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ORIGINAL ARTICLE

Repurposing of thalidomide and its derivatives for the treatment of SARS-coV-2 infections: Hints on molecular action

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Aims: The SARS-coV-2 pandemic continues to cause an unprecedented global destabilization requiring urgent attention towards drug and vaccine development. Thalidomide, a drug with known anti-inflammatory and immunomodulatory effects has been indicated to be effective in treating a SARS-coV-2 pneumonia patient. Here, we study the possible mechanisms through which thalidomide might affect coronavirus disease-19 (COVID-19).

Methods: The present study explores the possibility of repurposing thalidomide for the treatment of SARS-coV-2 pneumonia by reanalysing transcriptomes of SARS-coV-2 infected tissues with thalidomide and lenalidomide induced transcriptomic changes in transformed lung and haematopoietic models as procured from databases, and further comparing them with the transcriptome of primary endothelial cells.

Results: Thalidomide and lenalidomide exhibited pleiotropic effects affecting a range of biological processes including inflammation, immune response, angiogenesis, MAPK signalling, NOD-like receptor signalling, Toll-like receptor signalling, leucocyte differentiation and innate immunity, the processes that are aberrantly regulated in severe COVID-19 patients.

Conclusion: The present study indicates thalidomide analogues as a *better fit* for treating severe cases of novel viral infections, healing the damaged network by compensating the impairment caused by the COVID-19.

KEYWORDS
angiogenesis, COVID-19, endothelium, immune response, inflammation, lenalidomide, SARS-coV-2, thalidomide

1 | INTRODUCTION

Novel coronavirus, SARS-coV-2 has been posing devastating effects on a global scale with a soaring number of infections and an alarming rate of mortality. Along with the tremendous efforts to develop vaccines, repurposing of drugs with known safety and efficacy profiles is one of the viable choices for treatment. Coronavirus disease-19 (COVID-19) is clinically very challenging since the novel coronavirus triggers multiorgan turbulence devastating the homeostasis of the human system. Once the SARS-CoV-2 virus enters the respiratory tract, there are 4 different stages of the infection from symptoms to multiorgan failures. Phase I begins with the naso-oral viral entry followed by host immune system alert with active viral replication in the upper respiratory tract (Phase II). In Phase III, a minor cytokine storm occurs in the alveoli, releasing the inflammatory cytokines resulting in leaky blood vessels, which is followed by the second

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cytokine storm with uncontrolled inflammatory and life-threatening symptoms, acute respiratory distress syndrome (ARDS), seizure, severe hypoxia and severed organ toxicity (Phase IV).[1,2] Manifestation of the biphasic cytokine storm occurs through the activation of a series of cytokines including monocyte chemoattractant protein 1 (CCL2), macrophage inflammatory protein 1α (MIP-1α), tumour necrosis factor α (TNF-α), interleukin (IL)-2R and IL-6 overwhelming the system leading to indiscriminate damages in multiple organs.[3–5] There is an increased amount of blood vessel growth in the lungs of COVID-19 patients compared to severe influenza.[6] As COVID-19 is a multilayer problem, researchers around the world are desperately in search for a drug, which would able to tackle all or few of these COVID-19 hallmarks.

Thalidomide, a small molecule drug, with many years of history known to cause misery,[7] became a game changer for its multi-faceted pharmacological effects such as immunomodulation, anti-inflammation, antiangiogenesis and antiviral effects.[8] At present, the world needs a *smart* solution. Thalidomide increases the hope of treating COVID-19 patients.[9] Chen et al. report successful treatment of SARS-coV-2 associated pneumonia with combinatory treatment of thalidomide and a low-dose glucocorticoid.[10] Two clinical trials, NCT04273581 and NCT04273529 have been registered to check the efficacy of thalidomide in treating COVID-19 patients. The adverse effects of thalidomide and its analogues are well documented.[11] Extensive information available on thalidomide's mechanisms, its efficacy and safety in haemophagocytic syndrome-induced cytokine storm[12] and idiopathic pulmonary fibrosis,[13] severe H1N1, and paraquat poisoning lung injury[14,15] argues for the possible action of thalidomide on COVID-19 induced lung effects and cytokine storm. Recent reviews on thalidomide in COVID-19 treatment endorse the possibility of thalidomide and its analogues for the treatment of COVID-19 symptoms.[16,17]

Transcriptome-based approach to connect diseases with drug responses is a recognized strategy in drug repurposing.[18] With the fast-growing literature on SARS-coV-2 infections, we performed a combined analysis of whole transcriptome signatures of lungs, peripheral blood mononuclear cells (PBMC), bronchoalveolar lavage fluid (BALF) from SARS-coV-2 affected patients and A549 cells (transformed adenocarcinoma cells), and compared with the gene expression signatures of A549 cells treated with thalidomide or lenalidomide, haematopoietic cells, and human umbilical vein endothelial cells (HUVEC). We hereby provide possible mechanistic actions of thalidomide in treating the SARS-coV-2 pathology. In addition, we suggest that the derivatives of thalidomide, lenalidomide and CC-220 might also be effective in the treatment of SARS-coV-2.

What is already known about this subject

- Severe cases of COVID-19 infections show an aberrant surge in the host immune response and cytokine storm in lungs.
- There is an increased amount of angiogenesis observed in lungs of COVID-19 patients and the endothelium is heavily affected.
- A patient with severe pneumonia-associated with COVID-19 has been successfully treated with thalidomide.

What this study adds

- This study presents insights into the possible mechanisms by which thalidomide and lenalidomide would suppress the cytokine storm and immune response.
- Thalidomide and its derivative lenalidomide modulate expression of several genes and key pathways aberrantly regulated in SARS-coV-2 infected tissues.

2 | METHODS

2.1 | Data collection

A total of 16 gene expression datasets including 15 publicly available expression datasets were included in this study. Transcriptomes of SARS-coV-2 infected lung tissues matched with healthy control and SARS-coV-2 treated A549 cells were obtained from GEO (Accession ID: GSE147507).[19] Transcriptome data of BALF and PBMC were obtained from BIG Data Center (https://bigd.big.ac.cn/; Accession ID: CRA002390).[20] The expression profiles of systemic lupus erythematosus (Accession ID: GSE112087),[21] bone marrow cells treated with lenalidomide (Accession ID: GSE106748),[22] lymphoma cells treated with lenalidomide (Accession ID: GSE60618),[23] CD34-positive cells treated with pomalidomide (Accession ID: GSE144052), MERS infected PBMC (Accession: GSE1739),[24] lenalidomide-treated PBMC (Accession ID: GSE84251) and CC-122 treated lymphoma cells (Accession ID: GSE75420)[25] were procured from GEO and differentially expressed genes (DEGs) were identified using limma.[26] Library of Integrated Network-Based Cellular Signatures (iLINCS) is a database that contains the gene expression signatures of >21, 000 compounds (http://www.ilincs.org/ilincs/). We obtained the gene expression signatures for A549 cells treated with 10 μM thalidomide for 6 hours (LINCSCP_4683) and 24 hours (LINCSCP_4463), 100 μM lenalidomide for 6 hours (LINCSCP_4650) and 24 h (LINCSCP_4427).

