Chikungunya Virus Time Course Infection of Human Macrophages

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Introduction

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. (Yactayo, Staples, Millot, Cibrelus, & Ramon-Pardo, 2016). Chikungunya virus is an arbovirus spread through Aedes albopictus and Aedes aegypti mosquito vectors. 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 experience 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 37ºC. 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 at 37ºC 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 fastq files representing the RNA-sequencing data were then subjected to analyses including differential gene expression (DEG), enriched functional annotations, and modulated intracellular signaling pathways. The automated Snakemake-based ARMOR computational workflow program was used to produce DEG results using edgeR, functional enrichment using Camera, gene 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, NCI, BioCarta, and Panther yielded significant p-values, and predicted effect on 234 pathways regulation 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 Etancerpt). 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 exclusive to whether the drug has been through clinical trials or has FDA approval. Toxicity of the drugs was determined by FD50 values if they were provided, if not, toxicity was determined from recommended diet restrictions, developers' safety statements, requirements of administration, and long-term impacts or risks that are included in drug consumption (numerically 0-10, 0 being the most toxic). If previously published sources indicated a correlation between the drug and CHIKV this was included in the drug ranking.

Acknowledgements

Our sincere thanks to Brigham Young University for the funding and resources used in this study. *CHIKV-LR strain was provided by Dr. Jonathan Miner, Washington University, St. Louis, MO. *U937 cells were acquired from ATCC, cell line stocks have been authenticated at University of Utah DNA sequencing core and University of Arizona Genetics core facilities.

Results

Figure 1 and *Figure 2* display the results from the differential gene expression analysis. Critical genes that are impacted by the virus include GNAL 2774 (Golf) and OR4C (R membrane) of the Olfactory pathway 24 hours post-infection. While AGER 177(RAGE) and MAPK (ERK1/2, p38, and JNK) were modulated in the AGE pathway at 48 hours post-infection.

Results from further pathway analysis indicated that four pathways are impacted at the 24h timepoint (*Table 1*) and 235 pathways are impacted at the 48h. The top-ranking seven pathways that are impacted at 48 hpi were sorted by Bonferroni-corrected p-value and are displayed in Table 2. Afterward, we cross-referenced human proteins within each signaling 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, Etanercept, Vorinostat, Dasatinib, and Regorafenib are potential therapeutic drugs to treat an infection with Chikungunya virus.

Conclusions

Our ranked results indicated that Telmisartan, 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. Etanercept, common name Enbrel, is a Dimeric fusion protein with extracellular ligand-binding to TNFR. Enbrel's primary use is treating a variety of inflammatory conditions including rheumatoid arthritis. Vorinostat, market name Zolinza, influences Alcoholism 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 diabetic complications, and Ras signaling pathway all at 48h post-infection. These results provide direction in the pursuit of 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.

Name of Pathway	pSize	NDE	pGFWER	Status	Source Database
Olfactory Transduction	357	68	0.000151106	Inhibited	KEGG
Cytokine-Cytokine receptor Interaction	227	30	0.001848883	Inhibited	KEGG
Alcoholism	158	38	0.015071574	Activated	KEGG
HATs Acetylate Histones	139	26	0.000500798	Activated	Reactome

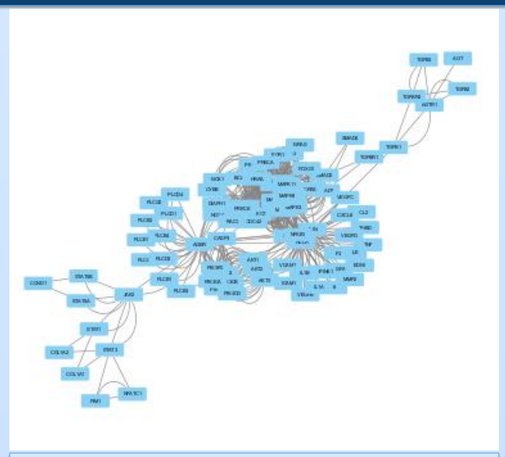
Table 1: Biological pathways impacted by CHIKV in U937 macrophages at 24-hour post-infection. The number of genes on the pathway (pSize), the quantity of DE genes per pathway (NDE), Bonferroni- correct p-values (pGFWER), the influence the virus has on the system compared to normal conditions.

