ORIGINAL RESEARCH

SLAMF8 Promotes Atherosclerosis by Activating the TLR4 Signaling Pathway in Rheumatoid Arthritis

Ziyu Lin, BM; Guiling Sun, MM; Zhihong Ou, MM; Yanjin Wei, MM; Yuqiang Wang, PhD

ABSTRACT

Objectives • Rheumatoid Arthritis (RA) can accelerate atherosclerosis (AS) plaque formation. High prevalence of AS has been demonstrated in early-stage RA patients. Therefore, there is an urgent need to investigate what mechanisms and key molecules accelerate AS in RA to improve the management of RA.

Methods • We retrieved gene expression data for RA (GSE45291) and atherosclerosis (GSE28829) from Gene Expression Omnibus (GEO). Seventeen key genes were identified, and the top one candidate hub gene was SLAM family member 8 (SLAMF8). To investigate the role of SLAMF8 in AS and RA, U937 cells were differentiated into macrophages using Phorbol 12-myristate 13-acetate (PMA) and further transformed into foam cells by oxidized low-density lipoprotein (ox-LDL) treatment and siRNA was manipulated to knock down SLAMF8. Flow Cytometry was employed to assess cell state. The mRNA and protein expressions of the genes were investigated using western blot and RT-qPCR.

Results • SLAMF8 was screened as a key gene by bioinformatic methods. Compared to Mφ, SLAMF8, TLR4 and inflammatory cytokines, tumor necrosis factor α (TNF-α), and Interleukin 6 (IL-6) were noticeably expressed in foam cells. Knockdown of SLAMF8 could remarkably curtail TLR4, TNF-α, and IL-6 protein levels. Antagonizing SLAMF8 could attenuate inflammatory factors and apoptosis of foam cells by inhibiting the TLR4 pathway, thus mitigating the severity of AS in RA.

Conclusions • Our work demonstrated that SLAMF8 promoted AS in patients with RA by inducing inflammation and apoptosis of foam cells via TLR4 signaling. Therefore, SLAMF8 could be a possible therapeutic spot for AS in RA patients. (*Altern Ther Health Med.* [E-pub ahead of print.])

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INTRODUCTION

Traditionally, atherosclerosis was considered a result of passive lipid deposition within the arterial wall.[1] It is now recognized as a persistent chronic inflammatory state manifestation. Concurrently, rheumatoid arthritis (RA), with the prototypical characteristic of joint involvement, is also classified as an autoimmune disorder characterized by systemic inflammation and the presence of autoantibodies.[2] This indicates that there exists a certain degree of similarity in the risk factor and pathological processes between the two diseases. Emerging research strongly suggests that individuals with RA are more inclined to develop cardiovascular disease (CVD), especially AS.[3] A meta-analysis from Juan et al. reported 48% increase of CVD in RA patients.[4] And CVD accounts for about 50% deaths of RA patients.[5] Besides, high prevalence of AS has been demonstrated in early-stage RA patients. Therefore, there is an urgent need to investigate what mechanisms and key molecules accelerate AS in patients with RA to improve the management of RA.

Recently, systematic bioinformatics analysis has been utilized integratedly to identify disease-related biomarkers and reveal the underlying mechanisms. On this basis, multiple studies have discovered various RA-related and AS-related molecules and signaling pathways, some of which have been proved to be closely involved in the development of the two diseases.[6-8] However, only few research have emphasized the biomarkers related to AS in RA. Liu et al. discovered six immune-related hub genes for diagnosis of AS in RA.[9] An exploratory analysis from Bathon et al. identified a number of molecules and integrated them into a new multimarker to predict and discriminate patients with AS in

RA more precisely.[10] In order to delineate pathogenesis for AS in RA, further investigations are warranted to uncover more relevant molecules and pathways.

