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LncRNA TUG1 Targets miR-222-3p to Take Part in Proliferation and Invasion of Breast Cancer Cells

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Abstract: This study aimed to explore LncRNA TUG1 targeted miR-222-3p in the proliferation and invasion of breast cancer (BC) cells. Seventy-six BC patients admitted to our hospital and 62 health check-ups at the same time were selected as the research objects. Among them, the former was seen as the observation group (OG), and the latter was considered as the control group (CG). The clinical significance of LncRNA TUG1 and miR-222-3p in BC was detected. Human BC cell MCF7 and normal human breast epithelial cell MCF-10A were purchased. After cells were transfected with LncRNA TUG1 and miR-222-3p, their proliferation, invasion, and apoptosis were observed, and the relationship between the two was detected. LncRNA TUG1 was highly expressed, while miR-222-3p was low expressed in BC. When the cut-off value (COV) was 2.109, the sensitivity and specificity of detecting LncRNA TUG1 expression in peripheral blood for BC diagnosis were 98.39% and 69.74%, respectively. When the COV was 0.760, the sensitivity and specificity of detecting miR-222-3p expression were 67.11% and 91.94%. The cell proliferation and invasion of the sh-TUG1 group were dramatically higher than those of the other two, while the apoptosis rate was lower ($p < 0.05$). The cell proliferation and invasion of the si-TUG1r group were lower than those of the TUG1-NC group, while the apoptosis rate was higher ($p < 0.05$). The cell proliferation and invasion of the miR-222-3p-inhibitor group were dramatically higher than those of the other two, while the apoptosis rate was lower ($p < 0.05$). The miR-222-3p-mimics group has lower cell proliferation and invasion but higher apoptosis rate than NC group. ($p < 0.05$) The LncRNA TUG1 and miR-222-3p had the same gene sequence after target gene verification by http://starbase.sysu.edu.cn/. Biological behavior tests showed no difference in cell proliferation, invasion and apoptosis between the sh-TUG1 + miR-222-3p-mimics group and the TUG1-NC group ($p > 0.05$). TUG1 promotes the proliferation, invasion and inhibit apoptosis of BC cells by targeted regulation of miR-222-3p.

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Keywords: LncRNA TUG1; miR-222-3p; breast cancer; proliferation; invasion

1 Introduction

Breast cancer (BC) is one of the most primary cancers threatening women's lives in the world [1]. According to statistics, the morbidity is second only to cervical cancer. With society's development, the patient population is getting younger [2,3], which seriously threatens women's life and health safety. BC is a malignancy appearing at mammary gland epithelial tissue, and the etiology is still not completely clear [4]. Early BC does not have typical symptoms and signs, so it is easily ignored [5]. Once examined, patients are often in the development stage [6]. However, the primary clinical treatment is radical mastectomy. However, due to the high recurrence rate and metastasis rate, the mortality is still high [7]. Therefore, early detection and diagnosis are the keys to improve BC efficacy [8]. Finding molecular markers with high sensitivity and specificity for early diagnosis is a hot research topic in BC clinical treatment and it has

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important practical significance.

Recently, long-chain non-coding LncRNA has been found to be relevant to human physiological and pathological functions and has gradually become the focus of clinical research [9]. Previous studies have shown that LncRNA takes part in regulating cell differentiation, apoptosis and other processes [10]. Dysregulation of its expression is tied to proliferation, migration, infiltration and apoptosis of tumor cells [11]. Studies have shown that in BC, some lncRNA has been identified as oncogene or tumor suppressor and has potential as a diagnostic or prognostic marker for BC patients [12]. Taurine upregulated gene 1 (TUG1) is a member of LncRNA family. Its dysfunction is tied to various malignancies. For example, TUG1 can participate in the development of multidrug resistance in hepatocellular carcinoma by regulating the expression of P-gp and MDR1 [13], indicating that it may be a valid biomarker for diagnosing malignancies.

Moreover, some studies have found that it has certain carcinogenicity in BC [14], but its specific mechanism is ambiguous. microRNA (miRNA) is a non-coding small RNA family involved in normal cells and pathological processes. Some studies have found that miR-222-3p takes part in various tumor pathological processes, such as gastric cancer, renal cell cancer, etc. [15]. We found that TUG1 and miR-222-3p have binding sites through ENCORI online website, so we speculated that TUG1 might have some relationship with miR-222-3p in BC development. To verify our conjecture, this experiment will analyze TUG1 and miR-222-3p's role in BC cells and the relationship between the two, providing new ideas for future clinical diagnosis and treatment.

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2 Materials and Methods

2.1 Patient Data

Seventy-six BC patients admitted to our hospital from June 2017 to June 2018 and 62 health check-ups at the same time were selected as the research objects for prospective analysis. Among them, the former was seen as the observation group (OG) and the latter were considered as the control group (CG).

