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ROLE OF MELANIN PRODUCTION IN FUNGAL PATHOGENESIS

Microbiology

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INTRODUCTION

Melanins are distinct high molecular weight dark brown or black pigments. They are stable polymers of phenolic compounds, insoluble in aqueous or organic fluids, resistant to concentrated acid and susceptible to bleaching by oxidizing agents[1,2,3]. Melanin production is the most universal adaptation of living organisms to cope with the variable conditions of the planet[4,5,6]. The evolutionary importance of melanogenesis is undisputable due to presence of melanins in almost every large taxon of the animal and plant kingdom. Of note, melanogenesis via the polyketide synthase pathway or catalyzed by phenoloxidases has been reported in diverse fungi, bacteria and helminths[7].The ability of certain microbes to produce melanin has been found to be playing important role in virulence and pathogenicity for their respective hosts. In this review, we will briefly discuss on the pathogenic importance of melanins in fungi.

Melanin synthesis in fungus

The most common precursor for fungal melaninsis1,8-dihydroxynaphthalene (DHN) [also known as DHN-melanins] and melanogenesis is via the polyketide pathway[8,9,10,11,12]. The DHN pathway hasbeen reported functional in several important human pathogenic fungi. Human pathogenic fungi, that produce melanin are, *Cryptococcus neoformans, Histoplasma capsulatum, Sporothrix schenckii, Aspergillus spp., Paracoccidioides brasiliensis* etc[13,14,15,16,17]. However, *Candida spp*. do notmelanize. The polymers that produce melanin can be of three main types: (a) Eumelanin (brown or black), Pheomelanins (yellow-red) and Allomelanins. Of the human pathogenic fungi, *C. neoformans* makes only eumelanin[18].Whereas, *H. capsulatum*[19], *P. brasiliensis*[16] and *Aspergillus spp*[17,20,21] are thought to synthesize DNH melanin.

Localization

The finding of melanin at the fungal cell wall is in contrast to localization of melanin in melanosomal vacuoles of mammalian melanocytes[22].The fungal cell wall is composed of dynamic construct of complex branched polysaccharides, mannoproteins and proteins[23,24]. Fungal melanin is typically located within the cell wall, but the distribution and quantity varies widely between species. In *C. neoformans,* the melanin occupies throughout the cell wall over time[25,26]. Incontrast, the melanin is either found along the outer regions of the cell wall and/or clustered on the cell wall surface of many pathogenic fungi, including *Candida albicans*[26,27], *Aspergillu s*sp.[28,29], *Sporothrix schenckii*[30], *Coccidioide s*sp.[31], and *Histoplasma capsulatum*[32]. In vitro melanized particles ('ghosts') are similar in dimension to their parental melanized cells[33,34]. As exception, melanin can also be deposited as granules at the surface of the cell wall, as with *V. dahlia*[35], *Sporothrix schenckii*[36], etc. Notably, in *Fonsecaea pedrosoi* melanin is stored in melanosome-like cytoplasmic bodies[37]. However, fungi are also known to produce extracellular melanins[38].

PATHOGENESIS

Apart from fungus, bacterial species having melanin are *Pseudomonas aeruginosa*, *Hypomonas sp. Shewanelia colwelliana*, *Legionella pneumophila, Burkholderia cepacia, Proteus mirabilis, Klebsiella pneumoniae, Escherichia coli, Bordetella pertussis, Campylobacter jejuni, Yersinia pestis* and *Mycobacterium* species. Melanin also play a role in pathogenesis of some protozoa like *Plasmodium*, *Tetrahymena thermophila* and *Dinoflagellata*. Out of these micro-organism, *Cryptococcus neoformans* has been studied extensively with regard to melanin synthesis and its virulence. In this paper, we will concentrate over the role of melanin in pathogenesis of fungal diseases only.

There are various correlational studies in relation to virulence of fungal melanin. Not all the pathogenic fungi are melanotic, however many fungal species have property of invasiveness which is common for production of melanin. These are called as dematiaceous or phaeohyphomycetous fungi[39].There are numerous melanizing and non-melanizing strains of *Basidiobolus* species, melanizing cultures were associated with human disease [40]. It has been also noted for melanizing species of *Cryptococcus* most common cryptococcal pathogen of humans [41].

Mel^- point mutants which is either UV-induced or spontaneous for *C. neoformans* have exhibited reduced virulence on several occasions as compare to Mel^+. These effect were seen on L-DOPA, dopamine, or L-norepinephrine agar, also exhibited an inability to grow at 37°C. These property were also demonstrated by three temperature tolerant Mel^- progeny did not kill any mice, while the Mel^+ progeny killed all mice inoculated under the conditions used [41].The gene which regulates the melanization were classified by meiotic genetic analysis[42].The drug glyphosate may also inhibit melanization, particularly in *C. neoformans*. However, the significance of their therapeutic effect is not clear, because the drug is thought to inhibit de novo synthesis of aromatic compounds (including precursors of melanin) which synthesize them[43]. Whereas on the other hand, *C. neoformans* makes melanin only from exogenous catecholamines[44].

