



UNIVERSITI PUTRA MALAYSIA

**RECOMBINANT ADENOVIRUS EXPRESSING ANTI-CANCER GENE
IN COLON CANCER CELL EXPLANT IN MICE**

TAN SEOK SHIN

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**DOCTOR OF PHILOSOPHY
UNIVERSITI PUTRA MALAYSIA
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COLON CANCER CELL EXPLANT IN MICE**

By

TAN SEOK SHIN

**Thesis Submitted to the School of Graduate Studies,
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fulfilment of the requirement for the degree of Doctor of Philosophy

**RECOMBINANT ADENOVIRUS EXPRESSING ANTI-CANCER GENE IN
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October 2010

Chairman : Zeenathul Nazariah Allaudin, PhD

Faculty : Institute of Bioscience

Gene therapy is an alternative method to cure or slow down the progression of malignant cancer. Recombinant adenovirus encoding viral protein 2 (VP2) (ADV-VP2) of very virulent infectious bursal disease virus (vvIBDV) was employed to eliminate cancer cells by apoptosis mechanism. Besides, another recombinant adenovirus encoding murine endostatin (ADV-endo) was constructed aiming to block the formation of new blood vessels that supply nutrients to tumor. Recombinant adenoviruses were found to express the VP2 gene at a significantly high level in cancer cells, especially adenocarcinomas, with the relative quantification (RQ) value from 149.58 to 233.12 fold 72 hour (hr) post-infection (p.i). However, only small traces of VP2 gene expression was found in non-cancer cells, with the RQ value ranging from 0.04 to 0.54 fold 72 hr p.i. The capacity of recombinant adenovirus to infect target cells is dependent on the level of coxsackievirus adenovirus receptor (CAR) available in each cell. DNA fragmentation test, TUNEL assay, FITC Annexin V/PI double staining quantification test and caspase



tests were carried out to determine the apoptosis induction level by recombinant adenovirus as well as the apoptosis related pathway. All four apoptosis tests were in agreement with recombinant adenovirus induced apoptosis in cancer cells, particularly in MCF-7, CT26 and HepG2, but not in non-cancer cells. CT26 cells demonstrated DNA fragmentation as early as 24 hr p.i, followed by MCF-7 and HepG2 cells, which showed DNA fragments during 48 and 72 hr p.i. These three cancer cells indicated significantly higher apoptotic cells proportion via TUNEL assay and FITC Annexin V/PI double staining test, with the percentage of apoptotic cells ranging from 78.0% to 60.0%. Caspase tests indicated that recombinant adenovirus activated apoptosis at the late stage of infection, through the intrinsic pathway by caspase 2 (initiator caspase), then led to the activation caspase 3 (effector caspase). No apoptosis was detected in cancer cells infected with mock adenovirus vector, thus apoptosis induction was solely contributed by the inserted gene. Colon cancer cells explanted mice were used as a model for cancer therapy in the present study. Tumor size regression was found in multiple doses of recombinant adenovirus treated mice but no regression was found in control mice. Partial tumor size regression was observed in mice treated with 1 dose of ADV-VP2. Complete regression of tumor mass was observed in 5 out of 6 mice and 2 out of 6 mice treated with 3 and 2 doses of ADV-VP2, respectively. Combined treatment of ADV-VP2 and ADV-endo demonstrated prolong mice survival time for up to one month as compared to control mice. Female mice can survive 15 days longer than male mice which suffered from similar large tumor mass. Mouse organs of recombinant adenovirus treated groups were comparable to the control group due to the nature of adenovirus which transiently expressed. The gene expression level in mouse intestines were significantly higher than other organs, 93.06 ± 1.82 fold in 3 doses ADV-VP2 treated



mice. Findings collectively justified the ability of ADV-VP2 to induce apoptosis effectively in tumor mass upon booster administration. In conclusion, the combined administration of recombinant adenovirus (ADV-VP2 and ADV-endo) had therapeutic potential against cancer. Further investigation on the optimal dosage of combined therapy need to be carried out in order to achieve the augmentative effect of these constructs on cancer therapy.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**ADENOVIRUS REKOMBINAN MENGEKSPRESKAN ANTI- KANSER GEN
PADA TIKUS YANG MENGALAMI SEL KOLON KANSER EKSPLAN**