2.2 Inclusion and Exclusion Criteria

Inclusion criteria: all patients in the OG were diagnosed as BC by pathological biopsy and were treated in our hospital for the first time; all the researchers had complete case data; the physical examination results of the CG were normal; those had no history of major diseases before and agreed to cooperate with and take part in the investigation work.

Exclusion criteria: patients had multiple tumors, other cardiovascular and cerebrovascular diseases, autoimmune diseases, or physical and mental diseases; patients had an estimated survival time of less than 1 month; patients had low treatment compliance; pregnant patients; patients had physical disabilities who could not take care of themselves or those lied in bed for a long time.

As to general data such as age, gender and BMI, there was no statistical difference between both groups ($p > 0.050$). Altogether 4 mL fasting venous blood was drawn from patients in the two groups in the morning. After standing 30 min at indoor temperature, the blood was centrifuged 10 min (400 × g) to obtain upper serum to be tested.

2.3 qRT-PCR Detection

The total RNA was extracted using extraction kit (Bioteke, BC1710213, China) and reverse transcribed into cDNA based on reverse transcription kit (Takara, China 16072315). Then, qRT-PCR was performed in line with SYBR GREEN (MIC091465, Kangwei Century, China). The reaction system was as follows: Mix 10 μL, upstream and downstream primers 0.8 μL, cDNA 2 μL, DEPC water 6.4 μL. Cyclic conditions were as follows: denaturation at 95°C for 5 min; 95°C for 30 s; 61°C for 30 s; 72°C for 30 s, 45 cycles, 3 multiple holes in each group. The experiment was conducted three times. The LncRNA TUG1 and miR-222-3p relative expression levels were counted by 2-ΔΔCt method.

Table 1: primer sequence table

Factor	Upstream primer	Downstream primer
LncRNA TUG1	5'-TAGCAGTTCCCCAATCCTTG-3'	5'-CACAAATTCCCATCATTCCC-3'
GAPDH	5'-AGTCACGACGCTCACGAGACC-3'	5'-GACGATCCCCGCGACTACCAAAC-3'
miR-222-3p	5'-GGGGAGCTACATCTGGCT-3'	5'-TGCGTGTCGTGGAGTC-3'
U6	5'-TGCGGGTGCTCGCTTCGGCAGC-3'	5'-CCAGTGCAGGGTCCGAGGT-3'

2.4 Cell Data and Culture

Both human BC cell MCF7 and normal human breast epithelial cell MCF-10A were bought from Beina Biology. They were cultured at a RPMI 1640 medium containing 10% fetal bovine serum (Gibco, USA) in a humid atmosphere at 37°C, 5% CO_2. Cells were transfected with over-expressing TUG1 expression lentivirus vector (sh-TUG1), inhibiting TUG1 expression expression lentivirus vector (si-TUG1), TUG1 empty vector (TUG1-NC), miR-222-3p inhibitor RNA (miR-222-3p-mimics), mimetic RNA (miR-222-3p-inhibitor) and negative control (NC) through Lipofectamine™ 2000 kit (Invitrogen, Carlsbad, USA).

2.5 Cell Proliferation (MTT Assay)

Cells were inoculated on 96-well plates. Then, 20 μL MTT solution (5 μmg/mL) (Shifeng Biotechnology Co., Ltd., Shanghai, China, R0335) was added into each hole at 24 h, 48 h, 72 h, and 96 h of incubation, respectively. Four hours later, 200 μL dimethyl sulfoxide was added. Finally, the OD value was determined at 450 mm wavelength using V-1200 spectrophotometer (Hengfei Biotechnology Co., Ltd., Shanghai, China).

2.6 Cell Invasion

The cell density was adjusted to 4 × 104 cell suspension, and then it was suspended in serum-free medium containing 1 μg/ml mitomycin C. Afterwards, they were inoculated into transwell upper chamber and 10% bovine fetal serum was added into lower chamber. Twenty-four hours later, the substrate and cells in the upper chamber that did not cross over the membrane surface were wiped, and then washed 3 times with PBS, fixed 10 min with paraformaldehyde, and dyed with 0.5% crystal violet. Ultimately, the cell invasion was surveyed with a microscope.

2.7 Apoptosis

The cells were digested with 0.25% trypsin (Gibco), cleaned twice with PBS, supplemented with 100 μL binding buffer, and prepared into 1×106 cells/mL suspension. AnnexinV-FITC (Yisheng biotechnology Co., Ltd., Shanghai, China) and PI were supplemented in turn, incubated 5 min at indoor temperature in the dark, and tested by FC500MCL flow cytometry. The test was conducted 3 times and results were averaged.

2.8 Western Blot (WB) Test

The cells were dissociated using RIPA buffer and the protein concentration was tested by BCA method (using bovine serum albumin as the standard). The protein in equal measure was separated for SDS-PAGE, and then it was transferred to another membrane and closed 1 h by 5% skim milk powder at indoor temperature. Next, it was incubated at 4°C all night supplemented with Bax, Bcl-2 and GAPDH primary antibodies. Then, HRP labeled goat anti-mouse IgG secondary antibody (1:10000) was supplemented to incubate it for 2 h, and the luminescent liquid was exposed and it developed.

