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Altered transcriptome and chromatin dynamics following short- and long-term Zika infections

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Introduction

The ability to synthetically reprogram human somatic cells into induced pluripotent stem (iPS) cells provides a near-limitless source of ES-like cells. However, the conventional reprogramming process faces challenges due to the original epigenetic memory of somatic cells, which may have been altered by invading pathogens like viruses. Viruses, such as SARS-CoV-2, can manipulate transcriptional regulation through methods like histone mimicry and cleavage. Viral manipulations of transcriptional regulation can lead to long lasting epigenetic changes in host cells. With rising global temperatures and increased vector (ticks and mosquitoes) populations, vector-borne diseases like flaviviruses are increasing. With outbreaks of locally-acquired Zika in the United States in the past decade, it is crucial to determine the lasting effects of flaviviruses on health. In this study, we use Zika as a model flavivirus to investigate changes in transcriptome and chromatin dynamics in susceptible and resistant cells and how this can influence host cells characteristics, an area that has received limited attention.

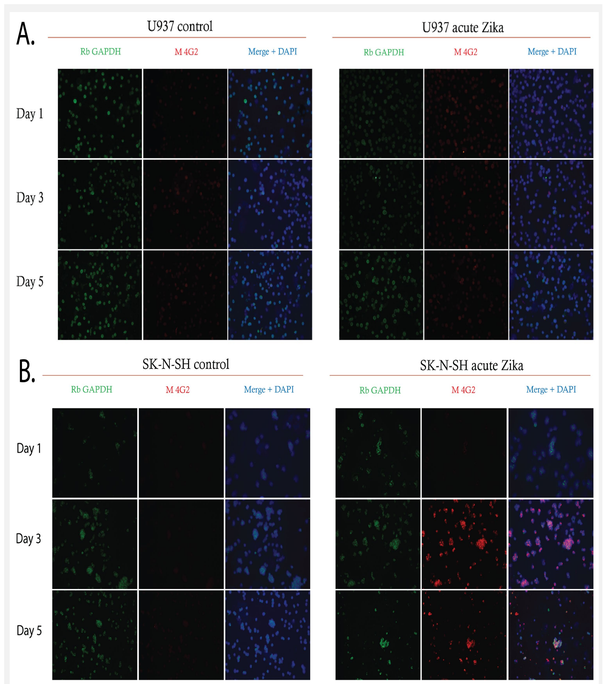

Figure 1. Comparison of ZIKV-resistant and ZIKV–susceptible cells via immunofluorescence assay. (A) U937 monocyte cells show resistance to flavivirus infection over 5 days post-infection with Zika MR766 virus. The housekeeping protein GAPDH (left column/green) signal shows similar protein levels in control vs. acute zika infected while anti-flavivirus antibody 4G2 (middle column/red) signal shows similar weak levels of infection. (B) SK-N-SH neuroblastoma cells show susceptibility to flavivirus infection as early as Day 3 post-infection. Anti-GAPDH (left column/green) shows similar protein levels in control vs. acute zika infected while anti-4G2 shows increasing signal which indicates flavivirus susceptibility.

Materials and Methods

U937 (monocyte) and SK-N-SH (neuronal) cells were used as flavivirus-resistant and -susceptible cells, respectively. For short-term (acute) infection, the cells were infected with the ZIKV-MR766 strain at multiplicity of infection (MOI) 1.0, or one virus per cell. Long-term (persistent) infections were generated as acute infections at MOI=0.01 and allowed to grow for 3+ months, passaging weekly. Cells were collected at different time points to investigate the transcriptomic changes during short- and long-term infection. Changes in histones and associated modifications were also studied via western blot (WB) and immunofluorescence assay (IFA). Samples were prepared for RNA-seq via phenol/chloroform extraction, rRNA-depleted, cDNA libraries were generated, and samples were sequenced.

Finally, we plan to study epigenetic landscape and three-dimensional genome organizational alterations using CUT&Tag and Hi-C assays.

