Mitochondria-Mediated Azole Drug

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In recent years, the role of mitochondria in pathogenic fungi in terms of azole resistance and fungal pathogenicity has been a rapidly developing field. In this review, we describe the molecular mechanisms by which mitochondria are involved in regulating azole resistance and fungal pathogenicity. Mitochondrial function is involved in the regulation of drug efflux pumps at the transcriptional and posttranslational levels. On the one hand, defects in mitochondrial function can serve as the signal leading to activation of calcium signaling and the pleiotropic drug resistance pathway and, therefore, can globally upregulate the expression of drug efflux pump genes, leading to azole drug resistance. On the other hand, mitochondria also contribute to azole resistance through modulation of drug efflux pump localization and activity. Mitochondria further contribute to azole resistance through participating in iron homeostasis and lipid biosynthesis. Additionally, mitochondrial dynamics play an important role in azole resistance. Meanwhile, mitochondrial morphology is important for fungal virulence, playing roles in growth in stressful conditions in a host. Furthermore, there is a close link between mitochondrial respiration and fungal virulence, and mitochondrial respiration plays an important role in morphogenetic transition, hypoxia adaptation, and cell wall biosynthesis. Finally, we discuss the possibility for targeting mitochondrial factors for the development of antifungal therapies.

azole resistance pathogenic fungi mitochondria calcium signaling

1. Introduction

Fungal infection is still an important medical issue worldwide and poses a significant threat to human health [1]. The severity of fungal infections and the concomitant importance of searching for new and better antifungal treatments are often underappreciated. The number of drugs available to treat fungal infections is limited, and those that are commonly used often suffer from being fungistatic rather than fungicidal [2][3]. Azoles, the first-line antifungals used in the clinic, are one of these fungistatic classes of drug [4][5][6]; they can decrease the production of ergosterol by inhibiting cytochrome P450 enzyme Erg11 and by damaging the cell membrane [7][8][9]. The fungistatic nature of the azoles coupled with their extensive use has resulted in azole resistance in populations of various pathogenic fungi [4][6][10][11]. Accordingly, there is an urgent need to unravel the molecular mechanisms of azole resistance to search for new and effective therapies.

Azole drug resistance and the fungal virulence are intimately intertwined with their metabolism, and mitochondria play a central role in that metabolism [12][13]. Mitochondria house and integrate multiple metabolic functions relating to lipids, iron metabolism, energy production, and cell wall biosynthesis [14][15][16][17][18][19], which are associated with fungal virulence and resistance to azoles. Mutations affecting mitochondrial functions including functioning of

the electron transport chain (ETC), protein import, calcium homeostasis, mitochondrial genome maintenance, and mitochondrial transcription can result in avirulence and azole resistance or susceptibility [17][20][21][22][23][24][25]. The functions of mitochondria in these pathways are complex, and research findings open avenues for new, mitochondria-targeted therapeutic approaches. In this review, we describe the molecular mechanisms through which mitochondria are involved in regulating azole resistance and fungal pathogenicity and discuss the possibility for targeting mitochondrial factors for the development of antifungal therapies.

2. Potential for Mitochondrial Factors as Novel Antifungal Therapeutic Targets

The classic antifungal drugs used to treat fungal pathogens do not rapidly inhibit fungal growth, and hence, mortality rates remain unacceptably high. To counter these problems, the development of new therapeutic approaches is essential. As described above, fungal pathogens require mitochondrial function for normal growth, azole drug resistance, and virulence. Given the central role of mitochondria in processes essential for adaptability, growth, and maintenance, coupled with the presence of fungal-specific characteristics, it may be possible to develop therapies based on inhibition of fungal mitochondria.

