UNIVERSITI PUTRA MALAYSIA

ANTIVIRAL EFFECT OF TRIPLE HELIX-FORMING OLIGONUCLEOTIDES ON FELINE INFECTIOUS PERITONITIS VIRUS INFECTION IN VITRO

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IB 2012 20

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science
May 2012

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

ANTIVIRAL EFFECT OF TRIPLE HELIX-FORMING OLIGONUCLEOTIDES ON FELINE INFECTIOUS PERITONITIS VIRUS INFECTION *IN VITRO*

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Feline infectious peritonitis virus (FIPV) is a feline coronavirus (FCoV) which causes a fatal immune-mediated disease called feline infectious peritonitis (FIP) in cats. The virus is classified under the family *Coronaviridae* which consist of a positive sense single stranded RNA genome positioned in helical symmetry. FIPV has been proven as mutants of feline enteric coronavirus (FECV) where the main transmission route for this virus is through faecal-oral route which target the monocytes and macrophage cells. Antiviral chemotherapy treatments using Ribavirin and interferon have been used to treat the disease symptomatically. However, these treatments are not effective to control the fatal progression of the disease.

Furthermore, the various vaccines that have been developed are ineffective to control FIP in cats. Hence, the development of new effective therapy against FIP is impelled.

Triple Helix-Forming Oligonucleotide (TFO) were chosen as a potential anti-viral therapy to inhibit FIP replication due to its ability to compete successfully with other DNA/RNA binders and sequence-specific binding. Specific TFOs targeted to the selected regions of virulent feline coronavirus (FCoV) strain FIPV WSU 79-1146 genomes were designed and tested in FIPV infected Crandell-Reef Feline Kidney (CRFK) cell line. Five different circular TFOs (TFO1 to TFO5) and one unrelated circular TFO (TFO7) were designed and tested for *in vitro* antiviral effects. TFO1 and TFO2 target the 5' and 3' untranslated region (UTR) of FIPV genome, respectively, while the TFO3, TFO4 and TFO5 target the different regions of open reading frame (ORF) 1a/1b of FIPV genome.

Results revealed that TFO1, TFO3, TFO4 and TFO5 were able to hybridize to the target regions and produced triplex, while TFO2 was unable to perform hybridization with its target region. *In vitro* antiviral assays were conducted to examine the ability of TFOs to inhibit virus replication in cell culture based on the presence of CPE and quantitation of viral RNA genome using qRT-PCR. Results from this study showed 50 to 100 nM of circular TFO1 is sufficient to inhibit virus replication. However, increasing the concentration of TFO1 to 500 nM does not enhance the ability of the TFO to inhibit FIPV replication. In the study of antiviral effect of TFOs, results showed the copies of viral RNA genome of cells treated with TFO1, TFO2, TFO3,

TFO4, TFO5 and TFO7 are 3.65 x 10^9, 2.23 x 10^{14}, 4.86 x 10^9, 5.01 x 10^9, 4.41 x 10^9 and 6.02 x 10^{14}, respectively. Hence, transfection with all the circular TFOs, except for TFO2 significantly reduced viral RNA genome up to 100,000 fold compared to mock transfected cells. As expected the mock transfected cells showed high copy number of viral RNA genome, 3.93 x 10^{14}. qRT-PCR study also showed that all linear TFOs and unrelated TFO7 were unable to show any antiviral properties towards the virus. In addition, circular TFO1 and TFO5 which effectively inhibit FIPV replication failed to show any antiviral properties in influenza virus subtype H1N1 infected cells.

In conclusion, all the circular TFOs except for TFO2 demonstrated antiviral effect on FIPV replication indicating the potential use of TFO as an antiviral agent against coronavirus such as FIPV in cats. Further studies are underway to demonstrate the therapeutic values of the designed TFOs towards FIPV infection in cats.

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

KESAN ANTIVIRAL OLIGONUKLEOTIDE YANG MEMBENTUK HELIKS GANDAAN TIGA DENGAN VIRUS PERITONITIS JANGKITAN FELIN IN VITRO

Oleh

CHOONG OI KUAN

Mei 2012

Pengerusi: Abdul Rahman Omar, PhD

Fakulti: Institut Biosains

Virus peritonitis jangkitan felin (FIPV) adalah virus korona felin (FCoV) yang boleh menyebabkan peritonitis jangkitan felin (FIP) iaitu penyakit maut akibat tindak balas sistem imun di dalam kucing. Virus tersebut dikelaskan di bawah keluarga *Coronaviridae* dan mengandungi genom RNA untai tunggal polar positif yang berbentuk simetri heliks. FIPV merupakan mutan virus korona felin (FECV) dan laluan penularan utama bagi virus ini adalah melalui tinja yang menjadikan monosit dan sel makrofaj sebagai sasaran. Rawatan antiviral kemoterapi yang melibatkan Ribavirin dan interferon telah digunakan untuk merawat penyakit ini dengan berpandukan simptom yang ada pada kucing. Namun, rawatan ini tidak dapat mengawal perkembangan

penyakit tersebut dengan berkesan. Bahkan vaksin juga tidak dapat mengawal FIP dalam kucing. Oleh yang demikian, penemuan terapi baru yang lebih berkesan untuk menangani masalah ini amatlah diperlukan.