2.2 | Transcriptome sequencing of HUVEC treated with thalidomide

Endothelitis is a common sign of COVID-19[6] and as thalidomide possesses well established vascular and anti-inflammatory effects, we

attempted to explore the effects of thalidomide and its derivatives on endothelium. HUVEC were subjected to 20 μM thalidomide or 20 μM lenalidomide or 20 μM pomalidomide or vehicle control treatment for 8 hours. RNA was isolated using TRIzol method and whole transcriptome sequencing was performed using Illumina HiSeq 2500 platform. The data can be accessed at GEO with the Accession ID GSE118979. The sequence reads were aligned with reference genome of *Homo sapiens* using TopHat2 (v2.0.8) and then followed by transcript compilation and gene identification was done using Cufflinks (v2.2.0).[27] The DEGs were identified using Cuffdiff program (v2.2.0).[27]

2.3 | Differential expression and enrichment analysis

The raw counts from the SARS-coV-2 transcriptomic profiles were subjected to differential expression analysis by DESEq2 v1.26.0.[28] Subsequently the genes were pre-ranked using the *P*-values from DESeq2 analysis and subjected to pre-ranked gene set enrichment analysis.[29] Gene sets with false discovery rate < 0.05 were considered to be statistically significant and were visualized using EnrichmentMap plugin[30] of Cytoscape.[31] For drug signatures from iLINCS, differentially expressed genes with $P < 0.05$ were considered to be statistically significant. Enrichment of kinase perturbation was carried out using Enrichr.[32]

2.4 | KINOMEscan kinase screening

We analysed our previously published KINOMEscan kinase screening dataset of thalidomide[33] in order to investigate how thalidomide affects the immune system. Kinases whose activities were reduced at least by 60% were considered for further enrichment analysis.

2.5 | Comparative analysis using Toppcluster

The DEGs ($Q < 0.05$ for transcriptome and $P < 0.05$ for drug signatures) from all the gene expression profiles were compared for overlapping genes and over-represented pathways using Toppcluster[34] and the networks were visualized using in Cytoscape.[31]

2.6 | Identification of protein targets using PharmMapper

For identifying the protein targets of thalidomide, we utilized the PharmMapper server (http://www.lilab-ecust.cn/pharmmapper/).[35] The server identifies possible physiological protein targets of any drug molecule by using a pharmacophore-based mapping approach. The 3-dimensional structure of thalidomide was obtained from PubChem and processed on the PharmMapper server choosing only human protein target sets. The top 100 target proteins were selected based on the ranking associated with a fit score (pKd value) for further enrichment analysis using Enrichr.[32]

3 | RESULTS

3.1 | Meta-analysis of SARS-coV-2 affected lung biopsies and PBMCs reveal enrichment of various pathways pertaining to immune response

We reanalysed and compared the SARS-coV-2 infected lung biopsies, A549 cells, PBMC and BALF transcriptomic profiles obtained from different studies. SARS-coV-2 infection showed a massive surge in inflammatory response, cytokine production and cytokine-mediated signalling. There was a substantial upregulation of immune response including the processes of haematopoietic development and lymphocyte activation (Figures 1A, 5A, S3A). Activation of viral life cycle and antiviral interferon signalling was observed in infected lungs (Figure 1A) and A549 cells (Figures 1B, S7). Over-representation of pathways including NOD-like receptor signalling, MAPK cascade, measles and influenza-A were seen in the infected lung as identified by gene set enrichment analysis (Figure 1A). Enrichr analysis revealed that many genes upregulated in SARS-coV-2 lung are the genes downregulated when SYK was knocked down or inhibited as supported by previous GEO studies (GSE43510, GDS3609 and GSE34176; Figure 1C). Expression to kinase (X2K) analysis showed the possible perturbation of various MAP kinases including ABL1 and JNK1 (Figures S1A, S1B). Human phenotype enrichment analysis shows thrombocytopenia, poor wound healing, abnormality of lymphatic system, serositis and abnormal anticoagulant pathways in SARS-coV-2 infected lungs (Figure S5).

3.2 | Similarity of SARS-coV-2 expression profile with that of systemic lupus erythematosus, lymphoma and multiple myeloma

Comparative enrichment analysis of gene expression profiles for disease-specific phenotypes revealed the similarity of SARS-infected tissues with pneumonia, influenza, lymphoma, systemic lupus erythematosus (SLE), multiple myeloma, asthma, auto-immune diseases, asthma, pneumonia and atherosclerosis while SARS-coV-2 PBMC showed exclusive overlap with lymphoma, multiple myeloma and chronic lymphocytic leukemia (Figure 2A). The gene expression profile of SARS-coV-2 infected lungs showed high resemblance to that of PBMC from SLE patients (Figure 2B). There was a significant overlap between SARS-coV-2 and SLE in upregulated genes involved in immune regulation (Figure S3A), interferon signalling (Figure S7B) and disease-specific phenotypes including lymphoma and multiple myeloma (Figures S2A, S2B, S2C). Targets of transcription factors, IFN-sensitive response element and IFN-regulatory factor (IRF) were upregulated in SARS-coV-2 affected lungs similar to upregulation observed in the PBMC of SLE patients (Figures 1B, S1C).

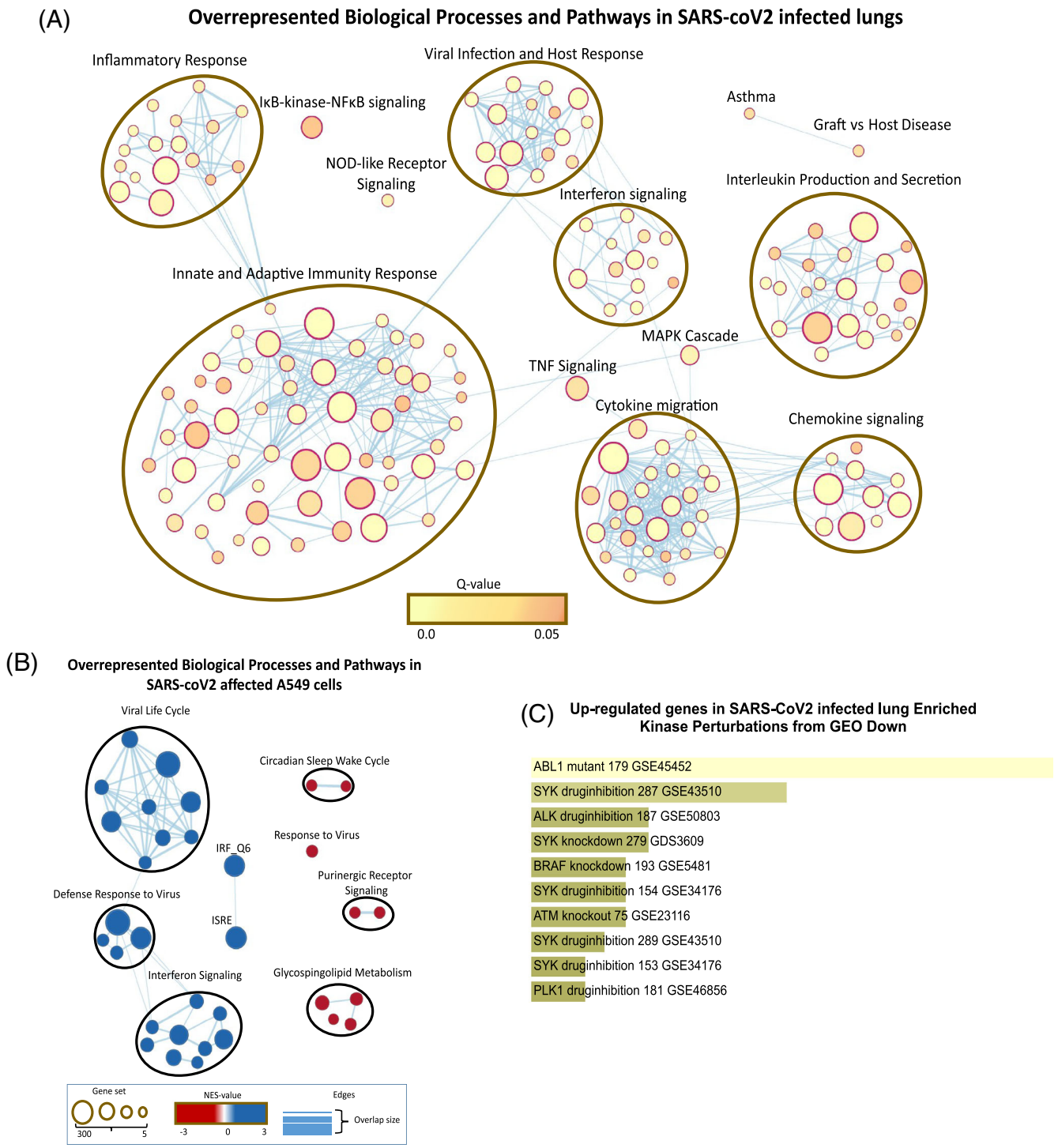

FIGURE 1 Characteristics of SARS-coV-2 infected lungs and A549 cells. Gene set enrichment analysis of genes modulated in (A) SARS-coV-2 infected lung and (B) A549 cells. (C) Genes activated in SARS-coV-2 overlapping with genes downregulated upon kinase perturbations

