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ORIGINAL ARTICLE

Dysregulation of miRNA-30e-3p targeting IL-1β in an international cohort of systemic autoinflammatory disease patients

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Abstract

Autoinflammation is the standard mechanism seen in systemic autoinflammatory disease (SAID) patients. This study aimed to investigate the effect of a candidate miRNA, miR-30e-3p, which was identified in our previous study, on the autoinflammation phenotype seen in SAID patients and to analyze its expression in a larger group of European SAID patients. We examined the potential anti-inflammatory effect of miR-30e-3p, which we had defined as one of the differentially expressed miRNAs in microarray analysis involved in inflammation-related pathways. This study validated our previous microarray results of miR-30e-3p in a cohort involving European SAID patients. We performed cell culture transfection assays for miR-30e-3p. Then, in transfected cells, we analyzed expression levels of pro-inflammatory genes; IL-1β, TNF-α, TGF-β, and MEFV. We also performed functional experiments, caspase-1 activation by fluorometric assay kit, apoptosis assay by flow cytometry, and cell migration assays by wound healing and filter system to understand the possible effect of miR-30e-3p on inflammation. Following these functional assays, 3'UTR luciferase activity assay and western blotting were carried out to identify the target gene of the aforementioned miRNA. MiR-30e-3p was decreased in severe European SAID patients like the Turkish patients. The functional assays associated with inflammation suggested that miR-30e-3p has an anti-inflammatory effect. 3'UTR luciferase activity assay demonstrated that miR-30e-3p directly binds to interleukin-1-beta (IL-1β), one of the critical molecules of inflammatory pathways, and reduces both RNA and protein levels of IL-1β. miR-30e-3p, which has been associated with IL-1β, a principal component of inflammation, might be of potential diagnostic and therapeutic value for SAIDs.

Key Messages

- miR-30e-3p, which targets IL-1β, could have a role in the pathogenesis of SAID patients.
- miR-30e-3p has a role in regulating inflammatory pathways like migration, caspase-1 activation.
- miR-30e-3p has the potential to be used for future diagnostic and therapeutic approaches.

Keywords Systemic autoinflammatory disease · Familial Mediterranean fever · Inflammation · MicroRNA · miR-30e-3p · IL-1β

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Background

Systemic autoinflammatory diseases (SAID) are mainly characterized by the dysregulation of the innate immune system [1]. This disease group is characterized by the activation of clinical and biological inflammatory components of the innate immune system with little or no involvement of the adaptive immune system [2]. The concept of autoinflammation, the primary mechanism of SAID, was first proposed in 1999 to explain recurrent fevers and various forms of systemic inflammation [3]. Nowadays, autoinflammation has been generally used for diseases in which innate immunity plays the primary role in pathophysiology [4]. The number of diseases defined in this group is increasing day by day.

Dysregulated inflammasome-mediated production of the cytokine IL-1β is one of the primary mechanisms linked to autoinflammation [5], thus to monogenic autoinflammatory diseases [4]. SAIDs; familial Mediterranean fever (FMF), TNF receptor-associated periodic syndrome (TRAPS), cryopyrin-associated periodic syndromes (CAPS), and mevalonate kinase deficiency (MKD) are the most common ones related to high interleukin-1 (IL-1β) production. Genes and pathways associated with monogenic autoinflammatory diseases have been elucidated mainly because of developments in genomics in recent years. In addition to genetic factors, epigenetic factors are also known to be effective in the pathophysiology of these diseases.miRNAs contribute to many biological processes, including inflammation, through their interference with mRNAs [6]. Many studies have been conducted to elucidate the relationship between SAID and miRNAs. However, most of these studies focused on FMF disease, the most common among these diseases. One of the recent studies identified differential expression of miR-20a-5p and miR-197-3p in homozygous FMF patients, which compares FMF patients and healthy controls [7]. Then, in another study, the anti-inflammatory effect of miR-197-3p, which targets the interleukin-1beta (IL-1β) receptor, type I (IL1R1) gene, was shown by inflammation-related functional assays [8]. Apart from these studies, many publications have been made in FMF, and the differential expression of several miRNAs that have the potential to be involved in immune processes has been demonstrated to date [9–11].

Although there are limited studies in this field, differential expression of miR-134, miR-17-5p, miR-498, miR-451a, miR- 572, and miR-92a-3p have been shown in a study related to TRAPS [12], and the role of miR-223, which was reduced in CAPS patients has been demonstrated in suppressing inflammation in another study [13]. We had previously shown that miR-30e-3p was decreased in Turkish FMF patients [14]. In this presented study, we validated miR-30e-3p in European SAID patients in addition to Turkish patients. The functional analysis related to inflammation, such as caspase-1 activation, apoptosis assay, and cell migration assays in miR-30e-3p transfected cells supported the anti-inflammatory effect of this miRNA. Finally, interleukin-1beta (IL-1β), one of the critical molecules of inflammatory pathways, was identified as miR-30e-3p's target gene, and this effect was confirmed on both RNA and protein levels of IL-1β.

