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Bioinformatics Analysis Of Differentially Expressed Genes And Pathway Enrichment In Nasopharyngeal Cells During COVID-19 Infection And Recovery

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Abstract

Bioinformatics approach undertaken in this study explores the differential expression of genes (DEGs) within nasopharyngeal epithelial cells during active SARS-CoV-2 infection and post-recovery. RNA sequencing (RNA-seq) data from 22 nasopharyngeal samples, categorized into COVID-19 positive individuals, individuals who had recovered, and unaffected controls, underwent comprehensive analysis. The results identified 1,845 DEGs among infected subjects and 138 DEGs among recovered subjects when compared to healthy controls. Pathway enrichment highlighted significant involvement in inflammatory responses, lipid metabolic processes, and protein synthesis during the infection phase. Furthermore, common DEGs observed in both infected and recovered participants indicated a prolonged inflammatory response even after symptomatic resolution. These findings contribute substantially to understanding the host response mechanisms and may guide future therapeutic interventions against COVID-19.

Keywords: SARS-CoV-2, Differential Gene Analysis, RNA-seq, Nasopharyngeal Tissue, Cytokine Response, Bioinformatics Pathway Analysis

Introduction

The rapid global transmission of COVID-19, caused by the SARS-CoV-2 virus, has posed severe health and socio-economic challenges worldwide. Clinical manifestations range from asymptomatic to severe respiratory distress, complicated by excessive inflammatory responses often termed cytokine storms. Bioinformatics analysis of gene expression in nasopharyngeal cellsthe primary infection sitecan significantly enhance our understanding of viral-host interactions and disease pathogenesis. While

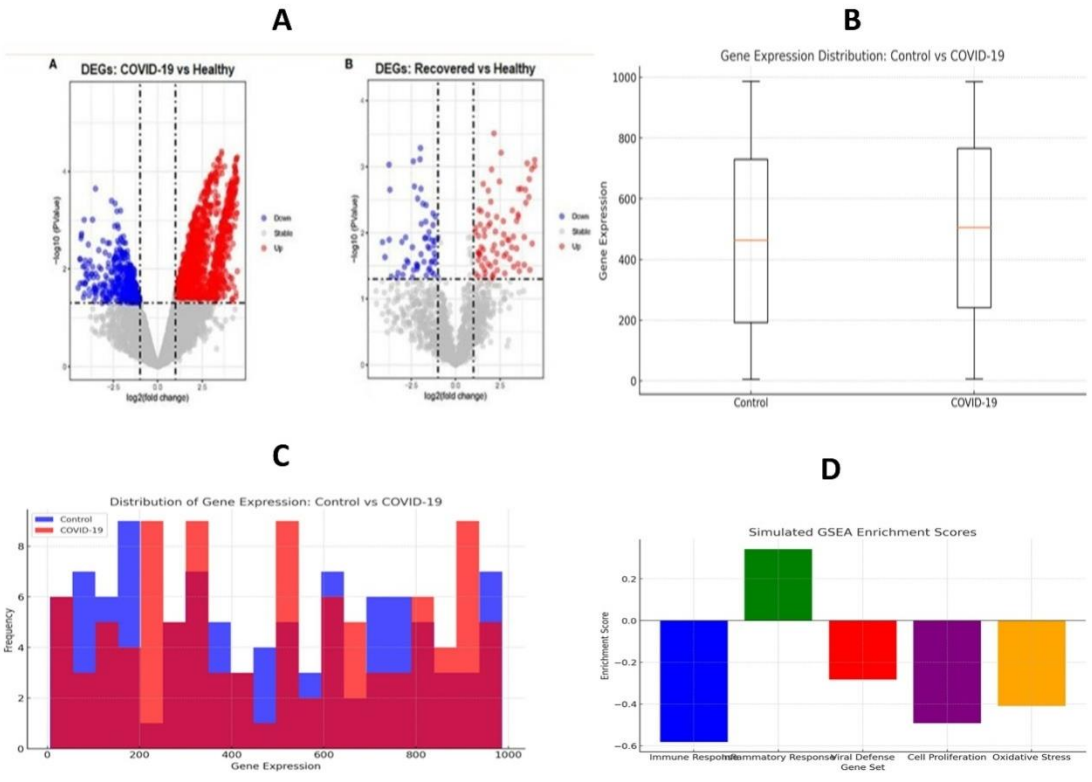
previous studies highlighted specific genes tied to inflammation, extensive analysis at the transcriptional level remains limited. Thus, this study employs RNA-seq data analysis techniques to determine DEGs in nasopharyngeal samples of COVID-19 patients and recovered subjects, identifying key molecular pathways involved.

