

Proteomic analysis of synovial tissue in rheumatoid arthritis, OLFM4 promotes fibroblast like synoviocytes proliferation in RA

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Research article

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Abstract

Background To understand the roles of synovial tissue in rheumatoid arthritis (RA), we comprehensively investigated the protein profiles of synovial tissues in patients with RA and osteoarthritis (OA).

Methods Protein were isolated from synovial tissues of 46 patients (22 with RA, 24with OA). Proteins were digested and labeled with TMT kit. All the peptide were send to LC-MS/MS analysis, and processed with Mascot and Proteome Discovery (version 1.4) search engines. All the protein data were analysis with Gene ontology (GO) and KEGG on line database. Four up-regulated protein were examined with western blotting and IHC. The RNAi were used for revealing the OLFM4 function in FLS.

Results In total, over 500 proteins were identified as the different expression protein compared RA and OA synovia tissues, including 239 proteins were up-regulated in RA, and 271 proteins were up-regulated in OA. GO and KEGG analysis of the different expressed protein mostly identified as developmental processes and protein processing in ER. RT-qPCR, western blotting and IHC confirmed that there was a strong over expressed OLFM4 in RA synovia tissues. OLFM4 could be up-regulated under inflammatory stimulation, and participate in fibroblast like synoviocytes proliferation.

Conclusion patients with RA possessed considerably different protein profiles of synovial tissues from with OA. The unique protein profiles of RA synovial tissues, such as the OLFM4 and MZB1 had been up-regulated compared to the OA, may reflect the pathophysiology of RA.

Background

Rheumatoid arthritis (RA) affects in 0.5%-1% of the world population, and is a leading cause of pain and loss of physical function [1]. The molecular mechanisms underlying RA remain incompletely understood, and the main therapy of RA is non-steroidal anti-inflammatory drugs and disease-modifying anti-rheumatic drugs [2–4]. RA progression culminates in joint replacement surgery. RA is a complex disease, with both heritable [5] and environmental factors[6] contributing to susceptibility.

Synovial tissue inflammation and proliferation is the key features of RA[7]. Synovial tissue could be readily accessible at join replacement surgery, providing an opportunity to characterize the molecular processes underpinning disease development in the right tissue, both to improve our fundamental understanding of disease biology and to identify novel therapeutic opportunities. In recent years, the -omics studies of RA disease pathogenesis or RA biomarkers were mainly in wet samples: synovial fluid[8], blood[9], urine[10], or fibroblast-like synoviocytes (FLS)[11]. However, there are some reports indicate that the synovial tissue from osteoarthritis (OA) patients was also proliferate.

Modern approaches and methodologies of system biology analysis are becoming increasingly important in clarifying disease mechanisms. Novel proteomics techniques, liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) technology are powerful tools to detect protein changes and low-abundance protein alternations in injured tissue samples and experimental disease models. Isobaric

tags for relative and absolute quantitation (iTRAQ), a MS-based proteomics approach, is helpful to compare protein expression levels between multiple samples, and can provide accurate mass measurement of critical changes in the amounts of proteins[12].

Using proteomic methods, previous studies have uncovered several novel pathophysiological processes, potential therapeutic targets, or biomarkers in rheumatoid arthritis [9, 13–15]. However, the differentially expressed protein between the OA and RA synovial tissues remains unknown.

Here, a quantitative proteomic profiling of synovial tissue obtained from RA and OA patients was carried out by using iTRAQ labeling followed by high resolution mass spectrometry analysis. We have identified 4822 proteins out of which 510 proteins were found to be differentially expressed by ≥ 1.2 fold change in the synovial tissue from rheumatoid arthritis verses osteoarthritis patients. Proteins not previously reported to be associated with rheumatoid arthritis including, Proteinase 3 (PRTN3), Olfactomedin 4 (OLFM4), and Elastase-2 (ELA2) were found to be upregulated in RA synovial tissue. Proteins such as ATP Binding Cassette Subfamily A Member 8 (ABCA8), Synuclein Gamma (SNCG), and Clusterin (CLU) were found to be upregulated in the synovial tissue of OA patients. We confirmed the up-regulation of OLFM4 in RA synovial fluid by IHC and immunofluorescence as well as western blotting on protein level. The intervention of OLFM4 could inhibit the FLS proliferation. Pathway analysis of differentially expressed proteins revealed a significant enrichment of genes involved in protein processing in endoplasmic reticulum (ER) in rheumatoid arthritis.

Methods

Patients and synovial tissue samples

All the synovial tissue specimens for Tandem Mass Tag (TMT) relative quantitative proteomics and further experiments were obtained from the patients with RA or OA undergoing surgical joint replacement at the clinical of joint surgery (Xi'an Hong Hui Hospital, Xi'an Jiaotong University, China). The diagnosis of the patients were accorded to the criteria of the American College of Rheumatology (ACR) and European League Against Rheumatism (EULAR) in 2010. This study was approved by the Ethics Committee in Hong Hui Hospital, and the Medical Ethics Committee of Xi'an Jiaotong University. The detailed patient clinical tests were presented in Table 1.

