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Transcriptional Control of PI3K/AKT/mTOR by piRNA-651 in Breast Carcinoma

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ABSTRACT

Background: Breast cancer is among the most prevalent malignancies in women worldwide, yet early diagnosis is associated with a high survival rate. The proliferation of BC is linked to the overexpression of genes within the PI3K/AKT/mTOR signaling pathway. piR-651 has been reported to be effective in the proliferation and metastasis of BC. This research sought to evaluate the impact of piR-651 inhibition on the PI3K/AKT/mTOR pathway in HUVEC, MCF-7, and MDA-MB-231 cells.

Methods: Anti-piR-651 and non-target sequences were introduced into HUVEC, MCF-7, and MDA-MB-231 BC cells by lipofectamine transfection. After 48 hours, total RNA was extracted, and qRT-PCR assessed the gene expression of *PI3K*, *AKT*, and *mTOR*.

Results: Anti-piR-651 treatment significantly increased *PI3K*, *AKT*, and *mTOR* gene expression in HUVECs ($p < 0.001$). In contrast, *PI3K* and *mTOR* expression decreased in MCF-7 and MDA-MB-231 cells ($p < 0.001$), while *AKT* expression remained unchanged in MDA-MB-231 cells ($p > 0.05$). Correlations between these genes varied by cell type, with significant associations observed at $p < 0.05$ or $p < 0.01$, depending on the group.

Conclusion: *piR-651* inhibition causes *AKT* to behave independently of *PI3K* and *mTOR*, particularly in MCF-7 cells, suggesting limited gene therapy potential for estrogen receptor-positive BC. Preliminary data indicate that piR-651 inhibition may reduce BC cell proliferation through effects on *PI3K* and *mTOR*. ***DOI: 10.61882/ibj.5229***

Keywords: Epigenetics, Piwi-interacting RNA, Triple negative breast neoplasms

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INTRODUCTION

Breast cancer is among the most prevalent malignant tumors in women globally, and its prognosis is greatly impacted by its metastatic dissemination[1]. In recent years, there has been an increasing focus on the role of small non-coding RNAs in cancer biology.

piRNAs are essential for regulating gene expression and maintaining genome stability[2]. These small non-coding RNAs, approximately 26-31 nucleotides long, interact with PIWI proteins to form the pi-RISC complex. Traditionally, piRNAs have been associated with transposon repression in germ cells, but they have been recently recognized as important regulators in somatic cells. In malignant diseases such as BC,

List of Abbreviations:
BC: breast cancer; **CDK4:** cyclin dependent kinase 4; **DMEM:** Dulbecco's Modified Eagle Medium; **FBS:** fetal bovine serum; **GAPDH:** gliseraldehide-3-phosphate dehydrogenase; **HUVEC:** human umbilical vein endothelial cell; **piR-651:** PIWI-interacting RNA-651; **piRNA:** PIWI-interacting RNA; **PIWI:** P-element Induced WImpy testis; **qRT-PCR:** quantitative real-time polymerase chain reaction; **RISC:** RNA-induced silencing complex

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abnormal piRNA expression has been associated with tumor progression and metastasis[3]. The influence of piRNAs in BC is facilitated by gene expression and epigenetic regulation. piR-932 has been shown to increase the methylation of the *Latexin* gene by interacting with PIWIL2 and promote the differentiation of BC stem cells through this mechanism[4]. Elevated expression of several piRNAs, such as piR-651, piR-4987, piR-20365, piR-20485, and piR-20582, has been associated with enhanced proliferative, invasive, and metastatic potential of cancer cells [5]. Among these piRNAs, piR-651 plays an important role in cancer biology, being highly expressed in BC cells where it inhibits apoptosis and promotes cell proliferation by regulating the G2/M phase of the cell cycle[6]. Studies have indicated that overexpression of piR-651 accelerates cell cycle progression into the G2/M phase, increasing the number of cancer cells[5].

