Review Article

Molecular Insights into Pathogenesis and Infection with *Aspergillus Fumigatus*

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Abstract

The virulence of fungi is dependent on multiple factors, including the immune status of patients and biological features of fungi. In particular, the virulence of *Aspergillus fumigatus* is due to the complex interaction among various molecules involved in thermotolerance (such as ribosomal biogenesis proteins, α-mannosyltransferase and heat shock proteins), pigment production (DHN-melanin), immune evasion (like melanin and hydrophobin) and nutrient uptake (such as siderophores and zinc transporters). Other molecules also play important roles in the virulence of *A. fumigatus*, including cell wall components and those which maintain its integrity (for instance β-1-3 glucan, α-1-3 glucan, chitin, galactomannan and mannoproteins) and adhesion (such as hydrophobins), as well as various hydrolytic enzymes (such as serine and aspartic protease, phospholipases, metalloproteinase and dipeptidyl peptidases). Signalling molecules (including G-protein, cAMP, Ras protein and calcineurin) also increase the virulence through altering the metabolic response to stress conditions and toxins (such as gliotoxin, fumitremorgins, fumagatin and helvolic acid).

Keywords: *Aspergillus fumigatus*, virulence, proteins, siderophores, G-protein

Introduction

According to Samson et al. (1), 344 species of fungi belonging to the genus *Aspergillus* have been identified, with nearly 20 of them being pathogenic to humans. Among these human pathogenic species, *Aspergillus fumigatus* is the most important causative agent of human infections (2). *A. fumigatus* is one of the filamentous, opportunistic and pathogenic species being widely spread in diverse environments due to its capability to utilise various organic substrates and adapt to various microenvironments.

A. fumigatus causes a severe infection known as Aspergillosis in people who have suppressed immune systems, such as those suffering from AIDS, neutropenia, cancer or those having undergone solid organ transplants (3). *A. fumigatus* can be created in clinical settings in vitro, as a hydrophobic matrix which would possess biofilm characteristics under all static conditions, namely agar polystyrene, bronchial epithelial cells and agar media (4). Its excellent property of being used as the hydrophobic matrix makes it more suitable for being used in clinical settings as against *A. niger*, *A. flavus* or *A. nidulans* (5). Furthermore, *A. fumigatus* is most widely available in the environment and gets well adapted to it. Aspergillosis is a group of diseases comprised of invasive Aspergillosis, Aspergilloma and allergic Bronchopulmonary Aspergillosis. In order to understand the infection process of *A. fumigatus*, virulence factors must be identified.

Molecular strategies employed to investigate the virulence-related characteristics of *A. fumigatus* include various profiling methodologies and selective gene inactivation as well as the specific alteration of the genome through the gene targeting process. Apart from such techniques, bioinformatics tools have

enabled the identification, quantification and detailed functional analysis of genes involved in the interaction of pathogenic fungi with their hosts.

By and large, to date, studies on the pathogenic mechanism of *A. fumigatus* have strongly suggested that the virulence of the fungus is dependent on various factors, including immunological conditions of patients, biological features of the fungus, its capacity for growth and response to stress conditions, its mechanism for dodging the immune complex and its capacity to cause impairment to the host. Recent studies have suggested that the biofilm formation by *A. fumigatus* may be one of the most important virulence factors in IPA and Aspergilloma (6). In this study, a general overview of the genes and molecules involved in the infection with *A. fumigatus* is presented.

The Virulence Factors of *A. fumigatus*

The virulence of *A. fumigatus* is due to the complex interaction among various molecules involved in the cell signalling process as well as structural proteins. These include the ones responsible for thermotolerance and cell wall components, the ones maintaining its integrity, adhesion and pigment production, those involved in immune evasion and nutrient uptake, various hydrolytic enzymes, and signalling molecules that play a role in increasing virulence through altering the metabolic response to stress conditions and toxins. Gliotoxin, an immunosuppressive mycotoxin, has been long suspected as a potential virulence factor of *A. fumigatus* (7). It is a mycotoxin that alters the hosts' defence system. Neutrophiles of the host are the prime target of gliotoxin. Gliotoxin induces the disruption of the pro-inflammatory response through the inhibition of NF-κB (8).

