



Dysregulation of Signaling Pathways Due to Differentially Expressed Genes From the B-Cell Transcriptomes of Systemic Lupus Erythematosus Patients – A Bioinformatics Approach

S. Udhaya Kumar¹, D. Thirumal Kumar¹, R. Siva¹, C. George Priya Doss^{1*},
Salma Younes², Nadin Younes², Mariem Sidenna² and Hatem Zayed^{2*}

OPEN ACCESS

Edited by:

Manoj Kumar Kashyap,
Amity University Gurgaon, India

Reviewed by:

Jian Yu,
Beihang University, China
Noor Ahmad Shaik,
King Abdulaziz University,
Saudi Arabia

*Correspondence:

C. George Priya Doss
georgepriyadoss@vit.ac.in
Hatem Zayed
hatem.zayed@qu.edu.qa

Specialty section:

This article was submitted to
Bioinformatics and Computational
Biology,
a section of the journal
Frontiers in Bioengineering and
Biotechnology

Received: 06 January 2020

Accepted: 16 March 2020

Published: 30 April 2020

Citation:

Udhaya Kumar S, Thirumal
Kumar D, Siva R, George Priya
Doss C, Younes S, Younes N,
Sidenna M and Zayed H (2020)
Dysregulation of Signaling Pathways
Due to Differentially Expressed Genes
From the B-Cell Transcriptomes
of Systemic Lupus Erythematosus
Patients – A Bioinformatics Approach.
Front. Bioeng. Biotechnol. 8:276.
doi: 10.3389/fbioe.2020.00276

¹ School of Biosciences and Technology, Vellore Institute of Technology, Vellore, India, ² Department of Biomedical Sciences, College of Health and Sciences, QU Health, Qatar University, Doha, Qatar

Systemic lupus erythematosus (SLE) is an autoimmune inflammatory disorder that is clinically complex and has increased production of autoantibodies. Via emerging technologies, researchers have identified genetic variants, expression profiling of genes, animal models, and epigenetic findings that have paved the way for a better understanding of the molecular and genetic mechanisms of SLE. Our current study aimed to illustrate the essential genes and molecular pathways that are potentially involved in the pathogenesis of SLE. This study incorporates the gene expression profiling data of the microarray dataset GSE30153 from the Gene Expression Omnibus (GEO) database, and differentially expressed genes (DEGs) between the B-cell transcriptomes of SLE patients and healthy controls were screened using the GEO2R web tool. The identified DEGs were subjected to STRING analysis and Cytoscape to explore the protein–protein interaction (PPI) networks between them. The MCODE (Molecular Complex Detection) plugin of Cytoscape was used to screen the cluster subnetworks that are highly interlinked between the DEGs. Subsequently, the clustered DEGs were subjected to functional annotation with ClueGO/CluePedia to identify the significant pathways that were enriched. For integrative analysis, we used GeneGo Metacore™, a Cortellis Solution software, to exhibit the Gene Ontology (GO) and enriched pathways between the datasets. Our study identified 4 upregulated and 13 downregulated genes. Analysis of GO and functional enrichment using ClueGO revealed the pathways that were statistically significant, including pathways involving T-cell costimulation, lymphocyte costimulation, negative regulation of vascular permeability, and B-cell receptor signaling. The DEGs were mainly enriched in metabolic networks such as the phosphatidylinositol-3,4,5-triphosphate pathway and the carnitine pathway. Additionally, potentially enriched pathways, such as the signaling pathways induced by oxidative stress and reactive oxygen species (ROS), chemotaxis and lysophosphatidic acid signaling induced via G protein-coupled receptors (GPCRs), and the androgen

receptor activation pathway, were identified from the DEGs that were mainly associated with the immune system. Four genes (*EGR1*, *CD38*, *CAV1*, and *AKT1*) were identified to be strongly associated with SLE. Our integrative analysis using a multitude of bioinformatics tools might promote an understanding of the dysregulated pathways that are associated with SLE development and progression. The four DEGs in SLE patients might shed light on the pathogenesis of SLE and might serve as potential biomarkers in early diagnosis and as therapeutic targets for SLE.

Keywords: systemic lupus erythematosus, protein–protein interactions, Metacore, microarray and bioinformatics, expression profiling data, biomarkers, functional enrichment analysis

INTRODUCTION

Systemic lupus erythematosus (SLE), also known as lupus, is a rare systemic autoimmune disease that mostly affects middle-aged women, mainly of Asian, African, American, and Hispanic origin (Costa-Reis and Sullivan, 2013; Cui et al., 2013; Gurevitz et al., 2013). SLE affects an estimated 5 million people across the world, with an incidence of 1–10 per 100,000 person-years (Pons-Estel et al., 2010). SLE is characterized by a wide range of different autoantibodies, deposition of immune complexes, and immune system infiltration and inflammation within damaged organs. SLE autoantibodies invade the patient's kidneys, heart, skin, joints, and brain, leading to various typical clinical symptoms. The most common clinical symptoms of lupus are rash, arthritis, and fatigue. Severe complications of SLE lead to nephritis, anemia, neurological symptoms, and thrombocytopenia, eventually leading to severe morbidity and mortality.

SLE is characterized by its clinical heterogeneity, with a wide range of clinical manifestations reflecting its complex etiopathogenesis (Tan et al., 1982). The clinical heterogeneity of SLE highlights the contribution of genetic and environmental factors to the susceptibility to the disease (Prokunina and Alarcon-Riquelme, 2004; Harley et al., 2009; Yang and Lau, 2015; Dang et al., 2016; Wang et al., 2017). To date, the reason for phenotypic variation in SLE is unknown. Understanding the molecular mechanisms behind the pathogenesis of SLE phenotypes could help in developing more efficient therapeutic approaches and preventive strategies.

With the extensive use of gene detection methods, high-throughput sequencing and extensive microarray data profiling studies on SLE have been conducted, and several differentially expressed genes (DEGs) and cellular pathways in SLE have been identified (Borrebæck et al., 2014; Zhu et al., 2015). Nevertheless, until now, no particular gene has been recognized to act as a potential marker for the diagnosis of SLE. In addition, a large amount of data obtained from microarray technology and high-throughput sequencing have not been fully used. Ducreux et al. (2016) collected blood samples from SLE patients and healthy volunteers to identify differentially expressed genes (Ducreux et al., 2016). However, the interactions among differentially expressed genes and key genes involved in the signaling pathways of SLE remain to be elucidated. In addition, previous studies of genetic factors primarily focused on single genes; nevertheless,

interactions among multiple genes may result in the multisystem invasion characteristics observed in SLE (Smith et al., 2017). Remarkably, studies have shown that disease-associated gene expression networks have a potential role in the immune response, which highlights their mechanism and therapeutic value for SLE (Deng and Tsao, 2010; Bentham et al., 2015).

Integrating and reanalyzing the data using bioinformatics methods may help in identifying gene regulatory pathways, essential genes, and their associated networks in SLE disease, which can provide new and valuable ideas for understanding the molecular mechanisms and identifying reliable diagnostic and therapeutic targets of SLE. Therefore, in this study, we first conducted a comprehensive collection of genes associated with SLE from the GEO dataset with ID GSE30153. Then, we performed a bioinformatics analysis of these genes with the MCODE (Molecular Complex Detection), GeneGo, and ClueGO tools. To further explore the pathogenesis of SLE in a more specific manner, functions and pathways identified by the modules were used to indicate the biological processes and biochemical pathways related to the immune system. Finally, the genes potentially associated with arthritis, pleurisy, and myocarditis, which are the common complications of SLE, were compared with SLE-related genes to identify common genes that participated in the development of SLE. To interpret the biological relevance of these changes in gene expression, we analyzed the microarray data via an integrated bioinformatic analysis expanding on traditional microarray analysis methods, namely, Gene Ontology (GO) and pathway analysis, thereby allowing the construction of interaction networks that might identify novel prognostic markers and therapeutic targets.

MATERIALS AND METHODS

Acquisition of Array Data and Processing

Gene expression profiling data from microarray array analysis of the GSE30153 dataset were downloaded from the NCBI GEO database (Gene Expression Omnibus database)¹. The database accommodates gene expression datasets from a variety of experiments, such as DNA-seq, ChIPs, RNA-seq, microarray, and high-throughput hybridization array (Edgar et al., 2002; Barrett et al., 2013). GSE30153 contains 26 samples, including 17 patients

¹<https://www.ncbi.nlm.nih.gov/geo/>

with SLE and 9 healthy controls of human sorted B-cells obtained by using the platform GPL570 (HG-U133_Plus_2) Affymetrix Human Genome U133 Plus 2.0 Array (Garaud et al., 2011). The downloaded gene expression profiling data are freely available in the public database, and there were no human or animal experiments conducted by any of the authors in this study.

Preprocessing of Data and DEG Identification

Using the robust multiarray standard model, the initial information from the dataset was subjected to quantile normalization, background correction, and log transition (Irizarry et al., 2003). Preprocessing included changing to gene symbols from probe IDs using the Gene ID converter from Entrez (Alibés et al., 2007). The statistical online tool GEO2R uses the R/Bioconductor, and limma package v3.26.8 was used to screen the raw gene expression data (Smyth, 2005; Barrett et al., 2013; Ritchie et al., 2015). We performed a Benjamini–Hochberg test (to determine the false discovery rate) and *T*-tests to compute the false discovery rate (FDR) and *p*-values to identify the DEGs between SLE patients and healthy control human sorted B-cells (Benjamini and Hochberg, 1995; Aubert et al., 2004). We set the primary criteria of $|\log(2 \text{ fold change})| > 1$ and $p < 0.05$ to obtain significant DEGs from the dataset, whereas cutoffs of $\log_2\text{FC} \geq 1$ and $\log_2\text{FC} \leq -1$ were used to denote upregulated and downregulated DEGs, respectively. For high-throughput sequencing, a logarithm to base 2 is widely used and in the initial scaling, the doubling is equivalent to a $\log_2\text{FC}$ of 1 (Love et al., 2014). A volcano plot was constructed using a web-based tool². The resulting DEGs were used for further analysis.

Constructing PPI Networks

To assess the relationships between the DEGs from the GSE30153 dataset, we constructed a protein–protein interaction (PPI) network by using Search Tool for the Retrieval of Interacting Genes (STRING v11.0)³ (Szklarczyk et al., 2017, 2019). The cutoff criterion was set to a high confident interaction score of ≥ 0.7 to eliminate inconsistent PPIs from the dataset. We then incorporated the results from the STRING database into Cytoscape software (v3.7.2)⁴ to envisage the PPIs within the statistically relevant DEGs (Shannon et al., 2003). The MCODE plugin from Cytoscape was utilized to identify the interconnected regions or clusters from the PPI network. The cluster finding parameters were adopted, such as a degree cutoff of 2, a node score cutoff of 0.2, a kappa score (K-core) of 5, and a max depth of 100, which limits the cluster size for coexpressing networks (Bader and Hogue, 2003). The top clusters from MCODE were subjected to ClueGO v2.5.5/CluePedia v1.5.5 analysis to obtain comprehensive GO and pathway results from the PPI network. ClueGO combines GO and pathway analyses from KEGG and BioCarta and provides a fundamentally structured GO or pathway network from the PPI network (Bindea et al., 2009).

²<https://paolo.shinyapps.io/ShinyVolcanoPlot/>

³<http://www.string-db.org/>

⁴<http://www.cytoscape.org/>

Metacore GeneGo Analysis of DEGs

Metacore, a Cortellis Solution software (Clarivate Analytics, London, United Kingdom)⁵, was used to perform curated pathway enrichment analysis and GO analysis. GeneGo facilitates the rapid assessment of metabolic pathways, protein biological networks, and pathway maps from high-throughput experimental data (MetaCoreLogin | Clarivate Analytics). Based on a significance threshold of $p < 0.05$, a pictorial representation of the molecular interactions of DEGs from the study groups is generated. Determination of a hypergeometric *p*-value enables the estimation of the chance that an intersection between DEGs and ontological elements is random. An FDR < 0.05 was used as a criterion to calculate if statistically significant DEGs constituted a processor pathway.

