Exploring Molecular Interactions between SARS-CoV-2 Infection and Diabetes: Transcriptomic Analysis of PBMCs Reveals Converging Pathways

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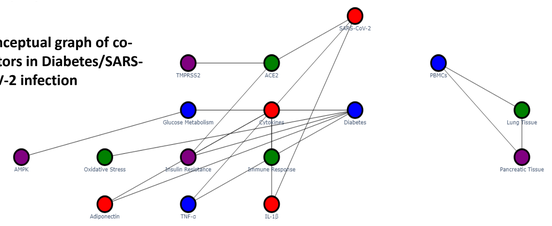
Background

- Studies have shown that individuals with Diabetes have a higher risk of hospitalization, intensive care unit (ICU) admission, and mortality when infected with SARS-CoV-2.
- The mechanisms underlying the vulnerability of diabetic patients to severe COVID-19 outcomes involve chronic inflammation, impaired immune responses, altered ACE2 receptor expression, and coexisting health conditions such as obesity.
- The "Cytokine Storm" involves an intense systemic immune response characterized by elevated levels of pro-inflammatory cytokines, such as interleukin-6 (IL-6). Elevated IL-6 levels are linked to poorer outcomes in COVID-19 patients.
- Recent clinical studies have also explored the bidirectional relationship between COVID-19 and diabetes. SARS-CoV-2 infection can lead to hyperglycemia and the onset of diabetes, possibly by disrupting metabolic processes and affecting insulin sensitivity.
- miRNAs have been increasingly recognized as critical regulators of the insulin signaling pathway. In diabetes, dysregulation of this pathway results in impaired insulin sensitivity and glucose homeostasis. miRNAs that modulate the insulin pathway may serve as molecular links between the two conditions.
- Identifying common molecular pathways may uncover potential therapeutic targets that could be modulated to mitigate the severity of COVID-19 in individuals with diabetes. This research can pave the way for targeted interventions to improve clinical outcomes in this high-risk population.

Conceptual graph of co-factors in Diabetes/SARS-CoV-2 infection



Aims

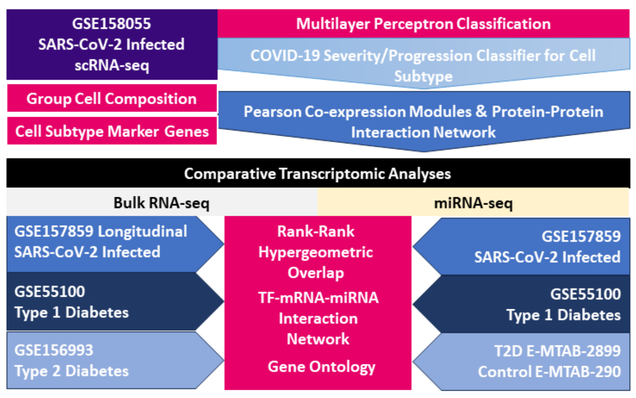
- While clinical observations have identified the association between diabetes and COVID-19 severity, the exact molecular mechanisms governing these interactions remain largely unknown.
- A multilayer perceptron classification model informed by cell composition, differential expression, and co-morbidities is used to distinguish significant features in altered metabolic signaling and immune profiles across measures of SARS-COV-2 infection severity and stage.
- This study involves comparative transcriptomic analysis for Type I and Type 2 Diabetes and SARS-CoV-2 infected datasets to identify significant overlapping miRNA–mRNA–TFs regulatory networks through co-modulated genes.

Methods

- **Data Collection:** Raw data is obtained from the Gene Expression Omnibus (GEO) repository, comprising scRNA-seq and bulk RNA-seq PBMC datasets.
- **Data Preprocessing:** Transcripts are aggregated based on sample IDs and cell types to ensure data uniformity. Feature selection is conducted using Mutual Information and Recursive Feature Elimination to identify relevant gene sets.

