Topical Review

A Novel Transporter, Pfcrt, Confers Antimalarial Drug Resistance

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Abstract. The elucidation of the molecular details of drug resistance phenomena is a very active area of research that crosses many disciplinary boundaries. Drug resistance is due to altered drug-target interaction, and/or dysregulated signaling related to cell growth and death. Since many drugs need to rapidly diffuse into and within cells in order to find their targets, and since transmembrane ion transport is an important facet of cellular signaling, it is not surprising that membrane transport phenomena have been implicated in the evolution of drug resistance in tumor cells, bacteria, and intracellular parasites such as Plasmodium falciparum, the causative agent of the most lethal form of human malaria. The most infamous membrane transport protein involved in drug resistance is “MDR protein” or “P-glycoprotein” (Pgp), 1 which was found to be overexpressed in drug-resistant tumor cells over 15 years ago, and which is representative of the ATP-binding cassette (ABC) superfamily that also includes the important cystic fibrosis transmembrane conductance regulator (CFTR) and sulfonyl urea receptor (SUR) ion channels. Availability of mouse and human Pgp cDNA rather quickly led to the identification of homologues in many species, including P. falciparum, and these were de facto assumed to be the ultimate determinants of drug resistance in these systems as well. However, research over the past 10 years has taught us that this assumption likely is wrong and that the situation is more complex. We now know that human Pgp plays a relatively minor role in clinically relevant tumor drug resistance, and that an integral membrane protein with no homology to the ABC superfamily, Pfcrt, ultimately confers chloroquine resistance in P. falciparum. Thus, the general hypothesis that membrane transport and membrane transport proteins are important in drug resistance phenomena remains correct, but at a genetic, biochemical, and physiological level we have recently witnessed a few very interesting surprises.

Key words: Chloroquine resistance — Plasmodium falciparum — Malaria

Introduction

Malaria is caused by intracellular parasites from the genus Plasmodium. Over 100 Plasmodia species are found, four infect humans, and P. falciparum accounts for most of the mortality. A variety of drugs are used to treat malaria, including antifolates that poison DNA synthesis, as well as quinoline and acridine-based drugs that inhibit crystallization of heme to hematin. Hemozoin formation is a key step in the parasite’s catabolism of hemoglobin (Hb), which is a chief source of food during the intraerythrocytic stage of the P. falciparum life cycle. Because of its efficacy, low cost and stability, the antimalarial of choice has historically been the 4-aminquinoline chloroquine (CQ). However, during worldwide use of CQ over the past 50 years, CQ-resistant (CQR) strains of P. falciparum and P. vivax have both evolved and spread to the point where currently more people die of malaria each year than die of AIDS. Knowing what we now know about drug resistance, it is curious that CQR parasites took this long to appear on a large scale, but, as detailed below, one attractive explanation is now apparent for P. falciparum.

1Abbreviations: MDR, multidrug resistance; P-gp, P-glycoprotein; ABC, ATP binding cassette; CFTR, cystic fibrosis transmembrane conductance regulator; SUR, sulfonyl urea receptor; Pfcrt, Plasmodium falciparum chloroquine resistance transporter; Hb, hemoglobin; CQ, chloroquine; CQR, chloroquine resistant; MQ, mefloquine; CQS, chloroquine sensitive; HF, halofantrine; QN, quinine; AQ, amodiaquine Pgh1, P-glycoprotein homologue; kDa, kiloDaltons; DV, digestive vacuole; kbp, kilobasepair; AO, acridine orange; FPIX, ferriprotoporphyrin IX; DHFR-TS, dihydrofolate reductase-thymidylate synthase.