2.9 Statistical Methods

All the data were analyzed through SPSS 20.0. The measurement data were represented by SD ± meas and analyzed by *t* test. The inter-group comparison was analyzed by independent-samples *T* test and represented by t. The multi-group comparison was analyzed by one-way analysis of variance (ANOVA),

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and post hoc pairwise comparison was assessed by LSD-*t* test. The multi-time point expression was analyzed by repeated measures ANOVA, and the diagnostic value was assessed through ROC curve analysis. Verification of the target gene was obtained by dual luciferase reporter enzyme on http://starbase.sysu.edu.cn/ website. The difference was statistically marked when $p < 0.05$.

3 Results

3.1 Clinical Significance of LncRNA TUG1 in BC

The LncRNA TUG1 expression level in the OG was obviously higher than that in the CG ($p < 0.05$). ROC curve analysis identified that when the COV was 2.109, the sensitivity and specificity of detecting LncRNA TUG1 expression in peripheral blood for BC diagnosis were 98.39% and 69.74%, respectively. (Fig. 1).

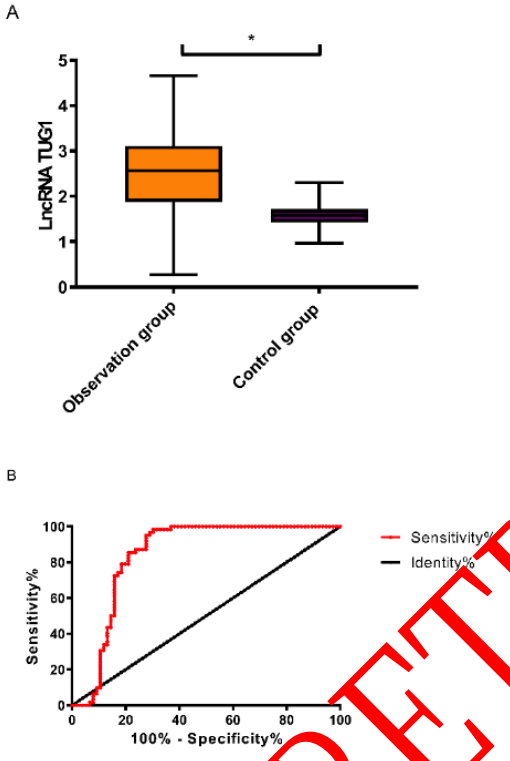

Figure 1: Clinical significance of LncRNA TUG1 in BC. A) LncRNA TUG1 expression level in peripheral blood of patients in both groups; B) ROC curve of LncRNA TUG1 expression on BC in patients

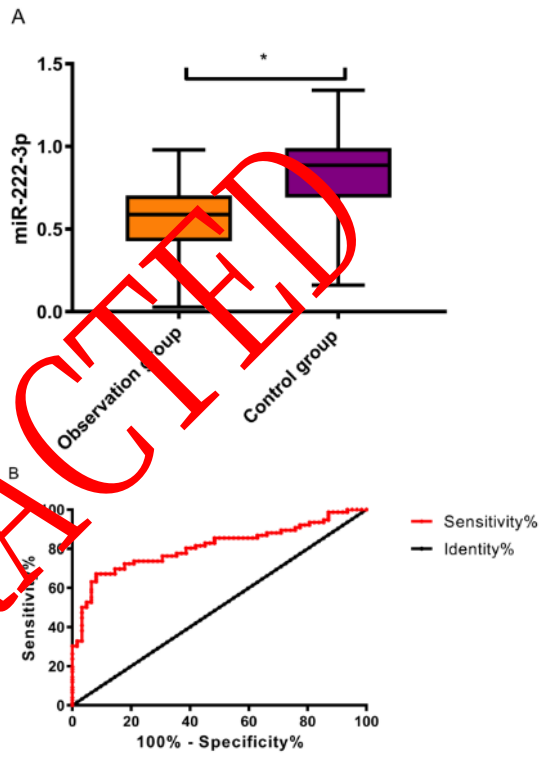

Figure 2: Clinical significance of miR-222-3p in BC. A) miR-222-3p expression level in peripheral blood of patients in both groups; B) ROC curve of miR-222-3p expression on BC in patients

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3.2 Clinical Significance of miR-222-3p in BC

The miR-222-3p expression level in peripheral blood of patients in the OG was dramatically lower than that in the CG ($p < 0.05$). ROC curve analysis identified that when the COV was 0.760, the sensitivity and specificity of detecting miR-222-3p expression were 67.11% and 91.94%, respectively. (Fig. 2).

