MiR-185 is involved in human breast carcinogenesis by targeting Vegfa

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ABSTRACT

MiR-185 expression has been associated with many cancers. However, the roles of miR-185 in human breast cancer remain elusive. Here, we found that miR-185 expression was decreased in human breast cancer tissues compared with healthy tissue controls. Up-regulation of miR-185 inhibited human breast cancer cell proliferation and invasion and vice versa. MiR-185 was shown to bind to the 3'-untranslated region (UTR) of vascular endothelial growth factor a (Vegfa), and a significant inverse correlation was found between miR-185 and Vegfa. Vegfa overexpression partially restored the inhibition of cell proliferation and invasion that was induced by miR-185, and vice versa. Additionally, Vegfa expression was found to be high in human breast cancer tissues. Thus, miR-185-mediated Vegfa targeting may be involved in breast cancer formation.

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1. Introduction

MicroRNAs (miRNAs) are small non-protein-coding RNAs that negatively regulate gene expression by inhibiting the translation or reducing the stability of target mRNAs [1]. Many miRNAs are differentially expressed in breast cancers tissues as compared to adjacent normal breast tissues by high-throughput miRNA expression profiling [2–4]. For example, miR-124 was dramatically downregulated in breast cancer tissues in human [5]. The proliferative and invasive ability in ERα-positive and ERα-negative breast cancer cells were inhibited by miR-196a2 expression and enhanced by miR-196a2 antagonism [6].

MiR-185 takes part in a series of cellular activities, such as cell proliferation and tumorigenesis [7–9]. In recent years, increasing studies mainly focus on its possible roles in tumorigenesis. Xiang et al. found that miR-185 was markedly down-regulated in the cisplatin-resistant ovarian cancer cells. Moreover, the up-regulation of miR-185 increased the sensitivity of ovarian cancer cells to cisplatin-induced apoptosis via altering the DMNT1 expression [7]. In poor survival and metastasis colorectal cancer, miR-185 was observably up-regulated [8]. Ectopic expression of miR-185 could suppress the growth of human non small cell lung cancer cell lines and induce G1 cell cycle arrest in H1229 cells [9]. However, little is known about the pathophysiological significance of miR-185 in breast cancer.

In order to investigate the molecular mechanisms by which miR-185 may execute in breast cancer, its target genes were searched. TargetScan and miRanda prediction algorithms revealed many possible miR-185 targets. Among them, we found that there was a highly conserved miR-185 responsive element in 3'-untranslated region (3'-UTR) of Vegfa. It was reported that Vegfa was a key regulator in angiogenesis and highly expressed in tumor tissues. Furthermore, it closely associates with tumor aggressive features [10,11]. Serum VEGFA concentrations had been elevated in patients with locoregional ductal cancers compared with women with benign breast cancer. The patients with metastatic breast tumor would receive the highest concentrations of serum VEGFA, in particular among patients who did not obtain cancer therapy [12]. Thus, we speculate that Vegfa may be the target gene of miR-185.

Abbreviations: miRNAs, MicroRNAs; Vegfa, vascular endothelial growth factor a; 3'-UTR, 3'-untranslated region; DMEM, Dulbecco’s Modified Eagle Medium; FBS, fetal bovine serum; qRT-PCR, quantitative reverse-transcriptase polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; FITC, fluorescein isothiocyanate; PI, phosphatidylinositol; PVDF, polyvinylidene fluoride membrane; HRP, horseradish peroxidase; ER, estrogen receptor; PR, progesterone receptor
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In this study, the expression pattern of miR-185 and its target gene Vegfa was evaluated in breast cancer tissues and adjoining normal breast tissues. The pathophysiological effects of miR-185 in breast cancer were researched via in vitro cell model. Our study reveals that the miR-185 may be a novel biomarker for the diagnosis and therapy to human breast cancer in the future.

