Identifying Main Genes and Pathways by Using Gene Expression Profiling in Primary Immunodeficiency HOIL-1/RBCK1 Disorder Patients

Khyber Shinwari[1], Guojun Liu[2], Mikhail Bolkov[3], Monib Ullah[4], Irina Tuzankina[3]

[1] *Department of Immunochemistry, Institute of Chemical Engineering, Ural Federal University, Yekaterinburg, Russia*
[2] *School of Life Science and Technology, Inner Mongolia University of Science and Technology, Baotou 014010, China*
[3] *Institute of Immunology and Physiology of the Ural Branch of the Russian Academy of Sciences, Yekaterinburg, Russia*
[4] *Institute of Microbiology, University of Agriculture Faisalabad, Faisalabad, Pakistan*

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Abstract- HOIL-1/RBCK1 deficiency is a new autosomal receiving disorder with dysfunctional cellular responses to pro-inflammatory cytokines, leading to auto-inflammation, pyogenic bacterial disease, and muscle amylopectinosis growth. Our study with integrated bioinformatics studies of the feature genes and the correlative gene functions, investigated the molecular mechanisms of RBCK1 deficiency. GSE31064 dataset expression profile was downloaded from the Omnibus Gene Expression database. Between RBCK1, MYDK88, NEMO deficient fibroblast, and healthy fibroblast specimens, differentially expressed genes (DEGs) were defined. Gene ontology (GO) gene role enrichment analysis and the Kyoto Encyclopedia of Gene and Genome (KEGG) pathway analysis were performed using the Annotation, Visualization and Integrated Discovery Database (DAVID). The protein-protein interaction (PPI) of these DEGs was visualized using Cytoscape. GO analysis revealed that the "Skeletal system development, Extracellular matrix organization, Positive regulation of cell migration, Negative regulation of canonical Wnt signaling pathway, Cell adhesion, Angiogenesis and Negative regulation of BMP signaling pathway, Serine-type carboxypeptidase activity, Polysaccharide binding, Calcium ion binding, frizzled binding, Neuropilin binding, and cell adhesion molecule binding, extracellular exosome, extracellular space, extracellular region, lysosomal lumen, endoplasmic reticulum lumen, cell surface and focal adhesion to BP, MF, and CC, respectively. The study of the KEGG pathway showed that the complement and coagulation cascade, interactions of the ECM receptor, PI3K-Akt signaling pathway, PPAR signaling pathway, TGF-beta signaling pathway, cancer pathway, viral carcinogenesis and focal adhesion pathway were closely correlated with the incidence of RBCK1 deficiency. Importantly, it has been predicted that TK1, AURKB, CDCA2, UBE2C, KIFC1, CEP55, CDCA3, GINS2, MCM6 and CDC45 are significantly associated with RCBK1 deficiency. Our study offers a record of damaged genes and pathways in RCBK1, which will boost the understanding of RBCK1 deficiency pathogenesis and other inherent immunodeficiency diseases. This research has the potential and can possibly use in the clinic for diagnosis and targeted therapy of HOIL-1/RBCK1 disorder and other inherent immunodeficiencies.

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Keywords: Primary immunodeficiency; RBCK1 deficiency; Differential expressed genes; Key pathways; Hub genes; Microarray

Introduction

In regulating TLR signaling, the Ubiquitin system plays an important role. The ubiquitin system is a system of posttranslational alteration that regulates protein function (1). In several situations, to form polyubiquitin chains, the ubiquitin molecule is bound to target proteins. In the synthesis of these polyubiquitin chains, C-terminal glycine residue serial conjugation involves glycine residue in one ubiquitin molecule conjugating to one of

Corresponding Author: Kh. Shinwari
Department of Immunochemistry, Institute of Chemical Engineering, Ural Federal University, Yekaterinburg, Russia
Tel: +79655189239, Fax: +73433740079, E-mail address: khybershinwari05@gmail.com

seven lysine residues in another ubiquitin molecule (2). Studies have shown that LUBAC-catalyzed linear ubiquitination, in response to TNF-alpha stimulation, is involved in activating the canonical NF-aB pathway and preventing cell death (1). RBCK1 (58 kDa, also known as HOIL-1) with two other proteins, SHANK associated RH domain-interacting protein (SHARPIN) and HOIL-1 Interacting Protein (HOIP-1) is best known to form a ~600 kDa complex (2,3). In addition to its position in muscle cells, HOIL-1 is also an integral component of the so-called Linear Ubiquitination Chain Assembly Complex (LUBAC), which regulates a number of important immune response mechanisms based on NF-μB. (4). Affected subjects can have both chronic autoinflammation and immunodeficiency, including recurrent septicemia, at the same time (5). The patients with RBCK1 mutations identified to date differ considerably in terms of their clinical outcome (i.e., skeletal muscle, heart muscle, autoinflammation or immunodeficiency). The explanation for this individual heterogeneity remains uncertain, although it was speculated that the exact position of the variant within the gene may be a predictor of the prevailing phenotype, with mutations mainly leading to immunological dysfunction in the N-terminal region of RBCK1 and mutations in the middle or C-terminal sections leading to a phenotype of (cardio) myopathy (6). M1-linked linear polyubiquitination is mediated by LUBAC, a complex modification that makes nuclear factor-kB (NF-kB) and its pleiotropic immune system-critical nuclear translocation and transcriptional control. RBCK1 and HOIP both contain a RING-between-RING domain (RBR). The LUBAC-mediated ubiquitination, however, is achieved through the catalytic domain of HOIP, with the position of RBCK1 in the complex apparently reduced to a crucial collaboration with the auto-inhibitory domain of HOIP, stopping the blockage and enabling HOIP to operate (2,3). RBCK1 is also known to target a diverse range of proteins, independent of LUBAC, most likely via its own RBR domain for ubiquitination and proteasomal degradation (7-11). Linear ubiquitin assembly complex (LUBAC), including HOIL-1-interacting protein (HOIP), Heme-oxidized IRP2 ubiquitin ligase-1 (HOIL-1) and SHANK-associated RH domain interactor (SHARPIN), often binds linear (Met1) ubiquitin chains in the canonical NF-kB pathway to many target proteins (12). The linear ubiquitin-specific OTULIN deubiquitinase counter controls the function of LUBAC. In mice and humans, immune dysregulation is noted when there are defects in the processes of linear and K63 ubiquitination and deubiquitination (13). The catalytic subunit of the linear ubiquitination chain assembly complex (LUBAC) is HOIP, which is important for NF-kB signaling and, hence, proper innate and adaptive immunity. As of now, with symptoms such as immunodeficiency, systemic lymphangiectasia, and autoinflammation, only one person has been identified with HOIP deficiency (14). Humans and mice missing RBCK1 are shown to have a distinct category of immune deficiency and hyper-inflammation that, depending on particular pathogen interaction and other factors, tends to irregularly enter clinical significance from zero to fatality. Both humans and mice exhibit advanced PB development in the heart and skeletal muscles, ending in skeletal myopathy and heart failure in humans (15,16,17). Amylopectinosis, cardiac and/or skeletal muscle, autoinflammation and immunodeficiency are observed in patients with flaws in the components of LUBAC (18,19). HOIP deficiency is also shown to consist of lymphangiectasia in the systemic edema, gastrointestinal tract, and hypoalbuminemia that can trigger malabsorption. Molecular research has established that the fibroblasts and B cells of patients who were not receptive to immune stimuli and were unable to steadily upregulate NF-kB activity with the immunodeficient phenotype observed by the patient. In comparison to immune responses in fibroblasts, HOIP and HOIL1-deficient peripheral blood mononuclear cells (PBMCs) were highly reactive to IL-1 stimulation and expressed IL-6 and MIP-1a proinflammatory cytokines (14). HOIL-1 deficiency in patient cells resulted in lower levels of IKK kinase phosphorylation, slower IIB alpha degradation and decreased NEMO ubiquitinity in response to either TNF or IL-1β stimulation, and lower levels of NF-aB activation in patient cells were associated with lower NF-aB transcriptional activity. HOIP, Uh, HOIP, in fibroblasts and B cells from HOIL-1 deficient patients, the catalytic center of LUBAC (19,20) was relatively imperceptible, indicating that these patients were LUBAC deficient. LUBAC is active in the NF-1B pathway and binds the linear chains of polyubiquitin to unique NEMO 16 remains of Lys. HOIL-1-deficient human fibroblasts showed weakened activation of NF-1B, resulting in poor transcription of the NF-1B-driven gene and development of cytokines in response to TNF and IL-1β, constant data in Hoil1 mouse knockdown or knockout cells (19). The use of bioinformatics to analyze gene expression profiles has demonstrated to be extremely effective in identifying potential important genes and pathways in complicated disorders (21). It could also be beneficial to find new genes and biological processes linked to HOIL-1/RBCK1 pathogenesis. As a result, we used rigorous bioinformatics analysis to identify DEGs and the related biological processes in AMI using the original data (GSE31064) from the publically available Gene Expression Omnibus database (GEO, http://www.ncbi.nlm.nih.gov/geo/). DEGs were analyzed for function enrichment and pathway analysis. In addition, a protein-protein interaction (PPI) network was built to find important gene nodes. Analysis of the biological activities and pathways in these illnesses may provide more molecular insights into HOIL-1/RBCK1 development and pave the way for a better understanding of probable disease pathogenesis mechanisms to aid diagnosis, prognosis, and therapeutic target identification.

