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Research

Hsa-circVIM regulates breast cancer tumor progression and tamoxifen sensitivity by sponging miR-1294 in hormone receptor-positive breast cancer cells

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Abstract

Background The progression and metastasis of breast cancer patients are regulated by genetics and epigenetics. Circular RNA (circRNA) plays a pivotal role in modulating the advancement of tumors. The study aimed to explore the clinical performance and regulatory role of hsa-circVIM in breast cancer and its modulatory effect on tamoxifen resistance in hormone receptor (HR) positive breast cancer cells.

Methods RT-qPCR was performed to detect hsa-circVIM expression in breast cancer tissues and cells.CCK-8 assay, Transwell assay, and flow cytometric analyses were performed to evaluate the effects of hsa-circVIM on cellular activities in breast cancer cells and TAM sensitivity in MCF7/TR cells. Bioinformatic analyses were conducted to make function and pathway enrichment analyses.

Results hsa-circVIM expression was raised in breast cancer and predicted unsatisfactory overall survival outcomes. Silencing of hsa-circVIM suppressed cell viability, and migration capacities, while simultaneously enhancing TAM sensitivity and inducing apoptosis of HR-positive breast cancer cells by targeting miR-1294.

Conclusion Elevated hsa-circVIM expression in breast cancer suggested its potential as a prognostic biomarker. hsa-circVIM functions as both a cancer-promoting molecule and a regulator of TAM responsiveness in HR-positive breast cancer cells by regulating miR-1294 expression. Therefore, hsa-circVIM serves as a potent biomarker for prognosis, plays a promoting role in breast cancer progression, and may offer a therapeutic avenue to overcome TAM resistance.

Keywords Hsa_circ_0017873 · TAM · Prognosis · Proliferation · Invasion

Ying Chen and Zhenhua Zhang contributed equally to the study.

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1 Introduction

For women, breast cancer surpassed lung cancer as the first commonly diagnosed tumor with an increasing incidence rate (about 0.5% per year since the mid-2000 s) [1]. Among breast cancer patients, approximately 70% of patients are hormone receptor (HR)-positive, and endocrine drugs are very important for ER-, PR-positive patients'treatment [2]. Tamoxifen (TAM) is one of the endocrine therapy drugs, but not all HR-positive patients are sensitive to TAM, in addition, some patients who are sensitive to drugs will develop drug resistance to TAM, resulting in unsatisfactory treatment effects[3, 4]. The NCCN Clinical Practice Guidelines in Oncology for breast cancer (version 3.2024) reported clinical systemic therapy options for breast cancer, such as immunotherapy, targeted therapy, adjuvant systemic therapy, and gene-based precision medicine[5]. For HR-positive breast cancer, the combination of cyclin-dependent kinase 4/6 (CDK4/6) inhibitors with endocrine therapies has emerged as a game-changer [5]. Despite the remarkable progress brought by the combination of CDK4/6 inhibitors with endocrine therapies in treating HR-positive breast cancer, the issue of endocrine resistance remains a significant challenge[6]. An in-depth exploration of the mechanism of endocrine resistance in HR-positive breast cancer is pivotal to enhancing the prognosis for patients.

With the continuous development of RNA sequencing technology and bioinformatics, more and more circular RNAs (circRNA) have been detected. CircRNAs are a novel kind of RNA molecule in eukaryotic cells that have a stable circular structure [7]. The circular structure gives circRNAs many other properties that RNA does not have, such as insensitivity to exonuclease enzymes, higher stability, and better bioconservatism [8]. A burgeoning body of studies demonstrated that circRNAs can regulate gene expression and take part in the onset and progression of diverse human diseases, including cancers and neurodegenerative diseases[9, 10]. CircRNAs can sponge miRNAs to regulate mRNA expression and thus exhibit either promoting role or suppressing function activities in cancers [10, 11]. In breast cancer, circRNAs, such as circCDYL[12], circFOXK2[13], and circRNF20 [14], are associated with tumorigenesis bounding with miRNAs. In addition, abnormal expressions of circRNA could affect the tumorigenesis of ER-positive breast cancer and TAM resistance by targeting miRNAs [15]. However, the precise roles and mechanisms of circRNAs in breast cancer and TAM-resistant HR-positive breast cancer remain largely uncharted and warrant further investigation.

Although many advances have been achieved in endocrine therapy for HR-positive breast cancer, new and acquired resistance during treatment in tumors remains an important clinical issue[16]. The regulatory effect of circRNA on endocrine therapy has gradually been recognized, such as circPGR and circPVT1, which participate in the regulation of TAM resistance in breast cancer [15, 17, 18]. Herein, we identified a new abnormally expressed circRNA termed hsa-circVIM (ID: hsa_circ_0017873, position: chr10: 17,275,585–17,279,592) in breast cancer. hsa-circVIM has prognostic significance in acute myeloid leukemia[19] and silencing its expression could inhibit immune escape and malignant phenotypes by regulating the miR-124/PD-L1 axis in esophageal cancer[20]. However, the clinical and functional role of circVIM in breast cancer remains uncertain. We attempted to carry out functional analyses and explore its regulatory abilities in breast cancer for investigating the underlying mechanism of circVIM in tumorigenesis in HR-positive breast cancer cells. While hsa-circVIM has been explored in acute myeloid leukemia and esophageal cancer, our research is the first to comprehensively investigate its role specifically in HR-positive breast cancer cells. This unique focus on a particular breast cancer subtype allows for a more targeted and in-depth understanding of the circRNA's function in a context that has been previously overlooked.

2 Materials and methods

2.1 Collection of tissue specimens

The procedures of the present study have been approved by the Ethical Committee of The Affiliated Hospital of Youjiang Medical University for Nationalities. A total of 135 breast cancer patients receiving surgical resection were enrolled in the current study. The inclusive criteria were: (1) patients were diagnosed with breast cancer and confirmed by three pathologists; (2) None of the patients had undergone any local or systemic breast cancer-related therapy prior to surgery; (3) all patients had complete clinicopathological information and five-year follow-up data. The exclusion criteria were: (1) patients diagnosed with other malignant tumors; (2) patients who have received local therapy and systemic therapy such as chemotherapy or radiotherapy before surgery. Paired tumor tissues and adjacent tissue specimens were obtained

during surgery or biopsy and partially stored in liquid nitrogen for further analysis. All patients were followed up for 60 months (from May 2017 to August 2022) after surgery.

