MicroRNA-21-5p are involved in apoptosis and invasion of fibroblast-like synoviocytes through PTEN/PI3K/AKT signal

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Abstract The function of *microRNA-21-5p* (*miR-21*) in fibroblast-like synoviocytes in RA was still unclear. In our study, we used tumor necrosis factor alpha (TNF α) (10 ng/ml) to mimic RA-FLSs and we found that normal FLS stimulated with TNF α caused the increasing expression of *miR-21*, a disintegrin and metalloproteinase with thrombospondin motifs 5 and matrix metalloproteinase 3, which were in accord with RA-FLSs changes. Our data showed that *miR-21* overexpression significantly increased cell invasion and decreased apoptosis in FLSs. Knockdown of *miR-21* in FLSs causes the opposite result. However, *miR-21* may not affect the proliferation of FLSs. Meanwhile, we showed that *miR-21* activated the PI3K/AKT signaling pathway to participate in RA by inhibiting PTEN expression. Taken together, our results suggested that *miR-21* may play a positive role

in RA and may be a promising new therapeutic target for RA.

Keywords Fibroblast-like synoviocytes · *MicroRNA-21-5p* · Apoptosis · Invasion · PTEN/PI3K/AKT signal

Introduction

Rheumatoid arthritis (RA) is a chronic and progressive autoimmune disease that mainly affects joints. Bone erosion and bone destruction are the characteristic features of RA. Fibroblast-like synoviocytes (FLSs), which can be cancerous and can destroy cartilage, participate in synovitis, are the main effectors of bone destruction in RA. The raised invasiveness of these cells is closely related to the pathogenesis and progression of RA (Zhuang et al. 2017). Numerous studies have shown that FLSs in RA patients can abnormally produce high levels of matrix metalloproteinases (MMPs), which are important effector molecules of bone destruction (Huang et al. 2017). Accumulating data indicate that the massive FLSs hyperplasia of RA results from reduced apoptosis (Korb et al. 2009); apoptosis is rarely found in the synovial tissues of either RA patients or animal models of RA, and RA-FLSs evaded apoptosis in vitro (Dharmapatni et al. 2009; Drynda et al. 2005; Korb et al. 2009; Perlman et al. 2001). However,

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the mechanisms that account for increased invasion and reduced apoptosis in RA synovium are not definitively known.

MicroRNAs (miRNAs) are small non-coding RNAs that inhibit translation of mRNA (Bartel 2009). *MicroRNA-21-5p* (*miR-21*) is a crucial regulator of cell proliferation, migration and apoptosis (Cappellesso et al. 2014; Chan et al. 2005; Meng et al. 2007; Venturutti et al. 2016). Studies have revealed that aberrant miRNA expression is involved in the pathogenesis of RA (Ammari et al. 2013; Chatzikyriakidou et al. 2012). *MiR-21*, the most commonly upregulated miRNA in various cancers, is upregulated in blood, and T cells of patients with RA (Smigielska-Czepiel et al. 2014; Wang et al. 2012). Studies have confirmed *miR-21* can suppress apoptosis of T cells (Meisgen et al. 2012; Smigielska-Czepiel et al. 2013), but few studies have reported on the role of *miR-21* in RA-FLSs. Additionally, the phosphatase and tensin homolog (PTEN)/phosphatidylinositol-3-OH kinase (PI3K)/AKT signaling pathway that regulates various cellular functions including cell apoptosis is involved in the pathogenesis of RA (Choy 2012). PTEN expression is lacking in RA synovium as well as in cultured RA-FLSs (Pap et al. 2000). M.D. Chanyshiev et al. demonstrated *miR-21* regulated mRNA and protein levels of the PTEN gene in MCF-7 cells as it is a proven *miR-21* target (Chanyshiev et al. 2018). Luo et al. detected that Overexpression or inhibition of *miR-21* in HK-2 cells activated or blocked the PI3K/Akt signaling via targeting PTEN (Luo et al. 2018). Then, does *miR-21* activate the PI3K/AKT pathway by inhibiting PTEN in RA-FLSs? This is our key concern.