Material and methods

Patient selection

This is a cross-sectional study. Children (< 18 years) with SAID were included from the Pediatric Rheumatology Units of Hacettepe University Faculty of Medicine, Ankara, Turkey; University Medical Center Utrecht, Utrecht, the Netherlands; Unit of Rheumatology and Autoinflammatory Diseases, IRCCS Istituto Giannina Gaslini, Genova, Italy; Department for Pediatric Rheumatology & Immunology, University Hospital Muenster Muenster, Germany. All FMF, MKD/HIDS, CAPS, and TRAPS patients met the Eurofever/PRINTO classification criteria [15]. A total of 63 SAID patients (FMF ($n=38$), MKD/HIDS ($n=8$); CAPS ($n=6$), TRAPS ($n=4$), and uSAID ($n=7$)) were included. All included FMF patients had two exon 10 *MEFV* mutations. Undefined SAID (uSAID) was defined as patients with inflammatory attacks who do not have any pathogenic mutation in the gene panel for common autoinflammatory diseases. For the decision of severe/mild phenotype, patients who all decided to be put on anti-IL-1 therapy were expected to have severe disease. Patients were sampled for miRNA isolation after a washout of 48 h after the last anakinra dose and at least 30 days after the last canakinumab dose to overcome the effect of the anti-IL1 drugs. All research was performed in accordance with relevant guidelines/regulations. The study was approved by the Hacettepe University Non-Interventional Clinical Research Ethics Board (GO 15/1744–19). Written informed consent was obtained from all participants and/or their legal representatives.

Whole blood miRNA isolation

MicroRNA isolation was performed using PreAnalytiX miRNA Kit and PAXgene Blood RNA tubes from patients and controls involved in the study group. The purity and quantities of RNAs were determined in NanoDrop 2000, and a 2.5% agarose gel electrophoresis technique was used for the quality control of the samples.

Microarray analysis

GeneChip miRNA 4.0 Array (Affymetrix) analysis determined the differential expression of miRNAs in patient groups. The raw data were analyzed with MeV (Multi Experiment Viewer) and Affymetrix Transcriptome Analysis Console (TAC). In addition to TAC analysis, Significance Analysis of Microarrays (SAM) analysis in the Multi Experiment Viewer (MeV) program was applied. miRNAs found as differentially expressed at least twofold change in both MeV-SAM analysis and TAC analysis were identified. Details of microarray results were explained in previously published article [14].

Pathway analysis and selection of target miRNA

Genes likely to bind miRNAs were identified using the miRWalk 2.0 database. Lists were created from genes determined by at least six programs for each miRNA using this database. Genes were classified according to the KEGG (Kyoto Encyclopedia of Genes and Genomes) and Geneontology-Panther programs' analysis results [16, 17]. Biological process analysis was performed by using the GeneOntology database with the help of enrichR/Appyters [18–20]. Cytoscape platform was used to visualize inflammation-related potential target genes of miR-30e-3p [21].

Cell culture and transfection

Synovial sarcoma cell line (SW982) (ATCC) and monocytic cell line (THP-1) were cultured in RPMI (Roswell Park Memorial Institute) 1640 (Gibco) medium containing heat-inactivated FBS (10%), Penicillin/Streptomycin (1%), and L-glutamine (1%). Both SW982 and THP-1 cells were transfected with three µl of Lipofectamine 2000 (Invitrogen) and 20 nM hsa-miR-30e-3p, mirVana® miRNA mimic (Ambion) or mirVana ™ miRNA Mimic, Negative Control # 1 (Ambion) according to the manufacturer's instructions. Transfection efficiency was determined by qRT-PCR (Fig. S2).

RNA isolation and quantitative real-time polymerase chain reaction (qRT-PCR) experiments for cell line and patient materials

The cDNA was synthesized with TaqMan® Advanced miRNA cDNA synthesis kit to validate microarray results. Amplification of miRNAs was performed by using the TaqMan® Advanced miRNA Assays. RNU48 snRNA was used for normalization.

For expression analysis of miR-30e-3p related genes, RNA isolation was performed with RNeasy® Plus Mini Kit (Qiagen) from pre-miR-30e-3p or mimic control transfected cells. The cDNA was synthesized with QuantiTect Reverse Transcription Kit (Qiagen). After cDNA synthesis, the qRT-PCR reaction was performed for *MEFV*, *IL-1β*, *TNF-α*, and *TGF-β* genes with iTaq Universal SYBR Green Supermix (BioRad) according to the manufacturer's instruction. *GAPDH* gene expression was used as a normalizer. The reactions were carried out on the BioRad IQ5, and each sample was run in triplicate.

Apoptosis assay

After transfection of the miRNAs, cell death analysis was performed by flow cytometry using Annexin V-FITC Apoptosis Detection Kit, APOAF, Sigma according to the manufacturer's guidelines and analyzed by BD FACS Aria II device. Also, the level of cell death was investigated in only-lipofectamine-treated cells.

Wound healing assay

In wound healing experiments, 1×10^6 SW982 cells were seeded into a 6-well plate at approximately 50% confluency and transfected with 20 nM pre-miR-30e-3p or mimic control at reaching 90% confluency. Two wounds for each well were formed by a 10-µl pipet tip. The same region of wounds (marked on the plate) was imaged by Leica IM50 fluorescence inverted microscope at 0, 6, 12, and 24 h after scratches. The areas of four regions of wounds of each well were calculated by using ImageJ 1.46 software, and the percentage of wound closure was calculated by comparing to the area of wounds at 0 h.

