Exosomes derived from circBCRC-3-knockdown mesenchymal stem cells promoted macrophage polarization

Qi SONG1; JUN ZHANG1; QIANG ZHANG1; JING LIU1; KE LV1; JIALU YAO1,2,3,*; YAFENG ZHOU2,3,*

1 Department of Cardiology, Suzhou Municipal Hospital Affiliated to Nanjing Medical University, Suzhou, 215000, China
2 Department of Cardiology, Dushuhu Public Hospital Affiliated to Soochow University, Suzhou, 215000, China
3 Department of Cardiology, The First Affiliated Hospital of Soochow University, Suzhou, 215000, China

Key words: Exosome, CircBCRC-3, Mesenchymal stem cell, Myocardial ischemia-reperfusion injury

Abstract: Macrophages play an essential role in the myocardial ischemia-reperfusion injury (MIRI), and the macrophage shifting from M1 to M2 phenotypes might be a potential strategy for the treatment of MIRI. It has been reported that miR-182 plays an important role in MSC-Exo-associated macrophage polarization. As circBCRC-3 is a newly discovered circle RNA that worked as a sponge of miR-182, this research aimed to find if circBCRC-3 plays a role in MSC-Exo-associated macrophage polarization. Firstly, circBCRC-3 was identified by divergent primers in mesenchymal stem cells (MSCs). Secondly, the exosome of MSCs was isolated and identified by transmission electron microscopy (TEM), nanoparticle-tracking analysis, and western blotting analysis. The expression level of circBCRC-3 in MSCexos was detected by RT-PCR. Finally, the polarization of the RAW264.7 cell phenotype was analyzed by flow cytometry. Moreover, we first identified circBCRC-3 in MSCs. The results further confirmed that MSCexo could effectively shift the macrophage polarization state from M1 towards the M2 phenotype, which indicated its role in MIRI cure.

Introduction

Authors Acute myocardial infarction (MI) has been one of the leading causes of death in the world. The reperfusion therapy, which is a common process to MI patients, could cause myocardial ischemia-reperfusion injury (MIRI) that triggers an inflammatory cascade reaction in the myocardial cells (Hausenloy and Yellon, 2013). The macrophage-associated immune response plays an important role in MIRI. After reperfusion, macrophages in M1 status create a pro-inflammatory environment and clear away dead cells. Later, macrophages in M2 status through anti-inflammatory cytokines, secrete growth factors, processing scar formation. Thus, the two macrophage phenotypes and the regulations of changing two statuses are important for infarct healing (Ong et al., 2018). By promoting earlier and more M2 macrophage infiltration, shifting the balance between M1 and M2 macrophages might be a potential way of treating MIRI.

Exosomes are membrane nanovesicles that exist in almost all biological fluids (Elahi et al., 2020; Kourembanas, 2015; Raposo and Stoorvogel, 2013). They were reported as the important mediator of paracrine mechanisms and potential clinic applications in lung injury, cardiovascular disease, regenerative medicine, and therapy of inflammatory diseases (Elahi et al., 2020; Liu et al., 2020; Ha et al., 2020; Harrell et al., 2019; Wang et al., 2020; Chen et al., 2020). Recent studies have shown that exosomes were associated with many pathological and physiological conditions (Chen et al., 2020; Elahi et al., 2020; Ha et al., 2020; Harrell et al., 2019; Liu et al., 2020; Wang et al., 2020). Emerging evidence suggests that exosome derived from a mesenchymal stem cell (MSC) (MSCexo) could exert beneficial effects on some diseases, including MI (Liu et al., 2017; Ma et al., 2018; Zhang et al., 2016; Zhao et al., 2015), hepatic fibrosis (Li et al., 2013; Jiang et al., 2018; Qu et al., 2017), and cancers (Phinney and Pittenger, 2017; Ono et al., 2014; Kim et al., 2018; Lee et al., 2013; Qi et al., 2017; Reza et al., 2016).

