ACCSCIENCE PUBLISHING

Cancer Plus

ORIGINAL RESEARCH ARTICLE

Long non-coding RNA DARS-AS1 facilitates breast cancer progression by modulating the miR-6835-3p/ATF3 axis

Krishnamoorthy Vignesh* and **Pattusamy Thangamalar**

Department of Noi Naadal, Government of Siddha Medical College, Tirunelveli, Tamil Nadu, India

Abstract

Long non-coding RNAs (lncRNAs) play crucial roles in various cellular processes associated with cancer progression, including invasion, proliferation, and metastasis. Despite this understanding, the specific role of DARS-AS1 in breast cancer remains underexplored. In this study, we employed quantitative reverse transcription-polymerase chain reaction to measure the expression levels of DARS-AS1 and miR-6835-3p. Functional assessments, including the cell invasion and CCK-8 assays, were conducted to investigate cellular behaviors. In addition, a luciferase reporter assay was employed to elucidate the mechanistic interaction between DARS-AS1 and miR-6835-3p. Notably, DARS-AS1 expression was elevated in breast cancer cell lines (MCF7 and MDA-MB-231) relative to the non-cancerous MCF-10A cells. Overexpression of DARS-AS1 enhanced cell growth and invasion in MDA-MB-231 breast cancer cells. Further investigation revealed that DARS-AS1 acts as a sponge for miR-6835-3p in breast cancer cells. Overexpression of miR-6835-3p inhibited luciferase activity, specifically in the presence of wild-type DARS-AS1, highlighting a direct interaction. Ectopic expression of DARS-AS1 suppressed miR-6835-3p in MDA-MB-231 cells. Concurrently, miR-6835-3p levels were downregulated in breast cancer cells, and miR-6835-3p exhibited a negative correlation with DARS-AS1 expression. Mechanistically, miR-6835-3p targeted ATF3 expression in breast cancer cells. Increased levels of DARS-AS1 were found to enhance cellular proliferation and invasion by modulating ATF3. Our findings indicate that DARS-AS1 acts as an oncogene in breast cancer, partially through regulation of the miR-6835-3p/ATF3 pathway. This study provides valuable insights into the molecular mechanisms contributing to breast cancer progression, offering potential targets for therapeutic interventions.

Keywords: miR-6835-3p; ATF3; LncRNA DARS-AS1; Breast cancer

***Corresponding author:**
Krishnamoorthy Vignesh
(vignesh02vg@gmail.com)

Citation: Vignesh K, Thangamalar P. Long non-coding RNA DARS-AS1 facilitates breast cancer progression by modulating the miR-6835-3p/ATF3 axis. *Cancer Plus*. 2024;6(2):2867.
doi: 10.36922/cp.2867

Received: February 1, 2024

Accepted: March 12, 2024

Published Online: June 28, 2024

1. Introduction

Breast cancer is the most prevalent form of cancer worldwide and a major contributor to cancer-related mortality.[1-3] Despite significant advancements in treatments, including radiation, multiagent chemotherapy, and precision tumor removal, the prognosis for breast cancer patients diagnosed at an advanced stage remains less than ideal.[4-9] Therefore, it is imperative to discover novel biomarkers and therapeutic targets to enhance the prognosis of breast cancer patients.

Long non-coding RNAs (lncRNAs) are a specific type of non-coding RNAs longer than 200 nucleotides. They have received considerable interest due to their capacity to regulate gene expression at the post-transcriptional or transcriptional levels.[10-13] Recent data highlight the crucial significance of lncRNAs in various essential cellular processes, such as proliferation, differentiation, invasion, migration, metastasis, and apoptosis.[14-18] Increasing evidence suggests dysregulation of lncRNAs in several types of cancer, including hepatocellular carcinoma, bladder cancer, gastric carcinoma, lung carcinoma, and breast cancer.[19-24]

DARS-AS1, among these lncRNAs, has been found to play a role in promoting cancer in various human cancer types, such as ovarian cancer, myeloma, lung cancer, and thyroid cancer.[25-28] Elevated levels of DARS-AS1 in thyroid tumor samples have been linked to poor prognosis, cancer metastasis, and heightened disease severity. In addition, DARS-AS1 has been implicated in promoting the migration and growth of thyroid tumor cells through modulation of miR-129.[27] In the advancement of non-small cell lung cancer, DARS-AS1 has been found to regulate the activity of miR-6835-3p.[28] Furthermore, DARS-AS1 has been linked to the development of myeloma tumors and their survival and attributed to interactions with RNA-binding motif protein 39 (RBM39). It is also increased through the action of HIF-1 in myeloma.[25-27] Moreover, in ovarian cancers, the overexpression of DARS-AS1 has been associated with increased cell invasion, migration, and proliferation.[25]

Nevertheless, the extent to which DARS-AS1 contributes to breast cancer has not been sufficiently investigated. To address this gap, this work reveals new evidence demonstrating an increase in DARS-AS1 expression in both breast cancer cell line cultures. Furthermore, we present evidence demonstrating that DARS-AS1 stimulates cell proliferation and infiltration in MDA-MB-231 breast cancer cells. Investigating the functional significance of DARS-AS1 in breast cancer enhances our comprehension of its role in the disease, potentially facilitating the discovery of therapeutic targets and enhancing clinical tactics.

2. Materials and methods

2.1. Cell Culture

The breast cancer cell lines MCF7 and MDA-MB-231, as well as the normal breast cell line MCF-10A, were obtained from the National Centre for Cell Science (NCCS, India). The cell lines were cultivated in DMEM media (Sigma-Aldrich, USA) containing fetal bovine serum (FBS) (Sigma-Aldrich, USA), penicillin (Sigma-Aldrich, USA), and streptomycin (Sigma-Aldrich, USA) to ensure optimal growth conditions.

2.2. Cell transfection

The miR-6835-3p mimic, control miRNA, siRNA-control, siRNA-ATF3, pcDNA-control, pcDNA-DARS-AS1, and siRNA-DARS-AS1 plasmids were obtained from RiboBio (China). The process of introducing foreign genetic material into cells, known as cell transfections, was carried out using a transfection reagent Lipofectamine 2000 (Invitrogen, USA), following the manufacturer's instructions. The transfection protocol was optimized to guarantee the effective transfer of genetic material into the cells.

