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Probing into the Network of Transcription Factor Twist-Related Protein 1 (*TWIST1*) in Breast Cancer Metastasis

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Abstract

Background: The transcription factor twist-related protein 1 (*TWIST1*) plays a major role in the prognosis of breast cancer. Our present study aimed to identify the network of *TWIST1* with related oncogenes and their associated miRNAs.

Method: This in silico study included the differential expression analysis of genes and miRNA associated with breast carcinoma. The breast cancer patients' data were retrieved from the Gene Expression Omnibus database and the differential expression analysis was done using GEO2R. Transfac analysis was performed to determine the binding sites of *TWIST1*. We predicted the target genes of MicroRNA-96 (miR-96) using miRBase. An integrated network was generated among *TWIST1* and target genes of miR-96 through Gene MANIA. Survival analysis was carried out for *TWIST1* using UALCAN. Experimental methods, including gene expression analysis, were performed in the MDA-MB-231 cell line for validating in silico findings.

Results: miR-96, the second differentially expressed miRNA among the top 250 miRNAs, was found to have eight binding sites for *TWIST1*. *TWIST1* was observed to be significantly correlated with patient prognosis. ACTN4, BCL2, and *FRMD4A* were upregulated and *CAMTA1*, *DAB2IP*, and E- Cadherin were downregulated in the expression studies carried out in the MDA-MB-231 breast cancer cell line.

Conclusion: A network between *TWIST1* and target genes of miR-96 was analyzed. Hence, targeting the genes linked with miR-96 could work toward an efficient therapeutic option for breast cancer metastasis.

Keywords: Twist-related protein 1, Gene expression, Omnibus datasets, MicroRNA-96, Survival analysis, MDA-MB-231 breast cancer cells

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Introduction

Breast cancer is one of the most prevalent invasive cancers found in women.[1] Several treatment options, namely mastectomy, radiation therapy, and chemotherapy, are currently available for breast cancer and these treatment options affect the normal cells too. Targeted therapy is one way of alleviating the

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undesirable side-effects of chemotherapy. Treatment of breast cancer at its early stage requires a combinational approach.[2] Few of the oldest methods of targeted therapy include targeting the receptors, such as estrogen receptor with tamoxifen, HER2 (human epidermal growth factor receptor with trastuzumab and vascular endothelial growth factor (VEGF) with bevacizumab, and so on.[3-5] Given the disappointing results of these combinations in the clinical studies, researchers are looking forward to identifying different molecular targets for treating breast cancer.[6]

One of the targets sought to achieve better control over metastatic spread is transcription factors.[7] Twist-related protein 1 (*TWIST1*) is one of such transcription factors which is overexpressed in breast, prostate, lung, and uterine cancer metastasis. *TWIST1*, belonging to the bHLH protein family, plays a significant role in cancer metastasis.[8] Upregulation of *TWIST1* promotes cancer cell invasion due to the loss of *E-Cadherin*. Thus *E-Cadherin* and *TWIST1* serve as potential markers to determine the metastatic potential of cancer cells. *TWIST1* also prevents cancer cell death through apoptosis, gives resistance to chemotherapeutic drugs, and controls the expression of many genes by regulating microRNAs .[9]

MicroRNAs (miRNAs) are small non-coding RNA molecules that are mostly involved in the modulation of gene expression, controlling cell growth, differentiation, and cell death mediated by transcription factors.[10-12] miRNAs are reported to be majorly involved in cancer progression by acting as tumor suppressors and oncogenic factors. For example, miR-10b which was highly expressed in breast cancer cells positively regulated the invasion and cell movement of tumor cells.[13] Many such miRNAs were found to be overexpressed in different cancers, but only a few of them are well characterized.[14] miR-96 is one such oncogenic microRNA that suppresses the expression of tumor-suppressing genes.[15] Complete information about miRNAs could be especially useful for an effective therapeutic

