Article

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The Role of Meniscus Inflammation Homeostatic Cells in Knee Osteoarthritis

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Background: Knee osteoarthritis (OA) is a prevalent, age-related form of arthritis, characterized by chronic inflammation. However, quantitative tools to assess inflammatory homeostasis are currently lacking. Thus, this study aimed to develop a tool for quantitatively evaluating inflammatory homeostasis in knee OA.
Methods: We developed the EntroInflame score, a quantitative algorithm integrating single sample gene set enrichment analysis (ssGSEA) scores and Shannon entropy of inflammation-associated genes from single-cell meniscus RNA-seq data (12 samples and 67,681 cells), to evaluate inflammatory homeostasis.
Results: Based on the algorithm, we identified a unique type of inflammation homeostatic cells (with EntroInflame score $<$ 0.5) in meniscus tissue. Through molecular characterization analysis, we found that signaling pathways, such as the phosphoinositide 3 kinase-protein kinase B (PI3K-Akt), mitogen activated protein kinase (MAPK) and longevity-regulating pathway, are closely associated with the functions of inflammation homeostatic cells. In addition, the macrophage migration inhibitory factor-atypical chemokine receptor 3 (MIF-ACKR3) was pinpointed as a vital link between inflammation and non-inflammation homeostatic cells, suggesting its therapeutic potential. Furthermore, we utilized computational biology methods to predict compounds targeting inflammation homeostatic cells and constructed a scoring system based on literature evidence to assess their credibility.
Conclusions: In summary, our study establishes inflammatory homeostasis as a critical regulatory mechanism in OA and provides a framework for precision interventions. Currently, inflammation homeostatic cells are inferred through calculation. In the future, we will further verify them through *in vivo* and *in vitro* experiments.

Keywords: meniscus; inflammation; homeostasis; entropy; osteoarthritis

Introduction

Osteoarthritis (OA) is the most common debilitating joint disease and is a leading cause of disability, which is characterized by chronic pain and a spectrum of arthropathies, including articular cartilage damage, subchondral bone remodeling, synovitis, and osteophyte formation, collectively leading to joint dysfunction and impaired mobility [1–3]. It imposes a considerable burden on individuals and healthcare systems. At present, the number of knee OA cases is continuously increasing worldwide owing to the growing elderly demographics [4]. Since no therapeutic and preventive measures are currently available for patients with OA, there is an urgent need for a deeper understanding of its etiopathogenesis [5]. Recently, bibliometric analysis has indicated that the meniscus has emerged as the focus of research on the occurrence and development of knee OA [6].

Many studies have shown that meniscal tears, extrusion and injury could play a critical role in facilitating the progression of knee OA [4,7,8]. Meanwhile, with the progression of knee OA, degenerative changes such as cartilage loss and synovial inflammation enhance the mechanical stress on the meniscus, thus increasing the risk of meniscal injury [9,10]. In addition, the repair of the meniscus has been shown to effectively alleviate OA [11–13]. These studies indicate that the meniscus, as a unique fibrocartilage structure within the knee joint, plays a dual role in biomechanical conduction and joint dynamic balance. Its pathological changes present a complex, bidirectional interaction with the occurrence and development of knee OA.

In 1963, Claude Shannon established the fundamentals of information theory and introduced the concept of Shannon entropy [14]. The Shannon entropy is established on a universal framework and can be applied to any gener-

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alized information transmission system. Because the expression of genes is an information-driven process, their randomness and uncertainty can be quantified using Shannon entropy. For instance, the Shannon entropy value of gene methylation can quantify the replication rate and aging state of stem cells [15], and cancers can be clustered through entropy analysis of gene expression profiles [16]. The single sample gene set enrichment analysis (ssGSEA) algorithm calculates the relative enrichment of a gene set within a sample by comparing its gene expression profile with that set [17]. The score from the ssGSEA algorithm reflects the degree to which the genes in that set are coordinately up- or down-regulated [18].

In our study, we developed the EntroInflame score, a quantitative tool for measuring inflammatory homeostasis by integrating the ssGSEA score and Shannon entropy value of inflammation-related genes. We used the EntroInflame score to identify a type of inflammation homeostatic cells in the meniscus from single-cell sequencing data. Based on the inflammation homeostatic cells, we identified a series of potential drugs for treating OA. Among these drugs, a substantial number have been experimentally validated to exert therapeutic efficacy against OA. Our findings suggest that the inflammation homeostatic cells could play an essential role in the occurrence and development of OA, and potentially serve as a novel therapeutic target for treating OA.

Materials and Methods

Data Collection

The single-cell data of human meniscus were sourced from Gene Expression Omnibus (GEO) dataset GSE220243 (https://www.ncbi.nlm.nih.gov/geo/), including 6 healthy meniscuses (GSM6797162, GSM6797163, GSM6797164, GSM6797165, GSM6797166, GSM6797167) and 6 meniscuses of patients with osteoarthritis (GSM6797168, GSM6797169, GSM6797170, GSM6797171, GSM6797172, GSM6797173). Quality control excluded low-quality cells with a gene count of less than 200 and a mitochondrial gene ratio greater than 5%. A total of 67,681 cells were retained for downstream analysis.

Single-Cell RNA Analysis

We performed single-cell RNA analysis of 67,681 human meniscus cells using R package Seurat (v5.1.0, Satija Lab, New York, NY, USA) [19]. Canonical correlation analysis (CCA) was used for sample integration and batch effect removal. K-nearest neighbor (KNN) and shared nearest neighbor (SNN) algorithms were used to assess the similarity of cells, followed by clustering cells with Louvain algorithm. Data were visualized in two dimensions using uniform manifold approximation and projection (UMAP) algorithm.