The specific sequences utilized in the transfection experiments were as follows:

(i) si-ATF3: 5'-AAGAGGCGACGAGAAAGAAAT-3

(ii) miR-6835-3p mimic: 5'-GACCCUCUGUCUUUUCACGAAAA-3'

These sequences were selected for their ability to target ATF3 and mimic miR-6835-3p expression, respectively. The siRNA-control and scramble sequences were used as negative controls in the respective experiments. By employing these cell lines and transfection procedures, we aimed to investigate the functional implications of ATF3, miR-6835-3p, and DARS-AS1 in breast cancer cells, shedding light on their roles in cellular processes such as growth and invasion.

2.3. RNA extraction

Total RNA extraction was performed using the TRIzol kit (Invitrogen, USA) according to the manufacturer's instructions. The TRIzol reagent was employed for its efficiency in isolating RNA, DNA, and proteins from a single sample, ensuring the preservation of cellular component integrity during extraction.

2.4. Complementary DNA synthesis and quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis

Complementary DNAs (cDNAs) were synthesized from the extracted RNA samples. For qRT-PCR analysis, SYBR Green (Bio-Rad, USA) was utilized on the ABI7500 real-time PCR instrument (Applied Biosystems, USA). The expression levels of lncRNA, miRNA (miR-6835-3p), and mRNA were normalized to the reference gene GAPDH using the $2^{-\Delta\Delta CT}$ method. The choice of GAPDH as a reference gene ensures robust normalization and accurate quantification of target gene expression.

The following qRT-PCR primers (Eurofins, India) were used for amplification:

(i) GAPDH:

Forward 5'-AGGTCCACCACTGACACGTT-3'
Reverse 5'-GCCTCAAGATCATCAGCAAT-3'
(ii) DARS-AS1:
Forward 5'-AGCCAAGGACTGGTCTCTTTT-3'
Reverse 5'-CTGTACTGGTGGGAAGAGCC-3'
(iii) miR-6835-3p
Forward 5'-GACCCTCTGTCTTTTCACGAAAA-3'
Reverse 5'-TTTTCGTGAAAAGACAGAGGGTC-3'
(iv) AFT3
Forward 5'-CCTCTGCGCTGGAATCAGTC-3'
Reverse 5'-TTCTTTCTCGTCGCCTCTTTTT-3'

The qRT-PCR reactions were carried out under the following conditions: initial denaturation at 95°C for 40 s, followed by 45 cycles of denaturation at 95°C for 12 s, and annealing and extension at 60°C for 40 s. These experimental conditions were optimized to ensure accurate and reproducible quantification of the expression levels of miR-6835-3p, DARS-AS1, and GAPDH for subsequent analysis of their roles in breast cancer.

2.5. Cell proliferation assay

To assess cell proliferation, cells were seeded into 96-well dishes at a density of 5×10^3 cells per well. The cell growth rate was monitored at different time points using the Cell Counting Kit-8 (CCK-8, Sigma-Aldrich, USA), following the manufacturer's protocol. The CCK-8 assay is based on the ability of cellular dehydrogenases to convert WST-8 to formazan, providing a quantitative measure of cell viability. Absorbance measurements were taken at 450 nm wavelength.

2.6. Cell invasion assay

Cell invasion was measured using Bio-Coat Matrigel invasion assay chambers (BD Biosciences, German). In this assay, cells were placed in the upper chamber of a Matrigel-coated filter in a serum-free solution. The lower chamber contained medium with 10% FBS, serving as a chemoattractant. After a 48-h incubation period, the cells that had migrated to the underside of the filter were immobilized and quantified. These assays were conducted to evaluate the impact of various experimental conditions including DARS-AS1, ki-67, and Cyclin D1 expression levels, on cell proliferation and invasion.

2.7. Luciferase reporter assay

To investigate the interactions between DARS-AS1, ATF3, and miR-6835-3p, a luciferase reporter assay was conducted. Cells were co-transfected with the miRNA plasmid vector containing either wild-type or mutant ATF3 and wild-type or mutant DARS-AS1, along with the pRL-TK control plasmid. In addition, cells were co-transfected with miR-6835-3p mimic or scramble control using Lipofectamine 2000. The pRL-TK control plasmid served as an internal control for normalization.

After a 2-day incubation period, cells were harvested, and luciferase activity was measured using the Dual Luciferase Assay kit (Promega, USA) following the manufacturer's protocol. This assay provided quantitative insights into the regulatory interactions between DARS-AS1, ATF3, and miR-6835-3p, as alterations in luciferase activity reflect changes in the binding and modulation of these molecular components.

2.8. Statistical analysis

Data were reported as mean values ± *SD* obtained from three independent experiments. Statistical analyses were conducted using SPSS version 12.0 software (SPSS, USA). A p-value of <0.05 was considered statistically significant. Statistical significance was determined using either one-way analysis of variance (ANOVA) or Student's t-tests, as appropriate for the specific comparison being made. These statistical methods were applied to assess the reliability and significance of the observed results across experimental replicates, ensuring robust and valid conclusions.

3. Results

3.1. DARS-AS1 expression levels were elevated in breast cancer cells

The qRT-PCR analysis was conducted to assess the expression levels of DARS-AS1 in breast cancer cell lines, specifically MCF7 and MDA-MB-231, compared to a normal breast cell line, MCF-10A. Figure 1 shows that the expression of DARS-AS1 was significantly increased in the MCF7 and MDA-MB-231 cell lines compared to the MCF-10A cell line. These data demonstrate that DARS-AS1 is significantly overexpressed in breast cancer cells, indicating a possible involvement of this lncRNA in the progression of breast cancer.

