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SITE-DIRECTED MUTAGENESIS OF NEWCASTLE DISEASE VIRUS V PROTEIN IN VIRUS PATHOGENICITY

THAM MAY LING

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By

THAM MAY LING

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SITE-DIRECTED MUTAGENESIS OF NEWCASTLE DISEASE VIRUS V PROTEIN IN VIRUS PATHOGENICITY

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THAM MAY LING

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Chair : Chia Suet Lin, PhD
Faculty : Biotechnology and Biomolecular Sciences

Newcastle disease virus (NDV) is an avian virus that is highly pathogenic in poultry causing severe economic losses during an outbreak. One of the virulence factors of NDV was identified as the V protein that antagonises the interferon of the host innate immunity in order to allow the virus to replicate successfully in the host cells. This protein is produced by RNA editing at the P gene through insertion of one guanine nucleotide at the conserved editing site. A conserved seven cysteine residues in the C-terminal of paramyxoviral V protein contributes to virus pathogenicity. However, no studies have been carried out to investigate the correlation between pathogenicity and the different lengths of the C-terminal of NDV V protein. This study aims to study the effect of V mutations on virus pathogenicity by mutating the V protein of AF2240-I, a local velogenic NDV strain. Site-directed mutagenesis was performed to introduce 396A>G399G>A at RNA editing site, and four premature stop codons: 456G>T, 537G>T, 624C>T and 642G>T in the V gene respectively. The NDV antigenome plasmids containing the mutated V genes were co-transfected with helper plasmids (plasmids containing NP, P, and L genes) into BSR T7/5 cells to produce the recombinant NDV (rNDV) by reverse genetics. The virus was then rescued and propagated in embryonated chicken eggs. Only three out of five rNDVs were successfully rescued. However, instead of having the substituted thymine in rNDV 456G>T, rNDV 624C>T and rNDV 642G>T, the thymine seemed to have mutated into cytosine. As a result, the stop codon was substituted with other amino acids and the V protein was no longer truncated. The viral pathogenicity was determined by mean death time (MDT) on 9-day old SPF embryonated chicken eggs. Results showed that rNDV 456G>T>C, rNDV 624C>T>C and rNDV 642G>T>C remained as mesogenic strains. It appears that an intact V protein is important for viral replication and pathogenicity. In conclusion, the pathogenicity of rNDVs were not reduced due to the substitution of the desired mutation into another nucleotide. This study warrants a further investigation on the mechanism of the viral RNA dependent RNA polymerase in introducing a mutation for successful virus replication.

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PENGAJIAN KEMUDARATAN NEWCASTLE DISEASE VIRUS DENGAN MUTAGENESIS TAPAK-TERARAH ATAS PROTEIN V

Oleh

THAM MAY LING

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Pengerusi : Chia Suet Lin, PhD
Fakulti : Bioteknologi dan Sains Biomolekul

Newcastle disease virus (NDV) adalah virus burung patogenik yang menyebabkan kerugian ekonomi besar di kalangan penternakan ayam semasa wabak. Salah satu faktor kevirulenan NDV adalah protein V di mana ianya adalah antagonis interferon keimunan inat perumah yang membolehkanya bereplikasi di dalam sel. Protein ini dihasilkan oleh penyuntingan RNA di gen P melalui pertambahan satu nukleotida guanina di laman penyuntingan terpelihara. Tujuh sistein residu terpelihara di terminal C protein V dalam kalangan paramiksovirus menyumbang kepada kemudaratan virus. Walau bagaimanapun, tiada kajian telah dijalankan untuk menyiasat korelasi antara kemudaratan dan kepanjangan terminal C protein V untuk NDV. Kajian ini bertujuan untuk menjalankan mutasi atas protein V AF2240-I, strain NDV velogenic tempatan untuk mengkaji kesan mutasi V ke atas kemudaratan. Mutagenesis tapak-terarah telah dilakukan untuk memperkenalkan mutasi 396A>G399G>A di laman penyuntingan terpelihara dan empat kodon berhenti pramatang: 456G>T, 537G>T, 624C>T dan 642G>T pada gen V masing-masing. Plasmid antigenom NDV dengan mutasi protein V ditransfek serentak dengan plasmid-pembantu (plasmid yang mengandungi gen NP, P, dan L) dalam sel BSR T7/5 untuk menghasilkan virus rekombinan (rNDV) dengan genetik terbalik. Virus yang dipulih akan disuntikkan dalam telur ayam berembrio untuk replikasi. Hanya tiga daripada lima rNDV telah berjaya dihasilkan. Walau bagaimanapun, nukleotida timina yang digantikan dalam rNDV 456G>T, rNDV 624C>T dan rNDV 642G>T telah mengalami mutasi kepada nukleotida sitosina. Akibatnya, kodon berhenti pramatang telah digantikan dengan asid amino yang lain dan protein V tidak lagi dipendekkan. Kepatogenan virus ditentukan oleh purata masa mati dengan menggunakan telur ayam berembrio yang berumur 9 hari. Hasil kajian menunjukkan bahawa rNDV 456G>T>C, rNDV 624C>T>C dan rNDV 642G>T>C adalah strain mesogenik. Ini menunjukkan bahawa protein V yang sempurna adalah penting untuk replikasi dan kepatogenan virus. Kesimpulannya, kepatogenan rNDV tidak dapat dikurangkan dengan

