ENTRY AND ASSEMBLY OF PATHOGENIC VIRUSES

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INDIAN INSTITUTE OF TECHNOLOGY DELHI

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by

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Submitted

in fulfilment of the requirements of the degree of ***Doctor of Philosophy***

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Dedicated to the Almighty Lord Ram…

My beloved parents, family members, my lovely wife and wonderful daughter for their unconditional love, care and endless support

CERTIFICATE

This is to certify that the thesis entitled **"Entry and assembly of pathogenic viruses"**, being submitted by **Mr. Chandra Shekhar Kumar** to the Indian Institute of Technology Delhi for the award ofthe degree of **"Doctor of Philosophy"** is a record of the *bonafide* research carried out by him, which has been prepared under my supervision and guidance in conformity with rules and regulation of the Indian Institute of Technology Delhi, India. The results described in it have not been submitted in part or full to any other University or Institute for the award of any Degree/Diploma.

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CHANDRA SHEKHAR KUMAR

ABSTRACT

Viral structural proteins are important therapeutic targets and are being used extensively for designing antivirals and vaccines against pathogenic viruses. Engineering of virus-like particles (VLPs), from one or more structural proteins from pathogenic viruses is a viable strategy for development of vaccines and for studies of the viral life cycle without using live virus. The present work is primarily focused on the generation and characterization of quadruple-antigen SARS-CoV-2 VLPs, which can serve as virus surrogates for multiple biomedical purposes. VLPs were generated by transient transfection of two expression cassettes in adherent HEK293T cells – one cassette containing M^{pro} for processing of three structural proteins (M, E and N), and the second cassette expressing the Spike protein. Further characterization revealed that the VLPs retain close morphological and antigenic similarity with the native virus, and also bind strongly to the SARS-CoV-2 receptor hACE-2 in an *in vitro* binding assay. Interestingly, the VLPs was found to internalize into U87-MG cells through cholesterol rich domains in a dynamin dependent process. Finally, our results showed that mice immunized with VLPs induce robust humoral and cellular immune response mediated by enhanced levels of IL-4, IL-17 and IFNγ. Taken together, our results demonstrate that VLPs mimic the native virus and induce strong immune response indicating possible use of these particles as an alternative vaccine candidate against SARS-CoV-2. VLPs can also be effective in mapping the initial stages of virus entry and screening of inhibitors. In the second part of this work, we have made an effort to structurally analyze the ion-channel forming 6K peptide from Chikungunya Virus, which is known to play a major role in viral egress. We found that recombinant CHIKV 6K peptide, expressed with a MBP fusion tag, exists primarily as an octamer and exhibits membrane interaction activity. The preliminary structural analysis of the *in silico* generated 3D octamer shows similarities with reference free 2D classes of MBP-6K generated from cryoEM studies. This presents an opportunity to solve a high-resolution structure that can be utilized to design inhibitors specific to Chikungunya Virus 6K, which will inhibit virus assembly and propagation

