MiR-143 Induces Expression of AIM2 and ASC in Jurkat Cell Line

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ABSTRACT

Background: Absent in Melanoma 2 (AIM2) is an intracellular microbial dsDNA sensor which plays an important role in production of proinflammatory cytokines through Apoptosis associated Speck-like protein containing a Caspase activation and recruitment domain (ASC) and Caspase-1. Micro-RNAs (miRNAs) play important roles in regulation of immune related genes. However, there is little information regarding the effects of miRNAs on the AIM2 and ASC expression. **Objective:** To determine the mRNA levels of AIM2 and ASC in Jurkat cell line following introducing miRNA-143 (MiR-143). **Methods:** MiR-143, a scrambled sequence and PBS were introduced separately, to the Jurkat cell lines and the mRNA levels of AIM2 and ASC were examined in parallel with beta-actin and GAPDH (as housekeeping genes) using Real-Time PCR technique. **Results:** The mRNA levels of AIM2 and ASC were significantly increased in the MiR-143 transfected Jurkat cells when compared to the scrambled sequence or PBS treated cells. **Conclusions:** MiR-143 can lead to increased expression of AIM2 and ASC mRNAs. Considering the significance of AIM2 and ASC in DNA sensing and inflammosome formation, it can be considered as a therapeutic agent for the treatment of chronic infectious diseases, especially viral infections.

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INTRODUCTION

AIM2, as an inflammasome, is a main intracellular receptor which recognizes microbial, viral, and host dsDNA to active pro-IL-1β and IL-18, the two inflammatory cytokines, via ASC and caspase-1 activation (1,2). Both activated IL-1β and IL-18 induce expression of several inflammatory molecules such as selectins, integrins and co-stimulatory molecules as well as neutrophils infiltration, fever, etc. (3). Therefore, altered expressions of AIM2 and ASC may affect immune responses during inflammatory based diseases including autoimmune and dsDNA bearing infectious diseases. Previous studies demonstrated that mRNA levels of AIM2 and ASC were significantly changed during autoimmune and chronic microbial infections (2,4). Therefore, regulation of expression of these transcripts can be considered as a potent future molecular therapy. MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression by alteration in the stability, longevity and translation of transcripts (5). Interestingly, previous bioinformatic approaches hypothesized that miRNAs may regulate the expression of several genes (6), but there is little information regarding the miRNA gene targets and their biological functions. According to the important roles which are played by AIM2 and ASC in the induction of immune responses during inflammatory diseases, it seems that the miRNAs which target AIM2 and ASC and alter their expression can be considered as potent molecular therapy to cure the immune related diseases including autoimmune and infectious diseases. Previous studies showed that the levels of miRNA-143 (MiR-143) are changed during inflammatory diseases (7,8), hence, inflammatory transcripts may be plausible targets of MiR-143. Therefore, the main aim of this study was to investigate the mRNA levels of AIM2 and ASC in a lymphocyte cell line (Jurkat) before and after MiR-143 introducing.

MATERIALS AND METHODS

Cell Culture and Viability. Jurkat cells (human T-cell leukemia) were obtained from the Iranian National Cell Bank (Pasteur Institute of Iran, Tehran) and were cultured in RPMI-1640 medium enriched with 2 mM L-glutamine and 10% (v/v) heat-inactivated FBS (Sigma, St. Louis, MO, USA) under 5% CO2 and an atmosphere of 95% air at 37°C. The viability of cells was identified using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. In order to examine apoptotic changes, cells were stained with annexin-V-FLOUS and propidium iodide using a commercial kit (Sigma, St. Louis, MO, USA) according to the manufacture's guidelines, and analyzed by the Partec flow cytometry PAS system.

MiR-143, Scramble Sequence and PBS Transfection of Jurkat Cells. Jurkat cells were seeded in 3 well plates (for each MiR-143, scramble sequence and PBS) at a concentration of 2×10^5 cells/ml/well at one day before the transfection. 20 nM of mixed mature MiR-143 and a scrambled sequence (miRNA with no known mRNA target) as well as a vehicle in PBS mock as controls were transfected to the Jurkat cells under the same conditions (Bioneer, South-Korea) was used for the transfection of the cells using Lipofectamine 2000 (Invitrogen, USA) according to the manufacturer's guidelines. In order to confirm the transfection process, a FITC-5` conjugated MiR-143 was transfected to Jurkat cells and then analysed by florescent microscopy (Nikon, Japan). The sequence of the mature miRNA-143 was obtained from mirbase database and was

as follows: UGAGAUGAAG-CACUGUAGCUCA. A scrambled sequence was also used as negative control. Previous studies demonstrated that the best time of MiR-143 functions is 48 hrs after cells transfections (9), hence, the expression of AIM2 and ASC was measured at 48 hrs after the transfection of Jurkat cells by mature MiR-143.

