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Deciphering Molecular Mechanisms of Cutaneous Leishmaniasis, Pathogenesis and Drug Repurposing through Systems Biology

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ABSTRACT

Background: Cutaneous leishmaniasis is a major health problem caused by an intracellular pathogen of the genus *Leishmania*. CL results in morphologically distinct skin injuries, ranging from nodules to plaques and ulcers, which persist as a recuperating incessant injury depending on the type of contaminating parasite. There is still no effective treatment to reduce the skin lesions in patients infected with CL. The aim of this study was to develop strategies to treat skin lesions in CL patients.

Methods: We retrieved the transcriptomic data of skin lesions from patients with CL and normal skin from the GEO database. The PPIN was constructed using the STRING database and Cytoscape v3.10.1 software. Critical genes were identified by topological network analysis and cluster detection. Finally, gene ontology and repurposing drugs for critical genes were determined.

Results: CD8A, IFNG, IL-6, PTPRC, CCR7, TLR2, GSTA5, CYBB, IL-12RB2, ITGB2, FCGR3A, CTLA4, and IFNG were identified as the critical genes in PPIN and subnetworks. Enrichment analysis revealed that T-cell receptor signaling, TLR signaling, cytokine-cytokine receptor interaction, graft-versus-host disease, leishmaniasis, chemokine signaling, primary immunodeficiency, and Th17 cell differentiation were the major pathways associated with critical genes. The drug repurposing results identified cyclosporine, rituximab, infliximab, blinatumomab, and methylprednisolone as candidates for treatment of CL.

Conclusion: After validating our model with available experimental data, we found that critical molecules and drug candidates play a crucial role in the treatment of skin lesions caused by Leishmania in prospective studies.

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Keywords: Cutaneous leishmaniasis, Gene ontology, Repurpose drug, Systems biology

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List of Abbreviations:
CL: cutaneous leishmaniasis; **CTLA4:** cytotoxic T-lymphocyte-associated protein 4 or CD15; **DAVID:** Visualization and Integrated Discovery; **DEGs:** differentially expressed genes; **DGIdb:** Drug-Gene Interaction Database; **GEO:** gene Expression Omnibus; **GRN:** gene regulatory networks; **IFN-γ:** interferon gamma; **L. major:** *Leishmania major*; **PPIN:** protein-protein interaction network; **PTPRC:** protein tyrosine phosphatase receptor type C; **Th1:** T helper type 1; **TLR:** toll-like receptor

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INTRODUCTION

Cutaneous leishmaniasis is a significant health issue caused by an intracellular pathogen belonging to the genus *Leishmania*[1]. Morphologically, CL results in different skin lesions, such as nodules, plaques, and ulcers, which can evolve into healing scars or chronic lesions, depending on the infecting parasite[2].

Treatment of CL is difficult for several reasons. First, there is no preventive vaccine. Second, treatment of CL worldwide is complicated by the toxicity, side effects, and low efficacy of available drugs. The first-line drugs for CL treatment, megluminantimonate and sodium stibogluconate, are toxic and their mechanisms of action in the body are not fully understood. In addition, the efficacy of these drugs is limited in certain countries such as Iran[3]. Second-line drugs such as miltefosine are effective for diffuse CL, but there is a possibility of relapse with new infections[4]. Amphotericin B and pentamidine, as second-line treatments, require long-term parenteral administration[5]. All these drawbacks force researchers to look for new strategies to combat this important neglected disease.

Therapeutic strategies in use today include drug repurposing, multidrug therapy, CO_2 laser administration, using immunomodulators to activate the immune system, topical drug therapies such as the use of NO compounds, intralesional drug administration, and nanotechnology-based drug delivery systems. In addition to these new strategies, the search for new targets for the development of potential new drugs is one of the most reliable alternatives to overcome the adversities of infection[6]. By using different branches of computational biology such as bioinformatics, chemoinformatics, and systems biology, researchers can find new molecular targets. In recent decades, systems biology has improved our knowledge of the molecular mechanisms underlying various human diseases. Furthermore, various network analysis techniques such as PPIN and GRN are employed to decipher the underlying molecular mechanisms and how interactions can be addressed[7].

In the present study, we determined hub-bottleneck, clusters, and seed nodes in the PPIN, identified gene ontology and functional pathways associated with hub-bottleneck and cluster nodes, repurposed drugs, and analyzed the DGIdb to reduce skin lesions. We finally confirmed critical genes and repurposed drug candidates based on the previous studies.

MATERIALS AND METHODS

This study consisted of four steps: (1) identifying DEGs of CL/normal skin (GSE63931), (2) detecting crucial genes and clusters of PPIN, (3) enrichment analysis of crucial genes and clusters, and (4) repurposing drug for crucial genes.

Data collection and raw data processing

The CL/normal skin dataset (GSE63931) was retrieved from the GEO database (https://www.ncbi.nlm.nih.gov/geo/). This study included eight samples of normal skin (non-infected) and eight samples of cutaneous *Leishmania braziliensis*. DEGs were analyzed using GEO2R and normalized using the GEO query and the R package limma v3.60.3. DEGs were identified according to $p < 0.001$ and log2 fold changes >3 and <-3.

PPIN construction and topological analysis

DEGs were entered into the STRING database (https://string-db.org/), and interactions with a confidence score >0.7 were selected for the protein-protein interaction network retrieval using Cytoscape v3.10.1 software. The Network Analyzer App was used for topological analysis and identification of the nodes with the highest degree, betweenness centrality and closeness centrality in PPIN. Finally, Venn diagram v.1.7.3 presented shared nodes with the above-mentioned characteristics in PPIN.

