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Aida H. Alobaidi
Entkhab M. A. Alanisi
Khalid A. Alyodawi

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ORIGINAL STUDY

Exploring PCAT3 Expression in Arthritis: Proinflammatory Marker Patterns and P53 Involvement

Aida H. Alobaidi [a,*], **Entkhab M. A. Alanisi** [b], **Khalid A. Alyodawi** [a]

[a] Department of Anatomy and Medical Biology, College of Medicine, Wasit University, Kut 52001, Iraq
[b] Department of Biology, College of Science, Mustansiriyah University, Baghdad P.O. Box 14022, Iraq

ABSTRACT

This study investigated the role of long non-coding RNA (lncRNA) PCAT3 in arthritis, focusing on its impact on proinflammatory markers. Researchers analyzed both mRNA and protein levels of various proinflammatory markers (TNF-α, IL-1β, IL-4, IL-6, IL-8, IL-10, and IL-13) in arthritis patients and compared them to healthy controls. Additionally, PCAT3 expression was assessed in both patient groups and further investigated using siRNA knockdown in the human synovial cell line SW982. Results revealed distinct expression patterns of proinflammatory markers in arthritis patients, with TNF-α and IL-6 showing the highest levels. PCAT3 expression was significantly elevated in arthritis patients compared to controls. Silencing PCAT3 in SW982 cells led to a significant reduction in the expression of several proinflammatory genes, including TNF-α, IL-1β, IL-6, and IL-10. These findings suggest that PCAT3 plays a role in the pathogenesis of arthritis by contributing to the upregulation of proinflammatory markers. Targeting PCAT3 may offer a potential therapeutic strategy for managing inflammation in arthritis.

Keywords: Arthritis, PCAT3, lncRNA, Inflammation, Proinflammatory markers, Cytokines

1. Introduction

The human genome is made up of regulatory components and functional genes that code for proteins, but it also contains non-coding sequences that serve a variety of purposes [1]. Recent research have shown many types of long non-coding RNA [2]. These types of non-coding RNAs can be categorized into two groups and possess various implied functions. There exist two classifications of non-coding RNAs: long non-coding RNA (lncRNA), consisting of more than 200 nucleotides, and short non-coding RNA, which is shorter than 200 nucleotides in length [3].

In addition, several RNA classes exist, including ribosomal RNA (rRNA) and transfer RNA (tRNA) [4, 5]. Although the exact roles played by ncRNA in several processes, including epigenetics and the control of transcription by ANRIL and HOTAIR, PRC1 and PRC2 are regulated by ncRNA [6, 7].

It been suggested that long noncoding RNAs (lncRNAs), specifically lincRNAs, may play a role in the pathophysiology of RA. LincRNAs have been found to be present in CD14(+) monocytes of RA patients, and a small number of lincRNAs have been shown to be regulated by cytokines such as IL-6 and TNF-alpha [8]. Additionally, lincRNA-p21 has been proposed as a regulator of cell proliferation, apoptosis, and DNA damage response, and has been implicated in the initiation and progression of human diseases, including RA [9]. These findings suggest that lincRNAs may be involved in the molecular mechanisms underlying RA and could potentially serve as diagnostic markers or therapeutic targets for the disease.

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* Corresponding author.
E-mail address: Aidah@uowasit.edu.iq (A. H. Alobaidi).

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The aim of the study is to investigate the role of PCAT3 (PCA3) in arthritis patients and its impact on the upregulation of proinflammatory cytokines.

Arthritis is a term that covers more than 100 conditions affecting joints. Although the specific causes are not known, many of these conditions are believed to arise from problems in the immune system, which leads to inflammation and other changes in the joints [10]. In most cases, these changes result in too little, rather than too much, cartilage in the joint (i.e., in the knee, between the femur and the patella), leading to bone-to-bone contact and to excessive "grinding" (i.e., under too much pressure). This condition is too painful for most people to function normally. PCAT3 is a gene on the X chromosome. PCAT3 have an important role in prostate cancer [11], however it may be involved in arthritis by regulating inflammation and immune responses. While research is still ongoing, its potential role in this condition suggests it could be a target for future therapeutic interventions.

