MEANDROS MEDICAL AND DENTAL JOURNAL
December 2024 (4):396-411

Meandros Medical and Dental Journal
doi: 10.69601/meandrosmdj.1557035

Research Article

DIFFERENTIALLY REGULATED MIRNAS BY TWIST1 IN TRIPLE NEGATIVE BREAST CANCER CELLS

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Received: 13 October 2024
Revised: 15 November 2024
Accepted: 19 November 2024
Published: 22 December 2024

ABSTRACT

Objective: Breast cancer (BC) is the most common cancer in women and the second leading cause of cancer-related deaths. MicroRNAs (miRNAs) are short, non-coding RNA molecules that regulate gene expression post-transcriptionally and play a central role in the dysregulation of gene expression associated with carcinogenesis, cancer cell proliferation and metastasis. Twist1 is a transcription factor that binds to E-box motifs and controls the transcriptional activity of genes as a positive or negative regulator decisive in the cellular mechanisms. Accordingly, Twist1 also regulates the expression of miRNAs that are associated with cancer progression. In the present study, we aimed to investigate the expression changes of possible miRNAs directly regulated by Twist1 in triple negative breast cancer (TNBC) MDA-MB-231 cells.

Materials and Methods: In this study, a total of 43 miRNA genes were evaluated that predicted might be associated with TNBC. To determine the Twist1-targeted miRNA genes, endogenous high-level Twist1 expression was suppressed through the antisense oligonucleotides in MDA-MB-231 TNBC cells. Differential miRNA expression levels were analyzed using quantitative real-time PCR (qRT-PCR) in Twist1-suppressed cells compared to the control group.

Results: Twist1 suppression resulted in increased expression of miR-1-1 and miR-210-3p, while the expression of miR-193b-3p, miR-181b-5p, and miR-148a-3p decreased.

Conclusion: This study shows that the expression levels of certain miRNAs linked to invasion, metastasis, and apoptosis are controlled by Twist1 in TNBC cells.

Keywords: Breast cancer, MDA-MB-231, microRNA, Twist1

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INTRODUCTION

According to global statistics, breast cancer (BC) is identified as the most frequently detected cancer worldwide, affecting individuals across all genders (1). Clinically, this type of cancer is divided into different subtypes based on the status of specific receptors: the human epidermal growth factor receptor 2 (HER2), which promotes aggressive tumor growth; the progesterone receptor (PR), associated with hormone-driven tumor development; and the estrogen receptor (ER), a key factor in hormone-dependent BC (2). The major subtypes include luminal A, which is generally hormone receptor-positive with a slower growth rate, luminal B, known for its higher proliferation rate and possible HER2 positivity, and triple-negative breast cancer (TNBC), which lacks hormone receptor expression. TNBC constitutes approximately 15-20% of total BC diagnoses and is marked by its aggressive behavior and poor prognosis (3). MDA-MB-231, which is derived from human breast adenocarcinoma, is an epithelial cell type that is classified as TNBC because of the absence of HER2, PR and PR expression on its cell membrane (4). The identification of breast cancer-specific miRNAs and the determination of their mechanisms of action in cellular signaling pathways are important for cancer diagnosis and treatment. The effects on miRNA profile in cancer cells are being studied and their importance is increasing day by day (5, 6).

MicroRNAs (miRNAs) are short, non-coding RNA sequences that exert pivotal functions in post-transcriptional gene regulation by modulating the degradation and translational efficiency of specific target messenger RNAs (7). These miRNAs participate in numerous biological functions, including development, cell differentiation, growth, and programmed cell death, acting as crucial modulators of gene activity (8). In healthy cells, miRNAs are tightly controlled to maintain homeostasis (9), while dysregulation of miRNAs occurs in many diseases, including cancer (10). Studies indicate that miRNA dysregulation contributes to cancer development and progression, with miRNAs acting either as oncogenes or tumor suppressors, influencing different stages of tumorigenesis. In the field of cancer gene therapy, focusing on particular miRNAs has demonstrated potential as an effective approach in the management, detection, and prediction of disease outcomes (11).