Entroinflame Score Calculation

In this study, we developed a novel method to evaluate the entropy and inflammation status of single cells using an integrated approach. The core of our methods consists of two key functions: CalculateEntropy and EntroInflame. The CalculateEntropy function is designed to quantify the entropy from a vector of single-cell gene expression. Initially, zero values were filtered from the vector to ensure the calculations reflect true expression levels. Subsequently, for vectors meeting a variability threshold, adaptive binning was conducted using the Freedman-Diaconis rule. After binning the expression vectors, the frequency of each bin was calculated. Then these frequencies were normalized to obtain probabilities, and the entropy was quantified according to the Shannon entropy formula. The EntroInflame function integrated the entropy calculation with pathway activity (calculating via ssGSEA) to provide a comprehensive assessment of the inflammatory state of single cells. The Entroinflame score integrated the entropy and pathway values into a unified metric of inflammatory state. The scores were normalized and combined using a dynamic weighting scheme based on their intercorrelation. This ensures that the final integrated score reflects both the variability and the inflammatory pathway activity. Finally, the calculated scores were integrated back into the Seurat object, enabling downstream analysis and visualization within the Seurat framework. This integrated approach provides a detailed understanding of the inflammatory landscape at the single-cell level, capturing both the variability and the functional activity of inflammation-related genes.

Milo Differential Abundance Analysis

To quantify changes in cell population abundance, we performed differential abundance analysis using Milo, based on miloR (v2.0.0, Marioni Lab, Cambridge, UK) package. Milo is a method designed for single-cell datasets and operates by constructing a KNN (k-nearest neighbors) graph from single-cell data to identify significant changes in cell type or state abundances between different experimental conditions [20].

Cell Response Analysis

We calculated and ranked the degree of cell response of cell types affected by experimental conditions/disease states based on Augur (v1.0.3, NeuroRestore, Lausanne, Vaud, Switzerland) R package [21]. The principle involves training machine learning models separately for each type of cell to trace the experimental conditions from which each cell originated. To quantitatively prioritize cell types, the accuracy of each cell type-specific classifier was cross-validated.

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Cell-Cell Communication Analysis

To explore the key signaling pathways mediating intercellular communication, we conducted a cell-cell communication analysis using CellChat (v1.6.1, Jin Lab, Wuhan, China) [22]. CellChat facilitates the quantitative deduction and examination of communication pathways among cells, utilizing single-cell RNA sequencing data. The R package predicts the key signaling pathways that cells receive and transmit, uses network analysis and pattern recognition to interpret these signals coordinate cellular functions.

Potential Drug Prediction and Scoring

We utilized DREEP (v0.0.0.9, Gambardella Lab, Pozzuoli, Naples, Italy) to predict potential drugs for treating OA that target specific cell types. DREEP is a bioinformatics analysis tool that uses large-scale cell-line viability screening results and enrichment analysis to predict drug sensitivity based on the cell transcriptional expression profile [23]. Subsequently, the selected drugs were evaluated through a comprehensive literature review. A score of 3 was assigned for studies clearly demonstrating an improvement effect on OA; a score of 1 was given for evidence of a therapeutic effect on either inflammation or bone-related diseases; a score of 0 indicated no relevant research supporting research.

Statistical Analysis

All statistical analyses were performed using R language (v4.4.3, R Foundation, Vienna, Vienna, Austria) and R studio software (v2025.09.2+418, Posit, Boston, MA, USA). Statistical significance was defined as a p-value $<$ 0.05 (*), with $p < 0.01$ denoted as ** and $p < 0.001$ as ***.

Results

The Landscape of Single-Cell Transcription of Human Meniscus Cells

Through canonical correlation analysis (CCA), we integrated the meniscus samples of 6 patients with osteoarthritis and 6 healthy volunteers and removed the batch effect (**Supplementary Fig. 1A**). We performed unsupervised clustering analysis on 67,681 single cells from 12 samples and mapped the 14 cell types to a two-dimensional plane using the uniform manifold approximation and projection (UMAP) algorithm (Fig. 1A). Based on literature reports and cell marker database [24,25], we identified 14 cell types, including regulatory chondrocytes-1, regulatory chondrocytes-2, effector chondrocytes-1, effector chondrocytes-2, proliferative chondrocytes-1, proliferative chondrocytes-2, fibrocartilage progenitor cells-1, fibrocartilage progenitor cells-2, pre-fibrocartilage chondrocytes, pre-hypertrophic chondrocytes, fibrocartilage chondrocytes, endothelial cells, macrophage and mature NK T cell (Fig. 1A,B). We calculated the cell numbers and proportions of different cell types in the osteoarthritis group (OA group) and the normal group (NO group) (Fig. 1C–E, **Supplementary Fig. 1B,C**). Fig. 1C shows that the total cell counts in the OA group and the NO group are balanced (33,415 and 34,266, respectively). Nevertheless, we found that the number and proportion of cell types changed visibly after osteoarthritis occurred (Fig. 1C–E, **Supplementary Fig. 1B,C**). We conducted a statistical analysis of the cell proportions between the OA and NO groups, and found significant differences in the proportions of regulatory chondrocytes-1 ($p < 0.05$), effector chondrocytes-2 ($p < 0.05$), pre-fibrocartilage chondrocytes ($p < 0.05$) and macrophages ($p < 0.01$) (Fig. 1E). Among them, the proportion of pre-fibrocartilage chondrocytes and macrophages increased significantly after the occurrence of osteoarthritis, while the proportion of regulatory chondrocytes-1 and effector chondrocytes-2 decreased significantly (Fig. 1E). In addition, we conducted a Milo analysis to further explore the abundance changes of various cell types in osteoarthritis. The results showed that mature NK T cells, macrophages, proliferative chondrocytes-2 and regulatory chondrocytes-2 are obviously enriched in patients with osteoarthritis, while endothelial cells and effector chondrocytes-2 are obviously enriched in healthy people (Fig. 1F).

