RESEARCH ARTICLE

Expression and regulatory function of miRNA-182 in triple-negative breast cancer cells through its targeting of profilin 1

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Abstract We aimed to evaluate the expression of microRNA-182 (miR-182) in triple-negative breast cancer (TNBC) tissues and the TNBC cell line MDA-MB-231 and to investigate the effects of mirR-182 on the cellular behavior of MDA-MB-231 and the expression of the target gene profilin 1 (PFN1), thus providing new methods and new strategies for the treatment of TNBC. Quantitative real-time PCR (qRT-PCR) was utilized to evaluate the expression of miR-182 in TNBC tissues, relatively normal tissues adjacent to TNBC and the TNBC cell line MDA-MB-231. Forty-eight hours after the MDA-MB-231 cells were transfected with the miR-182 inhibitor, qRT-PCR was utilized to detect the changes in miR-182 expression levels, and an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was utilized to determine the effects of miR-182 on cell viability. Flow cytometry was adopted to determine whether miR-182 affects the proliferation rates and apoptosis levels of the MDA-MB-231 cells. The transwell migration assay method was used to investigate the effects of miR-182 on the migration of the MDA-MB-231 cells. A luciferase reporter gene system was applied to validate that PFN1 was the target gene of miR-182. Western blot was used to measure the effects of miR-182 on the PFN1 protein expression levels in the cells. qRT-PCR results showed that compared with the relatively normal tissues adjacent to TNBC, miR-182 expression was significantly increased in the TNBC tissues and the MDA-MB-231 cells ($p<0.01$). Compared with the control group, MDA-MB-231 cells transfected with the miR-182 inhibitor and incubated for 48 h showed significantly decreased miR-182 expression ($p<0.01$). The results of an MTT assay showed that inhibition of miR-182 in MDA-MB-231 cells led to significantly reduced cell viability ($p<0.05$). Flow cytometry analysis indicated that inhibition of miR-182 expression resulted in significantly decreased cell proliferation ($p<0.05$) and significantly increased levels of apoptosis ($p<0.05$). The results of a transwell migration assay showed that after inhibited of miR-182 expression, the number of cells passing through the transwell membranes was significantly decreased ($p<0.05$). The results from a luciferase reporter gene system showed that compared with the control group, the relative luciferase activity of the group transfected with the miR-182 inhibitor was significantly increased ($p<0.05$). Western blot analysis showed that compared with the control group, PFN1 protein expression levels were significantly increased in the MDA-MB-231 cells transfected with the miR-182 inhibitor and incubated for 48 h ($p<0.05$). In conclusion, miR-182 is upregulated in TNBC tissues and cells. It promotes the proliferation and invasion of MDA-MB-231 cells and could negatively regulate PFN1 protein expression. Treatment strategies utilizing inhibition of miR-182 expression or overexpression of the *PFN1* gene might benefit patients with TNBC.

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Introduction

Triple-negative breast cancer (TNBC) specifically refers to any breast cancer tumor cells that do not express the estrogen receptor (ER), the progesterone receptor (PR), or epidermal growth factor receptor 2 (HER2) [1–3]. Patients with this type of breast cancer have similar characteristics, very high homology, and poor tissue differentiation. Most TNBC tumors are basal-like breast cancers [4]. TNBC accounts for approximately 10–20.8 % of patients with breast cancer and is most commonly found in young women. The prognosis for patients with TNBC is very poor, with the 5-year survival rate less than 15 % in certain populations [2, 5]. The pathological characteristics of TNBC include high histological grade, strong invasiveness, and often high expression of basal cytokeratin, C-Kit (v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog), epidermal growth factor receptor (EGFR), and CKS/6 [6]. Due to an absence of targets for endocrine and anti-HER2 therapies, these patients tend to have unsatisfactory treatment outcomes for endocrine therapies and targeted therapies, such as trastuzumab monoclonal antibody therapy. Currently, the systematic treatment approaches utilized in patients with TNBC rely solely on chemotherapy. Even for patients who are responsive to chemotherapy, the remission period is relatively short, and recurrence is expected within a short period of time [7, 8]. Due to the biological characteristics of TNBC itself, as well as the lack of effective systematic treatment approaches, the prognosis of TNBC is extremely poor, the risk of recurrence within 3 years is significantly higher than those with non-TNBC, and distant metastases occur rapidly in many patients, leading to deaths. Therefore, investigation of the pathogenesis of TNBC, identification of the indicators for susceptible population screening and early diagnosis, and the subsequent development of new therapeutic targets are all urgent necessities for the current TNBC field.

microRNAs (miRNAs) have been a major focus of tumor research in recent years [9, 10]. miRNAs are a type of small non-coding RNAs of 18-25 nucleotides in length and play important roles in a variety of evolutionary processes [11]. In animal cells, mature miRNAs, together with proteins, form the RNA-induced silencing complex (RISC) and bind to the 3′ untranslated region (3′UTR) of target gene mRNAs, subsequently leading to the degradation of target mRNAs or inhibition of their translation [12]. A single miRNA may have hundreds or thousands of target mRNAs, which allows these small RNAs to either significantly or slightly decrease the expression of a whole range of genes, depending on the characteristics of the target sites in the 3′UTR [13, 14]. These small RNA molecules with regulatory functions have been shown to be involved in the biological processes of cancer [15]. Researchers have found that many miRNAs are encoded in cancer-related gene regions, suggesting that changes in miRNA expression might have a causal relationship with tumorigenesis [16, 17].

miRNA-182 is a relatively new member of the miRNA family, as it was first identified in 2003, and it contains a 24-bp sequence. It is involved in the regulation of tumor proliferation and apoptosis and behaves like an oncogene [18, 19]. Aberrant expression of miRNA-182 can be detected in patients with a variety of tumors. Segura et al. utilized a luciferase reporter gene assay to confirm that forkhead box O3a (FOXO3a) was a direct target gene of miR-182. The authors also found that abnormally expressed miR-182 in multiple melanoma cell lines could accelerate tumor metastasis, whereas blocking miR-182 using antisense oligonucleotides resulted in decreased tumor migration, as indicated by a scratch wound healing experiment. Over time, miR-182 knockdown led to increased tumor cell apoptosis in a dose-dependent manner [20]. However, the relationship between miR-182 and TNBC has not been reported.