RNA Extraction, Reverse Transcription and Quantitative Real-Time PCR. Total RNA was extracted from Jurkat cells using RNX extraction kit from Cinnaclon Company (Iran). The extracted RNA quality was identified by electrophoresis on the ethidium bromide contained agarose gel and measured absorption on 260/280 nm by spectrophotometer. cDNA was synthesized using a cDNA synthesis kit (Parstous, Iran) with oligo(dT) primers (Aryatous, Iran). The following program was performed to cDNA synthesizes: 70°C for 10 min (without reverse transcription enzymes), 20°C for 1 min (cooling), 42°C for 60 min (added reverse transcription enzymes) and 95°C for 10 min (reverse transcription enzymes inactivation). Quantitative Real-time PCR was performed by adding of following materials: SYBR green master mix (Parstous, Iran), 200 ng of produced cDNA and 2 pg/µl of appropriate primers (Table 1).

Table 1. Primer sequences of AIM2 and ASC genes.

Target gene	Primer Sequences
Absent In Melanoma 2 (AIM2)	F: 5′-CAGGAGGAGAAGGAGAAAGTTG-3′ R: 5′-GTGCAGCACGTTGCTTTG-3′
Apoptosis-associated speck like protein containing a caspase recruitment domain (ASC)	F: 5′- AACCCAAGCAAGATGCG-3′ R: 5′-TTAGGGCCTGGAGGAGCAAG-3′
β-Actin	F: 5′- GGCACCCAGCACAATGAAG -3′ R: 5′- CCGATCCACACGGAGTACTTG -3′

The following program was run on the BIO-RAD CFX96 system (Bio-Rad Company, USA): one cycle of 95°C for 15 min, 40 cycles of 95°C for 30 s, 60°C for 30 s and 72°C for 30 s. Real-Time PCR were carried out in triplicate and the β-actin and GAPDH as housekeeping gene were used for normalization of amplification. The relative amounts of PCR product were calculated using the $2^{-\Delta\Delta Ct}$ formula. The quality of graphs, melting curves and quantitative analyses of the data were performed using CFX manager software version 1.1.308.111 (Bio-Rad, USA). PCR products were electrophoresed on 2% agarose gel containing 0.5 mg/ml ethidium bromide to recheck the size of PCR products.

Data Analysis and Statistical Methods. The parametric statistical analyses (*t-test*) under SPSS software version 18 were performed and p value less than 0.05 were considered as significant.

RESULTS

Cell Viability and Apoptotic Features. The MTT test revealed that the viability of Jurkat cells was decreased in MiR-143 transfected cells when compared to scrambled sequence and mock transfected cells ($p<0.001$) (Figure 1).
The results demonstrated that 1 ± 0.05, 1.1 ± 0.1 and 30 ± 2.4 percent of the mock, scrambled sequence and MiR-143 transfected Jurkat cells had apoptotic features using annexin V, respectively.