The virulence factor also depends upon penetration into the cells, particularly in *Wangiella dermatitidis,* in which growth rate is determined on the agar by hardness of the gel. Melanin is also an important in hyphal tip protrusion, which may be possible due to osmotic mechanism[45].It also act as a physiological redox buffer because multiple oxidation states for melanin were confirmed by electrochemical reduction of melanin with Ti (III) and oxidation with Ferric or oxygen [46]. It also catalyze the reduction of Ferric by NADH showing that melanin may act as a mediator of electron exchanges[47]. Interestingly, melanin did not protect yeast cells against hydrogen peroxide. Several studies suggests that (i) hydrogenperoxide is only 1/100 as fungicidal as hypochlorite; (ii) while melaninis extracellular, hydrogen peroxide is neutral and diffuses readily into the cell; and (iii) hydrogen peroxide reacts with melanin only under alkaline condition. Melanin may protect against several free radicals, which may be of either oxygen or nitrogen free radicals[48]. The growth for mutant melanin precursor L-DOPA increased resistance to nitric oxide but did not to hydroxyl radical, suggesting very small amount of melanin might suffice to protect cells against nitric oxide here as larger amount were required for protection against the oxygen radical-generating system. Some melanized fungi are also resistant to heat in some but not

in others[49]. But their mechanistic basis for resistance is not known. Exogenous L-DOPA supplied to a wild type culture of *C. neoformans* allowed melanization and slightly increased resistance to heating, while the same chemical supplied to a Mel^- mutant did not show its resistance[50].

Use of antioxidant pigment mutants in *A. fumigates* show that green, rough, melanin-like, antioxidant surface pigments participate in virulence of aspergillosis. Whereas pigment-less white mutants induced by UV have smooth external surface, although the pigments were responsible for surface texture. The sensitivity of white mutants were 10-fold to 12-fold more than that of exogenous oxidant wild type variety. Mutant variety of conidia were shown to have been damaged more extensively than wild-type. Whereas mutant conidia were less lethal when inoculated into mice[51]. DHN-melanin of dematiaceous fungi functions as an antioxidant. Melanized strains were found to resist oxidative killing by human neutrophils more than non-melanized strains. However normal strains whose melanization was blocked by growth in acidic medium, were made as sensitive to oxidative killing, while mutant melanized which were exposed with the DHN pathway intermediate, scytalone, was made resistant[52].

The interaction of melanin with metals also help in the virulence of the fungus, through binding of melanin with many metals[53]. Binding has been interpreted as in case of toxic metals. The concentrations of free radicals decreased in the case of essential metals, adjacent to the cell.

Melanin either oxidize or reduce metals and can either facilitate or inhibit single-electron transfers, leading to free radical formation, depending upon whether its binding to metal effectively or not. Ferrous ion (Fe^{++}), in the presence of the weak chelator ADP bound by melanin, inhibits hydroxyl (OH^-) radical formation from Fe^{++} and hydrogen peroxide(H_2O_2).Whereas in the presence of the strong chelator, EDTA, melanin neither bind to Fe^{++} and nor inhibit hydroxyl (OH-) radical formation. If Fe^{++} is chelated by EDTA, melanin do not bind to ferric ion (Fe^{+++}); thus resulting in $Fe^{++}$to react with H_2O_2 to produce OH^- radicals[54]. Thus, in the presence of the stronger chelator, EDTA, melanin does not bind Fe^{+++} effectively but does serve as a reducing agent for Fe (III). However, melanin does not protect against transition metals in all fungi.

The interaction of melanin on exposure to various drugs depends upon either it is melanized or non melanized. Melanin did not protect *W. dermatitidis* against antifungal drugs, since Mel^-mutants were no more susceptible to a variety of antifungals than was the Mel^+ wild type[55]. The reason for differential susceptibility is not known, but trifluperazine has been reported to be effective in the treatment of experimental murine cryptococcosis[56].

Interaction of melanin with extracellular enzymes depends upon pathogen, binding to organism's extracellular hydrolytic enzymes and to release them very slowly. This is for localizing the pathogenic factors to the site of the infection[57]. Cryptococcal melanin bind cryptococcal cellular proteins and provide some protection from leukocyte microbicidal proteins, binding them before they can reach the plasma membrane of the fungus[58]. These melanized fungi are more resistant to hydrolytic enzymes, possibly because of sequestration of enzymes on melanin, or because of cross-linking of cell wall polysaccharides by melanin[59] or possibly because of steric hindrance by melanin attached to the cell wall polysaccharides.

Melanin may also protect the fungi from UV rays. However UV resistance have no direct relation to the host-parasite relationship, but melanized strains of fungi are more resistant than nonmelanized strains to UV irradiation[60].

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