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Spotlight – CORRECTED MANUSCRIPT

Fungal melanin rewires macrophage metabolism

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Abstract (48 words)

Aspergillus fumigatus **is a deadly fungal pathogen in immunocompromised patients. A report by Gonçalves *et al.* reveals that melanin, a secondary metabolite present at the surface of infecting fungal spores, induces glycolysis in macrophages to promote inflammatory responses. This opens a window for considering innovative host-directed antifungal therapies.**

Keywords: Fungal infections, *Aspergillus*, glycolysis, macrophages, melanin

Core text (1,037 words)

While behaving as a saprophytic mold ubiquitously found in the environment, *Aspergillus fumigatus* remains an extremely worrisome fungal agent responsible for up to a hundred thousand deaths a year worldwide [1]. Among immunocompromised patients, neutropenia and general phagocytosis deficiencies are now well established as a predominant risk factors for these life-threatening fungal infections. This is consistent with the central role of lung epithelial cells and phagocytes (such as macrophages and neutrophils) in killing the large amount of *Aspergillus* spores (termed conidia) that we inhale daily [2].

In this regard, macrophages are considered as primarily sentinels that interact with infecting *Aspergillus* conidia. While the sensing and the phagocytosis of the conidia lead to a fine-tuned regulation of cellular metabolism required to induce a microbicidal activity of macrophages, processes involved in the orchestration of host response toward *Aspergillus* remains largely obscure [3]. In a striking new study, Gonçalves and colleagues decipher the molecular and biochemical mechanisms through which infection with *Aspergillus* reprograms macrophage metabolism for enrolling the energetic, anabolic, and functional activity during host defense [4].

They first demonstrated that macrophage metabolism is modulated by *Aspergillus* infection (Figure 1). Transcriptomic analysis showed that the phagocytosis of *Aspergillus* conidia, occurring within the first two hours, is accompanied, as expected, by a strong production of inflammatory cytokines and by a significant upregulation of genes involved in glycolysis. This metabolism shift towards glycolysis in infected macrophages is attested by recruitment of glucose transporters and of glycolytic regulators while suppressors of glucose uptake and metabolism appeared downregulated. The pathophysiological relevance is provided by the observation of the induction of several glycolytic enzymes in the lungs of mice from day 1 to day 3 following fungal infection. This was corroborated by an increase of lactate release and glucose consumption by macrophages along with increased levels of most glycolytic intermediates. Surprisingly, contrary to the pathogenic yeast *Candida* that hijacks macrophage glucose to promote their death during infection, the sugar depletion process seems not to be committed in the framework of *Aspergillus* infections [5]. This is illustrated by (i) the absence of significant loss of viability by human macrophages infected with *Aspergillus* conidia, (ii) no impact on the expression of fungal glycolytic enzymes during the infection process, and (iii) attested at the pathophysiological level by an unchanged number of macrophages in the lungs of infected mice. This data are really exciting in an evolutionary perspective since they imply that different human pathogenic fungi, belonging to unrelated clades, have independently fine-tuned distinct strategies to rewire metabolism of host immune cells for taking over.

The authors then nicely showed that a full glycolysis activity must operate in macrophages for immune responses to *Aspergillus* infection. Indeed *in vitro*, glycolysis inhibitors or substrates impairing glycolytic flux significantly affect the production of proinflammatory cytokines, phagocytosis and killing that are the major effector functions of macrophages during infection. Similar results were obtained by using these inhibitors in immunocompetent infected mice, reinforcing the idea of a central role played by glucose metabolism in the induction of host responses to *Aspergillus in vivo*.

Most importantly, the host glycolysis induction was shown to depend on fungal melanin (Figure 1). Together with rodlets, melanin is known for a while to stand as major components in outermost layer of dormant *Aspergillus* conidia [6,7]. This was evidenced by a decrease of mRNA levels of glycolysis-related genes in macrophages infected with conidia from an *Aspergillus* strain lacking the capacity to biosynthesize melanin. The pivotal role of fungal melanin in stimulating the macrophage glycolysis was

also attested by the inability of the *Aspergillus* mutant strain to efficiently trigger lactate secretion and glucose consumption in infected macrophages. Conversely, an *Aspergillus* mutant strain devoid of rodlets was able to induce the metabolic reprograming in macrophages in a way similar to *Aspergillus* wild-type strain. Further investigation gave evidence that melanin is only active in these processes when present in the conidial cell wall as fully mature melanin particles. Accordingly, the melanin precursor 1,8-dihydroxynaphthalene (DHN) did not influence glycolysis in macrophages and activation of glycolysis in infected macrophages was shown to be fully dispensable of the recently identified 1,8-DHN receptor MelLec [8]. Finally, beyond being only active in fully mature particles, authors provided evidence that melanin should be actively shed or removed during conidial germination for activating macrophage metabolic reprogramming.

The last part of this study was focused on the identification of the host cell signaling pathway that mediate rewiring by melanin of host metabolism. First, pharmacological approaches indicated that endoplasmic reticulum calcium stores, independently of calcium entry, are required for the activation of glucose metabolism by fungal melanin. Furthermore, transcriptomic together with phosphorylation analyses revealed the involvement of the canonical Akt/mammalian target of rapamycin (mTOR) signaling pathway in the melanin-induced macrophage reprogramming, leading to the activation of the transcription factor hypoxia-inducible factor 1 subunit alpha (HIF-1α). This factor is involved in the melanin-driven immune and metabolic regulations of macrophages during *Aspergillus* infection. This is consistent with previous studies that gave insight into a crucial role of this prominent intracellular sensor as a master regulator of glucose metabolism in myeloid cells [9]. Taken together, these experiments provide pioneering evidences that, during the *Aspergillus* infection process, the cell wall secondary metabolite melanin remodels intracellular calcium machinery and impairs cell circuitry via calmodulin [10]. This allows melanin to directly control an immunometabolic signaling axis towards glycolysis that relies on phagosomal recruitment of mTOR and the downstream activation of HIF-1α (Figure 1).

