ORIGINAL RESEARCH ARTICLE

Single-Cell Transcriptome Analysis Reveals the Role of Pancreatic Secretome in COVID-19 Associated Multi-organ Dysfunctions
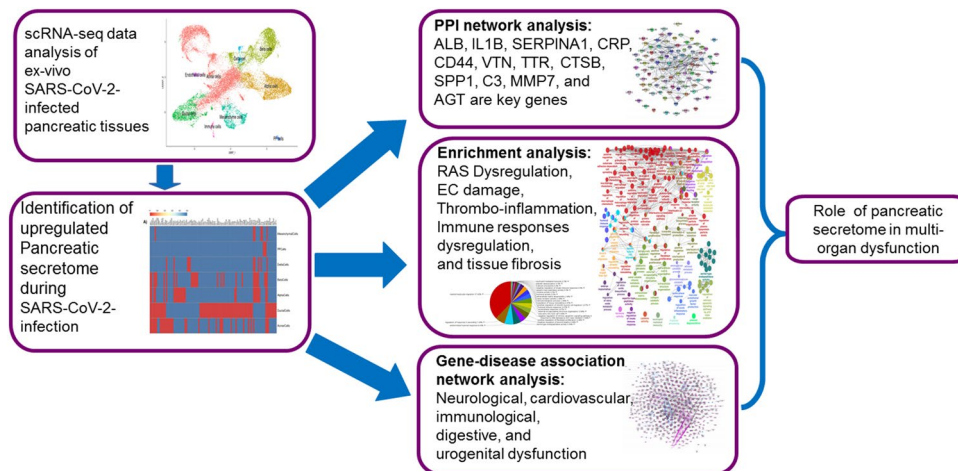
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Abstract

The SARS-CoV-2 infection affects the lungs, heart, kidney, intestine, olfactory epithelia, liver, and pancreas and brings forward multi-organ dysfunctions (MODs). However, mechanistic details of SARS-CoV-2-induced MODs are unclear. Here, we have investigated the role of pancreatic secretory proteins to mechanistically link COVID-19 with MODs using single-cell transcriptome analysis. Secretory proteins were identified using the Human Protein Atlas. Gene ontology, pathway, and disease enrichment analyses were used to highlight the role of upregulated pancreatic secretory proteins (secretome). We show that SARS-CoV-2 infection shifts the expression profile of pancreatic endocrine cells to acinar and ductal cell-specific profiles, resulting in increased expression of acinar and ductal cell-specific genes. Among all the secretory proteins, the upregulated expression of IL1B, AGT, ALB, SPP1, CRP, SERPINA1, C3, TFRC, TNFSF10, and MIF was mainly associated with disease of diverse organs. Extensive literature and experimental evidence are used to validate the association of the upregulated pancreatic secretome with the coagulation cascade, complement activation, renin-angiotensinogen system dysregulation, endothelial cell injury and thrombosis, immune system dysregulation, and fibrosis. Our finding suggests the influence of an upregulated secretome on multi-organ systems such as nervous, cardiovascular, immune, digestive, and urogenital systems. Our study provides evidence that an upregulated pancreatic secretome is a possible cause of SARS-CoV-2-induced MODs. This finding may have a significant impact on the clinical setting regarding the prevention of SARS-CoV-2-induced MODs.

Graphical abstract

Extended author information available on the last page of the article

1 Introduction

The ongoing pandemic of Coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) encompasses a myriad of pathologies [1]. In many patients, SARS-CoV-2 infection affects the lungs, heart, kidney, intestine, olfactory epithelia, liver, and pancreas, resulting in multi-organ dysfunctions (MODs) [2–9]. SARS-CoV-2 uses the ACE-2 receptor to enter the host cells and cause pancreatic injury [10, 11]. Acute pancreatitis (AP) is triggered in the pancreas in response to an inflammatory event, leading to deleterious local and systemic effects [12] and eventually multi-organ damage and dysfunction [13]. There are cases of pancreatitis associated with no respiratory symptoms [14, 15] and after the clearance of SARS-CoV-2 in the lungs [16] of the COVID-19 patients. While the precise mechanisms of SARS-CoV-2-induced acute pancreatitis remain unknown [17–19], AP pathogenesis is commonly attributed to trypsin activation and intracellular signalling [20], the release of proteolytic enzymes such as amylase and lipase [21], reactive oxygen species (ROS) [22], inflammatory elements, and the release of other mediators into the blood, all of which lead to the activation of the systemic inflammatory response [23].

Several aspects of SARS-CoV-2-induced organ damage have been studied [24–30]. However, the involvement of specific pathways, such as those centred on pancreatic infection of SARS-CoV-2, needs to be investigated. Here, to mechanistically link the multi-organ dysfunction with the COVID-19-infected pancreas, we have investigated the role of upregulated pancreatic secretory proteins (pancreatic secretome) in COVID-19-associated MODs using single-cell RNA-seq data of ex-vivo SARS-CoV-2-infected human pancreas. Furthermore, we validated that an upregulated pancreatic secretome is associated with coagulation cascade, complement activation, renin-angiotensinogen system dysregulation, endothelial cell injury and thrombosis, immune system dysregulation, and fibrosis using extensive literature and experimental evidence. Our finding suggests the influence of an upregulated pancreatic secretome on the nervous, cardiovascular, immune, digestive, and urogenital systems. In addition, we report that the secretory proteins IL1B, AGT, ALB, SPP1, CRP, SERPINA1, C3, TFRC, TNFSF10, and MIF are associated with diseases of diverse organs. Thus, our analysis suggests the role of the upregulated pancreatic secretome in MODs.

2 Materials and Methods

2.1 Data Sources

Ex-vivo SARS-CoV-2-infected human pancreas single-cell RNA-seq data were obtained from the Gene Expression Omnibus (GSE159556) [31], which contained two samples for mock- and SARS-CoV-2-infected tissues. As described by Tang, Xuming et al., the mock-infected pancreas served as a control [31]. scRNA-seq data analysis was performed using Seurat 4.0.2. [32]. The viral strain used in the study is SARS-CoV-2 isolate USA-WA1/2020 (NR-52281).

2.2 Single-Cell RNA-seq Data Analysis

2.2.1 Quality Control

For quality control, we have followed the standard preprocessing workflow given in the vignette of Seurat (v.4.0.2) [32]. We checked the quality control thresholds used in recent literature [31, 33, 34] and filtered the cells with fewer than 200 genes, greater than 20% mitochondrial genes, and less than 5% ribosomal genes based on our data. In addition, cells with genes expressed in fewer than three cells were also filtered. Then we removed the effects of the cell cycle on the transcriptome using CellCycleScoring. Before running CellCycleScoring, the data were normalised and logtransformed using NormalizeData. We then removed the doublets using DoubletFinder [35]. The doublet prediction was run on each sample separately with a 4–7.6% doublet rate based on the loading rate. After removing doublets, the two SARS-CoV-2-infected samples now have 5821 and 6661 cells, whereas the 2 mock-infected samples have 3726 and 7869 cells. Next, we used the QC-filtered data to identify the top 2000 variable genes using FindVariableFeatures with selection.method "vst". Next, ScaleData was used to scale and centre the data, where the number of genes and percentage of mitochondrial genes were the "vars. to.regress". After scaling, we performed principal component analysis (PCA) and Uniform Manifold Approximation and Projection(UMAP) for dimensionality reduction using the first 30 principal components. The UMAP plot coloured by COVID-19 and mock-infected was also generated and found that the batch effect was effectively removed (Supplementary figure S1).

