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Original Research Article

Inhibition of breast cancer cell growth and migration through siRNA-mediated modulation of circ_0009910/miR-145-5p/MUC1 axis

Maryam Abtin [a], Nahid Nafisi [b], Asghar Hosseinzadeh [a], Sepideh Kadkhoda [a], Ramesh Omranipour [c,d], Leyla Sahebi [e], Masoumeh Razipour [a], Soudeh Ghafouri-Fard [f,*], Abbas Shakoori [a,g,**]

[a] Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[b] Surgery Department, Rasoul Akram Hospital Clinical Research Development Center (RCRDC), Iran University of Medical Sciences, Tehran, Iran
[c] Breast Disease Research Center (BDRC), Tehran University of Medical Sciences, Tehran, Iran
[d] Department of Surgical Oncology, Cancer Institute, Tehran University of Medical Sciences, Tehran, Iran
[e] Maternal, Fetal and Neonatal Research Center, Family Health Research Institute, Tehran University of Medical Sciences, Tehran, Iran
[f] Department of Medical Genetics, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[g] Department of Medical Genetics, Cancer Institute of Iran, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Dr. Qarib St., Keshavarz Blvd, Tehran, Iran

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ABSTRACT

Circular RNAs (circRNAs) characterize a novel kind of regulatory RNAs distinguished by great evolutionary conservation and constancy. Although their exact role in malignancies is not fully understood, they mainly work through specific axes. Circular RNA/miRNA/mRNA axes affect the pathogenesis of human cancers including breast cancer. We assessed the expression and function of circ_0009910/miR-145-5p/MUC1 axis in Breast Cancer tissues and MCF-7 cells. Expression levels of circ_0009910 and MUC1 were notably increased in breast cancer tissues compared with control tissues, parallel with the down-regulation of miR-145-5p. Clinicopathological analysis indicated that up-regulation of circ_0009910 in breast tumors is related to invasion of the tumor to lymph node (P value = 0.011). Also, the downregulation of miR-145-5p was significantly correlated with tumor invasion to lymph nodes (P value = 0.04) and HER2-negative tumors (P value = 0.037). Finally, overexpression of MUC1 was correlated with age under 45 years (P value = 0.002). More importantly, circ_0009910-siRNA decreased the proliferation and migration ability of breast cancer cells, enhanced expression of miR-145-5p, and decreased levels of MUC1. Taken together, the circ_0009910/miR-145-5p/MUC1 axis has been demonstrated to affect the pathogenesis of breast cancer and might provide a target for breast cancer treatment.

1. Introduction

Globally, breast cancer is the first leading cause of cancer-related death in women [1,2]. In recent decades, prominent advance has been attained in breast cancer treatments, but morbidity and mortality are even now rising [3]. The common cause of this issue is the complex and multifactorial nature of breast cancer [4,5]. Hence, it is vital to recognize the molecular mechanisms that comprise the proliferation and progression of BC. It is therefore essential to investigate the possible targets for breast cancer treatment [6].

Circular RNAs (circRNAs) are a newly acknowledged group of long non-coding RNAs that are crucial regulators of breast tumorigenesis and progression [7]. Furthermore, circRNAs participate in chemoresistance and are linked with clinicopathological parameters of breast cancer [8]. Recent clinical studies have focused on the deregulation of these transcripts as diagnostic and prognostic biomarkers, particularly owing to the covalently closed configuration that renders them stable in body fluids [9,10]. Among the diverse functions of circRNAs, their sponging effect on miRNAs and subsequent modulation of expression of miRNA targets are more clarified [11].

Circ_0009910 is an example of circRNAs that act as an oncogene in some types of cancers, including gastric cancer [12], colorectal cancer

* Corresponding author.
** Corresponding author. Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
E-mail addresses: s.ghafourifard@sbmu.ac.ir (S. Ghafouri-Fard), shakooria@sina.tums.ac.ir (A. Shakoori).

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Table 1
List of primers used for quantitative real-time PCR.

Gene name	Primer type	Primer sequences (5' to 3')
Circ_0009910	Divergent Forward	TTTGGCCGCGCAATGTCC
	Reverse	GCATTCACCTCAGCCATGTGTC
miR-145-5p	Forward	GGCTTAGTCCAGTTTTCCCAG
	Reverse	GTGCAGGGTCCGAGGT
	Stem- Loop RT- primer	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAGGGAT
U6snRNA	Forward	GCTTCGGCAGCACATATACTAAAAT
	Reverse	CGCTTCACGAATTTGCGTGTCAT
	Stem- Loop RT- primer	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCA CTGGATACGACAAAAATAT
MUC1	Forward	TTTCCAGCCCGGGATACCTA
	Reverse	ACAAGTTGGCAGAAGTGGCT
GAPDH	Forward	AAGGCTGAGAACGGGAAGCT
	Reverse	CAGCATCGCCCCACTTGATT

[13], leukemia [14], hepatocellular carcinoma [15], and ovarian cancer [16]. However, there is not any consistent study about circ_0009910 role in breast cancer. Diverse miRNAs are sponged with this circRNA (Circular RNA Interactome database), among them miR-145-5p was the prominent one in breast cancer [13]. This miRNA is a tumor suppressor miRNA in most types of cancers, including breast cancer [17]. Many studies showed the inhibitory roles of miR-145-5p in cell proliferation, invasion, and migration. It plays this vital role by transcriptionally targeting a wide variety of oncogenes [18,19]. Among several targets of miR-145, MUC1 has an imperative function in stimulating breast cancer invasion and metastasis [20]. Notably, MUC1 is elevated in multiple subtypes of breast cancer types compared with the normal tissues, conferring poor prognosis in them [21]. Thus, circ_0009910/miR-145-5p/MUC1 axis is an appropriate candidate for further investigations in breast cancer with an especial focus on its involvement in cancer progression and its application as a tumor marker.

