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Circ-TFCP2L1 Promotes the Proliferation and Migration of Triple Negative Breast Cancer through Sponging miR-7 by Inhibiting PAK1

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Abstract

CircRNAs are essential factors that have been verified to regulate various forms of carcinogenesis. However, the role of circRNAs in triple negative breast cancer (TNBC) tumourigenesis is not well clarified. In this study, we explored the circRNA expression profiles and possible modulation mechanism of circRNAs on triple negative breast cancer tumourigenesis. We used three pairs of triple negative breast cancer tissues and adjacent noncancerous tissues to perform a human circRNA microarray for screening of circRNA expression patterns in TNBC. The results showed that circ-TFCP2L1 was significantly up-regulated in TNBC tissues and cells, tending to have a shorter disease-free survival of TNBC patients. In vitro loss-of-function experiments showed that knockdown of circ-TFCP2L1 significantly suppressed the proliferation and migration of TNBC cells. Moreover, the results showed that the proliferation and migration capabilities and PAK1 expression in TNBC cells treated with si-circ-TFCP2L1 + miR-7 mimics were significantly suppressed compared with the normal group. Therefore, circ-TFCP2L1 was identified as a sponge of miR-7 functionally targeting PAK1 and further promoting the proliferation and migration of TNBC cells. Taken together, the results from our study reveal a novel regulatory mechanism and offer novel insight into the role of circ-TFCP2L1 in progression of triple negative breast cancer.

Keywords circRNAs · Microarray · Sponge · Proliferation · Migration

Introduction

Breast cancer (BC) is the most common hormone-dependent tumour disease in women worldwide, with a significant diversity in genetic and genetic phenotype [1, 2]. Triple-negative breast cancer (TNBC) is a special type of breast cancer that accounts for 15% to 20% of total breast cancer diagnoses, with references to immunohistochemical staining of negative expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER-2) [3]. TNBC has unique biological characteristics and clinical features such as high recurrence, early metastasis and poor prognosis. Endocrine or anti-HER2 targeted therapies are never the treatment options for TNBC because there is no corresponding hormone receptor or HER-2 expression, but the use of chemotherapeutic agents such as paclitaxel sometimes holds disease progression [4]. To maximize therapy effectiveness and optimize treatment modalities, it is important to gain a better understanding of the molecular pathways involved in TNBC pathogenesis that cause proliferation and metastasis.

Qian Wang, Zhouxiao Li and Yun Hu contributed equally to this work.

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Circular RNAs (circRNAs) have been detected and studied in eukaryotic cells [5]. In addition to long noncoding RNAs (lncRNAs) and microRNAs (miRNAs), circRNAs are found to exist in the RNA world as well. Additionally, circRNAs are a special type of endogenous noncoding RNAs that derive from a noncanonical form of alternative splicing, which can originate from exons (exonic circRNA, ecircRNA), introns (intronic circRNA, ciRNA) or both (exon–intron circRNA, EIciRNA) [6]. In most cases, circRNAs are formed with exons, mainly correlated with circularization characteristics [7]. It is reported that RNAs can function as competitive endogenous RNAs (ceRNAs) that co-regulate each other via competition for shared microRNAs. CircRNAs in mammals have also been shown to function as miRNA sponges or ceRNAs [8, 9]. Yang C et al. showed that circRNA-ITCH functions as a miR-17/miR-224 sponge [10]. Zhou J et al. Found that hsa_circ_0011946 suppresses the migration and invasion of breast cancer by targeting RFC-3 [11]. Liu Y reported that hsa_circ_0008039 promotes breast cancer cell proliferation and migration by regulating the miR-432-5p/E2F3 axis [12]. All of these findings reveal that circRNAs could function as miRNA sponges to modulate tumour progression. Nevertheless, the roles of circRNAs in TNBC remain underfined at present and its features and functions require further investigation.

In this study, human circRNA microarray analysis was used to screen the circRNA expression profiles in TNBC tissues and paracancerous tissues from 3 TNBC patients. Among these circRNAs, circ-TFCP2L1 is the most remarkable in terms of expression level. Based on this information, a series of functional verification experiments were conducted to explore the role of circ-TFCP2L1 in the development of TNBC. The results showed that circ-TFCP2L1 promoted proliferation and migration of TNBC cells by "sponging" miR-7, thereby promoting transcription of p21 protein-activated kinase 1(PAK1), indicating that circ-TFCP2L1 could be used as a diagnostic biomarker and potential target in TNBC therapy.

Methods

CircRNA Microarray

The CapitalBio Technology Human CircRNA Array v2 was designed with four identical arrays on each slide. GeneSpring software V13.0 (Agilent) was used to analyse the circRNA array data for data summary, normalization and quality control. The threshold values of ≥2-fold and ≤ 2-fold changes and a Benjamini-Hochberg corrected p value of 0.05 were used to choose the differentially expressed genes. Log2 transformation of data was conducted using CLUSTER 3.0 software to adjust the data function, and gene-centred midranking was performed, followed by hierarchical clustering and average linkage analysis.