Profilin (PFN) was one of the first actin-binding proteins to be discovered and is found in all identified eukaryotic cells [21]. Previous studies have found that PFN plays important roles in a number of cellular processes, including membrane transport, GTPase signaling pathways, RNA splicing, pathogenesis of neurological diseases, and tumorigenesis [22]. PFN expression is decreased in metastatic tumor cells, and PFN overexpression in these cells can reduce the tumorigenicity of these cells, indicating that it might be a tumor suppressor protein. Decreased PFN expression might be associated with the pathogenic mechanisms of cancer, and overexpression of PFN might be an effective strategy for the inhibition of cancer cell migration [23]. Our previous studies utilized mass spectrometry to screen for proteins differentially expressed in TNBC tissue and the adjacent relatively normal breast tissues and had revealed that PFN was one of the proteins that was characterized by a relatively large expression difference.

In this study, we examined the expression of miR-182 in TNBC tissues and MDA-MB-231 cells. We then further investigated the effects of miR-182 on the expression of the target gene PFN1, as well as on the cell biological characteristics of MDA-MB-231 cells, thus providing new methods and strategies for the treatment of TNBC.

Materials and methods

Major reagents

The TNBC tissues and adjacent relatively normal tissues were collected from fresh specimens surgically resected from TNBC patients admitted to our hospital and were immediately placed in liquid nitrogen for storage until use. None of the patients had chemotherapy, radiotherapy, or other treatment history before the surgery, and the patients were not afflicted with other inflammatory diseases. Surgically resected TNBC tissue and adjacent relatively normal breast tissue (more than 5 cm away from the cancer tissue) were all verified by the Pathology Department of our hospital.

The TNBC cell line MDA-MB-231 was purchased from the American Type Culture Collection (ATCC). Fetal bovine serum (FBS), DMEM/F12 medium, L-glutamine, HEPES, and Lipofectamine™ 2000 were all purchased from Invitrogen Co. (USA). The cell culture plates or Petri dishes and the transwell invasion chambers were purchased from Corning Co.

The TaqMan miRNA Isolation Kit, TaqMan microRNA Assay Kit, and TaqMan Universal PCR Master Mix were purchased from Applied Biosystems. The miR-182 inhibitor (miRIDIAN microRNA hairpin inhibitor) and negative control (a non-targeting sequence) were purchased from Dharmacon.

Trypsin, phosphate-buffered solution (PBS), and MTT (3-(4,5)-dimethylthiahiazo(-z-y1)-3,5-di-phenytetrazoliumromide) were purchased from Sigma-Aldrich Co. (USA). Matrigel was purchased from BD Biosciences. Annexin V and propidium iodide (PI) were purchased from Roche. The primary antibodies consisting of rabbit anti-human PFN1 polyclonal antibody and mouse anti-human β-actin monoclonal antibody were purchased from Abcam (UK). The secondary antibodies consisting of IRDye 800-conjugated affinity purified goat anti-mouse IgG and IRDye 800-conjugated affinity purified goat anti-rabbit IgG were purchased from LI-COR. The protein extraction and quantification kits were purchased from Bio-Rad.

Detection of miR-182 expression in the TNBC tissues and MDA-MB-231 cells

The TNBC cell line MDA-MB-231 was cultured in DMEM/F12 medium containing 10 % FBS at 37 °C, 5 % CO_2, 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.

MDA-MB-231 cells cultured in vitro and the TNBC tissues and adjacent relatively normal breast tissues stored in liquid nitrogen were collected, and RNA was extracted using the TaqMan microRNA Isolation Kit. The expression of mature miR-182 was detected using the TaqMan miRNA Assay and the TaqMan Universal PCR Master Mix with U6 as the internal reference gene. Three duplicated wells were set up for all reactions. The CT (cycle threshold) value of the specimens in each reaction tube was recorded, and the experimental results were analyzed using the qRT-PCR relative quantification method. $2^{-\Delta\Delta CT}$ represented the fold change of the miR-182 expression level in MDA-MB-231 cells or TNBC tissues compared to that of adjacent relatively normal breast tissue (normal tissue), and $\Delta\Delta CT = (CT_{182} - CT_{U6})_{TNBC} - (CT_{182} - CT_{U6})_{normal\ tissue}$.

Detection of the effects of miR-182 inhibitor transfection on miR-182 expression in MDA-MB-231 cells

MDA-MB-231 cells were evenly seeded in 6-well plates at a concentration of 3×10^5 cells/ml in a volume of 1,000 μl per well. After the cells adhered, the transfection of the miR-182 inhibitor and negative control was performed according to the manufacturer's instructions using Lipofectamine™ 2000. An untransfected control group (MDA-MB-231) was set up at the same time. The miR-182 inhibitor and negative control were diluted in serum-free DMEM/F12 medium. The liposome Lipofectamine™ 2000 was then diluted in the DMEM/F12 medium, mixed gently, and incubated for 5 min at room temperature. The diluted Lipofectamine™ 2000 was then mixed with the diluted miR-182 inhibitor or the negative control, mixed gently, and incubated for 20 min at room temperature to form the transfection complexes, which were added to the culture plates containing the MDA-MB-231 cells and mixed. The cells were placed in incubators at 37 °C and with a CO_2 volume fraction of 5 %. The culture medium was replaced with DMEM/F12 medium containing 10 % FBS 5 h later, and the cells were incubated another 48 h. The miRNA Isolation Kit was used to extract the RNA from each group of cells, and qRT-PCR was conducted to detect the changes in miR-182 expression for each group of MDA-MB-231 cells.

Effects of miR-182 on cell viability using MTT assay

MDA-MB-231 cells were uniformly inoculated into 96-well culture plates at a concentration of 3×10^5 cells/ml in a volume of 100 μl per well. The transfection of the miR-182 inhibitor and negative control 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 µl 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_2. In each well, 100 µl of 20 % SDS (50 % dimethyl formamide as the co-solvent) was added, and the reaction continued for 24 h at 37 °C. The OD value at 570 nm was measured using a microplate reader (Bio-Tek). Each experimental group contained ten duplicated wells, and the experiment was repeated three times.

