miR-19a promotes proliferation and migration of lens epithelial cells by regulating AKT/pAKT signaling

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Abstract

Background: Posterior capsule opacification (PCO) occurs frequently following surgical treatment of cataracts, representing a common adverse outcome. PCO develops as a result of lens epithelial cell (LEC) transdifferentiation, migration, and proliferation. In the present report we sought to explore the role of microRNA (miR)-19a in this process and to establish the underlying molecular mechanisms. Methods: miR-19a was transduced into HLE-B3 LECs, with microscopy and RT-qPCR used to confirm transfection efficiency. We then employed MTT, wound healing, and transwell assay approaches to monitor the proliferation and migration of these LECs. We further assessed levels of the proteins PTEN, AKT, and phosphorylated AKT (pAKT) via western blotting in WT and miRNA-transfected cells. Results: HLE-B3 proliferation was markedly enhanced by miR-19a transduction, as well the migration activity of these cells (both $P<0.01$). Furthermore, overexpressing miR-19a failed to reduce PTEN expression whereas it did enhance pAKT levels within these LECs ($P<0.05$). Conclusions: This suggests that miR-19a can enhance LEC proliferation and migratory activity through a mechanism that may be linked with regulating AKT activation and signaling, thus highlighting a potential avenue for therapeutic treatment of PCO patients.

Background

Posterior capsule opacification (PCO) is a significant adverse event that occurs following surgical treatment of cataracts, impairing the vision of as many as 28% of individuals within 5 years of intraocular lens (IOL) implantation [1].

PCO develops as a result of tissue fibrosis that occurs as a result of some combination of inflammation induced by the surgical operation and an immunological response to the IOL. During PCO development and progression, lens epithelial cells (LECs), which are normally present in a single layer on the inner side of the anterior lens capsule, transdifferentiate, proliferate, and migrate in a pathogenic manner [2].

MicroRNAs (miRNAs) are short RNAs that lack coding potential but which play broad roles in the post-transcriptional regulation of a wide array of target genes, influencing processes including but not limited to proliferation, apoptotic cell death, and differentiation [3]. The miR–17–92 cluster encodes for 6 distinct oncogenic miRNAs with significant roles in regulating genes linked to cellular proliferation (miR–17, miR–18a, miR–19a, miR–19b, miR–20a, and miR–92) [4]. Of these, miR–19a overexpression has been detected in the context of colorectal cancer [5], and this miRNA is known to be induced in colorectal cancer cells by PRL3 whereupon it can target TG2 and thereby drive their metastasis and proliferation [6, 7].

miRNAs have been shown to play tissue-specific roles within the eye [8], with unique roles for multiple miRNAs including miR–19 in this setting [9, 10]. However, at present the specific understanding of the role of individual miRNAs in the eye is limited, and further investigation is thus warranted.

In this study, we assessed the role of miR–19 in regulating the ability of LECs to proliferate and migrate and we explored the underlying molecular mechanisms in an effort to highlight new potential targets for

treating PCO.

Methods

Cell Culture

The human HLE-B3 LC line was from Shanghai Genechem (Shanghai, China). Cells were grown in DMEM (Gibco, MA, USA) containing 15% FBS (Zhejiang Tianhang Biotechnology, Hangzhou, China), along with penicillin and streptomycin in a 37°C 5% CO_2 incubator.

Cell Transduction

HLE-B3 cells were transduced with control or miR–19-encoding lentiviral particles (Shanghai Genechem) in a 96-well plate, with $5x10^4$ cells/mL in 100 uL per well. Lentiviral particles were added to cells at a range of multiplicities of infection (MOIs), and then after 72 h the GFP expression in transduces cells was assessed via fluorescent microscope (Leica DMI6000B).

RT-qPCR

The RNAiso for Small RNA kit (TaKaRa, Dalian, China) was used to extract total RNA from WT or lentivirally-transduced cells, with a BioSpectrometer (Eppendorf, Germany) used to gauge RNA quality. A total of 0.25–8 μg RNA in a 3.75 μl volume was then used with the Mir-X miRNA First-Strand Synthesis Kit (TaKaRa) for reverse transcription based on provided directions, and the Step One Plus Real-time PCR System (Applied Biosystems) was used to measure relative miR–19a expression. TB Green Premix Ex Taq II (Tli RNaseH Plus; TaKaRa) was used for all reactions, which consisted of a total of 25 μl made up of 12.5 μl TB Green Advanced premix, 0.5 μl ROX, 0.5 μl of the appropriate miR–19a primer, 0.5 μl of the mRQ 3' primer, 2 μl cDNA, and 9 μl dd H_2O. Thermocycler settings were: 95°C for 30 sec; 40 cycles of 95°C for 5 sec, 60°C for 30 sec. PCR products were confirmed via melting curve analyses. Relative miR–19a levels were normalized to levels of the U6 RNA, with the $2^{-\triangle\triangle Ct}$ method used for quantification. miR–19a primer sequences were as follows: Forward - 5'-GGAACGATACAGAGAAGATTAGC–3'; Reverse - 5'-TGGAACGCTTCACGAATTTGCG –3' (TaKaRa).