Patients and Samples

This study was approved by the Ethics Committee of Nanjing First Hospital, Nanjing Medical University. A total of 32 triple negative breast cancer patients who underwent mastectomy were recruited for the study. Pairs of TNBC tissues and paracancerous samples were collected. The paracancerous tissue was taken from a location 5 cm away from the cancer tissue. None of the patients received radiotherapy or chemotherapy before the operation. Tissue samples were immediately preserved at −80 °C until use. Following the principle, the paired adjacent non-tumor tissues were confirmed to contain no tumour cells via pathological analysis. Written informed consent was obtained from all patients before participation in the research.

Follow-Up

For the disease-free survival (DFS) study, all patients saw their doctors during the follow-up period for assessment every 3 months in the first year, every 6 months in the second year and annually thereafter until June 2018, and dates of relapse were obtained from inpatient and outpatient records or from the patients' families through telephone follow-up.

Cell Lines and Transfection

Human TNBC cell lines SUM1315, HCC1937, MDA-MB-231 and MCF-10A were established from samples extracted from Shanghai Genechem Co.,Ltd. HCC1937 and MDA-MB-231 cells were transfected with 100 nM siRNAs or negative control (si-NC). After 48 h, knockdown of circ-TFCP2L1 was confirmed via quantitative real-time PCR (qRT-PCR). The target sequences for circ-TFCP2L1 siRNAs are listed as follows: siRNA-1: 5′- CCATCAAAGGCCGG TCAGT-3′; siRNA-2:5'-CAAAGGCCGGTCAGTCTTA-3′ and siRNA-3: 5′- GCCGGTCAGTCTTATGAAA-3′. The primer sequences of PAK1 are listed as follows: forward 5'-CAGCCCCTCCGATGAGAAATA-3′ and reverse 5'-CAAAACCGACATGAATTGTGTGT-3′. The sequence of the miR-7 is 5′- UGGAAGACUAGUGAUUUUGUUGUU -3′. The sequence of the miR-7 mimic is listed as follows: sense: 5'-UGGAAGACUAGUGAUUUUGUUGU-3′, antisense: 5'-AACAAAAUCACUAGUCUUCCAUU-3′.

RNA Isolation, Reverse Transcription and Quantitative Real-Time Polymerase Chain Reaction

We extracted total RNA from paired tissues using the TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instruction. All complementary DNAs (cDNAs) were obtained using the Goldenstar™ RT6 cDNA Synthesis Kit (TSINGKE, Beijing, China). The

expression level of circ-TFCP2L1 was determined by qRT-PCR via the following primer pair:5′-CTCCCATCAGCTTC GATCCA-3′ (Forward, or F) and 5′- TCATAAGACTGACC GGCCTT −3′ (reverse, R). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was selected as the internal control. Each experiment was repeated in triplicate.

Cell Counting Kit-8 Proliferation Assay

Cell proliferation was determined using the Cell Counting Kit-8 (CCK-8, Promega) assay according to the manufacturer's instructions. HCC1937 and MDA-MB-231 cells were transfected with si-NC or 100 nM si-circ-TFCP2L1 and seeded in 96-well plates at 2×10^3 per well and monitored. An amount of 10 μl of CCK-8 reagent was added to each well, and the cells were incubated for two hours in a 37 °C cell incubator. A microplate reader (MD, USA) at an absorbance of 450 nm was used to evaluate the cell viability every 24 h. Each experiment was repeated in triplicate.

Scratch Wound Assay

HCC1937 and MDA-MB-231 cells were transfected with 100 nM si-circ-TFCP2L1 or a negative control. When the cell confluence reached approximately 90% at 24 h post-transfection, wounds were created with a 200 μl pipette tip, and the cells were rinsed cells with medium to remove free-floating cells and debris. Medium was added, and the culture plates were incubated at 37 °C. Wound healing was surveyed at different time points and representative scrape lines were photographed. Each experiment was repeated in triplicate.

Luciferase Reporter Assay

The circ-TFCP2L1-WT, circ-TFCP2L1-Mut, PAK1-WT and PAK1-Mut binding sites were inserted into the KpnI and SacI sites of the pGL3 promoter vector (Realgene, Nanjing, China). HCC1937 and MDA-MB-231 cells were seeded on a 96-well plate and cultured in medium containing 10% FBS at 37 °C and 5% CO_2. The cells were co-transfected with luciferase reporters and miR-7 mimics. After incubation for 48 h, the firefly and Renilla luciferase activities were measured via a dual-luciferase reporter assay (Promega, Madison, WI, USA) according to the manufacturer's protocol. Each experiment was repeated in triplicate.

Biotin-Coupled Probe RNA Pull Down Assay

To detective the miRNA sponged by circRNA, we performed the biotin-coupled probe RNA pull down assay. HCC1937 and MDA-MB-231 cells transfected with miR-7 mimics were lysed and incubated with the biotin-coupled probe of circ-TFCP2L1, which was pre-bound on magnetic beads. For approximately 2 h, target RNA was pulled down by the RNeasy Mini Kit (QIAGEN, Germany). The pull-down product was extracted, reversed and quantitated using qRT-PCR. Each experiment was repeated three times to ensure the credibility of the results.

Fluorescence In Situ Hybridization (FISH)

The fluorescence in situ hybridization assay was applied to ascertain the presence of circ-TFCP2L1 and miR-7 via using the Fluorescence in Situ Hybridization Kit (RiboBio, Guangzhou, China). Circ-TFCP2L1 was captured with a Cy5-labelled probe, and separately, miR-7 was captured with a Cy3-labelled probe. After prehybridization, the circ-TFCP2L1 probe and miR-7 probe were hybridized in prepared hybridization buffer in the cells. Nuclei were stained using 4,6-diamidino-2-phenylindole (DAPI). All images were acquired using confocal microscopy.