3.2. miR-6835-3p expression was downregulated in breast cancer cells

In our investigation, we assessed the expression levels of miR-6835-3p in the MCF7 and MDA-MB-231 cell lines in comparison to the MCF-10A cell line (Figure 2). The results indicated a significant decrease in miR-6835-3p expression in breast cancer cell lines compared to the normal breast cell line. Furthermore, a correlation analysis was performed to explore the association between miR-6835-3p and DARS-AS1 expressions in breast cancer cell lines. As depicted in Figure 2, our findings revealed a negative association between miR-6835-3p and DARS-AS1 expression, suggesting a potential regulatory relationship between these two molecular components in breast

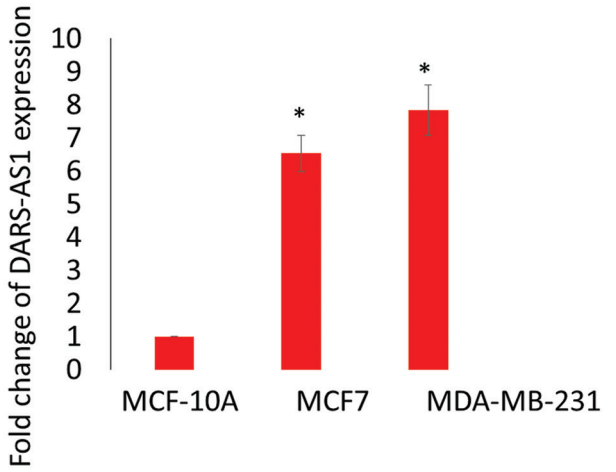

Figure 1. Expression of DARS-AS1 in breast cancer cells. The qRT-PCR technique was used to evaluate the relative expression levels of DARS-AS1 in breast cancer cell lines (MCF7 and MDA-MB-231) in comparison to a normal breast cell line (MCF-10A). The data demonstrate the upregulation of DARS-AS1 in breast cancer cell lines, emphasizing its increased expression in breast cancer cells relative to a normal breast cell line. *P < 0.05 indicates statistical significance compared to MCF-10A.
Abbreviation: qRT-PCR: Quantitative reverse transcription-polymerase chain reaction.

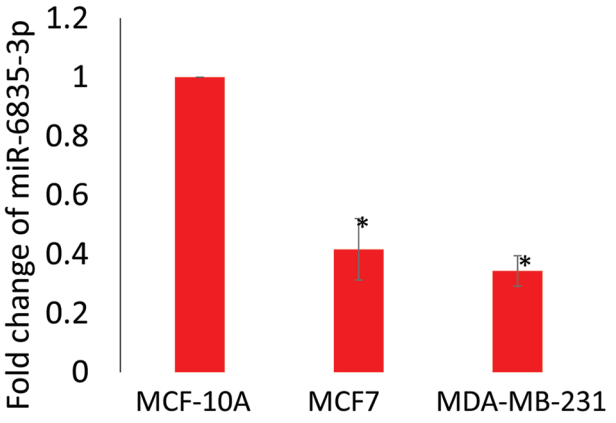

Figure 2. Decreased miR-6835-3p expression in breast cancer cells. The qRT-PCR technique was used to evaluate the relative amounts of miR-6835-3p expression in breast cancer cell lines (MCF7 and MDA-MB-231) in comparison to a normal breast cell line (MCF-10A). The data indicate that the expression of miR-6835-3p is reduced in the MCF7 and MDA-MB-231 cell lines compared to the MCF-10A cell line, emphasizing the reduced levels of miR-6835-3p in breast cancer cells. *P < 0.05 indicates statistical significance compared to MCF-10A.
Abbreviation: qRT-PCR: Quantitative reverse transcription-polymerase chain reaction.

cancer cells. These results provide valuable insights into the dysregulation of miR-6835-3p in breast cancer and its potential interplay with DARS-AS1 in this disease.

3.3. DARS-AS1 sponged miR-6835-3p in breast cancer cells

To investigate the potential interaction between miR-6835-3p and DARS-AS1, we identified putative binding sites of miR-6835-3p within the DARS-AS1 sequence (Figure 3A). Subsequently, we induced overexpression of miR-6835-3p in MDA-MB-231 breast cancer cells, confirming a significant upregulation of miR-6835-3p expression (Figure 3B). To validate the direct interaction between DARS-AS1 and miR-6835-3p, luciferase reporter assays were performed. The results indicated that overexpression of miR-6835-3p led to a decrease in luciferase activity, specifically in the presence of wild-type DARS-AS1, while no significant effect was observed with mutant DARS-AS1 in MDA-MB-231 cells (Figure 3C). This finding suggests a direct regulatory interaction between miR-6835-3p and DARS-AS1. Furthermore, we explored the impact of DARS-AS1 overexpression on miR-6835-3p levels. The expression of DARS-AS1 was increased in MDA-MB-231 cells after transfection with the pcDNA-DARS-AS1 plasmid (Figure 3D). Importantly, the ectopic expression level of DARS-AS1 resulted in the inhibition of miR-6835-3p levels in MDA-MB-231 cells (Figure 3E). These findings collectively indicate that DARS-AS1 functions as a sponge for miR-6835-3p in breast cancer cells, influencing the expression levels of both molecules and suggesting a regulatory axis in breast cancer progression.

3.4. miR-6835-3p directly targeted ATF3 expression in breast cancer cells

To investigate the regulatory connection between ATF3 and miR-6835-3p, we identified possible binding locations for miR-6835-3p within the ATF3 sequence (Figure 4A). Subsequently, luciferase reporter assays were performed to clarify the direct influence of miR-6835-3p on ATF3. The luciferase study revealed that the increased expression of miR-6835-3p significantly inhibited the luciferase activity of wild-type ATF3 in MDA-MB-231 cells, while no significant impact was observed with mutant ATF3 (Figure 4B). This finding provides evidence for the direct regulation of ATF3 by miR-6835-3p. In addition, the investigation examined the effect of increased expression of miR-6835-3p on ATF3 levels. Analysis of the findings demonstrates that the upregulation of miR-6835-3p resulted in a notable suppression of ATF3 expression in MDA-MB-231 cells (Figure 4C). Furthermore, we investigated the impact of DARS-AS1 on the expression of ATF3. The overexpression of DARS-AS1 led to a significant increase in ATF3 expression in MDA-MB-231 cells (Figure 4D). These findings indicate a regulatory relationship between miR-6835-3p, ATF3, and DARS-AS1 in breast cancer cells, providing valuable information on the complex molecular pathways that drive breast cancer growth.