Construction of PPIN clusters

PPIN clusters were identified using the MCODE App in the Cytoscape v3.10.1 software. We used the parameters degree cutoff = 2, node score cutoff = 0.2, K-core = 2, and max-depth = 100 to identify clusters. The clusters are regions with dense connectivity in PPIN. Finally, the clusters with score >3 were identified as significant clusters.

Gene ontology and pathways analysis

The shared nodes with the highest degree, betwenness centrality, and closeness centrality in PPIN, as well as the cluster nodes were selected for gene ontology analysis (biological process, molecular function, and cellular component) and functional pathways. The nodes were enriched using the Database for Annotation, DAVID, and webGestalt tools.

Drug-target interaction analysis

We selected shared nodes with the highest degree, betweenness and closeness centrality, and cluster nodes to identify drug-gene interaction networks. By using the drug-gene interaction database (https://www.dgidb.org/), we repurposed new drug candidates for critical proteins. In the end, the interaction network between the new repurposed drugs and the selected proteins was visualized using Cytoscape v3.10.1 software.

RESULTS

Raw data analysis

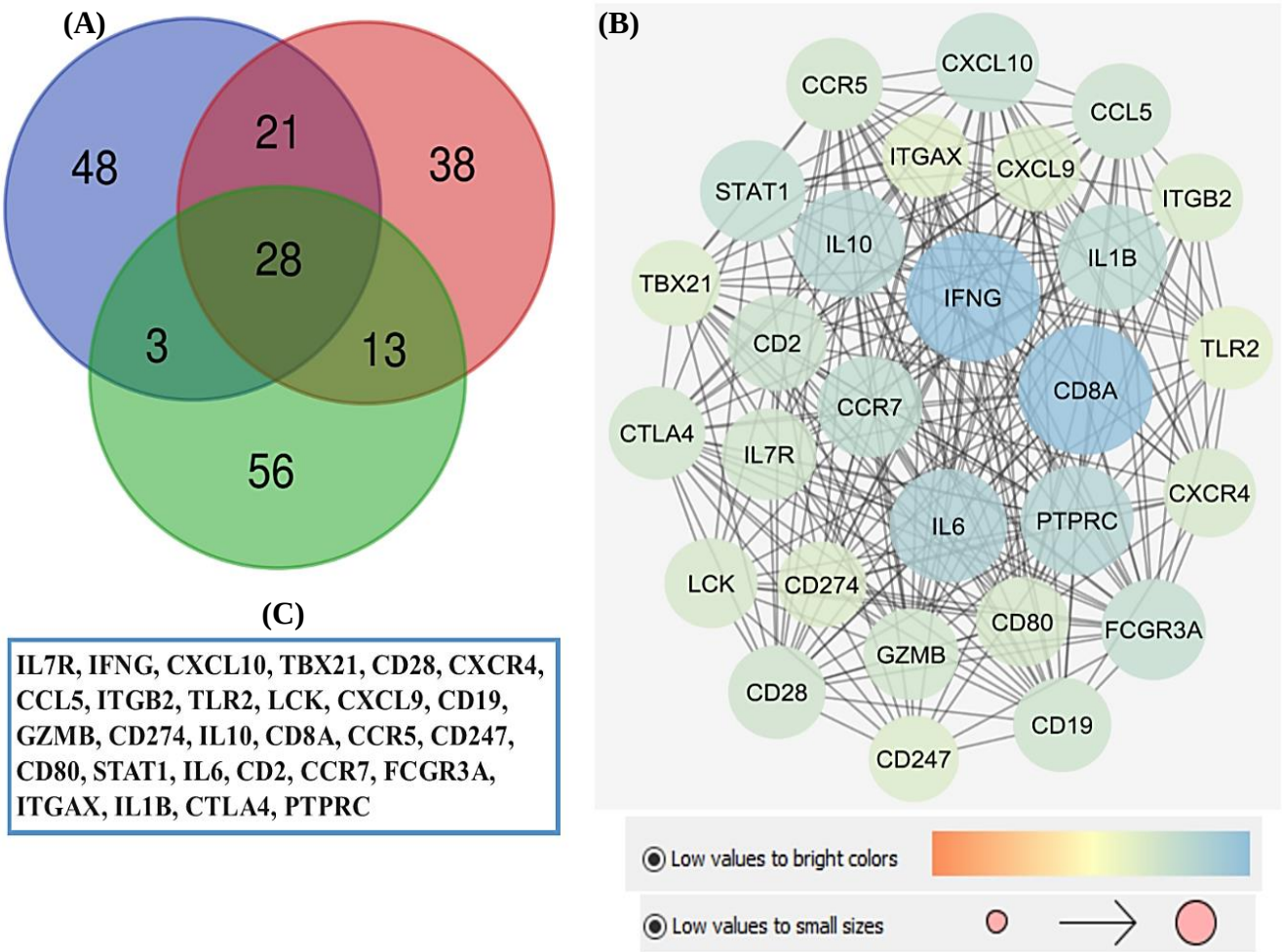
A total of 936 DEGs, including 665 upregulated and 271 downregulated genes, were retrieved from the analysis of GSE63931 (CL/normal skin dataset) using GEO2R. The filtering criteria applied were $p < 0.001$ and log2 fold change >3 and <-3. All DEGs are shown in Table S1.

Construction of PPIN and topological analysis

The STRING database was used to create the PPIN (score > 0.7). The visualization of this PPIN (interactions with score > 0.7) in the Cytoscape software showed a network with 495 nodes and 3134 edges. The parameters of this network included clustering coefficient of 0.411, network density of 0.026, characteristic path length of 3.228, and shortest paths of 73%. The top 100 nodes with the highest degree betweenness and closeness centrality were entered into the Venn diagram, and shared nodes were identified (Fig. 1A). The network of the shared nodes and name of proteins are shown in Figure 1B and 1C, respectively. Table 1 shows the top ten shared nodes with the highest degree, betweenness centrality, and closeness centrality.

