

MicroRNA expression analysis for identification of biomarkers in inflammatory bowel disease subtypes

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Research Article

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Abstract

The potential biomarkers in inflammatory bowel diseases (IBDs) were analyzed from GSE53867 dataset. Differentially expressed microRNAs (DEMs)-genes and protein-protein interaction networks were constructed, and hub genes selected using Cytoscape. Differentially expressed genes were analyzed for GO and Reactome-pathway. Seven DEMs were upregulated in Crohn's disease (CD), 4 downregulated in ulcerative colitis (UC), 8 upregulated and 2 downregulated in IBD. A 620, 2377, and 1821 target-genes were in CD, UC, and IBD, respectively. SOCS3, upregulated by miR-650, was hub gene in CD, induced by cytokines, through NF κ B-signalling pathway to mediate ubiquitin-proteasomal degradation. CIRH1A, downregulated by miR-16, was hub gene of UC, acted by impairing ribosome-biogenesis. SKP2 and ASB1, up- and downregulated, by miR-142 and miR-665, respectively, were hub genes of IBD, induced cytokines through activation of TLR- and TNF-signalling pathways to mediate ubiquitin-proteasomal degradation. SOCS3, CIRH1A, SKP2 and ASB1 genes might serve as valuable biomarkers to differentiate CD, UC and IBD.

1. Introduction

The inflammatory bowel diseases (IBDs), including ulcerative colitis (UC) and Crohn's disease (CD), are chronic, idiopathic inflammation of the gastrointestinal tract (GIT), with 6.8 million cases worldwide, as reported in 2017 [1]; 1.64 million cases in the USA, and 1.4 million in India, reported in 2010 [2]. The potential causes of IBD are genetic susceptibility, environmental factors, and dysregulation of immune system [3]. CD can affect any area of the GIT, including the small intestine and colon, has patchy transmural inflammation; while UC affects only the colon, has continuous superficial inflammation [4]. The overlapping symptoms and complications of the disease along with limited treatment options having side effects, highlights the significance of developing specific diagnostic and novel therapeutic strategies in IBD. Recent studies have indicated the role of microRNA (miRNA) in IBD pathogenesis with therapeutic prospects. MiRNAs are small (18–24 nucleotides), single stranded, conserved, noncoding RNAs that bind to complementary 3'-UTRs (untranslated regions) of mRNA (messenger RNA) target causing instability and inhibition of translation, rendering miRNAs as important epigenetic modifiers of gene expression [5]. For example, miR-21, miR-31, and miR-141, have been implicated in IBD pathogenesis by targeting respectively PDCD4 and Rho GTPase RhoB, IL-25, and CXCL5 and CXCL12 β genes mediated inflammatory responses [6]. However, the pivotal molecular interactions of IBD based on differentially expressed genes (DEGs) have not been extensively investigated. Therefore, the current study is an attempt to identify the potential biomarkers that distinguish IBD and its subtypes, investigate underlying molecular interactions based on DEGs profiling, functional enrichment and significant pathways associated with the DEGs, protein-protein interaction (PPI) network of common DEGs. To achieve this, the GSE53867 dataset was downloaded from the Gene Expression Omnibus (GEO) database (www.ncbi.nlm.nih.gov/geo/). Differentially expressed miRNAs (DEMs) were identified between and unique to UC and CD patients, compared with controls. The DEMs-gene network and protein-protein interaction (PPI) network were constructed to identify potential biomarkers and major regulated genes.

GO (Gene Ontology) Process and Reactome pathway analysis was performed with modules of PPI network to determine the functional enrichment and significant pathways associated with the DEGs, to gain further insight into the pathogenesis of UC and CD at the molecular level, which may help in the differential diagnosis of UC and CD and determine prospective molecular targets for the IBD subtypes.

2. Methods

2.1. Microarray data acquisition

The miRNA expression dataset GSE53867 was obtained from the gene expression omnibus database (www.ncbi.nlm.nih.gov/geo/), containing two CD (CD1 and CD2), two UC (UC1 and UC2) compared to a single non-IBD control (C) human colon biopsy samples.

2.2. Identification of DEMs

The DEMs were identified between UC and C (defined as UC group), CD and C (defined as CD group), UC and CD (defined as IBD group) groups, using GEO2R (<http://www.ncbi.nlm.nih.gov/geo/geo2r/>) for performing comparisons on GSE53867 raw data applying GEOquery, limma R, and Biobase packages from the Bioconductor project [7,8]. The raw microarray data was preprocessed to obtain “NA” filtered expression data and normalized using “normalizeBetweenArrays” in limma, with quantile method. The normalized microarray expression data was fit to a linear model and compared between groups using Empirical Bayes moderated t test, also in limma, to obtain log₂FoldChange (log₂FC) differential expression of CD, UC, IBD, Average Expression (AveExpr), F-statistics (F), P value (P.Value) and FDR (False Discovery Rate) adjusted P value (adj.P.Value). The threshold for DEMs were set as P value <0.05 and |log₂FC| >1.

