

## RESEARCH ARTICLE

# miR-485 Acts as a Tumor Suppressor by Inhibiting Cell Growth and Migration in Breast Carcinoma T47D Cells

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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-485 mimics in breast carcinoma T47D cells. Forty-eight hours after T47D cells were transfected with miR-485 mimics, an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was utilized to determine the effects on cell viability. Colony formation and cell migration assays were adopted to determine whether miR-485 affects the proliferation rates and cell migration of breast carcinoma T47D cells. Our results showed that ectopic expression of miR-485 resulted in a significant decrease in cell growth, cell colony formation, and cell migration. These findings suggest that miR-485 might play an important role in breast cancer by suppressing cell proliferation and migration.

**Keywords:** Breast cancer - microRNA - migration - tumor suppressor

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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). The mortality rate in developing countries is even higher because of limited medical infrastructure and awareness (Siegel et al., 2012). This substantiates the necessity to explore for novel biomarkers which can help in early detection and diagnosis of breast cancer for better cancer management.

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). Indeed, specific miRNA down-regulated has been shown to correlate with breast cancer (Harquail et al., 2012; Singh and Mo, 2013). For instance, 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). Also, miR-10b an inhibitor

of the cell cycle is down-regulated human breast tumors (Biagioni et al., 2012). On the other hand, miR-129 was found to significantly inhibit the cell mobility and migration in MDA-MB-231, BT549 and MDA-MB-435s breast cancer cell lines. Most importantly, miR-129 is down-regulated both in breast cancer tissues and cell lines (Wang et al., 2012).

In this study, we could demonstrate that miR-485 is pathologically downregulated in breast carcinoma T47D cells, and that ectopic expression of miR-485 reduced cell proliferation and migration. Thus, our results suggest that miR-485 could play an important role in breast cancer pathogenesis and may thus represent a novel therapeutic target.

### Materials and Methods

#### Cell culture

T47D cells (HTB-133, ATCC) a triple positive breast cancer cell line, were grown in RPMI-1640 medium, supplemented with 10% fetal bovine serum (FBS), 100 U/mL of penicillin, and 100 µg/mL of streptomycin at 37°C, 5% CO<sub>2</sub>, 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, T47D cells were plated in 96-well plates at a density of  $3 \times 10^5$  cells/ml in a volume of 100  $\mu$ l per well. The transfection with 100 nM of miR-485 mimics or negative control (both from Ambion) was performed according to the manufacturer’s instructions using Lipofectamine™ 2000. The untransfected control group was set up simultaneously. Forty-eight hours after transfection, 100  $\mu$ 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% CO<sub>2</sub> 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.

**Anchorage-independent colony formation assay**

Colony formation was evaluated using a soft agar colony formation assay. A total of 1.5 ml of RPMI 1640 medium containing 10% fetal bovine serum and 0.5% agar was plated on the bottom of six-well plates. The plates were stored at 4°C to allow the agar to solidify. The cells were first seeded in six-well plates for 24 h and then transfected with miR-485 mimics for 24 h. Subsequently, the cells were trypsinized. A number of cells per well were mixed with RPMI 1640 medium containing 10% fetal bovine serum and 0.35% agar and were plated on the prepared six-well plates (three wells per condition). The plates were then transferred to 37°C. After 14 days, the colonies were fixed with methanol for 30 min, stained with crystal violet for 20 min, and scored using a microscope. Colony formation for each condition was calculated in relation to the values obtained for the scramble-treated control cells.

**Cell migration assay**

The migration capacity of breast cancer cells transfected with miR-485 mimics were tested by using Corning transwell assay according to the user’s manual. Briefly, breast carcinoma T47D cells were transfected with miR-485 mimics or negative control for 48 h and then the transfected cells with serum-free medium (100  $\mu$ l containing  $1 \times 10^5$  cells) were seeded into the upper chamber of the system. Bottom wells in the system were filled with complete medium. After 12 h of incubation, the cells in the upper chamber were carefully removed with a cotton swab, and the cells that had migrated through the membrane and had stuck to the lower surface of the membrane were fixed with 90% methanol and stained with 0.1% crystal violet. For quantification, the cells were counted by photographing the membrane under a microscope in five predetermined fields at 200X magnification.