MCODE sub-networks

The MCODE App identified seven clusters with score >3 in PPIN using the Cytoscape software. The seed proteins of these clusters included CCR7 (cluster no.1), TLR2 (cluster no.2), GSTA5 (cluster no.5), CYBB (cluster no.6), and IL-12RB2 (cluster no.7). The characteristics and nodes related to clusters are depicted in Table 2 and Figure 2.



(C)

IL7R, IFNG, CXCL10, TBX21, CD28, CXCR4, CCL5, ITGB2, TLR2, LCK, CXCL9, CD19, GZMB, CD274, IL10, CD8A, CCR5, CD247, CD80, STAT1, IL6, CD2, CCR7, FCGR3A, ITGAX, IL1B, CTLA4, PTPRC

Fig. 1. PPIN. (A) The shared genes with highest degree, betweenness centrality, and closeness centrality obtained by Venn diagram v1.7.3; (B) PPIN of the shared proteins with the highest degree, betweenness centrality, and closeness centrality. The nodes with blue color and big size have the highest degree. (C) Name of the shared proteins.

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Table 1. The top 10 shared proteins with highest degree, betweenness centrality, and closeness centrality in PPIN

Gene	Degree	Betweenness centrality	Closeness centrality
CD8A	112	0.081918	0.487356
IFNG	111	0.084965	0.481271
IL-6	87	0.062358	0.454448
PTPRC	81	0.056818	0.468508
IL-10	79	0.025762	0.445846
IL-1B	75	0.050386	0.451544
STAT1	69	0.076388	0.431772
CCR7	69	0.013879	0.428716
FCGR3A	68	0.038007	0.452026
CXCL10	66	0.023928	0.414467

Table 2. Details of clusters obtained from PPIN using MCODE App with score >3

Cluster	Nodes	Edge	Score	Seed protein	Nodes IDs
1	25	268	22.333	CCR7	CXCL2, CXCR3, ICAM1, CXCL10, CXCL1, CXCL3, CCL4, IL-1B, CCL3, CCL27, CXCL11, CCL25, CCR1, CXCL9, CXCL8, CCR5, CCL8, CXCR4, CCL5, CCRL2, CCL2, CXCR6, CXCL13, CCR7, CXCR5
2	39	277	14.579	TLR2	EPSTI1, HERC6, IFIT3, CD38, IFIT2, RSAD2, IFI44L, IFI44, CSF3, LAG3, OASL, PDCD1LG2, XAF1, ICOS, MX1, PTPRC, ISG15, HAVCR2, IFI6, GZMB, CD28, TNFRSF4, CTLA4, IL-6, ITGAX, SELL, TBX21, GBP1, CD27, CMPK2, STAT1, IDO1, IL7R, IRF7, IFITM1, IL-2RB, OAS2, TLR2, MX2
3	20	79	8.316	--	IL-10, CD80, ITK, IFNG, FCGR3A, TIGIT, CD5, CSF2, NCR1, MMP3, TIMP1, CD68, MMP1, LCK, CD8A, CD19, CD163, PRF1, TNFRSF9, KLRD1
4	21	70	7	--	CD7, CD274, KLRB1, CD3G, KLRG1, NKG7, CD3E, GZMA, CD3D, ITGB2, ITGAL, ZAP70, CD247, KLRK1, CD6, LGALS9, EOMES, GNLY, BTLA, CD2, LCP2
5	7	13	4.333	GSTA5	CYP3A5, CYP1A1, GSTA5, GSTA3, CYP3A7, ADH1C, ADH1A
6	4	6	4	CYBB	CYBB, CYBA, NOX4, NCF1
7	7	11	3.667	IL-12RB2	OSM, CSF3R, IL-12RB2, IL-21R, LEPR, IL-24, JAK3

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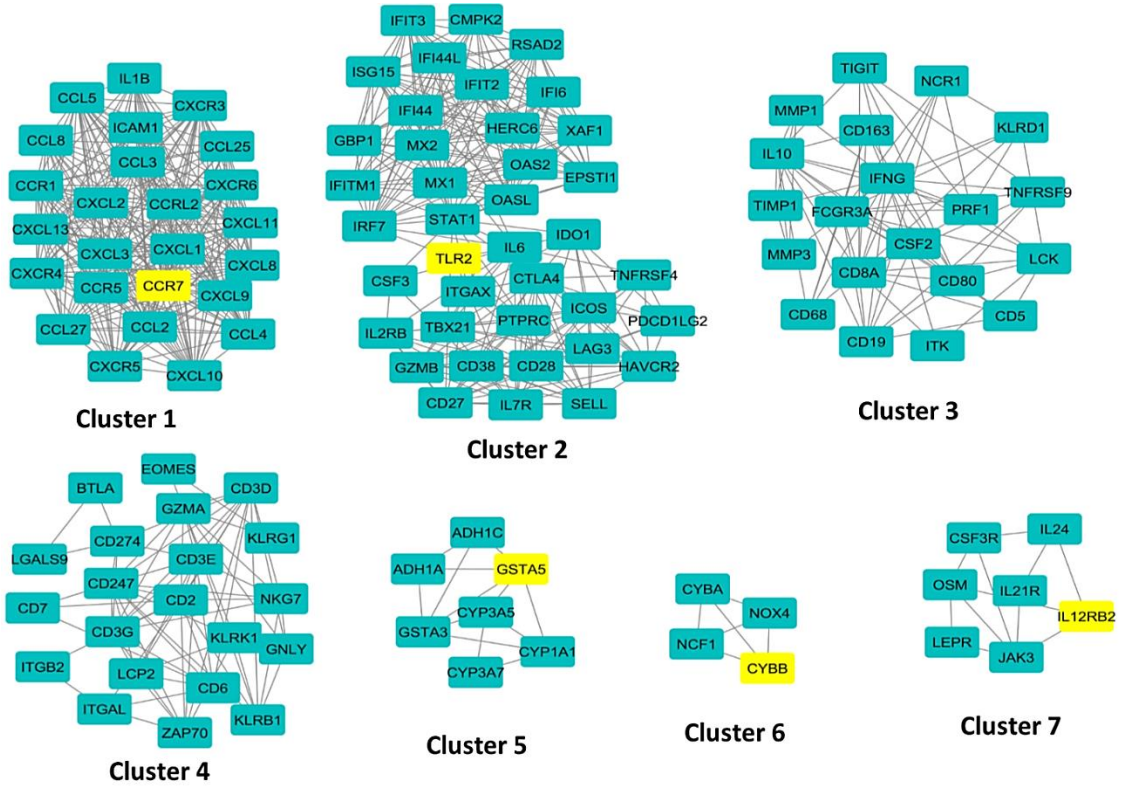

