UNIVERSITI PUTRA MALAYSIA

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DOCTOR OF PHILOSOPHY
UNIVERSITI PUTRA MALAYSIA
2010

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

By

TAN SEOK SHIN

**Thesis Submitted to the School of Graduate Studies,
Universiti Putra Malaysia, in Fulfilment of the Requirements for the
Degree of Doctor of Philosophy**

October 2010

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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

By

TAN SEOK SHIN

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

TAN SEOK SHIN

Oktober 2010

Pengerusi : Zeenathul Nazariah Allaudin, PhD

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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 segnifikan 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 caspase) 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 segnifikan 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 combinasi perlu dijalankan untuk mencapai kesan yang bertambah pada terapi kanser.

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation and gratitude to my supervisor, Dr. Zeenathul Nazariah Allaudin, for providing invaluable advice and untiring assistance in this PhD research. Her constant guidance, support and encouragement were the motivation that enabled me to complete my work.

I would also like to express my heartiest thanks and appreciation to Professor Dr. Mohd Azmi Mohd Lila and Associate Professor Dr. Noordin Mohamed Mustapha, as members of the supervisory committee, for their constructive suggestion, proper guidance and encouragement throughout my study.

Special thanks go to the staffs in Virology Lab, Histology Lab, Bacteriology Lab and Microscopic Unit for providing the research facilities and technical assistance. I would also like to thank my family, Virology and LIVES laboratory mates for their infinite support, and help.

Last but not least, I wish to thank Majlis Kanser National (MAKNA) and Graduate Research Fellowship for providing me the opportunity of further study.

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

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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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TAN SEOK SHIN

Date: 15 October 2010

TABLE OF CONTENTS