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Role of long non-coding RNAs MIR22HG, LNCTAM34A, and TP53TG1 in Breast Cancer

Short subject: lncRNAs, cell survival and apoptosis in breast cancer

Abstract

Long non-coding RNAs (lncRNAs) like MIR22HG, LNCTAM34A, and TP53TG1 are dysregulated in a variety of cancers. In this study, we looked into how these lncRNAs affect breast cancer. Analysis of 150 breast tissues revealed a significantly decreased expression of the selected lncRNAs in tumor tissues than TANs ($P < 0.05$); this lower expression was linked to a graver patient survival rate. Suppression of cell cycle-related genes in human breast cancers suggests that MIR22HG may have a tumor suppressor function. This is supported by the fact that silencing of MIR22HG, LNCTAM34A, and TP53TG1 induced both cell survival and decreased programmed cell death through downregulation of the P53. According to our research, MIR22HG, LNCTAM34A, and TP53TG1 are down-regulated in breast cancer, and their suppression encourages the growth of breast cancer cells while preventing apoptosis. In addition, our results imply that MIR22HG, LNCTAM34A, and TP53TG1 may be used as novel diagnostic and prognostic markers for breast cancer as well as therapeutic targets.

Keywords: *Long non-coding RNA, Breast Cancer, Gene Knockdown, MIR22HG, LNCTAM34A, TP53TG1.*

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1. Introduction

In the world, lung cancer is the leading cause of cancer death in women, and breast cancer (BC) is the most prevalent cancer in women. Each year, more than 268,000 new patients receive a diagnosis of BC, and the disease claims 41,760 lives [1]. Mastectomy, hormone therapy, surgery with adjuvant radiation therapy, chemotherapy, and immunotherapy are some of the latest BC treatments that can help affected people live longer [2], [3]. Although the TNM stage method is useful for classifying BC patients, it does not predict their prognosis. As a result, molecular indicators must be discovered to assess BC patients' chances of survival [4]. Despite significant early diagnosis and treatment progress over the previous 20 years, BC remains a serious public health concern as a complex heterogeneous malignancy.

According to improvements in next-generation sequencing technology and the results of several projects such as The Encyclopedia of DNA Elements (ENCODE), at least 75% of the human genome is effectively transcribed into RNAs, with just 2% of these transcripts being translated into proteins. Non-coding RNAs (ncRNAs) are divided into long ncRNAs (LncRNAs), which have lengths greater than 200 nucleotides, and short ncRNAs, which have lengths less than 200 nucleotide [5]. The biological significance of lncRNAs is not well understood, despite the fact that short ncRNAs, particularly miRNAs, have been extensively studied and many of their biological functions have been identified [6]. By controlling gene expression at the transcriptional, post-transcriptional, and epigenetic levels, lncRNAs are essential for a variety of biological processes that occur throughout life [7].

In a variety of human cancers, lncRNAs serve as regulators and controllers of cancer initiation and development, making them desirable therapeutic targets for new drug development and biomarkers for clinical diagnosis [8], [9]. Apoptosis suppression, invasion promotion, proliferation persistence, altered cellular kinetics, genomic instability, and evading of growth inhibitors are all lncRNA-related cellular characteristics that have been documented [10]. LncRNAs have also been discovered as important BC controllers. A growing number of lncRNAs have been linked to BC initiation and development, implying that elucidating the role of important lncRNAs in BC might give useful evidence for clinical surveillance and therapy.

The miR-22 host gene (MIR22HG) is transcribed from the cytogenic region of 17p13.3, the region that shows loss of heterozygosity, deletion, or hypermethylation [11], [12]. Many human cancers, such as hepatocellular carcinoma, thyroid carcinoma, colorectal cancer, lung cancer, and gastric cancer, exhibit decreased MIR22HG expression, while other cancers exhibit markedly elevated expression (glioblastoma and esophageal adenocarcinoma). As a host gene in carcinogenesis and tumor growth, MIR22HG functions as a competitive endogenous RNA (ceRNA), takes part in signaling cascades, interacts with proteins, and cooperates with miRNAs [13]. However, as far as we are aware, MIR22HG's contribution to the emergence of BC has never been studied. The genomic region 1p36.22 contains the long-non-coding transcriptional activator of miR34a (lncTAM34a), which is synthesized from the miR34a locus' antisense orientation. Various cellular stresses are believed to trigger lncTAM34a, and its expression reduces tumorigenic characteristics via a positive control of miR34a expression [14]. In order to aid the TP53 signaling pathway and the feedback to cellular damage, TP53 target 1 (TP53TG1), which can be found in the cytogenetic band 7q21.12, was first discovered in a colon cancer cell line [15]. In colorectal and gastric primary tumors, it was found that TP53TG1 was silenced under cancer-specific hypermethylation, which was linked to poor outcomes [16]. The methylation status-controlled gene TP53TG1 was recently found to be significantly correlated with BC outcomes [17].

They might value diagnostic, prognostic, and therapeutic cancer research in BC greatly given the differential expression and significant biological function of MIR22HG, lncTAM34a, and TP53TG1. As a result, this study evaluated the levels of specific lncRNA expression in tumor tissues from BC patients. Thereafter, MIR22HG, lncTAM34a, and TP53TG1 expression in BC cells were knocked down, and a functional study was performed. The current work discovered a link between MIR22HG, lncTAM34a, and TP53TG1 and BC, providing a new possibility for lncRNA-based BC detection and treatment.

2. Materials & Methods

2.1. Study population, breast tissue sampling, and clinical data

The national ethnic committee of Tehran University of Medical Sciences (TUMS) gave its approval to the study's experimental methods (ethical code IR.TUMS.Medicine.REC.1398.950). Moreover, an informed consent form containing an explanation of risks, benefits, and the purpose of the biopsy procedure was signed by each participant before inclusion in the study. From 1 February 2018 to 31 November 2020, 150 female patients who were referred to the TUMS Cancer Institute provided their paired normal adjacent tissues (NATs) and BC tissues. Patients with invasive ductal carcinoma confirmed by breast MRI and pathologic examination and patients with no experience of chemotherapy or radiotherapy before the surgery were included in the study. Until they were used, the excised tissues were kept at 80°C while being frozen in liquid nitrogen.