2.3. Analysis of target genes of DEMs

The target genes, possessing miRBase (Release 22.1, www.mirbase.org) [9] ID, of DEMs were analyzed using both validated and predicted targets obtained from the database miRWalk3.0 (<http://mirwalk.umm.uni-heidelberg.de>) [10]. Only those predicted targets were selected which overlapped all five databases including miRWalk, miRDB (www.mirdb.org) [11], miRanda (www.microrna.org › microrna) [12], RNA22 (version 2.0, <https://cm.jefferson.edu> › rna22) [13], and TargetScan (Release 7.2, www.targetscan.org) [14].

2.4. Construction of DEMs-gene network

A regulatory network of DEMs associated to genes was constructed using Cytoscape (<https://cytoscape.org/>) software, version 3.7.2 [15].

2.5. PPI network analysis

A PPI network was constructed with the predicted and validated target genes of DEMs using STRING (Search Tool for the Retrieval of Interacting Genes) (<http://string-db.org/cgi/input.pl>; version 1.5.0) App [16] of the Cytoscape software, version 3.7.2 (<https://cytoscape.org/>) [15].

2.6. Hub genes selection and identification of significant modules

The top 10 genes in the PPI network were selected using the Cytoscape plugin CytoHubba, version 0.1, ranked by Maximal Clique Centrality (MCC) method [17]. Genes possessing higher scores in the MCC analysis were considered hub genes.

The Cytoscape plugin MCODE (Molecular Complex Detection) version 1.5.1 was applied to obtain significant modules associated with the PPI network with degree cutoff 2, node score cutoff 0.2, k-core 2, and maximum depth 100 [18].

2.7. GO and Reactome pathway enrichment analyses

The DEGs were analyzed at the functional level for GO and Reactome pathway enrichment using the STRING Enrichment App of the Cytoscape software, with P value < 0.05 as the cutoff criterion.

3. Results

3.1. Normalization of raw data

The miRNA microarray signal intensity downloaded from NCBI GEO dataset GSE53867 before and after normalization have been indicated in Fig. 1a and Fig. 1b.

3.2. Identification of DEMs

Analysis of normalized GSE53867 expression data using GEO2R yielded 16 DEMs within CD types (CD1 and CD2), UC types (UC1 and UC2), and C samples as shown with a heatmap in Fig. 1c. There were 7 upregulated DEMs including hsa-miR-24-2-5p, hsa-miR-650, hsa-miR-24-1-5p, hsa-miR-491-3p, hsa-miR-891a-5p, hsa-miR-27a-5p, and hsa-miR-1204 in the CD group; 4 downregulated DEMs including hsa-miR-1973, hsa-miR-142-3p, hsa-miR-342-3p, and hsa-miR-16-5p in the UC group; 10 DEMs including 8 upregulated (hsa-miR-491-3p, hsa-miR-24-2-5p, hsa-miR-650, hsa-miR-24-1-5p, hsa-miR-523-3p, hsa-miR-422a, hsa-miR-142-3p, and hsa-miR-1973) and 2 downregulated (hsa-

miR-519c-5p and hsa-miR-665) in the IBD group; miRNAs expressed in order of descending log₂FoldChange (log₂FC) (P value<0.05 and |log₂FC| >1).

The upregulated and downregulated DEMs among CD, UC, and IBD groups have been represented with volcano plots in Fig. 2a, Fig. 2b, and Fig. 2c respectively. The results of the Empirical Bayes moderated t test have been represented graphically in terms of log₂FC, Average Expression (AveExpr), F-statistics (F), P value (P.Value) and FDR (False Discovery Rate) adjusted P value (adj.P.Value) in Fig. 2d.

3.3. Target genes of DEMs

The number of target genes obtained from the databases were 725 (478 validated and 247 predicted), 3313 (3003 validated and 310 predicted), and 2201 (1654 validated and 547 predicted), in CD, UC, and IBD, group respectively; after removal of duplicate target genes resulted into 620, 2377, and 1821 number of genes in the CD, UC, and IBD, group respectively, an amount of which were further used for construction of regulatory network between the miRNAs and their targets. The miRNAs, hsa-miR-886-3p, hsa-miR-886-5p, and hsa-miR-1979 were excluded from the DEMs-Gene network, due to non-availability of records in the miRNA database, miRBase and withdrawn status from Hugo Gene Nomenclature Committee (HGNC) (<https://www.genenames.org>); hsa-miR-519d was excluded from the network due to lack of mature sequence miRNA in the dataset. Fig. 1d represents the Venn diagram indicating a total of 620 DEGs in the CD group, 2377 DEGs in the UC group, and 1821 DEGs in the IBD group, out of which a total of 110 DEGs fitted all the three groups, while 149, 659, and 501 DEGs overlapped CD and UC, UC and IBD, CD and IBD respectively.

3.4. DEMs-Gene Network

The DEMs-Gene regulatory network of the CD, UC, and IBD, group constructed using Cytoscape, is depicted in Supplement Figures (Fig. 1S1, Fig. 1S2, and Fig. 1S3), respectively (confidence score cutoff 0.7).

3.5. PPI network

The PPI network visualized using Cytoscape showed 1147, 422, 1482, 1020 protein-protein interactions among 571, 300, 947, 762 proteins (confidence score cutoff 0.7 and P<0.05 as the cut-off criterion) in the CD, UC, upregulated and downregulated IBD, groups respectively.