PCAT3 may be involved in the inflammatory aspects associated with arthritis through regulation of inflammatory mediators and other immune regulatory molecules. In particular, PCAT3 may as a result have an impact on the activity of the NF-κB signaling pathways, which play an important role in arthritis development. Despite the more complete understanding of the specific pathways through which PCAT3 functions as a molecular switch in arthritis, its regulating role in inflammation and immune responses provides reasoning to be considered a potential target to treat arthritis.

2. Materials and methods

2.1. Cell Line and Culture Media

The human synovial cell line SW982 was obtain from American Type Culture Collection (Rockville USA). Cells were cultured in T-150 flasks and growing in Dulbecco's modified Eagle medium (DMEM) with 10% fetal bovine serum (Atlanta Biologicals), 50 units/mL penicillin, and 50 µg/mL streptomycin (ThermoFisher Scientific).

2.2. RNA isolation

Total RNA was extracted from cells using TRI reagent (Sigma). 500 µL of TRI reagent was added to the cells, followed by the addition of 100 µL of chloroform. The mixture was vigorously agitated for 15 sec and then centrifuged at 12,000 rpm for 15 min. After centrifugation, 500 µL of ice-cold isopropanol was added to the top aqueous layer containing the RNA, and the samples were centrifuged at 12,000 rpm for 10 min. The resulting RNA pellet was washed with 75% ethanol in DEPC-treated water and centrifuged again at 12,000 rpm for 10 min. After removing the ethanol, the tube was air-dried briefly. The RNA pellet was then resuspended in nuclease-free water, and the concentration was determined using a Nanodrop™ ND-1000 Spectrophotometer based on the optical density of the sample.

2.3. Real-time RT-PCR

To generate cDNA from 1 µg of total RNA, the iScript cDNA Synthesis Kit from Bio-Rad was used. The reaction mixture was prepared as follows: 1 µg of total RNA, 4 µL of the iScript reaction mix, 1 µL of the iScript reverse transcriptase, and 20 µL of nuclease-free water were combined. The reverse transcription process consisted of priming at 25°C for 5 min, followed by the reaction at 42°C for 30 min, and finally, inactivation of the reaction at 85°C for 5 min. The resulting cDNA reaction mixture was used in real-time PCR assays, performed using the StepOnePlus™ Real-Time PCR System from Applied Biosystems.

To obtain a total volume of 10 µL for each real-time PCR reaction mixture, the components were as follows: 0.3 µL of forward and reverse primers, 0.5 µL of cDNA, 5 µL of Fast SYBR™ Green Master Mix (Applied Biosystems), and 3.9 µL of nuclease-free water. The reactions underwent 40 cycles of the following thermal cycling conditions: denaturation at 95°C for 3 sec, followed by annealing/extension at 60°C for 30 sec, with a final hold at 95°C for 20 sec.

The experiments employed specific primer pairs for the mRNA expression levels were scaled to GAPDH levels. The presented information does not include the precise primer sequences, despite the fact that the primer sets were purchased from Invitrogen.

2.4. Measurement proinflammatory levels

The patients diagnosed with arthritis will be enrolled in the study, and matched to a similar number of normal subjects with no history of arthritis; both patients and normal subjects will have blood samples collected and pro-inflammatory markers assayed in a standardized manner. This study protocol was approved by the Institutional Review Board of AL-Zahra Educational Hospital (UW.MED.2024.0207, approved February 07th, 2024). For measurement of proinflammatory, blood samples were collected from patients and healthy in blood tubes, then stored at room temperature for 30 min. Tubes were centrifuged at 3000 rpm for 15 min, an aliquot of supernatant (serum) was collected in Eppendorf tubes then stored at −80°C. A panel of pro-inflammatory

markers were measured using a multiplex bead-based assay as described by the manufacturer (Bio-Plex Pro kit, Bio-Rad, Hercules, California). Cytokine concentrations that were below the assay limits of detection were assigned the minimal detectable concentration for purposes of statistical analysis.

For proinflammatory measurement in cell culture, cells after treatment were transferred precooled tubes. A micro homogenizing force was implied for 30 sec to destroy the cells. 500 µL of ice-cold sterile phosphate buffered saline containing Complete Protease Inhibitor Cocktail (Roche, Camarillo, CA) was added. Homogenates cells were centrifuged at 13,700 × g at 4°C for 10 min. An aliquot of supernatant was removed and assayed for protein concentration using the BCA protein assay (Pierce Scientific, Rockford, IL). The remaining supernatant was stored in aliquots at −80 C until analysis. A panel of proinflammatory markers were measured using a multiplex bead-based assay as described by the manufacturer (Bio-Plex Pro kit, Bio-Rad, Hercules, California).