CircRNA is a new member of non-coding RNAs, produced by a back-splicing event from pre-mRNA. CircRNA could tolerate the digestion of exonuclease for lacking 5’ cap and 3’ poly (A), which suggests that it is much more stable than linear RNA. Moreover, circRNA...
mainly locates in the cytoplasm and exerts its function by acting as a sponge of miRNAs (Hansen et al., 2013; Jeck et al., 2013). It only has been revealed that circDLPAG4 (Chen et al., 2020; Wang et al., 2019), circNCXI (Li et al., 2018), and circACR (Zhou et al., 2019) could play roles in MIRI. Recently, it was reported that miR-182 in MSCexo played a role in the macrophage polarization of MIRI (Zhao et al., 2019), and circBCRC-3 could bind to miR-182 (Xie et al., 2018). Therefore, we speculate that circBCRC-3 may play a regulatory role in the macrophage polarization of MIRI. In this study, we showed that an exosome derived from a circBCRC-3 knockdown mesenchymal stem cell could promote macrophage polarization indicating its potential in MIRI cure.

Materials and Methods

Isolation of exosome of mesenchymal stem cell (MSCexo) Two 6–8 weeks-old c57bl/6 mice were sacrificed. The femurs and tibias of the mice were collected through centrifugation (1000 rpm, 10 min). MSCs were resuspended with 5 mL PBS and washed twice. Then, MSCs were resuspended in DMEM and maintained cell culture as described. The ExoQuick™ Plasma Prep and Exosome Precipitation Kit (SB System Biosciences, USA) were used to isolate MSCexo from the MSC cell supernatants according to the manufacturer’s instructions.

Cell culture

RAW264.7 cells (from ATCC) were cultured in DMEM medium (Gibco, Gaithersburg, MD, USA) containing 10% fetal bovine serum (FBS) at a cell culture incubator (Thermo Scientific HeraCell 240i) at 37°C. For M1 macrophage induction, 500 ng/mL LPS was used.

Reverse Transcription polymerase chain reaction (PCR) assay Total RNA was extracted from cells using TRIzol Reagent (Invitrogen; Carlsbad, CA, USA) according to the manufacturer's instructions. Using a Primerscript RT reagent kit with gDNA Eraser, the cDNA was then synthesized with reverse transcriptase (RTase) following the manufacturer's instructions. Real-time-polymerase chain reactions (RT-PCR) were run using SYBR® Premix Ex Taq™ II (Tli RNaseH Plus) (TaKaRa), following manufacturer’s instructions. CircRNA BCRC-3 siRNA and transfection A siRNA against the reverse splicing site of Circular RNA BCRC-3 was designed with the online prediction tool (Siridict2), with the target sequence CTTTGGCTATAACGGTTGC. MSC cell transfection was performed according to the manufacturer’s instruction. 5 μL Lipofectamine 2000 (Invitrogen, USA) was added to 50 μL optiMEM (Invitrogen, USA), and 2 μg siRNA was added into 50 μL optiMEM; two solutions were mixed and stayed for 15 min. The siRNA mixture was then added into the serum-free cell culture; after incubating for 6 h, the cell culture was changed to the complete medium. After 48 h, the cell culture was collected to isolate exosomes as described.

Western blotting

Western blotting was performed using the ECL Western Blotting Substrate Kit (Abnova) and antibodies, Calnexin (Abcam, ab22595), CD63 (Abcam, ab216230), TSG101 (Abcam, ab30871).

Flow cytometry analysis

Cells were resuspended and adjusted to a concentration of 1 × 10⁶ cells/mL in staining buffer. After 48 h treatment, cells were stained for the antibody iNOS-FITC (FabGennix, P35228) and PI (50 μg/mL) and kept in the dark. All samples were then run on a BD Accuri™ C6 (BD Bioscience) with a four-color (FITC, PE, PerCP Cy5.5, and APC) fluorescence flow cytometry analysis.

Statistical analysis

All data analyses were completed using R Statistical Software (v 2.15.0, http://www.r-project.org/). Analysis of variance (ANOVA) was used to determine the differences in circBCRC-3 expression levels between groups. The p-values which are smaller than 0.05 were regarded as statistically significant.

