DECIPHERING THE MECHANISM OF A HOST CELLULAR FACTOR HIGH MOBILITY GROUP BOX1 PROTEIN IN DENGUE VIRUS PATHOGENESIS

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by

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Dedicated to Lord Shiva…

My beloved parents, brothers, sisters-in-law, nephews and husband

For their unconditional love, care, support, and endless sacrifices

CERTIFICATE

This is to certify that the thesis titled "**DECIPHERING THE MECHANISM OF A HOST CELLULAR FACTOR HIGH MOBILITY GROUP BOX1 PROTEIN IN DENGUE VIRUS PATHOGENESIS**", submitted by **Ms. Nidhi Chaudhary** 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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ABSTRACT

Dengue disease is a highly prevalent mosquito-borne infection-causing millions of infections annually. The global rapid emergence and distribution of dengue in tropical and sub-tropical regions are exposing almost half of the world's population to the threat. Dengue virus (DENV) causes the overexpression and secretion of many proinflammatory cytokines, including high-mobility group box-1 protein (HMGB1). HMGB1 is the most abundant, extremely conserved, ubiquitously expressed nuclear protein that executes the localization-dependent function. HMGB1 translocation and secretion have been implicated in many chronic infections, metabolic disorders, cancer, and viral and inflammatory diseases. In this study, we explored the contribution of HMGB1 in dengue virus replication in A549 cells. Results showed that HMGB1 is translocated and secreted out during dengue infection. Moreover, blockage of HMGB1 release using ethyl pyruvate resulted in enhanced dengue replication and the knockdown of HMGB1 abolished viral replication. *In-silico, in-vitro* assays, and co-immunoprecipitation revealed the binding of HMGB1 to both untranslated regions of viral RNA. This interaction further induces the expression of proinflammatory cytokines like TNF-α, IL-6, and IL-1β which contributes to the severity of DENV disease. Therefore, our study suggests that the DENV tweaks HMGB1 translocation and its interaction with the DENV genome to stimulate proinflammatory cytokines expression in A549 cells.

DENV exploits various cellular pathways including autophagy to assure enhanced virus propagation. The mechanisms of DENV-mediated control of the autophagy pathway are largely unknown. Our investigations have revealed a novel role for HMGB1 protein in the regulation of the cellular autophagy process in the DENV-2 infected A549 cell line. While induction of autophagy by rapamycin treatment resulted in enhanced DENV-2 propagation, the blockade of autophagy flux with bafilomycin A1 suppressed viral replication.

Furthermore, siRNA-mediated silencing of HMGB1 significantly abrogated DENV-induced autophagy, while LPS-induced HMGB1 expression counteracted these effects. Interestingly, silencing of HMGB1 showed a reduction of BECN1 and stabilization of BCL-2 protein. On the contrary, LPS induction of HMGB1 resulted in enhanced BECN1 and a reduction in BCL-2 levels. This study shows that the modulation of autophagy by DENV-2 in A549 cells is HMGB1/ BECN1 dependent. In addition, glycyrrhizic acid (GA), a potent HMGB1 inhibitor suppressed autophagy as well as DENV-2 replication. Altogether, our data suggest that HMGB1 induces BECN1-dependent autophagy to promote DENV-2 replication. Therefore, we postulate HMGB1 as a crucial host element promoting viral propagation and must be considered as an alternative approach for targeting DENV infection. The complex pathogenesis of DENV infection can be better resolved by understanding the complicated correlation between the DENV virus and its host factors.

Growing pieces of evidence demonstrate the importance of metabolic control on proinflammatory response, however, the regulatory mechanism is not known. Here, we suggest a novel mechanism for pyruvate kinase M2 (PKM2) in mediating HMGB1 release during DENV infection. Results showed that the PKM2 expression and nuclear localization were increased in the DENV-2 infection group. Inhibition of PKM2 nuclear translocation by DASA-58 and ML-265 results in decreased HMGB1 release in the supernatant. Furthermore, we observed that DASA-58 and ML-265 resulted in reduced viral protein expression and autophagy. Altogether, our results shed light on an important process for metabolic regulation of proinflammatory response by controlling HMGB1 release and emphasize the role of a metabolic enzyme in understanding the DENV disease pathogenesis.