2.5. siRNA sequences

The PCA3 siRNA was purchased from Thermofisher Scientific technology (CA, USA) (#siRNA ID 4390771), which includes a mix of two siRNA sequences against PCA3, the target of these siRNA is the mRNA coded from the location of Chromosome 9: 76764436–76787569. A non-targeting sequence siRNA for target-specific knockdown (scramble) was purchased from Dharmacon (Lafayette, CO).

2.6. Silencing of PCA3 gene

To investigate the role of PCA3 in arthritis pathogenesis, we employed siRNA-mediated silencing in the human synovial cell line SW982. Specific siRNAs targeting PCA3 mRNA were designed and synthesized, ensuring efficient and targeted knockdown. Delivery of these siRNAs was achieved using lipid nanoparticles, a method known for its efficacy in promoting cellular uptake. Following treatment, RNA isolation and quantitative real-time PCR (qPCR) were performed to confirm successful knockdown of PCA3 mRNA expression. Subsequent functional studies focused on analyzing the impact of PCA3 silencing on key aspects of arthritis, including cytokine production, cell proliferation, and cartilage degradation markers. This approach, employing lipid nanoparticle-mediated siRNA delivery, provides a robust and reliable method for investigating the role of PCA3 in the development and progression of arthritis.

2.7. Statistical analysis

Statistical analysis was performed using SPSS version 28 (IBM Corp., Armonk, NY). Data were analyzed to compare the effects of PCA3 silencing on various parameters between the control and experimental groups. Independent samples t-tests were used to assess significant differences in means between groups. A p-value greater than 0.05 was considered statistically non-significant, indicating no significant difference between the groups.

3. Results and discussion

The mRNA expression levels of various proinflammatory markers in patients with arthritis illustrated in Fig. 1. The investigation focused on assessing the mRNA expression of proinflammatory markers, including TNF-α, IL-1β, IL-4, IL-6, IL-8, IL-10, and IL-13. The mRNA expression levels were measured and quantified for each marker. The results revealed distinct patterns of mRNA expression among the markers. TNF-α exhibited the highest level, with a value of 3.7 ± 0.03. IL-6 showed a significant mRNA expression level as well, with a value of 2.1 ± 0.02. IL-1β, IL-4, and IL-10 displayed moderate mRNA expression levels, with values of 2.0 ± 0.02, 1.9 ± 0.02, and 1.9 ± 0.9 respectively. In contrast, IL-8 and IL-13 exhibited relatively lower mRNA expression levels, with values of 1.5 and 0.9, respectively. The obtained results offer significant insights into the mRNA expression patterns of proinflammatory markers in individuals with arthritis. These findings enhance our understanding of the molecular mechanisms that underlie the disease, contributing to a deeper comprehension of its pathogenesis.

An analysis of the protein levels of various proinflammatory markers in patients with arthritis presented in Fig. 2. The study focused on assessing the expression of proinflammatory markers, including TNF-α, IL-1β, IL-4, IL-6, IL-8, IL-10, and IL-13. Protein levels were quantified and measured for each marker using ELISA. The results indicated varying levels of expression among the markers. TNF-α exhibited the highest protein level, with a concentration of 3 units. IL-6 demonstrated a notable protein level as well, with a concentration of 1.8 units. IL-1β, IL-4, and IL-10 displayed moderate protein levels, with concentrations of 1.5, 1.7, and 1.7 units, respectively. In contrast, IL-8 and IL-13 showed relatively lower protein levels, both measuring 1.1 units. These findings provide valuable insights into the protein profile of proinflammatory markers in arthritis patients, which contributes to our understanding of the disease

