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EDITORIAL

Amino acid biosynthetic pathways as antifungal targets for fungal infections

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Aspergillus fumigatus is an opportunistic pathogen and ubiquitous in the environment. In humans, *A. fumigatus* can cause a wide range of infections whose symptoms are directly determined by the immunological status of the host.[1] Superficial infections are related to local trauma or overgrowth of the fungus in burns; under occlusive dressings; after corneal trauma (keratitis); or in the sinuses, mouth, nose, or ear canal. Allergic forms of the diseases are caused by an exaggerated response of the immune system to colonization of the airways with *Aspergillus*. In addition, invasive aspergillosis (IA), usually acquired through inhalation of conidia and further fungal growth in the lung, is a systemic infection that affects immunosuppressed patients. IA represents one of the main cause of morbidity and mortality for infection complications in patients with hematological malignancies, hemopoietic stem cell and solid organ recipients and patients with other immunodeficiencies.[2]

Currently, the treatment options for aspergillosis are limited to 3 classes of antifungal drugs that target essential structural components of fungal cells. Triazoles and polyenes are aimed at ergosterol, the predominant sterol within the fungal cell plasma membrane. In particular, triazole drugs (itraconazole, voriconazole and posaconazole) are inhibitors that target the ergosterol biosynthetic pathway by binding to the 14-α sterol demethylase enzyme while the polyene amphotericin B may kill fungi primarily via channel-mediated membrane permeabilization leading to fungal cell death.[3] On the other hand echinocandins represent the newest class of antifungal agents and include caspofungin, micafungin and anidulafungin.[4] Echinocandins interfere with fungal cell wall synthesis by blocking β-1,3-D-glucan synthase.[5]

Despite the availability of drugs to treat *A. fumigatus* infections therapy can be challenging for many patients. The reasons for that are associated to the modest efficacy of existing antifungals and host toxicity. In addition, continuous increasing of antifungal drug resistance encourages the scientific community to look for novel antifungals compounds. In this regard, targeting virulence determinants in *A. fumigatus* has been an important research area for many years. In particular, the identification of essential metabolic pathways that do not exist in humans and inhibit fungal metabolism during pathogenesis provide interesting therapeutic alternatives. Among them, fungal species as well as other microorganisms have developed specific pathways for the biosynthesis of all proteinogenic amino acids.[6] Of the 20 amino acids, 9 are essential for humans and therefore several steps of the amino acid biosynthetic pathways are catalyzed by enzymes that are absent in mammals. In addition, attenuated virulence of strains of human pathogenic fungi defective in genes encoding enzymes of these fungi-specific pathways and antifungal in vitro and in vivo activity of some chemicals targeting these enzymatic steps suggest that these pathways are potential targets for antifungal chemotherapy.[6]

For *A. fumigatus*, it is known that some biosynthetic pathways of amino acids are crucial determinants for its virulence. For instance, biosynthesis of lysine,[7,8] of aromatic amino acids[8] and sulfur-containing amino acids such as methionine and cysteine[9] have been evaluated for their role in *A. fumigatus* pathogenesis.

In this issue of Virulence Haas and collaborators performed the functional characterization of the histidine biosynthetic pathway in *A. fumigatus*.[10] Histidine is one of the 9 amino acids essential for humans and therefore it should be acquired from the diet to guarantee optimal growth and development. Regarding histidine biosynthesis, there are some evidences suggesting that imidazole glycerol phosphate synthase (His7p) or histidinol dehydrogenase (His4p) could be potential targets for antifungal drugs.[6] His7p has been described as essential for histidine biosynthesis in plant pathogens as well as in

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opportunistic human pathogens such as *Cryptococcus, Candida*, and *Ajellomyces* spp.[6] In *A. nidulans* deletion of the imidazole glycerol phosphate synthase (hisB) caused histidine auxotrophy and deleted strains were blocked from sexual fruiting body formation on medium containing low concentrations of histidine.[11]

Synthesis of histidine in *A. fumigatus* is encoded by 7 genes of which the *his*B was deleted (Δ*his*B) in this work.[10] Deletion of this gene function in *A. fumigatus* caused histidine auxotrophy. In addition *A. fumigatus* was susceptible to 3-amino-1,2,4-triazole a compound that target the imidazole glycerol phosphate synthase enzyme confirming that histidine is required for its in vitro growth.

Further analysis of histidine requirement in vivo was performed by testing the pathogenicity of the Δ*his*B strain in 4 models of *A. fumigatus* infection: murine pulmonary infection, murine systemic infection, murine keratitis and the wax moth larvae. To date, murine models are considered to be the gold standard to study fungal pathogenesis and analyze efficacy of antifungal drugs. To mimic pulmonary and systemic infections, animals are usually immunosuppressed before fungal challenge with spores of *A. fumigatus*. Besides immunosuppressed individuals, superficial *A. fumigatus* infections such as keratitis can occurred in immunocompetent hosts. In this sense the authors also tested the pathogenicity of Δ*his*B strain in an immunocompetent model of murine keratitis. The Δ*his*B strain showed reduced virulence in all murine models. Although virulence of fungal pathogens has traditionally been determined using mammalian hosts, invertebrate models of aspergillosis such as *Galleria mellonella* appears to be very useful to test microbial virulence and to screen the efficacy of antimicrobial agents.[12,13] The development these in vivo models of fungal infection allows a higher throughput than the conventional mammalian models and reduces the number of low added value experiments frequent in the drug development process, with a positive impact regarding ethical considerations. Using the *G. mellonella* the authors demonstrated that the disruption of histidine biosynthesis by the deletion of the *his*B gene or using imidazole glycerol phosphate synthase inhibitors increased larvae survival. Altogether this showed that in the absence of histidine biosynthesis, *A. fumigatus* would need alternative mechanisms to get this amino acid from the host.[10]

Previous investigations by the same research group have resulted in an extensive knowledge of how *A. fumigatus* adapts to iron starvation conditions within the host.[14] Control of iron uptake is considered to be the major iron homeostatic mechanism in *A. fumigatus*. For that *A. fumigatus* employs siderophores that consist on extracellular siderophores for iron uptake as well as intracellular siderophores for iron storage. Siderophores are central components of the fungal metabolism as they affect germination, sexual and asexual reproduction, oxidative stress resistance and they are crucial for *A. fumigatus* virulence. In addition recent studies have focused on its potential to improve therapy of fungal infections.[14]

In line, the group of Haas and collaborators discovered an additional mechanism that link histidine biosynthesis and *A. fumigatus* metal homeostasis. Authors described that histidine has the property to bind metals such as iron so it could contribute to regulate iron excess as well as iron starvation during infection. In addition this mechanism was further extended to other metals as copper and zinc.[10]

The finding that blocking histidine synthesis in *A. fumigatus* leads to reduce growth and attenuated virulence offers new possibilities for further investigations of this biosynthetic pathway as antifungal target. Considering the difficulty of treating fungal infections insights in fungal metabolic pathways that are absent in humans, such as synthesis of essential amino acids, are very promising.

Disclosure of potential conflicts of interest

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