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Original Article

Inhibitory Effect of miRNA-145 on PD-L1 Expression in Breast Cancer Cell Line

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Not Applicable

Abstract

Introduction: PD-L1 is one of the most important immune control molecules in breast cancer and plays an important role in suppressing the immune system against tumor cells. Controlling the expression of PD-L1 at mRNA level using miRNA inhibitors could be helpful strategy for cancer treatment. In this study, considering the possible role of miR-145 as a tumor suppression in breast cancer, its involvement in reducing PD-L1 expression in breast cancer cell lines has been investigated.

Methods: First, the targeting of miRNA-145 on 3 'UTR of PD-L1 gene was confirmed using bioinformatics software and then by luciferase dual reporter assay. The expression level of miRNA-145 was measured in breast cancer cell lines compared to normal line. After transfection of miRNA-145 into breast cancer cell lines, qRT-PCR was used to evaluate the effect of miRNA-145 on PD-L1 expression.

Results: we showed that decreased expression of miRNA-145 in breast cancer cell lines was directly related to increased PD-L1 expression (r =-0.6175, P value=0.0457). In addition, increased expression of miRNA-145 in breast cancer cell lines MDA-MB231, BT549 and MCF7 significantly reduced PD-L1 expression (1.938±0.212, 1.784±0.03 and 0.083±0.02 respectively).

Conclusion: Our findings suggest that miRNA-145, by targeting the PD1/PD-L1 pathway and reducing PD-L1 expression, may be a therapeutic agent to prevent the progression of breast cancer.

Keywords: PD-L1, miRNA-145, Breast Cancer

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Introduction

Breast cancer is the uncontrolled growth of abnormal cells that occur in different breast tissues. In recent years, the treatment of BC has made significant progress, leading to the development of targeted therapy [1]. One of the most likely candidates for tumor immune-microenvironment in the incidence of BC is the change of PD-L1 expression. Appearance evidence reports that the miRNA network entirely controls the PD-L1 and PD-1 levels, consequently having a profound regulatory effect on the expression of immune checkpoint-related genes through a complex regulatory mechanism [2]. The main purpose of this study was to reduce the expression of PD-L1 by increasing the expression of miRNA-145 as a tumor suppressor in three breast cancer cell lines.

Materials & Methods

The expression level of PD-L1 and miR-145 were examined in BC cell lines by real time PCR and the targeting effect of miR-145 on PD-L1 was evaluated by luciferase reporter system. In vitro overexpression of miR-145 was used to figure out whether this miR could decrease the PD-L1 expression in mRNA levels in BC cell lines.

Results

miRNA-145 significantly reduces luciferase activity compared to disrupted oligonucleotides (scrambled). Overexpression of miRNA-145 to MDA-MB231, BT549 and MCF7 reduced luciferase activity by 43.5% 61 0.61, 45.4% 0. 0.42 and 46.5% 41 0.41%, respectively (figure 2B, P<0.01).

The expression of miR-145 was significantly downregulated in BC cell lines compared to their control, and its downregulation was negatively correlated with PD- L1 overexpression (r= -0.6175, p value= 0.0457) (figure 3A, B, C). Increased expression of miRNA-145 decreased PD-L1 level in MDA-MB231 to 59 ± 0.537, BT549 to 25.9 0.5 0.541 and in MCF7 to 0.547 ± 9.3%, compared with untransfected cells (figure 3D).

