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Analysis of the Effect of GINS4 Regarding the Proliferation and Invasion of Breast Cancer Cells

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Abstract: *Objective:* To analyze the role and influence of the GINS4 gene in breast cancer progression and to explore its expression in triple-negative and non-triple-negative breast cancer cell lines. *Methods:* Single-gene analysis of GINS4 was performed by breast cancer RNA transcriptome data from The Cancer Genome Atlas (TCGA). Real-time quantitative polymerase chain reaction (PCR) was used to detect the expression of GINS4 in triple-negative and non-triple-negative breast cancer cell lines. The knockdown effects of GINS4 in MDA-MB-231 and MCF-7 cell lines on the proliferation and invasion of breast cancer cells were examined by cell counting kit 8 (CCK8) and Transwell assays. *Results:* Bioinformatics analysis showed that the expression of GINS4 in breast cancer was significantly higher than that in normal breast tissues ($P > 0.05$). At the same time, cell experiments confirmed that GINS4 was highly expressed in human breast cancer cell lines with normal breast cells as reference and in MDA-MB-231 and MCF-7 cell lines as reference, where the ability of proliferation and invasion of MDA-MB-231 and MCF-7 cells decreased after GINS4 knockdown. *Conclusion:* GINS4 is a gene associated with breast cancer malignancy, which can act as a novel tumor marker and has the potential as a new therapeutic target for breast cancer.

Keyword: Breast Cancer; GINS4; Proliferation; Tumor marker; Invasion

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1. Introduction

Breast cancer has the highest incidence among women worldwide [1]. Metastasis is the main cause of death in most cancers, with the exact molecular mechanisms of metastatic breast cancer yet to be fully understood. Thus, the impact of breast cancer-related genes on the malignant biological behavior of tumors requires more research. GINS4 is a member of the GINS family, which is essential for initiating deoxyribonucleic acid (DNA) replication in yeast and *Xenopus laevis* egg extracts [2,3]. GINS4 interacts with SIK1 and facilitates the recruitment of SIK1 to DNA replication sites during the S phase of the cell cycle [4]. The GINS complex also collaborates with DNA helicase to assist in the unwinding of DNA double helix, enabling DNA polymerase to synthesize new DNA strands on the single-stranded DNA template [5]. Through interactions with other subunits

and DNA helicase, it regulates DNA unwinding and replication, ensuring the efficiency and accuracy of DNA replication by stabilizing the formation of unwinding complexes. Hence, it plays a crucial role in maintaining the accuracy and efficiency of cellular DNA replication [6,7]. GINS4 plays a crucial role in various cancers, with its high expression promoting the malignant biological behavior of colorectal cancer [8]. Although bioinformatic studies have found that the GINS complex was overexpressed in breast cancer, the exact role of GINS4 in breast cancer is still not clear [9]. Furthermore, GINS4 can suppress ferroptosis by activating Snail, a key epithelial-mesenchymal transition (EMT) molecule, to inhibit p53 acetylation, playing a regulatory role in this programmed cell death process [10]. These various regulatory pathways and potential carcinogenic mechanisms make GINS4 an important focus of research in tumor progression, prognosis assessment, and as a potential therapeutic target. Therefore, this study explored the impact of GINS4 on the proliferation and invasion of breast cancer cells through bioinformatics analysis combined with cellular experiments.

2. Materials and methods

2.1. Analysis of public databases

Breast cancer RNA transcriptome data and clinical data were downloaded from The Cancer Genome Atlas (TCGA) database and analyzed using R software (Rx64 version 4.0.2), which included 113 normal samples and 1109 tumor samples. Differential gene expression analysis was performed using the Limma package, with the threshold for differentially expressed genes (DEGs) set at fold-change > 2 and a false discovery rate (FDR) of < 0.05. The Kaplan-Meier Plotter database, which contains 54675 genes and 10461 cancer samples (including 4929 breast cancer samples), was used to assess the impact of GINS4 on the survival rate of breast cancer patients.