3.3 | Effect of thalidomide on kinases implicated in immune response and MAPK signalling

The kinase screening assay on thalidomide identified key kinases involved in the regulation of immune response. The most kinases affected were **LCK** and **SYK** (Figure 3C), critical modulators of T cell receptor signalling. Many LCK substrates, SPI1, TBK1, FOXP3, EGR1, ESR1, IRF1, CBL and STAT1 as well as SYK phosphorylation targets such as OAS1 and MX1 were upregulated in SARS-coV-2 lung (Figure s4, S1). Various processes mediating immune response

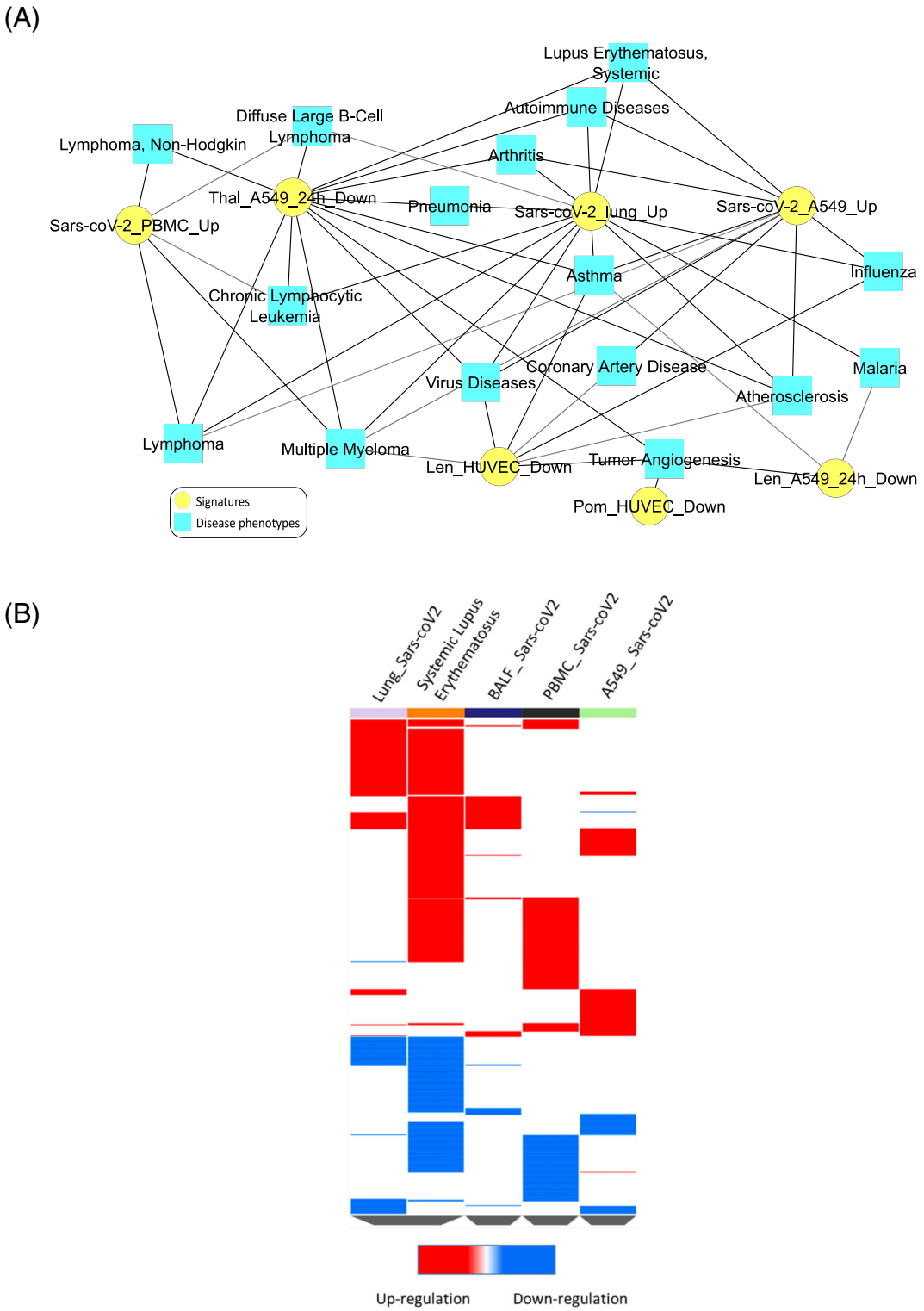

FIGURE 2 Enrichment of disease-specific phenotypes. (A) Similarity of SARS-coV-2 signatures with disease-specific phenotypes. (B) Overlapping of SARS-coV-2 expression profile of SARS-coV-2 affected lung with systemic lupus erythematosus

including JUN phosphorylation, IκB phosphorylation, JAK–STAT pathway, leucocyte-mediated immunity, neutrophil degranulation and activation, B-cell receptor signalling, and MAPK cascade were found to be affected by thalidomide (Figure 3D). We studied the effects of thalidomide and its derivatives on endothelium and identified the downregulation of several angiogenic genes (Figure 4B and Table 1).