Protein extraction, digestion and TMT labeling

The synovial tissues were extracted with SDT buffer (4 % (w/v) SDS, 100 mM Tris-HCl, pH 7.6, 0.1 M DTT). And then the proteins were digested with FASP after BCA quantification. Peptides were quantified with Nanodrop 2000 via OD₂₈₀. A total of 100 µg of peptides from each sample was labeled with a TMT kit following the kit instructions (ThermoFisher, USA). Briefly, acetonitrile and 0.1% TFA were used to balance the reversed-phase chromatography column, and then the equally mixed peptide fractionation was loaded onto the column with ddH₂O. After adding ddH₂O, the salt was desalted by low-speed centrifugation. Finally, the column-bound peptides were eluted with a high-pH acetonitrile solution with

sequentially increasing concentrations. Each eluted peptide was completely dried and resuspended in 12 μ L of 0.1% TFA.

LC-MS/MS analysis

Peptides were dissolved in 0.1% TFA and were directly loaded onto a reversed-phase precolumn (Acclaim PepMap 100, Thermo Scientific). Peptide separation was performed using a reversed-phase analytical column (Acclaim PepMap RSLC, Thermo Scientific) at a constant flow rate of 300 nl/min on an EASY-nLC 1000 UPLC system. The resulting peptides were analyzed by a Q Exactive hybrid quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific). The peptides were subjected to HCD followed by tandem mass spectrometry (MS/MS) in Q Exactive (ThermoFisher, USA). Intact peptides were detected in the Orbitrap at a resolution of 70,000. Peptides were selected for MS/MS using 28% NCE, ion fragments were detected in the Orbitrap at a resolution of 17,500. A data-dependent procedure that alternated between one MS scan followed by 20 MS/MS scans was applied for the top 20 precursor ions above a threshold ion count of $1.5E4$ in the MS survey scan with 60.0 ms dynamic exclusion. The electrospray voltage applied was 2.0 kV. Automatic gain control (AGC) was used to prevent overfilling of the ion trap; $5E4$ ions were used to generate the MS/MS spectra. For MS scans, the m/z scan range was 300 to 1800 Da.

Peptides data processing

The MS data were processed using Mascot and Proteome Discovery (version 1.4) search engines. Tandem mass spectra were searched against the UniProt_human database (161584 sequences) concatenated with the reverse Decoy Database. Trypsin/P was specified as a cleavage enzyme allowing up to 2 missed cleavages. Mass error was set to 20 ppm for precursor ions and 0.1 Da for fragment ions. Carbamidomethylation on cysteine was specified as a fixed modification, and oxidation and TMT were specified as variable modifications. A 1.0% false discovery rate (FDR) was used to filter the identified peptides. All the other parameters in Mascot were set to their default values.

GO analysis and KEGG pathway analysis

The online analysis database, Database for Annotation, Visualization and Integrated Discovery (DAVID) (<https://david.ncifcrf.gov/>, Version 6.8), to analyze the Gene Ontology (GO) annotation and the enrichment of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways for these data [18,19]. The *p* value < 0.05 and gene counts >2 were criteria for the both.

Western blotting assay

The synovial tissue from OA and RA patients were described in the synovial tissue segment. Such tissue samples were extracted in RIPA buffer with protease inhibitor cocktail. After 20 min incubation in ice, all the samples were centrifuged at 12000 rpm for 30 min at 4 °C and then quantified with BCA kit. 40 μ g protein of each samples were loaded on 8-10% SDS-PAGE gels. The antibodies used for western blotting were OLFM4 (NOVOS, NBP2-24535), MZB1 (Abcam-ab181205) and DERL3 (Abcam-ab78233), S100P (Abcam-ab225543), and LAP3 (Abclonal-A7101), rabbit anti-ACTB. All the first antibodies were diluted at

1:1000, and the HRP conjugated goat anti-rabbit IgG antibody was diluted at 1:10000. Signal intensity was determined by Supersignal[®] West Pico Kit (Thermo Scientific). And the images for western blotting were taken by Syngene G. All the density of the bands was measured and normalized to β -actin. Final data are expressed by showing one representative image and quantitative result of samples from various patients.

Immunohistochemistry

The paraffin-embedded RA and OA synovial tissues were sectioned and mounted on positively charged slides. All the slides were incubated at 60°C for overnight, followed by de-paraffinization and rehydration. These slides were transferred to the Leica Bond-IIITM and were treated for antigen retrieval at 100°C for 20 minutes in a retrieval solution. Endogenous peroxidase was blocked using peroxidase-blocking reagent, followed by primary antibody incubation for 60 minutes. The blocker buffer were used for blocking the unspecific reaction for 1 hour at room temperature. Then the first antibody of OLFM4, MZB1 (Marginal Zone B and B1 Cell Specific Protein) and DERL3 (Derlin 3), and S100P (S100 Calcium Binding Protein P) were diluted at 1:1000 and then applied to the tissue at 4 °C overnight. The next morning all the slides were taken out and balanced at room temperature for 30 min, then washed with PBS for times. Then the secondary antibody were used for 30min at room temperature, followed by the horseradish peroxidase reaction with DAB reaction.

