Gene-Epigene Regulation of the Host Response in Human Dendritic Cells Infected with Vaccinia and Venezuelan Equine Encephalitis Virus

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Abstract

Introduction:
To protect military personnel from viral threats like VEEV and VACV, understanding the host's regulatory response is crucial. While mRNA provides one view, microRNAs (miRNAs) offer deeper insight as post-transcriptional regulators, creating unique signatures upon infection. This study profiles mRNA and miRNA expression in infected human dendritic cells to identify novel biomarkers, enabling advanced, host-based diagnostics and therapeutics to ensure military health and readiness.

Materials and Methods:
Human dendritic cells (DCs) were infected with VEEV or VACV, and samples were collected at 1, 8, and 12 hours post-infection. RNA/miRNA was extracted and analyzed via microarrays. Data was statistically analyzed to identify significant expression changes, and pathway analysis was used to interpret the biological functions affected.

Results:
MicroRNA (miRNA) analysis revealed starkly different and non-overlapping regulatory responses. VACV induced a strong, time-dependent response, growing from 4 differentially expressed miRNAs at 1 hour to 90 by 12 hours, impacting inflammatory pathways. In contrast, VEEV triggered a brief, early burst of 40 miRNAs at 1 hour only, a response that vanished by 8 hours. This fundamental difference—a sustained VACV signature versus a transient VEEV signature—provides a clear basis for viral differentiation.

Methods

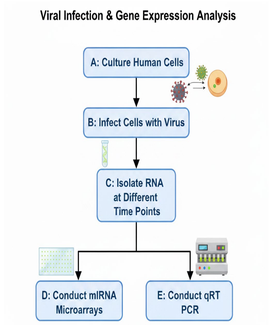

Figure 1:. Approach of the study

Results

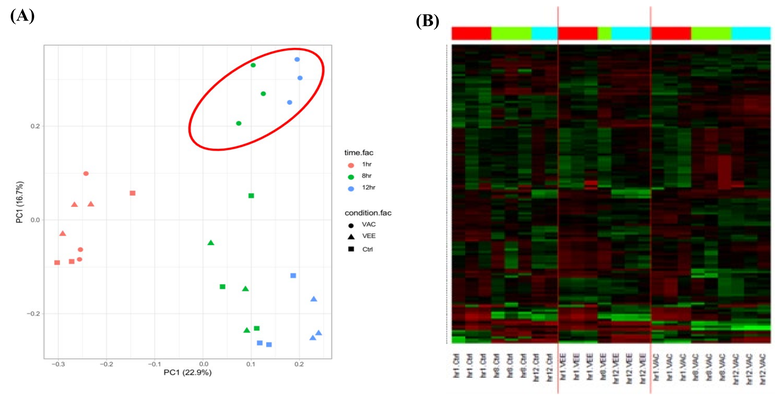

Figure 2: Experiment and miRNA expression patterns. (A) PCA plot for miRNA expression across all unique probes shows strong separation from controls in VACV infected cells by 8hrs and 12hrs respectively. (B) miRNA Heatmap on same probes grouped by condition then time confirm this pattern

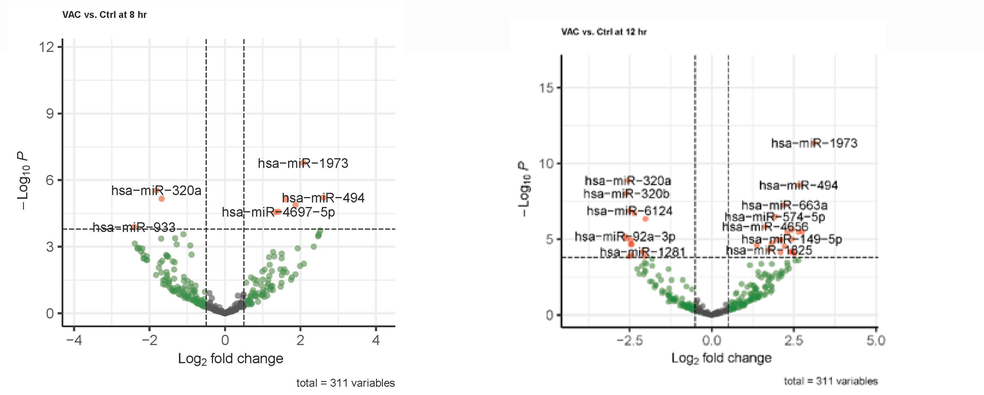

Figure 3: Experiment and Global gene expression patterns. Volcanic plot of DEGs, (A)VACV 8hpi (B) VACV 12hpi. Red dots represents DEGs that passed threshold for FDR and Log2FC, green dots represent genes that pass Log2FC. To the left of the horizontal line that delineates FDR p-value of 0.001, represents significantly up-regulated DEGs, to the left represents significantly down-regulated genes, and gray dots represents non-differentially expressed genes, the top genes by P value are labelled.

