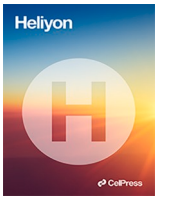
Heliyon 10 (2024) e24034

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Analysis of subsets and localization of macrophages in skin lesions and peripheral blood of patients with keloids

Xinyi Lv [a], Zhenghao He [a,b], Ming Yang [a], Lu Wang [a], Siqi Fu [c,*]

[a] Department of Dermatology, The Second Xiangya Hospital of Central South University, Hunan Key Laboratory of Medical Epigenomics, 139 Middle Renmin Road, Changsha, Hunan 410011, China
[b] Department of Plastic Surgery, Zhongshan City People's Hospital, Zhongshan, Guangdong, China
[c] Department of Dermatology, The Second Xiangya Hospital of Central South University, Hunan Key Laboratory of Medical Epigenomics, 139 Middle Renmin Road, Changsha, Hunan 410011, China

ARTICLE INFO

Keywords:
Keloid
Inflammation
Macrophage
Macrophage polarization

ABSTRACT

Keloids are a type of abnormal fibrous proliferation disease of the skin, characterized by local inflammation that lacks clear pathogenesis and satisfactory treatment. The phenomenon of distinct phenotypes, including M1 and M2 macrophages, is called macrophage polarization. Recently, macrophage polarization has been suggested to play a role in keloid formation. This study aimed to evaluate the relation between macrophage polarization and keloids and identify novel effective treatments for keloids. Differentially expressed genes were identified via RNA sequencing of the skin tissue of healthy controls and patients with keloids and validated using quantitative PCR. Multiplex immunofluorescence microscopy was used to detect different phenotypes of macrophages in keloid tissues. Finally, quantitative PCR validation of differentially expressed genes and flow cytometry were used to analyze macrophages in the peripheral blood of healthy controls and patients with keloids. Total and M2 macrophages were significantly increased in the local skin tissue and peripheral blood of patients with keloids compared with healthy controls. In addition, inflammation- and macrophage polarization-related differentially expressed genes in keloid tissue showed similar expression patterns in the peripheral blood. This study highlighted an increased frequency of total macrophages and M2 polarization in the local skin tissue and peripheral blood of patients with keloids. This systematic macrophage polarization tendency also indicates a potential genetic predisposition to keloids. These findings suggest the possibility of developing new diagnostic and therapeutic indicators for keloids focusing on macrophages.

1. Introduction

Keloids are clinically defined as benign skin tumors characterized by chronic inflammation-associated skin fibrosis with overgrowth and aggregation of immune cells and extracellular matrix (ECM) [1]. Keloid formation may occur secondary to minor skin injuries and wound healing procedures, such as acne or epifolliculitis [2]. Keloids generally manifest as persistent pain and pruritus, are often accompanied by local inflammation and infection, and lead to movement restriction and psychosocial impairment due to their scar-like appearance [3–5]. However, the pathogenesis of keloids remains unclear. Current studies and theories generally regard

* Corresponding author.
E-mail address: fusiqi@csu.edu.cn (S. Fu).

https://doi.org/10.1016/j.heliyon.2024.e24034
Received 29 May 2023; Received in revised form 19 December 2023; Accepted 2 January 2024
Available online 3 January 2024

heredity, immunity, mechanical microenvironment, local hypoxia, and lifestyle as the key factors of keloids; however, further studies are needed [1,3,6,7]. Although there are various treatment options for keloids, including surgical resection, drug injection, and radiotherapy, the results are unsatisfactory, and recurrence rates are high [4,8]. Limited treatments mainly focus on removing or inhibiting the uplifting keloid rather than preventing keloid formation from the etiology [4,8]. Therefore, clarifying the etiology of keloids and identifying novel and effective therapies are of great significance.

Macrophages play a key regulatory role in normal skin tissue homeostasis, wound healing, and later scar formation [9,10]. Local macrophages in the skin tissue are derived from the bone marrow, circulate in the blood as monocytes ($CD14^{+}CD16^{+}$), and are recruited to tissues under the influence of local chemokines [11]. In addition, macrophages (M0) can differentiate into different phenotypes under the influence of the microenvironment and produce different local immune effects, which can reflect and regulate the type 1 T helper/type 2 T helper immune response [12–14]. This phenomenon of distinct phenotypes is called macrophage polarization [15].

During the initial phase of tissue injury, local macrophages mainly manifest as classically activated macrophages (M1), also known as pro-inflammatory macrophages that secrete pro-inflammatory cytokines and chemokines to induce local inflammation and promote the type 1 T helper immune response [14,16]. Moreover, studies have shown that M1 macrophages can improve antigen presentation and stimulate the proliferation of fibroblasts and keratinocytes during the early stages of scar formation [17]. Under stimulation by interleukin (IL)-4, IL-13, or apoptotic neutrophils, monocytes are activated into alternatively activated macrophages (M2), which are divided into four subgroups: M2a, M2b, M2c, and M2d [14]. M2a macrophages, also called wound healing macrophages, can produce high levels of arginase-1 (ARG-1), platelet-derived growth factor, insulin-like growth factor 1 (IGF-1), IL-4, IL-13, and other cytokines and can produce collagen precursors to stimulate fibroblasts during tissue repair [18,19]. M2b macrophages produce high levels of IL-10 to inhibit inflammation; therefore, they are known as regulatory macrophages [20]. M2c macrophages have matrix remodeling abilities and are known as pro-decomposing macrophages [21]. M2d macrophages can produce high levels of IL-10, transforming growth factor beta-1 (TGF-β1), and vascular endothelial growth factor to inhibit inflammation [22]. M2 macrophages mainly appear in the late stage of scar formation and generally play a significant anti-inflammatory role, promoting wound healing, repairing tissue, and promoting scar formation [23,24].

Different phenotypes of macrophages show space-time specificity and ultimately decrease to the baseline during the normal wound healing process [9,25]. Studies have shown the infiltration of macrophages with an M2 polarization tendency in keloid tissue [26,27]. Limandjaja cocultured monocytes from the peripheral blood of patients with keloids with an in vitro culture model of keloids and found that monocytes differentiated into M2 macrophages [28]. Several studies also have shown increased expression levels of M2 polarization-related cytokines, such as ARG-1 [29], TGF-β1 [30], IGF-1 [30,31], IL-4, and IL-13 [32] in keloid tissue. These studies showed that macrophage differentiation may play a significant role in the formation of keloids.

Therefore, exploring the distribution of different macrophage phenotypes in patients with keloids helps elucidate their pathogenesis and inspire novel therapies. This study aimed to examine the conditions of macrophages and relevant gene expression in keloids to clarify the potential link between macrophage polarization and keloid formation.

2. Methods

2.1. Sample collection

In this study, skin tissues of healthy controls (HCs) and patients with keloids were obtained from the face (three HC samples and three keloid samples) and chest (three HC samples and three keloid samples) of discarded surgical specimens. Detailed information on these samples is provided in Table S1. Previous treatment of the surgical area within six months was defined as the exclusion criterion. The diagnostic and surgical procedures were performed at the Department of Dermatology at the Second Xiangya Hospital of Central South University. Blood specimens were collected from 15 patients with keloids recruited from the outpatient clinics of the Department of Dermatology, Second Xiangya Hospital of Central South University, and 15 sex- and age-matched HCs who underwent cosmetic surgery in the corresponding part. Detailed information on these samples is provided in Table S1.

This study was approved by the Ethics Committee of Second Xiangya Hospital of Central South University and the Chinese Clinical Trial Registry (ethics approval number ChiCTR2200065222). Written informed consent was obtained from all the participants.

2.2. RNA-sequencing and bioinformatics analysis of skin tissue

The skin tissues of HCs (six samples) and patients with keloids (six samples) were subjected to cutting, grinding, and storage in TRIzol Reagent (Meridian Life Science, US) for the following experiments. RNA extraction, sample detection, enrichment, amplification, library preparation, and RNA sequencing (RNA-seq) were performed by the BGI Company. All RNA-seq data were analyzed using R Studio. The R package limma was used to identify differentially expressed genes (DEGs), and the package clusterProfiler was used for Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis and Gene Ontology (GO) enrichment analyses.

2.3. Histology and multiplex immunofluorescence microscopy of skin samples

Skin tissue samples were fixed in 4 % paraformaldehyde solution and subjected to paraffin sectioning at a thickness of 4 mm. These sections were subjected to hematoxylin and eosin or multiplex immunofluorescence staining. For multiplex immunofluorescence, sections were deparaffinized and subjected to heat-mediated antigen retrieval buffer and staining cycles. Primary antibodies, including

anti-hCD68 (rabbit, ab213363, Abcam), anti-hCD40 (rabbit, ab224639, Abcam), and anti-mannose receptor/anti-hCD206 (rabbit, ab252921, Abcam), were incubated with tissue sections at 4 °C for 18 h, and anti-TGF beta 1 (rabbit, HA721143, HUABIO), anti-TGF beta 3 (rabbit, ER1917-64, HUABIO), and anti-collagen triple helix repeat containing-1 (CTHRC1; mouse, EM1701-36, HUABIO) were incubated with tissue sections at room temperature for 1 h. Then, the tissue slides were incubated with secondary antibodies (donkey anti-rabbit, ab205722, Abcam and goat anti-mouse, ab150113, Abcam) at room temperature for 30 min, followed by opal fluorophores at 1/300 dilution in tyramide signal amplification reagent (PerkinElmer). All primary and secondary antibody were diluted by 1%BSA. All multiplex immunofluorescence staining tests were performed by repeating the staining cycles and heat-mediated antigen retrieval (Servicebio, cat. G1203-250 ML, pH 9.0) between cycles. 4',6-Diamidino-2-phenylindole (DAPI) (PerkinElmer, EL801001KT) stained cell nuclei. Multiplex immunofluorescence was conducted in the following order: CD68 (Opal 520), CD40 (Opal 570), CD206 (Opal 650), and DAPI; TGF-β1(Opal 520), CD68 (Opal 650), and DAPI; TGF-β3 (Opal 520), CD68 (Opal 650), and DAPI; CTHRC1(Opal 520), CD68 (Opal 650), and DAPI. Tissue sections were scanned using a PerkinElmer Vectra multispectral imaging system (PerkinElmer), and images were analyzed using Form 2.3.1 (PerkinElmer).