Tumor necrosis factor alpha (TNF α) is a key proinflammatory factor and is also one of the major activators of permanent FLSs changes (Bartok and Firestein 2010; Scanzello and Goldring 2012). TNF α increases invasiveness of FLSs by inducing it to secrete high levels of extracellular matrix proteases, such as MMPs and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTSs). They are probably the primary enzymes involved in digestion of the extracellular matrix, which is a critical step in the tissue destruction in RA (Blom et al. 2007). In this present study, we used TNF α (10 ng/ml) to mimic RA-FLSs and we found that stimulated normal FLSs with TNF α caused the increasing expression of *miR-21*, ADAMTS5 and MMP3, which were in accord with RA-FLSs changes. Subsequently, we attempt to clarify

the impact of *miR-21* on FLSs proliferation, apoptosis and invasion, and explore the potential mechanism.

Materials and methods

Materials

Human TNF α , interleukin (IL)-1 β and IL-6 were purchased from Minneapolis (Minneapolis, MN). *MiR-21* inhibitor, *miR-21* negative control (NC), *miR-21* mimic and *miR-21* positive control (NC) were obtained from Biomics Biotechnologies (Nantong, China). CCK8 kit was provided by Dojindo (Kumamoto, Japan). Primary antibodies against PTEN, PI3K, AKT, p-akt (S473), Bax, Bcl-2 and GAPDH were all purchased from Cell Signaling Technology (Danvers, USA). Penicillin, streptomycin was bought from Gibco (MD, USA). MEM/EBSS was purchased from Hyclone (Utah, USA). Lipofectamine 2000 was provided by Invitrogen (CA, USA). Annexin V-Alexa Fluor 647/PI Apoptosis Assay Kit and Cell Cycle Assay Kit were bought from FcMACS (Nanjing, China). Primers for *miR-21*, U6, ADAMTS5, MMP3, MMP13 and GAPDH were all bought from Biomics Biotechnologies (Nantong, China).

Culture of FLSs

Normal human FLSs derived from three donors were purchased from Jennio Biotech Co.,Ltd (Guangzhou, China). FLSs were recovered in MEM/EBSS, 1% penicillin and streptomycin, 15% FBS at 37 °C, 5% CO₂. Cells at passage three to six were used for experiments.

Cell transfection

FLSs were plated 1×10^5 cells/well on six-well plates 12 h prior to experiment in MEM/EBSS, 1% penicillin and streptomycin, 15% FBS at 37 °C, 5% CO₂. Then, the FLSs were unpretreated or pretreated with 10 ng/ml TNF α for 24 h. Next cells were divided into four groups according to different transfection treatments: normal FLSs transfected with empty vector as the negative control (Normal NC) of that transfected with *miR-21* mimic (*miR-21*-up). TNF α pretreated FLSs transfected with empty vector as the

negative control (TNF α NC) of that transfected with *miR-21* inhibitor (TNF α + *miR-21*-down).

RNA extraction and qRT-PCR

Cells were collected and total RNA was isolated using Trizol reagents (Invitrogen, USA). The quantity of RNA was measured by Nanophotometer (Thermo Fisher Scientific, USA). 1 μ g of RNA were transcribed to cDNA by RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA). The converted cDNA samples were amplified by qRT-PCR (Roche cobas z 480) using SYBR Green (Vazyme Biotech, Nanjing, China). The expression levels of cells were evaluated by multiple comparison with control. GAPDH was used as an internal reference.

Cell proliferation assay

Cell proliferation viability was measured by CCK8 kit. Briefly, cells of each group were cultured at a concentration of 1×10^3 cells per well in 96-well plate. Cell viability was detected on days 1–6. The CCK-8 solution was diluted to 10% (v/v) in DMEM, and then a total of 100 μ l diluted CCK-8 was added to each well and incubated for 1.5 h. Absorbance was measured at 450 nm with a microplate reader (Thermo MK3, USA).

Cell cycle detection

Cell cycle was assessed by flow cytometry. In logarithmic growth phase, cells of each group were collected, washed twice in low temperature PBS, and fixed in 75% ethanol overnight at 4 °C. Next, cells were resuspended in PI and RNase A and incubated for 30 min at 37 °C in the dark. The samples were measured by Becton–Dickinson FACSCalibur Cytometer. Data were analyzed using ModFIT LT 2.0 software.

Cell apoptosis analysis

Cell apoptosis was also measured by BD FACSCalibur Cytometer after cells were collected, washed twice with PBS, stained with 100 μ l of binding buffer, 5 μ l Annexin V- Alexa Fluor 647 and 10 μ l PI for

15 min at room temperature and dark. Data were analyzed using FlowJo 10 software.