Cell proliferation assay

The MTT assay approach was used to quantify the proliferation of HLE-B3 cells, which were plated into 96-well plates ($2×10^4$ cells/ml, 100uL/well). After 24 h post-plating, cells were lentivirally transfected. After an additional 24, 48, 72, or 96 h, 20 μl MTT (5 mg/ml; Biofroxx, Germany) was added per well, and cells were incubated at 37°C for 4 h. Media was then exchanged for 150 μl DMSO (Beijing Solarbio, Beijing, China). A microplate reader (SepectraMax M3, Molecular Devices, Shanghai, China) was then used to assess absorbance values at 490 nm.

Wound healing assay

At 72 h post-lentiviral transduction, HLE-B3 cells were replated into 12-well plates at 3.5×10^5 cells/well. After an additional 24 h, a wound was generated in the monolayer within each well using a sterile 20μl pipette tip, and PBS was used to wash away non-adherent cells. After washing, fresh media was added and cells were imaged for 24 h, with the size of the wound being imaged at 0 and 24 h via an inverted microscope (Leica DMI6000B), with Image J used to quantify wound size changes over time.

Cell Migration Assay

At 72 h post-lentiviral transduction, HLE-B3 cells ($5x10^4$) were resuspended into 200 μl of serum-free DMEM, and were then added to the upper portion of a Transwell insert (Corning, High Wycombe, UK). This insert was then inserted into a well, with media supplemented with 20% FBS added to the lower chamber. Cells were incubated for 24 h, after which 4% paraformaldehyde was used to fix membranes prior to crystal violet (Beyotime) staining and counting of cells via Axio vert A_1 inverted fluorescence microscope.

Western blotting

After a 30 minute lysis step on ice, HLE-B3 cell lysates were spun at 12,000 rpm for 10 minutes at 4°C, with supernatants being collected and protein levels therein being quantified via a BCA assay. After boiling for 5 min in sample buffer, proteins from each sample were separated via SDS-PAGE, transferred to PVDF membranes, blocked for 4 h using 5% non-fat dried milk at room temperature, and probed overnight with primary antibodies specific for AKT, pAKT, and PTEN (1:1000, Wanlei Biotechnology, China) at 4°C. Secondary HRP-conjugated antibodies were then used for antigen detection for 1 h at room temperature. Blots were then washed using TBST, and an ECL detection kit (Beyotime) was used to quantify protein levels. Actin served as a loading control

Statistical analysis

Data are means ± SD. Values were compared via one-way ANOVAs with Bonferroni *post-hoc* testing for multiplecomparisons using SPSS. $P < 0.05$ was the significance threshold.

Results

Overexpression of miR–19a in HLE-B3 cells

We observed significantly enhanced GFP expression in HLE-B3 cells lentivirally transduced with viral particles encoding miR–19a, consistent with elevated miR–19a expression relative to control cells (Figure 1A), and this was confirmed via RT-qPCR (Figure 1B). We found an optimal MOI of infection to be 60, and as such this MOI was used for subsequent experiments.

Figure 1.miR–19a in lentivirally transduced HLEB3 cells. (A) GFP was used to assess miR–19a expression in HLE-B3 cells following lentiviral transduction. (B) miR–19a expression was assessed by RT-qPCR following lentiviral transduction. *P<0.05 vs. NC. miR, microRNA; NC, negative control

miR–19a regulates HLEB3 cell proliferation

We next used an MTT assay approach to assess how miR–19a impacted HLE-B3 cell proliferation. We observed a significant increase in the proliferation of these cells upon mIR–19a overexpression, with a significant increase in proliferation at 96 h post-transduction relative to NC cells (Figure 2).

