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Impact of HIF-1 α and hypoxia on fungal growth characteristics and fungal immunity

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Abstract

Human pathogenic fungi are highly adaptable to a changing environment. The ability to adjust to low oxygen conditions is crucial for colonization and infection of the host. Recently, the impact of mammalian hypoxia-inducible factor-1 α (HIF-1 α) on fungal immunity has emerged. In this review, the role of hypoxia and HIF-1 α in fungal infections is discussed regarding the innate immune response.

Keywords

Hypoxia; SREBP; HIF-1; Fungal immunity

1. Introduction

The impact of fungal infections surfaced when human immunodeficiency virus (HIV) became a pandemic threat, and recipient survival improved by immunosuppressive therapies as well as glucocorticoids became the first choice for the treatment of autoimmune diseases [1–4]. Mammalian pathogenic fungi share a feature which is unique inside the fungal kingdom: They are able to survive inside their hosts. The reason for this successful colonization is their ability to cope with temperatures above 30 °C. This thermal tolerance created an evolutionary benefit for host parasitism of mammals compared to pathogenic fungi of plants and insects [5]. Once inside the host, fungi are confronted by professional phagocytes such as macrophages (M Φ) that offer a large variety of antimicrobial activities to

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Conflict of interest
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thwart replication. While MΦ can cope with a low multiplicity of infection (MOI) [6], they need to be activated by cell-mediated immunity in order to finally restrict pathogen growth [7]. As a consequence, fungal pathogens are exposed to host microenvironmental challenges such as nutrient deprivation and low oxygen. Compared to atmospheric levels of oxygen (21% O₂), studies have shown that physiological oxygen levels are much lower and differ throughout the mammalian body. Starting in the lung, oxygen levels are ~14% in the alveoli and decrease to 5% O₂ in peribronchial tissues, whereas in organs such as the liver, the levels are less than 5% O₂ [8].

Regarding infections, tissue perfusion is impaired by disrupted blood flow and enhanced metabolic activity of host cells and pathogens. Within this setting, oxygen tension rapidly decreases. Oxygen levels below 3% O₂ are considered as hypoxia *in vitro* while *in vivo* hypoxia occurs every time oxygen consumption exceeds oxygen availability [9].

Mammalian cells cope with low oxygen environment by stabilizing proteins of the hypoxia-inducible factor family which are involved in maintaining cellular homeostasis. So far, there are three known members, hypoxia-inducible factor (HIF)-1α, HIF-2α and HIF-3α. While HIF-1α is ubiquitously expressed and plays a major role in immune cell function, HIF-2α and HIF-3α occur only in specific cell types [10–13]. Above 6% O₂, the abundance of HIF-1α protein is regulated via hydroxylation by oxygen-sensitive prolyl hydroxylases (PHD) and directed to proteasomal degradation by von Hippel Lindau tumor suppressor protein (pVHL) [14]. Under low oxygen conditions, HIF-1α is stabilized and translocates into the nucleus where it binds to constitutively expressed HIF-1β forming the heterodimer HIF-1 [15,16] which orchestrates the expression of genes involved in metabolic adaption [17] and innate immune responses [18,19]. Investigation of hypoxia adaption in fungal pathogens appeared within the last decade and revealed a homolog of mammalian sterol regulatory element binding protein (SREBP) which regulates expression of genes involved in hypoxia adaption [20].

Within this review, the current knowledge about the impact of HIF-1α and hypoxia on growth and survival of human pathogenic fungi is summarized and further discussed with regard to host–pathogen interactions in fungal immunity.

2. Hypoxia adaption in human pathogenic fungi

Human pathogenic fungi of the genera ascomycetes such as *Aspergillus fumigatus*, *Coccidioides immitis/posadasii*, and *Histoplasma capsulatum* as well as hemi-ascomycetic *Candida albicans* are highly adapted to a changing environment, since they exhibit an environment-dependent morphogenesis and a facultative intracellular lifestyle [21]. Usually, *C. albicans* is a commensal of the skin, mucosal surfaces and colonizes the gut in many healthy people [22,23]. During pathogenesis, *C. albicans* shows invasive hyphal growth, infiltrating deeper tissue leading to local, superficial infections up to severe systemic candidiasis. In the hypoxic microenvironment of the gut, virulence is usually suppressed [24,25]. Thermotrophic fungi such as *C. immitis* and *H. capsulatum* grow as a filamentous mold in soil at ambient temperatures. Inhaled spores switch into the pathogenic,

non-motile yeast phase (*Coccidioides species*: spherules) in the deeper alveoli at 37 °C causing acute pulmonary infections up to life-threatening systemic mycoses [26].

