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Article

# Gene expression profiling of MERS-CoV-London strain

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## SUMMARY

**Middle East Respiratory Syndrome-related coronavirus (MERS-CoV) is a variety of coronavirus identified in 2012. The purpose of our research was to identify unique regulation of protein coding genes in cells infected by the MERS-CoV-London strain (LoCov). To address this, we utilized genetic data from the National Center for Biotechnology Information (NCBI), analyzed differential gene expression, and performed analysis with other tools. We used GEO2R to run a comparison between mock and infected groups, then input the statistically significant genes into String-db, which provides a database of protein-protein interactions and associations that we used to analyze the biological interactions of our protein-encoding genes. Analysis of String interactions and the Log fold change (LogFC) values highlighted cellular interactions. From our String analysis, we learned how genes may connect to biological processes related to symptoms, the lasting effects of MERS strains, and classification into biological and Krypto Encyclopedia of Genes and Genomes (KEGG) pathways. We identified several significant genes, including *SSX2*, which is expressed in cancer cells. The upregulation of *SSX2* may be involved in the unregulated inflammatory responses seen in some MERS-CoV-2 patients. Contrastingly, the *HLA* genes, which are typically expressed in healthy cells, are downregulated in our infected group. A third group of upregulated genes included *MAK16* and *CDC25A* and showed decreased nuclear DNA function and apoptosis, indicating viral control of the cell for use in RNA processing. All these genes may potentially act as markers for infection, and given its expression levels, *SSX2* may also be a potential gene target.**

## INTRODUCTION

Viral infections are notoriously difficult to treat or identify early since the pathogenic process occurs intracellularly. This study examines MERS-CoV, which is a coronavirus that causes Middle Eastern Respiratory Syndrome (MERS) and has clinical features similar to those of severe acute respiratory syndrome (SARS) as well as a mortality rate of 60% (1). We examined MERS-CoV-London (LoCov) infected epithelial cells vs. mock infected cells to see if there were unique levels of gene expression that would allow us to identify infected cells or more easily target or enhance the immune response to infection. Through our gene expression analysis of the LoCov infected epithelial cells, we hypothesize that, owing to similar cellular functions in both cancerous cells and virally infected cells, genes whose upregulation act as markers for cancers may also be markers for viral infections. Current research into viral oncogenesis has revealed significant links between some cancers and infection that may provide opportunities for treatment or further research (2).

Previous studies have shown that viruses can stimulate a metabolism akin to that seen in tumor cells in their host cells and even in neighboring cells (3). Currently, researchers are working on developing vaccine-based immunotherapies using cancer-associated genes, though the technology is still relatively immature (4). If similar genes, meaning those that are always associated with abnormal processes such as unregulated cell proliferation, are indeed found in virally infected cells, it may be possible to synthesize similar treatments for persistent viral infections such as HIV/AIDS.

Two tenants that provoke unregulated and unhindered cellular proliferation are self-sufficiency in growth signals, as well as insensitivity to growth-inhibitory signals (5). Based on these hallmarks of cancer, our analysis into the gene expression of MERS-CoV-2 infected epithelial cells identified two genes as having implications in both combating malignant growths, as well as diagnosing and creating targeted treatments for certain viral infections. These genes were *SSX2*, which co-represses DNA transcription and promotes the innate immune response, and *TP53*, which controls cell growth and division. Virally infected cells, while not immortal like the cells found in many types of cancers, are seemingly trying to stimulate unregulated translation indirectly (6). *SSX2* represses DNA transcription and does not affect translation, meaning that the positive-sense single strand RNA (+ssRNA) of the Mers-CoV virus could more easily reproduce (7, 8). Because of these behaviors, *SSX2* may be able to act as a potential gene target in future diagnostic or treatment options in MERS-CoV-2 or other coronaviruses.

