Dissertation Evaluation Report

Report No.	Diploma Number: D-BIO1479	Applicant's Name	Chuang Huai

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Evaluation Report of Dissertation

1. Evaluation of the research purpose.
Plasmodium knowlesi infection is sometimes life-threatening in Southeast Asia. The sequestration of *P. knowlesi*-infected RBCs (iRBCs) in blood vessels has been reported in humans and monkeys. However, the responsible *P. knowlesi* ligands remain undetermined. Therefore, the study aimed to identify molecule(s) responsible for the cytoadhesion of *P. knowlesi*-iRBCs to human vascular endothelial cells. Thus, the purpose is clearly described and reasonable.

2. Evaluation of the research methods.
To obtain *P. knowlesi* lines with cytoadhesion phenotype, repeated panning with human umbilical vein endothelial cells (HUVECs) was applied. To identify molecules responsible for adhesion, genome-wide RNAseq was done. The transgenic *P. knowlesi* lines expressing a candidate molecule were generated. Cytoadhesion activity of infected monkey or human iRBCs with wild-type and transgenic *P. knowlesi* was compared together with the expression and localization of the molecule. The research methods were appropriate, well-designed, and soundly performed.

3. Evaluation of the analysis, interpretation and discussion.
P. knowlesi lines with increased cytoadhesion activity to iRBC were obtained by repeating panning against HUVECs. Transcriptome analysis revealed a schizont-infected cell agglutination (SICA) protein, herein termed SICA-HUVEC, that may be responsible for adhesion. Transgenic The monkey and human iRBCs with *P. knowlesi* expressing SICA-HUVEC increased cytoadhesion to HUVEC, confirming that SICA-HUVEC conveys activity to bind to HUVECs. However, the cytoadhesion activity of the transgenic *P. knowlesi*-iRBC was not as high as the *P. knowlesi* wild-type lines obtained by panning. The results were appropriately discussed.

As stated above, the dissertation will significantly contribute to gaining insights into the mechanism of sequestration of *P. knowlesi*-iRBCs in humans during blood-stage infection, and the evaluators uniformly agree that the dissertation is worthy of being approved for a Doctor of Philosophy in Medical Science.