Downregulation of NRARP exerts anti-tumor activities in the breast tumor cells depending on Wnt/β-catenin-mediated signals: The role of miR-130a-3p

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Abstract

The Notch-regulated ankyrin repeat protein (NRARP) functions as a molecular link between Notch and Wnt signaling pathways. Although it has recently been identified to be overexpressed in breast cancer (BC), the molecular mechanisms that regulate NRARP remain unknown. Since microRNAs (miRNAs) regulate gene expression post-transcriptionally, miRNA dysregulation could explain the abnormal gene expression. Here, we identified miR-130a-3p as an NRARP regulator and evaluated its effects on the behavior of BC cells. Quantitative real-time PCR was performed to assess the transcriptional levels of miR-130a-3p and NRARP in BC cells. Next, miR-130a-3p was transiently transfected into BC cells to assess its influence on NRARP expression. Owing to the positive regulatory effects of NRARP on the Wnt/β-catenin signaling pathway, we also analyzed the expression levels of five Wnt/β-catenin pathway genes and one downstream target gene in BC cells. We then assessed anti-tumor activities of miR-130a-3p in BC cells using the MTT proliferation assay, the soft agar colony formation assay for anchorage-independent growth (AIG), as well as scratch and transwell assays for cell migration. The results showed that miR-130a-3p was downregulated in BC cells, whereas NRARP was upregulated. Overexpression of miR-130a-3p inhibited the expression of NRARP and some Wnt/β-catenin signaling pathway genes, as well as exerted anti-tumor effects as evidenced by decreased cell proliferation, AIG, and migration of BC cells. In conclusion, the tumor-suppressive function of miR-130a-3p in BC may be mediated by inhibiting NRARP and Wnt/β-catenin signaling pathway. As a result, miR-130a-3p could be introduced as a therapeutic target for miRNA therapy in BC.
1 | INTRODUCTION

According to the data obtained from both developed and developing countries, breast cancer (BC) is the most common cancer in women, ranking first on the list of the most frequently diagnosed malignancies in 2020 (Raj et al., 2017; Sung et al., 2021). The complexity of the disease has led scientists to analyze the expression of many genes in breast tumors; for example, human epidermal growth factor receptor-2 (HER2) is overexpressed in 15%–20% of all breast tumors (Singh & Mo, 2013). Although drugs that target HER2 could reduce the severity of disease and mortality in these patients, drug resistance and tumor recurrence remain serious issues, and novel molecular targets to improve BC treatment are welcomed (Shah & Osipo, 2016).

The Notch and Wnt signaling pathways are essential for a variety of cellular functions such as cell proliferation, apoptosis, and differentiation (South et al., 2012; Yang et al., 2016). It has been demonstrated that abnormal activation of these pathways is associated with the development and progression of BC (Shah & Osipo, 2016). However, there is evidence that aberrant Wnt/β-catenin signaling pathway is sufficient to cause mammary cell transformation, and more than 50% of breast tumors are recognized to have an over-activated Wnt/β-catenin signaling pathway (Polakis, 2012). For instance, MCF-7 cells, which are ER-positive human BC cells, express high levels of some Wnt/β-catenin signaling pathway oncogenes including WNT1, AXIN2, LEF1, FZD4, and WISP1 (Chiang et al., 2015; Lamb et al., 2013; Wieczorek et al., 2008).

The Notch and Wnt signaling pathways interact in various ways; Notch-regulated ankyrin repeat protein (NRARP) is an important molecule that regulates both Notch and Wnt/β-catenin signaling pathways (Pinto et al., 2020). NRARP is expressed in response to increased Notch signals; however, it suppresses Notch signaling through feedback inhibition (Lamar et al., 2001). In the Wnt signaling pathway, NRARP functions as a potent inducer, increasing the expression of canonical Wnt pathway genes (Borggreve et al., 2016). NRARP has recently been demonstrated to act as an oncogene in a number of malignancies (Liao et al., 2018). In the context of BC, while microarray data indicate that NRARP is overexpressed in various rat models of BC, the molecular regulatory mechanisms of NRARP remain unknown (Imaoka et al., 2014).

