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Original Paper

Increased Expression of Circular RNA circ_0005230 Indicates Dismal Prognosis in Breast Cancer and Regulates Cell Proliferation and Invasion via miR-618/ CBX8 Signal Pathway

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Key Words
Breast cancer • Circular RNA • circ_0005230 • miR-618 • CBX8

Abstract
Background/Aims: Circular RNAs (circRNAs) are a class of non-coding RNAs. They have been proved to be critically involved in tumorigenesis and progression of malignancies through competing endogenous RNA (ceRNA) mechanism. Nevertheless, the exploration between circRNAs and pathogenesis of breast cancer (BC) is limited. Previously, circ_0005230 was identified upregulated in BC tissues screened by circRNA microarray. In the present study, we aimed to investigate the expression pattern, functional role, and mechanism of circ_0005230 in BC. ***Methods:*** qRT-PCR was conducted to elucidate the expression levels of circ_0005230 in BC tissues and cells. Additionally, the clinical severity and prognostic value were investigated. CCK-8, colony-forming, flow cytometric assays were performed. Animal study was conducted to validate the *in vitro* data. What's more, Transwell assays were induced to detect the cell metastatic properties of circ_0005230 exerts in BC cells. Luciferase reporter assay was used to measure the mechanism of circ_0005230. ***Results:*** circ_0005230 was overexpressed in BC tissue specimens and cell lines. The overexpression of circ_0005230 was related to adverse phenotypes in the patients with BC. In addition, circ_0005230 could be regarded as a prognostic predictor in BC patients. *In vitro* and *in vivo* data demonstrated the cell growth promoting role of circ_0005230. Moreover, circ_0005230 could also promote cell migratory and invasive capacities. For the mechanism investigation, circ_0005230 was proved to be a

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sponge of miR-618, and expression of miR-618 could regulate CBX8 expression via targeting the 3'UTR of CBX8. Rescue assays also illustrated an oncogenic function of circ_0005230 in BC via acting as a miR-618 sponge to promote CBX8 expression. ***Conclusion:*** circ_0005230/miR-618/CBX8 axis might play a key role in BC tumorigenesis and development.

Introduction

Breast cancer (BC) is a serious threat to the health of female worldwide. It ranks as the second leading cause of cancer-associated death according to public data [1, 2]. Treatment approaches for BC depend on the cancer's stage and personal general condition, as well as individual preferences. Although great progress in the diagnosis and treatment of BC has been made, the 5-year survival rate is still unfavorable [3]. The pathogenesis of BC is a long procedure related to dysregulation of multiple oncogenes/tumor suppressor genes. Thus, uncovering the underlying mechanism of BC tumorigenesis and development is essential to develop potential diagnostic markers and therapeutic approaches for BC patients.

Circular RNAs (CircRNAs) are a class of non-coding RNAs which constructed by a closed loop with no protein coding potential [4-6]. CircRNAs have been recognized as imperative regulators in multiple physiological/pathological processes. Although the exact roles of most circRNAs are still unclear, accumulating evidence has documented that some of them can function as oncogenic or tumor suppressive roles in human malignancies, including BC [7-9]. For example, Song et al. revealed that silencing of circ_0007534 attenuates BC cell progression via affecting miR-593/MUC19 axis [10]. What's more, circRNAs could function as a sponge to interact with miRNAs. For example, circGFRA1 could act as a competing endogenous RNA (ceRNA) in BC via binding to miR-34a [11]. CircRNAs are also able to bind to proteins to execute their biological behaviors [12]. The evidence above support the significance of investigating functional roles and mechanisms of circRNAs in BC.

Previously, Shi et al. reported that circ_0005230 expression was dramatically elevated in four paired BC tissue samples relative to their corresponding normal tissue samples screened by circRNA microarray [13]. Circ_0005230 was spliced from DNM3OS (DNM3 opposite strand/antisense RNA) gene. In this work, we measured circ_0005230 expression in a large cohort of BC patients and a panel of BC cells. Further Kaplan-Meier and Cox regression analysis confirmed circ_0005230 as a prognostic predictor in BC patients. Additionally, gain-of-function and loss-of-function experiments were performed to elucidate the functional roles of circ_0005230 in BC cells. *In vitro* results were further validated by the animal study. Mechanistically, circ_0005230 could directly sponge miR-618. Decreased miR-618 further releases its suppression of CBX8 mRNA, resulting in enhanced CBX8 protein levels. In summary, the present data uncover the participation of circ_0005230 in carcinogenesis and progression of BC cells, and may indicate a potential therapeutic target.

Materials and Methods

Tissue specimens

Clinical characteristics and tumor samples were collected from 76 patients diagnosed with BC and underwent resection from March 2011 to October 2013 in the Second Affiliated Hospital of Harbin Medical University. The project was approved by the Ethics Committee of Harbin Medical University. All the tissue samples were frozen in liquid nitrogen until RNA isolation.

Cell lines and culture

BC cells (MCF7, SKBR3, BT-20, T47D, MDA-MB-231, MDA-MB-436) and one normal mammary epithelial cell line (MCF10A) were obtained from ATCC (Manassas, VA, USA). All the cells were maintained in RPMI-1640 with 10% fetal bovine serum (FBS, Gibco, USA) in a humidified air at 37°C with 5% CO_2.

Cell transfection and qRT-PCR

The siRNA of circ_0005230 (si-circ_0005230-1/-2) and si-NC were obtained from GenePharma (Shanghai, China). For circ_0005230 overexpression, a pcDNA3.1 (+) circRNA mini vector was used to clone the whole circRNA sequence and artificial inverted repeats. miR-618 mimics/inhibitor were acquired from GenePharma (Shanghai, China). These oligonucleotides or vectors were transiently transfected into BC cells by Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) followed by the instruction of manufacturer.