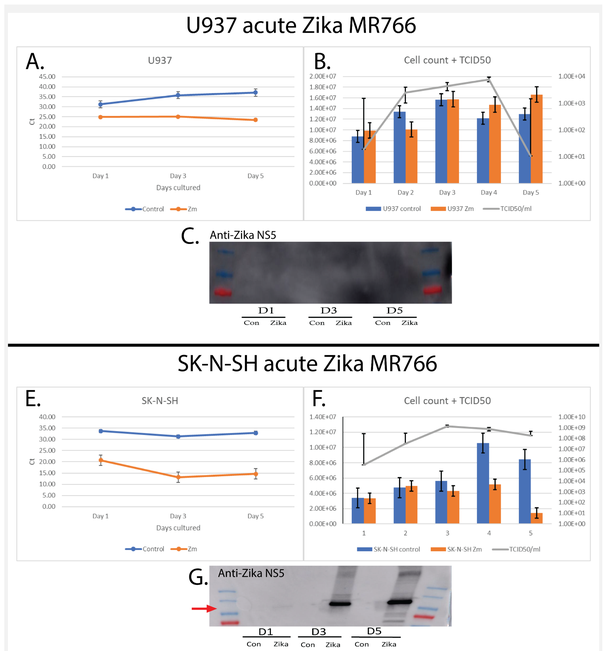

Figure 2. Comparison of U937 and SK-N-SH cell viability, TCID50, qRT-PCR, and western blot analysis. (A) Acute Zika-infected U937 samples show lower Ct values during all days of infection compared to control, indicating Zika presence. (B) TCID50 shows low levels of infectious virus with a decrease at Day 5 post-infection. (C) Western blot of acute infected U937 shows no defined bands for any day of infection. (D) Western blot of persistent infected U937 shows high levels of Zika virus still present after 3+ months of culturing. (E) Acute Zika-infected SK-N-SH samples show presence of Zika transcripts compared to control. (F) TCID50 results show high levels of Zika virus in infected samples. (G) This is complimented by increasing western blot bands on sequential days in infected SK-N-SH.

Results and Discussion

As anticipated, flavivirus-susceptible SK-N-SH cells exhibited higher viral load compared to flavivirus-resistant U937 cells, evident from IFA (Figure 1), TCID50 (Figure 2), qRT-PCR (Table 1), and WB analyses (Figures 3-5). This susceptibility led to increased cytopathic effects and reduced cell viability in SK-N-SH cells (Figure 2). However, we did not observe any such effects in U937 cells post-ZIKV infection. Notably, H3 and H4 histone levels rose in U937 cells during acute ZIKV infection. Similarly, ZIKV-infected Vero cells displayed changes to histone H3 cleavage and heightened histone H4 levels at 1-day post-infection. Furthermore, histone modification marks associated with active and repressed chromatin were elevated in U937 cells (Fig. 3). Interestingly, Zika long-term infection in U937 restores H3 levels but we observed reduction in total H4. The associated H3 and H4 modification marks stay reduced in these samples. We're currently analyzing RNA-seq data to delve deeper into these findings.

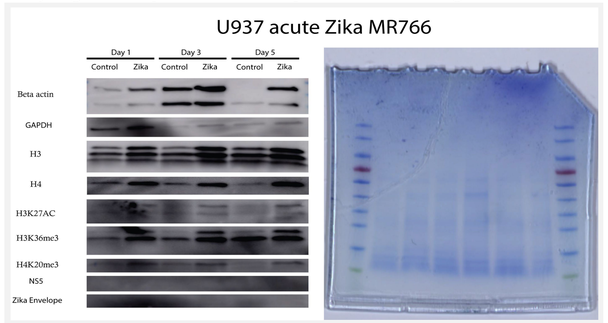

Figure 3. Overall Histone H3 and H4 levels were elevated during acute Zika infection in U937. Typical housekeeping protein levels can be altered during viral infection on a cell type basis as observed by beta actin and GAPDH. Histone modification marks corresponding to Histone H3 and H4 were similarly elevated. Interestingly, anti-Zika virus antibodies NS5 and Zika Envelope showed no bands on any day post-infection. On the right, Coomassie shows similar levels of protein loaded.

