

Background

The goal of this research is to explore the potential of identifying molecular changes (or biomarkers) in host cells resulting from exposure to biological agents.

There are well established diagnostic and prognostic biomarkers for chronic diseases such as prostate, breast and ovarian cancer, but none exist for early detection of exposure to biological agents.

Identifying biomarkers that are associated with exposure to specific biological agents is a novel approach that can lead to development of early diagnostic and prognostic methods, i.e., hours instead of days or weeks.

We used dendritic cells to study molecular changes due to infection with Vaccinia (VAC) and Venezuelan Equine Encephalitis (VEE) viruses. These viruses represent two different classes of viruses that are considered biological threat agents. Vaccinia virus (double-stranded DNA genome) is related to the smallpox variola virus. VEE (single-stranded positive RNA genome) is one of several viruses that cause serious encephalitic illnesses and can be weaponized.

Methods

Human dendritic cells were infected with VAC strain Lister or VEE TC-83 viruses and harvested at 1, 8 and 12 hour post-infection. Uninfected cells for the same time periods were used as controls.

Nucleic acids (RNA and DNA) were isolated from the infected and uninfected cells.

SurePrint G3 Human Gene Expression Microarray was used for determining the expression of over 50,000 unique biological features. SurePrint Human DNA Methylation Microarray was used to identify methylated DNA molecules using 237,227 biological probes.

Sureprint Human MicroRNA (miRNA) Microarray (Release 19.0, 8x60K) was used to determine the expression of miRNA molecules.

All array processing, normalization, comparative analysis and principal component analysis (PCA) was performed using custom R scripts. Gene Ontology (GO) term enrichment analysis and clustering was performed using the ClueGO plugin for Cytoscape.

Results

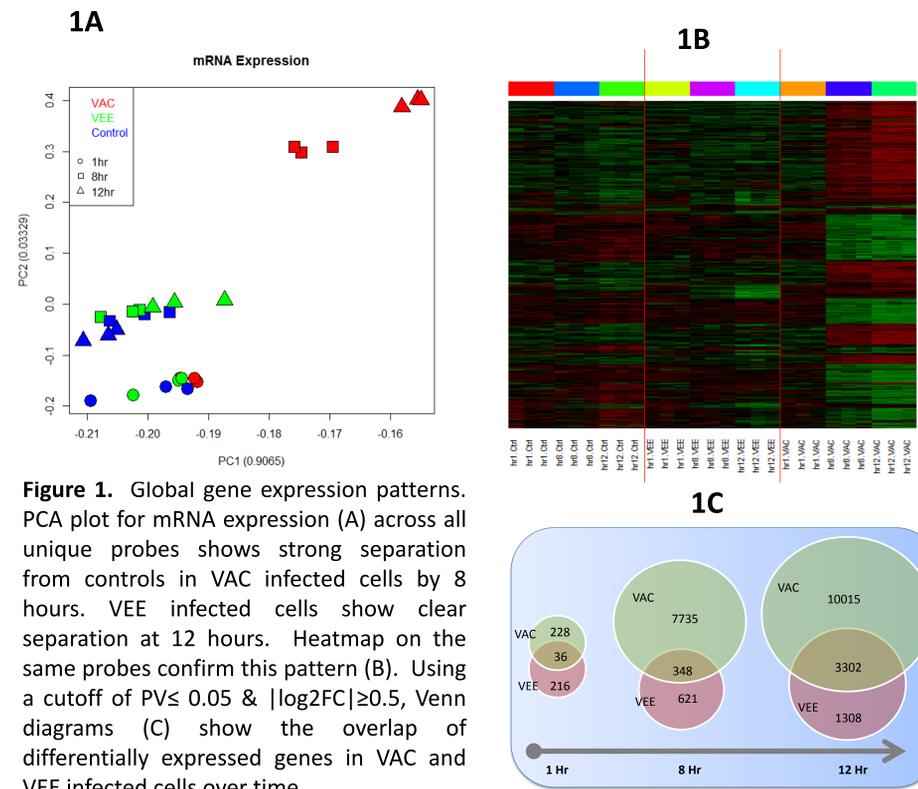


Figure 1. Global gene expression patterns. PCA plot for mRNA expression (A) across all unique probes shows strong separation from controls in VAC infected cells by 8 hours. VEE infected cells show clear separation at 12 hours. Heatmap on the same probes confirm this pattern (B). Using a cutoff of $PV \leq 0.05$ & $|\log_2FC| \geq 0.5$, Venn diagrams (C) show the overlap of differentially expressed genes in VAC and VEE infected cells over time.