**Statistics**

All experiments were repeated at least three times. The data are presented as means $\pm$ standard deviation. The

statistical analysis was performed using paired Student’s t-test and  $p < 0.05$  was considered statistically significant.

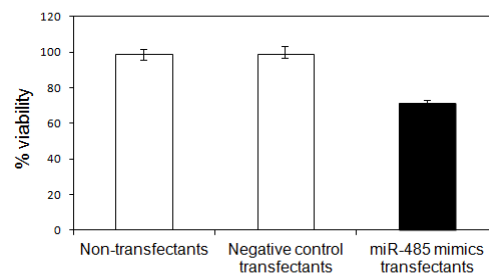
**Results**

**miR-485 inhibits cell growth of breast carcinoma T47D cells**

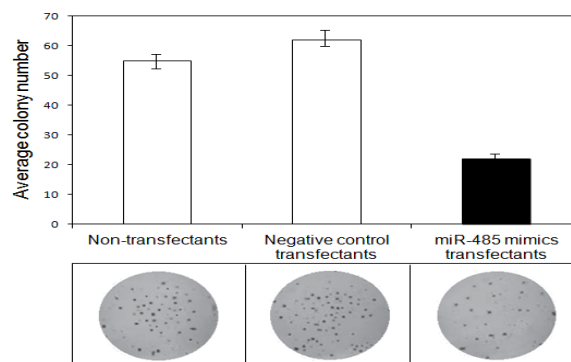
Using an MTT assay, we found that the cell viability of the miR-485 mimics transfection group was lower ( $71 \pm 2.8\%$ ) than that of the untransfected control group and the negative control group ( $98.2 \pm 1.5\%$  and  $98.7 \pm 2.3\%$ , respectively), suggesting that the ectopic expression of miR-485 reduced cell viability of breast cancer cell lines (Figure 1).

**miR-485 inhibits the colony formation efficiency of breast carcinoma T47D cells**

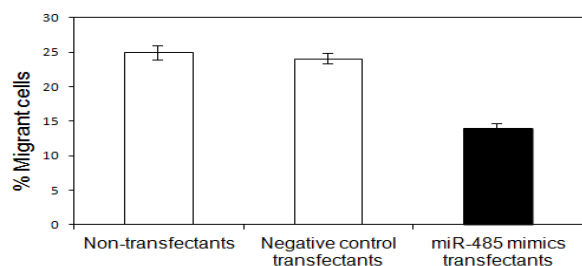
Colony formation assay essentially tests every cell in the population for its ability to undergo “unlimited” division. As shown in Figure 2, miR-485 mimics could inhibit (~65%) the colony formation efficiency of T47D cells. These results suggest that miR-485 attenuates the proliferation and anchorage-independent growth of breast cancer cell *in vitro*.



**Figure 1. Inhibition Proliferation Caused by miR-485 mimics in T47D Cells.** T47D cells were incubated during 48 hours in presence of miR-485 mimics or negative control. MTT assay was performed as detailed in materials and methods. Data represent the mean $\pm$ SD of three independent experiments ( $p < 0.05$ )



**Figure 2. miR-485 Mimics Inhibits the Colony Formation Efficiency of T47D Cells.** T47D cells were incubated during 48 hours in presence of miR-485 mimics or negative control. The cells were cultured for 14 days to form colonies. Colony formation assay was performed as detailed in material and methods. (Left panel) Crystal violet staining of T47D transfectants, (Right panel) The colony number of T47D transfectants. The results are presented as the mean $\pm$ SD of three independent assays ( $p < 0.05$ )



**Figure 3. miR-485 Mimics Inhibits the Migration of T47D Cells.** T47D cells were incubated during 48 hours in presence of miR-485 mimics or negative control. Cell migration assay was performed as detailed in material and methods. Data represent mean±SD from three independent experiments ( $p < 0.05$ )