3.6. Determination of hub genes and major modules

The top 10 hub genes in the CD, and UC groups are represented in Supplement Fig. 2Sa and Fig. 2Sb, respectively with MCC scores ranging from 363039–362884 and 175–85, respectively. The score of hub

genes by MCC was $2.09E+13$ and $1.31E+12$ in the upregulated and downregulated IBD group respectively, as shown in Supplement Fig. 2Sc and Fig. 2Sd, respectively. The top 10 upregulated hub genes of the CD group included SOCS3, WSB1, ASB6, UBE2D2, UBE2D3, CDC27, UBE2B, NEDD4L, UBR1, LTN1, and top 10 downregulated hub genes specific to the UC group included CIRH1A, PNO1, PWP2, UTP3, DNMT1, RSL1D1, RPSA, KDM6B, H2AFX. In the IBD group, the top 10 upregulated hub genes were SKP2, CDC27, SOCS3, CUL5, ASB4, WSB1, ASB6, UBE2D3, UBE2D2, UBE2B, while the top 10 downregulated hub genes included ASB1, WSB1, FBXW8, FBXW2, UBE2F, KLHL25, ASB11, UBA52, RPS27A, SMURF2. MCODE was used to establish major modules, which demonstrated 16, 7, 22, and 16 modules containing genes, number ranging from 22–5, 14–3, 44–3, and 33–3 respectively and score ranging from 10–2.5, 6.571–2.6, 17–2.8, and 16–3 in the CD, UC, IBD upregulated, and IBD downregulated groups respectively.

3.7. GO and pathway enrichment analyses of DEGs

A functional enrichment of the screened DEGs in the CD and IBD groups (PPI enrichment value $1.0E-16$), indicated that most of the DEGs were closely associated with protein ubiquitination GO Process (GO.0016567) and class I MHC mediated antigen processing Reactome pathway (HSA-983168) related to ubiquitination & proteasome degradation (Fig. 3). Among UC genes, GO Process (GO.0006364) and Reactome pathway (HSA-6790901) enrichment were significantly related to rRNA processing and modification respectively (PPI enrichment value $3.28E-10$) (Fig. 3). The representative KEGG pathways of statistically enriched DEGs in CD, IBD, and UC are represented in Fig. 4a, 4b, and 4c respectively. In the GO Process and pathway analysis, the top enriched pathways (PPI enrichment $1.0E-16$) in CD included respectively protein ubiquitination and class I MHC mediated antigen processing related to ubiquitination and proteasome degradation (FDR $9.69E-1$, Reactome pathways) (Fig. 3). However, other GO terms and pathways included post-translational protein modification, negative regulation of intracellular signal transduction, neddylation, synthesis of active ubiquitin including roles of E1 and E2 enzymes in CD exhibited significant expression.

In the GO and pathway analysis, the top enriched pathways in UC included rRNA processing and modification in the nucleus and cytosol. In addition, the GO terms in the category biological process significantly enriched by these genes were chromatin organization and remodeling, ribosomal small subunit assembly, double-strand break repair via nonhomologous end joining. However, other GO terms and pathways included oxidative stress induced senescence, DNA methylation, regulation of gene silencing, pallium development, positive regulation of DNA metabolic process in UC exhibited significant expression.

The top enriched pathways in upregulation of IBD included antigen processing related to ubiquitination and proteasome degradation; synthesis of active ubiquitin including roles of E1 and E2 enzymes. In addition, APC/C:Cdh1 mediated degradation of Cdc20 and other APC/C:Cdh1 targeted proteins in late mitosis/early G1 exhibited significantly enriched pathways. In GO analysis, the top enriched biological processes in upregulation of IBD included protein ubiquitination and mitotic cell cycle phase transition.

The top enriched pathways in downregulation of IBD included antigen processing related to ubiquitination & proteasome degradation, downregulation of SMAD2/3:SMAD4 transcriptional activity, josephin domain deubiquitins. In GO analysis, the top enriched biological processes in upregulation of IBD included protein ubiquitination, post-translational protein modification, nucleotide-binding oligomerization domain containing signaling pathway, regulation of catabolic process.

4. Discussion

Idiopathic IBD, which includes CD and UC, creates several diagnostic impediments owing to common clinical, radiographic, endoscopic, and histologic characteristics. Thus it is important to recognize the subtypes of IBD for developing specific diagnostic and novel therapeutic intervention in IBD. The miRNAs are potential candidates for therapeutic application owing to well-defined mechanism of action during dysregulation in human IBD and simple oligonucleotide design [19]. This paper investigated the expression pattern of miRNAs in IBD in comparison to the other IBD subtypes, namely CD and UC, in order to identify miRNAs as potential diagnostic and prognostic biomarkers to better delineate the IBD subtypes.

