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MINI-REVIEW

The role of melanins in melanotic fungi for pathogenesis and environmental survival

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Abstract

Melanins provide fungi protection from environmental stressors, support their ecological roles, and can confer virulence in pathogens. While the function, structure, and synthesis of melanins in fungi are not fully understood, they have been shown to have varied roles. Recent research has revealed a wide range of functions, from radiation resistance to increasing virulence, shedding light on fungal diversity. Understanding fungal melanins can provide useful information, from harnessing the properties of these various melanins to targeting fungal infections.

Key Points

- *Melanotic fungi are widespread in nature.*
- *Melanin functions to protect fungi in the environment from a range of stresses.*
- *Melanin contributes to pathogenesis and drug resistance of pathogenic fungi.*

Keywords Melanin · Fungi · Extremophile · Astrobiology · Phagocytosis · Antifungal

Introduction

Melanotic fungi are widespread, both in terms of phylogeny and ecology. They are included in both the *Ascomycota* and *Basidiomycota*, the two largest fungal phyla. Additionally, melanotic fungi are found growing in a variety of places, including radioactively contaminated soils, Antarctic rocks, dishwashers, ancient cave paintings, and spacecraft. Even some edible mushrooms contain melanins. In addition, several important pathogens of humans and plants produce melanin. The goal of this review is to highlight recent research from the literature (approximately the past 5 years) pertaining to the functions of melanin in fungi. In the environment, melanins enhance survival of fungi growing under numerous stresses. Environmental sampling shows that melanotic fungi are widespread and abundant in various ecosystems. Among human pathogens, melanin has been shown to enhance virulence and drug resistance in fungi. Herein, we discuss various functions of melanin in both the environment and in human pathogens and illustrate these functions with examples from the literature. A list of representative melanotic fungi with associated functions is provided in Table 1.

We begin with a brief review of melanin synthesis and its physical and chemical properties. This will provide a foundation for understanding melanin's functional roles. Melanins are polyphenol biopolymers with complex and often unspecified structures, and as such exhibit many different physicochemical properties and functions (Cordero and Casadevall 2017; Meredith and Sarna 2006; d'Ischia et al. 2019; Camacho et al. 2019; Panzella et al. 2018). Despite their complexity and varied structures, melanins absorb UV-Vis light (Meredith and Sarna 2006). Melanins are capable of not only absorbing light but dissipating the energy within the structure, which makes them effective as photoprotecting agents (Wolbarsht et al. 1981).

Absorbing light and transforming it into potential metabolic energy make melanins unique in terms of energy harvesting (Dadachova et al. 2007). By absorbing light and emitting almost no light back, melanins, in analogy to a blackbody, are basically

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Table 1 Melanotic fungal species and melanin function

Species	Common isolates	Melanin type(s)	Function	References
Alternaria infectoria	Environmental: saprotroph	DHN	Antifungal interactions	(Fernandes et al. 2015)
Aspergillus fumigatus	Clinical: aspergillosis	DHN and pyo-melanin	Inhibits LAP	(Akoumianaki et al. 2016)
			Dendritic cell interaction	(Bayry et al. 2014)
			Lectin interaction	(Stappers et al. 2018)
			Platelet activation	(Rambach et al. 2015)
			Monocyte interactions	(Mohebbi et al. 2016)
			Epithelial cell interactions	(Amin et al. 2014)
Aspergillus niger	Commercial: industrial fermentation	DHN and DOPA	Space survival	(Gomoiu et al. 2016)
Auricularia auricula	Commercial: edible mushroom	DHN and DOPA	Commercial/medicinal	(Prados-Rosales et al. 2015)
Cenococcum geophilum	Environmental: mycorrhizal	DHN	Osmotic stress and desiccation resistance	(Fernandez and Koide 2013)
			Fungal decomposition	(Fernandez and Koide 2014)
Cryomyces antarcticus	Environmental: endolithic	DHN	Space survival	(Onofri et al. 2008)
			Radiation resistance	(Pacelli et al. 2017)
Cryptococcus neoformans	Clinical: cryptococcosis	DOPA	Inhibits phagocytosis	(Wang et al. 1995)
Fonsecaea monophora	Clinical: chromoblastomycosis	DHN and DOPA	Macrophage interaction	(Shi et al. 2019; Li et al. 2016)
Fonsecaea pedrosoi	Clinical: chromoblastomycosis	DHN	Complement activation	(Pinto et al. 2018)
Histoplasma capsulatum	Clinical: histoplasmosis	DHN and DOPA	Antifungal resistance	(van Duin et al. 2002)
			Radiation resistance	(Dadachova et al. 2008)
Ochronis lascauxensis	Environmental: Lascaux cave	Unknown	Biodeterioration	(De la Rosa et al. 2017)
Paracoccidioides brasiliensis	Clinical: paracoccidioidomycosis	DHN and DOPA	Antifungal interactions	(Rossi et al. 2017)
Penicillium marneffei	Clinical: penicilliosis	DHN and DOPA	Inhibits phagocytosis	(Sapmak et al. 2015; Liu et al. 2014)
Rhizopus oryzae	Clinical: mucormycosis	DOPA	Inhibits LAP	(Andrianaki et al. 2018)