The signaling lymphocytic activation molecule family (SLAMF) proteins, which belong to CD2 family, are mainly expressed by immune cells and associated with the regulation of immune responses.[11] Several members of SLAMF have been proved to be intensely associated with inflammatory diseases. For instance, SLAMF1 was upregulated and SLAMF1-positive B cells were increased in systemic lupus erythematosus.[12] SLAMF7 expression was discovered to be elevated in sepsis patients and exerted a negative modulatory role in polymicrobial sepsis.[13] Of note, several SLAMF members have been reported to play important roles in AS or RA. SLAMF7, which was mainly regulated by IFN-γ and exaggerated inflammation in RA.[14] SLAMF1 was identified as a key biomarker in RA with integrated bioinformatics analysis.[6] Moreover, SLAMF8 was documented to alleviate inflammatory state in RA through interrupting ERK/MMPs signaling.[15] Besides, SLAMF8 was also discovered to be a hub gene in advanced AS with bioinformatics analysis.[16] However, whether SLAMF8 plays a role in the intersection of the two diseases is ambiguous.

Therefore, more studies are needed to comprehend the pathogenesis of AS development in RA fully. In this study, hub genes were firstly identified for AS in RA. Gene Expression Omnibus (GEO) is curated and managed by the National Center for Biotechnology Information (NCBI). And it is our primary information source for gene expression data.[17] Established in 2000, GEO houses large-scale gene expression data shared by science institutions worldwide. Two original datasets (GSE45291 and GSE28829) were retrieved from the GEO Database for this investigation. Each dataset was analyzed individually to identify their respective differentially expressed genes (DEGs) and potential molecular mechanisms. Subsequently, various bioinformatics analyses were conducted to stipulate the same DEGs and their roles. Then in vitro experiments were conducted to validate the role of key hub genes in both AS and RA.

MATERIALS AND METHODS

Data

GSE45291[7] and GSE28829[8] were procured from the GEO database (http://www.ncbi.nlm.nih.gov/geo). Selection standards: (1) the research specimens must be of human origin; (2) the two independent datasets selected do not need to be merged, allowing for the consideration of different platforms; (3) each dataset should consist of ten or more samples. Table. 1 offers comprehensive details.

Table. 1. General overview regarding the GEO microarray datasets

GEO	Platform	Tissues	Case	Control	Total	Case	Control
GSE45291	GPL13158	Whole Blood	Rheumatoid Arthritis (RA)	Healthy Control (HC)	513	493	20
GSE28829	GPL570	plaque	Advanced Atherosclerosis (AA)	Early Atherosclerosis (EA)	29	16	13

Identification of COMMON DEGs

"GEOquery" and "affy" Bioconductor package were implemented to interpret microarray data and normalize them respectively to retrieve DEGs, which were subsequently analyzed utilizing "limma". To rectify erroneous positives, we modified the *P* value. Our criteria for gene differential expression were set as "Adjusted $P < .05$". We extracted common DEGs by intersecting the DEGs identified via an online Venn diagram tool.

Functional Enrichment Studies for Respective DEGs

We converted the gene names of DEGs using "org.Hs.eg.db". Afterwards "clusterProfiler" was applied to forecast the respective DEGs' roles. "enrichplot" and "ggplot2" R packages made it easier to visualize the results of GO and KEGG studies. $P < .05$ was deemed as a significant change.

Functional Enrichment Analyses of Common DEGs

The KEGG Orthology Based Annotation System (KOBAS) (http://kobas.cbi.pku.edu.cn)[9] is a web-based platform for the analysis of gene enrichment through annotation, as well as the automatic mapping to genes with known annotations for the identification of putative pathways and disorders. Biological analyses were carried out using KOBAS. The top 24 items of GO and KEGG were rendered as bubble diagrams through "ggplot2". Statistical significance was determined to exist at a $P < .05$.

Screening of Hub Genes

We used Cytoscape (v3.9.1) and the CytoHubba plugin (version 0.1) to score each node gene using six filtering algorithms: MCC, MNC, DMNC, Closeness, and EPC. The R package "UpSet " facilitated the hub gene screening". Additionally, a versatile and intuitive website called GeneMANIA (http://www.genemania.org)[12] was used to build a biological co-expression network of these hub genes and anticipate the role of hub genes.

Hub Gene Expression Validation in Other Data Sets

We used dataset GSE55235[14] and dataset GSE100927[15] to confirm the expression information regarding hub genes that had been identified. The GSE55235 dataset comprises 20 healthy controls and 59 patients (26 with OA and 33 with RA). The GSE100927 dataset includes 66 AS lesions and 35 control arteries without atherosclerotic lesions. We used GraphPad Prism 9 (USA) to execute the unpaired *t* test. The established statistical significance is $P < .05$.