2.2 Cell culture and transfection

The noncancerous mammary gland cell line MCF-10 A, and human breast cancer cell lines (MCF-7, BT474, T47D, MDA-MB-361, SK-BR-3, and MDA-MB-231) were obtained from the Chinese Academy of Sciences (Shanghai Cell Bank; Shanghai, China). Tamoxifen-resistant cells (MCF7/TR) were established using tamoxifen-sensitive cell line MCF7 with 10 μM tamoxifen for 1 year as previously described [21]. The breast cancer cells were maintained in DMEM containing 10% FBS. The cells were cultivated in an incubator set to 5% CO_2 at 37 °C.

The siRNAs specifically targeting circVIM (si-circVIM-1, AACTCGATGTTGACAATGCGT; si-circVIM-2, TGGAAAAACTCGATG TTGACA), siRNA negative control (si-NC), miR-1294 mimic (miR-1294), mimic NC, miR-1294 inhibitor (in-miR-1294), and inhibitor NC (inhi-NC) were custom designed by RiboBio (Guangzhou, China). Then, the target plasmids or scrambled vectors were transfected or co-transfected into cells utilizing Lipofectamine 3000 (Invitrogen).

2.3 RNA separation and RT-qPCR

Total RNA was extracted from tissue specimens and cells with TRIzol (Invitrogen). A NanoDrop spectrophotometer was used to determine the purity and concentration of RNA (A260/A280 ratio between 1.8 and 2.0). After RNA purity and quality detection, implementation of reverse transcription was conducted using PrimeScript RT Reagent Kit (Takara, China) with primers. Subsequently, RT-PCR was carried out in triple with a SYBR Premix Ex Taq™ kit on an ABI 7900HT Real-Time PCR machine. The PCR cycling conditions comprised denaturation at 95 °C for 10 min, annealing at 58 °C for 20 s, and extension at 72 °C for 20 s, repeated for 40 cycles, and the melt curve analysis was done at a temperature range of 60–95 °C. The sequences used in PCR were as follows: circVIM, forward 5′-CCAGCAAGTATCCAACCAACTT-3′, reverse 5′-TCGTGGAGTTTCTTCAAAAAGGC-3′, GAPDH, forward 5′-GAGTCAACGGATTTGGTCGT-3′, reverse 5′-TTGATTTTG GAGGCATCTCG-3′, miR-1294, forward, 5′-TATGATCTCACCGAGTCCT-3′, reverse, 5′-CACCTTCCTAATCCTCAGTT-3′, and U6, forward 5′-GCTTCGGCAGCACATATACTAAAAT-3′and reverse 5′-CGCTTCACGAATTTGCGTGTCAT-3′. Relative expression was determined by applying the $2^{-\Delta\Delta Ct}$ calculation method with the internal reference of GAPDH and U6. The MIQE (Minimum Information for Publication of Quantitative Real-Time PCR Experiments) guidelines were strictly followed in the design and implementation of the experiments [22].

2.4 Luciferase reporter assay

The CircInteractome(https://circinteractome.nia.nih.gov/index.html), circBank(https://www.circbank.cn/#/home), and Starbase (https://rnasysu.com/encori/index.php) databases were utilized to search for the miRNAs downstream miRNAs of circVIM and predict interactions between circVIM and miR-1294.

To investigate the interaction between circVIM and miR-1294, hsa-circVIM 3′-UTR with miR-1294 binding sites were designed and inserted into the pGL3 promoter vector (constructs circVIM-wt, circVIM-mut). Then cells were cultured and co-transfected circVIM-wt or circVIM-mut with miR-1294 mimic, in-miR-1294, or NCs reagents with lipofectamine 3000 (Invitrogen). After 2 days of transfection culture, luciferase activities were measured using the dual-luciferase assay (Promega).

2.5 RNA immunoprecipitation (RIP)

The RIP assay was conducted using the Magna RIP RNA-binding Protein Immunoprecipitation Kit from Millipore. Anti-Ago2 or control IgG antibodies were obtained from Abcam. Total RNA in the immunoprecipitant complex was isolated for detection of hsa-circVIM expression by RT-qPCR.

2.6 Cell viability experiments

Cell Counting Kit-8 (CCK-8; Dojindo, Japan) was employed to assess cellular viability and cytotoxicity after different treatments. Transfected cells (MCF7, BT474, or MCF7/TR) were cultured in 96-well plates for cell proliferation detection.

For cell cytotoxicity assay, the medium was replaced with a complete medium containing various concentrations of TAM. The absorbance (450 nm) was measured using a microplate reader at the specified time points.

2.7 Transwell assays

Migration (without Matrigel) and invasion (with Matrigel) assays were performed using a Transwell chamber (Corning). Following transfection, breast cancer cells (1×10^5) in a serum-free medium were seeded into the top well, and a complete medium containing 10% FBS was added to the bottom well of the chamber. After 48 h of incubation, migrated or invaded cells were stained and photographed under a microscope (Olympus, Japan).

2.8 Flow cytometry assay

The Annexin V-Fluorescein Isothiocyanate (FITC)/Propidium Iodide (PI) Apoptosis Detection Kit (BD) was used to detect cell apoptosis abilities based on the manufacturer's protocol. After treatment, the cell samples were analyzed by flow cytometry on FACS Calibur (BD, NJ, USA).

2.9 Bioinformatic analysis

The online software TargetScan (https://www.targetscan.org/vert_80/), miRDB (https://mirdb.org/), and ENCORI (https://rnasysu.com/encori/index.php) were utilized to predict the potential targets of miR-1294. For the integration of protein–protein interactions, the STRING database (https://cn.string-db.org/cgi/input?sessionId=bEXsfMWsksEG&input_page_show_search=on) was employed, with a focus on high-confidence interactions (score ≥ 0.700) and excluding disconnected nodes from the network. The exploration of biomolecule interaction networks was conducted using Cytoscape software (version 3.7.1; Cytoscape Consortium, San Diego, CA, USA) with the short tabular text obtained from the STRING database. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses (*P*-value less than 0.05) were implemented using the DAVID database (https://david.ncifcrf.gov) and Bioinformatics (https://www.bioinformatics.com.cn/) to extract meaningful biological information from a large number of genes.

