Effect of miR-105-5p on Cell Proliferation, Colony Formation, and *hTERT/P53/P21* Gene Expression in Multiple Cancer Cell Lines[*]

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Abstract

Aim: MicroRNAs are small endogenous RNA molecules that do not code for proteins but have the capacity to influence cancer development by promoting or inhibiting it. They play normal biological roles in regulating cell metabolism, development, apoptosis, and metastasis. While numerous studies on the functional mechanisms of miRNAs exist, there is limited research in the literature on miR-105-5p, a member of the miR-105 family. This study investigated the effects of miR-105-5p on cell proliferation, colony formation, and the expression of *hTERT*, *P21*, and *P53* genes in five cancer cell lines: A549 (lung), Huh7 (liver), HCT116 (colon), MCF-7 (breast), and HeLa (cervix).

Method: The expression of miR-105-5p was both upregulated and downregulated through transfection in the selected cancer cell lines. After transfection, assays were performed to assess cell proliferation, colony-forming ability, and the expression of the *hTERT*, *P21*, and *P53* genes.

Results: Overexpression of miR-105-5p inhibited proliferation in all cancer cell lines. miR-105-5p suppressed colony formation in A549, MCF-7, HeLa, and Huh7 cells. Differences in the expression levels of *hTERT*, *P53*, and *P21* genes were observed in different cell lines due to the effect of the miR-105-5p mimic.

Conclusion: Direct or indirect differences in *hTERT* gene expression levels suggest that miR-105-5p may be useful in targeted therapy.

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Keywords: miR-105-5p, *hTERT*, *P53*, *P21*, transfection.

miR-105-5p'nin Çeşitli Kanser Hücre Hatlarında Hücre Proliferasyonu, Koloni Oluşumu ve *hTERT/P53/P21* Gen Ekspresyonuna Etkisi

Öz

Amaç: MikroRNA'lar, protein kodlamayan ancak kanser gelişimini teşvik ederek veya engelleyerek etkileme kapasitesine sahip küçük endojen RNA molekülleridir. Hücre metabolizması, gelişim, apoptoz ve metastazın düzenlenmesinde normal biyolojik roller oynarlar. MikroRNA'ların fonksiyonel mekanizmalarına ilişkin çok sayıda çalışma mevcut olmasına rağmen, miR-105 ailesinin bir üyesi olan miR-105-5p hakkında literatürde sınırlı araştırma bulunmaktadır. Bu çalışma, beş kanser hücresi hattında (A549 (akciğer), Huh7 (karaciğer), HCT116 (kolon), MCF-7 (meme) ve HeLa (serviks)) miR-105-5p'nin hücre çoğalması, koloni oluşumu ve *hTERT*, *P21* ve *P53* genlerinin ekspresyonu üzerindeki etkilerini araştırmıştır.

Yöntem: Seçilen kanser hücre hatlarında miR-105-5p ekspresyonu, transfeksiyon yoluyla hem yukarı hem de aşağı doğru düzenlenmiştir. Transfeksiyondan sonra, hücre çoğalması, koloni oluşturma yeteneği ve *hTERT*, *P21* ve *P53* genlerinin ekspresyonunu değerlendirmek için analizler yapıldı.

Bulgular: miR-105-5p'nin aşırı ekspresyonu tüm kanser hücre hatlarında çoğalmayı inhibe etti. miR-105-5p, A549, MCF-7, HeLa ve Huh7 hücrelerinde koloni oluşumunu baskıladı. miR-105-5p taklidinin etkisi nedeniyle farklı hücre hatlarında *hTERT*, *P53* ve *P21* genlerinin ekspresyon seviyelerinde farklılıklar gözlendi.

Sonuç: *hTERT* gen ekspresyon seviyelerindeki doğrudan veya dolaylı farklılıklar, miR-105-5p'nin hedefli tedavide yararlı olabileceğini düşündürmektedir.

Anahtar Sözcükler: miR-105-5p, *hTERT*, *P53*, *P21*, transfeksiyon.

Introduction

MicroRNAs (miRNAs), which regulate mechanisms such as differentiation, proliferation, and apoptosis within the cell, belong to the class of short non-coding RNAs. Genetic variations in miRNA genes can lead to differences in expression levels. Such miRNA-level differences may be linked to cancer and serve as biomarkers[1].

Differences in miRNA expression between normal and cancerous tissues are thought to play a role in cancer development, potentially arising from molecular damage in regions with low amplification[2]. miRNAs may function either as tumor suppressors or oncogenes depending on the cellular context, and a decrease in tumor-suppressive miRNAs that regulate oncogene expression can contribute to enhanced tumor development[3]. Variations in single nucleotide polymorphisms and mutations have been identified in pri-miRNA, pre-miRNA, and mat-miRNA regions in cancer patients through *in vivo* and *in vitro* studies. While some mutations do not affect miRNA function, mutations in pre-miRNA let-7e (G-A) can decrease miRNA expression[4]. Genetic variations identified in pre-miRNAs include miRNA-146a, miRNA-492, miRNA-499, miRNA-605, miRNA-631, and miRNA-633[5]. Identifying miRNA gene expression and variations in different cancer types is important when creating molecular diagnostics and targeted therapeutic approaches[6].