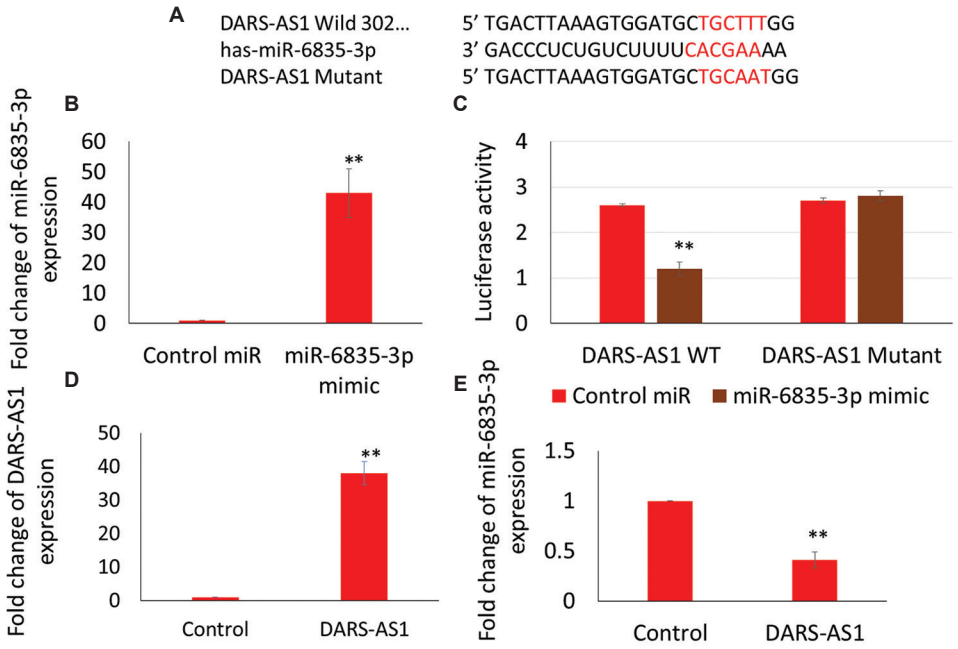

Figure 3. DARS-AS1 sponged miR-6835-3p in breast cancer cells. (A) The presence of putative binding sites for miR-6835-3p on DARS-AS1 suggests the possibility of an interaction. (B) Treatment with miR-6835-3p mimics resulted in a substantial increase in the expression of miR-6835-3p in MDA-MB-231 breast cancer cells. (C) The overexpression of miR-6835-3p led to a significant decrease in the luciferase activity of wild-type DARS-AS1 (DARS-AS1 WT), while the mutant DARS-AS1 (DARS-AS1 Mutant) did not exhibit any notable alteration in MDA-MB-231 cells. (D) The expression of DARS-AS1 was markedly increased in MDA-MB-231 breast cancer cells following the introduction of the pcDNA-DARS-AS1 plasmid. (E) Overexpression of DARS-AS1 resulted in the inhibition of miR-6835-3p expression in MDA-MB-231 breast cancer cells. $^{**}P < 0.01$ indicates statistical significance compared to the corresponding control.

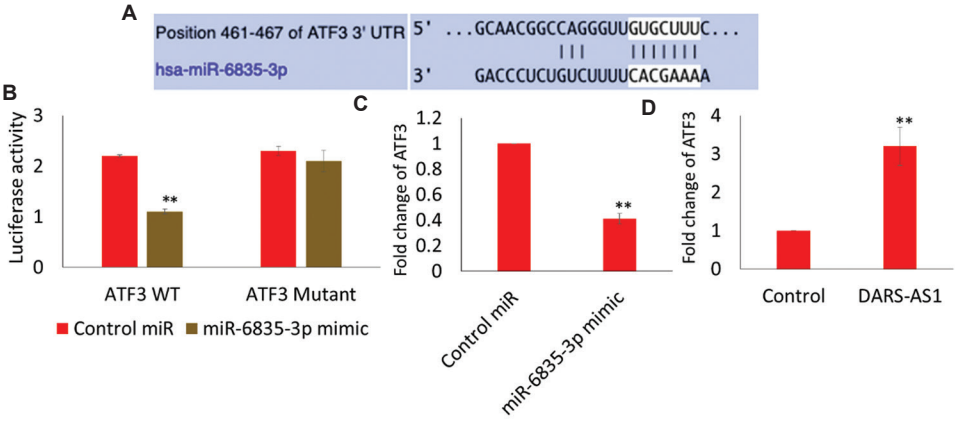

Figure 4. Direct regulation of ATF3 expression by miR-6835-3p in breast cancer cells. (A) ATF3 harbors potential binding sites for miR-6835-3p, suggesting a putative direct interaction. (B) Luciferase analysis demonstrated that overexpression of miR-6835-3p specifically suppressed the luciferase activity of wild-type ATF3 (ATF3-WT), whereas no significant effect was observed with mutant ATF3 (ATF3-Mutant) in MDA-MB-231 cells. (C) Elevated expression of miR-6835-3p resulted in a marked inhibition of ATF3 expression in MDA-MB-231 cells. (D) Ectopic expression of DARS-AS1 led to a notable promotion of ATF3 expression in MDA-MB-231 cells. $^{**}P < 0.01$ indicates statistical significance compared to the corresponding control.

3.5. DARS-AS1 increased breast cancer cell growth and invasion

The results of the CCK-8 assay demonstrated a notable increase in cell proliferation when DARS-AS1 was overexpressed in MDA-MB-231 cells (Figure 5A). This finding was supported by an elevation in ki-67 expression, a widely recognized indicator of cell growth, in MDA-MB-231 cells with artificially introduced DARS-AS1 expression (Figure 5B). Furthermore, increased levels of DARS-AS1 were shown to be linked with an increase in the expression of cyclin D1 in MDA-MB-231 cells (Figure 5C), providing additional evidence for its involvement