Oligonukleotida yang membentuk heliks gandaan tiga (TFO) telah dipilih sebagai terapi antivirus yang berpotensi untuk merencat replikasi FIPV oleh kerana kebolehan TFO untuk bersaing dengan pengikat DNA/RNA yang lain dan sifat pengikatan yang khusus dalam urutan genom. TFO khusus yang mensasarkan kawasan tertentu dalam genom FECV strain virulen FIPV WSU 79-1146 telah direka dan diuji dalam sel Crandell-Reef Feline Kidney (CRFK) yang dijangkiti oleh FIPV. Lima TFO bulat yang berbeza (TFO1 sehingga TFO5) dan satu TFO bulat yang tidak berkaitan (TFO7) telah dicipta dan diuji untuk kesan antivirus *in vitro*. TFO1 dan TFO2 masing-masing mensasarkan bahagian 5' dan 3' kawasan tidak diterjemahkan (UTR) dalam genom FIPV, manakala TFO3, TF04 dan TFO5 mensasarkan kawasan bacaan terbuka (ORF) 1a/1b bagi genom FIPV.

Keputusan menunjukkan TFO1, TFO3, TFO4 dan TFO5 mampu berinteraksi dengan sasaran masing-masing and membentuk gandaan tiga, manakala TFO2 tidak dapat melaksanakan hibridisasi dengan kawasan sasarannya. Kajian *in vitro* telah dijalankan untuk mengkaji keupayaan TFOs dalam menyekat replikasi virus di dalam kultur sel berdasarkan kehadiran CPE dan kuantifikasi genom RNA virus menggunakan qRT-PCR. Hasil daripada kajian ini menunjukkan 50 nM hingga 100 nM TFO1 bulat berupaya untuk menghalang replikasi virus. Walau bagaimanapun, peningkatan

kepekatan TFO1 kepada 500 nM tidak menambah keupayaan TFO untuk merencat replikasi FIPV. Untuk kajian kesan antiviral TFOs, keputusan menunjukkan bilangan salinan genom RNA virus bagi sel yang dirawat dengan TFO1, TFO2, TFO3, TFO4, TFO5 dan TFO7 masing-masing adalah 3.65×10^9, 2.23×10^{14}, 4.86×10^9, 5.01×10^9, 4.41×10^9 and 6.02×10^{14}. Maka, transfeksi dengan kesemua TFO bulat kecuali TFO2 telah mengurangkan secara signifikan bilangan salinan genom virus RNA sehingga 100,000 kali berbanding sel transfeksi maya. Seperti yang dijangkakan, sel transfeksi kawalan menunjukkan bilangan salinan genom virus RNA yang tinggi iaitu 3.93×10^{14}. Kajian qRT-PCR juga menunjukkan bahawa semua TFO linear dan TFO7 bulat tidak dapat menunjukkan kesan antivirus terhadap FIPV. Selain itu, TFO1 dan TFO5 bulat yang merencat replikasi FIPV dengan berkesan gagal menunjukkan ciri antivirus di dalam sel yang dijangkiti dengan virus influenza subtip H1N1.

Kesimpulannya, semua TFO bulat kecuali TFO2 menunjukkan kesan antivirus terhadap replikasi FIPV yang menandakan potensi penggunaan TFO sebagai agen antivirus terhadapt virus korona seperti FIPV di dalam kucing. Kajian lanjutan sedang dijalankan untuk menguji nilai terapeutik TFO tersebut di dalam kucing yang dijangkiti FIPV.

ACKNOWLEDGEMENT

This thesis would not have been possible without the guidance and the help of several individuals who in one way or another contributed and extended their valuable assistance in the preparation and completion of this study.

First and foremost, I would like to express my warmest gratitude and appreciation to my supervisor, Prof. Dr. Abdul Rahman Omar whose sincerity and encouragement I will never forget. Prof Dr. Abdul Rahman Omar has been my inspiration as I hurdled all the obstacles in the completion of this research work. My deepest gratitude also goes to Dr. Bimo Ario Tejo for his constructive suggestions and consideration regarding my academic requirements.

Thanks to all postgraduates and officers of Laboratory of Vaccines and Immunotherapeutics of Institute of Bioscience for being accommodating to my queries. I would like to thanks to my friends who support me morally during my study. Special thanks goes to Parvaneh Mehrbod who assisted me in a part of my research.

I would like to acknowledge and thanks to the financial support of National Science Fellowship (NSF) from Ministry of Science, Technology and Innovation (MOSTI).

Last but not the lease, I would like to express my highest appreciation and gratitude to my family. Thanks for their supports, concerns and encouragement during my study.

I certify that a Thesis Examination Committee has met on 21 May 2012 to conduct the final examination of Choong Oi Kuan on her thesis entitled "**Antiviral effect of triple helix-forming oligonucleotides on feline infectious peritonitis virus infection *in vitro***" 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 **degree of Master of Science**.

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

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