2.2. Cell culture

The MCF10A epithelial cell line was cultured in a specialized medium purchased from Procell Life Science and Technology Co., Ltd., Wuhan. MDA-MB-231 cells were cultured in 1640 medium (90% 1640 medium + 10% FBS), and MCF-7 cells were cultured in DMEM medium (90% DMEM medium + 10% FBS). All were placed in a cell culture incubator at 37°C and 5% CO_2.

2.3. Plasmid transfection

During transfection, cells were divided into a blank control group, a si-NC group, and a si-GINS4 group. The blank control group was cultured normally, the si-NC group used a negative control plasmid, and the si-GINS4 group selected three plasmids provided by the company for transfection according to the instructions in the lipo3000 manual.

2.4. GINS4 mRNA expression detection

Ribonucleic acid (RNA) was extracted from normal human mammary epithelial cells MCF-10A, breast cancer cells MCF-7, and triple-negative breast cancer cells MDA-MB-231. The RNA was reverse transcribed to synthesize copy DNA (cDNA), and quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed to detect expression levels, with GAPDH as the internal reference gene. RNA from cell samples of the blank control group, si-NC group, and si-GINS4 group was extracted 24 hours after transfection, and the operation was the same as above.

2.5. CCK-8 cell proliferation experiment

Twenty-four hours after transfection, the cells from each group were seeded into a 96-well plate, with a seeding

density of 3×10^3/well for MDA-MB-231 cells and $5\times10^{\wedge3}$/well for MCF-7 cells. After seeding, cell absorbance was measured according to instructions of the CCK-8 kit instructions, and growth curves were plotted.

2.6. Transwell invasion experiment

A matrix gel concentration of 1:6 was injected into the upper chamber of the Transwell and left overnight at 37°C. Each group was seeded with 3×10^4 cell suspension in the migration chamber, and 600 μL of complete medium was added to the lower chamber. After 24 hours, cells that did not penetrate the upper chamber were wiped off with a cotton swab, fixed, stained, and observed under a 200x microscope in each field of view (up, down, left, right, center) of each chamber. Photos were taken to record and calculate the average number of cells.

2.7. Statistical methods

Statistical analysis was carried out using the SPSS 23.0 software. Quantitative data were expressed as mean ± standard deviation. The *t*-test was used for comparison between two groups, and one-way ANOVA was used for comparisons among multiple groups. Results were considered statistically significant at $P < 0.05$.

3. Results

3.1. High expression of GINS4 in breast cancer

Through the analysis of breast cancer transcriptome data downloaded from the TCGA database for 1222 breast samples (N:113/T:1109), the differential analysis revealed that the expression of GINS4 in tumor samples was significantly higher than in normal samples ($P < 0.001$) (**Figure 1A**). Paired differential analysis showed that the expression of GINS4 differs between cancerous tissues and adjacent non-cancerous tissues of the same sample, with cancerous tissues having higher GINS4 expressions ($P \leq 1.1\times10^{-15}$) (**Figure 1B**).