2.10 Statistical analysis

The comparison was performed using SPSS software (version 26.0) and GraphPad Prism software (version 9.0) and data were displayed as mean ± SD. Statistical significance were assessed by Student's t-test, one-way analysis of variance (ANOVA), or two-way ANOVA, as appropriate. Kaplan–Meier curve and Cox analysis were used to assess the clinical prognostic value of hsa-circVIM in breast cancer. Values of *P* less than 0.05 were considered statistically significant.

3 Results

3.1 Raised hsa-circVIM expression was observed in breast cancer and its correlation with clinical parameters of breast cancer patients

The hsa-circVIM levels in breast cancer tissues were higher in tumor tissues in contrast to normal tissues (Fig. 1A). Based on the mean value of hsa-circVIM in tumor tissues as the cut-off value (2.273), the cohort of 135 breast cancer patients was grouped into two groups: those with low expression of hsa-circVIM and those with high expression of hsa-circVIM. (Table 1). Significant differences were found in histological Grade, lymph node metastasis, ER status, PR status, and TNM stage. In these cases, about 41% of ER and PR negative, and about 50% of HER negative tumors have high expression of hsa-circVIM in Table 1.

Consistently, hsa-circVIM expression was raised in breast cancer cells (MCF7, BT474, T47D, MDA-MB-361, SK-BR-3, and MDA-MB-231), especially in HR-positive breast cancer cells (MCF7, BT474, T47D, and MDA-MB-361) compared with normal MCF-10 A breast cell lines (Fig. 1B). Furthermore, the increased levels of hsa-circVIM were observed in MCF7/TR cells in comparison with MCF7 cells (Fig. 1C).

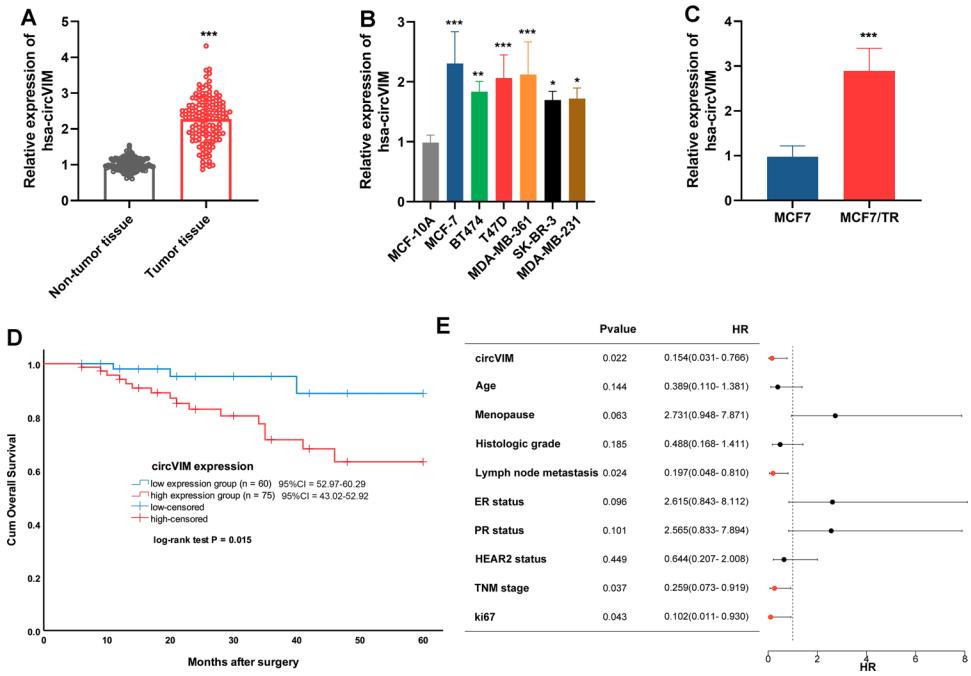


Fig. 1 hsa-circVIM expression increased in breast cancer and correlated with shorter overall survival outcomes. **A** hsa-circVIM expression was elevated in breast cancer tumor tissues (RT-qPCR). **B** hsa-circVIM expression in breast cancer cells. **C** The level of hsa-circVIM was enhanced in MCF/TR cells compared with TAM-sensitive MCF7 cells. **D**. Breast cancer patients (135 cases) with high hsa-circVIM showed a shorter overall survival rate. **E** Multivariate Cox regression analysis revealed that hsa-circVIM expression was one of the independent prognostic risk factors for patients with breast cancer. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

Table 1 Association between hsa_circVIM expression and clinical variables in patients with breast cancer

Variable	Cases (n = 135)	Hsa_circVIM expression		P value
		low	high	
Age(years)				0.463
≤ 50	74	35	39	
> 50	61	25	36	
Menopause				0.487
No	72	34	38	
Yes	63	26	37	
Histological Grade				0.048
I-II	75	39	36	
III	60	21	39	
Lymph node metastasis				0.009
Negative	80	43	37	
Positive	55	17	38	
ER				0.017
Negative	44	26	18	
Positive	91	34	57	
PR				0.016
Negative	48	28	20	
Positive	87	32	55	
HER2				0.165
Negative	105	50	55	
Positive	30	10	20	
TNM stage				0.003
I-II	87	47	40	
III-IV	48	13	35	
Ki67 level				0.405
Low	51	25	26	
High	84	35	49	

3.2 Correlation between hsa-circVIM expression and breast cancer patients' clinical outcome

The prognostic impact of hsa-circVIM was evaluated using clinical characteristics and survival information. Kaplan–Meier curve disclosed that high hsa-circVIM expression and shorter overall survival time go hand in hand (log-rank test $P = 0.015$, Fig. 1D). Further subgroup stratified analysis results indicated that patients with high hsa-circVIM expression had a shorter overall survival rate, but only the luminal A group had a statistical difference (Supplementary Fig. 1). Multivariate COX regression analysis results showed that the hazard ratio (HR) of low hsa-circVIM expression was 0.154, compared with high hsa-circVIM expression. These results manifested that low hsa-circVIM expression is a protective factor in this cohort, while high hsa-circVIM expression is a risk factor. High hsa-circVIM expression may be an independent prognostic marker in overall survival among all breast cancer patients (Fig. 1E).