Total RNA was extracted from cells or tissues by Trizol (Thermo Fisher Scientific, Waltham, MA, USA). RNA integrity number (RIN) analysis was performed using an Agilent 2100 Bioanalyser and RNA 6000 LabChip kit with Agilent 2100 Expert software (Agilent Technologies). Standard curves were constructed before quantifying gene expression. Amplification efficiencies were calculated from the slope of the standard curves. The Power SYBR Green (Takara Biotechnology) was used for qRT-PCR analysis. Expression of GAPDH served as an endogenous control. qRT-PCR results were calculated using the $2^{-\Delta\Delta Ct}$ method.

Cell counting kit detection

Cell counting kit-8 (CCK-8, Dojindo, Japan) was induced to detect the cell viability. The cell suspension was poured into the 96-well plates and maintained in 5% CO_2 at 37°C. 10μL of CCK-8 solution was required in each well without bubbles. After incubation for 2 hours, the absorbance was detected at the length of 450nm at five different time points (0h, 24h, 48h 72h and 96h) by a microplate reader (Tecan, Männedorf, Switzerland).

Colony forming assay

For clone forming assay, the same number of BC cells were counted and planted in plates and maintained in the cell incubator at 37°C with 5% CO_2 for about 2 weeks. Afterwards, the colonies were fixed, stained, counted and photographed.

Flow cytometric analysis

The apoptosis rate of BC cells was measured by flow cytometric analysis and analyzed through FACScan (BD, CA, USA). The detected cells were collected before washing with PBS buffer. The cells were then re-suspended with 1× Annexin V-FITC binding buffer containing 10mM Hepes/NaOH, pH 7.4, 140mM NaCl, 2.5mM $CaCl_2$. The staining procedure was finished with Annexin V-FITC and propodeum iodide (PI, BDBiosciences, USA). All the experiments were operated in triplicate under manufacturer's recommendations.

Transwell assay

Cell metastatic properties were determined by Transwell assays in accordance with the previous studies [14, 17].

Target prediction and dual-luciferase reporter assay

Circular RNA Interactome was induced to predict the potential miRNAs targeting circ_0005230. The targets of miR-618 was predicted by TargetScan database. BC cells were co-transfected with wild-type or mutated circ_0005230 reporter plasmids, and with miR-618 mimics or mimics-NC. The 3'UTR of CBX8 was amplified and sub-cloned to the pMIR-REPORT™ miRNA Expression Reporter Vector (ThermoFisher, US), which contained the luciferase coding sequence. The mutant 3'UTR of CBX8 was generated by using the Phusion Site-Directed Mutagenesis Kit (Thermo Scientific™) with the wild-type 3'UTR of CBX8 as the template. As for the co-transfection process, reporter construct and miR-618 mimics or negative control were utilized to transfer the cells by Lipofectamine 3000 according to the protocol of manufacturer. After 24h, the activity of luciferase was determined by Dual-Luciferase Reporter Assay System (Promega, USA).

Immunoblotting assay

Proteins were extracted from the cells with RIPA buffer (Thermo) and quantified by a BCA kit (Beyotime Biotechnology. Shanghai, China). 50μg proteins were analyzed by 10% separating gel and 5% stocking gel with SDS-PAGE, and then transferred onto PVDF membrane (Merck Millipore. Shanghai, China). After blocking with 5% skim milk (Millipore) at 25°C for 2h, protein bands were separately incubated with antibodies against CBX8 and GAPDH (Abcam, Cambridge, MA, USA) at 4°C, overnight. Then, the bands were

incubated with secondary antibody (CST, Danvers, MA, USA) at 25°C for 2h. An electrochemiluminescence (ECL) kit (Beyotime Biotechnology) was used for analyzing the proteins.

Animal study

The xenograft study was approved by the Animal Care and Use Committee of Harbin Medical University and conducted as previously described [14, 17]. Transfected MCF7 cells were used for injection.

Data analysis

The data are presented as the mean±standard deviation (SD) of 3 independent experiments and processed using SPSS 19.0 software (SPSS, Inc., Chicago, IL, USA). *t*-test was applied to analyze the significance of difference between groups. Fisher's exact test was used to analyze the association of circ_0005230 expression with clinical features of BC patients. Survival analysis was analyzed using the Kaplan-Meier method with log-rank test and Cox regression model. $P<0.05$ was considered statistically significant.

Results

circ_0005230 is significantly higher in BC specimens and cells and associated with clinical severity

qRT-PCR was conducted to determine circ_0005230 expression in BC tissues and adjacent normal tissue samples as well as BC cell lines and MCF10A. The results showed that circ_0005230 expression was elevated in BC tissues than their matched normal tissues (Fig. 1A). Moreover, circ_0005230 expression in BC cell lines (MCF7, SKBR3, BT-20, T47D, MDA-MB-231, MDA-MB-436) was higher than MCF10A cells. Among all the recruited cells, MCF7 has the highest expression of circ_0005230 (Fig. 1B). The recruited BC patients were divided into two groups (High/Low) according to the median expression level of circ_0005230. Fisher's exact test was induced to determine the relationship between circ_0005230 and clinical characteristics of BC patients and the results revealed that tumor size (p=0.002), TNM stage (p=0.011) and lymph node metastasis (p=0.019) were related with circ_0005230 expression (Table 1). The other parameters including age (p=0.821), Her-2 status (p=0.488), ER status (p=0.357), and PR status (p=0.167) were not associated with circ_0005230 expression. We further detected the 5-year survival rate by Kaplan-Meier and Cox regression analysis. As Fig. 1C demonstrated, the patients with elevated expression of circ_0005230 had a worse overall survival (p=0.004). As Table 2 indicated, advanced TNM stage (p=0.018) and circ_0005230 expression (p=0.005) were both adverse markers for BC patients proved