2.4. Human peripheral blood mononuclear cell separation

Human peripheral blood mononuclear cells (PBMCs) were separated using Ficoll-Paque Plus (GE Healthcare, cat. 17-1440-03) to obtain a single-cell suspension using density gradient centrifugation. Human peripheral blood samples were mixed with isovolumetric phosphate-buffered saline (PBS) and gently added to the Ficoll-Paque Plus density medium. After centrifugation (room temperature, 2000 rpm, 30 min with zero deceleration), the PBMCs were separated in the middle layer of the liquid. The collected PBMCs were washed twice with PBS and counted under a microscope.

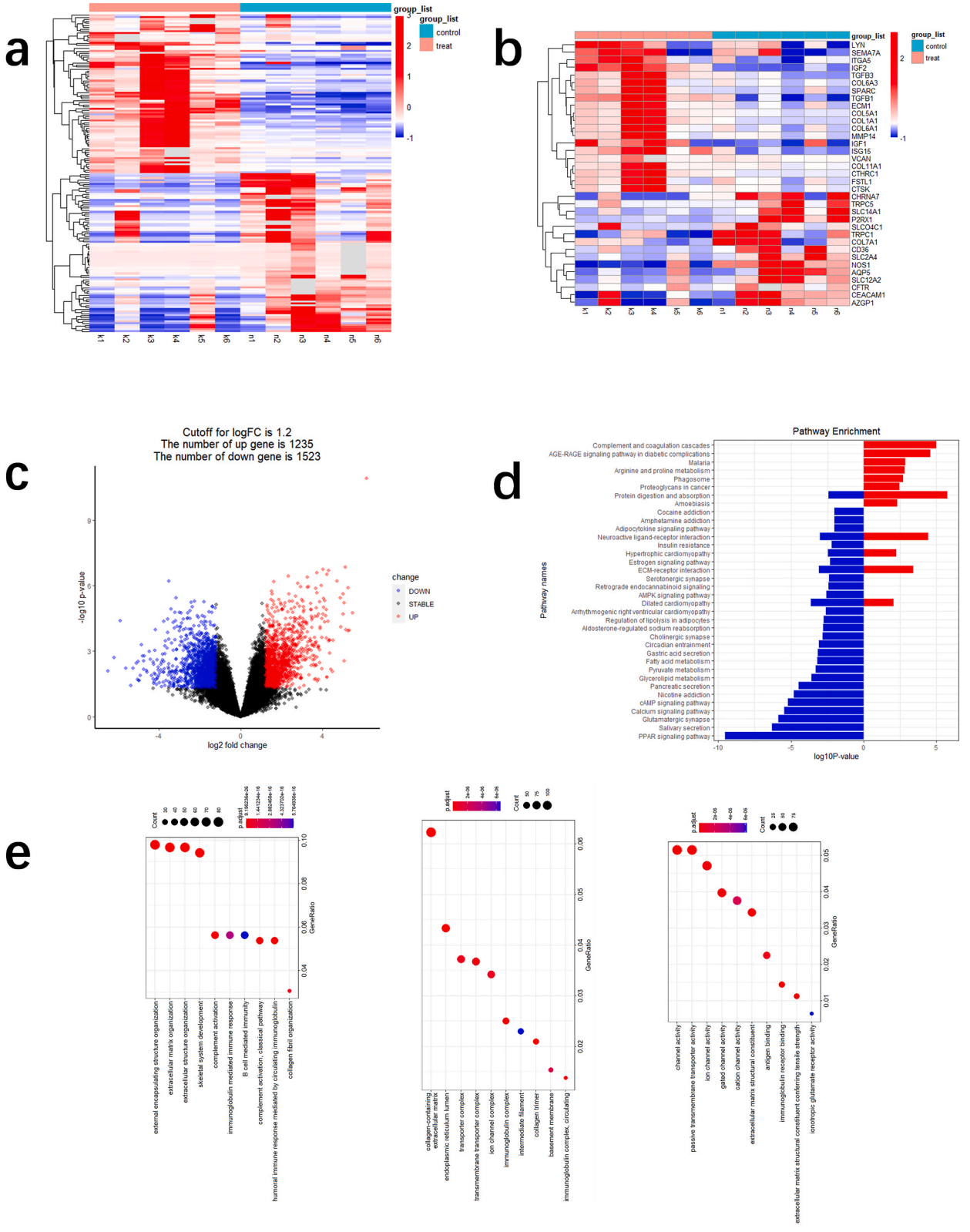
2.5. Flow cytometry analysis of PBMCs

Approximately 2×10^6 cells were suspended in PBS and incubated with surface marker antibodies at 4 °C for 45 min in the dark.

The following flow cytometric antibodies were used: PE-Cy7 anti-hCD80 (BioLegend, 305217), Alexa Fluor 488 anti-hCD68 (BioLegend, 333811), PE anti-hCD206 (BioLegend, 321105), PC5.5 anti-hCD45 (BioLegend, 3600503), APC anti-hCD86

Table 1
Primers for quantitative PCR.

Gene	Forward primer	Reverse primer
Cthrc1	CAATGGCATTCCGGGTACAC	GTACACTCCGCAATTTTCCCAA
Col1a1	GAGGGCCAAGACGAAGACATC	CAGATCACGTCATCGCACAAC
Col5a1	GCCCGGATGTCGCTTACAG	GCCCGGATGTCGCTTACAG
Col6a1	ACACCGACTGCGCTATCAAG	CGGTCACCACAATCAGGTACTT
Col6a3	ATGAGGAAACATCGGCACTTG	GGGCATGAGTTGTAGGAAAGC
Col11a1	ACCCTCGCATTGACCTTCC	TTTGTGCAAAATCCCGTTGTTT
Vcan	GTAACCCATGCGCTACATAAAGT	GGCAAAGTAGGCATCGTTGAAA
Ecm1	AGCACCCCAATGAACAGAAGG	CTGCATTCCAGGACTCAGGTT
Sparc	TGAGGTATCTGTGGGAGCTAATC	CCTTGCCGTGTTTGCAGTG
Tgfβ1	CTAATGGTGGAAACCCACAACG	TATCGCCAGGAATTGTTGCTG
Ctsk	GCAGAAGAACCGGGGTATTGA	GAAGGAGGTCAGGCTTGCAT
Tgfβ3	ACTTGCACCACCTTGGACTTC	GGTCATCACCGTTGGCTCA
Igf1	GCTCTTCAGTTCGTGTGTGGA	GCCTCCTTAGATCACAGCTCC
Igf2	GTGGCATCGTTGAGGAGTG	CACGTCCCTCTCGGACTTG
Itga5	GCCTGTGGAGTACAAGTCCTT	AATTCGGGTGAAGTTATCTGTGG
Lyn	TGCAGAGGGAATGGCATACAT	TGACTCGGAGACCAGAACATTAG
Mmp14	CATCTGTGACGGGAACTTTGA	GGCAGTGTTGATGGACGCA
Sema7a	CACCAAGACCAGGCTTACGAT	ACACGGGACACATTGAGAGGA
Isg15	CGCAGATCACCCAGAAGATCG	TTCGTCGCATTTGTCCACCA
Fstl1	GAGCAATGCAAACCTCACAAG	CAGTGTCCATCGTAATCAACCTG
Cftr	TGCCCTTCGGCGATGTTTTT	GTTATCCGGGTCATAGGAAGCTA
Chrna7	GCTGGTCAAGAACTACAATCCC	CTCATCCACGTCCATGATCTG
Aqp5	CGGGCTTTCTTCTACGTGG	GCTGGAAGGTCAGAATCAGCTC
P2rx1	ATGGTGCTGGTGCGTAATAAG	GGAAGACGTAGTCAGCCACA
Trpc1	AGGATAGCCTCCGGCATTC	TTCCACCTCCACAAGACTTAGT
Trpc5	CACACCGGACATCACTCCC	ACCTCTGAACTAGACACACACT
Slco4c1	TGAGCCATTTGCTGGTGTATC	GCATTACAAGGGGCTATCAAGTT
Azgp1	AACCAAGATGGTCGTTACTCTCT	CCTGCTTCCAATCCTCCATTC
Ceacam1	TGCTCTGATAGCAGTAGCCCT	TGCCGGTCTTCCCGAAATG
Slc2a4	ATCCTTGGACGATTCCTCATTGG	CAGGTGAGTGGGAGCAATCT
Slc14a1	ACTATGGTTAGAGTGGACAGCC	ACGGGTTTGTCTTTAAGCTGG
Cd36	AAGCCAGGTATTGCAGTTCTTT	GCATTTGCTGATGTCTAGCACA
Col7a1	TTACGCCGCTGACATTGTGTT	ACCAGCCCTTCGAGAAAGC
Nos1	TTCCCTCTCGCCAAAGAGTTT	AAGTGCTAGTGGTGTCGATCT
Slc12a2	TAAAGGAGTCGTGAAGTTTGGC	CTTGACCCACAATCCATGACA
Gapdh	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG



(caption on next page)

Fig. 1. Increased inflammation- and macrophage polarization-related gene expression in the skin tissue of HCs (n = 6) and patients with keloids (n = 6) (A) Heatmaps of RNA-seq of global genes. (B) Heatmaps of RNA-seq of curated genes that were relevant to inflammation and macrophages. (C) Volcano plot of RNA-seq of global genes. (D) Kyoto Encyclopedia of Genes and Genomes enrichment (KEGG) analysis of RNA-seq. (E) Gene Ontology (GO) enrichment analysis of RNA-seq, which was divided into biological process (BP), cell component (CC), and biological function (MF).