3.3 Effect of LncRNA TUG1 on Human BC Cells

Detection of LncRNA TUG1 expression in MCF7 and MCF-10A showed that the MCF7 expression level was higher than MCF-10A ($p < 0.05$). After MCF7 cells were transfected LncRNA TUG1, their biological behavior was detected. The cell proliferation and invasion of the sh-TUG1 group were dramatically higher than those of the other two, while the apoptosis rate was lower ($p < 0.05$). The cell

proliferation and invasion of the si-TUG1r group were lower than that of the TUG1-NC group, but the apoptosis rate was higher ($p < 0.05$). (Fig. 3).

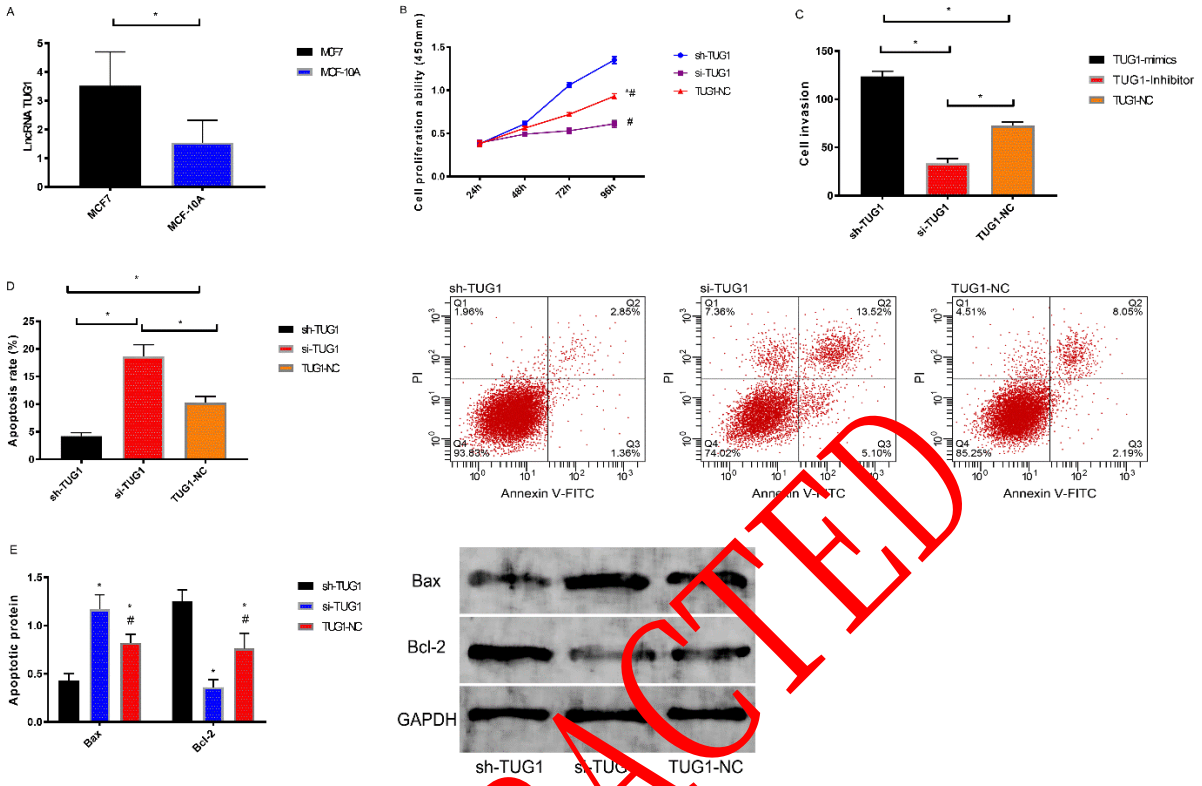

Figure 3: Effect of LncRNA TUG1 on human BC cells. A) LncRNA TUG1 expression in MCF7 and MCF-10A; B) MCF7 proliferation; C) MCF7 invasion; D) apoptosis rate and flow cytometry of MCF7; E) MCF7 apoptosis-related protein expression and WB. Note: *indicates the comparison with the sh-tug1group, and # indicates the comparison with the si-TUG1r group

3.4 Effect of miR-222-3p on Human BC cells

Detection of miR-222-3p expression in MCF7 and MCF-10A showed that the MCF7 expression level was lower than MCF-10A. ($p < 0.05$) After MCF7 cells were transfected with miR-222-3p, their biological behavior was assessed. The cell proliferation and invasion of the miR-222-3p-inhibitor group were dramatically higher than those of the other two while the apoptosis rate was lower ($p < 0.05$). The miR-222-3p-mimics group had lower cell proliferation and invasion but higher apoptosis rate than the NC group. ($p < 0.05$). (Fig. 4).

