

RESEARCH ARTICLE

miR-153 Silencing Induces Apoptosis in the MDA-MB-231 Breast Cancer Cell Line

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Abstract

MicroRNAs (miRNAs) are small, non-coding RNAs (18-25 nucleotides) that post-transcriptionally modulate gene expression by negatively regulating the stability or translational efficiency of their target mRNAs. In this context, the present study aimed to evaluate the *in vitro* effects of miR-153 inhibition in the breast carcinoma cell line MDA-MB-231. Forty-eight hours after MDA-MB-231 cells were transfected with the miR-153 inhibitor, an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was utilized to determine the effects of miR-153 on cell viability. Flow cytometry analysis and assessment of caspase 3/7 activity were adopted to determine whether miR-153 affects the proliferation rates and apoptosis levels of MDA-MB-231 cells. Our results showed that silencing of miR-153 significantly inhibited growth when compared to controls at 48 hours, reducing proliferation by 37.6%, and inducing apoptosis. Further studies are necessary to corroborate our findings and examine the potential use of this microRNA in future diagnostic and therapeutic interventions.

Keywords: Breast cancer - microRNA - apoptosis - triple negative - miR-153 silencing

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Introduction

Breast cancer is the most commonly diagnosed cancer, and the second leading cause of cancer-related deaths in US women (Siegel et al., 2012). Breast cancer is functionally classified based on molecular profiles. Estrogen receptor (ER), progesterone receptor (PR) and ErbB-2/human epidermal growth factor receptor 2 (HER-2) status are molecular markers used to determine breast cancer subtypes as well as targets for treatment (Lindström et al., 2012). In contrast, triple-negative breast cancer (TNBC) is negative for expression of estrogen and progesterone receptors and HER2 receptor. This form accounts for 15-25% of breast tumors and is an aggressive form with limited treatment options. Therefore, there is a critical need to enhance current systemic treatments and/or identify new targets for the treatment of TNBC.

miRNAs are a type of small non-coding RNAs of 18-25 nucleotides in length and play important roles in post-transcriptional gene expression regulation (Lee and Vasudevan, 2013). By binding to the 3' UTR (untranslated region) of target mRNAs, miRNA controls cell growth, proliferation, metabolism and apoptosis (Lages et al., 2012). Indeed, specific miRNA deregulation has been shown to correlate with breast cancer (Harquail et al., 2012; Singh and Mo, 2013). For instance, miR-21 is

overexpressed in breast cancer tissue (Ozguin et al., 2013; Mar-Aguilar et al., 2013). In the other hand, miR-132 is down-regulated in ductal carcinoma in situ of breast and acts as a tumor suppressor by inhibiting cell proliferation (Li et al., 2013). Therefore, identification of the molecular events leading to development and progression of cancers is the key to develop effective therapeutic strategies and prognostic markers.

In this study, we report that miR-153 silencing induces apoptosis on TNBC cell line MDA-MB-231. These results indicate that miR-153 functions as an oncogenic microRNA, whose dysregulation may be involved in the initiation and development of human breast cancer.

Materials and Methods

Cell culture

The TNBC cell line MDA-MB-231 was cultured in DMEM/F12 medium containing 10% FBS at 37°C, 5% CO₂, and saturated humidity. The growth of the cells was observed under an inverted microscope. When the cells reached 70-80% confluence, they were digested with 0.25% trypsin and passaged. The medium was changed every other day, and the cells were passaged every 3 to 4 days. Cells in the logarithmic growth phase were collected for experiments.

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Proliferation assay

A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich) assay was performed to determine cell proliferation. Briefly, MDA-MB-231 cells were plated in 96-well plates at a density of 3×10^5 cells/ml in a volume of 100 μ l per well. The transfection with 100 nM of miR-153 inhibitor or negative control (both from Ambion) was performed according to the manufacturer's instructions using Lipofectamine™ 2000. The untransfected control group (MDA-MB-231) was set up simultaneously. Forty-eight hours after transfection, 100 μ l of the MTT solution (0.5 mg/ml) was added to each well, and the plates were incubated for 4 h at 37°C with 5% CO₂ and then the plate was centrifuged to pellet the crystal. The medium was removed, and the crystal in each well was solubilized in DMSO. The absorption was measured at 562 nm. Each experimental group contained ten duplicated wells, and the experiment was repeated three times.

FACS analysis, PI and PI/Annexin V staining

In order to determine the effect of miR-153 inhibitor on MDA-MB-231 cells, FACS analysis was carried out. Forty-eight hours after the cells were transfected with the miR-153 inhibitor and negative control; the cells were washed one to two times with PBS and incubated with Annexin V-FITC and PI staining solutions in the dark for 15 min at room temperature. After filtration using mesh filters, the cells were analyzed by flow cytometry (BD Biosciences, USA) followed by analysis using CellQuest Pro software (BD Biosciences).

