Abstract of Dissertation submitted by Chuang Huai

Title: SICA-mediated cytoadhesion of *Plasmodium knowlesi*-infected red blood cells to human umbilical vein endothelial cells

二日熱マラリア原虫感染赤血球の SICA タンパク質を介したヒト静脈臍帯内皮細胞への細胞接着

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Introduction:

Zoonotic malaria due to *Plasmodium knowlesi* infection in Southeast Asia is sometimes life-threatening. Post-mortem examination of human *knowlesi* malaria cases showed sequestration of *P. knowlesi*-infected red blood cells (iRBCs) in blood vessels, which has been proposed to be linked to disease severity. This sequestration is likely mediated by the cytoadhesion of parasite-iRBCs to vascular endothelial cells. In *P. falciparum*, sequestration is known to be mediated by a parasite ligand, called *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1), which is expressed on the surface of iRBCs and confers adhesion to vascular endothelial cells. Sequestration can prevent the clearance of iRBCs by the spleen and serves as a major virulence factor. Sequestration of *P. knowlesi*-iRBCs in blood vessels has been reported both in humans and monkeys. However, in *P. knowlesi*, the responsible parasite ligands remain undetermined. To gain insights into the mechanism of sequestration in *P. knowlesi*-iRBCs in humans, I aimed to identify molecule(s) responsible for the cytoadhesion of *P. knowlesi*-iRBCs to human vascular endothelial cells.

Materials and Methods:

Plasmodium knowlesi parasite line with cytoadhesion phenotype was harvested by repeating panning selection with Human Umbilical Vein Endothelial Cells (HUVECs). Genome-wide RNA-seq analysis was performed to compare the transcripts' expression level before and after panning selection. A transgenic parasite line expressing identified protein tagged with myc epitopes was generated. The expression and the localization of the identified protein was evaluated by Western blot and indirect immunofluorescence assay (IFA), respectively. Exposure of the identified protein on the iRBC was validated with trypsin treatment of iRBCs followed by the detection of the cleaved protein products. Cytoadhesion

activity of monkey or human RBCs infected with the line expressing identified protein was examined by comparing with the wild-type parasite or a control parasite line expressing a fluorescent mCherry.

Results:

P. knowlesi lines with increased iRBC cytoadhesion activity were obtained by repeating panning against HUVECs. Transcriptome analysis revealed that the transcript level of one gene, encoding a Schizont Infected Cell Agglutination (SICA) protein, herein termed SICA-HUVEC, was more than 100-fold increased after the panning. Transcripts of a panel of other *P. knowlesi* proteins exported to the iRBC cytosol were also significantly increased, suggesting their potential roles in increasing cytoadhesion activity. Western blot with anti-myc antibody identified a protein of expected size. By IFA, the fluorescent signal was observed in the cytosol of the iRBC. Trypsin cleavage treatment clearly indicated the expression of SICA-HUVEC on the surface of iRBCs. Transgenic *P. knowlesi* parasites expressing myc-fused SICA-HUVEC increased cytoadhesion activity following infection of monkey as well as human RBCs, confirming that SICA-HUVEC conveys activity to bind to HUVECs.

Discussion:

This is the first time to identify the *P. knowlesi* ligand expressing on the iRBCs responsible for the cytoadhesion to human vascular endothelial cells. I also found that RBCs infected with transgenic *P. knowlesi* expressing myc-tagged SICA-HUVEC did not bind as much as the naturally selected HUVEC-binding lines. qRT-PCR revealed that a similar amount of the transcripts for the putative ligand were detected from the transgenic line compared to the naturally selected parasites, indicating that the transcript levels did not determine the difference in the cytoadhesion activity. It is possible that other alterations might be required for the stronger binding activity; for example, I found that the transcript levels of several *P. knowlesi* open reading frames encoding proteins that are exported to the iRBC cytosol were consistently changed in addition to SICA-HUVEC, which might contribute to the increased cytoadherence activity of the naturally selected lines. A further investigation is required to clarify this point. Identification of the host receptor of SICA-HUVEC would significantly increase our understanding of cytoadhesion in the pathogenesis of *knowlesi* malaria. A previous report proposed PECAM1, ICAM1, and VCAM1 as receptors for the *P. knowlesi*-iRBCs. Because these proteins are expressed on HUVECs, it is interesting if they are recognized by SICA-HUVEC. It is also of interest to determine if the identified SICA-HUVEC has a role in the sequestration of intraerythrocytic parasites in humans.

Notes: Summarize your dissertation with 2 pages of A4 (using 12 point, Times New Roman font, single space. Total number of words should not exceed 1000）