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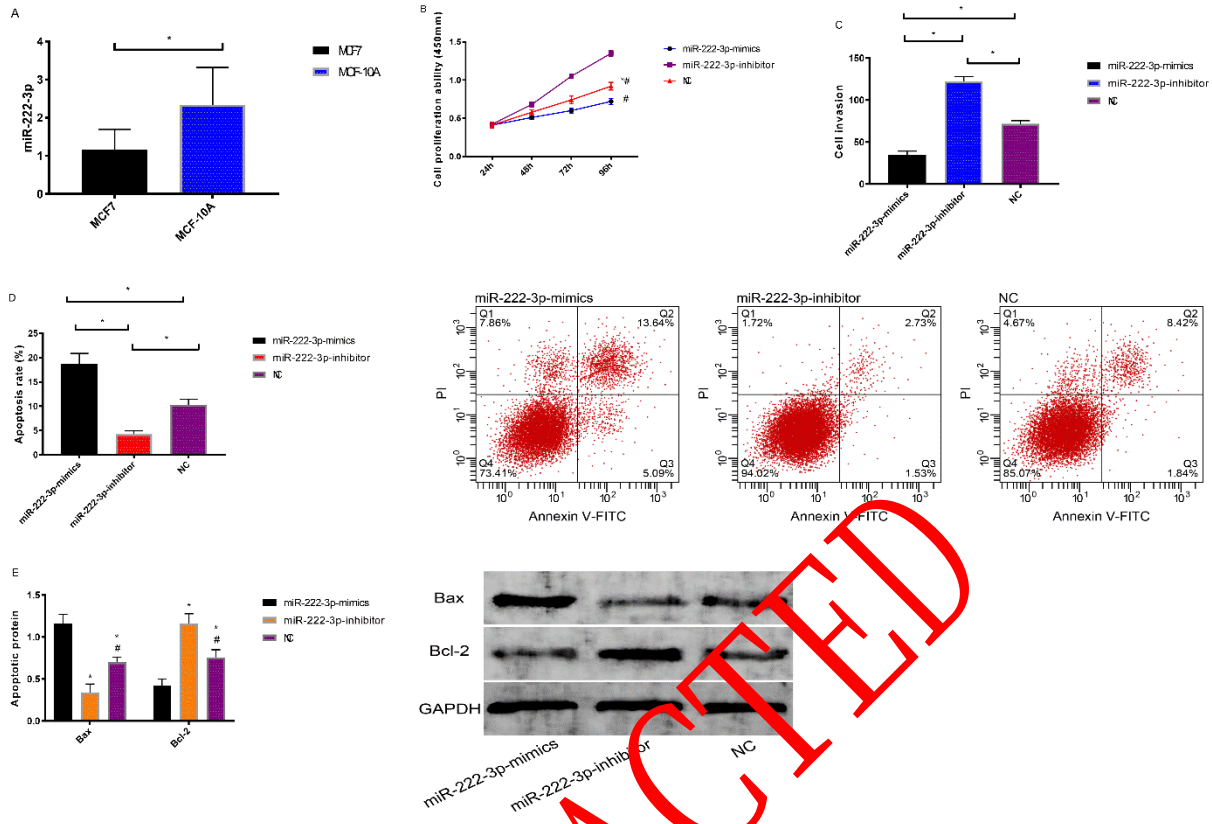

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Figure 4: Effect of miR-222-3p on human BC cells. A) miR-222-3p expression in MCF7 and MCF-10A; B) MCF7 proliferation; C) MCF7 invasion; D) apoptosis rate and flow cytometry of MCF7; E) MCF7 apoptosis-related protein expression and WB. Note: * indicates the comparison with the miR-222-3p-mimics group, and # indicates the comparison with the miR-222-3p-inhibitor group

3.5 Relationship between LncRNA TUG1 and miR-222-3p

ENCORI found that TUG1 and miR-222-3p had binding sites. Dual luciferase reporter enzyme experiment displayed that the fluorescence activity of TUG1-WT was inhibited by miR-222-3p-mimics ($p < 0.05$). (Fig. 5).

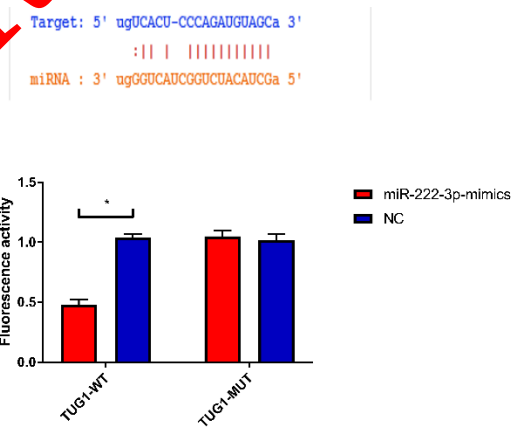

Figure 5: ENCORI analyzes the potential binding sites of LncRNA TUG1 and miR-222-3p and the fluorescence activity of dual luciferase reporter enzyme

3.6 Rescue Experiment

sh-TUG1 was co-transfected with miR-222-3p-mimics, and sh-TUG1 and TUG1-NC were separately transfected into MCF7. The biological behavior of cells in the sh-TUG1 + miR-222-3p-mimics group showed no difference in cell proliferation, invasion and apoptosis compared with the TUG1-NC group ($p > 0.05$). (Fig. 6).