MicroRNAs (miRNAs) are single-stranded RNA sequences of 20–22 nucleotides in length that function in messenger RNAs (mRNAs) silencing and post-transcriptional inhibition of gene expression (Peng et al., 2016). The miRNA research has gotten a lot of attention because of the wide range of target genes and the fact that they regulate almost every biological pathway. Therefore, oncogenic or tumor-suppressive miRNAs could be investigated for therapeutic purposes (Thorsen et al., 2012).

In the present study, we aimed at finding the putative miRNA that potentially targets NRARP in BC. Interestingly, miR-130a-3p was identified to be a potential miRNA involved in cell proliferation, migration, and drug resistance, as well as being dysregulated in BC cells (Zhang et al., 2017). It was then transfected into MCF-7, a human BC cell line that expresses estrogen and progesterone receptors, as well as SKBR3, a HER2 overexpressing BC cell line. Next, the effect of miR-130a-3p restoration on NRARP expression was investigated using quantitative real-time PCR (qRT-PCR). Besides this, as NRARP is a positive regulator of the Wnt/β-catenin signaling pathway, we also assessed the expression of pathway’s key oncogenic components. Among the scrutinized genes, Wnt2B, FZD6, LRP6, CTNNB1, TCF4, and a downstream target gene, ZEB1 were chosen for further investigation. Furthermore, reliable in vitro assays such as MTT, the formation of cell colonies on soft agar, scratch, and transwell cell migration assays were performed to investigate the mechanisms of miR-130a-3p’s involvement in BC cells.

2 | MATERIAL AND METHODS

2.1 | Bioinformatics prediction

TargetScan, miRvestigator, miRanda, miRDB, miRWalk, and miRmap were used to predict putative miRNA that potentially targets NRARP (Supplementary Material) (Karami et al., 2016). As shown in Figure 2a, with a perfect seed match model (7mer-m8), TargetScan indicated that miR-130a-3p potentially blocks NRARP. Other algorithms also predicted miR-130a-3p as a potential regulator of NRARP.

2.2 | Cell lines and culture

The MCF-7 and SKBR3 human BC cell lines, as well as MCF-10A as a non-cancerous breast epithelial cell line, were obtained from the National Cell Bank of Iran (NCBI). To verify that the cells are free of mycoplasma, a PCR test was performed. In order to culture BC cell lines,
complete cell culture medium containing high-glucose Dulbecco’s modified eagle medium (DMEM), 10% fetal bovine serum (FBS- Gibco), 100 units/ml penicillin, and 100 μg/ml streptomycin was used. A medium containing 10% horse serum (HBS- Gibco, USA) was utilized for the growth of MCF-10A cell line. The cells were incubated at 37°C with 90% humidity and 5% CO₂. Every 24 h, the cells were observed under an inverted microscope for cellular density, morphology, and contamination.

2.3 | RNA extraction

To assess the mRNA expression of miR-130a-3p, NRARP, and Wnt/β-catenin pathway genes in BC cells or normal breast epithelial cells, the cellular RNA was isolated using hybrid-R™ RNA extraction kit (GeneAll) according to the manufacturer’s protocol. Briefly, the cells were lysed with 1 ml of RiboEx™ lysis buffer for 5 min. Next, the cells were transferred to a microtube and each tube was mixed intensely with 0.2 ml of chloroform for 15 s and then centrifuged for 15 min at 12,000 g for phase separation. Afterward, the upper phase was moved to a small spin column. The column was centrifuged for 30 s (~10,000 g), and then passed-through material was removed. The spin column was washed twice with wash solutions, and then RNAse-free water was used to elute the purified RNA. The cellular RNA isolated from MCF-10A was considered as control sample. NanoDrop (Aosheng, China) and denaturing agarose gel electrophoresis were used to determine the concentration and integrity of isolated RNAs, respectively. Extracted RNAs were stored at −80°C for future experiments.