Figure 2. miR–19a impacts the proliferation of HLEB3 cells. * P<0.05 vs. NC.

miR, microRNA; NC, negative control

miR–19a regulates HLEB3 cell migration

We further explored the impact of miR–19a overexpression on HLEB3 cell migration using wound healing and Transwell assay approaches. We observed significantly enhanced rates of migration following miR–19a overexpression relative to NC cells in the wound healing assay (Figure 3A, 3B), and consistent with this the Transwell assay similarly confirmed enhanced migration of cells overexpressing miR–19a (Figure 3C, 3D).

Figure 3. miR–19a regulates HLEB3 cell migration. (A) Images and (B) quantification of relative cell migration in a wound healing assay. (C) Images and (D) quantification of cell migration and invasion in a Transwell assay. **P<0.01. miR, microRNA; NC, negative control.

miR–19a influences AKT signaling in HLE-B3 cells

We next measured the levels of proteins in HLE-B3 cells following lentiviral transduction via western blotting, revealing that miR–19a overexpression did not significantly impact PTEN levels, whereas it did significantly increase pAKT levels relative to NC cells (P<0.05; Figure 4).

Figure 4. miR–19a influences AKT signaling in HLE-B3 cells. (A) AKT, pAKT, and PTEN levels in miR–19a-transfected HLE-B3 and NC cells were assessed via western blotting; (B) AKT, pAKT, and PTEN levels were quantified. *P<0.05. miR, microRNA; NC, negative control.

Discussion

PCO frequently arises following surgical treatment of cataracts through a fibrotic process wherein LECs undergo proliferation, migration, and transdifferentiation within the lens capsule. While surgeons generally attempt to remove as many of these cells as possible during surgery, it is inevitable that some remain within the capsular bag. At 3–4 days post-surgery, these cells reach their maximal rate of proliferation in a manner that is age-dependent, such that younger individuals are at a higher risk of PCO. LECs are also able to contribute to PCO via migrating over the posterior lens capsule and towards the posterior visual axis through the use of a range of adhesion molecules, including integrins and intracellular adhesion molecule 1 (ICAM1), which allow them to adhere to other cells and to the extracellular matrix [11]. The specific mechanisms that initiate this LEC proliferation and migration, however, remain uncertain.

miR–19a is a member of the miR–17–92 family, which consists of a polycistronic locus encoding miRNAs that play key roles in regulating the survival, proliferation, and differentiation of many different cell types [12]. Multiple recent studies have detected both physiological and pathological roles for miRNAs within the eye [13]. In this report, we provided direct evidence of the role of miR–19a in LECs, suggesting it may contribute to their proliferation and migration after surgical treatment of cataracts.

LEC expression of miR–19a at baseline is very low, and as such to examine its function in this study we transduced HLE-B3 LECs with a lentivirus encoding this miRNA. We found that miR–19a overexpression significantly enhanced the migration and proliferation of these LECs, although the specific underlying mechanism still requires further clarification.

AKT proteins are kinases that play essential roles in central signaling events necessary to mediate the growth and differentiation of cells, such that the PI3K/AKT signaling pathway is central to cellular survival, proliferation, and migration [14]. Cancer cells with enhanced proliferative and migratory activity typically also exhibit increased AKT activation, such that suppressing AKT activity is a promising approach to suppressing the aberrant proliferation and invasion of tumor cells in a variety of cancers [15].

PTEN serves to negatively regulate PI3K/AKT signaling in humans [16], and it is known to be regulated by specific miRNAs. For example, miR–17–5p was recently found to be expressed at elevated levels in ovarian cancer cells, and to therein target and suppress PTEN signaling, thereby promoting the epithelial-mesenchymal transition process within these tumor cells [17]. Similarly, in lung cancer cells it has been shown that miR–10a overexpression can promote both proliferation and metastasis through activation of PTEN/AKT/ERK signaling *in vitro [*18]. In this report, we determined that miR–19a overexpression is linked to enhanced LEC proliferation and migration through a mechanism that correlates with enhanced AKT phosphorylation but without any corresponding change in PTEN levels, suggesting miR–19a does not target PTEN directly.

Conclusions

Together these findings clearly demonstrate that overexpressing miR–19a can drive enhanced proliferation and migration of LECs through a mechanism potentially linked with regulation of AKT signaling. This thus suggests that targeting miR–19a may be a viable therapeutic strategy for treating PCO.

Abbreviations

PCO: Posterior capsule opacification; miR: microRNA; LECs: lens epithelial cells; PTEN: phosphatase and tensin homolog.

Declarations

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on request.