Migration assay

Polycarbonate membrane filters containing 8 µm pores were placed in a 24-well cell culture plate. The filters used in this experimental system have two compartments, namely the upper and lower wells. Firstly, the medium was placed under and above the filter and incubated for 1 h at 37 °C in a 5% CO_2 incubator. Cells transfected with pre-miR-30e-3p or mimic control for 48 h were harvested and resuspended in a serum-free medium. Transfected cells were then transferred to the upper chamber at a density of 3×10^4 cells/well. After 24 h, non-migrated cells were removed by a cotton swab. The cells remaining at the bottom of the filter were treated with one mM calcein-AM and incubated for 30 min at 37 °C in a 5% CO_2 incubator. At the end of the incubation, three pictures were taken from the lower area of the filter with the Leica IM50 fluorescence microscope. Cells migrating to the lower well were quantitated using the "particle analysis" method found in ImageJ 1.46 software.

Caspase-1 activity assay

According to the manufacturer's guidelines, caspase-1 activity was analyzed in pre-miR-30e or mimic control transfected cells using the Caspase-1 Fluorometric Assay Kit (Abcam). Following the protocol, the samples were measured at Ex / Em = 400/505 nm using SpectraMax M2 Microplate Reader. Also, the level of cell death was investigated in only-lipofectamine-treated cells.

3'UTR luciferase activity assay

miR-30e-3p and its target genes were bioinformatically determined using TargetScanHuman (Release 7.2). TargetScanHuman database gives a target gene list sorted by cumulative weighted context score and based on 3P-seq tags. 3'UTR reporter construct for IL-1β was provided from the LightSwitch ™ 3'UTR Reporter GoClone® Collection catalog. The region determined by TargetScanHuman binds miR-30e-3p to the candidate gene was amplified by mutagenesis primer. Primers were designed using the QuikChange Primer Design Program (www.agilent.com/genomics/qcpd). Mutagenesis PCR was performed using the designed primers with QuikChange II XL Site-Directed Mutagenesis Kit (Agilent Technologies). IL-1β 3'UTR/mut IL-1β 3'UTR reporter constructs were co-transfected into SW982 cells with pre-miR-30e-3p miRNA or mimic control. At 24 h of post-transfection, luciferase activities were analyzed using the LightSwitch Luciferase Assay Reagent (SwitchGear Genomics). The luciferase signal from the cells was measured by a microplate reader (Molecular Devices, Spectramax i3x).

Western blotting

At 48 h post-transfection, cells were scraped from the dishes using protein lysis buffer supplemented with protease inhibitors. Protein concentrations were determined using a BCA assay (Thermo Scientific). Protein lysates were separated by SDS-PAGE, transferred to a nitrocellulose membrane, and subjected to immunoblotting analysis. The membranes were treated with 1/1000 anti-IL-1β/anti-GAPDH primer antibody (Cell Signaling Technology) and 1/1000 anti-rabbit/anti-mouse secondary antibody (Invitrogen), respectively. Super Signal™ West Femto Maximum Sensitivity Substrate (Invitrogen) was used for protein signal detection. Membranes were visualized at GeneGnome Western Blot Imager (Syngene International Limited).

Statistical analysis

Differential expression of the genes was normalized according to GAPDH. The results were calculated with the relative quantitation ($2^{-\Delta Ct}$) method by comparison with the control group. After determining the expression level of genes, the statistical significance of fold increase/decrease was analyzed using Student's *t* test using GraphPad Prism 5.01 software. $p<0.05$ values were considered as significant.

Results

Demographic and clinical characteristics of patients

A total of 63 SAID patients (M/F = 1.1) were included. The median (min–max) age was 14.25 (1.5–16) years. The diagnoses of the patients were as follows: FMF ($n=38$), MKD/HIDS ($n=8$); CAPS ($n=6$), TRAPS ($n=4$), and uSAID ($n=7$). All FMF patients were on colchicine, MKD/HIDS, TRAPS, five CAPS, eight FMF, and seven uSAID patients were on anti-IL-1 treatment when blood samples were drawn.

miR-30e-3p has shown a differential expression pattern in an international cohort of systemic autoinflammatory disease patients

miRNA-30e-3p expression levels were altered significantly among the groups in microarray, and bioinformatic analysis was performed for inflammation-related pathways. Our previous manuscript explains in detail that following the microarray analysis, candidate microRNAs affecting the genes in the inflammation-related pathways are determined because of pathway analyses. Then, array results of the candidate miRNAs among the bioinformatically selected groups were validated by qRT-PCR [14]. miR-30e-3p, one of the validated miRNAs, has shown a differential expression pattern in an international severe SAID patient group (AU, IT, NL, TR) compared with the mild SAID group (Fig. 1A). Potential target genes and possible biologic pathways of miR-30e-3p are given in Fig. 1B, C. It was decided to conduct functional studies for miR-30e-3p.

miR-30e-3p suppresses caspase-1 activation but not apoptosis in SW982 cells

Different death mechanisms, such as pyroptosis or apoptosis, are activated in cells in response to inflammation signals. In the apoptosis analysis, the cell death rate decreased in pre-miR-30e-3p transfected SW982 cells however the reduction was not significant (Fig. 2A). Active caspase-1 shows the pyroptosis process with inflammasome activation and maturation of interleukins. Compared to mimic control in pre-miR-30e-3p transfected cells, caspase-1 activity was significantly reduced (Fig. 2B). The reduction in caspase-1