photothermal agents (Meredith and Riesz 2004). Because of functional groups including carboxyl and phenolic groups present, melanins also have a rich chemistry, interacting with other compounds via covalent as well as non-covalent interactions. Furthermore, depending on pH, binding of the melanin polymer involves different functional groups. At pH < 7, carboxyl groups are the main sites available for binding, while at pH > 7 phenolic groups become the key locations for binding. These pH-dependent binding processes are likely mechanistically important to antifungal drugs in melanin-producing fungi.

Lastly, melanins can form radicals that are often persistent. Such radical species are detectable by electron paramagnetic spin resonance spectroscopy. Due to their radical character, melanins are antioxidants capable of scavenging of free radicals and protecting against oxidative stress, and also in quenching of compound excited states (Sarna and Plonka 2005).

Fungi usually produce melanin through one or both of the following pathways (Fig. 1). DOPA melanins are produced from tyrosine or L-3,4 dihydroxyphenylalanine (L-dopa). These substrates are oxidized to dopaquinone by a polyphenol oxidase enzyme such as laccase or tyrosinase, depending on the starting material and organism. Then, further steps produce the subunits dihydroxyindole or dihydroxyindole-2-carboxylic acid. These subunits polymerize into melanin. Fungi may also synthesize melanin via the dihydroxynaphthalene (DHN) pathway. First, a precursor molecule, acetyl coA or malonyl coA, is produced endogenously. Formation of 1,3,6,8-tetrahydroxynaphthalene (1,3,6,8-THN) is then catalyzed by a polyketide synthase (PKS) enzyme. A series of enzyme-catalyzed reduction and dehydration reactions produce various intermediates, then finally 1,8-dihydroxynaphthalene (DHN). Polymerization of DHN leads to formation of melanin. Many fungi have multiple melanin synthesis pathways (Eisenman and Casadevall 2012).

Melanized fungi in the environment

Melanotic fungi have been isolated from diverse environments and research on environmental melanotic fungi has far reaching implications on numerous topics, including survival in extreme conditions, response to nuclear disasters, space travel, and the global ecology. Fungi have many important roles in ecosystems. They are decomposers that recycle nutrients from dead organic matter, such as wood. Mycorrhizal symbionts form underground networks in forest soil connecting trees. Other fungi are pathogens of plants and animals that can have profound effects on biodiversity. Melanins have long been thought to have a protective role in fungi in the environment. However, much remains to be addressed regarding the specifics of function. Recent studies have addressed the fundamental biology and ecology of melanotic fungi. This includes basic questions on the abundance and distribution of melanotic fungi and their roles in ecosystems.

Melanin protects fungi in the environment

A lot of research has focused on the protective role of melanins to diverse environmental stressors, such as predation, hyperosmotic conditions, heavy metals, desiccation, and ultraviolet radiation. The studies described below show that inhibition of melanin production makes fungi more susceptible