2.2.2 Integration and Clustering

We then used FindIntegrationAnchors to identify anchors in Seurat objects and integrated the datasets with IntegrateData(). Then, the dataset was scaled using ScaleData. The PCA and UMAP were performed using the first 30 dimensions. Next, we used FindNeighbors to compute the nearest neighbour graph using the top 30 PCs. We then performed the graph-based clustering using FindClusters at a resolution of 4.5. The Clustree package was used to choose the final resolution [36].

2.2.3 Cell Type Identification

We first identified genes differentially expressed in a cluster with respect to other clusters using FindAllMarkers with logfc.threshold = 0.25, min.pct = 0.25, min.diff.pct = 0.25. The Benjamini–Hochberg false discovery rate (FDR) was 0.05. The test used was the Wilcoxon Rank Sum test, and the assay was "RNA". We used literature to manually curate the DEGs of each cluster to identify cell types. After identifying the cell type for clusters, we merged the same cell type cluster into one. This resulted in nine clusters of acinar cells, ductal cells, alpha cells, beta cells, delta cells, PP cells, endothelial cells, mesenchymal cells, and immune cells.

2.2.4 DEGs Across SARS-CoV-2-Infected and Mock-Infected Conditions

We have identified differentially expressed genes between *SARS-CoV-2-infected and mock-infected* conditions using FindAllMarkers with logfc.threshold = 0.2, min.pct = 0.1 and FDR = 0.05. We used the Wilcoxon Rank Sum test on the "RNA" assay.

2.3 Identification of Secretome

We identified the secretory proteins using the Human Protein Atlas [37]. Then, utilising this protein set as input, we regenerated the protein interaction network using STRING [38]. Finally, we used Cytoscape 3.8.2 to visualise and analyse the network [39].

2.4 Enrichment Analysis

We used g:Profiler for GO enrichment analysis and biological pathway enrichment analysis using KEGG, Reactome, and WikiPathways. Human Phenotype Ontology [40] was used to conduct the disease phenotype enrichment analysis. ClueGO, a Cytoscape plug-in, was used to identify the functionally grouped GO and pathways [41]. We used the DisGeNET Cytoscape app (7.3.0) for gene-disease associations (GDAs) [42]. We used EnhancedVolcano to generate a volcano plot [43]. The Pheatmap package was used for generating heatmaps [44].

3 Results and Discussion

Emerging evidence indicates an intricate relationship between SARS-CoV-2 infection and multi-organ dysfunctions (MODs), which affects the lungs, heart, kidney, intestine, olfactory epithelium, liver, and pancreas [24–30]. Acute pancreatitis (AP) is an inflammation of the pancreas that results in local and systemic complications, as well as multiple organ malfunctions and damage over time [12–16]. SARS-CoV-2-induced multi-organ dysfunctions are currently mechanistically unclear. The pancreatic secretome (the proteins secreted by the pancreas) was therefore investigated as a potential link between COVID-19 and multiple organ dysfunctions.

3.1 SARS-CoV-2 Infection Causes Pancreatic Endocrine Cells' Expression Profiles to Shift to Acinar and Ductal Cell-Specific Profiles

For this study, we obtained scRNA-seq data from the Gene Expression Omnibus under the accession code GSE159556 for mock-infected and SARS-CoV-2-infected pancreas [31]. The clustering analysis of the scRNA-seq data showed 45 different clusters. These clusters were merged to form nine clusters of different cell types, i.e., acinar cells, ductal cells, alpha cells, beta cells, delta cells, PP cells, endothelial cells, mesenchymal cells, and immune cells. As shown in Fig. 1, the cell type identification was based on marker genes PRSS2 (acinar cells), KRT19 (ductal cells), GCG (alpha cells), INS (beta cells), COL1A1 (mesenchyme cells), PPY (PP cells), SST (delta cells), ESAM (endothelial cells), and LAPTM5 (immune cells), using reported literature [45].

