Clinical Rheumatology (2019) 38:2629–2635
https://doi.org/10.1007/s10067-019-04533-1

ORIGINAL ARTICLE

CCL5 and related genes might be the potential diagnostic biomarkers for the therapeutic strategies of rheumatoid arthritis

Yinger Huang[1] · Songyuan Zheng[1] · Ran Wang[1] · Cuiping Tang[1] · Junqing Zhu[1,2] · Juan Li[1,2]

Received: 30 October 2018 / Revised: 7 March 2019 / Accepted: 25 March 2019 / Published online: 22 April 2019

Abstract

Objective Rheumatoid arthritis (RA) is a common disease of rheumatic diseases. The aim of this study was to identify gene signatures in RA and uncover their potential mechanisms.

Method Gene expression profiles of GSE1919, GSE55235, GSE55457, and GSE77928 were downloaded from GEO database. The above four series contained 76 samples, including 44 RA patients and 32 normal controls. The gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed, and protein–protein interaction (PPI) network of the differentially expressed genes (DEGs) was constructed by Cytoscape software.

Results Up-regulated DEGs were significantly enriched in biological processes, including immune response, positive regulation of immune system process and regulation of immune system process, while down-regulated DEGs were significantly enriched in biological processes, including response to oxygen-containing compound, cellular lipid metabolic process, and lipid metabolic process. KEGG pathway analysis showed the up-regulated DEGs were enriched in cytokine–cytokine receptor interaction, chemokine signaling pathway, and primary immunodeficiency. The 104 hub genes, which were significantly differently expressed between patients and normal controls in at least two datasets, were identified from the PPI network, and subnetworks revealed that these genes were involved in significant pathways, including cytokine–cytokine receptor interaction, chemokine signaling pathway, and primary immunodeficiency.

Conclusion The present study indicated that the identified DEGs and hub genes promote our understanding of molecular mechanisms underlying the development of RA, such as C-C motif chemokine 5 (CCL5), might have a negative impact in the development of RA. CCL5 and its related genes might be the potential diagnostic biomarkers for the therapeutic strategies of RA.

Keywords Bioinformatics analysis · Microarray · Rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is one of the commonest rheumatic diseases with many cases recorded [1]. RA is considered as a gene-related disease [2–6] with articular disorders. Accumulating evidence also has demonstrated that multiple genes and cellular pathways participate in the occurrence and development of RA [3, 7, 8]. To date, a lack of understanding the precise molecular mechanisms underlying RA progression limits the ability to treat advanced disease. In recent years, gene sequencing technology is now undergoing a revolution, which could be used on tissue or blood samples to identify biomarkers in individual person [9, 10]. Therefore, further knowledge of molecular mechanism involved in genetic expression disorder of RA which is extremely important for the future development of diagnosis and treatment could be learned through this up-to-date technology. Bioinformatics analysis [11] now is a popular approach to analyze the expression changes of gene in the development and progression of RA, comprehensively. In this present study, we downloaded the original data (GSE1919, GSE55235, GSE55457, GSE77298) from the Gene Expression Omnibus (GEO), which is repository leads to the archiving as a hub for microarray data deposit and retrieval. Gene expression profiles of

✉ Juan Li
lj40038@126.com

[1] The Department of Internal Medicine of Traditional Chinese Medicine, College of Traditional Chinese Medicine, Southern Medical University, Guangzhou 510515, Guangdong, China

[2] The Department of Rheumatology, Nanfang Hospital, Southern Medical University, Guangzhou 510515, Guangdong, China

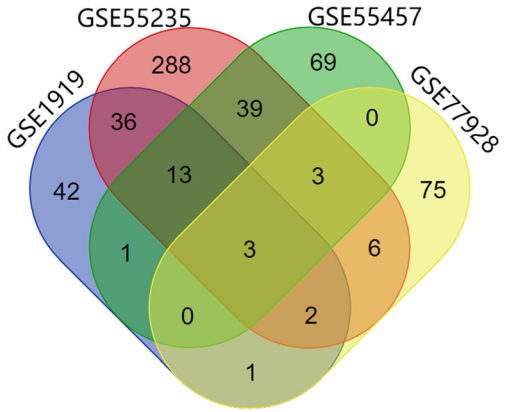

Fig. 1 The distribution of differential genes between GSE1919, GSE55235, GSE55457, and GSE77298

synovial tissue in patients with RA were compared with those in normal controls to identify the differentially expressed genes (DEGs). Subsequently, the selected DEGs were screened by gene ontology (GO) and pathway enrichment analysis. By way of analyzing their biological functions and pathways, we may take a further insight of RA at molecular level and explore the potential diagnostic biomarkers for the therapeutic strategies of RA.