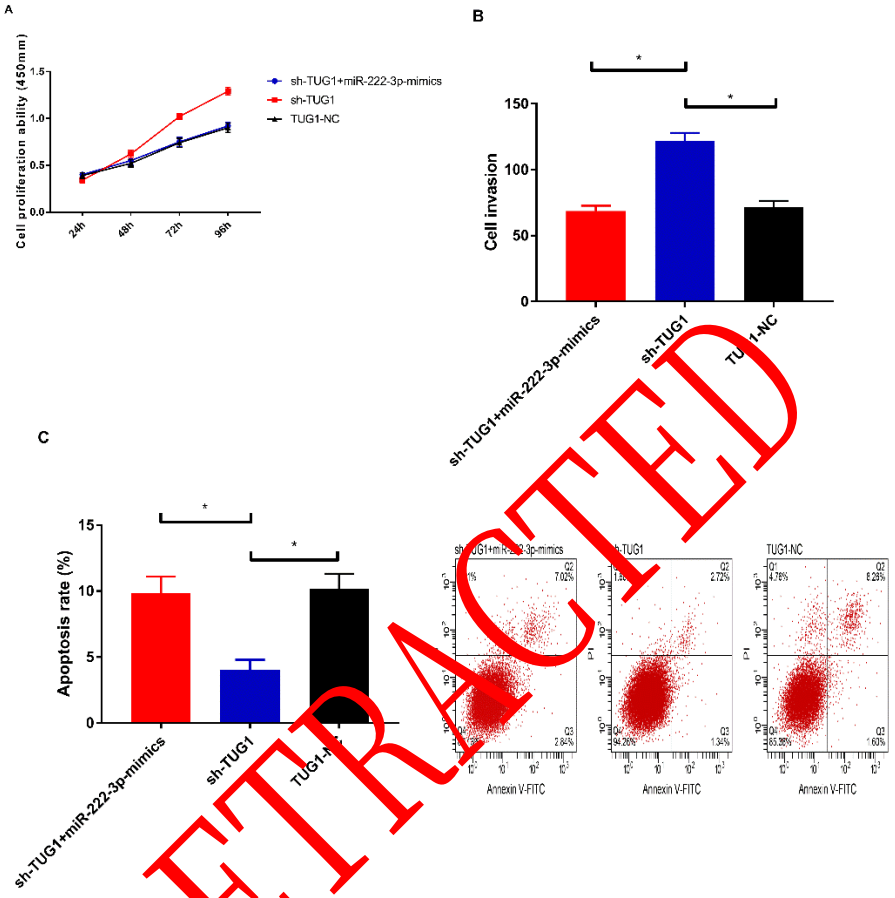

Figure 6: rescue experiment. A) Cell proliferation; B) Cell invasion; C) Apoptosis rate

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4 Discussion

BC is a great threat to patients' life and health [16]. At present, the treatment includes radiotherapy, chemotherapy, surgery and so on. Among them, surgery is the common [17]. Nevertheless, BC has no obvious clinical manifestations in the early stage and is easily ignored by patients, thus delaying the disease condition and reducing the efficacy [18]. Therefore, early detection and treatment of BC are of great significance to the survival rate of patients [19]. At present, lncRNA provides new hope for BC diagnosis, pathogenesis and treatment, which is quite significant for future clinical diagnosis and treatment [20]. Thus, this study explores the mechanism of LncRNA TUG1 on BC.

It showed that the expression level of LncRNA TUG1 in peripheral blood of BC patients increased dramatically, suggesting that it might be involved in asthma development or progression. According to previous studies, LncRNA TUG1 shows an upward trend in BC [21], which proves the accuracy of the experimental results. Moreover, LncRNA TUG1 is also highly expressed in tumor diseases such as esophageal cancer and gastric cancer [22], which also confirms the consistency of LncRNA TUG1 expression in tumor diseases. ROC curve analysis identified that LncRNA TUG1 had a sensitivity of 98.39% and specificity of 69.74% for BC diagnosis, which had good diagnostic value. This also indicated that it could be used as a clinical diagnostic index for BC and had great significance for future clinical

diagnosis. Meanwhile, we also detected miR-222-3p's clinical significance in BC. The miR-222-3p expression level in peripheral blood of BC patients also showed a decreasing trend. ROC curve analysis showed that the sensitivity and specificity of BC patients were 67.11% and 91.94%, respectively. This played a better auxiliary role for future clinicians to judge BC. Zhang et al. [23] pointed out that inhibiting miR-222-3p could promote the production of inflammatory factors in research, and Pan et al. [24] proposed that up-regulating miR-222-3p in exosomes could promote the occurrence of cervical cancer. All of these could support the results of this experiment. TUG1 was initially identified as an up-regulated transcript induced by taurine, which was highly conserved in mammals [25]. Some studies show that the TUG1 expression level in clear cell renal cell carcinoma tissue increases dramatically, and it is proved to be an independent predictor of adverse prognosis [26]. Other studies show that TUG1 can target Wnt/β- catenin signal to induce apoptosis of oral squamous cancer cells [27]. This further confirms the application prospect of LncRNA TUG1 in the future.