Name of Pathway	pSize	NDE	pGFWER	Status	Source Database
Stimuli-Sensing Channels	100	76	0.000271	Activated	Reactome
Wnt Signaling Pathway	219	139	0.000294	Activated	Panther
ECM-Receptor Interaction	79	58	0.000331	Inhibited	KEGG
IGF1 Pathway	30	22	0.000975	Activated	NCI
Role of Calcineurin-Dependent NFAT Signaling in Lymphocytes	36	26	0.001006	Activated	NCI
Stabilization and Expansion of the E-cadherin Adherens Junction	42	30	0.001025	Activated	NCI
HIF-1-alpha Transcription Factor Network	64	44	0.001294	Activated	NCI

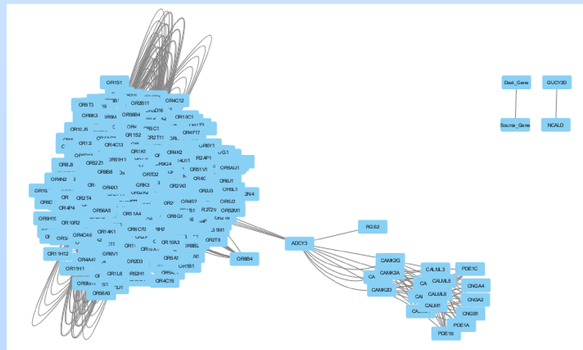
Table 2: Biological pathways impacted by CHIKV in U937 macrophages at 48-hour post-infection. The number of genes on the pathway (pSize), the quantity of DE genes per pathway (NDE), Bonferroni- correct p-values (pGFWER), the influence the virus has on the system compared to normal conditions.

Drug	In Clinical Trials	FDA Approved	Toxicity	Chikungunya Studies	Pathway	Grade
TELMISARTAN	X	X	7	X	AGE-RAGE signaling pathway in diabetic complications'	**23.85**
SUNITINIB	X	X	7	X	Cytokine-cytokine receptor interaction*/Ras signaling pathway'	**23.85**
ETANERCEPT	X	X	6	X	Cytokine-cytokine receptor interaction*/ Influenza A'/ AGE-RAGE signaling pathway in diabetic complications'	**23.83**
VORINOSTAT	X	X	6	X	Alcoholism*/Chronic myeloid leukemia'	**23.83**
DASATINIB	X	X	5	X	Fc epsilon RI signaling pathway'/ Chronic myeloid leukemia'/ Fc gamma R-mediated phagocytosis'/ Ras signaling pathway'	**23.80**
REGORAFENIB	X	X	2	X	Influenza A'/ Salmonella infection'/Fc epsilon RI signaling pathway'/Chronic myeloid leukemia'/ AGE-RAGE signaling pathway in diabetic complications'/ Ras signaling pathway'	**18.50**

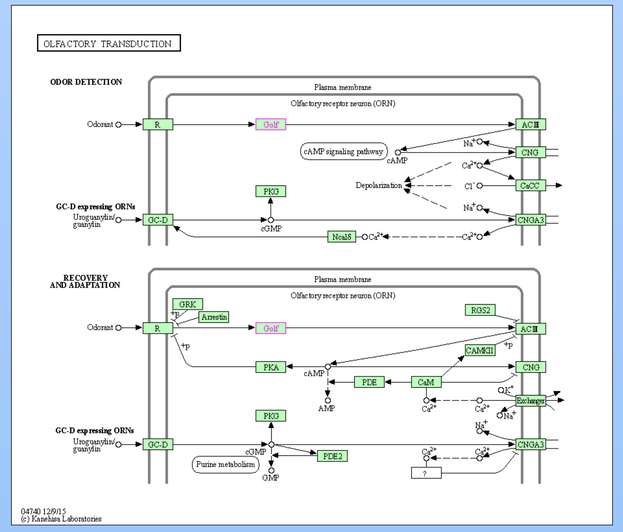
Table 3: Selected FDA-approved pharmaceutical drugs were investigated for their correlation to the 24 and 48-hour pathway results. Clinical trials and FDA approval information gathered from DrugBank (https://go.drugbank.com/). Toxicity taken into account; five components were used to create a numeric value. All the indicated medications have publications that indicate some correlation between them and CHIKV.

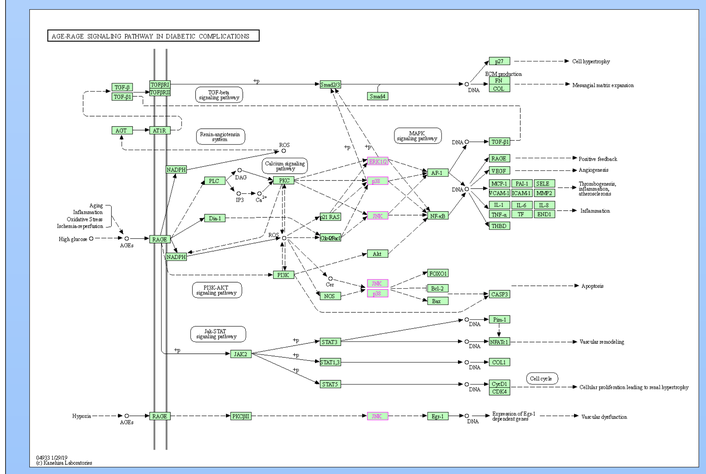


Gene map of infected with CHIKV in AGE-RAGE Pathway 48hpi



Gene map of infected with CHIKV in Olfactory Pathway 24hpi





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