Materials

U937, human myeloid leukemia cells, were supplied by the Institute of Chinese Cell Research. RPMI-1640 and fetal bovine serum (FBS) were acquired from Gibco BRL (USA). Ox-LDL was from Yuanye Biotech. Inc. (Shanghai, China). Sigma (USA) provided Phorbol 12-myristate 13-acetate (PMA), and anti-rabbit SLAMF8 was acquired by Biotechne

(USA), anti-rabbit interleukin-6, anti-rabbit tumor necrosis factor-α, and anti-rabbit TLR4 were supplied by Cell Signaling Technology (USA). Protein ladder, DAPI Staining Solution, BCA protein assay kit, Oil red O Staining Kit, and annexin V-FITC/PI double-staining apoptosis assay kit were both obtained from Beyotime Biotech. Inc. (Shanghai, China).

Foam Cell Model Establishment

The establishment of foam cells is based on the successful induction of macrophages by adding PMA to U937. After 8 hours of starvation in serum-free RPMI-1640, cells were assigned to different groups, consisting of the control and the model. The control were cells U937 cells with logarithmic growth cultured with 100μg/L PMA for 24h. The model was U937 cells with logarithmic growth phase inoculated in RPMI 1640 culture medium containing 100μg/L PMA and 100 mg/L ox-LDL. All of them were placed in an incubator at 37°C, saturated humidity for 72 hours in a 5% CO_2 environment.

Oil red O staining

After one gentle PBS rinse, cells were fixed in 10% formaldehyde for 30 minutes. Next, cells were treated with Oil Red O dilution, consisting of oil red o and deionized water(as a ratio of 3 to 2, respectively, for 10 minutes at room temperature. In the next phase, 60% isopropanol was added to remove the excess dye. Entering the final stage, the intracellular lipid contents were observed with a microscope.

Assessment of Apoptosis by Flow Cytometry

Cells were collected by centrifuging at 2000rpm for 5min and then were washed using precooled PBS(PH=7.4) two times. Subsequently, 2-5*105 cells were collected and 1x annexin-binding buffer was added, and then the resuspended cells were gently blown. Then cells were tinted with 5ul Annexin V-APC and 5ul 7-AAD successively, gently blown and mixed, and incubated at ambient temperature out of light for 5-15 minutes.

Western Blotting

Radioimmunoassay (RIPA) cell lysis buffer was used to extract protein, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was employed to isolate protein. The poly (vinylidene fluoride) membrane (Millipore, USA) encounters the isolated protein for further processing. The converted polyacrylamide membranes were placed into TBST buffer containing protein blockers. They incubated it overnight, mixed with matching antibodies at 4°C. After washing, the protein that binds to the first antibody was bound to the second antibody. After incubating for 1 hour, it was washed again. The bands were colored using a luminescent solution, and then were accessed and standardized of the target protein levels, with the help of Image-J.

Real-time PCR

TRNpure Total RNA Kit from NOBELAB BIOTECHNOLOGIES CO., LTD (Nobelab, Beijing, China) was applied to extract Total RNA from the cells. 2*SYBR Premix UrTaq II (Nobelab, Beijing, China) was used to reverse transcript RNA into cDNA. We performed qPCR with 2*UrTaq Master Mix 100 (Nobelab, Beijing, China). Primer sequences used are as follows: GAPDH receptors were 5'- TCAAGAAGGTGGTGAAGCAGG –3' (forward) and 5'- TCAAAGGTGGAGGAGTGGGT–3' (reverse).; SLAMF8 receptor were 5'- CCCAACATCAGCGAAAT –3' (forward) and 5'- CATCAGGAGCAGCGAGA –3' (reverse).

SLAMF8 Silencing

Small interfering RNA (siRNA) Oligonucleotide were purchased from Guangzhou Rebo Biotechnology Co., Ltd. SLAMF8-siRNA sequence is as follows: genOFFTM st-h-SLAMF8-001: 5'-CATGGACTTTGGTATGGAA-3'; genOFFTM st-h-SLAMF8-002: 5'-GGTACAAGTTTCATTG CT-3'; genOFFTM st-h-SLAMF8-003: 5'-GGACAGGT GCTGAGCATTT-3', and control siRNA: forward, 5'-UCUCUCCGAACGUCACGUTT-3', reverse, 5'-ACGUGACGUUCGGAGATT-3'. The siRNA and transfection reagent (GeneCopoeia, EndoFectin Max) were diluted in proportion to the manufacturer's instructions and transfected. RT-qPCR was used to detect the transfection efficiency of siRNA, the silencing efficiency of SLAMF8-siRNA was measured, and the sequence with the best interference rate was selected for subsequent verification experiments.