(BioLegend, 374207), and ACP-Cy7 anti-hCD40 (BioLegend, 334323). All samples were detected using Flow Cytometry DxP Athena™ (America, Cytek), and data were further analyzed with FlowJo software.

2.6. Quantitative PCR

Total RNA of PBMCs from HCs and patients with keloids was isolated using TRIzol Reagent (Meridian Life Science, US). Total RNA from skin tissues was extracted by BGI Company. RNA quality and quantity were determined using a NanoDrop ND-2000 spectrophotometer. Subsequently, total RNA was reverse-transcribed into complementary DNA using an RT−PCR Kit (Monad, MR05101) and stored at −20 °C. Quantitative PCR (qPCR) was performed using SYBR Premix Ex *Taq*II (TaKaRa Bio). Primers used (TSINGKE) are listed in Table 1. *Gapdh* was used as the internal control.

2.7. Statistical analysis

GraphPad Prism 8.0 was used to perform statistical analysis. All data are presented as the mean ± SEM. The statistical significance of groups was assessed using a two-tailed unpaired Student's t-test for comparisons between the two groups. The two-tailed Mann–Whitney *U* test was applied for statistical analysis when the data were not normally distributed. The correlation analysis of two indexes was performed using Pearson's r test or Spearman's r test (for abnormally distributed data).

3. Results

3.1. Differential inflammation- and macrophage polarization-relevant gene expression in keloid tissue using biological information data analysis

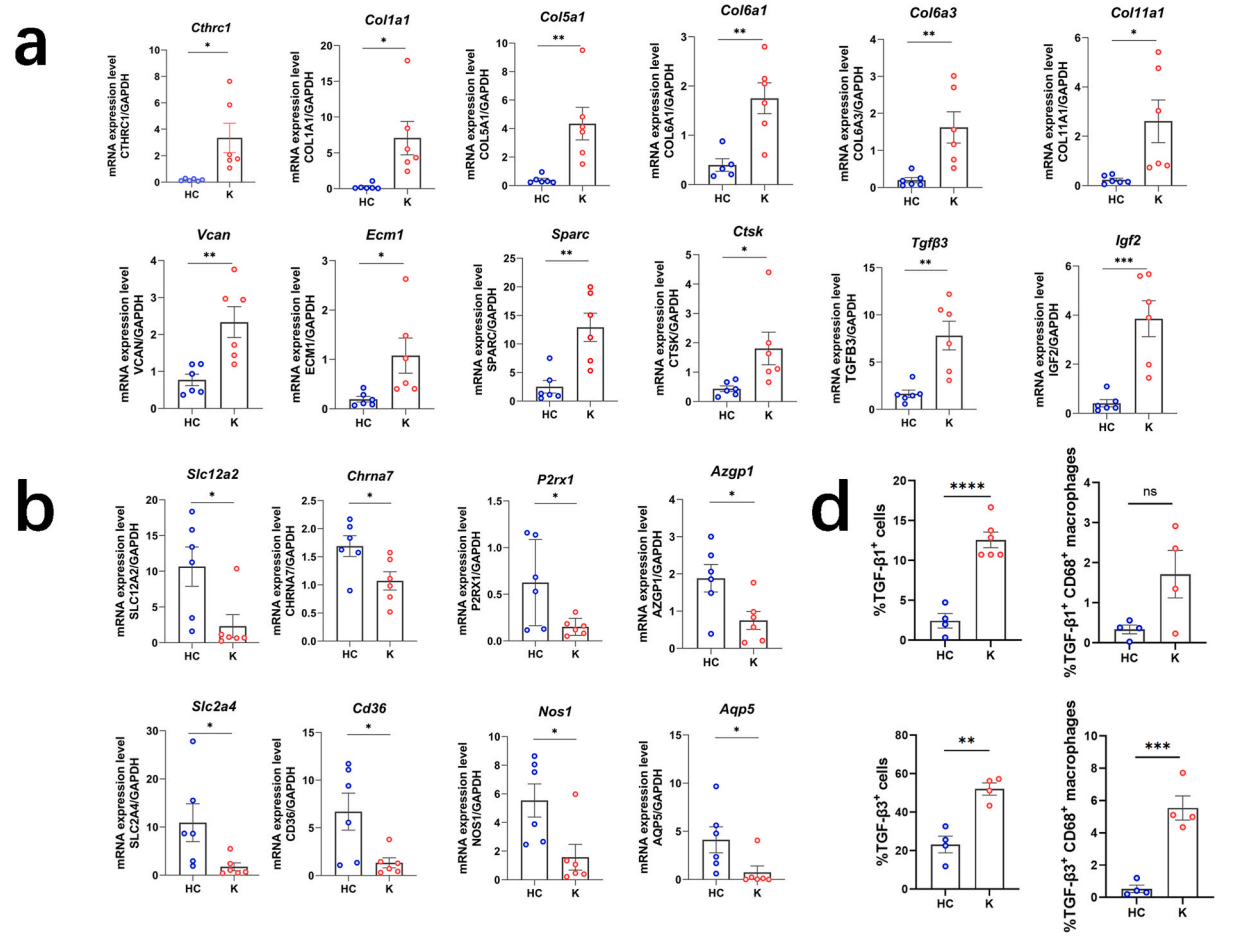
To explore the underlying inflammatory mechanisms in keloid pathogenesis, we performed RNA-seq assays and compared the transcriptional profiles of the skin tissues of six HCs and six patients with keloids matched in position, sex, and age. We detected 2758 DEGs (1235 highly expressed and 1523 lowly expressed) in keloid tissue compared with HCs using the criteria of logFC $\geq$1.2 and $p < 0.05$ to define high expression genes and logFC ≤ -1.2 and $p < 0.05$ as low expression genes (Fig. 1A and C).

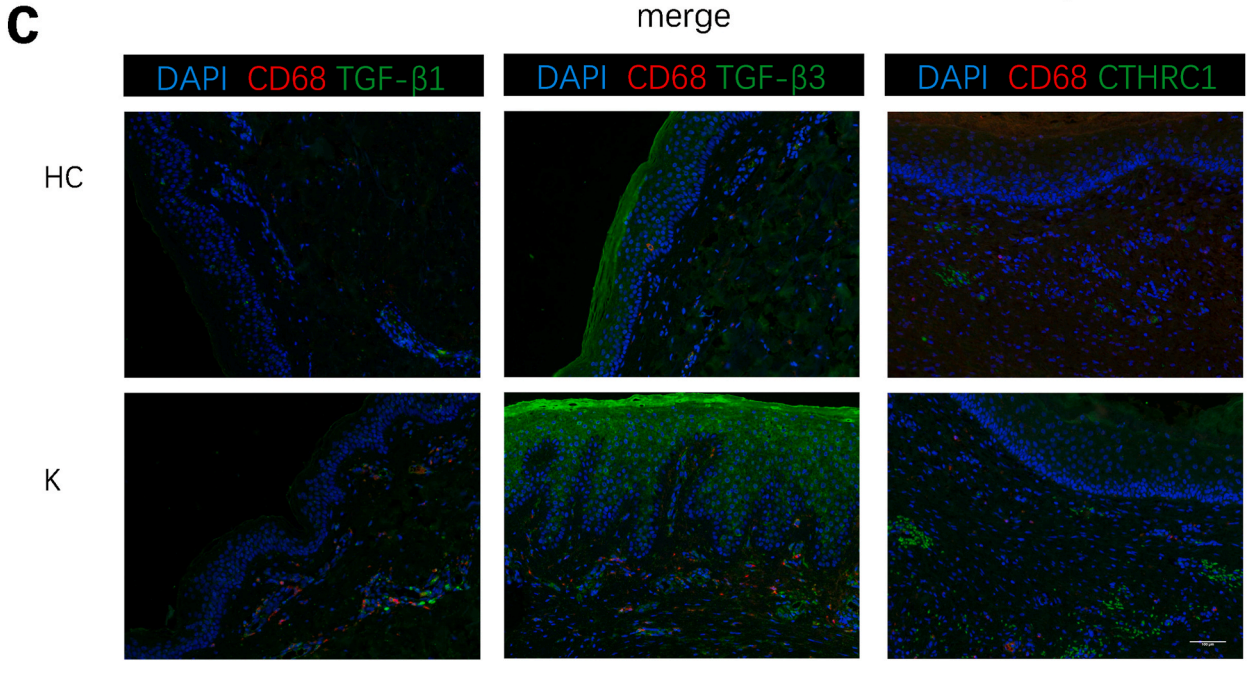
Various inflammation- and macrophage polarization-related genes were differentially expressed in keloid tissues, as shown in the curated heatmap (Fig. 1B). These genes with high expression in keloids (including CTHRC1, COL1A1, COL5A1, COL6A1, COL6A3, COL11A1, VCAN, ECM1, SPARC, TGFB1, CTSK, TGFB3, IGF1, IGF2, ITGA5, LYN, MMP14, SEMA7A, ISG15, and FSTL1) tended to promote inflammation and macrophage polarization to M2 macrophages, which was reversed by the lowly expressed genes (including CFTR, CHRNA7, AQP5, P2RX1, TRPC1, TRPC5, SLCO4C1, AZGP1, CEACAM1, SLC2A4, SLC14A1, CD36, COL7A1, NOS1, and SLC12A2). The highly expressed genes CTHRC1 [33,34], COL1A1 [35,36], COL5A1 [37], COL6A3 [37], COL11A1 [36], VCAN [38], TGFB1 [39], TGFB3 [40,41], IGF1 [42,43], MMP14 [44], and ISG15 [45] were demonstrated to promote macrophage affiliation and M2 polarization in the present study. This study also demonstrated that the poorly expressed genes SLC2A4 [46], CD36 [47,48], and NOS1 [49] were highly related to M1 polarization. CFTR [50], CHRNA7 [51], AQP5 [52], P2RX1 [53], and AZGP1 [54] also play important anti-inflammatory roles. Pathway enrichment and GO analyses revealed that the DEGs were highly enriched in pathways related to ECM formation and inflammation (Fig. 1D and E). The RNA-seq results suggest that the accompanying keloid markers may contribute to their pathogenesis by upregulating inflammation and active M2 polarization.