Identifying Highly Ordered Inflammation Homeostatic Cells Using the Entroinflame Score

By using the EntroInflame algorithm that integrates weighted entropy values to inflammatory signaling pathway scores, we successfully identified a novel cell subtype, which was named inflammation homeostatic cells. We calculated the EntroInflame scores for 67,681 cells, and compared the discrepancies of the scores between the OA and NO group among different cell types (Fig. 2A). Significant differences in EntroInflame score were observed in 9/14 cell types, including regulatory chondrocytes-1 ($p < 0.001$), effector chondrocytes-2 ($p < 0.001$), effector chondrocytes-1 ($p < 0.001$), proliferative chondrocytes-1 ($p < 0.001$), fibrocartilage progenitor cells-2 ($p < 0.001$), pre-fibrocartilage chondrocytes ($p < 0.001$), pre-hypertrophic chondrocytes ($p < 0.01$), macrophage ($p < 0.05$) and mature NK T cell ($p < 0.001$) (Fig. 2A), suggesting that the EntroInflame scores could reflect the inflammatory level of cells. Furthermore, we have discovered a cell subtype with low EntroInflame score (<0.5) in almost all cell types (Fig. 2A,B). The threshold of 0.5 was determined based on the bimodal distribution of the EntroInflame scores across all cells, with the trough around 0.5 naturally separating the two populations (**Supplementary Fig. 2**). These cells with low EntroInflame scores indicate that the activity of the internal inflammatory pathways within them is at a low level, while the expression of inflammation-related genes is highly ordered (with extremely small entropy values), maintaining a homeostatic

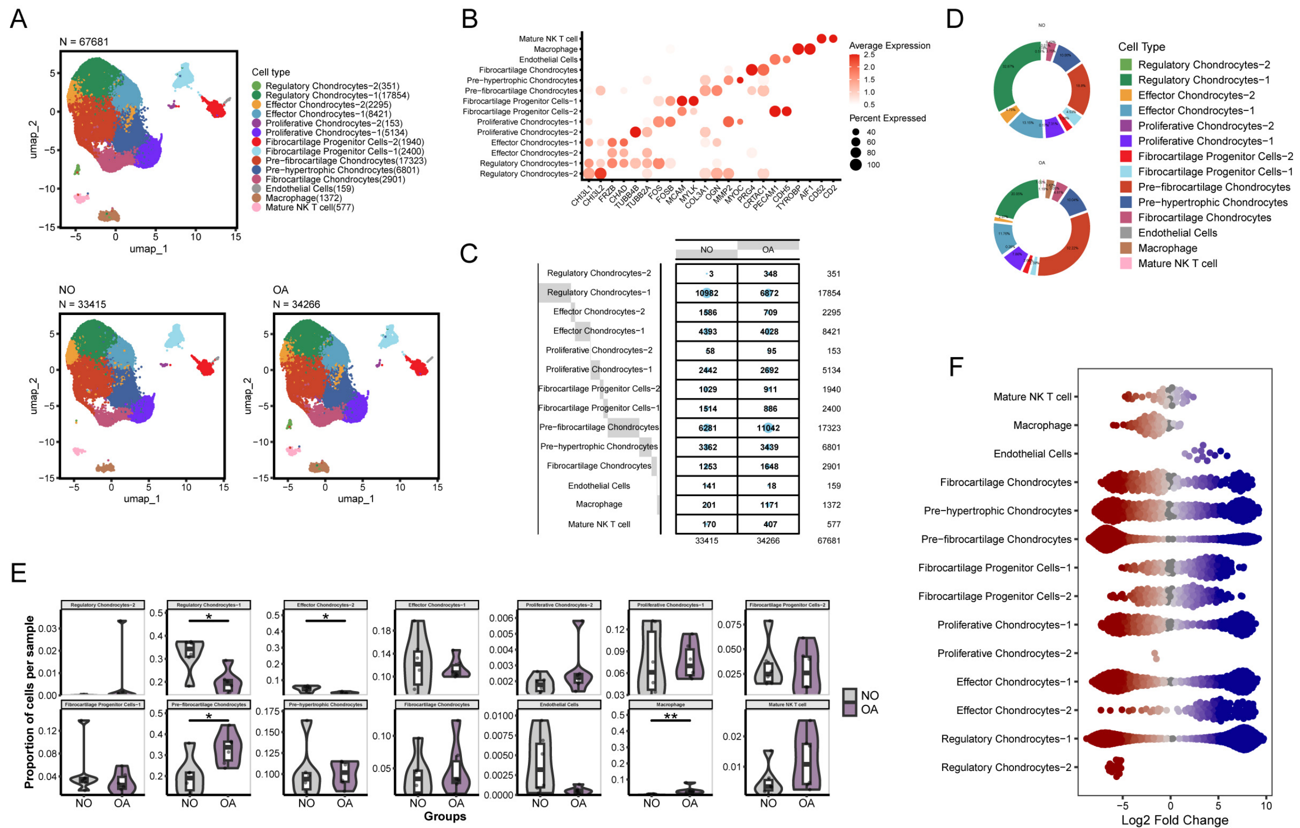

Fig. 1. Single-cell transcriptomic analysis of human meniscus cells. (A) The UMAP plots show the cell clustering and their projection in the two-dimensional space. (B) The bubble charts show the expression of marker genes. (C,D) The two charts display the number and proportion of different cell types in the OA and NO groups. (E) The violin plots show the differences in the cell proportion between the OA and NO groups. $*p < 0.05$, $**p < 0.01$. (F) The beeswarm plot shows the abundance of different cell types between the NO and OA groups. UMAP, uniform manifold approximation and projection; OA, osteoarthritis; NO, normal.

state. Hence, we designated these cells as inflammation homeostatic cells. In addition, the average number of genes and unique molecular identifier (UMI) in inflammation homeostatic cells (1347.569 and 3956.32, respectively) was lower than in non-inflammation homeostatic cells (2713.944 and 11271.16). This indicates that inflammation homeostatic cells are not low-quality cells.