Statistical analysis

All results were evaluated with SPSS 26.0; basic data was converted to mean ± standard deviation(SD). Continuous data was analysed using an independent-samples *t* test to obtain *P*-values. When $P < .05$ (*), $P < .01$ (**), $P < .0002$ (***), $P < .0001$ (****), the discrepancies between samples were statistically significant.

RESULTS

Identification and enrichment analysis of DEGs

Microarray data from the GSE45291 and GSE28829 datasets underwent standardization via a "Batch correction" process. Employing a cutoff established at FDR<0.05 and |log2(FC)|>1, we yielded DEGs from the datasets: 4159 from GSE45291 and 174 from GSE28829. Visualization of the DEGs' expression levels was accomplished through the use of volcano maps (Figure 1A, B), and we identified 52 common DEGs. Upon excluding genes demonstrating contradictory expression trends in GSE45291 and GSE28829, we discerned 49 common DEGs, which were all upregulated, indicating that these genes probably exert accelerating roles in AS and RA.

Respective DEGs underwent an examination involving GO and KEGG. We implemented the clusterProfiler package within the R software to conduct the GO and KEGG Pathway enrichment analysis. We classified GO annotations of DEGs into three categories: Biological Process (BP), Cellular Component (CC), and Molecular Function (MF). Within the BP category, DEGs from GSE45291 were predominantly

Figure 1. Identification and analysis of common DEGs. (A) The volcano plots of DEGs in GSE45291 (B)and in GSE28829. Upregulated genes were marked in light red ; downregulated genes were marked in light blue. (C) GO analysis of DEGs in GSE45291 presented by circle graph. (D) GOanalysis of DEGs in GSE28829 presented by cluster graph. (E)The GO analyses of common DEGs in GSE45291 were presented by bar plot. (F) KEGG pathway analyses of common DEGs in GSE28829 were presented by bar plot.

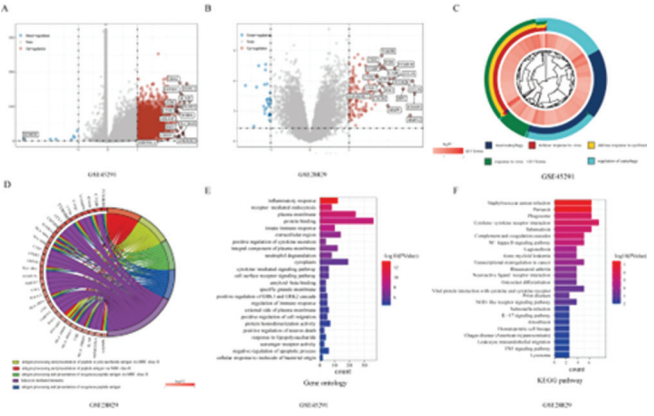
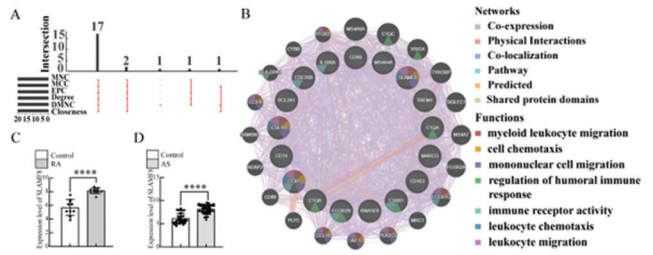


Figure 2. Acquisition and verification of the key gene-SLAMF8. (A)The UPSET diagram displayed 17 overlapping hub genes using seven closeness algorithms of CytoHubba. (B) The co-expression network of the hub genes conducted by GeneMANIA. (C) GSE55235 (D) GSE100927 were applied to validate SLAMF8 expression level using *t* test.