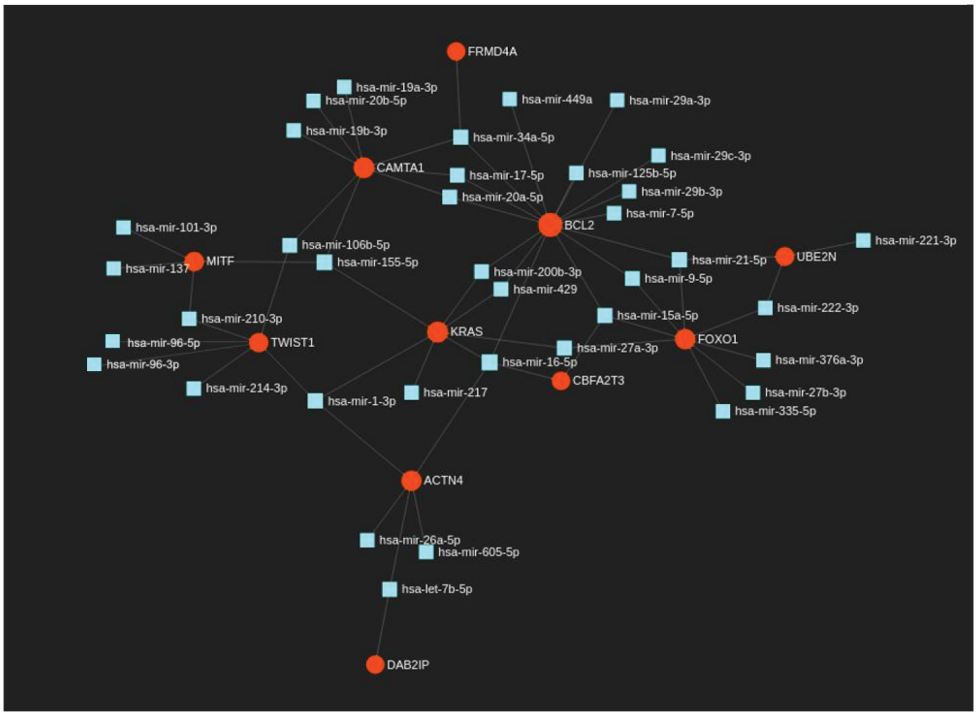

Figure 1. Top 20 differentially expressed miRNA and their target mRNA interaction network constructed using miRNet. The interaction between the genes *TWIST1* and ACTN4, *CAMTA1*, BCL2, *FRMD4A*, *DAB2IP*, FOXO1 along with the microRNAs regulating the *TWIST1* gene were observed from this miRNA-mRNA interaction network. The mRNA is represented in its human gene nomenclature form.

TWIST1: Twist-related protein 1; BCL2: B-cell lymphoma 2; *FRMD4A*: FERM domain-containing protein 4A; ACTN4: Alpha-actinin-4; *DAB2IP*: DAB2 Interacting protein; *CAMTA1*: Calmodulin binding transcription activator 1; FOXO1: Forkhead box protein O1

approach to breast cancer.

Understanding the correlation between transcription factors (TFs) and their associated miRNAs would provide a closer view of multiple levels of gene regulation and their biological functions.[16, 17] Though several studies have reported the regulatory role of *TWIST1* with miRNAs, the systematic correlation of *TWIST1* and miR- 96 in breast cancer progression is unexplored yet. Therefore, our study aims at identifying gene targets and exploring the functional correlation of *TWIST1* and its associated miRNAs in breast cancer metastasis.

Materials and Methods

In the current study, systematic bioinformatics analysis has been carried out for identifying the significant miRNA and mRNA in breast cancer progression. Further, the regulatory role of significant mRNA on the candidate miRNA targets was explored using experimental methods like gene expression studies.

Dataset collection

Expression datasets of miRNA and mRNA were obtained from GEO.[18] The miRNA data set GSE26659, "microRNA and cancer progression in breast cancer" with microarray platform (GPL8227, Agilent-019118 Human miRNA Microarray 2.0) and mRNA data set GSE30480 "Gene expression profile of purified tumor cells from primary breast cancer tumor and metastatic lymph nodes" with microarray platform (GPL6480, Agilent-014850 Whole Human Genome Microarray 4×44K) were used for the study. We downloaded the data and performed differential expression analysis through the GEO2R interface with *P*-values adjusted with the Benjamini and Hochberg (False Discovery Rate) method.