We assessed the expression and function of circ_0009910/miR-145-5p/MUC1 axis in breast cancer tissues and MCF-7 cell line. We also used circ_0009910-siRNA to down-regulate expression of this circRNA and assess the functional consequences of this intervention on cell viability, migration, and expressions of miR-145-5p and MUC1.

2. Materials and methods

2.1. Clinical samples

Expression assays were conducted on 50 pairs of cancer tissues and equivalent non-cancerous adjacent tissues obtained from breast cancer patients who underwent surgical resection at Imam Khomeini Hospital and one another center, in Tehran, Iran. Written informed consent was acquired from all patients. Tissue samples were collected in RNA Later solution (Biobasic, Canada) and stored based on instruction. No patient underwent preoperative chemotherapy or radiotherapy. This study was approved by the Ethics Committee of the Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran (IR.TUMS.MEDICINE.REC.1401.272).

2.2. Cell culture and transfection

Human breast cancer cell line MCF-7 was acquired from the Pasteur Institute of Iran. Cells were cultured in RPMI 1640 (Gibco) complemented with 1 % penicillin/streptomycin and 10 % fetal bovine serum (Gibco). The incubation condition was set at 37 °C with 5 % CO2.

Small interfering RNAs (siRNAs) targeting Circ_0009910 and scramble siRNAs were obtained (Metabion, Germany). MCF-7 cells were seeded at a density of 4×10^5 cells/well in a six-well plate. After 24h of incubation, cells were transfected with 10 nM of Si-Circ_0009910 or scramble using Lipofectamine3000 (Invitrogen, USA) in serum and antibiotic-free medium. After incubation times, the cells were tested by successive experiments. The sequences of used siRNAs are as follows: Circ0009910 inhibitor siRNA: 5′GGCUUUUUUUGGCCGCGCAAUTT3′ and Scramble: 5′UUGUACUACACAAAAGUACUG3'.

2.3. RNA extraction and cDNA synthesis

Extraction of total RNA was accomplished using TRIzol reagent (Invitrogen) as stated by the Company. The integrity and quantity of RNA were assessed through gel electrophoresis and spectrophotometry (NanoDrop 2000, Thermo Scientific), respectively. ExcelRT™ 1st Strand cDNA Synthesis Kit (SMOBIO) was used for the production of cDNA. The specific stem-loop RT primer for miR-145-5p and snRNA U6 was supplemented into the reaction mixture. The cDNA synthesis reaction was exposed to the following temperature circumstances: 5 min at 70 °C, 10 min at 25 °C, 50 min at 50 °C and then 5 min at 85 °C.

2.4. Quantitative real-time PCR (qRT-PCR)

Expression levels of circ_0009910, miR-145-5p, and MUC1 were identified in Light-Cycler96 Roche thermocycler by using RealQ Plus 2x Master Mix Green no Rox (Ampliqon, Denmark). GAPDH or U6 was applied for the normalization of expression data. Relative expression level of genes was computed using the $2^{-\Delta\Delta Ct}$ method with GAPDH or U6 as the internal reference. To specifically detect circ_0009910 rather than the linear form (*MFN2* gene), a divergent primer was designed by circprimer 2.0 software. The circular junction of circ_0009910 was confirmed by Sanger sequencing. The primer sequences are shown in Table 1.

2.5. MTT assay

To evaluate cell viability, 48h after transfection, 10000 MCF-7 cells were seeded in each well of 96-well plates. After 24 h, 48 h, and 72 h, MTT dye (Sigma-Aldrich) was added to wells, and cells were incubated for 3 h. After disposal of the supernatant, DMSO was added to dissolve formazan. Microplate spectrophotometer was used to perceive the absorbance at 570 nm.

2.6. Cell migration by wound–healing assay

A wound healing assay was implemented to assess cell migration capacity. MCF-7 cells were seeded at the density of 5×10^5 cells per well in a 6-well plate and transfected with siRNA. After 48h, a 200-μL pipette tip was expended to scratch wounds when cell confluence arrived at 80–90 %. After washing with PBS, an FBS-free RPMI medium was adjoined to fade the interference effect of cell proliferation. The healing wounds were photographed two times at 0 h and 24 h after scratching.