## RESULTS

We analyzed gene expression, biological functions, and cellular pathways in order to find novel gene targets for the purpose of diagnosing and potentially combating MERS-CoV. We used GEO2R gene expression analysis of mock-infected (control group) vs. LoCoV infected cells (infected group) to determine the top 250 genes by adj. *p*-value as well as the number of genes with significant adj. *p*-values ($p < 0.05$). Beyond adj. *p-values* we looked at Log Fold Change (LogFC)

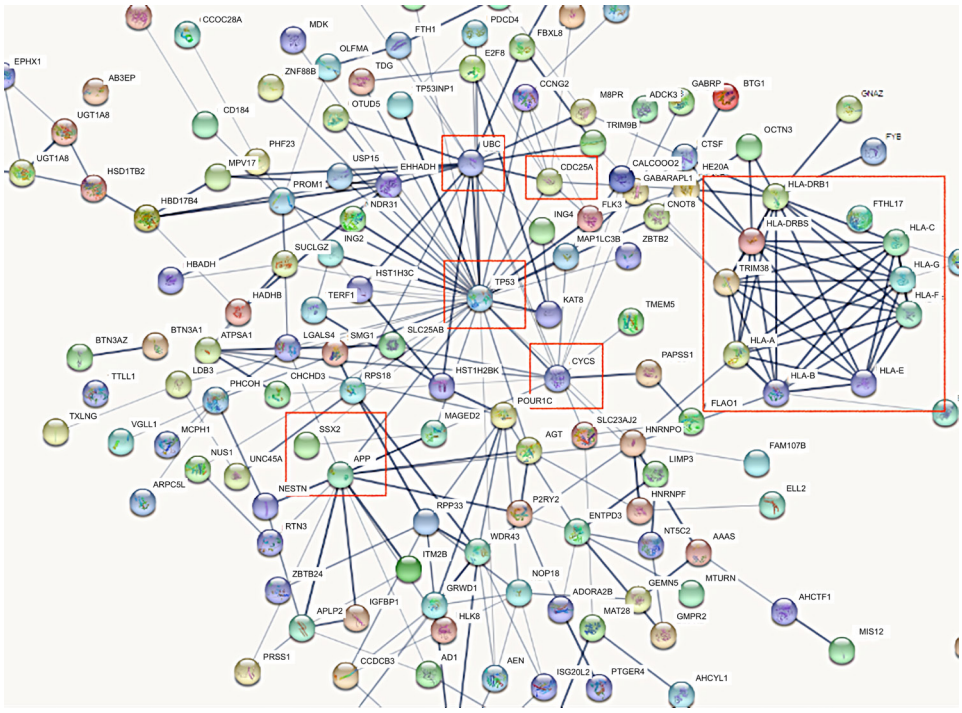


**Figure 1: String Analysis of Top 250 Genes.** Our String-db analysis shows connections between the top 250 statistically significant ($p < 0.05$) genes in the dataset GSE56677. The most prominent clusters are the ones on the right with the HLA genes and in the center with *TP53* and *UBC*.

values to determine the number of genes that were upregulated and downregulated. We did not have a threshold value for LogFC values. The dataset GSE56677 provided genomic expression data for Calu-3 2B4 cells infected with LoCov, as well as mock-infected at six time intervals. We put the top 250 differentially expressed genes, based on *p*-value, into String-db, and the String analysis of the genes showed clusters of highly associated genes, most of which were amongst the top upregulated or downregulated genes (**Figure 1**). GEO2R uses the limma library, coded in R, to generate microarray expression data that is used to determine the LogFC between the gene expression of our two groups. These values showed the difference in gene expression of a specific gene between a sample in our control. Downregulation was signified by negative LogFC values, meaning a lower expression level in the infected group than the control group, while positive LogFC values indicated upregulated genes and a higher expression level of a gene in the patient group over the control group (**Figure 2**).

Our results can be summarized in terms of three loosely defined groups of genes. Each group refers to infected cells versus the control cells. The first group was the three downregulated genes in infected cells that are involved in antigen presentation; the *HLA* class one series, the *HLA-BR* class two series, and *APP*.