We identified that *CoV2-N*, *CoV2-orf1ab*, *CoV2-M*, *CoV2-S*, *CoV2-ORF7a*, and *CoV2-ORF8* viral genes are expressed across all cell types in the single-cell expression analysis (Fig. 2A–H). Furthermore, we found 149 genes in acinar, 631 genes in ductal, 107 in alpha, 151 in beta, 28 in delta, 3 in endothelial, 11 in pp cells, and 22 genes in mesenchyme cells that were differentially expressed. Upregulated genes include 125 in acinar, 538 in ductal, 94 in alpha, 139 in beta, 16 in delta, 3 in endothelial, 9 in pp cells, and 18 in mesenchyme cells. There were a total of 712 genes found to be upregulated (Table S1). We found that *SPINK1*, *OLFM4*, *ISG15*, *REG1A*, *SPP1*, *REG3A*, *MMP7*, *ALB*, *IL32*, *PRSS2*, *REG1B* genes were upregulated in four or more cell types after COVID-19 infection (Table S1). Noticeably, we also found that acinar-specific genes *PRSS2*, *REG3A*, *REG1A*, *SPINK1*, and ductal-specific genes *SPP1*, *MMP7* were upregulated in pancreatic endocrine alpha, beta, delta, and mesenchyme cells (Fig. 2C–E, H). In contrast, the expression of the marker gene GCG does not alter significantly in alpha cells. However, INS expression in the beta cell is downregulated in the COVID-19 condition. Therefore, our analysis indicates that SARS-CoV-2 infection shifts the expression profile of pancreatic endocrine cells to acinar and ductal cell-specific profiles (Fig. 2A–H), resulting in increased expression of acinar and ductal cell-specific genes. We also identified and analysed the 127 downregulated genes (Table S4) for a possible role in the development of MODs. We found 29 genes encoding proteins that are
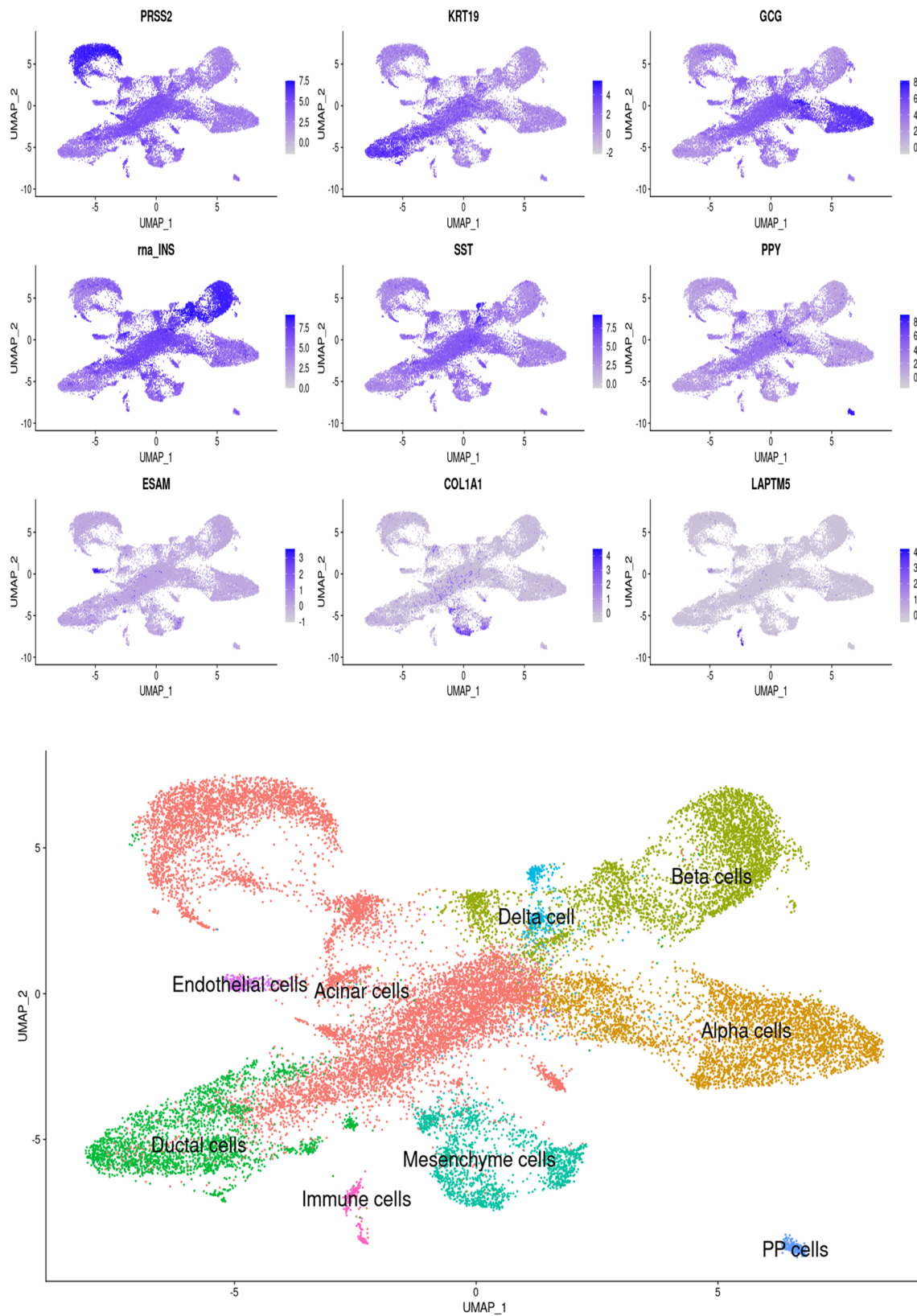
Fig. 1 Cell type identification. UMAP of cell marker genes PRSS2 (acinar cells), KRT19 (ductal cells), GCG (alpha cells), INS (beta cells), COL1A1 (mesenchyme cells), PPY (PP cells), SST (delta cells), ESAM (endothelial cells), and LAPTM5 (immune cells). UMAP of pancreatic cells showing cell types is depicted in bottom panel
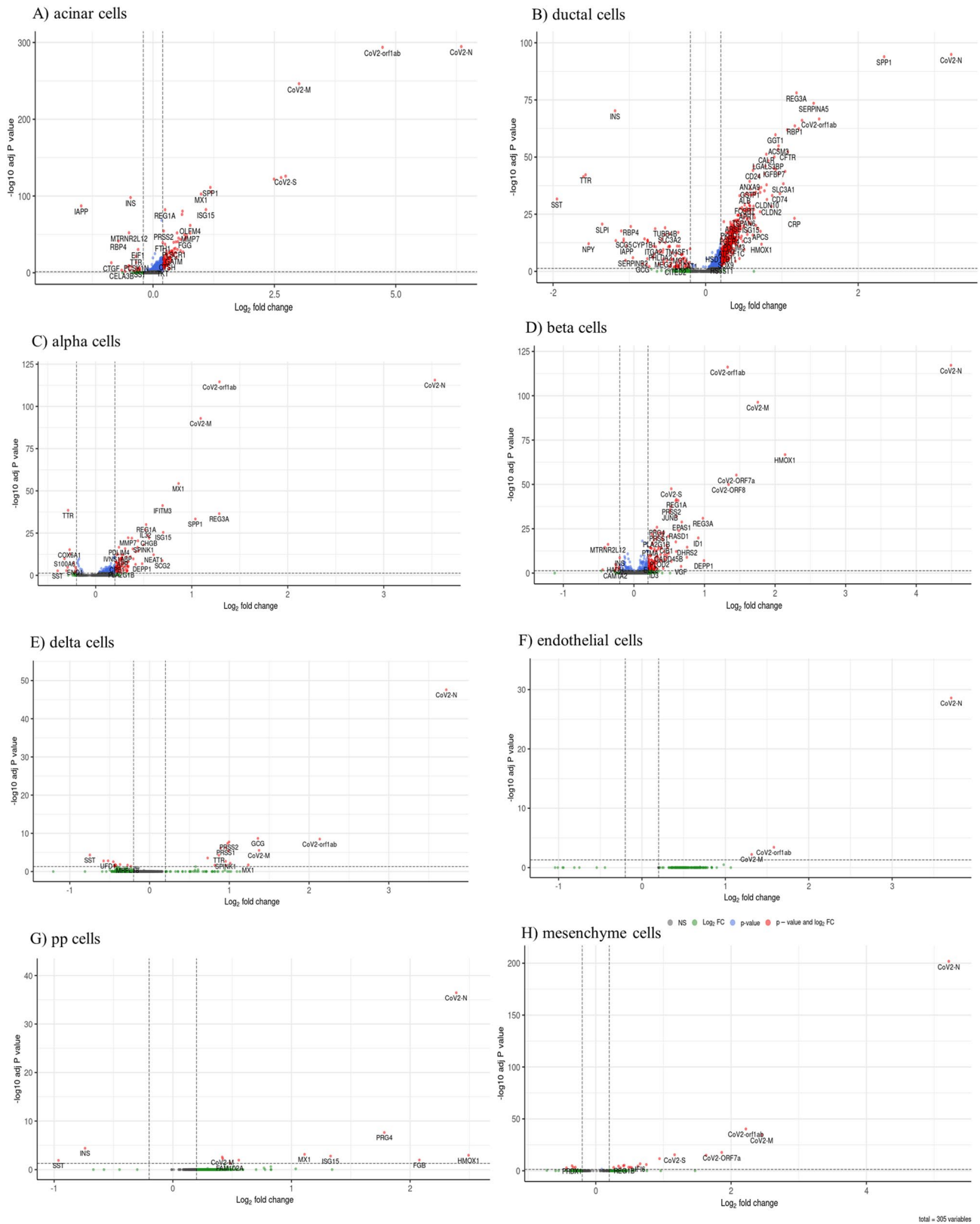
Fig. 2 Volcano plot showing differentially expressed genes. **A** Acinar cells, **B** ductal cells, **C** alpha cells, **D** beta cells, **E** delta cells, **F** PP cells, **G** endothelial cells, **H** mesenchyme cells