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Research indicates that miR-105 is involved in diverse oncogenic pathways and may function as a tumor suppressor or oncogene in cancers such as gastric cancer, hepatocellular carcinoma, prostate cancer, and non-small cell lung cancer[7-9]. The exosomal release of miR-105 by cancer cells has been linked to the promotion of metastasis at distant locations[10]. Moreover, miR-105 regulates cell proliferation by targeting CDK6 and plays a key role in the control of epithelial–mesenchymal transition (EMT)[11]. In breast cancer, it functions as a tumor promoter, contributing to metastatic progression. Its expression is elevated in metastatic lymph nodes and has been correlated with poor prognosis and the presence of lymph node metastasis. In breast cancer, miR-105 can act as a tumor promoter and contribute to metastasis[12]. miR-105-5p, a member of the miR-105 family, enhances metastatic potential in melanoma while suppressing proliferation in glioma cells[8,13]. This microRNA is located on the X chromosome, specifically on the p11.3–q22.3 region, within intron 1 of the GABRA3 gene (gamma-aminobutyric acid A receptor alpha 3 subunit). Owing to its tissue-specific expression and stability in body fluids such as serum, plasma, and urine, miR-105-5p has recently emerged as a promising biomarker for a range of clinical applications, particularly in cancer diagnostics.

The *P21* gene, also known as cyclin-dependent kinase inhibitor 1 or CDK-interacting protein 1, is regulated by the tumor suppressor protein p53. As a cyclin-dependent kinase inhibitor, *P21* can control the cell cycle by inhibiting various cyclin/CDK complexes. By stopping the cell cycle, *P21* allows the cell's DNA to be repaired or, in cases where repair is not possible, to direct the cell to apoptosis[14]. With this feature, it can prevent cancer by controlling cell growth. A mutation in the *P53* gene can cause uncontrolled cell growth by reducing *P21* gene expression[15]. In addition, *P21* suppresses *hTERT* by binding to its promoter region. *hTERT* is suppressed directly or indirectly via *P21*, and as a result, cell proliferation is inhibited and controlled cell death can be achieved[16].

In silico target prediction analyses using miRDB identified 213 putative targets of hsa-miR-105-5p with a prediction score ≥80, including genes involved in cell cycle regulation (CDC14A, CDC5L, CDK12), apoptotic signalling (FASLG), p53 regulation (MDM4), and oncogenic pathways (KRAS). Notably, MDM4, a key negative regulator of p53, was identified as a high-confidence predicted target (score: 87), providing a plausible mechanistic link between miR-105-5p and the *TP53/P21/hTERT* axis. Complementary analysis using TargetScan further identified SEL1L, FOXP2, and TNFAIP6 among predicted targets. Although TP53, CDKN1A, and TERT were not identified as direct targets, the predicted targeting of MDM4 suggests that miR-105-5p may indirectly modulate p53 activity and downstream *P21/hTERT* expression.

Despite growing interest in miR-105-5p across multiple cancer types, the specific effects of this miRNA on the *P53*/*P21*/*hTERT* regulatory axis remain poorly characterised in a systematic, multi-cell-line context. Notably, while Miliotis and Slack (2021)[17] reported an indirect relationship between miR-105-5p and telomerase activity in gastric cancer, their study did not directly investigate *hTERT*, *P53*, or *P21* gene expression across cancer types with distinct *P53* mutational backgrounds, leaving this axis underexplored. The

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P21 gene suppresses *hTERT* transcription by binding to its promoter region, and *P53* activates *P21* expression in response to cellular stress; thus, miR-105-5p-mediated regulation of *P53* and *P21* may indirectly influence *hTERT* expression and telomere maintenance. Investigating this axis across cancer cell lines with distinct *P53* mutational backgrounds — wild-type in A549, MCF-7, and HCT116; E6-mediated degradation in HeLa; and Y220C missense mutation in Huh7 — provides a biologically informative framework for evaluating the context-dependent activity of miR-105-5p and represents a comparative approach not previously described in the literature.

Rather than seeking a universal mechanism, this study aimed to compare the cancer-type-specific effects of miR-105-5p overexpression and inhibition on cell proliferation, colony-forming ability, and the expression of *hTERT*, *P21*, and *P53* across five cell lines with distinct *P53* mutational backgrounds, thereby evaluating the context-dependent potential of miR-105-5p as a target in cancer therapy.

Material and Methods

Cell Culture

In this study, hepatocellular carcinoma Huh7 (Cytion, Eppelheim, Germany), colon cancer cell line HCT116 (ATCC, Manassas, Virginia, USA), human non-small cell lung cancer A549 (ATCC, Manassas, Virginia, USA), breast cancer MCF-7 (ATCC, Manassas, Virginia, USA), and cervical cancer HeLa (ATCC, Manassas, Virginia, USA) cell lines were used. Huh7 and HCT116 cells were maintained in RPMI-1640 medium (HyClone, Cat. No: SH30027.01) supplemented with 10% fetal bovine serum (FBS; HyClone, Cat. No: SV30160.03) and 1% penicillin-streptomycin (HyClone, Cat. No: SV30079.01). A549, MCF-7, and HeLa cells were cultured in DMEM (Gibco™, Cat. No: 41966) supplemented with 10% FBS and 1% penicillin-streptomycin. All cell lines were maintained at 37 °C in a humidified incubator with 5% CO_2.

Transfection-Based Modulation of miR-105-5p in Cell Models

Transfection was performed using Lipofectamine 3000 (Invitrogen™, Cat No. L3000015) according to the manufacturer's protocol. Cells at 60–70% confluence were transfected with miR-105-5p mimic (Ambion™; Cat no: 4464066; final concentration: 50 nM), miR-105-5p inhibitor (Ambion™, Cat no: 4464084; 100 nM), or miR-105-5p negative control (Ambion™, Cat no: AM17010; 50 nM) in Opti-MEM™ reduced serum medium. Transfection efficiency was functionally assessed through downstream gene expression changes, which showed consistent and statistically significant responses to mimic and inhibitor conditions across all five cell lines, indirectly confirming successful modulation of miR-105-5p levels. Twenty-four hours after transfection, cells were propagated for proliferation, colony formation, and gene expression experiments. Each experiment was performed with three independent biological replicates (n=3), and all assays were conducted in technical triplicates.