2. Materials and methods

2.1. Tissue samples and breast cell lines

Human breast cancer tumor tissues and adjoining normal tissues were acquired from the Third Affiliated Hospital of Harbin Medical University (Harbin, China). Table 1 summarized the clinicopathologic characteristics of patients with breast tumors. Before initiation of the study, we obtained the approval of the Ethics Committee of National Research Institute for Family Planning (No. 2011–12) and informed consent from patients. Sixty pairs of human breast cancer tissues and adjoining normal tissues were collected. Partial tissues were fixed with 4% paraformaldehyde solution (Sigma–Aldrich) for in situ detection, and partial tissues were frozen in liquid nitrogen for RNA and protein analysis.

Human breast carcinoma cell lines T47D, MCF-7, MDA-MB-231 and MDA-MB-453 were acquired from Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China). These cells were cultured in Dulbecco’s Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS), 100 IU/ml penicillin and 10 mg/ml streptomycin. All cells were maintained at 37 °C and 5% carbon dioxide in a humidified atmosphere.

2.2. Plasmid construction and transfection

The Vegfa 3’-UTR were amplified from human genomic DNA by PCR using the primers in Table 2. The PCR product was double digested with Spe I and Pst I, and then cloned into pGL3 control vector (designated as Vegfa-pGL3). Mutating the binding sites of miR-185 in the 3’-UTR of Vegfa was used as control (designated as Vegfa-pGL3-Mutant). The VEGFA transcript was amplified from total mRNA of human T47D cells by RT-PCR using the primers in Table 2. The PCR product was double digested with Xba I and Sac I, and then cloned into pcAGGS-IRES-EGFP (PCA) control vector (designated PCA-Vegfa). All the constructs were verified by DNA sequencing.

Pre-miR control, miR-185 mimic, anti-miR control, miR-185 inhibitor, scramble siRNA control and Vegfa siRNA were synthesized by GenePharma (GenePharma). The sequences for scramble siRNA and Vegfa siRNA were as follows: scramble siRNA, UUCUCCGAACGUGUCACGUU; Vegfa siRNA, Sense: 5’-GGAGUACCUGUGAUCdTdT-3’; antisense: 5’-GAUCUCUACUGGGUAUCdTdT-3’.

2.3. Quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR)

Total RNAs were extracted from human breast tissues or cells using Trizol reagent (Invitrogen). The expression of miR-185 was detected by TaqMan miRNA RT-Real Time PCR. Total RNAs were reverse-transcribed by TaqMan Universal MicroRNA Reverse Transcription Kit (Applied Biosystems). The acquired single-stranded cDNA were amplified by TaqMan Universal PCR Master Mix (Applied Biosystems) and miRNA-specific TaqMan MGB probes: miR-185 and U6 (Applied Biosystems). The U6 was used for internal control. The expression of Stmn1 was detected by RT-Real-time PCR. Total RNAs were reverse-transcribed by iScript™ cDNA Synthesis Kit (Bio-Rad). The single-stranded cDNA were amplified by a FastStart Universal SYBR Green Master (Roche) with the primers in Table 2. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as control (designated GAPDH). Each sample in each group was detected in triplicate and the experiment was repeated three times.

2.4. Cell proliferation assay

In vitro cell viability was analyzed by MTS assay. MCF-7 and T47D were transfected with miR-185 inhibitor and anti-miR control, respectively. MDA-MB-231 and MDA-MB-453 was transfected with miR-185 inhibitor or anti-miR control. After 48 h of transfection,
20 μl MTT (5 mg/ml; Sigma–Aldrich) was added into each well and incubated for 4 h. The media was removed and each well was washed three times by phosphate buffered saline (PBS). 150 μl dimethyl sulfoxide (Sigma–Aldrich) was added into each well and the absorbance was measured by a 96-well plate reader (Bio-Rad 3550) at a wavelength of 570 nm. There were triplicate wells in each treatment group. The experiment was repeated for three times. The cell proliferation capacity was represented by the ratio of absorbance with miR-185 mimic or miR-185 inhibitor vs its corresponding control.