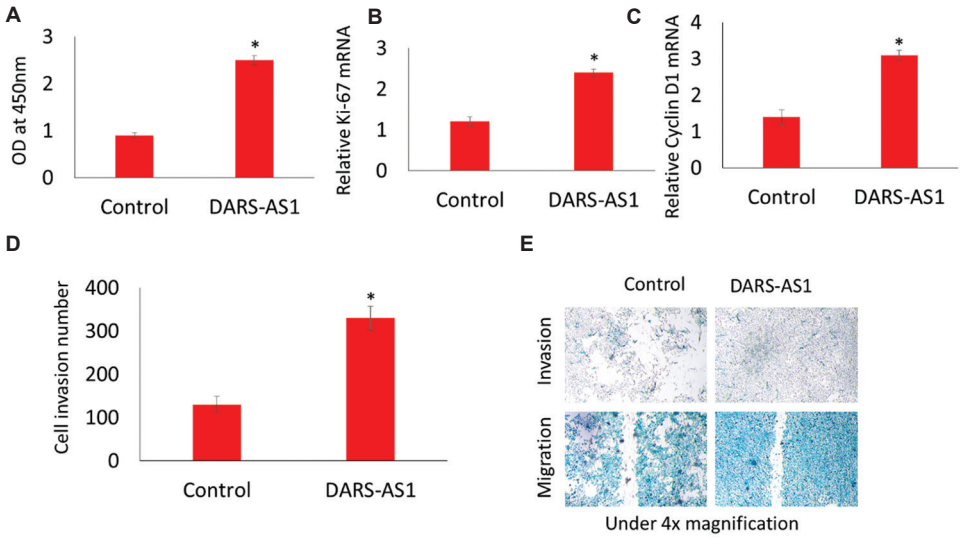

Figure 5. DARS-AS1 increased breast cancer invasion and growth. (A) The CCK-8 assay results indicate that the increased expression of DARS-AS1 significantly promotes cell proliferation in MDA-MB-231 cells. (B) Overexpression of DARS-AS1 results in an upregulation of ki-67 expression, indicating augmentation in cellular proliferation in MDA-MB-231 cells. (C) Increased expression of DARS-AS1 leads to an elevation in cyclin D1 expression, indicating its involvement in facilitating cell cycle progression in MDA-MB-231 cells. (D) Overexpression of DARS-AS1 induces cell invasion in MDA-MB-231 cells, as evidenced by functional invasion experiments. The bar graph displays the proportionate quantity of invasive cells, demonstrating a notable rise when DARS-AS1 is expressed in an abnormal location. (E) Invasion and Migration of DARS-AS1 and control. Magnification: ×4. *$P < 0.05$ indicates statistical significance compared to the corresponding control.

in encouraging the advancement of the cell cycle. Furthermore, functional experiments conducted to assess cell invasion revealed that the introduction of DARS-AS1 by ectopic expression notably enhanced cell invasion in MDA-MB-231 cells, as illustrated in Figure 5D and 5E. These data demonstrate that DARS-AS1 plays a role in promoting cell proliferation and invasion in breast cancer cells, indicating its potential as an oncogene in breast cancer progression.

3.6. Growth of MDA-MB-231 cells was inhibited by the downregulation of DARS-AS1 expression

To investigate the impact of reduced DARS-AS1 expression on cell growth, we employed si-DARS-AS1 to downregulate its expression in MDA-MB-231 breast cancer cells (Figure 6A). The results indicated a significant decrease in DARS-AS1 expression, indicating the effectiveness of the knockdown approach. Functional assays, specifically the CCK-8 assay, demonstrated that knockdown of DARS-AS1 led to a notable suppression of cell proliferation in MDA-MB-231 cells (Figure 6B). This observation was further supported by the downregulation of cyclin D1 (Figure 6C) and ki-67 (Figure 6D), indicating that downregulated DARS-AS1 expression inhibits cell cycle progression and cellular proliferation in MDA-MB-231 cells. These findings underscore the potential role of DARS-AS1 as an oncogenic driver in breast cancer cell growth.

3.7. Overexpression of DARS-AS1 promoted cell proliferation and invasion by regulating ATF3

To clarify the role of the DARS-AS1/ATF3 axis in the advancement of breast cancer, rescue experiments were performed. The findings demonstrated a notable decrease in ATF3 expression in MDA-MB-231 cells after administering si-ATF3 treatment (Figure 7A). Functional tests were used to evaluate the effect of reducing ATF3 expression on cell behavior in MDA-MB-231 cells that overexpress DARS-AS1. The results of the CCK-8 assay demonstrated that the inhibition of ATF3 significantly reduced cell proliferation in DARS-AS1-overexpressing cells (Figure 7B). Furthermore, the suppression of ATF3 led to a significant reduction in the expression of ki-67 (Figure 7C) and cyclin D1 (Figure 7D) in DARS-AS1-overexpressing MDA-MB-231 cells, highlighting the significance of ATF3 in controlling cell cycle progression. Furthermore, the inhibition of ATF3 resulted in a reduction of cell invasion in MDA-MB-231 cells overexpressing DARS-AS1, as evidenced by the data presented in Figure 7E and F. These findings indicate that the DARS-AS1/ATF3 axis plays a crucial role in promoting cell proliferation and invasion in breast cancer cells.[29-38]

4. Discussion

Our investigation illuminates the pivotal role of DARS-AS1 as an oncogenic lncRNA in the development of breast

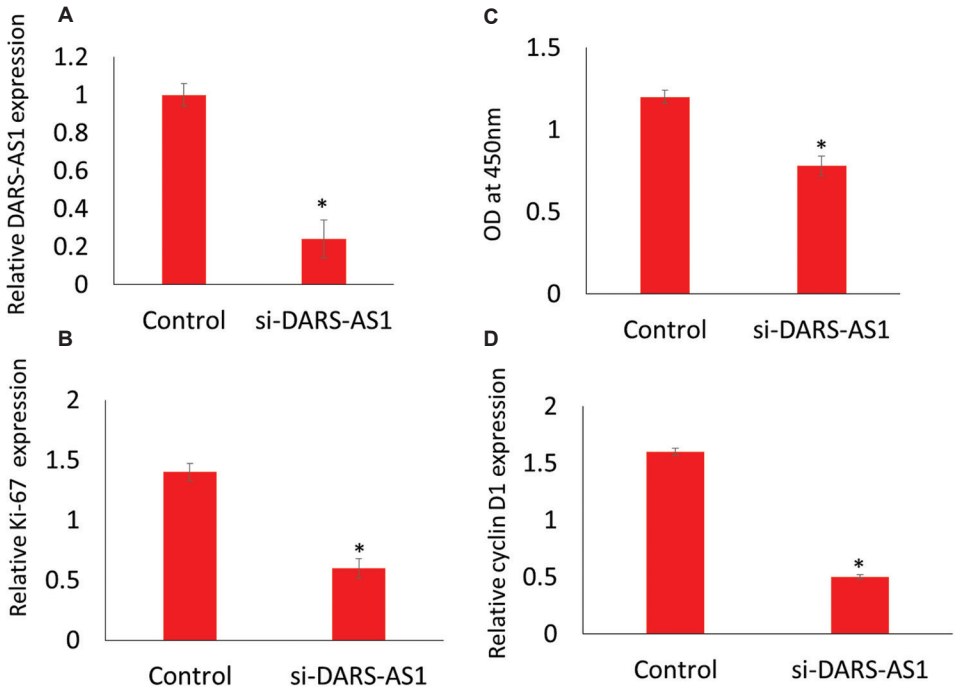

Figure 6. DARS-AS1 promotes MDA-MB-231 cell growth. (A) The qRT-PCR assay was employed to assess the expression of DARS-AS1, confirming successful knockdown in MDA-MB-231 cells. (B) Knockdown of DARS-AS1 resulted in significant suppression of cell proliferation in MDA-MB-231 cells, as demonstrated by the results of the CCK-8 assay. (C) The expression of cyclin D1, a pivotal cell cycle regulator, was evaluated using the qRT-PCR assay after DARS-AS1 knockdown in MDA-MB-231 cells. (D) Inhibition of DARS-AS1 expression led to a noteworthy suppression of ki-67 expression, a marker indicative of cellular proliferation, in MDA-MB-231 cells. *$P < 0.05$ indicates statistical significance compared to the corresponding control.
Abbreviation: qRT-PCR: Quantitative reverse transcription-polymerase chain reaction.