In the present study, an analysis of the GSE53867 expression dataset revealed 16 DEMs (P value <0.05 and $|\log_2FC| >1$), including 10 upregulated and 6 downregulated DEMs, in IBD and its subtypes. Among the 16 DEMs, 3 miRNAs including miR-891a-5p, miR-27a-5p, miR-1204 were unique to the CD group, 2 miRNAs such as miR-342-3p, miR-16-5p were unique to the UC group, 4 miRNAs including miR-523-3p, miR-422a, miR-665, miR-519c-5p belonged exclusively to the IBD group. However, the common miRNAs under the UC and IBD were miR-1973, miR-142-3p and those under CD and IBD were miR-24-2-5p, miR-650, miR-24-1-5p, miR-491-3p; no common miRNAs belonged concomitantly to both UC and CD groups. This finding supported the presence, in the colon tissues, of miRNAs: miR-891a-5p, miR-27a-5p, miR-1204, indicative of CD; miRNAs miR-342-3p, miR-16-5p could be applied as a biomarker to identify UC; miRNAs including miR-523-3p, miR-422a, miR-665, miR-519c-5p could implicate the possibility of IBD diagnosis, while the presence of common miRNAs within CD and IBD, UC and IBD could be suggestive of overlapping epigenetic modifiers targetable by combined therapeutic applications. Occurrence of a similar few signature miRNAs included elevated miR-16 and miR-24, decreased miRNA miR-650, in mucosal tissue of UC; similar miRNAs miR-16 and miR-422a have been found significantly upregulated in the mucosal tissue of CD compared to normal healthy controls [20–22]. The panel of microRNAs that have been found differentially expressed between UC and CD in colonic tissue included miR-19b, miR-23b, miR-100a-3p, miR-100b-5p, miR-106a, miR-150, miR-191, miR-196b, miR-223, miR-320a, and miR-629 [23]. The colonic microRNA panels reported by different studies attempting to delineate UC, CD, between UC and CD exhibited little overlap, due to discordance in the activity status of IBD during sampling, location of tissue, inflammation level, previous medication, platforms for micro-RNAs estimation [24].

In the current study, genes which concurrently fitted 5 databases (miRWalk, miRDB, miRanda, RNA22, and TargetScan) were chosen as predicted target genes, and validated target genes from miRWalk were taken,

amounting to 1673 upregulated and 3145 downregulated genes. The miRWalk database contains information about predicted interactions based on integration of several algorithms and experimentally validated interactions as well (<http://mirwalk.umm.uni-heidelberg.de>) [10]. Selecting interactions predicted by multiple tools ensures enhanced recovery of identical miRNA-gene interactions. Cytoscape tool was used to visualize the interaction data on the predicted and validated miRNA and their putative target gene regulatory network structure [15]. There were 620 DEGs identified in the CD group targeted by 6 upregulated DEMs including miR-24 (n = 98), miR-650 (n = 341), miR-491 (n = 92), miR-891 (n = 14), miR-27 (n = 62), and miR-1204 (n = 12) (n = DEGs) (Supplement Fig. 1S1); 2377 DEGs identified in the UC group targeted by 4 downregulated DEMs including miR-1973 (n = 10), miR-142 (n = 409), miR-342 (n = 358), and miR-16 (n = 1600) (Supplement Fig. 1S2); 1821 DEGs targeted by 9 DEMs amongst which 7 upregulated DEMs including miR-491 (n = 92), miR-24 (n = 98), miR-650 (n = 341), miR-523 (n = 4), miR-422 (n = 99), miR-142 (n = 409), miR-1973 (n = 10) and 2 downregulated DEMs including miR-519 (n = 95), miR-665 (n = 673) identified in the IBD group (Supplement Fig. 1S3). The UC and CD groups had 149 DEGs in common, while 110 DEGs fitted all the three groups (Fig. 1d), implying overlapping genetic constituent within CD, UC and IBD. The PPI network was visualized using Cytoscape among 571, 300, 947, 762 proteins in the CD, UC, upregulated and downregulated IBD, groups respectively, followed by analysis of the most connected nodes, considered as key regulators of pathways and biological functions (Supplement Fig. 2S). Among the eleven topological analysis methods in CytoHubba, the MCC method, which was based on the degree of connectivity of each protein and the size of the interactions connected to the rest of the network, was proposed to have superior accuracy of identifying hub genes encoding proteins in the network [17]. Hub genes, being central element of the PPI network, serve as prospective biomarkers, therapeutic targets and novel tool for analyzing crucial mechanisms regulating disease processes.

The top ranking hub gene of the CD group was SOCS3 (Suppressor Of Cytokine Signaling 3) upregulated by miR-650 ($\log_2FC = 1.745$, P value = 0.018561). Cheng et al reported the role of miR-19b in inhibiting inflammatory response by downregulating SOCS3 to alter chemokine production in CD [25]. PPI from cytoscape showed cytoplasmic expression of SOCS3 in most tissues and additionally in the plasma membrane; among blood cell types, it was specific to neutrophils. SOCS3 was expressed in the cells of epithelium and lamina propria in the colon in IBD, UC and CD cases [26]. SOCS3 as a member of the SSI (STAT-induced STAT inhibitor) family act to inhibit cytokine signal transduction through the JAK/STAT pathway [27]. SOCS3 also acted as a substrate recognition component of a SCF-like ECS (Elongin B/C-Cul2/Cul5-SOCS-box protein) E3 ubiquitin ligase complex to mediate ubiquitination and subsequent proteasomal degradation of cytokine receptors in inflammation [28].

