miR-149-5p mitigates tumor necrosis factor-α-induced chondrocyte apoptosis by inhibiting TRADD

Shi-cheng Xie¹, Lin Yang², Taipengfei Shu³, Qin Liu⁴, Wenbo Wang²

¹Department of Joint Surgery, the Affiliated Hospital of Jining Medical University, Jining, China
²Department of Orthopedics, the First Affiliated Hospital of Harbin Medical University, Harbin, China
³Department of Endocrinology, the First Affiliated Hospital of Harbin Medical University, Harbin, China
⁴Department of Radiology, Tancheng Traditional Chinese Medicine Hospital, Tancheng, China

Submitted: 17 July 2019; Accepted: 27 November 2019
Online publication: 17 January 2020

Arch Med Sci 2024; 20 (2): 602–611
DOI: https://doi.org/10.5114/aoms.2020.92324
Copyright © 2020 Termedia & Banach

Abstract

Introduction: Chondrocyte apoptosis as a prominent characteristic is usually accompanied by cartilage degeneration in osteoarthritis (OA). Herein, we aimed to determine the roles of miR-149-5p in tumor necrosis factor-α (TNF-α)-induced chondrocyte apoptosis.

Material and methods: Human chondrocytes were cultured with TNF-α to establish an apoptosis cell model in vitro. After transfection with miR-149-5p mimics or co-expression with TRADD in chondrocytes, cell viability, apoptosis, inflammatory cytokines, mRNA and protein expression were measured using CCK8, Annexin V-FITC double staining, ELISA assays, RT-qPCR and western blotting, respectively.

Results: TNF-α-induced chondrocyte apoptosis occurred in association with the inhibition of cell proliferation, the elevation of inflammatory cytokine levels and the activation of TRADD and caspase-3/8 signaling. The post-transcriptional regulatory mechanism suggested that TRADD was a direct target of miR-149-5p, and overexpression of miR-149-5p resulted in the down-regulation of TRADD protein expression in chondrocytes. In addition, miR-149-5p mimics had the ability to attenuate TNF-α-induced inflammation and apoptosis, while transfection with TRADD vector neutralized the protective effects of miR-149-5p on TNF-α-induced chondrocyte dysfunction.

Conclusions: miR-149-5p inversely regulated TNF-α-mediated chondrocyte damage by inhibiting TRADD-modulated caspases signaling. The miR-149-5p/TRADD signaling pathway might be a promising therapeutic target for the treatment of OA.

Key words: microRNA, post-transcriptional regulatory mechanism, chondrocyte apoptosis, TRADD, tumor necrosis factor-α.

Introduction

Chondrocyte apoptosis, known as programmed cell death, as a normal physiological phenomenon is responsible for modulating the homeostasis of articular cartilage, while excessive apoptosis of chondrocytes is a central pathological incident in articular cartilage injury or osteoarthritis (OA) [1, 2]. A substantial body of evidence reveals that mechanical over-
miR-149-5p mitigates tumor necrosis factor-α-induced chondrocyte apoptosis by inhibiting TRADD

Material and methods

Cell culture

Human primary chondrocytes were obtained as described previously [2] and were cultured in DMEM-F12 (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) with 5% CO₂ and 95% air as described previously [2].

Cell counting kit 8 (CCK8) assay

Cell viability was detected using a CCK-8 kit (Beyotime Institute of Biotechnology, Haimen, China). Absorbance was recorded at 450 nm.

Enzyme linked immunosorbent assay (ELISA)

The levels of IL-1β (Cat. no: E-EL-H0149c), IL-6 (Cat. no: E-EL-H0102c) and IL-18 (Cat. no: E-EL-H0253c) in the supernatant of cultured chondrocytes were measured using ELISA kits (Elabscience Biotechnology Co., Ltd, Wuhan, China) as described previously [23].

Annexin V-FITC double staining for apoptosis analysis

Annexin V-FITC kit (BD Biosciences, Franklin Lakes, NJ, USA) was used to evaluate the apoptotic cell proportion as described previously [23].

miR prediction

The bioinformatics algorithm was executed by TargetScan (www.targetscan.org) and miRDB (www.mirdb.org) to predict the potential miRNAs which could target modulation of TRADD expression via binding with its 3′-untranslated regions (3′-UTRs).