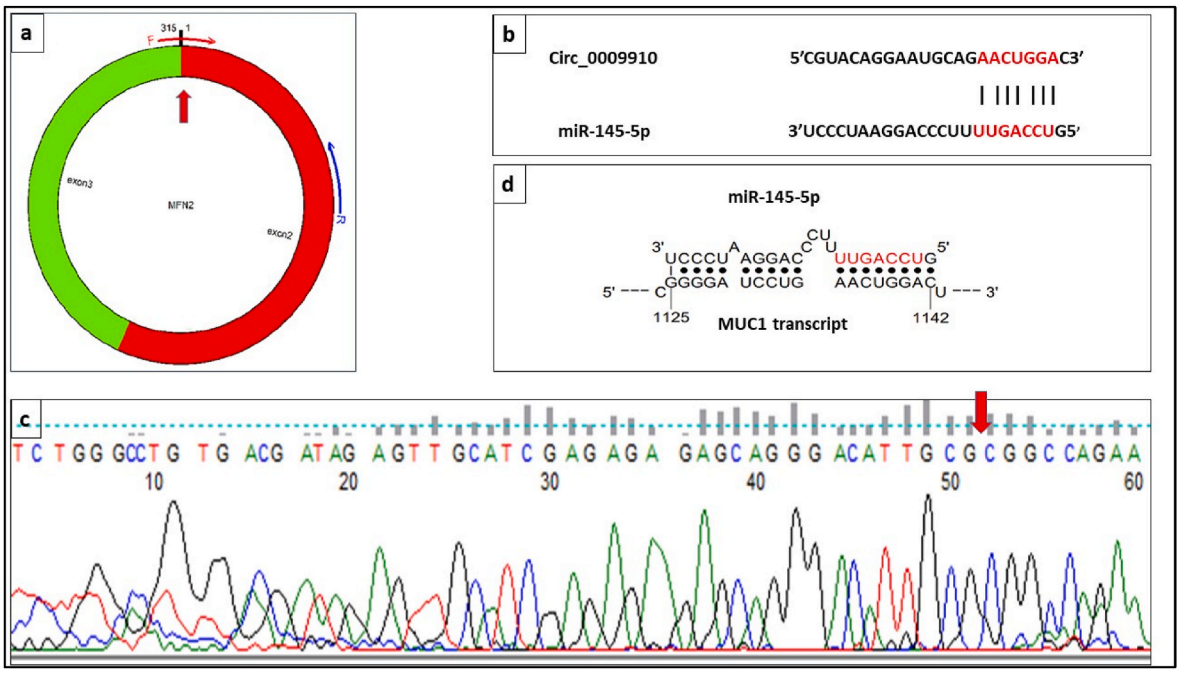

Fig. 1. a. Divergent forward primer for circ_0009910 junction site. b. Complementary sequences of miR-145 and circ_0009910 (circinteractome.nia.nih.gov). c. Junction site of circ_0009910 in Sanger sequencing chromatogram. d. complementary interaction site of the miR-145-5p and its target MUC1 (starmir database).

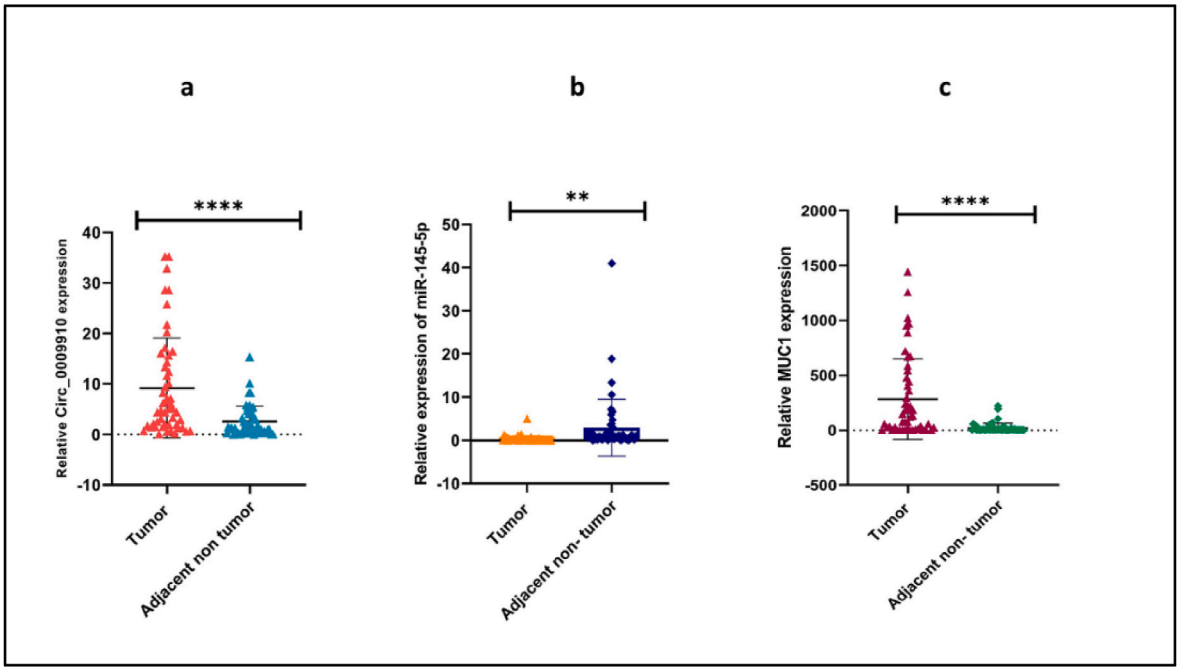

Fig. 2. a. Relative expression of circ_0009910 in breast cancer *vs*. control tissues. The expression level of circ_0009910 in breast tumor tissues was 3.65-fold higher than matched non-tumor tissues (P value < 0.0001). b. Relative expression of miR-145-5p in breast cancer *vs*. control tissues. The expression level of miR-145-5p in breast tumor tissues was 7.4-fold lower than matched non-tumor tissue (P value < 0.01). c. Relative expression of MUC1 gene in breast cancer *vs*. control tissues. The expression level of MUC1 in breast tumor tissues was 15.1-fold higher than that of non-tumor ones (P value < 0.0001).

2.7. Statistical analysis

Statistical analysis was done by GraphPad Prism 8.4.3 and IBM SPSS statistic v.26 software. Relative expression of circ_0009910, miR-145-5p, and MUC1 were compared between breast tumors and adjacent normal tissues using the paired sample *t*-test. The correlation of expression of these genes was computed *via* the Spearman correlation coefficient. The relationship between the expression of circ_0009910 and clinicopathological characters of patients was determined by Mann-Whitney and one-way ANOVA assessments (Kruskal-Wallis). Moreover, the charts of the MTT assay, wound healing assay, and receiver operating characteristic (ROC) curve were displayed by the GraphPad Prism v.8.4.3 software. The p-value <0.05 was contemplated to describe the statistical significance in all of the amounts.