Materials and methods

Data source

In GEO data, we selected synovial tissues from human sources to compare the expression differences of synovial tissues between rheumatoid patients and healthy people.

The gene profiles were downloaded from GEO database (https://www.ncbi.nlm.nih.gov/gds) according to the following keywords: (1) rheumatoid arthritis, (2) *Homo sapiens*, (3) synovial tissue, and (4) absence of drug intervention. Finally, we confirmed these four datasets: GSE1919, GSE55235, GSE55457, and GSE77298, which were based on the platform of GPL91 Affymetrix Human Genome U95A Array, GPL96[HG-U133A] Affymetrix Human Genome U133A Array, GPL96[HG-U133A] Affymetrix Human Genome U133A Array, and GPL570 Affymetrix Human Genome U133 Plus 2.0 Array, respectively. These four datasets were all derived from human synovial membrane, and GSE1919 contained 5 RA patients and 5 normal controls, GSE55235 contained 10 RA patients and 10 normal controls, GSE55457 contained 13 RA patients and 10 normal controls, and GSE77298 contained 16 RA patients and 7 normal controls. A total of 4 datasets including 44 RA samples and 32 normal controls were used for the

Table 1 The enriched GO terms of up-regulated and down-regulated expressed genes

Ontology	ID	Description	adjusted *P* value	Counts
The enriched GO terms of up-regulated genes				
BP	GO:0006955	Immune response	1.24E−22	35
BP	GO:0002684	Positive regulation of immune system process	2.01E−16	25
BP	GO:0002682	Regulation of immune system process	5.13E−16	28
BP	GO:0007166	Cell surface receptor signaling pathway	3.52E−14	34
BP	GO:0046649	Lymphocyte activation	3.77E−14	20
CC	GO:0009897	External side of plasma membrane	4.83E−09	11
CC	GO:0019814	Immunoglobulin complex	1.78E−08	6
CC	GO:0098552	Side of membrane	1.24E−07	12
CC	GO:0042571	Immunoglobulin complex, circulating	8.14E−07	5
CC	GO:0009986	Cell surface	2.45E−05	12
MF	GO:0003823	Antigen binding	1.27E−09	10
MF	GO:0005102	Receptor binding	4.32E−07	19
MF	GO:0008009	Chemokine activity	5.43E−07	6
MF	GO:0034987	Immunoglobulin receptor binding	1.73E−06	5
MF	GO:0042379	Chemokine receptor binding	1.93E−06	6
The enriched GO terms of down-regulated genes				
BP	GO:1901700	Response to oxygen-containing compound	2.60E−04	9
BP	GO:0044255	Cellular lipid metabolic process	3.13E−04	8
BP	GO:0006629	Lipid metabolic process	3.63E−04	9
BP	GO:0032787	Monocarboxylic acid metabolic process	6.38E−04	6
BP	GO:0006631	Fatty acid metabolic process	9.60E−04	5
CC	GO:0070062	Extracellular exosome	0.001755123	14
CC	GO:1903561	Extracellular vesicle	0.001859901	14
CC	GO:0043230	Extracellular organelle	0.001868868	14
CC	GO:0031988	Membrane-bounded vesicle	0.007192761	14
CC	GO:0044421	Extracellular region part	0.019276594	14
MF	GO:0031406	Carboxylic acid binding	0.014891981	3
MF	GO:0004806	Triglyceride lipase activity	0.032440976	2
MF	GO:0043168	Anion binding	0.05612219	3

GO gene ontology, *BP* biological process, *CC* cell component, *MF* molecular function

Table 2 The top five enriched KEGG pathways of up-regulated expressed genes

ID	Description	Adjusted *P* value	Counts
The top 5 enriched KEGG pathway of up-regulated genes			
hsa04060	Cytokine–cytokine receptor interaction	4.14E−06	9
hsa04062	Chemokine signaling pathway	1.22E−04	7
hsa05340	Primary immunodeficiency	4.04E−04	4
hsa04640	Hematopoietic cell lineage	0.005739321	4
hsa05323	Rheumatoid arthritis	0.006321026	4

hsa Homo sapiens

following analysis. All sample profiles of these four datasets were homogenized according to the statistics condition and requirement since all data were from Affymetrix Human Genome Array (a platform where data is preprocessed and normalized using the RMA method). Ethical approval was not necessary in our study because all data is from a public database and do not perform any experiments in patients or animals.

Identification of DEGs

DEGs in RA samples compared with normal controls were screened using *t* test method with the GEO2R online analysis tool (https://www.ncbi.nlm.nih.gov/geo/geo2r). The adjusted *P* value < 0.05 and |logFC| ≥ 2 were set as cutoff criteria. The coexpressed up-regulated and down-regulated DEGs of the two gene expression profiles were identified with a Venn diagram (http://bioinfogp.cnb.csic.es/tools/venny/index.html; Venny 2.1.0).