One can note that *HLA-A* (LogFC = -0.831, adj. *p*-value = 0.00164), *HLA-C* (LogFC = -0.812, adj. *p*-value = 0.00289), *HLA-E* (LogFC = -0.482, adj. *p*-value = 0.00289), *HLA-F* (LogFC = -0.831, adj. *p*-value = 0.00246), and *HLA-J* (LogFC = -0.945, adj. *p*-value = 0.00284) all had negative LogFC values, indicating that these five genes were downregulated in the infected group. String association shows a high degree of association between these genes (**Figure 3**). The LogFC values for *HLA-DRB1* (LogFC = -0.697, adj. *p*-value = 0.00295) and *HLA-DRB5* (LogFC = -0.731, adj. *p*-value = 0.00353) were also negative, indicating that both are downregulated.

The second grouped set of genes was downregulated in infected cells and related to nucleus function and DNA- or RNA-based activity (**Table 1**). In this second group, we found the following associations, which may limit cellular response to infection: *APP, UBC,* and *TP53* were all downregulated, though *MAK16* was upregulated. *APP* was downregulated in MERS-CoV London infected cells (LogFC = -0.794, adj. *p*-value = 0.00173).. *MAK16* (LogFC = 0.434, adj. *P*-value

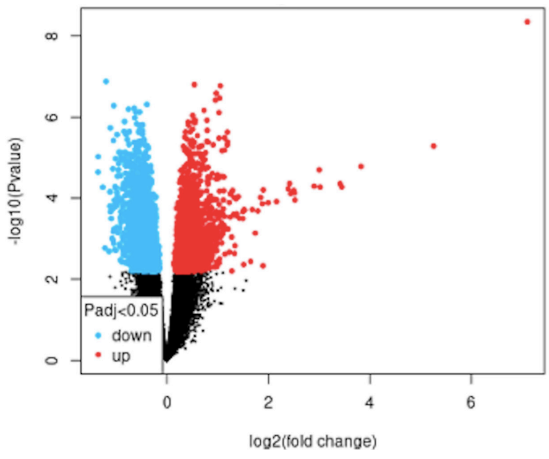


**Figure 2: Volcano Plot GSE56677: LoCov vs. Mock, Padj<0.05.** Volcano plot comparing the gene expression changes between the infected and control groups and shows a clear differentiation in gene expression. The extreme points represent the most highly up- or downregulated genes based on the largest or smallest LogFC values in the dataset. The red dot in the top right corner is *SSX2*.

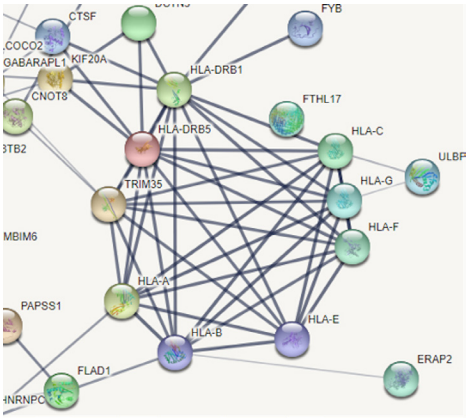


**Figure 3: String HLA Group Genes.** An excerpt from our String-db analysis shows the various connections between the HLA Class 1 and 2 genes within the network.

= 0.00387) was upregulated, in juxtaposition to *APP*. *UBC* (LogFC = -0.380, adj. *p*-value = 0.00205) was downregulated in the MERS infected cell. In this dataset, *TP53INP1* (LogFC = -0.552, adj. *p*-value = 0.00173) was also downregulated. Both *UBC* and *TP53* show significant associations through interconnectedness in the String diagram (**Figure 4**).