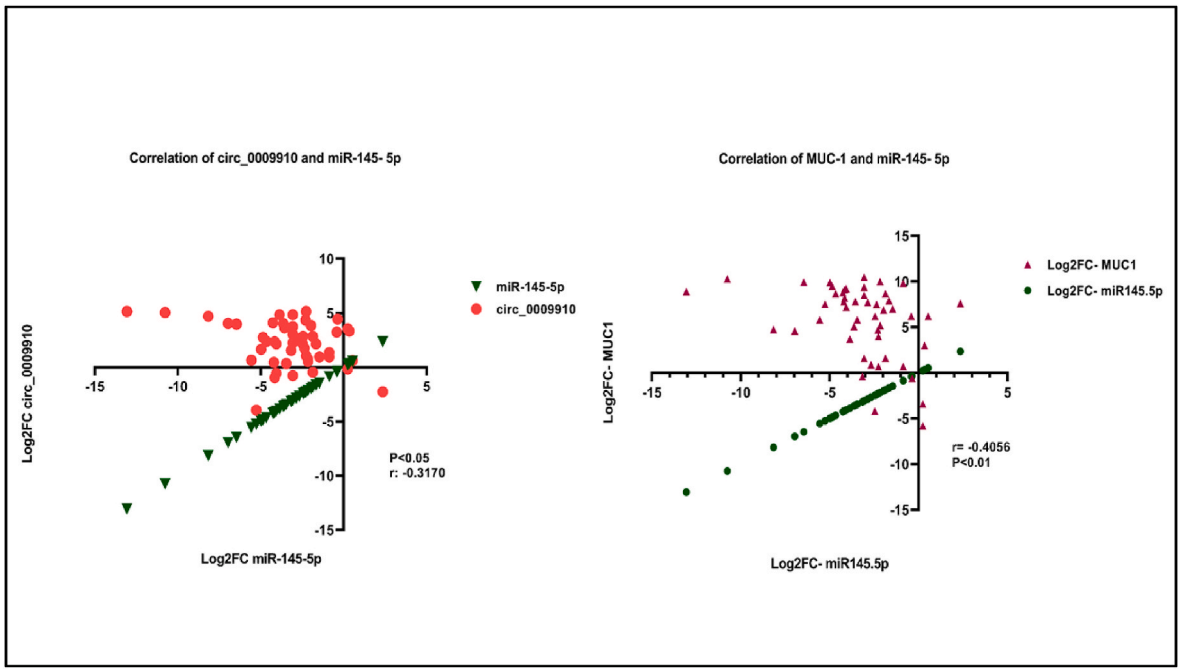

Fig. 3. a. Spearman correlation of circ_0009910 and miR-145-5p (P value < 0.05). b. Spearman correlation of miR-145-5p and MUC1 (P value < 0.01).

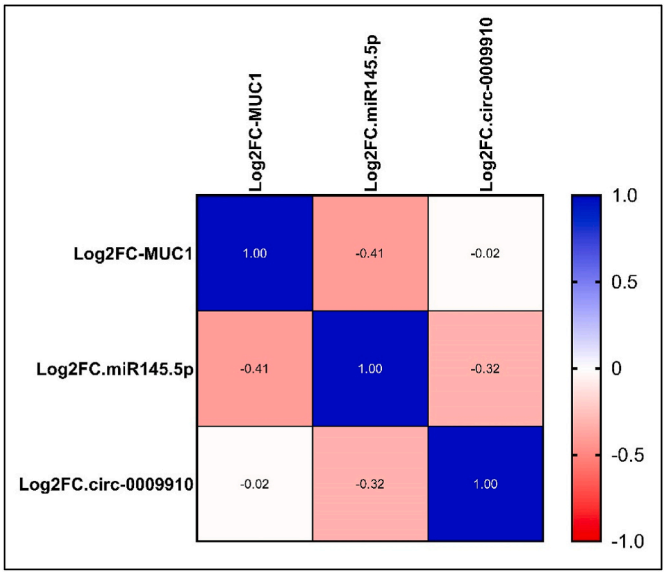

Fig. 4. Heat map demonstration of Spearman correlation of circ0009910-miR-145-5p- MUC1 (P value < 0.05 for circ0009910/miR-145-5p and miR-145-5p/MUC1 correlations and P value > 0.05 for circ0009910/MUC1 correlation).

3. Results

3.1. Confirmation of the sequence of junction site of circ_0009910

Fig. 1 shows the complementary sites for a divergent forward primer for circ_0009910 junction site and confirmation of the sequence of junction site of circ_0009910 by Sanger sequencing (Fig. 1 a and c). Based on the circBase database (https://circbase.org/), circ_0009910 is derived from back-splicing of exons 2 and 3 of the MFN2 gene, and is located at chr1:12049221-12052747 with 315 bp spliced sequence length.

3.2. Overexpression of circ_0009910 in breast cancer tissues

To uncover the primary function of circ_0009910 in breast cancer progression, circ_0009910 expression profile was investigated in 50 breast tumors and self-matched non-tumor adjacent tissues by qRT-PCR. This experiment showed a significantly increased expression level of this circRNA in the breast cancer tissues compared with controls (Fig. 2a, P value < 0.0001).

3.3. Correlation between circ_0009910 expression and expression levels of miR-145-5p and MUC1

The expression profile of miR-145-5p and MUC1 was also assessed in 50 paired samples. miR-145-5p was downregulated (Fig. 2b, P value < 0.01) and MUC1 was upregulated (Fig. 2c, P value < 0.0001) in tumor tissues compared with controls. Correlation analysis between expression amounts of circ_0009910, miR-145-5p, and MUC1 was judged by the Spearman correlation coefficient. Our results displayed a significantly negative correlation between miR-145-5p and circ0009910 ($r = -0.3170$ and P value < 0.05, Fig. 3a). Furthermore, our analysis showed a negative correlation between miR-145-5p and MUC1 levels ($r = -0.4056$ and P value = 0.01, Fig. 3b). However, there was no significant correlation between circ0009910 and MUC1 levels ($r = -0.02$ and P value > 0.05) (Fig. 4).