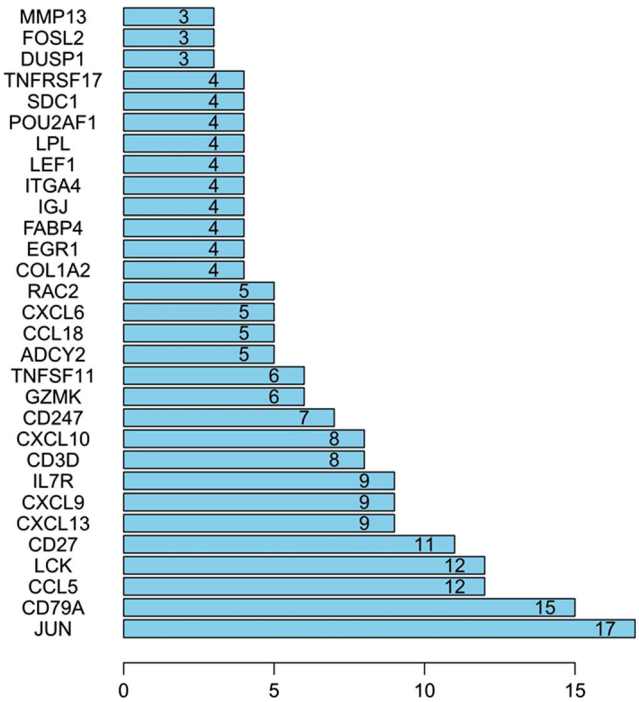

Fig. 2 The top 30 degree hub genes

Gene ontology and pathway enrichment analysis of DEGs

GO analysis (http://www.geneontology.org/) is increasingly applied for functional studies of large-scale genomic or transcriptomic data, which comprises three independent ontologies, including biological process (BP), molecular function (MF), and cellular component (CC). The Kyoto Encyclopedia of Genes and Genomes (KEGG: https://www.kegg.jp/) is a major public database containing the information of biochemistry pathways. The common up-regulated and down-regulated DEGs were analyzed using Database for Annotation, Visualization and Integrated Discovery version 6.7 (DAVID: https://david.ncifcrf.gov/), an online program that provides a comprehensive set of function annotation tools for researchers to understand the biological meaning lists of genes. GO enrichment and KEGG pathway analyses were performed using DAVID. $P < 0.05$ was considered to indicate statistically significant difference.

Integration of PPI network

The functional interactions between proteins can provide context in molecular mechanism of cellular processing. In present study, protein–protein interaction (PPI) network of DEGs was constructed using the Search Tool for the Retrieval of Interacting Genes (STRING, https://string-db.org/) database and subsequently was visualized using Cytoscape. And confidence score ≥ 0.4 was set as the cutoff criterion. Then, the molecular complex detection (MCODE) was performed to screen modules of PPI network with degree cutoff = 2, node score cutoff = 0.2, k-core = 2, and max depth = 100. The functional enrichment analysis of genes in each module was performed by STRING.

Results

Identification of DEGs

Forty-four cases of RA samples were compared with 32 cases of normal controls in GSE1919, GSE55235, GSE55457, and