2.2. Cell lines and cell culture

The Pasture Institute of Iran provided MCF-7, MDA-MB-231, MDA-MB-468, and MDA-MB-453 human breast cancer cell lines as well as the healthy MCF-10A breast epithelial cell line (Tehran, Iran). Breast cancer cells and a mammary epithelial cell line were cultured in DMEM (Gibco, Grand Island, NY, USA), which was supplemented with 10% fetal bovine serum (Gibco), 100 U/mL penicillin, and 100 mg/mL streptomycin (Invitrogen, Carlsbad, CA, USA) at 37°C in a humid incubator with 5% CO2. Cells were grown for no more than 25 passages in total for each experiment.

2.3. Reverse transcription and quantitative polymerase chain reaction (RT-qPCR)

The manufacturer's protocol was followed in extracting the total RNA from the samples using Trizol reagent (Invitrogen, Carlsbad, CA, USA). By using a spectrophotometer and gel electrophoresis, RNA quantity and quality were assessed. The Reverse Transcription System Kit (Applied Biosystems) was used to create first-strand cDNA from 2.0 g of RNA in accordance with the manufacturer's instructions. In a 20µl reaction containing 0µl of RealQ Plus 2x MasterMix Green (Amplicon, Denmark), 8µl DEPC water, 0.5µl of each primer, and 1µl diluted RT products, the expression of the target lncRNAs was assessed using a quantitative LightCycler® 96 real-time PCR instrument (Life Science, Roche, Germany) under the following thermocycling conditions: Denaturation at 95 °C for 15 min, 40 cycles of 95 °C for 15 s and 60 °C for 30 s, and a melting curve step from 65 °C to 95 °C with an increment of 0.5 °C/5 s are all necessary for denaturation.

B2M served as the internal standard for normalizing and quantifying mRNA and lncRNA expressions. The relative levels of gene expression were calculated using the $2^{-\Delta\Delta Ct}$ method, and the real-time PCR reactions were run in triplicate. The following information describes each of the other genes' primer sequences: F: 5′-GCTGGGTGCCGAGAAGAGACAG-3', R: 5′-CAGGTGAGGGCGTGAGAGGAAC-3' for MIR22HG, F: 5′-GCGAATGCTACCCAGATGTCTCAG-3', R: 5′-GATAGGTTCATTTGCCCGATGTGC-3' for LNCTAM34A, F: 5′-CAGCACCAGGCGAACACTTACAC, R: 5′-CTCAGACCTGCCAGCTCTCAGAG-3' for TP53TG1, and F:5′- AGATGAGTATGCCTGCCGTG -3′ and 5′-GCGGCATCTTCAAACCTCCA -3′ for B2M.

2.3. Small interfering RNA (siRNA)-mediated knockdown

In order to target breast cancer cells with siRNA oligonucleotides or non-targeting controls, trypsin solution was used to digest the cells before they were seeded onto 6-well plates and grown overnight at 37°C in a 5% CO2 environment. After reaching 80% confluence, cells were rinsed twice with PBS and added to 1.5 mL of fresh baseline DMEM. Knockdown was then carried out using 5µl Lipofectamine® 2000 transfection reagent (Invitrogen, USA), which was pre-mixed with 250µl OptiMEM medium in accordance with the manufacturer's instructions. The cells were then mixed with 10µM targeting siRNAs or negative control siRNAs. Guangzhou Forevergen Biosciences Co., Ltd. (Guangzhou, China) has created small interfering RNAs with the sequence Si-LNCTAM34A: 5'-GCGAATGCTACCCAGATGTCT-3'; Si-MIR22HG, sequence: 5'-GAGCCGCAGTAGTTCTTCAGT-3'; Si-TP53TG1, sequence: 5'-GGTGCCAAATGAGCTGTCCTA-3'). Eventually, cells were cultured for 48 hours in fresh DMEM before the tests, and knockdown effectiveness was evaluated by qRT-PCR.

2.4. Cell proliferation, colony formation, invasion, and migration assays

The Cell Counting Kit-8 (CCK-8) assay was used to determine the proliferation rates of breast cancer cells. To prepare a single-cell suspension, breast cancer cells were digested with a trypsin solution. Cells were seeded in 96-well plates (100µl/well) at a final density of 3104 cells/well and transfected with 10 nM of the corresponding siRNA oligonucleotides or non-targeting controls 2–24 h after plating, as directed by the manufacturer. After transfection with siRNA, the cells were cultured at 37 °C for 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, and 120 hours. After normalizing to the non-target control siRNA, cell proliferation rates were determined using a microplate reader to detect absorbance at 490 nm. At least three biological replications were performed for statistical analysis. The transfected BC cells were planted in a six-well plate at 200 cells per well with both the targeting and control siRNAs for the colony formation assay (CFA). The colonies were fixed and stained after 10 to 14 days of incubation at 37 °C with 0.1% crystal violet (Sigma-Aldrich) and 20% methanol as a dye solution. The number of colonies was counted in each well. Three separate experiments were carried out.

A 100-µl cell suspension of the diluted serum-free medium (1x105 cells/ml) was added to the upper chambers of a Transwell system to assess the migration of breast cancer cells. 600µl of brand-new DMEM were placed in the lower chambers of the Transwell system. Migrated cells were cultured normally for 48 hours before being discovered and measured under an inverted fluorescence microscope (Leica Microsystems GmbH, Wetzlar, Germany). Migrated cells were fixed with 4 percent paraformaldehyde for 15 minutes, stained with 1% crystal violet for 10 minutes, both at room temperature, and washed with PBS. Matrigel matrix (Corning Incorporation, Corning, NY, USA) was placed in the Transwell chambers after being treated with DMEM at a 1:3 ratio. It was then incubated for 2 hours at 37°C to measure cell invasion. Cells were cultured at 37°C for 48 hours before being fixed with 4% paraformaldehyde for 15 minutes at room

temperature, stained with 1% crystal violet for 10 minutes at room temperature, and analyzed for invasion under a microscope.