secretory in nature. However, we could not find their possible role in MODs.

3.2 Analysis of Pancreatic Secretome

The 712 upregulated genes were subjected to identification of the secretory proteins using The Human Protein Atlas (Fig. 3A). We found 34 secretory proteins in acinar cells, 65 in ductal cells, 26 in alpha cells, 28 in beta cells, 10 in delta cells, 3 in pp cells, and 6 in mesenchyme cells. Taken together, we found 102 upregulated pancreatic secretory proteins (pancreatic secretome). Interestingly, the genes that

were upregulated in four or more cell types after COVID-19 infection were also noted to be secretory proteins. Furthermore, the upregulated secretome was used to construct the protein–protein interaction network using the string database (Fig. 3B). Using network topological parameters, i.e., degree and closeness centrality, we revealed ALB, IL1B, SERPINA1, CRP, CD44, VTN, TTR, CTSB, SPP1, C3, MMP7, and AGT to be influential among all secretory proteins (Table S2).

We explored the roles of upregulated *PRSS2*, *REG3A*, *REG1A*, *SPINK1*, *OLFM4*, *ISG15*, *IL32*, *REG1B*, *ALB*, *IL1B*, *SERPINA*, *CRP*, *CD44*, *VTN*, *TTR*, *CTSB*, *SPP1*, *C3*,

Fig. 3 Secretome of pancreatic cells infected with SARS-CoV-2. **A** Heatmap depicting the up-regulation of secretory genes in COVID-19-infected pancreatic cells. Upregulated genes are shown in red. The blue colour indicates that genes are not upregulated. **B** Network of protein–protein interactions in the secretome of COVID-19 infected pancreatic cells
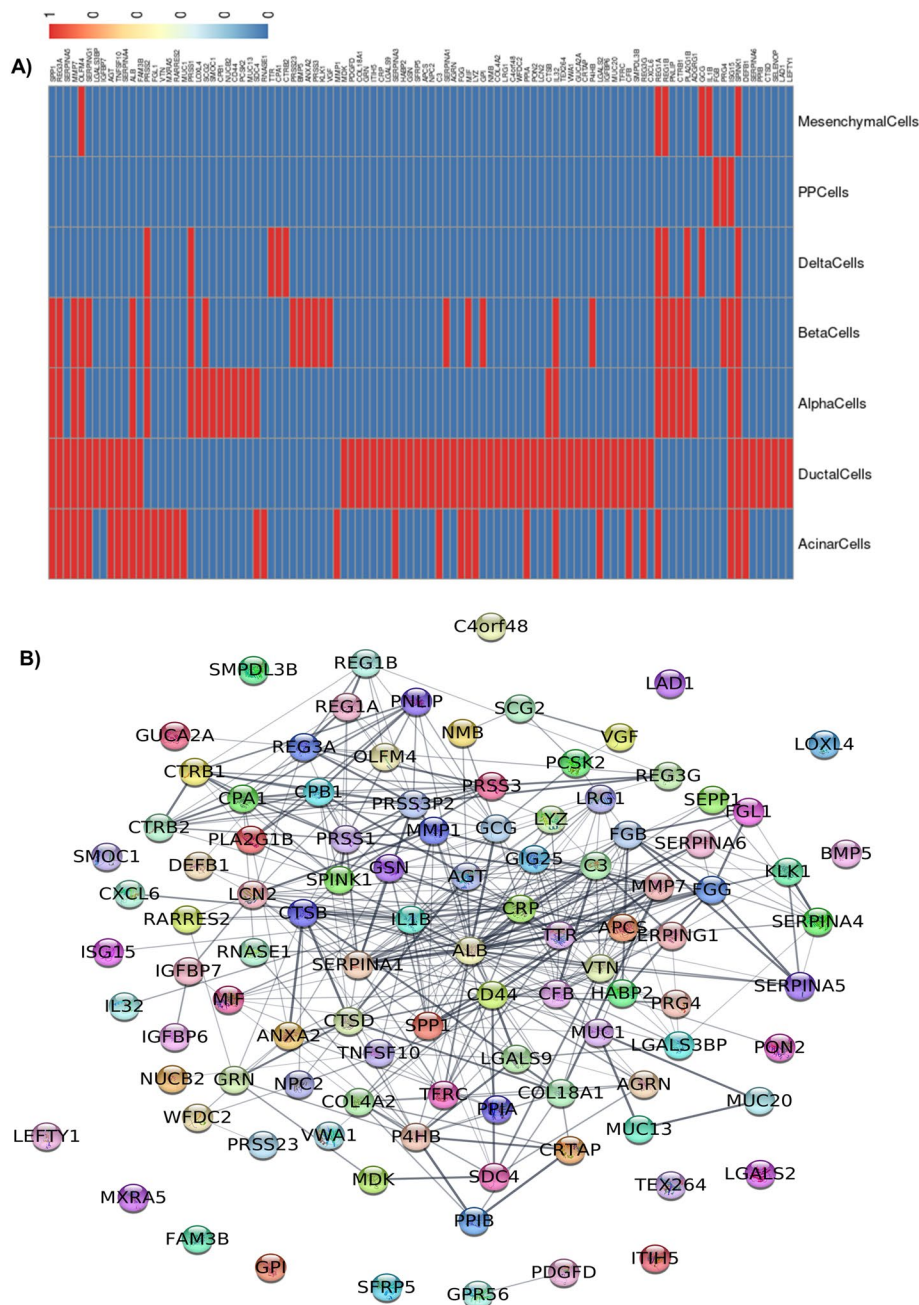
MMP7, and *AGT* genes using experimental evidence. The upregulation of PRSS1 and PRSS2 is a characteristic of pancreatitis that causes increased intra-pancreatic trypsin activity, resulting in pancreatic damage [46, 47]. *PRSS1* and *PRSS2* encode trypsin, a serine protease that can cleave complement components C3 into C3a and C3b and C5 into C5a and C5b. Inflammation is known to be mediated by C3a and C5a [48]. C3 is crucial for the activation of the complement system. In pancreatitis, C3 deposition occurs around injured acinar cells [49]. It causes neutrophil infiltration and the formation of neutrophil extracellular traps. Neutrophil infiltration is linked to tissue damage in severe acute pancreatitis [50, 51]. Activated trypsin causes pancreatic damage and haemorrhage. Trypsin has been linked to organ damage in several studies. It reaches other organs via the venous flow circulation [52]. Similarly, *SPINK1* is overexpressed in pancreatitis, and the elevation is associated with the disease severity [53]. During pancreatitis, *REG1A* and *REG3A* have increased expression. *REG1A* and *REG1B* are involved in islet cell regeneration and diabetogenesis. REG3A promotes cell growth and possesses antimicrobial properties [54]. SPP1 (osteopontin) is a hydroxyapatite-binding extracellular structural protein. It participates in efficient T-helper 1 cell immune responses and enhances mast cell responses to antigen [55]. SPP1 is a cytokine that upregulates the expression of IL-12 and IFN-γ. IL-12 stimulates T-helper 1 cell differentiation and IFN-γ release [56]. By activating T cell cytokine production, IFN-γ plays an important role in viral defense. However, a persistently elevated IFN-γ level exacerbates systemic inflammation, resulting in tissue damage and organ failure [57]. MMP7 degrades casein, gelatin, and fibronectin while also activating procollagenase [58]. MMP7, in association with MMP1, MMP9, and MMP12, can promote thrombosis in atherosclerotic plaques and alter the coagulation pathway in inflammatory disorders [58]. ALB is the main plasma protein and regulates the colloidal osmotic pressure of the blood [59]. IL32 is a cytokine that induces cytokines such as TNF-α and IL6 and chemokines IL8 and CXCL2 [60]. In addition, it activates the signal pathways of NF-kappa-B and p38 MAPK [60]. ISG15 induces the production of IFN-γ, as well as ubiquitination of newly-synthesized proteins [61]. It helps in the proliferation of natural killer cells and is a chemotactic factor for neutrophils. It inhibits viral replication and regulates the host's damage and repair response [61]. OLFM4 is a glycoprotein that assists in cell adhesion and is an antiapoptotic factor, promoting tumour growth [62]. AGT, a part of the renin-angiotensin system (RAS), regulates blood pressure. Inhibition of AGT reduces atherosclerosis and kidney dysfunction in polycystic kidney disease [63]. IL-1β, a pro-inflammatory cytokine [64], induces T and B-cell activation, cytokine and antibody production, neutrophil infiltration, and activation [65, 66]. IL-1β also induces prostaglandin