RESULTS

Identification of DEGs From the Dataset

Our study contained the gene expression profiles of the GSE30153 dataset from the GEO database, which were submitted by Garaud et al. (2011) based on analysis with the GPL570 platform (Affymetrix Human Genome U133 Plus 2.0 Array) (Garaud et al., 2011). The dataset encompasses 26 samples, including 17 patients with SLE and 9 healthy controls (Table 1). By utilizing the GEO2R online tool, we obtained the differentially expressed genes (DEGs) from the GSE30153 dataset by comparing the SLE samples with control samples. By calculating *p*-values and $|\log_2\text{FC}|$ values, the top 250 DEGs were identified. A volcano plot was constructed using the Rstudio web server ShinyVolcanoPlot to identify DEGs by comparing the SLE and control groups from the dataset. The volcano plot in Figure 1 depicts all the DEGs with a $\log_2\text{FC}$ against the $-\log_{10}(p\text{-value})$ between the two groups. With cutoffs of $p < 0.05$ and $\log_2\text{FC} \geq 1.0$ or ≤ -1 , we found 4 and 13 genes that were upregulated and downregulated, respectively, between the two groups (Table 2). The genes that were differentially expressed between the two groups are shown in Supplementary Table S1.

Screening of Module and Construction of Interlinking PPI Network

To assess the protein–protein connections among the DEGs, we used the STRING tool to compute the protein interactions and plotted them using Cytoscape v3.7.2. Figure 2 depicts the PPI network with 103 nodes and 201 edges. The DEGs are represented as nodes, and the edges are interactions between the DEGs. A combined node score of > 0.4 was considered to be significant. MCODE plugin v1.5.1 from Cytoscape was utilized to identify the densely interlinked regions within the protein network. As a result, we obtained the top two significant clusters from the DEGs protein network with MCODE scores of 5.043 and 3.625. A graphical representation of these clusters is shown in Figures 3A,B. The subnetworks, scores, number of nodes and edges, and node IDs are tabulated in Table 3.

⁵<https://clarivate.com/products/metacore/>

TABLE 1 | The primary characteristics of 26 studies in GSE30153 procured from the Gene Omnibus Expression database.

Group	Accession	Title	Organism	Disease state	Tissue	Cell type
Patient	GSM746726	Patient 1	Homo sapiens	Systemic lupus erythematosus (SLE)	Blood	Human sorted B cell
	GSM746727	Patient 2	Homo sapiens	Systemic lupus erythematosus (SLE)	Blood	Human sorted B cell
	GSM746728	Patient 3	Homo sapiens	Systemic lupus erythematosus (SLE)	Blood	Human sorted B cell
	GSM746729	Patient 4	Homo sapiens	Systemic lupus erythematosus (SLE)	Blood	Human sorted B cell
	GSM746730	Patient 5	Homo sapiens	Systemic lupus erythematosus (SLE)	Blood	Human sorted B cell
	GSM746731	Patient 6	Homo sapiens	Systemic lupus erythematosus (SLE)	Blood	Human sorted B cell
	GSM746732	Patient 7	Homo sapiens	Systemic lupus erythematosus (SLE)	Blood	Human sorted B cell
	GSM746733	Patient 8	Homo sapiens	Systemic lupus erythematosus (SLE)	Blood	Human sorted B cell
	GSM746734	Patient 9	Homo sapiens	Systemic lupus erythematosus (SLE)	Blood	Human sorted B cell
	GSM746735	Patient 10	Homo sapiens	Systemic lupus erythematosus (SLE)	Blood	Human sorted B cell
	GSM746736	Patient 11	Homo sapiens	Systemic lupus erythematosus (SLE)	Blood	Human sorted B cell
	GSM746737	Patient 12	Homo sapiens	Systemic lupus erythematosus (SLE)	Blood	Human sorted B cell
	GSM746738	Patient 13	Homo sapiens	Systemic lupus erythematosus (SLE)	Blood	Human sorted B cell
	GSM746739	Patient 14	Homo sapiens	Systemic lupus erythematosus (SLE)	Blood	Human sorted B cell
	GSM746740	Patient 15	Homo sapiens	Systemic lupus erythematosus (SLE)	Blood	Human sorted B cell
	GSM746741	Patient 16	Homo sapiens	Systemic lupus erythematosus (SLE)	Blood	Human sorted B cell
	GSM746742	Patient 17	Homo sapiens	Systemic lupus erythematosus (SLE)	Blood	Human sorted B cell
Control	GSM746743	Control 1	Homo sapiens	Control	Blood	Human sorted B cell
	GSM746744	Control 2	Homo sapiens	Control	Blood	Human sorted B cell
	GSM746745	Control 3	Homo sapiens	Control	Blood	Human sorted B cell
	GSM746746	Control 4	Homo sapiens	Control	Blood	Human sorted B cell
	GSM746747	Control 5	Homo sapiens	Control	Blood	Human sorted B cell
	GSM746748	Control 6	Homo sapiens	Control	Blood	Human sorted B cell
	GSM746750	Control 8	Homo sapiens	Control	Blood	Human sorted B cell
	GSM746751	Control 9	Homo sapiens	Control	Blood	Human sorted B cell
	GSM746752	Control 10	Homo sapiens	Control	Blood	Human sorted B cell

TABLE 2 | Significantly upregulated and downregulated DEGs between two groups from GSE30153 dataset are tabulated.

GENE SYMBOL	log2FC	p-value
Upregulating Genes		
<i>EGR1</i>	1.22	0.00074
<i>DSE</i>	1.125	0.00291
<i>CD1C</i>	1.068	0.00053
<i>GPM6A</i>	1.052	0.00097
<i>GPM6A*</i>	1.043	0.002981
Downregulating Genes		
<i>RRM2</i>	-2.406	0.0027527
<i>RRM2*</i>	-2.152	0.0030096
<i>TYMS</i>	-1.923	0.0032032
<i>CD38</i>	-1.702	0.0031747
<i>CAV1</i>	-1.516	0.0048324
<i>MIR7110</i>	-1.4	0.0027212
<i>ELL2</i>	-1.354	0.0035256
<i>SLC44A1</i>	-1.219	0.0035298
<i>SAR1B</i>	-1.176	0.0049953
<i>MAN1A1</i>	-1.111	0.004425
<i>CHAC2</i>	-1.071	0.004354
<i>ERAP1</i>	-1.047	0.005321
<i>ARF4</i>	-1.044	0.0058274
<i>PDIA4</i>	-1.014	0.0041988

*The asterisk denotes the DEGs with two different probes from the dataset.

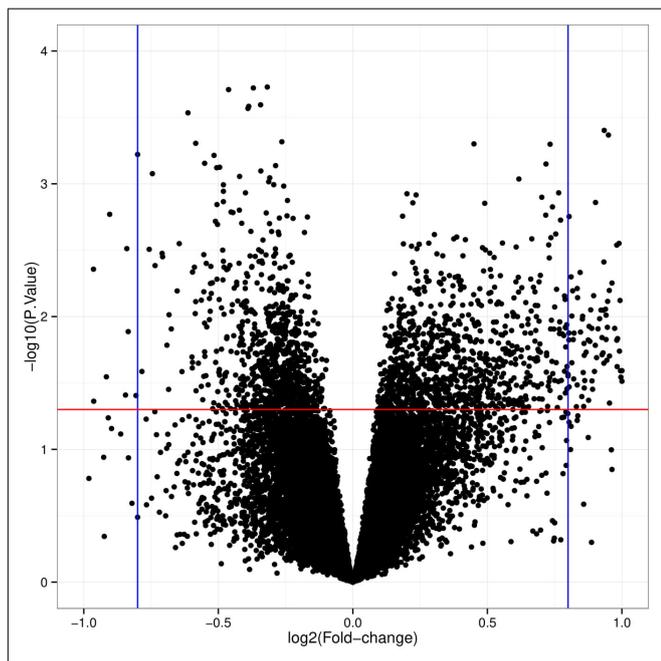


FIGURE 1 | Pictorial representation of volcano plot for differentially expressed genes (DEGs) in systemic lupus erythematosus (SLE) compared to controls from the GSE30153 dataset. The X-axis represents Log2FC, large magnitude fold changes; Y-axis represents $-\log_{10}$ of a p -value, high statistical significance. Each black dot represents one gene. Black dots above red and beside blue line (left-sided and right-sided) are $\log_2FC \geq 1$ and p -value < 0.05 , representing SLE related DEGs.

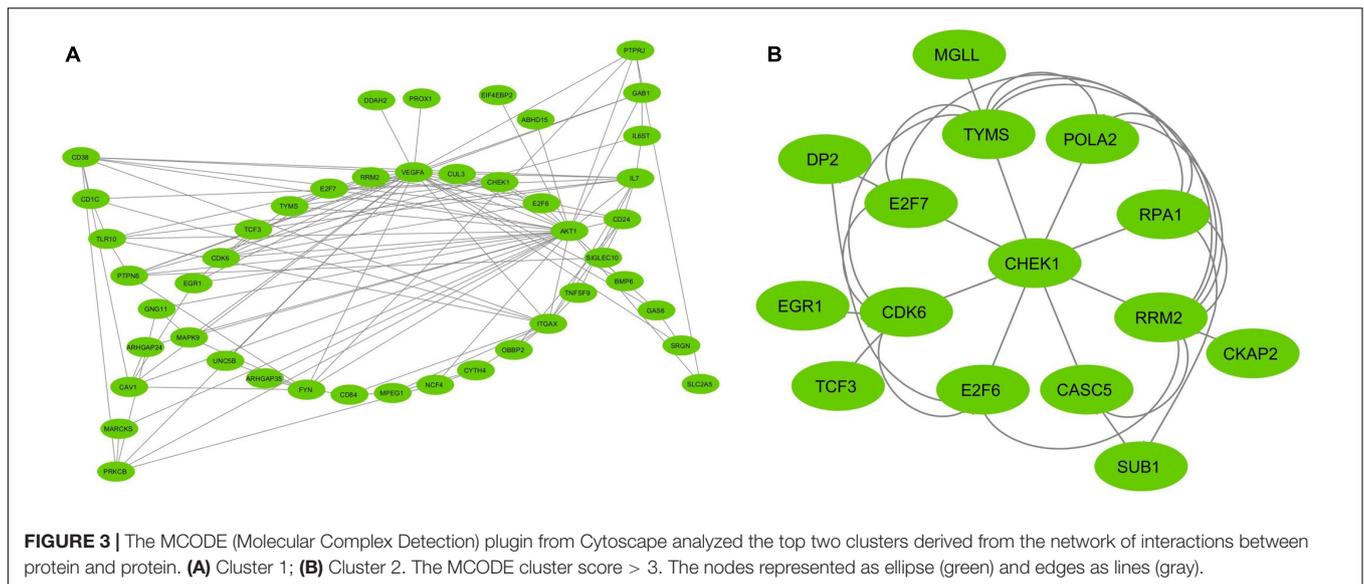


TABLE 3 | The interconnected regions are clustered from the GSE30153 dataset using MCODE plugin in Cytoscape.

