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Identification of key genes associated with rheumatoid arthritis with bioinformatics approach

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Abstract

We aimed to identify key genes associated with rheumatoid arthritis (RA).

The microarray datasets of GSE1919, GSE12021, and GSE21959 (35 RA samples and 32 normal controls) were downloaded from the Gene Expression Omnibus database. Differentially expressed genes (DEGs) in RA samples were identified using the *t* test in limma package. Functional enrichment analysis was performed using clusterProfiler package. A protein–protein interaction (PPI) network of selected DEGs was constructed based on the Human Protein Reference Database. Active modules were explored using the jActiveModules plug-in in the Cytoscape Network Modeling package.

In total, 537 DEGs in RA samples were identified, including 241 upregulated and 296 downregulated genes. A total of 24,451 PPI pairs were collected, and 5 active modules were screened. Furthermore, 19 submodules were acquired from the 5 active modules. Discs large homolog 1 (*DLG1*) and related DEGs such as guanylate cyclase 1, soluble, alpha 2 (*GUCY1A2*), N-methyl d-aspartate receptor 2A subunit (*GRIN2A*), and potassium voltage-gated channel member 1 (*KCNA1*) were identified in 8 submodules. Plasminogen (*PLG*) and related DEGs such as chemokine (C-X-C motif) ligand 2 (*CXCL2*), laminin, alpha 3 (*LAMA3*), complement component 7 (*C7*), and coagulation factor X (*F10*) were identified in 4 submodules.

Our results indicate that *DLG1*, *GUCY1A2*, *GRIN2A*, *KCNA1*, *PLG*, *CXCL2*, *LAMA3*, *C7*, and *F10* may play key roles in the progression of RA and may serve as putative therapeutic targets for treating RA.

Abbreviations: *ABL1* = c-abl oncogene 1, non-receptor tyrosine kinase, *APP* = amyloid beta (A4) precursor protein, *AR* = androgen receptor, BP = biological process, *C7* = complement component 7, *CAV1* = caveolin 1, caveolae protein, 22kDa, CC = cellular component, *CREBBP* = CREB-binding protein, *CXCL2* = chemokine (C-X-C motif) ligand 2, DEGs = differentially expressed genes, *DLG1* = discs large homolog 1, *DLG1* = discs large homolog 1, *DLG3* = discs large homolog 3, *EGF* = epidermal growth factor, *EGFR* = epidermal growth factor receptor, *ERBB2* = v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2, *ERBB4* = v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 4, *F10* = coagulation factor X, FC = fold change, GO = Gene Ontology, *GRB7* = growth factor receptor-bound protein 7, *GRIN2A* = N-methyl D-aspartate receptor 2A subunit, *GUCY1A2* = guanylate cyclase 1, soluble, alpha 2, HRPD = Human Protein Reference Database, IL-1α = interleukin-1α, *KCNA1* = potassium voltage-gated channel member 1, KEGG = Kyoto Encyclopedia of Genes and Genomes, *KLK2* = kallikrein-related peptidase 2, KNN = nearest neighbor averaging, *LAMA3* = laminin, alpha 3, MF = molecular function, *PLG* = Plasminogen, *PLG* = plasminogenl, PPI = protein–protein interaction, *PRKCA* = protein kinase C, alpha, *PTEN* = phosphatase and tensin homolog, RA = rheumatoid arthritis, *SRC* = v-src avian sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog, TCR = T cell receptor, TNFs = tumor necrosis factors.

Keywords: active modules, differentially expressed genes, microarray datasets, protein pairs, rheumatoid arthritis

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1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disorder that mainly affects the synovial membrane, cartilage, and bone.[1] It affects 1% of the population and is related to significant morbidity and increased mortality.[2] Because RA is incurable, it causes high economic burdens that severely reduce the quality of life.[3] The cause of RA is still unknown.[4] Reliable predictive biomarkers for RA prognosis and therapeutic response are limited.[4] Therefore, elucidation of molecular mechanisms underlying RA development would help in conceiving more effective therapeutic strategies than before for treating the disease.

