VIRAL-HOST PROTEIN INTERACTIONS IN CHANDIPURA VIRUS PATHOGENESIS

SYNOPSIS

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By

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BACKGROUND AND RATIONALE OF THE CURRENT STUDY

Viruses have co-evolved with their hosts developing effective approaches to establish a successful infection by hijacking and circumventing the host cellular processes to their own end. A complex network of specific protein-protein interactions is involved at the interface of viral infection and host response; with the host attempting to eradicate the invading viral pathogen, and the pathogen whilst evading the host immune surveillance continues to proliferate. The molecular mechanisms underlying the subversion of cell physiology mediated by viral infection can only be understood by uncovering how viral proteins perturb cellular protein interaction networks. Furthermore, identifying the host proteins targeted by viral proteins during infection provides significant insights into the mechanisms by which the virus manipulates the host and thereby several strategic targets for therapeutic interventions.

Chandipura virus (CHPV; Family *Rhabdoviridae* and genus *Vesiculovirus*), is an arthropod borne emerging pediatric encephalitic rhabdovirus associated with a number of acute and fatal epidemic outbreaks in the central states of India [1,2]. The 11 kb viral RNA genome codes for five different proteins – Nucleocapsid (N) protein, Phosphoprotein (P), Matrix (M) protein, Glycoprotein (G) and the Large (L) protein – through five different monocistronic mRNAs, in a sequential order and in decreasing amounts [3]. The interactions among these proteins are essential for the orchestration of key processes during virus replication. Alike other virus, CHPV is an obligate parasite depending on its host cell machineries to perform bulk of the functions that are necessary for its survival.

Among the five viral proteins, the M protein, also known as the viral weapon, is the most dynamic. This virion protein is known to play a critical role in virus assembly and budding of Vesicular Stomatitis Virus (VSV; Family *Rhabdoviridae*, Genus *Vesiculovirus*) and Rabies Virus (RV; Family *Rhabdoviridae*, Genus *Lyssavirus*) [4]. In assembled virions, M protein condenses the viral ribonucleoprotein (RNP) into the characteristic bullet shape and thereby helps in its packaging [5]. Apart from functioning as a structural component, M protein plays regulatory roles by controlling the switch between viral replication and transcription [6]. In vesiculoviruses, this protein is also responsible for most of the cytopathic effects (CPE) observed in the infected cells which includes the inhibition of host gene expression [7], disruption of the host

cytoskeleton [8], disablement of nucleo-cytoplasmic transport [9] and blocking the mRNA export [10]. Due to such multitude of roles that M protein plays in the replication and pathogenesis of the virus, it is anticipated that, alike in other rhabdoviruses, CHPV M protein must also be involved in a variety of interactions with its host that are responsible for its rapid and unique pathogenesis.

THESIS OBJECTIVES

The main objective of this study is to generate a viral-host protein interface in Chandipura virus (CHPV) mediated encephalitis. In order to attain this objective the work plan is devised with two different strategies: (1) Predicting the host interactors of CHPV by using a protein structure similarity-based computational approach and (2) Screening the human fetal brain cDNA library for interactors of CHPV using yeast two-hybrid system, followed by validating the identified interactions by GST pull down and protein interaction ELISA. Further objective is to computationally study the interaction interface of selected individual pair of viral-host proteins and design of peptides to inhibit their association.

THESIS CHAPTERS

The research work carried out for the achievement of specified objectives is divided into following chapters:

CHAPTER 1: *Introduction* deals with the basic background about CHPV and rationale of the study.

CHAPTER 2: *Review of Literature* includes the existing literature about various aspects of CHPV.

CHAPTER 3: *Materials and Methods* describe the details of the materials and various methods used in this study.

CHAPTER 4: *Structural Similarity-based Prediction of Protein Interactions Between Chandipura Virus and Human Host* identifies the putative host interactors of CHPV using a protein structure similarity based computational approach.

CHAPTER 5: *Yeast two-hybrid Screening to Identify the Candidate Host Protein Interactors of Chandipura Virus Matrix Protein* deals with the screening of human fetal brain cDNA library for identifying interactors of CHPV M protein using yeast two-hybrid system.

CHAPTER 6: *Validation of Viral-Host Protein Interactions* involves the confirmation of Y2H identified interactions using two independent assays – GST pull down and protein interaction ELISA.

CHAPTER 7: *Identification and Characterization of Linear Peptides Inhibiting the Host Interactions of Chandipura Virus Matrix Protein* explains the identification of amino acid residues involved in the interaction of CHPV M protein and selected host proteins. Further, the inhibitory potential of the peptides targeting the M-ABCE1 interaction has been tested by protein interaction ELISA.

CHAPTER 8: *Conclusion and Future Prospects* describes overall findings of the research work done in thesis and future prospects.

OVERVIEW OF THE RESEARCH

In the dearth of ample knowledge regarding the life cycle of CHPV, a structure-based computational approach was implemented to predict the interactions between CHPV and its human host proteins. In this approach, the human proteins with defined structure and known interactions were mapped to the viral proteins for structural similarity. This study is based on the principle that if two proteins have similar structural motifs, then their interaction dataset will be highly similar. Since the structures of CHPV proteins were not available in the PDB archives they were generated from I-TASSER or by Homology Modeling. Each of the CHPV protein was compared with human proteins of known structure for structural similarities using DaliLite v.3 web server. The known protein-protein interactions among these and other human proteins were obtained from Human Protein Reference Database (HPRD) Release 7, Biological General Repository for Interaction Datasets (BIOGRID), and STRING. The host interactors were shortlisted on the basis of their Gene Ontology (GO) analysis (functionality and cellular compartmentalization), which was performed using DAVID (Database for Annotation, Visualization and Integrated Discovery) and Reactome databases. The annotation of selected