Collectively, we have deciphered the proviral mechanism of HMGB1 in DENV virus replication by its interaction with the viral genome to induce a proinflammatory response and

inducing autophagy. Furthermore, we unravelled that release of HMGB1 during DENV infection is controlled metabolically by a glycolytic enzyme PKM2 expression and its nuclear translocation. Therefore, the molecular mechanisms associated with host factors that regulate inflammation must be further explored to understand the pathogenesis of severe DENV disease.

सारांश

डेंगू रोग सबसे अधिक प्रचलित मच्छर जनित अर्बोवायरल संक्रमण है जिसके कारण प्रतिवर्ष लाखों संक्रमण होते हैं। उष्णकटिबंधीय और उपोष्णकटिबंधीय क्षेत्रों में डेंगू का वैश्विक तेजी से उभरना और वितरण दुनिया की लगभग आधी आबादी को जोखिम में डाल रहा है। डेंगू वायरस (DENV) उच्च गतिशीलता समूह बॉक्स -1 प्रोटीन (HMGB1) सहित कई प्रोइंफ्लेमेटरी साइटोकिन्स की अधिकता और स्राव का कारण बनता है। HMGB1 सबसे प्रचुर मात्रा में, अत्यंत संरक्षित, सर्वव्यापी रूप से व्यक्त परमाणु प्रोटीन है जो स्थानीयकरण-निर्भर कार्य को प्रदर्शन करता है। HMGB1 अनुवादन और स्राव को कई पुराने संक्रमणों, चयापचय संबंधी विकारों, कैंसर, वायरल और सूजन संबंधी बीमारियों में फंसाया गया है। इस अध्ययन में, हमने A549 कोशिकाओं में डेंगू वायरस प्रतिकृति में HMGB1 के योगदान का पता लगाया। परिणामों से पता चला कि डेंगू संक्रमण के दौरान HMGB1 का स्थानान्तरण और स्राव होता है। इसके अलावा, एथिल पाइरूवेट का उपयोग करके HMGB1 स्राव के रुकावट के परिणामस्वरूप डेंगू प्रतिकृति में वृद्धि हुई और HMGB1 के नॉकडाउन ने वायरल प्रतिकृति को समाप्त कर दिया। इन-सिलिको, इन-विट्रो परीक्षण और सह-प्रतिरक्षादमन परीक्षण ने वायरल आरएनए के दोनों अनट्रांसलेटेड क्षेत्रों में HMGB1 के बंधन का खुलासा किया। यह आगे TNF-α, IL-6, और IL-1β जैसे प्रोइंफ्लेमेटरी साइटोकिन्स की अभिव्यक्ति को प्रेरित करती है जो डेंगू रोग की गंभीरता में योगदान करती है। इसलिए, हमारे अध्ययन से पता चलता है कि डेंगू वायरस HMGB1 ट्रांसलोकेशन और डेंगू जीनोम के साथ इसकी पारस्परिक क्रिया को A549 कोशिकाओं में प्रोइंफ्लेमेटरी साइटोकिन्स अभिव्यक्ति को प्रोत्साहित करने के लिए करता देता है।