Materials and Methods

Gene expression dataset

Gene Expression Omnibus (GEO, www.ncbi.nlm.nih.gov/geo/) was used to download GSE31064 dataset which is based on Illumina HumanHT-12 V4.0 expression bead-chip. Dataset contained four samples with 3 controls, Skin fibroblast cell lines were derived from controls (3 samples), patients with deficiencies for RBCK1 (HOIL1) (2 samples), MYD88 (1 sample), and NEMO (1 sample). Cell were cultured for 8 or 24 hours in the presence of TNF (5 ng/ml) or IL1B (5 ng/ml) or left unstimulated for the same length of time.

Data preprocessing and analysis of DEGs

GEO2R (http://www.ncbi.nlm.nih.gov/geo/geo2r /) is a handy web tool for comparing data from two GEO datasets (NCBI 2012) (22). To compare the FAMI group to the control group, GEO2R was utilized to analyze the released GSE24519 microarray dataset. DEGs were defined as genes with a P value less than 0.05 and a |log2FC (fold change)| greater than 2 while the results was confirmed through R language lima package. Online tool Morpheus (https://software.broadinstitute.org/morpheus/) was used to generate differential expressed genes heat map (23).

Gene ontology and kyoto encyclopedia of genes and genomes analyses of DEGs

DAVID http://david.ncifcrf.gov), web based analysis tool for KEGG and GO analyses was used with $P<0.05$ as statistically significant to understand about the DEGs gens pathways and gene ontologies (24,25,26)

Protein-protein interactions and module analysis

PPI network of the DEGs was constructed through STRING (version 10.0) (http://string-db.org) with a combined score >0.4 as the threshold for statistically significant interaction (27). To further examine the interactive network, the program Cytoscape (version 3.4.0) was used with the Molecular Complex Detection (MCODE) plugin to classify essential molecules in the PPI network while MCODE scores >5, degree cut-off=2, node score cut-off=0.2, Max depth=100 and k-score=2 were the recognition criteria (28,29)

Analysis of hub and core genes selection

Hub genes biological processes were visualized using the Cytoscape (30) plugin (BiNGO) (version 3.0.3) with a significance threshold of 0.01 and Homo sapiens as the selected organism. Subsequently, DAVID was used to conduct the KEGG and GO analyses for the genes in this module. Core genes were chosen by the level of connectivity and pictured by Metaphase software (31).

Results

Differentially expressed genes in HOIL1 (RBCK1)

We reported a total of 532 DEGs in GSE31064 after standardizing the microarray findings, in which 211 down-regulated and 321 up-regulated genes were seen in DEGS as table 1 top-down and up-regulated genes. Figure 1 displays the expression heat map of the DEGs, including the top 50 genes. GO analysis showed that in Skeletal system growth, Extracellular matrix organization, Positive cell migration control, Negative canonical Wnt signaling pathway regulation, Cell adhesion, Angiogenesis and Negative BMP signaling pathway regulation, the biological processes (BP) conditions of the DEGs were significantly enriched Figure 2a. The terms of molecular function (MF) were primarily enriched by the activity of serine-type carboxypeptidase, polysaccharide binding, calcium ion binding, frizzled binding, neuropilin binding and molecule binding cell adhesion Figure 2b. Finally, extracellular exosome, extracellular space, extracellular area, lysosomal lumen, endoplasmic reticulum lumen, cell surface and focal adhesion were primarily enriched by cell component (CC) terms Figure 2c. Study of the KEGG pathway showed that the DEGs were mainly enriched by complement and coagulation cascade, ECM receptor interactions, PI3K-Akt signaling pathway, PPAR signaling pathway, TGF-beta signaling pathway, cancer pathway, viral carcinogenesis, phagosome, lysosome, antibiotic biosynthesis, digestion and absorption of proteins, Axon guidance, metabolism of pyruvates, staphylococcus aureus infection, Malaria, proteoglycans in cancer , glycine, serine and threonine metabolism and Focal adhesion Figure 2d.

Protein-protein interactions and module analysis

The PPI network of DEGs was constructed Figure 3a and Cytoscape was used to obtain the most important module Figure 3b. The module's GO study showed substantial enrichment of cell cycle phase transition, cell cycle process control, cell cycle regulation, chromosome organization, cell organization, and organelle organization in the BP terms , MF terms of ATP binding, drug binding, and purine riboneuculotide triphosphate binding MF terms of DNA binding and CC terms of organelle, nucleoplasm, nucleus and nuclear lumen bound intracellular non-membrane. KEGG pathway study of hub genes showed that they were primarily enriched by drug metabolism-other enzymes, metabolism of pyrimidine, metabolic pathways, proteolysis mediated by ubiquitin, replication of DNA in the cell cycle and others. These hub genes (TK1, CDCA2, UBE2C, GINS2, and MCM6) also encode a MIRNA with GO hsa-miR-193b-3p and the results of module GO and KEGG are given in supplementary file 1.

Selection and analysis of hub gene

There were a total of 10 genes known as hub genes, and table 2 shows their names and MCODE ratings. A network of hub genes and their co-expression genes were analyzed using Cytoscape's BiNGO method Figure 4b and BP of all DEGS in Figure 4a.

Core genes analysis and selection

In the table 3, core genes were selected based on the degree of connectivity. The relationships between the terms were selected and made as a network map, a subset of enriched terms, where terms with a similarity >0.3 are linked by edges. From each of the 20 clusters, we pick the terms with the best *P*, with the restriction that there are no more than 15 terms per cluster and no more than 250 terms in total. Using Cytoscape5, the network is visualized where each node represents an enriched word and is first colored by its cluster ID Figure 5a and then by its *P* Figure 5b. The core genes were enriched as shown in figure 6 R-HSA-449147, Signaling by Interleukins, R-HSA-109582-Hemostasis, R-HSA-8957275- Post-translational phosphorylation of protein, GO(BP) extracellular structure organization, lipid transport control, mitotic spindle assembly, particle metabolic mechanism of low density lipoprotein receptor, peptide response, DNA biosynthesis regulation, positive regulation of organelle organization.