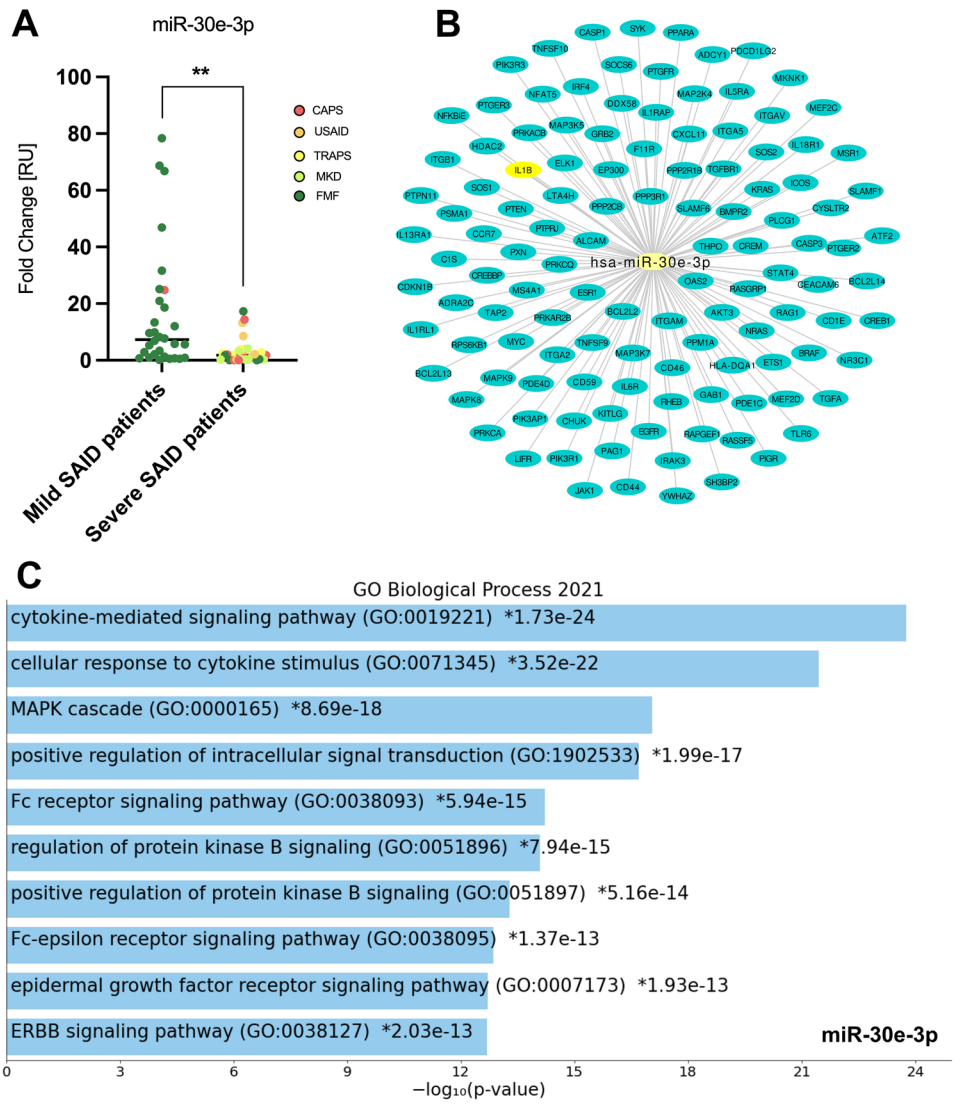

Fig. 1 Validation of the decrease in miR-30e-3p level in SAID patients, potential target genes, and possible biologic pathways of miR-30e-3p. **A** Decreased miR-30e-3p levels observed in severe Turkish SAID patients were also validated in cumulative patient groups (AU, IT, NL, TR) using qRT-PCR. A total of 31 mild SAID patients (30 FMF and 1 CAPS) and 32 severe SAID patients (8 FMF, 8 MKD/HIDS, 5 CAPS, 4 TRAPS, 7 USAID) were included. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. miRNA expression was normalized to U48 RNA. **B** Inflammation-related potential target genes (121 genes) of miR-30e-3p, which is visualized by CytoScape 5.0. **C** The inflammation-related pathway was determined according to the analysis results of the enrichR/GeneOntology-biological process program

activity in transfected cells supported the idea that this miRNA may have an anti-inflammatory effect.

miR-30e-3p regulates the gene expression level of *IL-1β*, *MEFV*, but not *TNF-α*, *TGF-β* in both SW982 and THP-1 cells

Compared to mimic control, expression levels of *IL-1β* and *MEFV* genes were significantly decreased in pre-miR-30e-3p transfected cells. On the other hand, *TGF-β* and *TNF-α* expression levels were also non-significantly decreased in pre-miR-30e-3p transfected cells (Fig. 3A, B). Moreover, blood IL-1β expression level decreased in Turkish severe SAID patients compared to mild SAID patients and control individuals (Fig. S1). The idea that this miRNA shows anti-inflammatory properties has been strengthened due to gene expression analysis in both SW982 and THP-1 cells. miR-30e-3p possibly suppresses inflammation through the potential target genes involved in cytokine release.

miR-30e-3p inhibits cell migration in both SW982 and THP-1 cells

Cell migration to the inflammatory region is one of the essential stages of inflammation which may be a critical marker in the pathogenesis of SAID. Compared to the mimic control, the number of migrated cells was found to be lower in cells transfected with pre-miR-30e-3p in the transwell assay system (Fig. 4B, C). pre-miR-30e-3p transfected cells were also found to have a lower percentage of wound closure (Fig. 4A). Obtaining similar results in two different cell migration analysis systems contributes to the potential anti-inflammatory effect of miR-30e-3p.