Cell transfection and plasmid constructs

miR-control (miR-Con; 5′-UACUUAGUCCGGAGUCCGAGUC-3′ and miR-149-5p mimics (5′-UCUGGCCGUGCUACUCC-3′) were synthesized by Guangzhou RiboBio Co., Ltd. (Guangzhou, China) and were transfected into chondrocytes using Lipofectamine 2000 (Invitrogen) as described previously [24]. A mammalian expression plasmid designed to specially express the full-length open reading frame of human TRADD without 3′-UTR, which did not contain the conserved complementary sequence binding with miR-149-5p, was purchased from GeneCopoeia, Inc. (Rockville, MD, USA). An empty plasmid served as the negative control. Vector-Con and vector-TRADD were transfected into chondrocytes using Lipofectamine 2000 (Invitrogen) as described previously [24].

Luciferase reporter assay

The wild-type (WT) or mutant-type (Mut) 3′-UTR of TRADD was inserted into the multiple cloning sites of the luciferase expressing pMiR-REPORT vector (Ambion; Thermo Fisher Scientific, Inc.). For the luciferase assay, chondrocytes containing the WT or Mut 3′-UTR of TRADD (0.5 μg) were co-transfected with miR-Con or miR-149-5p mimics using Lipofectamine 2000, according to the manufacturer’s protocols. The luciferase activ-
ity was measured using a luciferase reporter assay kit (Promega Corporation, Madison, WI, USA) according to the manufacturer’s protocols.

**Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)**

miRNasy Mini Kit (Qiagen, Inc., Valencia, CA, USA) was used to extract total RNA. TaqMan RT kit and TaqMan MicroRNA assay (Applied Biosystems) were used to detect miRNA expression levels using the Applied Biosystems 7300 Real-Time PCR system, according to the manufacturer’s protocol. U6 small nuclear RNA was used as an endogenous control. The relative expression levels of miRNAs were calculated using the 2^{-ΔΔCt} method [25]. The primers of miRs for qPCR are shown in Table I.

**Western blotting**

Western blotting were performed as previously described [26]. Membranes were cultured with primary antibodies of TNFR1 (Cat. no: sc-8436; dilution: 1 : 1,000; Santa Cruz Biotechnology, Inc., Dallas, TX, USA), TRADD (Cat. no: sc-46653; dilution: 1 : 1,000; Santa Cruz Biotechnology, Inc., Dallas, TX, USA), cleaved caspase-3 (Cat. no: 9661; dilution: 1 : 1,000; Cell Signaling Technology, Beverly, MA, USA) and cleaved caspase-8 (Cat. no: 9748; dilution: 1 : 1,000; Cell Signaling Technology, Beverly, MA, USA) at room temperature for 2 h. Then the membranes were incubated with an appropriate horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology, Inc., Dallas, TX, USA) and visualized with an enhanced chemiluminescence kit (Thermo Fisher Scientific, Inc.). Signals were analyzed with Quantity One software version 4.5 (Bio Rad Laboratories, Inc., Hercules, CA, USA). Anti-β-actin (Cat. no: sc-47778; dilution: 1 : 2,000; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) was used as the control antibody.

**Ethics**

The present study was approved by the First Affiliated Hospital of Harbin Medical University (Harbin, China).

**Statistical analysis**

Data were presented as the mean ± standard error of the mean. Statistical analysis was performed using SPSS software version 19.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism Version 7.0 (GraphPad Software, Inc., La Jolla, CA, USA). Student’s t-test was used to analyze the differences between the two groups. Differences between multiple groups were analyzed by one-way analysis of variance, followed by a post-hoc Tukey test. \(P < 0.05\) was considered to indicate a statistically significant difference.