Fundamental biological functions, such as cell growth, metabolism, migration, and apoptosis, are regulated by the PI3K/AKT/mTOR signaling pathways. In BC, abnormal activation of these pathways, particularly due to PIK3CA mutations, loss of PTEN, and *mTOR* overactivation, contributes to tumor progression and resistance to therapy[1,7,8].

Research on the role of piR-651 in the initiation and spread of BC has grown in the last several years. Its interaction with the PI3K/AKT/mTOR signaling pathway is essential for controlling the migration, proliferation, and apoptosis of cancer cells. Based on these observations, we designed this study to evaluate the effect of piR-651 inhibition on the PI3K/AKT /mTOR pathway in HUVEC, MCF-7, and MDA-MB-231 cells.

MATERIALS AND METHODS

Cell culture

MCF-7, MDA-MB-231, and HUVEC cells (all from ATCC, USA) were seeded in a growth medium consisting of DMEM (Gibco, USA), supplemented with 10% FBS (Gibco) and 1% penicillin/streptomycin (Gibco). HUVECs were used to investigate the interactions between metastatic cancer cells and the endothelial barrier, a critical step in the metastatic cascade[9]. These cells served as a "normal" counterpart, enabling researchers to elucidate differences in endothelial cell behavior and to assess how the tumor microenvironment alters this behavior. For example, HUVECs are used to compare angiogenic abilities along with interactions with BC cells under tumor conditions[10,11]. By using HUVECs, researchers can study how cancer cells interact with non-tumorigenic endothelium and how these healthy cells respond in diverse experimental setups, such as microfluidic tumor microenvironments[12]. In metastatic BC research, a crucial issue is to understand the differences between normal blood vessels and those formed within tumors. HUVECs provide a contrast to tumor-derived endothelial cells, which exhibit distinct phenotypes, genetic expression profiles, and functions, such as higher proliferative potential and immunosuppressive mechanisms that facilitate tumor progression[13]. Nevertheless, HUVECs remain a foundational healthy control model for investigating the complex interplay between cancer cells and the vascular system in metastatic BC[14].

Lipofectamine transfection

Lipofectamine (Takara, Japan) was used to transfect cells with non-target [5’-CAGUACUUUUGUGU AGUACAA-’3 (2’-O-metylated)] and anti-piR-651 [5’-GACGCUUUCCAAGGCACGGGCCCCUCUCU-’3 (2’-O-metylated)] sequences (GenScript, USA), once 80% cell confluency was observed non-target sequence is not specific to any region or gene. qRT-PCR was used to measure piR-651 expression in anti-piR-651-transfected HUVEC, MCF-7, and MDA-MB-231 cells to assess the effectiveness of transfection. Our previous research[15,16] indicated that the optimal time for piR-651 inhibition is 48 hours after transfection across all three cell lines (Fig. S1).

Total RNA isolation and qRT-PCR

Total RNA was extracted from HUVEC, MCF-7, and MDA-MB-231 cells 48 hours after transfection with non-target and anti-piR-651 sequences in accordance with the manufacturer's protocol (Nucleogene, Turkey). The qRT-PCR primers listed in Table 1 were obtained from BMLabosis (Ankara, Turkey). Following RNA isolation, reverse transcription was performed to convert

Table 1. Primer sequences used in qRT-PCR

Gene	Forward sequence	Reverse sequence
piR-651	5’-AGAGAGGGGCCCGTGCCTTG-3’	5’-CTTATGGAGCCTGGGACTCTGACC-3’
PI3K	5’-CAGAGCCAAAGGGAAGGAGG-3’	5’-AGCCAGTTCAGAAGGGCATC-3’
AKT	5’-AGAAGCAGGAGGAGGAGGAG-3’	5’-CGACCGCACATCATCTCGTA-3’
mTOR	5’-GCACAATGCAGCCAACAAGA-3’	5’-ATGTCCGTTGCTGCCCATAA-3’
GAPDH	5’-CGAGGGGGGAGCCAAAAGGG-3’	5’-TGCCAGCCCCAGCGTCAAAG-3’

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RNA into complementary DNA. After reverse transcription, the expression levels of *piR-651*, *PI3K*, *AKT*, and *mTOR* genes were measured by RT-PCR (Rotor-Gene, Qiagen, USA). *GAPDH* was used as an internal control. The ΔΔCT formula was used to compute gene expression levels for the experimental groups (n = 7 per group and per gene).