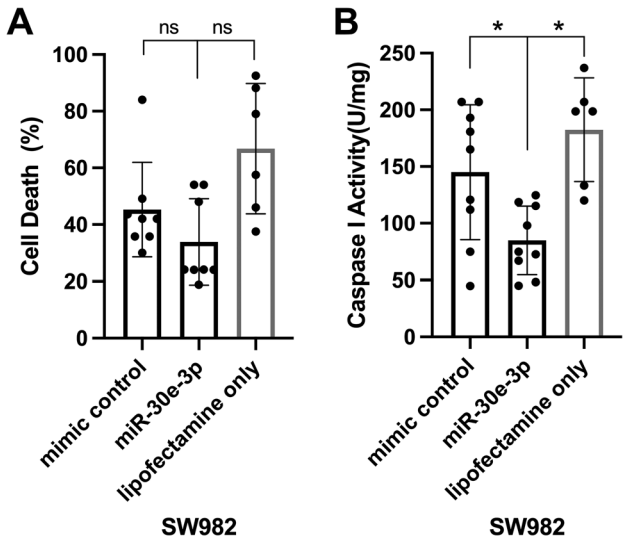

Fig. 2 miR-30e-3p regulates the caspase-1 activity and apoptosis. **A** Apoptosis rate in miR-30e-3p, mimic control-transfected, and only lipofectamine-treated SW982 cells. **B** Caspase-1 activity in miR-30e-3p, mimic control-transfected, and only lipofectamine-treated SW982 cells. Data represent the mean ± standard deviation from three independent experiments. Data represent the mean ± standard deviation from three independent experiments. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$, Student's *t* test (two-tailed)

miR-30e-3p targets IL-1β in both SW982 and THP-1 cells

According to the 3'UTR luciferase activity test results: When there is no mutation in the 3'UTR region of the IL-1β gene, it binds to the miR-30e-3p gene and the luciferase signal decreases. Where there is a mutation in this region, the binding site of miRNA disappears, and the expression of luciferase increases (Figs. 5A and S3). As a result, it has been demonstrated experimentally that miR-30e-3p can bind to the 3'UTR region of the IL1β gene. Both mRNA (Fig. 3A, B) and protein levels of IL-1β (Figs. 5B, C, S4 and S5) were decreased following pre-miR-30e-3p transfection in both SW982 and THP-1 cells.

Discussion

Systemic autoinflammatory diseases are defined as disorders of the innate immune system, and the role of the inflammasome in patients with autoinflammatory diseases is well-known [22]. Inflammasomes have essential roles in host defense against intracellular bacteria and viruses and regulate inflammation in the innate immune system, mainly of IL-1β and IL-18 cytokine production [5].

Mutations in the genes that encode protein components of the inflammasome and related pathways are associated with many autoinflammatory diseases [23]. In addition to their genetic background, epigenetic mechanisms have also been suggested to be important in the pathogenesis of these disorders. One of the epigenetic mechanisms, non-coding RNAs, mainly miRNAs, have been shown to regulate the inflammatory response by their effect on the expression levels of cytokines, transcription factors, and interleukin-related genes [24, 25]. In this context, the possible effects of a differentially expressed miRNA, miRNA30e-3p, on the pathogenesis of SAID were investigated.

Previously identified inflammasome-related miR-30e-3p [14] was analyzed in severe European SAID patients in this study. The samples of patients from the Netherlands, Germany, and Italy were transferred to our facility, and all the samples were analyzed in the same batch. The same epigenetic factor was observed in SAID patients with different genetic backgrounds and exposed to different environmental effects showed us that this miRNA may be important in inflammation. Further functional analysis, cell-based functional analysis on inflammation-related pathways such as caspase-1 activation, apoptosis assay, and cell migration assays in miR-30e-3p transfected cells supported the anti-inflammatory effect of this miRNA. Our hypothesis supported by these analyses were summarized in Fig. 6.

Caspase-1 activation induces processing and secretion of IL-1β and IL-18 in addition to gasdermin D-mediated pro-inflammatory programmed cell death. Hence, the downregulation of caspase-1 activity in miR-30e-3p transfected cells showed that at least one of the targets of miR-30e-3p is directly/indirectly responsible for the regulation of caspase-1 activation. In contrast to caspase-1-mediated pyroptosis, apoptosis is a general programmed cell death induced by various stimuli.

Inflammatory cell migration and motility of the immune cells to the inflamed body region have become one of the top research areas in autoimmune/autoinflammatory diseases [26]. The contribution of various inflammasome-dependent inflammatory cell migration dysregulation to the pathogenesis of many diseases has been discussed. Hence, the downregulation of migration in miR-30e-3p transfected cells has strengthened the emphasis on the anti-inflammatory properties of this microRNA.