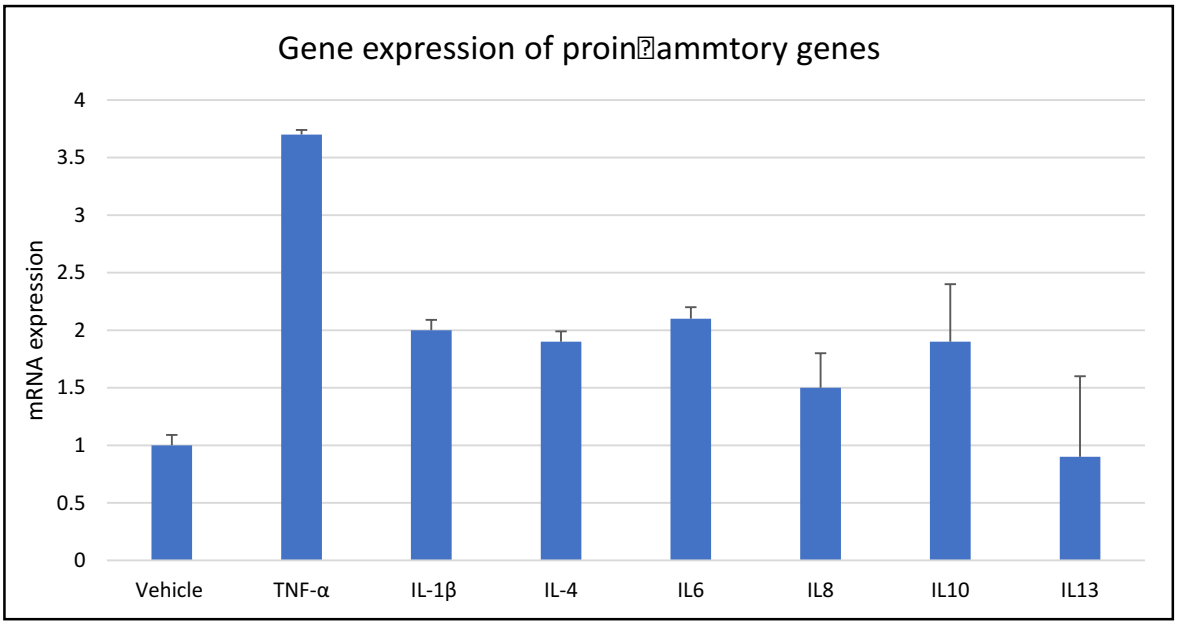

Fig. 1. The gene expression of using mRNA for proinflammatory genes in Arthritis Patients including TNF-α, IL-1β, IL-4, IL-6, IL-8, IL-10, and IL-13 in compared to vehicle.

pathogenesis and may potentially lead to the development of targeted therapeutic interventions.

The moderate upregulation of IL-10 observed following PCAT3 silencing in SW982 cells, despite its anti-inflammatory properties, could reflect a complex interplay of factors within the context of arthritis. While IL-10 typically acts to suppress inflammation, its moderate increase in this specific scenario might indicate a compensatory mechanism. This could be triggered by the initial reduction in pro-inflammatory cytokines like TNF-α, IL-1β, and IL-6, following PCAT3 silencing. The cells may respond by producing a moderate amount of IL-10 to counterbalance the decrease in pro-inflammatory signals and maintain a degree of inflammatory balance. Alternatively, the moderate upregulation of IL-10 could suggest a complex interplay between different inflammatory pathways, where the overall inflammatory response is still skewed towards a pro-inflammatory state, despite the reduction in specific pro-inflammatory markers. Further investigation is needed to fully elucidate the precise mechanisms driving IL-10 upregulation in this context and its implications for managing arthritis inflammation [12].

Insights into the expression levels of PAC3 gene using Real-time RT-PCR in control and arthritis patients are provided in Fig. 3 A. In the control group, PAC3 mRNA expression level was 1 ± 0.04 while in arthritis patients was 4.2 ± 0.3. These findings indicate a substantial upregulation of PAC3 gene expression in arthritis patients compared to controls. The statistical analysis using a t-test revealed a significant difference between the two groups, with a p-value of 0.009.

To evaluate the expression level of PCA3 in SW980 cell line, cells were transfected with siPCA3 while the control group was transfected with scramble. mRNA level for PCA3 gene was measured for both groups. For control group the PCA3 expression was normalized to 1 while PCA3 transcription level was abolished significantly in cells transfected with siPCAT3. These findings indicate a substantial downregulation of PAC3 gene using siRNA. The correlation between PCA3 and proinflammatory genes were tested using RT-PCR. Cells were transfected with siPCA3 showed significant inhibition of TNF-α, IL-6 and IL-10 while increase in IL-1β. The mRNA was 1, 04. $\pm$ 0.02, 0.3 $\pm$ 0.07, 0.5 $\pm$ 0.06, and 1.3 $\pm$ 0.1 for scramble, TNF-α, IL-6 IL-10, and IL-1β respectively (Fig. 4).