Table 1. Top up and down-regulated genes

Upregulated			**Down-regulated**		
Gene ID and symbol	***P***	**LogFC**	**Gene ID and symbol**	***P***	**LogFC**
ILMN_2148527 (H19)	3.41E-32	7.809	ILMN_2165753 (HLA-A)	5.80E-17	-6.298
ILMN_1726204 (SCRG1)	8.13E-28	5.798	ILMN_1777190 (CFD)	8.30E-11	-3.671
ILMN_1751062 (SCARA5)	3.42E-31	5.408	ILMN_1766925 (CDH13)	1.04E-16	-3.086
ILMN_1780349 (TPR)	3.66E-21	5.755	ILMN_1809537 (MASP1)	1.67E-11	-3.963
ILMN_2067656 (CCND2)	3.97E-17	6.119	ILMN_2141482 (SERPINF1)	8.54E-11	-2.392
ILMN_1659359 (SCUBE3)	3.82E-15	4.218	ILMN_1668134 (GSTM1)	3.22E-08	-2.646
ILMN_2387105 (OGN)	3.36E-14	4.051	ILMN_1765668 (IL20RB)	5.19E-06	-2.716
ILMN_2200836 (HSPB7)	1.79E-12	4.755	ILMN_1677603 (C1S)	8.09E-06	-2.5
ILMN_1707232 (EBF3)	2.98E-12	3.973	ILMN_2186806 (HLA-F)	3.88E-09	-4.203
ILMN_1848552 (NFIB)	1.16E-12	3.54	ILMN_1675797 (EPDR1)	1.10E-21	-3.88
ILMN_1812031 (PALM)	7.57E-18	3.868	ILMN_2049536 (TRPV2)	1.21E-18	-3.192
ILMN_1814333 (SERPINI1)	1.15E-15	3.732	ILMN_1806733 (COL18A1)	1.07E-16	-3.003
ILMN_1681515 (CRLF1)	1.78E-11	3.325	ILMN_1784459 (MMP3)	1.54E-11	-3.651
ILMN_1743445 (FAM107A)	3.46E-11	3.507	ILMN_2149164 (SFRP1)	1.10E-09	-3.079
ILMN_2071809 (MGP)	1.01E-10	4.323	ILMN_1659688 (LGALS3BP)	5.90E-09	-3.162
ILMN_1664861 (ID1)	1.69E-09	3.832	ILMN_1674386 (PITX1)	1.08E-07	-3.641
ILMN_1778991 (NFIB)	2.90E-09	3.679	ILMN_1761322 (FHOD3)	9.64E-10	-2.798
ILMN_1703926 (PTGER2)	4.70E-09	3.958	ILMN_2113490 (NTN4)	1.21E-07	-2.255
ILMN_1766264 (PI16)	4.656E-24	5.656	ILMN_1781155 (LYN)	1.73E-08	-2.499
ILMN_1778991 (NFIB)	1.61E-11	3.116	ILMN_1792885 (CTSC)	1.32E-07	-2.271
ILMN_2196328 (POSTN)	7.40E-09	3.761	ILMN_1741688 (HIST1H2BD)	3.97E-07	-2.201
ILMN_1710408 (LGR4)	4.80E-17	2.524	ILMN_1755537 (EIF1AY)	8.20E-07	-2.38
ILMN_2328094 (DACT1)	2.18E-07	2.369	ILMN_2339835 (PTGS1)	1.21E-06	-2.538
ILMN_3309404 (MIR503)	1.81E-09	1.993	ILMN_1767685 (SERPINB7)	1.58E-06	-2.222
ILMN_1687301 (VCAN)	1.32E-05	2.028	ILMN_1703852 (EFNB2)	9.62E-07	-2.111
ILMN_2071809 (MGP)	1.68E-06	2.419	ILMN_1707308 (IKBKG)	2.84E-22	-3.622
ILMN_2216637 (STK32B)	1.94E-06	2.347	ILMN_1809364 (NTF3)	3.48E-16	-3.066
ILMN_1731374 (CPE)	2.17E-07	1.9	ILMN_1788955 (PDLIM1)	1.79E-14	-3.506
ILMN_1735930 (KLF2)	2.00E-08	1.302	ILMN_1741688 (CPXM2)	3.91E-13	-4.647
ILMN_2142185 (CLEC14A)	3.36E-11	2.686	ILMN_1678493 (CHN1)	1.09E-12	-3.78
ILMN_1720710 (HSPB3)	6.24E-24	2.553	ILMN_1803213 (MXRA5)	1.78E-12	-3.533
ILMN_1765641 (SEMA3A)	2.57E-07	1.807	ILMN_2326509 (CASP1)	2.96E-08	-3.235
ILMN_2413158 (PODXL)	4.86E-06	1.936	ILMN_1676663 (TNFRSF11B)	1.58E-09	-3.665
ILMN_1772824 (WNT5B)	1.17E-05	1.419	ILMN_1669376 (DRAM1)	2.24E-11	-3.534
ILMN_2229379 (KIT)	1.20E-05	1.724	ILMN_1784459 (MMP3)	4.71E-11	-5.861