The third group included *SSX2, CYCS,* and *CDC25A,* which were all upregulated in infected cells (**Table 2**). All three genes also demonstrated decreased nuclear DNA function and decreased apoptosis. *SSX2* (LogFC = 7.0982, adj. *p*-value = 0.000128) was among the upregulated genes. Based on the magnitude of its LogFC value alone, *SSX2* was

| Downregulated Genes | | | |
|---|---|---|---|
| Gene ID | adj. *P* Value | LogFC | Gene |
| A_33_P3379962 | 0.00164 | -0.831 | *HLA-A* |
| A_23_P408353 | 0.00247 | -1.007 | *HLA-A* |
| A_33_P3330109 | 0.00289 | -0.812 | *HLA-C* |
| A_33_P3424803 | 0.00386 | -0.752 | *HLA-C* |
| A_32_P460973 | 0.00289 | -0.482 | *HLA-E* |
| A_33_P3379939 | 0.00246 | -0.831 | *HLA-F* |
| A_24_P418044 | 0.00284 | -0.945 | *HLA-J* |
| A_23_P168882 | 0.00173 | -0.552 | *TP53INP1* |
| A_24_P343233 | 0.00295 | -0.697 | *HLA-DRB1* |
| A_23_P45099 | 0.00353 | -0.731 | *HLA-DRB5* |
| A_33_P3296479 | 0.00388 | -0.794 | *APP* |
| A_33_P3508822 | 0.00173 | -1.111 | *APP* |
| A_24_P681301 | 0.00215 | -0.366 | *UBC* |
| A_23_P329740 | 0.00205 | -0.381 | *UBC* |
| A_24_P889720 | 0.00748 | -0.242 | *UBC* |

**Table 1: Significant downregulated genes by adj. p-Value and LogFC.** The genes of focus in our paper, along with their LogFC values and *p*-values, indicate the significant changes in expression that occurred in the MERS-CoV-London infected cells compared to the mock infected ones. The Gene IDs reference specific genetic isotypes. These tables were obtained through GEO2R analysis.

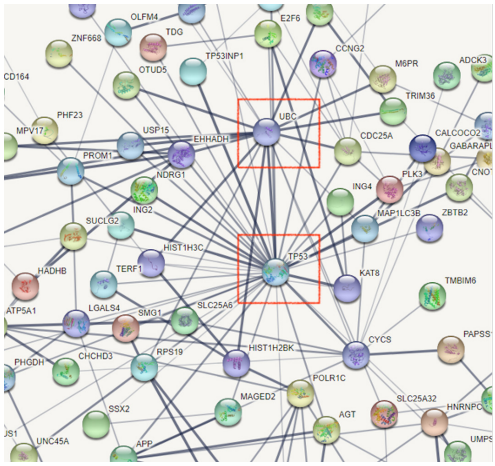


**Figure 4: String Diagram for *UBC* and *TP53*.** An excerpt from our String-db analysis highlights the connections formed between *UBC* and *TP53* within the rest of the network.

a significant gene despite having only one link to another gene in our String diagram. *CYCS* (LogFC = 0.544, adj. *p*-value = 0.00121) was also upregulated, but not to the same extent, as was CDC25A (LogFC = 0.733, adj. p-value = 0.00148). String-db revealed that *CYCS* had associations with many other genes (**Figure 5**).

| Upregulated Genes | | | |
|---|---|---|---|
| ID | adj. *P* Value | LogFC | Gene |
| A_24_P256243 | 0.000128 | 7.098 | *SSX2* |
| A_33_P3313401 | 0.00121 | 0.544 | *CYCS* |
| A_24_P397107 | 0.00148 | 0.733 | *CDC25A* |
| A_23_P111961 | 0.00387 | 0.434 | *MAK16* |

**Table 2: Significant upregulated genes by adj. *P*-value and LogFC.** The genes of focus in our paper, along with their LogFC values and *p*-values, indicate the significant changes in expression that occurred in the MERS-CoV-London infected cells compared to the mock infected ones. The Gene IDs reference specific genetic isotypes. These tables were obtained through GEO2R analysis.