Effects of miR-182 on cell proliferation, as detected by flow cytometry

MDA-MB-231 cells were uniformly inoculated into 6-well culture plates at a concentration of 3×10^5 cells/ml in a volume of 1,000 µl per well. The transfection of the miR-182 inhibitor and negative control 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, the cells were washed with PBS 1-2 times, digested with trypsin, washed twice with PBS, and collected by centrifugation. The PI staining solution was added, and cells were labeled in the dark for 30 min at 4 °C. After filtration using mesh filters, the cells were analyzed by flow cytometry (BD Biosciences, USA). The FCM CellQuest software was used to count the cells, and the Macquit software was used to analyze the data.

Effects of miR-182 on cell apoptosis, as detected by flow cytometry

Forty-eight hours after the MDA-MB-231 cells were transfected with the miR-182 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). The FCM CellQuest software was used to count the cells, and the Macquit software was used to analyze the data.

Effects of miR-182 on cell invasion using transwell invasion chambers

After the MDA-MB-231 cells were transfected with the miR-182 inhibitor and negative control for 24 h, the cells were seeded into transwell chambers and cultured under normal conditions for 24 h. The cells were then washed one to two times with PBS and stained with 0.1 % crystal violet. The number of cells passing through the polycarbonate membrane of the transwell chamber, which were considered to be the invasive cells, was

counted under a Leica microscope. Eight fields were randomly observed.

Verification of PFN1 as a miR-182 target gene by the luciferase reporter gene system

The luciferase reporter plasmid was constructed as follows: a fragment containing the target gene *PFN1* 3′UTR sequence that complementarily binds to the corresponding miR-182 was chemical synthesized, and the *PFN1* 3′UTR fragment containing the predicted miR-182 binding sites was cloned into the Xba I site of the pGL3-Luciferase vector.

MDA-MB-231 cells were uniformly inoculated into 6-well culture plates at a concentration of 3×10^5 cells/ml in a volume of 1,000 µl per well. The cells were cotransfected with the luciferase plasmid containing the *PFN1* 3′UTR, as well as the miR-182 inhibitor or the negative control. The untransfected control group (MDA-MB-231) was set up at the same time. The cells were collected 48 h after transfection, and a luciferase detection kit allowed for used for the detection of the reporter gene activity using a microplate reader.

Western blot detection of the effects of miR-182 on PFN1 expression

MDA-MB-231 cells cultured normally were uniformly inoculated into 6-well culture plates at a concentration of 3×10^5 cells/ml in a volume of 1,000 µl per well. The transfection of the miR-182 inhibitor and the negative control was performed according to the manufacturer's instructions using Lipofectamine™ 2000. The untransfected control group (MDA-MB-231) was set up at the same time. Forty-eight hours after transfection, Western blotting was used to measure the PFN1 protein expression levels in the cells.

Each well of the 6-well cell culture plates was lysed in 1 ml of Radio-Immunoprecipitation Assay (RIPA) lysis buffer [150 mM NaCl, 1 % NP40, 0.5 % sodium deoxycholate, 0.1 % SDS, 50 mM Tris (pH7.9), 10 mM NaF, 10 mM PMSF, and $1 \times$ protease inhibitors (Complete Protease Inhibitor Cocktail tablets, Roche)], and the cell lysates were transferred to 1.5-ml centrifuge tubes. After 30 min of centrifugation at $16,000 \times g$, the supernatant was collected, and the supernatant protein concentration was measured using the bicinchoninic acid (BCA) method. A 5 % stacking gel and 15 % separation gel was cast, and each lane was loaded with 50 µg protein, followed by electrophoretic separation. Proteins were wet transferred to PVDF membrane (Bio-Rad, USA), and the membrane was blocked in TBST (10 mM Tris-HCl, pH7.5, 150 mM NaCl, and 0.1 % Tween-20) containing 5 % non-fat milk at room temperature for 1 h. Subsequently, a rabbit anti-human PFN1 polyclonal antibody (1:500 dilution) and a mouse anti-human β-actin monoclonal antibody (1:1,000

dilution) were added, followed by overnight incubation at 4 °C. The corresponding IRDye 800-labeled secondary antibodies (1:2000 diluted in PBS) were added, followed by overnight incubation at 4 °C. After washing with TBST, the membrane was scanned using the Odyssey Infrared Imaging System (Rockland). The relative levels of PFN1 were represented by a PFN1/β-actin grayscale ratio, and the grayscale was analyzed using the QuantityOne software (Bio-Rad, USA).

Statistical analyses

The SPSS 17.0 statistical analysis software was used to statistically process the experimental data. Student's t test was applied for the comparison of two groups, and analysis of variance (ANOVA) was applied for the comparison of two or more sets of data. p values less than 0.05 indicated that the differences were statistically significant.

Results

Detection of miR-182 expression levels in TNBC tissues and MDA-MB-231 cells

Using qRT-PCR, we found that miR-182 expression levels in MDA-MB-231 cells and TNBC tissues were significantly higher than those in the adjacent relatively normal breast tissues ($p < 0.01$; Fig. 1).