In the miRNA dataset, the control group was set to include normal breast mammoplasty tissue samples and the test group was set to have samples that were derived from breast cancer tissues and stage 3 - lymph node-positive regions. Genomic sequences of miRNAs were retrieved from the UCSC browser. Information about chromosome number, start and end sites, ID, and miRNAs were obtained from miRBase. The table browser

```
Search for sites by WeightMatrix library: matrix.dat
Sequence file: vgs.seq
Site selection profile: sv1twist.prf prf to minimize false
positives

Inspecting sequence ID   hg19_ct_UserTrack_3545_hsa-miR-96

 V$TWIST_Q6              |     5653 (-) |  1.000 |  1.000 |
ccAGGTG
 V$TWIST_Q6              |     5750 (-) |  1.000 |  1.000 |
ccAGGTG
 V$TWIST_Q6              |     9139 (+) |  1.000 |  1.000 |
CACCTgg
 V$TWIST_Q6              |    12902 (+) |  1.000 |  1.000 |
CACCTgg
 V$TWIST_Q6              |    15752 (+) |  1.000 |  1.000 |
CACCTgg
 V$TWIST_Q6              |    19814 (-) |  1.000 |  1.000 |
ccAGGTG
 V$TWIST_Q6              |    20357 (+) |  1.000 |  1.000 |
CACCTgg
 V$TWIST_Q6              |    22476 (+) |  1.000 |  1.000 |
CACCTgg

 Total sequences length=50077

 Total number of found sites=8

 Frequency of sites per nucleotide=0.000160
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Figure 2. *TWIST1* binding site on hsa-miRNA-96. miR-96 (Second differentially expressed miRNA among the top 25) has eight binding sites for *TWIST1* and the frequency of the identified sites per nucleotide is 0.000160.
TWIST1: Twist-related protein 1

option in UCSC Genome Browser for Human Genome Build hg19 was utilized to retrieve the repeat-masked, genomic sequence (TSS ± 25 kb) for each miRNA ID entry. The miRNAs were filtered based on log FC value and taken for TRANSFAC analysis.

Network generation

A network connecting the top 20 differentially

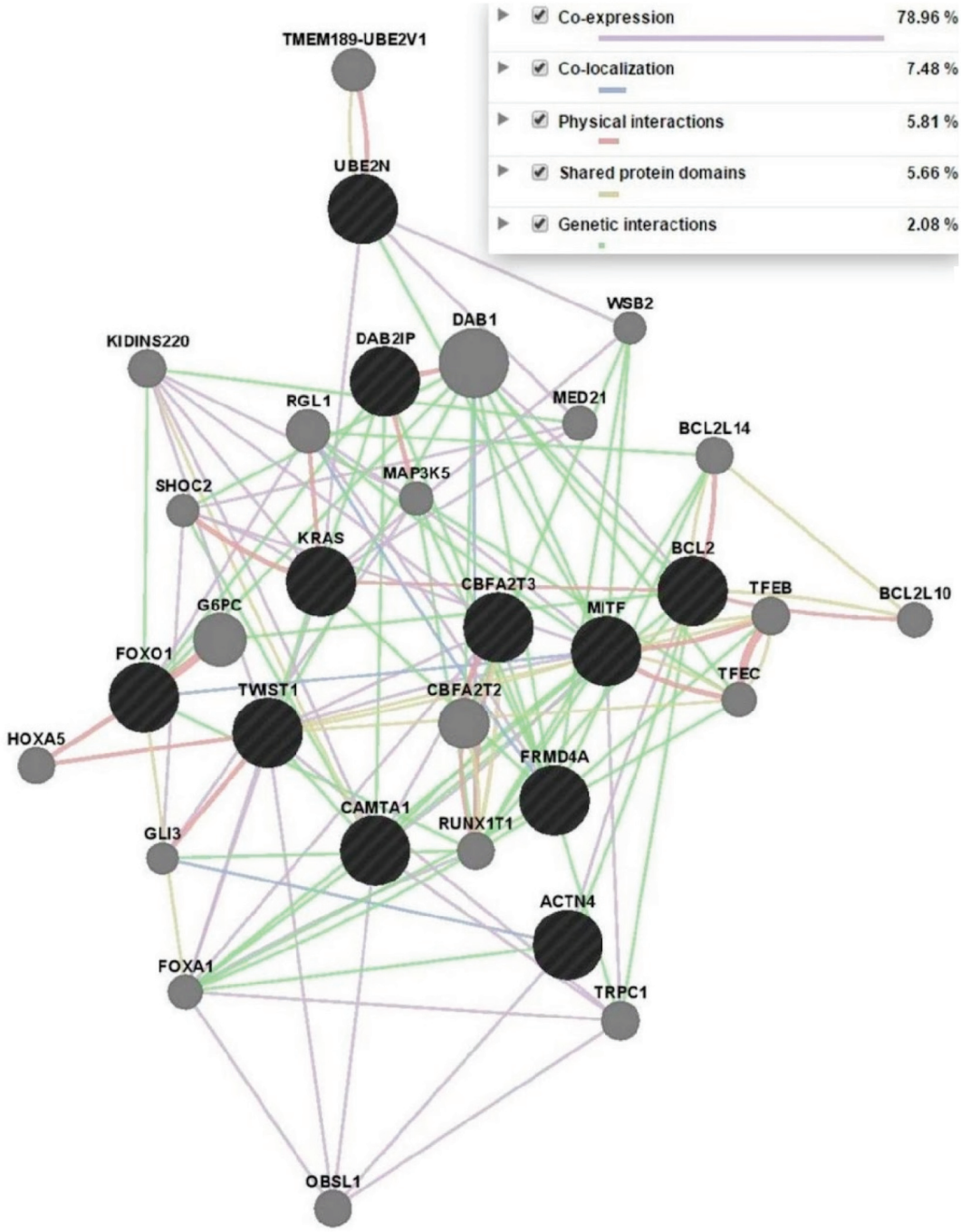

Figure 3. In-silico protein-protein interaction network of *TWIST1* and miR-96, the target genes were constructed using Gene mania at the highest confidence (0.9). Significantly interacting genes are highlighted in black color. The target genes are represented in their human gene nomenclature form.

TWIST1: Twist-related protein 1; BCL2: B-cell lymphoma 2; *FRMD4A*: FERM domain-containing protein 4A; ACTN4: Alpha-actinin-4; *DAB2IP*: DAB2 interacting protein; *CAMTA1*: Calmodulin binding transcription activator 1

Table 1. Primer sequences used for qRT-PCR

Primer name	Sequence	Product size (bps)	Annealing temperature (°C)
TWIST1-F	GGAGTCCGCAGTCTTACGAG	201	59
TWIST1-R	TCTGGAGGACCTGGTAGAGG		
E-Cadherin-F	GCTGCTCTTGCTGTTTCTTCG	108	59
E-Cadherin-R	CCGCCTCCTTCTTCATCTAG		
GAPDH-F	TGCCTCCTGCACCACCAACT	300	55
GAPDH-R	CTTCCACCACTTCGTCCG		
CAMTA1-F	AGTGCAGAAAATGAAGAATGCG	115	59
CAMTA1-R	CAAAATTCTCCTGCTTGATTCG		
ACTN-F	CATATCAGGGAGCGGTT	102	59
ACTN-R	GCAATAAAGTCCAGCGCT		
DAB2IP-F	TCCACACAGCACTGAGCAC	301	59
DAB2IP-R	ACCATGGAGAGGCTCTTGC		
FRMD4A-F	TGGCTTCTCACTTCAATCT	134	59
FRMD4A-R	CCACGGGTCCTGACTTTT		
BCL2-F	CCTGTGGATGACTGAGTACC	127	59
BCL2-R	GAGACAGCCAGGAGAAATCA		

F: Forward primer; R: Reverse primer; qRT-PCR: Quantitative real-time polymerase chain reaction; *TWIST 1*: Twist-related protein 1