Abbreviations: RA, rheumatoid arthritis; AS, atherosclerosis.

enriched in the 'proteasomal protein catabolic process' and 'response to the virus'. As for KEGG Pathway enrichment analysis, DEGs from GSE45291 significantly enriched in pathways like 'Amyotrophic lateral sclerosis', 'Salmonella infection', and 'Endocytosis' (Figure 1C). DEGs from GSE28829 mainly enriched in 'Staphylococcus aureus (SA) infection', 'Phagosome' and 'Tuberculosis' (Figure. 1D).

Through the KOBAS database, we analyzed 49 common DEGs. The GO analysis results indicated that these genes primarily enriched in inflammatory response (P = 5.61E-14), receptor-mediated endocytosis (P = 4.23E-11), plasma membrane (P = 1.66E-10), protein binding (P = 4.68E-10), and innate immune response (P = 1.82E-09)(Figure 1E). The KEGG Pathway analysis aimed to discover the connection between DEGs and signaling pathways. Common DEGs were primarily implicated in Staphylococcus aureus infection (P = 3.44E-08), Pertussis (P = 5.83E-08), Phagosome (P = 1.58E-06), and Cytokine-cytokine receptor interaction (P = 2.00E-06)(Figure 1F). Overall, the results strongly suggest that the common DEGs are primarily related to the inflammatory response and SA infection.

Identification and Analysis of the hub Gene-SLAMF8

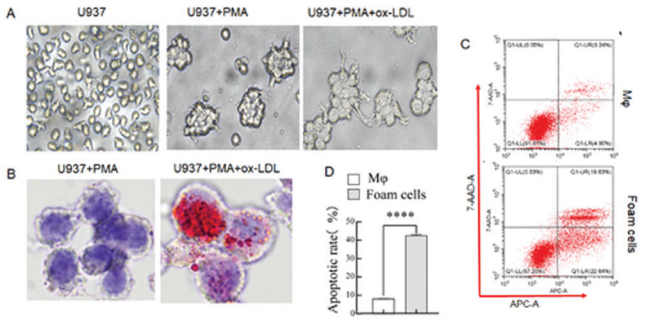
Utilizing the seven closeness algorithms of CytoHubba, we identified the top 20 hub genes. Overlapping Upset diagrams revealed 17 common hub genes, namely, SLAMF8, BCL2A1, C1QA, C1QB, C3AR1, CCR1, CD14, CD163, CD68, CSF1R, CSF2RB, FCGR2B, IL10RA, MARCO, MS4A4A, RNASE6, and TREM1 (Figure 2A). The GeneMANIA database facilitated the co-expression network drawing and related function analysis of these genes. The complex PPI network of these hub genes depicted the co-expression of 84.59%, physical interactions of 6.39%, co-localization of 4.85%, pathway involvement of 1.95%, and prediction of 1.41% (Figure 2B).

Based on the aforementioned bioinformatics analysis, we obtained the top one candidated hub gene, SLAMF8. Given the intense association between SLAMF8 and immune response, SLAMF8 was selected as a key hub gene and the role of SLAMF8 was further investigated in the following in vitro experiments. To enhance the reliability of our results, we undertook the verification of the pivotal gene SLAMF8 by leveraging two additional datasets, GSE55235 (associated with healthy joints and rheumatoid arthritis joints) and GSE100927 (which pertains to atherosclerotic lesions and control arteries). Our findings indicated a significant up-regulation of SLAMF8within diseased tissue samples compared to their normal counterparts (Figure 2C, D).

Foam cells are characteristic cells in the formation of atherosclerosis.

In the early stages of AS, monocytes enter blood vessels and become Mφ, where ox-LDL is engulfed by type scavenger receptors, forming foam cells. As a human lymphoma cell, U937 is essentially a monocyte with round nuclei, irregular cell bodies, and little cytoplasm (Figure 3A). It can be induced by PMA into Macrophages, into the growth of adhesion, a large number of scavenger receptors began to be expressed on the surface. And with the induction of ox-LDL, macrophages turns into foam cells. In this study, in U937+PMA group, U937 cells were treated with PMA for 24 h, the cell body was polygonal, and the nucleus was lobulated; cells began to adhere to the wall, suggesting that U937 has been converted from monocytes to Mφ after PMA stimulation (Figure 3A). In U937+PMA+ox-LDL group, U937 is polygonal and branched after 72 hours of incubation with PMA and ox-LDL, and the cytoplasm is filled with numerous lipid vesicles, which are characteristics of foam cells (Figure 3C). Oil Red O staining was used to observe a large number of red-stained lipid particles in the cytoplasm in U937+PMA+ox-LDL group (Figure 3E), whereas the U937+PMA group was U937 cells with no red-stained particles present in their cytoplasm (Figure 3B). These results