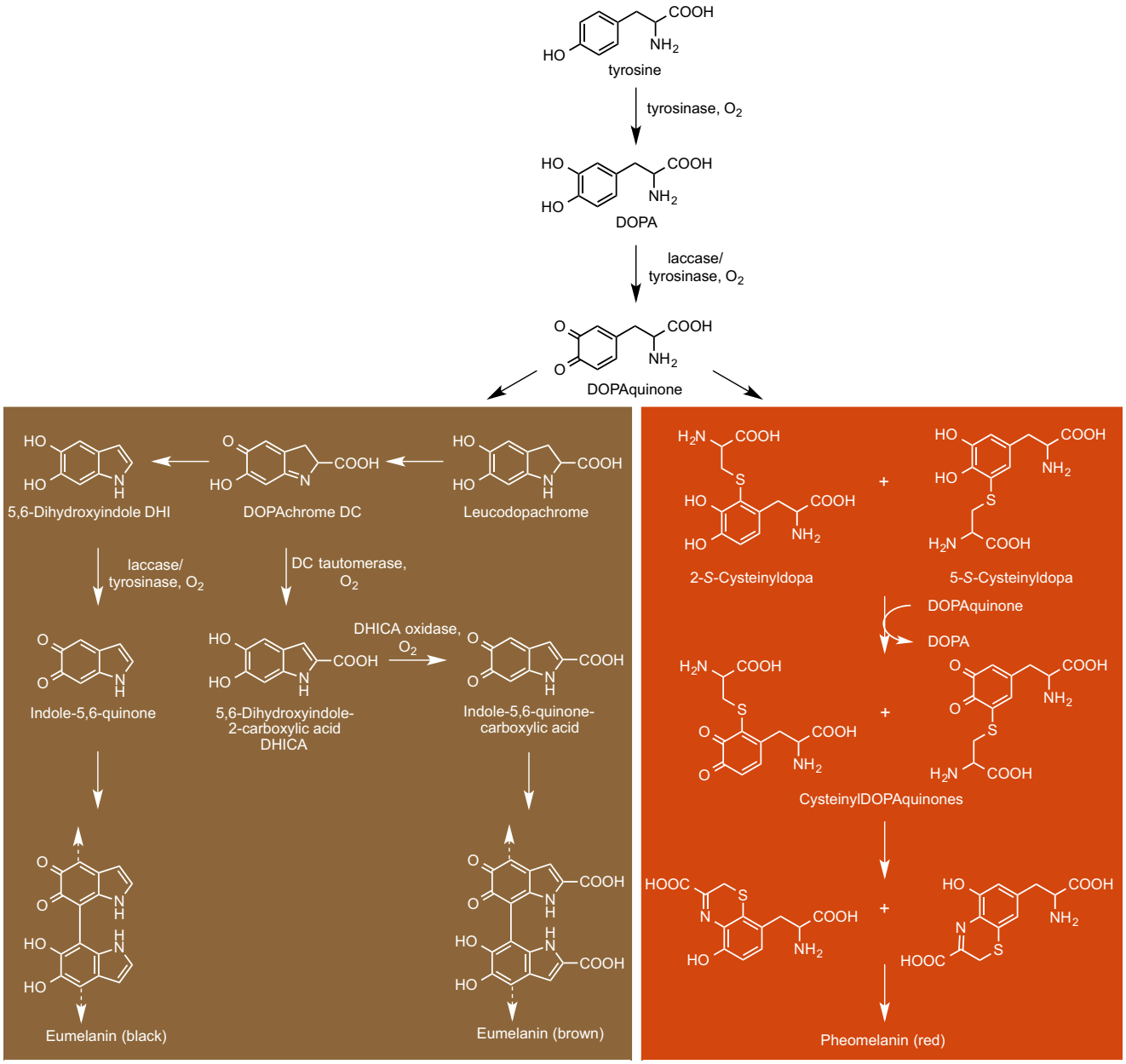

Fig. 1 DOPA melanin synthesis pathways. Scheme based on Sarna and Plonka (2005)

to environmental stress. Microbial fungi may be predated by other soil microbes. *Dictyostelium discoideum*, a soil amoeba, is able to phagocytose conidia of *Aspergillus fumigatus*. However, when components of the melanin synthesis pathway, such as *pksP*, are mutated, they become more susceptible to predation, having a significantly higher phagocytic index compared with wild-type fungi (Hillmann et al. 2015). Osmotic stress and desiccation are both likely scenarios faced by fungi in the environment. The melanotic ascomycete, *Cenococcum geophilum*, is an ectomycorrhizal fungus capable of withstanding osmotic stress when grown in the laboratory. However, addition of the melanin inhibitor tricyclazole reduces the growth rate under osmotic stress conditions. Likewise, tricyclazole also reduces the ability of the fungi to withstand desiccation in the laboratory (Fernandez and Koide 2013). *Hortaea werneckii*, a fungal isolate of salterns that produces DHN melanins, can survive salt concentrations that are near saturation levels. Addition of melanization inhibitors to growth medium reduced the growth rate of the fungus at high salt concentrations. The melanin inhibitor resulted in the release of pigment into culture supernatants and alteration in cell length, suggesting changes in the cell wall due to loss of melanin that affected ability to withstand osmotic stress (Kejžar et al. 2013). The protective role of melanins in the environment may enhance long-term survival of fungi. In one study of dozens of fungal species, melanization was

correlated with spore viability over a 5-year time period. Spores for the study were collected from field sites in northern California and analyzed for pigmentation and germination on malt agar (Nguyen 2018).

