



UNIVERSITI PUTRA MALAYSIA

***MOLECULAR IDENTIFICATION OF DENGUE VIRUS BINDING PROTEIN
ON FILOPODIA OF VERO CELLS***

ISAH ABUBAKAR ALIYU

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By

ISAH ABUBAKAR ALIYU

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

May 2017

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DEDICATION

This thesis was dedicated to my lovely father Alh. Abubakar Aliyu who passed away few weeks to the completion of this write-up (on 11th January 2017). May ALLAH (Subhanahu wata'ala) forgive his sins and make Jannatul Firdausi his final abode Amin.



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

**MOLECULAR IDENTIFICATION OF DENGUE VIRUS BINDING PROTEIN
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ISAH ABUBAKAR ALIYU

May 2017

Chair : Associate Professor Chee Hui Yee, PhD
Faculty : Medicine and Health Sciences

Despite much efforts to control dengue virus, there is still neither scientifically proven effective drug nor an effective internationally licensed vaccine to treat or control dengue. Recent evidence has demonstrated that dengue virus requires an active filopodia formation for successful infection. However, the precise mechanism for dengue virus interaction with filopodia to enhance virus infection is not fully understood. Therefore, the present study was designed to identify the dengue virus 2 binding protein that could be responsible for enhanced virus infection upon filopodia formation. Filopodia formation in Vero cells was induced with bradykinin and a combination of virus overlay protein binding assay (VOPBA) and LC-MS/MS was employed to identify annexin II as a dengue virus 2 binding protein on filopodia-induced cells. Translocation of annexin II to the external leaflet of plasma membrane following filopodia formation was demonstrated by immunocytochemistry staining and western blotting analysis. Down-regulation of plasma membrane annexin II was observed in response to dengue virus 2 infection. Moreover, anti-annexin II antibody inhibited virus binding in an antibody-dependent binding inhibition assay and a dose-dependent reduction in both dengue virus infection level and virus production was demonstrated in an antibody-mediated infection inhibition assay. Similarly, siRNA-mediated knockdown of annexin II gene significantly reduced virus infection level as well as virus production. Confocal microscopic examination showed extracellular and intracellular colocalization between annexin II and dengue virus 2 E glycoprotein, and co-immunoprecipitation assay confirmed that annexin II interacted with dengue virus 2 E glycoprotein. Furthermore, the putative annexin II interaction site on dengue virus 2 E glycoprotein was predicted using molecular docking and the interaction was likely to occur at residues Ile-380 to Leu-389 of E glycoprotein, with Ser-22 to Asn-32, Leu-300 to Tyr-317 and Lys-328 to Asp-338 of annexin II. Data from the present study reported for the first time the expression of a dengue virus 2 binding protein upon filopodia formation, which might be involved in dengue virus 2 binding, internalization and intracellular trafficking. Thus, the results could explain the likely reason behind enhanced virus infection observed upon filopodia formation and could be a potential target for the development of a novel antiviral.