In the IBD group, the key gene SKP2 (S-Phase Kinase Associated Protein 2) was upregulated by miR-142-3p ($\log_2FC = 1.145$, P value = 0.029848), while ASB1 (Ankyrin repeat and SOCS box protein 1) was downregulated by miR-665 ($\log_2FC = -1.03$, P value = 0.009245). Zhang et al demonstrated the role of miR-665 in promoting apoptosis and colitis in IBD by inhibiting ER stress components XBP1 and ORMDL3 [29]. SKP2 was tissue enhanced in placenta (<https://www.proteinatlas.org>) with nuclear and

cytoplasmic expression and low blood cell type specificity (PPI data); ASB1 was localized in the nucleoplasm (<https://www.proteinatlas.org>), had low tissue specificity, and the protein expression was mainly in the CNS, adrenal medulla, parathyroid gland and enteroendocrine cells (<https://www.proteinatlas.org>).

Among the top hub genes of each group, the top 7 upregulated hub genes of the CD group namely, SOCS3, WSB1, ASB6, UBE2D2, UBE2D3, CDC27, UBE2B were also expressed as the upregulated hub genes of the IBD group, while the WSB1 gene from the CD group was concomitantly present in the downregulated hub genes of the IBD group (Supplement Fig. 2S). The DEGs in both CD and IBD groups were significantly enriched (PPI enrichment $1.0E-16$) in pathways such as class I MHC mediated antigen processing related to ubiquitination & proteasome degradation (Fig. 3). Analysis of biological processes showed that the DEGs in both CD and IBD groups were significantly enriched in protein ubiquitination (Fig. 3). Intracellular antigens and cytokines induce proteasome, immunoproteasome containing PA 28 and other accessory particles, for further degradation of the ubiquitinated substrates by an ATP-dependent mechanism (Fig. 4a). Peptides in association with HSP chaperones are translocated by TAP to the endoplasmic reticulum (ER) lumen, where they are placed on the MHC class I complex, made of heavy chain and $\beta 2m$ (Fig. 4a). MHC I fold and assemble in ER, assisted by several ER chaperones. MHC class I complexes present peptides on the cell surface, for recognition by CD8+ T cells and NK cells. The SOCS3 and SKP2 genes, in the present study were up-regulated during the course of CD and IBD respectively concomitant with upregulation of pro-inflammatory cytokine TLR6 in both CD and IBD; upregulation of pro-inflammatory cytokines such as IL6, CCL22, IL-1A in IBD only, through activation of TLR- and TNF-signalling pathways via activation of NFKB (nuclear factor kappa B) pathway; JAK signaling pathway and cytokine-cytokine receptor interaction all leading to ubiquitin mediated proteolysis, by K63-linked polyubiquitin chains (Fig. 4b). The ubiquitin proteasome pathway, is essential for protein degradation carried out by three classes of enzymes E1, E2, and E3; E1 and E2 prepare ubiquitin chains that are then linked to proteins by the E3 [30]. The SOCS3, SKP2, and ASB1 are components of the SOCSbox in ECS complex of E3 ubiquitin ligase containing CUL5, RNF7/RBX2, Elongin BC complex and SOCSbox [28].

In TLR (toll-like receptor) mediated signaling (Fig. 4b), the microbial components like flagellin, peptidoglycan, and lipopolysaccharide are recognized by TLR6 (paralog of TLR2), which in association with TLR2, trigger the recruitment of the adaptor protein MyD88 (Myeloid Differentiation Primary Response 88), the TOLLIP (Toll Interacting Protein), and TIRAP (Toll/Interleukin-1 Receptor Domain-Containing Adapter Protein), through interleukin-1 receptor-associated kinases IRAK1 and IRAK4, in combination with RNF152 (Ring Finger Protein 152/ RING-Type E3 Ubiquitin Transferase RNF152) to stimulate K63-linked polyubiquitination. This complex, in turn, creates a binding platform for the assembly of the signaling genes, the TAK1, TAB1, TAB2, the TGF- β -activated kinase 1, TAK1-binding protein 1, 2 respectively, to cause the attachment of linear polyubiquitin chains on NFKB (Nuclear Factor Kappa B) essential modifier (NEMO) from the inhibitor of Kappa B kinase (IKBK) complex comprising CHUK (Conserved Helix-Loop-Helix Ubiquitous Kinase), IKBK- β /IKBKB (Inhibitor Of Nuclear Factor Kappa B Kinase Subunit Beta), and NEMO. The NEMO from the IKBK complex mediate K63-linked polyubiquitin binding on RNF152 to facilitate IKBK- β activation and successive phosphorylation of inhibitor of Kappa B

alpha (IKBA) for ubiquitination with K63 linkages by the E3 ligase ECS complex to induce IKB degradation. In ubiquitin-mediated JAK signaling (Fig. 4b), cytokine-induced receptor dimerization results in tyrosine phosphorylation of JAK2 followed by binding of SOCS protein at its SH2 domain leading to ECS complex mediated K63 polyubiquitination and proteasomal degradation of JAK2 and termination of JAK signaling process. In TNF signalling pathway (Fig. 4b), tumor necrosis factor (TNF) binds to the tumor necrosis factor receptor (TNFR)1, to trigger the recruitment of TRADD (TNFR1-Associated Protein), TRAF2/5 (TNF Receptor Associated Factor 2/5), RIP1 [Receptor (TNFRSF)-Interacting Serine-Threonine Kinase 1]. This, in turn, causes binding of TAK1, TAB1 and TAB2 ultimately leading to NFKB signaling pathway followed by K63-linked ubiquitin mediated proteolysis of IKBA.