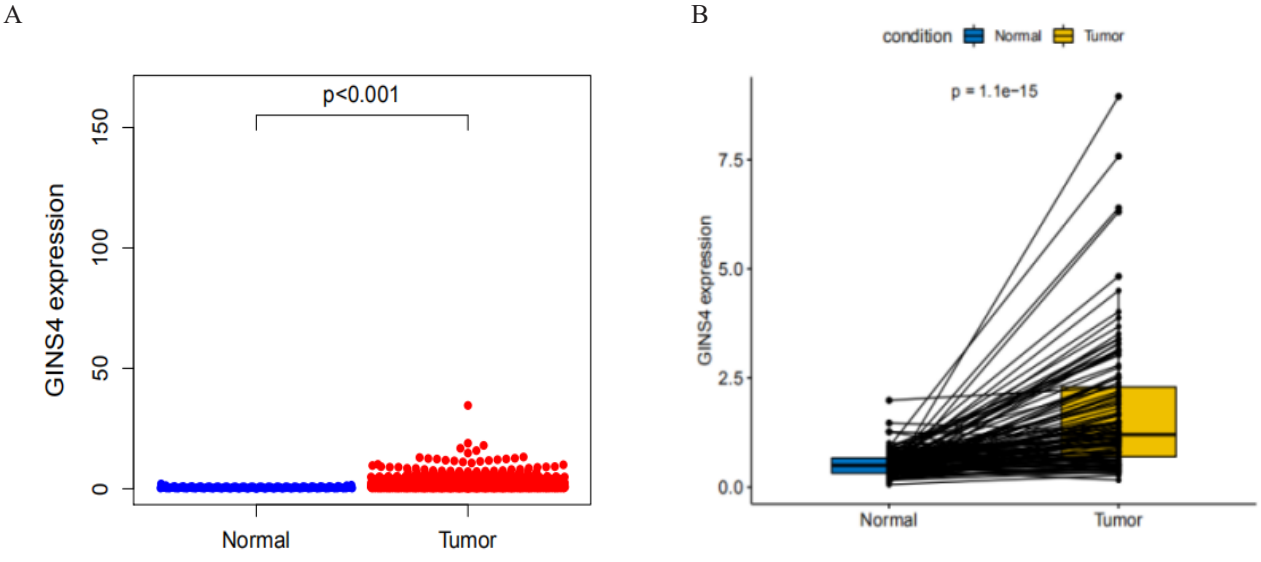


Figure 1. Differential expression of GINS4 in breast cancer. (A) Analysis of variance of GINS4 in TCGA database; (B) Analysis of paired variance of GINS4 in TCGA database.

3.2. GINS4 has higher expression in breast cancer cells than in normal mammary epithelial cells

qRT-PCR analysis confirmed the expression levels of GINS4 across different cell groups, showing that GINS4 expression in breast cancer cells MCF-7 and triple-negative breast cancer cells MB-231 was higher than in normal human mammary epithelial cells 10A (**Figure 2**).

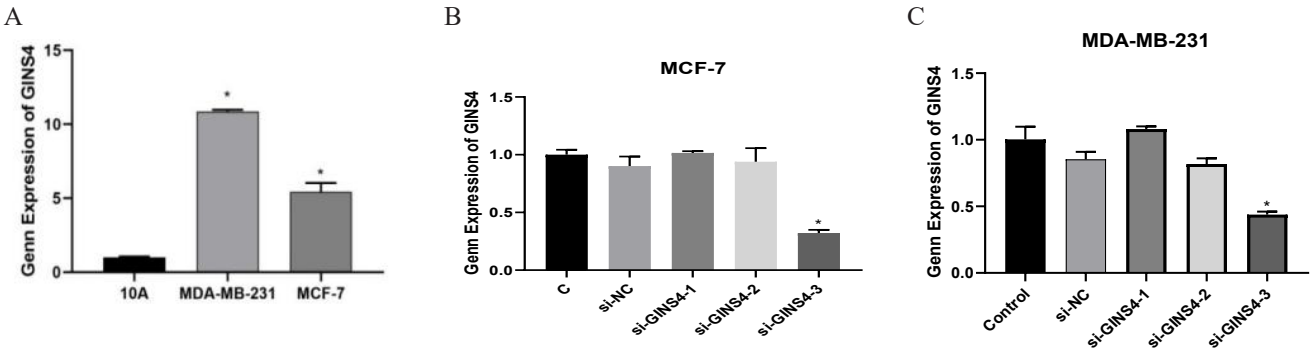


Figure. 2 Relative expression of GINS4 mRNA in breast cancer cell lines detected by qRT-PCR. (A) Relative expression of GINS4 in human breast cancer cell lines compared with MCF10A, $*P < 0.05$; (B) Knockdown effect of GINS4 in MCF-7 cell line, $*P < 0.05$; (C) Knockdown effect of GINS4 in MDA-MB-231 cell line, $*P < 0.05$.