The signaling pathway regulated by PCA3, leading to chondrocyte apoptosis, is not fully elucidated. However, emerging evidence suggests that PCA3 might contribute to chondrocyte apoptosis by interacting with the Wnt signaling pathway, a crucial regulator of chondrocyte differentiation and survival. PCA3 could potentially disrupt the Wnt pathway, leading to increased expression of pro-apoptotic genes and decreased expression of anti-apoptotic genes, ultimately promoting chondrocyte death. This mechanism could play a significant role in arthritis pathogenesis, where chondrocyte apoptosis contributes to cartilage degradation and joint destruction. Further investigation into the precise molecular

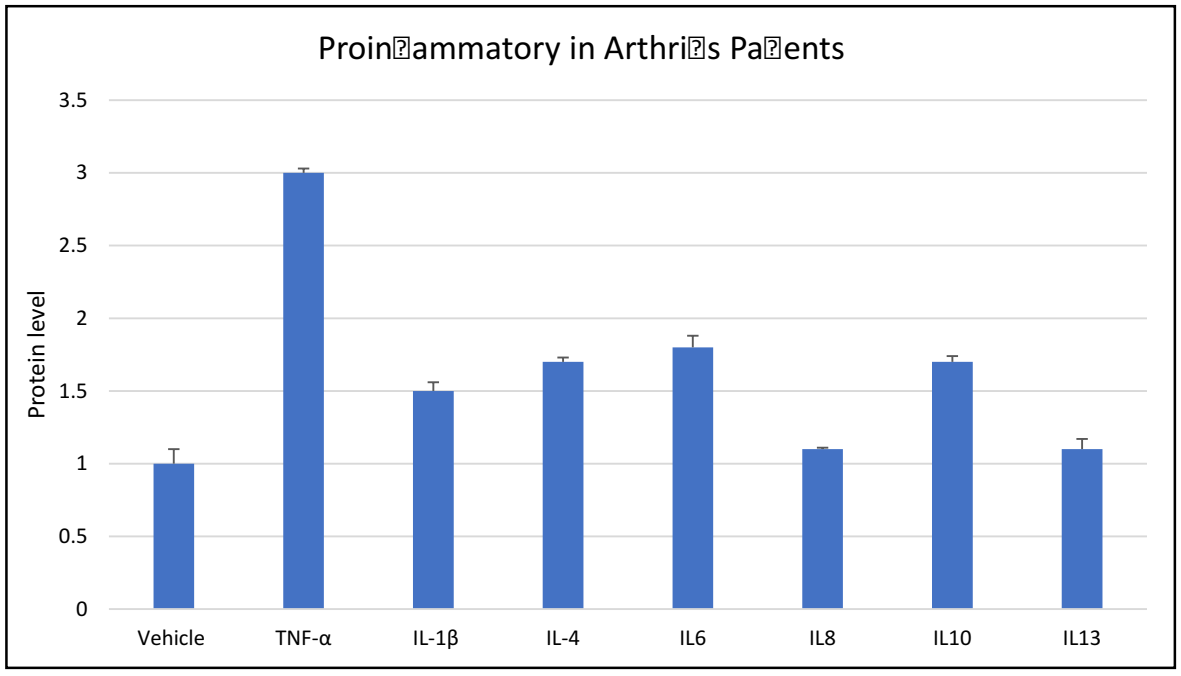

Fig. 2. Protein level of Proinflammatory markers including TNF-α, IL-1β, IL-4, IL-6, IL-8, IL-10, and IL-13 in compared to vehicle in Arthritis Patients using ELISA technique.