3.3 hsa-circVIM plays an oncogenic role in HR-positive breast cancer cells

To elucidate the functional role of hsa-circVIM in HR-positive breast cancer, two siRNAs were designed to knock down hsa-circVIM expression in HR-positive breast cancer cells. RT-PCR verified the transfection efficiency of si-circVIM-1 and si-circVIM-2 in MCF7 and BT474 cells (Fig. 2A). Because of the higher silencing efficiency, si-circVIM-1 was chosen for

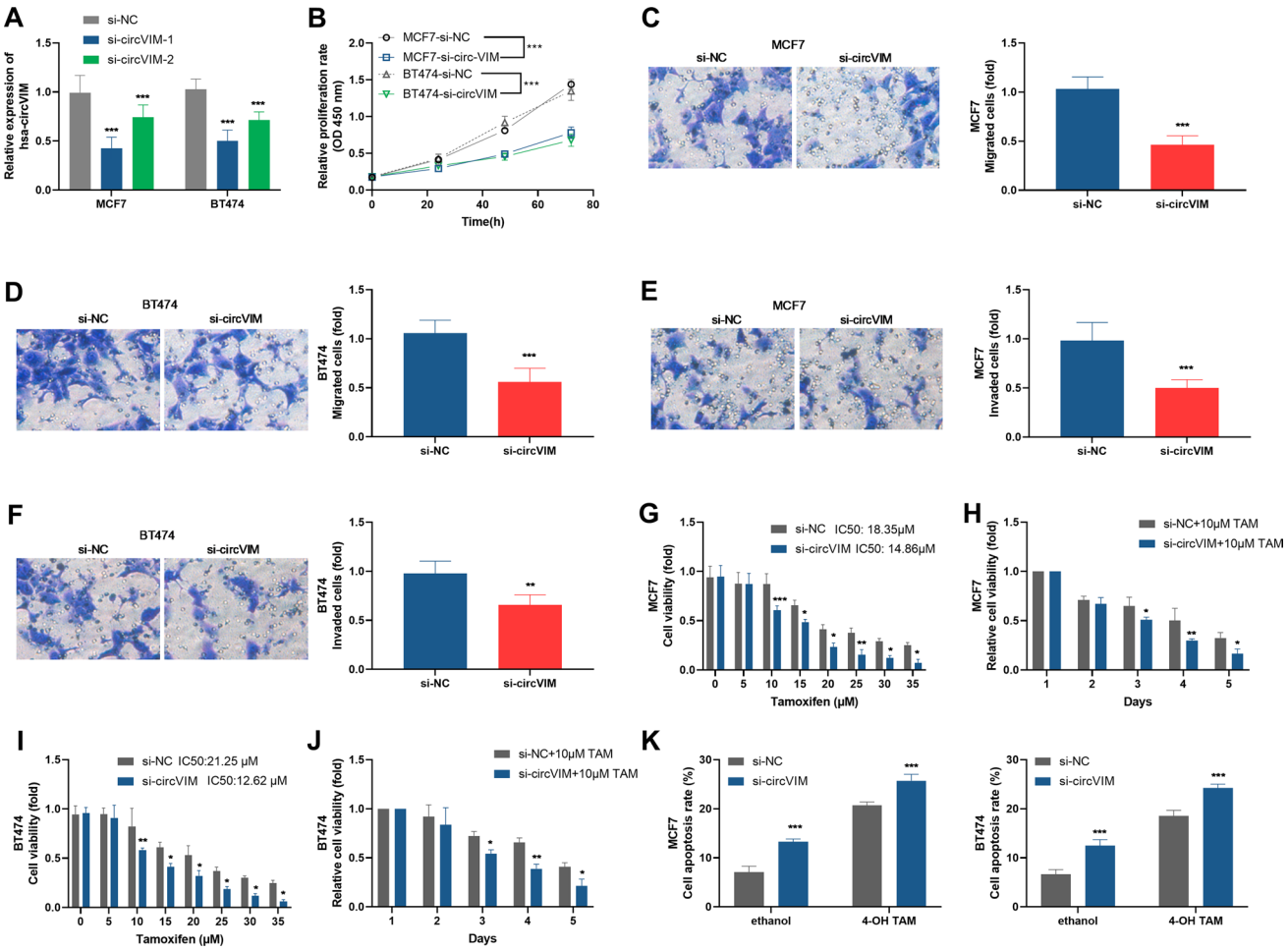


Fig. 2 Knockdown of hsa-circVIM restrains viability and invasion and promotes TAM sensitivity and apoptosis in HR-positive breast cancer cells. **A** si-circVIM decreased the expression of hsa-circVIM in cancer cells. **B** Decreased expression of hsa-circVIM weakened cell proliferation abilities. C-F. Silencing of hsa-circVIM repressed cell migration (**C** and **D**) and invasion capacities (**E** and **F**). **G** IC50 assays showed the effects of si-circVIM on TAM sensitivity in MCF7 cells. **H** The CCK-8 assay was performed to assess the viability of MCF7 cells following TAM treatment. **I** IC50 assays showed the effects of si-circVIM on TAM sensitivity in BT474 cells. **J** CCK-8 assay was conducted to evaluate the cell viability of BT474 cells treated with TAM. **K** Silencing of hsa-circVIM induced cell apoptosis. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

subsequent cellular experiments. The siRNA-mediated silencing of hsa-circVIM decreased the proliferative capacities (Fig. 2B), migration abilities (Fig. 2C and D), and invasive potential (Fig. 2E and F) of both MCF7 and BT474 cells. Cell sensitivity to TAM was increased in HR-positive breast cancer cells by si-circVIM (Fig. 2G–J). Silencing of hsa-circVIM-induced cell apoptosis (Fig. 2K).

3.4 Knockdown of hsa-circVIM can weaken the cellular behaviors and TAM resistance

To investigate the functional differences between MCF7 and MCF7/TR cells, a functional assay was performed. The results showed that MCF7/TR cells presented stronger resistance to TAM (Fig. 3A) and more enhanced proliferation abilities (Fig. 3B) than MCF7 cells. Subsequently, the functional role of hsa-circVIM was explored in MCF7/TR cells after transfection of si-circVIM (Fig. 3C). Downregulation of hsa-circVIM decreased MCF7/TR cell viability, migration behaviors, and invasion abilities (Fig. 3D–F). Flow cytometry results revealed that the knockdown of hsa-circVIM increased cell apoptosis rate (Fig. 3G).

