GENETIC AND FUNCTIONAL ANALYSIS OF THE PROTEOLYTIC CLEAVAGE AT THE JUNCTION OF THE NS1 AND NS2A PROTEINS OF MURRAY VALLEY ENCEPHALITIS VIRUS

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Dedicated to my parents Addis Addi & Rokiah Ali
&
To my husband Ahmad Faisal Mohamad Ayob

Statement

The work described in this thesis was performed by the author under the supervision of Assoc. Prof. Dr. Mario Lobigs, Dr. Jayaram Bettadapura and Dr. Eva Lee at The John Curtin School of Medical Research, Canberra, Australia. These studies were completed between Feb 2007 and July 2011 to fulfill requirements for the degree of Doctor of Philosophy in The Australian National University, Canberra, Australia. This thesis does not contain any material that has been accepted for the award of any other degree at this or any other University. To the best of my knowledge, this thesis does not contain any material that has been published previously, except where due reference is made in the text. The research described in this thesis is my own original work unless otherwise stated in the text.

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Pulau Pandan jauh ke tengah
Gunung Daik bercabang tiga
Hancur badan dikandung tanah
Budi yang baik dikenang juga

Abstract

Flaviviruses are a group of positive-strand RNA viruses of global significance. The flavivirus RNA genome encodes a single open reading frame that is directly translated into a single polyprotein and cleaved by host and virally encoded proteases prior to protein maturation. Hence, the proteolytic cleavage events play a central role in the process of viral gene expression in flaviviruses. Despite the importance of these events, the mechanism of proteolytic cleavage leading to the generation of the two non-structural proteins, NS1 and NS2A is poorly defined.

Sequence comparisons among the flaviviruses and experimental work on Dengue virus (DENV) NS1-NS2A cleavage revealed an octapeptide sequence motif at the C-terminus of NS1 predicted to allow recognition by a protease for the cleavage of the NS1 and NS2A proteins. Of the eight-residue recognition sequence, positions P1, P3, P5, P7 and P8 (with respect to N-terminus of NS2A protein) are highly conserved and substitutions in these positions influenced DENV NS1-NS2A cleavage efficiency. However, the role of this recognition sequence in NS1 and NS2A production of other flaviviruses has not been experimentally addressed to date. It is also unclear whether insight gained from subgenomic expression experiments carried out in DENV is applicable to other flaviviruses. In this thesis, investigations were carried out *in vitro* and *in vivo* to assess the role of the octapeptide motif, in the efficiency of cleavage at the Murray Valley encephalitis virus (MVEV) NS1-NS2A junction. Expression cassettes encoding NS1 and NS2A genes were engineered and used for the site-directed mutagenesis of residues in the octapeptide motif. Analysis from the mutagenesis studies showed that cleavage efficiency is influenced by mutations at conserved and non-conserved residues in the octapeptides of MVEV, putatively recognized by a host protease, although overall mutations at the conserved octapeptide residues impacted more on cleavage efficiency than mutations at the non-conserved positions.

Subsequently, four mutations in the octapeptide sequence (P2-Gly, P3-Gly, P8-Ala and P7,8-Ala) were introduced into an MVEV full-length infectious clone to

investigate the impact of the substitutions during virus infection. Analysis from this study demonstrates for the first time that the efficiency of NS1-NS2A cleavage tightly controls viral RNA replication, growth in mammalian and insect cells, and virulence in mice. Despite poor conservation of amino acid at the position P2 in the octapeptide sequence, a Gin to Gly substitution at this position dramatically reduced virus replication, as demonstrated by small plaque morphology, poor RNA replication, impaired protein processing and attenuation of virulence in IFN-α-receptor knock-out mice relative to the wild-type virus. On the contrary, non-conservative changes at highly conserved residue P3 (Val→Ala) and P8 (Leu→Ala), only slightly reduced NS1-NS2A cleavage efficiency relative to wild-type and did not markedly affect virus replication. These results clearly implicate a direct association of NS1-NS2A processing with viral replication, and suggest a vital role for the octapeptide motif in modulating NS1-NS2A proteolytic cleavage.

Finally, multiple growth passages of the two NS1-NS2A cleavage defective mutants, rP2-Gly and rP7,8-Ala in cell culture and mice had generated variants with revertant phenotypes. Interestingly, sequencing of the viral genome revealed that the variants had a second-site mutation in E protein in addition to the P2-Gly and rP7,8-Ala mutation at the NS1-NS2A junction. Introduction of compensatory mutation in codon 65 of E (V65A) together with the P2-Gly mutation restored the virus growth in cell culture. This result illustrates for the first time that growth deficiency of MVEV NS1-NS2A cleavage site mutants could be substantially repaired by compensatory mutations in E protein, suggesting an as-yet-unidentified role of the structural protein in NS1-NS2A cleavage or down-stream replication events. This proposition is further supported by the isolation of a putative E-NS1 polypeptide in rP2-Gly variant (containing the V65A change in E) infected cells, showing an interaction of E with NS1.