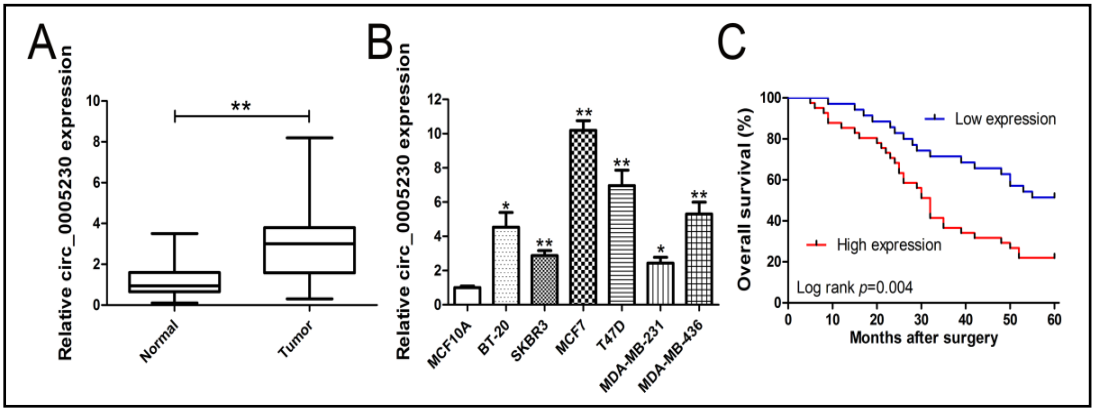

Fig. 1. Relative expression of circ_0005230 in BC tissues and cell lines and its clinical significance. (A) Relative expression of circ_0005230 in BC tissue samples and their paired non-cancerous tissue samples measured by qRT-PCR. (B) Relative expression of circ_0005230 in BC cell lines and normal cell line measured by qRT-PCR. (C) Kaplan-Meier survival analysis was used to assess circ_0005230 expression and BC patients' overall survival. B: The data are shown as mean ± SD (n = 3). *p<0.05, **p<0.01.

by univariate analysis. Further multivariate analysis verified circ_0005230 as a prognostic predictor for BC patients (p=0.042).

circ_0005230 modulates BC cell growth, apoptosis, and metastatic properties

qRT-PCR was applied to validate the knockdown efficiency of si-circ-1 and si-circ-2 in MCF7 cell lines. Circ_0005230 was markedly down-regulated in MCF7 cells relative to the si-NC group (Fig. 2A). MDA-MB-231 was used for gain-of-function assays due to its lowest expression of circ_0005230. Thus, we ectopically overexpressed circ_0005230 expression in MDA-MB-231 cells (Fig. 2B). Next, CCK-8 assays revealed that the cell proliferation in MCF7 cells were inhibited in si-circ-1/-2 groups compared to the controls. Moreover, overexpression of circ_0005230 dramatically enhanced cell viability in MDA-MB-231 cells (Fig. 2C). Subsequent colony-forming experiments indicated a similar result (Fig. 2D). We further determined the alteration of cell apoptotic rate by using flow cytometric analysis. As Fig. 2E showed, cell proliferation-promoting role of circ_0005230 is partly attributed to its suppression of cell apoptosis. The migration and invasion assays indicated that silencing of circ_0005230 evidently suppressed cell metastatic properties. Conversely, upregulated circ_0005230 could enhance the capacity of cell migration and invasion potential (Fig. 3). Animal assay was conducted to measure the effect of circ_0005230 on tumor growth. The data showed that the tumors formed from sh-circ transfected cells was smaller than the tumors formed from shCtrl transfected cells (Fig. 4A and B). Tumor weight was also lighter in sh-circ group (Fig. 4C). Tumors from shCtrl group had a higher Ki67 expression level compared to sh-circ group (Fig. 4D). These data revealed that circ_0005230 functions as an oncogene in BC cells.

Table 1. Circ_0005230 expression and clinicopathologic characteristics of breast cancer patients

Clinicopathologic features	No.	circ_0005230 expression		p-value
		High	Low	
Age (years)				0.821
≤50	36	20 (26.3%)	16 (21.1%)	
> 50	40	21 (27.6%)	19 (25.0%)	
Tumor size (cm)				0.002
≤2	29	9 (11.8%)	20 (26.3%)	
> 2	47	32 (42.1%)	15 (19.7%)	
TNM stage				0.011
I + II	35	13 (17.1%)	22 (28.9%)	
III	41	28 (36.8%)	13 (17.1%)	
Lymph node metastasis				0.019
Negative	30	11 (14.5%)	19 (25.0%)	
Positive	46	30 (39.5%)	16 (21.1%)	
Her-2 status				0.488
Negative	44	22 (28.9%)	22 (28.9%)	
Positive	32	19 (25.0%)	13 (17.1%)	
ER status				0.357
Negative	38	23 (30.3%)	15 (19.7%)	
Positive	38	18 (23.7%)	20 (26.3%)	
PR status				0.167
Negative	33	21 (27.6%)	12 (15.8%)	
Positive	43	20 (26.3%)	23 (30.3%)	

Table 2. Univariate and multivariate analysis of prognostic factors for overall survival in breast cancer patients

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	p-value	HR	95% CI	p-value
Overall Survival						
Age (> 50 vs. ≤50)	0.662	0.376-1.167	0.154			
Tumor size (> 2 vs. ≤2)	1.127	0.630-2.017	0.687			
TNM stage (III vs. I + II)	2.046	1.130-3.705	0.018	1.568	0.824-2.983	0.170
Lymph node metastasis (Positive vs. Negative)	1.856	0.996-3.459	0.052			
Her-2 status (Positive vs. Negative)	1.515	0.862-2.663	0.149			
ER status (Positive vs. Negative)	0.759	0.433-1.330	0.335			
PR status (Positive vs. Negative)	0.823	0.469-1.443	0.496			
circ_0005230 expression (High vs. Low)	2.327	1.286-4.211	0.005	1.945	1.023-3.699	0.042