2.5. Apoptosis Analysis

Apoptosis assays were carried out using the PI/Annexin V-FITC kit according to the manufacturer's protocol, the Apoptosis Detection Kit (BD Biosciences, Franklin Lakes, NJ, and USA). In brief, 100µl of singled BC cells transfected with targeting siRNAs and control siRNAs at a cell density of 1x106 cells/ml were incubated with 5µl Annexin V in the dark for 15 minutes at room temperature, then incubated with 400µl binding buffer for 1 hour before being analyzed by flow cytometry (Sysmex Partec GmbH). For statistical analysis, data from three biological repeats were used.

2.7. Western Blot Analysis

SDS-PAGE was used to separate total extracted proteins from treated BC cell lines. After blocking the membranes with 5% nonfat milk for 45 minutes, they were incubated overnight at 4°C with primary antibodies anti-P53 (1:3000), anti-Bax (1:2000), anti-BCL2 (1:2000), and anti-β-actin (1:10000). The membranes were then washed three times in PBS before being incubated for two hours with secondary antibodies conjugated to horseradish peroxidase (HRP). All experiments were conducted three times.

2.8. Statistical analysis

The information was given as the mean and mean standard error of the mean. Excel and GraphPad Prism 6 (software from GraphPad) were used to illustrate the data. The SPSS 20.0 program was used to perform a statistical analysis on it as well (SPSS, Inc., Chicago, IL, USA). To examine differences between two and more than two group comparisons, respectively, the Student's t-test or one-way analysis of variance are used. The statistically significant difference was defined as a p-value with two tails of 0.05.

3. Results

3.1 MIR22HG, LNCTAM34A, and TP53TG1 expression is decreased in breast cancer

To identify a new lncRNA associated with the etiology of breast cancer, 150 pairs of breast cancer and surrounding normal tissues had their expression levels of MIR22HG, LNCTAM34A, and TP53TG1 compared (Figure 1A). Breast cancer tissues had significantly lower levels of MIR22HG, LNCTAM34A, and TP53TG1 expression than NATs ($P<0.05$; Fig. 1A). These lncRNAs may play pathogenic roles in breast cancer, as suggested by the downregulation of MIR22HG, LNCTAM34A, and TP53TG1 in breast cancer tissues. Additionally, our clinical data demonstrated a significant correlation between the clinicopathological characteristics and the expression status of MIR22HG, LNCTAM34A, and TP53TG1 ($P<0.05$, Table 1). MIR22HG, LNCTAM34A, and TP53TG1 lncRNA expression was found to be lower in patients with advanced TNM stage and grade groups (P0.05) in Table 1. The overexpressed status of MIR22HG and TP53TG1 was significantly correlated with the older age at diagnosis (P 0.05). In subjects with tumors smaller than 5 cm in diameter, we discovered that MIR22HG expression was significantly higher, whereas LNM status did not significantly affect the expression levels of the lncRNAs under study ($P>0.05$).

MIR22HG, LNCTAM34A, and TP53TG1 expression were assessed in four breast cancer cell lines and a mammary epithelial cell line for additional validation. Our findings demonstrated that MIR22HG is overexpressed in the BC cell lines MDA-MB-231 and MCF-7, and that LNCTAM34A and TP53TG1 are upregulated in the BC cell lines MDA-MB-453 and MCF-7 compared to the mammary epithelial cell line MCF10A, but did not significantly overexpress in the other BC cell lines that were under investigation (Figure 1B).