RA is characterized by chronic inflammation, synovial hyperplasia, cartilage and bone destruction, and formation of pannus.[4,5] It involves a complex interplay among genotype, environmental triggers, and chance.[4] Numerous studies have confirmed that many activated cell types such as T and B cells, monocytes/macrophages, endothelial cells, and synovial fibroblasts play a role in the development and progression of RA.[6–8] Moreover, genome-wide analyses have verified that immune regulatory factors underlie the disease.[9] Cytokines such as interleukin (IL)-1α, IL-8, and tumor necrosis factors (TNFs) have

been directly implicated in a broad range of immune and inflammatory processes, thereby being responsible for joint damage and RA progression.[2] Extended analyses of gene expression profiles have shown that several gene groups such as activator protein-1, nuclear factor kappa B, and Smad family members show binding activity at their cognate recognition sites in the promoters of cytokines, and are, therefore, key players in RA development and progression.[10–12] Consequently, blocking TNFs and IL-1α has proved successful in treating RA.[13,14] In addition, increased levels of sphingosine kinase 1 in the synovium membrane constitute RA biomarkers.[15] However, identifying newer molecular targets remains necessary despite such advances in treating RA to foster the development of new therapeutics with improved outcomes and accuracy of diagnosis.

Previously, datasets GSE1919 and GSE12021 were used to decode differential biomarkers of RA and osteoarthritis for disease-specific therapeutics using statistical analysis.[15] These datasets were also used to identify a panel of potential molecular targets for diagnosis and treatment of RA.[16] The microarray dataset GSE21959 was used to show that gene expression is considerably altered in RA synovial fibroblasts compared with healthy synovial fibroblasts after hypoxia induction and to identify new factors and pathways relevant to the pathogenesis of chronic arthritis.[5] In comparison with these, we integrated the above 3 gene expression datasets in the present study and used comprehensive bioinformatics methods to screen differentially expressed genes (DEGs) associated with RA. Functional enrichment analysis, protein–protein interaction (PPI) network construction, and active module mining were then performed to identify key genes involved in the progression of RA. Our findings will help in the discovery of potential targets for therapeutic intervention in patients with RA.

2. Methods

2.1. Data resources

Data on the expression profile of GSE1919,[17] GSE12021,[1] and GSE21959[5] related to RA were acquired from the Gene Expression Omnibus repository by the National Center of Biotechnology Information based on the platform of GPL91 Affymetrix Human Genome U95A Array, GPL96 Affymetrix Human Genome U133A/B Array, and GPL4133 Agilent-014850 Whole Human Genome Microarray 4×44K G4112F (Feature Number version), respectively. GSE1919 datasets contained synovial membrane samples derived from 5 patients with RA and 5 normal controls; GSE12021 contained 9 RA samples and 12 normal controls; and GSE21959 contained 18 RA samples and 18 normal controls. Therefore, 67 datasets of synovial fibroblasts in total (35 RA samples and 32 normal controls) were used for subsequently analyses. Ethical approval was not necessary in our study because we downloaded the expression profiles from the public database and do not perform any experiments in patients or animals.

2.2. Data preprocessing

We downloaded the series matrix files of the 3 datasets, and the probe name of each series matrix was transformed into a gene symbol based on the Affy probe annotation files. If multiple probes corresponded to the same gene symbol, the mean value was calculated using the aggregate function[18] in R as the expression value of that particular gene. If the expression value of the probe was absent, the nearest neighbor averaging (KNN)[19] of the impute package[20] in R was used to supplement. Finally, quartile normalization was performed using the preprocessCore[21] in R, and the gene expression matrix of each sample was acquired.

All expression estimates were $\log_2$ transformed and merged using cross-platform normalization, which was performed using the CONOR[22,23] package in R. If different studies comprised similar or common gene symbols, 2 expression data of the same gene symbols were normalized for producing a new dataset. Then, the newly produced data were renormalized with the next data.

2.3. Identification of DEGs

DEGs in RA samples compared with normal controls were screened using the *t* test[24] method in the limma[24] package in R. Then, the *P* value was adjusted using the Benjamini and Hochberg method.[25] The $|\log_2$ fold change (FC)$| > 0.585$ and an adjusted *P* value of <0.05 were set as the threshold.

2.4. Functional enrichment analysis of DEGs

Gene ontology (GO) analysis is increasingly applied for functional studies of large-scale genomic or transcriptomic data,[26] which comprises 3 independent ontologies, including biological process (BP), molecular function (MF), and cellular component (CC). The Kyoto Encyclopedia of Genes and Genomes (KEGG) is the major public database containing the information of biochemistry pathways.[27]

We used the clusterProfiler[28] package in R to annotate and visualize GO terms (associated with BP, MF, and CC) and KEGG pathway of upregulated and downregulated genes, respectively.