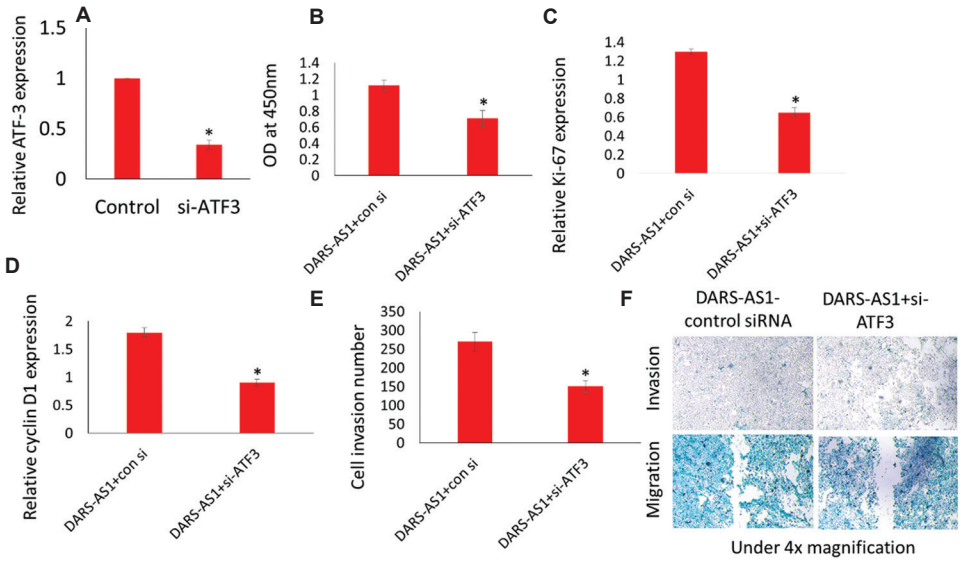

Figure 7. Increased DARS-AS1 expression promotes MDA-MB-231 cell proliferation and invasion by regulating ATF3. (A) The qRT-PCR experiment validated the decrease in ATF3 expression in MDA-MB-231 cells following si-ATF3 treatment. (B) Reduced ATF3 expression significantly inhibited cell proliferation in MDA-MB-231 cells overexpressing DARS-AS1, as evidenced by the results in the CCK-8 assay results, (C) the level of ki-67 expression measured using qRT-PCR, and (D) the quantification of cyclin D1 expression, a crucial controller of cell cycle advancement, performed using qRT-PCR. (E) Suppression of ATF3 expression hindered cell invasion in MDA-MB-231 cells overexpressing DARS-AS1. The bar graph displays the proportionate quantity of invasive cells, indicating a notable decrease when ATF3 is downregulated in DARS-AS1-overexpressing cells. (F) Invasion and migration of DARS-AS1 control siRNA and DARS-AS1+si-ATF3. Magnification: ×4. *$P < 0.05$ indicates statistical significance compared to the corresponding control.

cancer. Initially, we observed a significant upregulation of DARS-AS1 expression in breast cancer cell lines compared to a normal breast cell line, shedding light on its potential involvement in the pathogenesis of breast cancer. The functional assays conducted in MDA-MB-231 breast cancer cells demonstrated that DARS-AS1 plays a crucial role in promoting both cell growth and invasion. The chosen methodologies provide robust quantitative measurements, enabling a comprehensive understanding of the functional consequences of manipulating DARS-AS1 expression in breast cancer cells. Consistent with findings in other human tumors, DARS-AS1 has been identified as an oncogenic player in lung cancer, myeloma, thyroid cancer, and ovarian cancer. Previous studies have associated DARS-AS1 with adverse clinical outcomes, distant metastasis, and tumor progression in thyroid cancer, where it facilitated migration and proliferation through the modulation of miR-129. In non-small cell lung cancer, DARS-AS1-induced tumor progression through the regulation of miR-6835-3p. Similarly, in myeloma, DARS-AS1, upregulated through HIF-1, plays a role in tumorigenesis and cell survival through the binding of RBM39. In ovarian cancer, DARS-AS1 overexpression correlated with increased invasion, migration, and proliferation through modulation of miR-6835-3p.

Despite these established roles in various cancers, the function of DARS-AS1 in breast cancer remains unexplored. Our study fills this gap by demonstrating elevated expression of DARS-AS1 in breast cancer cell lines, accompanied by enhanced growth and invasion in breast cancer cells. These results underscore the oncogenic potential of DARS-AS1 in breast cancer, aligning with its established roles in other malignancies. Intriguingly, our study highlights the interaction between DARS-AS1 and miR-6835-3p in breast cancer cells. Acting as a sponge for miR-6835-3p, DARS-AS1 negatively regulated miR-6835-3p expression. The reciprocal relationship was evident as miR-6835-3p expression was lower in breast cancer cell lines compared to a normal cell line, and its expression correlated inversely with DARS-AS1 levels. Furthermore, we demonstrated that miR-6835-3p directly regulated ATF3 expression in breast cancer cells.