Caspase-Glo 3/7 assay

The influence of transfection of the miR-153 inhibitor on caspase 3/7 activity in MDA-MB-231 cell line was detected using Caspase-Glo 3/7 Assay kit (Promega). Cells cultured in DMEM/F12 were seeded in 96-well plates and treated with the miR-153 inhibitor or negative control. Forty-eight hours after the cells were transfected with the miR-153 inhibitor or negative control, 100 μ l of caspase 3/7 reagent were added to each well, mixed and incubated for 1 h at room temperature. Luminescence was measured using well Infinite M2000 Pro™ instrument (Tecan). Caspase 3/7 activity was expressed as percentage of the untreated control.

Statistics

All experiments were repeated at least three times. The data are presented as means±standard errors. The statistical analysis was performed using paired Student's t-test and $p < 0.05$ was considered statistically significant.

Results

miR-153 Inhibits Cell Growth of MDA-MB-231 cells

Effects of miR-153 on cell viability, as detected by MTT assay. The MTT assay revealed that cell proliferation was significantly inhibited in miR-153 inhibitor transfectants in comparison with non-transfectants and negative control transfectants in MDA-MB-231 cells ($62.4 \pm 3.2\%$, $98.2 \pm 1.5\%$ and $97.3 \pm 2.8\%$, respectively; $P < 0.001$)

suggesting that the silencing of miR-153 reduced MDA-MB-231 cell viability (Figure 1).

miR-153 Induces Apoptosis in MDA-MB-231 cells

To test cell death, apoptosis experiments were performed. We conducted apoptosis assays using an Annexin V-PI apoptosis detection kit to determine whether the miR-153 inhibitor transfectants induce cell apoptosis in breast cancer cells. The results shown in Figure 2 demonstrated that the apoptotic cells (%) of MDA-MB-231 transfected with the miR-153 inhibitor were higher than those transfected with the negative control. Interestingly, with miR-153 inhibitor transfectants MDA-MB-231 cells we revealed an apoptotic subpopulation of 42.4%, whereas negative control transfectants MDA-MB-231 showed an apoptotic subpopulation of 4.7%. These findings suggested that the process of death induced by miR-153 transfectants in MDA-MB-231 cells is via apoptosis pathway. Consistent with this observation, the caspase-3 activity in MDA-MB-231 cells transfected with the miR-153 inhibitor was eleven fold higher than in those negative control transfectants MDA-MB-231 (see Figure 3).

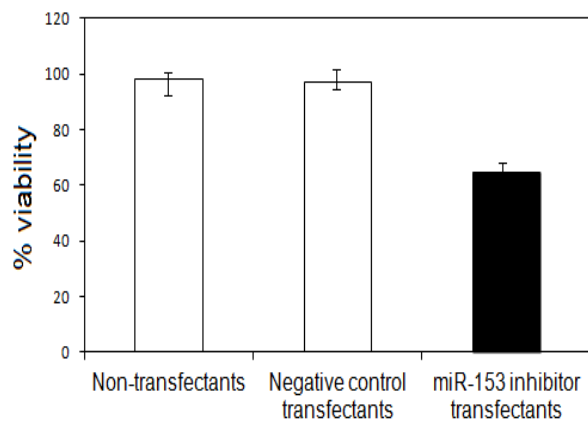


Figure 1. Inhibition Proliferation Caused by miR-153 Silencing in MDA-MB-231 Cells. MDA-MB-231 cells were incubated during 48 hours in presence of miR-153 inhibitor or negative control. MTT assay was performed as detailed in materials and methods. The results are shown as a percentage of the untreated control (+/- SE)

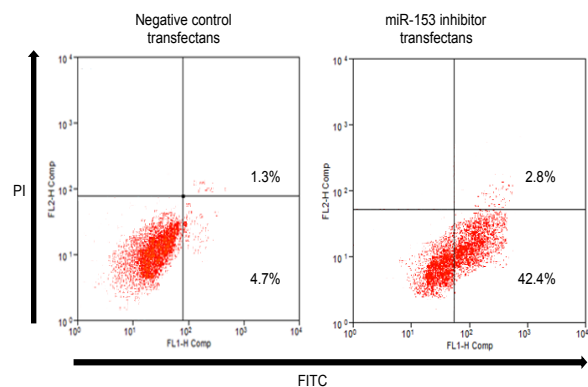


Figure 2. miR-153 Silencing Induces Apoptosis in MDA-MB-231 Cells. Untreated and treated MDA-MB-231 cells with miR-153 inhibitor during 48 h, as evidenced by PI/Annexin V double staining and FACS analysis