2.5. Construction of the PPnetwork and screening of active modules

Human Protein Reference Database (HPRD)[29] is a database containing curated proteomic information pertaining to human proteins. The human PPI data on HPRD (Release 9) consists of 39,240 interactions among 9617 genes. We used HPRD (Release 9) to identify PPI pairs of DEGs and construct the PPI network.

Active module hypothesis was proposed by Ideker et al[30] for clarifying underlying mechanisms governing observed changes in gene expression, which integrated expression profiling, large-scale proteomics, and PPI network construction along with using a rigorous statistical measure for identifying active modules with a search algorithm. Therefore, active modules were screened within the PPI network considering the active module hypothesis.

We also used the jActiveModules plug-in[31] available for the Cytoscape Network Modeling package[31] to identify active modules. At each step of the search, all adjacent proteins are considered for inclusion in the resultant network. Active modules were identified with a local search of "depth" = 2 and "max depth" = 2. Then, the connectivity degree of each node in modules was estimated to identify the hub nodes.

If many nodes were present in active modules, smaller submodules needed to be acquired. To produce smaller submodules, we repeated the active modules search within each original submodule with a local search of "depth" = 1 and "max depth" = 1 to identify singleton nodes with a significant number of neighbors.

Table 1

The enriched GO terms of differentially expressed genes.

Ontology	ID	Description	*P* adjust	Counts
The enriched GO terms of upregulated genes				
BP	GO:0006955	Immune response	1.09E-30	74
BP	GO:0050896	Response to stimulus	8.16E-30	159
BP	GO:0002376	Immune system process	6.51E-29	88
BP	GO:0045321	Leukocyte activation	8.68E-24	46
BP	GO:0001775	cell activation	1.39E-22	51
CC	GO:0044459	Plasma membrane part	1.42E-18	73
CC	GO:0005886	Plasma membrane	2.31E-16	109
CC	GO:0071944	cell periphery	2.31E-16	110
CC	GO:0005576	Extracellular region	9.32E-15	70
CC	GO:0005887	Integral to plasma membrane	3.60E-13	49
MF	GO:0003674	Molecular function	7.93E-13	195
MF	GO:0008009	Chemokine activity	3.05E-09	10
MF	GO:0005125	Cytokine activity	3.83E-09	17
MF	GO:0042379	Chemokine receptor binding	7.34E-09	10
MF	GO:0004872	Receptor activity	7.34E-09	45
The enriched GO terms of downregulated genes				
BP	GO:0032501	Multicellular organismal process	3.17E-27	174
BP	GO:0044699	Single-organism process	5.86E-27	238
BP	GO:0044707	Single-multicellular organism process	4.81E-26	168
BP	GO:0008150	Biological process	1.01E-20	260
BP	GO:0050896	Response to stimulus	5.32E-19	173
CC	GO:0044421	Extracellular region part	2.82E-11	52
CC	GO:0044459	plasma Membrane part	1.69E-09	67
CC	GO:0005576	extracellular region	2.02E-09	71
CC	G:0005615	Extracellular space	2.02E-09	40
CC	GO:0005886	Plasma membrane	8.15E-09	111
MF	GO:0003674	Molecular function	1.34E-17	262
MF	GO:0005488	Binding	2.04E-08	218
MF	GO:0005515	Protein binding	2.87E-08	158
MF	GO:0005102	Receptor binding	3.24E-08	46
MF	GO:0070851	Growth factor receptor binding	5.29E-05	10

Ontology represents the category of GO terms; Description represents the name of GO term; Counts represent the number of genes enriched in GO term. BP = biological process, CC = cellular component, GO = gene ontology, ID = identification number, MF = represents molecular function.

3. Results

3.1. Data preprocessing and identification of DEGs

Based on the merged information, 57 samples corresponding to 8453 gene expression levels were acquired. After preprocessing, data were successfully normalized and could be used for further study.

Using the limma package with $|\log_2 FC| > 0.585$ and an adjusted *P* value of <0.05 set as a threshold, we ultimately obtained 537 DEGs in RA samples compared with normal samples, including 241 upregulated and 296 downregulated genes.