3.2. Differential expression level of inflammation-related genes involved in macrophages in keloid tissue of patients

To validate the expression levels of the 35 curated DEGs in skin tissue, the total RNA of skin tissue from HCs and patients with keloids was subjected to qPCR. The results showed increased expression levels of CTHRC1, COL1A1, COL5A1, COL6A1, COL6A3, COL11A1, VCAN, ECM1, SPARC, CTSK, TGF-β3, and IGF2 (Fig. 2A) and decreased expression levels of SLC12A2, CHRNA7, P2RX1, AZGP1, SLC2A4, CD36, NOS1, and AQP5 (Fig. 2B) in keloid tissue, which matched the results of RNA-seq. The expression levels of TGFB1, IGF1, ITGA5, LYN, MMP14, SEMA7A, ISG15, FSTL1, CFTR, TRPC1, TRPC5, SLCO4C1, CEACAM1, SLC14A1, and COL7A1 were not significantly different between HCs and patients with keloids (Fig. S1). These validated, highly expressed genes tended to upregulate local inflammation. CTHRC1, mainly expressed in the ECM, was confirmed making sense in M2 macrophage polarization via TGF-β pathways [33,34,55]. The expression level of CTHRC1 is highly related to TGF-β1 and M2 macrophage populations in keloids [55]. Multiplex immunofluorescence microscopy was performed on skin tissue sections of HCs and patients with keloids with the cell surface marker CD68, cell-extracellular matrix, and secreted markers TGFB1, TGFB3, and CTHRC1 (Fig. 2C). CD68 was used as a pan-marker to determine total macrophages. The expression of TGFB1, TGFB3, and CTHRC1 was upregulated and was accompanied by macrophage infiltration (Fig. 2C and D).

Based on the results of RNA-seq, qPCR, and multiplex immunofluorescence microscopy validation, it was hypothesized that M2





(caption on next page)

Fig. 2. qPCR validation of DEGs in the skin tissue of HCs (n = 6) and patients with keloids (n = 6) (A) qPCR validation of CTHRC1, COL1A1, COL5A1, COL6A1, COL6A3, COL11A1, VCAN, ECM1, SPARC, CTSK, TGFB3, and IGF2. (B) qPCR validation of SLC12A2, CHRNA7, P2RX1, AZGP1, SLC2A4, CD36, NOS1, and AQP5. (C) Multiplex immunofluorescence microscopy of CD68, TGF-β1, TGF-β3, and CTHRC1 in the skin tissue sections of HCs and patients with keloids. (D) Statistical analysis of the percentage of TGF-$\beta1^+$ cells, $CD68^+$TGF-$\beta1^+$ macrophages, TGF-$\beta3^+$ cells, and $CD68^+$TGF-$\beta3^+$ macrophages. Bars represent the mean ± SEM. *$p < 0.05$. **$p < 0.01$, ***$p < 0.005$, ****$p < 0.0001$.

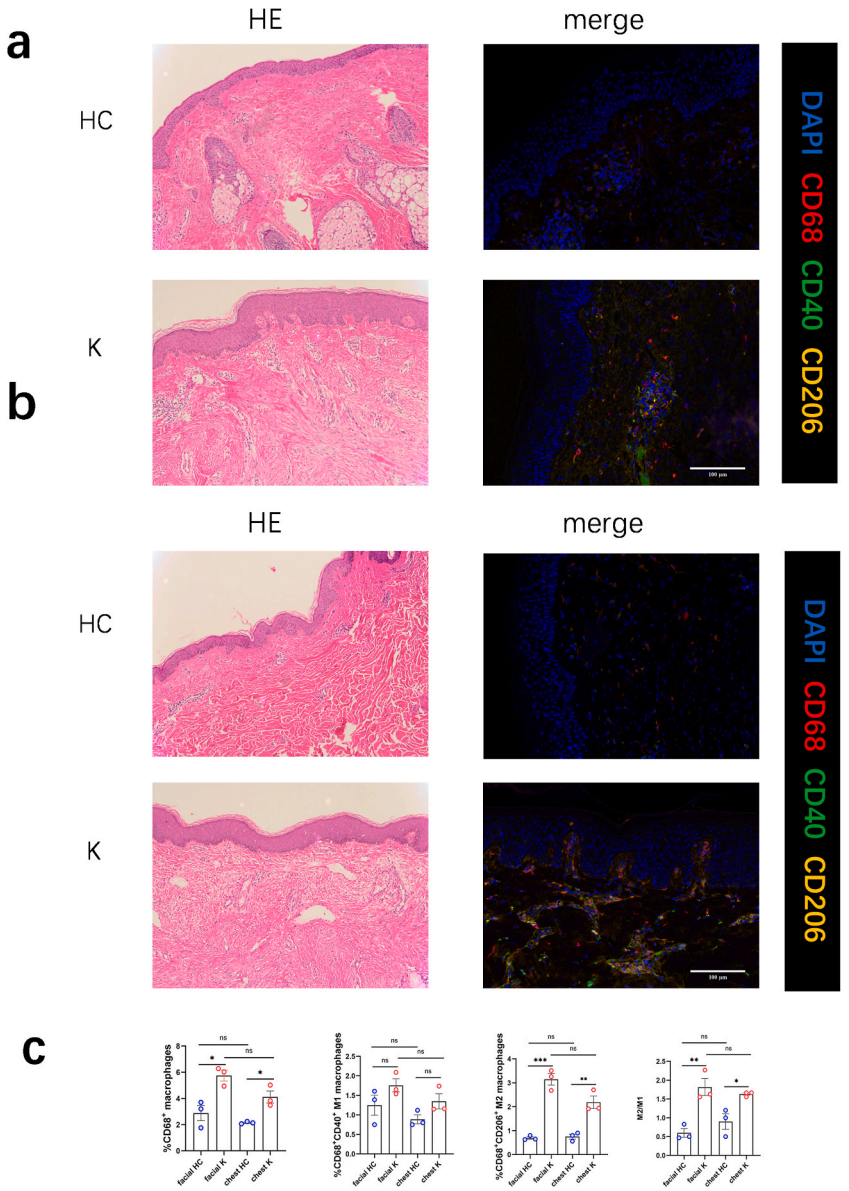


Fig. 3. Increased expression of CD68, CD40, and CD206 in HC skin tissues and keloid tissues (A) Hematoxylin and eosin (HE) and multiplex immunofluorescence microscopy of CD68, CD40, and CD206 in the facial tissue sections of HCs and patients with keloids. (B) HE and multiplex immunofluorescence microscopy of CD68, CD40, and CD206 in chest tissue sections of HCs and patients with keloids. (C) Statistical analysis of the percentage of total macrophages, M1 macrophages, M2 macrophages, and M2/M1 in the facial and chest skin tissue sections of HCs (facial n = 3, chest n = 3) and patients with keloids (facial n = 3, chest n = 3). Bars represent the mean ± SEM. *$p < 0.05$. **$p < 0.01$, ***$p < 0.005$, ****$p < 0.0001$.

polarization may play a role in keloid formation.

3.3. Infiltrated macrophages and M2 polarization in keloid tissues of patients compared with HCs

To understand the infiltrative status of total ($CD68^+$), M1 ($CD68^+CD40^+$), and M2 macrophages ($CD68^+CD206^+$) in the skin tissue of HCs and patients with keloids, multiplex immunofluorescence microscopy was performed on the facial and chest tissue sections of HCs and patients with keloids using the cell surface markers CD68, CD40, and CD206. CD68 was used as a pan-marker to detect total macrophages. A routine histopathological review of the corresponding hematoxylin and eosin slides of the mandibular and chest tissue sections of HCs and patients with keloids was performed before immunofluorescence analysis (Fig. 3A and B).