Fluorescence immunohistochemistry was performed using the primary antibodies, anti-OLFM4, and anti-Vimentin. DAPI (1:100000, Sigma-Aldrich, D9542) were used for staining the nucleic.

RT-qPCR

The total RNA were isolated from RA and OA patients' synovial tissues, and then quantified. The cDNA were reversed by using First Strand cDNA Synthesis Kit (Roche, USA). The synovial fibroblast were isolated from RA synovial tissues. The FLS were treated with different pro-inflammatory cytokines, 10 ng/mL TNF α , 2 ng/mL IL-1 β or 1 ng/mL LPS for 24 hours. All the RNA were extracted with Trizol, quantified and then the mRNA were reversed to cDNA by using First Strand cDNA Synthesis Kit (Roche, USA). The real time PCR was performed with Fast Start Universal SYBR Green Master (ROX) (Roche, USA) by Agilent Mx3000. PCR efficiency and specificity of each primer pair was examined by a standard curve of serially diluted cDNA and melting curve functionality respectively. Fold change was calculated based on $2^{-\Delta\Delta Ct}$ method after normalization to the transcript level of housekeeping gene ACTB. Primer sequences used in the RT-PCR could be found in Table 3.

RNAi

The siRNA kit for the target protein OLFM4 were purchased from Santa Cruz. The 20uM siRNA (siNC or siOLFM4, Santa Cruz) were transfected Lipofectamine[™] 2000 Transfection Reagent (Thermo Scientific, 11668019) to the FLS for 72 hours. Then the cell were collected either for RT-qPCR or for cell proliferation assay.

Cell proliferation assay

The transfected NC and siOLFM FLS cell were incubated with PE-labeled cell trace dye (diluted at 1:1000) for 72 hours. After 72 hours cultured, all the cell were collected and detected by flow cytometer. The unstained NC cell were used as a negative control. The MFI were measured to determine the cell proliferation rate after knocking down the OLFM4 gene.

Statistics

Statistics were carried out using SPSS and Graphpad Prism software. The student's *t*-test of the Mann-Whitney *U*-test and unpaired student's *t*-tests were used to determine statistical differences between two groups. The Fisher's tests were used to test for enrichment or one-way ANOVA depletion of specific annotation terms among members of the resulting protein cluster. $P < 0.05$ was considered to indicate a statistically significant difference. Data were presented as mean \pm SEM.

Results

Clinical characteristic of patients

Of the 22 patients with RA and 24 patients with OA from whom blood sample and synovial tissue were obtained. The age, sex, prevalence of rheumatoid factor (RF), anti-cyclic citrullinated peptide (CCP), anti-CRP (C reactive protein) and erythrocytesedimentationrate (ESR) were listed in Table 1. There were significant differences in patients' age, RF, anti-CCP, CRP and ESR.

Proteome profiles of RA and OA synovial tissues.

All the peptide collected in this experiment were mainly distributed in 10 ppm (Fig.1 A), the MASCOT score was over 20 (Fig.1 B), and the protein ratio from AR and OA groups were merely to 1 (Fig.1 C), which suggested the identification results were reliable could use for further analysis. Approximately 4822 proteins were found in synovial tissues via LC/MS, 239 proteins with a quantitative ration of more than 1.2 fold change were considered up-regulated, 271 proteins were considered down-regulated if the quantitative ratio was less than 1.2 fold change (Fig.1 D).

Differential protein expression, GO term and KEGG analysis in the synovial tissues

We summarized the up-regulated (Fig.2A) and down-regulated (Fig.2B) differential proteins (± 1.5 fold change) from RA and OA synovial tissues. The different expressed top 10 up-regulated and down-regulated proteins were summarized in Table 2. To fully understand the function of each protein as well as the cellular and biological processes involved, we performed GO term analysis. The GO term analysis (Fig.2C) showed that most significantly changed proteins were divided in 3 groups. Some were associated with biological process (BP), which were mainly in developmental processes, anatomical

structural development, extracellular structure organization, extracellular matrix organization, skeletal system development etc. Molecular function study showed calcium ion binding, glycosaminoglycan binding, RAGE receptor binding, metal ion binding, cation binding were changed significantly. As well as the extracellular matrix, proteinaceous extracellular matrix, extracellular region, collagen trimmer and fibrillar collagen trimmer were changed significantly in RA synovial tissues in cellular component analysis.

For further understanding the interaction and coordination of different protein function in the synovial tissues, the KEGG pathway analysis were carried out (Fig.3A). The KEGG pathway enrichment results illustrated that the significantly changed pathways were chemical carcinogenesis, drug metabolism-cytochrome P450, protein processing in ER, metabolism of xenobiotic by cytochrome P450, and ECM-receptor interaction (Fig.3B).