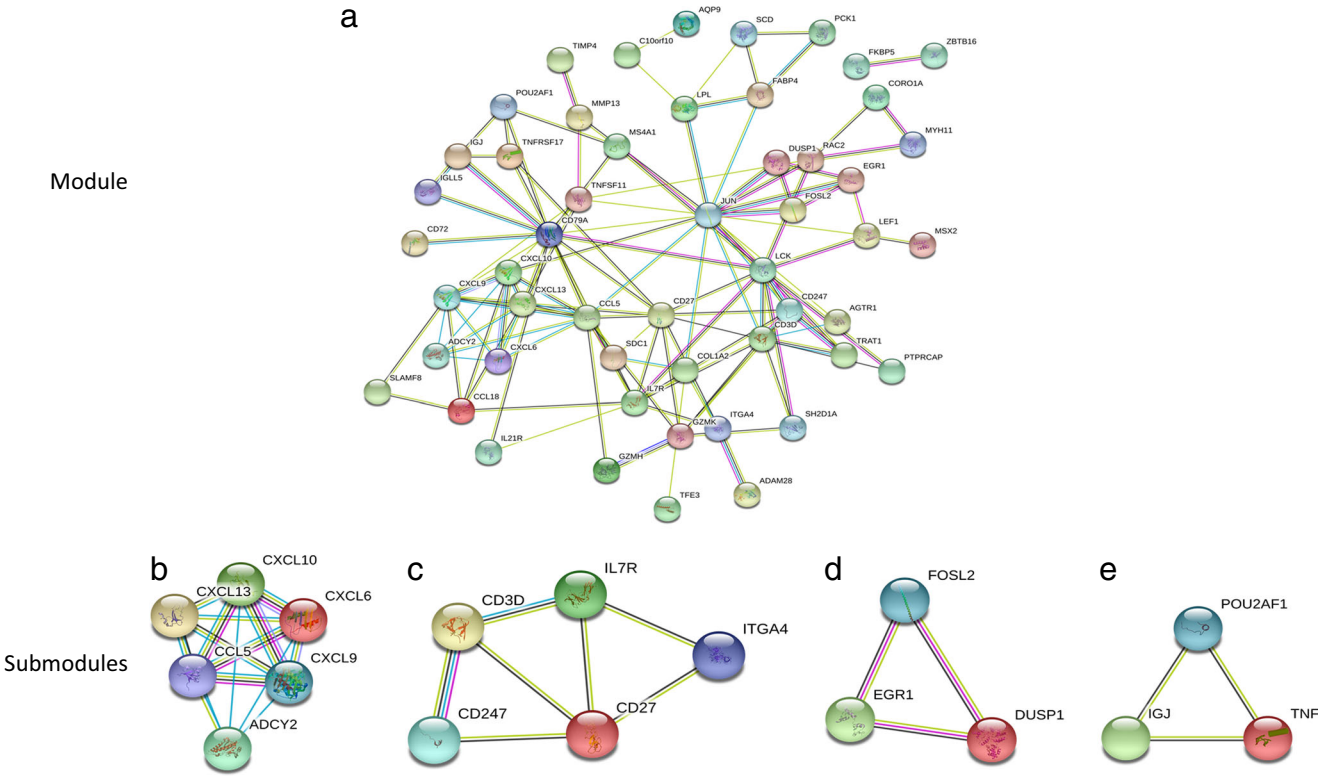

Fig. 3 Module (**a**) and 4 active submodules (**b**–**e**) of PPI network

GSE77298 [12–14] by using the GEO2R online analysis tool. Based on GEO2R analysis, we have used an adjusted $P < 0.05$ and log (fold change) (logFC) ≥ 2.0 criteria. In order to further confirm significant genes of similar tendency in expression in those four profiles, DEGs of the up-regulated and down-regulated gene expression profiles were identified with a Venn diagram, and the results are shown in Fig. 1. In total, there are 104 hub overlap genes (including 77 up-regulated and 27 down-regulated genes) which were significantly differently expressed between patients and normal controls in at least two datasets. Among the hub overlap genes, the top three hub genes were C-X-C motif chemokine 13 (CXCL13), immunoglobulin lambda-like polypeptide 5 (also called IGLC1), and immunoglobulin kappa (IGKC).

GO enrichment analysis

Among the 104 hub overlap genes (including 77 up-regulated and 27 down-regulated genes), we used the DAVID online analysis tool to identify statistically significantly enriched GO terms and KEGG pathways after uploading all up-regulated and down-regulated genes, respectively. GO analysis results showed that up-regulated DEGs were mainly involved regulation of immune system process in BP, external side of plasma membrane in CC, antigen binding in MF, and down-regulated DEGs were mainly involved response to oxygen-containing compound in BP, extracellular exosome in CC, and carboxylic acid binding in MF (Table 1).

KEGG pathway analysis

The significant enriched pathways of the 77 up-regulated DEGs, analyzed by KEGG analysis, are shown in Table 2. Up-regulated genes were enriched in the cytokine–cytokine receptor interaction, chemokine signaling pathway, primary immunodeficiency, hematopoietic cell lineage, and rheumatoid arthritis.

Module analysis and hub gene selection in the PPI network

Based on the information in the STRING database, the highest module was shown by using the MCODE plug-in, and the functional annotation of the genes involved in the module was analyzed. PPI network with DEGs (Fig. 3) and list the top ten degree hub genes: transcription factor AP-1 (JUN), B cell antigen receptor complex-associated protein alpha chain (CD79A), CCL5, tyrosine-protein kinase Lck (LCK), CD27 antigen (CD27), CXCL13, C-X-C motif chemokine 9 (CXCL9), interleukin-7 receptor subunit alpha (IL7R), T cell surface glycoprotein CD3 delta chain (CD3D), and C-X-C motif chemokine 10 (CXCL10) (Fig. 2). Four active