Among the genes screened from the differentially expressed genes in UC, CIRH1A (Cirrhosis, Autosomal Recessive 1A) gene, in the present study was downregulated by miR-16-5p ($\log_2FC = -1.195$, P value = 0.04479). CIRH1A showed low blood cell type and tissue specificity (PPI data) with nucleolar expression in several tissues (<https://www.proteinatlas.org>), mainly in neuronal cells. CIRH1A, encode WD40-repeat-containing protein, involved in nucleolar processing of small subunit pre-18S rRNA. In the GO and pathway analysis, the top enriched pathways in UC included rRNA processing and modification in the nucleus and cytosol (Fig. 3). The CIRH1A gene, specific to the UC group, was down regulated concomitant with downregulation of U3 snoRNP complex, 90S pre-ribosome components, pre-40S, pre-60S, and export factors components (Fig. 4c). Impairment of ribosome biogenesis induce ER stress leading to proinflammatory cytokine production via NFKB activation with IKK (IKBA kinase) activity maintained at basal level via IRE1 (inositol-requiring ER-to-nucleus signal kinase 1), an ER stress sensor [31]. The significantly downregulated genes in UC, in the present study, included receptor families such as TLR6 via NFKB signaling pathway; downregulation of pro-inflammatory cytokines such as IL6, IL-1A, IFNG through activation of JAK signaling pathway and cytokine-cytokine receptor interaction (Fig. 4b). IL-6 enhanced upregulation of rRNA transcription stimulated the MDM2 (mouse double minute 2 homolog)-mediated p53 proteasomal digestion, by reducing the availability of ribosome proteins for MDM2 binding [32].

5. Conclusions

It was found that the SOCS3, CIRH1A, SKP2, and ASB1 genes might play predominant role in the pathogenesis of CD, UC, IBD during upregulation, and downregulation respectively. The SOCS3 was upregulated by miR-650 in CD, induced by various cytokines, through the NFKB signalling pathway to mediate ubiquitination and subsequent proteasomal degradation of cytokine receptors in inflammation. CIRH1A gene downregulated by miR-16 was specific to the UC group, acted by impairing ribosome biogenesis to induce ER stress via NFKB signaling pathway, JAK signaling pathway and cytokine-cytokine receptor interaction. SKP2 was upregulated by miR-142 and ASB1 was downregulated by miR-665, to induce pro-inflammatory cytokines such as IL6, CCL22, IL-1A in IBD through activation of TLR- and TNF-signalling pathways via activation of NFKB pathway; JAK signaling pathway and cytokine-cytokine receptor interaction all leading to ubiquitin mediated proteolysis. The SOCS3, CIRH1A, SKP2 and

ASB1 genes might serve as valuable biomarkers to differentiate CD, UC and upregulated IBD and downregulated IBD respectively.

Declarations

Conflict of Interest: We declare that there is no conflict of interest.

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Supplement Figure Captions

Fig. 1S. Differentially expressed microRNA-gene regulatory network: 1. CD; 2. UC; 3. IBD.

Fig. 2S. Top 10 hub genes: *a.* CD; *b.* UC; *c.* IBD upregulated; *d.* IBD downregulated.