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Proliferation Assay

The cells were divided into the wells of 96 plates at $3x10^3$ cells/well in 200 µl medium, and their proliferation was observed at 0, 24, 48, and 72 hours. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; BioFROXX) was used for this purpose. 20 µL MTT (5 mg/mL) was added to each well, and the cells were incubated for 4 hours. Subsequently, the medium was removed, and 50 µl of isopropanol was added to each well. Following a 15-minute incubation, absorbance was measured at 570 nm using a microplate reader (Biochrom EZ 400).

Colony Formation Experiment

For the colony formation assay, 300 and 500 cells per well were seeded into 6-well plates containing either RPMI or DMEM medium, each supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Culture media were replenished every two days. After 14 days of incubation, colonies were fixed and stained with a methylene blue solution (0.4 g methylene blue in 50% methanol and 50% distilled water). Stained colonies were imaged under a light microscope using a 20× objective. Quantification of colony numbers was performed using ImageJ software (version 1.53a; NIH, USA). Statistical analysis was carried out using GraphPad Prism 8 (GraphPad Software, Inc.).

RNA İsolation from Transfected Cells

RNAs obtained from cells that reached sufficient density after transfection were isolated in accordance with the ELK Biotechnology TRIpure Total RNA Extraction Reagent (EP013), according to the kit protocol. The amounts of RNA obtained were measured and stored at -80°C until used.

Quantitative Polymerase Chain Reaction (PCR) Experiments

The amount of single-stranded RNA obtained from transfected cells was measured on the Quantus Fluorometer (Promega, TM396) using the QuanFluor® RNA Sample Kit (E3311). 1 µg of total RNA per sample was used for cDNA synthesis according to the protocol of the abm One Script® cDNA Synthesis Kit from each RNA sample. Primers were designed using the IDT PrimerQuest Tool (https://eu.idtdna.com/PrimerQuest/Home/Index). Polymerase chain reactions (PCR) were carried out in an Eppendorf Mastercycler using cDNA templates, SYBR Green mix (Sigma Aldrich), and the primers listed in Table 1. All reactions were performed in triplicate in a 96-well plate (100 µL) using a Bio-Rad iCycler system. The difference between the Ct (cycle threshold) value obtained for each well as a result of PCR and the conditions was calculated using the formula $2^{-\Delta\Delta CT}$ after normalizing with the 18S internal control. Statistical comparisons were performed using one-way ANOVA followed by Tukey's post-hoc test; $p<0.05$ was considered statistically significant.

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Table 1. Designed primers.

GEN	SEQUENCES
18S	forward: 5'-CTTAGAGGGACAAGTGGCG-3'
	reverse: 5'-ACGCTGAGCCAGTCAGTGTA-3'
P21	forward: 5'-CCCGTGAGCGATGGAACT-3'
	reverse: 5'-CGAGGCACAAGGGTACAAGA-3'
P53	forward: 5'-AGGGATGTTTGGGAGATGTAAG-3'
	reverse: 5'-CCTGGTTAGTACGGTGAAGTC-3'
hTERT	forward: 5'-CCCATTTCATCAGCAAGTTTGG-3'
	reverse: 5'-CTTGGCTTTCAGGATGGAGTAG-3'

In Silico Target Prediction Analysis

Putative targets of hsa-miR-105-5p were identified using miRDB (www.mirdb.org) and TargetScan (www.targetscan.org). Targets with a miRDB prediction score ≥ 80 were considered for further evaluation. Results from both databases were compared to identify overlapping predictions.

Results

miR-105-5p Mediates Growth Suppression in Various Cancer Cell Lines

The oncogenic or tumor-suppressive properties of miR-105-5p were determined by 24-, 48- and 72-hour proliferation analysis. Overexpression of miR-105-5p (mimic) was achieved by transfection in A549, HeLa, HCT116, Huh7, and MCF-7 cells. With miR-105-5p mimic transfection, proliferation levels of cancer cells decreased compared to negative control and inhibition groups ($P<0.05$) (Figure 1). In A549, HeLa, HCT116, Huh7, and MCF-7 cells, where miR-105-5p expression was suppressed by transfection (inhibition), proliferation levels increased significantly compared to mimic and negative control groups ($P<0.05$) (Figure 1).