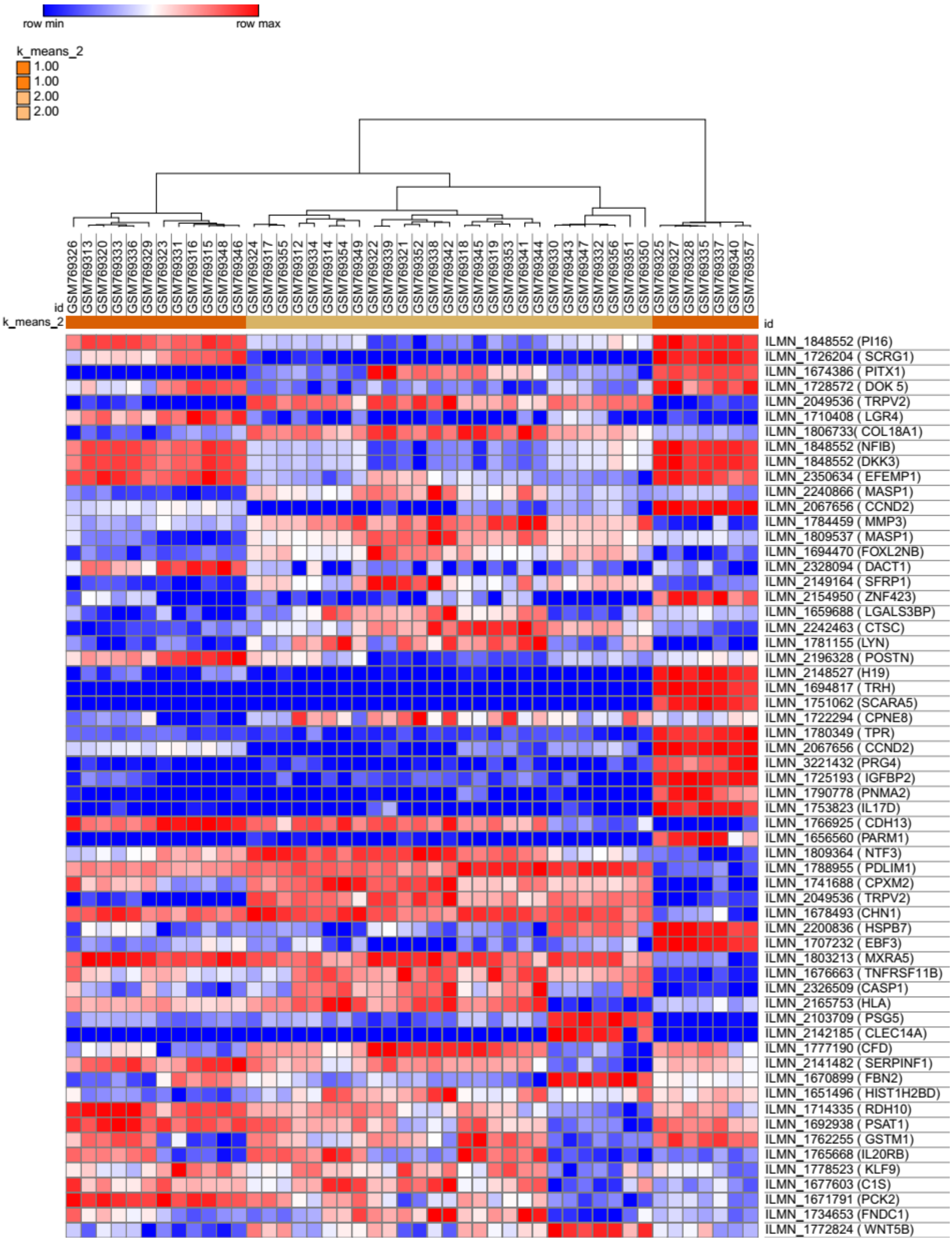
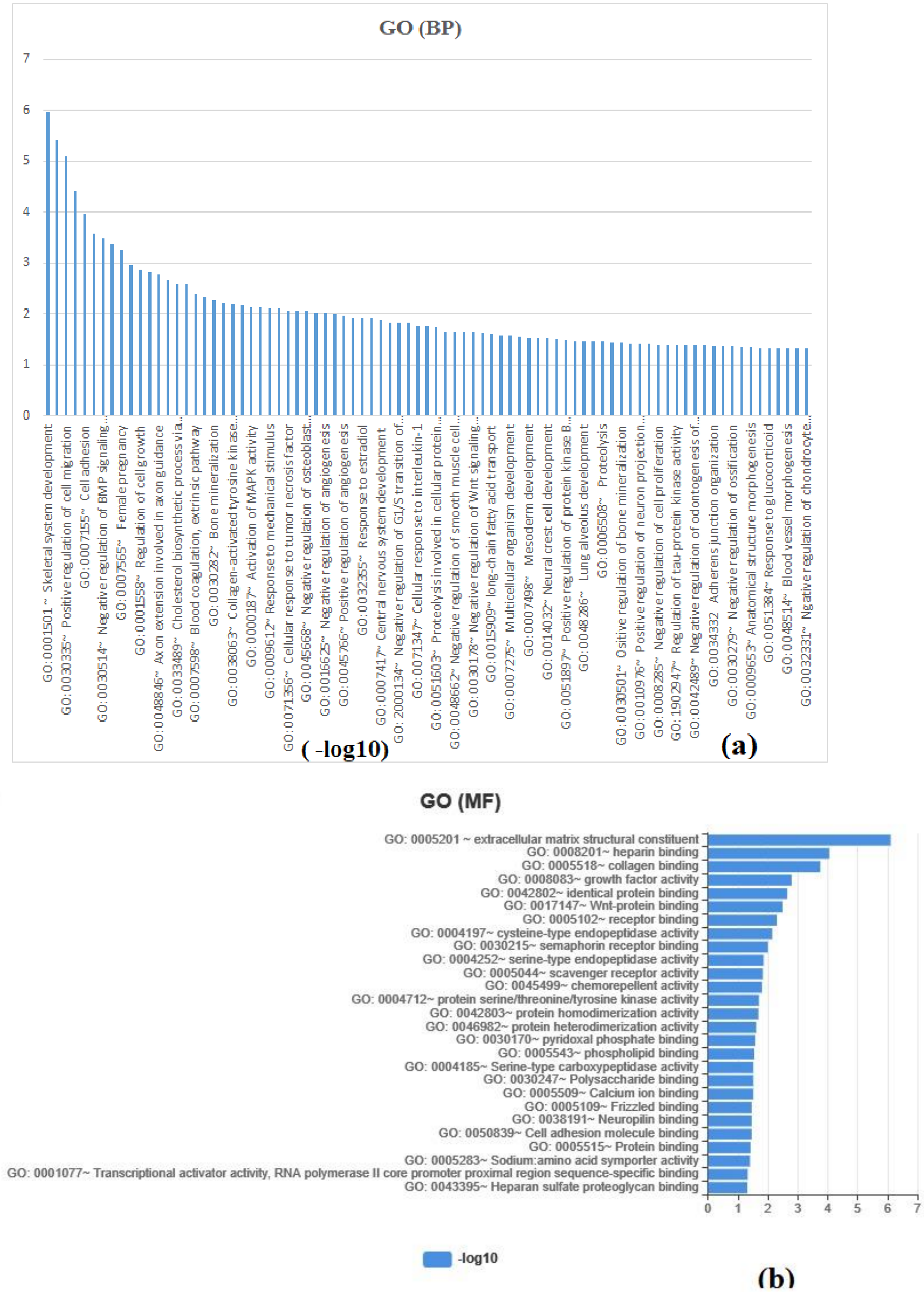
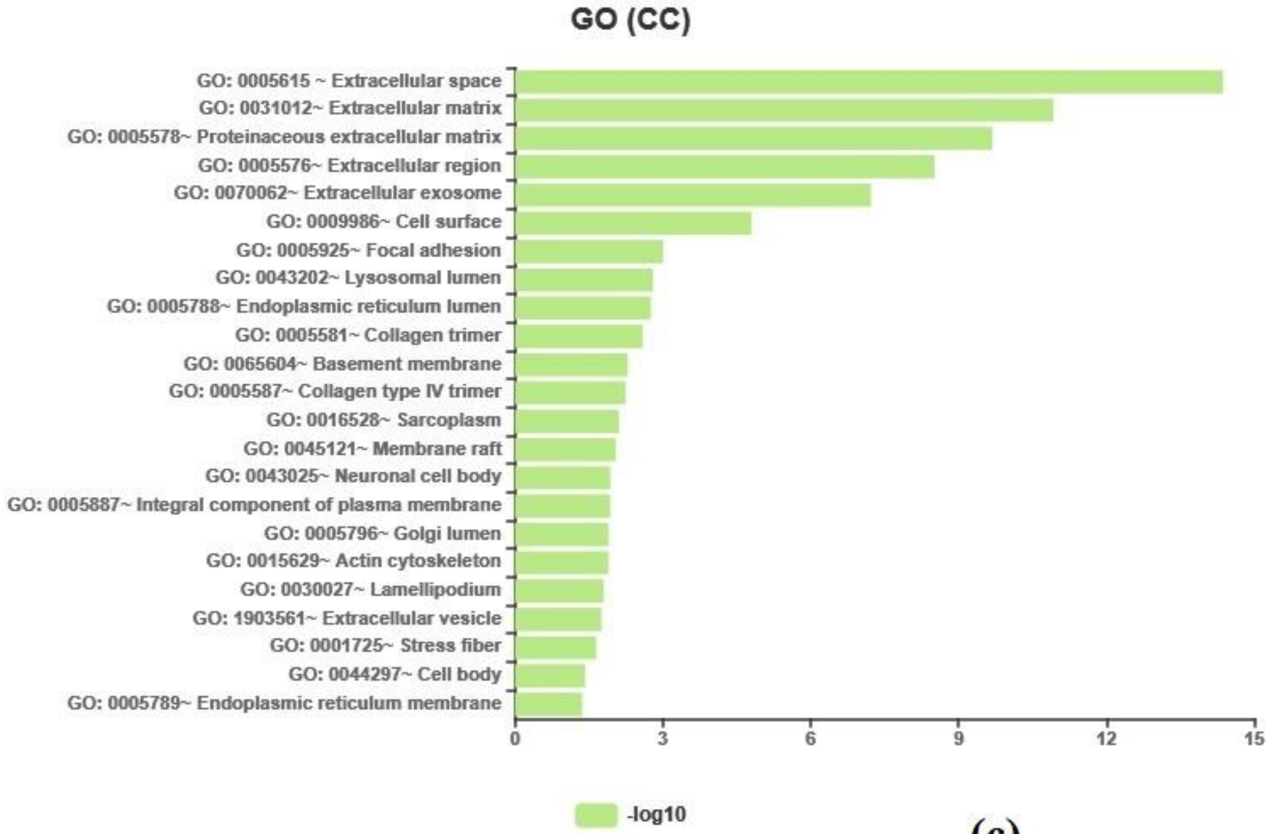
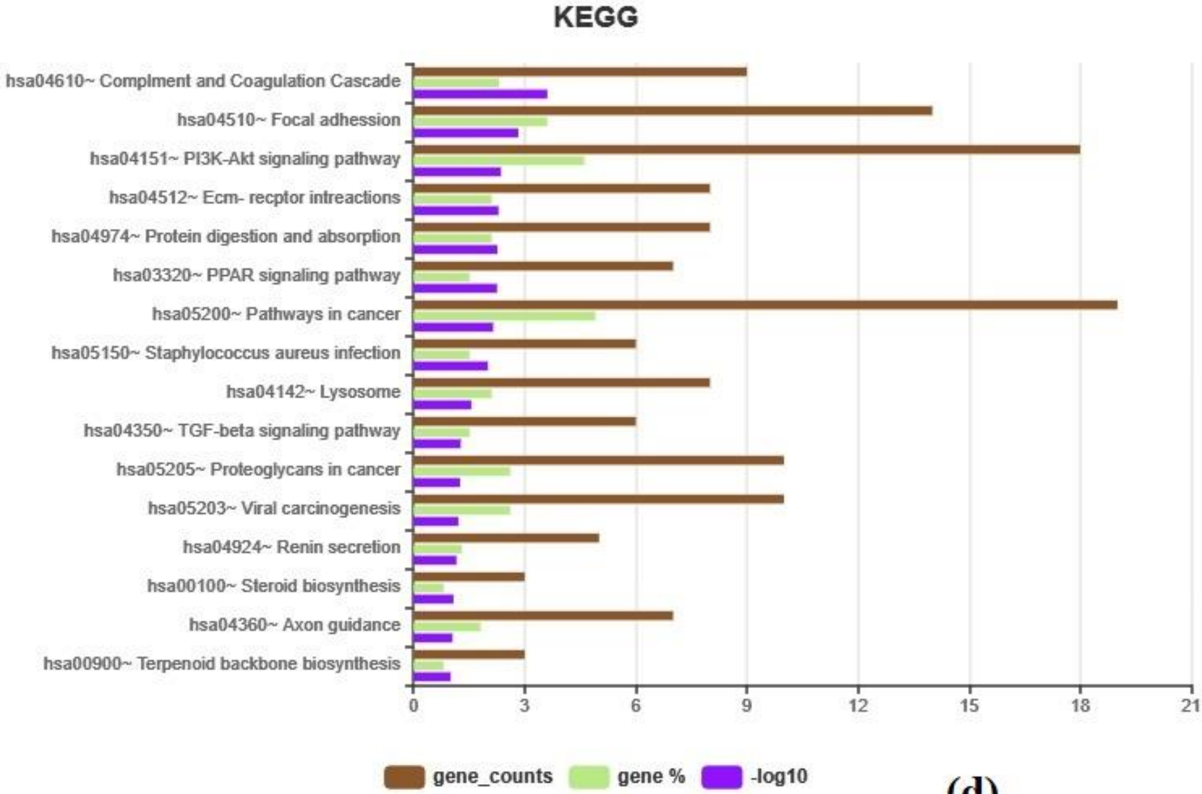