2.4 | Synthesis of cDNA and qRT-PCR

In a reaction catalyzed by the RevertAid enzyme (Thermo Scientific), cDNA was synthesized from isolated RNA in the presence of stem-loop primers for miR-130a-3p and SNORD47, and random hexamer primers for genes. The StepOne plus™ thermal cycler (ABI) was used to perform qRT-PCR using SYBR Green fluorescent dye and primers listed in Table 1. The PCR was carried out for 10 min at 95°C for initial denaturation. After that, 40 cycles of 95°C for 15 s, 60°C for 40 s, and 72°C for 50 s were performed. At the end of the experiment, the qRT-PCR amplification efficiency and cycle threshold (Ct) was analyzed using LinRegPCR program (J.M. Ruijter, Version 1.2). Expression values were normalized using Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a reference gene for the NRARP and Wnt/β-catenin pathway genes, and SNORD47 (U47) for the miR-130a-3p and presented as a relative expression using the 2−ΔΔCt method.

2.5 | Transient transfection

The miR-130a-3p miRNA mimics or scramble miRNA as negative control were purchased from Exiqon-Qiagen. Transfection of these sequences (5 nM) into BC cell lines was carried out using Lipofectamine 2000 (Invitrogen) in accordance with the manufacturer’s instructions. In brief, MCF-7 or SKBR3 cells from low passage were seeded in plates (24-well) and incubated at 37°C until 90% confluence was achieved. The complete medium in each well was then changed with FBS-free medium. Following that, the cells were exposed to respective sequences. Cells were processed 2 days after transfection for further testing. Non-transfected MCF-7 or SKBR3 cells (untreated cells) were investigated as control.

2.6 | qRT-PCR after transfection

Total RNA was extracted, cDNA was synthesized, and qRT-PCR was performed on either transfected or
untreated cells, as described above. For normalization of qRT-PCR data, SNORD47 and GAPDH were used as reference genes.

2.7 | Proliferation assay

The proliferation of BC cells after miR-130a-3p restoration was assessed using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. In brief, BC cells were dissociated and suspension of $3 \times 10^3$ cells for each well were transferred into a 96-well plate. The cells were transfected as described above or left untreated, and then were further grown for 24, 48, and 72 h. Following that, the cells were incubated in the MTT reagent (0.5 mg/ml in PBS) for 3 h at 37°C in order to develop formazan crystals. In the next step, the formazan crystals were dissolved using dimethyl sulfoxide (DMSO), and color intensity was quantified spectrophotometrically at 570 nm after 5–10 min shaking. Non-transfected MCF-7 or SKBR3 cells were investigated as control.

2.8 | Soft agar colony formation assay

The ability of BC cells to grow anchorage-independently in response to miR-130a-3p restoration was tested using a soft agar colony formation assay, which evaluates cellular transformation in vitro (Borowicz et al., 2014). First, the cells were exposed to respective treatments as described above or left untreated. Next, the experiment was carried out in a six-well plate with two layers of agar; the bottom layer containing 0.8% agar and 2× cell culture medium in a 1:1 ratio, and the upper layer containing 0.5% agar and the suspension of cells ($5 \times 10^3$ cells for each well) in a 1:1 ratio. After chilling and forming agar layers, plates were transferred to 37°C temperature and 5% CO$_2$ incubator. After 3 weeks, each well was stained with 0.2 ml of 70% ethanol (Sigma, Germany), exposed to the 0.05% crystal violet (Merck-Darmstadt), randomly different views were chosen, and the average of the cells per views was computed. Non-transfected MCF-7 or SKBR3 cells were investigated as control.