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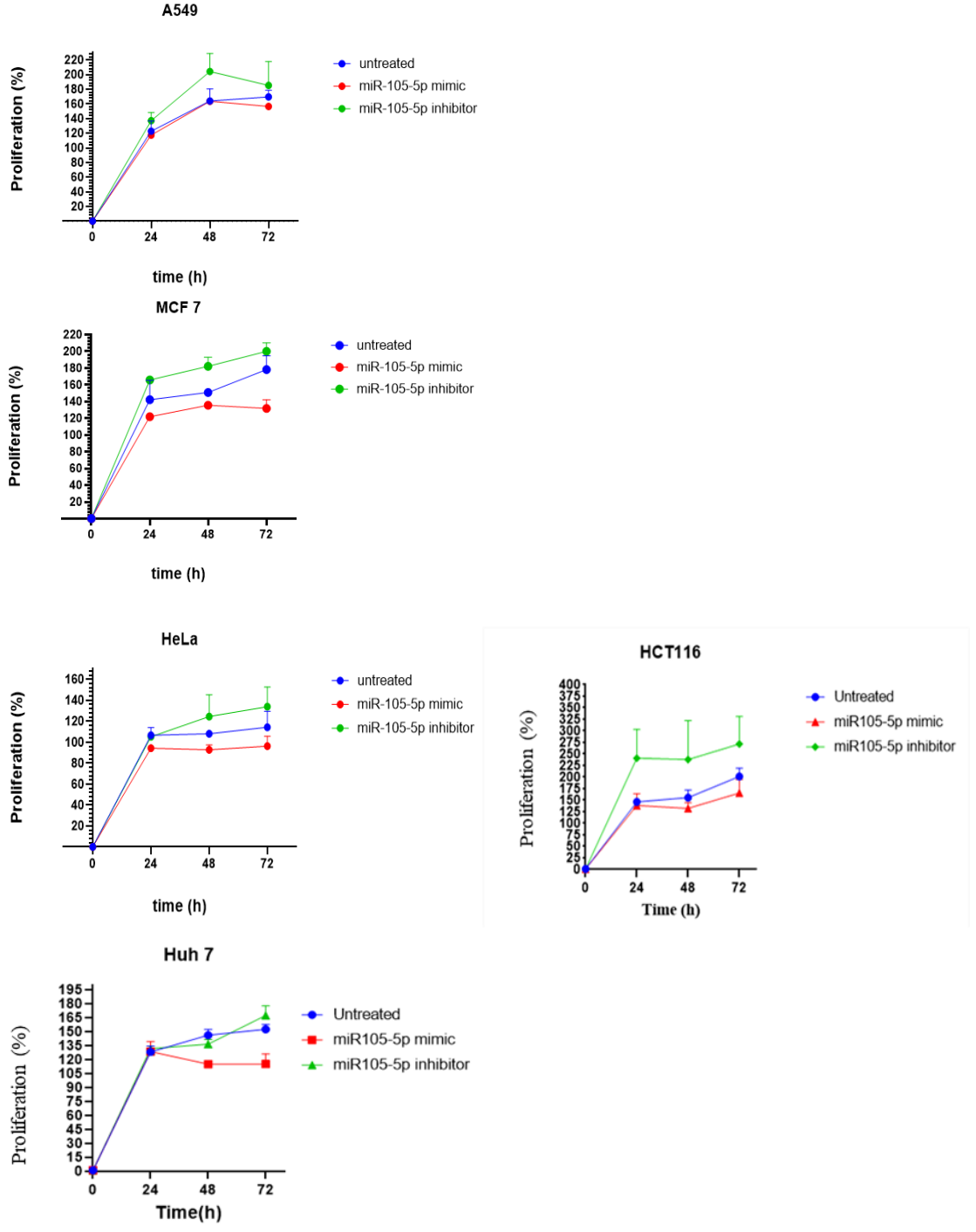
Figure 1. Researchers analyzed the proliferation rates of Huh7, HCT116, A549, MCF-7, and HeLa cells that were transfected with miR-105-5p mimic, miR-105-5p inhibitor, and negative control. Overexpression of miR-105-5p significantly decreased proliferation in all tested cancer cell lines.



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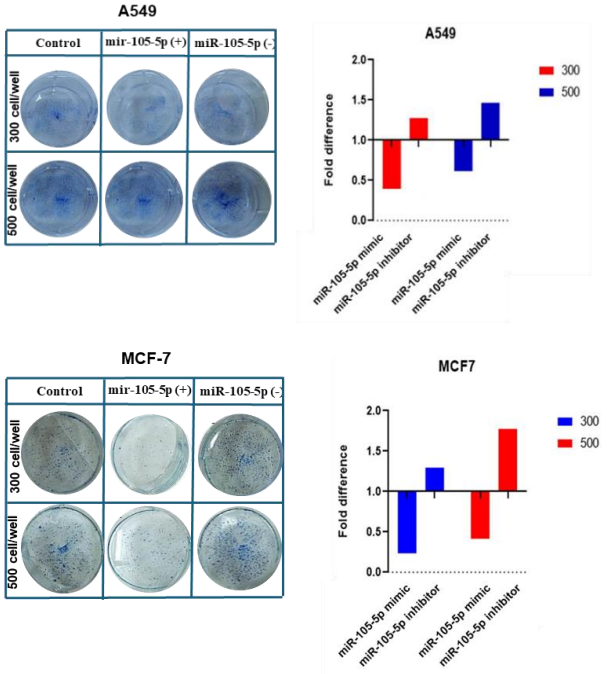
Inhibitory Role of miR-105-5p on Clonogenic Potential

The effects of overexpression and inhibition of miR-105-5p on the colony-forming properties of Huh7, HCT116, A549, MCF-7 and HeLa cells were examined. Colonies formed after incubation were analyzed using the GraphPad 9 program, and graphics were created. When miR-105-5p expression was increased, colony formation was suppressed in parallel with the proliferation of cells in A549, MCF-7 and HeLa cell lines (Figure 2). Inhibition of miR-105-5p resulted in enhanced colony formation across all cell lines relative to the negative control and miR-105-5p overexpression groups (Figure 2). In Huh7 cells, fewer colonies formed in the overexpression and inhibitor groups than in the negative control. In addition, colonization in the overexpression and inhibitor groups was close to each other, but inhibitor cells formed more colonies than mimic cells.