Effects of transfecting MDA-MB-231 cells with the miR-182 inhibitor on miR-182 expression

Transfection of MDA-MB-231 cells with the miR-182 inhibitor inhibited the expression of miR-182. Using qRT-
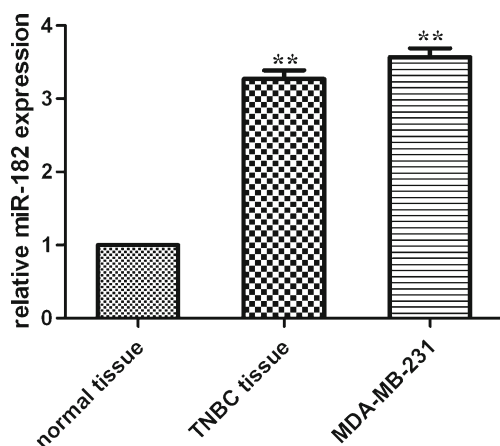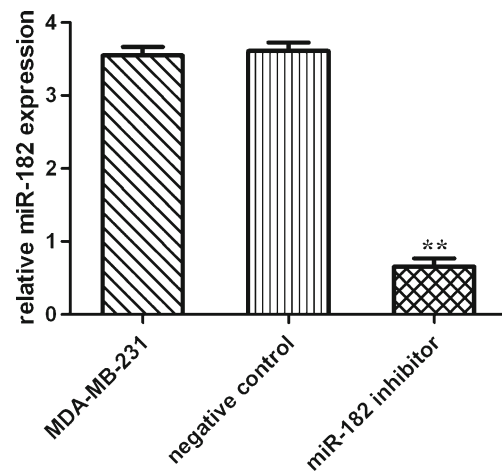

Fig. 2 Effects of transfecting MDA-MB-231 cells with the miR-182 inhibitor on miR-182 expression. **p<0.01 versus normal group or negative control group

PCR, we showed that the miR-182 expression levels in the miR-182 inhibitor transfection group were significantly lower than those of the untransfected control group (MDA-MB-231; $p < 0.01$) and negative control group (negative control; $p < 0.01$; Fig. 2).

Effects of miR-182 on cell viability, as detected by MTT assay

Using an MTT assay, we found that the cell viability of the miR-182 inhibitor transfection group was lower than that of the untransfected control group (MDA-MB-231; $p < 0.05$) and the negative control group (negative control; $p < 0.05$), suggesting that inhibition of miR-182 expression reduced MDA-MB-231 cell viability (Fig. 3).
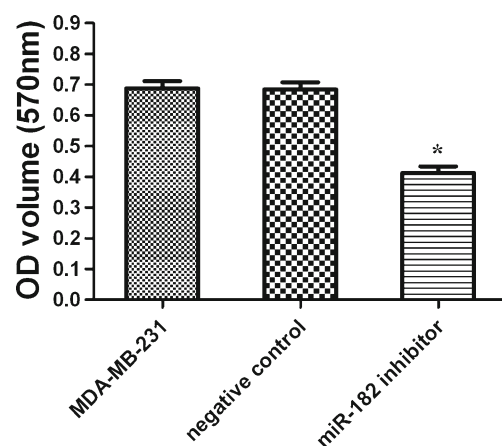

Fig. 1 miR-182 expression in the MDA-MB-231 cells, TNBC tissues, and adjacent relatively normal breast tissues. *p<0.01 versus normal tissue group

Fig. 3 MTT statistical results demonstrating the effects of miR-182 on MDA-MB-231 cell viability. *p<0.05 versus normal group or negative control group

Detection of the effects of miR-182 on cell proliferation using flow cytometry

We used the following equation to quantify proliferating cells: proliferation index $(PI)=(S+G2M)/(G0G1+S+G2M)$. Our flow cytometry analysis demonstrated that the PI of the miR-182 inhibitor transfection group was lower than that of the untransfected control group (MDA-MB-231; $p<0.05$) and the negative control group (negative control; $p<0.05$; Fig. 4).

Detection of the effects of miR-182 on apoptosis using low cytometry

Our flow cytometry analysis indicated that the percentage of apoptotic cells was significantly higher in the miR-182 inhibitor transfection group than in the untransfected control group (MDA-MB-231; $p<0.05$) or the negative control group (negative control; $p<0.05$; Fig. 5). These results suggest that inhibition of miR-182 expression promotes apoptosis.

Effects of miR-182 on cell invasion, as determined by the transwell migration assay

After transfection with the miR-182 inhibitor, the number of MDA-MB-231 cells passing through the transwell chambers was significantly reduced ($p<0.05$), suggesting that transfection of the miR-182 inhibitor could inhibit cell invasiveness (Fig. 6).

Verification of PFN1 as a target gene of miR-182 using the luciferase reporter gene system