3.2. Functional enrichment analysis of DEGs

GO terms and KEGG pathways were considerably enriched by upregulated and downregulated DEGs, respectively. Upregulated genes were significantly associated with immune response, response to stimulus, and immune system process (Table 1A). Downregulated genes were significantly associated with multicellular organism process, single-organism process, single-multicellular organism process (Table 1B). In addition, upregulated genes were significantly enriched in cell adhesion molecules, primary immunodeficiency, hematopoietic cell lineage, cytokine–cytokine receptor interaction, and *Staphylococcus aureus* infections (Table 2A). Downregulated genes were mainly

Table 2

The top 5 enriched KEGG pathways of differentially expressed genes.

ID	Description	*P* adjust	Counts
The top 5 enriched KEGG pathways of upregulated genes			
hsa04514	Cell adhesion molecules	7.72E-10	18
hsa05340	Primary immunodeficiency	4.43E-09	10
hsa04640	Hematopoietic cell lineage	4.25E-08	13
hsa04060	Cytokine-cytokine receptor interaction	3.36E-07	20
hsa05150	*Staphylococcus aureus* infection	3.23E-05	8
B: The top 5 enriched KEGG pathways of downregulated genes			
hsa00350	Tyrosine metabolism	6.01E-04	7
hsa05200	Pathways in cancer	3.70E-03	18
hsa04910	Insulin signaling pathway	6.97E-03	10
hsa04060	Cytokine-cytokine receptor interaction	9.94E-03	14
hsa05146	Amoebiasis	9.94E-03	8

ID = identification number of KEGG pathway. Description represents the name of KEGG pathway; counts represent the number of genes enriched in KEGG pathway.

enriched in tyrosine metabolism, pathways in cancer, insulin signaling pathway, cytokine–cytokine receptor interaction, and amoebiasis cancer (Table 2B).

3.3. PPnetwork construction and active module screening

Based on the information on HPRD, 24,451 PPI pairs corresponding to 8453 genes were acquired by mapping into HPRD-. Then, 5 active modules and 19 submodules were screened (Fig. 1). In active module (A), the hub genes with a higher degree were androgen receptor (*AR*), v-src avian sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (*SRC*), and CREB-binding protein (*CREBBP*); in active module (B), they were *SRC*, caveolin 1, caveolae protein, 22kDa (*CAV1*), and amyloid beta (A4) precursor protein (*APP*); in active module (C), they were c-abl oncogene 1, nonreceptor tyrosine kinase (*ABL1*),

