ROLE OF METHYLTRANSFERASE RV1515C DURING HOST-PATHOGEN INTERACTION IN MYCOBACTERIAL PATHOGENESIS

ANSHU RANI

Kusuma School of Biological Sciences
Indian Institute of Technology Delhi

September 2022

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by

ANSHU RANI

Kusuma School of Biological Sciences

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“Dedicated to my beloved parents for their unconditional love and blessings”

CERTIFICATE

This is to certify that the PhD thesis entitled "**ROLE OF METHYLTRANSFERASE RV1515C IN HOST-PATHOGEN INTERACTION DURING MYCOBACTERIAL PATHOGENESIS**", which is being submitted by **Miss ANSHU RANI (Entry No. 2016BLZ8133)** for the fulfilment of the requirements for the award of degree for **DOCTOR OF PHILOSOPHY, is a record of the student's own work carried out at Indian Institute of Technology, Delhi** under our supervision and guidance. The matter embodied in this report has not been submitted elsewhere to award any other degree or diploma.

Prof. (Dr.) Seyed E. Hasnain
(Supervisor)
National Science Chair & Honorary Professor,
Dept. of Biochemical Engineering & Biotechnology,
Indian Institute of Technology-Delhi,
New Delhi, India

Dr. Nasreen Z Ehtesham
(Co-Supervisor)
ICMR Emeritus Scientist,
National Institute of Pathology,
Safdarjung Hospital Campus,
New Delhi, India

Dr. Ashok Kumar Patel
(Acting Supervisor)
Associate Professor,
Kusuma School of Biological Sciences,
Indian Institute of Technology-Delhi,
New Delhi, India

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ABSTRACT

Tuberculosis (TB), caused by the intracellular pathogen *Mycobacterium tuberculosis* (*M.tb*), is responsible for around two million deaths per year worldwide**.** *M.tb* possesses myriad of mechanisms to infect and survive within the host. *M.tb* employs methyltransferases (MTases) to hijack the host transcriptional machinery through epigenetic regulation. Interestingly, around 3% of the *M.tb* genome encodes methyltransferases. Acquisition of the novel gene, Rv1515c, through horizontal gene transfer during reductive genomic evolution from non-pathogenic mycobacteria indicates its probable role in TB pathogenesis. Proteome analysis of the 121 methyltransferases of *M.tb* H_{37}Rv, investigated till date, shows that several of these MTases are present only in *M.tb*. Most of the MTases in *M.tb* still remain uncharacterised, and further research is required to explore their mechanistic role in TB pathogenesis. Among these MTases, the hypothetical protein Rv1515 is exclusively present in the *M.tb* complex (MTBC) and is absent in the non-pathogenic strain of mycobacteria. This study explores the functional role of the *M.tb* Rv1515c hypothetical protein in virulence and pathogenesis of *M.tb*.

In-silico analysis of *M.tb Rv1515c* revealed presence of S-adenosyl methionine binding site and conserved methyltransferase domain. *In-vitro* studies confirmed that Rv1515c exhibits DNA binding and DNA methyltransferase activity. Knock-in of *M.tb Rv1515c* gene in the non-pathogenic *M. smegmatis* strain lead to drastic morphological and physiological changes. The recombinant *M. smegmatis* was able to adapt and grow under extreme stress conditions, and exhibited pathogenicity. *In-vitro* treatment of recombinant *M. smegmatis* to macrophage showed enhanced uptake and survival by modulating phagolysosomal maturation. Recombinant *M. smegmatis* exhibited virulence by suppressing the host defence systems through lowering of NO (Nitric oxide), ROS (Reactive oxygen species) production and suppressing apoptosis in the macrophages. Rv1515c modulated pro-inflammatory cytokines, inhibited antigen presentation and suppressed co-stimulatory molecules.

Interestingly, recombinant *M. smegmatis* survived for longer duration and caused pathological conditions in several organs of the infected mice. We also observed that *M.tb* Rv1515c could reduce the effector T cells by increasing the Treg cells and downregulated activation markers on the macrophages. Notably, Rv1515c induced polarisation of macrophage towards M2 lineage in the peritoneal macrophage which favoured persistence of mycobacterial infection. Cumulatively, these results highlight the significant role of Rv1515c in *M.tb* pathogenesis and in modulating the host immune responses.

This thesis demonstrates the role of Rv1515c in host-pathogen interactions during mycobacterial pathogenesis which may aid in devising novel therapeutic interventions against TB.