Statistical Analysis

Student's t test was chosen for data analysis in the study. The paired t-test was used to test the significance of the difference between two pairs of paired data, and the independent sample t test was used to test the mean difference between two independent data. The total difference among the three groups was measured by the one-way analysis of variance. Kaplan-Meier plots and log-rank tests were applied to assess the relationship between circ-TFCP2L1 expression and disease-free survival time. Statistical analysis was performed with SPSS (Version 22.0, IBM, USA) and presented graphically in GraphPad Prism 5.0. A p value of 0.05 was considered to be statistically significant.

Results

CircRNA Expression Profiles in TNBC Tissues and the Biological Structure of Circ-TFCP2L1

The high-throughput human circRNA microarray was performed using cancer tissues and paracancerous tissues from 3 TNBC patients. As illustrated in Fig. 1a, the volcano plot revealed a total of 509 differentially expressed circRNAs in the case group vs control group: 339 were upregulated, and 170 were downregulated (Fig. 1a). The circRNA targets determined by profiling data were subjected to gene ontology (GO) (Supplement Figure 1a) and KEGG pathway analyses (Fig. 1b) based on their correlative mRNAs via Gene Ontology (http://www.geneongoloty.org/) and KOBAS software (KEGG Ontology-Based Annotation System). The results showed that many circRNAs were implied in the process of carcinogenesis. Top 10 significantly up-regulated circRNAs were listed in Supplement Table 1. We first verified