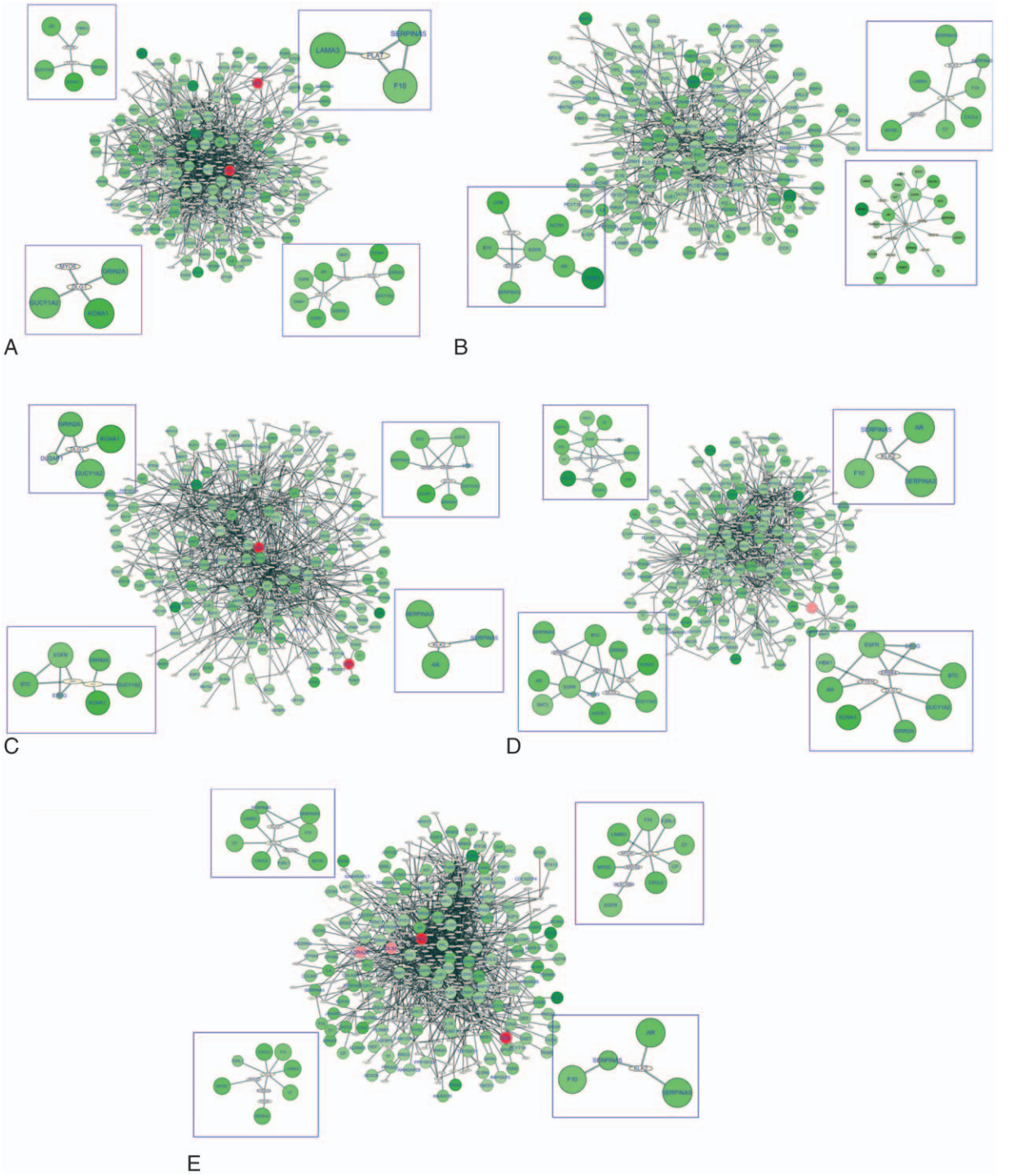

Figure 1. Five active modules and 19 submodules. The yellow nodes indicate normally expressed genes, red nodes indicate upregulated genes, and green nodes indicate downregulated genes. Color shades of nodes are proportional to the | $\log_2$ fold change (FC) |, and node size is inversely proportional to and adjusted *P* value. The edges between nodes indicate interaction between these genes.

CREBBP, and *SRC*; and in active module (D), they were *AR*, *SRC*, and epidermal growth factor receptor (*EGFR*). The hub genes in active module (E) were protein kinase C, alpha (*PRKCA*), *SRC*, and *EGFR*. Our results showed that most of DEGs present in active modules were downregulated genes rather than upregulated genes.

The connectivity degrees of many hub nodes of submodules were not <3, and they were not DEGs. These hub nodes of submodules were as follows: plasminogen (*PLG*), phosphatase and tensin homolog (*PTEN*), discs large homolog 1 (*DLG1*), v-erb-b2 avian erythroblastic leukemia viral oncogene homolog *4* (*ERBB4*), growth factor receptor-bound protein 7 (*GRB7*), v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2 (*ERBB2*), epidermal growth factor (*EGF*), discs large homolog 3 (*DLG3*), kallikrein-related peptidase 2 (*KLK2*), and *SRC*.

DLG1 and related DEGs such as guanylate cyclase 1 soluble alpha 2 (*GUCY1A2*), N-methyl D-aspartate receptor 2A subunit (*GRIN2A*), and potassium voltage-gated channel member 1 (*KCNA1*) were present in 8 submodules. *PLG* and associated DEGs such as chemokine (C-X-C motif) ligand 2 (*CXCL2*), laminin, alpha 3 (*LAMA3*), complement component 7 (*C7*), and coagulation factor X (*F10*) were present in 4 submodules.

4. Discussion and conclusions

We used a bioinformatics approach to identify key genes associated with RA progression. In total, 537 DEGs in RA samples were identified, including 241 upregulated and 296 downregulated genes. Five active modules and 19 submodules were also acquired. *DLG1* and related DEGs such as *GUCY1A2*, *GRIN2A*, and *KCNA1*, were present in 8 submodules. *PLG* and related DEGs such as *CXCL2*, *LAMA3*, *C7*, and *F10* were present in 4 submodules. Although *DLG1* and *PLG* were not DEGs, they were strongly associated with DEGs involved in RA progression.