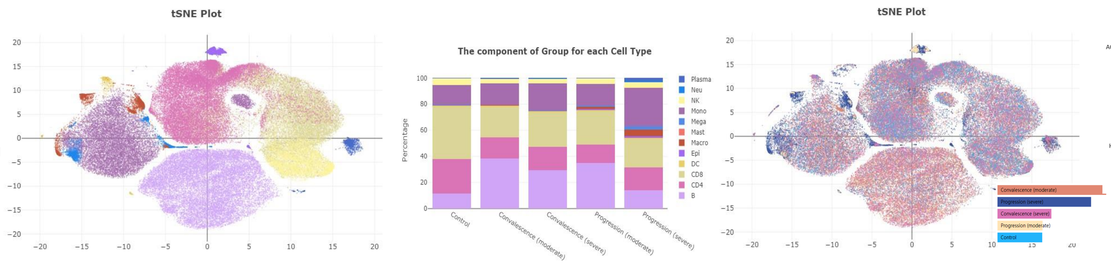
1. **Mutual Information Calculation:**
   - Calculate Mutual Information ($I(x_i;Y)$) for each feature x_i and the target variable Y.
   - $I(x_i;Y)$ for $i = 1, 2, \ldots, n$
2. **Feature Ranking based on Mutual Information:**
   - Rank features based on Mutual Information values.
   - $Rank(x_i) = \text{Sort}\ [I(x_i;Y)]$ in descending order
1. **Feature Selection with RFE:**
   - Select the top k features based on Mutual Information (e.g., k determined based on domain knowledge or experimentation).
   - SelectedFeatures $= [x_i$ for $i \leq k]$
2. **Recursive Feature Elimination (RFE):**
   - Perform Recursive Feature Elimination (RFE) on the selected features.

- **scRNA-seq Data:** Classification of severity, progression, and comorbidities matched samples is performed using selected features. Cell type-based classification is applied, validated using log fold change values, t-distributed stochastic neighbor embedding (tSNE), and Pearson Co-expression (>0.70) modules to confirm the robustness and specificity of selected genes.
- **Bulk RNA-seq Data:** Differential expression analysis is conducted using genes with false discovery rate (FDR) corrected p-values below 0.05. Concordant and discordant gene modules are identified using rank-rank hypergeometric overlap.
- **miRNA-mRNA Interactions and Transcription Factor Prediction:** Cytoscape is used to analyze miRNA-mRNA interactions and predict transcription factors employing a chance-corrected kappa score threshold of ≥0.5 to determine the reliability of associations. Subnetwork identification was accomplished using Steiner Tree methods.
- **Enrichment Analysis:** Enrichment analysis employing Enrichr is conducted to identify GO, KEGG, and other pathway/ontology/disease terms related to intersecting differentially expressed genes as well as miRNA associated targets across infection, convalescence, and recovery stages.