Cluster	Score (density x No. of nodes)	Nodes	Edges	Node IDs
1	5.043	45	116	<i>OBBP2, IL6ST, CD1C, ABHD15, PTPRJ, ITGAX, UNC5B, TLR10, CD38, GAS6, NCF4, MAPK9, DDAH2, PTPN6, GAB1, ARHGAP24, CUL3, PROX1, CYTH4, E2F6, TNFSF9, VEGFA, TYMS, IL7, RRM2, PRKCB, MPEG1, MARCKS, SLC2A5, ARHGAP35, BMP6, TCF3, AKT1, EIF4EBP2, GNG11, CAV1, FYN, EGR1, SIGLEC10, CD24, CHEK1, E2F7, CD84, CDK6, SRGN</i>
2	3.625	15	29	<i>RRM2, CKAP2, MGLL, TCF3, SUB1, EGR1, POLA2, RPA1, CHEK1, E2F7, CASC5, DP2, E2F6, CDK6, TYMS</i>

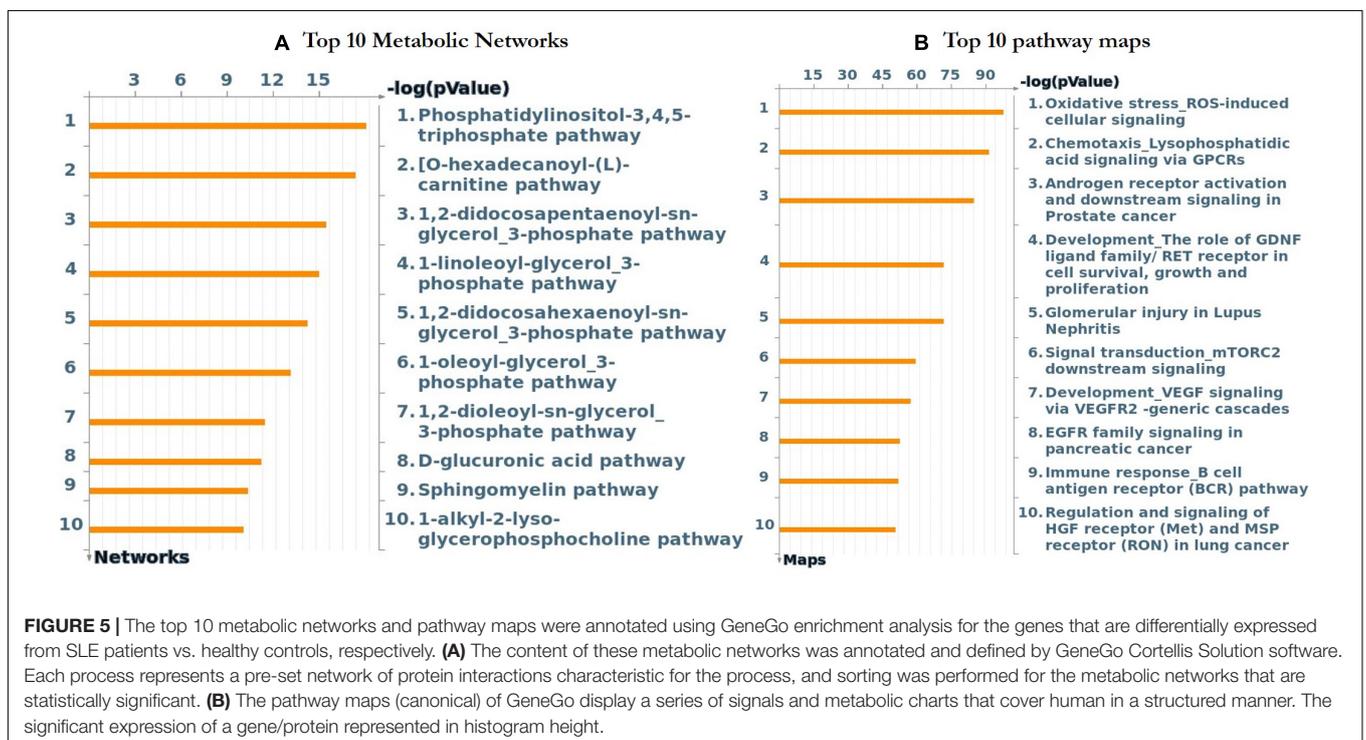
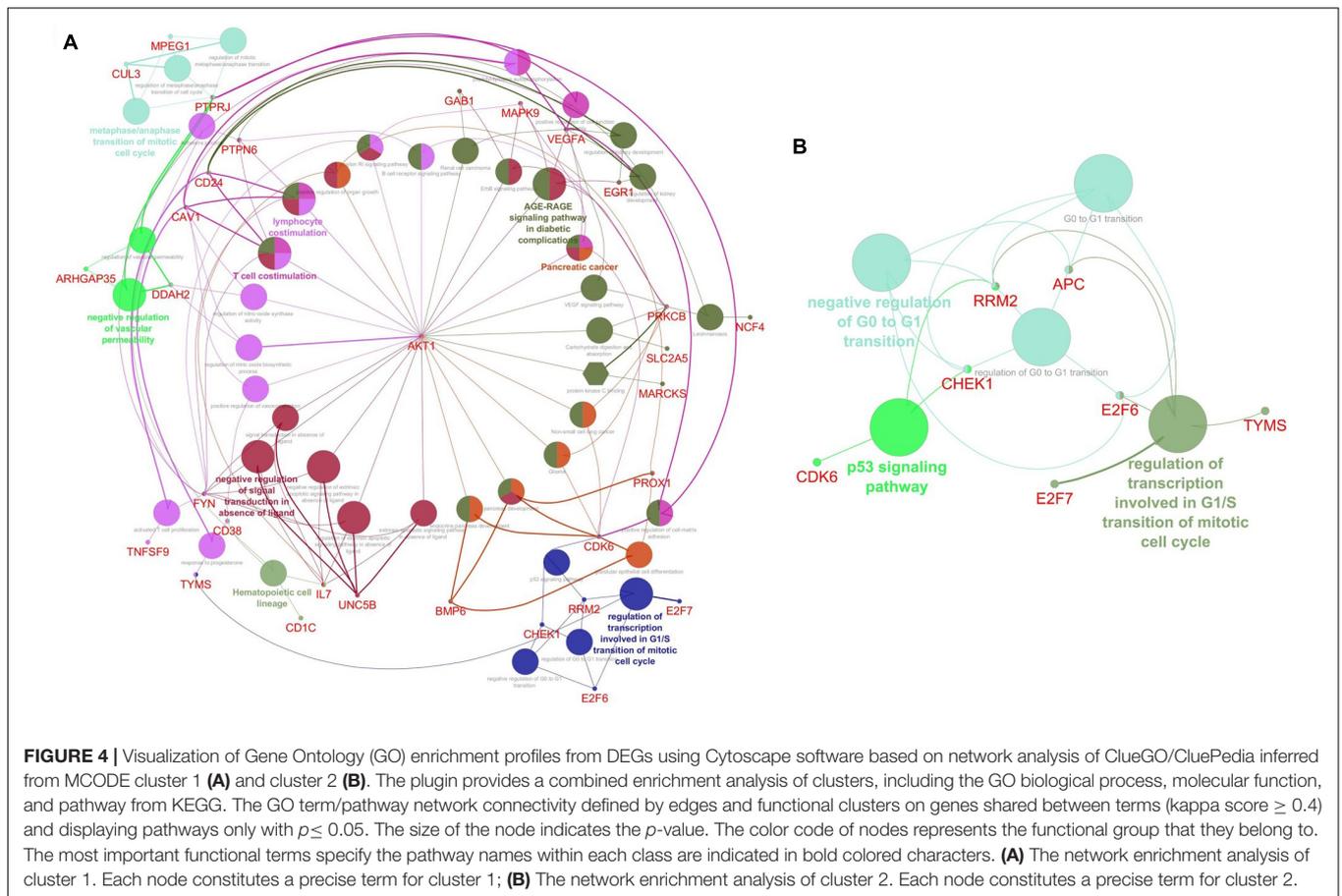
complications (**Figure 4A**). The DEGs from cluster 2 were mainly enriched in the regulation of the transcription involved in the G1/S transition of the mitotic cell cycle (GO: 0000083), the negative regulation of the G0 and G1 transitions (GO: 0070317),

and the p53 signaling pathway (KEGG: 04115) (**Figure 4B**). The pathways that were activated in the enrichment analysis were highly related to B-cell pathophysiology, resulting in events associated with the immune system, vasculopathy, and kidney.

Metacore™ GeneGo for Enrichment Analysis of DEGs

Further functional enrichment analysis was carried out using Metacore™ GeneGo software from Clarivate Analytics to comprehensively dissect the pathways associated with the DEGs. Using the functional ontology feature in GeneGo, the IDs of potential genes that were involved in the target pathways were identified. Based on hypergeometric *p*-values, the probability that the intersection of a gene set and associated ontological objects was random was evaluated. A decreased *p*-value indicated that the entity would be more significant to the DEGs, suggesting a better score. The functional enrichment analysis of the DEGs defined the top 10 metabolic networks, and canonical pathway maps are depicted in **Figures 5A,B**. For each classification, the significant statistical data rely on a low *p*-value. The pathway maps with the lowest *p*-value are shown in **Figures 6A–C**. These are the top-scoring signaling pathways based on the gene enrichment distribution, which emphasizes that the DEGs from human sorted B-cells are triggered via oxidative stress and ROS-induced cellular signaling (**Figure 6A**), chemotaxis and lysophosphatidic acid signaling via GPCRs (**Figure 6B**), and androgen receptor activation and downstream signaling in prostate cancer (**Figure 6C**). The well-distinguished proteins and complexes of proteins are shown as specific symbols⁶; all experimental data are displayed and have corresponding thermometer-like symbols on all the maps. The upregulated genes are indicated by a red thermometer facing upwards.

⁶https://portal.genego.com/help/MC_legend.pdf



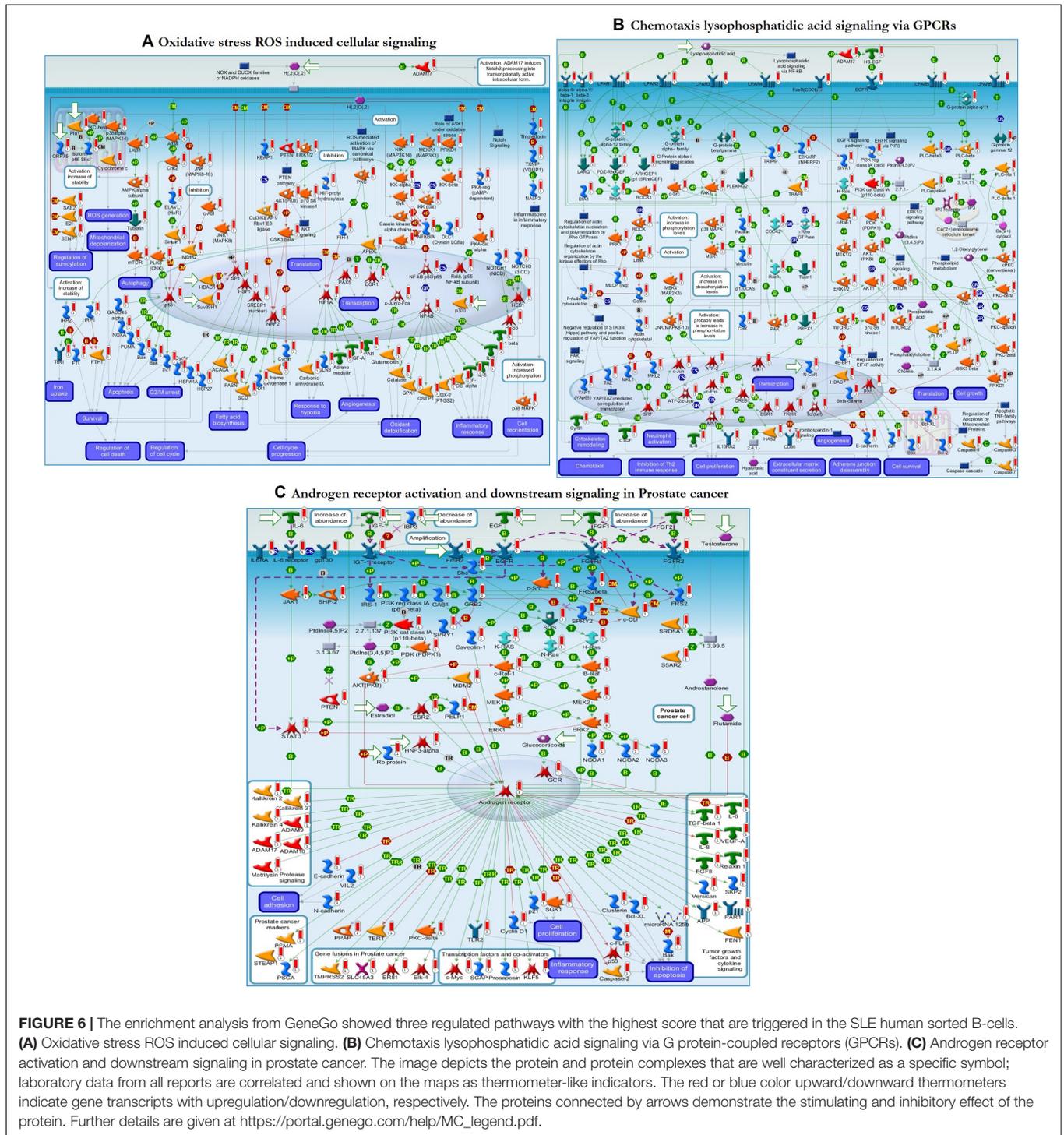


FIGURE 6 | The enrichment analysis from GeneGo showed three regulated pathways with the highest score that are triggered in the SLE human sorted B-cells. **(A)** Oxidative stress ROS induced cellular signaling. **(B)** Chemotaxis lysophosphatidic acid signaling via G protein-coupled receptors (GPCRs). **(C)** Androgen receptor activation and downstream signaling in prostate cancer. The image depicts the protein and protein complexes that are well characterized as a specific symbol; laboratory data from all reports are correlated and shown on the maps as thermometer-like indicators. The red or blue color upward/downward thermometers indicate gene transcripts with upregulation/downregulation, respectively. The proteins connected by arrows demonstrate the stimulating and inhibitory effect of the protein. Further details are given at https://portal.genego.com/help/MC_legend.pdf.