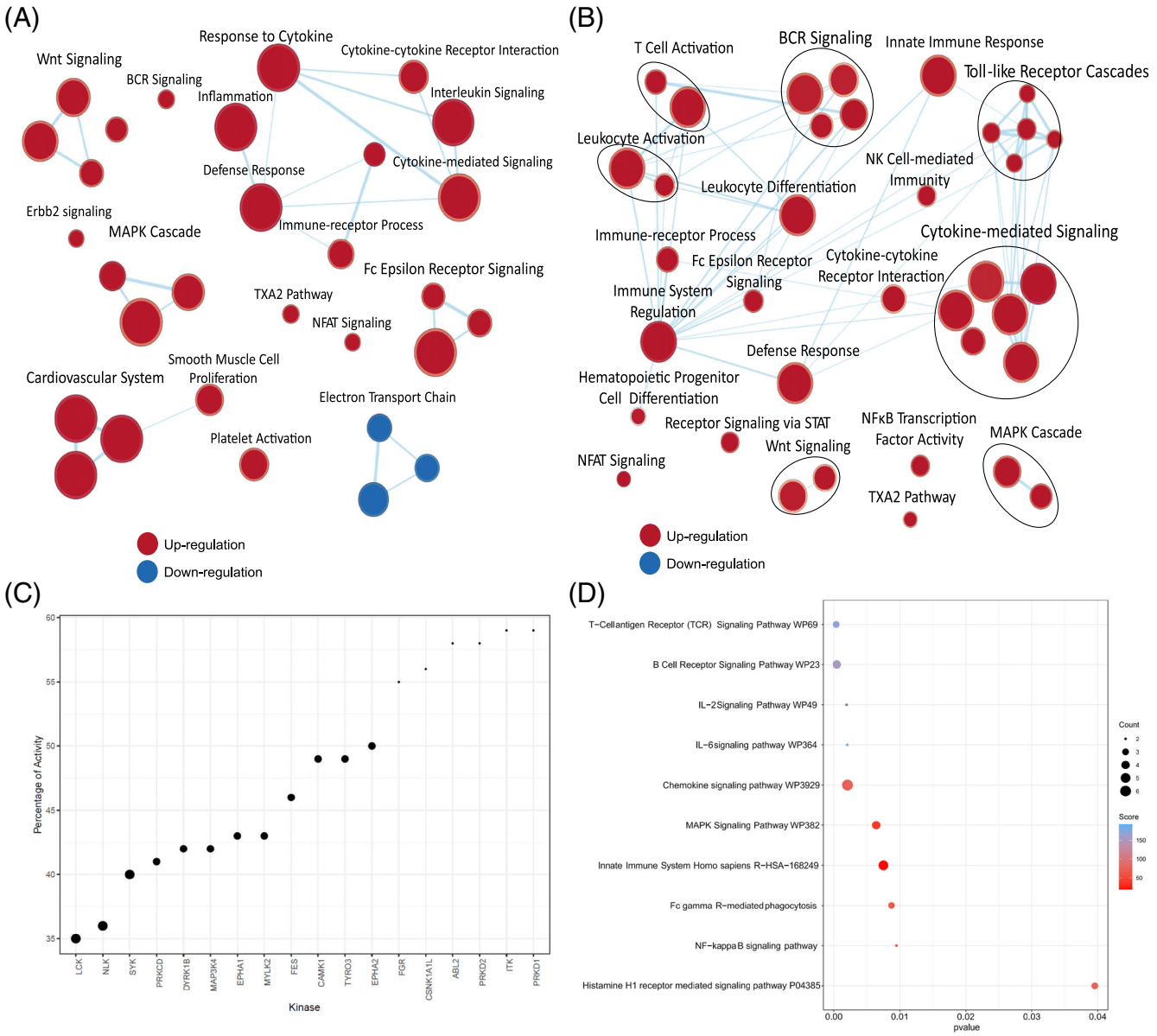

FIGURE 3 Effect of thalidomide and lenalidomide on immune system. Gene set enrichment analysis network of gene expression profiles of (A) thalidomide and (B) lenalidomide-treated A549 cells. (C) Activities of kinase involved in immunomodulation and MAPK signalling in the presence of thalidomide. (D) Biological pathways enriched by kinases affected by thalidomide

PharmMapper results showed strong affinity for LCK, HCK and SYK along with other proteins involved in innate and adaptive immune response (Table 2).

3.4 | Upregulated pathways and gene ontology biological processes in SARS-coV-2 infection suppressed by thalidomide and lenalidomide

Various genes aberrantly expressed in SARS-coV-2 affected lungs are known targets of thalidomide (Table 1). SARS-coV-2 infected lungs, PBMC and A549 cells showed significant upregulation of expression of genes involved in inflammation, cytokine signalling, MAPK signalling and activation of cells mediating the immune response whereas BALF exhibited a slightly different immune profile where leucocyte and neutrophil activation was suppressed (Figure 5A). Comparison of differentially expressed genes of all the signatures yielded interesting results. Many of the processes upregulated in SARS-coV-2 infected tissues were suppressed by thalidomide and lenalidomide in A549 cells and endothelial cells (Figures 3A,B, 5A). Thalidomide-treated A549 cells showed suppression of key genes including SYK, JUN, PIK3CA and HLA genes implicated in immune response (Figure S3A,B). Thalidomide and lenalidomide down-regulated various proinflammatory and angiogenic genes aberrantly expressed in SARS-cov-2 infected lungs including CCL2 and TSC22D3 which are NF-κB modulators in A549 cells (Figure 4A,B). Thalidomide

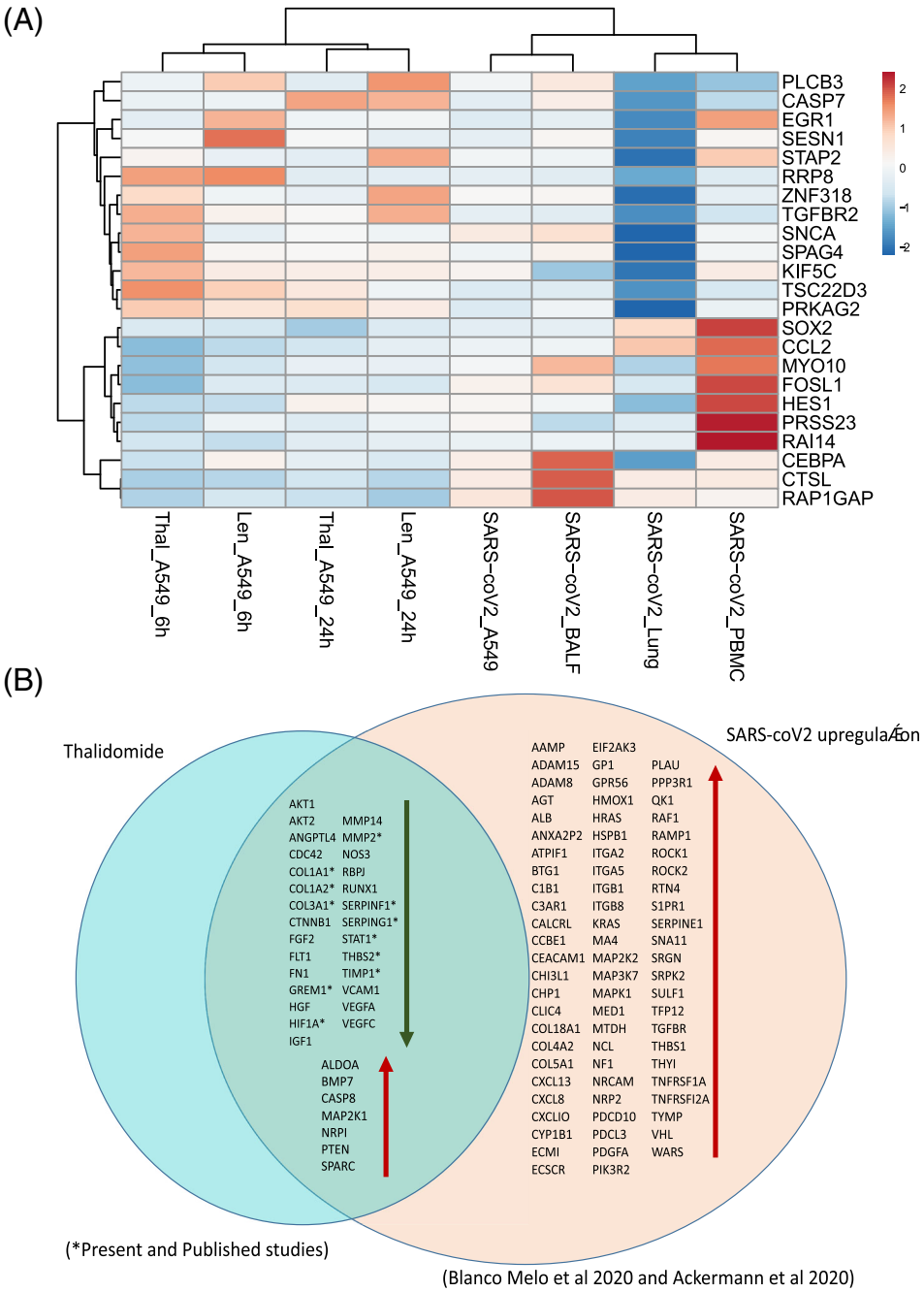

FIGURE 4 (A) Heatmap depicting the fold change of expression of inflammation and immunomodulatory genes implicated in SARS-coV-2 infected tissues and their regulation under thalidomide and lenalidomide treatment (log2FC > 2; false discovery rate < 0.05 for disease signatures). (B) Upregulation of genes implicated in angiogenesis in SARS-coV2 lung[6] and the modulation of those genes under thalidomide treatment from this study and published studies