Colony formation in HCT116 cells, where miR-105-5p was overexpressed and suppressed, increased compared to the negative control group ($P>0.0657$ and $P>0.1103$).

Figure 2. Colony formation assay results and representative images of A549, MCF-7, and HeLa cells transfected with miR-105-5p mimic, miR-105-5p inhibitor, and negative control. Overexpression of miR-105-5p significantly reduced colony formation in these cell lines.



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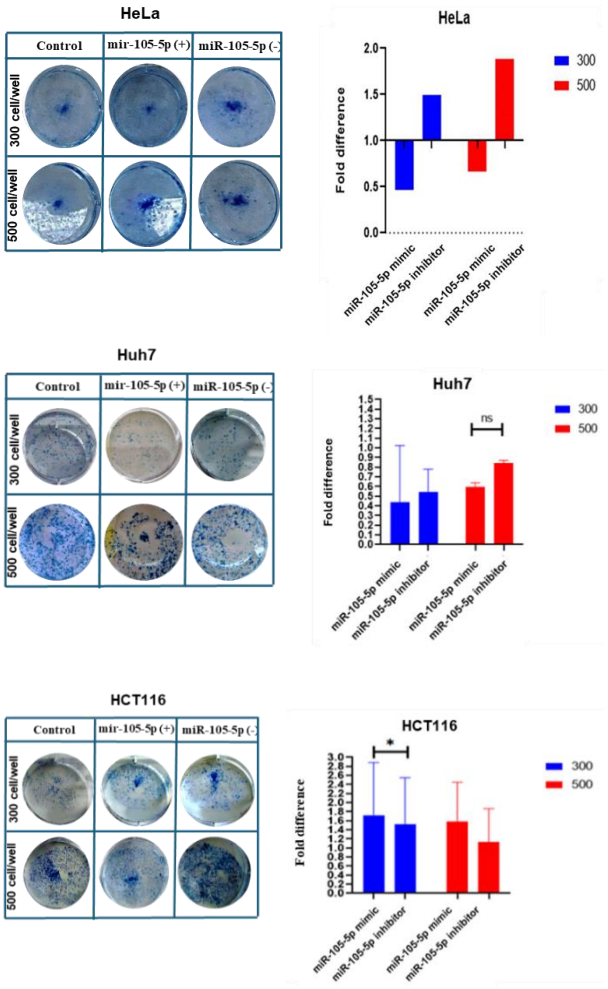

The expression levels of hTERT, P21, and P53 varied in cells transfected with miR-105-5p.

The effects of miR-105-5p expression on hTERT-associated gene regulation in A549, MCF-7, HeLa, Huh7, and HCT116 cell lines were investigated by examining changes in *hTERT*, *P53*, and *P21* gene expression levels. In A549 cells transfected with the miR-105-5p mimic, *hTERT* and *P53* gene expression levels increased, while *P21* expression levels decreased. Inhibition of miR-105-5p in A549 cells increased *P21* gene expression while decreasing *hTERT* gene expression (Figure 3).

In miR-105-5p mimic MCF-7 cells, *P21* and *P53* gene expression increased, but the *hTERT* gene expression level decreased. There was a decrease in *P21*, *P53* and *hTERT* gene expressions in cells where miR-105-5p was inhibited (Figure 3).

With overexpression of miR-105-5p, *P21*, *P53*, and *hTERT* gene expressions increased in HeLa cells. In HeLa cells where miR-105-5p was inhibited, *P21* and *P53* increased, while *hTERT* decreased relatively (Figure 3).

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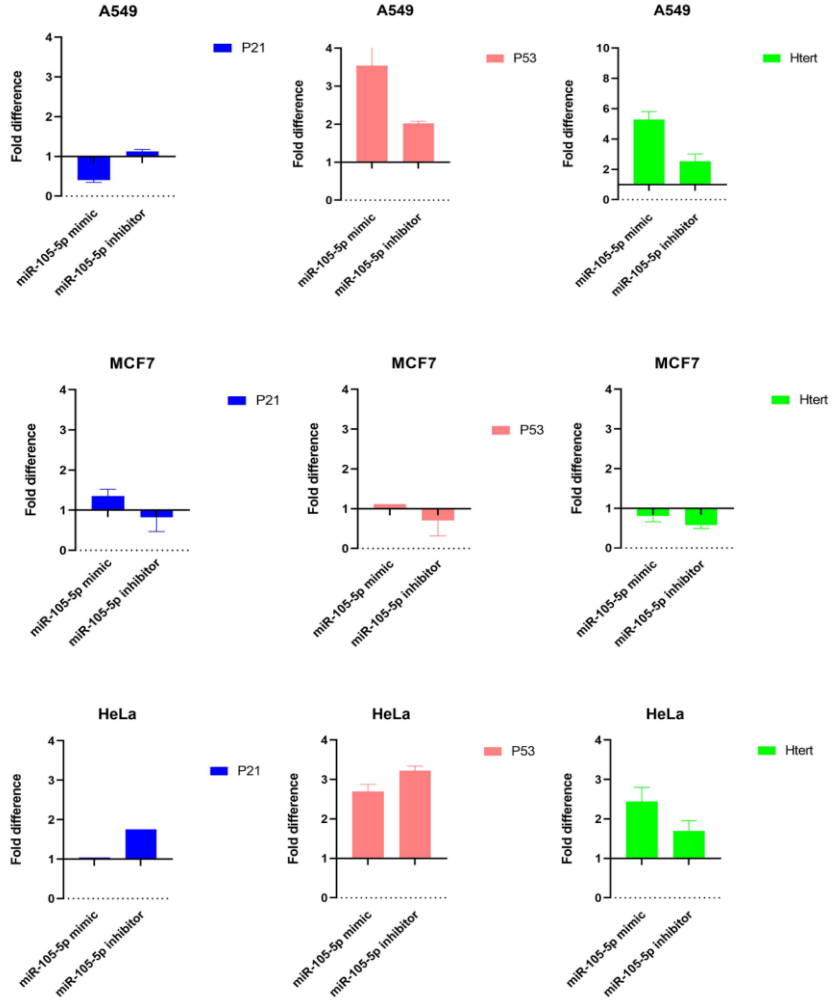
In Huh7 cells, *hTERT*, *P53*, and *P21* gene expression levels increased when miR-105-5p was overexpressed but remained at low levels when it was inhibited (Figure 3).