The association between miRNAs and lncRNAs has been well established in various cancers. In breast cancer, lncRNA, such as HOXA-AS2, NR2F1-AS1, SND1-IT1, and SPRY4-IT1, have been reported to influence cell behavior through miRNA modulation. In line with these findings, our study adds to the growing body of evidence supporting the intricate regulatory network between lncRNAs and miRNAs in breast cancer. The downstream effects of DARS-AS1 in breast cancer involve the regulation of ATF3, a transcription factor implicated in tumor migration and metastasis. By demonstrating that elevated expression of DARS-AS1 increases cell invasion and growth by modulating ATF3, our study unveils a novel axis – DARS-AS1/ATF3 – as a potential therapeutic target in breast cancer. This finding aligns with the established role of ATF3 in tumor progression and emphasizes the potential clinical relevance of targeting this axis in breast cancer treatment. Overall, this study uncovers the oncogenic role of DARS-AS1 in breast cancer, providing valuable insights into its molecular mechanisms and potential therapeutic implications. The DARS-AS1/ATF3 axis emerges as a promising avenue for further investigation and development of targeted therapies for breast cancer.

5. Conclusion

The current comprehensive study unveils compelling evidence supporting the pivotal role of DARS-AS1 as an oncogenic driver in breast cancer. Through meticulous examination of breast cancer cell lines, we successfully established a consistent pattern of upregulated DARS-AS1 expression in the breast cancer cells, providing a foundational understanding of its relevance in the context of breast cancer pathology. Crucially, functional assays conducted in breast cancer cells, specifically MDA-MB-231, revealed that elevated expression of DARS-AS1 is not merely a bystander but actively contributes to the malignant phenotype. The enhanced cell growth and invasion observed in response to DARS-AS1 overexpression underscore its functional significance in the progression of breast cancer. Current mechanistic investigations identified a regulatory axis involving DARS-AS1, miR-6835-3p, and ATF3. DARS-AS1 was found to act as a molecular orchestrator, modulating the expression of miR-6835-3p and ATF3. This intricate regulatory network sheds light on the underlying molecular events that drive breast cancer progression. Specifically, DARS-AS1, by regulating miR-6835-3p and ATF3, emerges as a key orchestrator in the complex molecular landscape of breast cancer.

The observed interaction between DARS-AS1 and miR-6835-3p adds an intriguing layer to our understanding of the regulatory mechanisms at play. Since DARS-AS1 functions as a sponge for miR-6835-3p, it exerts a fine-tuned control over miRNA expression. The reciprocal relationship, where miR-6835-3p also directly regulates ATF3 expression, adds depth to the intricate web of molecular interactions governing breast cancer progression. The identified role of ATF3 in the downstream effects of DARS-AS1 further establishes the functional consequences of this regulatory axis. ATF3, a transcription factor implicated in tumor migration and metastasis, emerges as a critical mediator of the enhanced cell growth and invasion induced by

elevated DARS-AS1 expression. In essence, our findings collectively support the assertion that DARS-AS1 serves as an oncogene in breast cancer. Its influence extends beyond mere overexpression, actively participating in the orchestration of a regulatory cascade involving miR-6835-3p and ATF3. The identification of this molecular axis not only enhances our understanding of breast cancer biology but also presents potential avenues for targeted therapeutic interventions.

Acknowledgments

None.

Funding

No funds were received for this study.

Conflict of interest

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

Author contributions

Conceptualization: All Authors
Format analysis: All Authors
Investigation: Krishnamoorthy Vignesh
Methodology: All Authors
Writing – original draft: Krishnamoorthy Vignesh
Writing – review & drafting: Krishnamoorthy Vignesh

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Data availability

Data will be made available with a reasonable request.

References

1. Polyak K. Heterogeneity in breast cancer. *J Clin Investig*. 2011;121:3786-3788.

   doi: 10.1172/JCI60534

2. Sørlie T, Perou CM, Tibshirani R, *et al*. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci U S A*. 2001;98:10869-10874.

   doi: 10.1073/pnas.191367098

3. Perou CM, Sørlie T, Eisen MB, *et al*. Molecular portraits of human breast tumours. *Nature*. 2000;406:747-752.

   doi: 10.1038/35021093

4. Onitilo AA, Engel JM, Greenlee RT, Mukesh BN. Breast cancer subtypes based on ER/PR and Her2 expression: Comparison of clinicopathologic features and survival. *Clin Med Res*. 2009;7:4-13.

   doi: 10.3121/cmr.2009.825

5. Brewster AM, Chavez-MacGregor M, Brown P. Epidemiology, biology, and treatment of triple-negative breast cancer in women of African ancestry. *Lancet Oncol*. 2014;15:e625-e634.

   doi: 10.1016/S1470-2045(14)70364-X

6. Gao Y, Shang S, Guo S, *et al*. Lnc2Cancer 3.0: An updated resource for experimentally supported lncRNA/circRNA cancer associations and web tools based on RNA-seq and scRNA-seq data. *Nucleic Acids Res*. 2021;49:D1251-D1258.

   doi: 10.1093/nar/gkaa1006

7. Han L, Yan Y, Zhao L, *et al*. LncRNA HOTTIP facilitates the stemness of breast cancer via regulation of miR-148a-3p/WNT1 pathway. *J Cell Mol Med*. 2020;24:6242-6252.

   doi: 10.1111/jcmm.15261

8. Hunt SE, McLaren W, Gil L, *et al*. Ensembl variation resources. *Database* (*Oxford*). 2018;2018:bay119.

   doi: 10.1093/database/bay119

9. Kong X, Duan Y, Sang Y, *et al*. LncRNA-CDC6 promotes breast cancer progression and function as ceRNA to target CDC6 by sponging microRNA-215. *J Cell Physiol*. 2019;234:9105-9117.

   doi: 10.1002/jcp.27587

10. Lakshmi S, Hughes TA, Priya S. Exosomes and exosomal RNAs in breast cancer: A status update. *Eur J Cancer*. 2021;144:252-268.

    doi: 10.1016/j.ejca.2020.11.033

11. Sun J, Zheng G, Gu Z, Guo Z. MiR-137 inhibits proliferation and angiogenesis of human glioblastoma cells by targeting EZH2. *J Neurooncol*. 2015;122:481-489.

    doi: 10.1007/s11060-015-1753-x

12. Cheetham SW, Gruhl F, Mattick JS, Dinger ME. Long noncoding RNAs and the genetics of cancer. *Br J Cancer*. 2013;108:2419-2425.

    doi: 10.1038/bjc.2013.233

13. Shin VY, Chu KM. MiRNA as potential biomarkers and therapeutic targets for gastric cancer. *World J Gastroenterol*. 2014;20:10432-10439.