Verification the candidate genes expression in RA and OA synovial tissues.

RT-qPCR were used to select the mRNA expression of different expressed genes in RA and OA synovial tissues. The results showed that, comparing with OA synovial tissues, only 4 genes, MZB1, OLFM4, DERL3 and S100P (Fig.4 A-D) were significantly up-regulated in RA synovial tissues. On the contrary, some candidates, like SEC61B (Fig.4 E) and RAC2 (Fig.4 K), only showed the increasing expression trend.

Verification of protein expression in RA and OA synovial tissues.

Western blotting and IHC were employed to further verify differential protein expression in order to ensure the reliability of proteomic results from iTraq. The protein expression of MZB1 and OLFM4 from RA and OA synovial tissues also showed significantly up-regulated (Fig.5 A-C), while the DERL3 and LAP3 were not that significantly up-regulated in RA synovial tissues than OA, when the western blotting was applied (Fig.5 D-E). What's more, the IHC results indicated that the expression levels of OLFM4, DERL3 and MZB1 were significantly higher in the RA synovial tissues compared with OA synovial tissues (Fig.5 F-J), which was consistent with proteomic analysis.

RA FLS cell proliferation via OLFM4

The expression of OLFM4 were determined in RA synovial tissue via IF, as the result showed in Fig.6A, the OLFM4 was mainly expressed in Vimentin positive cells. The FLS were isolated from synovial tissues of RA patients. The protein expression of OLFM4 could be significantly up-regulated after inflammation cytokine TNF- α , IL-1 β or LPS treatment (Fig.6B). Meanwhile the mRNA expression of OLFM4 and IL-6 were checked at different time points in TNF- α treated RA FLS. The results showed that the mRNA expression of OLFM4 was persistently up-regulated after 2 hours TNF- α priming until 48 hours, while the IL-6 mRNA expression was up-regulated by TNF- α stimulation from 1 hour to 4 hours, and then up-regulated again on 12 hours to 48 hours (Fig.6C). The function of OLFM4 were reported as an anti-apoptosis protein. So the function of OLFM4 in RA FLS were studied by RNAi. The OLFM4 could be down-regulated via siRNA successfully (Fig.6D). The proliferation of FLS was tested by CFSE method. As the

results showed in Fig.6E-F, after knocking down the expression of OLFM4, the PE labeled cells were significantly more than the NC group.

Discussion

Here, we compared the protein expression levels of synovial tissue from RA and OA patients by using iTRAQ and LC/MS based proteomics approach. As a result, over 500 protein were found different expression pattern in synovial tissues between RA and OA patients. The GO analysis and KEGG pathway analysis results also showed that, in RA synovial tissue, the protein processing in ER associated proteins was significantly up-regulated, as well as the extracellular matrix associated with the upregulated proteins in cell process. Some of the gene which related with these process and signal were also be checked. The candidates were confirmed by RT-qPCR and western blotting. Compared with OA synovial tissues, the *MZB1*, *OLFM4*, *DERL3* and *S100P* genes showed highly expressed in RA synovial tissues. However, the protein expression only showed MZB1 and OLFM4 were over expressed in RA patient's synovial tissues. Furthermore, the OLFM4 expression were evaluated via IHC. Since this gene was first reported in RA, a preliminary study was applied. The OLFM4 expression could be up-regulated after LPS, TNF α or IL1 β stimulation. After knocking the OLFM4 expression in FLS, the proliferation of FLS were inhibited, suggesting this gene might promote the cell proliferation.

The GO analysis of process networks suggested that the extracellular matrix associated with the upregulated proteins in RA synovial tissue compared to the OA synovial tissue. Moreover, the KEGG pathway analysis also revealed that the protein processing in ER associated with the upregulated proteins in RA. The ER is the place for folding the secreted and membrane proteins properly in the biosynthesis process [30]. It was also reported that in RA FLS, the ER stress associated genes, like DDIT3 and HSP5, were increased and involved in regulating the inflammatory genes expression in activated FLS from RA patients [31, 32].

To further understand the up-regulated proteins in RA, we focused on the 2 genes. Human OLFM4 (olfactomedin 4, also known as hGC-1, GW112), originally termed human cloned from myeloid precursor cells after granulocyte colony-stimulating factor stimulation[16], is a secreted glycoprotein more commonly known as the anti-apoptotic molecule GW112[17, 18]. It was first cloned in human hematopoietic myeloid cells and is also abundantly expressed in intestinal crypts.[16, 19] Recently it also was reported expressed in bone marrow, prostate, small intestine, stomach, colon and pancreas [16, 20]. More recently, up-regulated OLFM4 expression has been described in lung and breast[21], prostatic[18], gastric[22] and pancreatic cancers[21, 22] as well as in colorectal adenomas[19, 23–26], suggesting that this gene might be involved in the cell proliferating process. And the FLS also showed a rapid growth characteristic in RA [27]. In our latest results showed that OLFM4 were up-regulated in FLS from RA synovial tissues. Similarly, the increased OLFM4 levels were also found in the crypt epithelium of inflamed colonic mucosa of inflammatory bowel diseases [28] and in gastric biopsies infected with *Helicobacter pylori*[29, 30]. It has been suggested that OLFM4 is involved in cellular process such as apoptosis and tumor growth [17]. We also found that the OLFM4 gene expression could be up-regulated

