INVESTIGATING THE ROLE OF SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 3 (STAT3) IN DENGUE VIRUS PROPAGATION

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by

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Dedicated to my Parents…

For their unconditional love, support and sacrifices

CERTIFICATE

This is to certify that the thesis titled "**Investigating The Role of Signal Transducer and Activator of Transcription 3 (STAT3) in Dengue Virus Propagation**", submitted by **Ms. Shikha Srivastava** to the Indian Institute of Technology Delhi for the award of the degree of "**Doctor of Philosophy**" is a record of the bonafide research carried out by her, which has been prepared under my supervision and guidance in conformity with the rules and regulations of the Indian Institute of Technology Delhi, India. The results prescribed in it have not been submitted in part or full to any other University for the award of any degree or diploma.

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Shikha Srivastava

ABSTRACT

Dengue fever is an arthropod-borne viral infection affecting millions of people annually during the breeding season of its vector mosquito. To date, no specific antiviral drug or vaccination is invented to fight against dengue virus. Since viruses depend on host factors for their propagation, recent approaches to developing antivirals include targeting host factors for inhibiting viral propagation. DENV has developed several co-existing strategies to avoid eradication by the host antiviral mechanism. DENV modulates host interferon response by attenuating its components, inhibiting their activation or promoting their degradation. This dissertation summarizes the studies we carried out to identify the role of a host transcription factor; STAT3 in dengue virus propagation to identify its potential as a suitable antiviral target. We first wanted to understand how DENV manipulates STAT3 induced interferon response to its benefit. By utilizing various cell culture techniques, we found that STAT3 is increased and activated in DV-2 infected A549 cells and knocking it out resulted in a substantial decrease in viral protein production and viral replication. Our results also demonstrated that DV-2 purposefully manipulates STAT3, which negatively regulates type I IFN signaling, to avoid host interferon response. These results thus established the role of STAT3 as a proviral factor. Moving ahead, we explored the potential of STAT3 inhibitor stattic as an antiviral agent. For this we performed a whole proteome analysis of infected cells by high throughput liquid chromatography mass spectrometry (LC-MS), wherein we observed that treatment with stattic induces multiple host defense mechanisms to counteract dengue infection. We also observed that treatment with stattic downregulates several pathways involved in viral transcription. Taken together these results suggest that inhibiting STAT3 by stattic or its derivatives may prove to be an effective strategy for the development of antiviral interventions.

Furthermore, to identify residues of STAT3 for the development of novel therapeutics we explored its interaction with some of the viral proteins translocating into the nucleus e.g., capsid and NS5 we identified some critical residues which mediate the interaction of STAT3 with viral NS5 protein. Since both STAT3 and NS5 are important antagonists of human innate immune response we propose that targeting the residues which mediate their interaction may aid in the screening of alternative antivirals against dengue. Overall, this study strongly indicates the regulatory role of a host transcription factor STAT3 in modulating viral propagation that may be targeted for the development of effective antiviral therapeutics.

