Multidrug Resistance

Subjects: Others
Contributor: Rajendra Prasad

Multidrug resistance (MDR) transporters belonging to either the ATP-Binding Cassette (ABC) or Major Facilitator Superfamily (MFS) groups are major determinants of clinical drug resistance in fungi. The overproduction of these proteins enables the extrusion of incoming drugs at rates that prevent lethal effects. The promiscuity of these proteins is intriguing because they export a wide range of structurally unrelated molecules. Research in the last two decades has used multiple approaches to dissect the molecular basis of the polyspecificity of multidrug transporters. With large numbers of drug transporters potentially involved in clinical drug resistance in pathogenic yeasts, this review focuses on the drug transporters of the important pathogen *Candida albicans*. This organism harbors many such proteins, several of which have been shown to actively export antifungal drugs. Of these, the ABC protein *Ca*Cdr1 and the MFS protein *Ca*Mdr1 are the two most prominent and have thus been subjected to intense site-directed mutagenesis and suppressor genetics-based analysis. Numerous results point to a common theme underlying the strategy of promiscuity adopted by both CaCdr1 and CaMdr1.

Keywords: Candida albicans ; ABC transporters ; MFS transporters ; multidrug efflux pumps ; CaCdr1 ; CaMdr1 ; polyspecificity ; interdomain crosstalk ; mutagenesis ; suppressor genetics

1. Introduction

Human pathogenic yeast that include *Candida albicans* and non-*albicans Candida* (NAC) species are commensal pathogens infecting individuals with compromised immunity [1]. The superficial infections caused by *C. albicans* and NAC species can also extend to disseminated bloodstream and deep-tissue infections [1]. Although C. *albicans* and a few NAC species (e.g., *Candida glabrata*, *Candida tropicalis*, and *Candida parapsilosis*) are considered to be the most common fungi infecting immunocompromised patients, more recently, *Candida auris* has been recognized as a global health threat (https://www.cdc.gov/fungal/candida-auris/index.html) [2]. Besides the ability to spread nosocomially, its propensity to form adherent biofilms on medically relevant substrates has led to numerous hospital outbreaks of *C. auris* globally [3]. A higher percentage of clinical isolates resistant to multiple classes of antifungal agents is the greatest challenge posed by this recently emerged NAC species [4]. Apart from *C. auris*, the prophylactic or, prolonged use of antifungal drugs has allowed many other *Candida* species to manifest resistance to azoles, polyenes, echinocandins, and pyrimidine analogues [4][5]. Compared to other classes of antifungals, resistance to azole antifungals is much more common, presumably due to their fungistatic nature.

C. albicans and NAC species have used many strategies to deal with the onslaught of common antifungals [6]. One of the most prominent of these mechanisms is the ability of *Candida* species to rapidly efflux incoming drugs [6]. This feature is helped by a group of drug transporters belonging to the ATP-Binding Cassette (ABC) and Major Facilitator Superfamily (MFS) classes of proteins [7]. *Candida* cells harbor a battery of both ABC and MFS proteins; however, only a few have a well-established role in clinical drug resistance. While the ABC proteins are primary transporters that couple ATP-binding and hydrolysis to power drug extrusion, MFS transporters are secondary transporters that instead exploit the electrochemical gradient of protons to facilitate drug efflux [7]. Both ABC and MFS proteins are promiscuous transporters with the ability to export a diversity of substrates across membranes. Among the prominent transporters that have a proven role in azole resistance, the ABC proteins *Candida albicans* drug resistance protein 1 (*Ca*Cdr1) and *Candida albicans* drug resistance protein 2 (*Ca*Cdr2), and the MFS protein *Candida albicans* multidrug resistance protein 1 (*Ca*Mdr1) stand out in terms of their clinical relevance [6][7][8]. Most azole-resistant clinical isolates show overexpression of genes encoding these ABC and MFS proteins [6][9][10][11]. The rapid efflux of incoming drugs by these transporters prevents the retention of the drugs at detrimental concentrations, thus facilitating cell survival. These drug efflux proteins appear to undergo substantial conformational change during drug transport [7][12]. How ranges of diverse substrates are bound and transported are some of the questions that have been addressed significantly in several recent studies.

