Development of the method for identifying drug resistance gene from Malaria parasites

Department of Molecular protozoology, Research Institute for Microbial Diseases

Professor Shiroh Iwanaga

Researchmap https://researchmap.jp/shirohiwanaga?lang=en

Abstract

Drug resistance is one of the biggest threats to malaria control. The identification of drug resistance genes not only assists for elucidating the mechanism of resistance, but also is useful for monitoring the spread of resistance. We developed a method for rapid identification of drug resistance genes by functional screening using Plasmodium falciparum artificial chromosome technology. We further succeeded in identifying a new mefloquine resistance gene from the drug-resistant parasite isolated from malaria patients in the border area between Thai-Myanmar by using the developed method, demonstrating its feasibility.

Background & Results

Malaria patients are currently treated using artemisinin combination therapy (ACT), in which artemisinin is used as a first-line drug along with partner drugs. ACT has greatly contributed to the decrease in malaria deaths over the past two decades; however, since 2009, treatment failure caused by resistance to the drugs used during ACT has been reported in endemic areas including South-East Asia and Africa. Therefore, there is concern that the therapeutic effects of ACT may be decreasing.

Identification of drug-resistance genes contributes to not only the elucidation of molecular mechanisms of resistance but also the development of molecular markers for surveillance; thus, it offers one potential solution to the problem of drug resistance. However, current method for identifying drug resistance gene using next generation sequencing technology is laborious and time consuming. To solve this technical limitation, we developed a method to identify resistance genes by functional screening; a high-coverage genomic library of a drug-resistant strain is directly generated in a drug-sensitive strain, and the resistance gene is then identified from this library using drug screening (Fig. 1). In this method, a parasite clone, in which a drug-resistant gene from a resistant parasite is introduced, acquires newly resistance and can survive during screening with drug. In contrast, other clones, in which genes unrelated to resistance are introduced, remains susceptible and is killed by screening. Indeed, this method was applied to mefloquine-resistant Plasmodium parasites with unknown resistance genes, and a novel transporter gene (multidrug resistance protein 7, PfMDR7) was successfully identified as a resistance gene, demonstrating its utility (Fig. 2).

Significance of the research and Future perspective

The method developed is totally different in principle from the current method using next-generation sequencing technology. The rapidity of the method is particularly noteworthy; drug resistance genes can be identified within only 2 to 3 months. In addition, only one strain of drug resistant parasite from a patient is enogh to for gene identification, and it is expected to enable the monitoring and containment of resistance before it spreads globally. In the future, it will be essential to monitor the emergence of resistance along with the development of new drugs, which is expected to make a significant contribution to the advancement of malaria control.

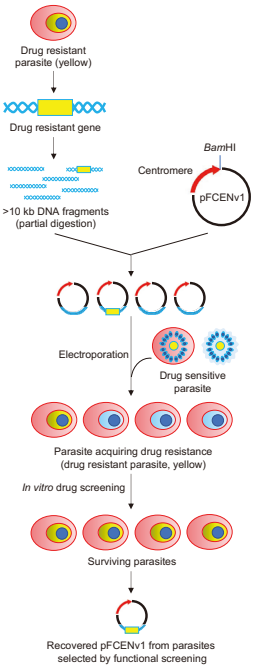

Figure 1: Schematic of the method for identification of drug resistance genes from P. falciparum.
Yellow and blue indicate drug-resistant and -susceptible parasites, respectively. In the presence of drug, the parasite (yellow), which acquires resistance newly by being introduced drug resistance gene, can survived. The drug resistance gene can be identified by analyzing the insert DNA fragment of artificial chromosome recovered from those survived parasites.

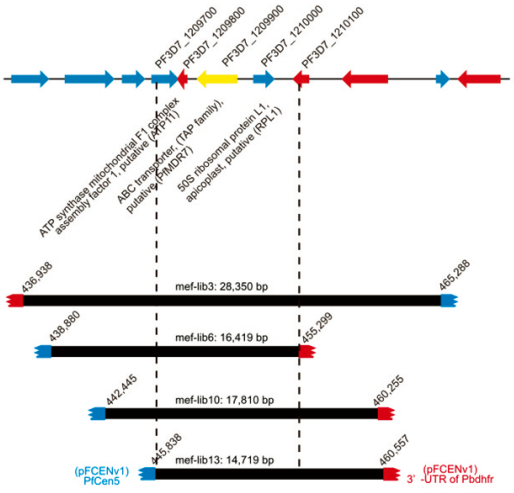

Figure 2: Identification of new mefloquine resistance genes.
Four independent DNA fragments were identified as a result of mefloquine screening, and all encoded the specific region on chromosome 12. Pf MDR7(yellow) was identified from the common region in those four fragments.

Patent Japanese Patent No.5773447

Treatise Iwanaga, Shiroh; Kubota, Rie; Nishi, Tsubasa et al. Genome-wide functional screening of drug-resistance genes in Plasmodium falciparum. Nat Commun. 2022,13(1):6163. doi: 10.1038/s41467-022-33804-w

URL

Keyword malaria, drug resistance gene, artificial chromosome