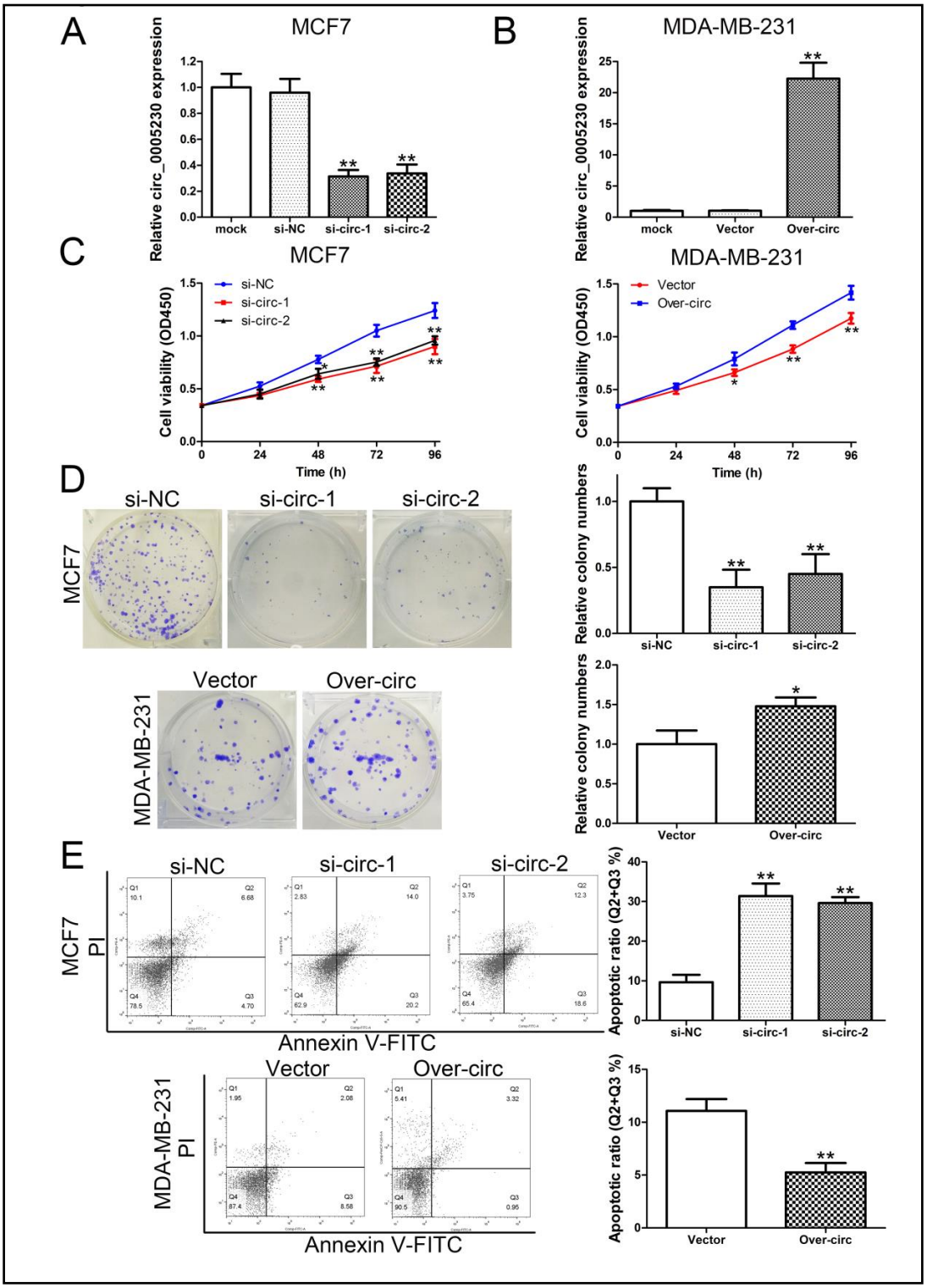

Fig. 2. Circ_0005230 regulates cell proliferation and apoptosis in vitro. (A,B) Circ_0005230 expression was detected after transfection in MCF7 and MDA-MB-231 cells by qRT-PCR. (C) CCK-8 assays were used to detect cell viability of MCF7 and MDA-MB-231 cells after transfection. (D) Colony formation assays were used to detect the clone ability of MCF7 and MDA-MB-231 cells after transfection. (E) Flow cytometric analysis were used to detect cell apoptosis of MCF7 and MDA-MB-231 cells after transfection. A-E: The data are shown as mean ± SD (n = 3). *p<0.05, **p<0.01.