To verify that the predicted target site of miR-182 is located in the *PFN1* 3′UTR, we constructed a luciferase plasmid containing the *PFN1* 3′UTR and co-transfected the plasmid into MDA-MB-231 cells together with the miR-182 inhibitor
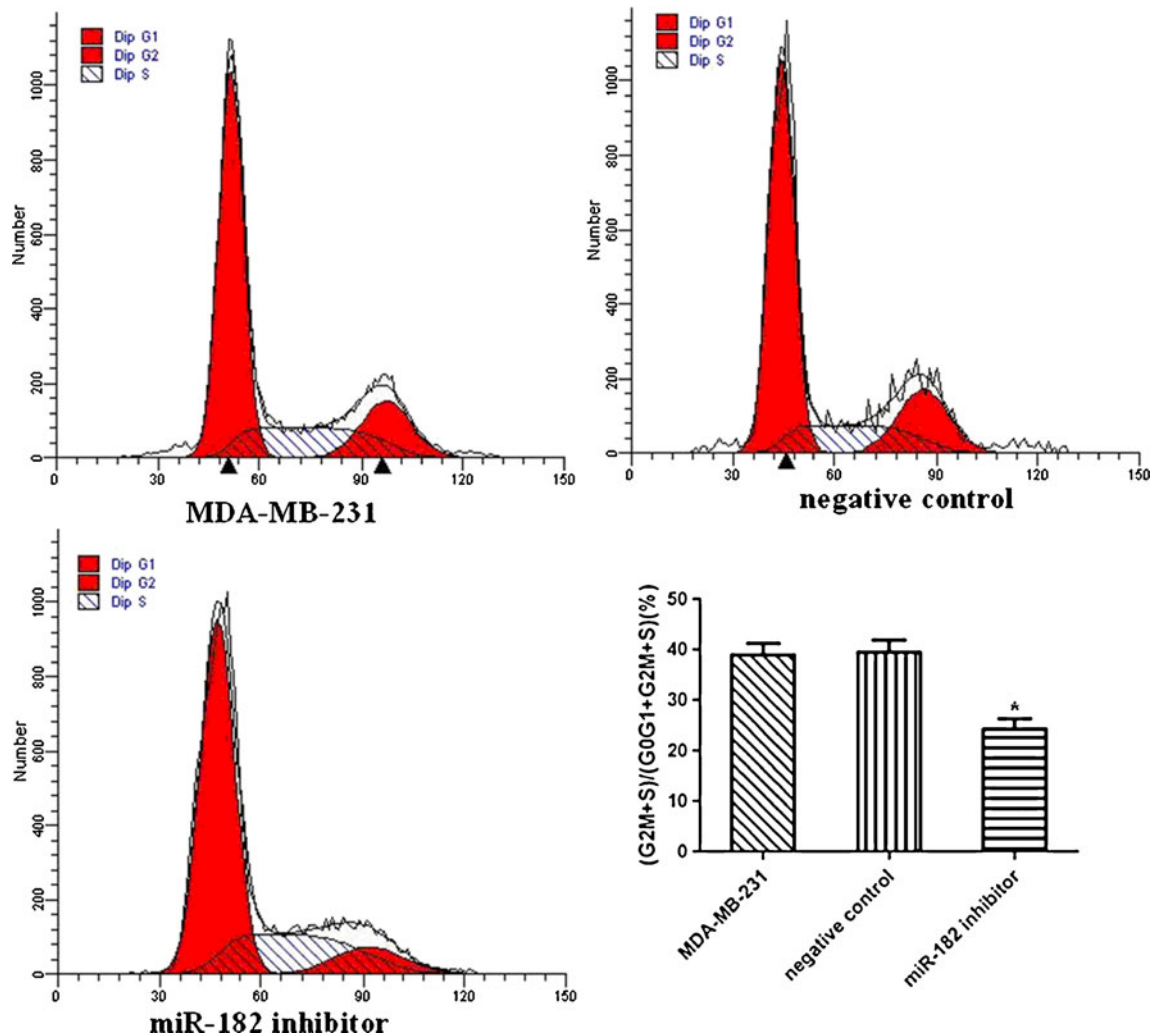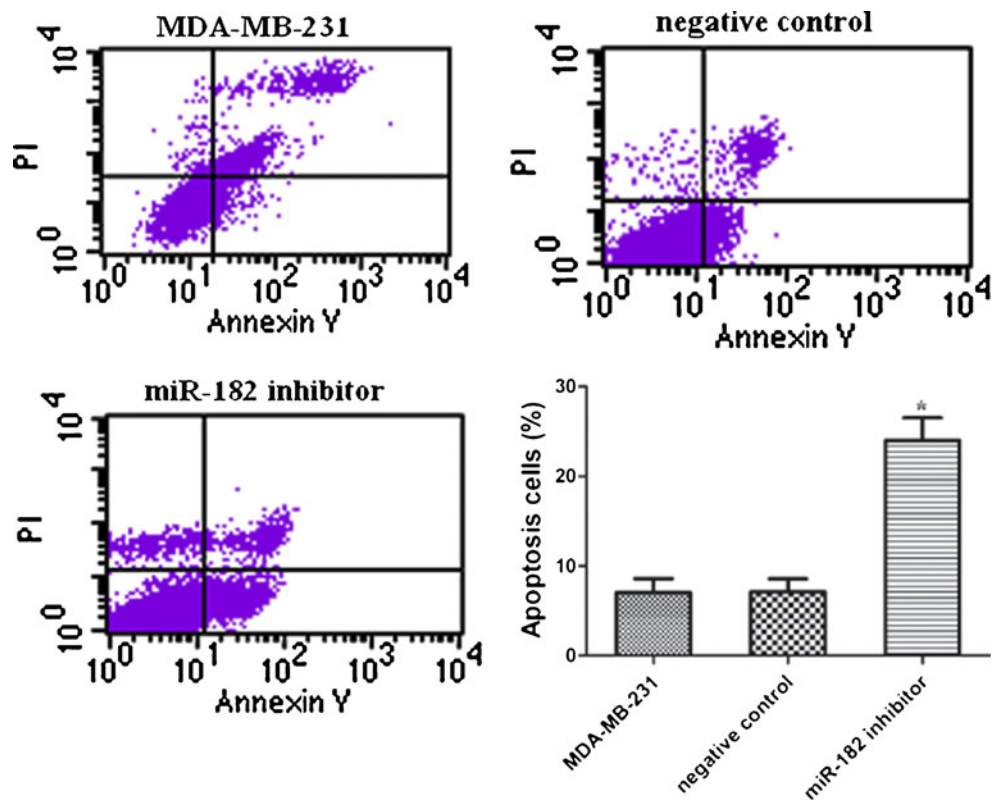

Fig. 4 Flow cytometry analysis results of the effects of miR-182 on cell proliferation. *$p<0.05$ versus normal group or negative control group
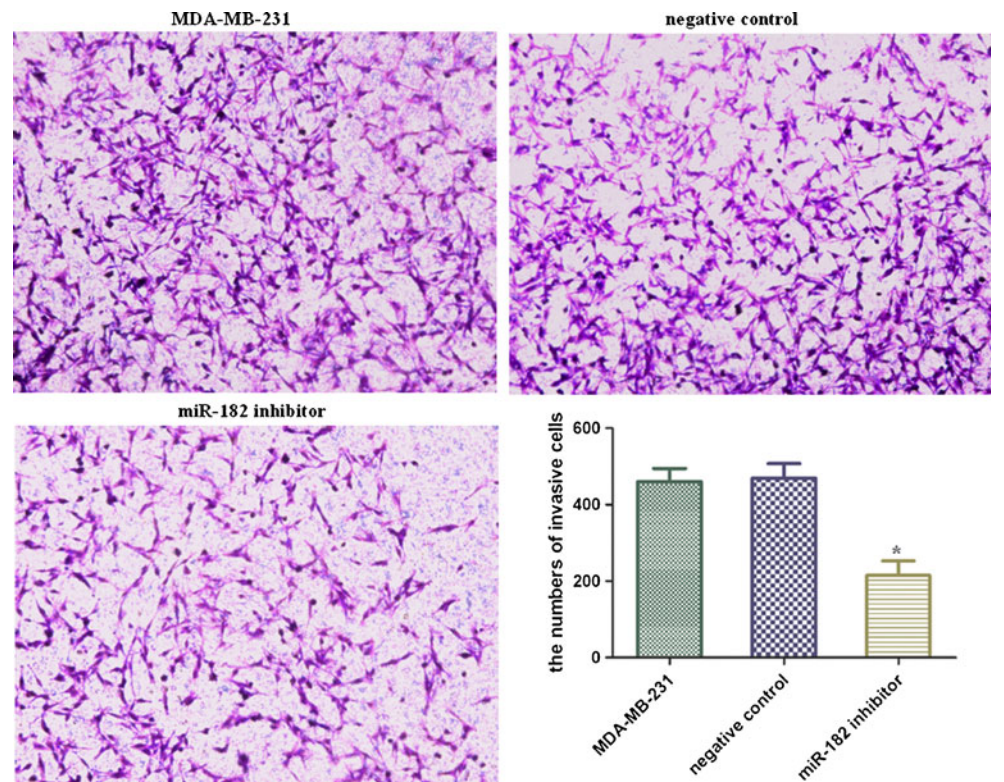
Fig. 5 Flow cytometry analysis results of the effects of miR-182 on apoptosis. *$p<0.05$ versus normal group or negative control group