and lenalidomide treatment resulted in significant suppression of cytokine response, angiogenesis, inflammation, Fc Epsilon receptor signalling and MAPK cascade (Figure 3A). In addition, lenalidomide downregulated STAT1 expression, leucocyte differentiation, TLR signalling as well as IRF activation (Figures 3B, S4B). Many genes implicated in NOD-like receptor signalling overexpressed in SARS-coV-2 were suppressed by lenalidomide in A549 and lymphoma cells (Figure S4C). B-cell receptor signalling was activated in SARS-coV-2 affected PBMC whereas T-cell activation was observed in SARS-coV-2 lungs (Figure S3B). Translation of viral mRNA was exclusively observed in SARS-coV-2 infected BALF whereas genes implicated in viral entry and life cycle were upregulated in SARS-coV-2 infected lungs, BALF and A549 cells. Genes involved in viral entry and type I interferon signalling were downregulated in thalidomide-treated A549 cells and lenalidomide-treated lymphoma, A549 and HUVEC (Figures S7A, S7C). Several aberrantly expressed genes involved in these disease phenotypes were downregulated in thalidomide and lenalidomide treated A549 and endothelium (Figure 2).

4 | DISCUSSION

SARS-coV-2 infection causes surge in a number of pathways related to inflammation, cytokine signalling, leucocyte and lymphocyte

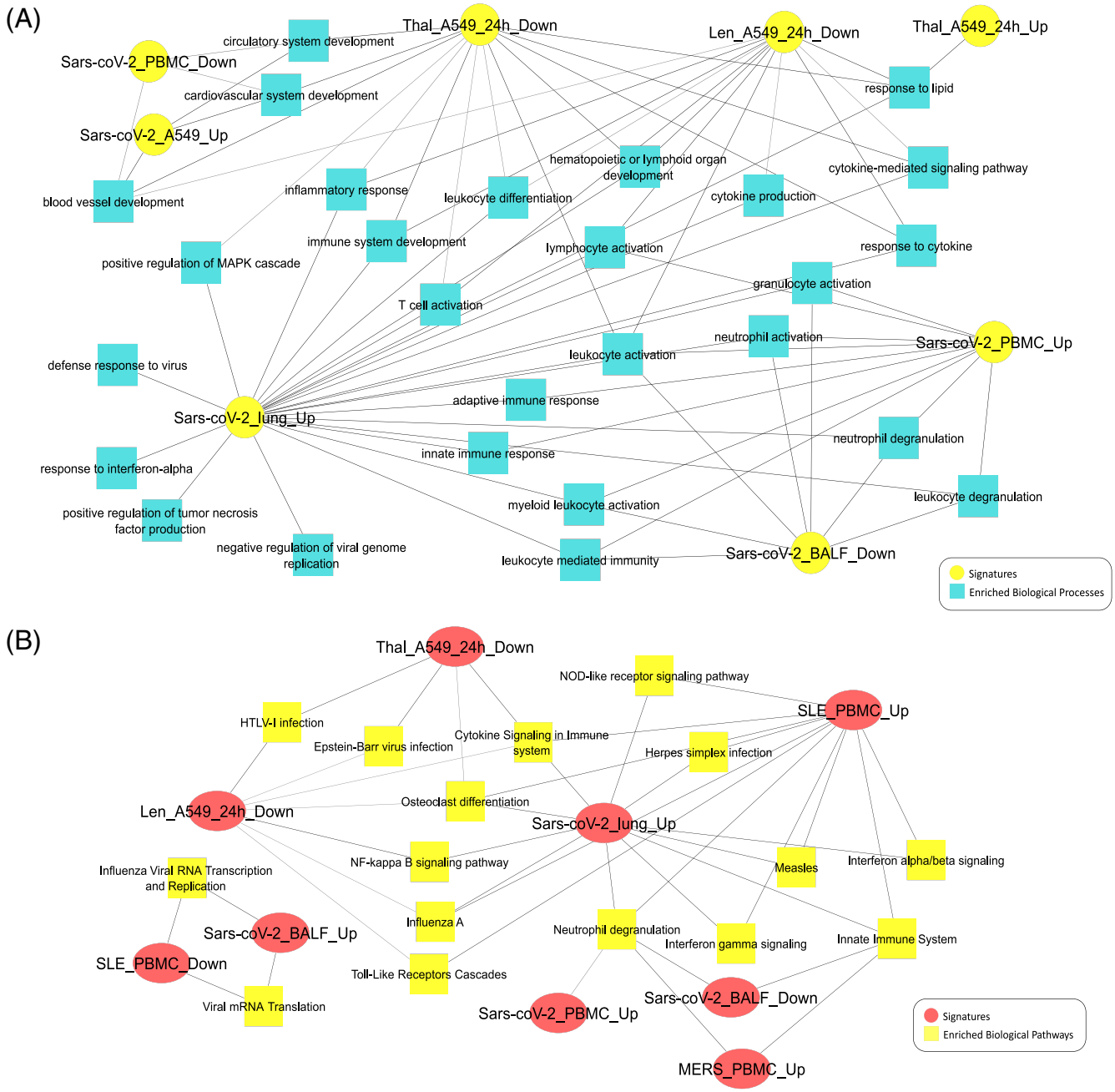

FIGURE 5 (A) Gene ontology biological process enrichment comparison of gene expression signatures. (B) Biological pathways enriched (false discovery rate < 0.05) in SARS-coV-2 infected tissues and their modulation by thalidomide and lenalidomide in A549 cells and lymphoma. The signatures of MERS-affected and systemic lupus erythematosus patients were used for comparison

activation, innate and adaptive immune response marking the phenomenon of *cytokine storm*. As the whole immune system is affected during the SARS-coV-2 infection, immunomodulators would be highly beneficial in treating the symptoms. A COVID-19 patient with pneumonia was treated successfully with thalidomide and low-dose glucocorticoid. There was a significant decrease in the inflammatory cytokines including IL1-, IL-6 and IFN-γ and increase in the CD4 + and CD8 + T cells and NK cells. Thalidomide reduced the severity of many COVID-19 symptoms such as lung lesions, exudation due to its pleiotropic effects on the human system.[10] Haemophagocytic syndrome, a hyperinflammatory disorder is another condition in which cytokine storm occurs. It is frequently present with extranodal natural killer/T cell lymphoma (ENKTL). Thalidomide was effective in suppressing the cytokine storm through inhibition of NF-κB based transcription of IFN-γ and TNF genes[12] and thalidomide along with P-Gemox was highly effective in treating ENKTL patients in a Phase II clinical trial.[58] Comparison of SARS-coV-2 expression profiles with drug signatures through enrichment analysis revealed striking actions of thalidomide and lenalidomide in A549 and endothelial cells. The results suggest that thalidomide and lenalidomide could reverse the devastating

TABLE 1 Genes overexpressed in SARS-coV-2 lungs and thalidomide's effects on gene expression