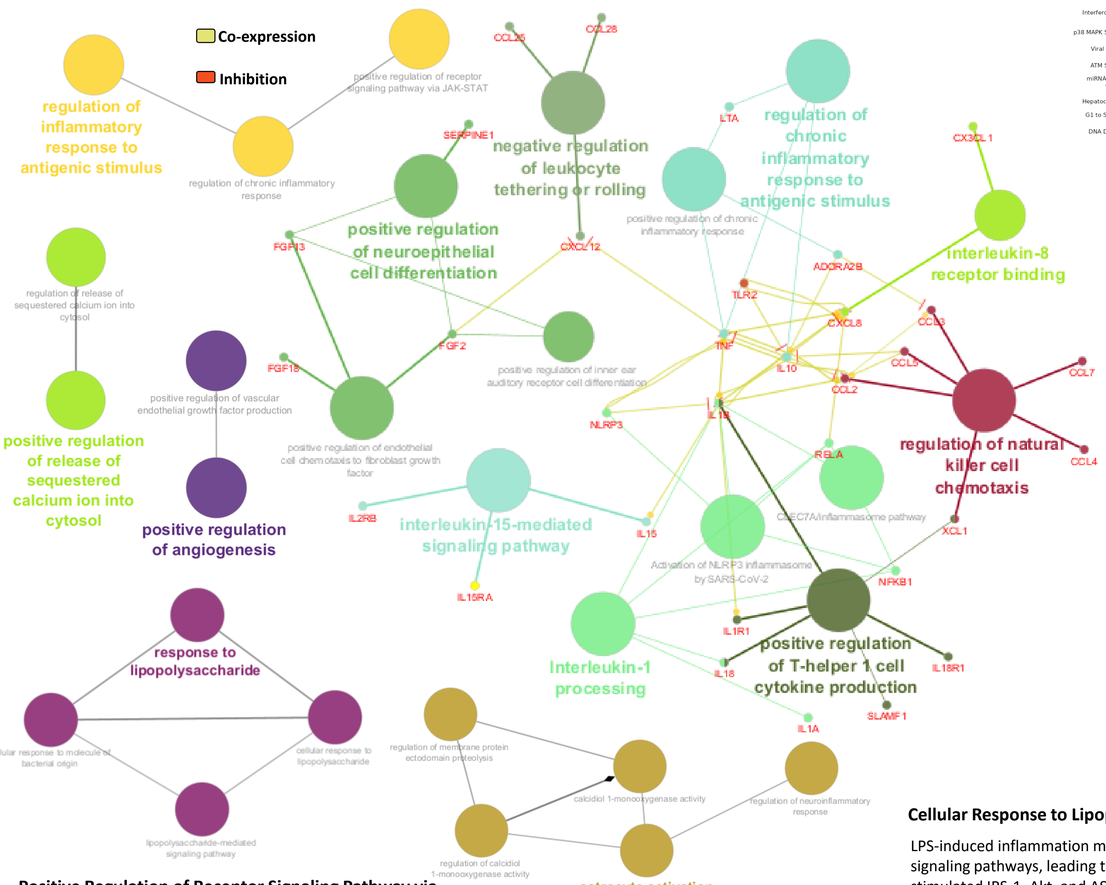


Results and Data Analyses

Severity/Progression Cell Type Composition

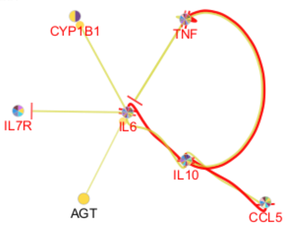


Pearson Co-Expression Modules & Protein-Protein Interaction Networks



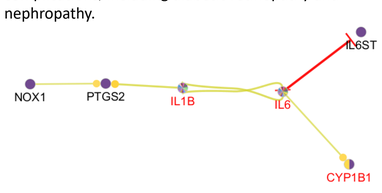
Positive Regulation of Receptor Signaling Pathway via JAK-STAT

Insulin signaling in the heart may activate the JAK 2-growth related pathway. The regulation of this pathway is crucial in maintaining normal cellular responses.



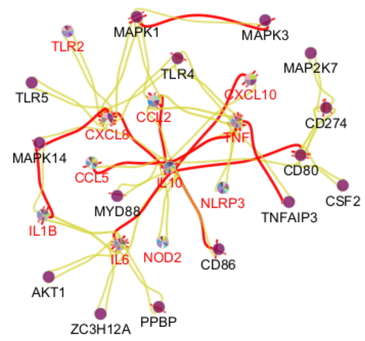
Positive Regulation of Vascular Endothelial Growth Factor Production

VEGF is a positive regulator of vascular endothelial growth and plays a central role in diabetic vascular complications, including diabetic retinopathy and nephropathy.

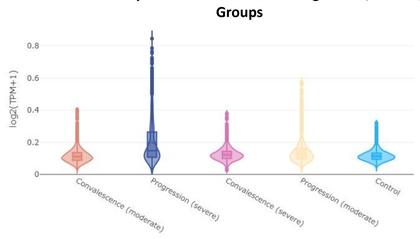


Cellular Response to Lipopolysaccharide

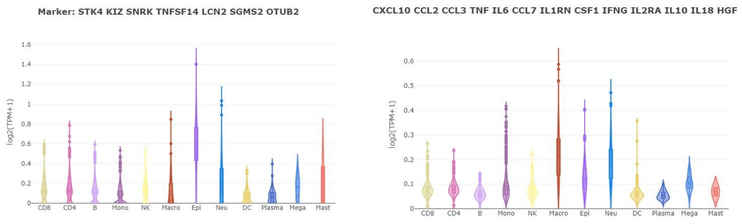
LPS-induced inflammation may impair insulin signaling pathways, leading to reduced insulin-stimulated IRS-1, Akt, and AS160 phosphorylation, ultimately affecting glucose transport.