2.5. Flow cytometry analysis

Cells apoptosis was detected by flow cytometry analysis using Annexin V-FLUOS staining kit (Roche, Mannheim, Germany). Cells suspension were added with 2.5 μl annexin V-fluorescein isothiocyanate (FITC) stock and 5 μl 20 μg/ml phosphatidylserinol (PI). The mixture was incubated 15 min at room temperature in the dark. The early and late apoptotic populations were analyzed by flow cytometry (BD Biosciences). Each sample acquired 8000 events. Each treatment was detected in triplicate and the experiment was repeated for three times.

2.6. In vitro migration and invasion assays

MCF-7 and T47D were transfected with miR-185 mimic or pre-miR control, respectively. MDA-MB-231 and MDA-MB-453 was transfected with miR-185 inhibitor or anti-miR control, respectively. After 48 h of transfection, the transfected cells were harvested and subjected to the following assays. For migration assays, the transfected cells (0.5 × 106 cells/ml) were seeded in the top of an 8.0-μm-pore membrane chamber (Corning). Cells were fixed by 4% paraformaldehyde solution (Sigma–Aldrich) after 17 h of incubation. For invasion assays, matrigel (BD Biosciences) was added in the top of an 8.0-μm-pore membrane chamber (Corning) and incubated at room temperature until the matrigel solidified. Cells (0.5 × 106 cells/ml) were seeded on the top of matrigel-coated membrane. Cells were fixed by 4% paraformaldehyde solution (Sigma–Aldrich) and stained with hematoxylin and eosin (Sigma–Aldrich) after 24 h of incubation. Cells through membrane were counted under a light microscope. The amount of cells passing through the membrane from five different fields per sample at 200× selected in a random manner was used to determine the capacity of cell migration or invasion.

2.7. Dual-luciferase activity assay

T47D cells were seeded in 48-well plates at a density of 1 × 105, and then transfected by lipofectamine 2000 (Invitrogen) according to the manufacture’s instruction. pRL-TK containing Renilla luciferase was used for normalization of gene expression in transiently transfected cells. After 48 h of transfection, cells were harvested. The luciferase activity was analyzed by the dual-luciferase kit (Promega). Each treatment was detected in triplicate and the experiment was repeated for three times. The results were expressed as relative luciferase activity (Firefly LUC/Renilla LUC).

2.8. Western blot

The extracted protein samples from human breast tissues or cells (40 μg) were separated on 12% sodium dodecylsulfate (SDS) polyacrylamide gels and electrotransferred onto polyvinylidene fluoride membrane (PVDF) (Amersham Pharmacia Biotech). The membrane was incubated with primary antibodies anti-VEGFA (Cell Signaling Technology) or anti-GAPDH (Santa Cruz Biotechnolog Inc.) overnight at 4 °C. Then the membrane was incubated with horseradish peroxidase (HRP) conjugated goat anti-rabbit IgG (Jackson Immunoresearch Laboratories, Inc.). The bands were visualized with the ECL kit (Millipore). GAPDH was used as an internal control. The experiment was repeated at least three times.

2.9. Statistical analysis

All statistical analyses were performed using SPSS 16.0. All values are reported as the mean ± S.E.M. One-way analysis of variance (ANOVA) was used to perform multiple group comparisons. A linear correlation assay was performed using Pearson correlation. Differences were considered statistically significant at *P < 0.05.

3. Results

3.1. The expression profiles of miR-185 in human breast cancer tissues

To investigate the role of miR-185 in the occurrence of human breast cancer, we analyzed the expression profiles of miR-185 in 60 pairs of human breast cancer tissues and adjoining normal breast tissues by qRT-PCR (Table 1 and Fig. 1). In order to reduce individual difference of the samples, we pooled cancer tissues from two patients with similar clinical characteristics as a sample (30 samples for 60 pairs). The expression level of miR-185 was dramatically reduced in 70% (21/30 samples) of breast cancer tissues (Fig. 1A). When compared with compared with adjoining normal tissues, miR-185 level was obviously diminished in all human breast cancer tissues (*P < 0.01; Fig. 1B).