Twist1, a transcription factor, has a significant function in several molecular pathways, especially contributing to cancer advancement. Twist1 participates in tumor formation, development, spread, and epithelial-mesenchymal transition (EMT) across different cancer types, including lung, breast, gastric, and prostate cancer (12). In addition, Twist1 has been identified as a master regulator in BC progression by controlling multiple genes in different metabolic pathways (13).

Previous research has examined how miRNAs contribute to controlling both metastasis and angiogenesis via the Twist1 gene in MDA-MB-231 TNBC cells. Nevertheless, the miRNA profile related to the antisense inhibition of Twist1 gene expression remains unexplored. This study seeks to analyze the alterations in miRNA expression levels in MDA-MB-231 TNBC cells after Twist1 gene activity is suppressed using an antisense approach.

MATERIALS AND METHODS

Cell culture and transfection

The TNBC cell line derived from humans, MDA-MB-231 (American Type Culture Collection, HTB-26), was preserved in Dulbecco's Modified Eagle Medium (DMEM) (Gibco™, catalog #11965092) supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, and L-glutamine. The cell line was cultivated at 37 °C in a humidified environment with 5% carbon dioxide. Before cell transfection, cells were plated one day earlier to achieve approximately 70% confluence.

A transfection reagent (Lipofectamine 2000, catalog no. 11668-027, purchased from Thermo Fisher Scientific, USA) was used for transfection of Twist1AS-pcDNA3.1 vector cloned with Twist1 anti-sense complementary DNA (cDNA), and pcDNA3.1 empty vector as a control into MDA-MB 231 TNBC cells. Following 72 hours post-transfection, cell selection was carried out using G-418 at a concentration of 0.4 mg/mL (Invitrogen #10131) until the wells reached 80-90% confluency. Then, the RNA samples of each well were obtained and quantified by Nanodrop.

miRNA Isolation

Total RNA, including miRNAs, was extracted and purified from control and Twist1-silenced cells using the RTA Total miRNA Isolation Kit (lot: AR2264118) according to the manufacturer's instructions. Briefly, 350 µl of lysis solution and 20 µl of proteinase K were added to each cell sample, followed by incubation in an IKA dry block heater at 60°C for 30 minutes. Afterward, 350 µl of binding solution was added, and the mixture was transferred to spin columns and centrifuged at 11,000 rpm for 1 minute. The washing step involved adding 500 µl of wash solution, followed by centrifugation at 10,000 rpm for 1 minute.

For elution, 40 µl of pre-warmed elution solution (65°C) was applied to the center of the miRNA spin column and incubated at room temperature (20-25°C) for 1-3 minutes. Eluted miRNA was collected by centrifugation at 8,000 rpm for 1 minute and stored at -20°C. Relative miRNA expression was evaluated using the comparative CT (ΔCT) method, with SNORD44 as a reference gene.

Quantitative Real-Time PCR (qRT-PCR)

qRT-PCR assays were conducted using a BrightGreen 2X Qrt-PCR Master Mix on a LightCycler® 96 real-time PCR system. Each reaction mixture (20 µl) contained 5 µl of 2X Master Mix, 0.5 µl of forward and reverse primers (300 nM), 1.75 µl of cDNA, and 2.25 µl of nuclease-free water.

The qRT-PCR program included an initial denaturation at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 10 seconds, annealing at 60°C for 15 seconds, and elongation at 72°C for 30 seconds. A melting curve analysis was performed to ensure the specificity of the amplification, increasing the temperature from 50°C to 95°C in 0.5°C increments every 30 seconds. The primers for Twist1 were; Forward: CGA CGA CAG CCT GAG CAA CA, Reverse: TGC AGC TCC TCG TAC GAC TG. The aim of this study was to compare miRNA expression levels between control cells and cells transfected with the Twist1 antisense clone (Twist1AS-pcDNA3.1 vector). miRNA analysis was performed in duplicate for both groups, with SNORD68 as the internal control for the 43 miRNAs analyzed.