Inflammatory Gene Modules Across Progression/Severity Groups

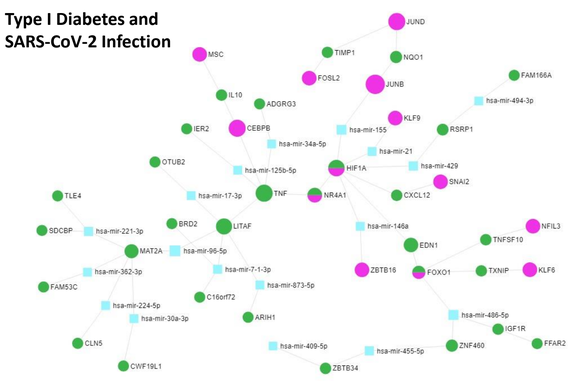


Inflammatory/Cytokine Genes Modules Across Cell Types



Transcription factor-miRNA-mRNA Interaction Networks

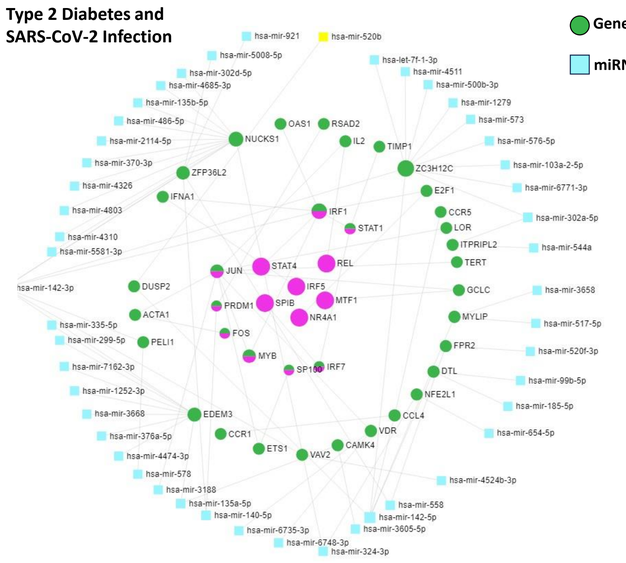
Type I Diabetes and SARS-CoV-2 Infection



Infection to Recovery Stages

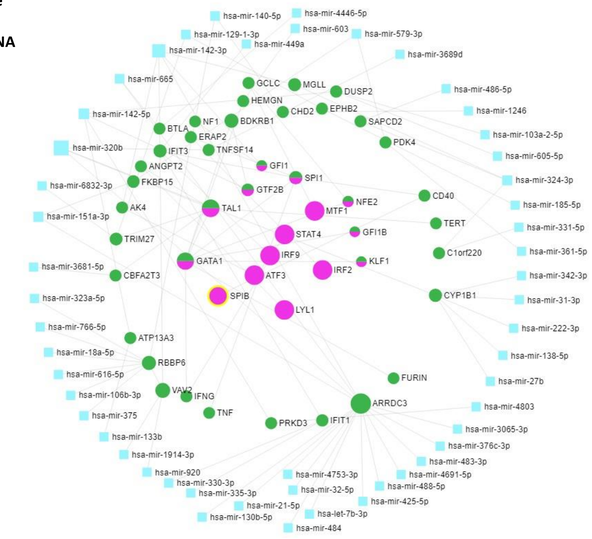
Convalescence to Recovery Stages

Type 2 Diabetes and SARS-CoV-2 Infection



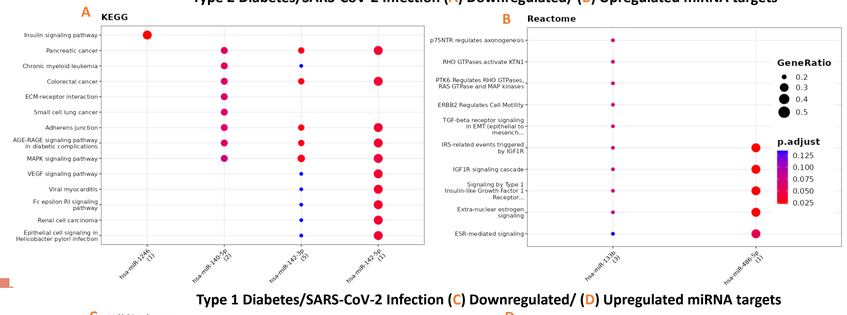
Infection to Recovery Stages

Transcription Factor
Gene
miRNA

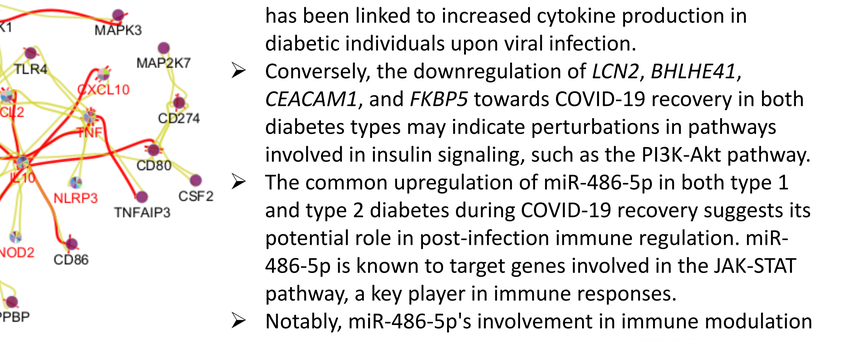


Convalescence to Recovery Stages

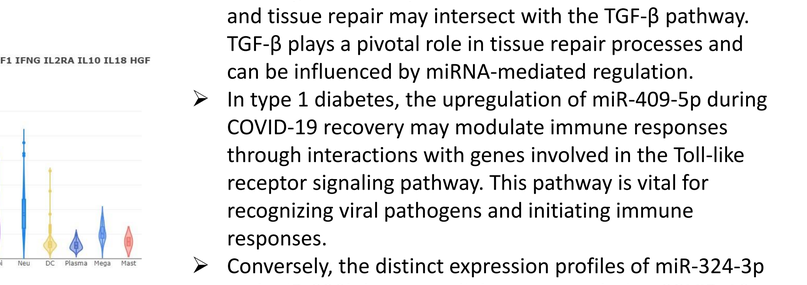
Type 2 Diabetes/SARS-CoV-2 Infection (A) Downregulated/ (B) Upregulated miRNA targets