DLG1 encodes a multidomain scaffolding protein that is required for T cell receptor (TCR)-induced activation of regulatory T cell function.[32] A previous study showed that T cells play an important role in the development of RA.[15] Factors involved in T cell activation, such as CD28 and T cell activation RhoGTPase-activating protein, are strongly linked to RA and, therefore, highlight the role of T cells in RA.[4] DLG1 is involved in the generation of memory T cells and regulation of T cell proliferation, migration, and Ag-receptor signaling.[33] Moreover, DLG1 functions as a negative regulator of TCR-induced proliferative responses, thereby decreasing the proliferation of T lymphocytes.[34] Thus, our results are consistent with those of previous studies and suggest that the downregulated *DLG1* may enhance the function of regulatory T cells, thereby exacerbating RA.

We also found that downregulated DEGs such as *GUCY1A2*, *GRIN2A*, and *KCNA* directly interacted with *DGL1* in several submodules. *GUCY1A2* is involved in EDNRB signaling, which may be a part of RA development.[35]*GRIN2A* (also known as *NMDAR2A*) helps increase the expression of proinflammatory molecules such as IL-1b and TNF-α following N-methyl D-aspartate-induced excitotoxicity in the postnatal brain.[36] These cytokines have been confirmed to play important roles in the pathogenesis of RA.[2] Furthermore, the expression of *GUCY1A2* and *GRIN2A* is downregulated in RA synovial fibroblasts compared with that in healthy synovial fibroblasts, specifically in hypoxia.[5]*KCNA1* is a member of potassium voltage-gated channel proteins, which regulate the membrane potential of T cells and thereby control the calcium signaling response necessary for lymphocyte activation.[37] The function of T cells is strongly associated with RA[15] and calcium signaling, which are few of the signaling pathways disrupted in RA.[38] Therefore, *GUCY1A2*, *GRIN2A*, and *KCNA1* may also be key molecules involved in the pathogenesis of RA. Our results also suggest that *DLG1* may be indirectly involved in RA progression owing to its interaction with the above 3 DEGs.

PLG was also found to be a crucial protein in several submodules. PLG induces the production of IL-1β and TNF-α in response to reactive oxygen species,[39] which are also important molecules in the development of RA. Moreover, plasmin is formed upon the cleavage of PLG by specific PLG activators, and further induces the expression of inflammatory cytokines.[40] Enhanced urokinase plasminogen activator activity is a key component of both the inflammatory and tissue remodeling processes occurring in the joints of patients with RA.[41] In other words, PLG has been shown to have both a positive and a negative influence with respect to RA, depending on joint location, in mice.[42] Therefore, downregulated *PLG* may be a joint-specific determinant playing a role in the progression of RA.

CXCL2, *LAMA3*, *C7*, and *F10* directly interacted with *PLG*. CXCL2 is considered a proinflammatory and matrix-destructive factor in RA.[43] CXCR2, the receptor of CXCL2, inhibits acute IL-8/CXCL8- or LPS-induced arthritis in rabbits.[44]*LAMA3*, which encodes an angiogenic protein, is induced by hypoxia in macrophages and effectively regulates angiogenesis.[45] Synovial hyperplasia in RA is accompanied by active angiogenesis and expansion of blood vessels,[5] highlighting the role of LAMA3 in RA progression. C7 is a key component of the complement system, which contributes to the pathogenesis of several autoimmune and inflammatory conditions, including RA.[46] C7 deficiency is strongly associated with the occurrence of RA.[47] F10, one of the blood coagulation factors, is associated with the activation of the coagulation cascade that widely occurs in RA, and reduced levels of coagulation factors have been found in the synovial fluids of patients with RA.[41] These DEGs are involved in different mechanisms underlying the pathogenesis of RA, and we speculate that they contribute to RA progression, albeit by playing different roles. Although current evidence on the direct association between *PLG* and DEGs is limited, we believe that PLG may be involved in the pathogenesis of RA via interacting with these DEGs based on our present findings.