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Bioinformatics Analysis Using DIANA Tools

To identify the miRNAs potentially regulated by Twist1, bioinformatics analyses were performed using the DIANA Tools platform (http://diana.imis.athena-innovation.gr). Specifically, the DIANA-microT-CDS tool was used to predict the interactions between miRNAs and their potential target genes, focusing on regulatory elements such as E-box motifs within the 3′ UTR regions. Additionally, the DIANA-miRPath v3.0 tool was employed to evaluate the impact of these miRNAs on biological pathways, using KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis. These analyses facilitated the identification of 43 miRNAs that may play significant roles in TNBC biology.

Statistical Analysis

Data are expressed as means ± standard errors. Statistical analysis was performed using Student's t-test in GraphPad Prism 8 software. The significance of the results was determined at a probability level of $p < 0.05$. Each experiment was conducted in triplicate across three independent experiments.

RESULTS

To investigate whether Twist1 drives miRNA expression under normal physiological conditions, a cloned Twist1 antisense oligonucleotide was utilized. Initially, an empty vector (pcDNA3.1) was used as a control, and the Twist1-AS vector was transfected into cells known to express high levels of endogenous Twist1. The transfected cells were then selected using G-418-containing medium. Subsequently, the cells were analyzed for endogenous Twist1 expression, and their morphology was examined using an inverted microscope.

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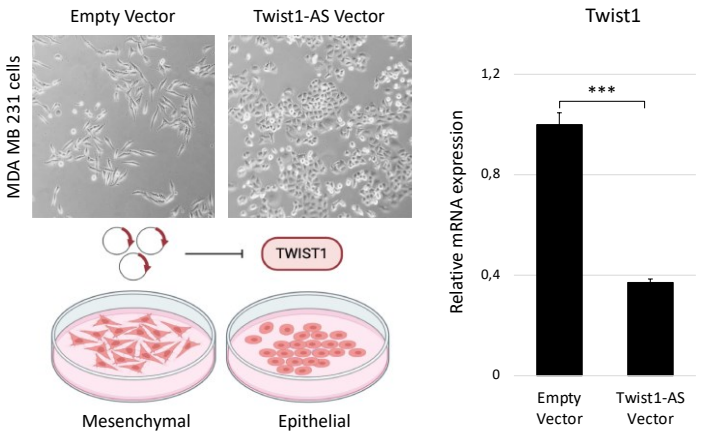

Figure 1 The Twist1 suppression level of Twist1-AS vector transfected cells compared to control cells (empty vector) resulted in the loss of their original mesenchymal morphology and transformation into epithelial morphology. (***p<0.001)

Cells with decreased Twist1 expression (a 2.7-fold reduction) due to antisense oligonucleotides lost their mesenchymal appearance and adopted an epithelial morphology. This finding aligns with the widely recognized role of Twist1 as a crucial regulator of epithelial-mesenchymal transition (EMT).

Table 1. CT values of up-regulated or down-regulated miRNAs in Twist1-silenced cells compared to the control, with a statistically significant difference ($p < 0.05$).

	CT average (control group)	CT reference average (control group)	ΔCT average (control group)	CT average (transfection group)	CT reference average (transfection group)	ΔCT average (transfection group)	-ΔΔCT
hsa-miR-1-1	33,7	24,02	9,67	29,28	24,08	5,2	4,47*
hsa-miR-193b-3p	25,19	24,02	1,17	26,77	24,08	2,69	-1,52*
hsa-miR-181b-5p	23,65	24,02	-0,37	25,7	24,08	1,62	-1,99*
hsa-miR-148a-3p	29,55	24,02	5,525	31,3	24,08	7,22	-1,69*
hsa-miR-210-3p	23,96	24,02	-0,06	22,11	24,08	-1,96	1,9*

*hsa, homo sapiens, *p<0.05*

These results suggest that efficient downregulation of Twist1 promotes the mesenchymal-epithelial transition (MET) process in cells (as shown in Figure 1). In this study, 43 miRNAs were analyzed (Supplement 1). Among them, 2 miRNAs (miR-1-1 and miR-210-3p) were upregulated, while 3 miRNAs (miR-193b-3p, miR-181b-5p, and miR-148a-3p) were downregulated. The cycle threshold (CT) was determined using the following formula (Table 1):

[ΔΔCT= ΔCT (target sample) - ΔCT (reference sample)] (14).