Oleh

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Terapi gen ialah satu kaedah alternatif untuk merawat atau memperlahankan perkembangan kanser malignan. Adenovirus rekombinan mengekodkan protein virus 2 (VP2) (ADV-VP2) daripada virus penyakit bursal berjangkit sangat virulen (vvIBDV) telah digunakan untuk menghapuskan sel-sel kanser dengan mekanisme apoptosis. Di samping itu, satu lagi adenovirus rekombinan yang mengekodkan endostatin murin (ADV-endo) dicipta dengan tujuan untuk menghalang pembentukan saluran-saluran darah baru yang membekalkan nutrien kepada tumor. Adenovirus rekombinan didapati mengekspresi gen VP2 di satu tahap tinggi yang amat signifikan dalam sel-sel kanser, terutamanya adenocarcinomas, dengan nilai ganda RQ dari 149.58 hingga 233.12 72 jam selepas jangkitan. Namun demikian, hanya sedikit kesan ekspresi gen VP2 didapati dalam sel tidak berkanser, dengan nilai julat RQ 0.04 hingga 0.54 72 jam selepas jangkitan. Keupayaan adenovirus rekombinan untuk menjangkiti sel sasaran bergantung kepada tahap reseptor adenovirus koksakievirus (CAR) yang terkandung dalam setiap

sel tersebut. Ujian fragmentasi DNA, ujian TUNEL, ujian kuantifikasi FITC Annexin V/PI perwarnaan berganda dan ujian kaspase telah digunakan untuk menentukan tahap induksi apoptosis oleh adenovirus rekombinan dan arah laluan berkaitan dengan apoptosis juga dikaji. Kesemua empat ujian apoptosis menepati antara satu dengan lain bahawa adenovirus rekombinan mengaruh apoptosis dalam sel-sel kanser, terutamanya dalam sel MCF-7, CT26 dan HepG2, tetapi tidak dalam sel-sel bukan berkanser. Sel CT26 menunjukkan fragmentasi DNA seawal 24 jam selepas jangkitan, diikuti oleh sel MCF-7 dan HepG2, yang menunjukkan fragmentasi DNA pada 48 dan 72 jam pasca jangkitan. Ketiga-tiga sel kanser ini menunjukkan kandungan sel apoptosis yang nyata lebih tinggi melalui ujian TUNEL dan ujian FITC Annexin V/PI pewarnaan berganda, dengan peratusan julat sel apoptotic antara 78.0% hingga 60.0%. Ujian kaspase menunjukkan adenovirus rekombinan mengaktifkan apoptosis pada lewat jangkitan melalui laluan intrinsik dengan pengaktifan kaspase 2 (pemula kaspase) dan seterusnya membawa kepada pengaktifan kaspase 3 (pengkesan kaspase). Tiada apoptosis didapati dalam sel-sel kanser yang dijangkiti dengan vektor adenovirus yang kosong, maka induksi apoptosis semata-mata disumbangkan oleh gen diselitkan. Tikus yang mengalami sel kolon kanser eksplan digunakan sebagai model untuk terapi kanser dalam kajian ini. Regresi saiz tumor telah dikesan pada tikus yang dirawat dengan adenovirus rekombinan berbilang dos tetapi tiada regresi saiz tumor pada tikus kontrol. Regresi saiz tumor separa dapat dicerap pada tikus yang dirawat dengan satu dos ADV-VP2. Regresi lengkap kelompok tumor didapati pada 5 daripada 6 tikus dan 2 daripada 6 tikus yang dirawat dengan 3 dan 2 dos ADV-VP2 masing-masing. Rawatan gabungan ADV-VP2 dan ADV-endo ke atas tikus menunjukkan perlanjutan masa kemandirian selama satu bulan berbanding dengan tikus kontrol. Tikus betina dapat melawan kanser 15 hari lebih