expressed miRNA with its target mRNA was built using an online tool miRnet.[19] We conducted a TRANSFAC analysis to determine the binding sites of *TWIST1* on selected miRNAs. Seven miRNAs had more than eight binding sites for *TWIST1* and miR-96 was selected further for network generation. Validated targets of mir-96 were obtained from mirTarbase.[20] By comparing the targets obtained from the miRTarBase and mRNA expression dataset, the candidate mRNA targets for miR-96 were picked up. An in-silico network was generated between *TWIST1* and the target genes of miR-96 using the Gene Mania tool.[21]

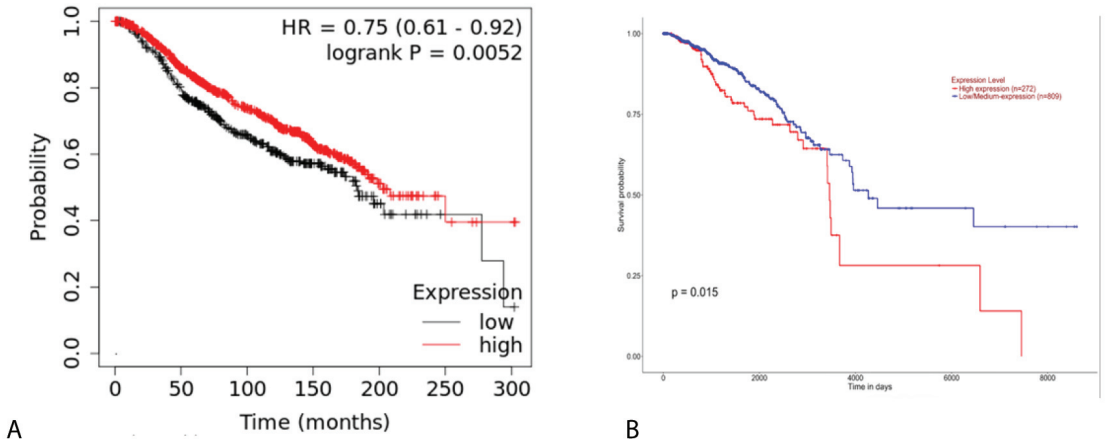

Figure 4. Survival analysis on the effect of miR-96 and *TWIST1* expression levels in BRCA patient survival A. Kaplan–Meier graph for miR-96 (Significant at $P \leq 0.05$) [high expression (n = 839) and low expression (n = 423)] B. Survival plot of *TWIST1* (Significant at $P \leq 0.05$) [high expression (n = 272) and low expression (n = 809)].
HR: Hazard ratio; *TWIST1*: Twist-related protein 1

Survival analysis

Survival analysis was carried out for the target genes of miR-96 and *TWIST1*. The analysis was performed with an online user-friendly interactive web resource UALCAN, which is integrated with OMICS data (TCGA and MET500). Moreover, 1097 patient samples and 114 control samples were employed to predict the level of their significance on the survival of breast cancer patients. Significant mRNAs were identified by examining the survival plots.[22] Kaplan-Meier graph was plotted for miR-96 with data from 1262 patients from the dataset METABRIC for assessing the significance of miR-96 on patient's survival using the online KM plotter server.[23]

Cloning of human TWIST-1 gene

Genomic DNA was isolated from cell lines (MDA-MB-231 breast cancer cells) with salting-out method.[24] The purity of the isolated genomic DNA was quantified in a nanodrop spectrophotometer with a lid factor of 10. We designed the forward (CGCGGATCCGCGATGATGCAGG ACGTGTCC) and the reverse primers (CCG-GAATTCCGGCTAGTGGGACGCGGACAT) for the *TWIST1* gene with restriction sites for gene-specific polymerase chain reaction (PCR). The amplified product was purified using Hi-Yield Gel/PCR DNA mini kit (HiMedia). The expression vector pcDNA3 Enhanced Green Fluorescent Protein (Invitrogen) was subjected to double digestion with EcoR1and BamH1. We then inserted the purified gene product and ligated it in a 1:3 ratio with the restricted vector (pcDNA3 EGFP). The mixture was used for transformation

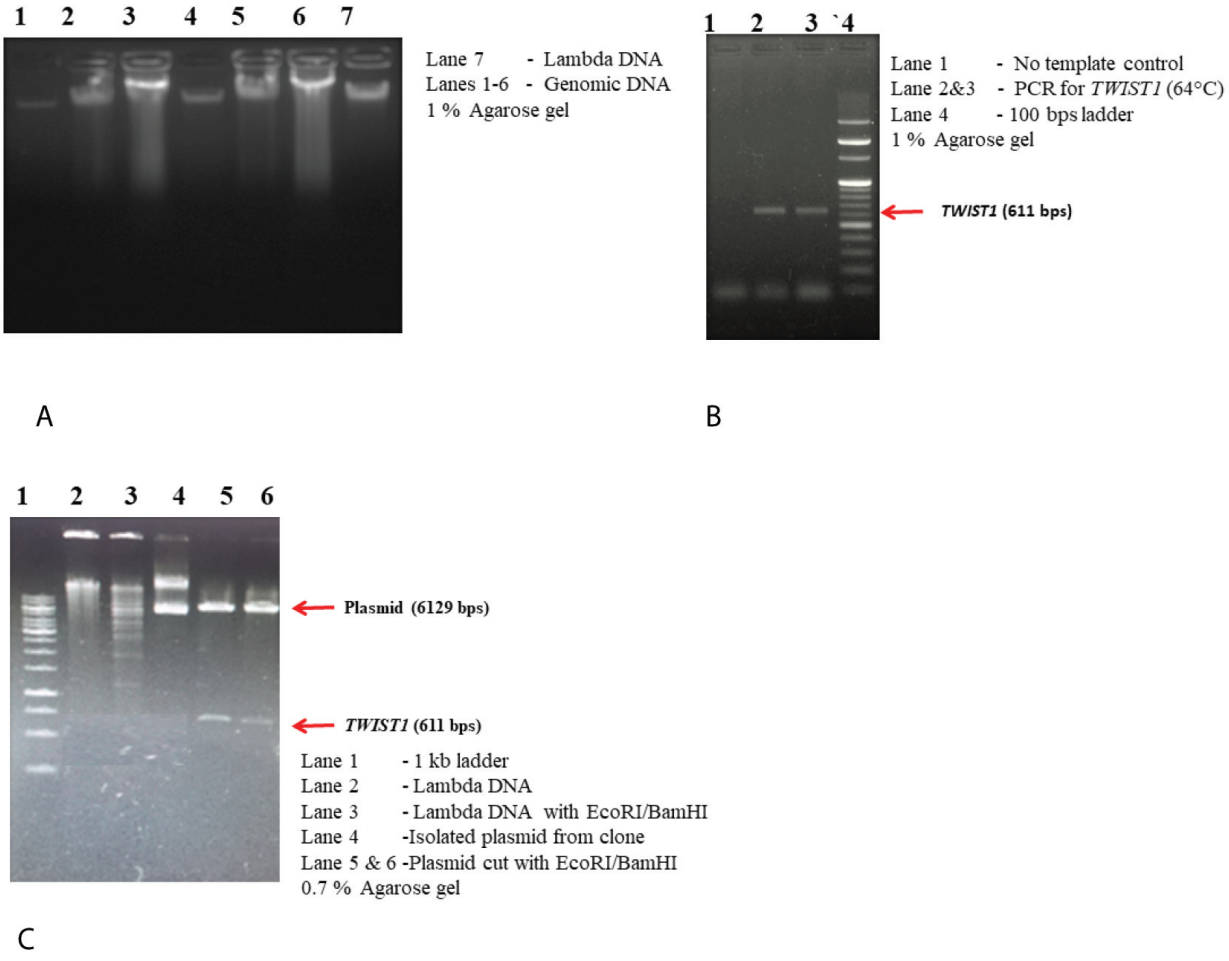

Figure 5. Cloning of human *TWIST1* gene. A. Genomic DNA run on 1 % agarose gel. B. PCR Amplification of human *TWIST1* gene (611 bps). C. Confirmation of the insert in the plasmid by double digestion.
Bps: Base pairs; kb: Kilobase; PCR: Polymerase chain reaction; *TWIST1*: Twist-related protein 1