Gene	SARS-coV2-lung	Thalidomide or lenalidomide treatment
AKT1, AKT2	Upregulation	Signalling—Down[36,37]
ANGPTL4	Upregulation	Down[38]
CDC42	Upregulation	Down[39]
COL1A1*	Upregulation	Down[15]
COL1A2*	Upregulation	Down[40]
MMP2	Upregulation	Down[41]
THBS2*	Upregulation	Down (present study)
VEGFA	Upregulation	Down[42,43]
VEGFC	Upregulation	Down[42,43]
FGF2*	Upregulation	Down[7,43]
FLT1	Upregulation	Down[44]
FN1	Upregulation	Down[45]
HIF1A	Upregulation	Down[46]
IGF1	Upregulation	Down[7]
MMP14	Upregulation	Down[41]
RBPJ	Upregulation	Down[47]
TIMP1	Upregulation	Down[36,37]
VCAM1	Upregulation	Down[38]
IFN-γ	Upregulation	Down[39]
IL-6	Upregulation	Down[15]
HGF	Upregulation	Down[39]
IL-10	Upregulation	Down[41]
IL-1β	Upregulation	Down[48]
CTNNB1*	Upregulation	Down[41,43]
MCP1*	Upregulation	Down[41,43]
NF-κB*	Upregulation	Down[7,41]
TNF-α	Upregulation	Down[49–52]
GREM1*	Upregulation	Down (present study)
STAT1*	Upregulation	Down[45]
NOS3	Upregulation	Down[53,54]
CASP8	Upregulation	Down[40]
MAP 2 K1	Upregulation	Down[46]
PTEN	Upregulation	Up[36]
IL-2	Upregulation	Up[55]
BMP7	Upregulation	Up[36]
MIP- α	Upregulation	Up/down[48,52]
SPARC	Upregulation	Up[56]
NRPI	Upregulation	Up[57]

*Downregulation of genes/pathways observed in the present study as well.

effects of SARS-coV-2 infections on immune system. We selected A549, an adenocarcinomic human alveolar basal epithelial cell line to test our hypothesis that thalidomide would be effective against the cytokine storms. The A549 cell line is an appropriate model for testing cytokine storm targeting drugs since a previous study established this model by infecting the cells with influenza A/H1N1 virus (PR-8) or nonstructural protein 1 plasmid to test the mechanisms behind inflammatory cytokines/chemokines mediated *cytokine storm*[59] Studies have utilized A549 cells to show the effects of thalidomide on lung fibrosis.[36,42,60] A limitation of this study is that only 978 genes called *landmark genes* are profiled in the iLINCS drug signatures. However, the profiles are highly reproducible and represent the whole transcriptome.[18,61] Our models of A549 and HUVEC effectively capture the effects of thalidomide in lungs as well as endothelium.

It is also emerging that SARS-coV-2 infections perturb vascular plexus significantly and there is a substantial increase in the growth of new blood vessels and evidence of intussusceptive angiogenesis with overexpression of angiogenesis and hypoxia genes in the lungs of COVID-19 patients.[6] Cytokine storm and atherosclerosis are tightly connected in SARS-coV-2,[62] which is consistent with our analysis revealing the enrichment of atherosclerosis in the SARS-coV-2 signatures (Figure 2A). Thalidomide is a renowned modulator of vascular system, and it is known to transcriptionally or functionally target various genes (Table 1) upregulated genes in the lungs of COVID-19 patients.[6,19] As SARS-coV-2 infection has a huge impact on the haematopoietic system[63] affecting the myeloid cell maturation, we reanalysed the effects of thalidomide and its derivatives on PBMC, bone marrow cells as well as lymphoma cells. Thalidomide and lenalidomide exhibited attenuation of cytokine signalling and inflammation in addition to its anti-angiogenic action (Figure 3A). The drugs affected most of the pathways upregulated in SARS-coV-2 affected lungs and PBMC (Figures 1A, 3A) in A549 cells, mandating direct investigations in SARS-coV-2 infected models.

COVID-19 coincides with a strong neuro-endocrine modulation because the disease devastates functions of the organs, and naturally the reciprocal communication between the organs of the endocrine stress system gets a set-back.[64] ACE2 is expressed along the hypothalamus, pituitary and adrenal axis which is implicated in the stress response and adrenal glands has the highest concentration of virus particles next to lung.[65] A high expression of ACE2 in brain is believed to be the reason for the possible infection of the central nervous system in SARS patients.[66] Chronic elevated stress levels have been reported in SARS and SARS-coV-2 patients even long after the outbreak.[10] Notably, thalidomide is also known for its neuro-endocrine modulation properties. Thalidomide modulates the central nervous system by reducing the generation of proinflammatory cytokines such as IL-1, IL-6, IL-8 and TNF-α through NF-κB inhibition.[67] There was a downregulation of genes involved in circadian wake cycle (Figures 1B, S2) including PER3 in the PBMC of COVID-19 patients, suggesting a reason for the possible sleep disturbances in SARS-coV-2 patients. Thalidomide, having a well-known antiemetic and sedative action on the neuroendocrine axis, would relax the patients, which is supported by the report that thalidomide was effective in treating the anxiety and digestive symptoms in COVID-19 patients.[10]

The anti-inflammatory properties of thalidomide and its analogues through reduction of IL-1β, TNF-α expression and NF-κB inhibition are well established.[49] SARS-coV-2 infections showing

TABLE 2 Biological processes and pathways enriched by Pharmmapper predicted protein targets of thalidomide, lenalidomide and pomalidomide

Pharmmapper identified protein targets of thalidomide and enriched biological processes and pathways			
Enriched biological pathways and processes	**Overlap***	**Adjusted *P*-value****	**Genes**
Innate immune system *Homo sapiens* R-HSA-168249	36/807	1.19E-10	ITK; GSK3B; SRC; CTSV; CTSS; EGFR; MAPK8; CTSL; AKT2; CTSK; ABL1; CASP1; AKT1; MAPK1; JAK3; HRAS; MAP 2 K1; HSP90AA1; SYK; PDPK1; FGG; MAPK14; PTK2; IL2; MAPK10; HCK; LCK; KIT; MAPKAPK2; BTK; MDM2; PRKCQ; BPI; TEK; RAF1; FGFR2
B-cell receptor signalling pathway WP23	13/97	7.96E-10	GSK3B; MAP 2 K1; SYK; PDPK1; MAPK14; MAPK8; LCK; BTK; RAC2; AKT1; MAPK1; RAF1; HRAS
MAPK signalling pathway	24/295	7.42E-13	HSPA8; MAP 2 K1; IGF1; MAPK14; EGFR; TGFBR1; IGF1R; MAPK10; MAPK8; AKT2; CASP3; KIT; APKAPK2; KDR; RAC2; AKT1; MAPK1; TEK; RAF1; HRAS; MET; HSPA1B; FGFR2; HSPA1A
Interleukin signalling pathway *Homo sapiens* P00036	9/86	3.40E-06	GSK3B; PDPK1; AKT2; MAPKAPK2; AKT1; MAPK1; RAF1; JAK3; IL2
Interferon-γ signalling pathway *Homo sapiens* P00035	4/28	1.09E-03	MAPK10; MAPK8; MAPK1; MAPK14
Inflammation mediated by chemokine and cytokine signalling pathway *Homo sapiens* P00031	9/188	1.05E-03	ROCK1; PDPK1; AKT2; AKT1; MAPK1; RAF1; ITGAL; IL2; RHOA
IL-3 signalling pathway WP286	9/49	2.60E-08	HCK; MAP 2 K1; MAPK8; SYK; SRC; AKT1; MAPK1; RAF1; HRAS
T-cell receptor signalling pathway	15/101	6.87E-12	ITK; GSK3B; MAP 2 K1; PDPK1; MAPK14; IL2; RHOA; ZAP70; LCK; AKT2; AKT1; MAPK1; PRKCQ; RAF1; HRAS
Melanoma	12/72	2.16E-10	MAP 2 K1; CDK6; AKT2; MDM2; AKT1; MAPK1; IGF1; RAF1; HRAS; MET; EGFR; IGF1R
Measles	14/138	3.11E-09	HSPA8; GSK3B; IL2; MAPK10; MAPK8; CDK6; AKT2; CASP3; CDK2; AKT1; RAB9A; JAK3; HSPA1B; HSPA1A
Osteoclast differentiation	13/127	9.88E-09	MAP 2 K1; SYK; MAPK14; TGFBR1; MAPK10; MAPK8; LCK; AKT2; CTSK; BTK; AKT1; MAPK1; PPARG
Chemokine signalling pathway	15/190	1.77E-08	ITK; GSK3B; MAP 2 K1; ROCK1; SRC; PTK2; RHOA; HCK; AKT2; RAC2; AKT1; MAPK1; RAF1; HRAS; JAK3
Pharmmapper identified protein targets of lenalidomide and enriched biological processes and pathways			
Enriched biological pathways and processes	**Overlap**	**Adjusted *P*-value**	**Genes**
Innate immune system *Homo sapiens* R-HSA-168249	22/807	7.02E-05	GSK3B; ITK; HSP90AA1; PDPK1; SRC; FGG; MAPK14; PTK2; CTSS; MAPK10; LCK; AKT2; CTSK; MAPKAPK2; KIT; BTK; ABL1; MAPK1; BPI; TEK; RAF1; HRAS
Toll-like receptors cascades *Homo sapiens* R-HSA-168898	8/140	7.97E-04	MAPK10; CTSK; MAPKAPK2; BTK; MAPK1; bpi; MAPK14; CTSS
Adaptive immune system *Homo sapiens* R-HSA-1280218	15/762	.016	GSK3B; ITK; PDPK1; SRC; KIF11; CTSS; ZAP70; LCK; AKT2; CTSK; kit; BTK; MAPK1; RAF1; HRAS
B cell activation *Homo sapiens* P00010	7/57	7.43E-06	MAPK10; RAC2; BTK; MAPK1; RAF1; MAPK14; HRAS
T cell receptor signalling pathway	11/101	3.16E-08	ITK; GSK3B; ZAP70; PDPK1; LCK; AKT2; MAPK1; RAF1; MAPK14; HRAS; RHOA
Fc epsilon RI signalling pathway	9/68	1.13E-07	MAPK10; PDPK1; AKT2; RAC2; BTK; MAPK1; RAF1; MAPK14; HRAS
IL-17 signalling pathway	10/93	1.15E-07	MAPK10; GSK3B; HSP90AA1; MMP13; MMP1; CASP3; MMP3; MAPK1; MAPK14; MMP9
MAPK signalling pathway	15/295	4.32E-07	MAPK14; MAPK10; AKT2; CASP3; MAPKAPK2; KIT; KDR; RAC2; MAPK1; TEK; RAF1; HRAS; MET; HSPA1B; HSPA1A
TNF signalling pathway	7/110	1.65E-04	MAPK10; AKT2; CASP3; MMP3; MAPK1; MAPK14; MMP9