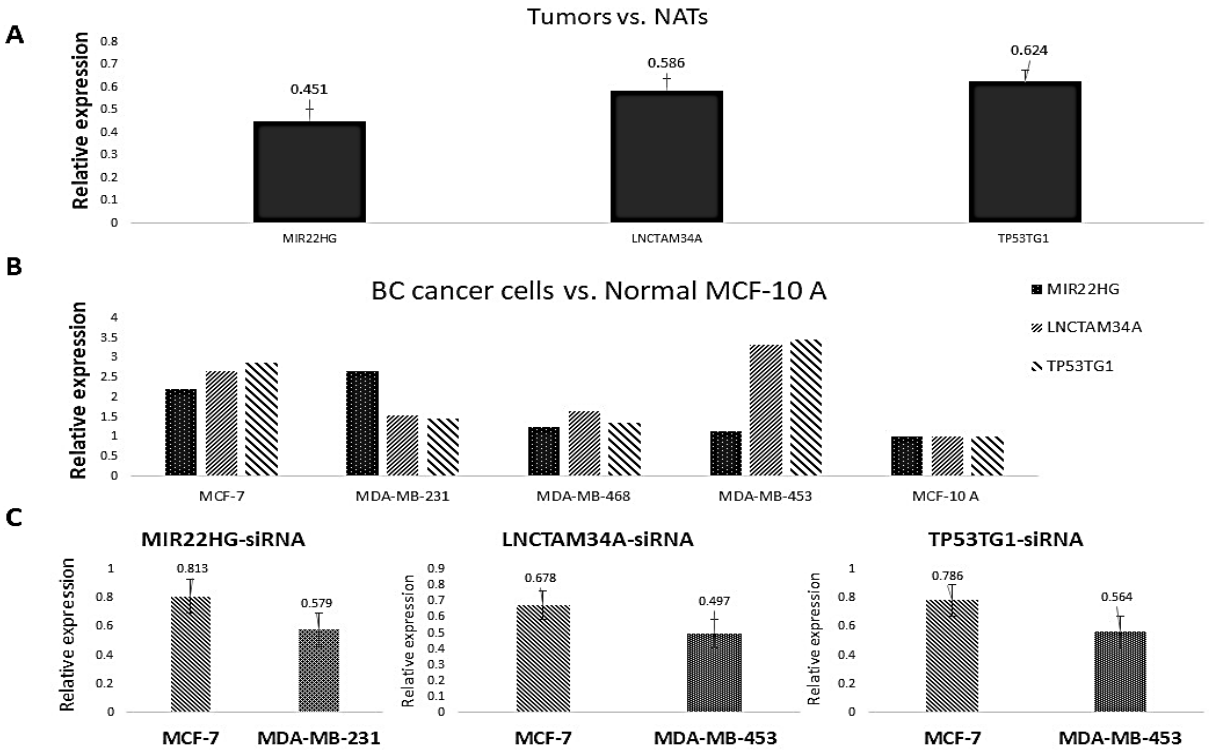

Figure 1. The expression status of MIR22HG, LNCTAM34A, and TP53TG1 in tumor tissues vs. NATs, in selected cell lines, and in treated cancer cells with targeting siRNA. (A) The lower MIR22HG, LNCTAM34A, and TP53TG1 expression levels in breast cancer tissues when compared to adjacent normal tissues. Reverse transcription-quantitative polymerase chain reaction was used to ascertain the expression of MIR22HG, LNCTAM34A, and TP53TG1 in MDA-MB-231, MDA-MB-453, and MCF-7 cells. Expression of TP53TG1, MIR22HG, and LNCTAM34A in MCF10A and four breast cancer cell lines. Four breast cancer cell lines and MCF-10A. MIR22HG expression in four breast cancer cell lines and MCF10A; (B) In contrast to the mammary epithelial cell line MCF10A, MIR22HG is overexpressed in the BC cell lines MDA-MB-231 and MCF-7, and LNCTAM34A and TP53TG1 are upregulated in MDA-MB-453 and MCF-7. (C) Expression of MIR22HG, LNCTAM34A, and TP53TG1 compared to MCF10A control cells in breast cancer cells transfected with targeting siRNA. Compared to the negative control group, qRT-PCR assays indicated that targeting siRNA significantly decreased the expression of MIR22HG, LNCTAM34A, and TP53TG1 by more than 80% after transfection with targeting siRNAs in MDA-MB-231, MDA-MB-453, and MCF-7 cells. NATs or MCF10A were compared at *P < 0.05 and **P< 0.001, respectively. NC is an abbreviation for negative control.

Table 1. Correlation between the MIR22HG, LNCTAM34A, and TP53TG1 lncRNAs expression with different clinicopathological features in BC patients.

Clinicopathological Characteristics		MIR22HG Mean FC**	P-value*	LNCTAM34A Mean FC**	P-value*	TP53TG1 Mean FC**	P-value*
Age	≤ 50	0.4	**0.020**	0.82	0.677	0.73	**0.012**
	> 50	0.5		0.83		0.83	
Tumor size	≤ 5 cm	0.46	0.082	0.84	**0.022**	0.77	0.891
	> 5 cm	0.36		0.73		0.77	
LNM status	Positive	0.43	0.251	0.81	0.264	0.76	0.317
	Negative	0.48		0.85		0.81	
Grade	Early grades (I & II)	0.53	**< 0.001**	0.88	**0.008**	0.83	**0.013**
	Late grades (III & IV)	0.38		0.78		0.73	

TNM stage	Early stages (I & II)	0.51	**0.005**	0.86	**0.027**	0.79	0.291
	Advanced stages (III & IV)	0.39		0.79		0.75	

* **Bolded** P-values < 0.05 were considered to show statistically significant values; ** FC: Fold Change; BC: breast cancer; LNM: lymph node metastasis

3.2. Knockdown of MIR*22HG*, LNCTAM34A, and TP53TG1 expression suppresses cell proliferation in BC cells

Four breast cancer cell lines and a mammary normal epithelial cell line were used to verify the expression of MIR22HG, LNCTAM34A, and TP53TG1. Statistically significant upregulation of these markers was seen in the MDA-MB-231 and MCF-7 cancer cell lines compared to the mammary epithelial cell line (Figure 1B). In order to investigate the potential role of these lncRNAs in the etiology of breast cancer, MDA-MB-231 and MCF-7 breast cancer cells that express target MIR22HG, as well as MDA-MB-453 and MCF-7 cancer cell lines that express LNCTAM34A and TP53TG1, were transfected with a specific targeting siRNA. After transfection with targeting siRNAs in MDA-MB-231, MDA-MB-453, and MCF-7 cells, qRT-PCR assays revealed that MIR22HG, LNCTAM34A, and TP53TG1 expression were significantly reduced by more than 80% compared to the negative control group. qRT-PCR in these cells confirmed the knockdown effectiveness (Figure 1C).

The proliferation of transfected MDA-MB-231, MDA-MB-453, and MCF-7 cells with targeting siRNAs was examined in order to investigate the potential roles of MIR22HG, LNCTAM34A, and TP53TG1 in the development and progression of breast cancer. Functionally, we discovered that, between 96 and 120 hours after MIR22HG was knocked down in MDA-MB-231 and MCF-7 breast cancer cells, the proliferation rates as measured by the CCK-8 assay significantly increased by 30% to 70%. (Figure 2A). Additionally, LNCTAM34A-siRNA and TP53TG1-siRNA-targeted MDA-MB-453 and MCF-7 cancer cells demonstrated noticeably higher cell proliferation compared to non-targeting control cells (Figure 2B and 2C). Colony formation assays further demonstrated that, contrary to the results of the CCK-8 assay, knocking down MIR22HG and LNCTAM34A significantly increased cell proliferation and TP53TG1 expression in MDA-MB-231, MDA-MB-453, and MCF-7 breast cancer cells when compared to the non-target control (Figure 2D). These findings suggested that MIR22HG, LNCTAM34A, and TP53TG1 may control BC cell growth through a tumor-suppressive mechanism.