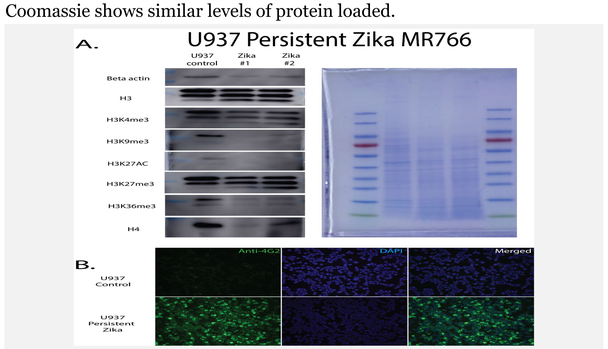

Figure 4. Histone H3 recovers, but Histone H4 decreases during persistent Zika infection in U937. (A) Beta actin appears altered by Zika infection despite Coomassie staining showing similar levels of protein loaded. Total Histone H3 recovers from acute infection levels, but Histone H4 levels are significantly reduced. The associated H3 and H4 modification marks stay reduced in these samples. (B) IFA data shows positive infection using anti-4G2 (anti-flavivirus, green, left column).

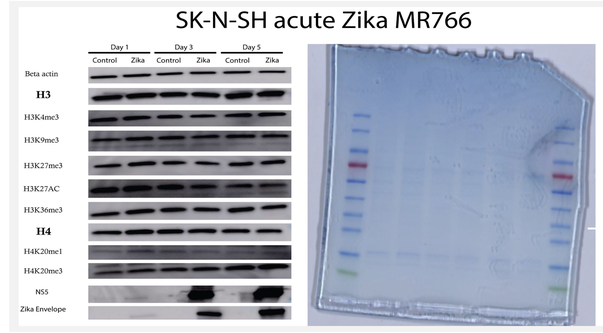

Figure 5. Histone levels were unchanged in SK-N-SH acute Zika infected samples compared to control. Anti-Zika virus antibodies NS5 and Zika Envelope show clear and growing infection of acute Zika-infected samples on Days 1, 3, and 5.

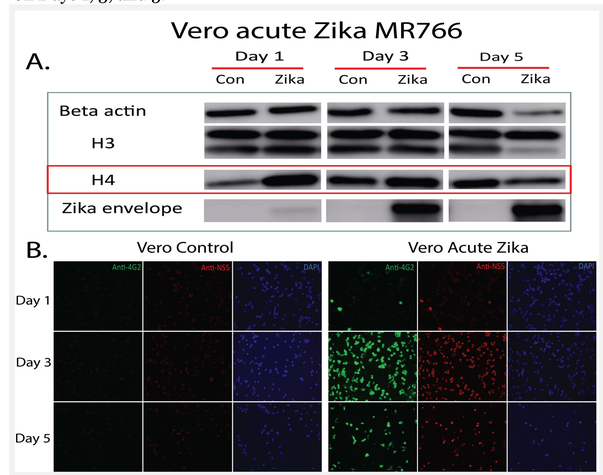

Figure 6. Comparison of histone levels in acute Zika-infected Vero (monkey kidney) cells. (A) Histone H3 cleavage decreases by Day 5 infected samples compared to control. Histone H4 increases in infected sample compared to control in Day 1 but decreases on both Day 3 and Day 5 for infected samples. Despite this, Zika envelope shows increasing infection from Day 1 through Day 5. (B) IFA data confirms a growing Zika infection in acute-infected samples compared to control.

Conclusion

Our data suggest histones, especially H4, may play a role in defending against ZIKV. We aim to validate this hypothesis in upcoming research. Samples have been submitted for RNA-Seq, and we are collaborating with HIVE and NCTR to analyze the data. We plan to study the epigenetic landscape and three-dimensional genome organizational alterations using CUT&Tag and capture Hi-C assays.