Materials and Methods

Sample Acquisition Nasopharyngeal swabs were collected from 22 subjects in Dhaka, Bangladesh, grouped into confirmed COVID-19 patients (8), recovered individuals (7), and healthy controls (7). Ethical clearance and informed consents were obtained in compliance with national guidelines.RNA Isolation and RT-qPCR Validation. The PureLink Viral RNA/DNA Mini Kit facilitated RNA extraction. The RT-qPCR assay detected SARS-CoV-2-specific genes ORF1ab and N, following standardized thermal cycling parameters.RNA-seq Analysis Libraries for RNA sequencing were constructed using Nextera DNA Flex methods. Bioinformatics tools analyzed sequencing data sourced from the NCBI BioProject PRJNA720904 database, facilitating DEG identification, generation of heatmaps, and Venn diagrams to visualize gene overlaps.

Results

Figure 1: Volcano plot, Gene expression distribution and GSEA simulated score, Figures A-D collectively present a comparative analysis of gene expression and pathway enrichment between Control and COVID-19 groups.



Results

Differential Expression Profiling RNA-seq data analysis demonstrated 1,845 DEGs in SARS-CoV-2 infected patients, including significant upregulation of genes indicative of inflammation and protein synthesis. Prominent DEGs identified included RPL4, MT-ND2, SCD5, MT-CYB, and EZR.Gene Expression in Recovered Individuals Recovered participants exhibited 138 DEGs, among which 85 were upregulated, suggesting ongoing post-infection inflammatory or healing processes. Analysis also revealed a subset of 34 DEGs common between infected and recovered subjects, potentially representing biomarkers of prolonged recovery.Functional Pathway Enrichment analyses pinpointed key cellular pathways related to inflammation, lipid metabolic processes, particularly ceramide metabolism, and epithelial cell differentiation as significantly altered during COVID-19 infection. Figure

Discussion

This comprehensive bioinformatics analysis reveals significant changes in gene expression profiles within nasopharyngeal epithelial cells during active COVID-19 infection and the recovery period. In infected individuals, 1,845 DEGs were identified, demonstrating extensive alterations primarily associated with immune activation, inflammatory responses, and metabolic processes. Such results substantiate the critical role of inflammation in the disease's severity, aligning closely with documented cytokine storm phenomena, where excessive production of inflammatory cytokines contributes to disease exacerbation. Notably, genes involved in protein synthesis (e.g., ribosomal protein L4 - RPL4), mitochondrial activity (e.g., MT-ND2, MT-CYB), and lipid metabolic pathways (e.g., Stearoyl-CoA desaturase 5 - SCD5) exhibited considerable expression shifts. These specific molecular changes suggest complex interactions between host cell metabolic reprogramming and viral pathogenesis, potentially driving sustained inflammation and tissue injury. Recovered individuals displayed fewer DEGs (138), with a majority (approximately 85 genes) continuing to show increased expression levels. Such persistence indicates ongoing biological processes post-infection, reflecting either gradual recovery or residual inflammation, potentially contributing to lingering symptoms commonly referred to as 'long COVID'. Importantly, 34 genes were consistently dysregulated across both actively infected and recovered groups, highlighting their potential as biomarkers for long-term monitoring and targeted therapeutic strategies. The enrichment of ceramide-related metabolic pathways points to a notable metabolic shift during infection. Ceramide has been implicated in inflammation and apoptosis; thus, its upregulated pathways might play a significant role in COVID-19 severity and resolution processes. Further investigation into these lipid metabolism changes could elucidate additional therapeutic targets. This analysis supports and expands upon previous findings that emphasize the significance of an extended inflammatory response even after clinical recovery. The detailed exploration of these molecular alterations advances the understanding of COVID-19's complex pathogenesis and provides potential biomarkers and therapeutic avenues worthy of exploration in subsequent studies.

Conclusion

Bioinformatics analysis has effectively elucidated key DEGs and associated pathways in nasopharyngeal epithelial cells during SARS-CoV-2 infection and recovery. These results provide valuable insights into the underlying biological processes of COVID-19, offering potential avenues for targeted treatments.

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