Authors' contributions

YBL, SJL and FLZ conceived and designed the study, YFW and FLZ drafted the manuscript. YFW, YBL, FLZ, SJL and YDP performed the experiments. YFW and FLZ analyzed the data. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors report no conflicts of interest.

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Figures

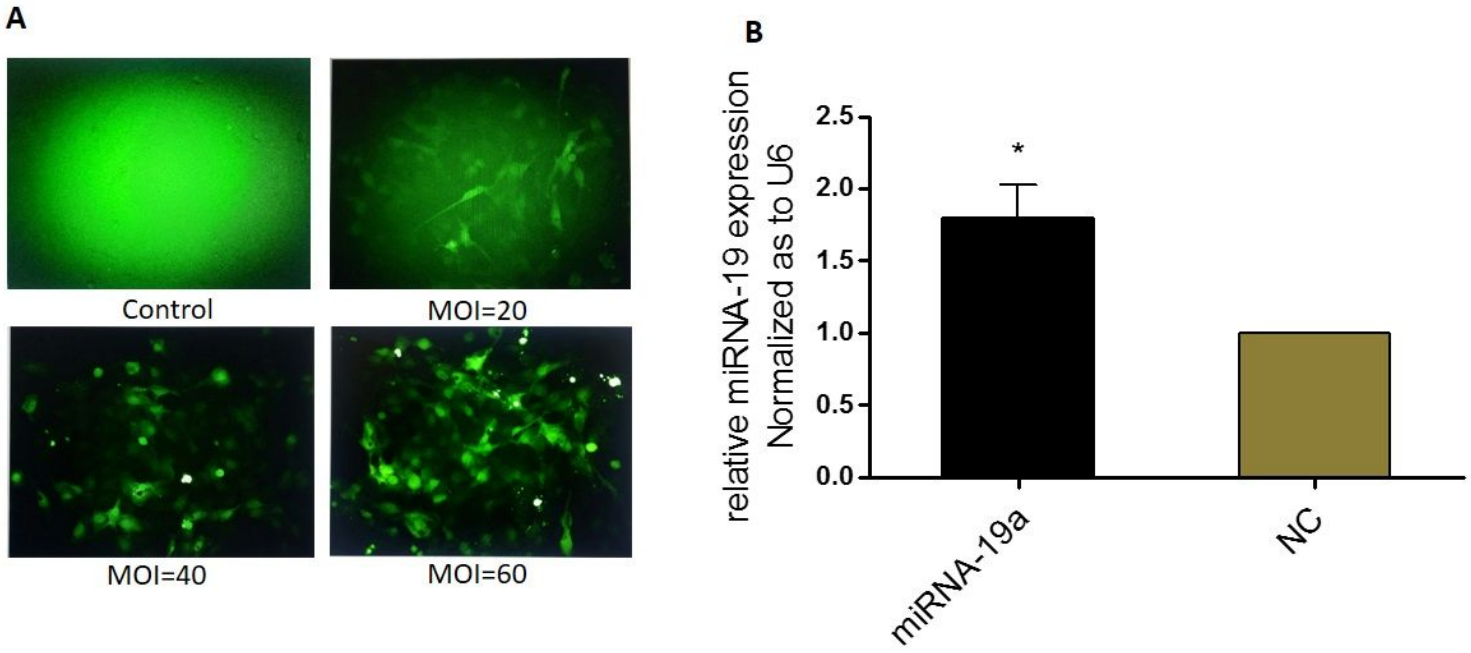

Figure 1

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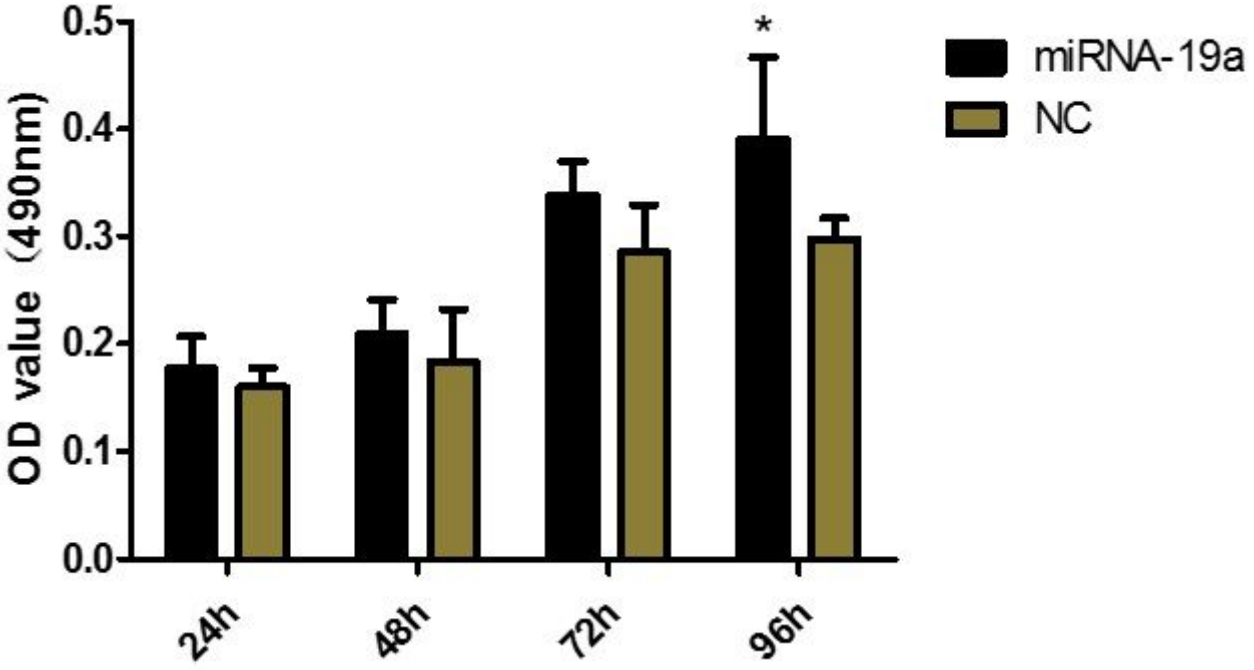
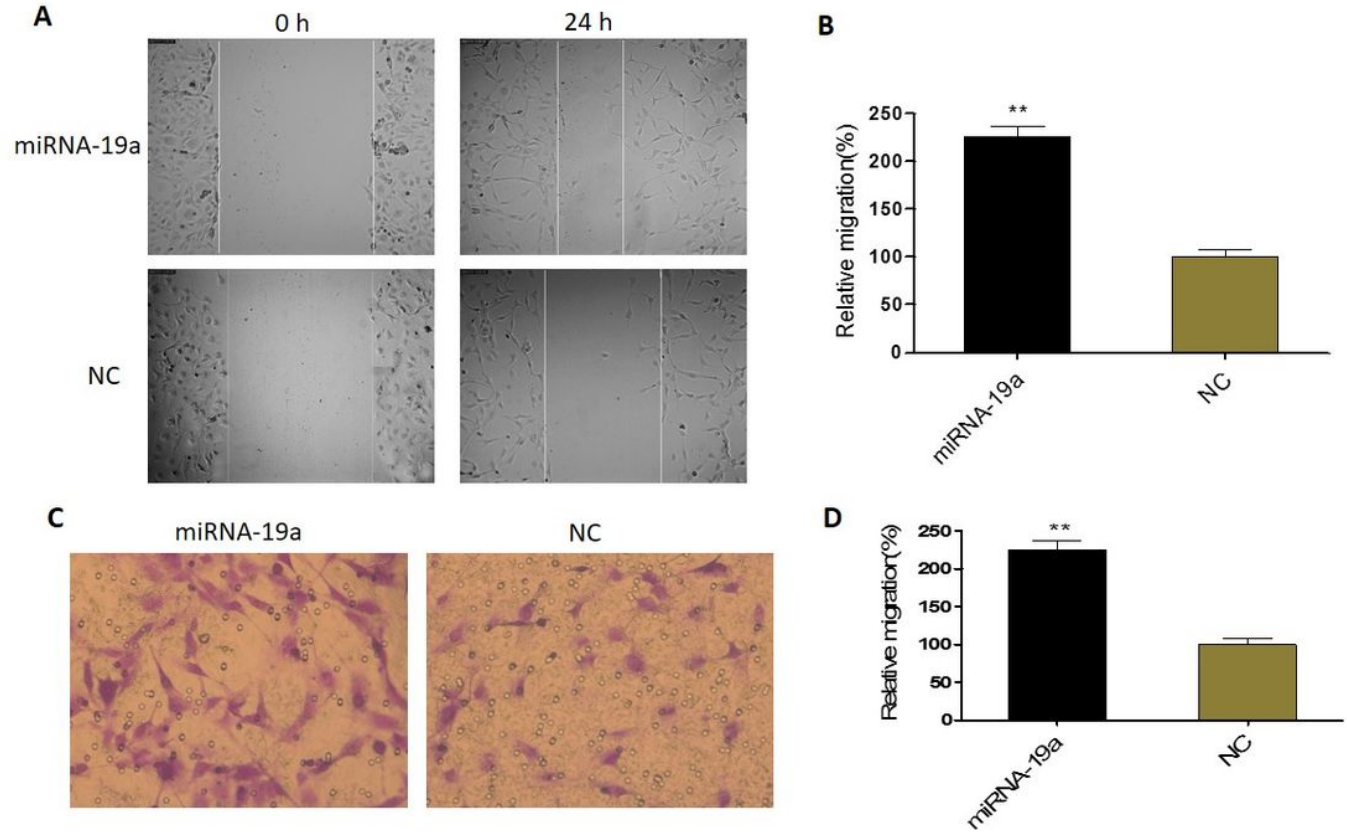


