral carcinoma Bresnick *et al.* [21], where it regulates proliferation, apoptosis, and migration. In this study, S100A14 expression was markedly reduced in pancreatic cancer tissues compared to adjacent normal tissues. This inverse relationship with miRNA-153-5p expression supports the hypothesis that S100A14 is a direct target of miRNA-153-5p, as predicted by bioinformatics analysis and confirmed by luciferase reporter assays.

Mechanistically, the tumor-suppressive role of S100A14 appears to be mediated through its involvement in regulating cytoskeletal dynamics and cell signaling pathways. Its downregulation in pancreatic cancer likely contributes to the enhanced invasive and migratory properties of cancer cells observed in this study. The ability of miRNA-153-5p to suppress S100A14 expression adds another layer to the understanding of how miRNAs can disrupt tumor-suppressive pathways in cancer [22].

The results of this study confirm a strong inverse correlation between miRNA-153-5p and S100A14 expression in pancreatic cancer tissues. This finding is consistent with the TargetScan prediction that S100A14 is a direct target of miRNA-153-5p. By binding to the 3′ UTR of S100A14 mRNA, miRNA-153-5p likely facilitates its degradation or inhibits its translation, thereby downregulating S100A14 expression [23]. This regulatory relationship is supported by the luciferase reporter assay results, which validated the direct interaction between miRNA-153-5p and S100A14.

Moreover, the functional assays demonstrated that inhibition of miRNA-153-5p not only restored S100A14 expression but also significantly impaired the malignant behavior of pancreatic cancer cells. These findings suggest that the oncogenic effects of miRNA-153-5p are at least partially mediated through its targeting of S100A14, emphasizing the critical role of this interaction in pancreatic cancer pathogenesis.

The identification of miRNA-153-5p and S100A14 as key players in pancreatic cancer progression presents opportunities for therapeutic development. miRNA-based therapies, such as miRNA inhibitors or mimics, are gaining traction as potential cancer treatments due to their ability to modulate gene expression with high specificity [24]. The use of a miRNA-153-5p inhibitor in this study highlights its potential to mitigate the aggressive characteristics of pancreatic cancer cells, providing a proof of concept for future therapeutic interventions.

Furthermore, restoring S100A14 expression through targeted therapies could also hold promise. Strategies that combine miRNA inhibition with agents that stabilize or enhance S100A14 expression may prove particularly effective in halting pancreatic cancer progression. However, the feasibility of such approaches in clinical settings requires further investigation, particularly with regard to delivery mechanisms, off-target effects, and long-term efficacy [25]. Specifically, miRNA-based therapies face several challenges, including the need for efficient and tissue-specific delivery systems, potential immune responses, and the risk of unintended interactions with non-target genes. Overcoming these barriers will be essential to translate miR-153-5p-targeted strategies into safe and effective treatments.

Despite the promising findings, this study has several limitations. The patient sample size was relatively small (n = 20), which may affect the generalizability of the findings. Additionally, the experiments were limited to *in vitro* and ex vivo models. Future studies using larger cohorts and *in vivo* validation, such as animal models, are needed to strengthen the clinical relevance of targeting miR-153-5p.

Conclusions

this study highlights the oncogenic role of miRNA-153-5p in pancreatic cancer and identifies S100A14 as a critical tumor suppressor that is directly targeted by miRNA-153-5p. The inverse correlation between these 2 markers underscores their significance in pancreatic cancer pathogenesis. By demonstrating the therapeutic potential of miRNA-153-5p inhibition and S100A14 restoration, this study provides a foundation for future research into targeted therapies for pancreatic cancer.

Declaration of Generative AI in Scientific Writing

The authors acknowledge the use of generative AI tools (e.g., QuillBot and ChatGPT by OpenAI) in the preparation of this manuscript, specifically for language

editing and grammar correction. No content generation or data interpretation was performed by AI. The authors take full responsibility for the content and conclusions of this work.

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Dian Jamel Salih: Validation, Writing, Data Curation, and Formal analysis.

Ghazwan Fawzi Ahmed: Data Curation, Validation, and Visualization.

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Saad Younis Saeed: Funding acquisition.

Khawla Abdalkarim Kasar: Project administration, Resources, and Supervision.

Hazhmat Ali: Methodology, Project administration, Resources, and Supervision.

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