Radiation resistance of melanotic fungi

Melanotic fungi are strikingly resistant to various types of electromagnetic radiation. The nuclear disaster site Chernobyl has a thriving community of black molds (Dighton et al. 2008). Recent studies have expanded upon the initial discoveries of melanized fungi in such sites, such as by testing the effects of ionizing radiation on different types of fungi. *Cryomyces antarcticus* and *Cryptococcus neoformans* differ in both growth rate and melanin type. *C. antarcticus*, an isolate of Antarctic rocks, is a slow-growing fungus that produces DHN melanin and is classified in phylum *Ascomycota*. *C. neoformans* is a fast-growing clinical fungus that produces DOPA melanin and is classified in phylum *Basidiomycota*. In the presence of densely ionizing deuteron radiation, melanin enhanced the growth of both fungi (Pacelli et al. 2017). Similarly, both *C. antarcticus* and *C. neoformans* are better able to survive radiation in the form of X-rays when melanized (Pacelli et al. 2018). These data suggest that melanization has widespread benefit across fungal types upon exposure to radiation. Research on radiation resistance has led to the hypothesis that fungi harvest energy of radiation via melanin. Evidence for "radiosynthesis" is summarized elsewhere (Casadevall et al. 2017).

Understanding the radiation resistance of melanized fungi has implications for nuclear disasters. Mycorrhizal fungi are critical to ecosystems and growth of most plant species. Thus, it is important to consider the response of mycorrhizal fungi for ecosystems contaminated with radioactivity. Lab studies suggest that melanization can protect mycorrhizal fungi from radiation. In the laboratory, mycorrhizal isolates exposed to extremely high-level ionizing radiation from a Cesium-137 source all grew, in some cases as well as in control conditions. Furthermore, some isolates of the genus *Suillus* produce more melanin in response to such radiation (Kothamasi et al. 2019).

Growth of melanotic fungi in space

Resistance to the wide range of environmental conditions discussed above allows melanotic fungi to survive in a variety of "extreme" environments with multiple sources of stress and/or severe pressures. For example, *C. antarcticus* grows in Antarctic rocks, where combined pressures include high ultraviolet radiations, extremely low temperatures, limited nutrients, and dry conditions (Sterflinger et al. 2014).

The discovery of melanized fungi in extreme environments has prompted researchers to consider whether melanotic fungi can survive in space and to test this in simulations as well as on actual space missions. Conditions in space are indeed extreme; they include microgravity, radiation, and temperature extremes. Melanized fungi have been found growing on the Mir Space Station and International Space Station (ISS). Recent studies have addressed fundamental questions about growth of melanotic fungi in space, such as the following: (1) How is viability and spore germination affected? (2) Does the microscopic appearance of the fungi change? (3) How long can fungi survive in space? (4) What happens to fungal metabolism in space? Researchers note this research has implications for space travel and colonization, extraterrestrial life, and ability to do research in space (Onofri et al. 2015; Selbmann et al. 2018; Gomoiu et al. 2016).

Spores of *Aspergillus niger*, *Cladosporium herbarum*, *Ulocladium chartatum*, and *Basipetospora halophile* were measured for viability on ISS space missions. Some of these were previously identified as contaminants on spacecraft. Two different survival time frames were tested, 2 weeks and 5 months, and viability was measured by culturing and electron microscopy. For three of the strains tested, spaceflight had little to no effect on viability. Only one species of fungi was affected by long flight in space in terms of viability. In some cases, only minor changes were observed in spore and colony morphology after spaceflight (Gomoiu et al. 2016). Other studies have used simulations to study the potential effects on fungal viability and metabolism. Isolates from Antarctica, including members of the *Cryomyces* genus, were placed in a Mars simulator and tested for viability by culturing. Conditions for spaceflight and Mars simulation included Martian atmosphere and pressure, temperature fluctuations between −20 and 20 °C, ultraviolet radiation, and vacuum. All of the fungal isolates survived at least some of conditions. One isolate, *Cryomyces minteri*, survived all tested conditions (Onofri et al. 2008). Proteomics analysis of fungi grown under these conditions has also been studied by two-dimensional gel electrophoresis. Three diverse organisms, *C. antarcticus*, *Knufia perforans*, and *Exophiala jeanselmei*, were grown in the Mars Simulation Facility in Germany for several days at near room temperature. Under these conditions, a decrease in protein expression was observed, followed by recovery. Together, these studies demonstrate the ability of melanotic fungi to not only survive in space but also undergo essential metabolism under such conditions (Zakharova et al. 2014).