synthesis, fibroblast proliferation, and vascular endothelial growth factor (VEGF) production [67–69]. SERPINA1 is a serine protease inhibitor and is reported as a potential prognostic marker for COVID-19 [70]. CRP is involved in inflammation and helps in complement binding to invaders and apoptotic cells and aids in opsonin-mediated phagocytosis, production of IL1B, IL6, and TNF-α, and the reduction of nitric oxide [71]. CD44 is a cellular adhesion molecule for the extracellular matrix (ECM) component hyaluronic acid [72]. VTN, an adhesive glycoprotein present in serum and ECM, repairs and remodels ECM in different tissues after trauma [73]. TTR transports thyroxin and the retinol-retinol binding complex to the brain and other parts of the body, thereby inducing oxidative stress in endoplasmic stress [74, 75]. CTSB is involved in extracellular matrix degradation [76]. Our analysis suggests that PRSS2, REG3A, REG1A, SPINK1 SPP1, MMP7, OLFM4, ISG15, ALB, IL32, and REG1B, AGT, IL1B, SERPINA, CRP, CD44, VTN, TTR, and CTSB are involved in the complement and coagulation cascade, extra-cellular matrix assembly, fluid balance, and immune response, and that their dysregulation may lead to sepsis.

3.3 Enrichment Analysis of Pancreatic Secretome: GO, Biological Pathway, Disease Phenotypes

The 102 upregulated pancreatic secretory proteins were examined further for GO keywords, biological pathways, and disease phenotypes. We found that serine-type peptidase activity, endopeptidase activity, glycosaminoglycan binding, and cytokine activity were among the top enriched molecular functions (Fig. 4A). Using ClueGO analysis, we found functionally grouped GO [41]. We noted that the top enriched biological processes were related to myeloid leukocyte migration (37.62%), regulation of response to wounding (7.43%), antimicrobial humoral response (6.93%), serine-type endopeptidase activity (5.94%), and positive regulation of fibroblast proliferation (4.46%) (Fig. 4B). Also, we found that the biological processes of metabolism of tetrapyrrole, cobalamin, hyaluronan, and retinoid, and the catabolism of collagen, aminoglycan, and glycosaminoglycan were enriched. We found that myeloid leukocyte migration was associated with neutrophil-mediated immunity, neutrophil chemotaxis, regulation of macrophage migration, positive regulation of protein secretion, endothelial cell apoptotic process, vascular endothelial growth factor production, interleukin-12-mediated signalling pathway, vasoconstriction, zymogen activation, platelet aggregation, and regulation of coagulation. The biological function of fibroblast proliferation was functionally linked to eicosanoid secretion and interleukin-8 production (Fig. 4C).