Pathway Map Interaction Results From Clarivate

From the Metacore™ results, we extracted the key genes from the enriched pathways that were differentially expressed, such as *EGR1*, *CD38*, *CAV1*, and *AKT1*. The differential expression of these genes was involved in the activation or inhibition of specific protein complexes in the enriched pathway maps

(Figure 6 and Table 4). Early growth response 1 (*EGR1*) is a transcription factor that interacts with the *IGF-2*, *APEX*, *SRD5A1*, *CD44*, and *EGFR* genes and activates them through transcriptional regulation. The cyclic adenosine diphosphate (ADP) ribose hydrolase *CD38* is an enzyme involved in the activation of the genes *Semaphorin 4D*, *CD19*, and *c-Cbl* through physical interactions. Caveolin 1 (*CAV1*) is a binding

TABLE 4 | The interaction reports of key genes from pathway maps by Clarivate Analytics.

Network object "from"	Object type	Network object "to"	Object type	Effect	Mechanism	Link info	Input IDs	Signal	P-value	PMID
<i>EGR1</i>	Transcription factor	<i>IGF-2, APEX, SRD5A1, CD44, EGFR</i>	Receptor ligand, generic enzyme, generic enzyme, generic receptor, receptor with enzyme activity	Activation	Transcription regulation	EGR1 increases IGF II expression, EGR1 binds to gene APEX promoter and activates APEX expression, Egr-1 trans-activates the 5alpha-R1 promoter via the Egr-1-binding site at position -60/-54, Putative EGR1 binding site is found in gene CD44 promoter, EGR1 binds to gene EGFR promoter and activates EGFR expression.	<i>EGR1</i>	1	0.0032807	8584025; 9925986; 10606246; 11336542; 16043101; 19276347; 29092905; 29170465; 15788231; 15936112; 17194527; 18215136; 8628295; 9300687; 12670907; 15155664; 15923644; 19195913; 20357818; 25673149; 1417865; 11830539; 16750517; 17230532; 19032775; 20190820; 23763269
<i>CD38</i>	Generic enzyme	<i>SEMA4D, CD19, c-Cbl</i>	Generic receptor, generic binding protein, generic enzyme	Activation	Unspecified, Binding	CD31-induced activation of CD38 up-regulates Semaphorin 4D cell-surface expression in B cells, CD19/CD81 complex interacts with CD38 but this interaction is not required to induce proliferation in mouse B-lymphocytes, Fluorescence resource energy transfer and coimmunoprecipitation showed that c-Cbl and CD38 bind each other.	<i>CD38</i>	1	0.0031747	15613544; 17327405; 20570673; 22564057; 8695807; 18974118; 19635790
<i>CAV1</i>	Generic binding protein	<i>ErbB2, MDR1, HTR2A, Androgen receptor</i>	Receptor with enzyme activity, transporter, GPCR, transcription factor	Unspecified, Inhibition, activation	Binding	HER2 physically interacts with caveolin-1, Caveolin-1 interacts with p-gp, Down-regulation of caveolin-1 by siRNA reduced the interaction between p-gp and caveolin-1, followed by a decrease in [3H]-Taxol and [3H]-Vinblastine accumulation in RBE4 cells, Caveolin-1 physically interacts with HTR2A and increases its activity, Highly conserved 9 amino acid motif in the ligand binding domains (E domains) was identified in human/mouse ER alpha and ER beta, progesterone receptors A and B, and the androgen receptor. The localization sequence mediated palmitoylation of each SR, which facilitated caveolin-1 association, subsequent membrane localization, and steroid signaling.	<i>CAV1</i>	1	0.0048324	9374534; 9685399; 11697880; 22389470; 14622130; 15239129; 15498565; 17326770; 18485890; 19099191; 22389470; 25788263; 15190056; 8703009; 11278309; 17535799; 17940184; 18786521; 19931639; 22771325; 24375805

(Continued)

TABLE 4 | Continued

Network object "from"	Object type	Network object "to"	Object type	Effect	Mechanism	Link info	Input IDs	Signal	P-value	PMID
AKT1, AKT (PKB)	Protein kinase	<i>FKHR, mTOR, Bcl-10, FOXO3A, HNF3-beta, GSK3 beta</i>	Transcription factor, protein kinase, generic binding protein	Inhibition, activation	Phosphorylation	AKT1 phosphorylates FKHR1 and decreases its activity, Increased AKT phosphorylation regulates different metabolic pathways in liver, including increases in protein synthesis through activation of mTOR/p70 (S6kinase), AKT1 phosphorylates Bcl-10 and increases its activity, AKT1 phosphorylates FOXO3A and decreases its activity, AKT1 phosphorylates HNF3-beta and decreases its activity, AKT (PKB) inhibits GSK3 alpha by phosphorylation at Ser-9.	<i>AKT1</i>	1	0.0010146	10102273; 10358014; 10358075; 10377430; 11030146; 12393870; 16076959; 16099987; 16230533; 16603397; 17186497; 18388859; 18391970; 18420577; 18687691; 18786403; 19703413; 20940043; 21106439; 21157483; 21238503; 21407213; 21440577; 21708191; 21779512; 26053093; 27966458; 30413788; 10567225; 10910062; 11357143; 11438723; 12767043; 14970221; 15208671; 15549092; 16818631; 17660512; 18505677; 18566586; 18566587; 18678273; 21097843; 21177249; 21302298; 21343617; 22084251; 22595285; 23686889; 23872070; 26958938; 29221131; 16280327; 10102273; 12130673; 12767043; 17570479; 17577629; 17957242; 17960591; 18391970; 18687691; 19703413; 20223831; 20399660; 21106439; 21157483; 21440577; 21621563; 21708191; 21775285; 21779512; 24518891; 27966458; 14500912; 11584303; 11701324; 12124352; 12750378; 12808085; 14966899; 14985354; 15016802; 15297258

protein shown to interact with the *ErbB2*, *MDR1*, *HTR2A*, and *androgen receptor* genes, and inhibition or activation is followed by specific binding to its corresponding proteins. RAC-alpha serine/threonine-protein kinase (AKT1) is a protein kinase that interacts with the *FKHR*, *mTOR*, *Bcl-10*, *FOXO3A*, *HNF3-beta*, and *GSK3 beta* genes via phosphorylation, resulting in inhibition or activation. These genes were differentially expressed between sorted B-cells from controls and sorted B-cells from SLE patients and result in transcriptional regulation and inhibition of genes/proteins within the top-scored pathway maps.

DISCUSSION

DNA microarrays and next generation sequencing (NGS) approaches are high-throughput technologies that have resulted in the emergence of new biomedical discoveries. Data from microarray and gene expression profiles have enabled a deeper understanding of the intrinsic molecular pathways of complex mechanisms of biological systems and their responses (Russo et al., 2003; Babu, 2004; Perez-Diez et al., 2013; Kumar et al., 2019). It is therefore highly relevant to examine the peripheral B-cell transcriptomes of SLE patients and healthy controls to determine genes that are differentially regulated and their target pathways. Our current study extracted DEGs from 17 SLE patients and 9 healthy controls from the GEO database (GSE30153) (Graud et al., 2011). The top 250 DEGs were identified, including 4 upregulated and 13 downregulated genes from the groups through bioinformatics strategies (**Table 2** and **Supplementary Table S1**). These identified DEGs were subjected to ClueGO and GeneGo Metacore™ analysis for GO and pathway annotation, and constructed the interacting networks of PPI and used for cluster analysis. In the network, the nodes were considered proteins, and the edges were their interactions. Using network topology features, the PPI network can be analyzed to distinguish the core proteins that are involved in the pathways (Barabási and Oltvai, 2004; Ideker and Sharan, 2008; Keskin et al., 2016; Kumar et al., 2019). The identified DEGs from the present study were analyzed with STRING to exploit the complex interactions between the DEGs via text mining, evidence from experiments, and repositories (**Figure 2**). We performed module screening of the PPI networks using the MCODE plugin from Cytoscape. As a result, we obtained significant clusters that are densely interlinked regions in the PPI network (**Figures 3A,B**). Screening of these clusters from the network might help to identify the essential genes that are involved in the pathogenesis and progression of SLE. The obtained clusters mostly contained protein complexes or proteins present in the pathways in the PPI network, and cluster visualization is essential for comprehending the properties of the network functionally and systematically (Krogan et al., 2006; Rahman et al., 2013).

Furthermore, to identify the functional enrichment of these subnetworks from MCODE, we implemented the ClueGO plugin for analysis. This revealed that the DEGs were enriched in most essential pathways, which are highly associated with the immune system. The GO and KEGG enrichment analyses of the DEGs from cluster 1 showed that they were mostly enriched

in T-cell costimulation, lymphocyte costimulation, negative regulation of vascular permeability, the metaphase/anaphase transition of the mitotic cell cycle, regulation of the transcription involved in the G1/S transition of mitotic cell cycle, the hematopoietic cell lineage, the B-cell receptor signaling pathway, the ErbB signaling pathway, the AGE-RAGE signaling pathway in diabetic complications, and pancreatic cancer. Interestingly, the costimulation of T-cell and lymphocyte receptors is recognized to be important in SLE pathogenesis by enabling communicating with B-cells for the production of autoantibodies (Shlomchik et al., 2001; Mak and Kow, 2014). In SLE, negative regulation of vascular permeability may be induced by different mechanisms; the dysregulated genes from the cluster 1 subnetwork might lead to endothelial cell damage and vasculopathy (Favero et al., 2014; Lee et al., 2019). The differential cell signaling results in the recruitment of various proteins and inappropriate activation of B-cells (Zhou et al., 2009; Comte et al., 2015). Oxidative stress is common in inflammatory disorders and results in the increased production of reactive carbonyl groups that are partially converted to AGEs, and the DEGs in the AGE-RAGE signaling pathway might also be involved in the accumulation of AGEs in SLE patients and lead to diabetic complications (de Leeuw et al., 2007; Li et al., 2007; Kurien and Scofield, 2008; Nienhuis et al., 2008). Interestingly, our enrichment analysis found that the identified differential expression of the genes (*AKT1*, *VEGFA*, *CDK6*, and *MAPK9*) that were involved in the risk of developing pancreatic cancer in SLE patients was due to chronic inflammation, suggesting that these genes might be involved in the pathogenesis of SLE. Our findings are therefore consistent with the roles of genes that are differentially expressed in SLE-causing pathways (**Figure 4A**). The enrichment analysis of the cluster 2 subnetwork showed that the DEGs were mostly enriched in the regulation of transcription involved in the G1/S transition of the mitotic cell cycle, the negative regulation of the G0 and G1 transitions, and the p53 signaling pathway. It has been reported that the proliferation of T-cells is followed by lowered levels of cyclin-dependent kinase (CDK) inhibitors, and alterations in the expression of CDKs in the G0/G1 phase were seen in the lymphocytes of SLE patients (Yamauchi and Bloom, 1997; Tang et al., 2009). The DEGs involved in the cluster 2 subnetwork might negatively regulate these pathways. Alterations in cyclin-CDK complex behavior and cyclin-dependent kinase inhibitors (CDKIs) have been reported to alter the proliferation of T-cells, oxidative stress, and immune responses (Santiago-Raber et al., 2001; Tang et al., 2009). p53 signaling is essential for various cellular mechanisms, and defects in this signaling pathway are associated with SLE development. Considerably elevated levels of p53 protein are found in SLE patients with active inflammatory disorders (Miret et al., 2003; Veeranki and Choubey, 2010). Apoptosis dysregulation appears to be another cause of SLE pathogenesis because the possible sources of autoantigens are cell debris from apoptosis in SLE, and excessive cellular senescence of the immune cells, especially T-cells, was reported in SLE patients with peripheral blood mononuclear cells (PBMCs) and skin lesions (Colonna et al., 2014; Sáenz-Corral et al., 2015). Thus, our identified DEGs (*RRM2*, *APC*, *CHEK1*, *E2F6*, *TYMS*, *E2F7*, and *CDK6*) from the