Presence and ecological roles of melanotic fungi in the environment

Quantifying and identifying melanized fungi present in the environment are complex and labor-intensive tasks. In one study, hundreds of endophytic fungi were collected from field sites in the region around the Sydney Basin region of Australia. A total of 118 out of 902 (13%) of these isolates produced dark pigments. The melanized fungi were isolated in

culture and inoculated onto wheat seeds to test their impact on plant growth as determined by changes in biomass compared with uninoculated controls. Twenty-five of the 118 isolates reduced the growth of inoculated plants after 7 weeks of growth, while the remainder had either no effect or mildly enhanced growth (Mugerwa et al. 2013). Employing an alternate approach to study melanized fungi in soils, Siletti et al. (2017) analyzed melanin content from dozens of species, primarily basidiomycetes, found in collections around the world. Species were grown in the laboratory and melanin production quantified in an Azure A dye binding assay. All but one of the samples produced some level of melanin in the assay. There was no correlation between ecological function and amount of melanization observed in the fungi. Saprotrophs, white and brown rot fungi, and ectomycorrhizal fungi were distributed among both the highest and lowest melanin producers. Similarly, no correlation was observed between melanin production and phylogeny. Together, these studies suggest that melanized fungi may be readily isolated from soils. However, no specific ecological role for them has been elucidated thus far.

If melanotic fungi are widespread in nature, then they likely have important roles in ecosystems. Recent studies have asked how melanized fungi affect ecosystem cycles. Fungi are abundant in soils and melanin can make up between 1 and 10% of fungal biomass (Frąc, et al. 2018; Revskaya et al. 2012; Heidrich et al. 2019). Hence, fungal melanin is a potential carbon sink in ecosystems. Given melanin's high chemical stability, Fernandez and Koide (2014) hypothesized that melanin may impact decomposition of fungi in soils. The decomposition of melanotic ectomycorrhizal fungi was tested by placing samples in mesh bags in soil field sites. A negative correlation between decomposition rate and melanin content was observed. Moreover, the addition of melanization inhibitors to the samples increased the rate of fungal decomposition. Laboratory research on fungal decomposition revealed similar effects. Over 200 fungal samples were collected from heathland in Belgium and measured for melanin content and decay rates in the laboratory. A correlation was again observed between melanin content and decomposition. Highly melanized fungi decayed slowly (Lenaers et al. 2018). Together, this evidence supports the hypothesis that melanin may be a carbon sink in ecosystems since it is slow to degrade.

The above studies have covered basic questions concerning melanotic fungi related to their abundance and ecological role. In examining the worldwide distribution of melanotic fungi, it was noted that darker fungi are associated with latitudes farther from the equator, in colder climates, and are less able to survive higher temperatures (37 °C and above). The effects of melanization on temperature tolerance were also tested in the laboratory with *C. neoformans*, a yeast that produces a range of melanin colors in the presence of different substrates. Darkly pigmented yeast absorbed more energy than light-colored cells when exposed to visible, infrared, and UV light. Correspondingly, the presence of melanin improves *C. neoformans* ability to survive cold temperatures (Cordero et al. 2018). These data underscore the importance of melanin's role in heat transfer and energy transfer in fungi and may point to widespread patterns in fungal distributions and biology in ecosystems.

Melanized fungi and pathogenesis

Melanin is a known contributor to pathogenesis in fungi infecting humans, animals, and plants. The list of pathogens in which melanin's connection to virulence has been established includes both basidiomycetes and ascomycetes and this list continues to grow. Examples include the human pathogens *Cryptococcus neoformans* (Salas et al. 1996), *Aspergillus fumigatus* (Tsai et al. 1999), and *Paracoccidioides brasiliensis* (Taborda et al. 2008) and the plant pathogens *Colletotrichum gloeosporioides* (Wei et al. 2017), *Verticillium dahliae* (Fan et al. 2017), and *Magnaporthe grisea* (Money and Howard 1996). In some cases, melanin genes are upregulated during infection (Poyntner et al. 2016). Below, we discuss research on fungal melanins' contribution to pathogenesis, which includes microbiological, biochemical, and genomics studies.

Immune blocking

Melanin's capacity to block host defense mechanisms is one way in which it increases virulence of human fungal pathogens. Reduced phagocytosis and pathogen killing are observed with melanized cells compared with non-melanized. For example, cells of *Penicillium marneffei* show reduced phagocytosis by macrophages when first melanized by culturing in the presence of L-dopa, a melanin substrate (Liu et al. 2014). Similar effects of fungal melanin on phagocytosis have been observed with other species, including *Fonsecaea pedrosoi* (Cunha et al. 2010) and *Sporothrix schenckii* (Romero-Martinez et al. 2000). Melanin is thought to protect the fungi from oxidative burst inside phagocytic cells. However, a more complex picture has emerged that suggest other mechanisms may be at work, such as by interfering with host cell signaling and autophagy or affecting recognition of fungal cells. Recent research has elucidated mechanisms and molecules that govern the interaction of fungal melanin with the host innate immune system.