As shown in Fig. 3A and B, stronger positive immunostaining for CD68, CD40, and CD206 was observed in keloid tissues than in HC skin tissues in both mandibular and chest tissue sections. Positive immunostaining was mainly observed in dermal sections rather than in epidermal sections. Compared with HC skin tissue, the percentages of total macrophages, M2 macrophages, and M2/M1 were

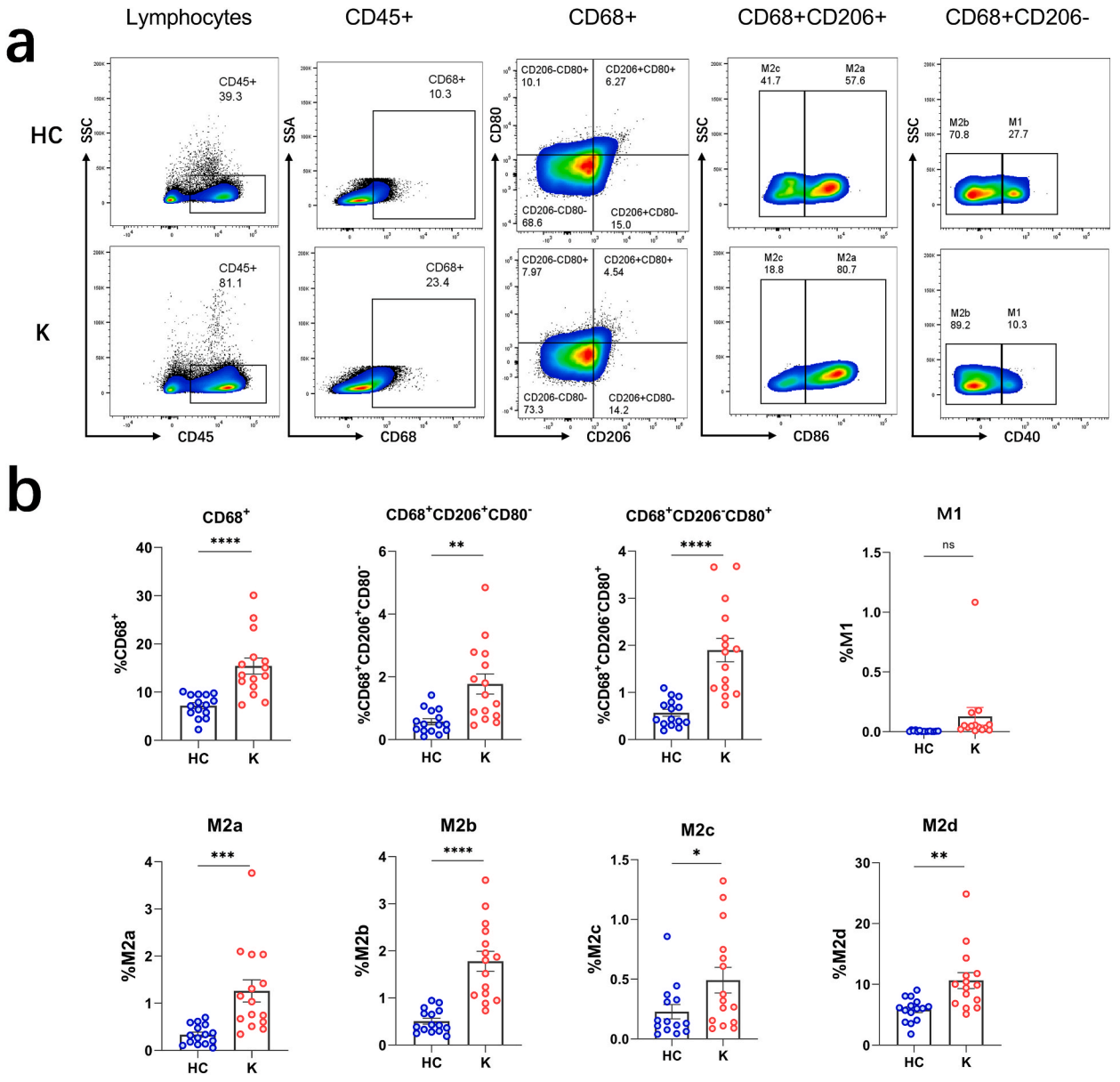


Fig. 4. Frequency of different macrophage phenotypes in human peripheral blood mononuclear cells (PBMCs) (A) Frequency of macrophages with different phenotypes from PBMCs of healthy controls (HCs) and patients with keloids analyzed using flow cytometry. (B) Statistical analysis of macrophages with different phenotypes from PBMCs of HCs (n = 15) and patients with keloids (n = 15). Bars represent the mean ± SEM. $^*p < 0.05$. $^{**}p < 0.01$, $^{***}p < 0.005$, $^{****}p < 0.0001$.

increased in keloid tissue. However, no significant difference was observed between the mandibular and chest tissue sections (Fig. 3C). These results indicate macrophage infiltration in keloids, which presented an M2 macrophage polarization tendency.

3.4. Increased frequency of all M2 macrophage phenotypes in the peripheral blood of patients with keloids

Flow cytometry analysis was performed on the PBMCs of HCs and patients with keloids to investigate the general macrophage status. Flow cytometry analysis showed that the frequency of total macrophages ($CD68^+$) was higher in the peripheral blood of patients with keloids than in that of HCs. No statistically significant difference was observed in the ratio of M1 macrophages ($CD68^+CD206^-CD80^+CD86^+CD40^+$) between patients with keloids and HCs. However, the frequencies of all M2 macrophage phenotypes, M2a ($CD68^+CD206^+CD80^-CD86^+CD40^-$), M2b ($CD68^+CD206^-CD80^+CD86^+CD40^-$), M2c ($CD68^+CD206^+CD80^-CD86^-CD40^-$), and M2d ($CD68^+CD206^-CD80^-CD86^-CD40^-$), were significantly higher in patients with keloids than in HCs (Fig. 4A and B). These data indicate an increased number of macrophages, especially M2 macrophages, in the peripheral blood of patients with keloids.

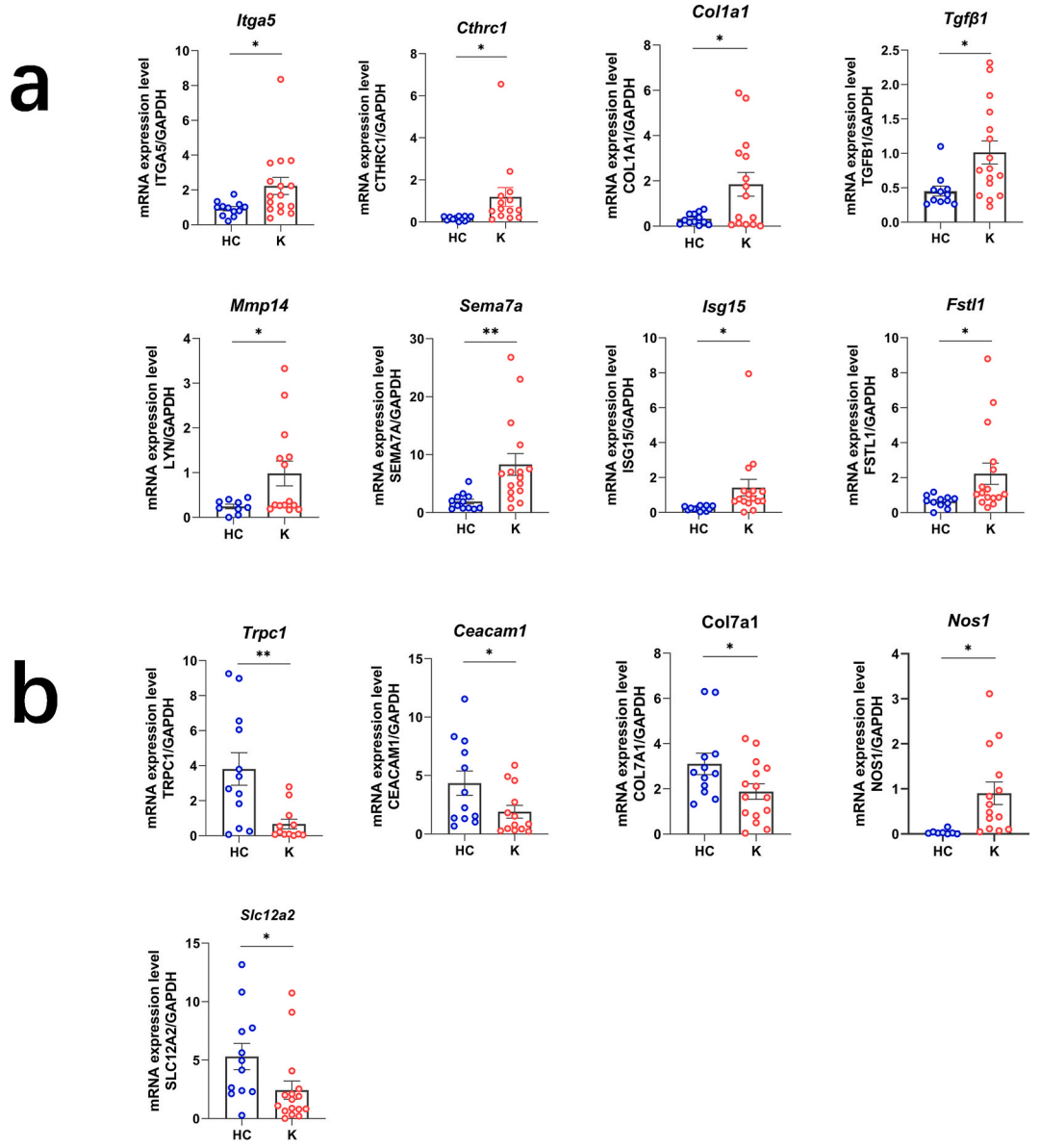


Fig. 5. qPCR validation of DEGs in the PBMCs of HCs (n = 15) and patients with keloids (n = 15) (A) qPCR validation of ITGA5, CTHRC1, COL1A1, TGFB1, MMP14, SEMA7A, ISG15, and FSTL1. (B) qPCR validation of SLC12A2, NOS1, COL7A1, CEACAM1 and TRPC1. Bars represent the mean ± SEM. *$p < 0.05$. **$p < 0.01$, ***$p < 0.005$, ****$p < 0.0001$.