सार

तपेदिक, अन्त:कोशिक रोगज़नक़ माइकोबैक्टीरियम ट्यूबरकुलोसिस (एम.टीबी) के कारण होने वाली सबसे घातक संक्रामक बीमारियों में से एक है, जो दुनिया भर में प्रति वर्ष लगभग 20 लाख मौतों के लिए जिम्मेदार है। माइकोबैक्टीरिया एपिजेनेटिक विनियमन के माध्यम से मेजबान ट्रांसक्रिप्शनल मशीनरी का अतिक्रमण करने के लिए मिथाइलट्रांसफेरेज़ को नियोजित करता है। यह महत्वपूर्ण बात है कि एम.टीबी जीनोम का लगभग 3% मिथाइलट्रांसफेरेज़ को कोड करता है। एम.टीबी H37Rv के 121 मिथाइलट्रांसफेरेज़ के क्रॉस प्रोटिओम विश्लेषण ने जांच की कि कई मिथाइलट्रांसफेरेज़ केवल एम.टीबी में मौजूद हैं। रोचक बात यह है कि *Rv1515c* विशेष रूप से एम.टीबी कॉम्प्लेक्स में मौजूद है और गैर-रोगजनक माइकोबैक्टीरिया में अनुपस्थित है। गैर-रोगजनक माइकोबैक्टीरिया से घटते संजीन के विकास के दौरान क्षैतिज जीन स्थानांतरण के जरिए नया जीन *Rv1515c* का अधिग्रहण टीबी रोगजनन में इसकी संभावित भूमिका को दर्शाता है। एम.टीबी में अधिकांश मिथाइलट्रांसफेरेज़ के ऊपर शोध नहीं किया गया है इसलिए टीबी रोगजनन में मिथाइलट्रांसफेरेज़ के बारे में विस्तार से अध्ययन करने की जरूरत है। थीसिस कार्य एम.टीबी के रोगजनन में *Rv1515c* के कार्यात्मक महत्व और भूमिका को प्रस्तुत करता है।

एम.टीबी *Rv1515c* विशेष रूप से एम.टीबी कॉम्प्लेक्स में मौजूद एक अद्वितीय मिथाइलट्रांसफेरेज़ है। इसके अलावा, *Rv1515c* के इन-सिलिको विश्लेषण से एस-एडेनोसिल मेथियोनीन बाइंडिंग साइट और संरक्षित मिथाइलट्रांसफेरेज़ डोमेन की उपस्थिति का पता चला। इन-विट्रो अध्ययनों से *Rv1515c* की डीएनए बाइंडिंग और डीएनए मिथाइलट्रांसफेरेज़ गतिविधि की पुष्टि की। एम.स्मेग्मेटिस में *Rv1515c* के दस्तक से कठोर रूपात्मक और क्रियात्मक परिवर्तन होते हैं। पुनः संयोजक एम.स्मेग्मेटिस अत्यधिक तनाव की स्थिति में अनुकूलन और जीवित रहने में सक्षम है। पुनः संयोजक एम. स्मेग्मैटिस मैक्रोफेज में लंबे समय तक जीवित अस्तित्व को दर्शाता है और NO (नाइट्रिक ऑक्साइड) और ROS (रिएक्टिव ऑक्सीजन प्रजाति)

उत्पादन को कम करके, एपोप्टोसिस को प्रतिबाधित करके और फैगोलिसोसोमल परिपक्वता से बचकर रक्षा प्रणालियों को दबाकर जीवाणु उत्तरजीविता में योगदान देता है। *Rv1515c* ने प्रो-इंफ्लेमेटरी साइटोकाइन को संशोधित किया और एंटीजन प्रस्तुति और सह-उत्तेजक संकेतों को बाधित किया।

रोचक बात यह है कि पुनः संयोजक एम. स्मेग्माटिस चूहों में लंबे समय तक जीवित रहे और एक्स्ट्रापल्मोनरी अंगों में विकृति का कारण बने। हमने यह भी देखा कि एम.टीबी *Rv1515c* ने प्रतिरक्षा अवरोधि T_{reg} कोशिकाओ को बढ़ाकर और मैक्रोफेज सक्रिय मार्कर अभिव्यक्ति को कम करके प्रभावकारी T कोशिकाओ को कम किया। विशेष रूप से, *Rv1515c* ने माइकोबैक्टीरियल के पक्ष में पेरिटोनियल मैक्रोफेज में M2 मैक्रोफेज ध्रुवीकरण में सहयोग किया। संचयी रूप से, ये परिणाम एम.टीबी रोगजनन और मेजबान प्रतिरक्षा रूपांतरण में *Rv1515c* की महत्वपूर्ण भूमिका को उजागर करते हैं।