Table 3 Characteristics of module and four submodules of PPI networks

Characteristics	Nodes	Edges	Average node degree	Average local clustering coefficient	PPI enrichment *P* value	Functional enrichments		
						BP	MF	CC
Module	94	221	4.7	0.528	< 1.0E−16			
Submodules								
1	6	15	5	1.000	1.38E−11	Chemokine-mediated signaling pathway	Chemokine activity	Extracellular space
2	5	7	2.8	0.767	4.07E−08	Positive regulation of immune system process	Signaling receptor activity	Alpha-beta T cell receptor complex
3	3	3	2	1.000	2.04E−05	NA	NA	NA
4	3	3	2	1.000	1.37E−08	Humoral immune response	NA	NA

GO gene ontology, *BP* biological process, *CC* cell component, *MF* molecular function, *NA* not available

submodules were required: (1) adenylate cyclase type 2 (ADCY2), CCL5, C-X-C motif chemokine 6 (CXCL6), CXCL9, CXCL10, and CXCL13; (2) CD27 antigen (CD27), integrin alpha-4 (ITGA4), IL7R, CD3D, and T cell surface glycoprotein CD3 zeta chain (CD247); (3) dual specificity protein phosphatase 1 (DUSP1), early growth response protein 1 (EGR1), and Fos-related antigen 2 (FOSL2); and (4) tumor necrosis factor receptor superfamily member 17 (TNFRSF17), immunoglobulin J chain (IGJ), POU domain class 2-associating factor 1 (POU2AF1) (Fig. 3b–e). Characteristics of active modules and submodules of PPI network are detailed in Table 3. Functional enrichment results of the first submodule revealed that the development of RA is associated with chemokine-mediated signaling pathway in biological process, chemokine activity in molecular function, and extracellular space in cellular component. Other submodules are detailed in Table 3.

Discussion

RA is an autoimmune disease which has brought a heavy financial burden to China [15]. According to previous studies, the inherited genetic architecture (that is, genomic DNA) significantly contributes to the etiology of RA [16–18]. Understanding the molecular mechanism of RA is of crucial importance for diagnosis and treatment. It has been widely used to predict therapeutic targets for RA since high-throughput sequencing can provide expression levels of genes in human genome simultaneously [19]. The aim of this study was to identify several hub genes with similar function highly expressed in RA compared to normal controls and uncover their potential mechanisms. In the present study, we extract the gene expression profiles of GSE1919, GSE55235, GSE55457, and GSE77928 downloaded from GEO database and identify 77 up-regulated and 27 down-regulated overlap DEGs between RA and normal control using bioinformatics analysis. Cumulative evidence has demonstrated that coexpression gene normally consists of a group of genes with similar expression profiles, which frequently participate in parallel biological process as well. In order to better understand the interactions of DEGs, we further performed GO and KEGG pathway analysis.

The GO term analysis showed that up-regulated DEGs were mainly involved regulation of immune system process in biological process, external side of plasma membrane in cell component, and antigen binding in molecular function. Since RA is a disease associated with immune dysfunction, genes are always involved in immune system process and antigen binding [20]. Previous studies have shown that immunoregulatory factors, CD28, CTLA-4, and CCL5 and their gene polymorphisms play a crucial role in the process of RA patients [21]. In addition, down-regulated DEGs were mainly involved response to oxygen-containing compound in biological process, extracellular exosome in cell component, and

carboxylic acid binding in molecular function. Recent evidences indicate that extracellular vehicles, including exosomes, are now recognized to play important roles in cell-to-cell communication, which are likely to play a prominent role in the pathophysiology of RA [22–25]. In the near future, genes related to extracellular exosomes might be therapeutic targets for modulation of RA.

Furthermore, the enriched KEGG pathways of up-regulated DEGs included cytokine–cytokine receptor interaction and chemokine signaling pathway. Abnormal levels of cytokines and chemokines often exist in patients with rheumatoid arthritis and other systemic autoimmune diseases; therefore, evaluating cytokine and chemokine profiles can be a useful method to diagnose RA [26]. At the same time, drugs for abnormal expression of cytokines or chemokines associated with RA are being developed.

We also constructed the PPI network with DEGs and list the top ten degree hub genes: JUN, CD79A, CCL5, LCK, CD27, CXCL13, CXCL9, IL7R, CD3D, and CXCL10. The CD79A gene encodes CD79A protein, which is known as B cell antigen receptor complex-associated protein alpha chain. It is reported that synovial infiltration with CD79a-positive B cells correlates with joint destruction in rheumatoid arthritis [27]. LCK, as a tyrosine kinase, is involved in inflammatory disorders. Selective LCK inhibitor might be the potential application in inflammatory disorders including RA [28]. CD27, a member of the tumor necrosis factor receptor superfamily, is a costimulatory immune checkpoint molecule. Current evidence suggests that CD27–CD70 pathway contributes to the pathophysiology of autoimmunity [29]. The use of TNF-inhibitor treatment affects B cell phenotype and IgD-CD27-memory B cells in circulation in RA patients [30]. As to IL7R (interleukin-7 receptor), it is a biomarker with potential applications in RA diagnosis and therapy [31]. The relationship between JUN, CD3D, and RA needs further clarification.