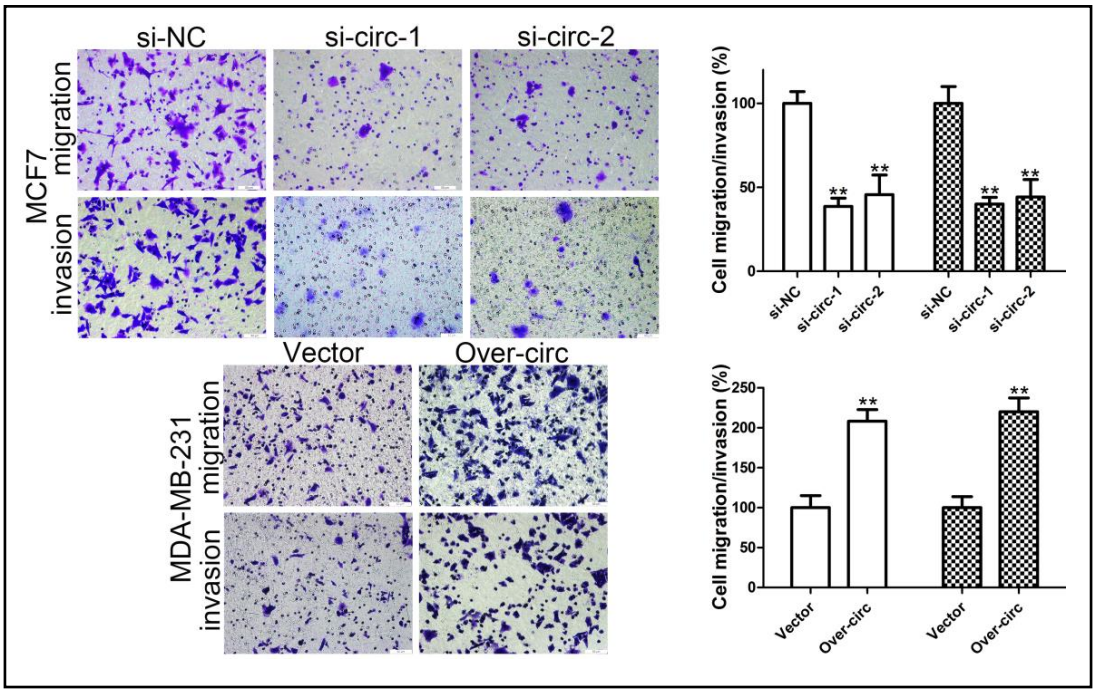

Fig. 3. Circ_0005230 promotes cell migration and invasion capacities by Transwell assays. The data are shown as mean ± SD (n = 3). *p<0.05, **p<0.01.

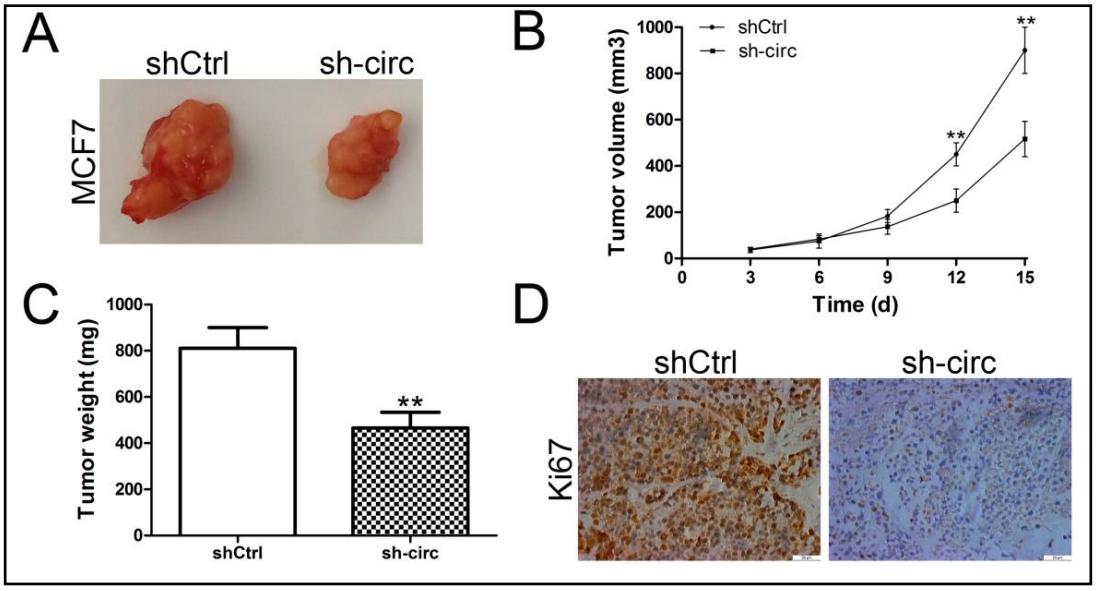

Fig. 4. Circ_0005230 promotes cell growth in vivo. (A) Tumors from nude mice after injection of transfected MCF7 cells. (B) Tumor volume was monitored every 3 days. (C) Tumor weights were measured. (D) Ki67 expression and positive cell numbers were determined by IHC. B-C: The data are shown as mean ± SD (n = 4). **p<0.01.

miR-618 can directly bind to circ_0005230 and CBX8 is a target of miR-618

To explore the mechanism of circ_0005230 execute in BC, we predicted several miRNAs (miR-145, miR-532-3p, miR-556-3p, miR-618, miR-623, miR-640, and miR-648) as potential downstream targets of circ_0005230. Circ_0005230 was knockdown by si-circ-1 and si-circ-2. As Fig. 5A documented, only miR-618 was statistically significantly elevated. Thus, miR-618