TABLE 2 (Continued)

Pharmmapper identified protein targets of lenalidomide and enriched biological processes and pathways			
Enriched biological pathways and processes	**Overlap**	**Adjusted *P*-value**	**Genes**
Natural killer cell mediated cytotoxicity	7/131	4.24E-04	ZAP70; LCK; CASP3; RAC2; MAPK1; RAF1; HRAS
Interferon-γ signalling pathway *Homo sapiens* P00035	3/20	7.82E-03	MAPK10; MAPK1; MAPK14
T-cell antigen receptor signalling pathway	8/90	1.10E-05	ITK; ZAP70; PDPK1; LCK; MAPK1; RAF1; MAPK14; HRAS
TGF-β signalling pathway WP366	9/132	1.80E-05	MMP12; MMP1; SRC; MAPK1; RAF1; MAPK14; met; PTK2; RHOA
IL-5 signalling pathway WP127	4/40	1.82E-03	GSK3B; BTK; MAPK1; RAF1
IL-2 signalling pathway WP49	4/42	2.10E-03	LCK; MAPK1; RAF1; HRAS
IL-3 signalling pathway WP286	4/49	3.46E-03	SRC; MAPK1; RAF1; HRAS
Neutrophil-mediated immunity (GO:0002446)	26/487	1.21E-10	CANT1; GSTP1; PYGL; CTSS; PLAU; MAPK1; CTSG; LTA4H; HSP90AA1; ACE; MME; NME2; RNASE3; MMP8; MAPK14; MMP9; RHOA; APRT; BST1; ADAM17; IMPDH1; IMPDH2; BPI; ALDOA; HSPA1B; HSPA1A
Neutrophil degranulation (GO:0043312)	24/479	1.94E-09	HSP90AA1; CANT1; MME; GSTP1; NME2; RNASE3; PYGL; MMP8; MAPK14; MMP9; CTSS; RHOA; APRT; BST1; PLAU; IMPDH1; IMPDH2; MAPK1; CTSG; BPI; LTA4H; ALDOA; HSPA1B; HSPA1A
Neutrophil activation involved in immune response (GO:0002283)	24/483	1.93E-09	HSP90AA1; CANT1; MME; GSTP1; NME2; RNASE3; PYGL; MMP8; MAPK14; MMP9; CTSS; RHOA; APRT; BST1; PLAU; IMPDH1; IMPDH2; MAPK1; CTSG; BPI; LTA4H; ALDOA; HSPA1B; HSPA1A
Regulation of inflammatory response (GO:0050727)	10/166	1.70E-04	ACE2; BST1; PDE2A; PLA2G2A; NR1H4; NR1H3; PPARG; TEK; PPARA; MAPK14
Myeloid leucocyte differentiation (GO:0002573)	5/50	3.14E-03	GLO1; kit; PPARG; MAPK14; MMP9
Myeloid leucocyte mediated immunity (GO:0002444)	9/20	.04	ADAM17; ace
Cellular response to cytokine stimulus (GO:0071345)	13/456	5.15E-03	GSK3B; HSP90AA1; MME; DAPK1; MAOA; MMP1; PDE2A; MMP3; MMP9; RHOA; CASP3; KIT; PIM1A44D61A45A44:D67
Pharmmapper identified protein targets of pomalidomide and enriched biological processes and pathways			
Enriched biological pathways and processes	**Overlap**	**Adjusted *P*-value**	**Genes**
T-cell receptor signalling pathway	15/101	9.64E-12	ITK; GSK3B; MAP 2 K1; PDPK1; MAPK14; IL2; RHOA; ZAP70; LCK; AKT2; AKT1; MAPK1; PRKCQ; RAF1; HRAS
MAPK signalling pathway	22/295	3.40E-11	MAP 2 K1; IGF1; MAPK14; EGFR; TGFBR1; IGF1R; MAPK10; MAPK8; AKT2; CASP3; KIT; MAPKAPK2; KDR; RAC2; AKT1; MAPK1; TEK; RAF1; HRAS; MET; HSPA1B; HSPA1A
Neutrophil-mediated immunity (GO:0002446)	29/487	6.52E-11	CDA; GPI; CANT1; ROCK1; GSTP1; ITGAL; CTSS; PLAU; MAPK1; CTSG; LTA4H; HSP90AA1; ACE; MME; NME2; MMP8; MAPK14; MMP9; RHOA; APRT; BST1; ADAM17; FABP5; IMPDH1; IMPDH2; BPI; ALDOA; HSPA1B; HSPA1A
T-cell antigen receptor signalling pathway	12/90	4.23E-09	ITK; ZAP70; MAP 2 K1; MAPK8; PDPK1; LCK; AKT1; MAPK1; PRKCQ; RAF1; MAPK14; HRAS
Osteoclast differentiation	13/127	1.26E-08	MAP 2 K1; SYK; MAPK14; TGFBR1; MAPK10; MAPK8; LCK; AKT2; CTSK; BTK; AKT1; MAPK1; PPARG
B-cell activation *Homo sapiens* P00010	21/94	1.49E-08	MAPK10; MAP 2 K1; MAPK8; SYK; RAC2; BTK; MAPK1; RAF1; MAPK14; HRAS
Chemokine signalling pathway	15/190	2.41E-08	ITK; GSK3B; MAP 2 K1; ROCK1; SRC; PTK2; RHOA; HCK; AKT2; RAC2; AKT1; MAPK1; RAF1; HRAS; JAK3
IL-3 signalling pathway WP286	9/49	3.05E-08	HCK; MAP 2 K1; MAPK8; SYK; SRC; AKT1; MAPK1; RAF1; HRAS
B-cell receptor signalling pathway	10/71	3.74E-08	GSK3B; MAP2 K1; SYK; AKT2; RAC2; BTK; AKT1; MAPK1; RAF1; HRAS