3.5 hsa-circVIM could sponge to miR-1294

CircInteractome, circBank, and Starbase databases were utilized to search for the downstream miRNAs of circVIM. Four miRNAs (miR-1294, miR-513a-5p, miR-574-5p, and miR-590-5p) were predicted among these three databases and the binding sites were shown in Fig. 4A. These miRNA expressions were measured in MCF7 cells and miR-1294 had higher expression levels after the knockdown of hsa-circVIM (Fig. 4B), which was chosen for the next analysis. Next, the levels of miR-1294 were found to be decreased in breast tumor tissues (Fig. 4C). The binding sites between hsa-circVIM and miR-1294 are shown in Fig. 4D. The luciferase reporter activities were reduced in MCF7 cells co-transfected with miR-1294 mimic and circVIM-wt while having no significant change with the mutant vector (Fig. 4E). Furthermore, RIP assay revealed that significantly higher levels of hsa-circVIM were detected in complex with Ago2 after transfection of miR-1294 mimic (Fig. 4F).

3.6 hsa-circVIM acted as a modulator in tumor cellular activities and TAM sensitivity by regulating miR-1294

To investigate the biological effects of hsa-circVIM/miR-1294 interaction, rescue cellular experiments were carried out using MCF7/TR cells. After transfection, miR-1294 levels were raised in cells transfected with si-circVIM, while its increased

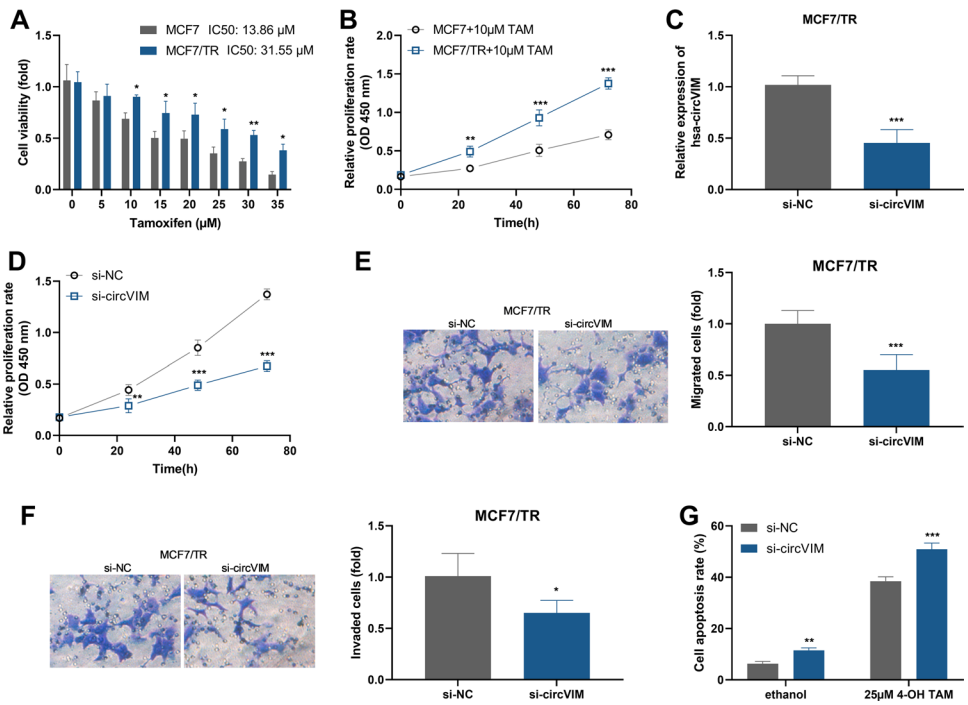
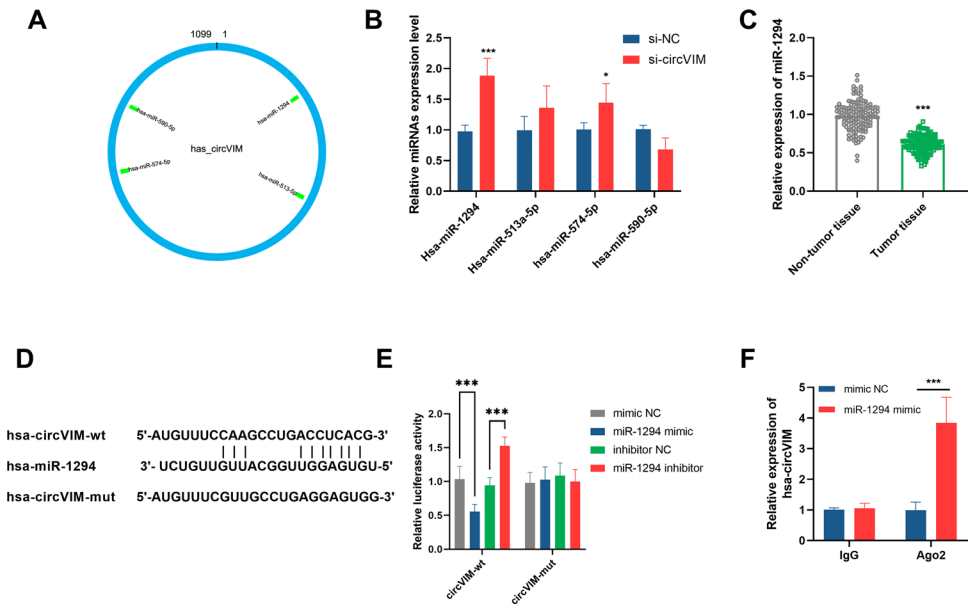


Fig. 3 The impact of reduced hsa-circVIM expression on the cellular functions and TAM resistance in MCF7/TR cells was investigated. **A** The difference of IC50 to TAM between MCF7 and MCF7/TR cells. **B** MCF7/TR cells have higher viabilities than MCF7 cells under the treatment of 10 μM TAM. **C** Transfection efficiency of si-circVIM in MCF7/TR cells. **D** Knockdown of hsa-circVIM decreased MCF7/TR cell viabilities. **E** and **F**. Silencing of hsa-circVIM weakened cell migration (**E**) invasion (**F**). **G** Decreased hsa-circVIM expression induced cell apoptosis in MCF7/TR cells treated with 25 μM TAM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$



expression was counteracted by the miR-1294 inhibitor (Fig. 5A). Silencing of circVIM reduced the viability capacities, migration abilities, and invasion rate (Fig. 5B-5D) and induced apoptosis (Fig. 5E). Importantly, inhibition of miR-1294 partially rescued these phenotypes in MCF7/TR cells co-transfected with si-circVIM and miR-1294 inhibitor (Fig. 5B-5E).

