Host Cellular Regulatory Networks in Dengue Virus-Human Interactions

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AUTHOR'S DECLARATION

I declare that, except where explicit reference is made to the contribution of others, this thesis is the result of my own work and has not been submitted for any other degree at the University of Glasgow or any other institution.

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ABSTRACTS

Dengue fever is one of the most important mosquito-borne diseases worldwide and is caused by infection with dengue virus (DENV). The disease is endemic in tropical and sub-tropical regions and has increased remarkably in the last few decades. At present, there is no antiviral or approved vaccine against the virus. Treatment of dengue patients is usually supportive, through oral or intravenous rehydration, or by blood transfusion for more severe dengue cases. Infection of DENV in humans and mosquitoes involves a complex interplay between the virus and host factors. This results in regulation of numerous intracellular processes, such as signal transduction and gene transcription which leads to progression of disease. To understand the mechanisms underlying the disease, the study of virus and host factors is therefore essential and could lead to the identification of human proteins modulating an essential step in the virus life cycle. Knowledge of these human proteins could lead to the discovery of potential new drug targets and disease control strategies in the future.

Recent advances of high throughput screening technologies have provided researchers with molecular tools to carry out investigations on a large scale. Several studies have focused on determination of the host factors during DENV infection in human and mosquito cells. For instance, a genome-wide RNA interference (RNAi) screen has identified host factors that potentially play an important role in both DENV and West Nile virus replication (Krishnan et al. 2008). In the present study, a high-throughput yeast two-hybrid screen has been utilised in order to identify human factors interacting with DENV non-structural proteins. From the screen, 94 potential human interactors were identified. These include proteins involved in immune signalling regulation, potassium voltage-gated channels, transcriptional regulators, protein transporters and endoplasmic reticulum-associated proteins. Validation of fifteen of these human interactions revealed twelve of them strongly interacted with DENV proteins. Two proteins of particular interest were selected for further investigations of functional biological systems at the molecular level.

These proteins, including a nuclear-associated protein BANP and a voltage-gated potassium channel Kv1.3, both have been identified through interaction with the DENV NS2A. BANP is known to be involved in NF-κB immune signalling pathway, whereas, Kv1.3 is known to play an important role in regulating passive flow of potassium ions upon changes in the cell transmembrane potential.

This study also initiated a construction of an *Aedes aegypti* cDNA library for use with DENV proteins in Y2H screen. However, several issues were encountered during the study which made the library unsuitable for protein interaction analysis. In parallel, innate immune signalling was also optimised for downstream analysis. Overall, the work presented in this thesis, in particular the Y2H screen provides a number of human factors potentially targeted by DENV during infection. Nonetheless, more work is required to be done in order to validate these proteins and determine their functional properties, as well as testing them with infectious DENV to establish a biological significance. In the long term, data from this study will be useful for investigating potential human factors for development of antiviral strategies against dengue.