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LIST OF ABBREVIATIONS

AdoMet	S-Adenosyl methionine
AFB	Acid Fast Bacillus
Ag	Antigen
BCG	Bacille Calmette-Guerin
BEI resources	Biodefense and Emerging Infections research resources repository
BDQ	Bedaquiline
BSA	Bovine Serum Albumin
CD	Circular dichroism spectroscopy
CFP	Culture Filtrate Protein
CFU	Colony Forming Unit
CISH	Cytokine-inducible Src homology 2(SH2) domain protein
Cm	Centimetre
COVID	Coronavirus disease
CTLA-4	Cytotoxic T lymphocyte antigen-*4*
°C	Degree Centigrade (Temperature)
DAPI	4',6-diamidino-2-phenylindole
DAT	Diacyl trehalose
DC	Dendritic cell
DLM	Delamanid
DMEM	Dulbecco's Modified Eagle Medium
DNA	Deoxyribonucleic acid
DPX	Dibutylphthalate Polystyrene Xylene
dsDNA	Double stranded DNA
EtBr	Ethidium bromide

ESAT	Early Secretory Antigenic Target
FADD	Fas-associated protein with death domain
FBS	Fetal bovine serum
FGM	Fast-growing mycobacteria
FITC	Fluorescein isothiocyanate
FSC	Forward scatter
HRP	Horseradish peroxide
H_2O_2	Hydrogen peroxide
HBHA	Heparin-binding hemagglutinin
HIV	Human Immunodeficiency Virus
IFN-γ	Interferon- γ
IL	Interleukin
IPTG	Iso-propyl thiogalactosidase
KHz	Kilo Hertz
LB	Luria-bertani
LTBI	Latent tuberculosis infection
M. africanum	*Mycobacterium africanum*
M. bovis	*Mycobacterium bovis*
M. canettii	*Mycobacterium canettii*
M. caprae	*Mycobacterium caprae*
M. kanasasii	*Mycobacterium kanasasii*

M. leprae	*Mycobacterium leprae*
M. microti	*Mycobacterium microti*
M. mungi	*Mycobacterium mungi*
M. pinnipedii	*Mycobacterium pinnipedii*
M. smegmatis	*Mycobacterium smegmatis*
M. tb	*Mycobacterium tuberculosis*
M. ulcerans	*Mycobacterium ulcerans*
Mb	Mega base
MDMs	Monocyte-derived macrophages
MDR	Multi-drug resistance
MIC	Minimum inhibitory concentration
min	Minute(s)
mm	millimeter(s)
mM	milli Molar
MMP-1	Matrix metalloprotease-1
MOI	Multiplicity of infection
MOM	Mycobacterial outer membrane
MP	Mega Pixel
MR	Mannose receptor
MRCA	Most recent common ancestor
MTases	Methyltransferases
MTBC	*Mycobacterium tuberculosis* complex
ng	Nanogram(s)
NK	Natural killer cell

NO	Nitric oxide
NOX	NADPH oxidase
nm	Nanometer
N/m	Newton per metre
OADC	Oleic- Albumin-Dextrose-Catalase
ºC	Degree Celsius
OD	Optical Density
OsO_4	Osmium tetraoxide
OXPHOS	Oxidative phosphorylation
PAT	Polyacyl trehalose
PBS	Phosphate buffer saline
PDIM	Phthiocerol dimycocerosates
PGE2	Prostaglandin E_2
PD-L1	Programmed death ligand-1
R	Rough
RD	Region of difference
ROS	Reactive oxygen species
rpm	Revolutions per minute
RR-DR	Rifampicin resistance- determining region
S	Smooth
SAM	S-adenosyl methionine
SD	Standard Deviation
SDS	Sodium dodecyl sulfate

SDS-PAGE	Sodium dodecyl sulfate-Polyacrylamide gel electrophoresis
Sec	Second(s)
SEM	Scanning electron microscopy
SGL	Sulphoglycolipid
SGM	Slow grower mycobacteria
SMRT	Single-molecule real-time (SMRT) sequencing
SSC	Side scatter
TB	Tuberculosis
TBST	Tris Buffer saline 0.1% Tween
TDM	Trehalose dimycolate
TEM	Transmission electron microscopy
TGF-β	Transforming growth factor
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TRAF	TNF receptor-associated factor
Tregs	Regulatory Tcells
UV	Ultraviolet
VDR	Vitamin D receptor
XDR	Extensively drug-resistant
α	Alpha
β	Beta
γ	Gamma
□	Delta
μM	Micromolar
μl	Microlitre