Figure 2

Figure 2. miR-19a impacts the proliferation of HLE B3 cells. * P<0.05 vs. NC. miR, microRNA; NC, negative control



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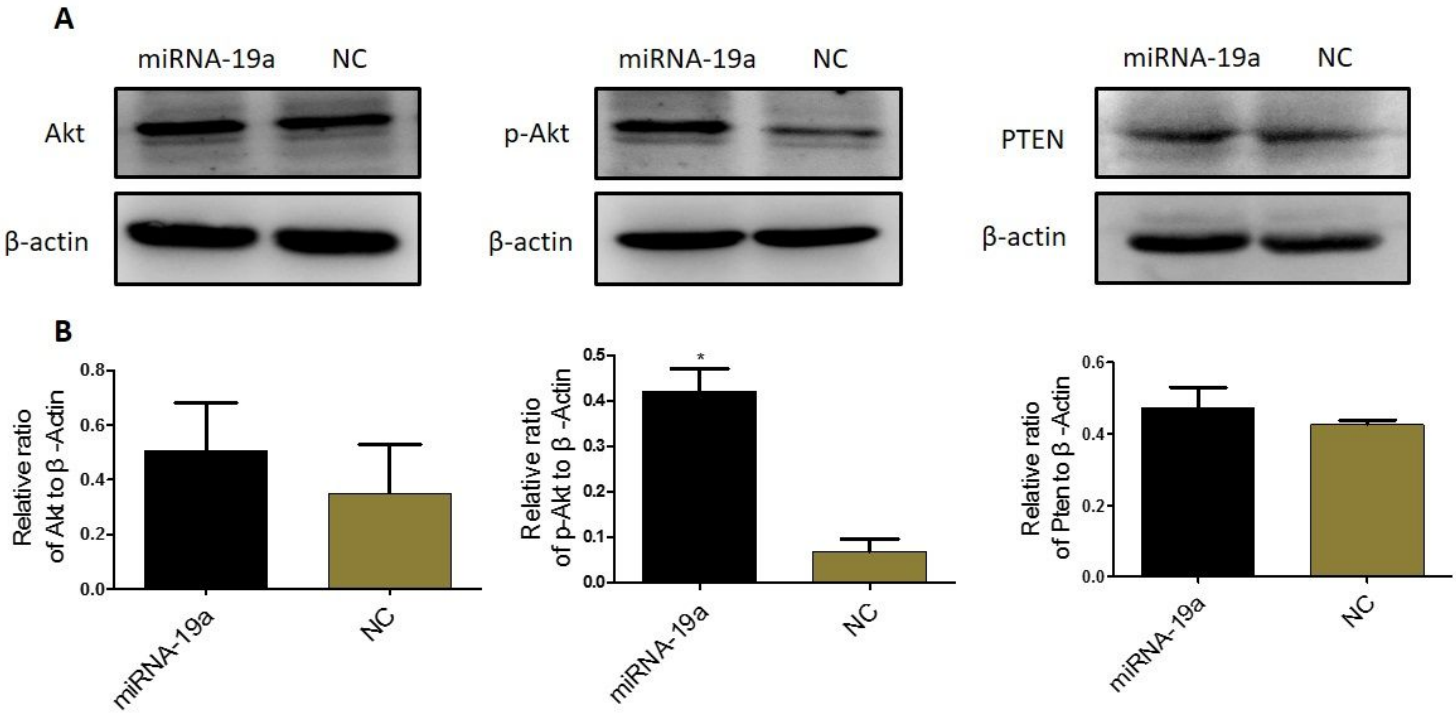

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