Statistical analysis

Gene expression studies were conducted using IBM SPSS Statistics v. 26.0. The Kolmogorov-Smirnov test was used to determine whether the continuous variables had a normal distribution. The findings were analyzed using One-way ANOVA, and multiple-group comparisons were conducted using Tukey's HSD. Data were displayed as mean ± standard deviation. The Spearman Rho Correlation test was used to evaluate gene correlations within each group. Statistical significance was defined as $p < 0.05$.

RESULTS

Comparison of PI3K, AKT, and mTOR gene expressions in HUVEC, MCF-7, and MDA-MB-231 cell lines

After 48-hour post-transfection, *PI3K* gene expression in anti-piR-651-transfected HUVECs (0.924 ± 0.003) was significantly increased compared to the HUVEC control group (-3.039 ± 0.22; $p < 0.001$). In contrast, *PI3K* expression in anti-piR-651-transfected MCF-7 (1.37 ± 0.102) and MDA-MB-231 (1.123 ± 0.031) cells were significantly lower than that in the control groups (MCF-7: 2.27 ± 0.123 and MDA-MB-231: 2.27 ± 0.123; $p < 0.001$; Fig. 1A). Similarly, *AKT* gene expression was elevated in anti-piR-651-transfected HUVECs (2.074 ± 0.052) compared to the HUVEC control group (-0.949 ± 0.018; $p < 0.001$). In MDA-MB-231 cells, *AKT* gene expression showed no significant difference between the anti-piR-651-transfected (2.683 ± 0.12) and control (2.65 ± 0.142) groups ($p > 0.05$; Fig. 1B). However, in MCF-7 cells, *AKT* expression level significantly increased in the anti-piR-651 transfected group (5.85 ± 0.041) compared to the control group (1.74 ± 0.13; $p < 0.001$). At 48 hours after transfection, *mTOR* gene expression was significantly increased in anti-piR-651-transfected HUVECs (0.504 ± 0.021) compared to the HUVEC control group (-2.599 ± 0.243; $p < 0.001$). Conversely, *mTOR* expression was reduced in both anti-piR-651-transfected MCF-7 (0.45 ± 0.011) and MDA-MB-231 (0.763 ± 0.013) cells compared to the MCF-7 (0.86 ± 0.036) and MDA-MB-231 controls (1.15 ± 0.02) groups ($p < 0.001$; Fig. 1C). At the 48th hour of transfection, *PI3K*, *AKT*, and *mTOR* gene expression levels were higher in MCF-7 (1.67 ± 0.078; 1.74 ± 0.129; 0.86 ± 0.036) and MDA-MB-231 (2.27 ± 0.123; 2.65 ± 0.142; 1.15 ± 0.02) control cells compared to HUVEC control cells (-3.039 ± 0.22; -0.95 ± 0.018; -2.599 ± 0.243; $p < 0.001$; Fig. 2). Furthermore, *PI3K* gene expression in MDA-MB-231 control cells was significantly higher than that of MCF-7 control cells ($p < 0.001$; Fig. 2A). In the anti-piR-651-transfected groups, *PI3K* gene expression in both MCF-7 (1.37 ± 0.102; $p < 0.001$) and MDA-MB-231 (1.123 ± 0.031; $p < 0.05$) cells were higher than that of HUVECs (0.924 ± 0.003). Similarly, *AKT* gene expression in both