or negative control. Compared with the negative control group or the untransfected control group (MDA-MB-231), the luciferase activity in the miR-182 inhibitor transfection group was significantly increased ($p<0.05$; Fig. 7). These result argue that miR-182 directly acts on the predicted target site located in the PFN1 3′UTR.

Fig. 6 Results of the transwell migration assay analyzing the effects of miR-182 on cell invasion. *$p<0.05$ versus normal group or negative control group
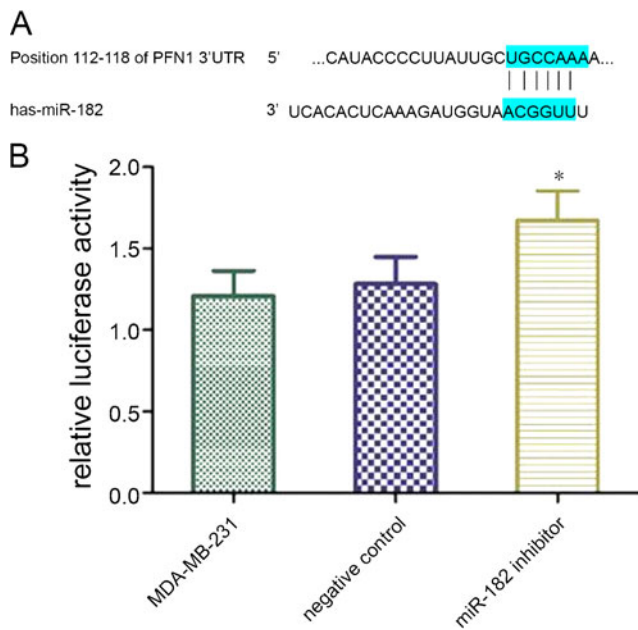
Fig. 7 **a** Schematic representation of the *PFN1* 3′UTR showing the putative miR-182 target site. **b** Statistical results of the relative luciferase activities of each group. *$p < 0.05$ versus normal group or negative control group

Western blot detection of the effects of miR-182 on PFN1 expression

Western blot analysis of PFN1 protein expression levels showed that PFN1 levels in the miR-182 inhibitor transfection group was significantly higher than those in the untransfected control group ($p < 0.05$) or the negative control group ($p < 0.05$; Fig. 8). These results indicate that inhibition of miR-182 expression can result in higher PFN1 levels.

Discussion

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 [24–26]. miRNAs act as "hackers" in the field of genetic research, as they regulate up to 30 % of the protein-coding genes in humans. Numerous studies have demonstrated that miRNA abnormalities are closely related to the tumorigenesis of multiple types of tumors [27, 28].

Carcinogenic miRNAs are often overexpressed in tumors and are termed "oncogenic" because they play similar roles as oncogenes during the process of cancer development. For example, overexpression of miR-21, miR-10b, miR-373, and miR-520 in tumor cells induces tumor invasion and metastasis [29]. In addition, the expression of tumor-suppressing miRNAs is often downregulated in tumors.
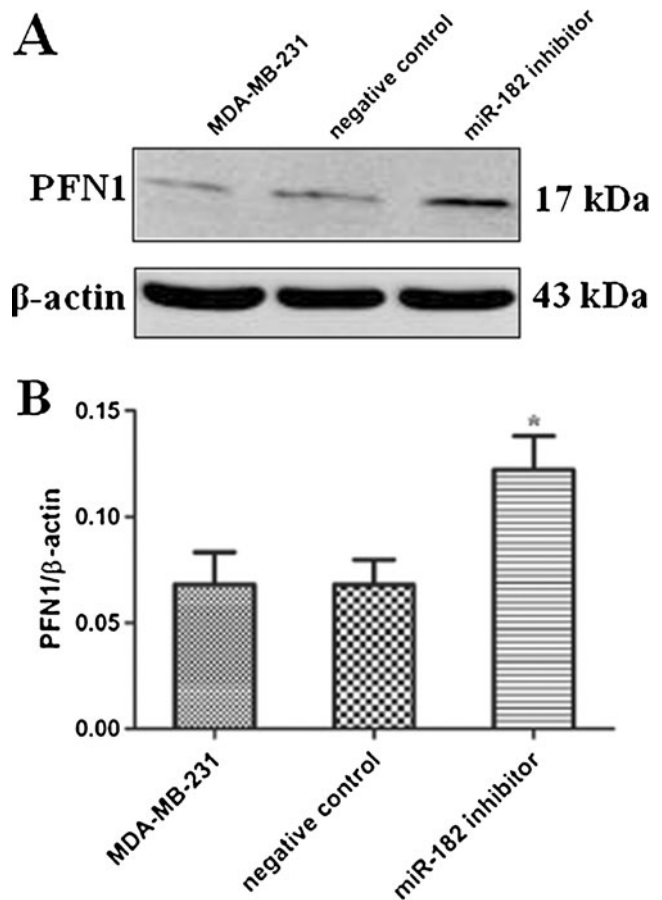

Fig. 8 **a** Western blot analysis of PFN1 protein levels in each group of MDA-MB-231 cells. **b** Changes in the relative PFN1 protein expression levels in each group of MDA-MB-231 cells. *$p < 0.05$ versus normal group or negative control group

These miRNAs are called tumor suppressor miRNAs because they function similarly to tumor suppressor genes. For example, downregulation of miR-126*, miR-335, miR146a, and miR-29c expression in tumor cells results in tumor occurrence, development, invasion, and metastasis [10].