in a competent *E.Coli* DH5α strain and was grown on ampicillin positive Luria Bertani (LB) plates. Transformed colonies were then picked up and colony PCR was performed for amplification of *TWIST1*. Double digestion of the isolated plasmid was carried out to confirm the successful cloning of the human *TWIST1* gene. The vector was then linearized using HindIII and transfected into MDA-MB-231 cells with Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. The cells were checked for green fluorescent protein expression after 24 hrs. The transfected MDA-MB-231 cell lines were maintained in a selection medium (geneticin - 500µg/ml) for generating a stable cell line expressing *TWIST1*.

Quantitative RT-PCR for network validation

Total RNA was isolated from MDA-MB-231 control and transfected cells via the trizol method. cDNA synthesis was carried out using Thermo scientific Revert Aid first-strand cDNA synthesis kit. RT-PCR (Applied Biosystems ABI Step One plus PCR system) was performed with the isolated cDNA samples using the primers listed in table 1. We analyzed the C_t values for the control and transfected samples for each gene and performed a comparative analysis to find the relative quantity of DNA present in each sample using GAPDH's Ct value as the internal control for normalization. Six major target genes (*ACTN4, BCL2, FRMD4A, CAMTA1, DAB2IP, E- Cadherin*) of *TWIST1* were analyzed for their expression in the *TWIST1* overexpressed cells and control cells. The quantity of gene expression was calculated using the formula:

Relative quantity to the control = $2^{-(\Delta\Delta Ct)}$

,Where $^{\Delta\Delta}Ct = (C_{tGOI} - C_{tnorm})$ treated - $(C_{tGOI} - C_{tnorm})$ control, GOI- Gene of Interest and normalizer (GAPDH).[25]

Ethical approval

The experimental studies were performed with the established breast cancer cell lines (MDA-MB-231) and hence do not necessitate ethical approval.

Results

Network generation

Top 250 differentially expressed miRNAs across the control and metastatic breast tumor samples were obtained. The top 25 differentially expressed miRNAs were sorted according to their log FC values and are listed in table 2. An interconnecting network was created between 20 differentially expressed miRNAs and their target mRNAs using miRNet. The network is represented in figure 1.

TRANSFAC analysis was carried out for

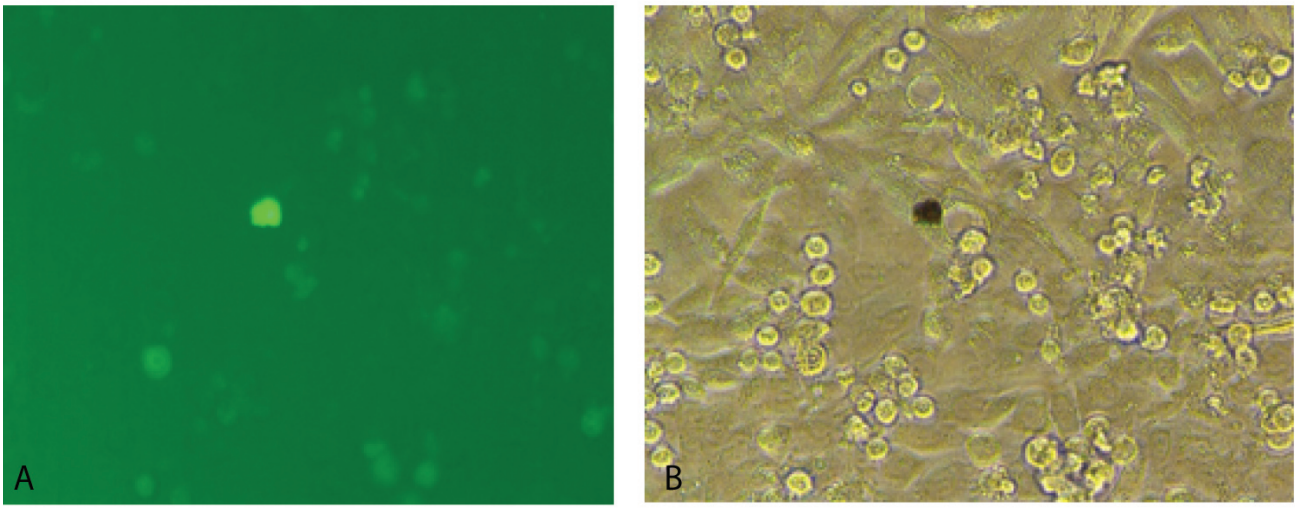

Figure 6. Transient transfection of *TWIST1* in MDA-MB-231 breast cancer cells. A. MDA-MB-231 cells expressing green fluorescent protein indicating the successful transfection of the vector B. Transfected MDA-MB-231 observed under Phase Contrast Microscope (400×).

TWIST1: Twist-related protein 1

analyzing the binding site of *TWIST1* among the top 250 differentially expressed miRNAs. We observed that miR-21 (first differentially expressed miRNA) had no binding sites for *TWIST1* whereas miR-96 has eight binding (Second differentially expressed miRNA) sites for *TWIST1*. Hence, further studies were carried out with hsa-miR-96. Binding sites of *TWIST1* on miR-96 are represented in figure 2. Figure 3 illustrates the interaction between the target genes of miR-96 and *TWIST1*. Some of the linkage parameters, such as co-expression and genetic and physical interactions, were also highlighted in the above-generated network with different color codes.

Survival analysis

