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DEVELOPMENT OF A RECOMBINANT RETROVIRUS EXPRESSING THE CHICKEN ANAEMIA VIRUS VP3 PROTEIN

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By
SURIA BINTI MOHD SAAD

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science

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Dedicated with love and gratitude to

My father Mohd Saad Suboh and my mother Hasnah Othman

Whose had supported me and were my constant source of encouragement and motivation. They are the ones who started me all those years ago on the journey of knowledge which has brought me to where I am today.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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September 2006

Chairman : Professor Mohd Azmi Mohd Lila, PhD

Faculty : Veterinary Medicine

Retrovirus is an infectious particle, hence it could be used as an efficient vector to deliver a desired gene product into mammalian cells. In this study, a recombinant viral vector was employed to carry a gene that induces apoptosis in various transformed and cancerous cell lines. The VP3 gene was cloned into pMSCV plasmid and the recombinant was used to transfect a packaging cell line to produce infectious replication-incompetent recombinant VP3-retrovirus. The sequence of the full length ORF encoding VP3 gene is similar to that of the reference CAV Cux-1 strain indicating that the VP3 gene was stably integrated into the RNA genome of the recombinant retrovirus. Real-time RT-PCR analysis showed virus production in packaging cells increased from day one, but gradually decreased on day three and day four and eventually were undetectable on day five post-infection. The number of packaging cells undergoing apoptosis was shown to be directly associated with recombinant VP3-retrovirus replication and the rate of cell-to-cell infection. Cells infected by recombinant

VP3-retrovirus expressed the VP3 protein in transformed and cancerous cell lines as confirmed by indirect immunoperoxidase assay using anti-VP3 monoclonal antibody. The VP3 protein was detected primarily in the nucleus of infected cells, the site in which the protein is believed to initiate the cascade of programmed cell death or apoptosis. Apoptotic genomic DNA cleavage of the transformed cells was observed. Terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL) assay confirmed the occurrence of apoptosis following infection by the recombinant VP3-retrovirus. This study demonstrated the potential application of recombinant VP3-retrovirus in cancer therapy. The current recombinant VP3-retrovirus construct may serve as an excellent prototype for the generation of alternative therapy to prevent the progressive growth of many types of cancer cells.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PEMBINAAN RETROVIRUS REKOMBINAN MENGEKSPRESKAN PROTEIN VP3 VIRUS ANAEMIA AYAM

Oleh

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Retrovirus adalah partikel berjangkit, maka ia boleh digunakan sebagai vektor efisien untuk membawa produk gen yang diingini ke dalam sel-sel mamalia. Dalam kajian ini, vektor virus rekombinan digunakan untuk membawa gen yang meransang apoptosis dalam pelbagai lapisan sel terubah dan sel kanser. Gen VP3 diklonkan ke dalam plasmid pMSCV dan rekombinan digunakan untuk menjangkiti lapisan sel pembungkusan untuk menghasilkan VP3-retrovirus rekombinan berjangkit tidak mampu-mereplikasi. Penjujukan panjang keseluruhan rangka pembacaan terbuka pengkodan gen VP3 adalah sama dengan baka CAV Cux-1 rujukan menunjukkan yang gen VP3 secara stabil disatukan ke dalam genom RNA retrovirus rekombinan. Analisis RT-PCR masa-sebenar menunjukkan penghasilan virus dalam sel pembungkusan meningkat daripada hari pertama, tetapi menurun secara perlahan-lahan pada hari ketiga dan keempat dan akhirnya tidak dapat dikesan pada hari kelima selepas jangkitan. Bilangan sel pembungkusan menjalani apoptosis berkadar lansung dengan replikasi VP3-retrovirus

rekombinan dan kadar jangkitan sel ke sel. Sel-sel yang dijangkiti oleh VP3-retrovirus rekombinan mengekspreskan protein VP3 seperti yang disahkan melalui ujian immunoperoksidase tidak lansung menggunakan antibodi monoklonal anti-VP3. Protein VP3 dikesan terutamanya dalam nukleus sel-sel terjangkit, tempat dimana protein dipercayai memulakan urutan kematian sel terprogram atau apoptosis. Pemotongan DNA genomik apoptotik sel-sel terubah diperhatikan. Ujian perlabelan hujung celah perantaraan-transferase deoksinukleotidil terminal dUTP (TUNEL) mengesahkan kejadian apoptosis berikutan jangkitan oleh VP3-retrovirus rekombinan. Kajian ini menunjukkan potensi aplikasi VP3-retrovirus rekombinan dalam terapi kanser. Pembinaan VP3-retrovirus rekombinan terkini boleh bertindak sebagai prototaip terbaik terapi alternatif untuk mencegah pertumbuhan progresif pelbagai jenis sel kanser.