In HCT116 cells, *P21*, *P53*, and *hTERT* gene expression levels increased with overexpression of miR-105-5p. By inhibiting miR-105-5p, *P21*, *P53*, and *hTERT* levels were expressed less than in the mimic group (Figure 3).

Figure 3. In all cancer cell lines transfected with miR-105-5p mimic or inhibitor, the expression levels of *hTERT*, *P53*, and *P21* were normalized to the 18S internal control, and data analysis was performed using GraphPad Prism 8.4.3. Overexpression and suppression of miR-105-5p elicited variable effects on gene expression across the different cell lines.



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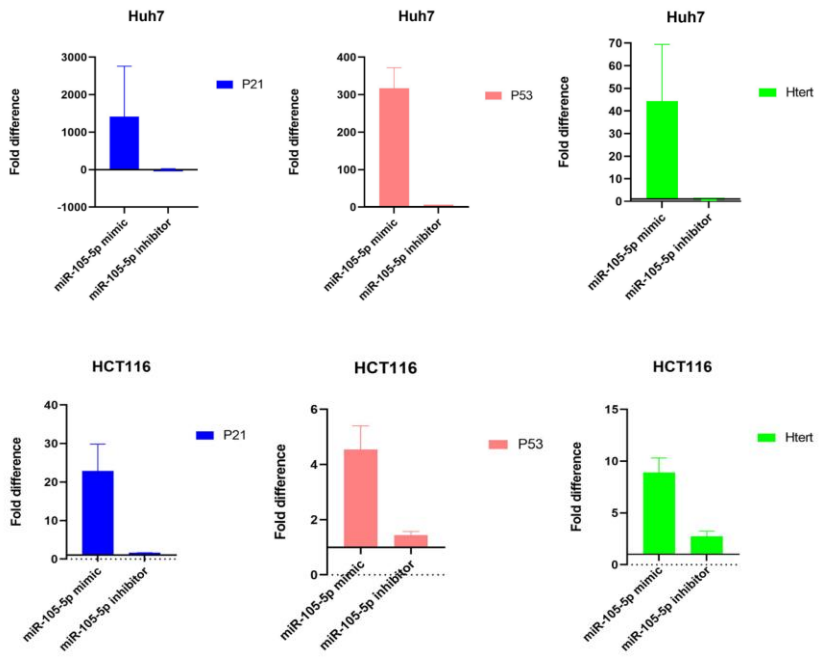

***Gene expression levels are presented as fold change relative to the negative control group ($2^{-\Delta\Delta CT}$ method). A value of 1.0 represents the negative control baseline.**

In Silico Target Prediction of miR-105-5p

miRDB analysis identified 213 putative targets of hsa-miR-105-5p with a prediction score ≥ 80. Among these, *MDM4* (score: 87), *FASLG* (score: 91), *CDC14A* (score: 86), *CDK12* (score: 87), and *KRAS* (score: 81) were identified as biologically relevant predicted targets. TargetScan analysis additionally predicted *SEL1L*, *FOXP2*, and *TNFAIP6* as putative targets. The predicted targeting of *MDM4* is of particular interest given its role as a negative regulator of p53, suggesting a potential indirect mechanism through which miR-105-5p may influence the *TP53*/*P21*/*hTERT* axis.

Discussion

MiRNAs are effective as oncogenic and tumor suppressors in many metabolic pathways associated with cancer (such as proliferation, differentiation, cell cycle, and apoptosis)[18-20]. In this study, the effects of miR-105-5p, which belongs to the miRNA family, on proliferation, colony formation, and especially the Human Telomerase Reverse Transcriptase (*hTERT*) gene expression levels in relation to *P21* and *P53* in various cancer cell lines were investigated.

Increasing miR-105-5p expression suppressed proliferation in hepatocellular carcinoma, colon, breast, and cervical cancer cell lines. When miR-105-5p expression was suppressed, the proliferation rate increased significantly compared to the control group.

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The antiproliferative effect of miR-105-5p in this study suggests that it may play a role as a tumor suppressor and may be a potential target for these cell lines in targeted treatment approaches. The level of miRNA should be evaluated individually for each type of cancer. Some types of cancer may develop due to overexpression of miRNAs. For our study, overexpression of miR-105-5p suppressed the proliferation of cancer cells. Numerous studies align with our findings, suggesting that cancer tissues exhibit lower expression of miRNAs and that carcinogenesis suppresses miRNAs[12,21]. Dong et al. also determined that upregulation of miR-105-5p suppressed proliferation in lung cell lines[22]. Contrary to this study, in another study, the increase of miR-105-5p in bladder cancer stem cells was associated with proliferation[23].