**Results**

TNF-α inhibited chondrocyte growth and elevated the production of inflammatory cytokines and apoptotic cell proportion

Chondrocytes were exposed to TNF-α for various durations at different concentrations, the CCK8

| Gene   | Forward primer (5’–3’)          | Reverse primer (5’–3’)          |
|--------|---------------------------------|---------------------------------|
| miR-4530 | ATCAGGACGGGACGAAAAA       | CAGTGCGGTTCGTGGAGT               |
| miR-6852-5p | GCCTCCGTTGGTCTCTGAG    | GTGCAGGGTCCGAGGT                |
| miR-149-5p | GGCTCTGCTTCCGTGTGTTT  | CAGTGCGGTTCGTGGAGT               |
| miR-515-5p | CCGGTGCTTCCAAGAAAGACA | CAGCAGGAGGGACTAAGC               |
| miR-519e-5p | GCCTCTTCCAAAAAGGGAG   | CAGTGCGGTTCGTGGAGT               |
| miR-4433a-5p | GCCTGGGCGTCCACCCCCCAC | CAGTGCGGTTCGTGGAGT               |
| miR-4804-3p | GCCTGGTCTTTACCTTGGCC  | CAGTGCGGTTCGTGGAGT               |
| miR-6840-3p | GCCGCCGCCAGGATGTGTCGTGGAGT | CAGTGCGGTTCGTGGAGT               |
| miR-1827 | GGGGGAGGAGCATGATCTGAG   | CAGTGCGGTTCGTGGAGT               |
| miR-3065-3p | GCCCGAGGAGCACGATGATGTGCCG | CAGTGCGGTTCGTGGAGT               |
| miR-4492 | GGGGGAGGAGCATGATCTGAG   | CAGTGCGGTTCGTGGAGT               |
| miR-432-3p | GGGGGAGGAGCATGATCTGAG   | CAGTGCGGTTCGTGGAGT               |
| U6     | CTCGACTGAGGACGACATATACCT | GTGCAGGGAGGAGGAGG                |

Table I. miRs primers for RT-qPCR
miR-149-5p mitigates tumor necrosis factor-α-induced chondrocyte apoptosis by inhibiting TRADD

Expression of post-transcriptional regulators-miRNAs

miRNAs as post-transcriptional regulators have been reported in the process of chondrocyte apoptosis [3]. However, TRADD-related miRNAs have not been described in TNF-α-induced chondrocyte apoptosis. We used the bioinformatics algorithms TargetScan (www.targetscan.org) and miRDB (www.mirdb.org) to predict TRADD-related miRNAs, and the results showed that a total of 13 miRNAs were filtered by both TargetScan and miRDB. Among these miRNAs, 7 miRNAs and 2 miRNAs were significantly down-regulated and up-regulated, respectively, in TNF-α-treated chondrocytes compared with those of the control group. Otherwise, 4 miRNAs showed no significant difference between the two groups (Figure 2). According to the |log2 (fold change)|, miR-149-5p was the maximum value. Therefore, we focused on miR-149-5p in our further experiments.

miR-149-5p directly targeted TRADD

First, we found a conserved complementary sequence between the 3′-UTR of TRADD and miR-149-5p, which was predicted by TargetScan (Figure 3 A). As shown in Figure 3B, miR-149-5p mimics reduced luciferase activity significantly compared with the control group, while the luciferase activity showed no obvious change in chondrocytes co-transfected with the Mut 3′-UTR of TRADD and miR-149-5p mimics. Western blotting analysis indicated that miR-149-5p mimics significantly attenuated the expression of TRADD compared with the control group (Figure 3 C). Meanwhile, we performed a rescue experiment in miR-149-5p mimic transfected chondrocytes by transfecting TRADD overexpressed plasmids, which did not contain the 3′-UTR of TRADD. Therefore, they were not targeted by miR-149-5p. As shown in Figure 3 C, miR-149-5p mimics induced the down-regulation of TRADD protein expression, which was reversed by the transfection of TRADD plasmids.

miR-149-5p mimics inhibited TNF-α-induced growth inhibition, inflammation and apoptosis in chondrocytes, which were reversed by overexpression of TRADD

After treatment with chondrocytes by TNF-α (100 ng/ml) for 48 h, CCK8 assays showed that overexpression of miR-149-5p alleviated TNF-α-caused chondrocyte growth inhibition (Figure 4 A). Moreover, the TNF-α-induced increase in the production of inflammatory cytokines, including IL-1β, IL-6 and IL-18, was also attenuated by overexpression of miR-149-5p (Figures 4 B–D). Furthermore, an elevation of apoptotic cell proportion in TNF-α-treated chondrocytes was partially mitigated by miR-149-5p mimics (Figures 5 A, B). However, the protective effects of miR-149-5p mimics on TNF-α-induced growth inhibition, inflammation and apoptosis in chondrocytes were reversed by overexpression of TRADD (Figures 4 A–D, 5 A, B).