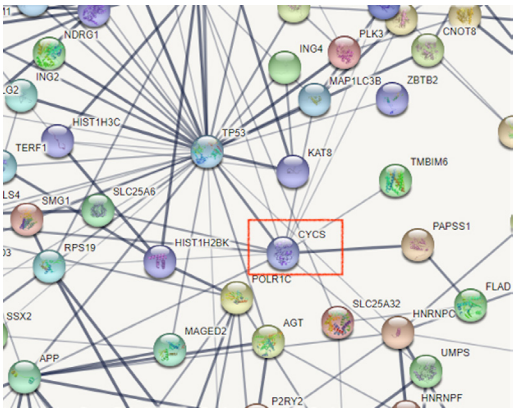


**Figure 5: Interactions with *CYCS*.** String-db analysis displays *CYCS*'s connections to genes including *PAPSS1*, *SLC25A6*, and *TP53*.

## DISCUSSION

Our results revealed there are three loosely defined groups of activity or associations with these genes. The first group was the three downregulated genes that were involved in antigen presentation. The components of this group were the *HLA* class one series, which stimulates the cytotoxic T-cells to destroy an infected cell, the *HLABR* class two series, which encode MHC proteins and stimulates the humoral response to antigens, and *APP*, which is involved in antigen presentation and due to its downregulation, may also decrease cellular pathways that lead to apoptosis (9, 10). Downregulation limits antigen presentation, preventing activated T-cells from recognizing the presence of the virus and taking part in the immune response. *HLA-A HLA-C, HLA-E, HLA-F, and HLA-J* are Class 1 Major Histocompatibility (MHC) genes that are responsible for presenting peptide fragments from within the cell to the cell surface (9). This activates the lymphocyte cell-mediated immune response, triggering cytotoxic T-cells to target and destroy an infected cell (11). Downregulation of these genes would limit the immune response to viral infection and could serve as nonspecific markers for MERS-CoV infection. This gene group allows noninfected cells to gather peptides or antigens found in the cellular environment and present them to the immune system to elicit an antibody response. Downregulation may decrease antigen presentation as a result.

The second grouped set of genes was downregulated compared to gene expression in our control samples and related to nucleus function and DNA- or RNA-based activity. In this second group, we found the following associations: *APP* functions as a cell surface receptor and is involved in transcriptional regulation through protein-protein interactions, and *UBC* is associated with DNA repair, cell signaling, and DNA damage responses (10, 12). Since *UBC* is downregulated, the cell's DNA repair and cell signaling responses may allow viral control of cellular processes to occur unimpeded; *MAK16*, which was also downregulated, is associated with the maturation of rRNA and results in decreased protein-encoding (13); *APP* was also downregulated and decreases apoptosis; *TP53INP1* is involved in RET (rearranged during transfection) signaling and DNA double-strand break repair. Downregulation of this gene would impact DNA repair and allow other processes to run unregulated (14).

We believe that, based on the functions of these genes, downregulation of this entire group would limit cellular responses to infection and would again serve as non-specific markers for MERS-CoV infection.The third group included *CDC25A, CYCS,* and *SSX2,* which were all upregulated, and serve to increase cell division, increase electron transport and cellular apoptosis, and decrease transcription of DNA, respectively. Furthermore, *SSX2* downregulates transcription of DNA and permits viral RNA production (8). *CYCS* is a component of the electron transport chain in mitochondria, associated with electron transfer and iron ion binding, and involved in apoptosis, and *CDC25A* encourages cell division through the G1- to S-phase transition and is overexpressed in natural killer (NK) cells (15, 16). *CDC25A* is found in the nucleus and the cytosol. Upregulation would be associated with protein kinase binding and increased cell cycle activity in virally infected cells (16)

Upregulation of *CYCS* could signify an increase in spontaneous cell death, but only in the case where its encoded protein, cytochrome c, leaks into the cytoplasm. In other cases, it is associated with mitochondrial activity (15). We believe that this increase in mitochondrial activity could facilitate viral RNA production.