Thermotolerance

A. fumigatus is a thermotolerant fungus, capable of thriving at temperatures up to 55 °C and withstanding temperatures up to around 70 °C. This characteristic feature of *A. fumigatus* may contribute to its virulence (9). Thus far, researchers have identified four genes related to thermotolerance, including *thtA*, *afpmt1* (mannosyl transferase, responsible for the growth of *A. fumigatus* at 48 °C), *crgA* (doing the coding for the ribosomal biogenesis proteins) *and ireA* (encoding the endoplasmic reticulum-transmembrane sensor protein) (10). The thermotolerence of these proteins may be due to amino acid changes, since sequence comparisons have shown that amino acid substitutions lead to thermostabilisation of the *A. fumigatus* phytase (11). According to a report on the temperature-regulated expression of elastase activity in *A. fumigatus,* the possibility of the presence of the networks of temperature-induced genes that could also be relevant to pathogenesis is raised. This issue can also elucidated by the genome-wide expression technology (12). *A. fumigatus* might also be activated as a part of the general response to in vivo stress, so it might lead to survival in the host (13).

In order to further understand the heat shock response in *A. fumigatus*, proteomic analyses were carried out during a temperature shift from 30 °C to 48 °C, using a differential gel electrophoresis (DIGE) technique (14). The results revealed that 64 different proteins were differently regulated out of 91 spots represented by these proteins. Further analysis showed that Hsp30/Hsp42 and Hsp90 were increased drastically. Additionally, the enzymes involved in NADPH-generation, biosynthesis of amino acids and fatty acids, as well as AspF3 (thioredoxin peroxidase) and Ccp1 (cytochrome c peroxidase) were upregulated (14). Moreover, enzymes involved in the oxidative stress response were increased, probably due to a higher respiration rate leading to the elevated reactive oxygen species (ROS) production (15). Since thermotolerance is most probably polygenic, there is the possibility that the other genes contributing to high temperature growth would still be involved in pathways that affect virulence (16).

Cell Wall Components and Integrity

The fungal cell wall is involved in dynamic interactions with the host cells, thereby modulating their responses. The cell wall of *A. fumigatius* is a complex network of β(1-3), β(1-4) and α(1-3)-glucans, chitins, galactofuran, galactomannan and proteins responsible for variability as against other fungi (17). The biosynthesis of α(1-3)-glucans, a major polysaccharide in the *A. fumigatus* cell wall, is encoded by the glucan synthase genes *ags1, ags2* and *ags3* (18). By eliminating these genes, some studies were carried out to examine their role in virulence. The results showed that the omission of *ags1* led to the deficiency of glycan

synthesis, yet it had no effect on virulence, while fungi with a mutant form of *ags3* showed hyper-virulence in an experimental mouse model. This kind of hyper-virulence may be attributed to the rapid germination, elevated melanin content or evasion of ROS by conidia (19). The galactomannans in the cell wall are in the form of a linear mannan backbone with short chains of (1, 5) galactofuranose residues (20). Galactomannan is responsible for tissue invasion and adhesion to host cells via fibronectin and laminin; it is also an important component of pathogen-associated molecular patterns (PAMP) for other surface receptors of macrophages, dendritic cells and Langerhans cells (20). During tissue intrusion, it shields the fungus and diverts the innate immune response of the host away from the site of the infection (3).

Apart from polysaccharides, glycosyl phosphatidyl inositol (GPI) motif proteins docked to the plasma membrane, encoded by the *ecm33* gene, also play an important role in the biosynthesis of the fungal cell wall, apart from maintaining its integrity. Rementaria et al. (21), showed that the absence of this protein increased the virulence of the fungus (22). In addition, mutations of *gel2* and *glfA* which encode glucanosyltransferase and UDP galactomutase, respectively, resulted in the hypo-virulence of *A. fumigatus* (23).

Nutrient Uptake: Iron Uptake

An essential requirement for the initiation of infection with pathogenic fungi in the host cells is the availability of nutrients and their uptake to adapt to shifting environments (23). For this condition to occur, *A. fumigatus* produces various hydrolytic enzymes, including different proteases like aspartic and serine proteases, phospholipases, metalloproteinases and dipeptidyl peptidases which contribute to fungal virulence by facilitating the tissue colonisation process (24).

Iron is one of the essential nutrients required for various cellular processes. As *A. fumigatus* has no mechanism for iron excretion, controlling iron uptake, metabolism and regulation can play a significant role in iron homeostasis. About 24 genes have been identified to be involved in iron homeostasis in *A. fumigatus* (25). Since *A. fumigatus* cannot directly utilise iron sources such as heme, ferritin or transferrin, it has developed two mechanisms to provide itself with iron, namely siderophore-mediated iron uptake and reductive iron assimilation (RIA) (26).