Four active submodules were required: (1) CCL5 CXCL6 CXCL9 CXCL10 CXCL13 ADCY2; (2) CD27 ITGA4 IL7R CD3D CD247; (3) DUSP1 EGR1 FOSL2; (4) TNFRSF17 IGJ POU2AF1. Among these four submodules above, CCL5 group reaches the highest degree. In this module, ADCY2 was a down-regulated gene, while other genes were up-regulated genes. Functional enrichment of this submodule revealed that the development of RA is associated with chemokine-mediated signaling pathway in biological process, chemokine activity in molecular function, and extracellular space in cellular component. Chemokines and chemokine receptors are involved in leukocyte recruitment and angiogenesis underlying the pathogenesis of RA and other inflammatory rheumatic diseases. Numerous chemokines, along with both conventional and atypical cell surface chemokine receptors, are found in inflamed synovia [32]. Previous study reported that strong expression of CCL5 and CXCL9/10 was observed in the RA synovial lining cells at mRNA and protein levels [33]. Yarilina et al. [34] reported that in RA patients, tumor necrosis factor (TNF) initiated a type I interferon β-mediated autocrine loop and that expression of CCL5, CXCL9, and CXCL10 was sustained and amplified by the sequential induction of IFNβ. Also, the levels of chemokines related to Th1 (CXCL9, CXCL10), T follicular helper, and B cells (CXCL13) were significantly higher in RA patients, pointing to the role of these chemokines and immune cells in early RA pathogenesis [35]. All these results suggest that CCL5 and its related genes contribute to the pathogenesis of RA. The relationship between CXCL6, ADCY2, and RA needs more study to clarify.

Nevertheless, there are several limitations to this study. First, the sample size in the current study was limited. Additionally, experiments such as quantitative real-time polymerase chain reaction and Western blot analysis were not performed to determine the expression levels of these hub genes. Therefore, further studies with more experimental validation need to verify our observation.

In summary, the present study reveals that some certain key molecules such as CCL5, CCL6, CCL9, CCL10, CCL13, and ADCY2 as well as their associated pathways may play key roles in the development and progression of RA. Findings in this study provided a new molecular understanding of RA and could pave the road for the discovery of new diagnostic biomarkers in the treatment of RA.

Compliance with ethical standards

Disclosures None.

Human participants and animal rights The article does not contain any studies with human participants or animals performed by any of the authors.

References

1. Siebert S, Lyall DM, Mackay DF, Porter D, McInnes IB, Sattar N, Pell JP (2016) Characteristics of rheumatoid arthritis and its association with major comorbid conditions: cross-sectional study of 502 649 UK Biobank participants. RMD Open 2(1):e267
2. McInnes IB, Schett G (2017) Pathogenetic insights from the treatment of rheumatoid arthritis. Lancet 389(10086):2328–2337
3. McInnes IB, Schett G (2011) The pathogenesis of rheumatoid arthritis. N Engl J Med 365(23):2205–2219
4. Brennan P, Ollier B, Worthington J, Hajeer A, Silman A (1996) Are both genetic and reproductive associations with rheumatoid arthritis linked to prolactin? Lancet 348(9020):106–109
5. Y Okada DW, Trynka G, Raj T, Terao C, Ikari K, Kochi Y, Ohmura K, Suzuki A, Yoshida S, Graham RR, Manoharan A, Ortmann W, Bhangale T, Denny JC, Carroll RJ, Eyler AE, Greenberg JD, Kremer JM, Pappas DA, Jiang L, Yin J, L Ye DFS, Yang J, Xie G, Keystone E, Westra HJ, Esko T, Metspalu A, Zhou X, Gupta N, Mirel D, Stahl EA, Diogo D, Cui J, Liao K, Guo MH, Myouzen K, Kawaguchi T, Coenen MJ, van Riel PL, van de Laar MA, Guchelaar HJ, Huizinga TW, Dieude P, Mariette X, Bridges SJ, Zhernakova A, Toes RE, Tak PP, Miceli-Richard C, Bang SY, Lee HS, Martin J, Gonzalez-Gay MA, Rodriguez-Rodriguez L, Rantapaa-

