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Identifying metabolite markers for detection of flaviviruses in human induced Pluripotent Stem Cells (hiPSCs)

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Introduction

Relevance:

- HCT/Ps are required to comply with the donor eligibility requirements as per 21CFR 1271 and applicable documents.
- The communicable diseases screening now includes screening donors for Zika (ZIKV) and West Nile virus (WNV).
- DENV and ZIKV viruses are mostly transmitted by mosquitoes and have been reported in 71 countries including Americas, Caribbean & Western Pacific regions. Moreover, rising global temperatures and expanding insect vector populations have led to a surge in vector-borne diseases, including flaviviruses.
- Zika infection associated with microcephaly and other neurological disorders is a major concern for Public Health.
- Among regulated cell therapy products, our research is focused on Induced pluripotent stem cells (iPSCs) which represents an excellent resource for generating cell therapy product candidates.
- Application of metabolomics in infectious disease diagnostics is an evolving area of science. In this study, we use flaviviruses (DENV & ZIKV) as a model to investigate changes in metabolites which can use as marker for early detection.

Purpose:

1. Compare the sensitivity, speed and specificity of metabolomics to the NATs and immunogenic detection assays.
2. Develop metabolite markers for detecting flaviviruses in hiPSCs.

Materials and Methods

- hiPSCs were infected with DENV3 and ZIKV-MR766 strains MOI 1. We assessed impact of viral infections on hiPSCs by examining cell viability, cytopathic effect (CPE), and viral load using immunofluorescence assay (IFA) and qRT-PCR.
- For metabolite extraction standardization, we analyzed 0.2, 0.8, 2, and 8 million control and infected cells using untargeted flow-injection mass spectrometry (FIA-MS). Ions were annotated by matching their inferred mass with compounds in the Human Metabolome Database.
- Currently, we are using untargeted LC-MS as a more sensitive and accurate method than FIA-MS to analyze cells infected by both viruses at 0, 8, 24, 48 and 96hpi to identify all metabolites and to confirm our FIA-MS data.

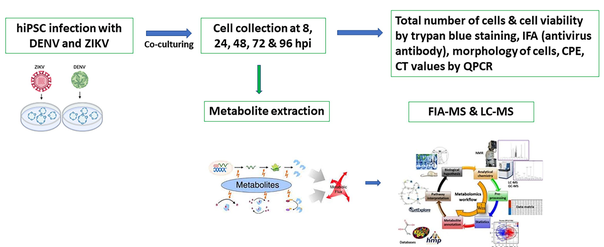

Figure 1. Experimental design.

Results and Discussion

Pilot study (hiPSC infected with DENV3 for FIA-MS)

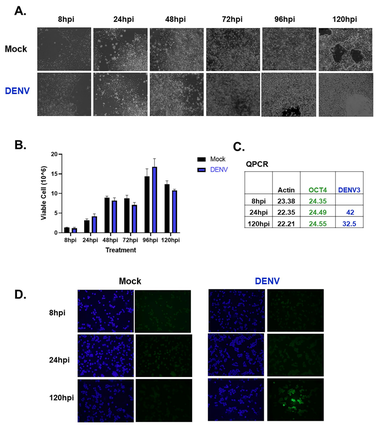

Figure 2. Cell growth (images were captured on an inverted phase contrast microscope-100x) (A). Viable cells were determined by trypan blue dye (B). qRT-PCR (CT values) for Actin, OCT4 & DENV3 (C). Immunofluorescent analysis (IFA) of flavivirus group antigen (green) and DAPI nuclear staining (blue) of cells. We did not observe significant differences in cell viability and CPE in hiPSCs infected with DENV3 vs control. qRT-PCR and IFA detected DENV3 in cells after 120hpi.

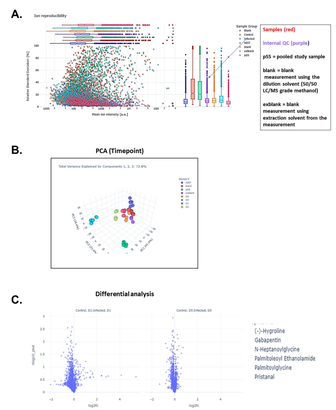

Figure 3. FIA-MS detected same number of annotated compounds (1658) in all tested samples concluding that 0.2 million cells are sufficient for extracting maximum number of metabolites (A). Biologically meaningful groups such as time, blanks and sample types separate well in PCA (B). Downstream analysis of the FIA-MS data identified 6 differentially expressed compounds in infected samples at 24hpi (Day1). These findings also confirmed sensitivity and consistency of FIA-MS.