3.4. The significance of circ_0009910, miR-145-5p and MUC1 as biomarkers in breast cancer

We used the expression values of circ_0009910, miR-145-5p, and MUC1 genes in tumor and non-tumor tissues for the illustration of ROC curves. This method tested the potential clinical proficiency of circ_0009910, miR-145-5p, and MUC1 genes in breast cancer as a unique biomarker. This assessment showed that circ_0009910 can be suggested as an appreciated biomarker for breast cancer (AUC = 0.7544; P value < 0.0001) with 78 % sensitivity and 76 % specificity (Fig. 5a). Moreover, in this study, we proposed miR-145-5p (AUC = 0.8524; P value < 0.0001, with 88 % sensitivity and 88 % specificity) (Fig. 5b), and MUC1 (AUC = 0.8166; P value < 0.0001, with 84 % sensitivity and 85 % specificity) (Fig. 5c) as potential strong biomarkers in breast

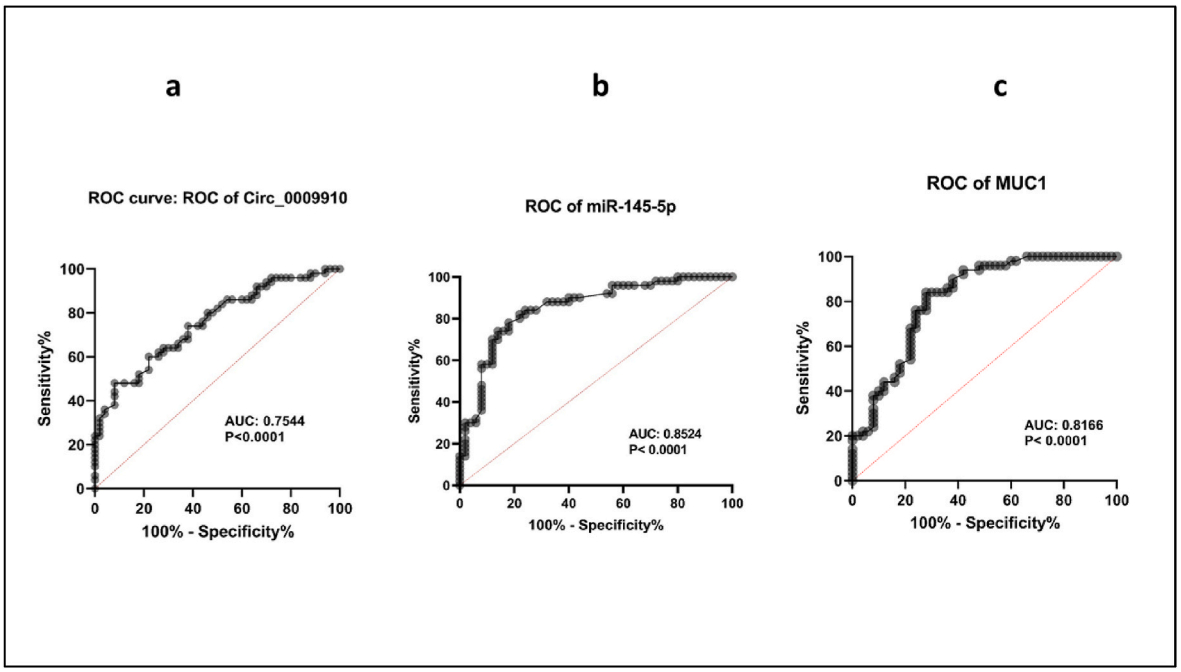

Fig. 5. ROC curves of circ_0009910, miR-145-5p, and MUC1 in breast cancer patients.

Table 2
Correlation of clinicopathological data of breast cancer patients with circ_0009910 expression.

	Subclass	Number of patients	Median of circ_0009910 expression	IQR	The mean of circ_0009910 expression	SD	P value
Age	<45 or 45	15	2.74	1.90	2.63	1.42	0.415
	>45	35	2.13	3.50	1.99	2.22	
Tumor diameter	<2	7	3.03	3.27	2.15	2.26	0.45
	2–5	38	2.50	3.12	2.31	2.05	
	>5	5	2.13	2.90	1.29	1.54	
Invasion to lymph	yes	29	2.8392	2.05	2.6771	2.17	**0.011**
	no	21	1.3392	2.70	1.5121	1.62	
Grade	1	13	3.96	3.70	2.97	2.15	0.1
	2	31	2.23	2.50	1.82	1.98	
	3	6	2.13	2.97	2.34	1.72	
Stage	1	11	3.2	3.27	2.49	2.04	0.81
	2	32	1.93	3.35	1.98	2.16	
	3	6	2.48	2.18	2.7	1.43	
	4	1					
ER	P	41	2.23	3.30	2.16	2.11	0.82
	N	9	2.58	2.93	2.07	1.65	
PR	P	38	2.23	3.30	2.14	2.15	0.84
	N	12	2.48	2.93	2.15	1.68	
HER2	P	7	2.13	2.80	1.76	2.31	0.54
	N	43	2.63	3.23	2.25	1.99	
KI67	<20	19	3.03	3.70	2.75	1.92	0.12
	20 or >20	31	2.13	2.80	1.83	2.03	
Family history	present	13	1.73	2.75	2.05	1.82	0.59
	absent	37	2.63	3.35	2.23	2.11	

cancer.