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

IDENTIFIKASI PROTEIN PENGIKAT VIRUS DENGGI DENGAN FILOPODIA DALAM SEL VIRO PADA PERIGKAT MOLEKULAR

Oleh

ISAH ABUBAKAR ALIYU

Mei 2017

Pengerusi : Profesor Madya Chee Hui Yee, PhD
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Walaupun pelbagai usaha telah diambil untuk mengawal virus denggi, namun masih tiada pembuktian secara saintifik ubat yang berkesan mahupun vaksin yang dilesenkan secara meluas untuk merawat atau mengawal denggi. Kajian terkini telah menunjukkan bahawa virus denggi memerlukan pembentukan filopodia yang aktif untuk sesuatu jangkitan itu berjaya. Walau bagaimanapun, mekanisme interaksi antara virus denggi dengan filopodia yang mengakibatkan peningkatan infeksi masih belum difahami sepenuhnya. Oleh itu, kajian ini adalah bertujuan untuk mengenal pasti protin pengikat virus denggi serotaip 2 yang mungkin bertanggungjawab dalam peningkatan infeksi berikutan pembentukan filopodia. Dalam kajian ini, kombinasi penggunaan teknik Virus Overlay Protein Binding Assay (VOPBA) dengan LC-MS/MS telah mengenalpasti annexin II sebagai protin pengikat virus denggi serotaip 2 dalam sel-sel Vero yang telah dirangsangkan pembentukan filopodia oleh bradykinin. Translokasi annexin II ke permukaan luaran membran plasma selepas pembentukan filopodia juga dapat diperhatikan dengan menggunakan pewarnaan immunositokimia dan pedapan Western. Sementara itu, ekspresi annexin II di permukaan membran plasma didapati menurun berikutan infeksi virus denggi serotaip 2. Antibodi anti-annexin II didapati merencatkan pengikatan virus dalam ujian perencatan pengikatan di mana jangkitan serta penghasilan virus denggi berkurang bersandarkan dos antibodi. Manakala, penggunaan siRNA yang boleh merencatkan pengekspresan gen annexin II juga didapati mengurangkan tahap jangkitan serta penghasilan virus secara ketara. Pemeriksaan mikroskopi konfokal menunjukkan persetempatan annexin II dengan protin E virus denggi serotaip 2 di luaran dan di dalaman sel. Selanjutnya, ujian pemendakan ko-immuno telah mengesahkan bahawa annexin II sememangnya berinteraksi dengan protin E virus denggi serotaip 2. Melalui program dok molekul, lokasi interaksi annexin II dengan protin E virus denggi serotaip 2 telah diramalkan berlaku pada lokasi Ile380 hingga Leu389 pada protin E dengan Ser-22 hingga Asn-32, Leu-300 hingga Tyr-317 dan Lys-328 hingga Asp-338 pada annexin II. Buat kali pertamanya kajian ini telah mengenalpasti pengekspresan protin pengikat virus denggi serotaip 2 berikutan pembentukan filopodia yang mungkin terlibat dalam pengikatan, internalisasi dan pengedaran selular virus denggi serotaip 2. Oleh itu, hasil kajian ini dapat menerang sebab peningkatan infeksi virus yang diperhatikan berikutan pembentukan filopodia dan ini boleh dijadikan sebagai sasaran yang berpotensi untuk pencarian antiviral yang novel.

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I certify that a Thesis Examination Committee has met on 15 May 2017 to conduct the final examination of Isah Abubakar Aliyu on his thesis entitled “Molecular identification of dengue virus binding protein on filopodia of vero cells” 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 (insert the name of relevant degree).

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LIST OF ABBREVIATIONS

ARP2/3	Actin-related protein 2/3
BHK	Baby hamster kidney cell line
C protein	Core protein
CD4	Cluster of differentiation 4
CMV	Cytomegalovirus
CPE	Cytopathic effect
DAPI	4',6-diamidino-2-phenylindole
DC-SING	Dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin
DHF	Dengue hemorrhagic fever
Dia2	Diaphanous-related formin-2
DMSO	Dimethyl sulfoxide
DSS	Dengue shock syndrome
E glycoprotein	Envelope glycoprotein
EMEM	Eagles minimal essential media
ENA/VASP	Enabled/vasodilator-stimulated phosphoprotein
FBS	Foetal bovine serum
FITC	Fluorescence isothiocyanate
HCV	Hepatitis C virus
HPV	Human papillomavirus
LC-MS/MS	Liquid chromatography- mass spectrophotometry/mass spectrophotometry
M/prM protein	Membrane protein, and precursor protein pr

MENA	Mammalian enabled
NSP	Non-structural protein
PI	Post infection
PT	Post transfection
PVDF	Polyvinylidene fluoride
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SiRNA	Small interference RNA
TCID50	Tissue culture infectious dose 50
VOPBA	Virus overlay protein binding assay
WASP	Wiskott-Aldrich syndrome protein
WAVE2	(WASP) family verprolin-homologous protein 2

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CHAPTER 1

INTRODUCTION

1.1 Background of the studies

Dengue virus is the most widespread mosquito-borne virus infection with immense global health importance. It is an etiologic agent of a mild fever known as dengue fever, or a more severe dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). The disease is ubiquitous in tropical and subtropical countries especially in Asia and Latin America, it is albeit endemic in more than 100 countries (Ligon, 2005). Dengue virus global prevalence was estimated using cartographic modelling to be 390 million cases annually, with about 96 million cases requiring hospitalization, and more than 200,000 annual mortality rates, 22,000 of which is among minor (Bhatt et al., 2013a; Pang and Loh, 2016; Rigau-Pérez et al., 1998).