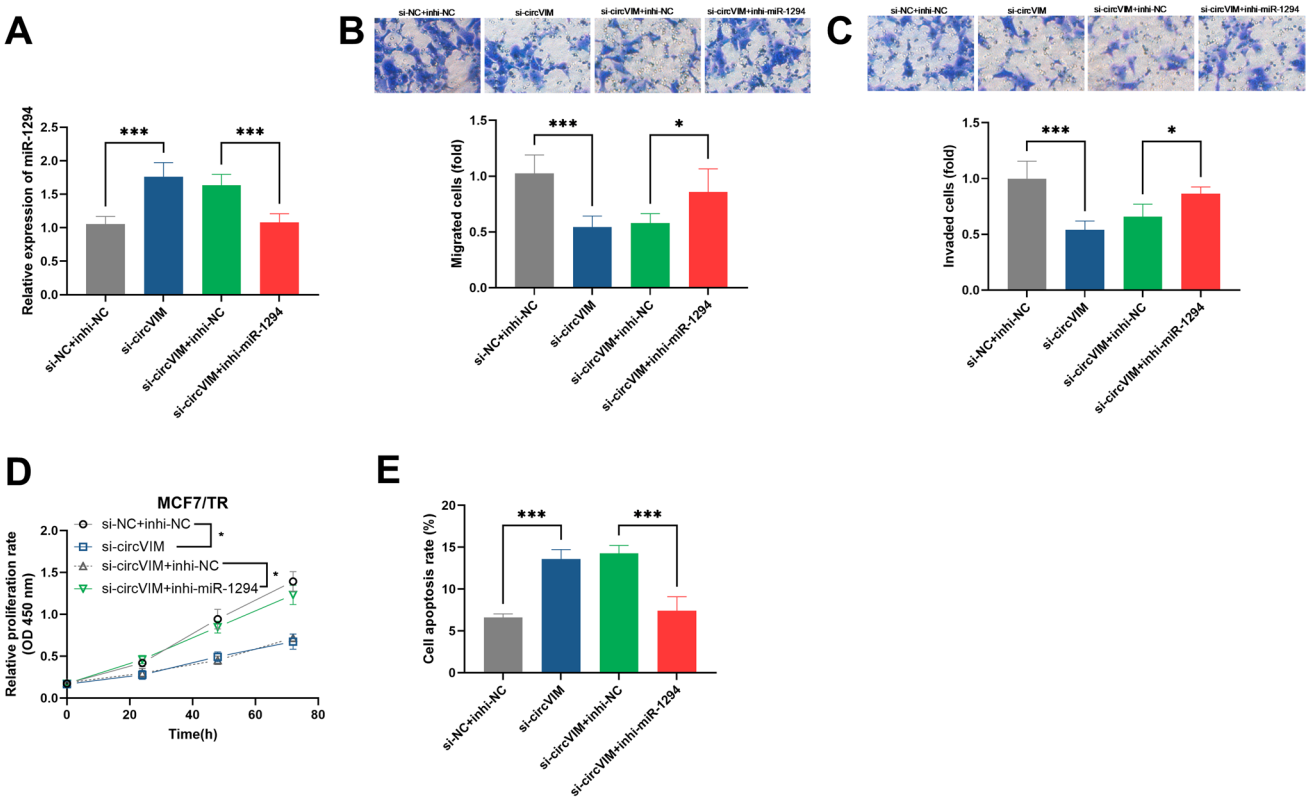


Fig. 4 hsa-circVIM could target miR-1294. **A** The predictive binding position of the miRNAs related to hsa-circVIM. **B** The expression of candidate miRNAs after transfection of si-circVIM. **C** Expression of miR-1294 in tissue samples was measured by RT-PCR. **D** The binding sites of hsa-circVIM and miR-1294. **E** The dual-luciferase reporter assay measured the luciferase activity of circVIM-wt or circVIM-mut after co-transfection with miR-1294 mimics in MCF7 cells. **F** RIP assay was conducted to measure the binding between hsa-circVIM and miR-1294. $^{*}P < 0.05$, $^{***}P < 0.001$

Fig. 5 hsa-circVIM targets miR-1294 to modulate the tumor cellular behaviors of MCF7/TR cells. **A** RT-qPCR analysis of miR-1294 expression in MCF7/TR cells transfected or co-transfected with si-circVIM or inhi-miR-1294. B and C. Transwell migration (**B**) and invasion (**C**) assays were performed in MCF7/TR cells co-transfected with si-circVIM and inhi-miR-1294. **D** The cell proliferation of MCF7/TR cells was measured at the indicated time. **E** Inhibition of miR-1294 partially eliminates the increased apoptosis caused by si-circVIM. $^{*}P < 0.05$, $^{***}P < 0.001$

3.7 Functional and pathway enrichment of target genes of miR-1294

To further understand the functional mechanism of circVIM in breast cancer, the downstream targets of miR-1294 were screened, and functional and pathway enrichment analyses were performed. Figure 6A results visualized the downstream