3.5. Correlation analyses of the expression levels of circ0009910, miR-145-5p, and MUC1 with clinicopathological features of breast cancer patients

The clinicopathological records of patients including age, tumor dimension, invasion to lymph nodes, and grade were documented, and correlations between them and the mentioned gene expression were demonstrated in Tables 2–4, respectively. Clinicopathological analysis indicated that up-regulation of circ_0009910 in breast tumors is related to invasion of the tumor to lymph node (P value = 0.011) (Table 2). Also, the downregulation of miR-145-5p was significantly correlated with tumor invasion to lymph nodes (P value = 0.04) and HER2 negative tumors (P value = 0.037) (Table 3). Finally, overexpression of MUC1 was correlated with age under 45 years (P value = 0.002) (Table 4).

3.6. Circ_0009910-siRNA decreased the proliferation of breast cancer cells

MCF-7 cell proliferation was examined by the MTT assay. As displayed in Fig. 6, the proliferation of the MCF-7 cells transfected with circ_0009910-siRNA was significantly decreased to 73.9 % and 83.6 % (P value < 0.05), 71.4 % and 75.7 % (P value < 0.01) and 68.2 % and 74.5 % (P value < 0.01) at 24, 48 and 72 h, compared to untransfected MCF-7 cells and scramble oligonucleotide transfected cells, respectively.

Table 3
Correlation of clinicopathological data of breast cancer patients with miR-145-5p expression.

	Subclass	Number of patients (%)	The median of miR-145-5p expression	IQR	Mean of miR-145-5p expression	SD	P value
Age	<45 or 45	15	−3.06	1.71	−3.76	2.8	0.27
	>45	35	−2.4	2.55	−2.96	2.6	
Tumor diameter	<2	7	−2.16	3.97	−1.72	2.33	0.39
	2–5	38	−3.1	2.81	−3.54	2.78	
	>5	5	−3.01	2.96	−2.67	1.77	
Invasion to lymph	yes	29	−3.06	2.50	−3.92	2.98	**0.04**
	no	21	−2.36	3.27	−2.20	1.84	
Grade	1	13	−4.06	3.93	−3.96	3.29	0.17
	2	31	−2.86	1.48	−3.05	2.26	
	3	6	−1.16	5.54	−2.29	3.33	
Stage	1	11	−2.45	3.85	−2.69	2.17	0.95
	2	32	−2.9	2.68	−3.44	3.03	
	3	6	−3.31	2.22	−2.82	1.74	
	4	1					
ER	P	41	−3.06	2.57	−3.33	2.87	0.44
	N	9	−2.7	2.25	−2.36	1.47	
PR	P	38	−3.6	2.57	−3.30	2.85	0.6
	N	12	−2.86	2.75	−2.80	2.26	
HER2	P	7	−2.15	1.60	−1.7	1.14	**0.037**
	N	43	−3.1	2.35	−3.4	2.7	
KI67	<20	19	−3.06	2.95	−3.67	3.68	0.39
	20 or >20	31	−2.46	2.20	−2.91	1.83	
Family history	present	13	−3.03	4.25	−3.80	4.24	0.8
	absent	37	−3.06	2.22	−2.98	1.90	

Table 4
Correlation of clinicopathological data of breast cancer patients with MUC1 expression.

	Subclass	Number of patients	Median of MUC1 expression	IQR	Mean of MUC1 expression	SD	P value
Age	<45 or 45	15	8.49	2.00	8.28	1.55	**0.002**
	>45	35	5.79	6.30	4.72	4.24	
Tumor diameter	<2	7	7.89	4.33	7.38	2.50	0.45
	2–5	38	6.54	5.49	5.54	3.86	
	>5	5	7.19	9.65	5.452	6.51	
Invasion to lymph	yes	29	7.79	4.35	6.72	2.95	0.10
	no	21	6.19	8.00	4.49	4.87	
Grade	1	13	6.89	5.50	5.93	3.89	0.9
	2	31	7.52	7.30	5.59	4.33	
	3	6	6.59	4.29	6.48	2.42	
Stage	1	11	6.19	6.40	5.53	4.56	0.40
	2	32	7.09	4.47	5.73	3.93	
	3	6	6.99	7.70	5.79	3.69	
	4	1					
ER	P	41	6.89	6.01	5.40	4.19	0.26
	N	9	8.14	3.87	7.36	2.37	
PR	P	38	6.99	4.81	5.75	3.87	0.8
	N	12	6.9	5.74	5.66	4.57	
HER2	P	7	3.99	6.90	3.66	4.50	0.11
	N	43	7.49	4.20	6.13	3.84	
KI67	<20	19	7.19	5.20	6.16	3.66	0.6
	20 or >20	31	6.99	4.66	5.55	4.21	
Family history	present	13	7.52	2.78	6.94	2.88	0.33
	absent	37	6.19	5.60	5.38	4.27	

No significant difference was detected between the untransfected MCF-7 cells and those transfected with scramble (P value > 0.05).

3.7. Circ_0009910 may be involved in MCF7 cell migration

To identify the roles of circ_0009910 in the migration and progression of breast cancer MCF7 cells, we performed a wound healing assay. In these assays, circ_0009910-siRNA transfected MCF7 cells showed a significant decrease in wound healing rate due to a reduction of cell migration ability compared with the scramble treated or untransfected cells after 24h (P < 0.05) (Fig. 7). There was no significant difference between scramble transfected cells and untreated cells (P value > 0.05).