सारांश

वायरल संरचनात्मक प्रोटीन महत्वपूर्ण चिकित्सीय लक्ष्य हैं और रोगजनक वायरस के खिलाफ एंटीवायरल और टीके डिजाइन करने के लिए व्यापक रूप से उपयोग किए जा रहे हैं। रोगजनक वायरस से एक या एक से अधिक संरचनात्मक प्रोटीन से वायरस जैसे कणों (वीएलपी) की इंजीनियरिंग टीकों के विकास और जीवित वायरस का उपयोग किए बिना वायरल जीवन चक्र के अध्ययन के लिए एक व्यवहार्य रणनीति है। वर्तमान कार्य मुख्य रूप से चौगुनी-एंटीजन सार्स-कोरोना-वायरस-2 वीएलपी के निर्माण और निरूपण पर केंद्रित है, जो कई बायोमेडिकल उद्देश्यों के लिए वायरस सरोगेट के रूप में काम कर सकता है। VLPs अनुयाई HEK293T कोशिकाओं में दो अभिव्यक्ति कैसेट के क्षणिक अभिकर्मक द्वारा उत्पन्न किए गए थे - एक कैसेट जिसमें तीन संरचनात्मक प्रोटीन (M, E और N) के प्रसंस्करण के लिए Mpro होता है, और दूसरा कैसेट स्पाइक प्रोटीन को व्यक्त करता है। आगे के लक्षण वर्णन से पता चला कि वीएलपी देशी वायरस के साथ निकट रूपात्मक और एंटीजेनिक समानता बनाए रखते हैं, और इन विट्रो बाइंडिंग परख में SARS-CoV-2 रिसेप्टर hACE-2 से मजबूती से जुड़ते हैं। दिलचस्प बात यह है कि वीएलपी को डायनामिन पर निर्भर प्रक्रिया में कोलेस्ट्रॉल समृद्ध डोमेन के माध्यम से यू87-एमजी कोशिकाओं में आंतरिक रूप से पाया गया था। अंत में, हमारे परिणामों से पता चला कि वीएलपी के साथ प्रतिरक्षित चूहों ने आईएल -4, आईएल -17 और आईएफएनγ के बढ़े हुए स्तरों द्वारा मध्यस्थता से मजबूत त्रिदोषन और सेलुलर प्रतिरक्षा प्रतिक्रिया को प्रेरित किया। एक साथ लिया गया, हमारे परिणाम प्रदर्शित करते हैं कि वीएलपी देशी वायरस की नकल करते हैं और मजबूत प्रतिरक्षा प्रतिक्रिया को प्रेरित करते हैं जो इन कणों के संभावित उपयोग को SARS-CoV-2 के खिलाफ वैकल्पिक वैक्सीन उम्मीदवार के रूप में दर्शाता है। वीएलपी वायरस के प्रवेश के प्रारंभिक चरणों और अवरोधकों की जांच के मानचित्रण में भी प्रभावी हो सकते हैं। इस काम के दूसरे भाग में, हमने चिकनगुनिया वायरस से 6K पेप्टाइड बनाने वाले आयन-चैनल का संरचनात्मक रूप से विश्लेषण करने का प्रयास किया है, जिसे वायरल निकास में एक प्रमुख भूमिका निभाने के लिए जाना जाता है। हमने पाया कि पुनः संयोजक CHIKV 6K पेप्टाइड, जिसे MBP फ्यूजन टैग के साथ व्यक्त किया गया है, मुख्य रूप से एक ऑक्टेमर के रूप में मौजूद है और झिल्ली संपर्क गतिविधि प्रदर्शित करता है। इन सिलिको जनित 3डी ऑक्टेमर का प्रारंभिक संरचनात्मक विश्लेषण क्रायोईएम अध्ययनों से उत्पन्न एमबीपी-6के के संदर्भ मुक्त 2डी वर्गों के साथ समानताएं दर्शाता है। यह एक उच्च-रिज़ॉल्यूशन संरचना को हल करने का अवसर प्रस्तुत करता है जिसका उपयोग चिकनगुनिया वायरस 6K के लिए विशिष्ट अवरोधकों को डिजाइन करने के लिए किया जा सकता है, जो वायरस असेंबली और प्रसार को रोक देगा।

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List of Abbreviations

The following list includes all abbreviations and acronyms used throughout the thesis

ACE2	Angiotensin-converting enzyme 2
ALIX	ALG-2 interacting protein-X
ANOVA	Analysis of variance
ARF6	ADP-ribosylation factor 6
BALB	Bagg and Albino
BAPTA	1,2-bis(*o*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid
BBB	Blood brain barrier
Bgp1	beta globin protein 1
BSA	Bovine serum albumin
BSL	Biosafety level
CAR	Chimeric antigen receptor
CCD	Charge-coupled devices
CCR5	Chemokine receptor-5
CD	Cluster of differentiation
cDNA	complementary Deoxyribonucleic acid
CHIKV	Chikungunya virus
CMV/R	Cytomegalovirus/ enhancer/promoter with the HTLV-1 R region
CNS	Central nervous system
CR2	Complement receptor type-2
Cryo-EM	Cryo-Electron microscopy
CTF	Contrast transfer function
CXCR4	CXC chemokine receptor-4
DAF	Decay accelerating factor
DAPI	4',6-diamidino-2-phenylindole