During host infection, pathogenic fungi face decreasing oxygen concentrations. Since they are eukaryotes, fungi utilize oxygen for cellular metabolism. Therefore, they need alternative pathways to sustain cellular homeostasis in hypoxic microenvironments. However, there are no homologs of mammalian HIF-1 α in fungi but homologs of the mammalian sterol pathways which are upregulated during hypoxia [20]. The mammalian sterol element binding protein (SREBP) pathway is found to be functionally conserved among fungi. In mammals, the transcription factor SREBP is activated by proteolytic cleavage and sustains cellular lipid levels by inducing gene expression involved in lipid uptake and synthesis [27,28].

Fungal homologs of SREBP, Sre1 and Sre2, were first discovered in *Schizosaccharomyces pombe* with homologs in other ascomycetes such as *A. fumigatus*, *H. capsulatum*, and *Paracoccidioides brasiliensis* [29e31]. In fungi, the major sterol produced by homologous SREBP pathways is ergosterol which is necessary for sustaining membrane integrity [32]. Since ergosterol synthesis is a highly oxygen consumptive pathway, it acts as an indirect oxygen sensor in fungi and is therefore a central pathway in hypoxia adaption [20].

In *A. fumigatus*, the loss of homologs of Sre1 and Sre2, SrbA and SrbB respectively, attenuates fungal growth under hypoxia (1% O₂) [33]. Knockdown of Sre1 via small-interfering RNA (siRNA) in *H. capsulatum* leads to increased production of the iron chelator siderophore during iron replete conditions and delayed filamentation in liquid culture [34]. Further, Hwang et al. demonstrate that Sre1 is a critical factor for *H. capsulatum* replication and virulence in murine bone marrow-derived M Φ (BMDM) *in vitro* and *in vivo* respectively using null mutants [35].

Cleavage proteins activating transcriptional activity of SREBP homologs were shown to impact hypoxia adaptations. Stewart et al. identified the defective for cleavage (Dsc) complex, a Golgi E3 ligase, which performs cleavage of fungal SREBP homologs and is constituted out of four subunits dsc1–4 [36]. In *A. fumigatus*, lacking all four subunits led to an impaired growth in hypoxia (1% O₂) and high susceptibility to triazole drugs. Deficiency in dscA and dscC, homologs of dsc1 and dsc3 respectively, led to reduced virulence in a murine model of pulmonary aspergillosis [37]. In *H. capsulatum*, dsc2 deficient strains were reduced in growth under hypoxic (0.5% O₂) and anoxic (0% O₂) conditions while it had no impact on the course of infection *in vivo* [38].

Thus, human pathogenic fungi are able to adapt to low oxygen environment by an array of genes regulated by a conserved sterol synthesis pathway which was shown to be crucial for hypoxic growth and contributes to fungal virulence.

3. HIF-1 α in fungal immunity

During infection, fungi invade tissues leading to recruitment and accumulation of cells of the innate immune system. These sites of infection are less perfused and rapidly acidify due to increased energy demands of activated immune cells and pathogen replication. Besides

oxygen levels as regulator of HIF-1 α there are also oxygen-independent mechanisms. Bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium* induce transcriptional upregulation of HIF-1 α transcription in M Φ by toll-like receptor (TLR) downstream signaling via nuclear factor 'kappa-light-chain-enhancer' of activated B-cells (NF κ B) under normoxia [19]. Parasites such as *Toxoplasma gondii* and *Leishmania donovani* upregulate HIF-1 α stabilization by inhibition of PHD2 activity or iron depletion, respectively [39,40]. Thus, HIF-1 α could be somewhat beneficial for pathogens by sustaining energy and nutrient supply but it also drives immune defense by generating antimicrobial peptides, nitric oxides and pro-inflammatory cytokines [41]. However, there are few studies dealing with the role of low oxygen levels and HIF-1 α during fungal infections to date.

Among innate immune cells, M Φ exert an important although ambivalent role in fungal infections. On the one hand, they are permissive for fungal growth before activation by granulocyte-macrophage colony-stimulating factor (GM-CSF) and interferon gamma (IFN- γ) via CD4+ T cell-mediated immunity [42–44]. On the other hand, they are essential for recruiting other immune cells and isolate foci of infection by forming granulomatous structures during infection [45–47].

In the following, the impact of HIF-1 α on fungal immunity is discussed on the basis of the so far investigated human pathogenic fungi. An overview of the current knowledge about HIF-1 α induction by pathogenic fungi is summarized in Table 1.