डेंगू वायरस प्रसार को सुनिश्चित करने के लिए स्वायत्तजीवी (ऑटोफैगी) सहित विभिन्न जीवकोषीय प्रक्रिया का शोषण करता है। ऑटोफैगी प्रक्रिया के DENV मध्यस्थता नियंत्रण के तंत्र काफी हद तक अज्ञात हैं। हमारी जांच ने डेंगू वायरस संक्रमण प्रेरित ऑटोफैगी प्रक्रिया के नियमन में HMGB1 प्रोटीन के लिए एक नई भूमिका का खुलासा किया है। रैपामाइसिन उपचार द्वारा ऑटोफैगी को वृद्धि करने से डेंगू वायरस प्रसार में वृद्धि हुई, जबकि बाफिलोमाइसिन A1 के साथ ऑटोफैगी की रुकाव ने वायरल प्रतिकृति को कम किया। इसके अलावा, HMGB1 की siRNA की मध्यस्थता वाली साइलेंसिंग ने डेंगू प्रेरित ऑटोफैगी को काफी हद तक निरस्त कर दिया, जबकि LPS ने HMGB1 अभिव्यक्ति को इन प्रभावों का प्रतिकार किया। दिलचस्प बात यह है कि HMGB1 की साइलेंसिंग ने BECN1 की कमी और BCL-2 प्रोटीन के स्थिरीकरण को दिखाया। इसके विपरीत, HMGB1 के LPS प्रेरण के परिणामस्वरूप

BECN1 में वृद्धि हुई और BCL-2 के स्तर में कमी आई। इस अध्ययन से पता चलता है कि A549 कोशिकाओं में डेंगू वायरस द्वारा ऑटोफैगी का नियंत्रण HMGB1 / BECN1 पर निर्भर है। इसके अलावा, एक शक्तिशाली HMGB1 अवरोधक ग्लाइसीराइज़िक एसिड (GA) ने ऑटोफैगी के साथ-साथ DENV-2 प्रतिकृति को भी दबा दिया। कुल मिलाकर, हमारा जांच से पता चलता है कि HMGB1 डेंगू वायरस प्रतिकृति को बढ़ावा देने के लिए BECN1 पर निर्भर ऑटोफैगी को प्रेरित करता है। इसलिए, हम HMGB1 को वायरल प्रसार को बढ़ावा देने वाले एक महत्वपूर्ण मेजबान तत्व के रूप में मानते हैं और इसे डेंगू संक्रमण को लक्षित करने के लिए एक वैकल्पिक दृष्टिकोण के रूप में माना जाना चाहिए। डेंगू वायरस और इसके मेजबान कारकों के बीच जटिल सहसंबंध को समझकर डेंगू संक्रमण के जटिल रोगजनन को बेहतर ढंग से हल किया जा सकता है।

बढ़ते सबूत प्रोइंफ्लेमेटरी प्रतिक्रिया पर चयापचय नियंत्रण के महत्व को प्रदर्शित करते हैं, हालांकि नियामक तंत्र ज्ञात नहीं है। यहां, हम डेंगू वायरस संक्रमण के दौरान HMGB1 स्राव के नियमन में पाइरूवेट काइनेज M2 (PKM2) के लिए नई भूमिका का सुझाव देते हैं। हमने देखा कि डेंगू वायरस संक्रमण के दौरान PKM2 अभिव्यक्ति और कोशिका केंद्रक स्थानान्तरण में वृद्धि हुई । DASA-58 और ML-265 द्वारा PKM2 कोशिका केंद्रक स्थानान्तरण को रोकने से कोशिका माध्यम में HMGB1 स्राव कम हो जाता है। इसके अलावा, हमने देखा कि DASA-58 और ML-265 के परिणामस्वरूप वायरल प्रोटीन की अभिव्यक्ति और ऑटोफैगी कम हो गई। कुल मिलाकर, हमारे परिणाम HMGB1 स्राव को नियंत्रित करके प्रोइंफ्लेमेटरी प्रतिक्रिया के चयापचय विनियमन के लिए एक महत्वपूर्ण प्रक्रिया पर प्रकाश डालते हैं और डेंगू वायरस रोग रोगजनन को समझने में चयापचय एंजाइम की भूमिका को उजागर करते हैं।