DISCUSSION

In this study, changes in the expression levels of 43 miRNAs, previously characterized as either oncogenic or tumor suppressive, were investigated following Twist1 gene knockdown. These miRNAs were identified through in silico analyses and a comprehensive literature review, focusing on their potential roles in TNBC biology. Among these, miR-193b-3p, miR-1-1, miR-210-3p, miR-181b-5p, and miR-148a-3p were selected for further analysis due to their significant expression changes following Twist1 inhibition. These miRNAs play crucial roles in key biological processes associated with TNBC, including metastasis, angiogenesis, and apoptosis. Notably, miR-210-3p and miR-1-1 are linked to adaptation to hypoxia and cellular energy metabolism, with miR-1-1 generally exhibiting tumor-suppressive effects by reducing proliferation and metastasis in cancer cells (15, 16). Similarly, miR-193b-3p has been reported to suppress metastatic processes (17), while miR-181b-5p exhibits either oncogenic or tumor-suppressive roles in BC (15, 16). miR-148a-3p acts as a tumor suppressor in various cancers, including BC, where its low expression levels are associated with cancer progression (17).

Given their significant expression changes and established roles in TNBC, this study focuses on these five miRNAs to investigate the biological impact of Twist1 suppression on their regulatory pathways. Ongoing research underscores the potential of miRNAs as biomarkers for early BC detection and prognosis determination, highlighting the importance of identifying specific miRNAs and understanding their mechanistic roles in cellular signaling pathways (18).

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Twist1 is an essential regulator of cancer progression and spread (19). It plays a key role in the promotion of EMT, cellular invasion, metastatic spread, and maintenance of cancer stem cell properties (20, 21). The importance of Twist1 has been highlighted in several cancers, including BC, prostate cancer and lung cancer (22-24). Additionally, Twist1 is associated with drug resistance in cancer cells (25). Twist1 promotes cancer cell invasion and metastasis by suppressing genes such as Foxa1, with its downregulation contributing to EMT, invasiveness, and metastasis in BC (13).

Twist1 functions as a regulatory factor in highly aggressive cells, including TNBC cells like the MDA-MB-231 line. Its interactions with specific DNA recognition sites can either upregulate or downregulate miRNAs. Twist1 activity influences the expression of miRNAs such as miR-34a, miR-373, miR-424, miR-129-5p, miR-20a, miR-448, miR-10, and miR-200, all of which are linked to invasion and metastasis. Research shows that Twist1 suppression reduces oncogenic miRNA expression while enhancing tumor-suppressive miRNA levels (26-29).

Li et al. found that inhibition of Twist1 affects apoptosis through nuclear factor kappa B (NF-κB)-dependent p53 activation by impairing the expression of miR-10a (26). Similarly, Yu et al. demonstrated that increased miR-129-5p levels suppress EMT, while its downregulation enhances EMT in BC cells. They showed that Twist1 directly represses miR-129-5p expression, with low miR-129-5p levels linked to poor clinical outcomes. Yeh et al. identified miR-151-3p, which targets Twist1 to limit BC cell motility, modulating Twist1 via its interaction with the 3′ untranslated region (3'UTR). This regulation inhibits motility and infiltration by promoting E-cadherin production (30). These investigations have shown that suppression of the Twist1 gene leads to a reduction in oncogenic miRNAs and an elevation in tumor-suppressive miRNAs.

miRNAs like miR-145a-5p, miR-300, miR-337-3p, miR-720, miR-151-5p have also been studied in relation to Twist1 inhibition (28, 29, 31). u et al. demonstrated that miR-300 directly targets Twist1 at its 3′ UTR, with elevated miR-300 levels inhibiting cell invasion in vitro and reducing experimental metastasis in vivo. An inverse relationship between miR-300 and Twist1 expression was observed in clinical samples, where reduced miR-300 levels correlated with higher metastatic potential (31). Activation of miR-720 significantly reduced BC cell migration both in vitro and in vivo. miR-720 directly targets Twist1, inhibiting metastasis by

suppressing its activity, with a negative correlation observed in human BC tissues (32). Nairismagi et al. reported that Twist1's 3′ UTR is highly conserved, with miR-151-5p significantly inhibiting Twist1 expression, particularly when paired with miR-337-3p or miR-145a-5p (29).