Results

Identification of circBCRC-3 in MSCs CircBCRC-3 (circBase ID: hsa_circ_0001110) is located at proteasome 26S subunit, non-ATPase 1 (PSMD1) gene locus, and its post-splicing sequence length is 1002 bp (Fig. 1A). Agarose gel electrophoresis was used to determine the specificity of the PCR product of circBCRC-3. Divergent

| Name of Primer | Primer’s Sequence |
|----------------|-------------------|
| divergent–H-BCRC3-F | GTCAGGAGGGCAACGATAGA |
| divergent–H-BCRC3-R | AACTCAATAGCCATTTCCAC |
| convergent–H-BCRC3-F | CTTTGGCTATAACGGTTGC |
| convergent–H-BCRC3-R | GAAATGGTGTAGGGATTTC |
| convergent–H-GAPDH-F | GAATGAGGCGTGCAGTC |
| convergent–H-GAPDH-R | CAAATGCCCTCATTGAGC |
| convergent–H-GAPDH-R | TTGATTTTGAGGGATCTCG |
primers (Tab. 1) detected circular RNA BCRC-3 in cDNA but not genomic DNA (gDNA) (Fig. 1B), which further characterize its circular form.

Characterization of exosome of MSC
Multiple approaches were employed to characterize the morphology features and molecular markers of the isolated extracellular vesicles of MSC in order to identify exosomes. The MSCexo were studied under transmission electron microscopy (TEM). The morphological features of exosomes could be clearly observed: a round or elliptical shape with a diameter range of 30–100 nm (Fig. 2A). Then, the size distribution of extracellular vesicles was assessed with the nanoparticle-tracking analysis, which showed that the mean size of extracellular vesicles was 132.5 ± 37.4 nm; and most of the extracellular vesicles were distributed within the range of the exosome diameter (30–150 nm) (Fig. 2B). Moreover, the expressions level of CD63 and TSG101 (molecular markers of exosomes) were determined using western blotting analysis. High levels of CD63 and TSG101 were detected in the isolated exosomes, whereas little calnexin (a molecular marker of the endoplasmic reticulum) could be found (Fig. 2C). Meanwhile, the expressions of circBCRC-3 in MSCexo and MSCs were compared. The RT-PCR results indicated that the expression of circBCRC-3 was much higher in purified exosomes than in donor MSCs (Fig. 2D). The difference was significant (p < 0.05*).

CircBCRC-3 involvement in MSCexo mediated macrophage polarization in vitro
To study the effects of circBCRC-3 on macrophage polarization, flow cytometry analysis was performed to detect the levels of M1 (iNOS-CD206+ and M2 (iNOS CD206−) markers. After the lipopolysaccharide (LPS) treatment, most raw264.7 cells transformed into M1 macrophage (iNOS-CD206−). With siRNA treatment, the percentage of M2 macrophage (iNOS CD206−, the second quadrant) was elevated (Fig. 3A). The results of Fig. 3A demonstrated that the polarization of macrophages from M1 to M2 under the inflammatory environment was facilitated by treating with circBCRC-3 siRNA, and it suggested that circBCRC-3 might be the key regulatory factor determining the macrophage polarization.
To confirm the role of circBCRC-3 in MSCexo, MSCs were transfected with circBCRC-3 siRNA, and the exosomes were subsequently isolated from the culture supernatants. As circBCRC-3 siRNA was fluorescein amidites (FAM)-labeled, it was seen under a fluorescence microscope (Fig. 3B). RT–PCR analysis revealed that the expression level of circBCRC-3 was significantly decreased in circBCRC-3 siRNA transfected MSCexo compared to negative control (NC) siRNA transfected MSCexo (Fig. 3C). LPS-stimulated macrophages were then treated with NC siRNA MSCexo or circBCRC-3 siRNA MSCexo for 48 h, and then the cells were collected for flow cytometry analysis. Compared to the LPS treatment group, myocardial macrophages treated with the circBCRC-3 siRNA transfected exosomes showed more percentage of M2 macrophage (iNOS<sup>−</sup>CD206<sup>+</sup>) (Fig. 3D). The result showed that the polarization of macrophages from M1 to M2 was significantly elevated by circBCRC-3 siRNA MSCexo (Fig. 3D), suggesting that MSCexo from circBCRC-3 knocked down MSC could be a key factor that affected macrophage polarization.