Effects on phagocytosis

Fungal melanin can block the autophagy pathway LAP (LC3-associated phagocytosis) and protect the fungi from being destroyed inside phagocytic cells. *Rhizopus oryzae* melanin

may increase virulence by this mechanism. Chemical treatment to remove melanin from *R. oryzae* resulted in phagosome maturation of macrophages, whereas melanized conidia arrest phagosome maturation (Andrianaki et al. 2018). Likewise, melanized *A. fumigatus* conidia are resistant to killing by monocytes and do not activate the LAP pathway. However, mutants lacking the *pksP* gene do activate the LAP pathway, resulting in phagolysosome maturation and killing of conidia (Akoumianaki et al. 2016).

A thorough analysis of melanin synthesis mutants in *A. fumigatus* revealed the importance of melanin to the overall surface structure of conidia. Wild-type conidia are coated with melanin and a rodlet protein layer. Analysis of mutants representing early, middle, and late stages of melanin formation revealed a range of effects on both conidial surface structure and dendritic cell activation. Mutants in the early stages of melanin synthesis had grossly malformed surface rodlet layers and correspondingly activated dendritic cells. By contrast, late pathway mutants appeared to produce a normal rodlet layer and did not activate dendritic cells, similar to wild-type conidia. These data suggest that *A. fumigatus* melanin may have an important structural role that ultimately affects interaction with the host immune system (Bayry et al. 2014).

Melanin affects the interaction of fungi with other cell types as well. Lung epithelial cells are capable of phagocytosing *A. fumigatus* conidia, albeit at low levels. Interestingly, fungal melanin increases phagocytosis, as *pksP* mutants show a lower phagocytosis percentage compared with wild type, the opposite pattern from what is seen with macrophages or monocytes. Melanin also inhibits apoptosis of epithelial cells. The study authors hypothesize that epithelial cells may be a site of extended conidial survival in the host and that melanin contributes such persistence (Amin et al. 2014). *A. fumigatus* conidia also interact with human platelets and potentially affect the inflammatory response to fungal infection. Melanin was identified as one component of conidia that contributes to the interaction (Rambach et al. 2015).

Effects on signaling in phagocytic cells

Other research supports the hypothesis that fungal melanin impacts host cell signaling in phagocytic cells. Fungal melanin affects gene expression in host phagocytic cells. Genome-wide transcriptional analysis of macrophages cultured with wild-type, albino, or hyper-pigmented strains of *Fonsecaea monophora* uncovered over 900 genes with altered expression when albino strains were compared with pigmented strains. Many of the genes represented known immune response signaling pathways (Shi et al. 2019).

Apoptosis of immune cells is an important part of host defense. Infected host cells can undergo apoptosis, thereby destroying the pathogen. Fungal melanin interferes with apoptosis of phagocytic cells. Hyperspectral imaging monitoring of single monocytes revealed that monocytes infected with wild-type *A. fumigatus* conidia recovered from apoptotic acidification. By contrast, non-melanized *pksP* mutants did not induce recovery from acidification and the monocytes completed apoptosis (Mohebbi et al. 2016).

The protection of fungal cells by phagocytic killing may be in part through effects of melanin on signaling. The mechanism by which fungal melanin inhibits LAP may be through inhibition of calcium signaling in host cells. Fungal melanin was found to inhibit calcium-calmodulin signaling in infected monocytes, possibly by binding and chelating calcium. Without normal calcium signaling, NADPH oxidase is not recruited to the phagosome in the monocytes and fungi are not destroyed (Kyrmizi et al. 2018). These data suggest that the inhibition of phagocytosis by melanin is not only due to its ability to neutralize oxidative radicals, but effects on signaling as well.

Immune activation and recognition of fungal melanin

In contrast to the above studies that focused on melanin's immune-blocking capacity, other research has revealed that melanin is immunologically recognized and that it activates certain components of host defense. A mammalian receptor for fungal melanin was identified with DHN melanin isolated from *A. fumigatus*. The receptor, MelLec, is a lectin that is widely expressed in epithelial tissues. Data suggests that MelLec plays a role in host defense against aspergillosis. Mice lacking MelLec are more susceptible to infection when immunosuppressed. Furthermore, a single-nucleotide polymorphism (SNP) in the human MelLec gene is associated with aspergillosis in transplant recipients when the donor has the SNP (Stappers et al. 2018). Lastly, melanized fungal species can activate the complement system, a molecular component of the innate immunity. In fact, C3, C4, and C5 fragments bind to purified fungal melanin from *F. pedrosoi*, indicating there is a direct interaction (Pinto et al. 2018).

Antifungal resistance

Besides increasing the virulence of fungal pathogens, melanins make fungal infections more difficult to treat by increasing resistance to antifungal drugs. Here, we discuss this facet in detail. We describe the apparent protection of fungus by melanins against drugs meant to actually kill fungus. Five examples are given by showing that fungi are protected by melanins, which by virtue of their antioxidant capacity and protective sheath decreases effectiveness of antifungal drugs. Furthermore, we discuss recent findings that some fungi actually increase melanin production in response to antifungals. Taken together, the research suggests that the development of

agents that selectively inhibit the melanin biosynthesis in fungi may enhance existing antifungal therapies.