Fig. 2. Clusters of PPIN obtained by MCODE App in Cytoscape v3.10.1software (score >3). The seeds are shown with yellow color.

Enrichment analysis

We performed gene ontology analysis for the shared genes with the highest degree, betweenness and closeness centralities, as well as clusters nodes using DAVID database. Tables 3 and 4 show gene ontology results. For the shared genes with the highest degree, betweenness centrality, and closeness centrality, the following biological process terms were included: immune response, immune system process, leukocyte activation, regulation of cell-cell adhesion of leukocyte, immune effector process. Receptor binding, signaling receptor activity, cytokine receptor binding, molecular transducer activity, and receptor activity were the top molecular functional terms. Most genes were mapped to outer side of the cell membrane. Inside the membrane, there were cell surface and plasma membrane. In cluster nodes, immune response, immune system process, defense response, response to other organisms, response to external biotic stimuli were the top important biological process terms. The top molecular terms included cytokine activity, cytokine receptor binding, chemokine receptor binding, chemokine activity, and receptor binding. Most genes were found in outer side of plasma membrane, cell surface, plasma membrane part, and integral component of plasma membrane. Analysis of KEGG pathway using WebGgestalt revealed that shared genes with the highest degree, betweenness centrality, and closeness centrality were involved in T-cell receptor signaling, TLR signaling, cytokine-cytokine receptor interaction, graft-versus-host disease, and leishmaniasis. Key pathway terms that were significantly enriched in cluster nodes included cytokine-cytokine receptor interaction, chemokine signaling pathway, T-cell receptor signaling pathway, primary immunodeficiency, and Th17 cell differentiation (Table 5).

Drug repurposing

We evaluated the shared proteins with the highest degree, betweenness and closeness centrality and clusterd the proteins according to their drug-protein interactions available in DGIdb. The drug-protein interaction was visualized using Cytoscape software. The results indicated that methylprednisolone interacted with CXCL10, IFNG, CD80, ITGB2, and CTLA4. Prednisone interacted with IFNG, ITGB2, CTLA4, and PTPRC. Cyclosporine interacted with CD80, ITGB2, CTLA4, IL-10, and FCGR3A. Thalidomide intracted with CTLA4, ITGB2, and FCGR3A. Also, the IFNG, STAT1, CXCR4, and IL-6 proteins were in interaction with cisplatin. Rituximab was associated with IL-6, PTPRC, IL-10, and FCGR3A. Infliximab interacted

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Table 3. Top biological process, molecular function, cellular component, and pathways related to 28 shared genes of highest degree, betweenness centrality, and closeness centrality in PPIN