Previous studies have shown that miR-182 functions as an oncogene and is associated with DNA damage repair, thus regulating the apoptosis and proliferation of cells. It is also involved in the regulation of the WNT signaling pathway and the p53 signaling pathway. The corresponding target genes of miR-182 that have been identified include forkhead box protein O1 (FOXO1), regulator of G-protein signaling 17 (RGS17), microphthalmia-associated transcription factor (MITF), and forkhead box protein O3 (FOXO3). Abnormal miR-182 expression has been detected in patients with a variety of tumors and has been confirmed to be associated with the tumorigenesis of numerous types of cancers, such as colon cancer, endometrial cancer, lung cancer, ovarian cancer, prostate cancer, medulloblastoma, malignant mesothelial tumor, and lupus. Additionally, abnormal upregulation of miR-182 can also be detected in the

sera of patients with blood disorders, including lymphoma, polycythemia vera, and myelofibrosis [30, 31]. Consistent with previous studies, in the present study, our qRT-PCR results demonstrated that the miR-182 expression levels in the MDA-MB-231 cells and TNBC tissues were significantly higher than those in the adjacent relatively normal breast tissues ($p < 0.01$). Our results suggest that miR-182 is upregulated in TNBC and might act as an oncogene in these tumors.

miR-182 regulates the apoptosis and proliferation of cells by functioning in the regulation of multiple intracellular signaling pathways [32]. Weeraratne et al. revealed that miR-182 regulated medulloblastoma survival, proliferation, and migration [33]. Kong et al. discovered that miR-182 inhibits the growth of human gastric cancer cells by regulating the target gene cAMP-responsive element-binding protein 1 (CREB1) [34]. Poell et al. demonstrated that miR-182 could inhibit the activity of human melanoma cells [35]. In this study, we utilized MTT assays, flow cytometry analyses, and transwell migration assays to determine the effects of miR-182 on MDA-MB-231 cells. Inhibition of the miR-182 expression inhibited cell viability, proliferation, and invasion, while it induced apoptosis. The results of this study are consistent with the previously reported effects of miR-182 in other types of tumors.

PFN1 is a small 12–15-kD protein that is expressed in all eukaryotic cells and plays important roles in normal cellular functions. It binds to the actin monomer at a ratio of 1:1 to form the profilin–actin complex that regulates signaling-dependent actin polymerization [36]. It has been revealed that PFN1 functions via the actin-associated cytoskeleton to participate in a variety of cellular functions, including cell adhesion and motility, growth and division, signal transduction and formation, and maintenance of actin-binding protein-dependent cell morphology [37]. Wang et al. demonstrated that miR-182 directly regulates PFN1 expression, thus affecting cell proliferation and migration [38]. Zou et al. showed that overexpression of PFN1 can inhibit the proliferation of MDA-MB-231 cells [39]. In the present study, we confirmed using a luciferase reporter gene system that PFN1 is a target gene of miR-182, while our Western blot results revealed that inhibition of miR-182 expression promotes PFN1 protein expression. Our results are consistent with the previous studies. Therefore, we argue that miR-182 affects MDA-MB-231 cell viability, proliferation, invasion, and apoptosis by regulating the expression levels of the PFN1 protein.

In summary, miR-182 expression was upregulated in TNBC tissues and cells, and miR-182 can negatively regulate PFN1 protein expression and inhibit the proliferation and invasion of MDA-MB-231 cells. Treatment strategies based on the inhibition of miR-182 expression or the overexpression of the *PFN1* gene are very promising and might greatly benefit TNBC patients.

Conflicts of interest   The authors have no commercial, proprietary, or financial interest in the products or companies described in this article.

References

1. Ishikawa T, Shimizu D, Kito A, Ota I, Sasaki T, Tanabe M, et al. Breast cancer manifested by hematologic disorders. J Thorac Dis. 2012;4(6):650–4.
2. Arnedos M, Weigelt B, Reis-Filho JS. Anti-HER2 therapies: when more is more. Transl Cancer Res. 2012;1(1):49–54.
3. Fausto P, Sandro B. Benefit of tamoxifen in estrogen receptor positive DCIS of the breast. Gland Surg. 2012;1(1):3–4.
4. Rashid OM, Takabe K. The evolution of the role of surgery in the management of breast cancer lung metastasis. J Thorac Dis. 2012;4(4):420–4.
5. Nakhlis F, Golshan M. Bevacizumab: where do we go from here in breast cancer? Transl Cancer Res. 2012;1(1):55–6.
6. Hartman AR, Kaldate RR, Sailer LM, Painter L, Grier CE, Endsley RR, et al. Prevalence of BRCA mutations in an unselected population of triple-negative breast cancer. Cancer. 2012;118(11):2787–95.
7. Gholami S, Chen CH, Lou E, De Brot M, Fujisawa S, Chen NG, et al. Vaccinia Virus GLV-1h153 is effective in treating and preventing metastatic triple-negative breast cancer. Ann Surg. 2012;256(3):437–45.
8. Gelmon K, Dent R, Mackey JR, Laing K, McLeod D, Verma S. Targeting triple-negative breast cancer: optimising therapeutic outcomes. Ann Oncol. 2012;23(9):2223–34.
9. Patnaik SK, Mallick R, Yendamuri S. MicroRNAs and esophageal cancer. J Gastrointest Oncol. 2010;1(1):55–63.
10. Link A, Kupcinskas J, Wex T, Malfertheiner P. Macro-role of microRNA in gastric cancer. Dig Dis. 2012;30(3):255–67.
11. Santovito D, Mezzetti A, Cipollone F. MicroRNAs and atherosclerosis: new actors for an old movie. Nutr Metab Cardiovasc Dis. 2012;22(11):937–43.
12. Pasquinelli AE. MicroRNAs and their targets: recognition, regulation and an emerging reciprocal relationship. Nat Rev Genet. 2012;13(4):271–82.
13. Ui-Tei K, Nishi K, Takahashi T, Nagasawa T. Thermodynamic control of small RNA-mediated gene silencing. Front Genet. 2012;3:101.
14. Yang JS, Maurin T, Lai EC. Functional parameters of dicer-independent microRNA biogenesis. RNA. 2012;18(5):945–57.
15. Chen J, Wang B. The roles of miRNA-143 in colon cancer and therapeutic implications. Transl Gastrointest Cancer. 2012;1(2):169–74.
16. Du L, Pertsemlidis A. microRNA regulation of cell viability and drug sensitivity in lung cancer. Expert Opin Biol Ther. 2012;12(9):1221–39.
17. Wall NR. Colorectal cancer screening using protected microRNAs. J Gastrointest Oncol. 2011;2(4):206–7.
18. Saus E, Soria V, Escaramis G, Vivarelli F, Crespo JM, Kagerbauer B, et al. Genetic variants and abnormal processing of pre-miR-182, a circadian clock modulator, in major depression patients with late insomnia. Hum Mol Genet. 2010;19(20):4017–25.