Cell migration

Cell migration was tested by scratch wound assay. About 2×10^4 cells of each group were collected and seeded on 6-well plates. 48 h after transfection, cells came to 100% confluence, we used sterile pipette tips to scratch wound across the middle of wells. Then cells were washed twice to remove the debris and incubated for 12 h. Migrated areas were photographed and measured using ImageJ software.

Cell invasion

The invasion of FLSs was detected by Transwell system coated with Matrigel (8.0 μ m pore size). Cells of each group in logarithmic growth phase were resuspended with basal medium. About 2×10^4 cells were seeded on the upper chambers. The lower chamber was added with medium containing 15% FBS as an inducer. After incubated for 48 h at 37 °C in 5% CO₂, cells transferred to the lower chamber were fixed with paraformaldehyde for 5 min, stained with 0.1% crystal violet for 2 min, washed in PBS and counted under the microscope (Nikon, Japan). Finally take the average number of any five fields as an indicator to evaluate invasive ability of cells.

Western blot analysis

Cells transfected for 72 h were collected and lysed on ice for 30 min using radioimmunoprecipitation (RIPA) solution. After centrifugation, the supernatant was extracted. Protein concentration was measured by BCA kit. Each group of protein to 100 μ g used for electrophoresis and transferred to PDVF membrane and incubated with primary antibody at 4 °C overnight. Then the membrane was incubated with secondary antibody for 2 h at room temperature. Expression of PTEN, PI3K, AKT, p-akt (S473), Bax, Bcl-2 were detected. Expression of GAPDH was used as an internal reference.

Statistical analysis

One-way ANOVA and *t* test were performed by SPSS 19.0 software to calculate *p* value of repeated

experiments. All values represented as the mean $\pm$ standard deviation (SD). p Values < 0.05 indicated significant differences between groups. Results were obtained from three independent experimental replicates.

Results

TNF α promotes the expression of *miR-21*, ADAMTS5, MMP3 in normal FLSs

To verify that normal FLSs stimulated by TNF α (10 ng/ml) demonstrate characteristics of RA-FLSs, we examined expression levels of *miR-21*, ADAMTS5, and MMP3. In RA-FLSs, *miR-21*, ADAMTS5 and MMP3 are upregulated (Bottini and Firestein 2013; Chen et al. 2016; Xiong et al. 2016). The expression of *miR-21*, ADAMTS5 and MMP3 were significantly increased in TNF α stimulated normal FLSs compared with normal FLSs (Fig. 1). These results suggest that TNF α simulated normal FLSs can mimic RA-FLSs. Therefore, we used TNF α

to induce conditions of RA-FLSs in vitro. During this phase of the current study, normal FLSs were also stimulated separately with IL-1 β (5 ng/ml) and IL-6 (100 ng/ml) which also play crucial role in RA and levels of *miR-21* were assessed. The results revealed that expression of *miR-21* was also increased in these FLSs following IL-1 β or IL-6 treatment (Fig. 1).

miR-21 has no apparent effect on FLSs proliferation

Cell proliferation viability were determined by CCK8 and Cell-cycle. Our data showed that all the four groups experienced slowing growth period, logarithmic multiplication period and growth stationary period. There was no significant difference of viability between each two groups (Fig. 2a). The results of cell cycle distribution indicated that the percentages of FLSs in G0/G1 phase of Normal (NC), *miR-21*-up, TNF α (NC), TNF α + *miR-21*-down group were $87.11 \pm 1.76\%$, $84.88 \pm 3.68\%$, $67.44 \pm 2.02\%$ and $69.66 \pm 0.64\%$, respectively. The S phase fraction in each group was $2.58 \pm 1.85\%$, $2.93 \pm 1.49\%$,

Fig. 1 In vitro expression of normal FLSs stimulated by TNF α , IL-1 β and IL-6. Normal FLSs were treated with TNF α (10 ng/ml), IL-1 β (5 ng/ml) or IL-6 (100 ng/ml) for 24 h separately. The expression of *miR-21*, ADAMTS5 and MMP3 was analyzed

by quantitative polymerase chain reaction. All the data were represented the mean $\pm$ SD from three independent replicate experiments and FLSs from three donor were used (* $p < 0.05$ vs. control)