after proinflammatory agent treatment, such as TNF α , IL1 β or LPS, which means OLFM4 could be up-regulated in RA proinflammatory environment. It also reported that OLFM4 transcription could be regulated by transcriptional factors, such as PU.1, NF- κ B, by Notch signaling or via retinoic acids treatment [31–34]. More reports show that, in human inflammatory bowel disease, OLFM4 expression is upregulated [35], and interacts with NOD1 and NOD2[29], which comprises ulcerative colitis and Crohn's disease [36, 37]. After knocking down the expression of OLFM4, could lead to an inhibition of proliferation in FLS, suggesting the OLFM4 might also participate in cell proliferation or cell anti-apoptosis. However, the study results from the function of OLFM4 in cell growth or apoptosis do not always coincident. For example, the overexpressed OLFM4 has been shown to facilitate mouse prostate tumor Tramp-C1 cells growth [17], while inhibits human prostate cancer PC-3 cell proliferation[38]. Moreover, up-regulated OLFM4 showed a strong anti-apoptotic activity in mouse lymphoid vein endothelial SVEC cells and human adenocarcinoma HeLa cells[17], whereas recent findings suggested a pro-apoptotic effect of OLFM4 in human myeloid leukemia HL-60 cells[31]. These evidences suggests that roles of OLFM4 in cell growth control and apoptosis may depend on the cell or tissue type.

Another gene, MZB1 was also found to be up-regulated in both the mRNA and protein expression from RA patients. As it reported, MZB1 is an ER-localized protein constitutively expressed in innate-like B cells, such as marginal zone (MZ) B cells and B1 cells, and highly up-regulated during plasma cell differentiation [39, 40] [41]. Meanwhile, the same up-regulation of DERL3 was found in RA synovial tissues from the proteomics data and the mRNA level. And DERL3 is also found as plasma cell or B cell signature genes [42, 43]. All these research results and our results suggested that there might be more B cell in RA synovial tissues than OA synovial tissues. Since the secondary germinal centers always were found in RA synovial tissues[44]. These germinal centers consider to have lots of B cell or plasma cell, which could produce some auto-antibodies.

Conclusion

In this paper, we used iTRAQ-labeled methods and found numerous changed proteins that were mainly in the pathways involved in phagosome, and protein processing in ER, and in the extracellular region and development process and indicated differentially expressed genes, including the first reported OLFM4 function in RA FLS. And the OLFM4 was upregulated in RA FLS, participated the cell proliferation progress. Taken together, we believe that our study could provide the opportunity for developing the new biomarker for diagnosis and treatment RA.

Abbreviations

RA, Rheumatoid arthritis; FLS, Fibroblast-like synoviocytes; OA, osteoarthritis; LC, liquid chromatography; MS, mass spectrometry; iTRAQ, isobaric tags for relative and absolute quantitation; OLFM4, Olfactomedin 4; IHC, Immunohistochemistry; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; ER, endoplasmic reticulum

Declarations

Ethics approval

This study (No.20161203) was approved by the Ethics Committee in Hong Hui Hospital, and the Medical Ethics Committee of Xi'an Jiaotong University.

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Authors' contributions

Ren XY, Xu K, Xu J and Xu P participated in the design of the study, carried out the experiments and statistical analysis, and drafted the manuscript. Ren XY, Xu K, Xu J, Geng MM, Lu C, Kong LB, Cai YS, Hou WK, Lu YF, Aihaiti Y, Wang R and Xu P collected data and blood samples, assisted carrying out the experiments, and provided a critical review of the manuscript. All authors read and approved the final manuscript

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Availability of data and materials

The datasets generated and/or analyzed during the current study are available in the supplementary data. Ethics approval and consent to participate. Written informed consent was obtained from each patient.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

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Tables

Table 1. Characteristic of patients

	RA	OA	<i>p</i> value
Number of patients	22	24	N.S.
Age, year	58.77 ± 1.829	67.63 ± 1.643	0.0008
Female, n (%)	72.7	87.5	N.S.
RF, mg/dl	76.59 ± 11.68	11.53 ± 3.726	< 0.0001
Anti-CCP antibody, U/ml	52.82 ± 13.19	23.61 ± 4.090	0.0336
Anti-CRP antibody, U/ml	33.47 ± 4.663	1.290 ± 0.2708	< 0.0001
ESR	57.95 ± 8.024	16.67 ± 3.280	< 0.0001

Values are expressed as the Mean ± SEM. Student's *t* test were used to compare variables between the two groups, respectively. *p* < 0.05 was considered statistically significant.

N.S, no significant, RF, rheumatoid factor, CCP, cyclic citrullinated peptide, CRP, C reactive protein, ESR, erythrocytesedimentationrate.