**Discussion**

Macrophages are central inflammatory mediators of the heart tissue, involving in both the initiation and resolution of the inflammatory process. Multiple reports have highlighted the significance of macrophages in MIRI models (de Couto et al., 2017; de Couto et al., 2015). Increasing evidence suggested that MSC could trigger the macrophage to switch to the anti-inflammatory M2 phenotype (Kudlik et al., 2016; Ben-Mordechai et al., 2013). Our work further confirmed that MSCexo could effectively shift the macrophage polarization state from M1 towards the M2 phenotype. Stem cells have a strong ability of proliferation, and multi-directional differentiation and could secrete chemokines, growth factors, microbubbles, cytokines, and exosomes to...
the injured site, which promotes the differentiation, proliferation, and chemotaxis of the injured site cells. Among these secretions, exosomes play an important role in signal transduction, intercellular transportation, and tissue regeneration (Zhang et al., 2015; Hu et al., 2015).

Mammalian macrophages are induced to a variety of phenotypes in response to different external stimuli. Some researchers have noted that the change of a subset of miRNA expression was repeatedly found to be involved in the macrophage polarization (Chen et al., 2009; Cheng et al., 2012; Forrest et al., 2010; Cai et al., 2012; Zhang et al., 2013; Rückerl et al., 2012; Chaudhuri et al., 2011). CircRNAs, always as miRNA sponges, are stable transcripts expressed from different genomic locations and have been recently recognized as important regulators for cellular miRNA abundance and thus are major players in the miRNA-mediated post-transcriptional regulatory network. With the interactions between circRNAs and miRNAs, circRNAs are potentially involved in many disease processes, cell processes, and gene expressions (Memczak et al., 2013; Ghosal et al., 2013).

As a circBCRC-3 is a sponge of miR-182, we testified that circBCRC-3 knockdown MSCexo could promote macrophage changed from M1 to M2, which indicated its role in MIRI therapy. Although our data provided macrophage polarization (Chen et al., 2009; Cheng et al., 2012; Forrest et al., 2010; Cai et al., 2012; Zhang et al., 2013; Rückerl et al., 2012; Chaudhuri et al., 2011). CircRNAs, always as miRNA sponges, are stable transcripts expressed from different genomic locations and have been recently recognized as important regulators for cellular miRNA abundance and thus are major players in the miRNA-mediated post-transcriptional regulatory network. With the interactions between circRNAs and miRNAs, circRNAs are potentially involved in many disease processes, cell processes, and gene expressions (Memczak et al., 2013; Ghosal et al., 2013).

Availability of Data and Materials: All data generated or analyzed during this study are included in this manuscript.

Funding Statement: This study was funded by the National Natural Science Foundation of China [81702235, 81873486], Natural Scientific Fund of Jiangsu province [BK20161226], Jiangsu Province’s Key Provincial Talents Program [ZDRC2016043], Jiangsu Province’s 333 High-Level Talents Project [BRA2017539]. The funders had no roles in study design, data collection, and analysis, decision to publish, or preparation of the manuscript.

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

References

Ben-Mordechai T, Holbva R, Landa-Rouben N, Harel-Adar T, Feinberg MS, Elrahman IA, Blum G, Epstein FH, Silman Z, Cohen S, Leor J (2013). Macrophage subpopulations are essential for infarct repair with and without stem cell therapy. Journal of the American College of Cardiology 62: 1890–1901. DOI 10.1016/j.jacc.2013.07.057.

Cai X, Yin Y, Li N, Zhu D, Zhang J, Zhang CY, Zen K (2012). Re-polarization of tumor-associated macrophages to pro-inflammatory M1 macrophages by miRNA-155. Journal of Molecular Cell Biology 4: 341–343. DOI 10.1093/jmcb/mjs044.

Chaudhuri AA, So AY, Sinha N, Gibson WS, Taganov KD, O’Connell RM, Baltimore D (2011). MicroRNA-125b potentiates macrophage activation. Journal of Immunology 187: 5062–5068. DOI 10.4049/jimmunol.1102001.

Chen L, Luo W, Zhang W, Chu H, Wang J, Dai X, Cheng Y, Zhu T, Chao J (2020). circDLPA.G4/HECTD1 mediates ischemia/reperfusion injury in endothelial cells via ER stress. RNA Biology 17: 240–253. DOI 10.1080/15476286.2019.1676114.

Chen T, Huang Z, Wang L, Wang Y, Wu F, Meng S, Wang C (2009). MicroRNA-125a-5p partly regulates the inflammatory response, lipid uptake, and ORP9 expression in oxLDL-stimulated monocyte/macrophage. Cardiovascular Research 83: 131–139. DOI 10.1093/cvr/cvp121.