3.2. MiR-185 regulates breast cancer cells proliferation in vitro

In order to figure out the possible functions of miR-185 in the pathological progression of breast cells, we detected the effect of miR-185 on the growth of breast cancer cells. To choose appropriate cell lines to execute over-expression and knockdown of miR-185, we firstly detected the endogenous miR-185 level in MCF-7,
The proliferation rates in T47D, MDA-MB-453 and MDA-MB-231 cells by qRT-PCR. The level of miR-185 in MDA-MB-231 was higher than that in MCF-7 (P < 0.01) and T47D (P < 0.01), but approximate with that in MDA-MB-453 (Fig. S1). After that, the effect of miR-185 mimic or inhibitor on miR-185 expression in these cancer cell lines was detected by qRT-PCR. Compared with pre-miR control, miR-185 mimic markedly enhanced miR-185 level (P < 0.01), and miR-185 inhibitor obviously reduced miR-185 expression compared with pre-miR control (Fig. S2; P < 0.01).

The cell proliferation in miR-185 mimic or inhibitor-treated cells was detected by MTT assay after 48 h of transfection (Fig. 2). Compared with pre-miR control, the relative proliferation rates in miR-185 mimic-treated MCF-7 and T47D cells were respectively decreased about 15.95% and 13.75% (P < 0.05); however, the relative proliferation rates in miR-185 inhibitor-treated MDA-MB-231 and MDA-MB-453 cells were respectively increased about 21.82% and 17.5% (P < 0.05). All these facts show that over-expression of miR-185 can inhibit the proliferation of breast cancer cells, while low expression of miR-185 obviously facilitates breast cancer cells viability.

3.3. MiR-185 affects breast cancer cells apoptosis in vitro

Cell apoptosis in four breast cancer cells was detected by flow cytometry in order to further investigate the role of miR-185 in regulating the growth of breast cancer cells (Fig. 3). Compared with pre-miR control, the number of early apoptotic cells were enhanced by miR-185 mimic in T47D (3.43% vs 1.62%), MCF-7 (13.94% vs 9.43%). The number of late apoptotic cells was augmented in T47D (4.57% vs 1.60%), while low expression of miR-185 obviously facilitates breast cancer cells viability.

3.4. MiR-185 regulates the capacity of breast cancer cells migration and invasion in vitro

The influence of miR-185 on migration and invasion of breast cancer cells were detected in order to further explore the role of miR-185 in modulating the of breast cancer cells metastasis (Figs. 4 and 5). Compared with pre-miR control, miR-185 mimic dramatically decreased the capacity of cell migration in T47D and MCF-7 cells (P < 0.05). The migration capacity was significantly higher in miR-185 inhibitor-treated MDA-MB-231 and MDA-MB-453 cells than corresponding control (Fig. 4; P < 0.05). Furthermore, the capacity of invasion assay revealed that miR-185 mimic significantly inhibited the invasive capacity of MCF-7 and T47D cells compared with pre-miR control (P < 0.05). However, the invasive ability was obviously increased in miR-185 inhibitor-treated MDA-MB-231 and MDA-MB-453 cells (Fig. 5; P < 0.05). These results indicate that the expression level of miR-185 may be closely related with breast cancer cells metastasis.

3.5. Vegfa is a target gene of miR-185

To confirm whether Vegfa is target gene of miR-185, the dual-luciferase assay was used to analyze the relationship between miR-185 and human Vegfa 3′-UTR fragment containing the binding sites of miR-185 (Fig. 6A), Vegfa-pGL3 and miR-185 mimic or inhibitor were co-transfected into to T47D cells (Fig. 6B). The luciferase activity was decreased about 44.19% by miR-185 mimic (P < 0.05) compared with the pre-miR control. Additionally, the luciferase activity was increased about 88.34% by miR-185 inhibitor when compared with the anti-miR control, (P < 0.01). These findings
show that miR-185 has effects on the binding ability of miR-185 and 3'-UTR of Vegfa.