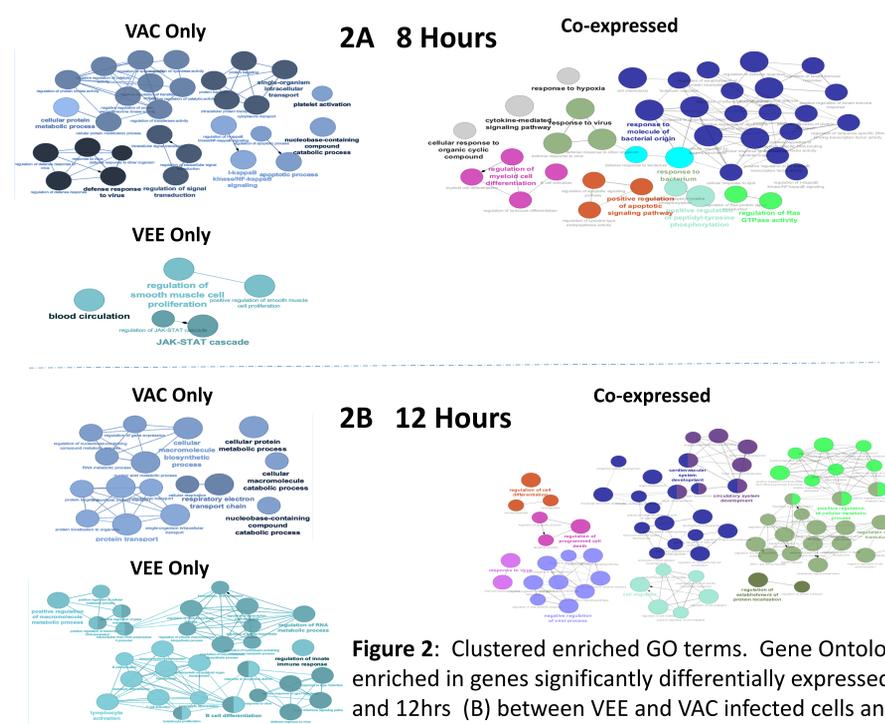


Figure 2: Clustered enriched GO terms. Gene Ontology terms enriched in genes significantly differentially expressed at 8hrs (A) and 12hrs (B) between VEE and VAC infected cells and uninfected controls.

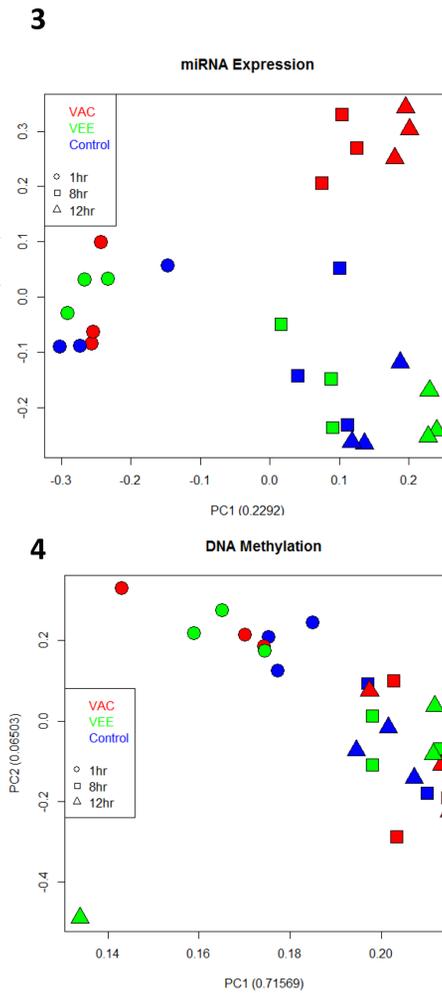


Figure 3. miRNA expression patterns. PCA plot of miRNA expression shows a similar pattern to that seen for mRNA expression. VAC and VEE infected cells show clear deviation from uninfected controls by 8hrs and 12hrs respectively.

Figure 4. DNA methylation patterns. PCA of DNA methylation shows similar patterns across time for both viruses infected cells and controls.

Conclusions

mRNA microarray expression showed overall stronger proinflammatory response in VEE relative to VAC at 8 and 12 h post infection (PI).

Both VAC and VEE viruses induced detectable changes in apoptotic immune response pathways at 8 and 12 h PI.

VAC-specific pathways included DNA damage at 1h, platelet activation at 8 h, and macromolecule biosynthesis at 12 h PI. VEE-specific pathways included blood circulation at 8 h and regulation of innate immune response at 12 h PI.

miRNA expression showed stronger VAC separation from uninfected controls, especially at 12 h PI.

Both virus-infected cells and uninfected controls showed similar DNA methylation patterns between 1 and 8 h PI, suggesting that global methylation changes are not a primary driver of host response within this time span.