3.3. Knockdown of GINS4 inhibits the proliferation of MCF-7 and MB-231 cells in vitro

qRT-PCR was used to screen the efficiency of the 3 siRNAs in knocking down GINS4. Results indicated that Si-siRNA-3 had the most significant knockdown efficiency (**Figures 3A** and **3B**), and Si-siRNA-3 was used for subsequent experiments. The CCK-8 assay was employed to assess the impact of GINS4 knockdown on the proliferation of MDA-MB-231 and MCF-7 cells. The results showed that the cell proliferation rate of the si-GINS4 group was significantly reduced for both MDA-MB-231 and MCF-7 cell lines (**Figure 4**).

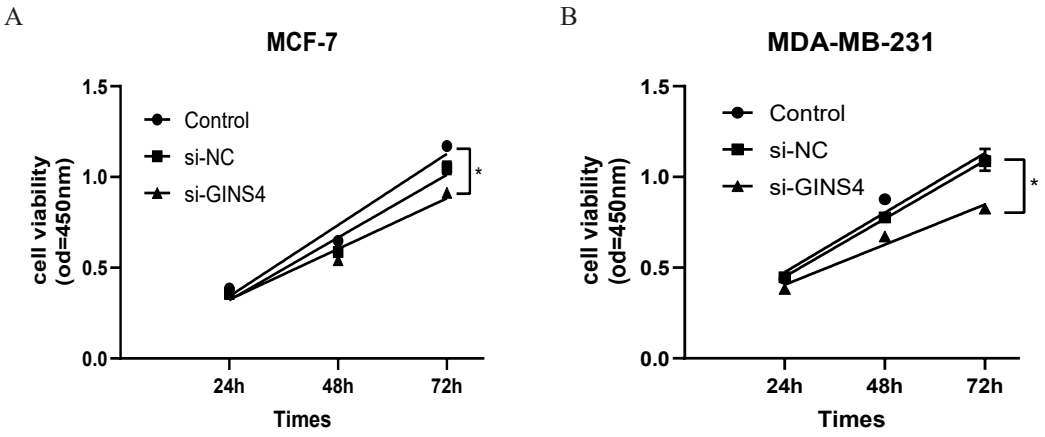
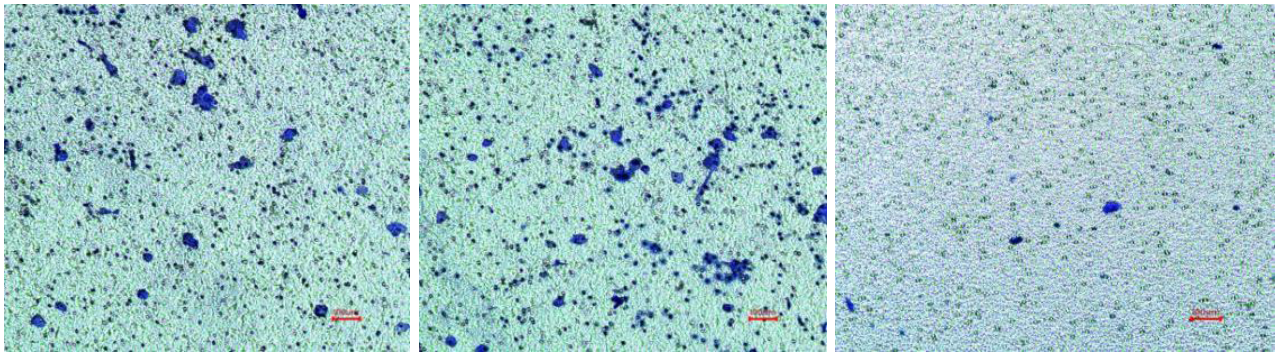


Figure 3. Proliferation ability of breast cancer cells after GINS4 knockdown detected by CCK-8 assay. (A) MCF-7 cell proliferation viability control group compared to si-GINS4 group, $*P < 0.05$; (B) MDA-MB-231 cell proliferation viability control group compared to si-GINS4 group, $*P < 0.05$.