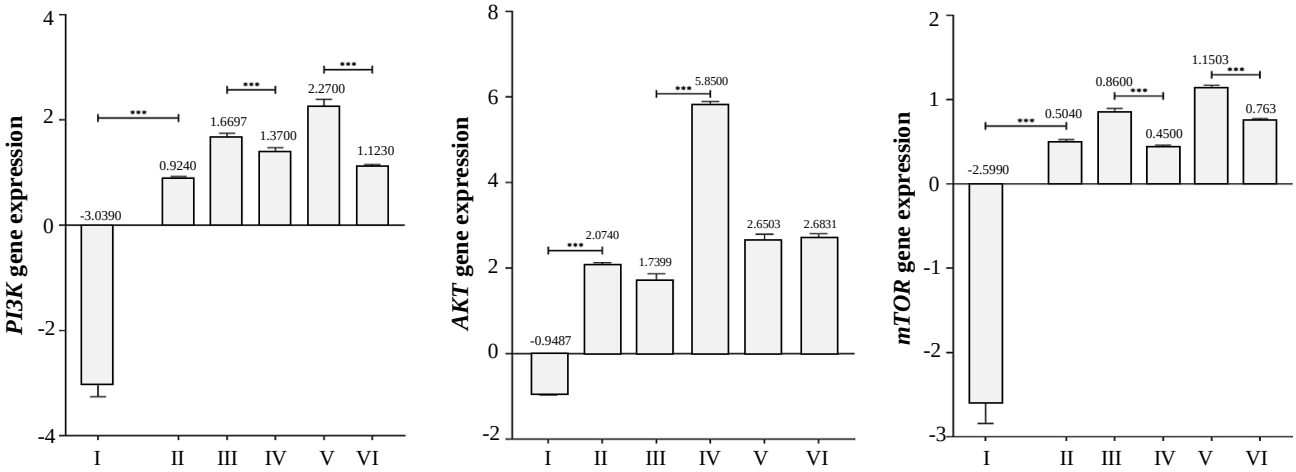

Fig. 1. Gene expression of (A) *PI3K*, (B) *AKT*, and (C) *mTOR* in HUVEC, MCF-7, and MDA-MB-231 cell lines ($^{***}p < 0.001$; n = 7 per group). I: HUVEC control; II: MCF-7 control; III: MDA-MB-231 control; IV: anti-piR-651 transfected HUVEC; V: anti-piR-651 transfected MCF-7; VI: anti-piR-651 transfected MDA-MB-231

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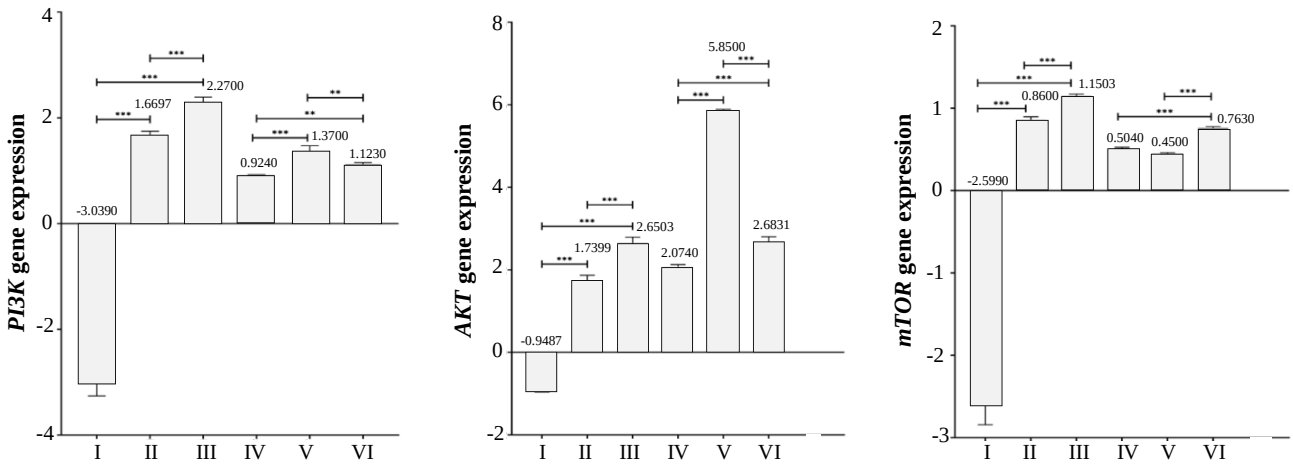