2.9 | Scratch test

Wound closure or scratch test is a type of whole-cell mass migration that is commonly used by researchers to determine the migratory potential of cancer cells (Grada et al., 2017). For performing the scratch test, the cells were seeded at a density of $1 \times 10^5$ cells per well in a 24-well plate and grown to form a cell monolayer. The scratch was created on the cell layer using a pipette tip. Following that, the cells were washed thoroughly twice with PBS, and a low-FBS medium (0.5%) was added to the wells. The first image of the scratch area was captured after it was formed and viewed under an inverted microscope, and subsequent images in the same area were captured at regular time intervals for up to 48 h. ImageJ software (v1.8.0, NIH) was used to quantify snapshots, and the following formula was used to determine the migration area:

\[
\text{Migration Area} = (\text{the scratch area measured after the scratch has formed}) - (\text{the scratch area measured at regular time intervals}).
\]

2.10 | In vitro cell migration assay

The ability of BC cells to migrate in response to attractant was appraised using an 8 μm pore transwell plate (SPL, Life Bioscience). To do so, at first. The cells were exposed to respective treatments as described above or left untreated. Next, the upper compartment of the insert was filled with 0.2 ml of FBS-free cell solution ($5 \times 10^4$ cells per well), and to the well of the plate (bottom of the lower chamber), 0.7 ml DMEM supplemented with 10% FBS as a chemoattractant was added. The cells were incubated for 24 h at 37°C, and then the cells that migrated from the upper to lower surface of the membrane were fixed with 70% ethanol (Sigma, Germany), exposed to the 0.05% crystal violet (Merck-Darmstadt), randomly different views were chosen, and the average of the cells per views was computed. Non-transfected MCF-7 or SKBR3 cells were investigated as control.

2.11 | Statistical analysis

GraphPad Prism version 8.0.1 (GraphPad) was used for statistical analysis. For multiple comparisons, analysis of variance (ANOVA) test was employed followed by Tukey post hoc tests. The results are presented as the mean ± SD of three independent experiments, and the p-value of less than .05 was considered statistically significant.

3 | RESULTS

3.1 | The expression of miR-130a-3p decreases in BC cell lines, whereas the expression of NRARP and Wnt/β-catenin pathway genes increase

To investigate the involvement of miR-130a-3p, NRARP, and Wnt/β-catenin pathway genes in the pathogenesis
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**FIGURE 1** The expression levels of miR-130a-3p, NRARP, and Wnt/β-catenin pathway genes determined by qRT-PCR. (a) The expression levels of miR-130a-3p in MCF-7 and SKBR3 cell lines (normalized to SNORD47, compared with MCF-10A). (b) The mRNA expression of NRARP in MCF-7 and SKBR3 cells. The mRNA expression of Wnt pathway genes in BC cell lines; (c) MCF-7 cell line; (d) SKBR3 cell line. GAPDH was used to normalize the gene expression data, and expression values were represented as the mean ± SD of the three independent experiments (*p < .05, **p ≤ .01, ***p ≤ .001 vs. MCF-10A) [Colour figure can be viewed at wileyonlinelibrary.com]

**FIGURE 2** Transient miR-130a-3p transfection in BC cell lines. (a) A screenshot of the matched miR-130a-3p and NRARP sequences is presented in accordance with TargetScan, (www.targetscan.org). (b) After transfection, qRT-PCR was used to evaluate relative miR-130a-3p expression in BC cells. Restoration of miR-130a-3p affects the expression of (c) NRARP, (d) Wnt pathway genes in MCF-7 cell line, and (e) Wnt pathway genes in SKBR3 cell line. Expression values were represented as the mean ± SD of the three independent experiments (*p < .05, **p ≤ .01, ***p ≤ .001, ****p ≤ .0001 vs. MCF-10A or control) [Colour figure can be viewed at wileyonlinelibrary.com]
of BC, their expression was analyzed using qRT-PCR. As indicated in Figure 1a, the mRNA level of miR-130a-3p was lower in MCF-7 and SKBR3 cells than in MCF-10A cells (193- and 17-folds, respectively). Furthermore, NRARP expression was 7.7 and 5.8-folds higher in MCF-7 and SKBR3 cells, respectively, than in normal breast epithelial cells (Figure 1b). Owing to the positive regulatory effects of NRARP on Wnt/β-catenin signaling pathway, we also analyzed the expression levels of the five Wnt/β-catenin pathway genes (Wnt2B, FZD6, LRP6, CTNNB1, TCF4) and a downstream target gene, ZEB1 in BC cells. As shown in Figure 1c, the expression levels of Wnt2B, FZD6, LRP6, and ZEB1 were higher in MCF-7 cells compared with normal breast epithelial cells (fold changes >2, p < .05). Furthermore, except for LRP6, the mRNA levels of Wnt pathway genes and ZEB1 were increased in SKBR3 cells than in normal breast epithelial cells, which is shown in Figure 1d (fold changes >2, p < .0001).