Fig. 2 Effect of *miR-21* on FLSs proliferation. **a** The cell proliferation viability was determined by CCK8 assay. A 450 absorption was assayed after culture from 1 to 6 days. The statistical tests were based on the normal (NC) group and TNFα (NC) group, respectively. **b** Cell cycle distribution of different group cells was measured using flow cytometry analysis. **c** The histogram showed the proportion of cells in each phase of the

cell cycle in different group. The statistical tests were based on the normal (NC) group and TNFα (NC) group, respectively. **d** The histogram showed the proliferation index in different groups. The statistical tests were based on the normal (NC) group and TNFα (NC) group, respectively. All the data were presented as the mean $\pm$ SD of three independent experiments and FLSs from three donor were used

$29.12 \pm 2.19\%$ and $26.72 \pm 2.19\%$, respectively. The G2/M phase fraction in each group was $7.31 \pm 2.44\%$, $9.18 \pm 1.42\%$, $2.12 \pm 0.83\%$ and $1.24 \pm 0.33\%$, respectively. Through statistical analysis, there was no significant difference between Normal (NC) and *miR-21*-up, also between $\text{TNF}\alpha$ (NC) and $\text{TNF}\alpha + \text{miR-21-down}$ in G0/G1, S and G2/M phase, respectively (Fig. 2b, c). As shown in Fig. 2d, the same results were obtained by using the proliferation index. In conclusion, we could conclude that *miR-21* has no apparent effect on FLSs proliferation viability.

miR-21 suppresses FLSs apoptosis

The apoptotic FLSs were detected by flow cytometry after transfected for 48 h. Total rate of apoptosis (Q2 + Q3) of Normal (NC), *miR-21*-up, $\text{TNF}\alpha$ (NC) and $\text{TNF}\alpha + \text{miR-21-down}$ were $31.2 \pm 2.49\%$, $15.43 \pm 2.15\%$, $3.10 \pm 0.82\%$ and $12.26 \pm 1.02\%$ respectively (Fig. 3a, b). Data displayed that compared to the normal (NC), apoptosis of normal FLSs transfected with *miR-21* mimic was significantly reduced ($p < 0.05$). Compared to the $\text{TNF}\alpha$ (NC), $\text{TNF}\alpha$ stimulated FLSs transfected with *miR-21* inhibitor promoted apoptosis significantly ($p < 0.01$). These results indicated *miR-21* could suppress FLSs apoptosis.

To explore the mechanism of this phenomenon, we analyzed the expression of apoptosis related genes at 72 h post-transfection by western blot (Fig. 3c, d). Compared with normal FLSs, *miR-21* mimic significantly increased the levels of antiapoptotic proteins Bcl-2 but inhibited the expression of proapoptotic gene Bax ($p < 0.05$). *miR-21* inhibitor increased the level of proapoptotic proteins Bax but reduced the level of antiapoptotic gene Bcl-2 compared to the $\text{TNF}\alpha$ (NC) group cells ($p < 0.01$). These results indicated *miR-21* suppresses cell apoptosis by regulating the expression of apoptosis related genes in FLSs.

miR-21 promotes FLSs migration and invasion

As Fig. 4a, b shown that *miR-21* promoted cell migration in a scratch wound assay. Cell invasion ability was detected by Transwell system coated with Matrigel. The number of FLSs that migrated to the lower chamber from the upper chamber of normal

(NC), *miR-21*-up, $\text{TNF}\alpha$ (NC) and $\text{TNF}\alpha + \text{miR-21-down}$ were 105.67 ± 8.50 , 205.33 ± 10.05 , 345.33 ± 4.51 , and 218.33 ± 8.57 respectively (Fig. 4c, d). These results showed that compared with the normal (NC), overexpression of *miR-21* promoted normal FLSs invasion ($p < 0.05$) and compared with the $\text{TNF}\alpha$ (NC), down-expression of *miR-21* of $\text{TNF}\alpha$ stimulated cells decreased FLSs invasion significantly ($p < 0.01$). These data indicated *miR-21* could promote invasion of FLSs.