Using the documented experimental evidence, we corroborated the role of enriched biological processes and
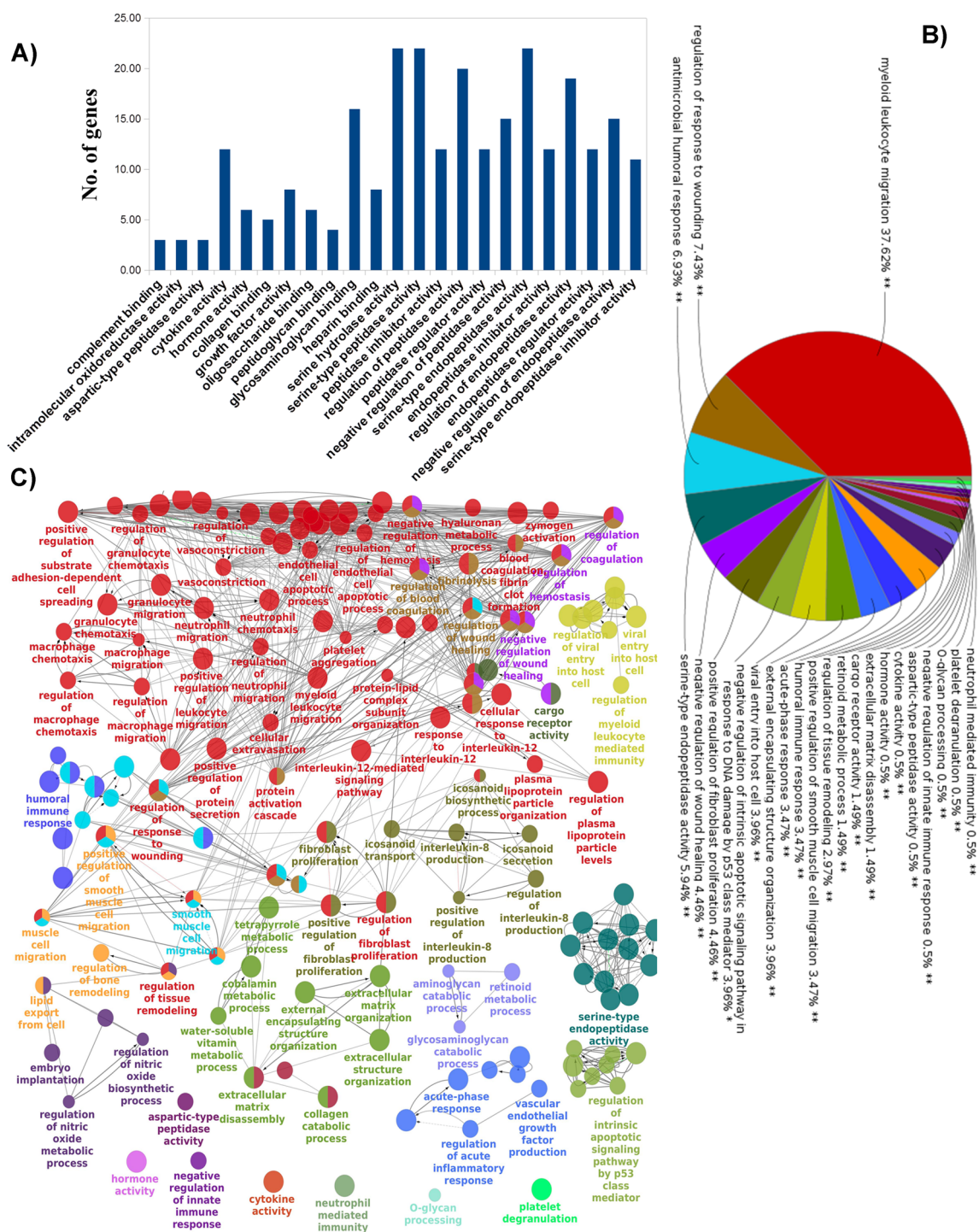
Fig. 4 Enrichment analysis of the secretome. **A** Molecular function: the *y* axis represents the number of upregulated genes enriched for a molecular function. **B** Representative biological processes: The percentage of biological processes was calculated using the ClueGO tool [41]. **C** Network of functionally grouped biological processes. The node represents the biological process. The nodes with two colours are shared between two groups of biological processes. The edge represents the connection between the functionally linked processes. The node size reflects the significance of the node. A larger node has more significance. The network was generated using ClueGO

molecular functions and their implications in MODs. Endothelial cells (ECs) regulate the coagulation cascade. EC activation and dysfunction have been reported in COVID-19

patients [77]. It interferes with vascular integrity and leads to EC apoptosis, activating the clotting cascade [78]. Platelets bind to cell adhesion molecules (CAM) displayed by
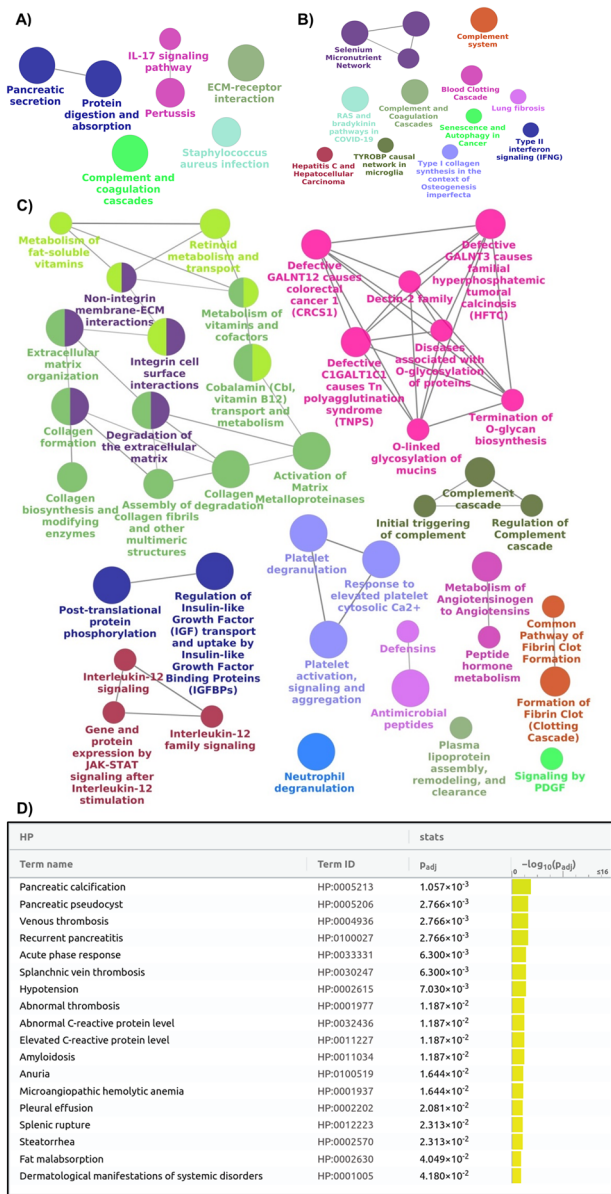
Fig. 5 Enrichment analysis of biological pathways and disease phenome of the secretome. The pathway enrichment analysis of the pancreatic secretome illustrates the network of functionally grouped pathways for **A** KEGG pathways, **B** WikiPathways, and **C** Reactome pathways. The node represents the biological pathway. The nodes with two colours are shared between two groups of pathways. The edge represents the connection between the functionally linked pathways. The node size reflects the significance of the node. A larger node has more significance. The figures were generated using the ClueGO tool [41]. **D** The disease phenotypes enrichment analysis of pancreatic secretome using Human Phenotype Ontology. The figure was generated using g:Profiler [41]