However, previous studies have not comprehensively investigated the full miRNA expression panel following Twist1 knockdown. This study addresses this gap by selecting 43 miRNAs not previously associated with Twist1 in BC to provide new insights for future research. The data revealed that Twist1 inhibition in MDA-MB-231 cells led to increased expression of miR-1-1 and miR-210-3p, while the expression of miR-193b-3p, miR-181b-5p, and miR-148a-3p decreased.

Yamasaki et al. identified miR-1-1 as a member of the miR-1 family and demonstrated that in carcinomas of epithelial origin, such as bladder cancer, the expression of miR-1 family members is significantly downregulated. They showed that protmosin-α and purine nucleoside phosphorylase are directly regulated by miR-1 family members. Silencing these two genes led to a marked inhibition of cell proliferation and invasion while significantly promoting apoptosis in cancer cells (33). Liu et al. reported decreased expression of miR-1 family members in BC tissues, which inhibited proliferation and impaired apoptosis in BC cells. miR-1 family members were shown to function as tumor suppressors by targeting K-ras and metastasis-associated lung adenocarcinoma transcript 1. Patients with low miR-1 family expression levels were found to have shorter survival times compared to those with high expression levels (34).

In a study analyzing miRNA expression profiles in metastatic BC cases, miR-1 was identified as a miRNA linked to distant metastasis, with its expression in breast carcinoma associated with aggressive cancer phenotypes, serving as a strong prognostic factor (35). In this research, silencing Twist1 gene expression resulted in a 4.47-fold increase in miR-1-1 expression levels. Using the TargetScan platform (http://www.targetscan.org/vert_72/) for target prediction, it was found that CXC chemokine receptor 4 (CXCR4), a metastasis-associated gene, may represent a common signaling pathway. It was hypothesized that Twist1 gene inhibition could upregulate miR-1-1 via this shared pathway (36).

The data obtained by silencing the Twist1 gene in this study showed that miR-1-1, previously identified as a tumor suppressor, was upregulated. It is hypothesized that Twist1 gene silencing inhibits metastasis, proliferation, motility, and apoptosis in cancer cell lines.

miR1-1 is primarily expressed in heart and skeletal muscle, where it is regulated by muscle differentiation factors during heart development. It functions within serum response factor-myocardin-dependent pathways in cardiac progenitor cells and is controlled by key muscle differentiation factors such as serum response factor, myoblast determination protein 1, and myocyte enhancer factor-2 (37). miR-1 regulates several critical genes involved in muscle and heart function, including kruppel-like factor 4, heat shock protein 60, heart and neural crest derivatives expressed 2 (a transcription factor essential for cardiomyocyte development), stanniocalcin 2, and components of the Transforming Growth Factor-beta (TGF-β) signaling pathway (38).

Dysregulation of miR-1-1 has been linked to various cancers, including liver cancer, lung cancer, BC, colon cancer, medulloblastoma, glioblastoma, and pancreatic cancer. In particular, downregulation of miR-1 is common in TNBC and is associated with poor prognosis and lower survival rates in BC. Additionally, miR-1 has been implicated in chemotherapy-induced cardiotoxicity, particularly in BC patients treated with anthracyclines, including epirubicin (38).

Functionally, overexpression of miR-1-1 in BC cells decreases cell proliferation and invasion while promoting apoptosis through the downregulation of B-cell lymphoma 2 (BCL2), Slug, and metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), ultimately reducing tumor growth (38). The expression of miR-1-1, which has been shown to play a tumor-suppressive role, increases upon the suppression of Twist1. Therefore, Twist1 may be considered a key regulator of miR-1-1 in TNBC progression.

miR-193b-3p is recognized as a tumor suppressor in BC and various cancer cell lines, playing a critical role in regulating metastatic processes. miR-193b-3p has been shown to be significantly downregulated in MDA-MB-231 and MCF-7 cells. Silencing of miR-193b-3p in BC abrogates the inhibition of several proteins associated with metastatic genes, potentially conferring metastatic properties to the cells. These findings are

supported by pathway analyses using KEGG (Kyoto Encyclopedia of Genes and Genomes) and IPA (Ingenuity Pathway Analysis) tools, which identify key signaling pathways linked to poor prognosis in patients (39).