penggantian mutasi keinginan kepada nukleotida yang lain. Siasatan lanjut atas mekanisme polimerase RNA bersandarkan RNA dalam memperkenalkan mutasi untuk menjamin replikasi virus yang berjaya boleh dilakukan pada masa hadapan.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Chia Suet Lin, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Khatijah Mohd Yusoff, PhD, FASc
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Siti Sarah Othman, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

_____
ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 9 March 2022

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Signature:	
Name of Chairman of Supervisory Committee:	Assoc. Prof. Dr. Chia Suet Lin
Signature:	
Name of Member of Supervisory Committee:	Prof. Datin Paduka Dr. Khatijah Mohd Yusoff, FASc
Signature:	
Name of Member of Supervisory Committee:	Assoc. Prof. Dr. Siti Sarah Othman

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LIST OF ABBREVIATIONS

α	alpha
APMV-1	avian paramyxovirus serotype 1
β	beta
BHK	baby hamster kidney fibroblast cell line
BSR T7/5	baby hamster kidney derived mammalian cell line stably expressing T7 RNA polymerase
C	cytosine
CacyBP/SIP	Calcyclin-Binding Protein and Siah-1 Interacting Protein
CEF	primary chicken embryo fibroblasts
CEK	chicken kidney cells
CPE	cytopathic effect
DAPI	4, 6-diamidino-2-phenylindole
DF1	chicken fibroblast cell line
DMEM	Dulbecco's Modified Eagle Medium
γ	gamma
G	guanine
GAF	gamma activation factor
GAS	gamma-activated sequence
GFP	green fluorescent protein
GMEM	Glasgow’s Minimum Essential Medium
F	fusion protein
HA	hemagglutination
Hep-2	human epithelial type-2 cell line
HN	haemagglutinin-neuraminidase protein

HPIV	human parainfluenza virus
HR	heptad repeats
ICPI	intracerebral pathogenicity index
IFN	interferon
IFNAR	interferon alpha receptor
IFNGR	interferon gamma receptor
IFIT1	interferon induced protein with tetratricopeptide repeats 1
IGS	intergenic sequences
IRAK-1	interleukin-1 receptor-associated kinase
IRF	interferon regulatory factor
ISG15	interferon stimulated gene 15
ISGF-3	interferon stimulated gene factor 3
ISRE	interferon stimulated response element
JAK	Janus family of tyrosine kinase
L	large protein
LPS	lipopolysaccharide
M	matrix protein
MDA-5	melanoma differentiation-associated gene-5
MEGA 7	Molecular Evolutionary Genetics Analysis version 7.0
MEM	Minimum Essential Medium
MV	measles virus
Mx	myxovirus resistance
MyD88	myeloid differentiation factor-88
ND	Newcastle disease

NDV	Newcastle disease virus
NiV	Nipah virus
NK	natural killer cells
NLS	nuclear localization signal
NP	nucleocapsid protein
OAS1	oligoadenylate synthetase 1
ORF	open reading frame
P	phosphoprotein
PAMP	pathogen-associated molecular patterns
PCR	polymerase chain reaction
pDC	plasmacytoid dendritic cell precursors
PKR	double-stranded RNA activated protein kinase
PRNTase	GDP polyribonucleotidyltransferase
PRR	pattern recognition receptors
RIG-I	retinoic acid-inducible gene-I
RNA	ribonucleic acid
RNAP	RNA-dependent RNA polymerase
RNP	ribonucleoprotein complex
Rpm	revolutions per minute
SeV	Sendai virus
SH	small hydrophobic protein
SPF	specific pathogen free
STAT	signal transducer and activator of transcription
SV	simian virus
TLR	toll-like receptors
TRAF6	tumour necrosis factor receptor-associated factor-6

