UNIVERSITI PUTRA MALAYSIA

TRANSCRIPTOME AND APOPTOSIS ANALYSIS OF FELINE INFECTIOUS PERITONITIS VIRUS-INFECTED CRANDELL REES FELINE KIDNEY CELLS

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

TRANSCRIPTOME AND APOPTOSIS ANALYSIS OF FELINE INFECTIOUS PERITONITIS VIRUS INFECTED CRANDELL REES FELINE KIDNEY CELLS

By

MOHAMMAD SYAMSUL REZA BIN HARUN

August 2012

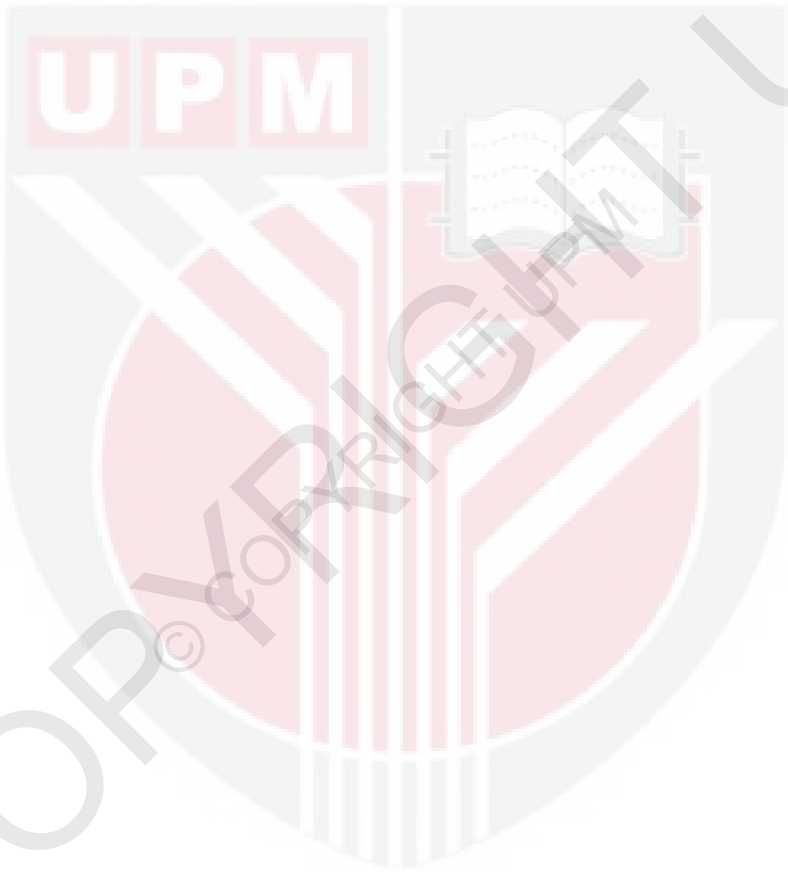
Chair: **Professor Dr. Abdul Rahman bin Omar, PhD**

Faculty: **Institute of Bioscience**

Feline infectious peritonitis (FIP) is a lethal systemic disease caused by FIP virus (FIPV), a virulent mutant of apathogenic feline enteric coronavirus (FECV). There are no effective diagnostic, vaccine and treatment available because the virus virulence determinants and pathogenesis are not fully understood. This study aims to elucidate the early host genes and the phenomenon of apoptosis associated with *in vitro* infection of FIPV in cell culture. RNA samples from FIPV serotype II strain 79-1146 infected Crandell Rees Feline Kidney (CRFK) cells at 3 hours post infection were sequenced using Illumina™ next generation sequencer platform then subsequently analysed with CLC bio Genome Workbench software. Sequencing reads were mapped to *Felis catus* 2X annotated shotgun reference genome and control versus infected cell reads expression analysis was conducted. Kal's Z-

test on expression proportions was used to determine significantly expressed genes. Genes expressed with false discovery rate (FDR) less than 0.05 and more than 1.99 fold change were considered for further analysis. RNA-seq analysis mapped both control and infected cell reads to 18899 genes out of 19046 annotated, while expression analysis revealed 61 genes were differentially expressed by both samples with 44 genes were up regulated while the rest were down-regulated. Among the genes is a chemokine for attracting monocytes, CCL8 that was expressed only in infected sample suggesting that early response against FIPV involves cell mediated immunity (CMI). In addition, 4 genes (CXCL10, PHF11, ATF3, IRF1) that associated with Th1 cytokines secretion were also up regulated in this study. Meanwhile, anti-apoptotic gene RNF7 and ribosomal gene RPL39 were expressed only in control sample indicating that FIPV initiate apoptosis and disturb host cell protein translation as early as 3 hours after infection. Besides that, 9 pro-apoptotic genes (CXCL10, MX1, RSAD2, UBA7, RNF19B, ESE1, BAK1, CASP7, PD-L1) were up regulated while another 3 anti-apoptotic genes (c-Kit, CKS2, ID-1) were down regulated. The detail role of those genes and other differentially expressed genes are discussed. The ability of the virus to induce apoptosis in CRFK cells was also analysed within 48 hours at 12 different time frames which are 3, 9, 12, 15, 18, 21, 24, 27, 30, 36, 42 and 48 hours by flow cytometry and annexin-V FITC staining. Apoptosis analysis confirmed that a significant number of cells undergo early apoptosis 18 hours post-infection and late-apoptosis 30 hours post-infection. This study

has succesfully identified several candidate genes that may play important role in FIPV pathogenesis and has characterized important events in cell death following FIPV infection in CRFK cells.