Cheng Y, Kuang W, Hao Y, Zhang D, Lei M, Du L, Jiao H, Zhang X, Wang F (2012). Downregulation of miR-27a* and miR-552-5p and upregulation of miR-146a and miR-155 in LPS-induced RAW264.7 macrophage cells. Inflammation 35: 1308–1313. DOI 10.1007/s10753-012-9443-8.

de Couto GD, Liu W, Tseliou E, Sun B, Makkar N, Kanazawa H, Arditi M, Marban E (2015). Macrophages mediate cardioprotective cellular postconditioning in acute myocardial infarction. Journal of Clinical Investigation 125: 3147–3162. DOI 10.1172/JCI81321.

de Couto G, Let Gal R, Lamberts E, Makkar N, Dawkins JP, Berman BP, Marban E (2017). Exosomal microRNA transfer into macrophages mediates cellular postconditioning. Circulation 136: 200–214. DOI 10.1161/CIRCULATIONAHA.116.024590.

Elahi FM, Farwell DG, Nolta JA, Anderson JD (2020). Preclinical translation of exosomes derived from mesenchymal item/stromal cells. Stem Cells 38: 15–21. DOI 10.1002/stem.3061.

Forrest AR, Kanamori-Katayama M, Tomaru Y, Lassmann T, Ninomiya N, Takahashi Y, de Hoon MJ, Kubosaki A, Kihara A, Suzuki M, Yasuda J, Kawai J, Hayashizaki Y, Hume DA, Suzuki H (2010). Induction of microRNAs, mir-155, mir-222, mir-424 and mir-503, promotes monocytic differentiation through combinatorial regulation. Leukemia 24: 460–466. DOI 10.1038/leu.2009.246.

Ghosal S, Das S, Sen R, Basak P, Chakrabarti J (2013). Circ2Traits: A comprehensive database for circular RNA potentially associated with disease and traits. Frontiers in Genetics 4: 283. DOI 10.3389/fgen.2013.00283.

Ha DH, Kim HK, Lee J, Kwon HH, Park GH, Yang SH, Jung YJ, Choi H, Lee JH, Sung S, Yi TW, Cho BS (2020). Mesenchymal stem/stromal cell-derived exosomes for immunomodulatory therapies and skin regeneration. Cells 9: 1157. DOI 10.3390/cells9051157.

Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK, Kjems J (2013). Natural RNA circles function as efficient microRNA sponges. Nature 495: 384–388. DOI 10.1038/nature11993.

Harrell CR, Jovicic N, Djonov V, Arsenijevic N, Volarevic V (2019). Mesenchymal stem cell-derived exosomes and other extracellular vesicles as new remedies in the therapy of inflammatory diseases. Cells 8: 1605. DOI 10.3390/cells8121605.

Hausenloy DJ, Yellon DM (2013). Myocardial ischemia-reperfusion injury: A neglected therapeutic target. Journal of Clinical Investigation 123: 92–100. DOI 10.1172/JCI62874.

Hu G, Li Q, Niu X, Hu B, Liu J, Zhou SM, Guo SC, Lang HL, Zhang CQ, Wang Y, Deng ZF (2015). Exosomes secreted by human-induced pluripotent stem cell-derived mesenchymal stem cells attenuate limb ischemia by promoting angiogenesis in mice. Stem Cell Research & Therapy 6: 10. DOI 10.1186/s13287-015-0346.

Jeck WR, Sorrentino JA, Wang K, Slevin MK, Burd CE, Liu J, Marzluff WF, Sharpless NE (2013). Circular RNAs are...
abundant, conserved, and associated with ALU repeats. RNA 19: 141–157. DOI 10.1261/rna.035667.112.

Jiang W, Tan Y, Cai M, Zhao T, Mao F, Zhang X, Xu W, Yan Z, Qian H, Yan Y (2018). Human umbilical cord MSC- derived exosomes suppress the development of CCl4-induced liver injury through antioxidant effect. Stem Cells International 2018: 6079642.

Kim R, Lee S, Lee J, Kim M, Kim WJ, Lee HW, Lee MY, Kim J, Chang W (2018). Exosomes derived from microRNA-584 transfected mesenchymal stem cells: Novel alternative therapeutic vehicles for cancer therapy. BMB Reports 51: 406–411. DOI 10.5483/BMRRep.2018.51.8.105.