3.2 Overexpression of miR-130a-3p is associated with NRARP downregulation in BC cell lines

To investigate how miR-130a-3p and NRARP are related, it was transiently transfected into BC cells, and transfection efficiency was assessed by qRT-PCR. As shown in Figure 2b, miR-130a-3p was upregulated in MCF-7 and SKBR3 transfected cells compared with control cells. Regarding NRARP, as shown in Figure 2c, the expression level of NRARP decreased significantly in the MCF-7 and SKBR3 cells after miR-130a-3p restoration compared with the MCF-10A cells. No statistically significant changes were detected between scramble-transfected cells and control cells in both cell lines (p > .05).

3.3 The expression of Wnt/β-catenin pathway genes in BC cell lines after miR-130a-3p restoration

We further want to explore whether miR-130a-3p restoration and downregulation of NRARP has any effects on Wnt/β-catenin signaling pathway. To address this question, the mRNA levels of some genes in the pathway were examined following transfection of miR-130a-3p into BC cells. As indicated in Figure 2d, all genes evaluated in the present study were downregulated in MCF-7 cells exposed to miR-130a-3p in comparison with control cells (fold change <0.5, p < .05). Similar findings were observed in SKBR3 cells, as the mRNA expression of these genes decreased significantly following miR-130a-3p restoration compared to control SKBR3 cells (fold change <0.5, p < .001), although TCF4 was not (Figure 2e). No statistically significant changes were detected between scramble-transfected cells and control cells in both cell lines (p > .05).

3.4 The proliferation and AIG of BC cell are both decreased by miR-130a-3p

As shown in Figure 3a, MCF-7 cells showed a decrease in cell proliferation 72h after exposure to miR-130a-3p in comparison with control cells (p < .01). Furthermore, 48 and 72h after miR-130a-3p restoration in SKBR3 cells, cell proliferation decreased significantly compared with control SKBR3 cells (p < .05). No statistically significant changes were detected between scramble-transfected cells and control cells in both cell lines (p > .05).

The results of AIG indicated that the number of cell colonies decreased significantly in MCF-7 cells exposed to miR-130a-3p in comparison with the cells exposed to scramble sequences and control cells (p < .0001 for both, Figure 3b). Furthermore, SKBR3 cells exposed to miR-130a-3p formed fewer colonies than cells exposed to scramble sequences or control cells (p = .0001 and p = .0003, respectively). No statistically significant changes were detected between scramble-transfected cells and control cells in both cell lines (p > .05).

3.5 Cell migration decreases in BC cell lines after miR-130a-3p restoration

The scratch test was performed by creating a line in a layer of cells and photographing it at regular intervals to follow whole-cell masses migration. As shown in Figure 4a–d, scratch closure decreased considerably, in both cell lines, within 24 and 48h after miR-130a-3p restoration compared with cells treated with scramble sequences (p = .0001 and p = .0007 in MCF-7 and SKBR3, respectively) or control cells (p < .0001 in both cell lines).