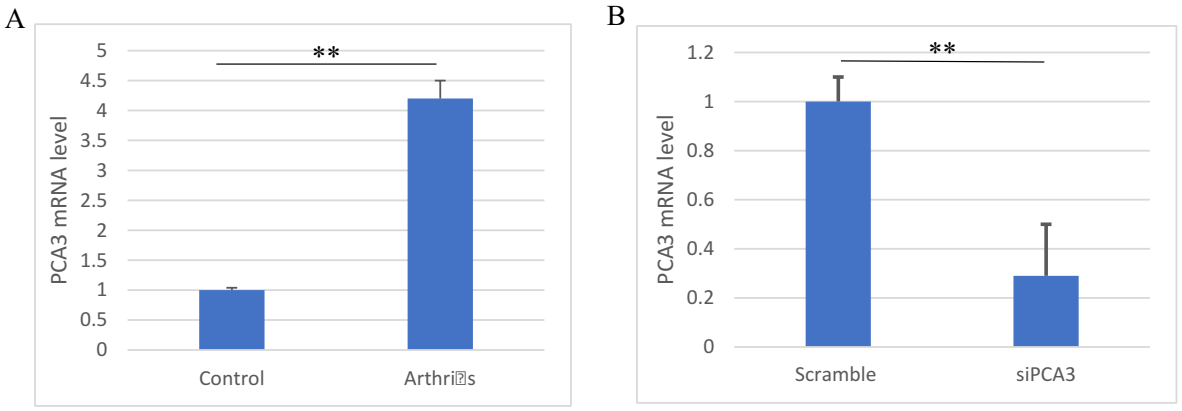

Fig. 3. Expression of PAC3 gene A. PCA3 gene was evaluate in healthy and arthritis people using Real-time RT-PCR. B. SW982 cells were infected with siPCA3 for 48 h then RNA was extracted and PCA3 gene expression was measured using RT-PCR. The control group was infected with scramble. (** $\leq$ 0.01).

interactions between PCA3 and the Wnt pathway is needed to clarify its role in chondrocyte apoptosis and its potential as a therapeutic target for arthritis [13].

The current study results present the mRNA and protein expression levels of various proinflammatory markers in patients with arthritis, including TNF-α, IL-1β, IL-4, IL-6, IL-8, IL-10, and IL-13, which were measured and quantified for each marker. The results revealed distinct patterns of mRNA and protein expression among the markers, with TNF-α exhibiting the highest expression level, followed by IL-6, while IL-8 and IL-13 exhibited relatively lower expression levels. The obtained findings offer valuable insights into the mRNA and protein expression profiles of proinflammatory markers in individuals with arthritis, contributing to our understanding of the underlying molecular mechanisms involved in the disease.

Additionally, the expression level of the PCAT3 gene was found to be higher in the arthritis group compared to the control group. This suggests a potential involvement of the PCAT3 gene in the regulation of inflammatory processes associated with arthritis. Furthermore, the study revealed a substantial