Breast cancer patient survival analysis was carried out for the genes using the UALCAN database. $P < 0.05$ was considered a significant score. Among the six genes (ACTN4, BCL2, *CAMTA1*, *FRMD4A*, *TWIST1*, *DAB2IP*), only *TWIST1* significantly ($P < 0.05$) affected the patient survival rate. Kaplan-Meier plot was also

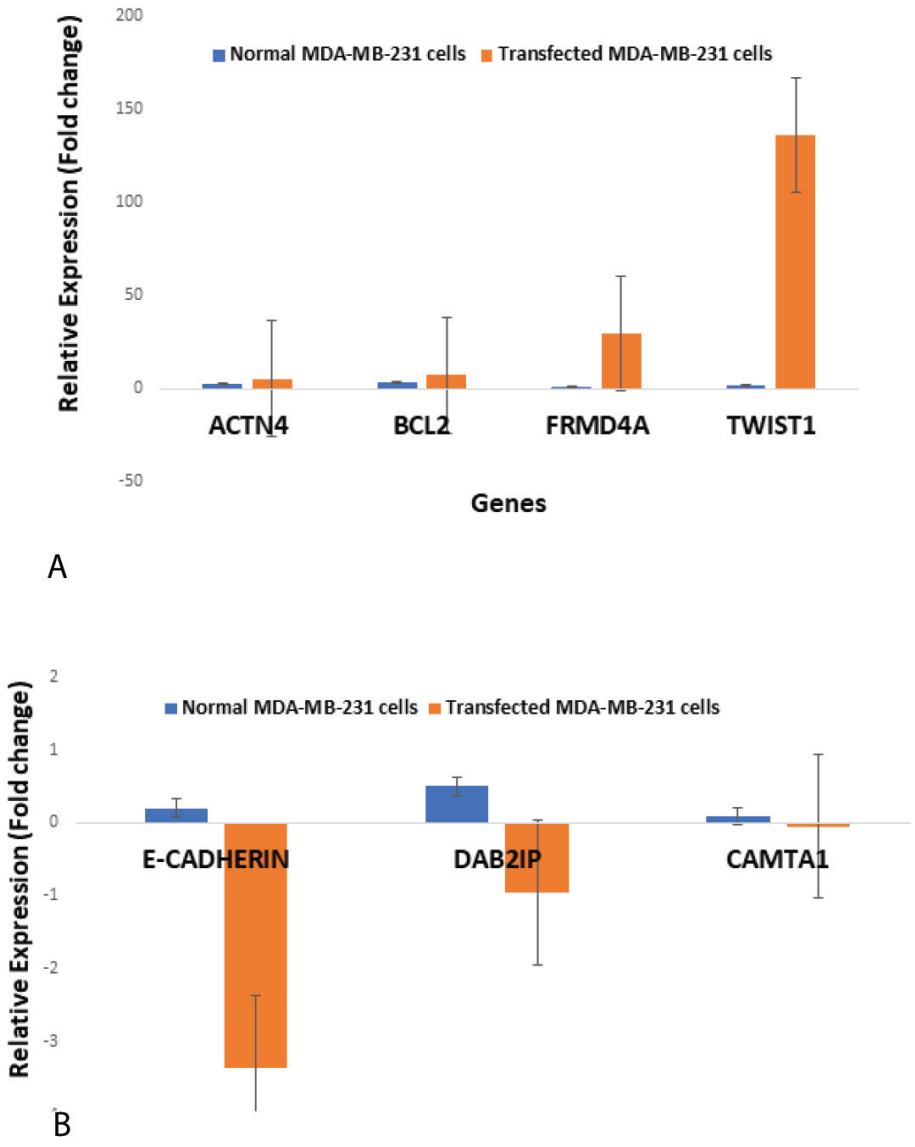

Figure 7. Relative expression of miR-96 targets genes and *TWIST1* in MDA-MB-231 and transfected MDA-MB-231*(P*-value < 0.05). A. Relative expression of upregulated genes (ACTN4, BCL2, *FRMD4A*, and *TWIST1*) B. Relative expression of downregulated genes (*E-Cadherin*, *DAB2IP*, *CAMTA1*). *Genes are represented in their human gene nomenclature form.

TWIST1: Twist-related protein 1; BCL2: B-cell lymphoma 2; *FRMD4A*: FERM domain-containing protein 4A; ACTN4: Alpha-actinin-4; *DAB2IP*: DAB2 interacting protein; *CAMTA1*: Calmodulin binding transcription activator 1

Table 2. Top 25 differentially expressed miRNAs in breast cancer

S. No	ID	adj.P.Val	*P*. Value	t	B	logFC	ORGANISM	miRNA_ID
1.	hsa-miR-21	3.29E-12	1.39E-14	12.06606	23.14585	3.001335	Homo sapiens	hsa-miR-21
2.	hsa-miR-96	6.69E -10	5.85E-12	10.25797	17.23006	4.06161	Homo sapiens	hsa-miR-96
3.	hsa-miR-331-3p	7.84E-10	9.92E-12	9.60986	16.59658	1.783096	Homo sapiens	hsa-miR-331-3p
4.	hsa-miR-15b	1.95E-09	3.03E-11	9.91904	15.3988	2.11635	Homo sapiens	hsa-miR-15b
5.	hsa-miR-222	2.37E-09	5.00E-11	-9.13222	15.00832	-2.100022	Homo sapiens	hsa-miR-222
6.	hsa-miR-140-3p	5.60E-09	1.65E-10	-8.64809	13.81715	-1.697103	Homo sapiens	hsa-miR-140-3p
7.	hsa-let-7e	5.60E-09	1.65E-10	8.63831	13.78831	1.589997	Homo sapiens	hsa-let-7e
8.	hsa-miR-200c	2.10E-08	7.08E-10	8.28969	12.39119	2.196712	Homo sapiens	hsa-miR-200c
9.	hsa-miR-145	2.82E-08	1.10E-09	-8.00494	11.89712	-2.517007	Homo sapiens	hsa-miR-145
10.	hsa-miR-130b	2.82E-08	1.19E-09	7.9795	11.82026	1.768419	Homo sapiens	hsa-miR-130b
11.	hsa-miR-155	8.99E-08	4.17E-09	7.56832	10.56911	2.278815	Homo sapiens	hsa-miR-155
12.	hsa-miR-378	1.04E-07	5.94E-09	-7.50749	10.24313	-2.187027	Homo sapiens	hsa-miR-378
13.	hsa-miR-26a	1.04E-07	5.79E-09	-7.46177	10.24238	-1.547842	Homo sapiens	hsa-miR-26a
14.	hsa-miR-324-5p	1.04E-07	6.12E-09	7.49771	10.21361	2.02085	Homo sapiens	hsa-miR-324-5p
15.	hsa-miR-101	1.08E-07	6.85E-09	-7.40712	10.07442	-1.356247	Homo sapiens	hsa-miR-101
16.	hsa-miR-484	1.52E-07	1.02E-08	7.32786	9.69946	1.391267	Homo sapiens	hsa-miR-484
17.	hsa-miR-125a-5p	1.78E-07	1.28E-08	7.20599	9.45428	1.774148	Homo sapiens	hsa-miR-125a-5p
18.	hsa-miR-183	2.92E-07	2.71E-08	7.80753	9.13685	4.707145	Homo sapiens	hsa-miR-183
19.	hsa-miR-197	2.46E-07	1.87E-08	7.08404	9.07681	1.697118	Homo sapiens	hsa-miR-197
20.	hsa-miR-202	2.53E-07	2.15E-08	-7.24491	9.04546	-2.293118	Homo sapiens	hsa-miR-202
21.	hsa-miR-768-3p	2.53E-07	2.10E-08	-7.04539	8.95698	-1.360845	Homo sapiens	hsa-miR-768-3p
22.	hsa-miR-425	2.53E-07	2.21E-08	7.02495	8.89356	1.717538	Homo sapiens	hsa-miR-425
23.	hsa-miR-103	5.65E-07	5.48E-08	6.7391	8.00419	0.960505	Homo sapiens	hsa-miR-103
24.	hsa-miR-601	7.25E-07	7.35E-08	-6.68654	7.73875	-1.627019	Homo sapiens	hsa-miR-601
25.	hsa-let-7d	3.51E-06	1.93E-08	-6.57012	7.65688	-1.210435	Homo sapiens	

adj.P.Va: Adjusted *P*-value; t: T value; B: Unstandardized Beta value; loFC: Log fold change; miRNA ID: MicroRNA ID