Figure 1. Heat map of differentially expressed genes for the GSE31064 dataset. A sample represents each column, and a gene represents each row. Red applies to genes up-regulated and blue down-regulated


GO (BP)
7
6
5
4
3
2
1
0
GO:0001501 ~ Skeletal system development
GO:0030335~ Positive regulation of cell migration
GO:0007155~ Cell adhesion
GO:0030514~ Negative regulation of BMP signaling...
GO:0007565~ Female pregnancy
GO:0001558~ Regulation of cell growth
GO:0048846~ Axon extension involved in axon guidance
GO:0033489~ Cholesterol biosynthetic process via...
GO:0007598~ Blood coagulation, extrinsic pathway
GO:0030282~ Bone mineralization
GO:0038063~ Collagen-activated tyrosine kinase...
GO:0000187~ Activation of MAPK activity
GO:0009612~ Response to mechanical stimulus
GO:0071356~ Cellular response to tumor necrosis factor
GO:0045668~ Negative regulation of osteoblast...
GO:0016525~ Negative regulation of angiogenesis
GO:0045766~ Positive regulation of angiogenesis
GO:0032355~ Response to estradiol
GO:0007417~ Central nervous system development
GO: 2000134~ Negative regulation of G1/S transition of...
GO:0071347~ Cellular response to interleukin-1
GO:0051603~ Proteolysis involved in cellular protein...
GO:0048662~ Negative regulation of smooth muscle cell...
GO:0030178~ Negative regulation of Wnt signaling...
GO:0015909~ long-chain fatty acid transport
GO:0007275~ Multicellular organism development
GO:0007498~ Mesoderm development
GO:0014032~ Neural crest cell development
GO:0051897~ Positive regulation of protein kinase B...
GO:0048286~ Lung alveolus development
GO:0006508~ Proteolysis
GO:0030501~ Ositive regulation of bone mineralization
GO:0010976~ Positive regulation of neuron projection...
GO:0008285~ Negative regulation of cell proliferation
GO:1902947~ Regulation of tau-protein kinase activity
GO:0042489~ Negative regulation of odontogenesis of...
GO:0034332 Adherens junction organization
GO:0030279~ Negative regulation of ossification
GO:0009653~ Anatomical structure morphogenesis
GO:0051384~ Response to glucocorticoid
GO:0048514~ Blood vessel morphogenesis
GO:0032331~ Negative regulation of chondrocyte...
(-log10)
(a)
GO (MF)
GO: 0005201 ~ extracellular matrix structural constituent
GO: 0008201~ heparin binding
GO: 0005518~ collagen binding
GO: 0008083~ growth factor activity
GO: 0042802~ identical protein binding
GO: 0017147~ Wnt-protein binding
GO: 0005102~ receptor binding
GO: 0004197~ cysteine-type endopeptidase activity
GO: 0030215~ semaphorin receptor binding
GO: 0004252~ serine-type endopeptidase activity
GO: 0005044~ scavenger receptor activity
GO: 0045499~ chemorepellent activity
GO: 0004712~ protein serine/threonine/tyrosine kinase activity
GO: 0042803~ protein homodimerization activity
GO: 0046982~ protein heterodimerization activity
GO: 0030170~ pyridoxal phosphate binding
GO: 0005543~ phospholipid binding
GO: 0004185~ Serine-type carboxypeptidase activity
GO: 0030247~ Polysaccharide binding
GO: 0005509~ Calcium ion binding
GO: 0005109~ Frizzled binding
GO: 0038191~ Neuropilin binding
GO: 0050839~ Cell adhesion molecule binding
GO: 0005515~ Protein binding
GO: 0005283~ Sodium:amino acid symporter activity
GO: 0001077~ Transcriptional activator activity, RNA polymerase II core promoter proximal region sequence-specific binding
GO: 0043395~ Heparan sulfate proteoglycan binding
0
1
2
3
4
5
6
7
-log10
(b)



(c)



(d)

Figure 2. GO and KEGG pathway enrichment analysis of DEGs. The color shows the –log10 of p-value and KEGG. The different colors indicated different parameters. (a) GO BP terms. (b) GO MF terms. (c) GO CC terms. (d) KEGG Pathway of DEGs

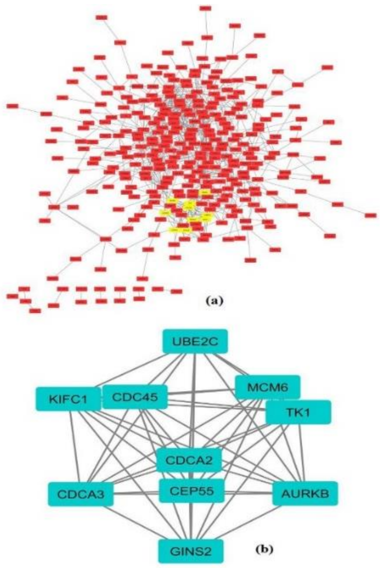


Figure 3. PPI network, and the most significant module of DEGs. (a)The PPI network of DEGs was constructed using Cytoscape. (b)The most significant module was obtained from the PPI network