The mirror density map visually presents the distribution of inflammation homeostatic cells and suggests that the number of inflammation homeostatic cells may change in osteoarthritis (Fig. 2B). Inflammation homeostatic cells accounted for 11.1% in the OA group and 12.7% in the NO group, and there is no significant difference in proportion between the two groups (**Supplementary Fig. 3C**). In addition, we also visualized the proportion of inflammation homeostatic cells in each cell type and sample, and found that there were numerical differences (**Supplementary Fig. 3A,B,D**). Moreover, in the comparison between the NO and OA groups of the 9 cell types, effector chondrocytes-1 ($p < 0.001$), proliferative chondrocytes-1 ($p < 0.01$), fibrocartilage progenitor cells-2 ($p < 0.05$), fibrocartilage progenitor cells-1 ($p < 0.001$), pre-fibrocartilage chondrocytes ($p < 0.001$), pre-hypertrophic chondrocytes ($p < 0.001$), fibrocartilage chondrocytes ($p < 0.001$), macrophages ($p < 0.01$) and mature NK T cells ($p < 0.001$), the proportion of inflammation homeostatic cells showed significant differences (Fig. 2C, **Supplementary Fig. 3**). These results suggest that inflammation homeostatic cells may play an important role in most cell types of the OA group.

Additionally, we calculated the correlation between the EntroInflame score and the Ucell score of Hallmark inflammatory response signatures, and found a significant correlation (r = 0.1975127). Furthermore, we validated this association in an independent meniscus sample (GSM6797160), where the EntroInflame score showed a similar correlation (r = 0.1975377) with the Seurat module score. These consistent correlations indicate that the EntroInflame score is robustly associated with the inflammatory response. Moreover, its unique algorithm captures information beyond conventional activity scores, providing a comprehensive metric of inflammatory homeostasis.

The Feature of Inflammation Homeostatic Cells

Based on the thresholds of |log2 fold change| >0.25 and adjusted p-value < 0.05, we identified 2712 (1362 up-regulated and 1350 down-regulated) differentially ex-