3.8. Downregulation of circ_0009910 was correlated with the up-regulation of miR-145-5p and downregulation of MUC1 expression in breast cancer cells

To explore the role of circ_0009910 in breast cancer, siRNA-mediated knockdown of circ_0009910 gene was accomplished in the MCF-7 breast cancer cell line. Then, expressions of circ_0009910, miR-145-5p and MUC1 were compared between siRNA transfected cells, scramble transfected cells, and untreated control cells. Circ_0009910 expression was significantly downregulated in MCF-7 following treatment with siRNA (0.31 and 0.177 after 24h and 48h, respectively (P value < 0.01) (Fig. 8a). Circ_0009910-siRNA was found to induce significant upregulation of miR-145-5p (2.65 fold in 24h and 2.95 fold in 48h, P value < 0.001) (Fig. 8b). Finally, MUC1 expression was not changed in time point 24h, but after 48h it was significantly

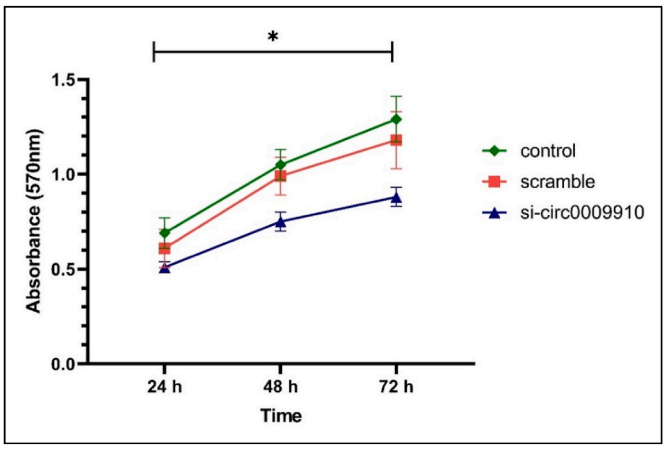

Fig. 6. MTT assay. Decrease in MCF-7 cell proliferation following transfection with circ_0009910-siRNA (P value < 0.05). Data from one of three independent trials is demonstrated.

downregulated compared to untransfected cells (fold change = 0.81, P value < 0.05) (Fig. 8c).

4. Discussion

CircRNAs play a key part in the progression of breast cancer [22]. For instance, circ_0001667 targeting miR-6838-5p/CXCL10 axis in breast cancer, is represented as a therapeutic biomarker in breast cancer because of its overexpression in tumor tissues [6]. Also, circDNAJC11 has been suggested as a predictive biomarker for breast cancer treatment depending on its high levels in malignant samples [2]. Circ_0001785 amounts have been described to be powerfully associated with histological grading, TNM staging, and metastasis, offering a reliable biomarker for breast cancer detection [23]. Furthermore, circ_0008673 is a therapeutic target for breast cancer, and is linked to distant metastases, tumor size, and estrogen receptors status [24].

The current study investigated the importance of circ0009910/miR-145-5p/MUC1 axis in the pathogenesis of breast cancer through functional cell line analyses and expression assays in clinical samples. These experiments revealed up-regulation of circ0009910 and MUC1, parallel with down-regulation of miR-145-5p. Moreover, correlation analyses confirmed a significantly negative correlation between miR-145-5p and circ0009910 as well as between miR-145-5p and MUC1 levels, which further support the existence and functionality of circ0009910/miR-145-5p/MUC1 axis in breast cancer. We also assessed the ability of the mentioned transcripts to separate malignant tissues from non-malignant ones through ROC curve examination. The highest AUC value was obtained for miR-145-5p highlighting its potential as a tissue tumor marker. It is worth mentioning that circulatory levels of this miRNA have been proven to efficiently discriminate breast cancer patients from healthy controls [25].

Among clinicopathological characteristics, we found correlations between circ_0009910 levels and invasion of the tumor to the lymph node. Similarly, the downregulation of miR-145-5p was significantly correlated with tumor invasion of lymph nodes. These verdicts are consistent with the oncogenic role of circ_000910 and the tumor suppressor role of miR-145-5p in breast cancer. Down-regulation of miR-145-5p in breast cancer tissues has been earlier reported to be associated with larger tumor size, distal metastases, and high Ki67 expression [19]. Moreover, this miRNA has been shown to decrease breast cancer cell migration and invasion [26].

We also reported a correlation between the downregulation of miR-145-5p and HER2 negative status. An earlier study has reported an association between MUC1-C silencing and higher sensitivity of HER2-positive cells to trastuzumab-induced growth inhibition [27]. Because MUC1 is a direct target of miR-145-5p, this finding may have a practical significance.

Besides, overexpression of MUC1 was correlated with lower age of breast cancer incidence in our study. Contrary to our study, a previous study has demonstrated higher levels of MUC1 in tumors of patients aged more than 51 years, versus patients aged ≤51 years [28].

We also assessed the effects of siRNA-mediated down-regulation of circ_0009910 on the migration and viability of MCF-7 cells. Consistent with the oncogenic roles of this circRNA in other types of cancers, migration and viability of MCF-7 cells were reduced after its silencing. Moreover, expression assays in MCF-7 cells confirmed the supposed