A



MDA-MB-231: Control MDA-MB-231:si-NC MDA-MB-231:si-GINS4

B

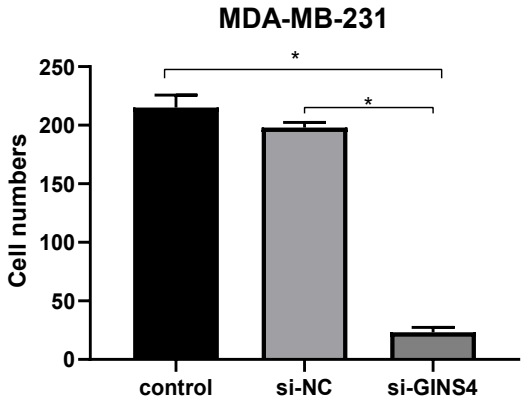
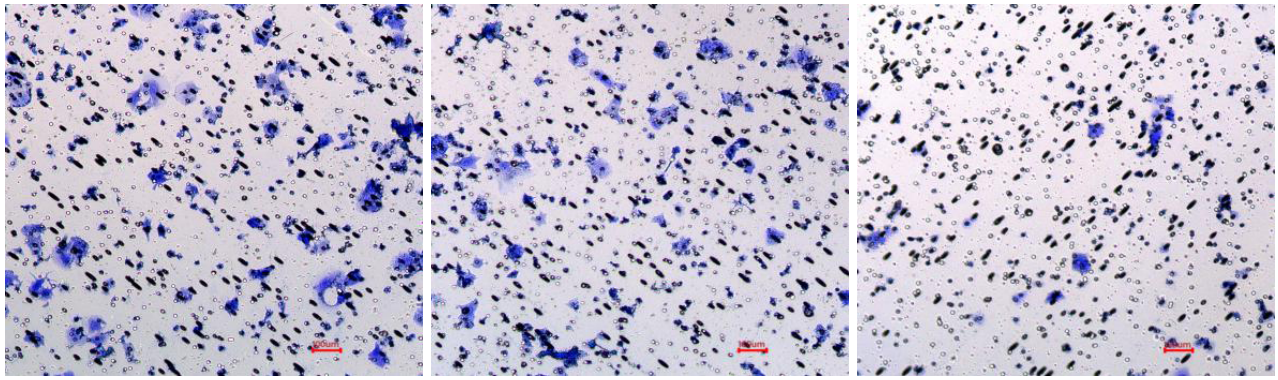


Figure 4. Effect of GINS4 knockdown on the invasive ability of MDA-MB-231 cells (SP×100). (A) Invasion of MDA-MB-231 groups after GINS4 knockdown; (B) Comparison of MDA-MB-231 cell control group with si-GINS4 number group, $*P < 0.05$.

3.4. Knockdown of GINS4 inhibits the invasion ability of MCF-7 and MB-231 cells

The impact of GINS4 knockdown on cell invasion was assessed using Transwell assays. The results showed that, compared to control cells, the invasion ability of the si-GINS4 group was significantly inhibited in both MDA-MB-231 and MCF-7 cells (**Figure 5**).