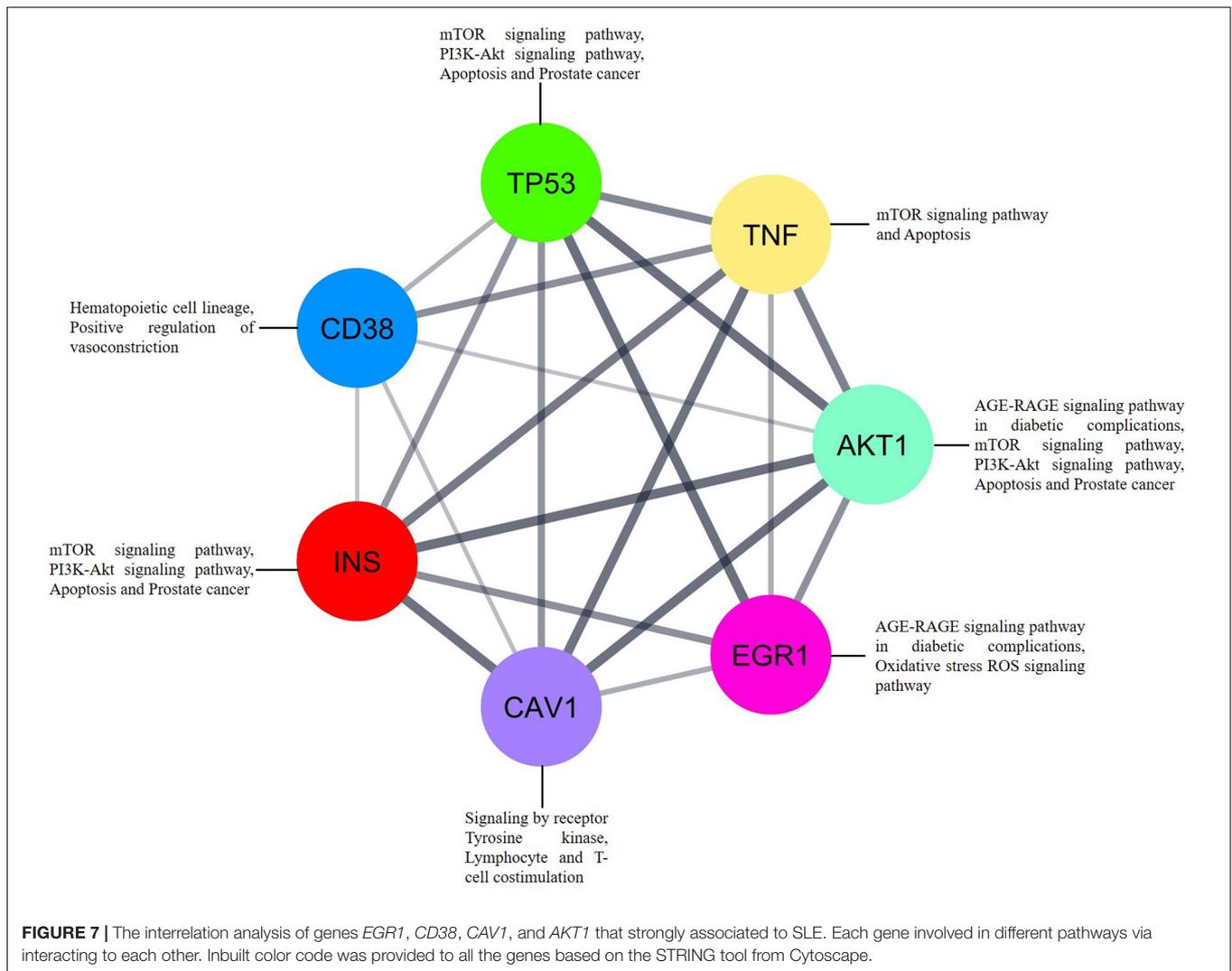
cluster 2 subnetwork are highly related to and consistent with the members of the signaling pathways associated with the immune system, apoptosis, the cell cycle, and vasculopathy.

To clearly define the interactions between the proteins and signaling pathways examined from the interpretation of STRING, Cytoscape, MCODE, and ClueGO analyses, we implemented the GeneGo Metacore software, which incorporates extensive data on metabolic signaling pathways and their regulatory mechanisms and contains accurately compiled networks of biological systems. By utilizing the GeneGo Metacore software, we obtained a detailed description of the DEGs that participate in SLE pathogenesis based on the determined *p*-values. Among the top 10 metabolic networks, the phosphatidylinositol-3,4,5-triphosphate pathway, O-hexadecanoyl-(L)-carnitine pathway, 1,2-didocosapentaenoyl-sn-glycerol 3-phosphate pathway, and 1-linoleoyl-glycerol-3-phosphate pathway were profoundly enriched and significant in the SLE DEGs (Figure 5A). The increased activity of phosphatidylinositol-3,4,5-triphosphate stimulates essential cell signaling pathways such as the pathways involved in cell division, survival, and the rapid increase in T-lymphocytes in SLE (Comte et al., 2015). PI3K (phosphatidylinositol 3-kinase) is a protein kinase that phosphorylates phosphatidylinositol 4,5-phosphate to regulate the signaling of T-lymphocytes; an increased amount of PI3K was also observed in an animal model of lupus (Liu et al., 1998; Grolleau et al., 2000; Niculescu et al., 2003; Joseph et al., 2014). Modification of the carnitine signaling pathway results in various organ failures by producing effective responses to pathogens (Famularo and De Simone, 1995; Famularo et al., 2004). Thus, the DEGs involved in the O-hexadecanoyl-(L)-carnitine pathway might lead to increased immune responses. In addition, the top three pathways associated with the DEGs of sorted B-cells from SLE patients were mostly enriched in oxidative stress- and ROS-induced cellular signaling (Figure 6A), chemotaxis and lysophosphatidic acid signaling via GPCRs (Figure 6B), and androgen receptor activation and downstream signaling in prostate cancer (Figure 6C). Recent findings have shown that oxidative stress and ROS induce molecular alterations that have adverse effects in SLE patients (Choi et al., 2016; Tsokos et al., 2016; Lightfoot et al., 2017). Elevated oxidative stress in SLE patients leads to the accumulation of higher amounts of oxidative lipoproteins, which are harmful in zebrafish models and cause additional oxidative damage to the system (Chung et al., 2007; Park et al., 2016; Lightfoot et al., 2017). Interestingly, our study identified the *EGR1* gene as downregulated in the SLE patients in comparison to controls, and it also plays a role in ROS signaling. This clearly indicates that *EGR1* might be required to maintain the oxidative stress and ROS signaling pathways.

Moreover, the DEGs involved in the oxidative stress signaling pathway might contribute to peripheral neuropathy, damage to blood vessels, and cardiovascular events, which are the prominent clinical conditions found in SLE patients. Chemotaxis and lysophosphatidic acid (LPA) signaling are essential pathways in autoimmune inflammatory disorders, and GPCRs are responsible for regulating immune cells via LPA receptors (Yang et al., 2005; Skoura and Hla, 2009). *G2A* gene knockout resulted in the hyperresponsiveness of T-cells to T-cell receptor stimulation, manifesting as an increased proliferation of T-cells, which may

promote inflammatory phenotypes in *G2A*-deficient mice (Le et al., 2001). Various studies have suggested that LPA plays a vital role in atherosclerosis progression and development by promoting neutrophil and monocyte adherence and enhancing inflammatory events (Siess et al., 1999; Smyth et al., 2008; Skoura and Hla, 2009). The androgen receptor (AR) is a transcription factor that is activated by a ligand and is essential for cells targeted by the androgen response (Robeva et al., 2013; Gubbels Bupp and Jorgensen, 2018). AR also regulates immune function in SLE via transcriptional regulation of various genes. Our study identified the transcription factor AR, which positively regulates the *c-Myc*, *SCAP*, *prosaposin*, and *KLF5* genes, which are responsible for inflammatory responses, and promotes tumor growth factors and cytokine signaling when activated (Figure 6C). The enriched terms from ClueGO modules and the GeneGo-identified terms correlated well in this study and validate the significance of the findings from the pathway maps. The combined results from these two enrichment analyses suggest that B-cells from SLE patients and B-cells from healthy controls undergo differential gene expression associated with positive regulation of kidney development, the hematopoietic cell lineage, positive regulation of vasoconstriction, T-cell costimulation, and regulation of the transcription involved in the G1/S transition of the mitotic cell cycle.

Furthermore, the interaction results from the GeneGo analysis provided the essential genes (*EGR1*, *CD38*, *CAV1*, and *AKT1*) from the pathway maps constructed from the DEGs. Among them, *EGR1* (early growth response 1) is a transcription factor shown to interact with the *IGF-2* (insulin-like growth factor 2), *APEX* (apurinic/aprimidinic endodeoxyribonuclease 1), *SRD5A1* (steroid 5 alpha-reductase 1), *CD44* (cell surface glycoprotein CD44), and *EGFR* (epidermal growth factor receptor) genes and transcriptionally regulate them by activating or promoting their expression in sorted B-cells from patients with SLE (Liu et al., 1995; Recio and Merlino, 2003; Lee et al., 2005; Pines et al., 2005; Blanchard et al., 2007; Rui et al., 2008; Cullen et al., 2010; Sauer et al., 2010). The cyclic ADP ribose hydrolase (*CD38*) is also known as cluster of differentiation 38 protein, can be found on several immune cells, and activates *SEMA4D* (semaphorin-4D or cluster of differentiation 100), *CD19* (B-lymphocyte antigen CD19 or cluster of differentiation 19), and *c-Cbl* (Casitas B-lineage lymphoma proto-oncogene) (Deaglio et al., 2005, 2007; Shen and Yen, 2008; Vences-Catalán et al., 2012). These interactions with *CD38* result in the activation of B-lymphocytes and increase immune responses in SLE patients. The protein caveolin 1 (*CAV1*) has been shown to interact with the *ErbB2* (Erb-B2 receptor tyrosine kinase 2), *MDR1* (multidrug resistance protein 1), *HTR2A* (5-hydroxytryptamine receptor 2A), and *AR* (androgen receptor) genes. Several studies have suggested that caveolin 1 physically interacts with *HER2*, *p-gp*, *HTR2A*, and *AR* and activates/inhibits them by binding to their specific caveolin-binding motif (Couet et al., 1997; Lu et al., 2001; Razani and Lisanti, 2001; Bhatnagar et al., 2004; Bennett et al., 2010, 2014; Yu et al., 2012). *AKT1* is a protein kinase that interacts with *FKHR* (Forkhead box protein O1), *mTOR* (mechanistic target of rapamycin), *Bcl-10* (B-cell lymphoma/leukemia 10), *FOXO3A* (Forkhead box O3),



HNF3-beta (hepatocyte nuclear factor 3-beta), and GSK3 beta (glycogen synthase kinase three beta). The protein kinase AKT1 inhibits FKHR via phosphorylation and decreases its activity (Biggs et al., 1999; Rena et al., 1999; Tang et al., 1999; Hay, 2011), whereas it increases its activity via phosphorylation of mTOR (Navé et al., 1999; Sekuliac et al., 2000; Ikenoue et al., 2008; Thirumal Kumar et al., 2019). Additionally, AKT1 phosphorylates Bcl-10 at the specific residues Ser231 and Ser218, increasing its activity (Yeh et al., 2006), while it inhibits the action of FOXO3A via phosphorylation and decreases its activity, increasing the survival of cells (Brunet et al., 1999; Linding et al., 2007; Calnan and Brunet, 2008; Li et al., 2008; Tzivion et al., 2011). AKT1 decreases HNF3-beta activity by phosphorylating it at Thr156 (Wolfrum et al., 2003), whereas phosphorylation of GSK3-beta by AKT1 occurs at Ser9 to inhibit its activity (Brazil and Hemmings, 2001; Salas et al., 2004; Kummerle, 2005; Shin et al., 2006; Markou et al., 2008). This suggests the vital genes we identified from the DEGs of patients with SLE play essential roles in the development and progression of SLE via different signaling pathways to increase autoimmune responses.