Fig. 2. The comparison of (A) *PI3K*, (B) *AKT*, and (C) *mTOR* gene expressions in HUVEC, MCF-7, and MDA-MB-231 cell lines ($^{**}p < 0.01$; $^{***}p < 0.001$; n = 7 for each group). I: HUVEC control; II: MCF-7 control; III: MDA-MB-231 control; IV: anti-piR-651 transfected HUVEC; V: anti-piR-651 transfected MCF-7; VI: anti-piR-651 transfected MDA-MB-231

MCF-7 (5.85 ± 0.041) and MDA-MB-231 (2.683 ± 0.12) cells was elevated compared to HUVECs (2.074 ± 0.0521; $p < 0.001$). In the anti-piR-651-transfected groups, *mTOR* gene expression was higher in MDA-MB-231 cells (0.763 ± 0.013) than that of HUVECs (0.504 ± 0.021; $p < 0.001$). Moreover, *mTOR* gene expression was significantly higher in anti-piR-651-transfected MDA-MB-231 (0.763 ± 0.013) compared to anti-piR-651-transfected MCF-7 (0.45 ± 0.0106) cells ($p < 0.001$; Fig. 2C).

Correlation analysis of genes

Correlation analysis showed a relationship between *PI3K* and *AKT* in the HUVEC controls, MCF-7 controls, and anti-piR-651-transfected MDA-MB-231 cells (r = 0.05). Significant correlations between *AKT* and *mTOR* were observed in MCF-7 controls (r = 0.05), anti-piR-651-transfected MCF-7 (r = 0.05), and MDA-MB-231 controls (r = 0.01). No correlations were detected among the genes in anti-piR-651-transfected HUVECs (Table 2).

DISCUSSION

piR-651 is a small RNA molecule that belongs to the piRNA family and exerts diverse effects on cellular mechanisms. A previous study has revealed that piR-651 plays important roles in gene expression regulation, epigenetic control, and cellular signaling pathways[17]. One study specifically investigated the effects of piR-651 and piR-823 on the metastatic and invasive properties of triple-negative BC. The inhibition of piR-651 led to decreased cell proliferation and motility, suggesting that piR-651 could be an important regulator of BC metastasis[16]. Additional investigation has displayed that piR-651 inhibition significantly reduces the proliferation, adhesion, and motility of MCF-7 cells. Moreover, the suppression of *Ki-67*, *MMP-2*, *ERα*, *HIF-1α*, and *hTERT* expressions demonstrated that piR-651 can alter the malignant properties of cancer cells[15]. These results highlight the crucial role of piR-651 and its potential as a biomarker candidate in cancer.

piR-651 has been shown to promote cell migration and proliferation, as well as accelerate tumor growth by inhibiting apoptosis. It has been identified as an oncogene implicated in multiple malignancies and influences various cellular processes, including apoptosis, proliferation, and migration[18]. piR-651 expression has been linked to different clinico-pathological features, including gender, age, tumor invasion, and tumor size. Research has shown that the inhibition of piR-651 reduces cell proliferation by arresting cells in the G2/M phase[19]. Another research revealed that in non-small cell lung cancer, piR-651 inhibition increased apoptosis and apoptosis-related proteins, while decreasing the growth[20]. Li et al. have found that high concentrations of piR-651 prolong the G2/M phase of the cell cycle in non-small cell lung cancer, both in vivo and in vitro, lowering the G0/G1 ratio and increasing cyclin D1 and CDK4 levels[21]. It has also been reported that piR-651 increases the expression of oncogenes, such as CDK4 and Cyclin D1, which promote cell cycle control and the growth of cancer cells[6]. Additionally, suppression of piR-651 arrests the cell cycle in MGC-803 gastric cancer cells at the G2/M phase[22]. Furthermore, piR-651 influences apoptosis through interactions with PARP-1, Bcl-2, and