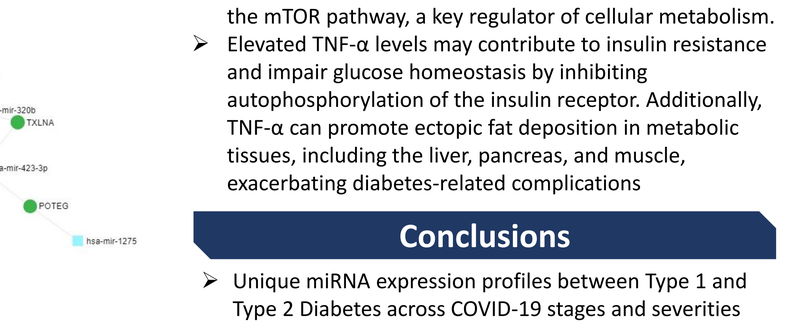
Type 1 Diabetes/SARS-CoV-2 Infection (C) Downregulated/ (D) Upregulated miRNA targets



Type 2 Diabetes/SARS-CoV-2 Infection (H) Downregulated/ (G) Upregulated Genes



Type 1 Diabetes/SARS-CoV-2 Infection (E) Downregulated/ (F) Upregulated Genes



Discussion

- The concurrent upregulation of *STK4*, *KIZ*, and *SNRK* in both type 1 and type 2 diabetes during COVID-19 infection suggests a common induction of proinflammatory pathways, such as the NF-κB pathway. Activation of NF-κB has been linked to increased cytokine production in diabetic individuals upon viral infection.
- Conversely, the downregulation of *LCN2*, *BHLHE41*, *CEACAM1*, and *FKBP5* towards COVID-19 recovery in both diabetes types may indicate perturbations in pathways involved in insulin signaling, such as the PI3K-Akt pathway.
- The common upregulation of miR-486-5p in both type 1 and type 2 diabetes during COVID-19 recovery suggests its potential role in post-infection immune regulation. miR-486-5p is known to target genes involved in the JAK-STAT pathway, a key player in immune responses.
- Notably, miR-486-5p's involvement in immune modulation and tissue repair may intersect with the TGF-β pathway. TGF-β plays a pivotal role in tissue repair processes and can be influenced by miRNA-mediated regulation.
- In type 1 diabetes, the upregulation of miR-409-5p during COVID-19 recovery may modulate immune responses through interactions with genes involved in the Toll-like receptor signaling pathway. This pathway is vital for recognizing viral pathogens and initiating immune responses.
- Conversely, the distinct expression profiles of miR-324-3p and miR-320b between diabetes types during COVID-19 recovery may be associated with differential regulation of the mTOR pathway, a key regulator of cellular metabolism.
- Elevated TNF-α levels may contribute to insulin resistance and impair glucose homeostasis by inhibiting autophosphorylation of the insulin receptor. Additionally, TNF-α can promote ectopic fat deposition in metabolic tissues, including the liver, pancreas, and muscle, exacerbating diabetes-related complications

Conclusions

- Unique miRNA expression profiles between Type 1 and Type 2 Diabetes across COVID-19 stages and severities underscore the importance of personalized therapeutic approaches, considering differences in immune and metabolic responses.
- The interplay between immune responses, insulin signaling, and cytokine modulation, particularly *TNF* and its upstream regulators, is central to understanding the heightened vulnerability of diabetic individuals to severe COVID-19 outcomes.
- These findings provide a foundation for targeted interventions aimed at mitigating the impact of COVID-19 in diabetic patients, potentially improving clinical outcomes and informing precision medicine strategies.

Bibliography

Shannon P, et al. (2003). Cytoscape; Chen EY, et al. (2013). Enrichr; Bindea G, et al. (2009). ClueGO; GSE157859; GSE158055; GSE157859; GSE55100; GSE55100; GSE156993; E-MTAB-2899; E-MTAB-2902

Acknowledgments

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