सामूहिक रूप से, हमने डेंगू वायरस प्रतिकृति में HMGB1 के वायरस समर्थक भूमिका को वायरल जीनोम के साथ परस्पर संपर्क द्वारा प्रोइंफ्लेमेटरी प्रतिक्रिया को प्रेरित करने और ऑटोफैगी को प्रेरित करने के नियमन को प्रदर्शित किया है। इसके अलावा, हमने यह खुलासा किया कि डेंगू संक्रमण के दौरान HMGB1 की स्राव को ग्लाइकोलाइटिक एंजाइम PKM2 अभिव्यक्ति और इसके कोशिका केंद्रक स्थानान्तरण द्वारा चयापचय रूप से नियंत्रित किया जाता है। इसलिए, गंभीर डेंगू रोग के रोगजनन को समझने के लिए परिचय प्रदाह को नियंत्रित करने वाले मेजबान कारकों से जुड़े आणविक तंत्र को और अधिक खोजा जाना चाहिए।

HIGHLIGHTS

The salient findings of this work are as follows:

- DENV-2 infection modulates HMGB1 protein expression and translocation in human lung epithelial A549 cell line.
- HMGB1 exerts a proviral effect to facilitate DENV-2 propagation via interaction with the untranslated regions of the viral genome.
- HMGB1-DENV-2 UTRs interaction mediates proinflammatory cytokine response which further contributes to DENV pathogenesis.
- HMGB1 also induces a BECN1-dependent cellular autophagy pathway to assure enhanced DENV replication.
- PKM2 is the metabolic key enzyme that regulates the HMGB1 release during DENV infection.
- Targeting HMGB1 is a promising treatment approach for DENV disease management.

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ABBREVIATIONS & SYMBOLS

%	Percent
~	Approximately
°C	Degree Celsius
A280	Absorbance measured at 280nm
Aa	Amino Acid
AaHMGB1	*Aedes aegypti* HMGB1
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
C	Capsid Protein
cDNA	Complementary Deoxyribonucleic Acid
CHIKV	Chikungunya Virus
CNS	Central Nervous System
CRP	C-Reactive Protein
DC-SIGN	Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Nonintegrin
DENV	Dengue Virus
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic acid
dsRNA	Double-stranded Ribo-Nucleic Acid
DTT	1, 4-Dithiothreitol

E	Envelope Protein
ELISA	Enzyme Linked Immunosorbent Assays
FBS	Fetal Bovine Serum
FL	Full-Length
g	Gram
H2O2	Hydrogen peroxide
HBV	Hepatitis B Virus
HCl	Hydrochloric Acid / Hydrochloride
HCV	Hepatitis C Virus
HDAC	Histone deacetylase
ICTV	International Committee on Taxonomy of Viruses
IFN	Interferon
Ig	Immunoglobin
IL	Interleukin
kb	Kilobase pair
kDa	Kilo Dalton
MAPK	Mitogen-Activated Protein Kinase
MD	Molecular Dynamics
mg	Milli gram
mM	Milli molar
mRNA	Messenger Ribonucleic acid
Na2HPO4	Sodium phosphate dibasic

NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
NH4HCO3	Ammonium bicarbonate
Ni-NTA	Ni- nitrilotriacetic acid
NLS	Nuclear Localization Signal
nM	Nanomolar
nm	Nanometer
NS/NSP	Non-structural Protein
NS5	Non-structural Protein 5
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffer saline
PCR	Polymerase Chain Reaction
PDB	Protein Data Bank
PKM2	Pyruvate kinase M2
PMSF	Phenyl methyl sulfonyl fluoride
PRM	Pattern Recognition Molecules
PRNT	Plaque Reduction Neutralization Test
RMSD	Root Mean Square Deviations
RNA	Ribo-Nucleic Acid
rpm	Revolutions per minute
RT-PCR	Real-Time Polymerase Chain Reaction
SDS	Sodium dodecyl sulphate

SS	Single-Stranded
STAT	Signal Transducer and Activator of Transcription (STAT)
TAE	Tris-acetate-EDTA
TBE	Tris/Borate/EDTA
TEMED	N, N, N', N'-Tetramethylethylenediamine
TNF	Tumor Necrosis Factor
WNV	West-Nile Virus
YFV	Yellow Fever Virus
ZIKV	Zika Virus
βME	β-mercaptoethanol
µg	Microgram
µl	Microliter
µM	Micromolar