19. Cai Y, Yu X, Hu S, Yu J. A brief review on the mechanisms of miRNA regulation. Genomics Proteomics Bioinformatics. 2009;7(4):147–54.

20. Segura MF, Hanniford D, Menendez S, Reavie L, Zou X, Alvarez-Diaz S, et al. Aberrant miR-182 expression promotes melanoma metastasis by repressing FOXO3 and microphthalmia-associated transcription factor. Proc Natl Acad Sci U S A. 2009;106(6):1814–9.

21. Santos A, Van Ree R. Profilins: mimickers of allergy or relevant allergens? Int Arch Allergy Immunol. 2011;155(3):191–204.

22. Gieni RS, Hendzel MJ. Actin dynamics and functions in the interphase nucleus: moving toward an understanding of nuclear polymeric actin. Biochem Cell Biol. 2009;87(1):283–306.

23. Rodriguez Faba O, Palou J. Predictive factors for recurrence progression and cancer specific survival in high-risk bladder cancer. Curr Opin Urol. 2012;22(5):415–20.

24. Qin XJ, Ling BX. Proteomic studies in breast cancer (Review). Oncol Lett. 2012;3(4):735–43.

25. Wei F, Xu J, Tang L, Shao J, Wang Y, Chen L, et al. p27(Kip1) V109G polymorphism and cancer risk: a systematic review and meta-analysis. Cancer Biother Radiopharm. 2012;27(10):665–71.

26. Wu Y, Sun WL, Feng JF. Antiangiogenic therapy in the management of breast cancer. Asia Pac J Clin Oncol. 2012. doi:10.1111/j.1743-7563.2012.01569.x.

27. Thorsen SB, Obad S, Jensen NF, Stenvang J, Kauppinen S. The therapeutic potential of microRNAs in cancer. Cancer J. 2012;18(3):275–84.

28. Cortez MA, Welsh JW, Calin GA. Circulating microRNAs as noninvasive biomarkers in breast cancer. Recent Results Cancer Res. 2012;195:151–61.

29. Vira D, Basak SK, Veena MS, Wang MB, Batra RK, Srivatsan ES. Cancer stem cells, microRNAs, and therapeutic strategies including natural products. Cancer Metastasis Rev. 2012;31(3–4):733–51.

30. Guan P, Yin Z, Li X, Wu W, Zhou B. Meta-analysis of human lung cancer microRNA expression profiling studies comparing cancer tissues with normal tissues. J Exp Clin Cancer Res. 2012;31(1):54.

31. Liu Z, Liu J, Segura MF, Shao C, Lee P, Gong Y, et al. MiR-182 overexpression in tumourigenesis of high-grade serous ovarian carcinoma. J Pathol. 2012;228(2):204–15.

32. Alshalalfa M, Bader GD, Goldenberg A, Morris Q, Alhajj R. Detecting microRNAs of high influence on protein functional interaction networks: a prostate cancer case study. BMC Syst Biol. 2012;6(1):112.

33. Weeraratne SD, Amani V, Teider N, Pierre-Francois J, Winter D, Kye MJ, et al. Pleiotropic effects of miR-183 96 182 converge to regulate cell survival, proliferation and migration in medulloblastoma. Acta Neuropathol. 2012;123(4):539–52.

34. Kong WQ, Bai R, Liu T, Cai CL, Liu M, Li X, et al. MicroRNA-182 targets cAMP-responsive element-binding protein 1 and suppresses cell growth in human gastric adenocarcinoma. FEBS J. 2012;279(7):1252–60.

35. Poell JB, van Haastert RJ, de Gunst T, Schultz IJ, Gommans WM, Verheul M, et al. A functional screen identifies specific microRNAs capable of inhibiting human melanoma cell viability. PLoS One. 2012;7(8):e43569.

36. Rawe VY, Payne C, Schatten G. Profilin and actin-related proteins regulate microfilament dynamics during early mammalian embryogenesis. Hum Reprod. 2006;21(5):1143–53.

37. Sagot I, Rodal AA, Moseley J, Goode BL, Pellman D. An actin nucleation mechanism mediated by Bni1 and profilin. Nat Cell Biol. 2002;4(8):626–31.

38. Wang D, Huang J, Hu Z. RNA helicase DDX5 regulates microRNA expression and contributes to cytoskeletal reorganization in basal breast cancer cells. Mol Cell Proteomics. 2012;11(2):M111 011932.

39. Zou L, Ding Z, Roy P. Profilin-1 overexpression inhibits proliferation of MDA-MB-231 breast cancer cells partly through p27kip1 upregulation. J Cell Physiol. 2010;223(3):623–9.