Table 2. Ten most upregulated and downregulated protein in RA synovial tissues.

Gene	RA vs OA ratio	adjusted <i>p</i> value
Upregulated protein		
PRTN3	3.01652	0.011597215
OLFM4	2.995775	0.000654509
ELA2	2.949639	0.000105424
S100A9	2.453185	0.001650958
MZB1	2.391361	0.002482689
CRP	2.365525	0.022693489
ISG20	2.352079	0.010584969
S100A8	2.215557	0.00102357
TPD52	2.197087	0.005925084
Downregulated protein		
AKR1C2	0.525669	0.001865633
GSN	0.503626	0.000233851
THBS4	0.490822	0.000561647
HBB	0.481889	0.004844073
MFAP5	0.47068	0.000765846
CHAD	0.465217	0.000170334
TPD52L1	0.444983	0.003863185
COL2A1	0.442412	0.007717312
STC2	0.44122	0.002503789
RLBP1	0.292805	0.029074331

Figures

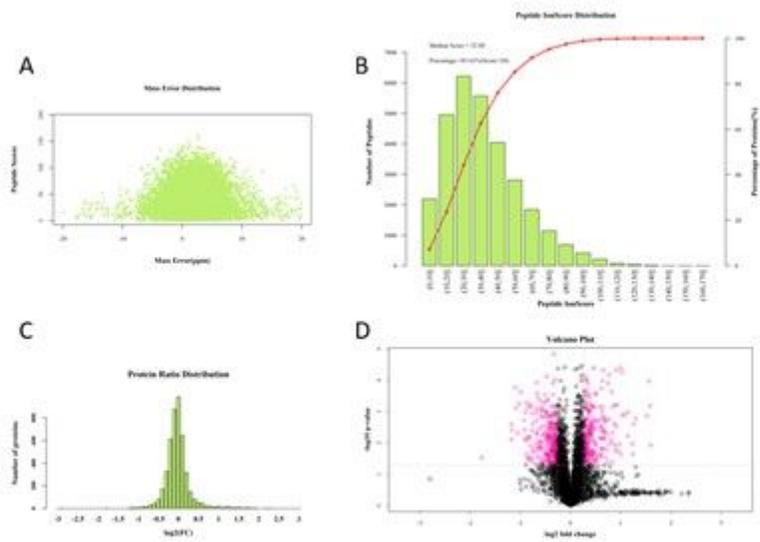


Figure 1

Proteome profiles of RA and OA synovial tissues. A. Peptide ion mass error distribution. B. Peptide ion score distribution. C. Protein Ratio distribution RA vs OA synovial tissue. D. Volcano plot RA vs OA synovial tissue (Red circles represent the significant fold change (>±1.2, and p<0.05) proteins between RA and OA synovial tissue).

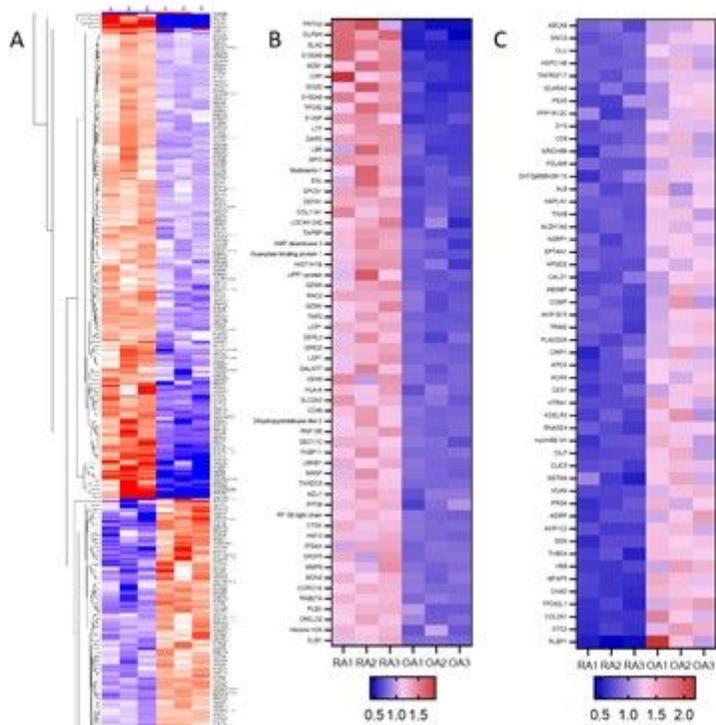


Figure 2

Different protein expression cluster in synovial tissues. Hierarchical cluster dendrogram of samples showed different protein expression in RA and OA synovial tissues. The heat map showed the fold change >1.2 and $p < 0.05$ proteins. (Red is suggesting the up-regulated proteins, while blue means the down-regulated proteins.)