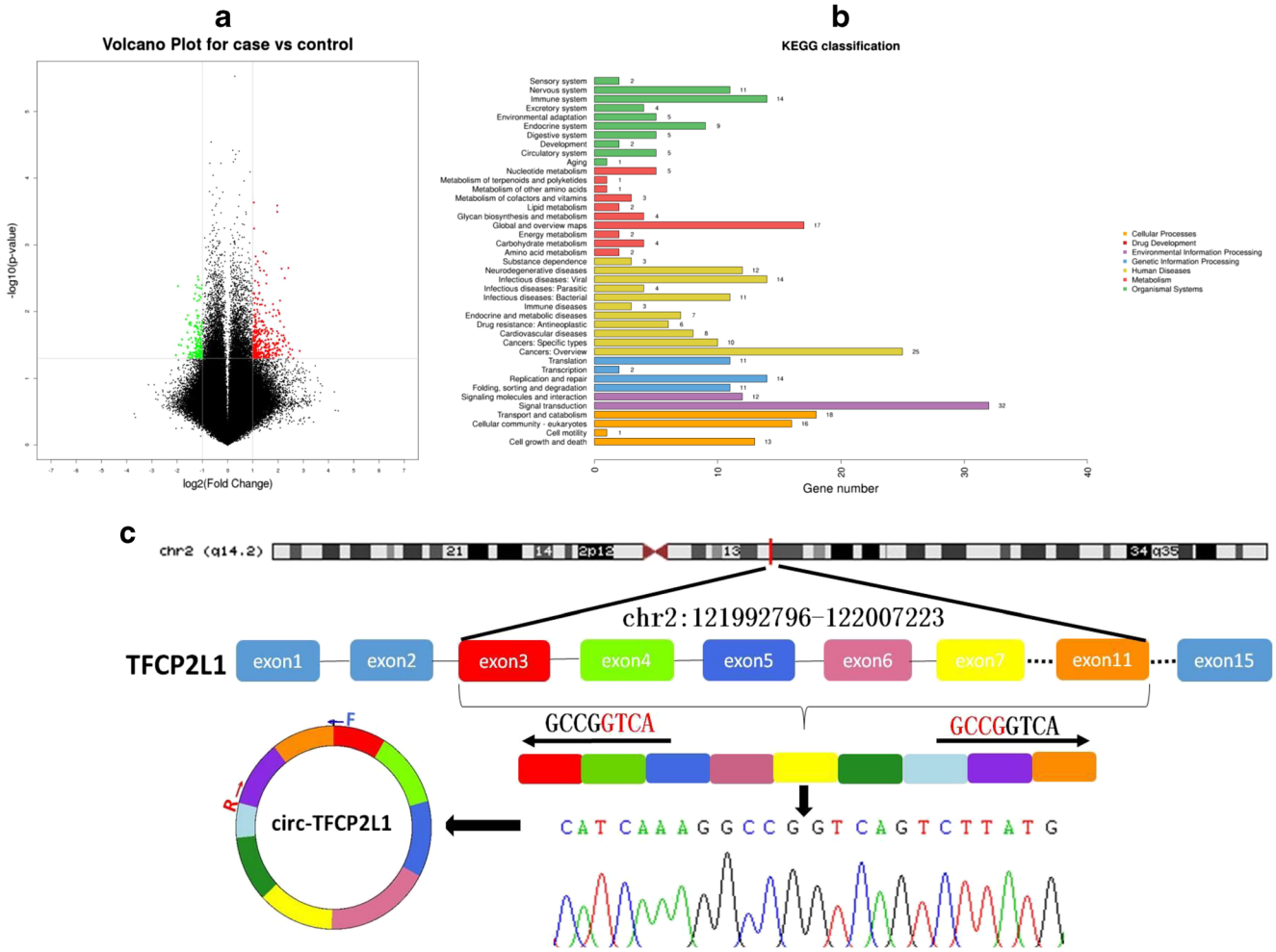

Fig. 1 CircRNA expression profiles in TNBC tissues and the biological structure of circ-TFCP2L1. **a** Volcano plot shows the upregulated and downregulated circRNAs in TNBC cancer vs control group. P<0.05. **b** KEGG classification in TNBC cancer vs control group. P<0.05. **c** Schematics show that circ-TFCP2L1 is derived from exons 3–11 of the TFCP2L1 gene

the circular structure of the up-regulated circRNAs and detected their expression levels in the TNBC tissues by qRT-PCR, finding that circ-TFCP2L1 showed the most significant difference.

As shown in Fig. 1c, circ-TFCP2L1 is derived from exons 3–11 of the gene TFCP2L1. The amplification products were inserted into a T-vector for Sanger sequencing to determine their full-length. Resistance to digestion with RNase R or actinomycin D in the HCC1937 cell line further confirmed that this RNA species was a stable circular RNA (Supplement Figure 1b-c).

Circ-TFCP2L1 Is over-Expressed in TNBC Tissues and Promotes the Proliferation and Migration of TNBC Cells

Using qRT-PCR, we detected the expression of circ-TFCP2L1 in 32 TNBC tissues and adjacent noncancerous tissues and found that circ-TFCP2L1 was significantly over-expressed in TNBC tissues compared with adjacent normal controls (Fig. 2a). The expression of circ-TFCP2L1 in 24 TNBC tissues was upregulated and was downregulated in 8 TNBC tissues (Fig. 2b). As shown in Supplement Table 2, circ-TFCP2L1 level was not associated with age, differentiation, or tumour size in patients with TNBC. However, upregulated expression of circ-TFCP2L1 level was positively associated with TNM stage ($P = 0.031$). We focused on the prognostic power of circ-TFCP2L1. The Kaplan-Meier disease-free survival curve revealed that TNBC patients with higher circ-TFCP2L1 expression showed a reduced DFS (Fig. 2c). The expression of circ-TFCP2L1 in 5 TNBC cell lines was examined by qRT-PCR, revealing that HCC1937 and MDA-MB-231 cells expressed the highest levels of circ-TFCP2L1 expression compared with MCF-10A (Fig. 2d). Additionally, although the expression level of circ-TFCP2L1 was found higher in MCF-7 and SUM185 cells than in the normal cell line, the result had no statistical significance (Supplement Figure 2). HCC1937 and MDA-MB-231 cells were transfected with si-circ-TFCP2L1 or si-NC using the Lipofectamine 2000 transfection reagent (Supplement Figure 1d). The effects of circ-TFCP2L1 on the proliferation,