Kourembanas S (2015). Exosomes: Vehicles of intercellular signaling, biomarkers, and vectors of cell therapy. Annual Review of Physiology 77: 13–27. DOI 10.1146/annurev-physiol-021014-071641.

Kudlik G, Hegyi B, Czibula A, Monostori E, Buday L, Uher F (2016). Review of exosomes derived from bone marrow stem cells. Stem Cells and Development 25: 817–827. DOI 10.1089/scd.2015.761643.

Li T, Yan Y, Wang B, Qian H, Zhang X, Shen L, Wang M, Zhou Y, Zhu W, Li W, Xu W (2013). Exosomes derived from human umbilical cord mesenchymal stem cells alleviate liver fibrosis. Stem Cells and Development 22: 845–854. DOI 10.1089/scd.2012.0395.

Liu A, Zhang X, He H, Zhou L, Naito Y, Sugita S, Lee JW (2020). Extracellular vesicles: Exosomes, microvesicles, and friends. Journal of Cell Biology 200: 373–383. DOI 10.1083/jcb.201211138.

Wang R, Ji Q, Meng C, Liu H, Fan C, Lipkind S, Wang Z, Xu Q (2020). Role of gingival mesenchymal stem cell exosomes in macrophage polarization under inflammatory conditions. International Immunopharmacology 81: 106030. DOI 10.1016/j.intimp.2019.106030.

Wang S, Chen J, Yu W, Deng F (2019). Circular RNA DLGAP4 ameliorates cardiomyocyte apoptosis through regulating BCL2 via targeting miR-143 in myocardial ischemia-reperfusion injury. Journal of Cardiology 279: 147. DOI 10.1016/j.jccard.2018.09.023.

Xie F, Li Y, Wang M, Huang C, Tao D, Zheng F, Zhang H, Zeng F, Xiao X, Jiang G (2018). Circular RNA BCRC-3 suppresses bladder cancer proliferation through miR-182-5p/p27 axis. Molecular Cancer 17: 144. DOI 10.1186/s12943-018-0892-z.

Zhao J, Li X, Hu J, Chen F, Qiao S, Sun X, Gao L, Xie J, Xu B (2019). Mesenchymal stromal cell-derived exosomes attenuate myocardial ischaemia-reperfusion injury through miR-182-regulated macrophage polarization. Cardiovascular Research 115: 1205–1216. DOI 10.1093/cvr/cvz040.

Zhao Y, Sun X, Cao W, Ma J, Sun L, Qian H, Zhu W, Xu W, Sliujter J (2015). Exosomes derived from human umbilical cord mesenchymal stem cells relieve acute myocardial ischemic injury. Stem Cells International 2015: 12. DOI 10.1155/2015/761643.

Zhang J, Guan J, Niu X, Hu G, Guo S, Li Q, Xie Z, Zhang C, Wang Y (2015). Exosomes released from human induced pluripotent stem cells-derived MSCs facilitate cutaneous wound healing by promoting collagen synthesis and angiogenesis. Journal of Translational Medicine 13: 49. DOI 10.1186/s12967-015-0417-0.

Zhang Z, Yang J, Yan W, Li Y, Shen Z, Asahara T (2016). Pretreatment of cardiac stem cells with exosomes derived from bone marrow mesenchymal stem cells rescue myocardial ischaemia-reperfusion injury by inducing cardiomyocyte autophagy. Journal of Cellular and Molecular Medicine 21: 2491–2502. DOI 10.1111/jcmm.13170.
from mesenchymal stem cells enhances myocardial repair. *Journal of the American Heart Association* 5: e002856.

Zhou LY, Zhai M, Huang Y, Xu S, An T, Wang YH, Zhang RC, Liu CY, Dong YH, Wang M, Qian LL, Ponnusamy M, Zhang YH, Zhang J, Wang K (2019). The circular RNA ACR attenuates myocardial ischemia/reperfusion injury by suppressing autophagy via modulation of the Pink1/FAM65B pathway. *Cell Death & Differentiation* 26: 1299–1315. DOI 10.1038/s41418-018-0206-4.

Zhang Y, Zhang M, Zhong M, Suo Q, Lv K (2013). Expression profiles of miRNAs in polarized macrophages. *International Journal of Molecular Medicine* 31:797–802. DOI 10.3892/ijmm.2013.1260.