To understand LncRNA TUG1's effect on BC in depth, we purchased human BC cell MCF7 and normal human breast epithelial cell MCF-10A and analyzed. The results displayed that LncRNA TUG1 was also highly expressed in human BC cells, further confirming our above results. Moreover, over-expressing LncRNA TUG1 can promote the proliferation and invasion of human BC cells and reduce their apoptosis rate, while inhibiting LncRNA TUG1 is the opposite. It is suggested that low expression of LncRNA TUG1 acts as a tumor suppressor gene in BC. However, looking up previous data, we found that LncRNA TUG1 played the same role in prostate cancer [28], which could support our experimental results. We speculate that LncRNA TUG1 may be a potential therapeutic target for BC, but this requires further experimental proof. The above experiments have confirmed the effect of LncRNA TUG1 on BC, but we do not know by what route it affects human BC cells. Before the study, we found that TUG1 and miR-222-3p had binding sites through ENCORI online website. We speculated that LncRNA TUG1 might be related to miR-222-3p in BC. Therefore, we tested the miR-222-3p expression in human BC cell MCF7 and normal human breast epithelial cell MCF-10A, and we discovered that it was low expressed in human BC cell MCF7. It was coincided with the description of miR-222-3p in previous studies [29]. Further transfection of miR-222-3p into human BC cell MCF7 showed that the miR-222-3p cell proliferation was inhibited, the invasion increased and apoptosis decreased. The regulatory effect of miR-222-3p on BC was confirmed. Subsequently, we found that the fluorescence activity of TUG1-WT was inhibited by miR-222-3p-mimics through dual luciferase reporter assay, while the rescue experiment found that the effect of transfected sh-TUG1 on the biological behavior of BC cells was completely reversed by transfected miR-222-3p-mimics, confirming the targeted relationship between the two.

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5 Conclusion

To sum up, we know that TUG1 promotes the proliferation, invasion and apoptosis of BC cells by targeted regulation of miR-222-3p.

Nevertheless, we did not analyze other types of BC cells and lacked nude mice tumorigenesis experiments, so we did not know the signaling pathway of TUG1 targeting miR-222-3p. We need to conduct a longer follow-up survey on patients to judge the long-term prognosis of BC.

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Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

References

1. DeSantis, C. E., Ma, J., Goding Sauer, A., Newman, L. A., Jemal, A. et al. (2017). Breast cancer statistics, 2017, racial disparity in mortality by state. *CA: A Cancer Journal for Clinicians, 67(6),* 439–448.
2. Singh, S. K., Singh, S., Lillard, Jr. J. W., Singh, R. (2017). Drug delivery approaches for breast cancer.

International Journal of Nanomedicine, 12, 6205.