In our study, miR-193b-3p expression levels decreased by 1.52-fold. Given the data, this unexpected reduction in tumor suppressor miRNA aligns with previous findings suggesting that miRNA expression levels can exhibit oncogenic or tumor-suppressive behavior depending on their mechanisms of action. The results from this study further support the hypothesis that miR-193b-3p can induce diverse behaviors in cancer cells.

miR-193b targets estrogen receptor-alpha to inhibit estrogen-induced growth in BC cells, with its target genes involved in cell signaling and steroid hormone production. This suggests that miR-193b plays a role in inhibiting steroid-dependent growth in BC (40). Additionally, miR-193b reduces lipid accumulation and adipogenic marker expression in cultured cells, though its knockdown after differentiation has minimal impact. miR-193b expression also inhibits the differentiation of mouse C2C12 myoblasts into multinucleated myotubes and serves as a key regulator of brown fat differentiation, partially by repressing myogenesis (41). Based on these results, Twist1 may potentially regulate miR-193b-mediated myogenic differentiation in BC cells.

miR-181 is aberrantly expressed in tumor tissues and plays a significant role in cancer progression. The overexpression of miR-181b-5p downregulates E-cadherin by interacting with various transcription factors, thereby promoting invasion and metastasis. These findings indicate that miR-181b-5p modulates TGF-β1-induced EMT by directly targeting E-cadherin (42, 43). In this study, a marked decrease in miR-181b-5p expression was observed following the suppression of Twist1 gene expression. Mechanistic analysis revealed that the interleukin 6 (IL-6) pathway and NF-κB activity were particularly prominent. The signal transducer and activator of transcription 3 (STAT3), along with miR-21-5p and miR-181b-5p, has been shown to directly influence IL-6 expression (44). The absence of a significant change in miR-21-5p expression levels in this study suggests that Twist1 affects miR-181b-5p through a pathway other than IL-6.

Previous research has demonstrated that miR-181b-5p, which exhibits epigenetic oncogenic behavior, can inhibit NF-κB activity, potentially mediating the epigenetic link between inflammation and cancer. It is hypothesized that a shared pathway exists between the epigenetic mechanisms of Twist1 and the regulation

of NF-κB activity by miR-181b-5p. This shared pathway indicates that the inhibition of Twist1 may downregulate miR-181b-5p expression.

miR-181b is a critical regulator of the phosphatidylinositol 3-kinase (PI3K) pathway, playing a key role in cellular metabolic adaptations that support high proliferation rates during development. It modulates the expression of phosphatase and tensin homolog (PTEN) to regulate PI3K signaling. Mice deficient in miR-181b exhibit severe defects in lymphoid development and T-cell homeostasis, which are linked to impaired PI3K signaling (45). Based on this literature, we propose that the Twist1 transcription factor could regulate the PI3K pathway through miR-181b by modulating PTEN expression in TNBC cells.

miR-148a-3p can function as either an oncogene or a tumor suppressor, depending on the context (46, 47). In our study, a significant decrease in miR-148a-3p expression was observed following the suppression of Twist1. The miR-148a/152 family has been identified as a key regulator of DNA methyltransferase 1 (DNMT1), playing a crucial role in epigenetic modifications and gene expression control (48). Our findings suggest that DNMT1 expression may be influenced by the epigenetic mechanisms of Twist1, potentially affecting the regulation of miR-148a-3p expression. This aligns with Xu et al.'s (2013) findings, which demonstrate DNMT1's role in epigenetic modifications and gene expression control (40). Inhibition of the Twist1 gene may impact cancer cell differentiation and survival by altering the expression of key regulatory miRNAs, including miR-148a-3p.