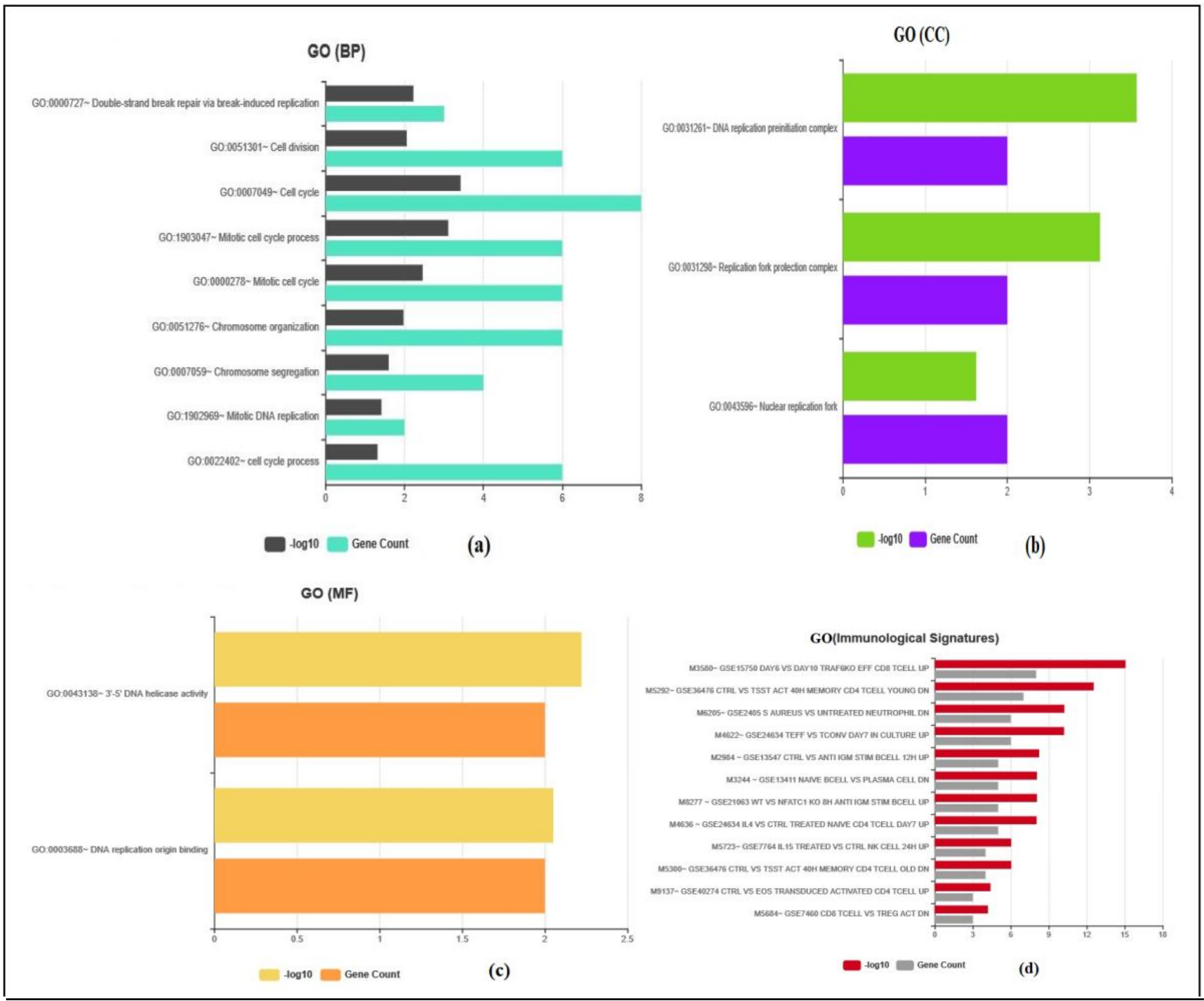


Figure 4. GO and Immunological Signature enrichment analysis of hub genes. Two colors one color refers to the *P*, and the other representative to the numbers of genes. (a) GO BP terms. (b) GO CC terms. (c) GO MF terms. (d) GO Immunological Signature of hub genes

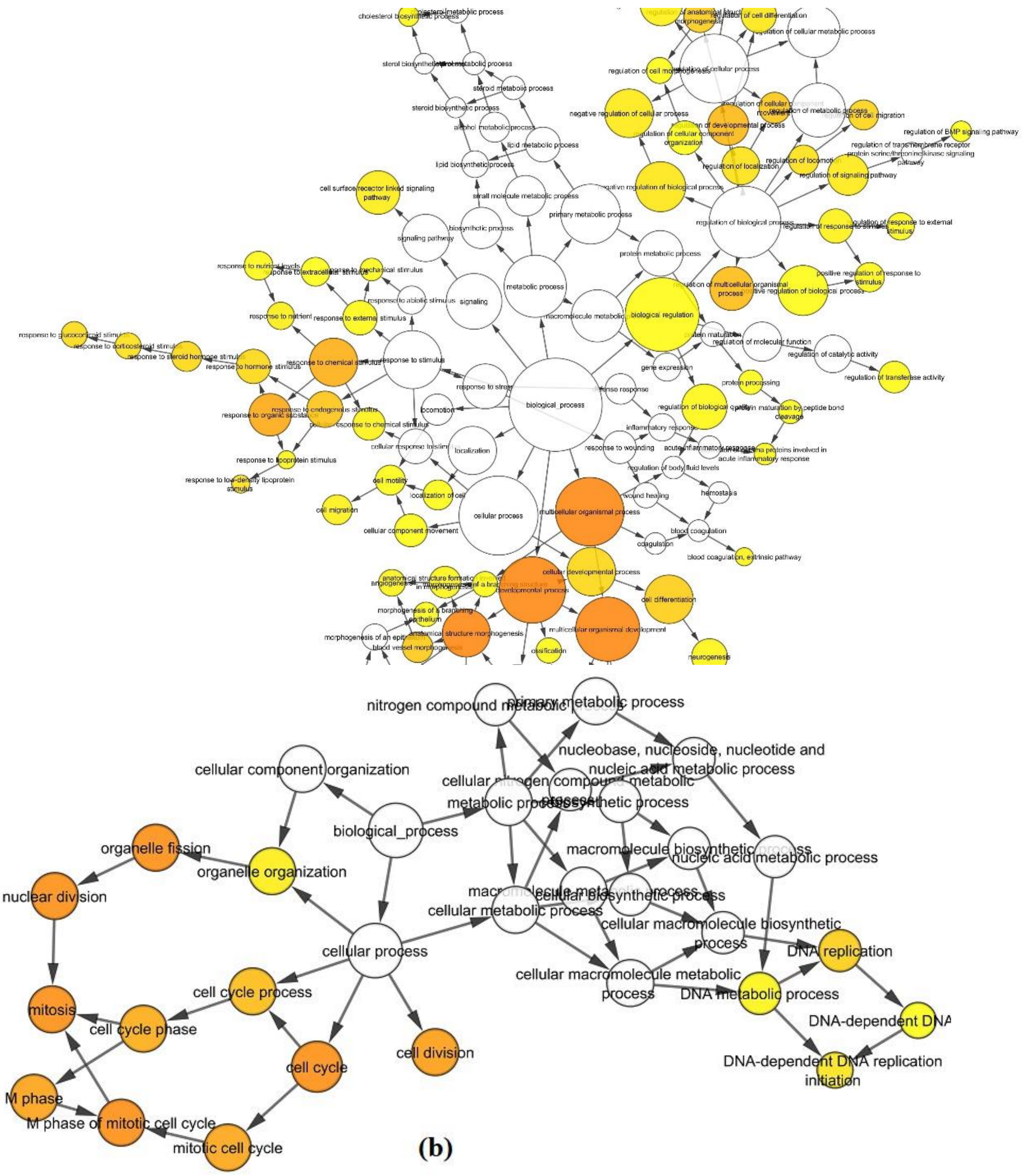


Figure 5. Interaction network and biological process analysis of the hub genes. The color depth of nodes refers to the P-value. The size of nodes refers to the number of genes. (a) BP analysis of the DEGs (b) Hub genes and their co-expression genes were analyzed using BiNGO