3.5. Differential expression levels of inflammation-related genes involved in macrophages in the PBMCs of patients with keloids

To explore the relation between gene expression and macrophage polarization, we validated the expression levels of 35 curated DEGs. As shown in Fig. 5A and B, the expression levels of ITGA5, CTHRC1, COL1A1, TGFB1, MMP14, SEMA7A, ISG15, and FSTL1 increased, and the expression levels of SLC12A2, NOS1, COL7A1, CEACAM1, and TRPC1 decreased in the PBMCs of patients with keloids than in HCs, which showed the same tendency as in the local skin tissue.

The expression levels of COL5A1, COL6A1, COL6A3, COL11A1, VCAN, ECM1, SPARC, CTSK, TGFB3, IGF1, IGF2, LYN, CFTR, CHRNA7, AQP5, P2RX1, TRPC5, SLCO4C1, AZGP1, SLC2A4, SLC14A1, and CD36 in PBMCs were not significantly different between HCs and patients with keloids (Fig. S2). These results indicate that the expression of these genes may have positive effects on peripheral blood macrophage polarization in patients with keloids.

4. Discussion

Keloids are a type of abnormal fibrous proliferation in the skin that is characterized by local inflammation and severely restricts the quality of life and aesthetic appearance of patients [1,56,57]. Unsatisfactory treatment accompanied by a high recurrence rate and intense inflammation has resulted in limited recognition of the keloid etiology thus far [4,8]. Previous studies have revealed the infiltration of major immune cells in wound healing, including mast cells, macrophages, and lymphocytes, in keloid tissue [58], highlighting the significance of local inflammation in the pathogenesis of keloids. M2 polarization is strongly associated with keloid formation [26,27]. Moreover, the increasing expression levels of M2 polarization-related cytokines, such as ARG-1 [29], TGF-β1 [30], IGF-1 [30,31], IL-4, and IL-13 [32] in keloid tissue also prompted the correlation of M2 polarization and keloids. The present study aimed to investigate the immune-relevant etiology of keloids and identify novel effective treatments for keloids.

In the current study, we identified an increased frequency of macrophages in the local skin tissue and peripheral blood of patients with keloids, which showed a tendency toward M2 polarization. Various inflammation- and macrophage polarization-related genes were differentially expressed in keloid tissues via RNA-seq, qPCR, and multiplex immunofluorescence microscopy. These curated DEGs mainly showed positive effects on promoting inflammation and M2 polarization, as confirmed in previous studies. The validated highly expressed genes CTHRC1 [33,34], COL1A1 [35,36], COL5A1 [37], COL6A3 [37], COL11A1 [36], VCAN [38], TGFB1 [39], TGFB3 [40,41], IGF1 [42,43], MMP14 [44], and ISG15 [45] in keloid tissue and HC skin tissue were demonstrated to promote macrophage affiliation and M2 polarization in the present study. For example, CTHRC1, mainly expressed in ECM, was demonstrated to promote M2 polarization in the wound repair process via TGF-β pathways [33,34,55]. The expression level of CTHRC1 was highly related to TGF-β1 in keloids [55]. Qin found that CTHRC1 increased the M2 macrophage population and the TGF-β expression level as a result of the activation of the TGF-β pathways and Notch pathways, which eventually contributed to the promotion of wound healing [34]. ITGA5 [59] and SEMA7A [60] are also significant inflammatory factors in the skin and endothelium. The present study also demonstrates that the poorly expressed genes SLC2A4 [46], CD36 [47,48], and NOS1 [49] are highly related to M1 polarization. CFTR [50], CHRNA7 [51], AQP5 [52], P2RX1 [53], and AZGP1 [54] also play important anti-inflammatory roles. Based on these results, multiplex immunofluorescence microscopy of the skin tissue confirmed macrophage infiltration, especially M2 macrophage infiltration, in keloid tissue, which was in accordance with existing studies [26,27,61]. In addition, the infiltration of macrophages was less related to the location of the keloid tissue in the present study and our study [61]. Seoudy found decreased levels of M1 macrophages in keloid tissue compared to those in normal scar tissue, which differed from our results [61]. In addition to the different detection methods and surface marker choices, the duration and status of the keloid tissue may also account for this inconsistency. In addition, different macrophage phenotypes are the extremes of a dynamic process and are interchangeable under the influence of the microenvironment [62]. The obvious aggregation of total and M2 macrophages and the increased rate of M2/M1 macrophages indicate macrophage infiltration and an M2 polarization tendency in keloid formation.

Previous studies on keloid macrophage polarization have been limited to immune cell infiltration into the local skin tissue [26–28,61,63]. For example, Seoudy analyzed the expression level of specific markers of M1 and M2 macrophages via enzyme-linked immunosorbent assay [63], Limandjaja cocultured monocytes with reconstructed keloid and normal skin in vitro and analyzed monocyte differentiation [28].

Given the genetic predisposition to keloids, we speculated general inflammation and increased macrophage levels. Based on this hypothesis, flow cytometry and qPCR validation were performed on PBMCs from HCs and patients with keloids. The increasing frequency of total macrophages and all phenotypes of M2 macrophages and the similar expression tendency of DEGs indicate systematic inflammation and M2 polarization in the pathogenesis of keloids. For example, the positive regulatory function of the highly expressed genes in keloid tissue, CTHRC1 [33,34], COL1A1 [35,36], COL5A1 [37], COL6A3 [37], and COL11A1 [36], on M2 polarization was demonstrated in the present study. These results also suggest a potential link between systemic inflammation and the accompanying markers in keloids.

Our study has some limitations. First, the relationship between the selected DEGs and keloid pathogenesis requires further investigation. Second, information on the duration and activity level of the keloids was neglected during skin tissue collection. Due to the space-time specificity of macrophage phenotypes during the wound healing process [9,25], disease duration and activity level may influence macrophage infiltration and polarization. Normal scar tissue could also be considered as the control group.

Based on these studies, macrophage aggregation and M2 polarization are of great significance in the pathogenesis of keloids and provide a new direction for keloid treatment. The differentiation and polarization of macrophages are primarily regulated by cytokines in the microenvironment, including granulocyte-macrophage colony-stimulating factor (GM-CSF) and macrophage colony-stimulating factor (M-CSF) [15,64]. Wang summarized the effects of several cytokines and naturally occurring compounds with potential

regulatory effects on macrophage polarization [65]. Systematic or topical supplementation with these relevant cytokines or compounds may be a new keloid therapy. Nevertheless, M2 macrophages dominate the late stage of normal wound healing, exert anti-inflammatory effects, and stimulate collagen production [18]. Inhibition of M2 polarization may aggravate local inflammation. Therefore, the balance between inflammation and macrophage phenotypes is a key factor in treating keloids and warrants further studies.

5. Conclusion

This study highlighted an increased frequency of total macrophages and M2 polarization in the local skin tissue and peripheral blood of patients with keloids. This systematic macrophage polarization tendency also suggests a potential genetic predisposition to keloids. These findings suggest the possibility of developing new diagnostic and therapeutic indicators for keloids focusing on macrophages.

6. Data availability statement

Data are available on reasonable request. All data are available in the manuscript or supplementary documents. Raw data of RNA-seq has been submitted to the SRA database with the accession number PRJNA1042670.

Declaration of Ethics

This study was approved by the Ethics Committee of Second Xiangya Hospital of Central South University and the Chinese Clinical Trial Registry (ethics approval number ChiCTR2200065222). Written informed consent was obtained from all the subjects.

CRediT authorship contribution statement

Xinyi Lv: Writing - review & editing, Writing - original draft, Software, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Zhenghao He:** Software, Methodology. **Ming Yang:** Formal analysis, Data curation. **Lu Wang:** Formal analysis. **Siqi Fu:** Writing - review & editing, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.

Acknowledgment

This work was supported by the Hunan Provincial Natural Science Foundation of China (No. 2022JJ30805) and the Project of Health Commission of Hunan Province (No. D202304126844).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e24034.