3.1. *H. capsulatum*

H. capsulatum-induced granuloma are hypoxic in the liver of mice during disseminated histoplasmosis while overall HIF-1 α was transcriptionally upregulated in tissue homogenates of the liver and lung during disseminated and pulmonary histoplasmosis respectively. Analysis of liver granuloma extracts revealed CD4+, CD8+ T cells and M Φ as the major cell populations. While the CD4 β T cells are dominant cell population early during infection, M Φ constituted the main cell population in liver granuloma in the later course of time. 95% of these M Φ were hypoxic [31] and therefore might deploy the vast majority of HIF-1 α protein in granulomatous tissue. Phagocytosis of *H. capsulatum* by M Φ is partially mediated by complement receptor 3 (CR3) [48] as shown for other pathogenic fungi [49]. CR3 synergizes with Dectin-1, the major β -glucan receptor, and orchestrates a pro-inflammatory cytokine response via spleen tyrosine kinase (Syk) [50]. Herein, Syk signaling might contribute to elevated HIF-1 α protein levels in infected M Φ .

Subsequent studies demonstrated that the presence of HIF-1 α in M Φ is critically important for a functional innate immune response to *H. capsulatum*. Mice deficient in myeloid HIF-1 α showed high mortality, despite the production of pro-inflammatory cytokines such as (interleukin) IL-1 β and tumor necrosis factor alpha (TNF- α) known to be important for effective immune response. Fungal burden were elevated as well as the inflammation sites increased in size. The lack in host controlling the infection was due to increased levels of the anti-inflammatory IL-10 produced for the most part by M Φ . IL-10 ameliorated the anti-histoplasma effect of interferon gamma (IFN- γ) which could be recapitulated *in vitro* using

BMDM [51]. Thus, HIF-1 α is critical for maintaining responsiveness to activating cytokines by blocking excess production of IL-10 during histoplasmosis.

3.2. *A. fumigatus*

A. fumigatus shows enhanced exposure of β -glucan on the cell wall in areas of hypoxia in lungs of infected mice which facilitates immune recognition by Dectin-1, the major β -glucan receptor [52]. HIF-1 α is transcriptionally upregulated in human monocyte-derived dendritic cells (DCs) which is even enhanced by hypoxia (1% O₂) [53]. While HIF-1 α protein stabilization is partially Dectin-1 dependent during infection under normoxia Dectin-1 signaling is dispensable for enhanced HIF-1 α protein levels under hypoxia. The authors claim that this might be due to additional oxygen-dependent HIF-1 α stabilization which ameliorates the reduction by knockdown of Dectin-1, since the reduction in Dectin-1 does not diminish hypoxic-induced HIF-1 α accumulation in control cells. HIF-1 α induces cell activation by metabolic switch from oxidative phosphorylation (OXPHOS) to glycolysis indicated by elevated glucose consumption as well as lactate production. Furthermore, HIF-1 α enhanced the pro-inflammatory profile of infected DCs by promoting the release of IL-1 α , IL-6 under hypoxia and IL12p70 in normoxia [54]. The downstream effect of increasing IL-12 is exaggerated priming of naïve T cells for IFN- γ production by infected DCs [55].

In a murine model of invasive pulmonary aspergillosis (IPA), HIF-1 α was essential for immune cell recruitment to the lungs of infected mice. In the absence of HIF-1 α in the myeloid cells, mice succumbed to infection. Further, HIF-1 α is abrogated by corticosteroid treatment, resembling a predisposing factor for aspergillosis infection in patients. While M Φ and neutrophils are able to phagocytose and kill *A. fumigatus* in the absence of HIF-1 α , neutrophil recruitment to the lung is impaired. HIF-1 α is crucial for transcriptional upregulation of the C-X-C motif chemokine ligand 1 (CXCL1) which is the main chemoattractant for neutrophils [53].

Another model of IPA revealed HIF-1 α as a central player in a positive feedback loop of cell activation by hypoxia and inflammatory cytokines. IL-1 receptor (IL-1R) signaling induced HIF-1 α and downstream targets in the lungs. Hypoxia and inflammation were reduced by blockade of IL-1R but required neutrophil recruitment for fungal clearance [56]. Further, IL-1R was essential for anti-aspergillus activity by IFN- γ production of CD4⁺ T cells [57]. HIF-1 α might mediate autocrine and paracrine cell activation by IL-1 α and IL-1 β production which in turn induce HIF-1 α transcription via IL-1 receptor (IL-1R) signaling.

Thus, *A. fumigatus* adapts to low oxygen levels but shows enhanced susceptibility to host immune response. Immune recognition by Dectin-1 initiated an efficient antifungal response wherein HIF-1 α played a central role in early cell recruitment, activation and fungal clearance.