Melanins in *Penicillium marneffei* provide protection against the treatment of antifungal drugs amphotericin B, clotrimazole, ketoconazole, itraconazole, and fluconazole (Fig. 2). These drugs were less effective against melanized cells compared with non-melanized cells of *P. marneffei*, when studies were carried out in the presence of L-dopa. The study suggests that the spontaneous synthesis of melanin decreases susceptibility of *P. marneffei* to antifungal agents (Kaewmalakul et al. 2014). More examples of antifungal drugs not performing as well as expected, due to the presence of melanins, are described, as we will see next. It was also shown that melanins protect *Sporothrix brasiliensis* and *Sporothrix schenckii* against the fungal cell-wall synthesis inhibitor drug, terbinafine. When *Sporothrix* spp. were subjected to the mixture of terbinafine and tricyclazole, the minimal inhibitory concentration (MIC) values for terbinafine were reduced. The decrease of the MIC values was attributed to the ability of tricyclazole to inhibit the synthesis of DHN-melanins consequently reducing the amount of melanin and viability of fungal cells (Almeida-Paes et al. 2016).

Melanin also protects fungi from a drug functioning by photodynamic action. *Paracoccidioides brasiliensis* was

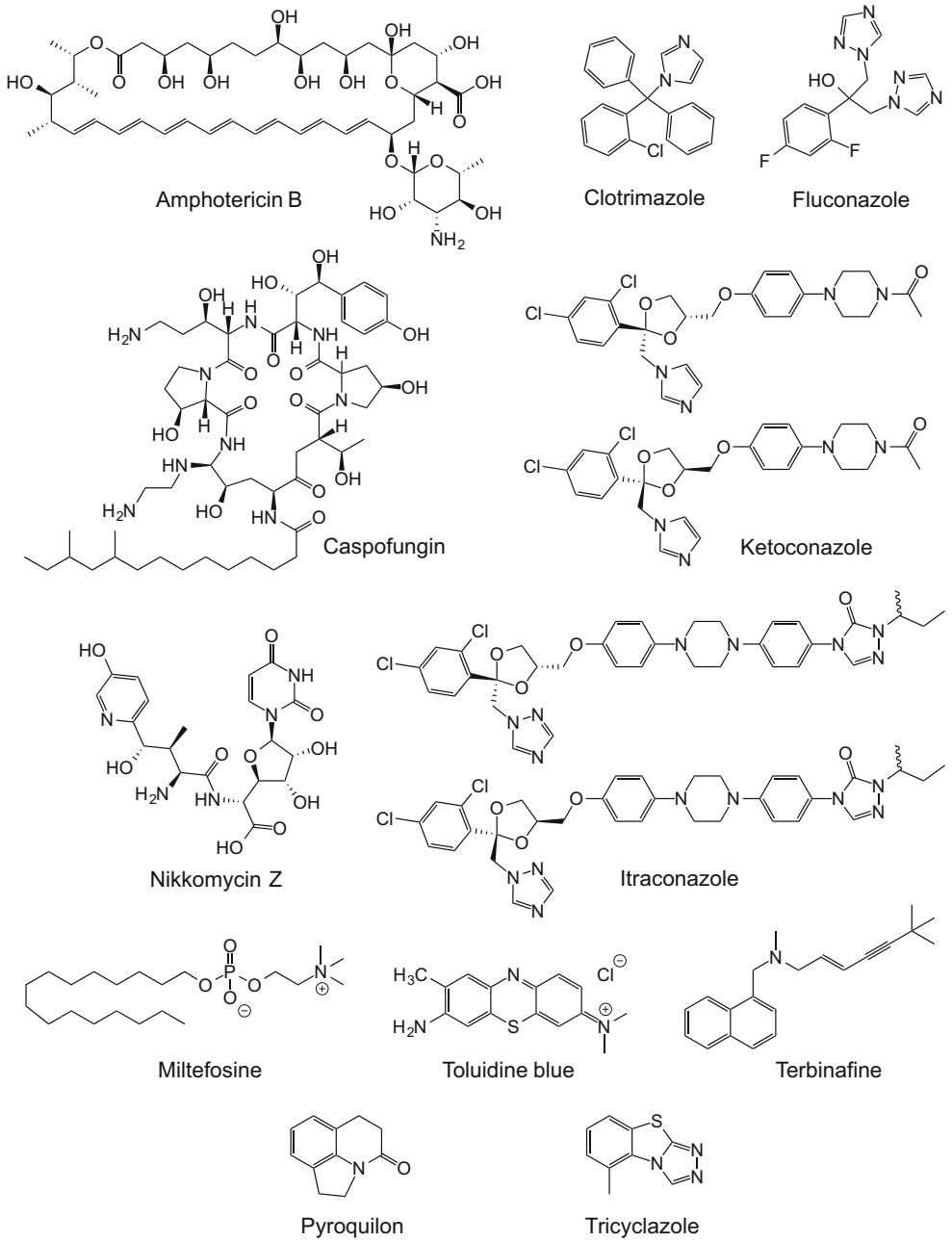

Fig. 2 Chemical structures of antifungal drugs

subjected to antimicrobial photodynamic inhibition (aPDI) with toluidine blue O (TBO) as a photosensitizer with oxygen and a 630-nm LED light (Baltazar et al. 2015). Drugs itraconazole and amphotericin B were also tested for comparison. These studies indicated that melanin served as a radical scavenger against many ROS and RNS produced with the exception of peroxynitrite $ONOO^-$. This pointed the authors to suggest that $ONOO^-$ is a key species for reducing fungal survival. Furthermore, the minimal inhibitory concentrations (MIC) were increased for itraconazole, and for the amphotericin B when melanized cells were tested. Thus, the MIC results show that melanized yeast cells are less susceptible to itraconazole and amphotericin B. The study supports growing body of evidence that melanin serves as a radical scavenging mechanism protecting yeast from the destructive effect of ROS, RNS, and itraconazole and amphotericin B antifungal drugs.

Paracoccidioides brasiliensis and *Paracoccidioides lutzii* produced melanin when treated with miltefosine at the subinhibitory concentration of 0.5 μg/mL (Rossi et al. 2017), which was confirmed by flow cytometry involving labeling with antibodies to melanin. The analysis revealed that yeast treated with miltefosine actually increased the melanin content compared with untreated cells. Similarly, the synthesis of 1,8-dihydroxynaphthalene (DHN)-melanin was found to increase during growth of fungus *Alternaria infectoria* in response to any of the following: itraconazole, nikkomycin Z, and caspofungin. In a control reaction, the inhibition of DHN-melanin synthesis by pyroquilon lowered minimum effective concentration (MEC) of caspofungin (Fernandes et al. 2015).