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Pf MDR 1

Similar to drug-resistant tumor cells and some drug-resistant bacteria, early studies of CQR *P. falciparum* showed that drug resistance was associated with decreased cellular accumulation of drug [21] and that reduced drug accumulation was reversed by the ion channel blocker verapamil [23, 33]. Similar phenomena had been seen for over a decade in drug-resistant tumor cells known to overexpress Pgp encoded by the mdr gene. Thus, Wirth and colleagues searched for *P. falciparum* genes homologous to the mammalian multidrug resistance *mdr* genes that encode Pgps and identified *pf mdr1* and *pf mdr2* [42]. While both *pf mdr1* and *pf mdr2* proteins are endogenously expressed in drug-sensitive strains of *P. falciparum*, a mefloquine (MQ)-resistant strain was found to have elevated *pf mdr1* expression [42]. MQ is similar to CQ, so it might be expected that resistance pathways overlap for the two drugs. Thus, this result initially enticed drug resistance researchers to assume similar mechanisms operated in both tumor cells and malarial parasites, namely, that overexpression of an *mdr* gene was responsible for pleiotropic drug resistance (“multi drug resistance”). In support of this notion, another group also isolated *pf mdr1* and found it to be upregulated in some CQR strains of *P. falciparum* [15], but subsequent experiments by the same group [2] showed that *pf mdr1* overexpression did not necessarily correlate with CQR. This was not surprising since Wellesm and colleagues had shown earlier that the CQR phenotype did not necessarily segregate with the *pf mdr1* chromosomal locus in progeny from a genetic cross [40]. Arguments against a dominant role for Pgp in tumor drug resistance were also being made during this period, in part because other ion transporters were found to be alternatively expressed during evolution of resistance well before significant overexpression of Pgp [32], but for whatever reasons, these arguments were viewed as particularly controversial at the time.

However, polymorphisms in *pf mdr1* were also associated with CQR early on [16; Table 1]. Chloroquine-sensitive (CQS) isolates (strains D10 and 3D7) and CQR isolates (strains K1, ITG2 and 7G8) were fully sequenced and while the sensitive isolates had identical *pf mdr1* sequences, there were five changes in CQR isolates [16]. In K1 and ITG2, N86Y was the only amino-acid change [16]. On the other hand, 7G8 had four amino-acid changes; Y184F, S1034C, N1042D and D1246Y [16]. The 184F mutation was postulated as not likely involved in CQR, since it had also been found in CQS strains [16]. Thus, the *mdr* overexpression hypothesis was revised to suggest that CQR strains expressed mutant *pf mdr1* with either 86Y or 1034C/1042D/1246Y codons, but did not necessarily overexpress *pf mdr1* [16]. Interestingly, the occurrence of the K1 versus 7G8 polymorphisms appeared to be geographically biased [16]. This principle is very important, as elaborated upon in a recent study that identifies an unusual CQR phenotype for Papua New Guinea [24].

Subsequently, when MQ-resistant strains of *P. falciparum* were selected to higher levels of MQ-resistance, *pf mdr1* was found to be amplified [7], and there was an inverse relationship between MQ and CQ resistance in the series of strains. Also, halofantrine (HF) and quinine (QN) resistance increased with increasing *pf mdr1*, whereas amodiaquine (AQ) resistance did not [7]. However, when the CQR strain K1 was selected for resistance to halofantrine, it did not result in MQ resistance or amplification of *pf mdr1* [31]. In the most recent study, which used allelic exchange of the *pf mdr* locus to probe these questions, incorporation of *pf mdr1* 7G8 polymorphisms into a CQS strain not previously exposed to drug had no effect on CQR, but incorporating wild type *pf mdr1* into a CQR strain expressing mutant *pf mdr1* did decrease the level of drug resistance by half [30]. On the other hand, the CQS strains expressing mutant *pf mdr1* alleles showed some resistance to the related quinoline antimalarial QN [30]. Incorporation of the 7G8 mutations also appeared to alter sensitivity to MQ and HF [30].

This brings us to our current understanding of the role of *pf mdr1* in antimalarial drug resistance. Namely, in the clinic, there is considerable controversy as to whether specific mutations in *pf mdr1* actually confer drug resistance in the field (e.g., [9]; Table 1). In addition, we are left with yet another modified theory now suggesting that while mutations in *pf mdr1* cannot induce CQR in CQS strains in and of themselves, they can provide a modulatory effect in the presence of essential additional gene(s) to perhaps induce higher levels of CQR and/or altered drug resistance profiles [30]. With regard to CQR, the additional essential gene has now recently been identified (see below).

The open reading frame of *pf mdr1* encodes a large polypeptide with 12 hydrophobic regions similar to hu *mdr1*-encoded Pgp [42]. This *P. falciparum* Pgp homologue (P gh1) is a 160 kDa protein that localizes to the membrane of the parasite digestive vacuole (DV) where hemoglobin is proteolyzed [6]. It is fitting that a protein implicated in quinoline antimalarial drug resistance resides within the DV membrane since quinoline antimalarial drugs accumulate within the acidic DV where they bind to their principal target (again, hemeb released from Hb). Similar to hu Pgp, Pf P gh1 has two homologous halves and two predicted ATP binding sites [42]. Like hu Pgp, it is a fascinating and important membrane protein that merits considerable additional study.

As described above, most of what is known about P gh1 is limited to genetics. While it appears that P gh1-mediated physiology alone does not