In addition to the interaction analysis, we carried out interrelation analysis for the essential genes to determine the relationships between the genes, which implicitly or explicitly interacted with each other. Interestingly, the identified genes indirectly communicated with each other via molecular signaling pathways related to mTOR signaling, apoptosis, PI3K-Akt signaling, the hematopoietic cell lineage, positive regulation of vasoconstriction, signaling by receptor tyrosine kinases, AGE-RAGE signaling, and lymphocyte and T-cell costimulation (Figure 7). *EGR1* and *AKT1* are directly involved in oxidative stress via ROS and AGE-RAGE signaling, whereas *CAV1* is directly involved in tyrosine kinase receptor signaling and lymphocyte and T-cell costimulation. *CD38* is directly associated with the hematopoietic cell lineage and positive regulation of vasoconstriction. Overall, the dysregulation of the indicated pathways in SLE patients is a result of differential gene expression. The essential genes are differentially expressed between cells from patients with SLE and cells from healthy controls and are present in important signaling cascades, which could be a crucial factor for SLE development.

CONCLUSION

Taken together, the results of our comprehensive bioinformatics analysis showed that the DEGs identified between sorted B-cells from patients with SLE sorted B-cells from controls could play a significant role in the growth, progression, and development of SLE. This study identified 4 upregulated and 13 downregulated genes, including essential genes (*EGRI*, *CD38*, *CAVI*, and *AKT1*), from the pathway enrichment analysis. Indeed, the identified pathways from the enrichment analysis were strongly related to the immune system, vasculopathy, cardiovascular functions, and inflammatory responses, which are processes that can lead to the development of SLE. The broad understanding of SLE pathophysiology from this study will allow us to identify and develop therapies targeting SLE and contribute to personalized treatment strategies. Collectively, the study findings could aid in enhancing our understanding of the fundamental molecular processes of SLE and provide possible strategies for early diagnosis in SLE; in addition, combinatorial therapeutic strategies using oxidative stress and ROS cellular signaling and lysophosphatidic acid signaling via GPCRs might have symbiotic effects on the molecular events in SLE.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE30153>.

REFERENCES

- Alibés, A., Yankilevich, P., Cañada, A., and Díaz-Uriarte, R. (2007). IDconverter and IDClight: conversion and annotation of gene and protein IDs. *BMC Bioinformatics* 8:9. doi: 10.1186/1471-2105-8-9
- Aubert, J., Bar-Hen, A., Daudin, J. J., and Robin, S. (2004). Determination of the differentially expressed genes in microarray experiments using local FDR. *BMC Bioinformatics* 5:125. doi: 10.1186/1471-2105-5-125
- Babu, M. M. (2004). *An Introduction to Microarray Data Analysis*. Cambridge: Horizon Bioscience, 225–249.
- Bader, G. D., and Hogue, C. W. (2003). An automated method for finding molecular complexes in large protein interaction networks. *BMC Bioinformatics* 4:2. doi: 10.1186/1471-2105-4-2
- Barabási, A.-L., and Oltvai, Z. N. (2004). Network biology: understanding the cell's functional organization. *Nat. Rev. Genet.* 5, 101–113. doi: 10.1038/nrg1272
- Barrett, T., Wilhite, S. E., Ledoux, P., Evangelista, C., Kim, I. F., Tomashevsky, M., et al. (2013). NCBI GEO: archive for functional genomics data sets—update. *Nucleic Acids Res.* 41, D991–D995. doi: 10.1093/nar/gks1193
- Benjamini, Y., and Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Statist. Soc. Ser. B (Methodol.)* 57, 289–300.
- Bennett, N. C., Gardiner, R. A., Hooper, J. D., Johnson, D. W., and Gobe, G. C. (2010). Molecular cell biology of androgen receptor signalling. *Int. J. Biochem. Cell Biol.* 42, 813–827. doi: 10.1016/j.biocel.2009.11.013
- Bennett, N. C., Hooper, J. D., Johnson, D. W., and Gobe, G. C. (2014). Expression profiles and functional associations of endogenous androgen receptor and caveolin-1 in prostate cancer cell lines. *Prostate* 74, 478–487. doi: 10.1002/pros.22767
- Bentham, J., Morris, D. L., Cunninghame Graham, D. S., Pinder, C. L., Tomblinson, P., Behrens, T. W., et al. (2015). Genetic association analyses implicate aberrant

AUTHOR CONTRIBUTIONS

SU, DT, HZ, and CG were involved in the design of the study and the acquisition, analysis, and interpretation of the data. SU, DT, CG, SY, NY, MS, and HZ were involved in the interpretation of the data and drafting the manuscript. CG, RS, and HZ supervised the entire study and were involved in study design, the acquisition, analysis, and interpretation of the data, and drafting the manuscript. The manuscript was reviewed and approved by all the authors.

FUNDING

This work was supported by Qatar University Grant# QUST-1-CHS-2020-2.

ACKNOWLEDGMENTS

We would like to take this opportunity to thank the management of VIT for providing the necessary facilities and encouragement to carry out this work.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fbioe.2020.00276/full#supplementary-material>

- regulation of innate and adaptive immunity genes in the pathogenesis of systemic lupus erythematosus. *Nat. Genet.* 47, 1457–1464. doi: 10.1038/ng.3434
- Bhatnagar, A., Sheffler, D. J., Kroeze, W. K., Compton-Toth, B., and Roth, B. L. (2004). Caveolin-1 interacts with 5-HT_{2A} serotonin receptors and profoundly modulates the signaling of selected Gαq-coupled protein receptors. *J. Biol. Chem.* 279, 34614–34623. doi: 10.1074/jbc.M404673200
- Biggs, W. H., Meisenhelder, J., Hunter, T., Cavenee, W. K., and Arden, K. C. (1999). Protein kinase B/Akt-mediated phosphorylation promotes nuclear exclusion of the winged helix transcription factor FKHR1. *Proc. Natl. Acad. Sci. U.S.A.* 96, 7421–7426. doi: 10.1073/pnas.96.13.7421
- Bindea, G., Mlecnik, B., Hackl, H., Charoentong, P., Tosolini, M., Kirilovsky, A., et al. (2009). ClueGO: a cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. *Bioinformatics* 25, 1091–1093. doi: 10.1093/bioinformatics/btp101
- Blanchard, Y., Seenundun, S., and Robaire, B. (2007). The promoter of the rat 5α-reductase type 1 gene is bidirectional and Sp1-dependent. *Mol. Cell. Endocrinol.* 264, 171–183. doi: 10.1016/j.mce.2006.11.007
- Borrebaeck, C. A. K., Sturfelt, G., and Wingren, C. (2014). “Recombinant antibody microarray for profiling the serum proteome of SLE,” in *Systemic Lupus Erythematosus: Methods and Protocols* Methods in Molecular Biology, eds P. Eggleton and F. J. Ward (New York, NY: Springer), 67–78. doi: 10.1007/978-1-4939-0326-9_6
- Brazil, D. P., and Hemmings, B. A. (2001). Ten years of protein kinase B signalling: a hard Akt to follow. *Trends Biochem. Sci.* 26, 657–664. doi: 10.1016/S0968-0004(01)01958-2
- Brunet, A., Bonni, A., Zigmond, M. J., Lin, M. Z., Juo, P., Hu, L. S., et al. (1999). Akt promotes cell survival by phosphorylating and inhibiting a forkhead transcription factor. *Cell* 96, 857–868. doi: 10.1016/S0092-8674(00)80595-4
- Calnan, D. R., and Brunet, A. (2008). The FoxO code. *Oncogene* 27, 2276–2288. doi: 10.1038/onc.2008.21