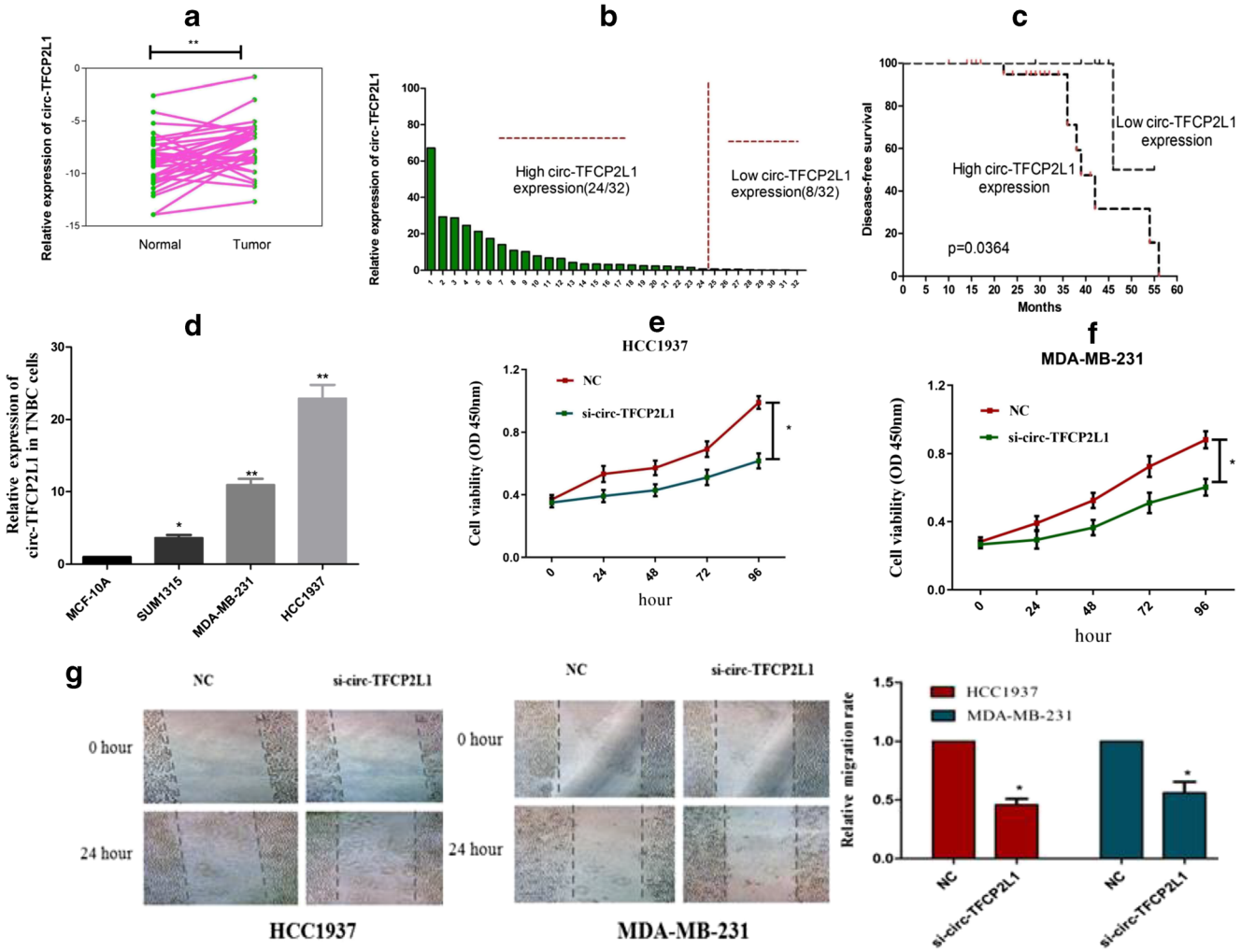

Fig. 2 Circ-TFCP2L1 is over-expressed in TNBC tissues and promotes the proliferation and migration of TNBC cells. **a** The expression of circ-TFCP2L1 levels was significantly higher in TNBC tissues than in adjacent noncancerous tissues. **b** A total of 24 of 32 TNBC tissues were over-expressed, and the other 8 were under-expressed. **c** The Kaplan-Meier survival curves revealed that TNBC patients with high circ-TFCP2L1 expression showed a reduced DFS. **d** The expression of circ-TFCP2L1 in TNBC cell lines was significantly higher than that in MCF-10A cells. **e** Knockdown of circ-TFCP2L1 significantly inhibited cell proliferation in HCC1937 cells. **f** Knockdown of circ-TFCP2L1 significantly inhibited cell proliferation in MDA-MB-231 cells. **g** Knockdown of circ-TFCP2L1 inhibited migration in HCC1937 and MDA-MB-231 cells. * P<0.05, ** P<0.01

migration and invasion behavior of TNBC cell lines were assessed. The results showed that knockdown of circ-TFCP2L1 significantly inhibited cell proliferation in both HCC1937 and MDA-MB-231 cells (Fig. 2e–f). The scratch-wound assay demonstrated that HCC1937 and MDA-MB-231 cells transfected with si-circ-TFCP2L1 exhibited a notably lower scratch closure rate compared with those transfected with si-NC (Fig. 2g). However, the effects of circ-TFCP2L1 on the invasion behavior of TNBC cell lines did not show obvious differences.

Circ-TFCP2L1 Directly Binds to miR-7 to Further Target PAK1

An in situ RNA hybridization experiment demonstrated that the circular form of TFCP2L1 was primarily localized in the cytoplasm (Fig. 3a). Increasing evidence has indicated that circRNAs in cytoplasm can regulate transcription and pathways by manipulating miRNAs in cancer cells [13–15]. The circular RNA interactome (https://circinteractome.nia.nih.gov/) was used to predict the circ-TFCP2L1 binding sites with miRNAs (Supplement Table 3) and miR-7 had a relatively higher context score percentile. The target genes of miR-7 were predicted by MIRDB (http://mirdb.org/) and PAK1 has a relatively higher target score (Supplement Table 4). To confirm the website prediction, the biotin-coupled probe pull-down assay was performed, and the results showed that miR-7 and circ-TFCP2L1 were detected in the circ-TFCP2L1 pulled-down pellet compared with the control group (Fig. 3b). The luciferase reporter assay was conducted, and the result revealed that the miR-7 mimics induced a