Figure 3. Foam cell formation induced by PMA and ox-LDL. (A) Cell morphology of U937 cells under inverted microscopy in U937, U937+PMA and U937+PMA+ox-LDL groups. (B) Oil red staining of U937 cells in U937+PMA and U937+PMA+ox-LDL groups. (C)Flow cytometry was used to detect apoptosis in macrophages and foam cells. (D) The apoptosis rates of macrophages and foam cells were compared by *t* test. Mean ± SD, n = 3. macrophages :Mφ.



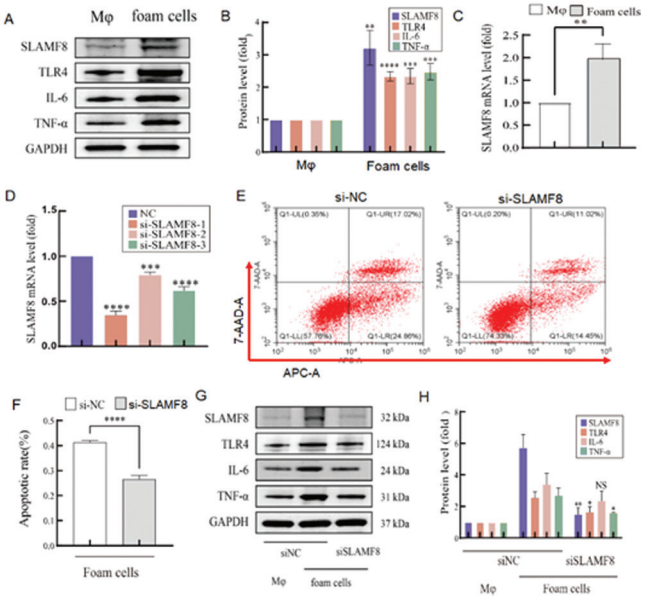
indicated the successful construction of foam cell model in U937+PMA+ox-LDL group. Apoptosis induction of foam cells was assessed using the Annexin v-apc7-aad double-staining apoptosis detection kit and Flow cytometry, with live cell numbers of 91.81% and 57.20% in the U937 and foam cell groups, respectively (Figure 3C and D). Furthermore, the number of apoptotic cells in the macrophages and foam cell groups was 8.14% and 42.72%, respectively (Figure 3F, G). These data indicated that the apoptosis rate of foam cells was significantly increased compared with macrophages (Figure 3H).

SLAMF8 can increase the expression of IL-6 and TNF-α through the TLR4 pathway in U937 foam cells

U937 foam cells show an elevated expression of SLAMF8, TLR4, IL-6, and TNF-α compared to Mφ. SLAMF8 is one of eight key genes uncovered by bioinformatic studies, but whether it can be used as a target gene for atherosclerosis has not been studied in the literature. Foam cells are the basis of AS plaque formation. We used U937 to construct Mφ and foam cells. Western blot analysis and q-PCR showed that, compared with macrophages, SLAMF8, TLR4, and the inflammatory factor IL-6, TNF-α was significantly expressed in foam cells (Figure 4A-C). These results suggest that SLAMF8 is involved in the development of AS in U937 foam cells by increasing inflammatory factors such as IL-6 and TNF-α via the TLR4 pathway.