Nevertheless, there was no experimental validation, such as quantitative real-time PCR and western blot analysis to determine the expression levels of these key genes we identified. Moreover, the significant role of these candidate genes in the development of RA was not investigated. More studies with high throughput data and experiment validation are still needed to verify our observation and speculation.

To summarize, *DLG1*, *GUCY1A2*, *GRIN2A*, *KCNA1*, *PLG*, *CXCL2*, *LAMA3*, *C7*, and *F10* may play a key role in the progression and development of RA. These genes may be promising as therapeutic targets for treating RA.

References

[1] Huber R, Hummert C, Gausmann U, et al. Identification of intra-group, inter-individual, and gene-specific variances in mRNA expression profiles in the rheumatoid arthritis synovial membrane. Arthritis Res Ther 2008;10:R98 (1–16).

[2] McInnes IB, Schett G. Cytokines in the pathogenesis of rheumatoid arthritis. Nat Rev Immunol 2007;7:429–42.

[3] Wong JB, Ramey DR, Singh G. Long-term morbidity, mortality, and economics of rheumatoid arthritis. Arthritis Rheum 2001;44:2746–9.
[4] McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N EnglJ Med 2011;365:2205–19.
[5] Del Rey MJ, Izquierdo E, Usategui A, et al. The transcriptional response of normal and rheumatoid arthritis synovial fibroblasts to hypoxia. Arthritis Rheum 2010;62:3584–94.
[6] Abeles AM, Pillinger MH. The role of the synovial fibroblast in rheumatoid arthritis: cartilage destruction and the regulation of matrix metalloproteinases. Bull NYU Hosp Joint Dis 2005;64:20–4.
[7] Weyand CM, Seyler T, Goronzy JJ. B cells in rheumatoid synovitis. Arthritis Research and Therapy 2005;7:S9.
[8] Firestein GS. Immunologic mechanisms in the pathogenesis of rheumatoid arthritis. JCR: Journal of Clinical Rheumatology 2005;11:S39–44.
[9] Burton PR, Clayton DG, Cardon LR, et al. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007;447:661–78.
[10] O'Neill LA. Targeting signal transduction as a strategy to treat inflammatory diseases. Nat Rev Drug Discov 2006;5:549–63.
[11] van der Pouw Kraan TC, van Gaalen FA, Kasperkovitz PV, et al. Rheumatoid arthritis is a heterogeneous disease: evidence for differences in the activation of the STAT-1 pathway between rheumatoid tissues. Arthritis Rheum 2003;48:2132–45.
[12] Roman-Blas J, Jimenez S. NF-κB as a potential therapeutic target in osteoarthritis and rheumatoid arthritis. Osteoarthritis Cartilage 2006;14: 839–48.
[13] McInnes IB, Liew FY. Cytokine networks—towards new therapies for rheumatoid arthritis. Nat Clin Pract Rheumatol 2005;1:31–9.
[14] Scott D, Kingsley G. Tumor necrosis factor inhibitors for rheumatoid arthritis. N Engl J Med 2006;355:704–12.
[15] Biswas S, Manikandan J, Pushparaj PN. Decoding the differential biomarkers of Rheumatoid arthritis and Osteoarthritis: a functional genomics paradigm to design disease specific therapeutics. Bioinformation 2011;6:153.
[16] You S, Cho C-S, Lee I, et al. A systems approach to rheumatoid arthritis. PloS One 2012;7:e51508.
[17] Ungethuem U, Haeupl T, Witt H, et al. Molecular signatures and new candidates to target the pathogenesis of rheumatoid arthritis. Physiol Genom 2010;42:267–82.
[18] Li X-S. An aggregate function method for nonlinear programming. Sci China (A) 1991;34:1467–73.
[19] Altman NS. An introduction to kernel and nearest-neighbor nonparametric regression. Am Stat 1992;46:175–85.
[20] Hastie T, Tibshirani R, Narasimhan B, et al. Impute: imputation for microarray data. Bioinformatics 2001;17:520–5.
[21] Bolstad B. preprocessCoreA collection of pre-processing functions. R package version 2013;1:
[22] Shabalin AA, Tjelmeland H, Fan C, et al. Merging two gene-expression studies via cross-platform normalization. Bioinformatics 2008;24: 1154–60.