Figures

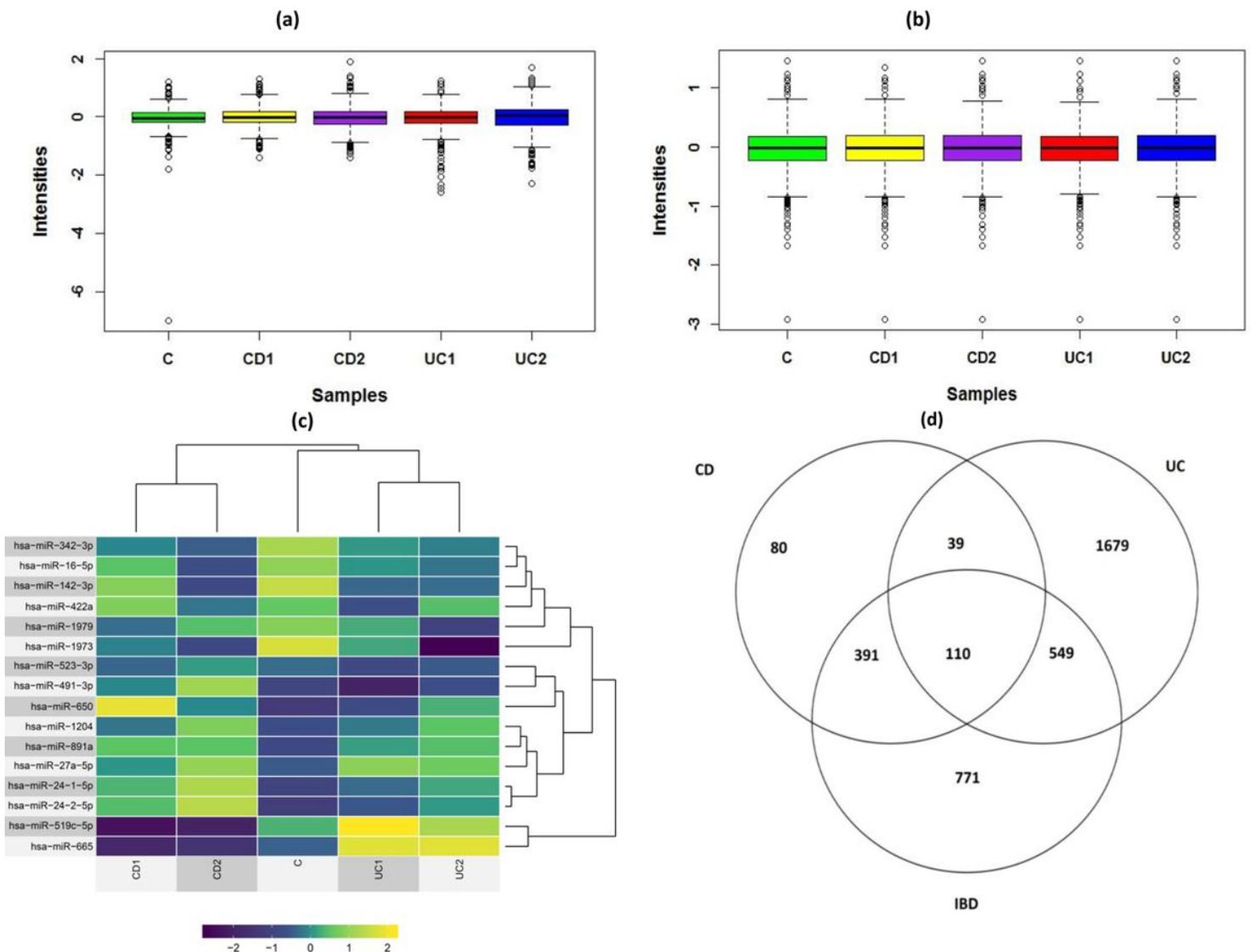


Figure 1

a. miRNA microarray signal intensity before normalization. b. miRNA microarray signal intensity after normalization. c. Heatmap of normalized expression dataset. d. Venn diagram of differentially expressed genes in CD, UC, and IBD.