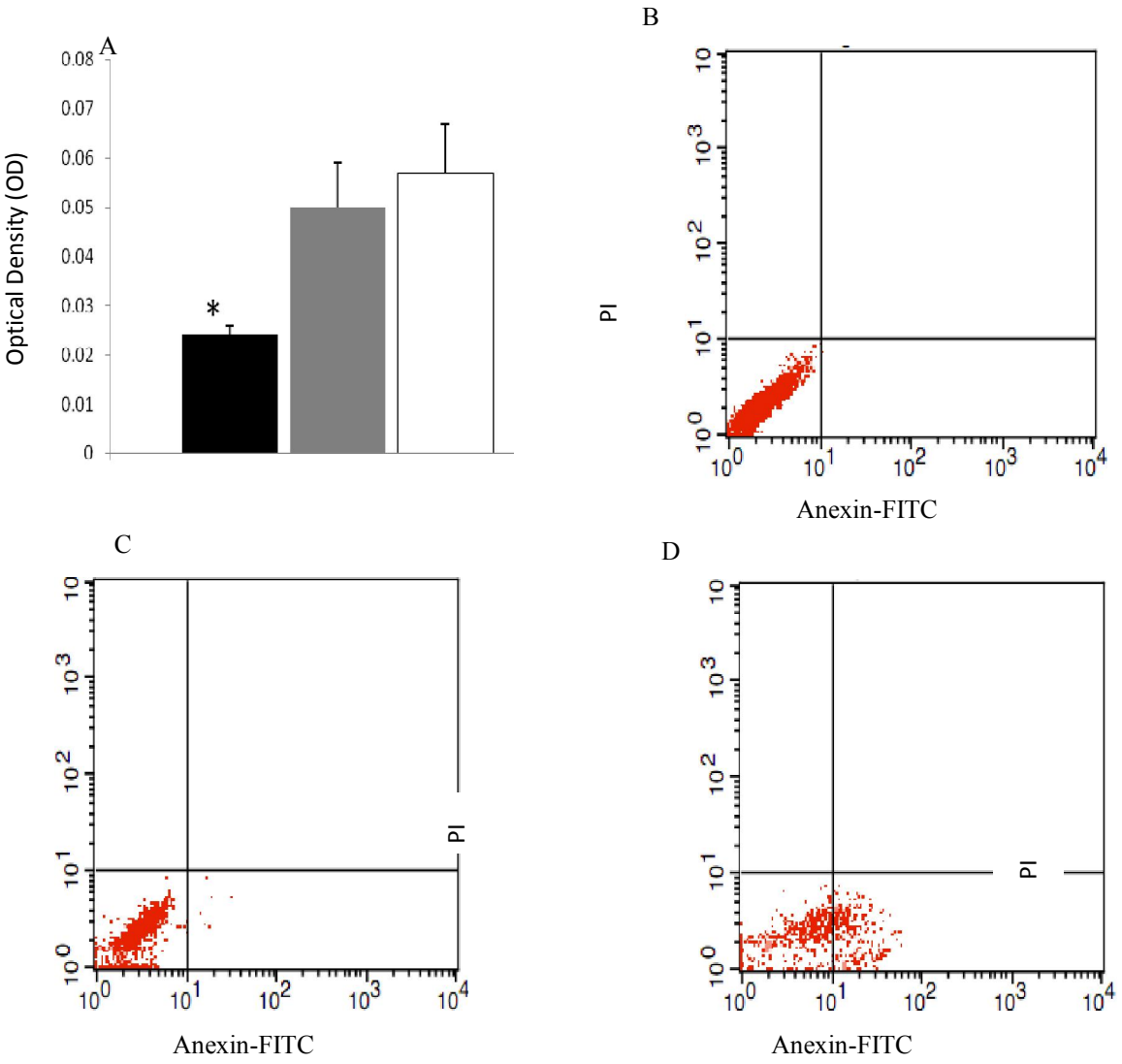

Figure 1. The figure shows MiR-143 transfected cells had lower viability in compare to both control groups. Figure 1A illustrates that the optical density (OD) of formazan was decreased in MiR-143 transfected cells (black column) when compared to the scrambled (gray column) and mock (white coloumn) control cells). Figure 1B, 1C and 1D illustrate the percent of apoptotic Jurkat cells in MiR-143 transfected cells, scrambled and mock control cells, respectively. Figure 1C shows that 30% of MiR-143 transfected cells cells had apoptotic features.
*Significant different between MiR-143 transfected cells with both control groups.

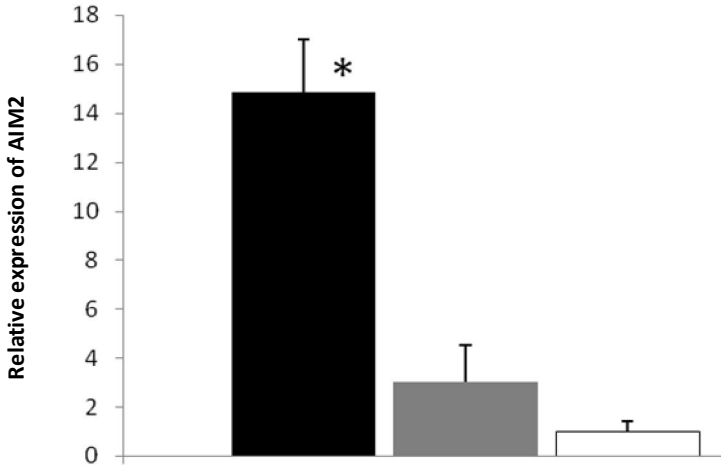

Figure 2. The figure illustrates mRNA levels of AIM2 were significantly increased in MiR-143 transfected cells in compare to both control groups (the scrambled (gray column) and mock (white coloumn) transfected cells).
*Significant different between MiR-143 transfected cells with both control groups.

Expression of Target Genes. Our results showed that expression of AIM2 in the Jurkat cell lines was increased 14.8 ± 2.2 and 3.02 ± 1.5 fold in MiR-143 and the scrambled sequence transfected Jurkat cells respectively, in comparison to controls (1 ± 0.4) (Figure 2). Statistical analyses revealed that the differences between MiR-143 transfected and other groups were significant ($p<0.001$), while the differences between scrambled sequence transfected and control (PBS transfected) cells were not significant ($p=0.62$). The results also demonstrated that the transcripts levels of ASC were upregulated 1.35 ± 0.1 fold ($p=0.04$) in MiR-143 transfected and 0.96 ± 0.01 ($p=1.0$) fold in scrambled sequence transfected Jurkat cells in comparison to controls (1 ± 0.05) (Figure 3).