Initiation of RIA is accomplished by the reduction of the ferric iron into the soluble ferrous iron by metalloreductases present in the plasma membrane (27). Subsequently, it is re-oxidised by ferroxidase FetC and imported aided by the protein complex iron permease FtrA (28).

Studies on metalloreductases led to the identification of 15 putative metalloreductases in the genome of *A. fumigatus*, from among which, *FreB* was found to be involved in RIA (27). Siderophores play an important role both during iron starvation and when iron is superfluous. *A. fumigatus* synthesises four types of siderophores and is dependent on the availability of arginine, ornithine and ergosterol transitional metabolite mevalonate (26, 29, 30). The four types of siderophores include fusarinine C (FsC), triacetylfusarinine C (TAFC) for iron uptake, ferricrocin (FC) and hydroxyferricrocin (HFC) for iron circulation and iron reserve in hyphal and conidia (26). The ferri-forms of FsC and TAFC are absorbed by siderophore iron transporters (SIT), with ten putative SITs being present in *A. fumigatus*. After the uptake, the release of the intracellular iron from TAFC and FsC occurs due to the hydrolysis of the siderophore backbones by esterase EstB (31).

Iron metabolism is regulated by two master transcription factors, i.e. HapX (during starvation) and SreA (during iron satiated conditions) (26, 29, 30). L-ornithine N5-oxygenase (encoded by *sidA*), nonribosomal peptide synthetase (NRPS, encoded by *sidC*) and fusarinine C-acetyltransferase (encoded by *sidG*) are involved in ferricrocin siderophore biosynthesis; they are positively regulated by HapX, but repressed by SreA during the iron starvation phase. In iron excess conditions, SreA induces CccA (an iron transporter of the vacuolar membrane, involved in the vacuolar iron reserve) but represses HapX (26).

Extracellular fusarinine C (FsC), its derivative triacetylfusarinineC (TAFC), intracellular ferricrocin (FC) and hydroxy-ferricrocin (HFC) siderophores are essential for virulence as the complete elimination of siderophore biosynthesis (Δ*sidA* mutant) results in the absolute avirulence of *A. fumigatus* in a murine model of invasive pulmonary aspergillosis (32). However, the insufficiency of either extracellular (Δ*sidI*, Δ*sidH*, Δ*sidF* or Δ*sidD* aberrations) or intracellular siderophores

(Δ*sidC* anomalies) decreases virulence partially (33).

The other transcription factor, i.e. AcuM (a putative mitochondrial transporter), is required for the gluconeogenesis and transcriptional inhibition of SreA, thereby reducing siderophore generation and attenuating virulence of *A. fumigatus* (34). This, in turn, involves iron in the post-translational regulation of both SreA and HapX transcription factors aided by phosphatases (26). In summary, studies indicate that the inactivation of ten out of the 19 *A. fumigatus* genes has induced defects in virulence during the iron starvation phase (25).

Zinc Uptake

Zinc is another important element for fungal growth due to its role in various cellular processes. For the optimal growth of fungi, the availability of free zinc in living tissues is limited, since they are bound to proteins. For the uptake of zinc from a living host, *A. fumigatus* uses zinc transporters (35). The genome of *A. fumigatus* consists of three putative zinc transporter-encoding genes (*zrfA, zrfB* and *zrfC*). Both pH and environmental concentration of zinc regulate the expression of these genes (3).

Among these transporters, *zrfC* gene encodes a transporter for collecting zinc from alkaline zinc-limiting media. The other two transporters, encoded by genes *zrfA* and *zrfB*, are required for receiving zinc from the acidic medium and fungal growth (36). *ZafA* upregulates the expression of *zrfC* and *aspf2*, an allergen produced by *A. fumigatus,* regardless of the environmental pH and under a limited supply of zinc. The growth and germination capacity of *A. fumigatus* is impaired by the omission of the transcriptional regulator *ZafA* gene, thereby affecting its virulence (3).

Hosts inhibit the growth of *A. fumigatus* by the zinc starvation process, a process known as "nutritional immunity" (37). Hosts have developed two different strategies to reduce the free available zinc, with the first one being the release of calprotectin (CP) and a zinc/magnesium (Zn/Mn)-chelating protein by neutrophils in abscesses and the other being by binding zinc to metallothioneins (MTs) in activated macrophages (38).

