UNIVERSITI TEKNOLOGI MARA

ELUCIDATING PROTEIN-PROTEIN INTERACTIONS BETWEEN HOST CELL RECEPTORS AND OUTER MEMBRANE PROTEIN A (OMPA) OF *ORIENTIA TSUTSUGAMUSHI* IN THE PATHOGENESIS OF CELLULAR INVASION

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ABSTRACT

Scrub typhus, caused by *Orientia tsutsugamushi*, remains an underdiagnosed and re-emerging infectious disease. Its antigenic variability complicates vaccine and diagnostic development. Outer membrane protein A (OtOmpA), highly conserved across strains and detected during various stages of infection, has been proposed as a promising target. However, its specific role in the pathogenesis of scrub typhus, particularly in human endothelial cells, the primary target cells, remains poorly understood. This study aimed to investigate the interaction between OtOmpA and proteins from human pulmonary (HPAEC) and cerebral microvascular endothelial cells (HBEC-5i). DNA sequence alignment using Jalview and ligand-binding pocket prediction with AutoDockFR-AGFR, visualised by PyMOL, confirmed OtOmpA's suitability as a study target and guided sequence selection for cloning. The Gilliam strain DNA sequence was cloned into three expression vectors (pET-14b(+), pET-20b(+), and pET-28a(+)). Positive colonies were identified by PCR and agarose gel electrophoresis, and DNA sequencing confirmed the inserts. Recombinant vectors were expressed in *E. coli* BL21(DE3) pLysS, and OtOmpA production was verified via SDS-PAGE, Western blotting, LC-MS/MS, and positive serum from scrub typhus a patient. The adherence and invasion capabilities of OtOmpA were assessed by immunofluorescence microscopy. Protein–protein interactions were analysed through a poly-His-tag pull-down assay and 1D-LC-MS/MS. Molecular docking and binding site prediction were performed using HDOCK and visualised in PyMOL, while MEMSAT-SVM predicted transmembrane helix topology. In this study, although all vectors contained the correctly oriented OtOmpA sequence, only pET-20b(+) successfully expressed the protein. OtOmpA enhanced *E. coli* association with endothelial cells, suggesting a role in adhesion and invasion. An integrated *in vitro* and *in silico* approach identified several host interacting proteins: heat shock protein β, integrin alpha-X, caspase-4, baculoviral IAP repeat-containing protein 6, serine/threonine-protein kinase ATR, glucose-6-phosphate dehydrogenase, oxysterol-binding protein-related protein 8, and phosphatidylinositol 4-kinase beta. Two OtOmpA regions, V_{68}–D_{112} and R_{124}–T_{157}, were predicted as key binding domains. In conclusion, this study provides novel insights into OtOmpA's role in scrub typhus pathogenesis, offering potential guidance for developing targeted diagnostics, vaccines, and therapeutic strategies.

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CHAPTER 1
INTRODUCTION

1.1 Research Background

Scrub typhus is one of the most underdiagnosed and underreported febrile illnesses worldwide (Luce-Fedrow et al., 2018). Despite being easily treated with antibiotics, such as chloramphenicol and doxycycline, scrub typhus is still considered life-threatening, with a fatality rate being as high as 70 % (Yang et al., 2019). The complications vary depending on the individual. Some patients only experience high fevers and rashes, with symptoms mimicking other common febrile illnesses such as malaria, dengue, chikungunya, and leptospirosis (Chaudhry et al., 2019; Prakash, 2017). Due to these similar symptoms, patients are often misdiagnosed with another disease, leading to delayed or inaccurate antibiotic treatment (Lin et al., 2019). As a result, patients may develop serious complications such as severe headache, lymphadenopathy (abnormal size of lymph nodes due to swelling or enlargement), myalgia (muscle pain), pneumonitis, meningitis, and hepatosplenomegaly (Bonell et al., 2017). In certain cases, patients may also develop life-threatening conditions such as acute respiratory distress syndrome which leads to lung injury (Trent et al., 2019), or serious neurological complications (Mahajan & Mahajan, 2017) that could lead to multi-organ failure (Rungta, 2014).

The only visible remarkable symptom during early infection of scrub typhus is the presence of eschar. Eschar forms on the patient's skin surface due to the bite of infected *Leptotrombidium* chiggers (larval mites) (Vincent, 2016). *Leptotrombidium* chiggers have been known for decades as the primary vector that transmits *Orientia tsutsugamushi*, the causing agent of scrub typhus, to humans (Paris et al., 2013). However, in certain cases, eschar is hardly discovered as it is commonly present in hidden folds of the skin, such as the fold of the anterior cervical and auricle skin, scrotum, inguinal area, and the base of the thigh (Lin et al., 2019). Multiple broad-spectrum antibiotics were given to a group of children with scrub typhus who were misdiagnosed with another disease because the eschar was invisible during the first physical examination (Lin et al., 2019). The high fever experienced by the children did not subside until they received appropriate antibiotic treatment (chloramphenicol and