Discussion

The present study attempted to investigate the underlying molecular mechanisms involved in TNF-α-induced chondrocyte apoptosis. We discovered that TNF-α stimulation resulted in the reduction of miR-149-5p expression, which had the ability to relieve the inhibitory effect of miR-149-5p on its target gene. Therefore, the activity of TRADD and its downstream target caspase-3/8 signaling were activated in TNF-α-treated chondrocytes. Next, we transfected miR-149-5p mimics into chondrocytes, and overexpression of miR-149-5p repressed TNF-α-induced growth inhibition, an increase in the secretion of IL-1β, IL-6 and IL-18 in the supernatant liquid, and the elevation of apoptotic cell proportion, which were reversed by overexpression of TRADD. These results demonstrated that TNF-α exacerbated the secretion of inflammatory cytokines, including IL-1β, IL-6 and IL-18, and apoptosis in chondrocytes could be attenuated by miR-149-5p via the inhibition of TRADD signaling (Figure 6).

TRADD as a transducer of TNF-α/TNFR1 signaling modulates TNF-α-induced apoptosis by recruiting RIP, TRAF2, and FADD to activate caspase-8 and caspase-3 [27]. TRADD is expressed at high levels in many different cellular models of TNF-α-induced apoptosis [11, 13, 28]. Other studies reveal that TNF/TNFRI signal transduction and their downstream targets, TRADD, caspase-8 and caspase-3, are involved in injurant-induced chondrocyte apoptosis in vitro [5, 14]. In the present study, we found that TNF-α treatment dramatically suppressed cell viability and elevated the produc-
Figure 1. TNF-α induced chondrocyte damage. A – CCK8 assays were performed to detect cell viability with or without TNF-α (100 ng/ml) treatment. B – ELISA assays were used to measure inflammatory cytokines. C – TNF-α-induced apoptosis in chondrocytes was analyzed by flow cytometry. D, E – Protein expression was measured by western blotting. n = 3 in each group, * p < 0.05 compared with control group.
miR-149-5p mitigates tumor necrosis factor-α-induced chondrocyte apoptosis by inhibiting TRADD

**Figure 2.** Expression of TRADD-associated miRNAs. Bioinformatics algorithms, TargetScan and miRDB, were used to predict TRADD-related miRNAs, and the expression of 13 miRNAs was measured using RT-qPCR in chondrocytes with or without TNF-α (100 ng/ml) treatment. n = 3 in each group, *p < 0.05 compared with control group

**Figure 3.** miR-149-5p directly targeted TRADD. A – The conserved complementary sequence between the 3'-UTR of TRADD and miR-149-5p was predicted by TargetScan. B – Luciferase activity was measured in chondrocytes after transfection with WT or Mut 3'-UTR of TRADD combined with miR-149-5p mimics or miR-Con. C – After transfection with miR-149-5p mimics or miR-149-5p mimics combined with TRADD vector into chondrocytes, the protein expression of TRADD was measured by western blotting. n = 3 in each group, *p < 0.05 and **p < 0.001
Figure 4. miR-149-5p mimics inhibited TNF-α-induced growth inhibition and inflammation. After transfection with miR-149-5p mimics or miR-149-5p mimics combined with TRADD vector into chondrocytes with or without TNF-α treatment, cell viability was measured by CCK8 assays (A); the production of inflammatory cytokines, including IL-1β, IL-6 and IL-18, was detected by ELISA assays (B–D). n = 3 in each group, * p < 0.05.
miR-149-5p mitigates tumor necrosis factor-α-induced chondrocyte apoptosis by inhibiting TRADD

**Figure 5.** miR-149-5p mimics inhibited TNF-α-induced apoptosis. A, B – After transfection with miR-149-5p mimics or miR-149-5p mimics combined with TRADD vector into chondrocytes with or without TNF-α treatment, cell apoptosis was measured by flow cytometry. n = 3 in each group, p < 0.05
findings suggest that miR-149-5p may function as an anti-apoptotic mediator in extrinsic pro-apoptotic stimulus-triggered cell death. However, in contrast to its anti-apoptotic role in normal cells, acute myeloid leukemia cell line THP-1 apoptosis is decreased by miR-149-5p overexpression and increased by miR-149-5p suppression; the underlying molecular mechanism was modulated by targeting the fas ligand [32]. These results indicate that miR-149-5p may perform different functions in the process of apoptosis with different diseases. In our research, up-regulation of miR-149-5p attenuated TNF-α-evoked chondrocyte apoptosis through post-transcriptional repression of TRADD. These results suggest that the miR-149-5p/TRADD pathway might be a novel therapeutic strategy for the treatment of OA.