Main study (hiPSC infected with DENV3 & ZIKV for LC-MS

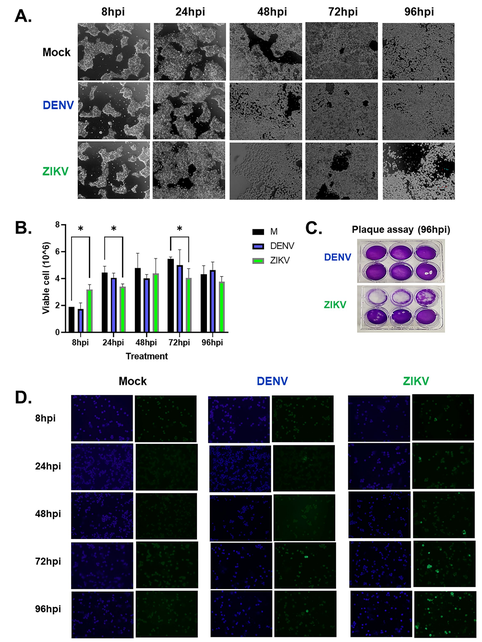

Figure 4. Changes in morphology and viability in DENV vs Mock and ZIKV vs Mock (A & B). Plaque assay (C). Immunofluorescence assay (IFA) with anti-4G2 antibody shows staining to DENV3 infection after 96 hours post-infection and staining to ZIKV infection as early as 48 hours post-infection (D). These results confirmed results from pilot study. The hiPSC showed more susceptiblity to ZIKV than DENV.

Metabolomics

LC-MS: We identified 271 annotated metabolites including a large number of unique metabolites in the samples in mock, DENV and ZIKV infected samples. The 148 and 123 metabolites were acquired using negative and positive modes, respectively. The raw data was normalized to the caffeine standard, and "differential metabolites" analysis was done using two-way ANOVA for each metabolite between mock and the two treatments (DENV & ZIKV)

FIA-MS: the annotation algorithm from "General Metabolics" identified 1658 annotated metabolites. Only 6 of these annotated metabolites were differentially expressed in DENV3 infected iPSC after 24hpi (Day1). Palmitoleoyl ethanolamide (POEA), N-palmitoyl glycine, Pristanal, Hygroline (Tropane alkaloids), Gabapentin, N-Heptanoglycine (Figure 3C).

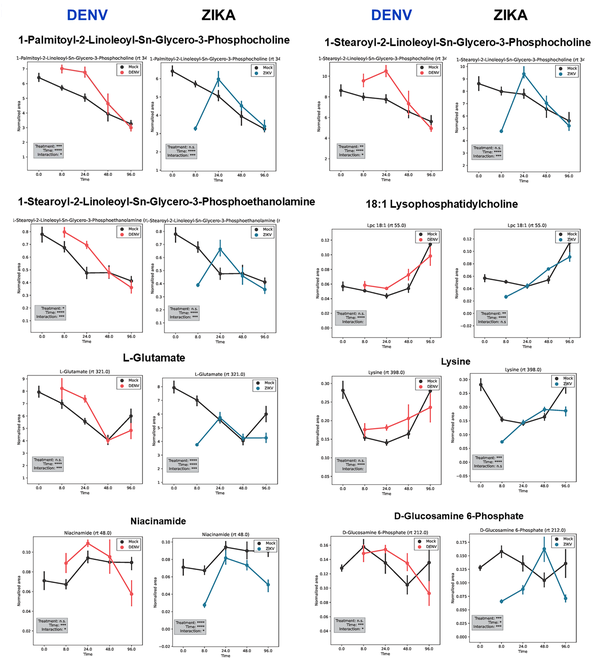

Figure 5. LC-MS/MS results for specific metabolites between mock and DENV and mock and ZIKV (statistical significance was calculated using two-way ANOVA).

Conclusion

We demonstrated that 'Metabolomics' is a rapid, reproducible, and sensitive method for detecting flaviviruses in infected cells. Our subsequent objective is to validate the 'specificity' of the identified metabolites and investigate the applicability of this technology to detect other infectious diseases.

Future directions:
Validation of selected metabolites; Transcriptomics and Metabolomics integrated data analyses; Study metabolomics in CMV latent vs acute infection and identify markers for CMV latent infection.

Acknowledgement

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