

UNIVERSITI PUTRA MALAYSIA

RAT CYTOMEGALOVIRUS GENOME SCAFFOLD AND A033 GENE AS INFECTION MARKER IN RATS

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FPV 2013 13



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By

SITI NAZRINA CAMALXAMAN

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

May 2013

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DEDICATIONS

Dedicated with great love and extreme gratitude to

My parents: Camalxaman & Che Nu

> My husband: Adi Suria



My children: Adi Irfan & Adi Reza

My siblings: Nazri, Nazrul, Nazmi & Nastiti

"The love of a family is life's greatest blessing"

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

RAT CYTOMEGALOVIRUS GENOME SCAFFOLD AND A033 GENE AS INFECTION MARKER IN RATS

By

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May 2013

Chair: Associate Professor Zeenathul Nazariah Allaudin, PhD.

Faculty: Veterinary Medicine

A local preliminary study has documented the prevalence of rat cytomegalovirus (RCMV) in the wild population to be as high as 96%. Hence, periodic screening and surveillance of laboratory rats is vital, since they may also harbor the viral agent, posing challenges for experimental usage. The lack of sequence information in RCMV ALL-03 strain however, has impeded its detection and prevented its assessment in vitro. This thesis describes the reactivation of RCMV ALL-03 from predilected sites, the establishment of rat brain endothelial cells (RBEC) as alternative target cells for viral replication and the identification of cross-reactive viral proteins with human CMV (HCMV). In addition, the draft genome for RCMV ALL-03 was generated using Next Generation Sequencing technology and assembled using CLC Genomics Workbench. This has led to the identification, analysis and primer design for the A033 gene, an infection determinant in RCMV. RCMV ALL-03 was reactivated from the brain, salivary gland and uterus of infected tissues and identified based on morphologic criteria classical of herpesvirus. RBEC primary cells were successfully established and deemed receptive for RCMV ALL-03 with concomitant production of plaques following cytopathogenic studies.



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Preliminary serological screening of HCMV in Selangor and Kuala Lumpur revealed 92% endemicity. Protein profiles of RCMV ALL-03 were compared to a local RCMV strain (RCMV UPM/Sg) and RCMV-E (Rat2; ATCC CRL-1764TM) reference strain, revealing eight common protein bands in the range of 44-231 kDa. The detection of a 61-68 kDa cross reactive protein by Western Blot raises the possibility of an immunological cross-reactivity between RCMV and HCMV. The RCMV ALL-03 draft genome was sequenced alongside RCMV-E, generating six contigs for RCMV ALL-03 and 11 contigs for RCMV-E. The sizes of RCMV ALL-03 and RCMV-E draft genome sequences were ~198,895 bp and ~175,071 bp respectively, with a total of 136 genes for RCMV ALL-03 as opposed to 112 genes for RCMV-E. From this, only 46 genes were annotated for RCMV ALL-03 and 43 genes in RCMV-E. This includes the A033 gene, identified as gene 21 (1,173 bp) in RCMV ALL-03 and gene 20 (1,187 bp) in RCMV-E. Specific primer for the A033 gene, a marker for RCMV infection has been proposed, and its specificity validated using PCR against other viral strains. To conclude, this study confirms that RCMV ALL-03 is endotheliotropic, justifying its use as an alternative cell culture system that could be further exploited to study the effects of cellular activation of RCMV in the brain. Furthermore, this study addresses the lack of sequence information in RCMV ALL-03, by reporting the first draft of the genome, providing new genomic data acquisition which has not been disclosed to date. Primers for the A033 gene is being proposed to replace existing ones that were rendered non-specific, paving way towards the establishment of a more accurate and sensitive detection assay to screen for RCMV. Once completed, the genome sequence could be further developed as a recombinant vector for delivering human chimeric or antifertility genes.

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

SKAFOLD GENOM SITOMEGALOVIRUS TIKUS DAN GEN A033 SEBAGAI PENANDA JANGKITAN DALAM TIKUS

Oleh

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Laporan saringan sitomegalovirus tikus (RCMV) sebelum ini menunjukkan 96% prevalensi dalam populasi liar. Oleh itu, pemeriksaan dan pengawasan tikus makmal secara berkala adalah penting, kerana virus tersebut boleh juga didapati dalam tikus-tikus ini, menimbulkan cabaran untuk kegunaan eksperimen. Kekurangan jujukan data dalam RCMV ALL-03 banyak menghalang pengesanannya secara in vitro. Tesis ini menerangkan pengaktifan semula RCMV ALL-03 dari kawasan tertentu, pengasingan sel-sel endothelial otak tikus (RBEC) sebagai sel-sel sasaran alternatif untuk replikasi virus dan pengenalpastian protein yang terlibat dalam tindak balas-silang dengan sitomegalovirus manusia (HCMV). Di samping itu, draf genom untuk RCMV ALL-03 telah dijana menggunakan teknologi Next Generation Sequencing dan disusun menggunakan CLC Genomic Workbench. Hasil susunan genom membawa kepada pengenalpastian, analisis dan penghasilan primer bagi gen A033, sejenis penentu jangkitan dalam RCMV. RCMV ALL-03 telah diaktifkan semula dari tisu yang telah dijangkiti iaitu tisu otak, kelenjar air liur dan uterus dan dikenalpasti berdasarkan ciri-ciri morfologi klasik herpesvirus. Sel primer RBEC telah berjaya diasingkan dan didapati sesuai bagi pembiakkan RCMV ALL-03