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Table 2. Gene correlation data for HUVEC, MCF-7, and MDA-MB-231 cells

Groups	Genes	Analysis	*PI3K*	*AKT*	*mTOR*
		Pearson Correlation	1	-0.766*	-0.240
		Sig. (2-tailed)		0.045	0.604
		N	7	7	7
		Pearson Correlation	-0.766*	1	0.011
		Sig. (2-tailed)	0.045		0.981
		N	7	7	7
		Pearson Correlation	-0.240	0.011	1
		Sig. (2-tailed)	0.604	0.981	
		N	7	7	7
		Pearson Correlation	1	0.488	0.590
		Sig. (2-tailed)		0.267	0.163
		N	7	7	7
		Pearson Correlation	0.488	1	-0.017
		Sig. (2-tailed)	0.267		0.971
		N	7	7	7
		Pearson Correlation	0.590	-0.017	1
		Sig. (2-tailed)	0.163	0.971	
		N	7	7	7
		Pearson Correlation	1	-0.389	0.793*
		Sig. (2-tailed)		0.389	0.033
		N	7	7	7
		Pearson Correlation	-0.389	1	-0.809*
		Sig. (2-tailed)	0.389		0.027
		N	7	7	7
		Pearson Correlation	0.793*	-0.809*	1
		Sig. (2-tailed)	0.033	0.027	
		N	7	7	7
		Pearson Correlation	1	-0.057	0.546
		Sig. (2-tailed)		0.904	0.205
		N	7	7	7
		Pearson Correlation	-0.057	1	0.800*
		Sig. (2-tailed)	0.904		0.031
		N	7	7	7
		Pearson Correlation	0.546	0.800*	1
		Sig. (2-tailed)	0.205	0.031	
		N	7	7	7
		Pearson Correlation	1	0.432	0.342
		Sig. (2-tailed)		0.392	0.507
		N	7	7	7
		Pearson Correlation	0.432	1	0.975**
		Sig. (2-tailed)	0.392		0.001
		N	7	7	7
		Pearson Correlation	0.342	0.975**	1
		Sig. (2-tailed)	0.507	0.001	
		N	7	7	7
		Pearson Correlation	1	0.763*	-0.062
		Sig. (2-tailed)		0.046	0.895
		N	7	7	7
		Pearson Correlation	0.763*	1	-0.528
		Sig. (2-tailed)	0.046		0.223
		N	7	7	7
		Pearson Correlation	-0.062	-0.528	1
		Sig. (2-tailed)	0.895	0.223	
		N	7	7	7

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed).

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Bax[23]. In highly metastatic 95-D human lung cancer cells, piR-651 inhibition increased *caspase 3* and *Bax* expressions, induced apoptosis, and promoted tumor growth[17]. The interaction of piRNAs with critical signaling pathways, such as PI3K/AKT/mTOR, is also important. piR-651 has been reported to increase the *PTEN* methylation, contributing to the overactivation of these pathways, which promotes the survival of cancer cells[24]. As a result, the inhibition of *PTEN* by piR-651 leads to PI3K/AKT/mTOR overactivation, inhibiting apoptosis, accelerating cell migration, and increasing cell proliferation[6]. By inducing cell cycle arrest in the G2/M phase, piR-651 inhibition suppresses the growth of gastric cancer cells[22]. Additionally, the piR-651/PIWIL1 complex inhibits gastric cancer invasion[25]. In BC cell lines, increased expression of piR-651 has been linked to enhanced cellular motility, invasion, and disease progression[6]. Due to their high expression in BC, piR-651, piR-4987, piR-20365, piR20485, and piR-20582 have been shown to exhibit oncogenic characteristics[26].