Conclusions and future prospects

Research on melanotic fungi has implications for multiple fields of study, from microbiology, ecology and the environment, medicine to astrobiology. The initial discoveries of melanin's fascinating roles in fungi are now being further explored in more nuanced studies that address questions such as (1) what factors affect melanin production? (2) what are the genomes of melanotic fungi like? and (3) how is melanization related to other cellular processes?

QTL mapping of more than 200 *C. neoformans* strains identified 5 genomic regions that were associated with altered levels of melanin production (Vogan et al. 2016). In another study, fifty-four different *C. neoformans* isolates were analyzed for melanin production levels. The level of melanin production varied by as much as fifty-two fold in the isolates. The influence of both environmental (e.g., temperature, oxidative stress) and genetic factors on melanin levels was tested. The researchers found that genetic factors had a predominant role in determining the level of melanin produced (Samarasinghe et al. 2018).

Our understanding of the biology of fungal melanin is growing due to advances in transcriptomics and genomics research. Transcriptomics research on melanization-associated genes in fungi has revealed relationships between melanization and various cellular processes. Transcriptomic analysis of an albino mutant of *F. monophora* identified over 2000 differentially expressed genes (DEGs) compared with melanized strains. DEGs included genes in the DHN and DOPA melanin pathways, cell wall, light sensing, and stress response, as well as cell growth and metabolic pathways. No obvious single gene created the albino phenotype (Li et al. 2016). Genome-wide transcriptional analysis of *C. neoformans* genes upregulated in the presence of L-dopa identified a number of stress response genes associated with melanization (Eisenman et al. 2011).

Over 1000 fungal genomes have been sequenced (Araujo and Sampaio-Maia 2018). Recently, the genome of *Wangiella dermatitidis*, a melanotic fungus, was sequenced and compared with other known fungi. A number of melanin pathways were identified in this fungus and many of the melanin genes were expressed at high levels (Chen et al. 2014). In analyzing the genomes of melanotic fungi, no clearly distinctive or trademark patterns have yet emerged. For example, the extremophile *C. antarcticus* has a genome very much like other fungi in terms of length, GC content, and encoded proteins (Sterflinger et al. 2014). Continued sequencing and analysis of melanotic fungal genomes will provide researchers with tools for understanding these fascinating organisms.

Dedication The authors wish to dedicate this article to the memory of Dalchand (Neil) Rampaul (1957–2019), a beloved colleague and friend.

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