The single-cell migration of BC cells after ectopic expression of miR-130a-3p was more assessed by performing transwell cell migration assay. As shown in Figure 5a,c, MCF-7 cells exposed to miR-130a-3p exhibited a significant reduction in the migrating cells compared with the cells exposed to scramble sequences or control cells (p = .0002 and p = .0001, respectively). In SKBR3 cells, restoration of miR-130a-3p resulted in a significant reduction in single-cell migration when compared to cells exposed to scramble sequences or control cells (p = .0008, and p = .0019, respectively, Figure 5b,d).
4 | DISCUSSION

Given the lack of a radical cure and the limitations of current therapies, human cancers should be extensively investigated at the molecular level and signaling pathways, where many potential opportunities for oligonucleotide-based therapies may emerge (Xiao et al., 2016). In this study, we evaluated the molecular regulatory mechanism of NRARP, which is a molecular link between the Notch and Wnt signaling pathways. Our results indicated that miR-130a-3p is a regulator of NRARP in BC and suggested that downregulation of NRARP could be followed by the suppression of Wnt/β-catenin signaling pathway, as well as decreased cell proliferation, AIG, and cell migration.

The existence of functional crosstalk between Wnt and Notch signaling has been confirmed in several studies; NRARP could be involved in tumorigenesis as a result of this crosstalk (Hayward et al., 2008). To date, only a few reports on NRARP’s function have been published. It was first shown to be a downstream target of Notch signaling, in which luciferase reporter results revealed that Notch signals increase the expression of NRARP. When expressed, it suppresses Notch signaling by increasing the degradation of Notch intracellular domain (Krebs et al., 2001; Pirot et al., 2004). Our findings demonstrated that NRARP expression was higher in BC cell lines than in non-malignant cells, and that NRARP expression was dramatically reduced after exposure to miR-130a-3p. In line with our findings, Imaoka et al. showed that NRARP was overexpressed in the two human BC cell lines, namely MCF-7 and T47D, and that NRARP silencing inhibited cell growth and decreased the expression of cell cycle-related genes (Imaoka et al., 2014). Another study in MCF-7 cells revealed that combining Emodin with 5-Fluorouracil (5-FU) causes NRARP to be downregulated and that suppressing NRARP with siRNA technology causes cellular...
senescence (Zu et al., 2018). These findings give support to the hypothesis that \textit{NRARP} plays an oncogenic role in BC. In the current study, \textit{NRARP} was predicted as a target gene for the miR-130a-3p using several bioinformatics algorithms (Supplementary Material). Furthermore, qRT-PCR results demonstrated that exposure to miR-130a-3p can diminish \textit{NRARP} expression.

In this study, the expression of certain genes of \textit{Wnt/β-catenin} signaling was higher in the BC cell lines than in normal cells. The \textit{Wnt/β-catenin} signaling pathway is abnormally activated in the human BC through different mechanisms (Howe & Brown, 2004). Ishitani et al. (2005) reported that zebrafish Nrarp inhibits the ubiquitination and subsequent degradation of \textit{Wnt/β-catenin} signaling transcription factor, lef1, resulting in the pathway activation. Also, Phng et al. (2009) reported that Nrarp knockdown inhibits the function of Wnt3a, a \textit{Wnt/β-catenin} signaling ligand and that induction of Nrarp expression by Dll4/Notch promotes Wnt/ Ctnnb1 signaling in the endothelial stalk cells through interactions with Lef1. Supporting this concept, Pinto et al. (2020) have recently demonstrated that \textit{NRARP} interacts with \textit{LEF1} in T-cell acute lymphoblastic leukemia cells and that \textit{NRARP} overexpression results in elevated levels of β-catenin, a main player in the pathway; so, they suggested that these findings could be extended to other malignancies in which Notch and Wnt are involved. Accordingly, it is reasonable to assume that the aberrant \textit{Wnt/β-catenin} signaling pathway in BC cells could be a result of \textit{NRARP} upregulation. We also observed that the expression of some \textit{Wnt/β-catenin} pathway genes was significantly decreased after miR-130a-3p restoration. As illustrated in Figure 6, it is possible that by downregulating \textit{NRARP}, its inducing effects on the \textit{Wnt/β-catenin} signaling pathway have been reduced. Another possibility is that miR-130a-3p directly inhibited the \textit{Wnt/β-catenin} signaling pathway, as it has been suggested in our previous study in the triple-negative BC (Poodineh et al., 2020).