The results of functional analysis, which represent inflammation phenotype, led us to crucial molecules in inflammation. Deep bioinformatic analysis revealed that most of the potential targets of miR-30e-3p belong to the cytokine-mediated signaling pathway. Hence, IL-1β, which has a high binding score among potential targets of miR-30e-3p and is known to be an important factor in inflammation, was selected for analysis. Both RNA and protein levels of IL-1β decreased following miR-30e-3p transfection, and direct interaction of miR-30e-3p – IL-1β was identified by 3'UTR luciferase assay.

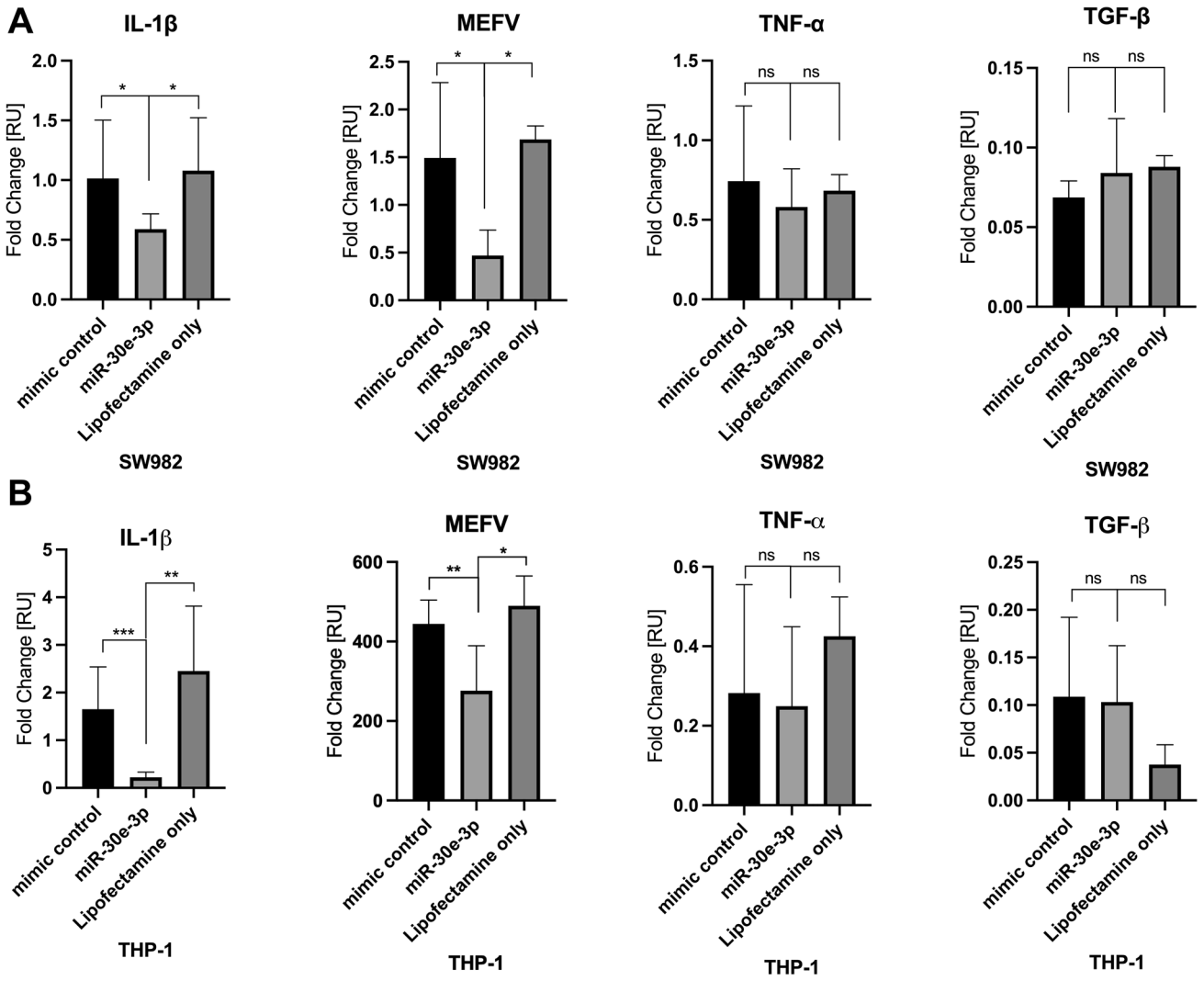

Fig. 3 miR-30e-3p suppress pro-inflammatory gene expression. **A** *IL-1β*, *MEFV*, *TNF-α*, and *TGF-β* expression in miR-30e-3p, mimic control-transfected, and only lipofectamine-treated SW982 cells. **B** *IL-1β*, *MEFV*, *TNF-α*, and *TGF-β* expression in miR-30e-3p, mimic control-transfected, and only lipofectamine-treated THP-1 cells. Data represent the mean ± standard deviation from three independent experiments. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$, Student's *t* test (two-tailed)