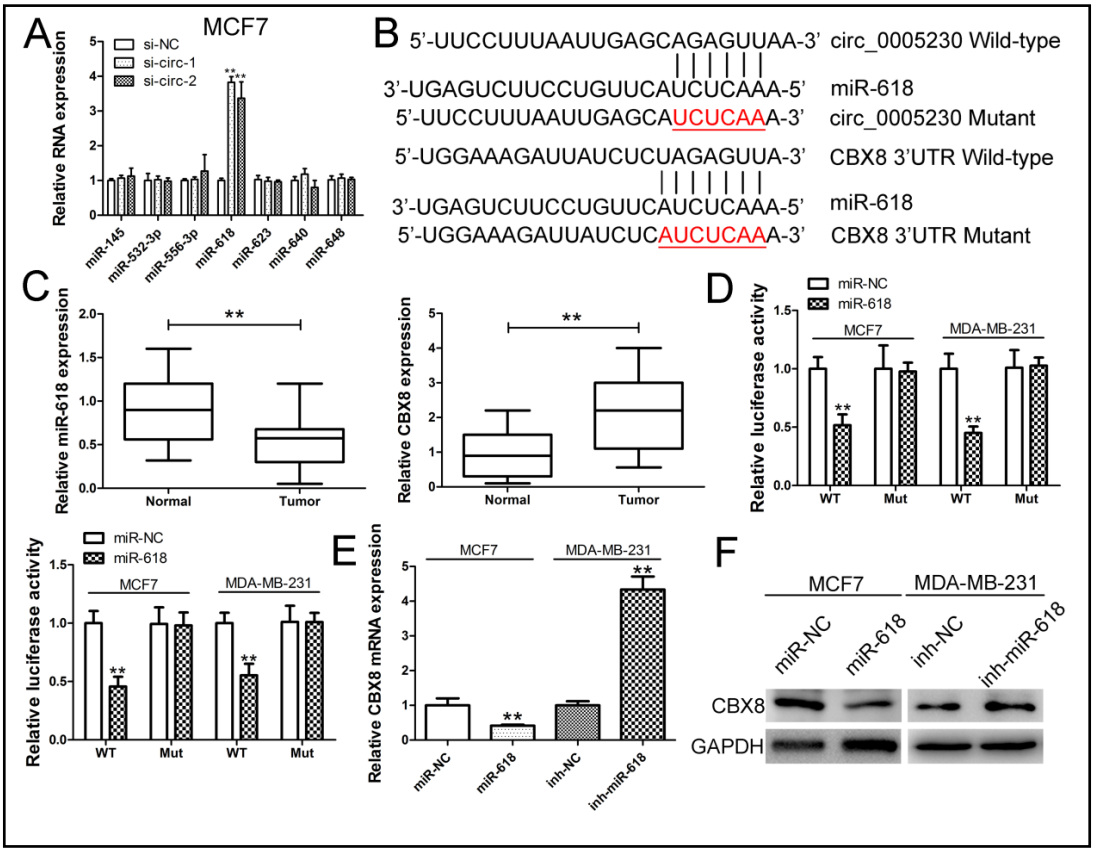

Fig. 5. Circ_0005230 directly binds to miR-618, and miR-618 targeted CBX8. (A) Relative miRNAs expression was detected by qRT-PCR after transfection in MCF7 cells. (B) The seed sequences of circ_0005230, miR-618, CBX8 3'UTR, circ_0005230 mutant, and CBX8 3'UTR mutant. (C) Relative miR-618 and CBX8 expression was detected by qRT-PCR in BC tissue samples. (D) Dual-luciferase reporter assays were performed to detect the correlation between miR-618 and circ_0005230, as well as between miR-618 and CBX8 3'UTR. (E, F) CBX8 expression was detected by qRT-PCR and Western Blot assays after transfection. A,D,E: The data are shown as mean ± SD (n = 3) **p<0.01.

might be an inhibitor target of circ_0005230. Furthermore, miR-618 may bind to the 3'-UTR of CBX8 predicted by TargetScan database (Fig. 5B). What's more, miR-618 was decreased in BC tissues compared to the matched non-tumorous tissue samples, which is opposite to circ_0005230 expression. On the contrary, CBX8 was upregulated in the tumor tissues (Fig. 5C). To determine whether circ_0005230 was a functional target of miR-618, luciferase reporter assay was performed. The results illustrated that transfection with miR-618 mimics remarkably inhibited the intensity of the luciferase reporter containing circ_0005230/CBX8 wild-type relative to the miR-NC. As expected, miR-618 mediated inhibition of luciferase activity was abolished in the mutated circ_0005230 and CBX8 construct relative to the wild-type vector (Fig. 5D). The above findings suggested that the miR-618 binding site within circ_0005230 and 3'UTR of CBX8 was functional. Then, we measured the effect of miR-618 on the expression of CBX8 by qRT-PCR and Western Blot experiments. The data documented that miR-618 negatively modulates CBX8 expression (Fig. 5E and F).

circ_0005230 promotes BC cell progression by targeting miR-618/CBX8 signal pathway

After validating that circ_0005230 could directly bind to miR-618 and miR-618 releases its inhibition of CBX8 mRNA, resulting in elevated CBX8 protein levels, it is important to understand whether circ_0005230/miR-618/CBX8 axis played an imperative role in the progression of BC cells. qRT-PCR was induced to determine the effect of circ_0005230