The most unique finding in this study was the significant upregulation (LogFC = 7.1) of *SSX2*. *SSX2* produces a protein that belongs to the synovial sarcoma X family and functions as a transcriptional repressor. The SYT-SSX fusion protein is also known to produce a transcriptional coactivator and directly downregulate the expression of *COM1*, a regulator of cell proliferation (17). Additionally, *SSX2* is known to elicit humoral and cellular immune responses in cancer patients. *SSX2* is not expressed in normal, healthy cells, but it is expressed in a variety of cancers, specifically sarcoma and melanoma (17, 18). There is evidence that in 12% of cancer formation, seven different viruses have been linked, such as Merkel cell cancer caused by Merkel cell polyomavirus (19). Virally infected cells may show upregulation of several genes that are associated with cancer as in EBV infection-causing mononucleosis (20). *SSX2* is not only expressed in specific tumors but also has been associated with eliciting a cellular immune response in some tumors, making it a potential target for cancer immunotherapies (21).

As previously mentioned, the *SSX* family is associated with synovial sarcomas, as well as with RNA-virus-infected cells, according to our analysis (17). Cancer is broadly characterized by unregulated cell growth (which eventually forms cancerous tumors), as well as by immortal cells since apoptotic processes are hindered in cancer (22). Though the virally infected cell would eventually lyse, it would ideally remain an immortal cell to allow more viruses to be created since MERS-CoV releases new virions via exocytosis rather than cell lysis (7). For this reason, a competitor process, namely the transcription of host DNA and its subsequent demand for RNA translation, would no longer be possible because they are inhibited by *SSX2*. This is in turn due to the fact that DNA transcription requires protein synthesis using RNA, but DNA would not be active in this case, as *SSX2* is a co-repressor of DNA transcription (23).

*SSX2* serves to co-repress transcription with polycomb group proteins RING1 or BMI1, but it may also activate a cell-mediated immune response. If *SSX2* co-represses transcription, it effectively shuts down DNA activity while permitting RNA translation to continue. As MERS-CoV is a +ssRNA virus, this would mean that *SSX2* could be a marker for increased viral activity in the cell (7). If *SSX2* elicits cell-mediated immune response directly, then it could be associated with the cytokine storm seen later in MERS-CoV infection. Cytokine storms are particularly associated with MERS-CoV, influenza, and other coronavirus infections (24). Upregulation of SSX could result in decreased transcription allowing for viral translation to proceed unimpeded. If this increases cytotoxic T-cell activity, it may also play a role in the unregulated inflammatory response seen in some MERS-CoV patients. *SSX2*'s appearance as an upregulated gene in our analyses suggests further reaching implications of this gene, beyond cancers or tumors, as our study now suggests that upregulation is associated with MERS-CoV infection. Additionally, if increased expression of *SSX2* directly elicits a cell-mediated response, then it could be an indicator of patients at risk for severe disease.

These results motivate the question of whether *SSX2* is

upregulated in other coronaviruses beyond the London strain of MERS-CoV. If so, then we believe targeting cells expressing *SSX2* for treatment purposes would allow for selective targeting of infected cells versus healthy cells that do not express this gene. Additionally, since *SSX2* is not expressed in healthy cells, it can be used as an early marker for RNA virus infection, which includes several strains of coronaviruses. COVID-19, which is caused by another coronavirus, and cancers are linked by a few strong factors, such as *ACE2*, the cytokine storm, and perhaps age and coagulopathy, and this link may help find novel antiviral and anticancer therapeutics (25). In this paper, we have further identified another link: *SSX2*. Downregulation of the tumor suppressor *TP53* and upregulation of *SSX2* may act as a basis for future studies looking into these two genes in early detection of MERS-CoV infected cells. Furthermore, additional analyses of this gene and its chimeric transcripts may provide insight into new possibilities for viral and oncogenic therapeutics (26).

There is a study relating to gene expression in severe acute respiratory syndrome-associated coronavirus (SARS-CoV) and MERS-CoV that draws connections to genes. including *SSX2* and *TP53,* using GO analysis, which we also found to be significant in our research (26). That study, however, did not examine the HLA Group, but otherwise its findings are consistent with our data (27). Another study examined the association of HLA class I and II genes with MERS-CoV through genotyping with polymerase chain reaction sequence-based typing and concluded that one allele of *HLA-DQB1* is significantly associated with mild cases of MERS-CoV (6). Our study did not look at specific alleles, but *HLA-DQB* was present and downregulated in our analysis.