DC-SIGN	Dendritic cell-specific ICAM 3-grabbing nonintegrin
DDM	n-Dodecyl-B-D-Maltoside
DLS	Dynamic light scattering
DMEM	Dulbecco's Modified Eagle's Medium
DTT	Dithiothreitol
E.coli	Escherichia coli
EDTA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
ER	Endoplasmic reticulum
ESCRT	Endosomal Sorting Complex Required for Transport
FACS	Fluorescence-Activated Cell Sorting
FBS	Fetal Bovine Serum
FEG	Field Emission Gun
FITC	Fluorescein Isothiocyanate
FMDV	Foot-and-Mouth Disease Virus
GAGs	Glycosaminoglycans
GFP	Green fluorescent protein
H1N1	Hemagglutinin type 1 and Neuraminidase type 1
HBc	Hepatitis B virus Core
HBs	Hepatitis B virus Surface
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HEK	Human Embryonic Kidney
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HEV	Hepatitis E virus
HIV	Human immunodeficiency virus
HPV	Human papilloma virus

HRP	Horse radish peroxidase
HveA	Herpesvirus entry mediator A
ICAM-1	Intercellular adhesion molecule-1
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
ILC	Innate lymphoid cells
IP	Intraperitoneal
IPTG	Isopropyl beta-D-1 thiogalactopyranoside
JEV	Japanese encephalitis virus
KRM1	Kringle Containing Transmembrane Protein 1
LDLR	Low-density lipoprotein receptor
L-SIGN	Lymph node-specific ICAM-3-grabbing Nonintegrin
MBP	Maltose binding protein
MCAT-1	Murine Cationic Amino Acid Transporter-1
MERS-CoV	Middle East respiratory syndrome-related coronavirus
MHV	Mouse hepatitis virus
mRNA	messenger Ribonucleic acid
MXRA8	Matrix remodeling associated protein 8
NaCl	Sodium chloride
NEDD	Neural precursor cell expressed developmentally down-regulated
Nsp	Non-structural protein
NLRP3	Nucleotide-binding Oligomerization Domain-like Receptor Protein 3
OD	Optical density
ORF	Open reading frame
PBS	Phosphate buffered saline
PBST	Phosphate buffered saline with Tween

PCR	Polymerase chain reaction
PerCp	Peridinin Chlorophyll Protein
PMSF	Phenylmethylsulfonyl fluoride
PiT-2	Inorganic phosphate transporter protein-2
POPC	Phosphatidyl choline
POPE	Phosphatidyl ethanolamine
PS	Phosphatidyl serine
RBD	Receptor-Binding Domain
RdRp	RNA dependent RNA polymerase
RELION	REgularised LIkelihood OptimisatioN
SARS-CoV	Severe acute respiratory syndrome-associated coronavirus
SCARB1	Scavenger Receptor Class B Type 1
SDS-PAGE	Sodium dodecyl sulfate Polyacrylamide Gel Electrophoresis
SLAM	Signaling lymphocytic activation molecule
SM	Sphingomyelin
SulfoB	SulforhodamineB
SV40	Simian virus 40
SYG1	Synaptogenesis protein-1
TEM	Transmission Electron Microscopy
TIM-1	T cell immunoglobulin and mucin domain-1
TMB	Tetramethylbenzidine
TMPRSS	Transmembrane Protease, Serine
TNF	Tumor necrosis factor
TSG	Tumor susceptibility gene
U87-MG	Uppsala 87 Malignant Glioma
VLP	Virus like particle
Vpu	Viral Protein U