The potential of piR-651 as a possible biomarker is prompted by its involvement in BC development and progression. PTEN methylation mediated by piR-651 promotes cancer cell survival and overactivates the PI3K/AKT/mTOR pathways. Thus, targeting piR-651 may lead to the development of new biomarker candidates or therapeutic approaches for BC. Our results showed that anti-piR-651-transfected HUVECs had higher levels of *PI3K* gene expression than the control group ($p < 0.001$), suggesting that piR-651 may activate the *PI3K* pathway and increase cell proliferation in normal endothelial cells. However, MCF-7 and MDA-MB-231 cells showed reduced *PI3K* gene expression ($p < 0.001$), exhibiting that piR-651 may reduce proliferation in cancer cells by suppressing the *PI3K* pathway. Furthermore, high *PI3K* gene expression was observed in anti-piR-651-transfected MCF-7 cells compared to the MDA-MB-231 cells ($p < 0.05$).

Treatment of HUVEC and MCF-7 cells with anti-piR-651 resulted in elevated *AKT* expression ($p < 0.001$). In the comparison between MCF-7 and MDA-MB-231 control cells, MDA-MB-231 cells exhibited an elevated *AKT* expression than MCF-7 cells ($p < 0.001$), proposing that piR-651 may increase cell proliferation by activating the *AKT* pathway in these cells. However, no statistically significant difference was found in *AKT* gene expression of MDA-MB-231 cells ($p > 0.05$), indicating that piR-651 does not affect the *AKT* pathway in these cells, or its effect has been limited. This transcriptional separation violates the conventional sequential logic of the signaling cascade, where PI3K is the upstream of AKT, which is upstream of mTOR, with mTOR positioned downstream. Elevation in AKT mRNA expression and decrease in PI3K and mTOR mRNA levels suggest that the PI3K/mTOR-independent mechanism could regulate AKT transcription in MCF-7 cells, potentially linking to estrogen receptor signaling or unique feedback loops. In anti-piR-651-transfused HUVECs, *mTOR* expression increased significantly ($p < 0.001$, implying that piR-651 may activate the mTOR pathway and increase cell proliferation in healthy cells. However, in MCF-7 and MDA-MB-231 cells, *mTOR* expression decreased ($p < 0.001$). Notably, *mTOR* gene expression was higher in the MDA-MB-231 than the MCF-7 control cells ($p < 0.001$). These changes in gene expression indicate that piR-651 may decrease cell proliferation by suppressing the mTOR pathway in cancer cells. Moreover, high *AKT* expression was observed in anti-piR-651-transfected MCF-7 cells compared to the MDA-MB-231 cells ($p < 0.001$). These findings provide crucial preliminary transcriptional insights into how the PI3K/AKT/mTOR pathway is regulated differently across BC subtypes.

The PI3K/AKT/mTOR pathway is critical for cell growth, metabolism, and survival. Its overexpression in healthy cells following piR-651 suppression may represent a protective or adaptive response. However, if uncontrolled, this overexpression could offer hazards, such as increasing angiogenesis or aberrant cell proliferation. Furthermore, the action of piR-651 may be context-dependent, behaving differently in healthy and malignant cells. In healthy endothelial cells, piR-651 blockage may activate compensatory mechanisms that increase survival and proliferation pathways (PI3K/AKT/mTOR), perhaps as a cellular stress response or to maintain homeostasis. In cancer cells, however, piR-651 is a component of oncogenic machinery; its inhibition affects signaling and disrupts signaling pathways that are essential for tumor development and survival. Moreover, the increase in *PI3K*/*AKT*/*mTOR* gene expression in healthy HUVECs after piR-651 inhibition suggests that piR-651 may have a suppressive role in healthy cells, and its inhibition triggers the upregulation of survival pathways. This context-dependent effect underscores the complexity of targeting piR-651 in cancer therapy and the need for further research to understand its role in different cell types. These findings from our study overlap with the effects of piR-651 in other solid tumors, as it has been reported to promote metastasis by increasing cyclin D1 and CDK4 expression in lung carcinoma[21]. Regarding the molecular mechanism, piR-651 has been shown to act via DNMT1[6]. This mechanism is important because the loss of PTEN function results in the suppression of the PI3K/AKT/mTOR pathways. According to the research, PI3K/AKT/mTOR activation is crucial for BC metastasis and proliferation, and PIK3CA mutations,