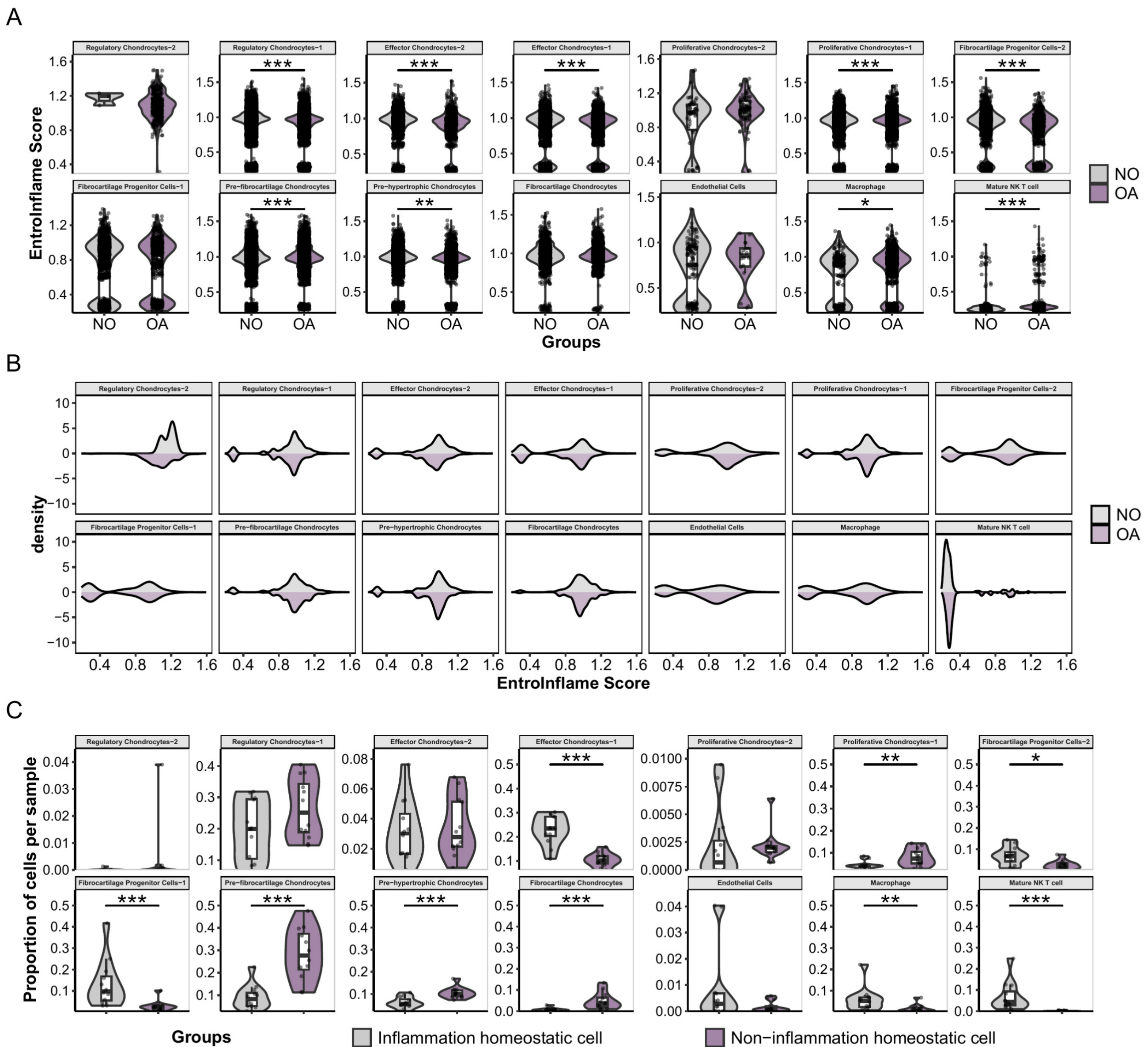

Fig. 2. The Entroinflame score and the inflammation homeostatic cells. (A) The violin plots show the Entroinflame score of each cell. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. (B) The mirror density plots show the distribution of the Entroinflame score between the NO and OA groups in each cell type. (C) The violin plots show the differences in the cell ratio between inflammation homeostatic cells and non-inflammation homeostatic cells in each cell type. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

pressed genes (DEGs) from inflammation homeostatic cells versus non-inflammation homeostatic cells (Fig. 3A, **Supplementary Table 1**). We conducted Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis on these genes and found that the top 15 significantly enriched pathways are phosphoinositide 3 kinase-protein kinase B (PI3K-Akt) signaling pathway, mitogen activated protein kinase (MAPK) signaling pathway, autophagy, lysosome, longevity regulating pathway (Fig. 3B). Moreover, we identified 1677 (991 up-regulated and 686 down-regulated) DEGs from OA-inflammation homeostatic cells versus NO-inflammation homeostatic cells (Fig. 3C, **Supplementary Table 2**). The top 15 significantly enriched pathways include PI3K-Akt signaling pathway, lysosome, cellular senescence, apoptosis, autophagy, mitophagy, and ferroptosis (Fig. 3D). These results indicate that meniscus inflammation homeostatic cells play an important role in the occurrence and development of osteoarthritis through the signaling pathways of cell cycle and programmed cell death.

To evaluate the sensitivity of inflammation homeostatic cells, we carried out cell response analysis using the Augur method. A variety of external stimuli lead to changes (temporary/permanent) in the state, function and shape of cells, which can be generically referred to as "perturbation". A smaller value of AUC (calculated by Augur) indicates

