ABSTRACT

In this study, we applied Plasmodium Artificial Chromosome (PAC) in the identification of genetic markers conferring resistance to dihydroartemisinin (DHA). Six artemisinin-resistant *P. falciparum* reference lines from the parasite depository at Malaria Research and Reference Reagent Resource Center (MR4), USA, were re-assessed for their level of resistance to DHA using the Ring-stage Survival Assay (RSA). We confirmed that the parasites line number MRA1236, MRA1240, and MRA1241 exhibited various degrees of resistance to the DHA treatment whereby MRA1240 displayed the highest artemisinin resistance among the resistant isolates in accordance with the published report. Gene library of MRA1240 isolate has been constructed using PAC technology. Using modified protocol, matured schizont-stage of the artemisinin-sensitive *P. falciparum* laboratory strain 3D7 parasites were purified and transfected with the PAC plasmid containing the MRA1240 gene library. The transgenic parasites harboring the pPAC1240 gene library plasmids containing the DNA fragments (library) were obtained as confirmed by Southern analysis. These transgenic parasites were assessed for their artemisinin sensitivity using the RSA. The results showed that all of them were sensitive to DHA, at the similar level to the 3D7 parental strain. However, for selection of DHA resistant transgenic parasites, another selection strategy using SAAR method was employed. After 4 rounds of low dose DHA treatment, we could obtain DHA-resistant 3D7_PAC1240 transgenic parasites which were subsequently isolated to single clones. One of the transgenic clones, 2G2, was analyzed for its inserted MRA1240 gene library using genome walking technique. The Southern, BLAST and chromosome analyses showed that the inserted MRA1240 gene library include a genome fragment of approximately 20 kB down-stream of the PF3D7_1448300.1 gene (coding for conserved protein with unknown function), which includes PF3D7_148200.1 (coding for condensin-2 complex subunit G2, putative), PF3D7_148100.1 (coding for conserved protein, unknown function), PF3D7_148000.1 (U3 small nucleolar RNA-associated protein 12, putative), and interestingly PF3D7_147900.1 (coding for multidrug resistance protein 2). From all the results and analysis, multidrug resistance protein 2 (MDR2) is the most promising gene candidate that may contribute to drug resistance to DHA in our study. We are in the process of reconfirming the role of PfMDR2 in DHA resistance by over-expression of PfMDR2 in the DHA-sensitive 3D7 parasite. We hypothesize that over-expression of MDR2 will make sensitive parasite become more resistance to DHA comparing to its parental parasite.

Although not proposed in the original proposal, as another approach to study the artemisinin resistance mechanism, we decided to study the currently proposed molecular marker for artemisinin resistance, PfKelch13, in more details by generating transgenic parasites harboring double and triple mutations of the K13 proteins and examine whether they have an effect on the degree of artemisinin resistance. Plasmids for introduction of different K13 mutation combinations to the parasites, pCas.BSgK13F and a series of pL6.SgK13B.K13mut, have been constructed and transfected to *k13* wild-type K1 parasite. At present, we have obtained transgenic parasites transfected with the plasmids. We are in the process of confirming sequences of the *k13* locus and assessing artemisinin sensitivity of these transgenic parasites.

In conclusion, this study has proposed PfMDR2 as a promising candidate that may contribute to the artemisinin resistance, although more studies are needed to confirm this finding. We hypothesize that over-expression of PfMDR2 will make sensitive parasite become more resistance to DHA comparing to its parental parasite. Once confirmed, PfMDR2 would be proposed as the marker for monitoring the progress of drug resistant parasites that will allow the public health authorities to plan effective treatment and control the spread of resistant parasites in the endemic areas.

Keywords: Artemisinin, drug resistance, biomarkers, Plasmodium Artificial Chromosome (PAC), *Plasmodium falciparum*