activated EC [79]. Platelets secreted Vascular endothelial growth factors (VEGF) induce tissue factor and matrix metalloproteinase production in endothelial cells, leading to thrombus formation and degradation of the underlying basement membrane, which causes vascular permeability [79]. A clinical study shows elevated levels of VEGF in COVID-19 patients [80]. High levels of VEGF lead to plasma extravasation, edema, and increased tissue hypoxia, and are also involved in atherosclerosis [81]. As a result of increased endothelial permeability, neutrophil migration occurs [82]. In COVID-19, over-activation of neutrophils in response to infection leads to excessive reactive oxygen species (ROS) production, thereby degrading the tetrapyrrole rings such as hemoglobin's heme and nitric oxide synthase (NOS), as well as vitamin B12's corrin ring [102]. The destruction of haemoglobin leads to hypoxia and protein aggregation, and the destruction of NOS leads to a deficiency of nitric oxide (NO) and ultimately to vasoconstriction [102]. The destruction of the corrin ring results in vitamin B12 deficiency, leading to oxidative stress, hypercoagulation, and vasoconstriction [83]. Low levels of NO, oxygen, and vitamin B12 deficiency are reported in COVID-19 patients [83]. ROS also increases matrix metalloproteinase (MMP) expression, which increases the production of chemokines and cytokines [84]. We observed the upregulation of matrix metalloproteinase (Fig. 3A). The high molecular weight glycosaminoglycan polymer, hyaluronan (HMW-HA), in acute inflammation, binds with fibrin and fibrinogen, which leads to increased clot formation [85]. HMW-HA is broken down into low molecular weight hyaluronan (LMW-HA), and oligo-HA by neutrophils producing ROS [85]. LMW-HA increases the vascular permeability, and both oligo-HA and LMW-HA act as damage-associated molecular patterns (DAMPs) leading to aggravated cytokine storms [85]. High levels of hyaluronans are reported in critical COVID-19 patients [86]. SARS-CoV-2 infection causes retinol and retinoic acid deficiency due to an increased catabolic process that results in retinoid signalling defects. It causes excessive cytokine secretion, leading to systemic effects and MOD [87]. Eicosanoids are arachidonic acid-derived chemicals are involved in physiological processes such as fever, allergy, and pain [88, 89]. Eicosanoids are dramatically upregulated in nonsurvivors of sepsis-induced multi-organ dysfunction [90]. An increased prostaglandin (eicosanoid) level contributes to the cytokine storm [91].

The pathway enrichment analysis of the pancreatic secretome revealed that the biological pathways were associated with the pancreatic secretion, RAS and bradykinin pathways in COVID-19, complement and coagulation cascades, IL-17 signalling pathway, ECM-receptor interaction, protein digestion, and absorption, Type II interferon signalling (IFNG), Vitamin B12 and folate metabolism, lung fibrosis, hepatitis C and hepatocellular carcinoma, Interleukin-12 family signaling, platelet activation, signalling and aggregation, and gene and protein expression by JAK-STAT signalling (Fig. 5A–C).
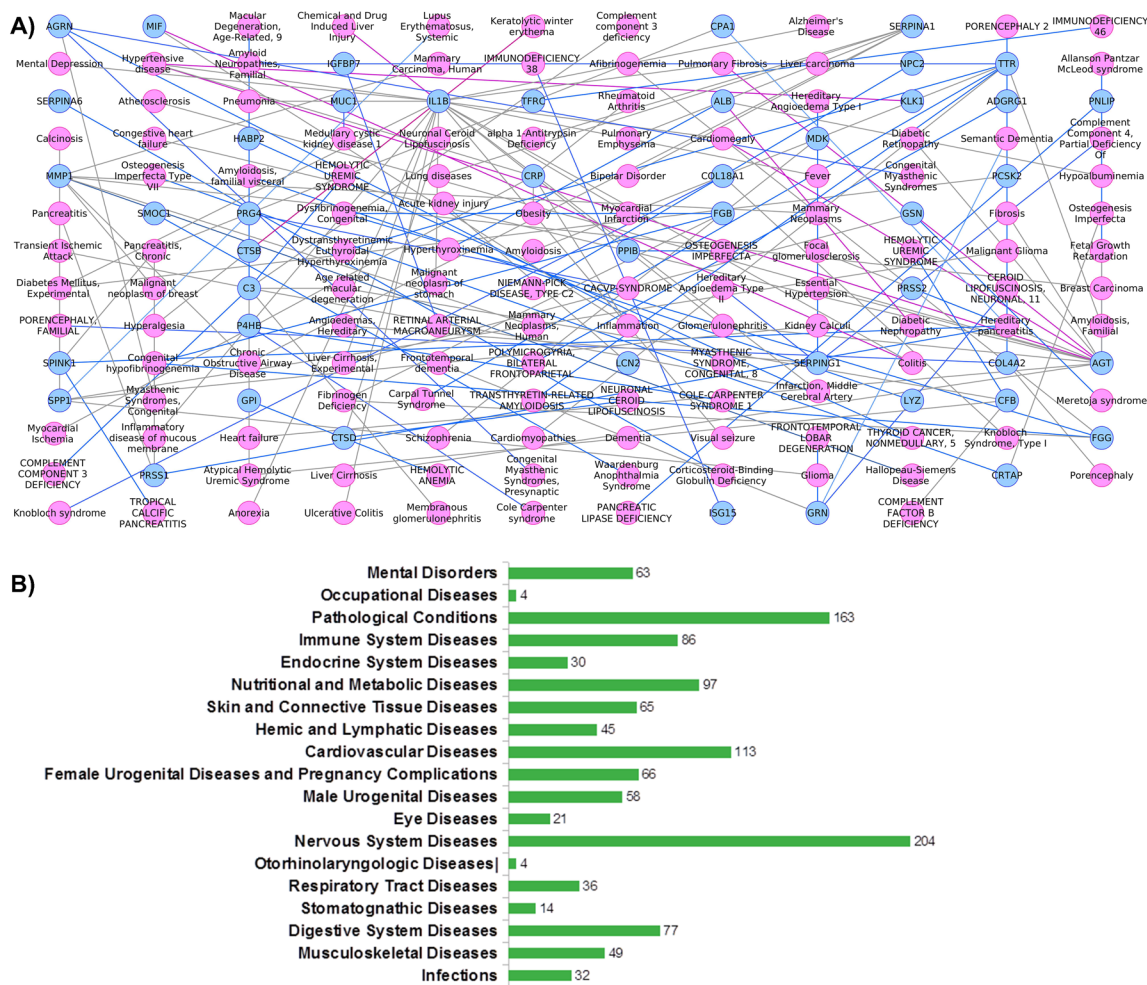
Fig. 6 **A** Gene-disease association network of secretome genes. The figure illustrates a glimpse of the complex network of disease (pink nodes) and the upregulated genes (blue nodes). For fuller details and an enlarged view, see the Supplementary figure S3. **B** Enriched disease classes for secretome. The top enriched disease classes are associated with nervous, cardiovascular, metabolic, immune, and digestive diseases