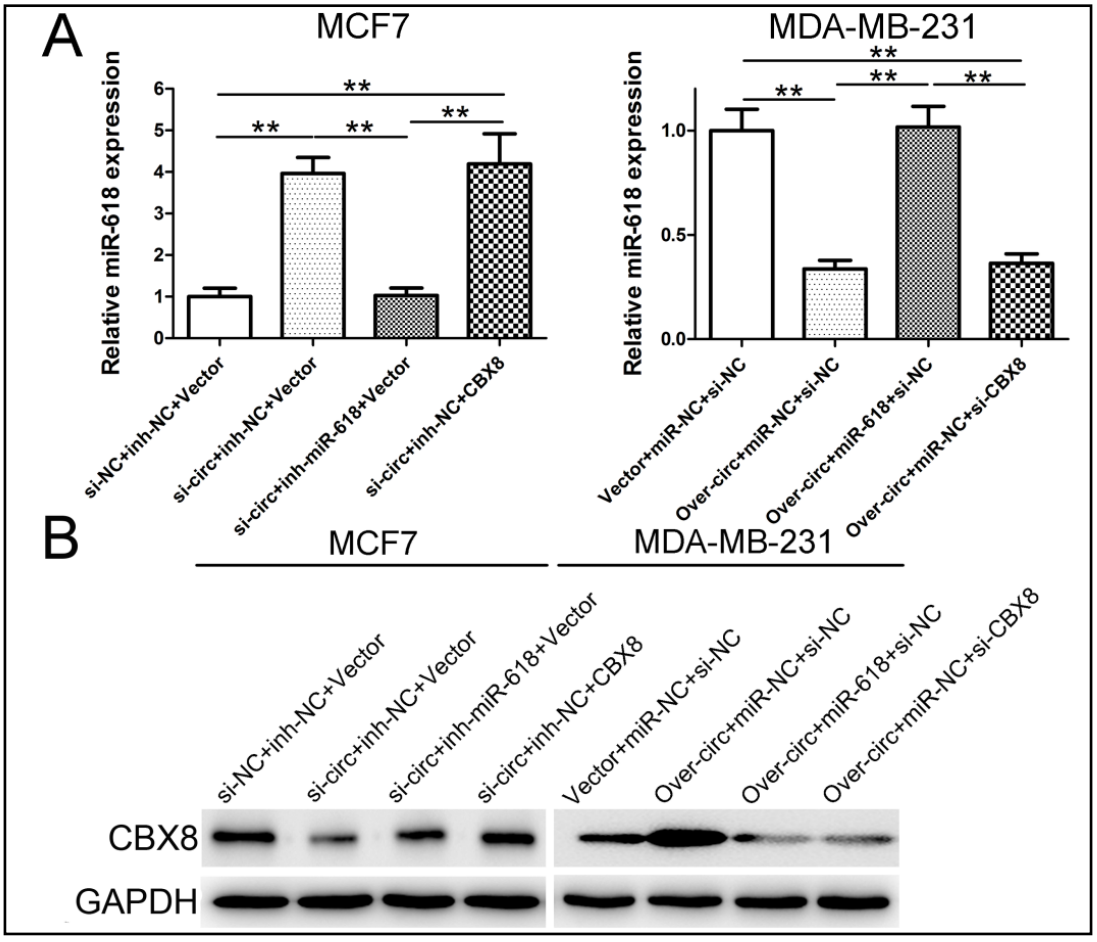

Fig. 6. The expression of miR-618 and CBX8 were regulated by circ_0005230. (A) Relative expression of miR-618 was measured by qRT-PCR after transfection. (B) The protein level of CBX8 was measured by Western Blot after transfection. A: The data are shown as mean ± SD (n = 3). **p<0.01.

expression on miR-618 and the data illustrated that downregulated circ_0005230 could increase the expression of miR-618. Moreover, after co-transfected with inh-miR-618, miR-618 was strikingly inhibited. In MDA-MB-231 cells, overexpressed circ_0005230 decreased miR-618 expression and co-transfected with miR-618 mimics could markedly reverse this effect (Fig. 6A). Afterwards, immunoblotting analysis was conducted to determine the effect of circ_0005230 and miR-618 on CBX8 expression. Inhibited circ_0005230 decreased the expression of CBX8 and after co-silencing of miR-618, CBX8 expression was restored. The expression of CBX8 was also enhanced in the si-circ+inh-NC+CBX8 co-transfection group. In MDA-MB-231 cells, upregulated circ_0005230 markedly increased CBX8 expression. Furthermore, either co-transfected with miR-618 mimics or si-CBX8 could dramatically suppress CBX8 expression (Fig. 6B). Next, we found that knockdown of miR-618 or ectopically upregulation of CBX8 could partially rescue the tumor suppressing effect of circ_0005230. Furthermore, miR-618 mimics and si-CBX8 could partly attenuate the adverse phenotypes of MDA-MB-231 cells caused by circ_0005230 proved by CCK-8, flow cytometry and Transwell experiments (Fig. 7A-C).