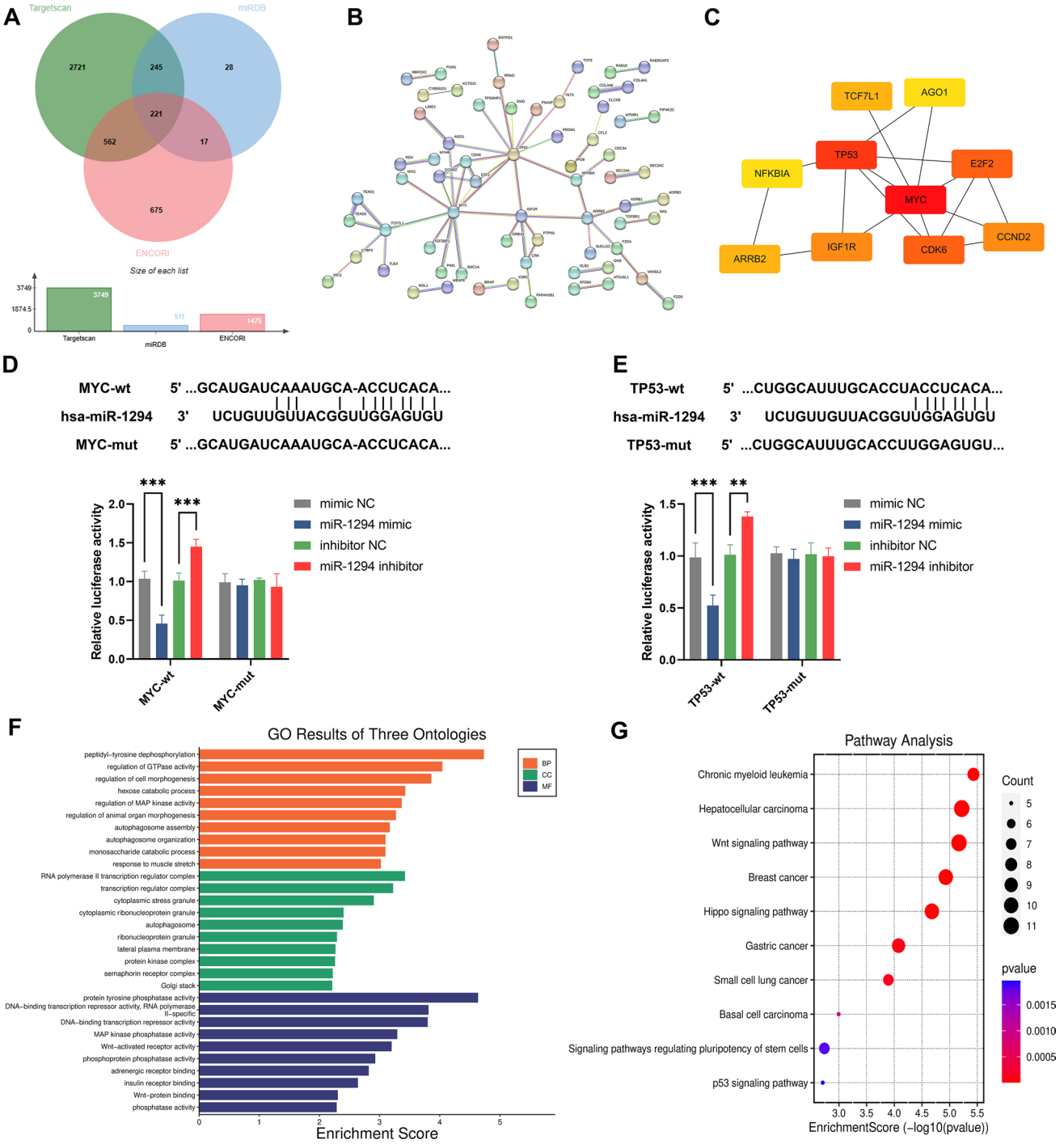


Fig. 6 Bioinformatic analyses of target genes of miR-1294. **A** Venn diagrams of target genes of miR-1294 using TargetScan, miRDB, and ENCORI databases. **B** STRING database was used to visualize the PPI interaction. **C** The top ten hubgenes were obtained using Cytoscape with PPI interaction data. **D** and **E** Dual-luciferase reporter assay was performed to verify the target relationship among miR-1294, MYC, and TP53. **F** GO enrichment results of three ontologies. **G** Top ten enriched pathways using KEGG enrichment analysis

genes from TargetScan, miRDB, and ENCORI databases and screened 221 genes. String database and Cytoscape visualized the PPI network (Fig. 6B) and top ten hubgenes, including MYC and TP53 (Fig. 6C). The dual-luciferase reporter assay preliminary verified the targeting relationship between miR-1294 and MYC, as well as TP53(Fig. 6D and E). The top ten GO term enrichment and KEGG enrichment patterns of these differently expressed genes were enriched and visualized in Fig. 6F and 6G. The GO-BP terms were associated with signaling transduction, cell migration, and differentiation, such as "peptidyl-tyrosine dephosphorylation", "regulation of GTPase activity", and "regulation of cell morphogenesis". For GO-MF, top terms were involved in the regulation of signal transduction, negative regulation of gene expression, and signaling pathways regulation, such as "protein tyrosine phosphatase activity", "DNA-binding transcription repressor activity", "MAP kinase phosphatase activity", and "Wnt-activated receptor activity". The top GO-CC enriched terms were highly related to nuclear and cytoplasm transcription, such as "RNA Polymerase II transcription regulator complex", "transcription regulator complex", and "cytoplasmic stress granule". The KEGG enrichment analysis was associated with breast cancer and some pathways that were reported to play a crucial role in cancer progression, such as the "Wnt signaling pathway" and "Hippo signaling pathway". The significant resulting GO enrichment terms and KEGG pathway enrichment terms are listed in Supplementary Tables.

4 Discussion

In this study, we identified that hsa-circVIM was raised in breast cancer tissues and was associated with HR-positive breast cancer, TAM resistance, and tumor metastasis. Clinical analysis revealed that dysregulation of hsa-circVIM was positively related to HR status, lymph node metastasis, grade, and TNM stage, revealing its promoting and crucial regulator role in breast cancer. Function studies indicated that loss of hsa-circVIM inhibited cell viability, migration abilities, and invasion capacities, while simultaneously promoting cell apoptosis, and enhancing TAM sensitivity by regulating miR-1294.