2. Historical Background of the MDR Pumps in Yeast

ABC proteins were first identified in bacteria as prominent nutrient importers and came to center stage when their homologues were shown to cause multidrug resistance in cancer cells [13][14][15]. In yeast, Rank and Bech-Hansen identified a point mutation in a gene (later named Pleiotropic drug resistance 1 (*PDR1*)) that led to increased xenobiotic resistance [16]. Goffeau's group subsequently established that it conferred resistance to a number of antifungal drugs including ketoconazole and cycloheximide [17]. Since then, numerous additional *PDR1* gain-of-function mutations have been reported [18]. Golin's group identified the first target of *PDR1* from a genomic DNA library. It was found within a DNA fragment that conferred resistance to cycloheximide and sulfometuron methyl and was named the Pleiotropic drug resistance 5 (*PDR5*) gene [19]. Myers and colleagues then demonstrated that deletion of the *PDR5* gene led to marked hyper-susceptibility to a number of antifungal compounds and some in vitro inhibitors including chloramphenicol [20]. Goffeau's group demonstrated that the gene belonged to the ABC superfamily and had the ability to transport a number of molecules including some anticancer drugs and rhodamines [21]. A *PDR5*-like gene was soon identified by a functional complementation of *PDR5* using a *C. albicans* genomic library. Sequencing of the complementing genomic fragment revealed an open reading frame (ORF) with close homology with *PDR5*, and was designated as *Candida* Drug Resistance 1 gene (*CDR1*) [22]. This was a turning point as *CDR1* was soon established as one of the major determinants of antifungal resistance in *C. albicans* [10]. *CaCDR1* identification quickly led to the identification of other homologues such as *CaCDR2*, *CaCDR3*, and *CaCDR4*. Of these, only *Ca*CDR2 was shown to play a role, albeit minor, in antifungal resistance due to its export of antifungals including azole drugs [23][24][25][26].

Based on sequence similarity, the ABC proteins in all organisms are divided into nine subfamilies, from ABCA to ABCI, according to the Human Genome Organization (HUGO) nomenclature [27]. An initial inventory by Gaur et al. of *C. albicans* ABC proteins contained 28 putative members [28]. Subsequent modifications in the genome assembly found 26 members that can be clustered into six subfamilies designated ABCB/MDR, ABCC/MRP, ABCD/ALDP, ABCF/YEF3, ABCE/RLI, and ABCG/PDR [6]. Since the members of ABCB/MDR, ABCC/MRP, ABCD/ALDP, and ABCG/PDR possess transmembrane domains (TMDs), they are putative membrane-localized transporters. The ABCF/YEF3 and ABCE/RLI representatives lack transmembrane components and have been shown to participate in non-transport functions such as translation initiation and regulation, ribosome biogenesis, etc. [6][29]. The ABCG/PDR subfamily is the largest among all *Candida* species: 9 among 26 in *C. albicans* [6], 7 among 25 in *C. glabrata* [30], and 7 among 28 in *C. auris* [31]. Characterization of the four PDR subfamily members in *C. albicans* (*CaCDR1-4*) showed that only *CDR1* and *CDR2* encode drug and phospholipid transporters. *CDR3* and *CDR4* do not encode drug transporters but instead translocate phosphoglycerides between the two lipid monolayers of plasma membrane [32][33][34].

The MFS superfamily is a vast family of transporters that is ubiquitous in the Kingdom of Life. Its members function as uniporters, antiporters, and symporters for a wide range of substrates from nutrients to drugs [35][36]. Yeast, including *Candida* species, are no exception and harbor large numbers of MFS proteins [37]. While the role of MFS proteins as transporters was well recognized in bacteria, the first realization of MFS protein involvement in drug resistance came when Fling et al. identified the *C. albicans* MFS transporter encoding the gene designated *BEN*^{r} (for benomyl resistance) [38]. It conferred resistance to benomyl and methotrexate in a susceptible *Saccharomyces cerevisiae* strain and had similarity to genes encoding antibiotic resistance in prokaryotes and eukaryotes. This included a high degree of identity to the cycloheximide resistance gene in *C. maltosa* [39]. *BEN*^{r} was also shown to confer resistance to many structurally and functionally unrelated compounds including cycloheximide, benzotriazoles, 4-nitroquinoline-N-oxide, and sulfometuron methyl [40]. As increased Ben^r levels conferred resistance to diverse substrates and thus functioned as a multidrug transporter, its gene was redesignated as *CaMDR1* [39]. Subsequent research identified *CaMDR1* homologues in other *Candida* species.

MFS proteins typically consist of 400–600 amino acids and analysis of their primary sequences revealed that within each family, sequence similarity is highly significant [41]. In the *S. cerevisiae* genome, sequences encoding a total of 22 MFS proteins have been identified belonging to either Drug:H^+ Antiporter family 1 (DHA1) or Drug:H^+ Antiporter family 2 (DHA2), which differ in number of transmembrane helices (TMHs) [41]. Members of the DHA1 family have 12 TMHs, while DHA2 members possess 14 TMHs. Bioinformatics analysis of *C. albicans* MFS proteins identified 95 members in 17 families, with DHA1 and DHA2 as the major families, comprising of 22 and 9 representatives, respectively [37]. The well characterized MFS drug transporter *Ca*Mdr1 belongs to the DHA1 family [41].

Despite the large number of PDR subfamily and DHA1 family members within the ABC and MFS superfamilies, respectively, only *Ca*Cdr1, *Ca*Cdr2, and *Ca*Mdr1 have demonstrated clinical significance as multidrug transporters [42]. What structural features enable this select group of proteins to be promiscuous transporters that are able to bind and release ranges of unrelated xenobiotics? Researchers have addressed such questions for over two decades. The TMD

mutagenesis studies carried out with *Ca*Cdr1 and *Ca*Mdr1 have shed light on the molecular basis of substrate promiscuity in these pumps. In both cases, a central binding pocket formed by certain helices of the TMDs is augmented by certain residues situated at the periphery of the central core in order to confer polyspecificity.

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