- Choi, S.-C., Titov, A. A., Sivakumar, R., Li, W., and Morel, L. (2016). Immune cell metabolism in systemic lupus erythematosus. *Curr. Rheumatol. Rep.* 18:66. doi: 10.1007/s11926-016-0615-7
- Chung, C. P., Avalos, I., Oeser, A., Gebretsadik, T., Shintani, A., Raggi, P., et al. (2007). High prevalence of the metabolic syndrome in patients with systemic lupus erythematosus: association with disease characteristics and cardiovascular risk factors. *Ann. Rheum. Dis.* 66, 208–214. doi: 10.1136/ard.2006.054973
- Colonna, L., Lood, C., and Elkon, K. (2014). Beyond apoptosis in lupus. *Curr Opin Rheumatol.* 26, 459–466. doi: 10.1097/BOR.0000000000000083
- Comte, D., Karampetsou, M. P., and Tsokos, G. C. (2015). T cells as a therapeutic target in SLE. *Lupus* 24, 351–363. doi: 10.1177/0961203314556139
- Costa-Reis, P., and Sullivan, K. E. (2013). Genetics and epigenetics of systemic lupus erythematosus. *Curr. Rheumatol. Rep.* 15:369. doi: 10.1007/s11926-013-0369-4
- Couet, J., Sargiacomo, M., and Lisanti, M. P. (1997). Interaction of a receptor tyrosine kinase, EGF-R, with caveolins. caveolin binding negatively regulates tyrosine and serine/threonine kinase activities. *J. Biol. Chem.* 272, 30429–30438. doi: 10.1074/jbc.272.48.30429
- Cui, Y., Sheng, Y., and Zhang, X. (2013). Genetic susceptibility to SLE: Recent progress from GWAS. *J. Autoimm.* 41, 25–33. doi: 10.1016/j.jaut.2013.01.008
- Cullen, E. M., Brazil, J. C., and O'Connor, C. M. (2010). Mature human neutrophils constitutively express the transcription factor EGR-1. *Mol. Immunol.* 47, 1701–1709. doi: 10.1016/j.molimm.2010.03.003
- Dang, J., Li, J., Xin, Q., Shan, S., Bian, X., Yuan, Q., et al. (2016). Gene-gene interaction of ATG5, ATG7, BLK and BANK1 in systemic lupus erythematosus. *Int. J. Rheum. Dis.* 19, 1284–1293. doi: 10.1111/1756-185X.12768
- de Leeuw, K., Graaff, R., de Vries, R., Dullaart, R. P., Smit, A. J., Kallenberg, C. G., et al. (2007). Accumulation of advanced glycation endproducts in patients with systemic lupus erythematosus. *Rheumatology (Oxford)* 46, 1551–1556. doi: 10.1093/rheumatology/kem215
- Deaglio, S., Vaisitti, T., Bergui, L., Bonello, L., Horenstein, A. L., Tamagnone, L., et al. (2005). CD38 and CD100 lead a network of surface receptors relaying positive signals for B-CLL growth and survival. *Blood* 105, 3042–3050. doi: 10.1182/blood-2004-10-3873
- Deaglio, S., Vaisitti, T., Billington, R., Bergui, L., Omede, P., Genazzani, A. A., et al. (2007). CD38/CD19: a lipid raft-dependent signaling complex in human B cells. *Blood* 109, 5390–5398. doi: 10.1182/blood-2006-12-061812
- Deng, Y., and Tsao, B. P. (2010). Genetic susceptibility to systemic lupus erythematosus in the genomic era. *Nat. Rev. Rheumatol.* 6, 683–692. doi: 10.1038/nrrheum.2010.176
- Ducreux, J., Houssiau, F. A., Vandepapelière, P., Jørgensen, C., Lazaro, E., Spertini, F., et al. (2016). Interferon α kinoid induces neutralizing anti-interferon α antibodies that decrease the expression of interferon-induced and B cell activation associated transcripts: analysis of extended follow-up data from the interferon α kinoid phase I/II study. *Rheumatology (Oxford)* 55, 1901–1905. doi: 10.1093/rheumatology/kew262
- Edgar, R., Domrachev, M., and Lash, A. E. (2002). Gene expression omnibus: NCBI gene expression and hybridization array data repository. *Nucleic Acids Res.* 30, 207–210. doi: 10.1093/nar/30.1.207
- Famularo, G., and De Simone, C. (1995). A new era for carnitine? *Immunol. Today* 16, 211–213. doi: 10.1016/0167-5699(95)80159-6
- Famularo, G., Simone, C., de Trinchieri, V., and Mosca, L. (2004). Carnitines and its congeners: a metabolic pathway to the regulation of immune response and inflammation. *Ann. N. Y. Acad. Sci.* 1033, 132–138. doi: 10.1196/annals.1320.012
- Favero, G., Paganelli, C., Buffoli, B., Rodella, L. F., and Rezzani, R. (2014). Endothelium and its alterations in cardiovascular diseases: life style intervention. *Biomed. Res. Int.* 2014:801896. doi: 10.1155/2014/801896
- Garaud, J.-C., Schickel, J.-N., Blaison, G., Knapp, A.-M., Dembele, D., Ruer-Laventie, J., et al. (2011). B cell signature during inactive systemic lupus is heterogeneous: toward a biological dissection of lupus. *PLoS One* 6:e23900. doi: 10.1371/journal.pone.0023900
- Grolleau, A., Kaplan, M. J., Hanash, S. M., Beretta, L., and Richardson, B. (2000). Impaired translational response and increased protein kinase PKR expression in T cells from lupus patients. *J. Clin. Invest.* 106, 1561–1568. doi: 10.1172/JCI9352
- Gubbels Bupp, M. R., and Jørgensen, T. N. (2018). Androgen-Induced Immunosuppression. *Front. Immunol.* 9:794. doi: 10.3389/fimmu.2018.00794
- Gurevitz, S., Snyder, J., Wessel, E., Frey, J., and Williamson, B. (2013). Systemic lupus erythematosus: a review of the disease and treatment options. *Consult. Pharm.* 28, 110–121. doi: 10.4140/TCP.n.2013.110
- Harley, I. T. W., Kaufman, K. M., Langefeld, C. D., Harley, J. B., and Kelly, J. A. (2009). Genetic susceptibility to SLE: new insights from fine mapping and genome-wide association studies. *Nat. Rev. Genet.* 10, 285–290. doi: 10.1038/nrg2571
- Hay, N. (2011). Interplay between FOXO, TOR, and Akt. *Biochim. Biophys. Acta (BBA) Mol. Cell Res.* 1813, 1965–1970. doi: 10.1016/j.bbamcr.2011.03.013
- Ideker, T., and Sharan, R. (2008). Protein networks in disease. *Genome Res.* 18, 644–652. doi: 10.1101/gr.071852.107
- Ikenoue, T., Inoki, K., Yang, Q., Zhou, X., and Guan, K.-L. (2008). Essential function of TORC2 in PKC and Akt turn motif phosphorylation, maturation and signalling. *EMBO J.* 27, 1919–1931. doi: 10.1038/emboj.2008.119
- Irizarry, R. A., Bolstad, B. M., Collin, F., Cope, L. M., Hobbs, B., and Speed, T. P. (2003). Summaries of affymetrix genechip probe level data. *Nucleic Acids Res.* 31:e15. doi: 10.1093/nar/gng015
- Joseph, N., Reicher, B., and Barda-Saad, M. (2014). The calcium feedback loop and T cell activation: How cytoskeleton networks control intracellular calcium flux. *Biochim. Biophys. Acta (BBA) Biomemb.* 1838, 557–568. doi: 10.1016/j.bbamem.2013.07.009
- Keskin, O., Tuncbag, N., and Gursoy, A. (2016). Predicting protein-protein interactions from the molecular to the proteome level. *Chem. Rev.* 116, 4884–4909. doi: 10.1021/acs.chemrev.5b00683
- Krogan, N. J., Cagney, G., Yu, H., Zhong, G., Guo, X., Ignatchenko, A., et al. (2006). Global landscape of protein complexes in the yeast *Saccharomyces cerevisiae*. *Nature* 440, 637–643. doi: 10.1038/nature04670
- Kuemmerle, J. F. (2005). Endogenous IGF-I protects human intestinal smooth muscle cells from apoptosis by regulation of GSK-3 β activity. *Am. J. Physiol. Gastroin. Liver Physiol.* 288, G101–G110. doi: 10.1152/ajpgi.00032.2004
- Kumar, S. U., Kumar, D. T., Siva, R., Doss, C. G. P., and Zayed, H. (2019). Integrative bioinformatics approaches to map potential novel genes and pathways involved in ovarian cancer. *Front. Bioeng. Biotechnol.* 7:391. doi: 10.3389/fbioe.2019.00391
- Kurien, B. T., and Scofield, R. H. (2008). Autoimmunity and oxidatively modified autoantigens. *Autoimmun. Rev.* 7, 567–573. doi: 10.1016/j.autrev.2008.04.019
- Le, L. Q., Kabarowski, J. H., Weng, Z., Satterthwaite, A. B., Harvill, E. T., Jensen, E. R., et al. (2001). Mice lacking the orphan G protein-coupled receptor G2A develop a late-onset autoimmune syndrome. *Immunity* 14, 561–571. doi: 10.1016/s1074-7613(01)00145-5
- Lee, W.-F., Wu, C.-Y., Yang, H.-Y., Lee, W.-I., Chen, L.-C., Ou, L.-S., et al. (2019). Biomarkers associating endothelial Dysregulation in pediatric-onset systemic lupus erythematosus. *Pediatr. Rheumatol.* 17:69. doi: 10.1186/s12969-019-0369-7
- Lee, Y.-S., Jang, H.-S., Kim, J.-M., Lee, J.-S., Lee, J.-Y., Kim, K. L., et al. (2005). Adenoviral-mediated delivery of early growth response factor-1 gene increases tissue perfusion in a murine model of hindlimb ischemia. *Mol. Ther.* 12, 328–336. doi: 10.1016/j.ythm.2005.03.027
- Li, J.-T., Hou, F.-F., Guo, Z.-J., Shan, Y.-X., Zhang, X., and Liu, Z.-Q. (2007). Advanced glycation end products upregulate C-reactive protein synthesis by human hepatocytes through stimulation of monocyte IL-6 and IL-1 β production. *Scand. J. Immunol.* 66, 555–562. doi: 10.1111/j.1365-3083.2007.02001.x
- Li, Y., Wang, Z., Kong, D., Li, R., Sarkar, S. H., and Sarkar, F. H. (2008). Regulation of Akt/FOXO3a/GSK-3 β /AR signaling network by isoflavone in prostate cancer cells. *J. Biol. Chem.* 283, 27707–27716. doi: 10.1074/jbc.M802759200
- Lightfoot, Y. L., Blanco, L. P., and Kaplan, M. J. (2017). Metabolic abnormalities and oxidative stress in lupus. *Curr. Opin. Rheumatol.* 29, 442–449. doi: 10.1097/BOR.0000000000000413
- Linding, R., Jensen, L. J., Ostheimer, G. J., Vugt, M. A. T. M., van Jørgensen, C., Miron, I. M., et al. (2007). Systematic discovery of in vivo phosphorylation networks. *Cell* 129, 1415–1426. doi: 10.1016/j.cell.2007.05.052
- Liu, K.-Q., Bunnell, S. C., Gurniak, C. B., and Berg, L. J. (1998). T cell receptor-initiated calcium release is uncoupled from capacitative calcium entry in itk-deficient T cells. *J. Exp. Med.* 187, 1721–1727. doi: 10.1084/jem.187.10.1721
- Liu, Z., Mittanck, D. W., Kim, S., and Rotwein, P. (1995). Control of insulin-like growth factor-II/mannose 6-phosphate receptor gene transcription by proximal