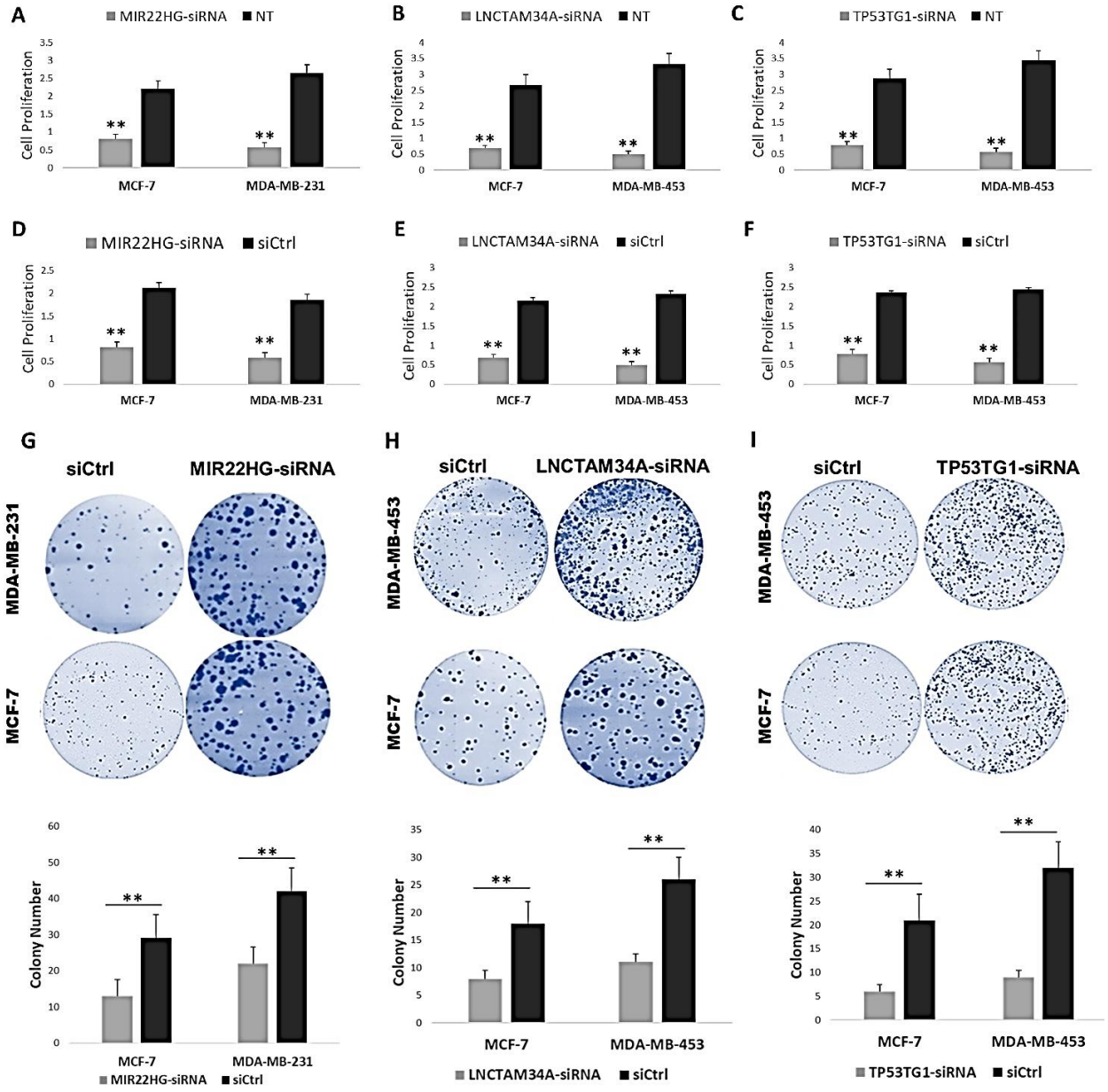

Figure 2. MIR22HG, LNCTAM34A, and TP53TG1 knockdown alter the cellular characteristics of MDA-MB-231, MDA-MB-453, and MCF-7 cells. (A and D) MDA-MB-231 and MCF-7 cell growth following MIR22HG knockdown (B and E) MDA-MB-231 and MCF-7 cell growth following LNCTAM34A knockdown (C and F). The growth of MDA-MB-453 and MCF-7 cells following TP53TG1 knockdown. (G-I) MIR22HG, LNCTAM34A, and TP53TG1 knockdown compared to the non-knocked-down group in colony formation assays of MDA-MB-231, MDA-MB-453, and MCF-7 cells. A MTS assay was used to measure cell proliferation. The values were the average plus standard deviation across three separate experiments. siCtrl: control group for non-targeting siRNA; **P< 0.01.

3.3. Silencing MIR22HG, LNCTAM34A, and TP53TG1 triggers BC cell migration and invasion

Additionally, using Transwell and Matrigel assays, the migration and invasion of knocked-down MDA-MB-231, MDA-MB-453, and MCF-7 cells for MIR22HG, LNCTAM34A, and TP53TG1 were examined. In contrast to cells treated with non-target control siRNA, we discovered that transfection with siRNAs targeting MIR22HG, LNCTAM34A, and TP53TG1 significantly increased the migration and invasion of BC cells (Figure 3). Our findings also showed that MIR22HG, LNCTAM34A, and TP53TG1 depletion significantly facilitated BC cell invasion, which was consistent with the migration assay's invasion assay results (Figure 3). These findings suggested that MIR22HG influences colony formation, invasion, and migration by influencing proliferation/cell cycle regulation.