lama daripada tikus jantan yang menderita jisim tumor serupa yang lebih besar. Organ-organ tikus dalam kumpulan yang dirawat dengan adenovirus rekombinan setanding dengan tikus dalam kumpulan kontrol disebabkan oleh sifat adenovirus yang mengekspresi secara sementara. Ekspresi gen yang signifikan tinggi dalam usus tikus berbanding organ lain, 93.06 ± 1.82 kali ganda dengan rawatan 3 dos ADV-VP2. Hasil kajian secara kolektif telah menekankan keberkesanan induksi apoptosis oleh rawatan berbilang dos ADV-VP2 dalam kelompok tumor. Secara kesimpulan, rawatan gabungan adenovirus rekombinan (ADV-VP2 dan ADV-endo) mempunyai potensi terapi dalam menentang kanser. Siasatan lanjut pada dos optimum untuk terapi kombinasi perlu dijalankan untuk mencapai kesan yang bertambah pada terapi kanser.

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I certify that a Thesis Examination Committee has met on 15 October 2010 to conduct the final examination of Tan Seok Shin on her thesis entitled “Recombinant Adenovirus Expressing Anti-Cancer Gene in Colon Cancer Cell Explant in Mice” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Doctor of Philosophy.

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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Date: 15 October 2010



TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	v
ACKNOWLEDGEMENTS	viii
APPROVAL	ix
DECLARATION	xi
LIST OF TABLES	xviii
LIST OF FIGURES	xx
LIST OF ABBREVIATIONS	xxvi
CHAPTER	
1 INTRODUCTION	1
1.1 Background	1
1.2 Problem Statements	3
1.3 Objectives	4
1.4 Hull Hypothesis	5
2 LITERATURE REVIEW	6
2.1 Cancer and Malaysian Scenario	6
2.1.1 Colon Cancer	8
2.1.2 Breast Cancer	9
2.1.3 Cervical Cancer	9
2.1.4 Liver Cancer	10
2.1.5 Lung Cancer	10
2.1.6 Prostate Cancer	11
2.1.7 Ovarian Cancer	11
2.2 Cancer Gene Therapy	12
2.3 Viral Vectors for Gene Delivery	13
2.3.1 Adenovirus Vector	14
2.3.2 Retrovirus Vector	16
2.4 Infectious Bursal Disease Virus	22
2.5 Apoptosis in Cancer	25
2.6 Angiogenesis in Cancer	26
2.7 Endostatin in Cancer Therapy	27
2.8 Apoptosis and Caspases	28
2.8.1 Intrinsic Pathway	31
2.8.2 Extrinsic Pathway	33
2.8.3 Caspases Activation in Cancer Therapy	36
3 DEVELOPMENT OF RECOMBINANT ADENOVIRUS ENCODING MURINE ENDOSTATIN	38
3.1 Introduction	38
3.2 Materials and Methods	40
3.2.1 Human Adenovirus and pShuttle-CMV Vector	40
3.2.2 pCR 2.1-TOPO Vector	40