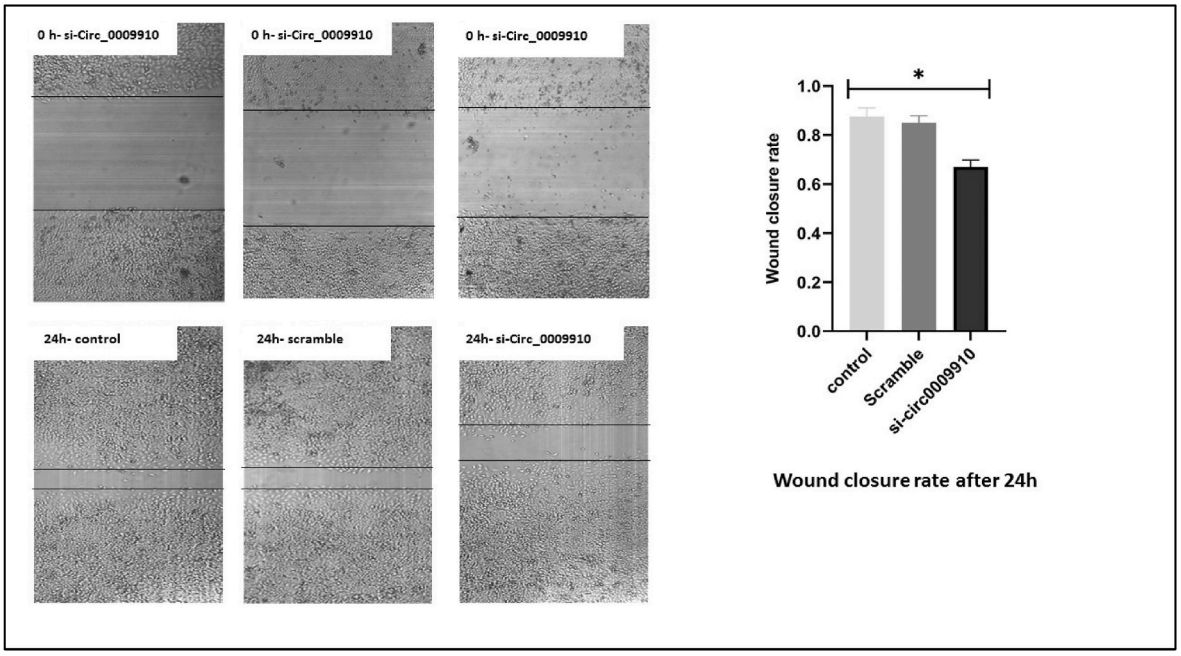

Fig. 7. Wound healing assay. There was a 17.7 % decrease (P < 0.05) in wound closure of circ_0009910-siRNA-transfected MCF7 cells compared with untransfected or scramble-transfected ones.

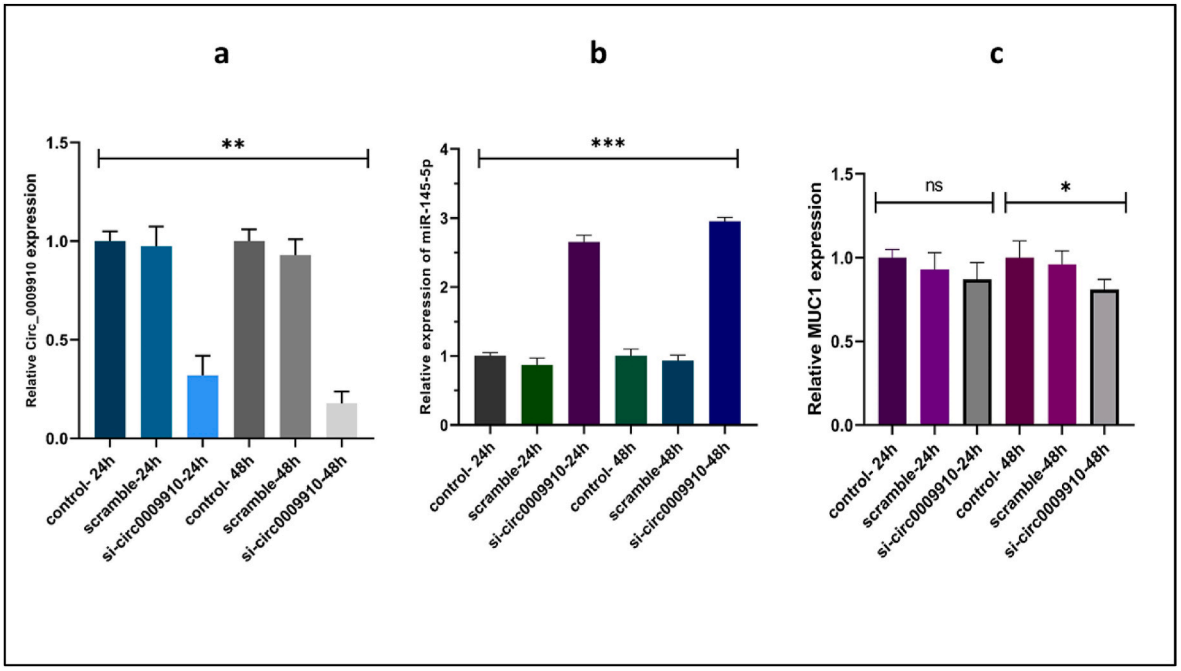

Fig. 8. Relative expression of a. circ_0009910, b. miR-145-5p and c. MUC1 after transfection with circ_0009910-siRNA in 24 and 48h after transfection.

correlations between this circRNA, miR-145-5p, and MUC1.

Cumulatively, our study supports the role of circ_0009910/miR-145-5p/MUC1 axis in the pathogenesis of breast cancer.

CRediT authorship contribution statement

Maryam Abtin: Writing – original draft, Methodology, Formal analysis, Data curation. **Nahid Nafisi:** Data curation. **Asghar Hosseinzadeh:** Conceptualization. **Sepideh Kadkhoda:** Formal analysis. **Ramesh Omranipour:** Data curation. **Leyla Sahebi:** Investigation. **Masoumeh Razipour:** Conceptualization. **Soudeh Ghafouri-Fard:** Writing – review & editing, Conceptualization. **Abbas Shakoori:** Validation, Supervision, Conceptualization.

Declaration of competing interest

Authors declare no conflicts of interests.

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