The overexpression of miR-1298, miR-3180-3p, miR-545, and miR-186 led to the inhibition of cell proliferation in non-small cell lung cancer (NSCLC) cells[24-28]. MiR-1298 reduced the proliferation of MCF-7 cells [24]. Researchers have reported that miR-423-5p enhances programmed cell death and reduces the proliferative, migratory, and invasive capabilities of HCT116 cells[29]. Similarly, miR-200b and miR-214 suppressed proliferation in HeLa cells[30,31]. Huh-7 cells overexpressed miR-296-3p, which inhibited proliferation, migration, and invasion while promoting cell death[31].

Researchers determined how the suppression and overexpression of miR-105-5p affected colony formation in lung, hepatocellular carcinoma, colon, breast, and cervical cancer cell lines using a colony formation assay. Overexpression of miR-105-5p significantly suppressed colony formation, except in the HCT116 cell line. Colony formation increased significantly with suppression of miR-105-5p (except for the HCT116 cell line). In cases where miR-105-5p was suppressed and overexpressed in HCT116 cells, the colony increase was higher than in the control groups. Additionally, overexpression increased colony formation, unlike other cell lines. In HCT116 cells, both suppression and overexpression of miR-105-5p lead to increased colony formation, highlighting the context-specific and complex role of this miRNA in colorectal cancer cell proliferation. This dual effect is believed to be related to miR-105-5p's interaction with multiple target genes and signaling pathways that regulate cell growth and colony formation[28]. In HCT116 cells, miR-423-3p promoted colony formation, while inhibiting it significantly reduced this process[32]. MiR-214 inhibited the colony-forming ability of HeLa cells, suggesting its potential role in reducing the malignancy of cervical cancer cells[31]. Furthermore, the overexpression of miR-296-3p inhibited colony formation in HCC (Huh7) cells[33]. These findings underscore the important role of miRNAs in regulating colony formation and cancer cell proliferation.

The relationship between miR-105-5p expression and the regulation of *hTERT*, *P53*, and *P21* was examined across lung, hepatocellular carcinoma, colon, breast, and cervical cancer cell lines. The induction of *hTERT* can significantly stabilize telomeres and trigger malignant processes like migration, metastasis, and angiogenesis[34]. Therefore, targeted treatment of *hTERT* offers an option in many types of cancer. Accordingly, increasing-decreasing expressions of miRNAs can suppress tumor growth by affecting *hTERT* activity[35]. The *P21* gene, whose expression is controlled by the *P53* gene, serves as the

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control mechanism of a cell cycle. As a result of abnormalities in cell division, *P53* allows *P21* gene expression to increase, thus stopping the cell cycle and allowing the control mechanism to work[36]. Elevated levels of *P21* can bind to the promoter region of the *hTERT* gene, leading to direct repression of its expression. *P53*, a well-known tumor suppressor gene, is frequently mutated across various cancer types[37]. Such mutations may result in altered expression—either upregulation or downregulation—of genes regulated directly or indirectly by *P53*. Consequently, these changes can disrupt critical cellular processes, including cell cycle regulation, apoptosis, and DNA repair mechanisms[38].

In A549 cells, the miR-105-5p mimic increased the expression levels of *hTERT* and *P53* genes while simultaneously suppressing the expression of the *P21* gene. Induction of the *P53* gene is directly proportional to suppression of proliferation and colony formation. However, decreased *P21* expression prevented suppression of *hTERT*. It is obvious that *P53* does not lead to cell death by binding to *P21*. Like some other miRNAs, miR-105-5p changed the expression levels of *P21*. The finding that miR-105-5p binds to the gene via the 3'UTR and down-regulates *P21* is consistent with another study in the literature[39]. The lack of a significant difference in the level of *P21* regulated by *P53* in the presence of miR-105-5p suggests that it may be mutated. In lung cancer, in the absence of miR-105-5p, *P21* acts as an oncogene; in the presence of miR-105-5p, *P53* and *hTERT* function as tumor suppressors.

In the breast cancer cell line MCF-7, miR-105-5p suppressed the expression level of *hTERT* while simultaneously increasing the expression levels of *P21* and *P53*. Increased *P53* levels are known to upregulate *P21* gene expression, leading to cell cycle arrest and potentially apoptotic signalling, though direct apoptosis was not assessed in this study. Additionally, *P21* suppressed *hTERT*, thereby inhibiting cell proliferation. The decreased *hTERT* level in the absence of miR-105-5p should be supported by additional experiments. In breast cancer, miR-105-5p did not affect *hTERT* gene expression, while the *P53* and *P21* genes acted as tumor suppressors in its presence.

MiR-105-5p increased the expression levels of *hTERT*, *P53*, and *P21* genes in the human cervical cancer cell line HeLa. However, either directly or indirectly, increased *P53* could not suppress *hTERT* levels. It is postulated that *P53* may indirectly suppress proliferation and colony formation through pro-apoptotic signalling, though this was not directly assessed in the present study. In cervical cancer cells, when miR-105-5p is expressed, *hTERT* shows a tumor suppressor effect, while when miR-105-5p is inhibited, the *P53* and *P21* genes show an oncogene effect.