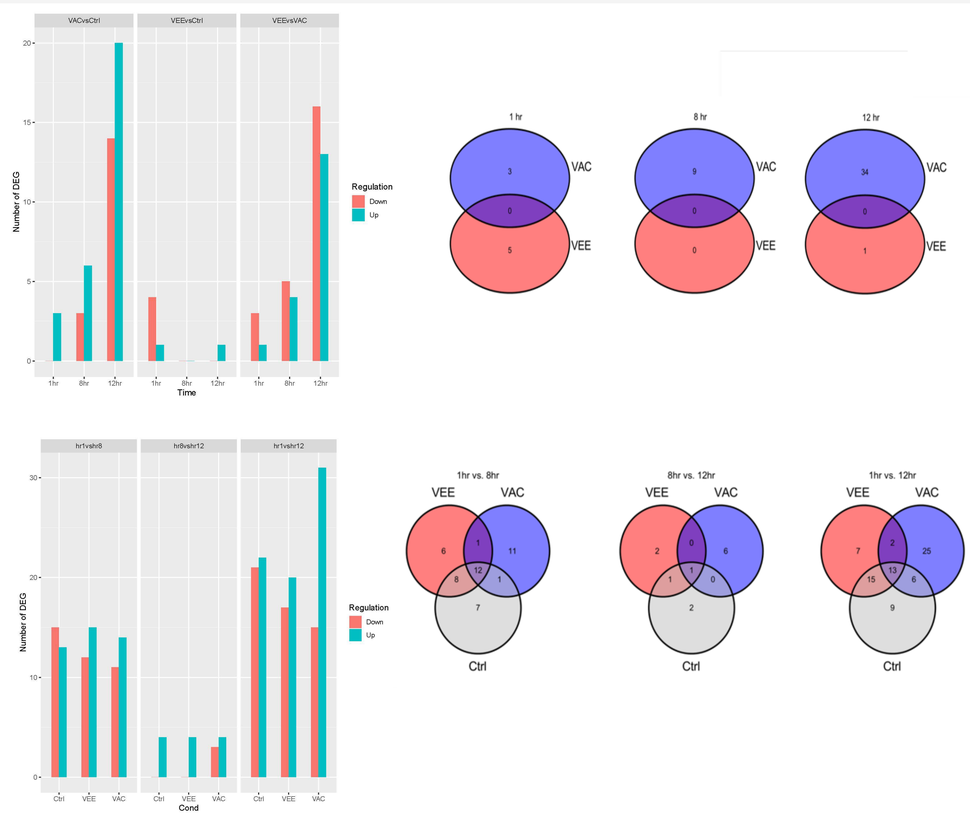

Figure 4: Differentially expressed genes (DEGs). Changes between VAC and VEE- infection over time. **(A)** Number of miRNAs targeted DEGs by VAC and VEE over time. **(B)** Venn diagram showing the overlap of the three targeted DEGs groups from. **(C)** Bar graph showing changes differentially expressed miRNAs across time due to VAC and VEE infection. **(D)** Venn diagram revealing the differentially expressed miRNAs in the three comparison groups. The overlapping parts indicates the number of differentially expressed miRNAs shared by groups. Differentially expressed miRNAs were selected with a setting of $P < 0.001$ and $|\log2 \text{ (fold change)}| > 1$. Red circle is the VEE group, and blue circle is the VAC group.

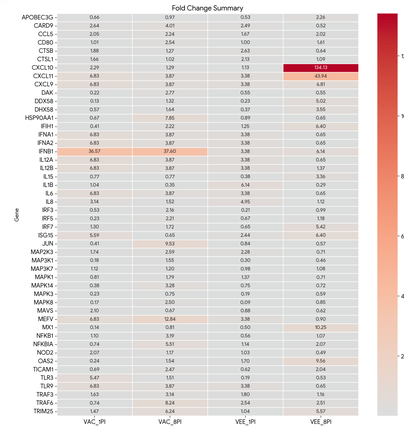

Figure 5: This heatmap provides a clear visual summary of the PCR-validated mRNA microarray expression fold changes for each gene under the different experimental conditions. The heatmap visually represents the fold change data, with colors indicating the magnitude of the change. Red indicates upregulation, blue indicates downregulation, and white indicates little to no change.

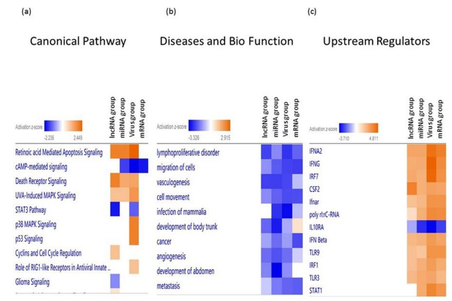

Figure 6: Comparison analysis heat map showing the top 10 canonical pathways, diseases and biofunction and upstream regulators. The colors indicating the magnitude of the change, orange indicates activated/upregulation, blue indicates inhibited/downregulation, and white indicates little to no change.

Conclusions

- While overall miRNA expression patterns shows very similar trends to previously reviewed mRNA expression, the fewer number of miRNA along with the large number of potential target genes makes functional interpretation of these changes more difficult.
- Our miRNA analysis reveals distinct regulatory signatures for VEEV and VACV infections.
- VEEV elicits a rapid, transient miRNA response, while VACV induces a sustained, escalating one.
- These unique miRNA fingerprints serve as highly specific biomarkers, providing a powerful foundation for developing next-generation rapid diagnostics and targeted therapies to protect military forces against diverse biological agents.
- By matching differently expressed genes to specific miRNA changes, we will hopefully gain a better understanding of the temporal regulatory dynamics of host-virus response.

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