ID	Category	Gene	*p* value
Biological process			
GO:0006955	Immune response	*CD274, CXCL9, CD80, ITGB2, CXCR4, FCGR3A, CCL5, CD19, TBX21, CTLA4, CCR7, CCR5, IL-10, STAT1, GZMB, CD2, CXCL10, IL-6, PTPRC, IFNG, LCK, CD8A, IL-1B, CD28, CD247, IL7R, TLR2*	1.58E-24
GO:0002376	Immune system process	*CD274, CXCL9, CD80, ITGB2, CXCR4, FCGR3A, CCL5, CD19, TBX21, ITGAX, CTLA4, CCR7, CCR5, IL-10, STAT1, GZMB, CD2, CXCL10, IL-6, PTPRC, IFNG, LCK, CD8A, IL-1B, CD28, CD247, IL7R, TLR2*	3.18E-22
GO:0045321	Leukocyte activation	*IL-10, CD274, CD80, ITGB2, CD2, FCGR3A, IL-6, PTPRC, IFNG, LCK, CD8A, IL-1B, CD19, CCL5, TBX21, CD28, CTLA4, CCR7, CD247, IL7R, TLR2*	3.40E-20
GO:1903037	Regulation of leukocyte cell-cell adhesion	*IL-10, CD274, CD80, ITGB2, CD2, IL-6, PTPRC, IFNG, LCK, IL-1B, CCL5, TBX21, CD28, CTLA4, CCR7, IL7R*	1.44E-18
GO:0002252	Immune effector process	*IL-10, CXCL9, STAT1, CD80, ITGB2, GZMB, CD2, FCGR3A, IL-6, PTPRC, IFNG, CD8A, IL-1B, CD19, TBX21, ITGAX, CD28, IL7R, TLR2*	7.60E-18
Molecular function			
GO:0005102	Receptor binding	*IL-10, CXCL9, STAT1, CD80, ITGB2, CD2, CXCL10, IL-6, PTPRC, IFNG, LCK, CD8A, IL-1B, CCL5, ITGAX, TLR2*	9.28E-10
GO:0038023	Signaling receptor activity	*CD80, ITGB2, CXCR4, CD2, FCGR3A, PTPRC, CD8A, ITGAX, CD28, CCR7, CD247, CCR5, IL7R, TLR2*	3.18E-08
GO:0005126	Cytokine receptor binding	*IL-10, CXCL10, IL-6, CXCL9, IFNG, STAT1, CCL5, IL-1B*	7.57E-08
GO:0060089	Molecular transducer activity	*CD80, ITGB2, CXCR4, CD2, FCGR3A, PTPRC, CD8A, ITGAX, CD28, CCR7, CD247, CCR5, IL7R, TLR2*	1.03E-07
GO:0004872	Receptor activity	*CD80, ITGB2, CXCR4, CD2, FCGR3A, PTPRC, CD8A, ITGAX, CD28, CCR7, CD247, CCR5, IL7R, TLR2*	1.03E-07
Cellular component			
GO:0009897	External side of plasma membrane	*CD274, CXCL9, CD80, ITGB2, CXCR4, CD2, CXCL10, FCGR3A, PTPRC, CD8A, CD19, ITGAX, CD28, CTLA4, CCR7, CCR5, IL7R*	8.72E-20
GO:0009986	Cell surface	*CD274, CXCL9, CD80, ITGB2, CXCR4, CD2, CXCL10, FCGR3A, PTPRC, CD8A, CD19, ITGAX, CD28, CTLA4, CCR7, CCR5, IL7R, TLR2*	6.94E-16
GO:0005886	Plasma membrane	*CD274, CXCL9, CD80, ITGB2, CXCR4, GZMB, CD2, CXCL10, FCGR3A, IL-6, PTPRC, LCK, CD8A, CD19, ITGAX, CD28, CTLA4, CCR7, CD247, CCR5, IL7R, TLR2*	3.14E-07

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Table 4. Top biological process, molecular function, cellular component, and pathways related to clusters in PPIN

ID	Category	Proteins	*p* value
Biological process			
GO:0006955	Immune response	IFITM1, CSF3, CSF2, NCF1, CD80, PRF1, CXCL13, IFIT3, IFI44L, ICAM1, IFIT2, CCRL2, LGALS9, TNFRSF4, EOMES, LAG3, RSAD2, CYBB, CYBA, etc.	6.88E-73
GO:0002376	Immune system process	IFITM1, CSF3, CSF2, CSF3R, NCF1, CD80, PRF1, CXCL13, IFIT3, IFI44L, ICAM1, IFIT2, CCRL2, LEPR, LGALS9, TNFRSF4, HERC6, EOMES, LAG3, RSAD2, CYBB, CYBA, CD8A, IDO1, CD274, …	1.41E-69
GO:0006952	Defense response	ITK, CXCL9, IFITM1, CSF3R, CXCL8, NCF1, ITGB2, PRF1, CXCL1, CXCL13, ITGAL, CXCL3, CXCL2, IFI44L, IFIT3, OASL, IFIT2, FCGR3A, …	1.29E-45
GO:0051707	Response to other organism	CSF3, CXCL9, IFITM1, CSF2, CXCL8, CD80, IL-24, PRF1, CXCL1, CXCL13, CXCL3, CXCL2, IFI44L, IFIT3, OASL, IFIT2, GNLY, ITGAX, CCR7, …	3.59E-42
GO:0043207	Response to external biotic stimulus	CSF3, CXCL9, IFITM1, CSF2, CXCL8, CD80, IL-24, PRF1, CXCL1, CXCL13, CXCL3, CXCL2, IFI44L, IFIT3, OASL, IFIT2, GNLY, ITGAX, CCR7, …	4.15E-42
Molecular function			
GO:0005125	Cytokine activity	IL-10, CCL25, CSF3, CXCL9, CXCL8, CSF2, IL-24, OSM, CXCL1, CXCL13, CXCL3, CXCL2, CXCL10, CXCL11, IL-6, CCL8, IFNG, IL-1B, CCL5, CCL4, …	6.89E-21
GO:0005126	Cytokine receptor binding	CSF3, CXCL9, CSF2, CXCL8, CXCL1, CXCL13, CXCL3, CXCL2, CCL8, CCL5, CCRL2, CCL4, CCL3, CCL2, JAK3, IL-10, CCL25, STAT1, OSM, …	1.38E-20
GO:0042379	Chemokine receptor binding	CCL25, CXCL9, CXCL8, STAT1, CXCL1, CXCL13, CXCL3, CXCL2, CXCL10, CXCL11, CCL8, CCL5, CCRL2, CCL4, CCL3, CCL2, CCL27	1.85E-20
GO:0008009	Chemokine activity	CCL25, CXCL9, CXCL8, CXCL1, CXCL13, CXCL3, CXCL2, CXCL10, CXCL11, CCL8, CCL5, CCL4, CCL3, CCL2, CCL27	5.85E-20
GO:0005102	Receptor binding	CSF3, CXCL9, CSF2, CXCL8, CD80, IL-24, ITGB2, CD3G, CXCL1, CXCL13, CD3E, ITGAL, CXCL3, CXCL2, ICAM1, OASL, KLRK1, CCL8, CCL5, …	4.19E-17
Cellular component			
GO:0009897	External side of plasma membrane	CD274, CXCL9, CSF3R, CD80, ITGB2, CXCR5, CXCR4, CD3G, CXCR6, CD3E, ITGAL, CD3D, ICAM1, FCGR3A, KLRK1, CXCR3, CD19, …	1.39E-37
GO:0009986	Cell surface	CD274, CXCL9, CSF3R, KLRB1, CD80, ITGB2, CXCR5, CXCR4, CD3G, CXCR6, CD3E, ITGAL, CD3D, ICAM1, FCGR3A, KLRK1, CXCR3, …	4.21E-29
GO:0044459	Plasma membrane part	CXCL9, CSF2, CSF3R, NCF1, CD80, ITGB2, PRF1, CD3G, CD3E, ITGAL, CD3D, ICAM1, FCGR3A, CCRL2, ITGAX, LEPR, CD38, CTLA4, CCR7, …	1.59E-20
GO:0005887	Integral component of plasma membrane	CSF2, CSF3R, NCF1, ITGB2, CXCR5, CD3G, CXCR6, CD3E, ITGAL, ICAM1, FCGR3A, KLRK1, CXCR3, CD19, CCRL2, BTLA, ITGAX, CTLA4, …	3.93E-15