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PTEN loss, or upstream signals like EGFR/HER2 could hyperactivate the cascade[27]. In this context, piR-651 indirectly activates this pathway by downregulating *PTEN* expression through epigenetic targeting and promotes tumor progression by increasing metastatic potential. Its redirection to the PTEN promoter via DNMT1 creates a new target for molecular therapies.

Despite the advantages of our study, its limitations should also be considered. Future work is needed to confirm the role of piR-651 in tumor progression and metastasis using in vivo models and patient samples. The use of only three cell lines—MCF-7, MDA-MB-231, and HUVEC—may not fully capture the diversity of BC or healthy endothelium cells. Furthermore, the effect of piR-651 on PI3K/AKT/mTOR subcomponents (e.g., mTORC1 vs. mTORC2 discrimination and AKT isoform specificity) should be examined in greater depth. A genomic assessment of the subject is produced by examining piR-651 inhibition just at the gene expression level. This study was focused solely on gene expression changes (mRNA levels) after piR-651 inhibition and did not assess protein expression levels or activity, which are crucial for understanding the functional impact on the PI3K/AKT/mTOR pathway. In the future, Western blotting or immunohistochemical evaluation of protein expression in the PI3K/AKT/mTOR pathway following piR-651 silencing will provide proteomic confirmation of our data. Even though our study suggested that piR-651 could be a therapeutic target or a possible biomarker for BC and its metastatic features, a comprehensive evaluation of piR-651's clinical significance, including its role in combination therapy, was not carried out. Future studies should comprehensively evaluate piR-651 as a potential biomarker candidate, its use as a therapeutic target, and its role in combination regimens.

CONCLUSION

The interaction of piR-651 with the PI3K/AKT/mTOR signaling pathway through PTEN emerges as a central motif that potentiates metastasis and progression in BC. These findings help understand the effects of piR-651 on PI3K/AKT/mTOR pathways and its role in healthy and cancer cells. However, the study is based solely on in vitro experiments with a limited number of cell lines; hence, the findings should be considered preliminary. The hypothesis about the role of piR-651 in PI3K/AKT/mTOR signaling in BC is important, but these findings still do not provide definitive evidence that can be directly translated to clinical settings. The tumor microenvironment, immune interactions, and genetic heterogeneity present in vivo are not captured in these models. Therefore, the effects of piR-651 inhibition on the PI3K/AKT/mTOR pathway may differ in animal models or human tissues. These observations imply that piR-651 may be considered a possible biomarker candidate for BC, but do not provide comprehensive evidence for its clinical utility. Further research is needed to validate these suggestions. The biological roles of this molecule and its potential therapeutic importance remain a key area of study in cancer biology and treatment.

DECLARATIONS

Acknowledgments

Artificial intelligence (AI) was not used in the preparation of this study.

Ethical approval

Not applicable.

Consent to participate

The authors declare that no patients were used in this study.

Consent for publication

All authors reviewed the results and approved the final version of the manuscript.

Authors' contributions

SK, BB, EBŞ, DD, AÇ, and AK: conceptualization, methodology, software, validation, investigation, visualization, writing-reviewing, and editing; DK and ÇÖ: conceptualization, methodology, software, validation, investigation, visualization, supervision, writing-reviewing, and editing.

Data availability

All relevant data are provided in the manuscript.

Competing interests

The authors declare that they have no competing interests.

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Supplementary information

The online version contains supplementary material.

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