proteins for biological processes and molecular functions revealed that in humans, CHPV mainly targets intracellular signaling cascades, protein trafficking, programmed cell death, macromolecular metabolic processes, intracellular transport, kinase activity, protein complex binding, cytoskeleton protein binding and transcription factor binding. Because there is not much literature support available for CHPV-human host interactions, interactome dataset of related rhabdoviruses such as Vesicular Stomatitis Virus (VSV) and Rabies Virus (RV) and those of other encephalitic viruses were used to validate the interactions of CHPV. The study predicted 50 % of host proteins known to associate with VSV as interactors of CHPV. Based on these interactions, the possible modes of cellular replication and neuroinvasion of CHPV was speculated.

The results from the computational approach and studies on other rhabdoviruses suggested that the myriad roles played by the M protein in the virus biology makes it a critical player in viral pathogenesis. Discerning the interactions of this protein with host can greatly facilitate our understanding of virus infections, ultimately leading to both improved therapeutics and insight into cellular processes. Thus, CHPV M protein was selected for screening of human fetal brain cDNA library cloned in pGADT7-Rec vector and transformed in *Saccharomyces cerevisiae* strain Y187. Mating of *S. cerevisiae* strain AH109 (transformed with pGBKT7-M [BD-M] construct) with Y187 cells (transformed with the cDNA library) was followed by the selection of the diploid progeny yeast cells (harboring both bait [BD] and prey [library plasmids/pGADT7-Rec/AD] plasmids that code for viral and host proteins, respectively) first on triple (TDO; SD -Trp/-Leu/-His [Synthetic Dropout media lacking amino acids tryptophan, leucine and histidine]) and then on quadruple dropout medium (QDO; SD -Trp/-Leu/-His/-Ade [Synthetic Dropout media lacking amino acids tryptophan, leucine and histidine, and the nitrogen base adenine]) supplemented with X-α-Gal. Consequently, the positive high stringency clones were subjected to yeast colony PCR coupled with blue-white screening (to eliminate multiple prey plasmids) and *Hae* III digestion (to eliminate duplicate prey plasmids) before they were sequenced using T7 forward (5’ TAATACGACTCACTATAGGGC 3’) and AD specific reverse primers (5’ AGATGGTGCACGATGCACAG 3’). Subsequently, the sequenced prey plasmids were subjected to a Blastn search on the human genomic and transcript database of National Centre for Biotechnology Information (NCBI). The amino acid sequences of the proteins encoded by these library inserts were determined using ExPASy (Expert Protein Analysis System) translation tool and were compared to the human protein sequences on the NCBI website using

Blastp. Blastn identified 10 clones which were found to encode either complete ORF (three clones) or a region of known human protein (seven clones) as potential candidate interactors of CHPV M protein. The three full length candidate host protein interactors, ATP binding cassette sub family E1 (ABCE1), CTD nuclear envelope phosphatase 1 (CTDNEP1) and Developmentally regulated GTP binding protein 1 (DRG1) were selected to further validate their interaction with M protein. The validation study was performed by using two independent *in vitro* assays - GST pulldown and protein interaction ELISA. This objective was achieved by cloning of host genes in pGEX-4T3 vector and CHPV-M gene in pCAK vector, followed by expression and solubilisation analysis. The interaction of selected host protein was found positive after pulldown and ELISA.

The interaction surface of selected individual pair of viral-host proteins (M-ABCE1, M-CTDNEP1 and M-DRG1) was further studied computationally. Since the full length structures of neither the viral nor the host protein were available in PDB, they were generated either by comparative modeling procedure using MODELLER software package (9v4) or I-TASSER together with Fragment guided-molecular dynamics simulations (FD-MD). The initial stage docking models were generated by ZDOCK ver 3.0.2 followed by in silico docking of the generated host structures to the homology-modeled CHPV M protein. Subsequently, linear peptides with potential to inhibit the viral-host interactions were designed based on the interface residues identified with DIMPLOT and Accelrys Discovery Studio ver 4.0, and modified for increased stability. Finally, the inhibitory potential of peptides targeting the M-ABCE1 interaction was displayed by protein interaction ELISA.

CONCLUSIONS FROM THE PRESENT RESEARCH

The identification of potential CHPV interactors revealed that viral proteins mainly associate with the cellular hub proteins which are involved in multiple pathways of the cell thus hijacking the cellular machinery with only five viral proteins. The computational study also investigated the biological assembly of CHPV-N protein and predicted the putative host proteins involved in CHPV life cycle along with speculating the possible modes of neuroinvasion by CHPV. The yeast two-hybrid screening of human brain cDNA library revealed ten host protein interactors of CHPV M protein. Based on a thorough literature search with regard to the

pathogenesis of CHPV, functions of M protein and the prominent roles played by these ten host proteins in the life cycle of other viruses, three of these interactions which included those of ABCE1, CTDNEP1 and DRG1 were shortlisted and further tested by GST pull down and protein interaction ELISA. Linear peptides with potential to inhibit the viral-host protein interactions were designed based on the interface residues computationally identified and the inhibitory potential of peptides targeting the M-ABCE1 interaction was tested by protein interaction ELISA.

FUTURE PROSPECTS

The network of putative associations presented in the computational study puts forth a set of potential interactions that can serve as testable hypotheses for future experimental validation. A better perspective and deeper experimental investigations of the identified human host interactions of CHPV M protein and linear peptides could complement the understanding of CHPV biology and further aid in the development of effective antiviral strategies.

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