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Table 5. Top five KEGG pathways related to 28 shared genes of highest degree, betweenness centrality, and closeness centrality and clusters in PPIN

ID	Category	*p* value
28 shared genes		
hsa04660	T-cell receptor signaling pathway	2.59E-09
hsa04620	Toll-like receptor signaling pathway	3.28E-09
hsa04060	Cytokine-cytokine receptor interaction	4.56E-09
hsa05332	Graft-versus-host disease	7.55E-09
hsa05140	Leishmaniasis	8.46E-09
Clusters		
hsa04060	Cytokine-cytokine receptor interaction	<2.2e-16
hsa04062	Chemokine signaling pathway	<2.2e-16
hsa04660	T-cell receptor signaling pathway	5.55E-13
hsa05340	Primary immunodeficiency	5.84E-11
hsa04659	Th17 cell differentiation	2.50E-10

with IL-1B, IL-6, PTPRC, and FCGR3A. Additionally, CD2, FCGR3A, and CD28 were related to Aldesleukin, while CXCL10, ITGB2, and CTLA4 were assosiated with antibiotic. Blinatumomab also interacted with CD19, CD3G, and CD247. The ITGB2, FCGR3A, CTLA4, and IFNG proteins showed the highest degree in drug-protein interaction network. The results are shown in Figure 3.

DISCUSSION

Due to the diverse clinical manifestations, CL ranges from small nodules on the skin to massive destruction of mucosal tissues. The backbone of treatment for CL is pentavalent antimonials with modern oral and topical treatment options that have been available in recent years. Nonetheless, a vaccination is not currently accessible. Infection prevention and control are problematic due to the complexity of the epidemiology of CL and the limited alternatives available for effective vector control[8].

Molecular and Omics information can serve as strategies to repurpose new potential drugs for the diseases. The identification of important therapeutic targets and drug suggestions can significantly help to reduce skin lesions. Therefore, in this study, we proposed therapeutic strategies to reduce skin lesions using in silico studies. We identified critical nodes, functional clusters, and molecular mechanisms and ultimately repurposed drugs for crucial genes. Our results showed that CD8A, IFNG, IL-6, and PTPRC were critical proteins with the highest degree, betweenness centrality, and closeness centrality in the PPIN, all of which were upregulated in CL skin lesions.

Cytokines play an important role in the immunopathogenesis of *Leishmania* infection. IFN-γ, encoded by the *IFNG* gene, is a key inflammatory cytokine against CL driving the early development of Th1 and Th2 responses[9]. Pinheiro and Rossi-Bergmann investigated the role of IFN-γ in the relative resistance of C57Bl/6 mice to *Leishmania amazonensis* infection and showed that IFN-γ deficiency resulted in the formation of larger lesions and increased parasite burden compared to wild-type mice[10]. Moskowitz et al. found that C57BL/6 mice with IL-6 deficiency exhibited an enhanced Th1 response when infected with *L. major*, resulting in similarly effective infection control compared to wild-type mice[11].

PTPRC, also known as T200 glycoprotein, leukocyte-common antigen, or CD45, is a hematopoietic-specific transmembrane glycoprotein that is upregulated in skin lesions. This protein regulates T- and B-cell antigen receptor-mediated activation[12]. *CD45* has been reported to be expressed in sentinel cells of the skin of *Leishmania infantum*-infected dogs, which may be related to its activation[13]. Subnetwork analysis using PPIN identified CCR7, TLR2, GSTA5, CYBB, and IL-12RB2 as seeds in clusters. CCR7 (C-C chemokine receptor type 7), which is upregulated in skin lesions, is a transmembrane protein that drives immune cells to immune organs. *CCR7* expression is mainly reduced in effector cells[14] but is increased in human CL lesions compared to healthy controls. *CCR* expression is correlated negatively with lesion size[15]. TLR2 is another important protein involves in the recognition of pathogens and the activation of innate immunity. *TLR2* expression increases in macrophages and monocytes of patients with CL, leading to adverse outcomes[16]. Carneiro et al. showed that blocking TLR2 with its antagonists decreases inflammation and parasite load in the infected monocytes and cells obtained from skin lesions of CL patients[17].