The epithelial–mesenchymal transition (EMT) is a dynamic process by which cancer cells acquire an aggressive phenotype. Zinc finger E-box-binding homeobox 1 (\textit{ZEB1}) is the main transcription factor that regulates EMT (Brabletz, 2012; Preca et al., 2015). In this study, following miR-130a-3p overexpression, the expression of \textit{ZEB1} was significantly decreased. There are three possible explanations for the \textit{ZEB1} downregulation (Figure 6): First, Fazio et al. (2016) found that colon cancer cells that express high...
transcriptional levels of ZEB1, also express high levels of NRARP. Besides this, Zhu et al. (2015) reported that by knocking down NRARP, the stemness and EMT are decreased in liver tumor cells. These findings suggest a functional link between NRARP and ZEB1 as an EMT marker, and our results could be evidence that downregulation of NRARP may be resulted in the inhibition of ZEB1.

Second, ZEB1, as a direct downstream target gene, is regulated by the Wnt/β-catenin signaling pathway, in which β-catenin promotes the expression of ZEB1 by binding to its promoter. Moreover, ZEB1 expression is reduced when β-catenin is knocked down (Simeone et al., 2019). Hence, the downregulation of β-catenin could be another explanation for the significant decrease in the ZEB1 expression after treatment with the miR-130a-3p. Third, as reported by Chi et al. (Chi et al., 2018) miR-130a-3p has target sequences in the 3′-UTR of ZEB1. Thus, the decreased transcriptional levels of ZEB1 found in the current study could be a direct impact of miR-130a-3p.

We also assessed cell proliferation, AIG, single-cell, and whole-cell mass migrations of BC cells after exposure to miR-130a-3p. Our results showed that ectopic miR-130a-3p expression could reduce the AIG in BC cells. Cancer cells manifest adherent-independent cell growth, a process by which cells separate from the extracellular matrix and attach to an inappropriate matrix, resulting in colonization in distant organs (Paoli et al., 2013). The Wnt/β-catenin is one of several molecular pathways that regulate the expression of downstream genes that are involved in the adherent-independent growth of cancer cells (Malagobadan & Nagoor, 2015). Therefore, the decrease in the AIG observed in this study could be a result of inhibition of Wnt/β-catenin signaling pathway following miR-130a-3p overexpression and/or NRARP downregulation. In addition, ectopic miR-130a-3p expression reduced both single-cell and whole-cell mass migrations in BC cells. Because NRARP, FZD6, LRP6, and ZEB1 have been linked to cell migration and invasion, the reduced
cell migration seen after miR-130a-3p restoration could be attributed to miR-130a-3p directly inhibiting these genes. (Corda et al., 2017; Ibrahim et al., 2013; Yang et al., 2015).

5 | CONCLUSION

Overall, the purpose of this study was on deciphering the processes by which NRARP and Wnt/β-catenin signaling pathway are regulated in BC. Our results showed that miR-130a-3p is an NRARP regulator, and its overexpression exerts anti-tumor activities. Although qRT-PCR results indicated that overexpression of miR-130a-3p and NRARP downregulation could be followed by the inhibition of some Wnt/β-catenin signaling pathway genes and ZEB1, the exact molecular regulatory mechanisms by which these genes are regulated were not fully elucidated in this study, emphasizing that more studies such as siRNA technology, dual-luciferase reporter assay, and miR-130a-3p antagomiR are needed.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding this article.

DATA AVAILABILITY STATEMENTS

The data that support the findings of this study are openly available in Research Square at https://www.researchsquare.com/article/rs-1365854/v1, reference number 10.21203/rs.3.rs-1365854/v1.

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