3.2.3	ADV-VP2	41
3.2.4	Murine Endostatin	41
3.2.5	Agarose Gel Purification	43
3.2.6	Gene Clean Gel Purification	44
3.2.7	cDNA Cloning of VP2 or Murine Endostatin into pCR 2.1-TOPO Vector	44
3.2.8	Plasmid Extraction	45
3.2.9	Screening of The Correct Clone in pCR 2.1-TOPO Vector	46
3.2.10	Verification of ADV-VP2 Clone	48
3.2.11	Cloning of Murine Endostatin into pShuttle-CMV Vector	49
3.2.12	Homologous Recombination-Murine Endostatin	50
3.2.13	Screening and Analysis	51
3.2.14	Amplification and Propagation of Recombinant Adenovirus	51
3.2.15	Preparation of HEK-293A cells	52
3.2.16	Transfection of Recombinant Adenovirus into HEK-293A cells	52
3.2.17	Screening of the Transfected Recombinant Plasmid	53
3.2.18	Purification of Recombinant Adenovirus	54
3.2.19	Median Tissue Culture Infective Dose (TCID ₅₀)	55
3.2.20	Transmission Electron Microscope (TEM)	56
3.3	Results	57
3.3.1	Verification of ADV-VP2 Clone	57
3.3.2	RT-PCR Amplification of Murine Endostatin	58
3.3.3	Cloning of VP2 IBDV or Murine Endostatin into pCR 2.1-TOPO Vector	59
3.3.4	Analysis of the Insert (VP2 IBDV) in pCR 2.1-TOPO Vector	59
3.3.5	Analysis of the Insert (Murine Endostatin) in pCR 2.1-TOPO Vector	61
3.3.6	Confirmation of the Correct Clone by Direct Sequencing	63
3.3.7	Cloning of Endostatin into pShuttle-CMV Vector and Adenoviral Vector	64
3.3.8	Plasmid, PCR and RE Double Digestion of Murine Endostatin in pShuttle-CMV Vector	67
3.3.9	Homologous Recombination of pShuttle-CMV Vector with Human Adenoviral Vector	68
3.3.10	Transfection of the Recombinant Adenovirus into HEK-293A cells	69
3.3.11	Screening of Recombinant Adenovirus Transfected into HEK-293A Cells	69
3.3.12	Recombinant Adenovirus Titer Determination	71
3.3.13	TEM of Recombinant Adenovirus	71
3.4	Discussion and Conclusion	73

4	GENE AND PROTEIN EXPRESSION OF RECOMBINANT ADENOVIRUS	76
4.1	Introduction	76
4.2	Materials and Methods	78
4.2.1	Preparation of Cancer and Non-Cancer Cells	78
4.2.2	Cells Count	79
4.2.3	Cells Subculture	79
4.2.4	Cells Proliferation Assay	80
4.2.5	Infection of Recombinant Adenovirus to Cancer and Non-Cancer Cells	81
4.2.6	Gene Expression of Recombinant Adenovirus	81
4.2.7	RNA Extraction	82
4.2.8	Primers for Gene Expression of VP2 IBDV	83
4.2.9	Quantitative Reverse Transcriptase Real Time PCR	83
4.2.10	Reverse Transcriptase Real Time PCR Efficiency	84
4.2.11	Analysis of Gene Expression	85
4.2.12	Quantitative Levels and Comparative C _T (2 ^{-ΔΔCT}) Method	85
4.2.13	SDS-PAGE Electrophoresis	86
4.2.14	Western Blotting for VP2 IBDV	87
4.2.15	Western Blotting for Murine Endostatin	88
4.2.16	Statistical Analysis	89
4.3	Results	89
4.3.1	Cells Proliferation Assay	89
4.3.2	Recombinant Adenovirus Infection to Cancer and Non-Cancer Cells	90
4.3.3	Real Time PCR Efficiency and Linearity	94
4.3.4	Relative Quantification Method	96
4.3.5	Gene Expression of VP2 in Cancer and Non-Cancer Cells	96
4.3.6	Protein Size Estimation by SDS-PAGE	99
4.3.7	VP2 Protein Expression	100
4.3.8	Protein Expression of VP2 in Cancer and Non-Cancer Cells	100
4.3.9	Endostatin Protein Expression	104
4.4	Discussion and Conclusion	106
5	APOPTOSIS INDUCTION AND ASSOCIATED PATHWAY BY RECOMBINANT ADENOVIRUS ENCODING VP2 IBDV IN CELL LINES	111
5.1	Introduction	111
5.2	Materials and Methods	113
5.2.1	DNA Fragmentation Test	113
5.2.2	TUNEL Assay	114
5.2.3	Quantification of Apoptotic Cells Stained with TUNEL Assay	116