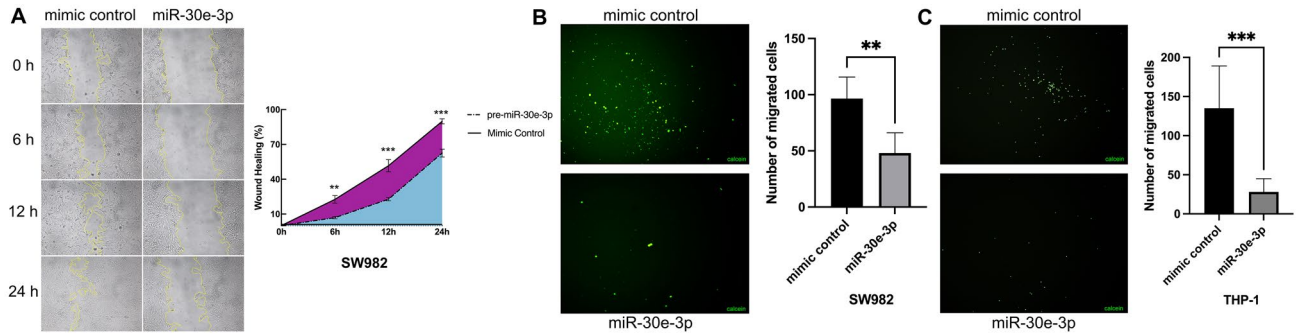

Fig. 4 miR-30e-3p suppresses inflammatory cell migration. **A** Wound healing assay results of pre-miR-30e-3p-transfected or mimic control-transfected SW982 cells in time courses (0, 6, 12, 24 h). **B** Filter assay results of pre-miR-30e-3p-transfected or mimic control-transfected SW982 cells. **C** Filter assay results of pre-miR-30e-3p-transfected or mimic control-transfected THP-1 cells. Representative images of live cells that have migrated through the filter visualized using the green fluorescent dye, Calcein (green). Data shows the mean ± standard deviation from three independent experiments. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and Student's *t* test (two-tailed)

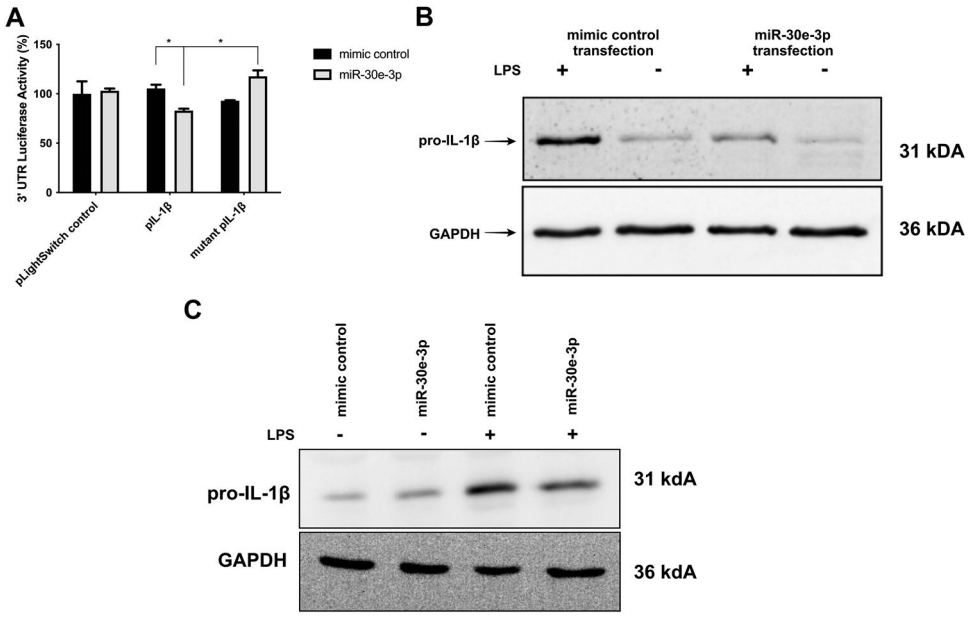

Fig. 5 miR-30e-3p targets IL-1β. **A** Wild type IL-1β 3'UTR and mutant IL-1β 3'UTR reporters were constructed, and a luciferase reporter assay was performed in SW982 cells after miR-30e-3p transfection. miR-30e-3p-binding site in IL-1β 3'UTR and the sequences of wild-type and mutant IL-1β 3'UTR used in the 3'UTR luciferase experiments are shown in Fig. S3. *$p<0.05$, **$p<0.01$, *** $p<0.001$, Student's t test (two-tailed). **B** pre-miR-30e-3p transfection reduced IL-1β protein level in both LPS-induced and non-stimulated SW982 cells. **C** pre-miR-30e-3p transfection reduced pro-IL-1β protein level in LPS-induced THP-1 cells. GAPDH was used as a loading control

Similar to our findings, Chen et al. showed that miR-30e-3p is a part of inflammation and apoptosis regulator complex with CircNFIC and DENND1B and regulates IL-1β secretion in chicken macrophage cell lines [27]. Also, the role of miR-30e-3p on drug resistance was reported in different cancer types [28, 29]. Wang et al. showed that cell invasion and migration are regulated by miRNA-30e-3p in clear-cell renal cell carcinoma [30]. Moreover, neuroinflammation and neuronal apoptosis can be regulated by miR-30e-3p with KCNQ1OT1- NLRP3 axis in HMC3 cells [31]. These findings show that miR-30e-3p takes a role in different cellular processes in addition to inflammation-related pathways.