TXNL1	thioredoxin-like protein 1
Tyk	tyrosine kinase

CHAPTER 1

INTRODUCTION

Newcastle disease (ND) is a highly contagious viral disease among avian species that can cause severe economic losses in poultry industries affecting many countries. ND was first discovered in Java, Indonesia and in Newcastle upon Tyne in England in the year 1926 and 1927, respectively (Doyle, 1927; Kraneveld, 1926). Examples of avian species that are susceptible to ND are chicken, turkey, pigeons, ducks, pet birds and wild birds (Alexander, 2000; Cattoli et al., 2011). ND is caused by Newcastle disease virus (NDV) which is an avian paramyxovirus serotype 1, APMV-1 (Ganar et al., 2014). NDV is the member of the newly classified genus *Orthoavulavirus* within the subfamily *Avulavirinae*, family *Paramyxoviridae*, and the order *Mononegavirales* in the current taxonomy accepted by International Committee on Taxonomy of Viruses (ICTV) (Amarasinghe et al., 2019). It is a pleomorphic virus that varied from spherical to ellipsoidal (Battisti et al., 2012). The diameters of the spherical virions ranged from 100 to 250 nm whereas the ellipsoidal virions were in between 125 nm and 350 nm (Battisti et al., 2012). The envelope of the virus is derived from the host cell membrane (Yusoff and Tan, 2001).

NDV isolates are categorised into three groups based on the severity of disease they caused. Lentogenic strains are non-virulent whereas mesogenic strains have intermediate virulence. Lentogenic strains such as LaSota and Hitchner B1 are used as live vaccines worldwide to protect poultry against ND (De Leeuw and Peeters 1999; Hitchner and Johnson, 1948). Viruses that cause the highest mortality rate in poultry are in the velogenic group (Huang et al., 2003). Velogenic NDV can be further classified into viscerotropic and neurotropic based on the clinical signs of infected poultry. Infection by viscerotropic NDV are characterised by acute lethal infections, and often come together with haemorrhagic gut lesions in dead avian. On the other hand, neurotropic viruses cause respiratory and neurological disease (Alexander, 2000; Cattoli et al., 2011; Miller et al, 2010). The pathogenicity of NDV can be measured in numerical value through mean death time (MDT), intravenous pathogenicity index (IVPI) and intracerebral pathogenicity index (ICPI) (Cattoli et al., 2011).

AF2240-I is a local viscerotropic velogenic strain that has a total genome length of 15,192 nucleotides and normally used as a vaccine challenge virus in Malaysia to study the vaccine efficacy (Murulitharan et al., 2013). This virus has an MDT of 48, ICPI of 1.90 and IVPI of 2.56 therefore classified into pathotype that causes highest mortality rate (Abdul Rahman et al., 1976; Lai and Ibrahim, 1987; Lee et al., 2006). The pathogenicity of this virus is contributed by the presence of the amino acid sequence 112-R-R-Q-K-R-F-117 at the cleavage site of fusion (F) protein. In addition, the virulent factor of the virus is also contributed by the V protein which is an interferon (IFN) antagonist (Murulitharan et al., 2013). A conserved seven cysteine residue region at the C terminal of V protein

contributes to the virus pathogenicity (Huang et al., 2003; Park et al., 2003a, Qiu et al., 2016). However, no studies have been carried out to mutate the carboxyl terminal of V protein from a velogenic NDV. We have chosen five mutations which are 396A>G399G>A to disrupt the RNA editing site, as well as four other mutations 456G>T, 537G>T, 624C>T and 642G>T to introduce a stop codon. It is hypothesised that truncation of the V protein will result in the inability of the recombinant virus to interfere with the antiviral system of the avian cell, and hence be less virulent than the wild type NDV strain AF2240-I. This study aims to study the virulence of recombinant NDV strain AF2240-I expressing GFP (rAF-GFP) by mutating the V gene on the pOLTV5 (rAF-GFP) plasmid that harbours the full-length of NDV antigenome. The specific objectives are:

1) To mutate the Newcastle disease virus (NDV) antigenome plasmid using site-directed mutagenesis

2) To recover the virus using reverse genetics system

3) To determine the virulence of the recombinant NDV (rNDV) using mean death time (MDT)

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