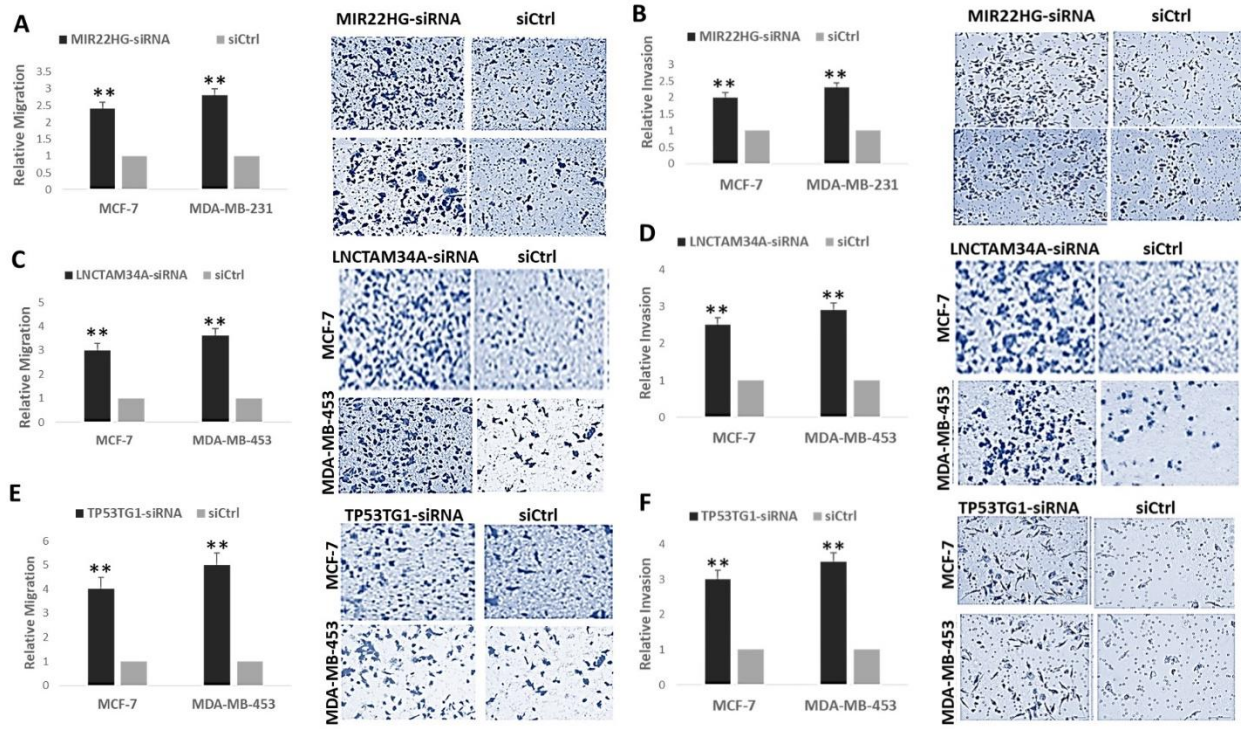

Figure 3. MIR22HG, LNCTAM34A, and TP53TG1 lncRNAs were knocked down by targeting siRNAs, changing the cellular characteristics of MDA-MB-231, MDA-MB-453, and MCF-7 cells. (A and B) Cells transfected with MIR22HG-siRNA exhibit migration and invasion. (C and D) Cells transfected with LNCTAM34A-siRNA exhibit migration and invasion, including MDA-MB-453 and MCF-7. (E and F) Cells transfected with TP53TG1-siRNA exhibit migration and invasion. In order to study cell invasion and migration, transwell assays were used. negative control, NC, with a **P<0.001.

3.3. MIR22HG, LNCTAM34A, and TP53TG1 knockdown decreases apoptosis

To investigate the function of MIR22HG, LNCTAM34A, and TP53TG1 in the apoptosis in BC, we studied transfected MDA-MB-231, MDA-MB-453, and MCF-7 cell lines by targeting silencing siRNAs at 72 h post-transfection. When MIR22HG was overexpressed, more cell apoptosis occurred in the target lncRNAs-overexpressing groups, whereas less cell apoptosis appeared in the MIR22HG-siRNA, LNCTAM34A-siRNA, and TP53TG1-siRNA groups (**Figure 4A**). Western blotting shows that the cell apoptosis-related protein further supported the above finding (**Figure 4B**).