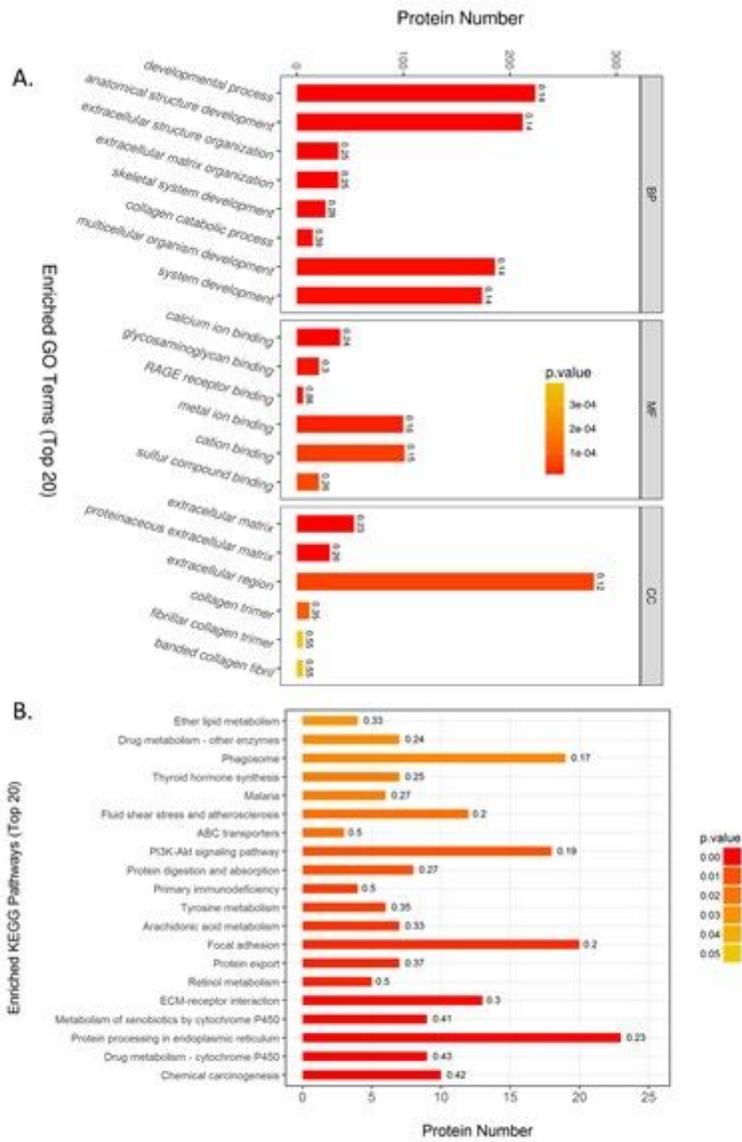


Figure 3

GO term enrichment and KEGG pathway analysis in synovial tissue. A. The top 20 GO term enrichment for different proteins in Biological process (BP), Molecular function (MF) and Cellular component (CC) of OA and RA synovial tissues. B. The top 20 KEGG enriched pathways.

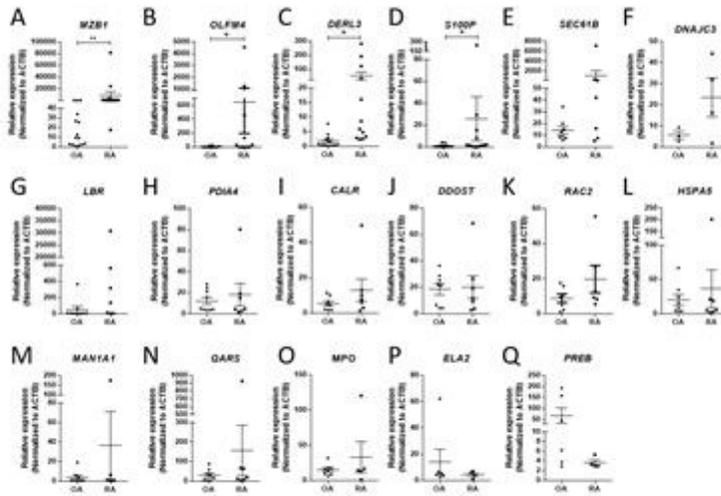


Figure 4

Different gene mRNA expression in synovial tissues from RA and OA patients. The mRNA of different expressed candidate genes were checked in synovial tissues from RA and OA patients. The gene expression of MZB1(OA n=15, RA n=13), OLFM4(OA n=10, RA n=9), DERL3(OA n=12, RA n=12), S100P(OA n=8, RA n=12), SEC61B(OA n=8, RA n=7), DNAJC3(OA n=3, RA n=4), LBR(OA n=9, RA n=7), PDIA4(OA n=9, RA n=7), CALR(OA n=8, RA n=7), DDOST(OA n=8, RA n=7), RAC2(OA n=7, RA n=6), HSPA5(OA n=8, RA n=7), MAN1A1(OA n=8, RA n=5), QARS(OA n=8, RA n=7), MPO(OA n=8, RA n=5), ELA2(OA n=6, RA n=4) and PREB(OA n=6, RA n=4) were checked via RT-qPCR in synovial tissues from RA and OA patients. Data are presented as mean \pm SEM. *p < 0.05

