Nuclear Shuttling Proteins of Paramyxoviruses: Identification of Previously Unknown Function of Respiratory Syncytial Virus Matrix Protein in Viral Proliferation and Potential Localisation Signals in Human Metapneumovirus Nucleocapsid Protein

A thesis submitted for the

Degree of Master in Applied Science (Research)

October, 2012

Wahyu Nawang Wulan

Biomedical Sciences

Faculty of Applied Science

University of Canberra

Canberra, Australia

Abstract

Respiratory syncytial virus (RSV) and human metapneumovirus (hMPV) are the major causes of viral lower respiratory tract infections (LRTIs). There are currently no licensed vaccines or antivirals for RSV and hMPV; and so research is directed towards the development of live-attenuated vaccines. One pathway to attenuation is to disrupt the nucleocytoplasmic transport of structural proteins of a virus. The nuclear localisation of a structural protein is a strategy used by many viruses to inhibit host antiviral responses.

RSV matrix (M) protein shuttles between the cytoplasm and the nucleus, with nuclear localisation occurring early in infection (around 18 – 24 hours post infection) and cytoplasmic localisation later. The disruption of RSV M nuclear import decreases the titre of infectious particles while the disruption of nuclear export ceases the production of infectious particles. The nucleocytoplasmic transport of RSV M is regulated by CK2 phosphorylation although the detailed mechanism is not well understood. RSV M(205) (T), which is adjacent to the nuclear export signal, has been predicted as a putative regulatory site. The nucleocytoplasmic transport of hMPV nucleocapsid (N) protein, however, has never been described. The hMPV N has been shown to localise in the nucleus late in the replication cycle (day 5 post infection), but further research is required to fully understand the mechanism.

Published literature (RSV M) and unpublished results (hMPV N) from the Ghildyal group led to a hypothesis that disruption of the nucleocytoplasmic transport of a viral structural protein can lead to attenuation. In this study, two hypotheses were tested: (1) mutation in RSV M205 reduces the production of infectious progeny and the induction of pro-inflammatory responses; and (2) the hMPV N has inherent nucleocytoplasmic transport ability. The hypotheses were tested in two aims. Firstly, to investigate the role of the RSV M(205) (T) in virus growth and induction of pro-inflammatory response in Vero E6 and A549 cells; and secondly, to investigate the nucleocytoplasmic transport ability of hMPV N in transfected Cos-7 cells.

To understand the role of RSV M(205) (T), cells were infected with recombinant RSV strain A2 having threonine (T) substitution to alanine (A) at M205 (rRSV A2 M(T205A)). Viral titres and expression of IL-8 and RANTES were determined using plaque assay and ELISA,

following single cycle and multiple cycle replication assays. To define hMPV N nucleocytoplasmic transport, full-length and truncated hMPV N constructs fused to green fluorescent protein were expressed in transfected Cos-7 cells.

rRSV A2 M(T205A) was found to be capable of infecting Vero E6 and A549 cells, but was unable to transmit the infection between cells. The inability to spread leads to a reduced induction of IL-8 and RANTES expression, which was significantly suppressed in the interferon (IFN)-α/β-producing A549 cells, compared to the wild type rRSV A2 M205 (T). hMPV N was found to have inherent ability of nucleocytoplasmic transport, with a potential nuclear export signal (NES) identified at amino acid 1 – 15 and a region containing a nuclear localisation signal (NLS) identified at amino acid 192 – 250.

This study shows for the first time that the RSV M(205) (T) is an important site for the success of transmission of infection and RSV M has a role in the suppression of IFN-α/β. This study was also the first to show that hMPV N is capable of nucleocytoplasmic transport, with potential nuclear transport motifs have been identified. By describing the nucleocytoplasmic transport function of the RSV M and hMPV N proteins, this study contributes to attempts to develop live-attenuated vaccines for RSV and hMPV.

Table of Contents

List of Tables and Figures

Form B

Certificate of Authorship of Thesis

Except where clearly acknowledged in footnotes, quotations and the bibliography, I certify that I am the sole author of the thesis submitted today entitled –

Nucleocytoplasmic Transport of Respiratory Syncytial Virus Matrix Protein and Human Metapneumovirus Nucleocapsid Protein in Infection

I further certify that to the best of my knowledge the thesis contains no material previously published or written by another person except where due reference is made in the text of the thesis.

The material in the thesis has not been the basis of an award of any other degree or diploma except where due reference is made in the text of the thesis.

The thesis complies with University requirements for a thesis as set out in *Gold Book Part 7: Examination of Higher Degree by Research Theses Policy, Schedule Two (S2).* Refer to http://www.canberra.edu.au/research-students/goldbook

31 October 2012

Signature of Candidate

Wahyu Nawang Wulan

Signature of chair of the supervisory panel

Date: ……..