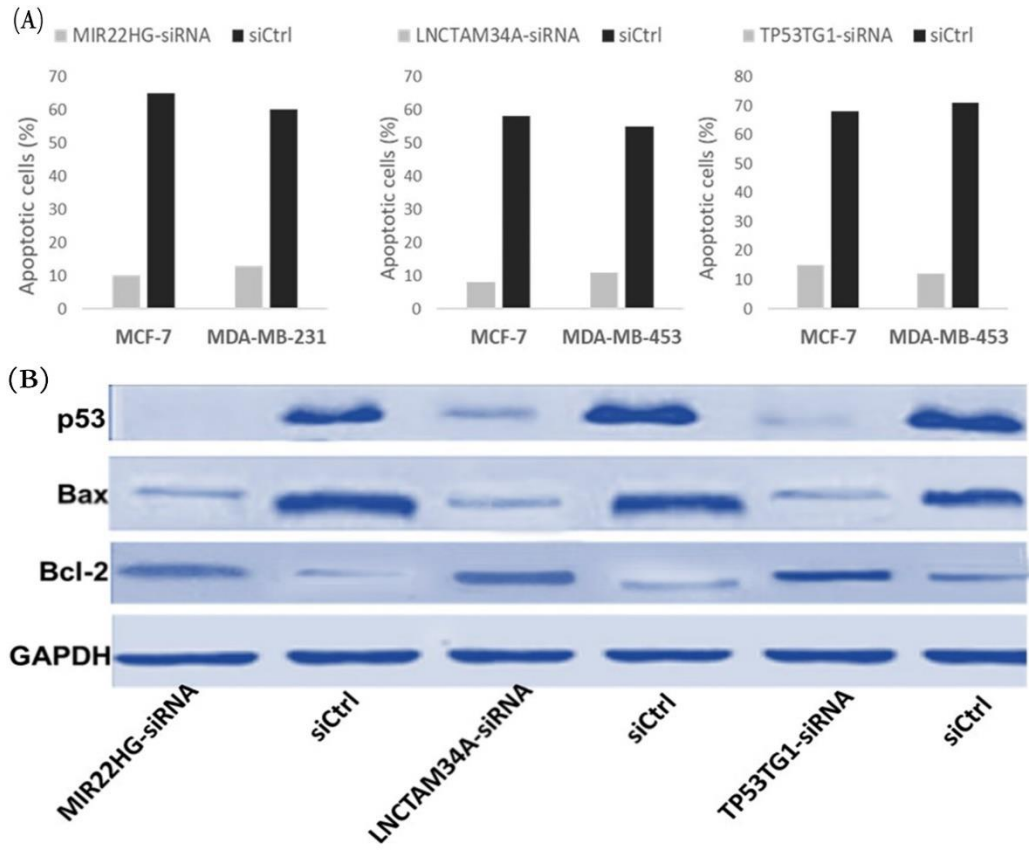

Figure 4. Reduced tumor cell apoptosis is a result of the knockdown of MIR22HG, LNCTAM34A, and TP53TG1. (A) A bar graph demonstrating the reduction in early and late apoptotic cells in MIR22HG, LNCTAM34A, and TP53TG1 targeting siRNA BC cell groups when compared to target lncRNA-expressing groups (B) Western blotting of the cell apoptosis-related protein in transfected cells with targeting siRNAs versus control groups. The information was the mean SD of two separate experiments. (*P<0.05).

4. Discussion

A significant portion of the human genome is made up of non-coding elements, and it has previously been discovered that these elements play a role in physiological and pathological processes like carcinogenesis. Therefore, it is encouraged to conduct clinical trials of their application in the diagnosis and prognosis of breast cancer [8], [18]. LncRNAs are important tumor suppressors and oncogenes in many cancers, although only a small number have been thoroughly studied [19]–[22].MIR22HG, LNCTAM34A, and TP53TG1 were substantially downregulated in breast cancer in this investigation, and the expression status of selected lncRNAs was linked to patients' clinicopathological characteristics. Furthermore, a number of in vitro experiments were conducted to assess the roles of MIR22HG, LNCTAM34A, and TP53TG1 in the tumorigenesis characteristics of the BC cell lines MDA-MB-231, MDA-MB-453, and MCF-7. In this study, we found that knockdown of MIR22HG, LNCTAM34A, and TP53TG1 induced cell proliferation, colony formation, invasion, and migration in the BC cell lines MDA-MB-231, MDA-MB-453, and MCF-7. Additionally, the examined lncRNAs reduced mechanistically the apoptosis in the aforementioned BC cell lines. These results demonstrate a novel role for these lncRNAs in BC that may be utilized to create lncRNA-based therapies.

In order to identify a new candidate for breast cancer detection and treatment, the current study examined the expression levels of the lncRNA MIR22HG in 150 pairs of BC tissues and adjacent normal tissues obtained from patients with breast cancer. MIR22HG expression was shown to be substantially lower in cancer tissues, indicating that it may have a role in the genesis of breast cancer. Similarly, MIR22HG is downregulated in several cancers [23], [24]. Additionally, abnormal MIR22HG expression has been linked to important clinical traits in a number of different types of human cancer, including advanced tumor size, TNM stage, and overall survival [24]–[26]. The overall survival of BC patients has been linked to clinicopathological factors. Therefore, we investigated whether any of these characteristics were correlated with either high or low MIR22HG expression. Low MIR22HG expression was significantly correlated with patient age (50 years; P = 0.020), early TNM stage (P = 0.005), and grade (P 0.001), but not with lymph node metastasis (LNM) or tumor size (P > 0.05). It has been discovered that MIR22HG, a well-researched lncRNA, acts as a key regulator in a number of cancers, suggesting that it may play a significant role in

tumorigenesis, involving proliferation, apoptosis, invasion, and metastasis [24], [27], [28].

In the current study, MIR22HG was knocked down in MDA-MB-231 and MCF-7 BC cell lines using targeting siRNAs compared to a control non-targeted-siRNA to explore its putative role in BC pathogenesis. Colony formation, cell proliferation, migration, and invasion were all evaluated. When compared to control siRNA, we discovered that colony formation increased two-fold. Furthermore, we observed that MIR22HG silencing impacted cell proliferation, with a 70 percent increase between 48 and 72 hours. MIR22HG suppression substantially enhanced cell migration in transwell experiments when compared to control. Compared to the non-targeting control group, the number of cells entering the matrigel and the area invaded by BC cells was considerably higher in the targeting MIR22HG-siRNA group. Flow cytometry apoptosis tests revealed that cell growth induction might be caused by an increase in apoptotic cell death (twofold compared to controls). A reduction in proteins involved in apoptosis processes, including the apoptotic biomarker P53 and BAX, and an increase in BCL-2 were correspondingly induced. This suggests that MIR22HG may be used as a tumor suppressor and prognostic biomarker in BC. It also suggests that MIR22HG may play a critical role in tumorigenesis by preventing tumor cell proliferation, invasion, and metastasis. The molecular mechanisms by which MIR22HG promotes cell cycle progression, migration, and proliferation in breast cancer cells require more investigation. Among other actions implicated in the MIR22HG-mediated regulation of cancer growth, it has been demonstrated that MIR22HG functions as a competitive endogenous RNA (ceRNA), participates in signaling pathways, interacts with proteins, and interacts with miRNAs as a host gene [13].