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LIST OF ABBREVIATIONS

%	Percentage
µg	Microgram
µl	Microlitre
µM	Micromole
Amp^r	Ampicillin resistance
APC	Antigen-presenting cell
ATV	Antibiotic-trypsin-versen
Bcl-2	B cell leukemia/lymphoma 2
β-ME	Beta-mercaptoethanol
bp	Base pair
BSA	Bovine serum albumin
$CaCl_2$	Calcium chloride
CAV	Chicken anemia virus
cm	Centimetre
cm^2	Centimetre square
CO_2	Carbon dioxide
CPE	Cytopathic effect
CT	Colon tumour
C_T	Threshold cycle
cyt-c	Cytochrome c
C-26	Murine colon carcinoma
DAB	3,3'-diaminobenzidine

DC	Dendritic Cell
ddH_2O	Deionized distilled water
DMEM	Dulbecco's modification of Eagle's medium
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
Dnase	Deoxyribonuclease
dNTP	Deoxyribonucleotide
E.coli	Escherichia coli
EC	Embryonic carcinoma
e.g	For example
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
ES	Embryonic stem
EtBr	Ethidium bromide
FasL	Fas ligand
FBS	Fetal bovine serum
ffu	Focus-forming units
G418	Neomycin
G-CSF	Granulocyte-colony stimulating factor
GFP	Green fluorescent protein
GM-CSF	Granulocyte macrophage-colony stimulating factor
g	Gram
HAT	Hypoxanthine/amethopterin/thymidine

HCl	Hydrochloric acid
HEK	Human embryonic kidney
HIV	Human immunodeficiency virus
HRP	Horseradish peroxidase
HT	Hypoxanthine/thymidine
H_2O_2	Hydrogen peroxide
ID_{50}	50% inhibitory dose
IFN-γ	Interferon gamma
IgG	Immunoglobulin G
IL	Interleukin
kb	Kilobase
kDa	Kilo dalton
LB	Luria bertani
LM-PCR	Ligation-mediated PCR
LTR	Long terminal repeat
MCF-7	Human mammary adenocarcinoma
MCS	Multiple cloning site
MDA-MB	Human, causian, breast adenocarcinoma
mg	Milligram
ml	Millilitre
mM	Millimole
mm	Millimetre
Mo-MLV	Moloney murine leukaemia virus

MTT	3-[4,5-dimethylthiazol-2-yl]-2,5diphenyltetrazolebromide
N	Normality
NaOH	Sodium hydroxide
Neo^r	Neomycin resistant
ng	Nanogram
NGF	Neural growth factor
^0C	Degree celcius
ORF	Open reading frame
PBS	Phosphate-buffered saline
PCD	Programmed cell death
PCR	Polymerase chain reaction
PCMV	PCC4-cell passage myloproliferative sarcoma virus
PDGF	Platelet-derived growth factor
pH	Hydrogen-ion activity
PKA	Protein kinase A
PKG	Phosphoglycerate kinase
P_{PKG}	Phosphoglycerate kinase promoter
psi	Packaging sequence
PT67	Mouse fibroblast derived cell
MSCV	Murine stem cell virus
RE	Restriction endonuclease
RLT	RNeasy lysis buffer
RNA	Ribonucleic acid

RNase	Ribonuclease
RPE	RNeasy precipitate buffer
ROPS	Random oligonucleotide primed synthesis
rpm	Revolution per minute
RPMI 1640	Roswell park memorial institute
RT-PCR	Reverse transcript PCR
RW1	RNeasy wash buffer
SDS	Sodium dodecyle sulphate
SDSC	San Diego Supercomputer Centre
TAA	Tumour-associated antigen
TAE	Tris-acetate-EDTA buffer
$TCID_{50}$	Tissue culture infective dose at 50%
TdT	Terminal deoxynucleotidyl transferase
TE	Tris-EDTA
TGFβ	Transforming growth factor-beta
TNFα	Tumour necrosis factor-alpha
TUNEL	TdT-mediated dUTP Nick-End Labeling
U	Unit
UPM	Universiti Putra Malaysia
UV	Ultraviolet
V	Volt
VERO	African green monkey kidney cell
v/v	Volume per volume

VP	Viral protein
w/v	Weight per volume
WT	Wild type

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