References

[1] G.C. Limandjaja, F.B. Niessen, R.J. Scheper, S. Gibbs, The keloid disorder: heterogeneity, histopathology, mechanisms and models, Front. Cell Dev. Biol. 8 (2020) 360.
[2] H.J. Lee, Y.J. Jang, Recent understandings of biology, prophylaxis and treatment strategies for hypertrophic scars and keloids, Int. J. Mol. Sci. 19 (3) (2018) 711.
[3] U. Betarbet, T.W. Blalock, Keloids: a review of etiology, prevention, and treatment, J Clin Aesthet Dermatol 13 (2) (2020) 33–43.
[4] S. Ud-Din, A. Bayat, New insights on keloids, hypertrophic scars, and striae, Dermatol. Clin. 32 (2) (2014) 193–209.
[5] D.D. Balci, T. Inandi, C.A. Dogramaci, E. Celik, DLQI scores in patients with keloids and hypertrophic scars: a prospective case control study, J Dtsch Dermatol Ges 7 (8) (2009) 688–692.
[6] B. Zhou, Z. Gao, W. Liu, X. Wu, W. Wang, Important role of mechanical microenvironment on macrophage dysfunction during keloid pathogenesis, Exp. Dermatol. 31 (3) (2022) 375–380.
[7] Y. Yu, H. Wu, Q. Zhang, R. Ogawa, S. Fu, Emerging insights into the immunological aspects of keloids, J. Dermatol. 48 (12) (2021) 1817–1826.
[8] I. Khansa, B. Harrison, J.E. Janis, Evidence-Based scar management: how to improve results with technique and technology, Plast. Reconstr. Surg. 138 (3 Suppl) (2016) 165s–178s.
[9] B. Mahdavian Delavary, W.M. van der Veer, M. van Egmond, F.B. Niessen, R.H. Beelen, Macrophages in skin injury and repair, Immunobiology 216 (7) (2011) 753–762.
[10] T.A. Wynn, A. Chawla, J.W. Pollard, Macrophage biology in development, homeostasis and disease, Nature 496 (7446) (2013) 445–455.
[11] C.V. Jakubzick, G.J. Randolph, P.M. Henson, Monocyte differentiation and antigen-presenting functions, Nat. Rev. Immunol. 17 (6) (2017) 349–362.

[12] A. Vishwakarma, N.S. Bhise, M.B. Evangelista, J. Rouwkema, M.R. Dokmeci, A.M. Ghaemmaghami, N.E. Vrana, A. Khademhosseini, Engineering immunomodulatory biomaterials to tune the inflammatory response, Trends Biotechnol. 34 (6) (2016) 470–482.
[13] A. Shapouri-Moghaddam, S. Mohammadian, H. Vazini, M. Taghadosi, S.A. Esmaeili, F. Mardani, B. Seifi, A. Mohammadi, J.T. Afshari, A. Sahebkar, Macrophage plasticity, polarization, and function in health and disease, J. Cell. Physiol. 233 (9) (2018) 6425–6440.
[14] S.C. Funes, M. Rios, J. Escobar-Vera, A.M. Kalergis, Implications of macrophage polarization in autoimmunity, Immunology 154 (2) (2018) 186–195.
[15] Y. Yang, J. Qin, L. Lan, N. Li, C. Wang, P. He, F. Liu, H. Ni, Y. Wang, M-CSF cooperating with NFκB induces macrophage transformation from M1 to M2 by upregulating c-Jun, Cancer Biol. Ther. 15 (1) (2014) 99–107.
[16] M. Orecchioni, Y. Ghosheh, A.B. Pramod, K. Ley, Macrophage polarization: different gene signatures in M1(LPS+) vs. Classically and M2(LPS-) vs. Alternatively activated macrophages, Front. Immunol. 10 (2019) 1084.
[17] D.Y. Vogel, J.E. Glim, A.W. Stavenuiter, M. Breur, P. Heijnen, S. Amor, C.D. Dijkstra, R.H. Beelen, Human macrophage polarization in vitro: maturation and activation methods compared, Immunobiology 219 (9) (2014) 695–703.
[18] M.E. Ogle, C.E. Segar, S. Sridhar, E.A. Botchwey, Monocytes and macrophages in tissue repair: implications for immunoregenerative biomaterial design, Exp. Biol. Med. 241 (10) (2016) 1084–1097.
[19] A. Gopalakrishnan, J. Joseph, K.A. Shirey, A.D. Keegan, M.S. Boukhvalova, S.N. Vogel, J.C.G. Blanco, Protection against influenza-induced Acute Lung Injury (ALI) by enhanced induction of M2a macrophages: possible role of PPARγ/RXR ligands in IL-4-induced M2a macrophage differentiation, Front. Immunol. 13 (2022) 968336.
[20] M. Hesketh, K.B. Sahin, Z.E. West, R.Z. Murray, Macrophage phenotypes regulate scar formation and chronic wound healing, Int. J. Mol. Sci. 18 (7) (2017) 1545.
[21] P. Krzyszczyk, R. Schloss, A. Palmer, F. Berthiaume, The role of macrophages in acute and chronic wound healing and interventions to promote pro-wound healing phenotypes, Front. Physiol. 9 (2018) 419.
[22] C.B. Anders, T.M.W. Lawton, H.L. Smith, J. Garret, M.M. Doucette, M.C.B. Ammons, Use of integrated metabolomics, transcriptomics, and signal protein profile to characterize the effector function and associated metabotype of polarized macrophage phenotypes, J. Leukoc. Biol. 111 (3) (2022) 667–693.
[23] K.E. Martin, A.J. García, Macrophage phenotypes in tissue repair and the foreign body response: implications for biomaterial-based regenerative medicine strategies, Acta Biomater. 133 (2021) 4–16.
[24] Z. Zhu, J. Ding, Z. Ma, T. Iwashina, E.E. Tredget, Systemic depletion of macrophages in the subacute phase of wound healing reduces hypertrophic scar formation, Wound Repair Regen. 24 (4) (2016) 644–656.
[25] L. Chen, J. Wang, S. Li, Z. Yu, B. Liu, B. Song, Y. Su, The clinical dynamic changes of macrophage phenotype and function in different stages of human wound healing and hypertrophic scar formation, Int. Wound J. 16 (2) (2019) 360–369.
[26] Q. Jin, L. Gui, F. Niu, B. Yu, N. Lauda, J. Liu, X. Mao, Y. Chen, Macrophages in keloid are potent at promoting the differentiation and function of regulatory T cells, Exp. Cell Res. 362 (2) (2018) 472–476.
[27] X. Li, Y. Wang, B. Yuan, H. Yang, L. Qiao, Status of M1 and M2 type macrophages in keloid, Int. J. Clin. Exp. Pathol. 10 (11) (2017) 11098–11105.
[28] G.C. Limandjaja, T. Waaijman, S. Roffel, F.B. Niessen, S. Gibbs, Monocytes co-cultured with reconstructed keloid and normal skin models skew towards M2 macrophage phenotype, Arch. Dermatol. Res. 311 (8) (2019) 615–627.
[29] L.R. da Cunha Colombo Tiveron, I.R. da Silva, M.V. da Silva, A.B. Peixoto, D.B.R. Rodrigues, V. Rodrigues, High in situ mRNA levels of IL-22, TFG-β, and ARG-1 in keloid scars, Immunobiology 223 (12) (2018) 812–817.
[30] C. Guo, L. Liang, J. Zheng, Y. Xie, X. Qiu, G. Tan, J. Huang, L. Wang, UCHL1 aggravates skin fibrosis through an IGF-1-induced Akt/mTOR/HIF-1α pathway in keloid, Faseb. J. 37 (7) (2023) e23015.
[31] Z.C. Hu, B. Tang, D. Guo, J. Zhang, Y.Y. Liang, D. Ma, J.Y. Zhu, Expression of insulin-like growth factor-1 receptor in keloid and hypertrophic scar, Clin. Exp. Dermatol. 39 (7) (2014) 822–828.
[32] G.A. Zhu, K.J.W. Kang, J.K. Chen, R.A. Novoa, R.A. Brown, A.S. Chiou, J.M. Ko, G. Honari, Inflammatory alopecia in patients on dupilumab: a retrospective cohort study at an academic institution, J. Eur. Acad. Dermatol. Venereol. 34 (4) (2019) e159–e161.
[33] X.L. Zhang, L.P. Hu, Q. Yang, W.T. Qin, X. Wang, C.J. Xu, G.A. Tian, X.M. Yang, L.L. Yao, L. Zhu, H.Z. Nie, Q. Li, Q. Xu, Z.G. Zhang, Y.L. Zhang, J. Li, Y.H. Wang, S.H. Jiang, CTHRC1 promotes liver metastasis by reshaping infiltrated macrophages through physical interactions with TGF-β receptors in colorectal cancer, Oncogene 40 (23) (2021) 3959–3973.
[34] S. Qin, J.H. Zheng, Z.H. Xia, J. Qian, C.L. Deng, S.L. Yang, CTHRC1 promotes wound repair by increasing M2 macrophages via regulating the TGF-β and notch pathways, Biomed. Pharmacother. 113 (2019) 108594.
[35] C.P. Hans, N. Sharma, S. Sen, S. Zeng, R. Dev, Y. Jiang, A. Mahajan, T. Joshi, Transcriptomics analysis reveals new insights into the roles of Notch1 signaling on macrophage polarization, Sci. Rep. 9 (1) (2019) 7999.
[36] S. Gu, H. Dai, X. Zhao, C. Gui, J. Gui, AKT3 deficiency in M2 macrophages impairs cutaneous wound healing by disrupting tissue remodeling, Aging (Albany NY) 12 (8) (2020) 6928–6946.
[37] X. Liao, Y. Bu, Z. Xu, F. Jia, F. Chang, J. Liang, Q. Jia, Y. Lv, WISP1 predicts clinical prognosis and is associated with tumor purity, immunocyte infiltration, and macrophage M2 polarization in pan-cancer, Front. Genet. 11 (2020) 502.
[38] E.B. Lurier, D. Dalton, W. Dampier, P. Raman, S. Nassiri, N.M. Ferraro, R. Rajagopalan, M. Sarmady, K.L. Spiller, Transcriptome analysis of IL-10-stimulated (M2c) macrophages by next-generation sequencing, Immunobiology 222 (7) (2017) 847–856.
[39] M. Jung, Y. Ma, R.P. Iyer, K.Y. DeLeon-Pennell, A. Yabluchanskiy, M.R. Garrett, M.L. Lindsey, IL-10 improves cardiac remodeling after myocardial infarction by stimulating M2 macrophage polarization and fibroblast activation, Basic Res. Cardiol. 112 (3) (2017) 33.
[40] X. Chen, B. Yang, J. Tian, H. Hong, Y. Du, K. Li, X. Li, N. Wang, X. Yu, X. Wei, Dental follicle stem cells ameliorate lipopolysaccharide-induced inflammation by secreting TGF-β3 and TSP-1 to elicit macrophage M2 polarization, Cell. Physiol. Biochem. 51 (5) (2018) 2290–2308.
[41] L. Zhang, Y. Dong, Y. Dong, J. Cheng, J. Du, Role of integrin-β3 protein in macrophage polarization and regeneration of injured muscle, J. Biol. Chem. 287 (9) (2012) 6177–6186.
[42] O. Spadaro, C.D. Camell, L. Bosurgi, K.Y. Nguyen, Y.H. Youm, C.V. Rothlin, V.D. Dixit, IGF1 shapes macrophage activation in response to immunometabolic challenge, Cell Rep. 19 (2) (2017) 225–234.
[43] L. Li, H. Gan, H. Jin, Y. Fang, Y. Yang, J. Zhang, X. Hu, L. Chu, Astragaloside IV promotes microglia/macrophages M2 polarization and enhances neurogenesis and angiogenesis through PPARγ pathway after cerebral ischemia/reperfusion injury in rats, Int. Immunopharm. 92 (2021) 107335.
[44] A. Klose, P. Zigrino, C. Mauch, Monocyte/macrophage MMP-14 modulates cell infiltration and T-cell attraction in contact dermatitis but not in murine wound healing, Am. J. Pathol. 182 (3) (2013) 755–764.
[45] W. Zhao, J. Hu, Q. He, The effect of the WKYMVm peptide on promoting mBMSC secretion of exosomes to induce M2 macrophage polarization through the FPR2 pathway, J. Orthop. Surg. Res. 16 (1) (2021) 171.
[46] A. Sarsenbayeva, P. Dipta, M. Lundqvist, K.E. Almby, B. Tirosh, G. Di Nunzio, J.W. Eriksson, M.J. Pereira, Human macrophages stimulate expression of inflammatory mediators in adipocytes; effects of second-generation antipsychotics and glucocorticoids on cellular cross-talk, Psychoneuroendocrinology 125 (2021) 105071.
[47] X. Wang, S. Chen, J. He, W. Chen, Y. Ding, J. Huang, J. Huang, Histone methyltransferases G9a mediated lipid-induced M1 macrophage polarization through negatively regulating CD36, Metabolism 114 (2021) 154404.
[48] F.Y. Chen, J. Zhou, N. Guo, W.G. Ma, X. Huang, H. Wang, Z.Y. Yuan, Curcumin retunes cholesterol transport homeostasis and inflammation response in M1 macrophage to prevent atherosclerosis, Biochem. Biophys. Res. Commun. 467 (4) (2015) 872–878.
[49] M. Srivastava, U. Saqib, A. Naim, A. Roy, D. Liu, D. Bhatnagar, R. Ravinder, M.S. Baig, The TLR4-NOS1-AP1 signaling axis regulates macrophage polarization, Inflamm. Res. 66 (4) (2017) 323–334.