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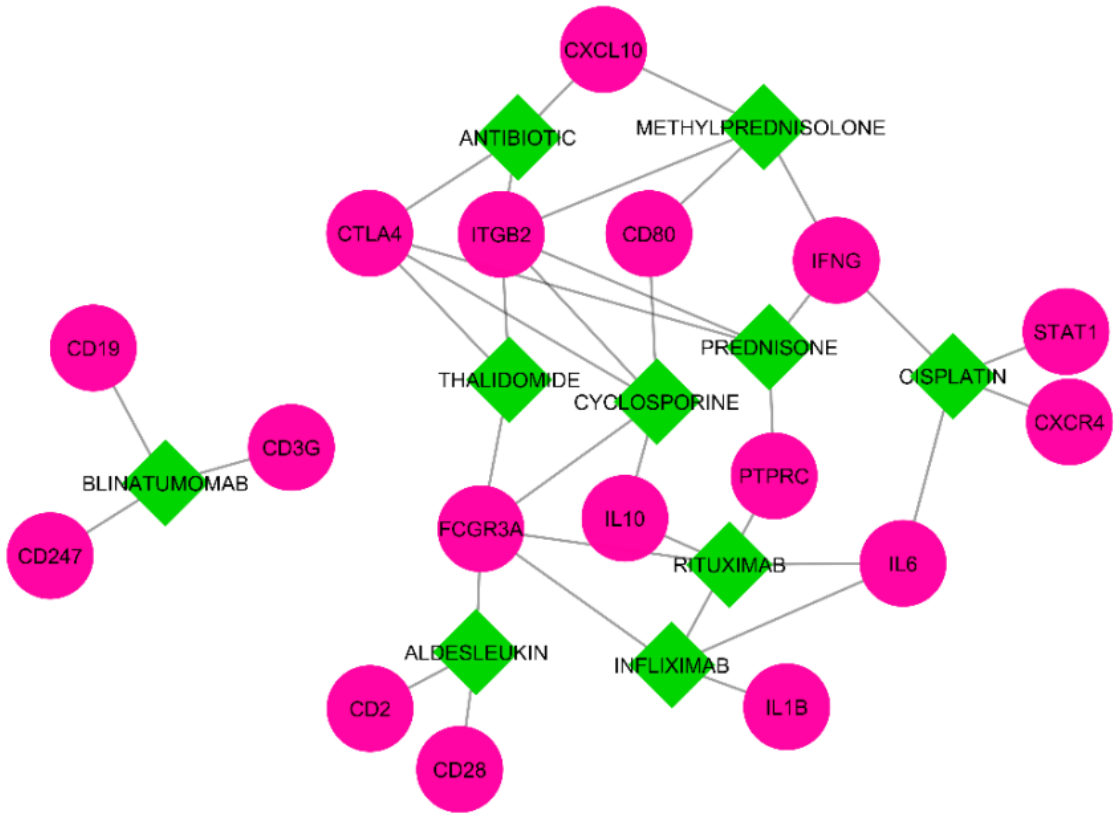

Fig. 3. The gene-drug interaction network. Drugs from DGID were extracted for hubs/bottlenecks and seeds. The final gene-drug interaction network obtained based on drugs with highest degree. The nodes with circular shape and pink color show genes, and the nodes with diamond shape and green color represent drugs.

Glutathione is a significant antioxidant modulator that plays a crucial dual role in the parasite-host relationship during infection with *L. major*. First, it protects *Leishmania* from the toxicity of the nitrogen-derived reactive species. Second, it regulates the immune response by promoting NO production and leishmanicidal activity of macrophages[18]. This protein is among the seed proteins that are downregulated in skin lesions. The relationship between the *GSTA5* expression and CL has not yet investigated. Therefore, further studies are needed to understand the role of *GSTA5* in CL lesions. CYBB, another seed protein, is upregulated in skin lesions. This protein encodes the cytochrome b-245 beta chain (also known as gp91phox). As a subunit of the phagocyte enzyme NADPH oxidase, CYBB produces the reactive oxygen species to kill parasite and activate immune cells. The production of reactive oxygen species derived from NOX2 by gp91phox has been shown to control parasite burden and inflammation in mice infected with *Leishmania amazonensis*[19]. IL-12RB2, an IL-12 receptor subunit beta-2, serves as a receptor for IL-12 and IL-23, cytokines that activate the immune cells such as T cells and natural killer cells to fight infection and tumors[20]. This receptor is upregulated in skin lesions of CL. It has been proposed that IL-12 has an active participation in triggering the Th1 response and the protective immune response to *Leishmania* infection[21]. Currently, there is no study showing the direct effect of *IL-12RB2* expression on CL lesions. Enrichment analysis revealed that T-cell receptor signaling, TLR signaling, cytokine-cytokine receptor interaction, graft-versus-host disease, leishmaniasis, chemokine signaling, primary immunodeficiency, and Th17 cell differentiation were the major pathways related to critical proteins;i.e. the shared proteins between the highest degree, betweenness centrality, closeness centrality, and clusters proteins. T-cell receptor signaling is important for antigen recognition via the connection between T cells and the antigen-presenting cells and subsequent, development and activation of T cells[22]. The TLRs are critical signaling pathways in the defense against infectious diseases, linking innate immunity to adaptive immunity by regulating the activation of antigen-presenting cells and the production of cytokines and chemokines[23]. Chemokine signaling and the interactions between cytokine-cytokine receptors are responsible for regulating the entire

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immune cell populations against infections, representing important potential drug targets[24]. Graft-versus-host disease is activated when the immune cells from the donor attack tissues and organs of the recipient, resulting in host tissue damage[25]. The primary immunodeficiency pathway is associated with disorders of cellular and humoral immunity or non-specific host defense mechanisms[26]. The Th17 cell differentiation pathway is a critical part of the immune response against extracellular pathogens, particularly at the mucosal and epithelial barriers, by which naïve T cells differentiate into Th17 effector cells[27].