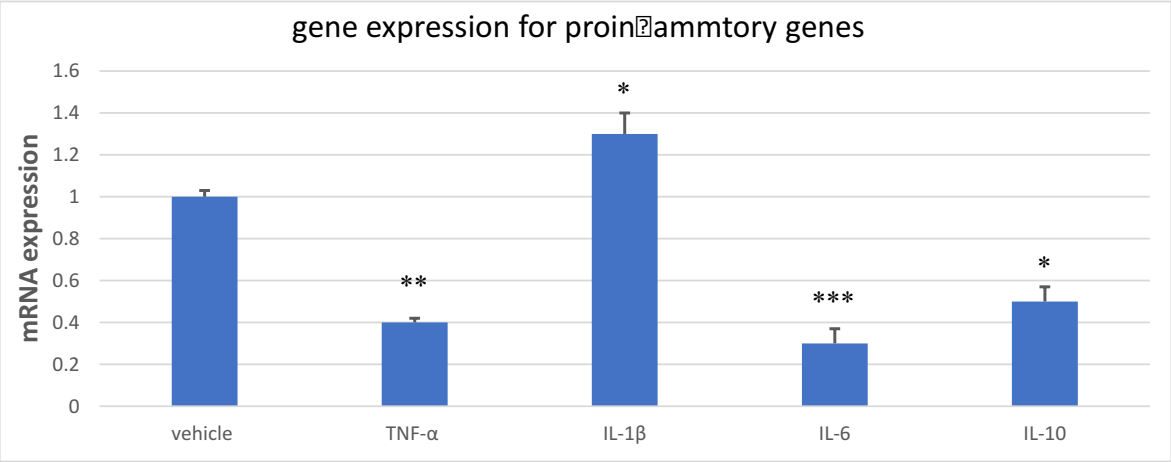

Fig. 4. Effect of siPCA3 on proinflammatory gene expression in SW982 cells. SW982 cells were treated with siPCA3 or scramble siRNA (vehicle) for 48 hours. Total RNA was extracted, and the expression of proinflammatory genes (TNF-α, IL-1β, IL-6, IL-8, IL-10, and IL-13) was measured using RT-PCR. Data are presented as fold change relative to the vehicle control group, normalized to GAPDH expression. Error bars represent the standard error of the mean (SEM) of three independent experiments. * = significance compared to vehicle (* $\leq$ 0.05, ** $\leq$ 0.01, and *** $\leq$ 0.001).

upregulation of PCA3 gene expression in arthritis patients compared to controls, indicating their potential involvement in the disease pathogenesis.

The given results are supported by several studies that have investigated the role of proinflammatory cytokines in arthritis pathogenesis [14, 15]. A study conducted by Vasilev et al. emphasized the significant role of IL-18, among various newly discovered cytokines, in the development of erosive and inflammatory arthritis [16]. In a study conducted by Debnath et al., it was demonstrated that during the early stages of macrophage activation, translational derepression plays a crucial role as a major mechanism in inducing feedback inhibitors that help regulate and suppress inflammation. This finding suggests an important regulatory pathway that functions to dampen the inflammatory response [17]. Moreover, Yu et al. revealed that TNF-α-induced chondrocyte apoptosis is a central pathological incident in articular cartilage injury or osteoarthritis [18].

Inflammation is a central driver of pain, stiffness, and joint damage in arthritis. The immune system mistakenly attacks the tissues surrounding joints, leading to an accumulation of inflammatory cells and the release of pro-inflammatory mediators like cytokines. These substances cause swelling, redness, and heat in the joints, further damaging cartilage and bone. This cycle of inflammation and tissue destruction can lead to irreversible joint deformities and reduced mobility [19].

The high expression of TNF-α in arthritis is a direct consequence of the activation and recruitment of immune cells, particularly macrophages and T cells, to the inflamed joint. Upon activation, these cells release a cascade of inflammatory mediators, including TNF-α, which plays a central role in perpetuating the inflammatory cycle. TNF-α acts as a potent proinflammatory cytokine, promoting the recruitment of more immune cells, increasing vascular permeability, and stimulating the production of other proinflammatory mediators. This self-amplifying loop, triggered by the initial activation of immune cells, results in the sustained high expression of TNF-α, contributing significantly to the development and progression of arthritis [20].

Several clinical trials have targeted specific proinflammatory markers in arthritis patients, including 14-3-3η, IL-6, TNF, IL-17, IL-18, and oxidative stress [21–24]. These studies have shown promising results in reducing disease activity and symptoms, but further research is needed to determine the long-term efficacy and safety of these treatments. The development of targeted therapeutic interventions that can effectively modulate the expression of proinflammatory cytokines and genes may provide a promising approach for the treatment of arthritis.

4. Conclusion

In conclusion, our study demonstrates that PCAT3 is significantly upregulated in arthritis patients and that its silencing in SW982 cells leads to a significant reduction in the expression of several proinflammatory genes, including TNF-α, IL-1β, IL-6, and IL-10. These findings suggest that PCAT3 plays a role in the pathogenesis of arthritis by contributing

to the upregulation of proinflammatory markers. These findings highlight the potential of targeting PCAT3 as a therapeutic strategy for managing inflammation in arthritis. Further investigations are needed to elucidate the precise mechanisms by which PCAT3 influences inflammation and to explore the potential for developing PCAT3-specific therapeutic interventions.

Conflicts of interest

The authors have no competing interests to declare that are relevant to the content of this article.

Ethics approval

This study protocol was ethically approved by the Institutional Review Board of AL-Zahra Educational Hospital (UW.MED.2024.0207, approved February 07th, 2024).

Data availability

Data generated from this study will be made available upon request.

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Author contributions

Conception: A,H,A; E,M,A,A; K,A,A
Design: A,H,A; E,M,A,A
Supervision: A,H,A
Literature: K,A,A
Review: A,H,A; K,A,A
Writing: K,A,A
Critical Review: A,H,A

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