[50] S. Hu, J.O. Russell, S. Liu, C. Cao, J. McGaughey, R. Rai, K. Kosar, J. Tao, E. Hurley, M. Poddar, S. Singh, A. Bell, D. Shin, R. Raeman, A.D. Singhi, K. Nejak-Bowen, S. Ko, S.P. Monga, β-Catenin-NF-κB-CFTR interactions in cholangiocytes regulate inflammation and fibrosis during ductular reaction, Elife 10 (2021) e71310.
[51] Y. Pu, Y. Tan, Y. Qu, L. Chang, S. Wang, Y. Wei, X. Wang, K. Hashimoto, A role of the subdiaphragmatic vagus nerve in depression-like phenotypes in mice after fecal microbiota transplantation from Chrna7 knock-out mice with depression-like phenotypes, Brain Behav. Immun. 94 (2021) 318–326.
[52] B. Ijaz, A. Shabbir, M. Shahzad, A. Mobashar, M. Sharif, M.I. Basheer, R.B. Tareen, N.I. Syed, Amelioration of airway inflammation and pulmonary edema by Teucrium stocksianum via attenuation of pro-inflammatory cytokines and up-regulation of AQP1 and AQP5, Respir. Physiol. Neurobiol. 284 (2021) 103569.
[53] X. Wang, X. Yuan, Y. Su, J. Hu, Q. Ji, S. Fu, R. Li, L. Hu, C. Dai, Targeting purinergic receptor P2RX1 modulates intestinal microbiota and alleviates inflammation in colitis, Front. Immunol. 12 (2021) 696766.
[54] H.S. Na, J.E. Kwon, S.H. Lee, J. Jhun, S.M. Kim, S.Y. Kim, E.K. Kim, K. Jung, S.H. Park, M.L. Cho, Th17 and IL-17 cause acceleration of inflammation and fat loss by inducing α(2)-glycoprotein 1 (AZGP1) in rheumatoid arthritis with high-fat diet, Am. J. Pathol. 187 (5) (2017) 1049–1058.
[55] J. Li, J. Cao, M. Li, Y. Yu, Y. Yang, X. Xiao, Z. Wu, L. Wang, Y. Tu, H. Chen, Collagen triple helix repeat containing-1 inhibits transforming growth factor-β1-induced collagen type I expression in keloid, Br. J. Dermatol. 164 (5) (2011) 1030–1036.
[56] E. Bijlard, R. Timman, G.M. Verduijn, F.B. Niessen, S.E.R. Hovius, M.A.M. Mureau, Intralesional cryotherapy versus excision with corticosteroid injections or brachytherapy for keloid treatment: randomised controlled trials, J. Plast. Reconstr. Aesthetic Surg. 71 (6) (2018) 847–856.
[57] S. Tan, N. Khumalo, A. Bayat, Understanding keloid pathobiology from a quasi-neoplastic perspective: less of a scar and more of a chronic inflammatory disease with cancer-like tendencies, Front. Immunol. 10 (2019) 1810.
[58] S.A. Shaker, N.N. Ayuob, N.H. Hajrah, Cell talk: a phenomenon observed in the keloid scar by immunohistochemical study, Appl. Immunohistochem. Mol. Morphol. 19 (2) (2011) 153–159.
[59] E.P. Béguin, B.L. van den Eshof, A.J. Hoogendijk, B. Nota, K. Mertens, A.B. Meijer, M. van den Biggelaar, Integrated proteomic analysis of tumor necrosis factor α and interleukin 1β-induced endothelial inflammation, J. Proteonomics 192 (2019) 89–101.
[60] A. Körner, A. Bernard, J.C. Fitzgerald, J.C. Alarcon-Barrera, S. Kostidis, T. Kaussen, M. Giera, V. Mirakaj, Sema7A is crucial for resolution of severe inflammation, Proc. Natl. Acad. Sci. U. S. A. 118 (9) (2021) e2017527118.
[61] W.M. Seoudy, S.M. Mohy El Dien, T.A. Abdel Reheem, M.M. Elfangary, M.A. Erfan, Macrophages of the M1 and M2 types play a role in keloids pathogenesis, Int. Wound J. 20 (1) (2022) 38–45.
[62] Y. Feng, Z.L. Sun, S.Y. Liu, J.J. Wu, B.H. Zhao, G.Z. Lv, Y. Du, S. Yu, M.L. Yang, F.L. Yuan, X.J. Zhou, Direct and indirect roles of macrophages in hypertrophic scar formation, Front. Physiol. 10 (2019) 1101.
[63] W.M. Seoudy, S.M. Mohy El Dien, T.A. Abdel Reheem, M.M. Elfangary, M.A. Erfan, Macrophages of the M1 and M2 types play a role in keloids pathogenesis, Int. Wound J. 20 (1) (2023) 38–45.
[64] J.L. Hood, Melanoma exosome induction of endothelial cell GM-CSF in pre-metastatic lymph nodes may result in different M1 and M2 macrophage mediated angiogenic processes, Med. Hypotheses 94 (2016) 118–122.
[65] Y. Wang, W. Smith, D. Hao, B. He, L. Kong, M1 and M2 macrophage polarization and potentially therapeutic naturally occurring compounds, Int. Immunopharm. 70 (2019) 459–466.