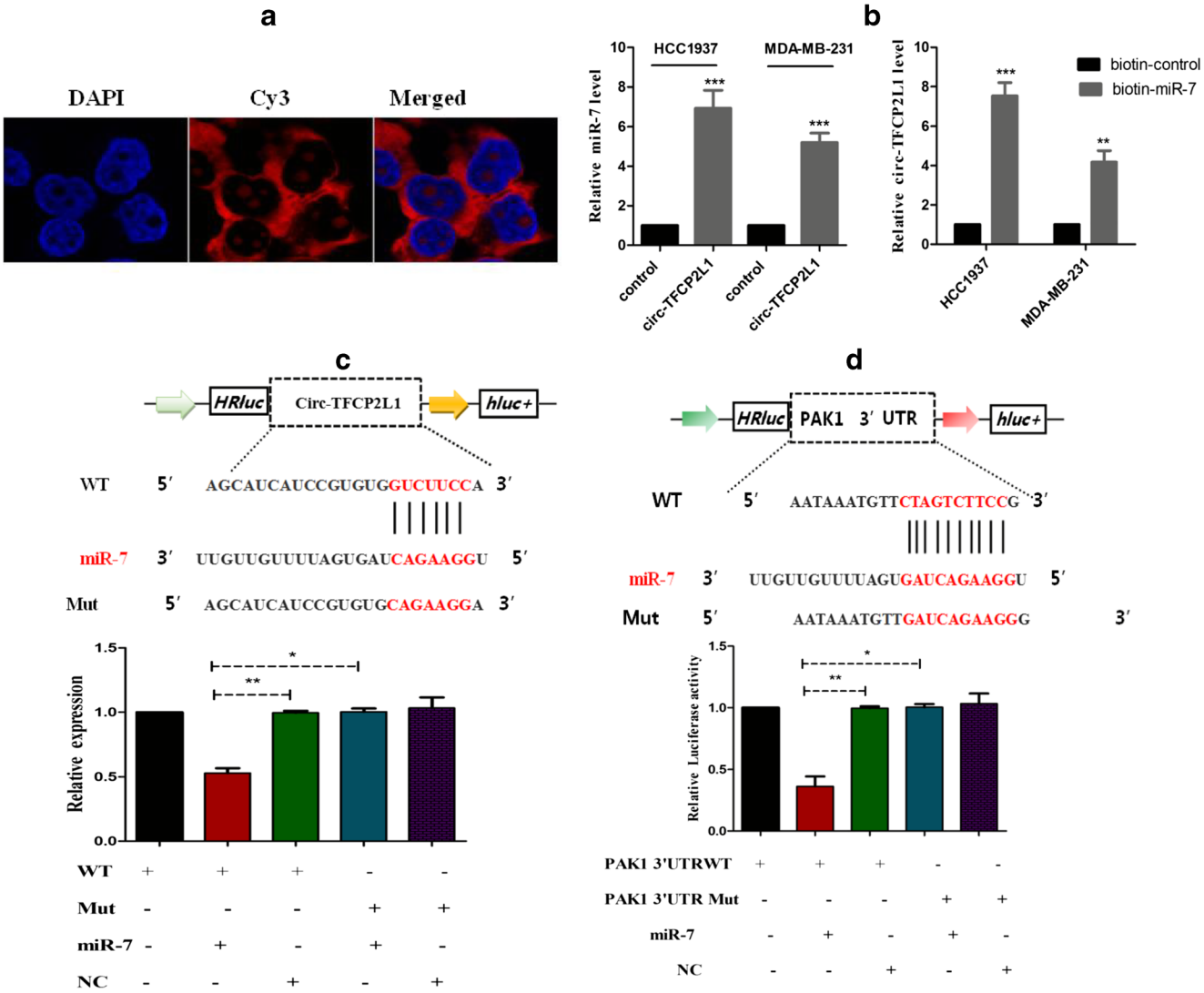

Fig. 3 Circ-TFCP2L1 directly binds to miR-7 to further target PAK1. **a** The FISH experiment confirmed that circ-TFCP2L1 was localized mainly in the cytoplasm. **b** The biotin-coupled probe pull-down assay was performed, and the results showed that miR-7 and circ-TFCP2L1 were detected in the circ-TFCP2L1 pulled-down pellet compared with the control group. **c** MiR-7 mimics induced a reduction in relative luciferase expression in circ-TFCP2L1-WT compared with the negative control in TNBC cells. **d** MiR-7 mimics induced a decrease in relative luciferase expression in PAK1-WT compared with the control.* P<0.05, ** P<0.01, *** P<0.001

decrease in relative luciferase expression in the circ-TFCP2L1-WT group compared with the negative control (Fig. 3c). However, no difference was observed in the circ-TFCP2L1-Mut group between the miR-7 mimics and the control. The luciferase reporter assay revealed that miR-7 mimics induced a decrease in relative luciferase expression in PAK1-WT compared with the control (Fig. 3d).

Circ-TFCP2L1 Promotes the Proliferation and Migration of TNBC by Sponging miR-7 to Regulate PAK1

We explored whether the circ-TFCP2L1-miR-7-PAK1 regulatory loop participated in the proliferation and migration of TNBC cells. The results showed that the proliferation and migration ability of cells treated with si-circ-TFCP2L1 + miR-7 were significantly suppressed compared with those treated with miR-7 in TNBC cells (Fig. 4a–c). Furthermore, the mRNA expression levels of PAK1 treated with miR-7 or si-circ-TFCP2L1+ miR-7 were significantly decreased compared with the si-circ-TFCP2L1+ miR-NC group in HCC1937 and MDA-MB-231 cell lines (Fig. 4d–e). Above all, these results illustrate that circ-TFCP2L1 takes part in the progression of TNBC through crosstalk with PAK1 by competing for shared miR-7 (Fig. 4f).