    doi: 10.3748/wjg.v20.i30.10432

14. Huang J, Zhang SY, Gao YM, *et al*. MicroRNAs as oncogenes or tumour suppressors in oesophageal cancer: Potential biomarkers and therapeutic targets. *Cell Prolif*. 2014;47:277-286.

doi: 10.1111/cpr.12109

15. Tay HL, Plank M, Collison A, Mattes J, Kumar RK, Foster PS. MicroRNA: Potential biomarkers and therapeutic targets for allergic asthma? *Ann Med*. 2014;46:633-639.

doi: 10.3109/07853890.2014.958196

16. Xiong DD, Li ZY, Liang L, *et al*. The LncRNA NEAT1 accelerates lung adenocarcinoma deterioration and binds to Mir-193a-3p as a competitive endogenous RNA. *Cell Physiol Biochem*. 2018;48:905-918.

doi: 10.1159/000491958

17. Zhang S, Ma H, Zhang D, *et al*. LncRNA KCNQ1OT1 regulates proliferation and cisplatin resistance in tongue cancer via miR-211-5p mediated Ezrin/Fak/Src signaling. *Cell Death Dis*. 2018;9:742.

doi: 10.1038/s41419-018-0793-5

18. Chi Y, Wang D, Wang J, Yu W, Yang J. Long non-coding RNA in the pathogenesis of cancers. *Cells*. 2019;8:1015.

doi: 10.3390/cells8091015

19. Cai Y, Yu X, Hu S, Yu J. A brief review on the mechanisms of miRNA regulation. *Genomics Proteomics Bioinformatics*. 2009;7:147-154.

doi: 10.1016/S1672-0229(08)60044-3

20. Cuperus JT, Fahlgren N, Carrington JC. Evolution and functional diversification of MIRNA genes. *Plant Cell*. 2011;23:431-442.

doi: 10.1105/tpc.110.082784

21. Reddy KB. MicroRNA (miRNA) in cancer. *Cancer Cell Int*. 2015;15:38.

doi: 10.1186/s12935-015-0185-1

22. Hill M, Tran N. miRNA interplay: Mechanisms and consequences in cancer. *Dis Model Mech*. 2021;14:dmm047662.

doi: 10.1242/dmm.047662

23. Pereira DM, Rodrigues PM, Borralho PM, Rodrigues CM. Delivering the promise of miRNA cancer therapeutics. *Drug Discov Today*. 2013;18:282-289.

doi: 10.1016/j.drudis.2012.10.002

24. Wang KJ, Zhao X, Liu YZ, *et al*. Circulating MiR-19b-3p, MiR-134-5p and MiR-186-5p are promising novel biomarkers for early diagnosis of acute myocardial infarction. *Cell Physiol Biochem*. 2016;38:1015-1029.

doi: 10.1159/000443053

25. Feng Z, Zhang L, Wang S, Hong Q. Circular RNA circDLGAP4 exerts neuroprotective effects via modulating miR-134-5p/CREB pathway in Parkinson's disease. *Biochem Biophys Res Commun*. 2020;522:388-394.

doi: 10.1016/j.bbrc.2019.11.102

26. Chi J, Liu T, Shi C, *et al*. Long non-coding RNA LUCAT1 promotes proliferation and invasion in gastric cancer by regulating miR-134-5p/YWHAZ axis. *Biomed Pharmacother*. 2019;118:109201.

doi: 10.1016/j.biopha.2019.109201

27. He J, Yang T, He W, *et al*. Liver X receptor inhibits the growth of hepatocellular carcinoma cells via regulating HULC/miR-134-5p/FOXM1 axis. *Cell Signal*. 2020;74:109720.

doi: 10.1016/j.cellsig.2020.109720

28. Zhong BZ, Wang Q, Liu F, He JL, Xiong Y, Cao J. Effects of miR-384 and miR-134-5p acting on YY1 signaling transduction on biological function of gastric cancer cells. *Onco Targets Ther*. 2020;13:9631-9641.

doi: 10.2147/OTT.S259988

29. Bartel DP. MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell*. 2004;116:281-297.

doi: 10.1016/S0092-8674(04)00045-5

30. Chen JF, Mandel EM, Thomson JM, *et al*. The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. *Nat Genet*. 2006;38:228-233.

doi: 10.1038/ng1725

31. Alvarez-Garcia I, Miska EA. MicroRNA functions in animal development and human disease. *Development*. 2005;132:4653-4662.

doi: 10.1242/dev.02073

32. Hu K, Liang M. Upregulated microRNA-224 promotes ovarian cancer cell proliferation by targeting KLLN. *In Vitro Cell Dev Biol Anim*. 2017;53:149-156.

doi: 10.1007/s11626-016-0093-2

33. Wang Y, He X, Yu Q, Eng C. Androgen receptor-induced tumor suppressor, KLLN, inhibits breast cancer growth and transcriptionally activates p53/p73-mediated apoptosis in breast carcinomas. *Hum Mol Genet*. 2013;22:2263-2272.

doi: 10.1093/hmg/ddt077

34. Wang Y, Radhakrishnan D, He X, Peehl DM, Eng C. Transcription factor KLLN inhibits tumor growth by AR suppression, induces apoptosis by TP53/TP73 stimulation in prostate carcinomas, and correlates with cellular differentiation. *J Clin Endocrinol Metab*. 2013;98:E586-E594.

doi: 10.1210/jc.2012-3490

35. Zou A, Liu X, Mai Z, *et al*. LINC00472 acts as a tumor suppressor in NSCLC through KLLN-mediated p53-signaling pathway via MicroRNA-149-3p and MicroRNA-4270. *Mol Ther Nucleic Acids*. 2019;17:563-577.

doi: 10.1016/j.omtn.2019.06.003

36. David CJ, Massagué J. Contextual determinants of TGFβ action in development, immunity and cancer. *Nat Rev Mol Cell Biol*. 2018;19:419-435.

doi: 10.1038/s41580-018-0007-0

37. Feng XH, Derynck R. Specificity and versatility in tgf-beta signaling through Smads. *Annu Rev Cell Dev Biol*. 2005;21:659-693.

doi: 10.1146/annurev.cellbio.21.022404.142018

38. Jiang M, Chen J, Zhang W, *et al*. Interleukin-6 trans-signaling pathway promotes immunosuppressive myeloid-derived suppressor cells via suppression of suppressor of cytokine signaling 3 in breast cancer. *Front Immunol*. 2017;8:1840.

doi: 10.3389/fimmu.2017.01840