Acknowledgment

I would like to thank my supervisors, Dr Reena Ghildyal and Dr Michelle Gahan for great supervision, guidance, patience, and encouragement. Their vast knowledge tells that they are world class scientists, and it is truly a great honour for me to be under their supervision.

Other than my supervisors, I would like to thank all the people who have helped me accomplishing my study, in particular Dr Erin Walker and Dijana Townsend for huge research assistance. A huge thank you to Parisa, Raj, Robert, James, Hamid, and Kylia from the Ghildyal lab, for providing technical assistance and making the lab a great place to work.

I would also like to thank the BMS students for sharing the experience of research works and the friendship, particularly the members of room 3D29: Guifang Shang, Khloud, Bilquis, Sandy, Matt, Jade, Jessica, Rebecca, Fan, Sherry, and Andrew. Also, a big thank you to Ke Jun, Marwa, Rafat, and other BMS staffs/ post-docs; the 3D level is a supportive place to work because of them.

My master's education could not have been carried out without the Australian Development Scholarship, for which I am greatly indebted. I am particularly grateful to Rozana Muir, the AUSAid Liaison Officer, for her invaluable assistance during my study.

Thank you to my family, relatives, and friends, for the support and encouragement. I am particularly indebted to the lengthy support and encouragement given by my mother and my aunt Djarwani, who have inspired me to pursue my dreams and kept on cheering me through the ups and downs.

Finally, I would like to thank God for giving me the strength in accomplishing this study.

List of Abbreviation

A	Alanine
BDV	Borna disease virus
BRAP2	BRCA1 binding protein 2
BRCA1	breast cancer antigen 1
CARD	caspase recruitment domain
Cdk	cyclin-dependent kinase
cDNA	complementary deoxyribonucleic acid
CDV	canine distemper virus
CK2	casein kinase 2
CLSM	confocal laser scanning microscopy
cp	cold passaged
CPE	cytopathic effect
Crm	chromosomal region maintenance
CTD	carboxy terminal domain
D	aspartic acid
d.p.i	day post infection
DEN	dengue virus
dsRNA	double-stranded ribonucleic acid
EAV	equine arteritis virus
ELISA	enzyme-linked immunosorbent assay
F	phenylalanine; fusion protein
FG	phenylalanine glycine
FI	formaline-inactivated
G	glycine; attachment glycoprotein
GAP	GTPase-activating protein
GDP	guanosine diphosphate
GE	gene end
GEF	guanine–nucleotide exchange factor
GFP	green fluorescent protein
GS	gene start
GTP	guanosine triphosphate
h.p.i	hour post infection
HCMV	human cytomegalovirus
hMPV	human metapneumovirus
I	isoleucine
IFN	interferon
IKK	inhibitor of κB kinase
IL	interleukin
ILI	influenza-like illness

Imp	importin
IPS1	interferon-β promoter stimulator 1
IRAK	IL-1 receptor-associated kinases
IRF	interferon regulatory factor
ISG	interferon-stimulated gene
ISGF	IFN-stimulated growth factor
IκB	inhibitor of κB
JAK	Janus kinase
K	lycine
kDa	kilo dalton
L	leucine
LRTIs	lower respiratory tract infections
M	methionine; matrix
MAVS	mitochondrial antiviral signalling protein
MOI	multiplicity of infection
MV	measles virus
N	nucleocapsid; asparagine
NES	nuclear export signal
NF-κB	nuclear factor kappa B
NLS	nuclear localisation signal
$NODS^{H+}$	nucleolar detention signal regulated by H^+
NoLS	nucleolar localisation signal
NPC	nuclear pore complex
NS	non-structural protein
NTD	amino terminal domain
NTF	nuclear transport factor
Nups	nucleoporins
ORF	open reading frame
PAMP	pathogen-associated molecular pattern
PKC	protein kinase C
ppp	triphosphate
PRR	pathogen recognition receptor
Q	glutamine
R	arginine
RABV	rabies virus
RANTES	regulated upon activation, normal T-cell expressed, and secreted
RC	recombinational cloning
RCC	regulator of chromosome condensation
RIG-1	retinoic-acid inducible gene
RPV	rinderpest virus
rRNA	ribosomal RNA
rRSV	recombinant respiratory syncytial virus

RSV	respiratory syncytial virus
RTIs	respiratory tract infections
S	serine
SEM	standard error of the means
SH	small hydrophobic
ssRNA	single-stranded ribonucleic acid
T	threonine
TAB	TAK1-binding protein
TAK	tumour growth factor-β-activated kinase
TLR	Toll-like receptor
TNF	tumour necrosis factor
TRAF	tumour necrosis factor receptor-associated factor
ts	temperature sensitive
Tyk	tyrosine kinase
URTIs	upper respiratory tract infections
UV	ultraviolet
V	valine
VISA	virus-induced signalling adaptor
VSV	vesicular stomatitis virus
ZFD	zinc finger domain