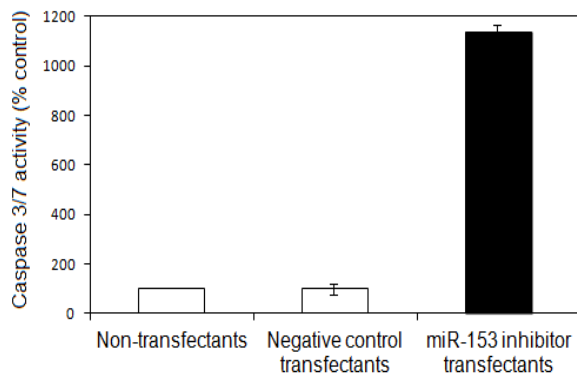


Figure 3. miR-153 Silencing Induces Apoptosis via caspases-3 and -7 in MDA-MB-231 Cells. Untreated and treated MDA-MB-231 cells with miR-153 inhibitor during 48 h. Caspase 3/7 activity was expressed as percentage of the untreated control (+/- SE)

Discussion

Woman breast cancer is the most important cause of mortality in the world (Haghighat et al., 2012; Yang et al., 2012). The occurrence, development, and metastasis of breast cancer are closely associated with mutations in a variety of genes, alterations in cell signaling pathways, and neovascular dysplasia, all of which involve multiple critical steps (Huang et al., 2011; Connolly and Stearns, 2012).

Although much remains to be learned, emerging evidence now suggests that in addition to protein-encoding genes, miRNAs play a important role in the pathogenesis of tumor development by functioning as agents of the RNA interference pathway (Kala et al., 2013; Parpart and Wang, 2013). miRNAs are a type of small non-coding RNAs of 18-25 nucleotides in length and play important roles in post-transcriptional gene expression regulation (Lee and Vasudevan, 2013). By binding to the 3' UTR (untranslated region) of target mRNAs, miRNA controls cell growth, proliferation, metabolism and apoptosis (Lages et al., 2012). Carcinogenic miRNAs are often overexpressed in tumors and are termed "oncogenic" because they play similar roles as oncogenes during the process of cancer development. In addition, the expression of tumor suppressing miRNAs is often downregulated in tumors; these miRNAs are called tumor suppressor miRNAs because they function similarly to tumor suppressor genes (Tang et al., 2012).

Recent studies have shown the participation of miR-153 in diverse cancer, including those from ovarian, glioblastoma, prostate, and epithelial cancer (Kim et al., 2010; Wu et al., 2012; Xu et al., 2010; 2013). These studies have shown that miR-153 functions as an oncogenic regulator and tumor suppressor. For example, miR-153 is overexpressed in prostate cancer cell lines and promotes cell migration and invasion by PTEN tumor suppressor gene (Wu et al., 2012). Xu et al. (2011) revealed that miRNA-153 expression to suppress Irs-2 in glioblastoma cell lines.

In the other hand, miR-153 act as tumor suppressor to modulate cell proliferation in glioblastoma cell line DBTRG-05MG (Xu et al., 2010). This study showed

that in vitro transfection of miR-153 decreased cell proliferation and increased apoptosis. Furthermore, antiapoptotic protein Bcl-2 and induced myeloid leukemia cell differentiation protein (Mcl-1) were downregulated by miR-153 upregulation. Zhao et al. (2013) discovered that transient transfection of miR-153 in glioblastoma stem cells induces apoptosis. Furthermore, low expression level of miR-153 was found to be significantly related to metastasis and poor prognosis in oral cancer patients by targeting SNAIL and ZEB2 (Xu et al., 2013). Additionally, abnormal downregulation of miR-153 can also be detected in human ovarian tumors and was correlated significantly with advanced clinical stage (Kim et al., 2010).

Consistent with previous studies, in the present study, our results suggest that miR-153 is upregulated in MDA-MB-231 cells and might act as an oncogene in breast tumors. In this study, we utilized MTT assays, flow cytometry analyses, and caspase assays to determine the effects of silencing of miR-153 on MDA-MB-231 cells. The silencing of miR-153 inhibited cell viability and proliferation, while it induced apoptosis. The results of this study are consistent with the previously reported effects of miR-153 in other types of tumors.

In summary, our results showed that miR-153 could be a tumor regulator in breast cancer and playing an important role in breast cancer development and progression. Consequently, miR-153 might serve as potential biomarker for predicting breast cancer progression, metastatic behavior and poor prognosis. However, the work on miR-153 is still in its infancy and requires additional research before being introduced in the clinic.

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