Despite the prominence and growing incidence of dengue virus infection, there is no specific antiviral agents or widely accepted licensed vaccines available for prevention or treatment of dengue virus infection. Generally, viral enzyme targeted drugs are used to treat viral infections, however the main problem in the use of these types of drugs is the emergence of resistance (Kieffer and George, 2014; Pennings, 2012; van der Vries et al., 2013). Research for dengue virus vaccines has been hampered by many factors including antibody-dependent enhancement of infection, original antigenic sin hypothesis and poor immunogenicity of subunits vaccines. Because of these factors, antiviral drug discovery has been tilted toward the possibility of developing host-oriented molecules that act on the cellular functions which are essential for virus entry and replication (de Chasse et al., 2014). This therapeutic strategy has successfully been used with promising result and has been approved for clinical use in HIV infection (Haqqani and Tilton, 2013).

Viral entry-associated proteins on the surface of target cells are important basic information for designing prevention and treatment strategies for viral diseases. Hence there is need to employ this therapeutic strategy in dengue virus by targeting virus binding molecules on susceptible host cells. Blockage of this molecules will prevent virus binding, entry and establishment of infection. However, to date, there is no detailed information on dengue virus interacting molecule which is indispensable for dengue virus attachment, trafficking, and internalization. This makes it difficult or almost impossible to address dengue virus entry-associated proteins for the development of dengue virus therapeutic.

Many studies have been carried out to identify molecules involved in the dengue virus host interaction. Independent studies showed that different proteins, including high-affinity laminin receptor (Thepparit and Smith, 2004; Tio et al., 2005), CD14-associated protein (Chen et al., 1997), and uncharacterized proteins (de Jesús Martínez-Barragán and del Angel, 2001) may be involved in dengue virus-host cell interaction, detailed of these molecules have been reviewed by Cruz-Oliveira et al. (2014). However, it has been

reported therein, that these molecules were not involved in dengue virus high-affinity interaction and internalization.

A possible candidate route for dengue virus entry into the host cell via interaction with cellular protrusion like filopodia has also been reported. Viruses have been observed to induce cytoskeletal rearrangement, which is associated with cellular signals that lead to the formation of filopodia (Smith et al., 2008). This is seen to be crucial in viral infection. After virus attachment, filopodia has been reported to play an important role in the virus confinement to the plasma membrane for internalization (Ewers et al., 2005; Pelkmans et al., 2002). Dengue virus was also reported to induce filopodia formation on host cells for successful infection (Zamudio-Meza et al., 2009). However, the molecular mechanism and protein interaction associated with virus-filopodia interaction has never been investigated. Hence, this research was designed to investigate the molecular mechanism and to identify dengue virus 2 interacting protein on filopodia of host cells involved in virus binding, trafficking, and internalization. It has been hypothesized in the present study that filopodia formation results in the expression of key molecules on the cells which are important for dengue virus interaction and internalization.

1.2 Objectives

The main objective of this study is to identify dengue virus 2 binding proteins on filopodia.

Specific objectives were to:

- i. chemically induce filopodia formation, investigate and identify dengue virus 2 binding protein in plasma membrane fraction of filopodia-induced cells.
- ii. determine the expression of this dengue virus 2 binding protein in filopodia-induced cells, and whether the expression of this protein is altered in response to dengue virus 2 infection
- iii. determine whether this dengue virus 2 binding protein plays a receptor role in dengue virus 2 infection
- iv. investigate and determine intracellular interaction between this protein and dengue virus 2 E glycoprotein.

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