Using antisense RNA, it is possible to target intracellular genes for therapeutic purposes (28). Subsequent analysis of the differences in expression, function, and their mechanisms could reveal clinically useful information. We believe that possible outcomes could include mechanisms to identify infected cells uniquely, cellular targets to enhance cell-mediated or humoral immune response, or gene sequences that could be turned off to slow viral replication or spread.
These findings provide a possible marker for MERS-CoV infection and a potential explanation for the mediator of the cytokine storm seen with these infections. This study brings up the question as to whether *SSX2* elevation is found in other coronavirus or +ssRNA virus infections, whether *SSX2* elevation is associated with cytokine storm or its severity, and whether this maker could be used as a potential diagnostic or therapeutic target, which future studies could explore. While other genes explored in this paper may be good markers for infection, with the exception of *SSX2*, these genes would be difficult to target therapeutically because they are also expressed in normal healthy cells and are not unique to infection.

## MATERIALS AND METHODS

We used the dataset GSE56677 from the NCBI Gene Expression Omnibus (GEO) database, which provided microarray expression profiling data on MERS-CoV-London and time matched mock infected cells at 0-, 3-, 7-, 12-, 18-, and 24-hours post-infection on a human airway epithelial cell line (Calu-3 2B4 cells) (29). We set our control group to mock-infected cells and our infected group to epithelial cells infected with MERS-CoV at the same time points. We analyzed both groups through GEO2R to identify trends in genetic expression between the two groups. GEO2R allowed us to select the LogFC and GENE_SYMBOL categories for inclusion in our resulting data table. We used GEO2R to calculate both the *p*-values, adjusted *p*-values, and LogFC values. We included adjusted *p*-values in our analysis, which were calculated using the Benjamini-Hochberg procedure, which serves to decrease the false discovery rate, and ensures the statistical significance of the differences in gene expression (30).

We selected the top 250 enriched gene symbols with the lowest *p*-values for further analysis. We looked through the LogFC column and took note of genes that showed significant upregulation or downregulation. We took the top 250 enriched gene symbols and put them in the “Multiple proteins” tab of String-db (31). We then selected *Homo sapiens* as the organism and opened our String map. From there, we looked for and took note of the genes with the most pathways and connections to other genes.

We further researched the noted genes and opened KEGG pathways in String (31, 32). KEGG pathways allowed us to find how the genes of interest interact to yield an overall function of a particular pathway as a result of the upregulation and downregulation (**Table 3)**. Further research with GeneCards regarding the purpose and functions of each of our selected genes allowed us to gain a better idea of the significance of our selected genes (33).

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| KEGG Pathway | Gene Groups Associated |
|---|---|
| Cell Adhesion Molecules | HLA Groups |
| Antigen Processing and Presentation | HLA Groups |
| Viral Myocarditis | HLA Groups, CYCS |
| Graft VS Host Disease | HLA Groups |
| Herpes Simplex Infection | HLA Groups, CYCS, TP53 |
| Cellular Senescence | TP53, CDC25A, HLA-A to F |
| Epstein Barr Infection | HLA Groups, TP53 |
| Viral Carcinogenesis | HLA- A TO F, TP53 |
| Kaposi sarcoma associated herpesvirus | HLA-A TO F, TP53 |
| HTLV1 Infection | HLA Groups, TP53 |

**Table 3: KEGG Pathways.** The table above was taken from our String-db analysis and lists the various KEGG pathways that were identified in our network, along with the gene groups associated with them. The gene groups mentioned are within the HLA Groups, *CYCS*, *TP53*, and *CDC25A*, and the KEGG Pathways associated include Antigen Processing, Viral Myocarditis, Viral Carcinogenesis, and various infections.

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