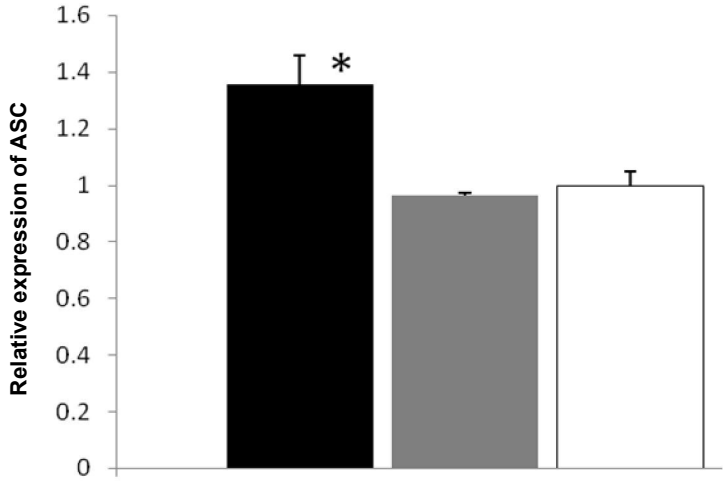

Figure 3. The figure shows mRNA levels of ASC were significantly increased in MiR-143 transfected cells in compare to both control groups (the scrambled (gray column) and mock (white coloumn) transfected cells).
*Significant different between MiR-143 transfected cells with both control groups.

DISCUSSION

An intriguing immune response against microbial infections and inflammation during immune related diseases starts via cytokine production (10). The IL-1β and IL-18 are the main cytokines which are produced in pro-cytokine forms and are activated subsequently via cleavage by caspase-1 activated by AIM2 and other inflammasome pathways (11). Additionally, previous studies identified that AIM2 and its corresponding molecular signaling play important roles in activation of IL-1β and IL-18 during viral and bacterial infection including *Vaccinia* virus (12), *Cytomegalovirus* (12) and *Listeria monocytogenes* (13). Our results showed that the transcripts levels of AIM2 and ASC were significantly increased in the MiR-143 transfected Jurkat cell lines when compared to both PBS mock and the scrambled sequence transfected controls. Therefore, based on our results it can be concluded that MiR-143 may increase half-life of AIM2 and ASC mRNAs and their expression in Jurkat cell line. Interestingly, our previous studies demonstrated that transcript levels of AIM2 and ASC were significantly decreased during chronic hepatitis B infections (unpublished data). Our results are in accordance with several studies that demonstrated a decrease in the expression of AIM2 and ASC during chronic infections (14,15). Hence, it can be concluded that MiR-143 may be considered as a target for future anti-microbial molecular-therapy which results in increased transcripts levels of AIM2 and ASC. To the best of our knowledge, there were neither *in vivo* nor *in vitro* published studies regarding the effects of miRNAs on the expression of AIM2 and ASC transcripts. However, in parallel with our results Schmidt et al., demonstrated that expression of MiR-143 was significantly increased in response to *Escherichia coli* lipopolysaccharide (LPS) (7). Interestingly, elevation levels of MiR-143 in the Enterovirus and Coxsackie virus infected patients are also reported by Cui et al. (16). Based on our results and aforementioned studies, it may be concluded that increased expression and/or half-life of AIM2 and ASC transcripts following MiR-143 expression may be a main mechanism of immune responses against bacterial and viral infections. Interestingly, the current results revealed that MiR-143 lead to 30 % apoptosis in the MiR-143 transfected Jurkat cell line when compared to controls; hence, it seems that this miRNA can also be used in cancer molecular therapy. In consistent with our results several studies reported that MiR-143 induces apoptosis in several cell lines including Jurkat cells (9,17-19). Additionally, down-regulation of MiR-143 in several cancers also has been documented by several researchers (20,21). Collectively, based on the results it can be concluded that MiR-143 not only led to increased expression of AIM2 and ASC but also resulted in apoptosis in Jurkat cell line. It is therefore suggested that MiR-143 induces apoptosis in Jurkat cells via either directly targeting the anti/pro-apoptotic molecules or indirectly through up-regulation of inflammatory molecules, including AIM2 and ASC. In other words, AIM2 and ASC may be involved in apoptosis of Jurkat cell line. It would be interesting to evaluate the apoptosis induced by MiR-143 after knocking down of AIM2 and ASC in future studies.

The results of the current study present a model for future chronic infectious diseases and cancer therapy in which up-regulation of MiR-143 is of paramount importance. This may be based on the increased expression of inflammatory molecules and subsequently cytokine activation via these molecules and also up-regulation of pro-apoptotic molecules.

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