UNIVERSITI PUTRA MALAYSIA

TRUNCATED CHICKEN ANEMIA VIRUS-VP3 AS A POTENTIAL ANTITUMOUR VACCINE

LIM SHEN NI

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TRUNCATED CHICKEN ANEMIA VIRUS-VP3 AS A POTENTIAL ANTI-TUMOUR VACCINE

By

LIM SHEN NI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia

in Fulfillment of the Requirement for the Degree of Master of Science

April 2012

Dedicated with love and gratitude

to:

My beloved parents and family members

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

TRUNCATED CHICKEN ANEMIA VIRUS -VP3 AS A POTENTIAL ANTI-TUMOUR VACCINE

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LIM SHEN NI

April 2012

Chairperson : Zeenathul Nazariah Allaudin, PhD

Faculty : Institute of Bioscience

Chicken Anemia Virus (CAV) VP3 protein (also known as Apoptin), a basic and proline-rich protein has been shown to act as an anticancer tool. Its special feature in inducing apoptosis in cancer cells but not in normal cells enables it to be widely studied for gene and protein therapies. When produced by *in vitro* protein synthesis system as a recombinant fusion with maltose-binding protein, the recombinant protein was able to migrate into the nucleus of tumour cells and caused apoptosis in the cells. In principle, minimizing the antigenic regions of a protein could reduce unnecessary side effects of the protein to a cell. The aim of this study was determining the minimal selective domain of Apoptin for apoptotic effect. The full length of Apoptin had been mutated by segmental deletion at the N' terminal and

linking it with nuclear localization sites (NLS1 and NLS2) to develop five truncated constructs. An *in vitro* protein expression system, RTS was used to express Apoptin and truncated Apoptin proteins. Microinjection method was applied to deliver all the purified proteins into human breast cancer cells, MCF7 and normal human liver cells, Chang cells. The cells were demonstrated for efficient protein delivery when using 2 mg/ml of purified proteins at 60 hPa of injection pressure for 0.5 s. Cellular microinjections of truncated Apoptin proteins, PrVP3A1-69N1N2, PrVP3A1-46N1N2 and PrVP3A1-31N1N2, retained the characteristics of ectopically expressed wild-type full length Apoptin in cancerous cells. Annexin V was used to detect the presence of early apoptosis in the cells and the result showed occurrence of apoptosis in the cell nucleus. All the three truncated complexes were successfully translocated to the nucleus of human breast cancer cells (MCF cells) and induced apoptosis, whereas in normal Chang cells they remained in the cytoplasm with no apparent toxicity. However, two other mutagens, PrVP3A32-69N1N2 and PrVP3A32-62N1N2, induced apoptosis in both cancerous and noncancerous cells, thus lost their specific selectivity to cancerous cells. Compared to the other three truncated Apoptin proteins, both latter proteins had deletion spanning a.a. 1-31, the upper region of leucine-rich sequence (LRS). LRS at the a.a. 33-46 can act as nuclear retention signals. Therefore, the findings suggested that the minimal selective domain for Apoptin resides in the N-terminal specifically within the deletions spanning a.a. 1-33, which could be the leucine-rich sequence (LRS) enhancer and the contributing factor to the non-selectivity of truncated Apoptin proteins, PrVP3A32-69N1N2 and PrVP3A32-62N1N2. This new finding associated to the selectivity of LRS upstream probably could play a role in cancer cells selectivity for apoptosis induction by Apoptin, In conclusion, out of the five truncated Apoptin proteins, three

of them (PrVP3A1-69N1N2, PrVP3A1-46N1N2 and PrVP3A1-31N1N2) were functioning similar to the full length wild type Apoptin, in their ability to induce apoptosis selectively to the targeted cancer cells.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan Ijazah Saujana Sains