SLAMF8 knockdown can inhibit the TLR4 pathway, thus lowering the inflammatory cytokine. In order to establish the role of SLAMF8 in the development of AS via the TLR4 pathway, we first activated U937 into macrophages using PMA and transfected Mφ with si-NC to create a blank group. The foam cells were then activated by ox-LDL and transfected with si-NC and si-SLAMF8 And the cells in si-SLAMF8-1 had the highest transfection efficiency by PCR (Figure 4D). According to Flow cytometry, SLAMF8 knockdown could drastically lower the apoptotic rate of foam cells compared to

Figure 4. SLAMF8, TLR4, IL-6, and TNF-α were expressed at higher levels in U937 foam cells than in Mφ. (A) SLAMF8, TLR4, IL-6, and TNF-α proteins in U937 foam cells and Mφ were depicted in representative gel blots, with GAPDH serving as the loading control. Representative protein lysates from Western blots were shown. The result of one of three separate tests. (B) Protein bands were assessed in grayscale using Image J, their expression levels were normalized, and their differences were represented as pictures. (C) Using quantitative RT-PCR, the mRNA expression values of SLAMF8 were identified (n = 3/group). (D) The mRNA level of SLAMF8 after three si-SLAMF8 transfecting foam cells. (E and F) Apoptosis of transfected Mφ and foam cells. The optimal si-SLAMF8 was chosen to transfect foam cells and macrophages, respectively. (G and H) The protein expression of SLAMF8, TLR4, IL-6, and TNF-α of transfected Mφ and foam cells. Using GAPDH as the reference gene (n = 3/group), presented as mean ± SD.



si-NC group (Figure 4E and F). TLR4 and the inflammatory cytokine TNF-α protein levels were significantly reduced in foam cells that had SLAMF8 knocked down (Figure 4 G and H). All the data indicated that SLAMF8 could promote AS in RA by inducing inflammation via TLR4 pathway.

DISCUSSION

In this study, we firstly detected 49 common genes in AS-related datasets and the 49 genes were all up-regulated in both AS and RS. GO and KEGG analyses revealed that these common genes were most associated with inflammatory response. These results implied the importance of inflammation in the two diseases, which was in accordance with the published studies. Besides, the 49 common genes were also significantly enriched in SA infection. Infection has reported to be a risk factor for the development of AS, and various pathogens were detected in atherosclerotic plaque, such as Streptococcus mutans, Streptococcus sanguis and

Fusobacterium nucleatum.[24] However, whether SA exists in atherosclerotic plaque hasnot been explored. A recent report from Frodermann et al. only demonstrated that the heat-killed SA could inhibited the progression of AS.[25] Moreover, SA superantigen were detected in synovial and blood of RA patients.[26] Therefore, SA infection probably play a role in pathogenesis of AS in RA. And our study provided a direction for the investigation of SA infection in AS in RA.

Utilizing CytoHubba and overlapping upset diagrams, 17 hub genes were finally obtained, among which SLAMF8 was the top one candidated hub gene. Then the up-regulation of SLAMF8 was validated in another one RA-related dataset (GSE55235) and AS-related dataset (GSE100927). Further, to elucidate the role of SLAMF8 in AS in RA, foam cell model was first established using U937 and oil red O staining revealed successful development of foam cell model. And SLAMF8 was identified to be upregulated in foam cell model. As a member of SLAMF, SLAMF8 is mainly expressed by myeloid cells.[27] SLAMF8 has been reported to play a role in various inflammatory diseases. For example, SLAMF8 in macropahages was uprelated and facilitated the progression in acute rejection in kidney transplantation (ARKT).[28] Deficiency of SLAMF8 inhibited release of cytokines and the infiltration of inflammatory cells in acute hepatic injury.[29] Moreover, there are a number of studies have focused on the role of SLAMF8 in RA. SLAMF8 was detected to be upregulated and SLAMF8 depletion could attenuate joint arthritis and synovial hyperplasia in RA mice model, implying that SLAMF8 contributed to the onset of RA.[30] The studies emphasized the role of SLAMF8 in AS are limited. Only one report detected SLAMF8 as a hub gene in AS with bioinformatics analysis.[16] Our study first reported SLAMF8 as a hub gene for accelerated AS in RA.

Monocytes in blood circulation were recruited and turned into Macrophages in the impaired vascular endothelium. The macrophages promoted the oxidation of low-density lipoprotein(LDL) to form ox-LDL, which in turn engulfed by macrophages. Then, the foam cells were formed.[31] The formation and deposition of foam cells are key pathological characteristics and important for the onset and progression of coronary AS.[32] Therefore, in vitro foam cell model was usually constructed from myeloids or smooth muscle cells to investigate the pathogeneis of AS.[31] U937, a human myeloid leukemia cell line, which induced by PMA to turn into Macrophages was widely used to construct foam cell model.[33]

Ox-LDL uptake of foam cells will lead to the cell apoptosis, which in turn promotes the release of proinflammatory cutokines such as IL-6 and TNF-α, thereby aggravating AS.[34] Consistently, in our study, increased apoptotic rate of foam cells were detected compared to macrophages. Recently, retarding foam cell apoptosis is seen as a promising therapeutic strategy for AS.[34] Importantly, in this study, SLAMF8 knockdown inhibited foam cell apoptosis. Therefore, developing antagonist of SLAMF8 may be a novel treatment for AS in RA.