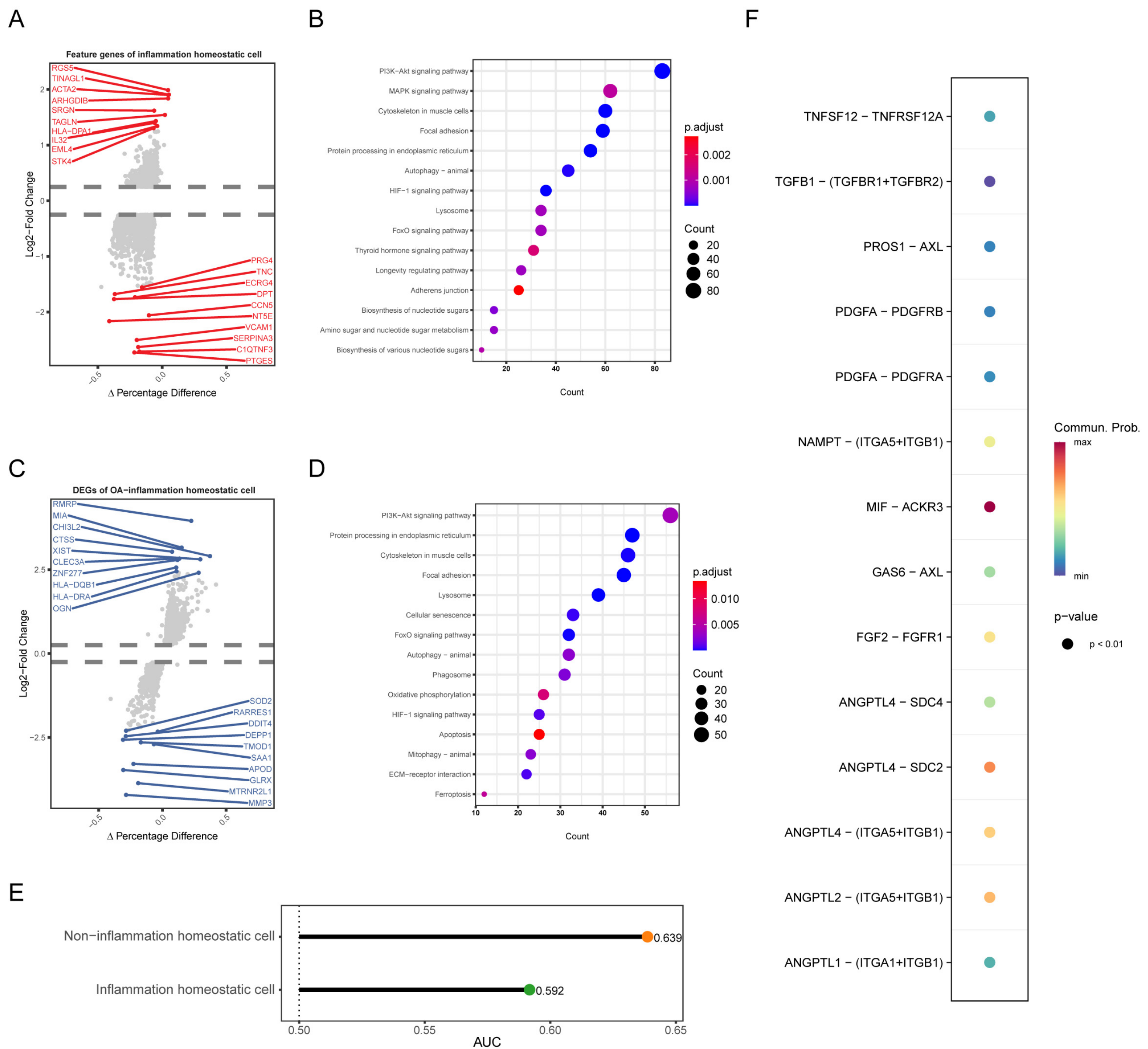

Fig. 3. Characterization analysis of inflammation homeostatic cells. (A,C) Two volcano plots separately display the differentially expressed genes between inflammation homeostatic cells and non-inflammation homeostatic cells (red), as well as those between OA-inflammation homeostatic cells and NO-inflammation homeostatic cells (blue). (B,D) Two dot plots respectively display the top 15 most significantly enriched signaling pathways of feature genes of inflammation homeostatic cells and DEGs of OA-inflammation homeostatic cells. (E) The lollipop chart shows the extent of perturbation in inflammation homeostatic cells and non-inflammation homeostatic cells. (F) The bubble plot shows the signal intensity of the ligand-receptor pairs involved in cell-cell communication between inflammation homeostatic cells and non-inflammation homeostatic cells.

that the cells are less affected by the disease state, showing the higher stability of the cells. Fig. 3E shows that inflammation homeostatic cells have higher stability than non-inflammation homeostatic cells. In addition, cell-cell communication analysis further revealed that the macrophage migration inhibitory factor-atypical chemokine receptor 3 (MIF-ACKR3) pathway is the most significant signaling axis between inflammation homeostatic cells and non-inflammation homeostatic cells (Fig. 3F), evidenced by the relatively high expression levels of MIF and ACKR3 in various cell types (**Supplementary Fig. 4**).

Drug Screening for Inflammation Homeostatic Cells Sensitivity

To investigate potential therapeutic agents targeting inflammation homeostatic cells, we employed DREEP to calculate drugs that are sensitive to OA-inflammation homeostatic cells. In the GDSC2, CTRP2, and PRISM