5.2.4	FITC Annexin V/PI Double Staining	117
5.2.5	Caspases Colorimetric Apoptosis Assay	118
5.2.6	Caspases Activity Calculation	119
5.2.7	Statistical Analysis	120
5.3	Results	121
5.3.1	DNA Fragmentation	121
5.3.2	Staining of Apoptotic Cells with TUNEL Assay	125
5.3.3	Quantification of TUNEL Apoptotic Cells	135
5.3.4	Quantification of Apoptotic Cells using Flow Cytometry	137
5.3.5	Caspases Related Pathway Induced by ADV-VP2 in Cancer Cells	146
5.3.6	Determination of Caspases Activity	147
5.4	Discussion and Conclusion	155
6	GROSS AND MICROSCOPIC EVALUATION OF TREATED TUMOR MASS	161
6.1	Introduction	161
6.2	Materials and Methods	162
6.2.1	Tumor Induction in Balb/C Mice	162
6.2.2	Recombinant Adenovirus Treatment for Colon Cancer Cells Explanted Mice	163
6.2.3	DNA Extraction	164
6.2.4	Blood DNA Extraction	165
6.2.5	PCR	166
6.2.6	Serum Separation	167
6.2.7	ELISA	167
6.2.8	Combined Treatment for Colon Cancer Cells Explanted Mice	168
6.2.9	Survival Day for Male and Female Mice	169
6.2.10	Tissue Preparation for Light Microscopy	170
6.2.11	Cancer Lesion Scoring	170
6.2.12	Statistical Analysis	171
6.3	Results	171
6.3.1	Implantation of CT26 Cells to Mice	171
6.3.2	Tumor Growth Rate of Recombinant Adenovirus Treated Mice	172
6.3.3	Tumor Growth Rate of Mice in Combined Treatment	175
6.3.4	Comparison of Tumor Growth Rate among Mice of Different Sex	177
6.3.5	Mice Survival Day	179
6.3.6	Screening of the Treated Mice Blood and Organs	180
6.3.7	Antibody Titer of Treated Mice	182
6.3.8	Histopathology Study of the Organs of ADV-VP2 Treated Mice	183

6.3.9	Histopathology Study of the Organs in ADV-endo and Combined Treated Mice	192
6.3.10	Lesion Score of the Treated Tumors	197
6.4	Discussion and Conclusion	198
7	APOPTOSIS IN TREATED TUMOR MASS	203
7.1	Introduction	203
7.2	Materials and Methods	204
7.2.1	Tumor Induction and Treatment in Balb/C Mice	204
7.2.2	RNA Extraction	205
7.2.3	Reverse-Transcriptase PCR	206
7.2.4	Primers for Gene Expression	206
7.2.5	Quantitative Reverse-Transcriptase Real Time PCR	207
7.2.6	Analysis of Gene Expression	207
7.2.7	Quantitative Levels and Comparative C _T ($2^{-\Delta\Delta C_T}$) Method	207
7.2.8	Transmission Electron Microscope (TEM)	207
7.2.9	DNA Fragmentation Test	208
7.2.10	TUNEL Assay	209
7.2.11	Quantification of Apoptotic Cells Stained with TUNEL Assay	209
7.2.12	FITC Annexin V/PI Double Staining	209
7.2.13	Statistical Analysis	210
7.3	Results	210
7.3.1	VP2 Expression in Treated Mice Organs	210
7.3.2	Real Time PCR Efficiency and Linearity	213
7.3.3	Relative Quantification Method	214
7.3.4	Gene Expression of VP2 and Endostatin in Treated Mice Organs	215
7.3.5	TEM Examination of Treated Mice Tumor Mass	218
7.3.6	DNA Fragmentation of Treated Mice Tumors	225
7.3.7	Staining of Apoptotic Tumor	226
7.3.8	Estimation of Apoptotic Cells	228
7.3.9	Quantification of Apoptotic Cells in Treated Mice Tumor via Flow Cytometry	229
7.4	Discussion and Conclusion	231
8	GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATION	235
8.1	General Discussion and Conclusion	235
8.2	Future Prospects and Recommendations	239
	REFERENCES	240
	APPENDICES	263
A	Buffers, Chemicals and Reagents	263
B	Virus Titer and Calculation	267

C	RQ Value Calculation	269
D	AUP Approval Letter	275
E	Mice Tumor Mass	276
F	Mice Tumor Size	278
G	Marker Size	286
H	Gel Dot Diagrams for DNA Fragmentation	289
BIODATA OF STUDENT		294
PUBLICATIONS		295