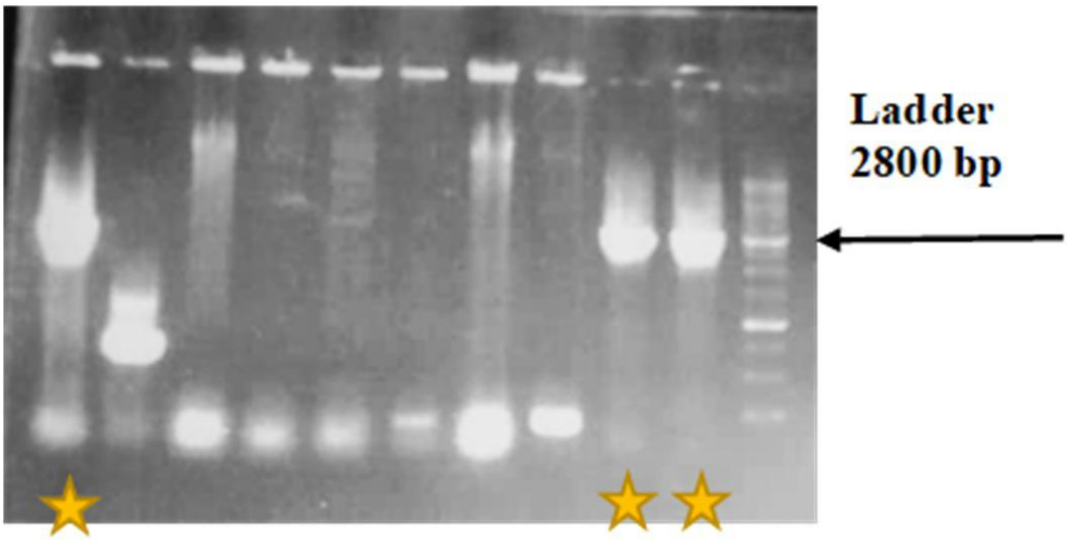

Figure 1: Detection of PD-L1 3'UTR PCR Cloning Product. Row 1 from Right: Ladder, Rows 2, 3, and 10: Positive Colonies Containing 3'UTR (2800bp) Fragment of the PD-L1 Gene Inside the PsiCHECK-2 Vector Grown in Top 10 F 'Bacteria

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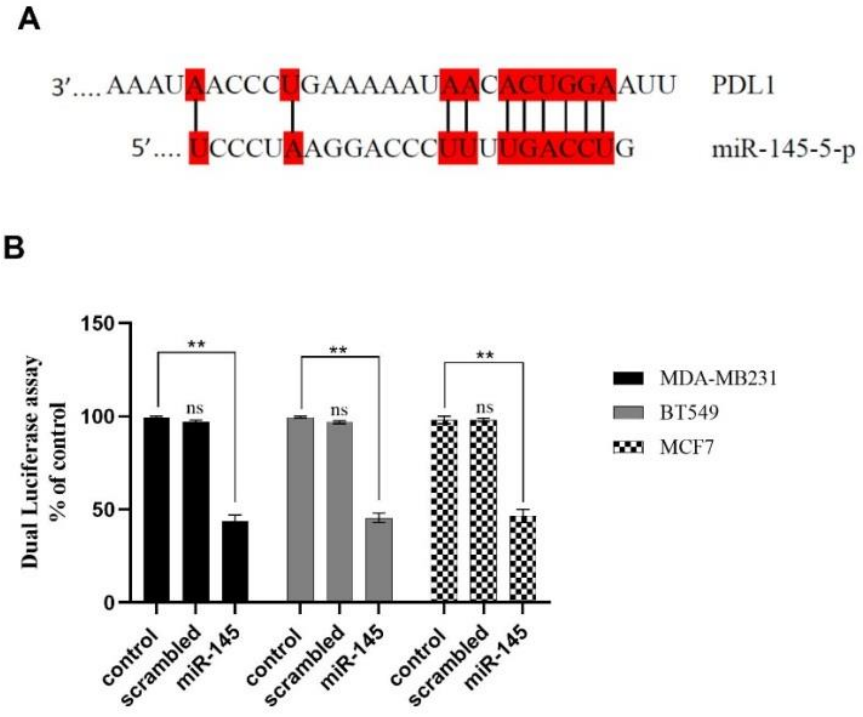