generated for miR-96 by the KM plotter and it was found to be significant ($P < 0.01$). The survival curves of miR-96 and *TWIST1* are depicted in figures 4 A and B, respectively.

Overexpression of TWIST1

TWIST1 was amplified from human genomic DNA (Figures 5 A and B). The PCR product was purified and cloned in pcDNA 3 enhanced green fluorescent protein (EGFP) vector by the restriction-ligation method of cloning (Figure 5 C and D). The cloned vector was then transfected in MDA-MB-231 cells. Successful recombination was confirmed through the expression of GFP, as observed through a phase-contrast microscope (Figure 6).

Validation of the expression of target genes

The target genes of miR-96, indicated in the generated network were validated by evaluating the expression levels of some of the genes by performing a real-time PCR. Three of the genes *ACTN4, BCL2,* and *FRMD4A* were found to be significantly upregulated and the other genes *CAMTA1, DAB2IP,* and *E- Cadherin* were observed to be downregulated in *TWIST1* transfected MDA-MB-231 cells, as compared with the non-transfected cells. The differential gene expression profile of the genes is shown in figure 7.

From the graph, it is seen that there is around a two-fold increase in the upregulated gene expression and a one-fold decrease in the downregulated gene expression levels in the *TWIST1* overexpressed cells, as compared with the control cells. From these results, it is evident that the overexpression of the *TWIST1* gene regulated the expression levels of the target genes of miR-96.

Discussion

In this study, the top differentially expressed genes and miRNAs between breast tumor cells and normal breast cells were identified and their correlation with *TWIST1* was explored. Around 250 differentially expressed miRNAs were observed between the control and the sample groups. The Transfac analysis of *TWIST1* on the differential expressed miRNAs revealed that the gene of interest (*TWIST1*) had eight binding sites with miR-96 (second differentially expressed miRNA). On the other hand, the top differentially expressed miRNA (miR-21) had no binding sites for *TWIST1*. The constructed protein-protein

interaction network identified the major interaction between *ACTN4, BCL2, FRMD4A, CAMTA1, DAB2IP*, and *TWIST1*. These outcomes suggested that miR-96 could regulate *TWIST1* and its associated genes.

The generated gene network was then validated by the expression studies. Over-expression of *TWIST1* in breast cancer cells (MDA-MB-231) led to an increase in the expression of *ACTN4, BCL2*, and *FRMD4A* which are the validated targets of miR-96. *TWIST1* expression was directly correlated to the aggressiveness of the tumor and therefore, the expression of *ACTN4, BCL2*, and *FRMD4A* increases as the tumor becomes aggressive thereby leading to metastasis. The other target genes of miR-96 like *CAMTA1, DAB2IP,* and *E- Cadherin* were observed to be downregulated in *TWIST1* transfected MDA-MB-231 cells. Moreover, *TWIST1* (*P*-value = 0.015) and miR-96 (*P*-value = 0.0052) were significantly associated with breast cancer patient survival. These results demonstrate that *TWIST1* and miR-96 are correlated with each other in the progression of breast cancer metastasis.