सार

डेंगू बुखार एक महत्वपूर्ण मच्छर जनित वायरल बीमारी है जो हर साल लाखों लोगों को प्रभावित करती है। आज तक डेंगू वायरस के खिलाफ कोई विशिष्ट एंटीवायरल या टीका उपलब्ध नहीं है। चूंकि वायरस अपने प्रसार के लिए मेजबान कारकों पर निर्भर करते हैं, हालिया दृष्टिकोण में एंटीवायरल विकसित करने के लिए तथा वायरल प्रसार को रोकने के लिए मेजबान कारकों को लक्षित करना शामिल है। एक सह-मौजूदा तंत्र के रूप में, डेंगू वायरस मेजबान एंटीवायरल प्रतिरक्षा प्रणाली द्वारा उन्मूलन से बचने के लिए विकसित हुआ है। डेंगू वायरस को होस्ट इंटरफेरॉन प्रतिक्रिया को प्रभावित करने के लिए दिखाया गया है उन क्षीणन घटकों द्वारा जो इंटरफेरॉन प्रतिक्रिया को मध्यस्थ करते हैं, उनकी सक्रियता को रोककर या उनके क्षरण को बढ़ावा देकर। यह शोध प्रबंध एक मेजबान प्रतिलेखन कारक की भूमिका जैसे कि STAT3 की भूमिका डेंगू वायरस के प्रसार और STAT3 की भूमिका एक उपयुक्त एंटीवायरल लक्ष्य के रूप में किए गए अध्ययनों को सारांशित करता है । यहां हमने पहले यह समझने का लक्ष्य रखा था कि कैसे डेंगू वायरस अपने स्वयं के लाभ के लिए STAT3 मध्यस्थता इंटरफेरॉन प्रतिक्रिया को नियंत्रित करता है। हमने यह दिखाने के लिए विभिन्न तकनीकों को नियोजित किया कि STAT3 डेंगू वायरस-2 प्रसार के लिए एक प्रो-वायरल कारक के रूप में कार्य करता है। हमारे परिणामों के अनुसार, STAT3 को डेंगू वायरस-2 संक्रमित A549 कोशिकाओं में फॉस्फोराइलेशन द्वारा सक्रिय होने के साथ-साथ विस्तार किया जाता है और इसके नॉकडाउन से वायरल प्रोटीन की अभिव्यक्ति के साथ-साथ वायरल प्रतिकृति में उल्लेखनीय कमी आई है। हम दिखाते हैं कि डेंगू वायरस-2 रणनीतिक रूप से STAT3 को बदल देता है, जो कि टाइप I इंटरफेरॉन प्रतिक्रिया का एक नकारात्मक नियामक है। डेंगू वायरस-2 मेजबान के टाइप I और टाइप III इंटरफेरॉन प्रतिक्रिया से बचने के लिए STAT3 की अभिव्यक्ति और सक्रियण को बढ़ाता है। हमारे परिणाम डेंगू वायरस-2 प्रसार के लिए STAT3 की अनंतिम भूमिका को प्रदर्शित करते हैं जो कि टाइरोसिन फॉस्फोराइलेशन द्वारा सक्रियण से संबंधित है। इसके बाद, हमने एंटीवायरल एजेंट के रूप में STAT3 के अवरोधक स्टेटिक की क्षमता का पता

लगाया। हमने उच्च थ्रूपुट तरल क्रोमैटोग्राफी मास स्पेक्ट्रोमेट्री (एलसी-एमएस) द्वारा संक्रमित कोशिकाओं का संपूर्ण प्रोटिओम विश्लेषण किया, जिसमें हमने देखा कि स्टेटिक के साथ उपचार डेंगू संक्रमण का मुकाबला करने के लिए कई मेजबान रक्षा तंत्र को प्रेरित करता है। इसके अलावा, हमने देखा कि स्टेटिक के साथ उपचार वायरल ट्रांसक्रिप्शन में शामिल कई मार्गों को डाउनग्रेड करता है। इन परिणामों को एक साथ लेने से पता चलता है कि स्टेटिक या इसके डेरिवेटिव द्वारा STAT3 को रोकना एंटीवायरल हस्तक्षेपों के विकास के लिए एक प्रभावी रणनीति साबित हो सकती है।

इसके अलावा, उपन्यास चिकित्सा विज्ञान के विकास के लिए STAT3 के अवशेषों की पहचान करने के लिए हमने कुछ वायरल प्रोटीनों के साथ इसकी परस्पर क्रिया का पता लगाया, जैसे, कैप्सिड और NS5 जो नाभिक में पाए जाते हैं। हमने कुछ महत्वपूर्ण अवशेषों की पहचान की जो वायरल NS5 प्रोटीन के साथ STAT3 की परस्पर क्रिया में मध्यस्थता करते हैं। चूंकि STAT3 और NS5 दोनों मानव जन्मजात प्रतिरक्षा प्रतिक्रिया के महत्वपूर्ण विरोधी हैं, इसलिए हम प्रस्ताव करते हैं कि उन अवशेषों को लक्षित करना जो उनकी परस्पर क्रिया में मध्यस्थता करते हैं, डेंगू के खिलाफ वैकल्पिक एंटीवायरल की जांच में सहायता कर सकते हैं। कुल मिलाकर, यह अध्ययन वायरल प्रसार को संशोधित करने में एक मेजबान प्रतिलेखन कारक STAT3 की नियामक भूमिका को दृढ़ता से इंगित करता है जिसे प्रभावी एंटीवायरल चिकित्सीय के विकास के लिए लक्षित किया जा सकता है।