Dahlqvist S, Arlestig L, Choi HK, Kamatani Y, Galan P, Lathrop M, Eyre S, Bowes J, Barton A, de Vries N, Moreland LW, Criswell LA, Karlson EW, Taniguchi A, Yamada R, Kubo M, Liu JS, Bae SC, Worthington J, Padyukov L, Klareskog L, Gregersen PK, Raychaudhuri S, Stranger BE, De Jager PL, Franke L, Visscher PM, Brown MA, Yamanaka H, Mimori T, A Takahashi HX, Behrens TW, Siminovitch KA, Momohara S, Matsuda F, Yamamoto K, Plenge RM (2014) Genetics of rheumatoid arthritis contributes to biology and drug discovery. Nature 506(7488):376–381

6. Gavrila BI, Ciofu C, Stoica V (2016) Biomarkers in rheumatoid arthritis, what is new? J Med Life 9(2):144–148
7. Harley JB, Chen X, Pujato M, Miller D, Maddox A, Forney C, Magnusen AF, Lynch A, Chetal K, Yukawa M, Barski A, Salomonis N, Kaufman KM, Kottyan LC, Weirauch MT (2018) Transcription factors operate across disease loci, with EBNA2 implicated in autoimmunity. Nat Genet 50(5):699–707
8. Ishigaki K, Kochi Y, Suzuki A, Tsuchida Y, Tsuchiya H, Sumitomo S, Yamaguchi K, Nagafuchi Y, Nakachi S, Kato R, Sakurai K, Shoda H, Ikari K, Taniguchi A, Yamanaka H, Miya F, Tsunoda T, Okada Y, Momozawa Y, Kamatani Y, Yamada R, Kubo M, Fujio K, Yamamoto K (2017) Polygenic burdens on cell-specific pathways underlie the risk of rheumatoid arthritis. Nat Genet 49(7):1120–1125
9. Park PJ (2009) ChIP–seq: advantages and challenges of a maturing technology. Nat Rev Genet 10(10):669–680
10. Zipporah EB, Govarthanan K, Shyamsunder P, Verma RS (2018) Expression profiling of differentially regulated genes in Fanconi anemia. Methods Mol Biol 1783:243–258
11. Sedlazeck FJ, Lee H, Darby CA, Schatz MC (2018) Piercing the dark matter: bioinformatics of long-range sequencing and mapping. Nat Rev Genet 19(6):329–346
12. Woetzel D, Huber R, Kupfer P, Pohlers D, Pfaff M, Driesch D, Haupl T, Koczan D, Stiehl P, Guthke R, Kinne RW (2014) Identification of rheumatoid arthritis and osteoarthritis patients by transcriptome-based rule set generation. Arthritis Res. Ther. 16(2):R84
13. Ungethuem U, Haeupl T, Witt H, Koczan D, Krenn V, Huber H, von Helversen TM, Drungowski M, Seyfert C, Zacher J, Pruss A, Neidel J, Lehrach H, Thiesen HJ, Ruiz P, Blass S (2010) Molecular signatures and new candidates to target the pathogenesis of rheumatoid arthritis. Physiol Genomics 42A(4):267–282
14. Broeren MG, de Vries M, Bennink MB, Arntz OJ, Blom AB, Koenders MI, van Lent PL, van der Kraan PM, van den Berg WB, van de Loo FA (2016) Disease-regulated gene therapy with anti-inflammatory interleukin-10 under the control of the CXCL10 promoter for the treatment of rheumatoid arthritis. Hum Gene Ther 27(3):244–254
15. Jin S, Li M, Fang Y, Li Q, Liu J, Duan X, Liu Y, R W, Shi X, Wang Y, Jiang Z, Wang Y, C Y, Wang Q, Tian X, Zhao Y, Zeng X (2017) Chinese Registry of rheumatoid arthritis (CREDIT): II. Prevalence and risk factors of major comorbidities in Chinese patients with rheumatoid arthritis. Arthritis Res. Ther. 19(1):251
16. Shafia S, Dilafroze FAS, Rasool R, Javeed S, Shah ZA (2014) Rheumatoid arthritis and genetic variations in cytokine genes: a population-based study in Kashmir Valley. Immunol Investig 43(4): 349–359
17. Glant TT, Mikecz K, Rauch TA (2014) Epigenetics in the pathogenesis of rheumatoid arthritis. BMC Med 12:35
18. Klein K, Gay S (2015) Epigenetics in rheumatoid arthritis. Curr Opin Rheumatol 27(1):76–82
19. Chen YJ, Chang WA, Hsu YL, Chen CH, Kuo PL (2017) Deduction of novel Genes potentially involved in osteoblasts of rheumatoid arthritis using next-generation sequencing and bioinformatic approaches. Int J Mol Sci 18(11):pii:E2396