- promoter elements. *Mol. Endocrinol.* 9, 1477–1487. doi: 10.1210/mend.9.11.8584025
- Love, M. I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 15:550. doi: 10.1186/s13059-014-0550-8
- Lu, M. L., Schneider, M. C., Zheng, Y., Zhang, X., and Richie, J. P. (2001). Caveolin-1 interacts with androgen receptor a positive modulator of androgen receptor mediated transactivation. *J. Biol. Chem.* 276, 13442–13451. doi: 10.1074/jbc.M006598200
- Mak, A., and Kow, N. Y. (2014). The pathology of T cells in systemic lupus erythematosus. *J. Immunol. Res.* 2014:419029. doi: 10.1155/2014/419029
- Markou, T., Cullingford, T. E., Giraldo, A., Weiss, S. C., Alsaifi, A., Fuller, S. J., et al. (2008). Glycogen synthase kinases 3 α and 3 β in cardiac myocytes: regulation and consequences of their inhibition. *Cell. Signal.* 20, 206–218. doi: 10.1016/j.cellsig.2007.10.004
- Miret, C., Molina, R., Filella, X., García-Carrasco, M., Claver, G., Ingelmo, M., et al. (2003). Relationship of p53 with other oncogenes, cytokines and systemic lupus erythematosus activity. *TBI* 24, 185–188. doi: 10.1159/000074428
- Navé, B. T., Ouwens, M., Withers, D. J., Alessi, D. R., and Shepherd, P. R. (1999). Mammalian target of rapamycin is a direct target for protein kinase B: identification of a convergence point for opposing effects of insulin and amino-acid deficiency on protein translation. *Biochem. J.* 344, 427–431.
- Niculescu, F., Nguyen, P., Niculescu, T., Rus, H., Rus, V., and Via, C. S. (2003). Pathogenic T cells in murine lupus exhibit spontaneous signaling activity through phosphatidylinositol 3-kinase and mitogen-activated protein kinase pathways. *Arthr. Rheum.* 48, 1071–1079. doi: 10.1002/art.10900
- Nienhuis, H. L., de Leeuw, K., Bijzet, J., Smit, A., Schalkwijk, C. G., Graaff, R., et al. (2008). Skin autofluorescence is increased in systemic lupus erythematosus but is not reflected by elevated plasma levels of advanced glycation endproducts. *Rheumatology (Oxford)* 47, 1554–1558. doi: 10.1093/rheumatology/ken302
- Park, J. K., Kim, J.-Y., Moon, J. Y., Ahn, E. Y., Lee, E. Y., Lee, E. B., et al. (2016). Altered lipoproteins in patients with systemic lupus erythematosus are associated with augmented oxidative stress: a potential role in atherosclerosis. *Arthr. Res. Ther.* 18:306. doi: 10.1186/s13075-016-1204-x
- Perez-Diez, A., Morgun, A., and Shulzhenko, N. (2013). *Microarrays for Cancer Diagnosis and Classification. Landes Bioscience.* Available online at: <https://www.ncbi.nlm.nih.gov/books/NBK6624/> (accessed July 19, 2019).
- Pines, A., Bivi, N., Romanello, M., Damante, G., Kelley, M. R., Adamson, E. D., et al. (2005). Cross-regulation between Egr-1 and APE/Ref-1 during early response to oxidative stress in the human osteoblastic HOBIT cell line: Evidence for an autoregulatory loop. *Free Radical Res.* 39, 269–281. doi: 10.1080/10715760400028423
- Pons-Estel, G. J., Alarcón, G. S., Scofield, L., Reinlib, L., and Cooper, G. S. (2010). Understanding the epidemiology and progression of systemic lupus erythematosus. *Sem. Arthr. Rheum.* 39, 257–268. doi: 10.1016/j.semarthrit.2008.10.007
- Prokunina, L., and Alarcon-Riquelme, M. (2004). The genetic basis of systemic lupus erythematosus—knowledge of today and thoughts for tomorrow. *Hum. Mol. Genet.* 13, R143–R148. doi: 10.1093/hmg/ddh076
- Rahman, K. M. T., Islam, M. F., Banik, R. S., Honi, U., Diba, F. S., Sumi, S. S., et al. (2013). *Changes in Protein Interaction Networks Between Normal and Cancer Conditions: Total Chaos or Ordered Disorder? Network Biology.* Available online at: <http://agris.fao.org/agris-search/search.do?recordID=CN2013200002> (accessed November 19, 2019).
- Razani, B., and Lisanti, M. P. (2001). Caveolins and caveolae: molecular and functional relationships. *Exp. Cell Res.* 271, 36–44. doi: 10.1006/excr.2001.5372
- Recio, J. A., and Merlino, G. (2003). Hepatocyte growth factor/scatter factor induces feedback up-regulation of CD44v6 in melanoma cells through Egr-1. *Cancer Res.* 63, 1576–1582.
- Rena, G., Guo, S., Cichy, S. C., Unterman, T. G., and Cohen, P. (1999). Phosphorylation of the transcription factor forkhead family member FKHR by protein kinase B. *J. Biol. Chem.* 274, 17179–17183. doi: 10.1074/jbc.274.24.17179
- Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., et al. (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res.* 43:e47. doi: 10.1093/nar/gkv007
- Robeva, R., Tanev, D., Andonova, S., Kirilov, G., Savov, A., Stoycheva, M., et al. (2013). Androgen receptor (CAG) n polymorphism and androgen levels in women with systemic lupus erythematosus and healthy controls. *Rheumatol. Int.* 33, 2031–2038. doi: 10.1007/s00296-013-2687-2
- Rui, C., Li, C., Xu, W., Zhan, Y., Li, Y., and Yang, X. (2008). Involvement of Egr-1 in HGF-induced elevation of the human 5 α -R1 gene in human hepatocellular carcinoma cells. *Biochem. J.* 411, 379–386. doi: 10.1042/BJ20071343
- Russo, G., Zegar, C., and Giordano, A. (2003). Advantages and limitations of microarray technology in human cancer. *Oncogene* 22:6497. doi: 10.1038/sj.onc.1206865
- Sáenz-Corral, I., Vega-Memije, M. E., Martínez-Luna, E., Cuevas-González, J. C., Rodríguez-Carreón, A. A., de la Rosa, J. B.-G., et al. (2015). Apoptosis in chronic cutaneous lupus erythematosus, discoid lupus, and lupus profundus. *Int. J. Clin. Exp. Pathol.* 8, 7260–7265.
- Salas, T. R., Kim, J., Vakar-Lopez, F., Sabichi, A. L., Troncoso, P., Jenster, G., et al. (2004). Glycogen synthase kinase-3 β is involved in the phosphorylation and suppression of androgen receptor activity. *J. Biol. Chem.* 279, 19191–19200. doi: 10.1074/jbc.M309560200
- Santiago-Raber, M.-L., Lawson, B. R., Dummer, W., Barnhouse, M., Koundouris, S., Wilson, C. B., et al. (2001). Role of cyclin kinase inhibitor p21 in systemic autoimmunity. *J. Immunol.* 167, 4067–4074. doi: 10.4049/jimmunol.167.7.4067
- Sauer, L., Gitenay, D., Vo, C., and Baron, V. T. (2010). Mutant p53 initiates a feedback loop that involves Egr-1/EGF receptor/ERK in prostate cancer cells. *Oncogene* 29, 2628–2637. doi: 10.1038/onc.2010.24
- Sekuliac, A., Hudson, C. C., Homme, J. L., Yin, P., Otterness, D. M., Karnitz, L. M., et al. (2000). A Direct linkage between the phosphoinositide 3-kinase-AKT signaling pathway and the mammalian target of rapamycin in mitogen-stimulated and transformed cells. *Cancer Res.* 60, 3504–3513.
- Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., et al. (2003). Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* 13, 2498–2504. doi: 10.1101/gr.1239303
- Shen, M., and Yen, A. (2008). c-Cbl Interacts with CD38 and promotes retinoic acid-induced differentiation and G0 arrest of human myeloblastic leukemia cells. *Cancer Res.* 68, 8761–8769. doi: 10.1158/0008-5472.CAN.08-1058
- Shin, S. Y., Chin, B. R., Lee, Y. H., and Kim, J.-H. (2006). Involvement of glycogen synthase kinase-3 β in hydrogen peroxide-induced suppression of Tcf/Lef-dependent transcriptional activity. *Cell. Signal.* 18, 601–607. doi: 10.1016/j.cellsig.2005.06.001
- Shlomchik, M. J., Craft, J. E., and Mamula, M. J. (2001). From T to B and back again: positive feedback in systemic autoimmune disease. *Nat. Rev. Immunol.* 1, 147–153. doi: 10.1038/35100573
- Siess, W., Zangl, K. J., Essler, M., Bauer, M., Brandl, R., Corrinth, C., et al. (1999). Lysophosphatidic acid mediates the rapid activation of platelets and endothelial cells by mildly oxidized low density lipoprotein and accumulates in human atherosclerotic lesions. *Proc. Natl. Acad. Sci. U.S.A.* 96, 6931–6936.
- Skoura, A., and Hla, T. (2009). Lysophospholipid receptors in vertebrate development, physiology, and pathology. *J. Lipid Res.* 50, S293–S298. doi: 10.1194/jlr.R800047-JLR200
- Smith, S., Fernando, T., Wu, P. W., Seo, J., Ni Gabhann, J., Piskareva, O., et al. (2017). MicroRNA-302d targets IRF9 to regulate the IFN-induced gene expression in SLE. *J. Autoimmu.* 79, 105–111. doi: 10.1016/j.jaut.2017.03.003
- Smyth, G. K. (2005). “limma: linear models for microarray data,” in *Bioinformatics and Computational Biology Solutions Using R and Bioconductor Statistics for Biology and Health*, eds R. Gentleman, V. J. Carey, W. Huber, R. A. Irizarry, and S. Dudoit (New York, NY: Springer), 397–420. doi: 10.1007/0-387-29362-0_23
- Smyth, S. S., Cheng, H.-Y., Miriyala, S., Panchatcharam, M., and Morris, A. J. (2008). Roles of lysophosphatidic acid in cardiovascular physiology and disease. *Biochim. Biophys. Acta (BBA) Mol. Cell Biol. Lipids* 1781, 563–570. doi: 10.1016/j.bbalip.2008.05.008
- Szklarczyk, D., Gable, A. L., Lyon, D., Junge, A., Wyder, S., Huerta-Cepas, J., et al. (2019). STRING v11: protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res.* 47, D607–D613. doi: 10.1093/nar/gky1131
- Szklarczyk, D., Morris, J. H., Cook, H., Kuhn, M., Wyder, S., Simonovic, M., et al. (2017). The STRING database in 2017: quality-controlled protein–protein association networks, made broadly accessible. *Nucleic Acids Res.* 45, D362–D368. doi: 10.1093/nar/gkw937
- Tan, E. M., Cohen, A. S., Fries, J. F., Masi, A. T., Mcshane, D. J., Rothfield, N. F., et al. (1982). The 1982 revised criteria for the classification of systemic

- lupus erythematosus. *Arthr. Rheum.* 25, 1271–1277. doi: 10.1002/art.1780251101
- Tang, E. D., Nuñez, G., Barr, F. G., and Guan, K.-L. (1999). Negative regulation of the forkhead transcription factor FKHR by Akt. *J. Biol. Chem.* 274, 16741–16746. doi: 10.1074/jbc.274.24.16741
- Tang, H., Tan, G., Guo, Q., Pang, R., and Zeng, F. (2009). Abnormal activation of the Akt-GSK3 β signaling pathway in peripheral blood T cells from patients with systemic lupus erythematosus. *Cell Cycle* 8, 2789–2793. doi: 10.4161/cc.8.17.9446
- Thirumal Kumar, D., Jain, N., Evangeline, J., Kamaraj, B., Siva, R., Zayed, H., et al. (2019). A computational approach for investigating the mutational landscape of RAC-alpha serine/threonine-protein kinase (AKT1) and screening inhibitors against the oncogenic E17K mutation causing breast cancer. *Comput. Biol. Med.* 115:103513. doi: 10.1016/j.combiomed.2019.103513
- Tsokos, G. C., Lo, M. S., Reis, P. C., and Sullivan, K. E. (2016). New insights into the immunopathogenesis of systemic lupus erythematosus. *Nat. Rev. Rheumatol.* 12, 716–730. doi: 10.1038/nrrheum.2016.186
- Tzivion, G., Dobson, M., and Ramakrishnan, G. (2011). FoxO transcription factors; Regulation by AKT and 14-3-3 proteins. *Biochim. Biophys. Acta (BBA) Mol. Cell Res.* 1813, 1938–1945. doi: 10.1016/j.bbamcr.2011.06.002
- Veeranki, S., and Choubey, D. (2010). Systemic lupus erythematosus and increased risk to develop B cell malignancies: role of the p200-family proteins. *Immunol. Lett.* 133, 1–5. doi: 10.1016/j.imlet.2010.06.008
- Vences-Catalán, F., Rajapaksa, R., Levy, S., and Santos-Argumedo, L. (2012). The CD19/CD81 complex physically interacts with CD38 but is not required to induce proliferation in mouse B lymphocytes. *Immunology* 137, 48–55. doi: 10.1111/j.1365-2567.2012.03602.x
- Wang, J., Liu, Y., Zhao, J., Xu, J., Li, S., and Qin, X. (2017). P-glycoprotein gene MDR1 polymorphisms and susceptibility to systemic lupus erythematosus in Guangxi population: a case-control study. *Rheumatol. Int.* 37, 537–545. doi: 10.1007/s00296-017-3652-2
- Wolfrum, C., Besser, D., Luca, E., and Stoffel, M. (2003). Insulin regulates the activity of forkhead transcription factor Hnf-3 β /Foxa-2 by Akt-mediated phosphorylation and nuclear/cytosolic localization. *Proc. Natl. Acad. Sci. U.S.A.* 100, 11624–11629. doi: 10.1073/pnas.1931483100
- Yamauchi, A., and Bloom, E. T. (1997). Control of cell cycle progression in human natural killer cells through redox regulation of expression and phosphorylation of retinoblastoma gene product protein. *Blood* 89, 4092–4099. doi: 10.1182/blood.V89.11.4092
- Yang, L. V., Radu, C. G., Wang, L., Riedinger, M., and Witte, O. N. (2005). Gi-independent macrophage chemotaxis to lysophosphatidylcholine via the immunoregulatory GPCR G2A. *Blood* 105, 1127–1134. doi: 10.1182/blood-2004-05-1916
- Yang, W., and Lau, Y. L. (2015). Solving the genetic puzzle of systemic lupus erythematosus. *Pediatr. Nephrol.* 30, 1735–1748. doi: 10.1007/s00467-014-2947-8
- Yeh, P. Y., Kuo, S.-H., Yeh, K.-H., Chuang, S.-E., Hsu, C.-H., Chang, W. C., et al. (2006). A pathway for tumor necrosis factor- α -induced Bcl10 nuclear translocation Bcl10 is up-regulated by nf- κ b and phosphorylated by Akt1 and then complexes with bcl3 to enter the nucleus. *J. Biol. Chem.* 281, 167–175. doi: 10.1074/jbc.M511014200
- Yu, J., Akishita, M., Eto, M., Koizumi, H., Hashimoto, R., Ogawa, S., et al. (2012). Src kinase mediates androgen receptor-dependent non-genomic activation of signaling cascade leading to endothelial nitric oxide synthase. *Biochem. Biophys. Res. Commun.* 424, 538–543. doi: 10.1016/j.bbrc.2012.06.151
- Zhou, Y., Yuan, J., Pan, Y., Fei, Y., Qiu, X., Hu, N., et al. (2009). T cell CD40LG gene expression and the production of IgG by autologous B cells in systemic lupus erythematosus. *Clin. Immunol.* 132, 362–370. doi: 10.1016/j.clim.2009.05.011
- Zhu, H., Luo, H., Yan, M., Zuo, X., and Li, Q.-Z. (2015). Autoantigen microarray for high-throughput autoantibody profiling in systemic lupus erythematosus. *Genom. Proteom. Bioinformatics* 13, 210–218. doi: 10.1016/j.gpb.2015.09.001

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Udhaya Kumar, Thirumal Kumar, Siva, George Priya Doss, Younes, Younes, Sidenna and Zayed. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.