MMPs play crucial roles during RA-FLS invasion. We detected the effect of *miR-21* on the relative levels of MMP3 and MMP13 by qRT-PCR (Fig. 4e). Expression of MMP3 and MMP13 in *miR-21*-up group cells obviously increased compared with the normal (NC) group ($p < 0.05$). Compared with the $\text{TNF}\alpha$ (NC) group cells, $\text{TNF}\alpha$ stimulated FLSs transfected with *miR-21*inhibitor decreased the expressions of MMP3 and MMP13 ($p < 0.01$). These data indicated that *miR-21* could promote FLSs invasion via regulating the expression of MMP3 and MMP13.

miR-21 regulates the PTEN/PI3K/AKT signal in FLSs

An increasing number of evidence showed that PTEN/PI3K/AKT modulated cell apoptosis and invasion in various cancers and were involved in the pathogenesis of rheumatoid arthritis (Choy 2012; Hennessy et al. 2005) and PTEN is a proven *miR-21* target (Chanyshiev et al. 2018). By searching the miRTarBase database, we found 3'-UTR of human PTEN contained a putative region (position 1990–2013) could match to the sequence of *miR-21* (Fig. 5a). Then, gain-and-loss function assay was used to verify whether PTEN was the target of *miR-21*. The expression of PTEN was downregulated in *miR-21*-up group and upregulated in *miR-21*-down group compared to NC group ($p < 0.05$) (Fig. 5a). PI3K/AKT pathway could be negatively regulated by PTEN, therefore we studied the relationship between *miR-21* and PI3K/AKT signal by Western blot. Compared with normal (NC) group, *miR-21* mimic reduced the expression level of PTEN but promoted the level of PI3K and p-akt (S473) ($p < 0.05$). Compared to $\text{TNF}\alpha$ (NC) group *miR-21* inhibitor increased the level of PTEN and suppressed the level of PI3K and p-akt (S473) ($p < 0.01$). Furthermore, LY294002, a specific

Fig. 3 The effect of *miR-21* on FLSs apoptosis. **a** After transfected FLSs for 48 h, cells were collected and stained with Annexin V- Alexa/Fluor 647 and then examined by flow cytometry. **b** The histogram showed the apoptosis rate obtained from each group. **c** After transfected FLSs for 72 h, cells were collected and total proteins were extracted for western blot. The protein levels of Bax and Bcl-2 were analyzed by western blot.

d The relative levels of Bax and Bcl-2 were quantitatively analyzed by measuring bands density using Quantity One software. GAPDH was the internal control. All statistical tests were based on the normal (NC) group and TNFα (NC) group, respectively (* $p < 0.05$; ** $p < 0.01$). All the data were presented as the mean $\pm$ SD of three independent experiments and FLSs from three donor were used

Fig. 4 The effect of *miR-21* on FLSs invasion. **a** Cells of each group were collected and seeded on 6-well plates. 48 h after transfection, cells came to 100% confluence, we used sterile pipette tips to scratch wound across the middle of wells. Then cells were washed twice to remove the debris. After 12 h for incubation pictures were taken. **b** Migrated area was quantified as the average of relative length between margins of three different fields. **c** Cells that passed through the Matrigel-coated polycarbonate membrane were stained with crystal violet and observed under an inverted phase contrast microscope. **d** The

histogram showed the number of cells that passed through Transwell invasion chambers. **e** After transfected FLSs for 48 h, cells were collected and total RNA were extracted for quantitative polymerase chain reaction. The mRNA levels of MMP3 and MMP13 were analyzed by qPCR. All statistical tests were based on the normal (NC) group and TNF α (NC) group, respectively (* $p < 0.05$; ** $p < 0.01$). All the data were presented as the mean $\pm$ SD of three independent experiments and FLSs from three donor were used

inhibitor of Akt, blocked the p-akt (S473) expression in *miR-21*-up group ($p < 0.05$) (Fig. 5b, c). These results suggested *miR-21* regulated PTEN/PI3K/AKT signal in FLSs.

Discussion

Rheumatoid arthritis is featured by abnormal hyperplasia of FLSs, leading to gradual destruction of the