It was determined that miR-105-5p caused a significant increase in *hTERT*, *P53*, and *P21* levels in hepatocellular cancer cell lines. Although increased *P53* and *P21* expressions prevented proliferation in cells, there was no significant decrease or increase in colony formation compared to control groups. However, when miR-105-5p was suppressed, *hTERT* levels decreased. This suggests that miR-105-5p has oncogenic properties for Huh7 cells. The Y220C missense mutation in *TP53* in Huh7 cells results in a structurally destabilised protein with impaired transactivation capacity, explaining the inability of

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mutant *P53* to effectively suppress *hTERT* expression via *P21* upregulation. In this context, mutant *P53* may acquire gain-of-function oncogenic properties that sustain *hTERT* expression and colony formation, consistent with the gain-of-function mutant *P53* phenotype described in the literature.

In colon cancer cells, miR-105-5p induced an increase in the expression levels of the *hTERT*, *P53*, and *P21* genes. The upregulation of *P53* and *P21* significantly inhibited proliferation in HCT116 cells, whereas proliferation was enhanced when miR-105-5p was absent. Interestingly, the observed increase in *hTERT*—contrary to the expected decrease—suggests that miR-105-5p may exert anti-proliferative effects through an alternative mechanism. Furthermore, the elevated *hTERT* expression might explain the lack of a significant impact of miR-105-5p on colony formation. The unexpected increase in *hTERT* expression in HCT116 cells, despite concurrent upregulation of *P53* and *P21*, may reflect the activation of alternative *hTERT* regulatory pathways operating independently of the *P53*/*P21* axis — such as c-Myc-mediated transcriptional activation or epigenetic deregulation of the *hTERT* promoter — and warrants further mechanistic investigation. The simultaneous increase in colony formation upon both overexpression and inhibition of miR-105-5p in HCT116 cells suggests that this cell line may be particularly sensitive to perturbations in miRNA homeostasis, regardless of direction. One possible explanation is that miR-105-5p targets multiple genes with opposing functions in HCT116 cells, such that both its overexpression and its inhibition disrupt the balance of pro- and anti-tumorigenic signals, ultimately favouring colony growth. Furthermore, HCT116 cells harbour wild-type *TP53* but also carry an activating *KRAS* mutation, which is a predicted target of miR-105-5p (miRDB score: 81). It is plausible that miR-105-5p-mediated suppression of *KRAS* signalling in the mimic condition partially counteracts the expected tumour-suppressive effects of elevated *P53* and *P21*, resulting in the paradoxical increase in colony formation despite reduced proliferation. The concurrent elevation of *hTERT* in this context may reflect *KRAS*-driven transcriptional activation of the *hTERT* promoter, which is known to operate independently of the *P53*/*P21* axis. Taken together, the HCT116 data highlight the complex, context-dependent nature of miR-105-5p function in colorectal cancer and underscore the need for further mechanistic studies in this cell line.

This study has a number of limitations that should be taken into account when interpreting the findings. To begin with, direct qPCR quantification of miR-105-5p levels after mimic and inhibitor transfection was not carried out, so successful modulation of miR-105-5p could not be formally verified. Instead, transfection efficacy was inferred from the downstream changes in *hTERT*, *P21*, and *P53* expression, which were consistent and statistically significant across all five cell lines. Beyond this, apoptosis was not directly measured; the upregulation of *P53* and *P21* alongside downregulation of *hTERT* were taken as indirect indicators of a potential pro-apoptotic response, rather than as definitive evidence. Telomerase enzymatic activity was likewise not assessed; *hTERT* mRNA levels served as a proxy, which may not accurately reflect protein-level activity. Protein expression was not examined by western blotting or similar methods, meaning transcript-level changes may not correspond directly to changes in protein abundance.

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In addition, baseline miR-105-5p expression in these cell lines relative to normal counterparts was not measured before transfection, which limits the physiological relevance of the findings. Finally, the variable responses across cell lines, particularly in HCT116 and Huh7, most likely reflect the distinct *TP53* mutational backgrounds and signalling contexts of each model, and should not be generalised as a uniform miR-105-5p mechanism.

Conclusion

In some cancer cells, miR-105-5p promotes cell cycle arrest, anti-proliferative effects, and senescence, while in others, it can have oncogenic effects. In A549, MCF-7, and HCT116 cells, miR-105-5p caused anti-proliferative effects associated with increased non-mutant *P53* levels, while it prevented colony formation. These findings are consistent with a potential role for miR-105-5p in modulating *P53* expression, though this warrants further experimental validation. There are studies reporting that some miRNAs exhibit high expression in tumors, bind to the 3'UTR of *P53*, and inhibit *P53* levels[39,40]. However, the same is not the case for miR-105-5p. In cervical cancer, where the E6 oncoprotein-dependent *P53* degradation pathway is active[41], mutated P53 was already inhibited together with miR-105-5p expression and could not show tumor suppressor properties. The elevated level of mutant P53, resulting from the mutation that changed tyrosine 220 to cysteine in Huh7 cells, was unable to prevent colony formation and did not act as a suppressive element on *hTERT*. Inhibition of miR-105-5p was associated with increased mutated *P53* levels in HeLa cells, consistent with a potential gain-of-function oncogenic role of mutant *P53* in this context; however, this interpretation requires further experimental validation. These findings suggest that the expected *P53*-mediated tumour suppressor response was not observed in HeLa cervical cancer cells, most likely because the HPV-encoded E6 oncoprotein constitutively targets *P53* for proteasomal degradation in this cell line, preventing its accumulation regardless of miR-105-5p modulation. The substantial heterogeneity observed across cell lines underscores that miR-105-5p does not act through a single universal mechanism, and its effects are likely shaped by the specific molecular background of each cancer type, including *P53* mutational status and downstream regulatory networks.

In light of the data of this study, miR-105-5p gene expression, which varies in different types of cancer, suggests that it may be a beneficial marker in targeted therapy.

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