Research has substantiated a strong association between the emergence and growth of tumors and the dysregulated expression of certain circular RNAs. For instance, circ_0001785 represses the proliferation, migration, and invasion of breast cancer cells by regulating miR-942/SOCS3 axis expression[23]. Circ_001783 facilitated the proliferation and invasion of breast cancer cells by sponging miR-200c-3p and might serve as a prognostic biomarker for breast cancer[24]. Continuous studies have confirmed that the expression of circRNA has obvious disease specificity, especially the abnormal expression in different malignant tumors[25]. Herein, increased hsa-circVIM expression in breast cancer tissues and MCF7/TR cells suggest hsa-circVIM may play an oncogenic role in breast cancer, especially in HR-positive breast cancer, and is associated with tamoxifen-resistant. However, it remains unclear whether this high expression also occurs specifically within breast cancer cells in clinical tissue samples, which will be verified in our future studies. Consistently, Liang Gao and co-workers performed microarray analysis and identified that hsa-circVIM was one of the abnormally expressed circRNAs correlated with tamoxifen-resistant breast cancer, revealing that hsa-circVIM expression might be strongly linked to TAM resistance in breast cancer[26]. hsa-circVIM was reported to be upregulated in acute myeloid leukemia (AML) patients (vs. healthy control) and might be a promising predictor for diagnosis and prognosis for AML [19]. Thus, the clinical significance of hsa-circVIM was further explored. High expression of hsa-circVIM in breast cancer revealed a positive correlation between high expression levels and several clinical parameters, as well as patients' shorter overall survival rate, revealing that hsa-circVIM expression might be an important regulator in breast cancer progression. Moreover, high levels of hsa-circVIM lead to shorter survival outcomes in breast cancer patients. However, the enrolled patients included a few cases at stage IV, which is a limitation in the current study. This may have a biased effect on its clinical significance, which needs to be confirmed in future studies in a large cohort of cases on stage 3 or below. Because circRNAs possess a high degree of sequence stability, this suggests that hsa-circVIM might be a prognostic predictor for breast cancer patients.

A previous study has pointed out that hsa-circVIM is overexpressed in esophageal cancer, and reducing its expression has been shown to inhibit immune evasion and the diverse malignant characteristics of esophageal cancer cells [20]. The clinical evaluation revealed a relationship between hsa-circVIM expression levels and the status of ER and PR, indicating that dysregulated hsa-circVIM expression may play a role in the advancement of HR-positive breast cancer. However, the influence of hsa-circVIM on the progressive phenotype of HR-positive breast cancer remains elusive. Functionally, silencing of hsa-circVIM could inhibit cellular behaviors, induce cell apoptosis in breast cancer cells, and sensitize cells to TAM treatment, revealing that hsa-circVIM might have a promoting impact on breast cancer. Furthermore, the same effects were observed in MCF7/TR cells after knockdown hsa-circVIM, enhancing that hsa-circVIM could affect HR-positive breast cancer cell viability, invasion, and sensitivity to ATM. These results suggested that hsa-circVIM had an essential promoting functional role in regulating the progression of HR-positive breast cancer and TAM resistance.

The roles of circRNAs in various cancers are exerted through regulating miRNA expression as sponges and modulating the activity of target genes [13, 27, 28]. For instance, circRNA_102002 could promote papillary thyroid cancer cell metastasis by modulating miR-488-3p [29]. CircRNA WHSC1 sponges miR-646 to facilitate the progression of endometrial cancer [30]. Herein, we implied that hsa-circVIM may adsorb and regulate miR-1294. Abundant studies have demonstrated the suppressive function of miR-1294 in many tumors[31–33]. For instance, miR-1294 correlates with dismal prognosis and has an anti-tumor role in esophageal squamous cell carcinoma by targeting c-Myc [31]. Consistent with our finding of decreased miR-1294 expression in breast cancer tissues, a recent study pointed out that miR-1294 was decreased in breast cancer tissues and played an inhibitory role in tumorigenesis[34]. Moreover, functional experiments in MCF7/TR cells indicated that miR-1294 inhibition partially reverses the influence of silencing hsa-circVIM on cell viability, apoptosis, and sensitivity of TAM. These data suggested that hsa-circVIM participated in breast cancer progression and modulates the TAM response in HR-positive breast cancer cells affecting the miR-1294 expression.

Using bioinformatic analysis, 221 target genes of miR-1294 were overlapped, and the top ten hub genes were obtained after PPI interaction, especially MYC, and TP53. MYC and TP53 had vital effects on breast cancer [35–37]. The present study preliminarily verified the targeting relationship between miR-1294 and MYC, as well as miR-1294 and TP53. GO and KEGG enrichment analyses of target genes were mainly enriched in metabolism (such as peptidyl-tyrosine dephosphorylation and protein tyrosine phosphatase) and Wnt signaling pathway and breast cancer, which are correlated with the progression and tamoxifen-resistant breast cancer go hand and hand [38, 39]. Thus, silencing circVIM may exhibit enhanced sensitivity to tamoxifen through dephosphorylation of ER. The putative functional pathway involving hsa-circVIM and miR-1294 in HR-positive breast cancer is displayed in Supplementary Fig. 2.

It should be noted that this study has some limitations. Firstly, the sample size is limited for stratified studies, especially among different molecular subtypes of breast cancer, which may lead to underrepresentation of the results. Secondly, due to the limitations of time, funds, and equipment, a deeper and more comprehensive study could not be carried out. More advanced experiments or more rigorous study designs could be used to overcome the shortcomings. Finally, the functional role of circVIM and its detailed mechanism in HR-positive breast cancer are not fully understood. The specific mechanism of hsa-circVIM in HR-positive breast cancer will continue to be investigated with in vivo experiments.

5 Conclusion

In sum, hsa-circVIM expression was increased in breast cancer and associated with HR status, as well as patients' poorer survival outcomes. Silencing of hsa-circVIM could affect breast cancer cell proliferation, invasion, apoptosis, and sensitivity to TAM by affecting miR-1294 expression in HR-positive cells. Taken together, hsa-circVIM may be a putative biomarker for prognosis and TAM resistance. hsa-circVIM may participate in breast cancer tumorigenesis and regulate TAM response in HR-positive breast cancer by affecting miR-1294 expression, highlighting its potential role as a therapeutic target in TAM-resistant HR-positive breast cancer.

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate The study protocol was approved by The Ethics Committee of The Affiliated Hospital of Youjiang Medical University for Nationalities and followed the principles outlined in the Declaration of Helsinki. In addition, informed consent has been obtained from the participants involved.

Competing interests The authors declare no competing interests.

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