Afterwards, we conducted base mutation of seed sequence to further validate the binding sites for miR-185 (Fig. 6C). PGL3 empty vector and Vegfa-pGL3-Mutant that mutated the miR-185 binding sites in the 3'-UTR of Vegfa were used as control. Compared with Vegfa-pGL3-Mutant or pGL3 empty vector, the enzyme activity was significantly decreased in cells co-transfected with miR-185 mimic and Vegfa-pGL3 (P < 0.05). These results show that Vegfa may be a direct target gene of miR-185 that inhibits Vegfa expression through binding to seed sequence in Vegfa 3'-UTR.

3.6. MiR-185 affects the expression of Vegfa in breast cancer cells in vitro

T47D cells were transfected with miR-185 mimic or inhibitor to analyze the effects of miR-185 expression disorder on endogenous VEGFA expression. VEGFA protein level was significantly down-regulated by miR-185 mimic compared with pre-miR control (P < 0.05), while miR-185 inhibitor markedly promoted VEGFA expression (P < 0.05) (Fig. 6D). Furthermore, qRT-PCR was used to detect Vegfa mRNA level. miR-185 mimic significantly decreased Vegfa mRNA level (P < 0.05), while miR-185 inhibitor markedly increased Vegfa mRNA level (P < 0.05) (Fig. 6E). These findings show that the mRNA and protein level of endogenous Vegfa can be regulated by miR-185.

3.7. Vegfa is involved in the influence of mir-185 on cell viability and metastasis

We assessed the influences of Vegfa on miR-185-mediated cell growth and metastasis in order to further validate whether miR-185 executed tumor-suppressive functions by targeting Vegfa. The influence of Vegfa constructs on Vegfa expression was detected by qRT-PCR and Western blot. The mRNA and protein level of Vegfa was obviously increased by PCA-Vegfa (P < 0.01) and markedly reduced by Vegfa siRNA (Fig. S3; P < 0.01). Co-transfection of PCA-Vegfa and miR-185 mimic increased the ability of proliferation, migration and invasion and decreased the level of apoptosis compared with miR-185 mimic alone in T47D and MCF7 cells (Figs. 2A, 3A, 4A and 5A), implying that the up-regulation of Vegfa could partially rehabilitate the inhibition of cell proliferation and metastasis mediated by miR-185 overexpression. Additionally, Co-transfection of Vegfa siRNA and miR-185 inhibitor reduced cell proliferation, migration and invasion ability and enhanced cell apoptosis level compared miR-185 inhibitor alone in MDA-MB-
231 and MDA-MB-435 cells (Figs. 2B, 3B, 4B and 5B), displaying that miR-185 knockdown-mediated the facilitation of cell viability and metastasis was partly attenuated by down-regulation of Vegfa.

These findings reveal that miR-185 carries out functions in breast cancer cells partly by regulating Vegfa.

3.8. Vegfa expression in human breast cancer tissues

We detected the protein and mRNA level of Vegfa in breast cancer tissues by Western blot and qRT-PCR in order to further investigate the expression pattern of miR-185 target gene Vegfa in vivo, (Fig. 7). The protein level of VEGFA was dramatically enhanced in breast cancer tissues (Fig. 7A; *P < 0.05). Additionally, Vegfa mRNA level was obviously augmented in 80% (24/30 samples) of breast cancer tissues (Fig. 7B). When compared with that in adjacent normal breast tissues, the general trend of Vegfa in all human breast cancer tissues was increased (Fig. 7B1; *P < 0.05). The expression profiles of Vegfa is inverse with miR-185 in breast cancer tissues, suggesting that miR-185 may take part in the occurrence of breast cancer by targeting Vegfa in vivo.
3.9. The correlation between miR-185 and Vegfa in human breast cancer tissues

In order to further demonstrate the relationship between miR-185 and Vegfa, a linear correlation assay was carried out by SPSS16.0. Different housekeeping genes were used as control for normalization of miR-185 and Vegfa expression. In order to decrease experiment error, we adopted the ratio of miR-185 or Vegfa in breast cancer tissues vs adjacent normal breast tissues to execute the linear correlation assay. A significant inverse correlation was found between miR-185 and Vegfa on human breast cancer tissues (Fig. 8; \(P < 0.01\)). The Pearson Correlation Coefficient is \(-0.573\).