Table 2. Functional roles of 10 hub genes by MCODE Score

NO	Gene Symbol	MCODE Score
1	TK1	09
2	AURKB	09
3	CDCA2	09
4	UBE2C	09
5	KIFC1	09
6	CEP55	09
7	CDCA3	09
8	GINS2	09
9	MCM6	09
10	CDC45	09

Table 3. Top 20 Core gene by Degree

Gene Name	Betweenness Centrality	Degree	Gene Name	Betweenness Centrality	Degree
APP	0.11157344	46	CDH2	0.04917757	28
THBS1	0.05539869	34	KIF11	0.01013162	27
APOE	0.04564229	31	TPX2	0.00960601	26
TIMP1	0.02844216	31	ITGAV	0.02242985	25
DCN	0.02824711	31	KIFC1	0.00286402	24
PPARG	0.10510128	31	VCAN	0.02595851	24
POSTN	0.03629329	31	MMP3	0.01121897	23
UBE2C	0.02546135	30	EPRS	0.03779798	22
AURKB	0.01854466	28	MCM6	0.01010529	22
HGF	0.04908112	28	CFL1	0.06682249	22

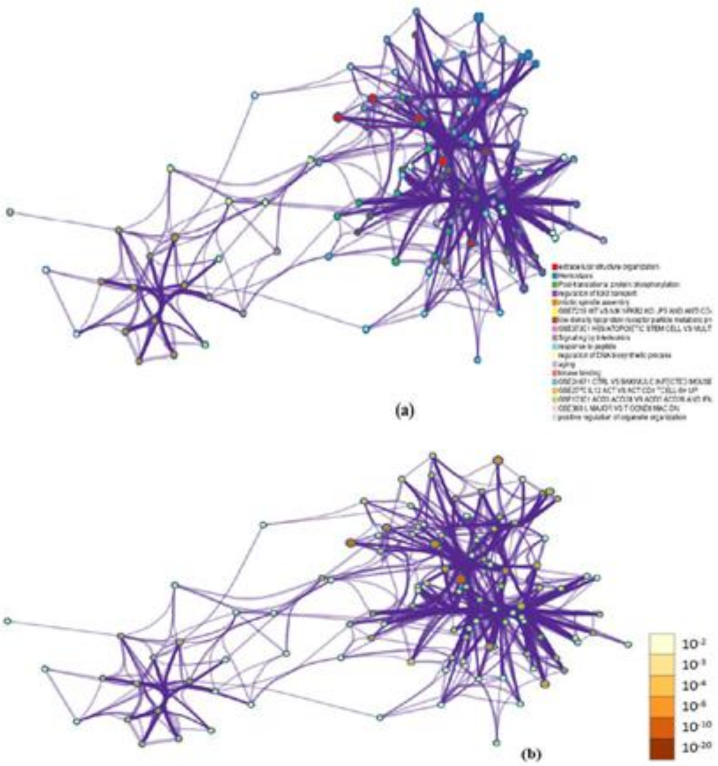


Figure 6. The network of enriched terms: (a) colored by cluster-ID, where nodes that share the same cluster-ID are typically close to each other; (b) colored by p-value, where terms containing more genes tend to have a more significant *P*