20. Rombouts Y, Willemze A, van Beers JJ, Shi J, Kerkman PF, van Toorn L, Janssen GM, Zaldumbide A, Hoeben RC, Pruijn GJ, Deelder AM, Wolbink G, Rispens T, van Veelen PA, Huizinga TW, Wuhrer M, Trouw LA, Scherer HU, Toes RE (2016) Extensive glycosylation of ACPA-IgG variable domains modulates binding to citrullinated antigens in rheumatoid arthritis. Ann Rheum Dis 75(3):578–585
21. Luterek-Puszynska K, Malinowski D, Paradowska-Gorycka A, Safranow K, Pawlik A (2017) CD28, CTLA-4 and CCL5 gene polymorphisms in patients with rheumatoid arthritis. Clin Rheumatol 36(5):1129–1135
22. Withrow J, Murphy C, Liu Y, Hunter M, Fulzele S, Hamrick MW (2016) Extracellular vesicles in the pathogenesis of rheumatoid arthritis and osteoarthritis. Arthritis Res. Ther. 18(1):286
23. Cosenza S, Ruiz M, Maumus M, Jorgensen C, Noel D (2017) Pathogenic or therapeutic extracellular vesicles in rheumatic diseases: role of mesenchymal stem cell-derived vesicles.30. B-cell phenotype and IgD-CD27- memory B cells are affected by TNF-inhibitors and tocilizumab treatment in rheumatoid arthritis. Int J Mol Sci 18(4)
24. Song J, Kim D, Han J, Kim Y, Lee M, Jin EJ (2015) PBMC and exosome-derived Hotair is a critical regulator and potent marker for rheumatoid arthritis. Clin Exp Med 15(1):121–126
25. Yoo J, Lee SK, Lim M, Sheen D, Choi EH, Kim SA (2017) Exosomal amyloid A and lymphatic vessel endothelial hyaluronic acid receptor-1 proteins are associated with disease activity in rheumatoid arthritis. Arthritis Res Ther 19(1):119
26. Wallace DJ, Gavin IM, Karpenko O, Barkhordar F, Gillis BS (2015) Cytokine and chemokine profiles in fibromyalgia, rheumatoid arthritis and systemic lupus erythematosus: a potentially useful tool in differential diagnosis. Rheumatol Int 35(6):991–996
27. Mo YQ, Dai L, Zheng DH, Zhu LJ, Wei XN, Pessler F, Shen J, Zhang BY (2011) Synovial infiltration with CD79a-positive B cells, but not other B cell lineage markers, correlates with joint destruction in rheumatoid arthritis. J Rheumatol 38(11):2301–2308
28. Farag AK, Elkamhawy A, Londhe AM, Lee KT, Pae AN, Roh EJ (2017) Novel LCK/FMS inhibitors based on phenoxypyrimidine scaffold as potential treatment for inflammatory disorders. Eur J Med Chem 141:657–675
29. Han BK, Olsen NJ, Bottaro A (2016) The CD27–CD70 pathway and pathogenesis of autoimmune disease. Semin Arthritis Rheum 45(4):496–501
30. Moura RA, Quaresma C, Vieira AR, Goncalves MJ, Polido-Pereira J, Romao VC, Martins N, Canhao H, Fonseca JE (2017) PLoS One 12(9):e182927
31. Burtea C, Laurent S, Sanli T, Fanfone D, Devalckeneer A, Sauvage S, Beckers MC, Rorive S, Salmon I, Vander EL, Lauwerys BR, Muller RN (2016) Screening for peptides targeted to IL-7Rα for molecular imaging of rheumatoid arthritis synovium. Arthritis Res. Ther. 18(1):230
32. Szekanecz Z, Koch AE (2016) Successes and failures of chemokine-pathway targeting in rheumatoid arthritis. Nat Rev Rheumatol 12(1):5–13
33. Yoshida S, Arakawa F, Higuchi F, Ishibashi Y, Goto M, Sugita Y, Nomura Y, Niino D, Shimizu K, Aoki R, Hashikawa K, Kimura Y, Yasuda K, Tashiro K, Kuhara S, Nagata K, Ohshima K (2012) Gene expression analysis of rheumatoid arthritis synovial lining regions by cDNA microarray combined with laser microdissection: up-regulation of inflammation-associated STAT1, IRF1, CXCL9, CXCL10, and CCL5. Scand J Rheumatol 41(3):170–179
34. Yarilina A, Park-Min KH, T Antoniv XH, Ivashkiv LB (2008) TNF activates an IRF1-dependent autocrine loop leading to sustained expression of chemokines and STAT1-dependent type I interferon–response genes. Nat Immunol 9(4):378–387
35. Pandya JM, Lundell AC, Andersson K, Nordstrom I, Theander E, Rudin A (2017) Blood chemokine profile in untreated early rheumatoid arthritis: CXCL10 as a disease activity marker. Arthritis Res Ther 19(1):20