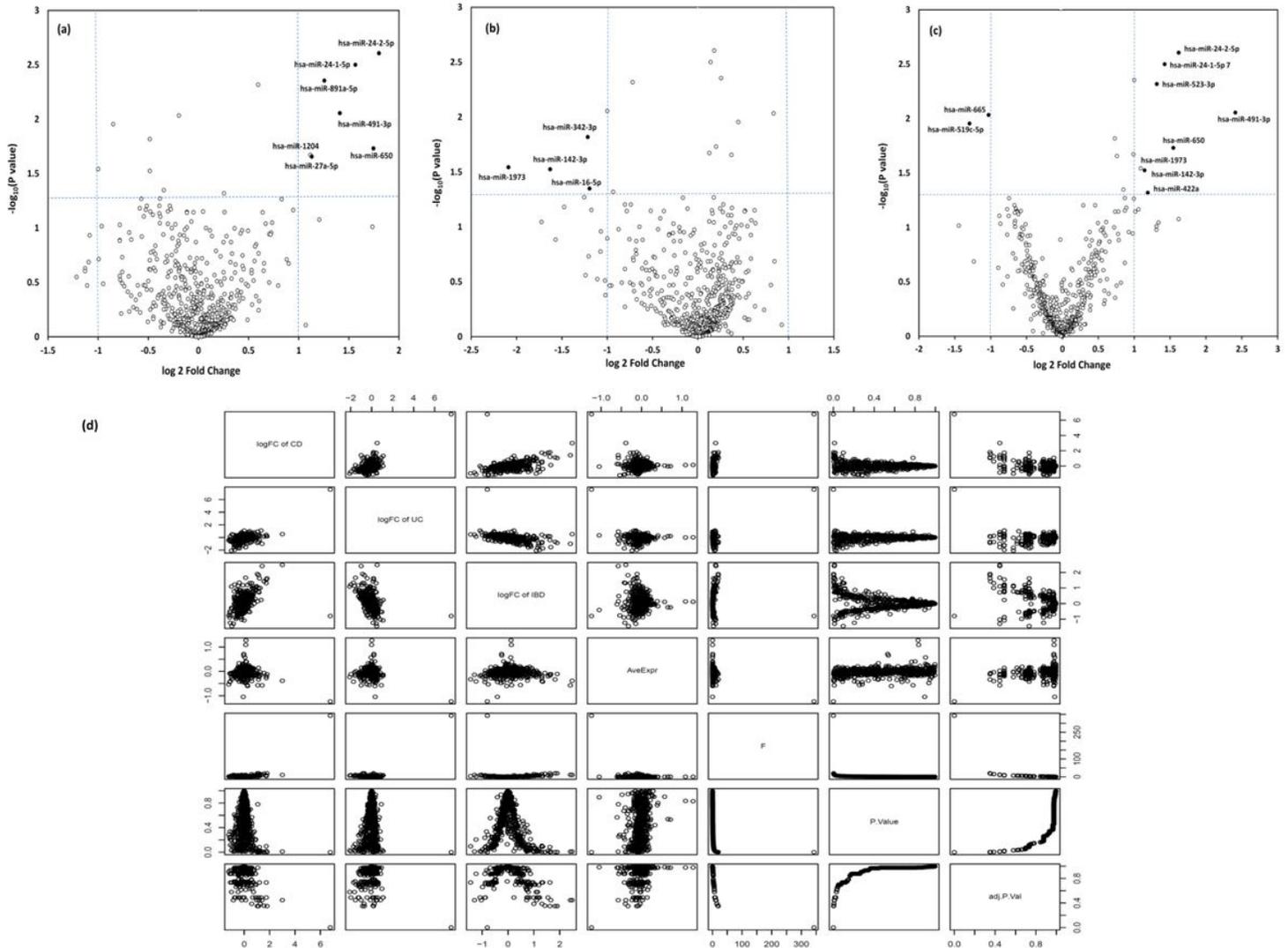


Figure 2

Volcano plots of the upregulated and downregulated differentially expressed microRNAs a. CD. b. UC c. IBD, and d. Plot of Empirical Bayes moderated t test.

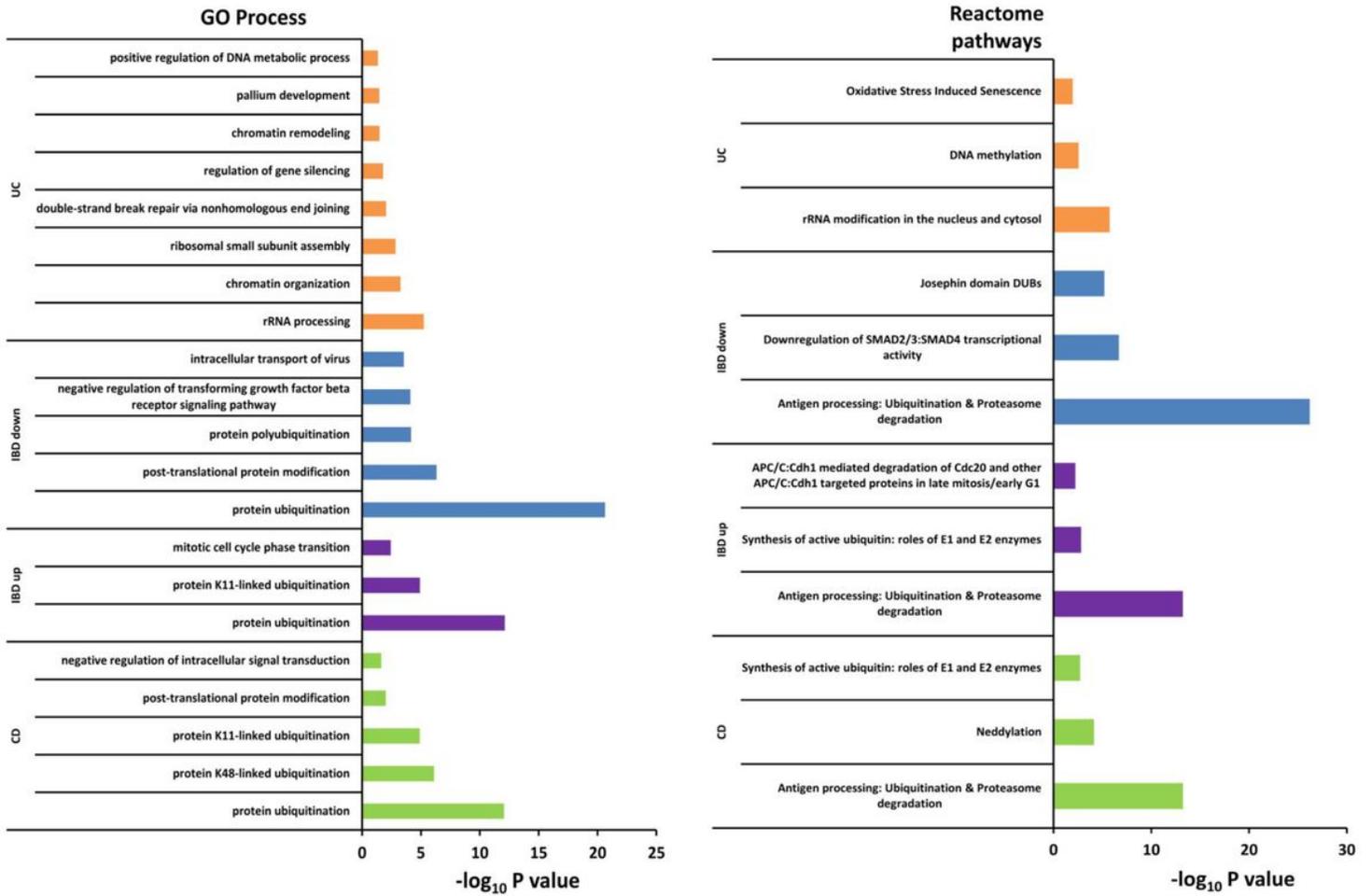


Figure 3

Gene Ontology and pathway enrichment analyses of differentially expressed genes.

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