Discussion

HOIL1 (RBCK1) loss-of-expression and loss-of-function mutations result in the disruption of the complex linear ubiquitination assembly (LUBAC). Impairment of LUBAC results in impaired activation of NF-kB in fibroblastic cells in response to TNF and IL-1b, but also in improved pro-inflammatory responses to TNF and IL-1b in leukocytes. A new condition with unbalanced cellular responses to pro-inflammatory cytokines is autosomal recessive total human HOIL-1/RBCK1 deficiency, resulting in the paradoxical association of auto-inflammation and pyogenic bacterial disease, as well as the unexpected development of muscular amylopectinosis (32). For the diagnosis and treatment of RBCK1 and other innate immunodeficiencies, many attempts and advances have been made, although the production of clinically validated useful markers poses enormous challenges. Recent developments in gene microarray technology and the study of bioinformatics will give new possibilities for some diseases to identify possible main genes. To scan for DEGs in RBCK1 gene mutant patients and identify possible biomarkers, we analyzed RBCK1 microarray datasets from the GEO database. A total of 536 DEGs were obtained and the DEGs genes were mainly enriched by complement and coagulation cascade, ECM receptor interactions, PI3K-Akt signaling pathway, PPAR signaling pathway, TGF-beta signaling pathway, Cancer pathway, Viral carcinogenesis, Phagosome, lysosome, Antibiotic biosynthesis, digestion and absorption of proteins, Axon guidance, metabolism of pyruvates, infection of staphylococcus aureus, Malaria, proteoglycans in cancer , glycine serine and threonine metabolism, and Focal adhesion. In addition, high-connectivity genes were obtained using a PPI network, and TK1, AURKB, CDCA2, UBE2C, KIFC1, CEP55, CDCA3, GINS2, MCM6 and CDC45 research modules were recognized as hub nodes. The most important sub-modules of DEGs were extracted from the PPI network, and through cytoscap Bingo, we also carried out gene function and pathway analysis. Receptor tyrosine kinases, integrins, B and T cell receptors, cytokine receptors, G-protein-coupled receptors and other stimuli activate the Akt signaling cascade by inducing phospha-tidylinositol (3,4,5) trisphosphate (PIP3) development by phosphoinositide 3-kinase (PI3K). If these receptors have issues, the PI3K/Akt pathway dysregulation does not activate this pathway and is involved in a variety of human diseases, including cancer, diabetes, cardiovascular disease, and neurological diseases (33). An intracellular signaling pathway essential to control the cell cycle is the Pi3k/akt pathway. Phosphorylates and activates AKT by PI3K activation, locating it in the plasma membrane (34). A strong association of HOIL1 (RBCK1) and other innate development and prognosis of the immune system with such pathways has been shown in previous studies. The signaling pathway of phosphatidylinositol 3 kinase/serine/threonine kinase B (PI3K/Akt) plays a key role in the regulation of in vitro and in vivo immune response and inflammatory factor release by controlling the activation of downstream signaling molecules. In particular, gene coding mutations for phosphoinositide3-kinase (PI3K)/AKT/mTOR/S6 kinase (S6K) signaling cascade members or for molecules interacting with this pathway have been associated with various PIDs that are also characterized by the coexistence of both immune deficiency and autoimmunity (35). As a key regulator of immune responses, the serine/threonine kinase mechanistic/mammalian target of rapamycin (mTOR) acting downstream of PI3K and AKT is emerging. To regulate cell development, proliferation, and metabolism, it incorporates a range of signals from the microenvironment. Therefore, mTOR plays a central role in regulating the differentiation and function of immune cells (36). In most patients with ovarian cancer, phosphoinositol 3 kinase (PI3K)/protein kinase B (AKT)/mammalian rapamycin target (mTOR) and nuclear factor-and light chain activated B cell enhancer (NF afB) pathways are highly mutated and/or hyperactivated (37). Recent discovery of phosphoinositide-3-kinase (PI3K) signaling pathway mutations that can induce primary immunodeficiency provides useful insight into the role of PI3K signaling in the maturation and lytic function of human NK cells (38). Mutations that decrease PI3K activity often contribute to flawed production and function of lymphocytes; thus, too little or too much PI3K activity leads to immunodeficiency (39). During the differentiation process, the signaling pathway consisting of PI3K/Akt-NF-βB-Bcl-xL controls cell survival. PI3K/Akt-mediated activation of NF-βB plays a key role in the survival of macrophage differentiation by precisely preserving anti-apoptotic expression of Bcl-xL (40). Human immunodeficiency in certain PI3K-encoding genes may result from either loss or gain-of-function mutations (41). The family of nuclear hormone receptors that control immune and inflammatory responses are peroxisome proliferator-activated receptors (PPARs) (42). Activation of members of the peroxisome proliferator-activated receptor (PPAR) family has been shown to have beneficial effects in these interlinked pathologies, and these improvements are mainly due to the anti-inflammatory effects of activation of PPAR. Recent research has shown that PPARs play an important role in regulating different forms of inflammatory response. These functions are largely mediated by the ability of the isoforms PPAR alpha and PPARγ, using agonist-dependent mechanisms, to TRANS-REPRESS the activities of several activated transcription factors, including nuclear factor-zB (NF-zB), transcription signal transducers and activators (STATs), activator protein 1 (AP1) and activated T cell nuclear factor (NFAT). It is known that PPAR alpha and PPARγ are expressed by macrophages and dendritic cells (DCs), as well as by cells B and T. Most of the studies conducted have come to the common conclusion that PPAR activation can negatively control the induction of inflammatory responses, whether it is PPAR alpha or PPARγ specific (43, 44). In response to the chemokine CCL21, peroxisome proliferator-activated gamma receptor (PPARγ) and liver X receptor (LXR) prevented pro-inflammatory cytokine development by DCs and inhibited DC migration by preventing TLR-induced upregulation of CCR7 (45). A key enforcer of immune homeostasis and tolerance, transforming growth factor (TGF)-β inhibits the expansion and function of several components of the immune system. TGF-β is also essential to tumor microenvironment immune suppression, and recent studies have reported roles in tumor immune evasion and weak cancer immunotherapy responses (46). Patients are usually predisposed to infection by primary immunodeficiencies (PIDs) that mainly affect phagocytes (neutrophils and macrophages) (47). Staphylococcal infections in children with a variety of immunodeficiencies, including chronic granulomatous disease, are also prominent pathogens (48). Recent research has shown that the human malarial parasite Plasmodium falciparum has been shown to more specifically modulate host defenses by altering antigen-presenting cell function (49). We selected 8 DEGs as the hub genes and 20 core genes by degree of connectivity using STRING and MCODE. The gene card showed that complement component 7 deficiency and methotrexate-related lymphoproliferation diseases were associated with one of the hub genes UBE2C (50). The anaphase-promoting complex/cyclosome (APC/C) and the ubiquitin conjugating enzyme, E2C (UBE2C), are involved in the ubiquitin-proteasome pathway in initiating ubiquitin chain formation on substrates of APC/C. On these substrates, UBE2C produces mainly Lys-11 (K11)-linked polyubiquitination and then APC/C and another E2 enzyme, UBE2S, elongates and branches ubiquitin, generating more powerful signals of proteolytic degradation (i.e., on mitotic cyclins) for the proteasome receptor, S5A, controlling the progression of mitosis. The ubiquitin system controls various cellular processes; its dysregulation is therefore predicted to result in human diseases, including cancers. HOIL1 (RBCK1) results in linear ubiquitination assembly complex (LUBAC) disruption, so this gene can be used and screened for this RCBCK1 or some other immunodeficiencies as a biomarker (51). Previous research has established Aurora-A (AURKA) as the gene center of the genome wide association analysis focused on the gastric cancer linkage network (eGWAS). In addition, the siRNA knockdown of UBE2C significantly reduced the phosphorylation level of AURKA (p AURKA) via Wnt/β catenin and PI3K/Akt signaling pathways, suppressing the occurrence and development of gastric cancer (52). In the study, H19 was the top overexpressed gene and the study stated that H19 was the first imprinted non-coding transcript to be recognized, the function of this retained RNA remained unclear. Research has identified an H19-derived 23-nucleotide microRNA that is endogenously expressed in human keratinocytes and neonatal mice and overexpressed in cells transfected with plasmids of human or mouse H19 expression. H19 may act as a primary precursor of microRNA and indicate that during vertebrate development, H19 expression results in the posttranscriptional down-regulation of specific mRNAs (53). All immune system cells rely on a highly integrated and dynamic program of gene expression regulated by both transcriptional and post-transcriptional mechanisms. Lately, in various biological contexts, noncoding RNAs, including long non-coding RNAs (lncRNA), have emerged as major regulators of gene expression. By modulating transcription or by post-transcriptional mechanisms targeting the splicing, stability or translation of mRNAs, long non-coding RNAs regulate gene expression in the nucleus (54). Neurodegenerative changes observed in transmissible spongiform encephalopathies are associated with scrapie-responsive gene 1. In the host response to prion-associated infections, it can play a role (gene card reference). Inoue, M stated that through ERK1/2 activation in mouse macrophage Raw264.7 cells, SCRG1 suppresses LPS-induced development of CCL22 (55). In this research, the top down-regulated gene was HLA-A, as HLA antigens in lymphocyte differentiation are strongly indicated by the presence of a newly described bare lymphocyte immunodeficiency syndrome associated with the lack of expression of HLA-A, B, and C antigens as well as /gB2 microglobulin on different cells of hematopoietic origin (56). Genomics England (https://panelapp.genomicsengland.co.uk/panels/398/gene/CFD/#!) panel includes CFD in the Green List of Primary Immunodeficiency. Scoring of genes submitted on behalf of North West GLH by Tracy Briggs, David Gokhale and Abigal Rousseau for the GMS Immunology specialist test group. As, on 20 June 2019, the Specialist Test Group confirmed in a follow-up email that all agreed that there is enough evidence to rate this gene Green on PID causing genes. Wet lab testing of these genes is required to check their relationship with the other syndromes of innate immune deficiency and HOIL1 (RBCK1) (57). DNA replication is impaired by autosomal recessive partial GINS1 deficiency and is caused by intrauterine (and postnatal) growth retardation, chronic neutropenia, and NK cell deficiency (58). Thrombospondin-1 (THBS1), which regulates inflammation by involving multiple cell surface receptors and by modulating the activity of other secreted factors, plays a novel role in modulating the production and activation of proinflammatory cytokine IL-1β by human and murine macrophages in core genes with a high degree of connectivity. Thrombospondin-1 demonstrates the ability to disrupt the interaction between CD47 and CD14, thereby limiting lipopolysaccharide activation of

NFforB/AP-1 (59). Periostin (POSTN), a biomarker for systemic eosinophilic airway inflammation and subepithelial fibrosis, needs to be checked for other primary immunodeficiency disorders in asthmatic patients (60). Significant regulators of the actin cytoskeleton and mutations in CORO1A are members of the coronin family of proteins, encoding Coronin-1A, the predominant coronin expressed in lymphocytes, causing variable levels of T cell lymphopenia, susceptibility to infection and immune dysregulation in mice and humans (61). In addition, some pathways and genes with high degrees of differential expression may lead to progress and merit further discussion of RCBK1 deficiency and other innate immunodeficiency disorders. In this study, TK1, AURKB, CDCA2, UBE2C, KIFC1, CEP55, CDCA3, GINS2, MCM6 and CDC4 and genes identified as core genes, down-regulated and up-regulated may play a vital role in the development and progression of RBCK1 deficiency and other primary innate or adoptive immunodeficiency disorders. For the diagnosis and treatment of RBCK1 plus primary immunodeficiency disorders, they may be considered as possible biomarkers. In addition, in developing RBCK1 deficiency and other distinct innate primary immunodeficiency disorders, the PPAR signaling pathway and the Pi3k-Akt signaling pathway could also play critical roles in innate immune system remodeling. However, in order to confirm our current findings, more studies and further experiments with tissue or cells are required to verify the degree of expression of these DEGs. The current results have important implications for future research and could lead to the design of new therapies for patients with intrinsic immunodeficiency and RBCK1 deficiency. In the future, we expect more high-quality research to be performed.

Summery

Such microarray data and bioinformatics analyses, in short, provide a valuable tool for identifying key genes and pathways linked to RBCK1 deficiency. In addition, some essential DEGs identified in down-regulated, up-regulated, hub genes and core genes may play a critical role in the development and progression of RBCK1 and various other inherent immunodeficiency disorders.

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