In conclusion, these findings highlight new mechanistic insights into the interaction between *Aspergillus* and sugar metabolism in immune cells. Of course, it is highly likely that additional metabolic pathways should intervene in the regulation of antifungal immunity. Unfortunately, currently available technologies are still limiting in acquiring a broad view of the cell circuitries, metabolic pathways, and their interconnections that underlie such complex immune processes. Nevertheless, this enlightening article provides unprecedented data suggesting a central role for glycolysis and represents a promising step toward a better comprehension of the metabolic features involved in the regulation of antifungal immunity. Immunomodulatory approaches or metabolic adjuncts should thus be considered in the near future to reorient host cells towards immune protection against infections. This is of course in line with the current trend that aims at developing innovative host-directed therapies.

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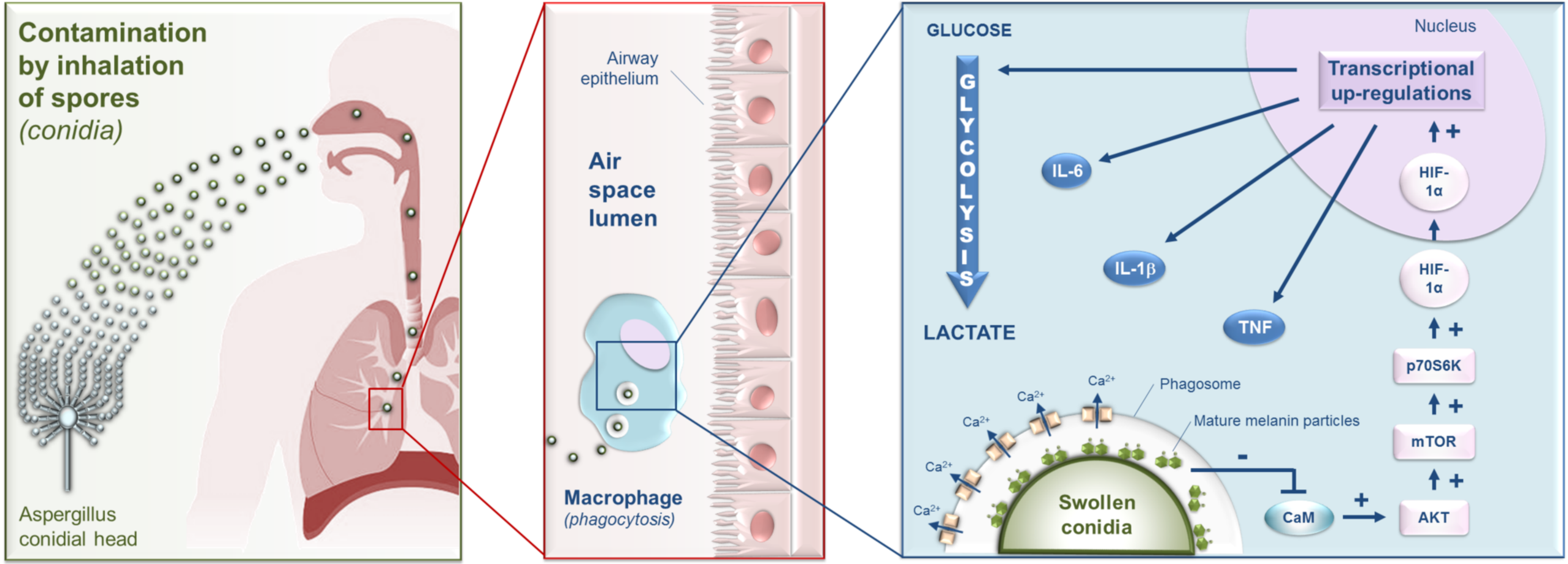
Figure legend

Figure 1. Fungal melanin rewires macrophage metabolism. The human lung is contaminated daily by a huge amount of *Aspergillus* fungal spores (termed conidia). At the level of the airway epithelium, macrophages are considered as primarily sentinels that phagocyte infecting *Aspergillus* conidia. Fully mature particles of melanin present at the surface of the conidia are probably removed during the swelling process and this inhibits the calcium/CaM signaling in macrophages. The melanin-mediated restraining of calcium signaling positively affects the mTOR pathway leading to an activation of HIF-1α. HIF-1α participates in the transcriptional up-regulation of a set of genes for immunometabolic responses. These include genes encoding enzymes involved in the glycolysis pathway but also those encoding some proinflammatory cytokines (IL-6, IL-1β, and TNF).


Contamination by inhalation of spores (conidia)
Aspergillus conidial head
Airway epithelium
Air space lumen
Macrophage (phagocytosis)
GLUCOSE
GLYCOLYSIS
LACTATE
Nucleus
Transcriptional up-regulations
IL-6
IL-1β
TNF
HIF-1α
HIF-1α
p70S6K
mTOR
AKT
CaM
Ca2+
Phagosome
Mature melanin particles
Swollen conidia