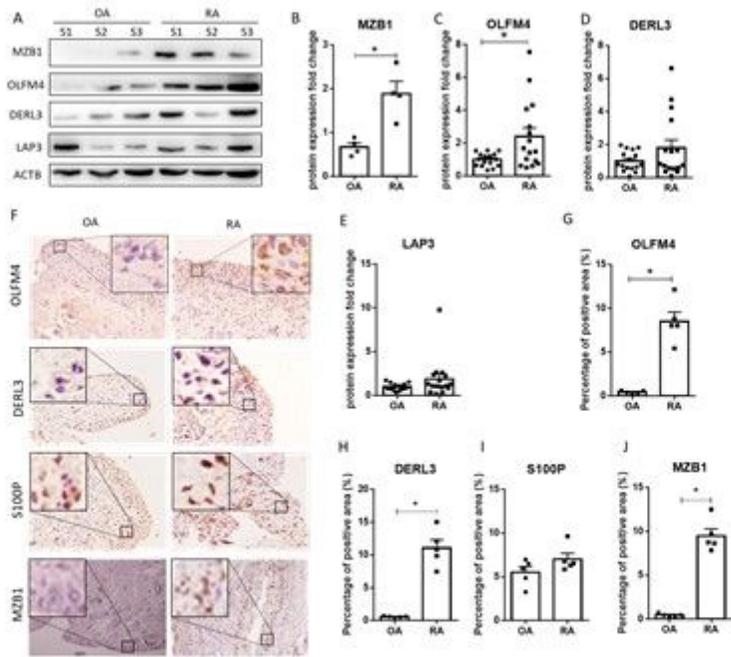


Figure 5

The proteins expression in OA and RA synovial tissues. A. The representative protein expression of MZB1, OLFM4, DERL3 and LAP3 in OA and RA synovial tissues. B-E. The relative fold change of MZB1 (n=4), OLFM4 (n=16), DERL3 (n=16) and LAP3 (n=16) in OA and RA synovial tissues. F. The representative IHC of OLFM4, DERL3, S100P and MZB1 in OA and RA synovial tissues. G-J. The percentage of positive OLFM4, DERL3, S100P and MZB1 expressed area in OA and RA synovial tissues (n=5). Data are presented as mean ± SEM. *p < 0.05

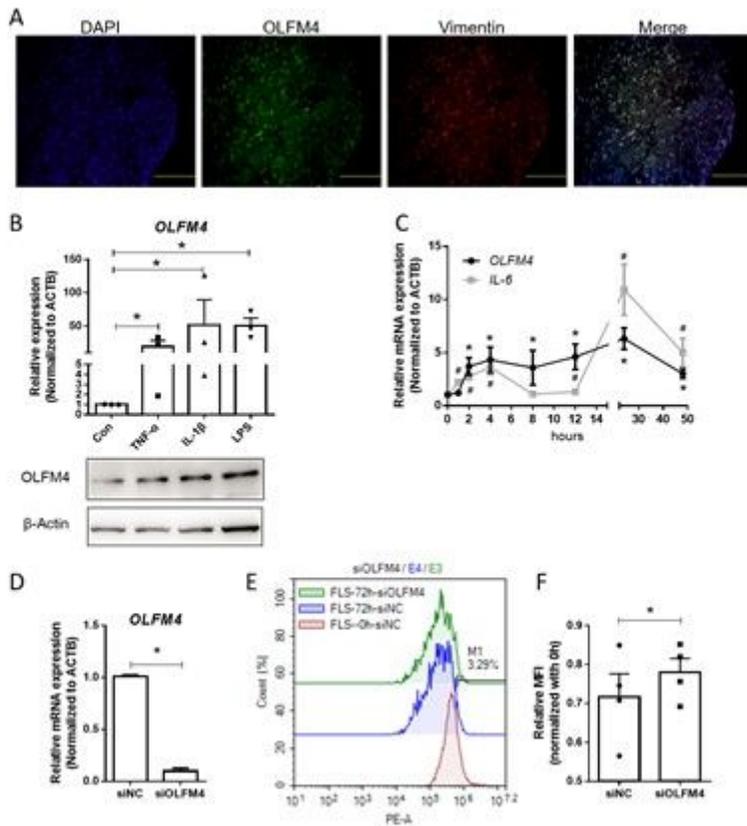


Figure 6

Up-regulated OLFM4 participated in cell proliferation in RA FLS. A. The OLFM4 protein mainly expressed in Vimentin positive cells (Red, represented as Vimentin, Green, represented as OLFM4, and Blue, represented as nucleic). B. The OLFM4 expression in different cytokine and LPS treatment. C. The relative mRNA expression of IL-6 and OLFM4 at different time points after TNF α treatment. D. The efficient of OLFM4 RNAi in RA FLS. E-F. The proliferation of FLS in NC and siOLFM4 FLS (n=4). Data are presented as mean \pm SEM. * $p < 0.05$