(Continues)

TABLE 2 (Continued)

Pharmmapper identified protein targets of pomalidomide and enriched biological processes and pathways			
Enriched biological pathways and processes	**Overlap**	**Adjusted *P*-value**	**Genes**
IL-17 signalling pathway	11/93	4.19E-08	MAPK10; GSK3B; HSP90AA1; MAPK8; MMP13; MMP1; CASP3; MMP3; MAPK1; MAPK14; MMP9
Influenza A	14/171	4.60E-08	FDPS; GSK3B; MAP 2 K1; MAPK14; MAPK10; MAPK8; DDX39B; AKT2; CASP1; AKT1; MAPK1; RAF1; HSPA1B; HSPA1A
Viral carcinogenesis	15/201	4.62E-08	SYK; SRC; HDAC8; RHOA; CCNA2; KAT2B; CDK6; CASP3; CHEK1; MAPKAPK2; CDK2; MDM2; MAPK1; HRAS; JAK3
Regulation of inflammatory response (GO:0050727)	15/166	1.01E-07	PDE2A; PLA2G2A; NR1H4; XIAP; NR1H3; MAPK14; IL2; ACE2; BST1; HCK; CASP1; PPARG; TEK; PPARA; PPARD
IL-5 signalling pathway WP127	8/40	1.06E-07	GSK3B; MAP 2 K1; SYK; BTK; AKT1; MAPK1; RAF1; IL2
TNF signalling pathway	11/110	1.99E-07	MAPK10; MAP 2 K1; CASP7; MAPK8; AKT2; CASP3; MMP3; AKT1; MAPK1; MAPK14; MMP9
Interleukin signalling pathway *Homo sapiens* P00036	9/86	3.96E-06	GSK3B; PDPK1; AKT2; MAPKAPK2; AKT1; MAPK1; RAF1; JAK3; IL2
ACE inhibitor pathway WP554	5/17	5.33E-06	ACE2; ACE; CTSG; REN; NR3C2
Natural killer cell mediated cytotoxicity	10/131	7.39E-06	ZAP70; MAP 2 K1; SYK; LCK; CASP3; RAC2; MAPK1; RAF1; ITGAL; HRAS
Fc γ R-mediated phagocytosis	8/91	2.57E-05	HCK; MAP 2 K1; SYK; AKT2; RAC2; AKT1; MAPK1; RAF1
Toll-like receptor signalling pathway	8/104	6.29E-05	MAPK10; MAP 2 K1; MAPK8; AKT2; CTSK; AKT1; MAPK1; MAPK14
Interferon-γ signalling pathway *Homo sapiens* P00035	4/20	.001	MAPK10; MAPK8; MAPK1; MAPK14
Inflammation mediated by chemokine and cytokine Signalling pathway *Homo sapiens* P00031	9/188	.001	ROCK1; PDPK1; AKT2; AKT1; MAPK1; RAF1; ITGAL; IL2; RHOA
JAK–STAT signalling pathway	8/162	0.001	AKT2; PIM1; AKT1; RAF1; HRAS; JAK3; IL2; EGFR
Toll-like receptor signalling pathway (GO:0002224)	7/86	0.001	CTSL; CTSK; FGG; MAPKAPK2; BTK; NR1H4; CTSS

*Overlap = the number of genes enriched in the category vs the total no of proteins contributing to the particular pathway/biological process.
**Adjusted *P*-value denotes the *P*-value obtained after multiple testing.

elevated NF-κB signalling and rampage activation of immune response. Unlike other RNA viruses, SARS-coV-2 suppresses TNF receptor-associated factors 3 activation, inhibiting NF-κB and IRFs, leading to suppression of early proinflammatory and antiviral responses. Whereas later stages of the infection show an enhanced expression of IRF targets in the lungs with an activation of IL-1, IL-6 and TNF-α expression and inhibition of type I interferon signalling.[68] Activation of IRF and IFN-sensitive response element transcriptional targets in SARS-coV-2 affected lungs is in agreement with previous studies reporting the SARS biology.[69] Thalidomide inhibited LCK activity affecting STAT1 phosphorylation, cytokine mediated signalling, NF-κB signalling, osteoclast differentiation and MAPK signalling through modulation of various upstream activators and downstream effectors. Lenalidomide, in addition, suppressed leucocyte differentiation, TLR signalling along with IRF activation in A549 and lymphoma cells. The effects of thalidomide and lenalidomide observed in our study are consistent with the previous studies where thalidomide and lenalidomide has been shown to inhibit IRF and STAT1 phosphorylation resulting in the downregulation of interferon expression and TLR signalling.[70,71]

The expression profile of SARS-coV-2 infected lungs, PBMC as well as A549 cells show resemblance with profiles of lymphoma, multiple myeloma and SLE (Figure 2A); however, we have focused on SLE as there was a striking similarity of the SLE expression profile and enriched pathways with that of lungs affected by COVID-19 (Figure 2B). Interestingly, our findings showing the similarity of COVID-19 infected lung with SLE strongly support a recent study that identified the resemblance between severe cases of COVID-19 and SLE.[72] We chose PBMC from SLE patients as it also captures the immune activity relatively better, and for better comparison with PBMC from COVID-19 patients and thalidomide-treated PBMC. Therefore, drugs that are effective in treating SLE, lymphoma and multiple myeloma might be effective against SARS-coV-2 infection. Thalidomide and its derivatives show impressive efficacy in treating

multiple myeloma and certain forms of lymphoma.[49] Notably, hydroxychloroquine, an Food and Drug Administration-approved SLE drug is currently being used in the management of critically ill SARS-coV-2 patients.[73] CC-220, another thalidomide analogue shows very promising results in phase I/II clinical trials against SLE.[74,75] CC-220 through suppression of Ikaros and Aiolos expression,[76] transcription factors that are essential for differentiation of leucocyte and NK cells, thus modulates the innate immune system. As innate immune system pathways are deregulated in SARS-coV-2 infected lung and PBMC, further studies are warranted to investigate the efficacy and safety of CC-220 in treating COVID-19.[62]

Any treatment strategy with thalidomide and its analogues including repurposing thalidomide for COVID-19, should consider thalidomide-induced adverse effects including neuropathy and venous thromboembolism.[77] There have been many reports on COVID-19 patients develop blood clots,[62] a dangerous issue that might be aggravated with the use of thalidomide and lenalidomide. In addition, lenalidomide might cause cytokine release syndrome in chronic lymphocytic leukaemia patients.[78] Therefore, a very careful dosage regimen has to be followed with all these drugs as serious adverse effects have been observed during dose escalation.

5 | CONCLUSION

Our study sheds light on the possible mechanisms through which thalidomide and lenalidomide might be effective in the management of SARS-coV-2 pathology. Thalidomide and derivatives effectively modulating various aberrantly regulated pathways infections with abundant pharmacological information available make them promising candidates for the treatment of novel coronavirus infections.

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CONTRIBUTORS

L.S., S.G., H.S. and S.C. contributed to study design, experiments and data collection while L.S., S.G. and S.C. helped in manuscript preparation.

COMPETING INTERESTS

The authors have none to declare.

DATA AVAILABILITY STATEMENT

The data generated in this study have been deposited to Gene Expression Omnibus. The datasets that support this study are available in GEO at https://www.ncbi.nlm.nih.gov/geo/, BIG Data Center at https://bigd.big.ac.cn/and iLINCS at http://www.ilincs.org/ilincs/. The appropriate references have been mentioned in the Methods section.

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SUPPORTING INFORMATION

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