As described above, Fzo1 is a major player in mitochondria-related azole resistance and virulence [14]. Although Fzo1 proteins are highly conserved in fungi, plants, and animals, they have specific characteristics in fungi. Notably, the *C*-terminal region of Fzo1 proteins in animals and fungi has no detectable sequence similarity and cannot be reliably aligned. Bioinformatic predictions showed that fungal Fzo1 proteins carry two predicted *C*-terminal TMDs whereas Fzo1 proteins in animals have only a single predicted *C*-terminal TMD. Thus, any compound inhibiting the function of fungal Fzo1 by targeting its *C*-terminal TMD without affecting the human homolog would be theoretically valid. Taken together, these data indicate that these specific *C*-terminal TMD of Fzo1 may serve as targets for developing novel antifungal therapies.

The mitochondrial respiratory pathway is an effective target for fungicides to control fungal growth. Additionally, the presence of fungal-specific respiratory components and the recent discovery of the association between respiration and pathogenesis in several fungal pathogens have promoted the development of new mitochondria-targeted fungicides [20]. Recently, a study identified seven genes (*Nuo3*, *Nuo4*, *Nue1*, *Nue2*, *Qce1*, *Coe1*, and *Coe2*) that are unique to the CTG fungal clade, which is so named because they generally translate CTG as serine rather than leucine. The CTG fungal clade contains multiple important human pathogens, including *C. albicans*, and showed that they are required for full mitochondrial respiratory metabolism and fungal virulence [13], implying that these clade-specific mitochondrial factors might represent novel antifungal therapeutic targets. The mitochondrial respiratory chain is composed of four large multi-subunit enzymes, complexes I to IV. Of these, complex I has the highest molecular weight and, energetically speaking, is responsible for generation of approximately half of the ATP [35]. Complex I is present in most fungal pathogens, and recent work has identified two subunits (Nuo1 and Nuo2) of complex I itself as well as the complex I regulator Goa1 to be fungus-specific [21]. Loss of these proteins can lead to deficiencies in respiration and virulence [21]. Similarly, deletion of *Nue1*, *Nue2*, *Nuo3*, or *Nuo4* can impair complex I function, causing deficiencies in respiration and virulence [13], making them attractive antifungal drug

targets. Additionally, dysfunction of complex I can lead to ROS accumulation in mitochondria, which in turn promotes fungal cell death. Thus, inhibitors of complex I have fungicidal activity by increasing mitochondrial ROS levels. Taken together, fungal-specific mitochondrial factors regulating the function of complex I may represent novel antifungal therapeutic targets.

In addition to the classical ETC, many fungal pathogens possess a cyanide-insensitive alternative oxidase (AOX), which can permit respiration when the classical electron transport chain is inhibited, thus maintaining growth and viability. As AOX is absent from mammals, it has been investigated as a potential antifungal target. Because AOX is dispensable for virulence in some fungal pathogens, AOX inhibitors as antifungal agents may not be universally successful, at least not as monotherapy. Therefore, a combination of alternative respiration and classical pathway inhibitors may be the most effective antifungal strategy. For example, a combination of the AOX inhibitor salicylhydroxamic acid (SHAM) and fluconazole displayed synergistic antifungal activity against *C. albicans*. The only AOX protein structure available is of that from the human parasite *Trypanosoma brucei* (a kinetoplastid, not a fungus). On the basis of AOX structural information, some new inhibitors, such as ascofuranone, have been discovered. However, because of the lack of structural information about fungal AOX, the development of fungal AOX inhibitors has been hampered. Recently, novel fungal AOX inhibitors, optimized *N*-phenylbenzamide derivatives, were shown to effectively inhibit spore germination of the phytopathogen *Moniliophthora perniciosa*. Of course, it is necessary to further study the structure and physiological activity of AOX in fungi and the structure–activity relationships of existing AOX inhibitors, which will promote the development of effective fungal AOX inhibitors to control fungal reproduction.

