MiR-22-3p Suppresses Cell Migration and Invasion by Targeting PLAGL2 in Breast Cancer

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ABSTRACT

Objective: To investigate the expression of *miR-22-3p* in breast cancer and the mechanism of targeting *PLAGL2* to inhibit the invasion and migration in human breast cancer.

Study Design: An experimental study.

Place and Duration of Study: Department of Oncology and Department of General Surgery, The People's Hospital of China Three Gorges University, China, from March 2019 to December 2020.

Methodology: The *miR-22-3p* expression level in 41 paired human primary breast invasive ductal carcinoma tissues and para-cancer tissues was obtained by real-time fluorescence quantitative reverse transcriptase PCR (qRT-PCR). The effect of *miR-22-3p* on the proliferation of breast cancer cells was detected by growth curve method. Online software TargetScan was used to predict the target genes of *miR-22-3p*. The prediction results were verified by luciferase reporter gene assay and qRT-PCR.

Results: *MiR-22-3p* expression was significantly decreased in the breast cancer tissues than in para-carcinoma normal breast tissues ($p < 0.05$). Over-expression of *miR-22-3p* can inhibit the proliferation of MCF-7 cells significantly. Pleomorphic adenoma gene-like protein 2 (*PLAGL2*) is the predicted target gene of *miR-22-3p*. *MiR-22-3p* binds to its predicted target gene *PLAGL2* 3'UTR. The expression of *miR-22-3p* was negatively correlated with *PLAGL2* in MCF-7 cells.

Conclusion: *MiR-22-3p* could suppress the proliferation of breast cancer by targeting *PLAGL2*. This suggests that *miR-22-3p* may be a strategy of choice for targeted therapy of breast cancer.

Key Words: Breast cancer, *MiR-22-3p*, *PLAGL2*, Cell proliferation.

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INTRODUCTION

The latest global cancer burden data released in 2020 by the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) showed that the number of new cases of breast cancer increased rapidly, replacing lung cancer as the world's highest incidence cancer. Studies showed that approximately 50% of breast cancer patients, who receive systemic treatment, develop distant meta-stases.^{1,2}

MiRNAs play a key role in the occurrence and development of breast cancer by binding to the 3'-untranslated region (3'-UTR) of target genes and silencing gene expression after transcription; and participate in the regulation of tumor proliferation and metastasis.^{3,4}

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The aim of this study was to investigate the expression level of *miR-22-3p* in breast cancer and the regulatory mechanism of targeting *PLAGL2* to inhibit the invasion and migration in human breast cancer, so as to provide a theoretical basis for the development of therapeutic agents with molecular targets of *miR-22-3p*.

METHODOLOGY

This study was conducted at the Department of Oncology and Department of General Surgery, The People's Hospital of China Three Gorges University, China from March 2019 to December 2020. Human breast cancer cell line MCF-7 was obtained from the Typical Cultures Preservation Committee Cell Bank, Chinese Academy of Sciences. Cell medium: DMEM, 10% FBS, 100 IU/mL penicillin and 100 µg/mL streptomycin sulfate were cultured at 37°C with 5% CO₂. To determine the expression level of *miR-22-3p* in breast cancer tissues, breast cancer tissues and para-cancerous tissues were collected from 41 patients, who underwent surgical treatment in the First People's Hospital of Yichang from March 2019 to December 2020. None of these patients received preoperative chemotherapy.

Figure 3: The effect of miR-22-3p on the expression of *PLAGL2* in MCF-7 cells.

TargetScan found that miR-22-3p has one binding site with the highly expressed oncogene *PLAGL2* at 3'UTR (Figure 2) in breast cancer.

QRT-PCR results showed that after transfection of miR-22-3p mimics, the expression of *PLAGL2* in MCF-7 cells decreased, while after transfection of miR-22-3p inhibitor, the expression of *PLAGL2* in MCF-7 cells increased and the difference was statistically significant ($p=0.001$ and $p<0.001$, Figure 3).

DISCUSSION

MiR-22-3p is derived from the 3' end arm of miR-22 precursor, and its expression is not consistent in tumors. The expression of miR-22-3p decreased in lung cancer,⁵ cervical cancer,⁶ osteosarcoma,⁷ and hepatocellular carcinoma;⁸ but increased in urine of patients with bladder cancer.⁹ In this study, expression of miR-22-3p was detected in breast cancer tissues and adjacent tissues. The results showed that the expression of miR-22-3p in breast cancer tissues was lower than that in adjacent tissues. This is consistent with previous literature reports.¹⁰ However, the mechanism of miR-22-3p targeting regulation of breast cancer cells is not fully understood. In this study, the growth curve experiment showed that increasing the expression of miR-22-3p in breast cancer cells MCF-7 inhibited the growth of MCF-7 cells. This study confirmed that miR-22-3p can inhibit the proliferation of breast cancer cells and play an anti-tumor role in breast cancer.

Subsequently, it was predicted that the target gene of miR-22-3p using the online software TargetScan and found that pleomorphic adenoma gene-like protein 2 (*PLAGL2*) might be the target gene of miR-22-3p. *PLAGL2* is a class of Zinc finger proteins derived from the pleomorphic adenoma gene (*PLAG*) family which composed of three members (*PLAG1*, *PLAGL1*, and *PLAGL2*) with highly conserved structure and function exhibiting antiproliferative effects on tumor cells.¹¹ More and more evidence has shown that *PLAGL2* is involved in the progression of a variety of cancers, including liver cancer, stomach cancer, colorectal cancer, lung adenocarcinoma, neuroblastoma and bladder urothelial carcinoma malignant glioma, and plays a central role in tumorigenesis.¹¹⁻²⁰ In addition,

the authors used the online software TargetScan for predictive analysis and found that miR-22-3p could bind to *PLAGL2* gene 3'UTR. Importantly, the overexpression of *PLAGL2* in breast cancer may be related to the low expression of miR-22-3p. It was proposed a new modulator mechanism that miR-22-3p suppresses proliferation, migration and invasion of breast cancer cells by regulating *PLAGL2*, which may provide an effective strategy for breast cancer treatment.

The qRT-PCR results showed that the expression levels of *PLAGL2* was dramatically downregulated in overexpression of miR-22-3p in human breast cancer cells (MCF-7). Furthermore, overexpression of *PLAGL2* could significantly reduce the number of miR-22-3p in human breast cancer cells (MCF-7). In other words, miR-22-3p can affect breast cancer cells migration and invasion via targeting *PLAGL2*. However, the results of this study need to be further confirmed in subsequent studies.

CONCLUSION

MiR-22-3p was frequently downregulated in human breast cancer samples and cell lines. Overexpression of miR-22-3p inhibited the migration and invasion of breast cancer cell through regulation of *PLAGL2* expression. MiR-22-3p might be a novel target for treatment of breast cancer patients.

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ETHICAL APPROVAL:

This study had prior formal approval of the Ethical and Research Committee of People's Hospital of China Three Gorges University.

PATIENTS' CONSENT:

Informed consents were obtained from the patients.

CONFLICT OF INTEREST:

The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:

TF, CQW: Conception, design and drafted of the work.

XTL, HY, JZ: Design and of the work, acquisition, analysis, interpretation of data.

YJS: Drafted and reviewed the paper, advices and final approval.

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