3. Michailidou, K., Lindström, S., Dennis, J., Beesley, J., Hui, S. et al. (2017). Association analysis identifies 65 new breast cancer risk loci. *Nature, 551(7678),* 92.
4. Mahtani, R., Holmes, F. A., Badve, S., Caldera, H., Coleman, R. et al. (2019). A roundtable discussion of the breast cancer therapy expert group (BCTEG): clinical developments and practice guidance on human epidermal growth factor receptor 2 (HER2)-positive breast cancer. *Clinical Breast Cancer, 20(3),* e251–e260.
5. De Nonneville, A., Goncalves, A. (2019), Triple-negative breast cancer: current data and future prospects. *Oncologie, 21(1–4),* 33–39.
6. Sachs, N., de Ligt, J., Kopper, O., Gogola, E., Bounova, G. et al. (2018). A living biobank of breast cancer organoids captures disease heterogeneity. *Cell, 172(1–2),* 373–386.
7. Couch, F. J., Shimelis, H., Hu, C., Hart, S. N., Polley, E. C. (2017). Associations between cancer predisposition testing panel genes and breast cancer. *JAMA Oncology, 3(9),* 1190–1196.
8. Yates, L. R., Knappskog, S., Wedge, D., Farmery, J. H. R., Gonzalez, S. et al. (2017). Genomic evolution of breast cancer metastasis and relapse. *Cancer Cell, 32(2),* 169–184.
9. Peng, W. X., Koirala, P., Mo, Y. Y. (2017). LncRNA-mediated regulation of cell signaling in cancer. *Oncogene, 36(41),* 5661–5667.
10. Wang, Z., Yang, B., Zhang, M., Guo, W., Wu, Z. et al. (2018). lncRNA epigenetic landscape analysis identifies EPIC1 as an oncogenic lncRNA that interacts with MYC and promotes cell-cycle progression in cancer. *Cancer Cell, 33(4),* 706–720.
11. Koch, L. (2017). Functional genomics: screening for lncRNA function. *Nature Reviews Genetics, 18(2),* 70.
12. Li, Z., Hou, P., Fan, D., Dong, M., Ma, M. et al. (2017). The degradation of EZH2 mediated by lncRNA ANCR attenuated the invasion and metastasis of breast cancer. *Cell Death & Differentiation, 24(1),* 59–71.
13. Ding, B., Lou, W., Xu, L., Fan, W. (2018). Non-coding RNA in drug resistance of hepatocellular carcinoma. *Bioscience Reports, 38(5),* BSR20180915.
14. Gradia, D. F., Mathias, C., Coutinho, R., Cavalli, I. J., Ribeiro, E. M. S. F. et al. (2017). Long non-coding RNA TUG1 expression is associated with different subtypes in human breast cancer. *Non-coding RNA, 3(4),* 26.
15. Tan, X., Tang, H., Bi, J., Li, N., Jia, Y. et al. (2018). MicroRNA-222-3p associated with Helicobacter pylori targets HIPK2 to promote cell proliferation, invasion, and inhibits apoptosis in gastric cancer. *Journal of Cellular Biochemistry, 119(7),* 5153–5162.
16. Giuliano, A. E., Connolly, J. L., Edge, S. B., Mittendorf, E. A., Rugo, H. S. et al. (2017). Breast cancer—major changes in the American joint committee on cancer eighth edition cancer staging manual. *CA: A Cancer Journal for Clinicians, 67(4),* 290–305.
17. Curigliano, G., Burstein, H. J. P., Winer, E., Gnant, M., Dubsky, P. et al. (2017). De-escalating and escalating treatments for early-stage breast cancer: the St. gallen international expert consensus conference on the primary therapy of early breast cancer. *Annals of Oncology, 28(8),* 1700–1712.
18. Cardoso, F., Costa, A., Senkus, E., Aapro, M., André, F. et al. (2017). 3rd ESO–ESMO international consensus guidelines for advanced breast cancer (ABC 3). *Annals of Oncology, 28(1),* 16–33.
19. Waks, A. G., Winer, E. P. (2019). Breast cancer treatment: a review. *Jama, 321(3),* 288–300.
20. Xu, S., Kong, D., Chen, Q., Ping, Y., Pang, D. (2017). Oncogenic long noncoding RNA landscape in breast cancer. *Molecular Cancer, 16(1),* 129.
21. Li, T., Liu, Y., Xiao, H., Xu, G. (2017). Long non-coding RNA TUG1 promotes cell proliferation and metastasis in human breast cancer. *Breast Cancer, 24(4),* 535–543.
22. Xie, C., Chen, B., Wu, B., Guo, J., Cao, Y. (2018). LncRNA TUG1 promotes cell proliferation and suppresses apoptosis in osteosarcoma by regulating miR-212-3p/FOXA1 axis. *Biomedicine & Pharmacotherapy, 97,* 1645–1653.
23. Zhang, Y., Yang, J., Zhou, X., Wang, N., Li, Z. et al. (2019). Knockdown of miR-222 inhibits inflammation and the apoptosis of LPS-stimulated human intervertebral disc nucleus pulposus cells. *International Journal of Molecular Medicine, 44(4),* 1357–1365.
24. Pan, Z. X., Zhang, X. Y., Chen, S. R., Li, C. Z. (2019). Upregulated exosomal miR-221/222 promotes cervical cancer via repressing methyl-CpG-binding domain protein 2. *European Review for Medical and*

RETRACTED

Pharmacological Sciences, 23(9), 3645–3653.

25. Zhao, L., Sun, H., Kong, H., Chen, Z., Chen, B. et al. (2017). The Lncrna-TUG1/EZH2 axis promotes pancreatic cancer cell proliferation, migration and EMT phenotype formation through sponging Mir-382. *Cellular Physiology and Biochemistry, 42(6),* 2145–2158.
26. Wang, P. Q., Wu, Y. X., Zhong, X. D., Liu, B., Qiao, G. (2017). Prognostic significance of overexpressed long non-coding RNA TUG1 in patients with clear cell renal cell carcinoma. *European Review for Medical and Pharmacological Sciences, 21(1),* 82–86.
27. Liang, S., Zhang, S., Wang, P., Yang, C., Shang, C. et al. (2017). LncRNA, TUG1 regulates the oral squamous cell carcinoma progression possibly via interacting with Wnt/β-catenin signaling. *Gene, 608,* 49–57.
28. Xu, T., Liu, C. L., Li, T., Zhang, Y. H., Zhao, Y. H. (2019). LncRNA TUG1 aggravates the progression of prostate cancer and predicts the poor prognosis. *European Review for Medical and Pharmacological Sciences, 23(11),* 4698–4705.
29. Zhang, F., Lu, Y., Wang, M., Zhu, J., Li, J. et al. (2020). Exosomes derived from human bone marrow mesenchymal stem cells transfer miR-222-3p to suppress acute myeloid leukemia cell proliferation by targeting IRF2/INPP4B. *Molecular and Cellular Probes, 51,* 101513.

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