Moreover, TLR4 and the inflammatory factor IL-6, TNF-α were also identified to be upregulated in foam cell model. And SLAMF8 depletion restrained the expression of TLR4, IL-6 and TNF-α. Toll-like receptors (TLRs) are sequence identification receptors found on plasma membranes of stem cells. They are members of the TLR family.[35] TLR4 overactivation produces inflammatory chemicals implicated in RA and AS. The level of TLR4 was elevated in synovial tissues and immune cells in RA patients. And the activation of TLR4 promoted the damage of joint by inducing inflammatory state and destroying extracellular matrix in RA.[36] Similarly, multiple studies have indicated that TLR4 play a key role in the onset and progress of AS by regulating the inflammatory response and initiating dysfunction of endothelial cells in AS.[37] Several investigations have documented that SLAMF8 could promote inflammation via TLR4 in a number of inflammatory diseases, such as ARKT[28] and RA.[30] Recently, developing drugs targeting TLR4 has attracted scientists' attention for treating RA and AS.[38] By identifying molecular patterns linked to pathogens, TLRs mediate inflammatory reactions. TLR4 recruits MyD88, an adapter protein, and promotes IKB phosphorylation, resulting in ubiquitination, proteasome degradation, and inhibition of NF-kB. NF-kB translocates into the nucleus and upregulates the levels of pro-inflammatory substances, specifically IL-6 and TNF-α, thereby further stimulating the inflammatory response.[39,40] In vitro studies demonstrate that TLR4 inhibitors exhibit potent inhibitory activity against TLR4, contributing to the suppression of TNF-α and IL-6 release.[41,42] TNF-α and IL-6, particularly IL-6, are indispensable in the pathological mechanisms observed in patients suffering from RA complicated by AS. In our study, the levels of TNF-α and IL-6 were decreased after SLAMF8 depletion, suggesting that the inflammatory state was inhibited in foam cells. Therefore, SLAMF8 may promote AS in RA by inducing inflammation and apoptosis of foam cells via TLR4 signaling.

Consequently, this study may inform future research trajectories. These include the role of SA infection in the correlation between AS and RA, the potential for hub gene-associated drugs to ameliorate atherosclerosis and RA, especially SLAMF8. This work does have several notable drawbacks, though. Primarily, the work relies on public databases for microarray data and for conducting a portion of the analyses rather than on experimental data. In this study, the role of SLAMF8 was only validated in vitro, and in vivo experiments are needed to be performed further. Moreover, comprehensive, large-scale verifications need to be carried out to elucidate the roles of these key hub genes, which constitute the central point of future tasks.

CONCLUSION

In total, this study discovered 49 DEGs, all of which are upregulated, through bioinformatics analyses. Following this, 17 hub genes were isolated through seven closeness algorithms of CytoHubba. The study also conducted

enrichment analyses and PPI network analyses. SLAMF8 was identified as top one candidate hub gene and upregulated in foam cell model. Of note, in vitro experiments revealed that SLAMF8 inhibited inflammation and apoptosis of foam cells via TLR4 signaling. Our findings indicated that SLAMF8 may promote AS in patients with RA by inducing inflammation and apoptosis of foam cells via TLR4 signaling. These advances our knowledge of the mechanisms of RA that are complicated by AS. SLAMF8 was discovered to be a new therapeutic target for AS in patients with RA And our study provides a novel idea for management and therapy of AS in patients with RA.

CONFLICT OF INTEREST

None.

FUNDING

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AUTHOR CONTRIBUTIONS

Z-Y L and Y-Q W designed the study and performed the experiments, G-L S collected the data, Y-J W and Z-H O analyzed the data, and Z-Y L prepared the manuscript. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data material in this study is available from the corresponding author upon reasonable request.

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