Chikungunya virus (CHIKV) has been an enigma for decades before the 2005 epidemic in India and 2015 in the Western Hemisphere. CHIKV infects 1.1 million people per year in 113 countries around the world. (Vackay, Staples, Milik, Citron-Shein, & Ramon-Pardo, 2016). CHIKV is an arbovirus spread through Aeolis albopictus and Aeolis aegypti mosquitoes. The name ‘chikungunya’ means ‘that which bends up’ termed after the assumed posture of suffering patients. Patients who are infected with CHIKV could contract life-altering arthralgia and myalgia. As of today, there is no cure, treatment, or vaccine for most alphaviruses.

Chikungunya virus is an enveloped positive-sense single-stranded RNA virus with an incubation period of 1 to 12 days. Recent research on CHIKV indicates human macrophages contribute to successful virus replication. A rapid immune response is stimulated after the virus is transferred to a human. In response, macrophages begin phagocytosing CHIKV, which induces macrophage apoptosis. Macrophages infected with CHIKV express increased expression of MHC and co-stimulatory molecules (Nayak et al., 2017). The sudden apoptosis of the strained macrophage catalyzes the spread of the virus in the body. The question that remains is when, during the course of infection, does the macrophage generate apoptosis signals?

Understanding when the infection tipping point occurs can provide additional insight into the underlying mechanisms of pathogenesis for Chikungunya virus, enabling the development of effective prophylactics, therapeutics, and/or vaccines.

Methods

U937 cells were propagated with FBS, Penicillin/Streptomycin, L-glutamine, and HEPEs buffer. The cells were then cultured in T-75 culture flasks at 37°C. Monocytes were transferred to 6-well tissue culture plates with macrophage cells and incubated for 24 hours. The CHIKV-LR strain was diluted and incubated to allow virus infection. PMA-differentiated U937 macrophages were infected using CHIKV-LR and incubated with 5% CO2 for two hours. Duplicate samples were taken from mock-infected and infected monocyte-derived macrophages at 24 and 48 hours post-infection (hpi), after which RNA was extracted and stored. An illumina Novaseq instrument housed at Intermountain Precision Genomics in St. George, Utah synthesized the cDNA, prepared the libraries, and generated 30-60 million RNA-sequencing reads for each of the duplicate samples. The raw sequencing data were then subjected to analyses including differential gene expression (DEG), enriched functional annotations, and modified intracellular signaling pathways. The automated Snakemake-based ARMOR computational workflow program was used to produce DEG results using edger, functional enrichment using GeneGo, and isoform usage with DRIMseq, and visualization using R Shiny. The significant results from the differential gene expression analysis identified host transcripts that were influenced during CHIKV infection. These results were subjected to a pathway enrichment analysis using SPIA. The pathway results from KEGG, Reactome, NC1, BioCarta, and Panther yielded significant p-values, and predicted effect on the viral infection. The RAW sequencing was impacted at 48 hours post-infection. The regulatory patterns from these analyses were then used to predict existing drugs that could be used as therapeutics for CHIKV, which generated a list of 136 different drugs.

To reduce the results several statistical strategies were implemented. For this study, only small molecule results were taken into consideration (except for EnzCheck). To simplify and demonstrate the variables that were considered in the ranking of the results, a numerical grade was assigned to each result and is included in Table 3. Requirements include but are not limited to predicted effect on the viral infection and predicted effect on the pathway against a database with existing drug targets. Table 3 includes the top five therapeutic drug results and the pathways that they affect. Our ranked results indicated that Telmisartan, Sunitinib, Erlotinib, Vorinostat, Dasatinib, and Regorafenib are potential therapeutic drugs to treat an infection with Chikungunya virus. These therapeutics will require validation experiments to augment ongoing efforts to develop an effective prophylactic or therapeutic treatment for CHIKV.

Conclusions

Our ranked results indicated that Telmsartan, common name Micardis, modulates the AGE-RAGE signaling pathway at 48hpi. Sunitinib, market name Sutent, modulates the cytokine-cytokine receptor interaction at 24hpi and the Ras signaling pathway at 48hpi. Erlotinib, common name Tarceva, is a Dimere fusion protein with extracellular ligand that binds to EGFR. Erlotinib’s primary use is treating a variety of inflammatory conditions including rheumatoid arthritis. Vorinostat, market name Zolinza, influences Alcoholic signaling pathway at 24h and Chronic myeloid leukemia pathway at 48h. Dasatinib, trade name SPRYCEL, impacts the Fc epsilon RI signaling pathway, Chronic myeloid leukemia, Fc gamma R-mediated phagocytosis, and Ras signaling pathway at 48h. Regorafenib, common name Stivarga, modulates Influenza A, Salmonella infection, Fc epsilon RI signaling pathway, Chronic myeloid leukemia, AGE-RAGE signaling pathway in diabatic complications, and Ras signaling pathway all at 48h post-infection. These results provide direction in the pursuit for potential treatments for Chikungunya infection. These therapeutics will require validation experiments to augment ongoing efforts to develop an effective prophylactic or therapeutic treatment for CHIKV.