In conclusion, we elucidated a novel regulatory mechanism in which miR-149-5p as a post-transcriptional regulator inversely regulated TNF-α-mediated chondrocyte growth inhibition, apoptosis and the inflammatory response by inhibiting TRADD cascade signaling.

Conflict of interest

The authors declare no conflict of interest.

References

1. Wei Y, Zheng D, Guo X, Zhao M, Gao L, Bai L. Transient receptor potential channel, vanilloid 5, induces chondrocyte apoptosis in a rat osteoarthritis model through the mediation of Ca2+ influx. Cell Physiol Biochem 2018; 46: 687-98.
2. Jin L, Zhao J, Jing W, et al. Role of miR-146a in human chondrocyte apoptosis in response to mechanical pressure injury in vitro. Int J Mol Med 2014; 34: 451-63.
3. Li J, Huang J, Dai L, et al. miR-146a, an IL-1beta responsive miRNA, induces vascular endothelial growth factor and chondrocyte apoptosis by targeting Smad4. Arthritis Res Ther 2012; 14: R75.
4. Zhang C, Lin S, Li T, et al. Mechanical force-mediated pathological cartilage thinning is regulated by necrosis and apoptosis. Osteoarthritis Cartilage 2017; 25: 1324-34.
5. Zhang FT, Ding Y, Shah Z, et al. TNF/TNFRI(1) pathway and endoplasmic reticulum stress are involved in oxifloxacin-induced apoptosis of juvenile canine chondrocytes. Toxicol Appl Pharmacol 2014; 276: 121-8.
6. Kayal RA, Siqueira M, Albowi J, et al. TNF-alpha mediates diabetes-enhanced chondrocyte apoptosis during fracture healing and stimulates chondrocyte apoptosis through FOXO1. J Bone Miner Res 2010; 25: 1604-15.
7. Chen H, Wang J, Hu B, et al. miR-34a promotes Fas-mediated cartilage endplate chondrocyte apoptosis by targeting Bcl-2. Mol Cell Biochem 2015; 406: 21-30.
8. Ito K, Maruyama Z, Sakai A, et al. Overexpression of Cdk6 and Cdc1 in chondrocytes inhibited chondrocyte maturation and caused p53-dependent apoptosis without enhancing proliferation. Oncogene 2014; 33: 1862-71.
9. Zou J, Li XL, Shi ZM, Xue JF. Effects of C-myc gene silencing on interleukin-1beta-induced rat chondrocyte cell proliferation, apoptosis and cytokine expression. J Bone Miner Metab 2018; 36: 286-96.
10. Peng M, Wang Y, Qiang L, et al. Interleukin-35 inhibits TNF-alpha-induced neuronal cell apoptosis through miR-485-5p/TRADD signaling. Biochem Biophys Res Commun 2016; 478: 1304-9.
11. He KL, Ting AT. A20 inhibits tumor necrosis factor (TNF) receptor-associated death domain (TRADD)-TRAF2 to TRADD-Fas-associated death domain by JAK1/STAT1. Front Immunol 2018; 9: 1417.
12. Chand Z, Zhang Z, Zhang D, Li H, Sun Z. Hydrogen sulfide protects against TNF-alpha-induced neuronal apoptosis via shifting the activation from TNF receptor-associated death domain (TRADD)-TRAF2 to TRADD-Fas-associated death domain by JAK1/STAT1. Front Immunol 2018; 9: 1417.
13. Gaud G, Guillermot D, Jacob Y, Favre M, Vuiller F. EVER2 protein binds TRADD to promote TNF-alpha-induced apoptosis. Cell Death Dis 2013; 4: e499.
14. He KL, Ting AT. A20 inhibits tumor necrosis factor (TNF) alpha-induced apoptosis by disrupting recruitment of TRADD and RIP to the TNF receptor 1 complex in Jurkat T cells. Mol Cell Biol 2002; 22: 6034-45.
15. Qin J, Shang L, Ping AS, et al. TNF/TNF signal transduction pathway-mediated anti-apoptosis and anti-inflammatory effects of sodium ferulate on IL-1beta-induced rat osteoarthritis chondrocytes in vitro. Arthritis Res Ther 2012; 14: R242.
16. Soroosh A, Koutsoumpa M, Pothoulakis C, Ilopoulos D. Functional role and therapeutic targeting of microRNAs in inflammatory bowel disease. Am J Physiol Gastrointest Liver Physiol 2018; 314: G256-62.
17. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-97.
18. Alvarez-Garcia I, Miska EA. MicroRNA functions in animal development and human disease. Development 2005; 132: 4653-62.
miR-149-5p mitigates tumor necrosis factor-α-induced chondrocyte apoptosis by inhibiting TRADD