Our previous study showed that miR-197-3p, a down-regulated miRNA in severe Turkish FMF patients, had an

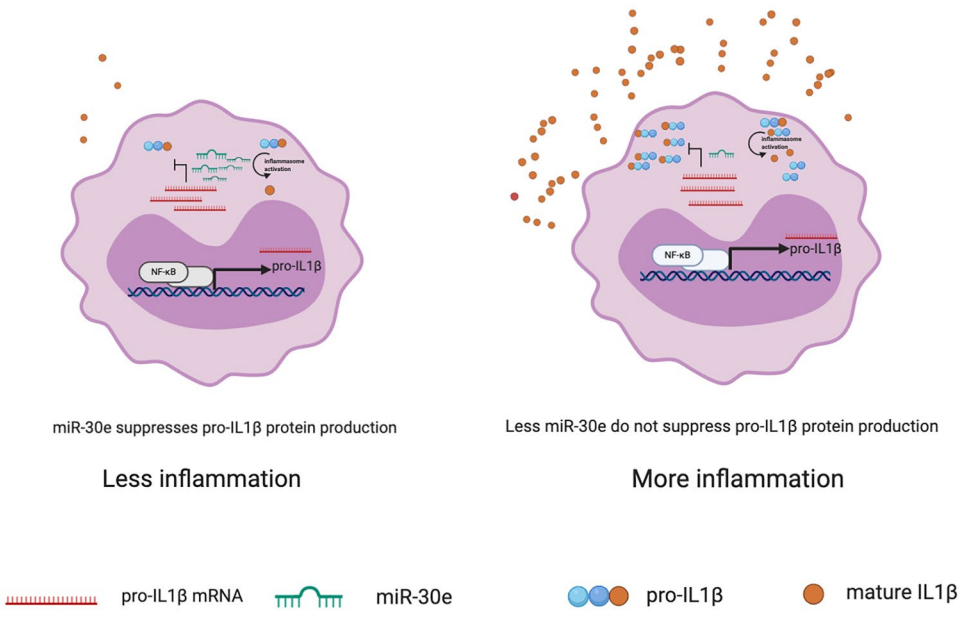

Fig. 6 miR-30e-3p suppresses inflammation by targeting IL-1β. Our hypothesis supported by the functional analysis results is summarized in this figure. Under inflammatory conditions, decreased miR-30e-3p, which targets IL-1β, could not suppress pro-IL-1β, so this may have a role in the pathogenesis of SAID patients

anti-inflammatory effect on inflammation-related pathways. Functional assays of miR-197-3p demonstrated significant results on pathways related to inflammation, and more importantly, the gene target of miR-197-3p was the IL1R1 gene, confirmed by bioinformatic analysis and 3'UTR luciferase activity assay [8]. Finally, we suggest that the miRNAs targeting IL-1β and its receptor are essential for inflammation's epigenetic regulation and thus SAID.

This study has some limitations. The study was a multicenter study, and we do not have all the demographic details of the patients except that they were genetically confirmed patients and had a washout of anti-IL1 drugs as specified before. Also, one of the target genes of miR-30e-3p was identified as IL-1β, but other potential target genes should be studied. The effect of miR-30e-3p was shown by transfection assays, but further functional studies in animal models may strengthen our data. Dysregulation of miR-30e-3p in SAID patients was shown in total blood samples, but the presence of these differences should be confirmed in other body fluids (serum, plasma, and urine or microvesicles) in addition to enlarging the study population.

Confirming the anti-inflammatory role of miR-30e-3p in severe SAID patients makes this miRNA a valuable therapeutic target. Since it exerts its anti-inflammatory effect by regulating the gene expression levels of different pro-inflammatory cytokines, it might become an effective treatment strategy to control exaggerated inflammation in severe SAID patients. We demonstrated that it decreased in these patients. Thus, miRNA mimics could be used to restore the loss of miR-30e-3p functions. Currently, there are no studies in SAID addressing therapeutic options targeting miRNAs.

Conclusion

This study suggests that miR-30e-3p is a critical epigenetic factor in various monogenic autoinflammatory diseases. This may be since the target gene of this miRNA is the IL-1β gene, and IL-1β is a crucial cytokine in the monogenic SAIDs studies in our cohort. Thus, miR-30e-3p may serve as a biomarker to define the course of the disease and may offer new therapeutic strategies for monogenic SAIDs associated with IL-1β activation.

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Authors' contributions THA, ZYAU, SO, and BBP: conceptualization. THA and ZYAU: investigation. EDB, FP, HW, BK, MEVG, DF, MG, and SO: resources. THA and BBP: writing the original draft. THA, EDB, BBP, and SO: writing the review and editing. MEVG, DF, MG, SO, and BBP: funding acquisition. The authors have read and approved the final manuscript.

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Availability of data and materials The graphical abstract was created with BioRender.com. The microarray data generated or analyzed during this study are included in this published article [14], but are available from the corresponding author on reasonable request.

Declarations

Ethics approval The study was approved by the Hacettepe University Non-Interventional Clinical Research Ethics Board (GO 15/1744–19). Written consent was obtained from all parents and children.

Consent for publication All authors agree on publishing.

Conflict of interest The authors declare no competing interests.

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