Figure 2: Targeting of PD-L1 by miRNA-145 in Breast Cancer Cells

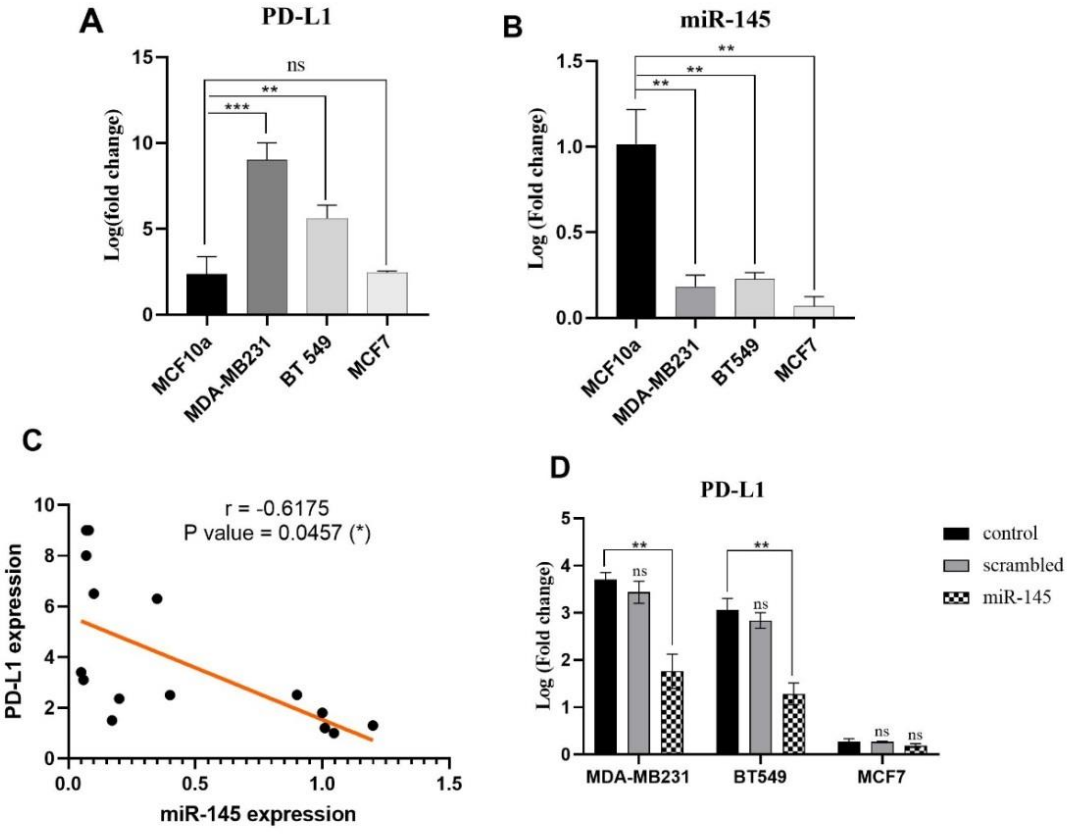

Figure 3: Expression of PD-L1 and miRNA-145 in Breast Cancer Cell Lines

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Figure 1: Detection of 3UTR PCR Product of PD-L1 Gene on Gel

Gene	Sequence of oligonucelotides from 5' to 3' for PCR analysis	Product
PD-L1	Forward: CCGCTCGAGCAGATACACATTTGGAGGAGA Reverse: ATAAGAATGCGGCCGCTGTGCAGGATTCAAAAGCAT	2800 bp
Gene	**Sequence of oligonucelotides from 5' to 3' for real time-PCR analysis**	**Product**
PD-L1	Forward: GTGATCCGCTGCATGATCAG Reverse: GGTAGCCCTCAGCCTGACATG	151 bp
GAPDH	Forward: GAAAGCCTGCCGGTGACTAA Reverse: GCGCCCAATACGACCAAATC	152 bp
miR-145-5p	Forward: GTCCAGTTTTCCCAGGAATCCC Reverse: AGACTGCACCTGTCCGG	180 bp
Stem loop miR-145-5P	CAATTAGACTACACCTGTCCGGTCCCTGCGTCCTGTAGTCTAATTGAGGGAT	64 bp

Discussion

Breast carcinogenesis is a process involving the dysregulation of tumor suppressor genes and oncogenes [3]. The PD- 1/PD- L1 pathway is important as an immunosuppressant, and it is deregulated in a wide range of human cancers [4], including BC. PD-L1 is involved in tumorigenesis with anti-tumor immunosuppression; obstruction makes it a valuable therapeutic target for a variety of human cancers. Identifying the molecular mechanisms involved in regulating the PD- 1/PD- L1 pathway through breast tumorigenesis could help improve new strategies aimed at the antitumoral immune response against BC. miRs are also important regulators of gene expression and have been deregulated with an increasing number of cancer [5]. It is important to achieve efficient methods to increase the expression of tumor suppressor miRNAs for the treatment and control of cancer or its complications. One of the tumor suppressor miRNAs that has been reduced in many cancers, including ovarian, breast, lung, and colon cancers, is miRNA-145 [6].

In the present study, using bioinformatics analysis, we identified region 3'UTR of the PD- L1 gene as a potential target for miRNA-145, suggesting its possible role in escaping tumor cells from the immune system. Subsequently, we showed that misregulation of miRNA-145 in breast cancer cells was directly related to increased PD-L1 expression, suggesting that miRNA-145 may play a role in post-transcriptional regulation of PD-L1 expression.

In this study, dual luciferase assay confirmed that miRNA-145 could target specific regions of PD-L1 ′3′UTR and significantly reduce its expression ($P < 0.01$). Also, the results of qRT-PCR and a significant decrease in PD-L1 expression after miRNA-145 transfection in the breast cancer metastatic cell line (MDA-MB231, BT549) by 1.938 ± 0.212 and 1.784 ± 0.03, respectively Compared with the non-metastatic cell line (MCF7, 0.083 ± 0.02) of breast cancer, indicates that a significant decrease in miR-145 is involved in an increase in PD-L1 and ultimately breast cancer metastasis. According to this study, increasing miRNA-145 expression in breast cancer cells significantly reduced PD-L1 expression at the mRNA level, reducing the function of miRNA-145 in PD-L1 gene expression in BC.

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Conclusion

Our findings suggest that miRNA-145, by targeting the PD1/PD-L1 pathway and reducing PD-L1 expression, may be a therapeutic agent to prevent the progression of breast cancer.

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