Using the experimental evidence, we validated the mechanistic role of enriched biological pathways and their implications in MODs. The imbalance in the Renin-Angiotensin System (RAS) has been widely associated with COVID-19 [92]. The RAS regulates blood pressure and fluid and electrolyte balance. The kidney secretes renin, which acts on angiotensinogen (AGT) to form angiotensin I (Ang I) [93]. Here, we found upregulation of AGT in SARS-CoV-2-infected pancreatic cells. Angiotensin-converting enzymes (ACE), present in the endothelial cells of the heart, lung, brain, and kidney, convert Ang I to a vasoconstrictor and proinflammtory Ang II [93]. Ang II induces macrophage and IL-8-mediated neutrophil recruitment into the tissues through the endothelial lining of blood vessels [94, 95]. Ang II increases the production of cytokines such as TNF-α, IL-1, and IL-6, and CAM [96]. CAM aids in the initiation of atherosclerosis and thrombus formation [96, 97]. Ang

II mediates transcytosis of plasma low-density lipoprotein (LDL) particles across endothelial barriers, marking the start of atherosclerosis [98]. In addition, Ang II plays a role in tissue fibrosis through angiotensin type 1 receptor (AT1) in cardiac, renal, pulmonary, and abdominal tissues as well as systemic sclerosis [99]. Pulmonary fibrosis impairs pulmonary function, affecting the oxygen exchange in COVID-19 patients [100, 101]. Therefore, our pathway analysis indicates that pancreatic secretions are associated with the immune system's hyperactivation and coagulation abnormalities, leading to multi-organ failure.

Here, the disease phenotype enrichment analysis of the pancreatic secretome using Human Phenotype Ontology revealed pancreatic calcification, pancreatic pseudocyst, venous thrombosis, recurrent pancreatitis, pleural effusion, splenic rupture, acute phase response, hypotension, abnormal thrombosis, elevated C-reactive protein level, amyloidosis, anuria, microangiopathic

Fig. 7 Enriched disease classes for secretory proteins. IL1B, AGT, ALB, SPP1, CRP, SERPINA1, C3, TFRC, TNFSF10, and MIF proteins are associated with diverse disease terms in the gene-disease association network

hemolytic anemia, and fat malabsorption disease phenotypes (Fig. 5D, and Supplementary Figure S2). The documented experimental evidence indicates that pancreatitis leads to acute phase response, coagulation, thrombus formation, hemolytic anemia, and amyloidosis [86]. Amyloid deposition in the heart, kidneys, liver, spleen, nervous system, and digestive tract induces inflammation, thrombosis, and immune dysfunction that causes systemic complications [86]. Thus, we suggest the role of the upregulated pancreatic secretome-associated disease phenotypes in MODs. Interestingly, we found that FGB, FGG, ANXA2, MDK, AGT, VTN, SERPING1, CD44, and IL1B are involved in many processes (Table S3). For example, blood coagulation and complement cascade: FGB, FGG, and SER-PING1[103], vasoconstriction: AGT [63], pro-inflammatory response: IL1B [64], host-virus interaction: ANXA2 [104], cytokine and growth factor: MDK [105], cell adhesion and extracellular matrix organization: CD44 [72] and VTN [73]. Furthermore, experimental evidence suggests that SARS-CoV-2-induced tissue damage, renin-angiotensin system (RAS) dysregulation, EC damage, thrombo-inflammation, immune response dysregulation, and tissue fibrosis are fundamental processes of viral sepsis and MODs in COVID-19 [57]. Therefore, our finding of an upregulated pancreatic secretome presents strong indications of the sepsis-mediated MODs.

3.4 Gene-Disease Association Network Analysis of the Pancreatic Secretome

We generated a gene-disease association network to further understand the implications of the upregulated pancreatic secretome in MODs (Fig. 6A). The top enriched disease classes were associated with nervous, cardiovascular, metabolic, immune, and digestive diseases (Fig. 6B), suggesting a multi-organ impact of the upregulated pancreatic secretome. In addition, our analysis revealed that IL1B, AGT, ALB, SPP1, CRP, SERPINA1, C3, TFRC, TNFSF10, and MIF proteins are associated with diverse disease terms in the gene-disease association network (Fig. 7). In addition, we found that IL1B, AGT, ALB, SPP1, CRP, SERPINA1, C3, TFRC, TNFSF10, and MIF were influential as they were linked to 31, 42, 47, 17, 24, 24, 17, 8, and 6 neighbouring secretory proteins, respectively, in the protein interaction network (Fig. 3B). As shown in Fig. 7, we noted that IL1B was associated with 231 disease terms and 17 disease classes, mainly with nervous system and cardiovascular diseases. AGT was associated with 146 diseases and 15 classes, mainly with the cardiovascular, nervous system, and digestive systems. ALB was linked to 123 diseases and 17 classes, most notably urinogenital disease and pregnancy complications, immune system, digestive system, and cardiovascular diseases. was linked to 81 diseases and 13 classes, primarily nervous system, digestive, respiratory tract, and cardiovascular diseases, whereas CRP was linked to 80 diseases and 16 classes, primarily cardiovascular,

digestive, metabolic disease, and mental disorders. SERPINA1 was associated with 59 diseases in 14 categories, primarily with respiratory tract and digestive system diseases. C3 was linked to 54 diseases of 13 different types, primarily cardiovascular disease and nervous system and immune system diseases. TFRC was associated with 52 diseases of 13 classes, mainly hemic and lymphatic diseases and immune system diseases. TNFSF10 was associated with 49 diseases across eight different categories. Urogenital diseases, pregnancy complications, and digestive system diseases were among the top enriched disease classes. MIF is associated with 49 diseases of 11 classes. Skin and connective tissue diseases, mental disorders, and immune system diseases were the top enriched disease classes. Thus, our analysis suggests that upregulation of *IL1B*, *AGT*, *ALB*, *SPP1*, *CRP*, *SERPINA1*, *C3*, *TFRC*, *TNFSF10*, and *MIF* genes may have systemic effects and may impact MODs.

4 Conclusion

The single-cell RNA-seq data analysis of SARS-CoV-2-infected pancreatic cells provides evidence of the potential role of the pancreatic secretome in SARS-CoV-2 associated multi-organ dysfunction. Acinar-specific *PRSS2*, *REG3A*, *REG1A*, *SPINK1*, and ductal-specific *SPP1*, *MMP7* genes are upregulated in alpha, beta, delta, and mesenchyme cells. We discovered several key secretory proteins that are linked to neurological, cardiovascular, immunological, digestive, and urogenital dysfunction. Our study suggests that the coagulation cascade, complement activation, renin angiotensinogen system dysregulation, endothelial cell injury and thrombosis, immune system dysregulation, and fibrosis are potentially associated with a dysregulated pancreatic secretome. This study may have a significant impact on clinical settings in terms of preventing SARS-CoV-2-induced MODs.

Author contributions EP conceived and designed the research; EP performed literature survey, single-cell RNAseq data analysis and prepared the illustrations; EP, and RM analyzed the data; EP and RM wrote the manuscript. NA assisted in the analysis, critically read and helped in improving the manuscript. RM designed and supervised the whole study. All the authors approved the final version of the manuscript before submission.

Declarations

Conflicts of interest The authors declare that there are no conflicts of interest with the contents of this article.

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