3.3. *C. albicans*

HIF-1 α is involved in priming innate immune system by *C. albicans* infection. Challenging mice with *C. albicans* or β -glucan protected mice from detrimental effects of secondary lethal infection which was independent of B-cell and T-cell mediated immunity. Pro-

inflammatory cytokines such as TNF- α were strongly upregulated in primed monocytes upon restimulation compared to naïve monocytes [58] which elicited fungicidal effects in a murine model of oral candidiasis [59]. Innate immunity was achieved by Dectin-1 downstream signaling via the mammalian target of rapamycin (mTOR)-HIF-1 α pathway inducing a metabolic shift from OXPHOS to aerobic glycolysis [60]. The study pointed out glycolysis is the essential factor for an effective innate immune cell priming/activation with a significant contribution of HIF-1 α .

In the gastro-intestinal tract of antibiotic-treated mice, commensal bacteria induced HIF-1 α which was substantial in mediating resistance to *C. albicans* colonization by promoting cathelicidin-related antimicrobial peptide (CRAMP) [61], a homolog of the human cathelicidin LL-37 which both elicited killing of *C. albicans in vitro* [62]. In summary, HIF-1 α mediates innate memory and antimicrobial properties during *C. albicans* infection.

3.4. *C. immitis*

So far, to our knowledge, there is only one study dealing with the potential role of HIF-1 α in *C. immitis* infection. Differences between the immune functions of *C. immitis* susceptible and protected mice strains were investigated. Transcription of HIF-1 α and pro-inflammatory cytokines such as IFN- γ and TNF- α was elevated in the resistant mice strain which might promote fungal immunity [63] since IFN- γ was shown to be protective against *Coccidioides* infection [42]. Nevertheless, a direct link between HIF-1 α and host protection has not been shown so far.

4. Conclusion

Since studies have uncovered a significant impact of hypoxia on fungal growth and virulence, the role of mammalian hypoxia adaption via HIF-1 α in fungal immunity is gaining more and more traction. However, there are only few studies out so far. Current investigations shed light on the genetic basis of fungal adaption to low oxygen environments which could be linked to successful colonization of the host. Nevertheless, hypoxia adaption bears the risk for enhanced susceptibility such as facilitated immune recognition by elevated β -glucan levels seen in *A. fumigatus* or diminished growth in *H. capsulatum*. These fungi are very successful in colonizing their host which might be due to their hypoxia adaption. Thereby, fungi might be able to persist in a latent state inside granuloma until reactivation as soon as the immune system is compromised. Regarding host cells, low oxygen levels seem to facilitate immune responses by promoting cell activation and initiating pro-inflammatory responses by elevated HIF-1 α stabilization (Fig. 1). There is evidence that HIF-1 α is part of the major pathways involved in antifungal events during innate immunity. HIF-1 α promoted metabolic activation as well as cell recruitment with a major contribution to elicit effector cell function in M Φ . In future, more studies on hypoxia-driven pathways and HIF-1 α downstream targets during fungal infections will be needed in order to find suitable targets for potential antifungal therapies.

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Fig. 1.

HIF-1 α in innate fungal immunity. HIF-1 α is a downstream target of the major β -glucan receptor Dectin-1 and regulates distinct innate immune cell functions involved in antifungal immunity. In the present models of fungal infections, hypoxia diminished (red) fungal growth but enhanced (green) β -glucan exposure on fungal cell walls facilitating recognition by Dectin-1 which might collaborate with CR3 in upregulation of HIF-1 α via Syk (dotted arrows). A positive feedback loop of hypoxia and inflammation is fueled by IL-1 receptor (IL-1R) signaling and HIF-1 α stabilization. HIF-1 α drives the pro-inflammatory phenotype via metabolic activation. The concomitant release of cytokines leads to recruitment of further innate immune cells and effector cells of the cell-mediated immunity. Herein, CD4+

T cells enhance production of antimicrobial peptides (AMPs) via GM-CSF and IFN- γ resulting in fungistasis/fungal killing.

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Table 1HIF-1 α induction by human pathogenic fungi.

Fungi	HIF-1 α regulation	References
<i>Aspergillus fumigatus</i>	Transcriptional upregulation/protein stabilization	[52–54]
<i>Candida albicans</i>	Transcriptional reduction/protein stabilization	[61,64]
<i>Coccidioides immitis</i>	Transcriptional upregulation	[63]
<i>Histoplasma capsulatum</i>	Transcriptional upregulation/protein stabilization	[30,51]