Fig. 5 Target gene of *miR-21* and potential pathway were detected. **a** Predicted *miR-21* binding sites within the 3'-UTR of PTEN mRNA by miRTarBase Release 7.0 (<http://mirtarbase.mbc.nctu.edu.tw>) and western blot was used to detect the expression of PTEN in FLSs after different treatment. **b** After transfected for 72 h, FLSs were collected and total proteins were extracted for western blot. The protein levels of PTEN, PI3K and p-akt (S473) were analyzed by western blot. **c** LY294002 blocked p-akt (S473)

expression. Akt was used to be a control. **d** The relative levels of PTEN, PI3K and p-akt (S473) were quantitatively analyzed by measuring bands density using Quantity One software. GAPDH was the internal control. All statistical tests were based on the normal (NC) group and TNF α (NC) group, respectively (* $p < 0.05$; ** $p < 0.01$). All the data were presented as the mean $\pm$ SD of three independent experiments and FLSs from three donor were used

joint and ultimately disability (Shin et al. 2018). RA-FLSs display ‘tumor-like’ features, including increased migration, invasion and reduced apoptosis (Nakamura et al. 2018). To better understand the molecular mechanism of RA progression, the dysregulation of miRNAs in FLSs is an interesting area (Maeda et al. 2017). Previous studies reported that *miR-21*, which is upregulated in a variety of diseases, is aberrantly elevated in blood, T cells and FLSs of patients with RA (Smigielska-Czepiel et al. 2014; Wang et al. 2012; Xiong et al. 2016). However, little was reported about the mechanism by which *miR-21* was involved in the progression of RA. In this study, on one hand, we demonstrated that *miR-21* suppressed apoptosis and promoted invasion of FLSs in RA via the activation of PTEN/PI3K/AKT signaling; On the other hand, we supplied new evidence that *miR-21* inhibitor might be used as a novel therapeutic drug for RA.

In RA-FLSs, the balance between proliferation and apoptosis is disturbed. Impaired apoptosis results in overabundance of FLSs. Studies have not found RA-FLS have significant abnormal proliferation ability in vivo and vitro (Pap et al. 2000). A major checkpoint of cell apoptosis pathway is the ratio of pro-apoptotic protein to anti-apoptotic protein. Bax, as a pro-apoptotic protein, had the ability to promote cell apoptosis. Bcl-2, as an anti-apoptotic protein, had the ability to suppress cell apoptosis (Sims et al. 2017). In our study, we confirmed *miR-21* has no apparent effect on FLSs proliferation but suppresses FLSs apoptosis. Additionally, we first found that *miR-21* mimic induced the expression of bcl-2 to inhibit apoptosis but surpassed the Bax level in FLSs. Knocking down *miR-21* of FLSs could get an opposite result. These results were in accordance with the previous study that *miR-21* suppressed apoptosis by decreasing Bax/Bcl-2 ratio in human cancer cells (Lv et al. 2016; Shi et al. 2010).

Invasive phenotype is the second important feature of RA-FLS. RA-FLSs secrete high levels of MMP13, MMP3, ADAMTS5 and cathepsins, which break the balance between protease and protease inhibitor, result in bone destruction (Tang et al. 2018). TNF α , as the most prominent mediators of RA, can induce the expression of MMPs, ADAMTSs and other catabolic enzymes in FLSs (Bottini and Firestein 2013), which was coincident with our data. In our present study, *miR-21* mimic promoted the mRNA levels of MMP3

and MMP13 in normal FLSs. Moreover, *miR-21* inhibitor reduced mRNA expression of MMP3 and MMP13 in TNF α stimulated normal FLSs which was regarded as the analog of RA FLSs. So, we demonstrated that *miR-21* induced FLSs invasion through the activation of MMP3 and MMP13 in RA.

The PTEN/PI3K/AKT signal regulates multiple cellular functions including cell survival and apoptosis. Similar to tumor cells, RA-FLSs also have abnormal viability (Malemud 2015). Previous studies have demonstrated that PTEN expression is significantly reduced in synovial tissue and FLSs in RA patients (Pap et al. 2000). PTEN, which was the direct target of *miR-21*, was suppressed in human cancers (Salmena 2016). In our subject, we first found *miR-21* inhibitor promoted the level of PTEN but suppressed the expression of PI3K and p-akt (S473) in RA-FLSs. Overexpression of *miR-21* of FLSs generated an opposite result. Taken together, *miR-21* modulated PTEN/PI3K/AKT signal in FLSs. However further experiments need to be carried out for the mechanism.

In conclusion, our study showed *miR-21* suppressed apoptosis and promoted invasion of FLSs but may not affect the proliferation of FLSs. Meanwhile, we indicated that *miR-21* participated in the development of RA via activating PI3K/AKT signal by targeting PTEN. Altogether, *miR-21* may act as a potential diagnostic indicator and drug treatment target for RA.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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