PEMENGGALAN VIRUS ANEMIA AYAM-VP3 SEBAGAI VAKSIN ANTI-TUMUR YANG BERPOTENSI

Oleh

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Protin virus yang ketiga (VP3) virus anemia ayam (CAV) (juga dikenali sebagai Apoptin) merupakan protin alkali yang kaya dengan prolin telah menunjukkan keupayaannya bertindak sebagai alat anti-kanser. Ciri khas Apoptin dalam mendorong apoptosis dalam sel-sel kanser tetapi tidak pada sel-sel normal telah mendorong protin ini dikaji secara meluas dalam kajian terapi gen dan protin. Apabila protin Apoptin yang terpotong digabungkan bersama protin pengikat maltosa dan dihasilkan melalui sistem sintesis protin secara *in vitro*, protin rekombinan ini berupaya beralih ke nukleus sel-sel tumour dan mengakibatkan berlakunya apoptosis dalam sel. Secara prinsip, meminimumkan kawasan antigen protin boleh mengurangkan kesan sampingan yang tidak perlu kepada sel. Tujuan kajian ini dijalankan adalah untuk menentukan domain selektif minimum Apoptin

bagi tujuan apoptotik. Apoptin asal telah bermutasi dengan penghapusan segmen di terminal N dan menghubungkan segmen-segmen yang tinggal dengan tapak penyetempatan nuklear (NLS1 dan NLS2) untuk membina lima kompleks yang baru. Sistem pengekspresan protin secara *in vitro*, RTS telah digunakan untuk mengekspres Apoptin dan Apoptin yang dipenggal. Kaedah mikro-suntikan telah digunakan untuk menyuntikkan semua protin yang ditulenkan ke dalam sel-sel kanser payudara manusia, sel MCF7 dan sel hati manusia, sel Chang. Kepekatan protin yang ditulenkan sebanyak 2 mg/ml telah digunakan untuk dimikro-suntik ke dalam sel pada tekanan suntikan 60 hPa selama 0.5 s. Apoptin protin yang dipenggal, PrVP3A1-69N1N2, PrVP3A1-46N1N2 dan PrVP3A1-31N1N2 yang telah dimikro-suntik ke dalam sel kanser mengekalkan ciri-ciri asal Apoptin. Annexin V telah digunakan untuk mengesan kehadiran apoptosis awal dalam sel dan hasilnya menunjukkan berlakunya apoptosis dalam nukleus sel. Kesemua tiga kompleks Apoptin yang terpotong telah berjaya dipindahkan ke nukleus sel kanser payudara manusia (sel MCF) dan mendorong berlakunya apoptosis dalam sel, sedangkan dalam sel Chang, protin ini kekal di dalam sitoplasma tanpa kesan toksik pada sel. Walau bagaimanapun, dua mutagens lain, PrVP3A32-69N1N2 dan PrVP3A32-62N1N2 telah mendorong berlakunya apoptosis dalam kedua-dua sel kanser dan sel normal, dengan itu ia telah kehilangan selektiviti khususnya pada sel kanser sahaja. Berbanding dengan tiga protin Apoptin lain yang terpotong, kedua-dua protin ini kekurangan a.a. 1-31, rantau atas jujukan-kaya dengan leusin (LRS). LRS yang berkedudukan pada a.a. 33-46 bertindak sebagai isyarat pengekalan nuklear. Oleh itu, kajian ini mencadangkan bahawa domain selektif minimum untuk Apoptin terdapat di terminal N, khususnya pada a.a. 1-33, yang bertindak sebagai pendorong jujukan-kaya leusin berkemungkinan mengakibatkan kehilangan keupayaan selektif pada sel

kanser bagi protin Apoptin terpotong, PrVP3A32-69N1N2 dan PrVP3A32-62N1N2. Penemuan baru ini dikaitkan dengan kepilihan rantau atas LRS mungkin memainkan peranan dalam pemilihan sel kanser untuk induksi apoptosis oleh Apoptin, Kesimpulannya, tiga daripada lima kompleks protin Apoptin terpotong (PrVP3A1-69N1N2, PrVP3A1-46N1N2 dan PrVP3A1-31N1N2) telah memberi kesan apoptosis hanya pada sel kanser sahaja yang mana setanding dengan Apoptin asal.

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I certify that a Thesis Examination Committee has met on 19th April 2012 to conduct the final examination of LIM SHEN NI on her thesis entitled "Truncated Chicken Anemia Virus -VP3 Protein – A Potential Candidate for Anti-Tumour Vaccine" in accordance with the Universities and University Colleges Act 1971 and the Constitution of Universiti Putra Malaysia [P.U.(A) 106] 15 march 1998. The Committee recommends that the student be awarded the relevant degree.

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This thesis was submitted to the Senat 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:

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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 other institutions.

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LIM SHEN NI

Date:19 April 2012

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