HIGHLIGHTS

The following are the most significant findings of this study:

- Dengue virus upregulates and activates STAT3 by its phosphorylation at its 705 tyrosine residue to block Type I and Type III interferon responses.
- STAT3 knockdown adversely affects dengue viral transcription, translation and viral release.
- Inhibiting STAT3 by stattic upregulates some of the important immune responsive pathways to counteract DENV infection.
- Stattic treatment on virus infected cells downregulates cellular pathways hijacked by DENV for its transcription.
- Inhibiting STAT3 by stattic or "stattic-like" molecules may serve as an alternate strategy to counteract DENV severity in humans.
- STAT3 translocates into the nucleus of DENV infected cells and interacts with DENV-NS5 protein.

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ADE	Antibody Dependent Enhancement
BHK	Baby Hamster Kidney
CARD	Caspase activation recruitment domains
CCL5	Chemokine (C-C motif) ligand 5
CNS	Central Nervous System
COP 9	Constitutive photomorphogenesis 9
CTCF	Corrected Total Cell Fluorescence
CXCL	C-X-C motif chemokine ligand
DAPI	4',6-diamidino-2-phenylindole
DAVID	Database for Annotation, Visualization, and Integrated Discovery
DEP	Differentially Expressed Protein
DENV	Dengue Virus
DF	Dengue Fever
DHF	Dengue High Fever
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl Sulfoxide
DSS	Dengue Shock Syndrome
FBS	Fetal Bovine Serum
FDA	Food and Drug Administration
FFU	Focus Forming Unit
GAS	Gamma Activated Sequence
GO	Gene Ontology
GPCR	G-Protein- Coupled Receptors
HRP	Horseradish peroxidase
ICAM	Intercellular Adhesion Molecule

IRF	Interferon regulatory factor
IFN	Interferon
IL	Interleukin
IKK	IB kinase
ISG	Interferon Responsive Gene
ISRE	Interferon-stimulated response element
JAK	Janus Kinase
KEGG	Kyoto Encyclopedia of Genes and Genomes
LC/MS	Liquid Chromatography/mass spectrometry
MAVS	Mitochondrial antiviral signaling
MFI	Mean Fluorescence Intensity
MHC	Major histocompatibility complex
MOI	Multiplicity of infection
MTT	3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide
Mx2	Myxovirus resistance 2
NaCl	Sodium Chloride
NAD	Nicotinamide adenine dinucleotide
NFκB	Nuclear factor kappa B
NLS	Nuclear Localization Signal
NTD	N-Terminal domain
OAS2	2'-5'-oligoadenylate synthetase 2
ORF	Open Reading Frame
PAGE	Polyacrylamide gel electrophoresis
PCNA	Proliferating cell nuclear antigen
PDB	Protein Data Bank
PKR	Protein Kinase R
PMSF	Phenyl methyl sulfonyl fluoride

PRR	Pathogen Recognition Receptor
p-Y705	Phospho-Tyrosine 705
RCSB	Research Collaboratory for Structural Bioinformatics
RdRP	RNA-dependent RNA polymerase
RIPA	Radio-immunoprecipitation assay
RMSD	Root Mean Square Deviations
RNAi	RNA interference
RT-PCR	Reverse Transcription- Polymerase Chain Reaction
SAM	S-adenosyl-methionine
SDS	Sodium dodecyl-sulfate
SOCS	Suppressor of Cytokine Signaling
STAT	Signal Transducers and Activators of Transcription
STRING	Search Tool for the Retrieval of Interacting Genes/Proteins
TBK	TANK-binding kinase
TCA	Tricarboxylic Acid Cycle
TLR	Toll like Receptor
TNF	Tumor Necrosis Factor
Tyr	Tyrosine
UPR	Unfolded Protein Response
UTR	Untranslated region
VP	Vesicle Packets
WHO	World Health Organization