melalui penghasilan plak sejurus selepas kajian sitopatogenik. Saringan serologi awal HCMV di Selangor dan Kuala Lumpur menunjukkan 92% endemisiti. Perbandingan profil protein RCMV ALL-03 dengan strain tempatan (RCMV UPM/Sg) dan strain rujukan RCMV-E (Rat2; ATCC CRL-1764TM) menunjukkan lapan jalur protein yang sama dalam julat 44-231 kDa. Pengenalpastian protein yang terlibat dalam tindak balas-silang RCMV dan HCMV telah dilaksanakan melalui pengesanan protein reaktif bersaiz 61-68 kDa menggunakan kaedah Western Blot. Genom RCMV ALL-03 telah disusun bersama strain RCMV-E menghasilkan enam contigs untuk RCMV ALL-03 dan 11 contigs untuk RCMV-E. Saiz pencirian jujukan genom RCMV ALL-03 dan RCMV-E separa adalah masing-masing ~198,895 bp dan ~175,071 bp, dengan jumlah 136 gen untuk RCMV ALL-03 berbanding 112 gen untuk RCMV-E. Dari jumlah ini, hanya 46 gen beranotasi untuk RCMV ALL-03 dan 43 gen untuk RCMV-E. Ini termasuk gen A033, yang telah dikenalpasti sebagai gen 21 (1,173 bp) dalam RCMV ALL-03 dan gen 20 (1187 bp) dalam RCMV-E. Primer khusus untuk gen A033, telah direka dan spesifikasinya disahkan dengan kaedah PCR menggunakan strain virus yang berlainan. Kesimpulannya, kajian ini mengesahkan bahawa RCMV ALL-03 adalah endoteliotropik. Penemuan ini menunjukkan kewajaran penggunaan RBEC sebagai sistem kultur sel alternatif yang berpotensi untuk kajian kesan pengaktifan selular RCMV dalam otak. Tambahan pula, kajian ini menangani kekurangan maklumat jujukan dalam RCMV ALL-03, dengan melaporkan draf pertama genom, secara langsung menghasilkan data baru genomik yang belum pernah didedahkan sehingga hari ini. Primer khusus untuk gen A033 dijangka dapat digunakan untuk menghasilkan asai pengesanan untuk RCMV. Setelah selesai, jujukan genom seterusnya boleh dijadikan sebagai vektor rekombinan untuk menyampaikan gen kimera manusia atau gen antifertiliti.

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ACKNOWLEDGEMENTS

In the name of Allah, the Most Gracious and the Most Merciful.

Syukur Alhamdulillah, all praises to Allah, for granting me the strength, courage and blessings to conclude the writing of my thesis. I would not have accomplished this without the help and support from so many people. Firstly, I must acknowledge my sincere gratitude to my supervisor Assoc. Prof. Dr. Zeenathul Nazariah Allaudin. *Thank you* for your guidance, supervision and assistance throughout these years. I have learnt a great deal from you and for that I am forever indebted. My sincere appreciation also goes to my co-supervisors: Assoc. Prof. Dr. Zuridah (my dearest mentor), Assoc. Prof. Dr. Sandy Loh Hwei San, and Prof. Dato' Dr. Sheikh Omar for all your constructive suggestions, valuable guidance and fruitful discussions that have aided my study.

To Yi Wan, you are the best partner one could ever have. *Thank you* for dividing the task and multiplying the success. To the rest of Dr Zeenat's group members: Noraini, Hidayah, Morvarid, Homayoun, Ruzila, Tan, Nik, Lo, Tam, Caryn, Parisa, Faezah, Fatemah, Noushin and Tarlan, *thank you* for making my challenges less challenging, and my problems less problematic. To Iffah, Fahmi, En. Din, En. Nazri, and Miza, *thank you* for sharing your technical expertise and for teaching me from scratch. To my best friend Dr. Amer and virology lab mates Dr. Mayada, Dr. Saeed, Dr. Faruku, Dr. Rohaya, Afzal and Dr. Majed, *thank you* for the warmth of your friendship. *Thank you* to UiTM and MOHE for granting me the scholarship and the opportunity to pursue my dreams in becoming a better educator. To my colleagues: En. Zed, Ann Erynna, Dr. Emida, Hisham, Nisa, Hadi, Dr. Maimunah, Dr. Mazlina, Dr. Jack, Pn. Azlin, Hartini, Dr. Mazura, Dr. Izham, Dr. Roslinah and Tn Hj Nazri *thank you* for the uncountable support and motivation during the hard and stressful moments.

To my beloved parents Camalxaman Md Nor & Che Nu Hashim: *thank you* for helping me in all ways imaginable, for praying for me day and night, and for taking care of the kids while I was busy taking care of my virus. I am truly blessed. To my parents-in-law Jamaludin Mohamad & Zaini Badiuzzaman, siblings Nazri, Nazrul Nazmi & Nastiti and extended family, Mazuin, Iya, Elia, Aca, Ainur, Ayu, Sarah and Wonder, *thank you* for your love and support. To my soul mate Adi Suria, *thank you* for being with me throughout this roller-coaster ride and for being my pillar of strength during my most difficult times. Without you, I would have crumbled and I'm certain this work would have never occurred. To Irfan & Reza, you are both the reasons why I kept on going and never gave up. I apologize for spending more time in the lab than I did at home, but I promise to catch up on lost time. I hope that my struggles will inspire you both to reach your fullest potential and to fight for your dreams.

Last but not least, *thank you* to the members of my Thesis Examination Committee who took their time to go through, deliberate and examine my thesis. At last, my hard work, perseverance and tenacity paid off, and for that I thank Allah. I certify that a Thesis Examination Committee has met on 10 May 2013 to conduct the final examination of Siti Nazrina Camalxaman on her thesis entitled "Rat Cytomegalovirus Genome Scaffold and A033 Gene as Infection Marker in Rats" 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 institutions.



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