A



MCF-7: Control MCF-7-si-NC MCF-7-si-GINS4

B

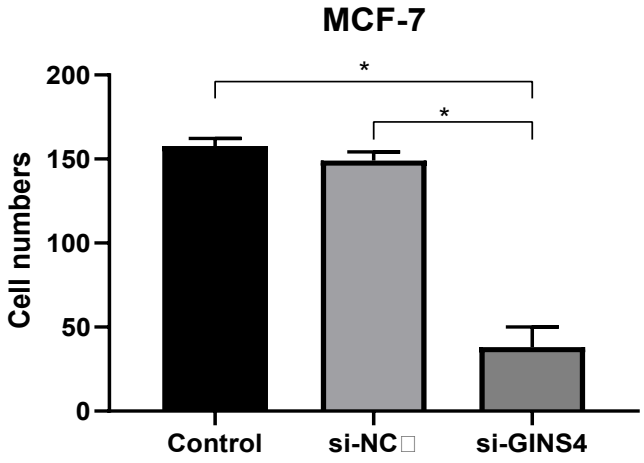


Figure 5. Effect of invasive ability of MCF-7 cells after GINS4 knockdown (SP×100). (A) MCF-7 invasion in each group after GINS4 knockdown; (B) Invasive ability of MCF-7 cells after GINS4 knockdown Control group compared with si-GINS4 group, $*P < 0.05$.

4. Discussion

Breast cancer is the most prevalent cancer among women worldwide [1]. By improving prevention and conducting early diagnosis, the mortality rate of breast cancer patients can be effectively reduced. However, breast cancer exhibits enhanced drug resistance and high recurrence rates, with the efficacy of traditional treatment methods being less than ideal. As the molecular mechanisms of cancer are further explored, it is gradually recognized that molecular diagnostics, immunotherapy, and adjuvant therapy may become the future directions of breast cancer treatment [11]. Understanding the specific mechanisms of breast cancer development at the molecular biology level is of great importance for improving patient prognosis [12]. Metastasis of cancer cells is a major cause of death related to most cancers, and the molecular mechanisms underlying the development and progression of breast cancer require further in-depth study [13].

GINS4 is a member of the GINS protein family, including GINS1, GINS2, GINS3, and GINS4. These proteins form a heterotetrameric complex that is essential for DNA replication [4,5]. GINS4 is overexpressed in various cancers and plays a role in promoting cancer [14]. It can regulate the proliferation and invasion of gastric cancer cells through the control of RAC1 and CDC42, participate in the development of glioma, be

overexpressed in lung cancer, and upregulate the expression of Snail to promote the malignant biological behavior of lung cancer cells [15–17]. GINS4 is significantly correlated with the prognosis and immune cell infiltration levels of esophageal squamous cell carcinoma (ESCC) and other cancers [18]. Moreover, high expression of GINS4 is associated with poor prognosis in endometrial cancer [19]. Experiments have shown that GINS4 may affect DNA damage in breast cancer and interact with cancer-related non-coding RNAs such as ribonuclease (RNAse) and MIR578 [20,21]. However, the impact of GINS4 on breast cancer cells is yet to be fully understood.

Our research group discovered through bioinformatics that GINS4 was overexpressed in breast cancer tissues. RT-qPCR confirmed that the mRNA expression level of GINS4 in cancer cells was higher than in normal mammary epithelial cells. Compared to MCF-10A cells, the expression of GINS4 was significantly increased in MDA-MB-231 and MCF-7 human breast cancer cell lines, consistent with the previous bioinformatics results. We successfully established a human breast cancer cell model with a decreased expression of GINS4. The effects of GINS4 on the proliferation and invasion capabilities of breast cancer cells were assessed using CCK8 and Transwell assays. Reducing the levels of GINS4 can effectively inhibit the proliferation, migration, and invasion abilities of breast cancer cells.

5. Conclusion

Bioinformatics analysis has verified that GINS4 is overexpressed in breast cancer and related to prognosis. It was found for the first time that knocking down GINS4 in MDA-MB-231 and MCF-7 cells reduced their proliferation and invasion capabilities. However, there is a lack of understanding regarding its mechanisms of action. This research group plans to further investigate the mechanisms by which GINS4 affects the invasion and metastasis of breast cancer in subsequent research.

Disclosure statement

The authors declare no conflict of interest.

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