#### miR-485 inhibits the migration and invasion in breast carcinoma T47D cells

To investigate the role of miR-485 in breast cancer metastasis, we detected the migrant capacity of T47D cells transfected with miR-485 mimics or negative control. Through transwell assay, we found that the percentage of cells travelled through the micropore membrane was significantly decreased in cells transfected with miR-485 mimics ( $14 \pm 1.2\%$ ) as compared to those cells transfected with negative control ( $24 \pm 1.4\%$ , Figure 3). These results indicate that miR-485 could inhibit the migration and invasion of breast cancer cells.

## Discussion

Breast cancer is the most commonly diagnosed malignancy and leading cause of cancer mortality among women in the world (Haghighat et al., 2012; Yang et al., 2012). Ninety percent of all cancer deaths arise from tumor metastases, the process in which abnormal tumor cells proliferate, expand, reorganize, degrade and migrate through the surrounding stroma's microenvironment into the circulation to invade other tissues.

Although much remains to be learned, emerging evidence now suggests that in addition to protein-encoding genes, miRNAs play an important role in the pathogenesis of tumor metastasis by functioning as agents of the RNA interference pathway (Kala et al., 2013; Parpart and Wang, 2013). Recent studies have shown the participation of miRNAs in breast cancer metastasis (Harquail et al., 2012). These studies have shown, for example, that miR-let-7, miR-584, and miR-204 inhibits cell motility by regulating the genes in the actin cytoskeleton pathway, implicating a central role for it in cytoskeletal dynamics (Imam et al., 2012; Fils-Aimé et al., 2013; Hu et al., 2013).

In the other hand, recent studies have shown that the expression of tumor suppressing miRNAs is often down-regulated in tumors. For example, has been shown that miR-9 is down-regulated in MCF-7 and MDA-MB-231 breast cancer cells and have tumor suppressor-like activity breast via MTHFD2 (Selcuklu et al., 2012). Additionally, miR-206 has a crucial tumour suppressive role in the progression of breast cancer, via up-regulation of the expression of cyclinD2 (Zhou et al., 2013). Furthermore, has been shown that miR-132 is frequently down-regulated in ductal carcinoma in situ (DCIS) of breast and

acts as a tumor suppressor by inhibiting cell proliferation (Li et al., 2013).

Recently, the expression of miR-485 was correlated with clinicopathological parameters in four major histotypes of ovarian cancers, which suggests that miR-485 might be of potential importance as diagnostic biomarkers (Kim et al., 2010). Consistently, miR-485-5p is down-regulated in pediatric central nervous system tumor (Costa et al., 2011). Moreover, up-regulation of miR-485-3p contributes to lower methionine adenosyltransferase 1A expression in hepatocellular carcinoma, and enhanced tumorigenesis may provide potential targets for hepatocellular carcinoma therapy (Yang et al., 2013). Interestingly, miR-485-3p has been shown to mediate topoisomerase II $\alpha$  down-regulation in part via altered regulation of the transcription factor nuclear factor YB and to have a role in drug responsiveness in CEM and CEM/VM-1-5 cells (human leukemic lymphoblastic cells) and in Rh30 and Rh30/v1 cells (human rhabdomyosarcoma cells) (Chen et al., 2011). Similarly, has been shown that miR-485 was up-regulated in serum from prostate cancer patients compared to normal donor sera (Lodes et al., 2009).

In the present study, our results suggest that miR-485 is down-regulated in T47D cells and might act as a suppressor in breast tumors. In this study, we utilized MTT, colony formation and cell migration assays, to determine the effects of the ectopic expression of miR-485 on T47D cells. The expression inhibited cell viability, colony formation and cell migration. In summary, our results showed that miR-485 could be a tumor regulator in breast cancer and playing an important role in breast cancer development and progression. Consequently, miR-485 might serve as potential biomarker for predicting breast cancer progression, metastatic behavior and poor prognosis. However, the work on miR-485 is still in its infancy and requires additional research before being introduced in the clinic.

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