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CLONING AND CHARACTERIZATION OF SOME REP20 DNA FRAGMENTS FROM THE GENOME OF THE HUMAN MALARIA PATHOGEN *PLASMODIUM FALCIPARUM*

By

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Abstract

Chromosomes of the human malaria parasite *Plasmodium falciparum* contain long subtelomeric repeat sequences and little is known about them. In this study, we have cloned 10 fragments of the non-coding rep20 sequence from the genome of *Plasmodium falciparum* 3D7 and HB3 strains. Analysis of these fragments showed that they represent 4 different 3D7 fragments and 2 different HB3 ones. Blasting the sequence of these fragments to the PlasmoDB revealed a varying degree of identity to the released rep20 sequence. One of these fragments was found to contain 27 degenerate repeats and show the highest consistency with the rep20 consensus sequence. This fragment was inserted into a plasmid construct containing the green fluorescence gene and a stably transfected *plasmodium* cell line was established. Our data show that this rep20 fragment enhances the establishment of drug-resistant parasite populations after transfection, however it restricts the expression of the green fluorescence transgene. These results attract attention to an in-depth study of the role that some rep20 sequences may play between the telomeres and the differentially expressed virulence-related genes.

Key words: *Plasmodium falciparum*, Subtelomere, rep20, Transfection, gene expression.

Abbreviations: hdhfr, human hydrofolate reductase; Myc, cellular homologue of myelocytomatosis virus 29 oncogene; rhoph2, rhoptry protein 2; gfp, green fluorescent protein.

Introduction

Human malaria is known to be caused by either *Plasmodium falciparum, P. vivax, P. ovale* or *P. malariae*. A naturally acquired infection of human with the simian malaria parasite, *Plasmodium knowlesi*, was recorded by Jongwutiwes et al. (2004) and a widespread distribution of such infection in Thailand was recently highlighted by Putaporntip et al. (2009). Among these 5 human malaria species, *P. falciparum* is the most potent and deadly parasites, and at least one-third of the world's population is at risk of infection, with over 300 million people developing clinical disease each year and at least 2 million deaths (Snow et al., 2005). Thus, malaria puts a huge economic burden upon the affected countries in addition to the suffering the infected individuals endure.

Plasmodium falciparum is transmitted by female *Anopheles* mosquitoes when sporozoites of the parasite are injected into human host during a blood meal. During its life cycle, *plasmodium* undergoes three cycles of replication; asexual pre-erythrocytic cycle in the liver, asexual erythrocytic cycle in the red blood cells and sexual cycle in the mosquito midgut. The high morbidity and mortality associated with *falciparum* malaria are related to the intraerythrocytic stages of the parasite (Miller et al, 2002).