[23] Rudy J, Valafar F. Empirical comparison of cross-platform normalization methods for gene expression data. BMC Bioinformatics 2011;12: 467.
[24] Smyth GK. Limma: linear models for microarray data. Bioinformatics and computational biology solutions using R and Bioconductor. 2005; Springer, 397–420.
[25] Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J Royal Stat Soc B (Methodological) 1995;289–300.
[26] Hulsegge I, Kommadath A, Smits MA. Globaltest and GOEAST: two different approaches for Gene Ontology analysis. BMC Proc 2009;3 (suppl 4):S10.
[27] Kanehisa M. The KEGG database. Novartis Found Symp 2002;247: 91–101.
[28] Yu G, Wang L-G, Han Y, et al. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS 2012;16: 284–7.
[29] Prasad TK, Goel R, Kandasamy K, et al. Human protein reference database—2009 update. Nucleic Acids Res 2009;37(suppl 1): D767–72.
[30] Ideker T, Ozier O, Schwikowski B, et al. Discovering regulatory and signalling circuits in molecular interaction networks. Bioinformatics 2002;18(suppl 1):S233–40.
[31] Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 2003;13:2498–504.
[32] Zanin-Zhorov A, Lin J, Scher J, et al. Scaffold protein Disc large homolog 1 is required for T-cell receptor-induced activation of regulatory T-cell function. Proc Natl Acad Sci 2012;109:1625–30.
[33] Gmyrek GB, Graham DB, Sandoval GJ, et al. Polarity gene discs large homolog 1regulates the generation of memory T cells. Eur J Immunol 2013;43:1185–94.
[34] Stephenson LM, Sammut B, Graham DB, et al. DLGH1 is a negative regulator of T-lymphocyte proliferation. Mol Cell Biol 2007;27: 7574–81.
[35] Galligan C, Baig E, Bykerk V, et al. Distinctive gene expression signatures in rheumatoid arthritis synovial tissue fibroblast cells: correlates with disease activity. Genes Immun 2007;8:480–91.
[36] Acarin L, González B, Castellano B. Decrease of proinflammatory molecules correlates with neuroprotective effect of the fluorinated salicylate triflusal after postnatal excitotoxic damage. Stroke 2002;33: 2499–505.
[37] Chandy KG, Gutman GA, Grissmer S. Physiological role, molecular structure and evolutionary relationships of voltage-gated potassium channels in T lymphocytes. Semin Neurosci 1993;5:125–34.
[38] Torkamani A, Topol EJ, Schork NJ. Pathway analysis of seven common diseases assessed by genome-wide association. Genomics 2008;92: 265–72.
[39] Min K-j, Jou I, Joe E. Plasminogen-induced IL-1β and TNF-α production in microglia is regulated by reactive oxygen species. Bioch Biophys Res Commun 2003;312:969–74.
[40] Syrovets T, Jendrach M, Rohwedder A, et al. Plasmin-induced expression of cytokines and tissue factor in human monocytes involves AP-1 and IKKbeta-mediated NF-kappaB activation. Blood 2001;97:3941–50.
[41] Busso N, Hamilton JA. Extravascular coagulation and the plasminogen activator/plasmin system in rheumatoid arthritis. Arthritis Rheum 2002;46:2268–79.
[42] Raghu H, Jone A, Cruz C, et al. Plasminogen is a joint-specific positive or negative determinant of arthritis pathogenesis in mice. Arthritis Rheumatol 2014;66:1504–16.
[43] Neumann E, Lefèvre S, Zimmermann B, et al. Rheumatoid arthritis progression mediated by activated synovial fibroblasts. Trends Mol Med 2010;16:458–68.
[44] Koch AE. Chemokines and their receptors in rheumatoid arthritis: future targets? Arthritis Rheum 2005;52:710–21.
[45] White JR, Harris RA, Lee SR, et al. Genetic amplification of the transcriptional response to hypoxia as a novel means of identifying regulators of angiogenesis. Genomics 2004;83:1–8.
[46] Ballanti E, Perricone C, Di Muzio G, et al. Role of the complement system in rheumatoid arthritis and psoriatic arthritis: relationship with anti-TNF inhibitors. Autoimmun Rev 2011;10:617–23.
[47] Mizuno M. A review of current knowledge of the complement system and the therapeutic opportunities in inflammatory arthritis. Curr Med Chem 2006;13:1707–17.