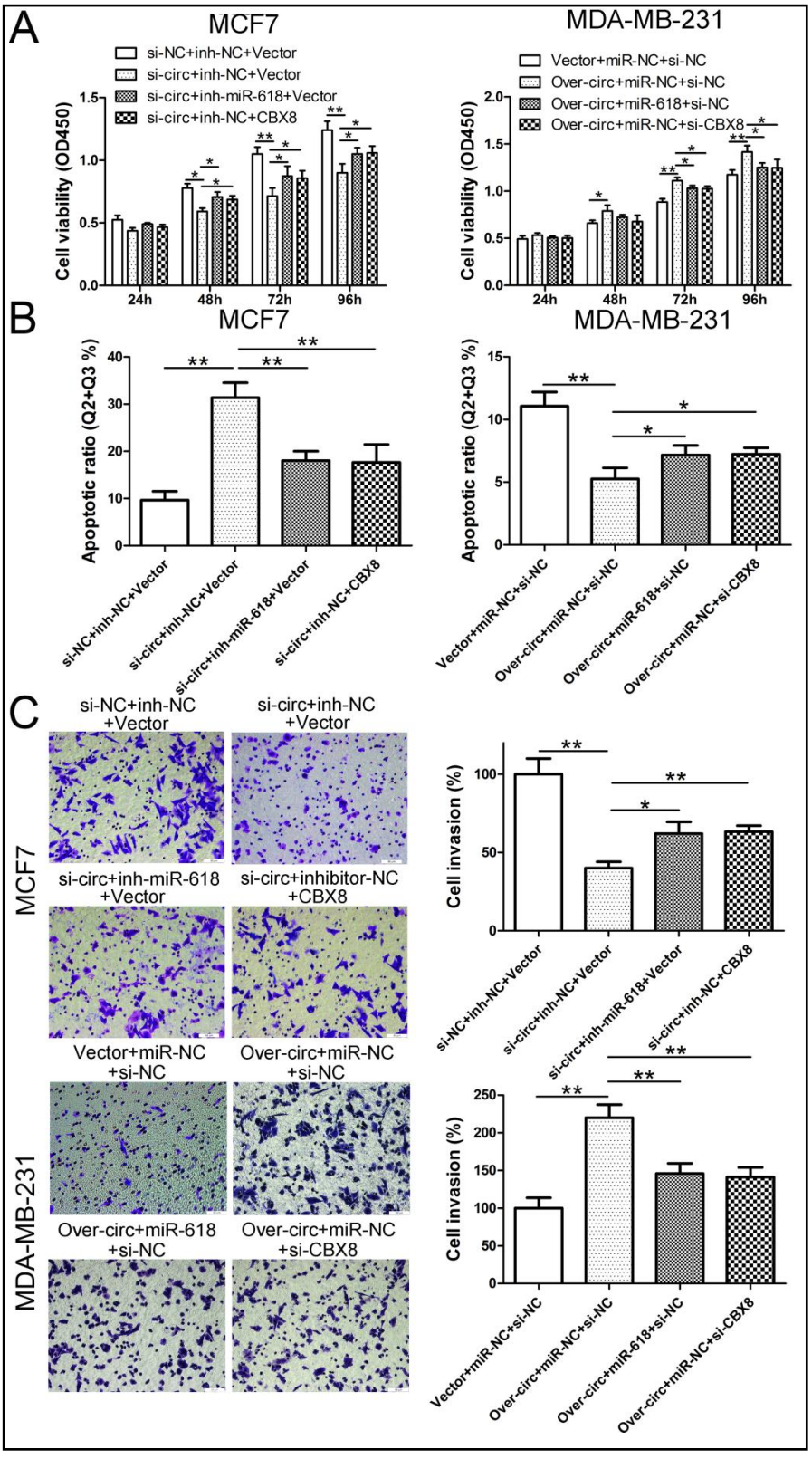

Fig. 7. Circ_0005230 facilitates BC cell growth and invasion via upregulating CBX8 expression by sponging miR-618. (A) CCK-8 assay was performed to analyze the cell viability of MCF7 and MDA-MB-231 cells after transfection. (B) Flow cytometric assay was performed to detect cell apoptosis of MCF7 and MDA-MB-231 cells after transfection. (C) Transwell assay was performed to detect cell migration and invasion of MCF7 and MDA-MB-231 cells after transfection. A-C: The data are shown as mean ± SD (n = 3). *p<0.05, **p<0.01.

Discussion

With the existence of intrinsic resistance, BC is quite resistant to available therapeutic methods [15]. CircRNAs were discovered over 30 years ago, but has only recently attracted investigators' attention due to their important functional roles in diseases [16]. Recently, accumulating evidence has indicated that circRNAs could function as a biomarker to predict the clinical severity in cancer patients [17-20]. Previously, circ_0005230 was found upregulated in BC tissues revealed by the human circRNA microarray [13]. However, its expression profiles in BC cells, clinical features, biological functions, and mechanisms were not explored yet. In the present study, we found circ_0005230 was not only overexpressed in human BC tissues but also in a panel of BC cells relative to the corresponding normal tissues and normal mammary epithelial cells, respectively. Moreover, the clinical implications of circ_0005230 expression in BC patients were further explored. The fisher's exact test documented that the expression of circ_0005230 was closely associated with larger tumor size, advanced TNM stage, as well as positive lymph node metastasis in the patients with BC. Also, multivariate analysis identified circ_0005230 as an effective prognostic biomarker in BC patients who underwent surgery.

The results above promoted us to further elucidate the functional behavior of circ_0005230 exerts in BC. We selected MCF7 (highest expression of circ_0005230) and MDA-MB-231 (lowest expression of circ_0005230) cells for the subsequent study. As a result, downregulation of circ_0005230 by siRNAs could strikingly attenuate BC cell proliferation and metastatic properties. Additionally, cell apoptotic rate was boosted after circ_0005230 silenced in MCF7 cells. The xenograft study further confirmed the *in vitro* data. On the contrary, ectopically expressed circ_0005230 promotes cell growth and metastatic properties in MDA-MB-231 cells. The rate of apoptosis was remarkably decreased. Therefore, the oncogenic functions of circ_0005230 in BC carcinogenesis and development was identified.

Since the first report of circRNAs functioning as miRNA sponges, various studies supports this hypothesis in multiple diseases, including cancers. For instance, Li et al. revealed that Cdr1as executes anti-oncogenic functions in bladder cancer via sponging miR-135a [21]. Thus, we predicted that the biological behaviors of circ_0005230 in BC were mediated by downstream miRNAs. qRT-PCR and dual luciferase reporter analysis further demonstrated that circ_0005230 directly sponges miR-618 and inhibits miR-618 expression. Previously, Song et al. revealed that miR-618 could inhibit prostate cancer metastatic properties through targeting FOXP2 [22]. In addition, miR-618 regulates cell proliferation by targeting PI3K/Akt signaling in thyroid cancer [23]. MiR-618 could also inhibit anaplastic thyroid cancer by suppressing XIAP [24]. Nevertheless, the biological functions and mechanisms of miR-618 in BC were not explored. In the current work, we found a decrease of miR-618 in BC tissues and illuminate its tumor suppressing role in BC cells. Additionally, CSX8 was predicted and verified as the target of miR-618. What's more, circ_0005230 significantly boosted CBX8 expression via inhibiting miR-618. CSX8 is known as a member of CBX family and exerts oncogenic functions in some cancers. Liu et al. reported that CBX8 could be regulated by miRNAs (miR-518d-5p/519-5p) at post-transcriptional level [25]. In hepatocellular carcinoma, CBX8 exhibits oncogenic properties by affecting AKT/β-catenin pathway [26]. In this study, rescue assays demonstrated that silencing of miR-618 or enforce the expression of CBX8 could partially suppress the tumor suppressing functions of si-circ_0005230. In addition, either increased miR-618 or si-CBX8 partly rescued the oncogenic functions induced by circ_0005230 in MDA-MB-231 cells. These data documented that circ_0005230/miR-618/CBX8 signal pathway might help the progression of BC.

Conclusion

This study illustrated that circ_0005230 might a prognostic biomarker for BC patients. Additionally, silencing of circ_0005230 could strikingly repress BC progression by reducing miR-618 to facilitate CBX8 production. In summary, the data support that circ_0005230 could modulate BC cell progression by miR-618/CBX8 axis.

Acknowledgements

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Disclosure Statement

The authors declare that they have no competing interests.

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