Discussion

With the emergence of high-throughput circRNA microarray and the development of bioinformatics, circRNAs have

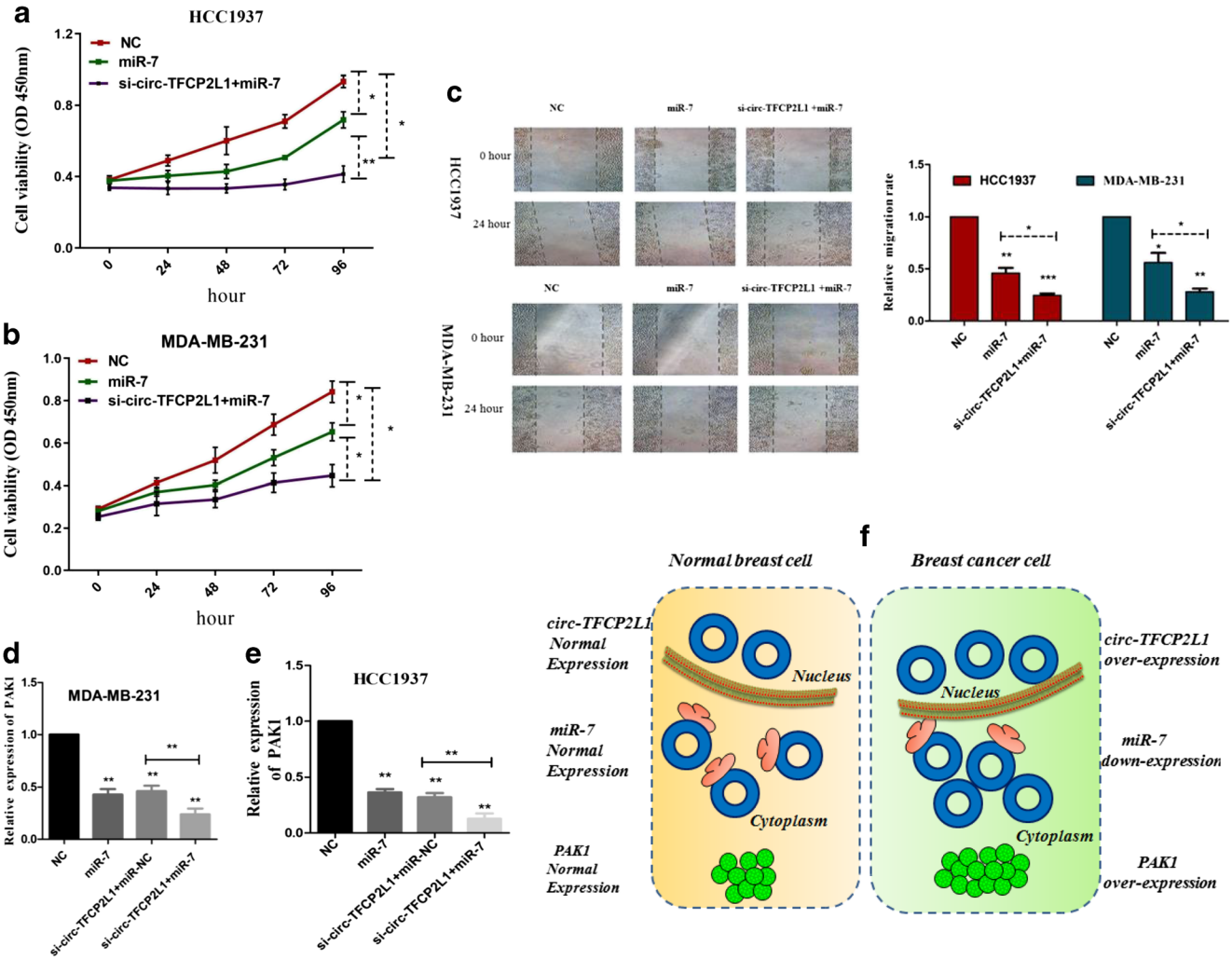

Fig. 4 Circ-TFCP2L1 promotes the proliferation and migration of TNBC by sponging miR-7 to regulate PAK1. **a** The proliferation ability of cells treated with si-circ-TFCP2L1 + miR-7 mimics was significantly suppressed compared with that treated with miR-7 in HCC1937 cells. **b** The proliferation ability of cells treated with si-circ-TFCP2L1 + miR-7 mimics was significantly suppressed compared with that treated with miR-7 mimics in MDA-MB-231 cells. **c** The migration ability of cells treated with si-circ-TFCP2L1 + miR-7 mimics was significantly suppressed compared with that treated with miR-7 in TNBC cells. **d** The mRNA expression levels of PAK1 treated with miR-7 or si-circ-TFCP2L1 + miR-7 mimics were significantly decreased compared with the control group in HCC1937 cell lines. **e** The mRNA expression levels of PAK1 treated with miR-7 mimics or si-circ-TFCP2L1 + miR-7 mimics were significantly decreased compared with the control group in MDA-MB-231 cell lines. **f** Circ-TFCP2L1 takes part in the progression of TNBC through crosstalk with PAK1 by competing for shared miR-7. * P<0.05, ** P<0.01, *** P<0.001

attracted increasing attention and have become an emerging research hotspot in recent years [16]. The number of publications investigating circRNAs in various disciplines accelerating, given the vital role of these elements as regulators and valuable diagnostic biomarkers for diseases, including cardiovascular disease, diabetes mellitus and multiple tumours [17, 18]. In this study, we used a human circRNA microarray to assess the differences in the circRNAs expression profiles for TNBC patient tissues and non-tumour tissues and chose the observably upregulated circRNA known as circ-TFCP2L1 for further research. Using qRT-PCR, we found that circ-TFCP2L1 was significantly over-expressed in TNBC tissues compared with normal controls. The Kaplan-Meier disease-free survival curve revealed that TNBC patients with higher circ-TFCP2L1 expression showed a shorter DFS. In vitro experiments indicated that circ-TFCP2L1 might be closely associated with proliferation and migration of TNBC.