Although the role of miR-96 in breast cancer metastasis is not clear, previous studies have shown that miR-96 might be responsible for increasing cancer cell proliferation and migration of breast cancer cells.[26] miR-96 was recognized as the second differentially expressed miRNA with a highly significant *P*-value between the tumor and normal breast cancer cells in our differential mRNA expression analysis. *TWIST1*, a target of miR-96, was also found to be significantly associated with patient survival in an earlier study.[27] Similarly, the KM plot of *TWIST1* denotes the significance of this gene on breast cancer patients' survival.

TWIST1 directly targets miRNA (miR-151-3p and miR-33b) and may play a negative regulatory role in the metastasis of breast cancer.[28, 29] Few target genes of miR-96 (*ACTN4, BCL2, CAMTA1, DAB2IP, FOXA1, FRMD4A*) were predicted to interact with *TWIST1* significantly. The *ACTN4* gene which is highly interacted with *TWIST1* plays a significant role in breast cancer tumorigenesis and functions as a versatile promoter for breast cancer.[30] Similarly, the *BCL2* gene was found to be a prognostic and a predictive marker for breast cancer.[31] *FRMD4A* gene was upregulated in human squamous cell carcinoma promoting tumor growth and metastasis.[32] *CAMTA1* was found to be a tumor suppressor gene in neuroblastoma.[33] *DAB2IP* is also a tumor suppressor gene that gets downregulated by methylation in breast cancer.[34] *E-Cadherin* expression is directly affected by *TWIST1* expression as *TWIST1* promoted metastasis with the conversion of *E-Cadherin* to *N-Cadherin*.[35] Moreover, it has been reported that *TWIST1* suppresses the transcriptional activity of FOXA1 promoter and inhibits *TWIST1*-promoted breast cancer progression.[36] Furthermore, *TWIST1* largely inhibits the expression of co-expressed EMT driving transcription factors and blocks breast tumor cell intravasation and metastasis.[37]

Our study highlights the role of micro-RNA (miR-96) in the progression of breast cancer. The network analysis also shed light on the highly interacted targets of miR-96. In addition, the expression studies explore the regulatory role of *TWIST1* on the identified gene targets of miR-96. Overexpression of *TWIST1* expression results in the altered expression of key miR-96 target genes. This might be due to the positive regulation of *TWIST1* on the oncogenic miR-96. Hence, miR-96 would act as a potential candidate for diagnostic and therapeutic leads. The regulation of *TWIST1* on miR-96 must be probed by quantifying the miR-96 levels in the *TWIST1* overexpressed cells.

Conclusion

To conclude, the correlation between *TWIST1* and certain target genes of miR-96 was explored. We found a significant association between the expression levels of *TWIST1* and miR-96 target genes. Further investigations are needed to understand the mechanism of regulation of *TWIST1* and miR-96. The present study supports the idea that miR-96 may act as a prognostic marker and a useful therapeutic target for breast cancer metastasis.

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Conflict of Interest

None declared.

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