19. Baek D, Lee KM, Park KW, et al. Inhibition of miR-449a promotes cartilage regeneration and prevents progression of osteoarthritis in in vivo rat models. Mol Ther Nucleic Acids 2018; 13: 322-33.

20. Liu P, Chen Y, Wang B, Wang Z, Li C, Wang Y. Expression of microRNAs in the plasma of patients with acute gouty arthritis and the effects of colchicine and etoricoxib on the differential expression of microRNAs. Arch Med Sci 2019; 15: 1047-56.

21. Hu G, Zhao X, Wang C, et al. MicroRNA-145 attenuates TNF-alpha-driven cartilage matrix degradation in osteoarthritis via direct suppression of MKK4. Cell Death Dis 2017; 8: e3140.

22. Zhang G, Sun Y, Wang Y, Liu R, Bao Y, Li Q. MiR-502-5p inhibits IL-1beta-induced chondrocyte injury by targeting TRAF2. Cell Immunol 2016; 302: 50-7.

23. Zou Y, Kong M. Tetrahydroxy stilbene glucoside alleviates palmitic acid-induced inflammation and apoptosis in cardiomyocytes by regulating miR-129-3p/Smad3 signaling. Cell Mol Biol Lett 2019; 24: 5.

24. Yin T, Liu MM, Jin RT, Kong J, Wang SH, Sun WB. miR-152-3p Modulates hepatic carcinogenesis by targeting cyclin-dependent kinase 8. Pathol Res Pract 2019; 215: 152406.

25. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 2001; 25: 402-8.

26. Yu FY, Xie CQ, Sun JT, Peng W, Huang XW. Overexpressed miR-145 inhibits osteoclastogenesis in RANKL-induced bone marrow-derived macrophages and ovariectomized mice by regulation of Smad3. Life Sci 2018; 202: 11-20.

27. Jin Z, El-Deiry WS. Distinct signaling pathways in TRAIL-versus tumor necrosis factor-induced apoptosis. Mol Cell Biol 2006; 26: 8136-48.

28. Chang X, Wang L, Wang Z, et al. TRADD mediates the tumor necrosis factor-induced apoptosis of L929 cells in the absence of RIP3. Sci Rep 2017; 7: 16111.

29. Li YF, Li SH, Liu Y, Luo YT. Long noncoding RNA CIR promotes chondrocyte extracellular matrix degradation in osteoarthritis by acting as a sponge for Mir-27b. Cell Physiol Biochem 2017; 43: 602-10.

30. Ruan Q, Liu Y, Wang X, Yang D, Sun Y. miR-149-5p protects against high glucose-induced pancreatic beta cell apoptosis via targeting the BH3-only protein BIM. Exp Mol Pathol 2019; 110: 104279.

31. Grieco FA, Sebastiani G, Juan-Mateu J, et al. MicroRNAs miR-23a-3p, miR-23b-3p, and miR-149-5p regulate the expression of proapoptotic BH3-only proteins DPS and PUMA in human pancreatic beta-cells. Diabetes 2017; 66: 100-12.

32. Tian P, Yan L. Inhibition of microRNA-149-5p induces apoptosis of acute myeloid leukemia cell line THP-1 by targeting Fas ligand (FASLG). Med Sci Monitor 2016; 22: 5116-23.