CircRNAs are endogenous non-coding RNAs (ncRNAs) with a stable structure of covalently closed continuous loops that do not have 3′ and 5′. The structure is more stable than linear RNA. Circular transcripts are generated from pre-mRNA, which could also form linear transcripts. Thus, many linear transcripts are manufactured as circRNA synthesized by “back- splicing” [19]. For now, although an increasing body of evidence has indicated that circRNAs functions as a regulator in various

physical and pathological regulation processes, the specific biological mechanism still remains unknown. Many previous studies have demonstrated that multiple types of RNAs containing mRNAs, lncRNAs and circRNAs could serve as ceRNAs by competitively binding to miRNAs and modulating gene expression post-transcriptionally [20]. Tang YY screened the circRNA expression profiles in breast cancer tissues using circRNA microarray analysis. In total, 1705 circRNAs were identified as significantly aberrant. Among these dysregulated circRNAs, hsa_circ_0001982 was markedly overexpressed in breast cancer tissues and cell lines. Bioinformatics analysis predicted that miR-143 acted as a target of hsa_circ_0001982, which was confirmed by the dual-luciferase reporter assay. Loss-of-function and rescue experiments revealed that hsa_circ_0001982 knockdown suppressed breast cancer cell proliferation and invasion and induced apoptosis by targeting miR-143. In summary, those studies preliminarily investigated circRNA expression in breast cancer tissue and explored the role of the competing endogenous RNA (ceRNA) mechanism in progression, offering a novel insight for breast cancer tumourigenesis [21]. Similar to our study, hsa_circ_0001982 was highly expressed in triple-negative breast cancer cell lines, and brought new therapeutic hopes for targeted therapy with triple-negative breast cancer, but the difference with our study is that hsa_circ_0001982 in hormone receptor-positive breast cancer also showed an upward trend. This evidence demonstrates that circRNAs could serve as miRNA sponges to regulate breast cancer progression. In our study, the in situ RNA hybridization experiment demonstrated that the circular form of TFCP2L1 was preferentially localized in the cytoplasm.

We have proven that circ-TFCP2L1 takes part in the progression of TNBC through crosstalk with PAK1 by competing for shared miR-7. The biotin-coupled probe pull-down assay and luciferase reporter assay were performed to prove the binding site among circ-TFCP2L1, miR-7, and PAK1.

A previous study indicated that miR-7 plays a critical role in governing cell growth and apoptosis in pancreatic cancer cells [22]. Other studies showed that miR-7 serves as a tumour suppressor in hepatocellular carcinoma and breast cancer [23, 24]. For PAK1, our prediction on the GEPIA website showed that PAK1 was significantly overexpressed in BC tissues compared with normal tissues (Supplement Figure 1e). Previous research has showed that high-expression of PAK1 reverses proliferation and clonogenic inhibition as well as migration and invasion suppression in BC cells [25, 26]. More importantly, the expression level of PAK1 was related to survival rate in BC patients [27]. In addition, Yue K found that miR-7 functions as a tumour suppressor by targeting PAK1 directly and thus might present a novel therapeutic target for treatment of thyroid cancer, further confirming our results [28]. Our results showed that the proliferation and migration ability of cells treated with si-circ-TFCP2L1 + miR-7 was significantly suppressed compared with that treated with miR-7 in TNBC cells. Furthermore, the mRNA expression levels of PAK1 treated with miR-7 or si-circ-TFCP2L1+ miR-7 were significantly decreased compared with the normal group. Overall, these results illustrate that circ-TFCP2L1 promotes the proliferation and migration of triple negative breast cancer through sponging of miR-7 by inhibiting PAK1.

It is well known that early diagnosis and treatment can significantly improve the survival rate of breast cancer patients. Therefore, it is necessary to develop effective and specific breast cancer diagnostic markers. Considering stability and resistance to decomposition, circRNAs are considered an ideal biomarker for early diagnosis and detection. Lu J reported that plasma hsa_circ_0006848 may be a novel noninvasive biomarker in early gastric cancer diagnosis [29]. Wang C found that hsa_circ_0077837 and hsa_circ_0001821 could serve as potential biomarkers for both lung adenocarcinoma and squamous cell carcinoma [30]. Additionally, Gao D analyzed a cohort of 97 patients and found that circ_0006528 expression was significantly up-regulated in human breast cancer tissues compared with that in adjacent non-tumorous tissues and was significantly associated with advanced tumor-node-metastasis stage and poor prognosis [31]. We found that upregulated expression of circ-TFCP2L1 level was positively associated with TNM stage. The findings of this study may provide some insight into the molecular mechanisms of breast cancer development potential and may therefore be expected to be used for treatment.

Conclusion

In summary, our findings suggest that circ-TFCP2L1 is identified as a sponge of miR-7 and that downregulated miR-7 functionally targets PAK1, further promoting the proliferation and migration of triple negative breast cancer. Our study reveals a novel regulatory mechanism and offers a novel insight for circ-TFCP2L1 in the progression of triple negative breast cancer.

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Compliance with Ethical Standards

Conflict of Interest The authors report no conflicts of interest in this work.

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