The results of drug repurposing revealed methylprednisolone, prednisone, cyclosporine, thalidomide, cisplatin, rituximab, infliximab, aldesleukin, antibiotic, and blinatumomab as candidates for the treatment of CL. Prednisone and methylprednisolone are synthetic glucocorticoids with anti-inflammatory and immunosuppresive effects[28,29]. Cyclosporine is recognized as an imunosuppresive drug. In this regard, Solbach et al. have shown that cyclosporine suppresses the development of cutaneous lesions in susceptible BALAB/C mice infected with *Leishmania tropica*[30]. Thalidomide, a synthesis product of glutamic acid, is an immunomodulator that acts through various mechanisms, such as stimulating T cells and reducing TNF-α production. Thalidomide can be used as an effective conjunctive therapy with antimony in *L. major*-infected BALB/c mice[31]. To date, there has been no report on the effect of thalidomide on skin lesions of CL patients.

Cisplatin is a platinnum-based chemotherapeutic agent that binds to DNA[32]. Akhtari et al. showed in vitro antileishmanial activity of carbon nanotubes bound to cisplatin against both promastigotes and amastigotes of *L. major*, a cause of CL in vitro[33]. Rituximab, infliximab, and blinatumomab are monoclonal antibodies acting on the immune system. Rituximab targets $CD20^+$ B cells and is used for treating some cancers and autoimmune diseases. There is one case report of the fatal outcome of visceral leishmaniasis treated with rituximab[34], and no report has yet been found on the side effect of rituximab in CL. Schwartz et al. have highlighted that the combination of infliximab, a TNF-α inhibitor, and paromomycin reduces the inflammatory response of the skin in CL lesions of BALB/c mice[35]. Blinatumomab is a bispecific monoclonal antibody that targets the CD3 protein on T cells and CD19 on B cells[36]. Aldesleukin is a recombinant analog of IL-2 that stimulates the immune system to fight against cancer cells. Akuffo et al. have reported that intra-nodular injection of recombinant IL-2 in three cases of disseminated CL, increased cellular immunity, consequently decreased parasites at the cutaneous site[37]. Antibiotics are the most important medicines against bacterial infections. They are sometimes used to treat the protozoan infections. It has been found that some antibiotics such as pentamidine and paromomycin can be used in combination with gentamicin for treating CL[38,39]. The upregulated proteins ITGB2, FCGR3A, CTLA4, and IFNG indicated the highest level of interaction with these drugs. ITGB2 is correlated with antibiotics, thalidomide, methylprednisolone, and cyclosporine. It encodes integrin beta chain-2, also known as CD18, in leukocytes, endothelial cells, and epithelial cells. Thalidomide has been displayed to increase the expression of *CD18* in the neutrophils of mice[40]. Conversely, cyclosporine decreases its expression in gingival fibroblasts[41].

The *FCGR3A* gene has been associated with thalidomide, cyclosporine, rituximab, infliximab, and aldesleukin. It encodes immunoglobulin gamma Fc region receptor III-A, which is involved in antibody-dependent cellular cytotoxicity in natural killer cells. *FCGR3A* SNP genotype affects NK cell-mediated lysis of $CD20^+$ cells by rituximab[42], the pharmacokinetics of infliximab[43], and the response to IL-2[44]. CTLA4 interacts with thalidomide, antibiotics, cyclosporine, and prednisone. CTLA4 is a receptor on T cells that acts as an immune checkpoint for downregulation of immune responses[45]. Thalidomide and cyclosporine have been shown to increase *CTLA4* expression[46,47]. Cisplatin, prednisone, and methylprednisolone has been interacted IFNG. Treatment of macrophages with cisplatin promotes the transcription of *IFNG*[48], and prednisone enhances the expression of *IFNG* mRNA[49]. Methylprednisolone also suppresses the *IFNG* expression[50]. Considering the above-mentioned data, cyclosperin, rituximab, infliximab, blinatumomab, and methylprednisolone are suitable drugs for reducing skin lesions in CL infections.

CONCLUSION

Systems biology and network-based approaches (PPIN) can help predict the mechanisms involving in diseases and also find the repurposed drugs for disease treatment. This study shows that *CD8A*, *IFNG*, *IL-6*, *PTPRC*, *CCR7*, *TLR2*, *GSTA5*, *CYBB*, *IL-12RB2*, *ITGB2*, *FCGR3A*, *CTLA4*, and *IFNG* can be applied as biomarker panels and drug targets. These critical genes regulate functional signaling pathways such as T-cell receptor signaling, TLR signaling, cytokine-cytokine receptor interaction, graft-versus-host disease, leishmaniasis, cytokine-cytokine receptor interaction, chemokine signaling pathway, T-cell receptor signaling

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pathway, primary immune deficiency, and Th17 cell differentiation. Our study identified methylprednisolon, prednisone, cyclosporine, thalidomide, cisplatin, rituximab, infliximab, aldesleukin, antibiotic, and blinatumomab as repurposed drug candidates that interact with critical genes in CL infection. Since previous treatments have not helped reduce skin lesions in patients infected with Leishmani, identifying important genes to target skin lesions and repurposing drugs can contribute to the treatment of these lesions. We propose investigating the repurposed drugs both in vitro and in vivo for their potential role in treating skin lesions in CL infection. Validation of these studies with additional datasets and expremental research can yeild more reliable results.

DECLARATIONS

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Ethical approval

All the experimental procedures in this study were approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran (ethical code IR.SBMU.RETECH.REC.1400.785).

Consent to participate

Not applicable.

Consent for publication

All authors reviewed the results and approved the final version of the manuscript.

Authors' contributions

SF, DZ, PT, and TZ: writing manuscript; DZ and ZH: data collection and analysis.

Data availability

All relevant data can be found within the manuscript.

Competing interests

The authors declare that they have no competing interests.

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Supplementary information

The online version contains supplementary material.

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