miR-148a acts as a negative regulator of the innate immune response and the antigen-presenting capacity of mouse dendritic cells (DCs). Its expression is upregulated in mouse DCs during maturation and activation induced by agonists of Toll-like receptor (TLR) 3, TLR4, and TLR9. Overexpression of miR-148a inhibits the production of cytokines, including IL-6, IL-12, tumor necrosis factor (TNF), and interferon-β. It also suppresses the upregulation of major histocompatibility complex class II (MHC II) expression and DC-initiated, antigen-specific T-cell proliferation. This inhibition occurs through the targeting of Camk2a expression. Liu et al. (2010) proposed that miR-148a fine-tunes the innate immune response (49). In light of these findings, further analyses are warranted to investigate the potential roles of Twist1 in the innate immune system and its implications for breast cancer progression.

miR-210-3p may have an effect on cancer cell survival and metastasis (50). In this research, suppression of the Twist1 gene led to a significant increase in the expression level of miR-210-3p. Based on previous studies, this increase in miR-210-3p expression may be mediated by the fibroblast growth factor receptor through the RAS signaling pathway, which modulates the RAS-mitogen-activated protein kinase signaling cascade (51, 52). It is proposed that the inhibition of Twist1 through this common signaling pathway may elevate miR-210-3p expression. Notably, miR-210-3p has previously been classified as a tumor suppressor. Therefore, suppression of the Twist1 gene may hinder cancer cell survival and metastasis by upregulating miR-210-3p.

miR-210 expression progressively increases in human umbilical vein endothelial cells (HUVECs) upon exposure to hypoxia. Overexpression of miR-210 in normoxic endothelial cells stimulates the formation of capillary-like structures in a 3D gel and enhances vascular endothelial growth factor (VEGF)-induced cell migration. Conversely, miR-210 inhibition via anti-miRNA transfection suppresses hypoxia-induced capillary-like structure formation and reduces cell migration in response to VEGF. While miR-210 overexpression does not affect endothelial cell growth, anti-miR-210 transfection inhibits cell growth and induces apoptosis under both normoxic and hypoxic conditions (53).

miR-210 downregulates ephrin-A3 expression, and the expression of an miR-210-resistant ephrin-A3 allele prevents miR-210-mediated stimulation of tubulogenesis and chemotaxis. Fasanaro et al. (2008) concluded that miR-210 plays a key role in the endothelial cell response to hypoxia (53). Antisense-mediated downregulation of miR-210 has been reported to suppress cell viability, induce G0/G1 phase arrest, increase the apoptotic rate, and enhance radiosensitivity in hypoxic human hepatoma cells. Reporter assays indicate that miR-210 targets the 3′ UTR of apoptosis-inducing factor, mitochondrion-associated 3 (AIFM3), leading to the downregulation of AIFM3 expression. Human hepatoma cells expressing antisense miR-210 and AIFM3 small interfering RNA exhibit reduced apoptosis following irradiation compared to control cells, suggesting that miR-210 typically downregulates AIFM3-mediated apoptosis (54).

Based on this information, a detailed investigation into the hypoxia-related angiogenesis and cancer development processes regulated by miR-210, modulated by Twist1 in TNBC cells, could elucidate the precise role of Twist1 in miR-210-mediated pathways.

CONCLUSION

In summary, the findings of this research provide novel insights into the regulatory role of Twist1 in modulating miRNA expression patterns within TNBC cells. The miRNAs identified in connection with Twist1 signaling pathways represent potential candidates for the development of new therapeutic strategies targeting TNBC. Further investigation is required to elucidate the underlying molecular mechanisms and to validate the clinical relevance of these findings in patients with TNBC.

Acknowledgments

We would like to thank Selçuk University Scientific Research Projects Coordination Office.

Authorship contributions

BO and SP contributed to the study conception and design. AK, BO and SP conducted the cells experiments. FSB drafted the first manuscript. BO and SP revised the manuscript. All authors contributed to the data interpretation, critically reviewed each draft of the manuscript, and approved the final version for submission.

Data availibity statement

The data that support the findings of this study are available on request from the corresponding author.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics

This study protocol was reviewed and approved by Selcuk University Faculty of Medicine Ethics Committee, approval number 2024/113.

Funding

Funding for this study was provided by Selçuk University Scientific Research Projects Coordination Office, grant number 17202072.

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