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

ANALISIS TRANSKRIPTOM DAN APOPTOSIS KE ATAS SEL GINJAL KUCING CRANDELL REES YANG DIJANGKITI VIRUS PERITONITIS BERJANGKIT FELIN

Oleh

MOHAMMAD SYAMSUL REZA BIN HARUN

Ogos 2012

Pengerusi: **Profesor Dr. Abdul Rahman bin Omar, PhD**

Fakulti: **Institut Biosains**

Peritonitis berjangkit felin (FIP) adalah penyakit sistemik yang mematikan disebabkan oleh virus FIP (FIPV), mutan virulen daripada virus korona kucing enterik (FECV) yang tidak berbahaya. Tiada terdapat diagnostik, vaksin dan rawatan yang berkesan kerana faktor penentu virulensi virus dan patogenesis tidak difahami sepenuhnya. Penyelidikan ini bertujuan untuk mengkaji gen awal sel CRFK yang diaktifkan semasa jangkitan untuk menjelaskan gen awal yg penting kepada jangkitan FIPV dan pertahanan perumah. Dalam kajian ini, sampel RNA sel ginjal kucing Crandell Rees (CRFK) yang dijangkiti FIPV serotype II strain 79-1146 selama 3 jam dijujuk menggunakan platform penjujuk generasi terkini, Illumina™ kemudian

dianalisis dengan perisian CLC bio Genomic Workbench. Bacaan penjujukan dipetakan kepada genom rujukan 'shotgun' *Felis catus* 2X beranotasi dan analisis ekspresi sel kawalan melawan sel yang dijangkiti dijalankan. Ujian Kal's Z perkadaran ekspresi digunakan untuk menentukan gen yang diekspresikan secara signifikan. Gen yang dinyatakan dengan kadar penemuan palsu (FDR) kurang daripada 0.05 dan lebih daripada 1.99 perubahan kali ganda telah dipertimbangkan untuk analisis lanjut. Analisis RNA-seq memetakan bacaan sel kawalan dan sel yang dijangkiti kepada 18899 gen daripada 19046 gen beranotasi, manakala analisis ekspresi gen mendedahkan 61 gen berbeza kawal atur oleh kedua-dua sampel dengan 44 gen dinaik kawal atur manakala selebihnya diturun kawal atur. Di antara gen tersebut adalah kimokin untuk menarik monosit, CCL8 yang diekspresi hanya di dalam sampel yang dijangkiti mencadangkan bahawa tindak balas awal terhadap FIPV melibatkan sel imun berantara (CMI). Di samping itu, 4 gen (CXCL10, PHF11, ATF3, IRF1) yang dikaitkan dengan sitokin rembesan Th1 juga dinaik kawal atur dalam kajian ini. Sementara itu, gen anti-apoptotic RNF7 dan gen ribosom RPL39 diekspresi hanya dalam sampel kawalan menunjukkan bahawa FIPV memulakan apoptosis dan mengganggu penterjemahan protein sel perumah seawal 3 jam selepas jangkitan. Selain itu, 9 gen pro-apoptotic (CXCL10, MX1, RSAD2, UBA7, RNF19B, ESE1, BAK1, CASP7, PD-L1) telah dinaik kawal atur manakala lagi 3 gen anti-apoptotic (c-Kit, CKS2, ID-1) diturun ekspresinya. Peranan terperinci gen tersebut dan gen berbeza kawal atur yang lain dibincangkan.

Keupayaan virus untuk menyebabkan apoptosis dalam sel CRFK juga dianalisis dalam tempoh 48 jam pada 12 rangka masa yang berlainan iaitu 3, 9, 12, 15, 18, 21, 24, 27, 30, 36, 42 dan 48 jam menggunakan aliran sitometri dan pewarnaan annexin-V FITC. Analisis apoptosis mengesahkan bahawa sel menjalani apoptosis secara signifikan seawal 18 jam selepas jangkitan dan apoptosis lewat 30 jam selepas jangkitan. Analisis apoptosis mengesahkan bahawa virus menyebabkan apoptosis pada sel CRFK 18 jam selepas infeksi. Penemuan kajian ini dijangka memberi maklumat asas berkenaan patogenesis jangkitan FIPV. Kajian ini telah berjaya mengenal pasti beberapa calon gen yang bermungkinan memainkan peranan penting dalam patogenesis FIPV dan telah memperincikan peristiwa penting kematian sel berikutan jangkitan FIPV dalam sel-sel CRFK.

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I certify that an Examination Committee has met on 14 August 2012 to conduct the final examination of Mohammad Syamsul Reza Bin Harun on his Master of Science thesis entitled "Transcriptome and Apoptosis Analysis of Feline Infectious Peritonitis Virus Infected Crandell Rees Feline Kidney cells" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Master of Science.

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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 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 at any other institution.

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MOHAMMAD SYAMSUL REZA BIN HARUN

Date: 14 August 2012

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