4. Discussion

In this study, we provided the first evidence of the association of miR-185 and breast cancer, although some studies had showed that
miR-185 participated in ovarian cancer, colorectal cancer, non-small cell lung cancer and so on [7–9,13,14].

In this study, we found that miR-185 was dramatically increased in breast cancers tissues when compared with that in adjoining normal tissues. It suggests that miR-185 may take part in breast carcinogenesis.

We further explore the roles of miR-185 in the occurrence of breast cancer. Over-expression of miR-185 in human breast cancer cell lines accelerated apoptosis and suppressed cells proliferation, invasion and migration, and vice versa. It had been reported that overexpression of miR-185 in ovarian cells could inhibit the growth of cells and promote cellular apoptosis [7]. The differential expression of miR-185 inhibited the growth of non-small lung cancer cells, lead to the arrest of cell cycle [9]. The roles miR-185 in breast cancer are similar with that in other cancers. All of these results imply that miR-185 may act as tumor suppressor in the occurrence of cancer.

It is generally accepted that miRNAs carry out their function by modulating the expression of their target genes. Vegfa is a member of the Vegf family, which is the most critical driver of vascular formation and required to initiate the formation of immature vessels in tumor [15–17]. The expression of Vegf was up-regulated in human breast tumors tissues and negatively influenced survival [10,11]. Overexpression of Vegf dramatically reduced tumor uptake and enhanced tumor growth in a murine model of breast cancer [16]. Vegf could prevent apoptosis induced by serum starvation and induce expression of the anti-apoptotic proteins Bcl-2 and A1 [18,19]. All these facts indicate that Vegfa may be closely related with the occurrence of breast tumor.

In this study, we explored the target genes of miR-185 and found a conservative seed sequence in the 3′-UTR of Vegfa predicted by miranda and Targetscan. miR-185 mimic and inhibitor regulated Vegfa 3′-UTR-mediated luciferase activity. Mutating seed sequence in the 3′-UTR of Vegfa obviously suppressed the binding
miR-185 and 3'-UTR of Vegfa. Additionally, up-regulation of miR-185 decreased the expression of Vegfa and down-regulation of miR-185 increased the protein and mRNA level of Vegfa in vitro. A significant inverse correlation was also found between miR-185 and Vegfa in human breast cancer tissues. Furthermore, the up-regulation of Vegfa could partially regain the overexpression of miR-185-mediated the suppression of cell growth and metastasis. The influence of knockdown of miR-185 on cell growth and metastasis was weakened down-regulation of Vegfa. Therefore, we confirm that Vegfa is the target gene of miR-185 and miR-185 may execute its tumor suppressor function partially by targeting Vegfa.

Divertingly, our results indicated that miR-185 level in breast cancer cells and breast cancer tissues was all oppositely related with the Vegfa mRNA level. Usually, miRNAs were supposed to exert function by inhibiting mRNA translation [20]. However, recent reports indicated that the alteration in protein level mediated by a miRNA were usually related with the changes of mRNA level, indicating that mRNA degradation might be a main element of mammalian miRNA suppression [21,22]. Our findings were also consistent with this reports.
Because a specific miRNA usually targets to multiple genes, miR-185 exert its function may get through synergistic action of its target genes. In the future, besides Vegfa, other targets of miR-
185 still require to be confirmed in breast cancer. Additionally, animal experiment may be needed in order to further validate the function of miR-185 and the relationship between miR-185 and Vegfa in vivo.

In conclusion, miR-185 is obviously diminished in human breast cancer tissues, which facilitates breast cancer cell proliferation, invasion and migration and suppresses cell apoptosis. MiR-185 excerts these functions in breast cancer cell lines partly by regulat-
ing Vegfa. Our data will not only show the potential roles of miR-
185 in breast carcinogenesis, but also provide a clue for diagnosis and therapeutics of breast cancer.

Conflict of interest statement

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2014.09.045.

References