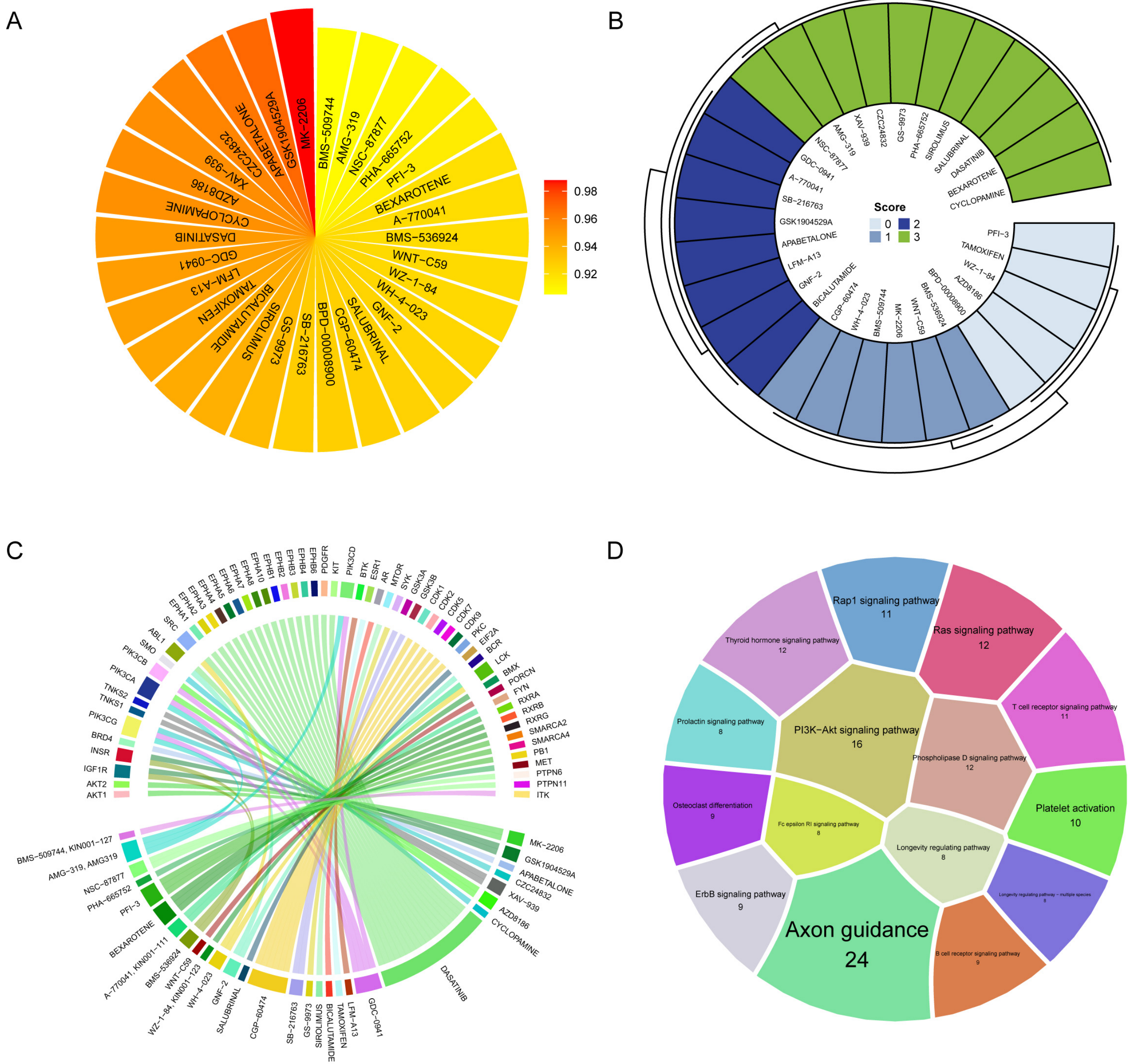

Fig. 4. Potential drugs targeting the inflammation homeostatic cells. (A) The ranking of 30 candidate compounds with drug sensitivity greater than 0.9 from highest to lowest. (B) The heatmap shows the clustering of drugs based on the literature scoring system. (C) The chord diagram illustrates the mapping relationship between potential compounds and their targets. (D) The top 15 enrichment results demonstrate the key biological processes regulated by the drug targets, with the size of the area corresponding to the count of enriched targets.

databases, we calculated and found that the sensitivity of 30 drugs to inflammation homeostatic cells was above 0.9 (Fig. 4A). Although the 30 candidate agents showed high sensitivity in inflammation homeostatic cells, the foregoing analysis alone is insufficient to support the conclusion that these drugs possess therapeutic effects on OA. Consequently, we proceeded to assess the therapeutic potential of these compounds in OA through a scoring system based on literature review (3 = clear OA improvement, 1 each = inflammation/bone disease therapy, 0 = no relevant research) (Fig. 4B). Experimental analyses demonstrated that 11 types of compounds exhibit therapeutic effects on OA (Fig. 4B). Additionally, 14 types of compounds have been shown to have therapeutic effects on inflammation or bone-related diseases (Fig. 4B). A total of 59 potential drug targets were identified by DREEP (v0.0.0.9) (Fig. 4C). KEGG enrichment analysis was performed on these targets, and the top 15 significantly enriched functions were presented (Fig. 4D). We found that the targets were also enriched in inflammation homeostatic cells related signaling pathways, such as the PI3K-Akt signaling pathway and longevity regulating pathway.

Discussion

In this study, through high-resolution single-cell transcriptome analysis, we systematically elucidated the dynamic characteristics of the cellular composition in the meniscus tissue of patients with OA and its pathological implications. By conducting an integrated analysis of 67,681 single cells from 6 OA patients and 6 healthy controls, we discovered a notable remodeling of cellular heterogeneity in the OA meniscus. Specifically, the proportions of pre-fibrocartilage chondrocytes and macrophages increased significantly, whereas the proportions of regulatory chondrocytes-1 and effector chondrocytes-2 decreased remarkably. Many studies have shown that chondrocyte-like cells play an important role in the regeneration process of the meniscus [26,27]. From the perspective of cell function, the significant enrichment of pre-fibrocartilage cells in the OA group may reflect the regenerative compensatory mechanism of meniscus tissue. These progenitor cells may form fibrocartilage tissue through differentiation to partially replace the degenerated area, which is consistent with the previous study, which proposed a reparative fibrotic process after OA injury [28]. However, it is concerning that the significant reduction of regulatory and effector chondrocytes may disrupt the homeostatic balance of extracellular matrix (ECM) synthesis and degradation [29–31], leading to excessive activation of matrix metalloproteinases (MMPs) and collagen network disorder [32]. This imbalance may be a contributor to the ultimate failure of meniscus fibrosis repair. At the level of the immune microenvironment, the abnormal expansion of macrophages and the enrichment of mature NK T cells suggest the presence of persistent chronic inflammation in OA menisci. Macrophages may enhance the local inflammatory cascade reaction by secreting pro-inflammatory factors such as tumor necrosis factor α (TNF-α), interleukin 1 β (IL-1β), interleukin 6 (IL-6) and matrix metalloproteinases (MMPs), thereby inhibiting chondrocyte function and accelerating ECM degradation [33]. Meanwhile, NK T cells serve an immunoregulatory function by releasing cytokines, such as interleukin 2 (IL-2), Interferon γ (IFN-γ), TNF-α, and granulocyte-macrophage colony-stimulating factor [34], but they cannot promote cartilage healing or enhance joint integrity, and could even trigger osteoclastogenesis and bone destruction in OA [35,36]. These findings imply that during the progression of OA, the meniscus may actively engage in the pathological remodeling of the joint microenvironment by activating progenitor cell differentiation and inflammatory mechanisms, rather than simply serving as a passive target organ of damage.

Despite the considerable heterogeneity among individual cells, grouping them into functional types is a foundational step in biology. It enables the systematic study of new biological phenomena, the clarification of underlying mechanisms and the identification of therapeutic targets. In the study, we developed the EntroInflame score, an algorithm that integrating weighted entropy with inflammatory pathway activity, to assess the inflammatory homeostasis of various cell types. Intriguingly, we discovered a distinct subtype of cells. These cells exhibit a unique biological signature characterized by both low inflammatory pathway activation and highly ordered expression of inflammation-associated genes (EntroInflame score <0.5). Their broad distribution across nearly all examined cell types highlights a fundamental mechanism for maintaining inflammatory homeostasis. Since these cells have similar functional changes in some signaling pathways related to inflammation, we designated the cell subtype as the inflammation homeostatic cell. While the overall proportion of inflammation homeostatic cells remained relatively stable between the OA (11.1%) and NO (12.7%) groups, their distribution across specific cell types altered dramatically in OA (Fig. 2C, **Supplementary Fig. 2**) group. This cell type-specific disruption, particularly in chondrogenic lineages and immune cells, suggests that inflammation homeostatic cells may play a role in maintaining joint homeostasis.