A few examples of tightly controlled cellular processes that are almost always dysregulated in cancer include DNA damage, nutrient deficiency, and persistent oncogene expression. TP53, which regulates the transcription of coding and non-coding downstream targets like miR34a, frequently activates various cell signaling pathways in response to these stressors [29]. By down-regulating target genes involved in cellular pathways like growth factor signaling, apoptosis, differentiation, and cellular senescence, MiR34a is made more abundant in response to TP53 activation [30], [31]. MiR34a is consequently frequently deleted or down-regulated in human malignancies, indicating its tumor-suppressive effect and making it a helpful prognostic marker in a number of cancers [32] [33]. The lncTAM34a is functionally characterized, and it has been discovered that it acts as an antisense RNA to positively control miR34a production, reducing various tumorigenic characteristics.

Furthermore, in the absence of TP53, lncTAM34a-mediated up-regulation of miR34a is sufficient to activate endogenous cellular processes that combat a variety of stressors. [14]. The expression of lncTAM34a was significantly lower in BC tissues than in paired normal adjacent tissues in the current study, and the difference was statistically significant [14]. Due to the association between TP53 mutations, reduced expression of miR34a, and a poor prognosis in cancer, we also looked at the association between clinicopathological characteristics of patients with low expression of lncTAM34a and control samples. In the included samples, our data demonstrated a significant ($P<0.05$) correlation between the higher mean expression of LNCTAM34A and smaller tumor sizes, early grade, and TNM stage. Similarly, it has been reported that LNCTAM34A could be a favorable prognostic factor and better overall survival in endometrial cancer [34]. The above results indicate that LNCTAM34A may act as a tumor suppressor in BC, and its elevated expression could have good prognostic effects.

We silence LNCTAM34A in MDA-MB-453 and MCF-7 cell lines to examine the effects of LNCTAM34A expression on tumorigenesis-related processes in order to verify this theory. Compared to the non-targeting control group, BC cancer cells' capacity for colony formation, proliferation, migration, and invasion increased significantly when LNCTAM34A was depleted by targeting LNCTAM34A-siRNA. The targeting siRNA-transfected cells' apoptosis rate was also significantly higher than the cells in the mimic control group, according to flow cytometry ($P<0.05$), which was another finding. In addition, we carried out a Western blot analysis to assess the levels of a number of apoptosis-related proteins. P53 and BAX expression were downregulated, and bcl2 expression was elevated when LNCTAM34A was silenced. Cell cycle, apoptosis, senescence, and DNA repair are all under the control of the TP53 gene, which performs as a significant tumor suppressor gene. It is estimated that 20-30% of cancer cases, including breast cancer, have somatic TP53 mutations [35], [36]. Accordingly, these results suggest that decreased expression of LNCTAM34A observed in the BC tissues in the current study induces proliferation, migration, and invasion, probably via influence on the P53/ miR34a/ LNCTAM34A. This data suggest that LNCTAM34A may act as a tumor suppressor lncRNA in breast cancer.

It has been shown that the TP53TG1 expression may be enhanced in a wild-type TP53-dependent mechanism under circumstances of cellular stress [17]. It has been discovered that TP53TG1 has tumor suppressor properties and that suppression of TP53TG1 via DNA methylation is associated with aggressive gastrointestinal cancers resistant to cellular death [17]. Furthermore, TP53TG1 transcription was substantially increased during glucose restriction, and

upregulation of TP53TG1 enhanced cell proliferation and migration while inhibiting cell death [16]. TP53TG1 was also shown to be significantly expressed in nasopharyngeal carcinoma and to enhance malignant characteristics in this kind of cancer [16]. Even though multiple reports have indicated that TP53TG1 is dysregulated in several cancers, the role of TP53TG1 in the carcinogenesis of BC is unknown [16], [37], [38].

The expression level of TP53TG1 was assessed using RT-qPCR analysis in the current study using 150 pairs of BC and nearby normal tissue samples. The findings demonstrated that TP53TG1 is significantly down-regulated in BC tumor tissues, which is consistent with MIR22HG and LNCTAM34A. Recent research has revealed that TP53TG1, which has oncogenic or tumor-suppressing properties, is either highly expressed or downregulated in many cancers.

Checking whether TP53TG1 was involved in the development of BC tumors was the next step. By using anti-TP53TG1 siRNAs to transfect the MDA-MB-453 and MCF-7 BC cell lines, we produced a TP53TG1 knockdown model. In this study, we found that TP53TG1 knockdown increased the proliferation, migration, and invasion of BC cells. Additionally, the effect of TP53TG1 deletion on apoptosis was investigated. Few early apoptotic particles were observed in transfected cells that were specifically targeting TP53TG1-siRNA, as expected. However, Annexin V staining revealed a significant increase in early apoptotic cells in the negative control (NC)-treated cells. According to the apoptosis assay, the protein levels of P53 and BAX were lower in the TP53TG1-siRNA-targeted groups than in the non-targeting siRNA control group. In contrast, there was an increase in the BCL-2 protein level..

TP53TG1 was also discovered to be underexpressed in hepatocellular carcinoma in the past, and its knockdown increased proliferation, induced apoptosis, and induced migration and invasion [39]. However, in pancreatic ductal adenocarcinoma, downregulation or depletion of TP53TG1 had the opposite impact [40]. Our findings show that TP53TG1 depletion/downregulation triggers BC cell proliferation as a regulator of BC development.

Conclusion

We discovered that MIR22HG, LNCTAM34A, and TP53TG1 knockdown could stimulate tumor growth and apoptosis inhibition, suggesting that down-regulation of these lncRNAs in BC may also contribute to increased cell proliferation and poor prognosis. Our findings suggest that a novel therapeutic approach may be offered by the tumor-suppressive function of MIR22HG, LNCTAM34A, and TP53TG1 in a lncRNA-mediated regulatory mechanism.

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