During the infection process, fungal pathogens are challenged by massive changes of the environmental conditions, e.g., by nutrient depletion, elevated temperatures, and hypoxia. Oxygen availability drops from 21% in the atmosphere to less than 1% in inflammatory and necrotic tissue. Thus, hypoxic microenvironments were often found to exist at the site of infection in mice infected with fungal pathogens, and the adaptation to hypoxia might be a critical factor in the ability of fungal pathogens to cause lethal disease. Currently, a series of studies have confirmed that functional mitochondria play an essential role in the adaptation process towards hypoxia of several pathogenic fungi. The mitochondrial aerobic respiration is active during hypoxia and the protein levels of all respiratory complexes also increased under hypoxic growth conditions to increase the respiratory capacity of mitochondria. Recently, proteome analysis identified a mitochondrial protein HorA to be highly upregulated in *A. fumigatus* during hypoxic adaptation. HorA is associated with biosynthesis of coenzyme Q, which is involved in mitochondrial respiration and maintenance of cellular redox homeostasis. Therefore, the loss of HorA displayed an impaired response to both oxidative and reductive stress and showed significantly attenuated virulence. Moreover, an increased resistance against azole drugs was also observed. Taken together, HorA plays a critical role in the virulence of *A. fumigatus*. Noteworthily, due to its absence in mammals, the HorA may represent a promising target for the development of novel antifungal drugs.

As described above, mitochondrial function is associated with ergosterol biosynthesis, indicating that mitochondrion inhibitors have the potential to enhance the effects of current azole drugs that target ergosterol biosynthesis. For example, inhibition of mitochondrial aerobic respiration with tetrandrine can cause increased

susceptibility to azole drugs, fungal-specific inhibitors of complex III can reverse azole resistance [17], and a combination of the AOX inhibitor SHAM and fluconazole displayed synergistic antifungal activity. Taken together, because of the connection of mitochondria to ergosterol metabolism, mitochondrial inhibitors may prove to be effective against fungal pathogens in combination with current azole drugs. It has been reported that the mitochondrial outer membrane Sorting and Assembly Machinery (SAM) complexes Sam35 and Sam37 are required for mitochondrial biogenesis and dynamics. Sam35 is required for growth and virulence of *C. albicans*, and Sam37 is critical for cell wall integrity and virulence. Importantly, there are significant structural differences in fungal Sam35 and Sam37 compared with their animal counterparts [23], and thus, they could be explored as targets for antifungal drug development. Future research will focus on screening and developing small molecule compounds that can inhibit these proteins.

3. Conclusions and Future Prospects

Because of the connections of mitochondria to azole resistance, lipid metabolism, pathogenesis, and cell wall regulation, pharmacological disruption of mitochondrial function may prove to be effective against fungal pathogens. Combining a mitochondrial function inhibitor with one or more current antifungals (azole, polyene, and echinocandin) could increase efficacy, reduce toxicity, and prevent the emergence of antifungal drugs resistance better than monotherapy regimens. Additionally, fungal pathogens must be able to resist oxidative stress in phagocytes to survive, and mitochondrial function plays an important role in oxidative stress resistance. Therefore, the pharmacological damage of mitochondrial function will lead to impaired capacity to cope with the oxidative stress from host cells and inhibit the growth of fungal pathogens in the host. Taken together, the regulation of mitochondrial function provides promising therapeutic targets for combating fungal infection. Furthermore, by targeting mitochondrial function with a specific combination of two antifungal drugs, the possibility that fungal pathogens will develop mutations that increase azole drug resistance is significantly lessened. Therefore, we hope that future efforts will focus on finding new compounds that specifically block mitochondrial function. An important challenge for future mitochondria-targeting therapy is developing selectivity for mitochondrial factors. In this regard, structural analysis of mitochondrial factors would be an important next step to help guide medicinal chemistry efforts. Although some studies have emphasized the importance of mitochondrial function for azole resistance and fungal virulence, the exact molecular mechanisms are not fully understood. Thus, it is important to understand the underlying mechanisms of mitochondria-mediated azole drug resistance and fungal pathogenicity. Taken together, it is worthwhile to study how mitochondria promote azole drug resistance and fungal pathogenicity.

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