Based on transcriptomic analysis, our study elucidated the key role of inflammation homeostatic cells in the meniscus tissue during the pathogenesis of OA. The identification of OA feature genes and their enrichment in PI3K-Akt and MAPK signaling pathways highlighted the profound impact of inflammatory mechanisms in these cells. These pathways are well-established drivers of synovial inflammation and cartilage degradation in OA [37,38]. Notably, the enrichment of autophagy, lysosome, and longevity-regulating pathways indicates that they may play a significant role in maintaining inflammatory homeostasis. For instance, a recent study revealed that a kind of senescent cell type with zinc-finger E-box-binding homeobox 1 (ZEB1) transcription factor promotes OA in the meniscus [25]. Further analysis of OA-inflammation homeostatic cells revealed 1677 DEGs enriched in cell senescence, apoptosis, autophagy, mitophagy and ferroptosis. This highlights the dominance of programmed cell death pathways in OA, potentially explaining the irreversible tissue degeneration observed clinically [39]. The Augur-based cell response analysis, revealing higher stability in inflammation homeostatic cells, suggested these cells exhibit adaptive resilience to inflammatory stimuli. Interestingly, our cell-cell communication analysis identified the MIF-ACKR3 axis as the primary mediator between inflammatory and non-inflammation homeostatic cells. Macrophage migration inhibitory factor (MIF) is a potent pro-inflammatory cytokine, and its interaction with Atypical chemokine receptor 3 (ACKR3) likely modulates immune cell recruitment and activation [40]. In addition, the MIF-ACKR3 acts as a key factor influencing the differentiation of adipose stem and progenitor cells, driving them toward pro-fibrogenic and pro-inflammatory differentiation [41], suggesting an important role in the fibrosis

process of cartilage tissue. The dominance of MIF-ACKR3 implies its potential as a therapeutic target to disrupt inflammatory cascades in early OA.

Using DREEP, our analysis identified 30 compounds with high sensitivity against OA-inflammation homeostatic cells. This finding, consistent across three pharmacogenomic databases, indicates that these cells are promising therapeutic targets for knee OA. Although MK-2206, an AKT inhibitor, was identified as the most sensitive drug, there is no direct evidence of its direct therapeutic effect on OA. However, recent research showed that MK-2206 alleviates atherosclerosis by suppressing inflammation and lipid accumulation [42]. Sirolimus, also known as rapamycin, is an mTOR inhibitor and a potential anti-aging compound. In our study, sirolimus exhibited a sensitivity of up to 0.94 against inflammation homeostatic cells. Evidence showed that administering rapamycin-loaded microparticles via joint injection prevents cellular senescence and efficiently addresses OA [43], suggesting that inflammation homeostatic cell is likely to be one of the key targets. Furthermore, we implemented a literature-based scoring system to explore the anti-OA potential of these cell-sensitizing drugs. KEGG enrichment of the 59 identified drug targets further validated their involvement in inflammation homeostatic cell-relevant pathways, including PI3K-Akt signaling pathway and longevity-regulating pathway. In addition, we have noted that these drugs may also target the osteoclast differentiation pathway. Osteoclasts act as the crucial mediator in the subchondral bone, accelerating the occurrence and development of OA [44]. These results indicate that the candidate drugs targeting inflammation homeostatic cells, which were identified through computational biology, show potential therapeutic effects in the treatment of OA.

This study innovatively develops the EntroInflame score to quantify inflammatory homeostasis, identifies meniscal inflammation homeostatic cells, and pinpoints the MIF-ACKR3 axis as well as candidate drugs using computational analyses. Limited by a small sample size and reliance on bioinformatics, its findings lack experimental validation. Subsequent research must be conducted *in vitro* and *in vivo* experiments to confirm the functional roles of these cells, pathways, and drugs for their clinical application.

Conclusions

In summary, our comprehensive study on inflammation homeostatic cells in meniscus tissue during OA pathogenesis provides several critical insights. We developed the EntroInflame score to assess inflammatory homeostasis across various cell types, revealing a distinct subtype of inflammation homeostatic cells characterized by low inflammatory pathway activation and highly ordered expression of inflammation-associated genes. These cells, broadly distributed across examined cell types, highlight a fundamental mechanism for maintaining inflammatory homeostasis. Future research should focus on validating these findings and exploring the clinical applications of targeting inflammation homeostatic cells in OA treatment.

Abbreviations

OA group, Osteoarthritis group; NO group, Normal group; DEGs, differentially expressed genes; ssGSEA, single sample gene set enrichment analysis; CCA, canonical correlation analysis; KNN, K-nearest neighbor; SNN, shared nearest neighbor; UMAP, uniform manifold approximation and projection; UMI, unique molecular identifier; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Availability of Data and Materials

All generated data are within this article. The sequencing data can be downloaded from the GEO database (GSE220243).

Author Contributions

YH, ZFC, JQM and ZLL contributed to the study conception and design. JQM, HW, and LCW were responsible for data acquisition, formal analysis, and visualization. ZLL, JQM, LCW and HW drafted the original manuscript. YH, LCW and ZFC critically revised the manuscript for important intellectual content. HW and ZFC acquired funding for the research. YH, ZFC, and ZLL provided overall supervision of the research project. All authors have reviewed the final version of the manuscript, provided their approval for publication, and agreed to be accountable for all aspects of the work, ensuring that questions related to its accuracy or integrity are appropriately addressed.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.24976/Discov.Med.202638204.7.

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