Recent studies indicate that calprotectin creates a Zn/Mn-deprived microenvironment around fungal cells to inhibit their growth. Gene *zrfC* is required to overcome the inhibitory effect of calprotectin (39). Thus, *zrfC* plays a dual role in fungal virulence; in other words, it is important for the uptake of zinc and also counteracting the inhibitory effect of CP (35).

Adhesins (Hydrophobins)

The cell surface of dormant conidia in *A.fumigatus* is covered with a rodlet layer composed of systematically organised RodA hydrophobin proteins. These hydrophobins are from a conserved family of surface-active hydrophobic proteins secreted by fungi. They have a low molecular weight of approximately 20 kDa (40). These hydrophobins can be classified into two types, i.e. class I and class II. Class I proteins form sturdy amyloid-like rodlets at the air-water junction, whereas class II proteins form ordered lattices at this interface, helping with reducing the surface tension (40). In both classes, eight conserved cysteine residues are available. These residues form a series of disulfide bridges that restrict the mobility of the polypeptide chain by establishing a rigid framework (40).

The unique attribute of these proteins is their capability of spontaneously getting assembled into amphipathic monolayers at hydrophobic-hydrophilic junctions (41). Asexual spores (conidia) and aerial hyphae are covered with a hydrophobin rodlet layer which decreases the outward pressure of the medium or the surface on which fungi grow. As a result, this layer breaks the air-water interface, which in turn prevents the water-logging process while maintaining permeability to the gaseous exchange.

These hydrophobins play an important role in the completion of the fungal biological cycle. They make the conidial surface hydrophobic and water-resistant, thus facilitating the spore dispersal process in the air. Furthermore, these layers are covered with a mucilaginous extracellular matrix that helps the conidia stick to the substrate (41). Hydrophobins also provide the spores with immunological inertness by preventing the pathogen-associated molecular patterns (PAMPs) from being recognised by innate and adaptive immune cells, thereby preventing the activation of the host immune system.

In *A. fumigatus*, the rodlet-layer made up of RodA prevents NETosis, a process by which a mixture of nuclear DNA and a granular content is released by the disruption of neutrophil-

membranes content, operating as a neutrophil extracellular trap (NET). However, studies show that the removal of RodA and RodB do not affect the pathogenicity of *A. fumigatus* (41).

Role of Melanin

Two varieties of melanin pigment, i.e. pyomelanin and di-hydroxyl-naphthalene (DHN) melanin, are synthesised by *A. fumigatus*. Melanins formed by the polyketide synthase (*pksP*) pathway are called DHN melanins. Melanins synthesised by the polymerisation of phenolic and/or indolic compounds produce high molecular weight and negatively charged hydrophobic pigments (42).

In *A. fumigatus*, the grayish-green colour of conidia is due to DHN melanin. Mutations in the pathway involved in the biosynthesis of DHN melanin cause colour variation in conidia, resulting in the production of brown, yellow, reddish or white conidia. The *pksP* gene, coding for a polyketide synthase, is the first essential gene identified for the conidial pigment synthesis. In *A. fumigatus*, apart from the *pksP* gene, another cluster of genes, namely *ayg1*, *arp1*, *arp2* and *abr2* are essential for the production of the DHN melanin (43).

The function of melanin pigments is to provide protection against UV radiation to maintain the genomic stability of the cells and spores (44). These pigments modify cytokine responses, decrease phagocytosis by reducing intracellular trafficking to acidified compartments, reduce the toxicity of microbicidal peptides and provide increased fungal resistance to ROS, antifungal drugs and cell lysis. They also have a pivotal role in maintaining the mechanical strength of the fungal cell wall (44, 45). Moreover, melanins function as a physiological redox buffer by being bound to metal ions and free electrons (46).

Researchers examining the role of genes essential for melanin biosynthesis in *C. neoformans* have noted that the disruption of these genes essential for melanin production reduces lethality and fungal dissemination in murine infection models. Heinekamp et al. (43) reported similar results in *A. fumigatus* on the disruption of genes associated with the melanin synthesis.

Studies show that during the infection period, the host's immune system is exposed to several fungal morphotypes, namely resting conidia, swollen conidia, germlings and hyphae. As soon as *A. fumigatus* conidia find an appropriate environment, it begins to swell, inducing the loss of the rodlet layer that contains the melanin pigment with an immunomodulatory capacity (47). Disruption of *pksP* function modifies the cell surface of conidia, thereby exposing glucan (1, 3). Glucan is a polysaccharide present in the fungal cell wall; it forms the dominant target of the mammalian innate immune system (48).

To examine the role of melanin in *A. fumigatus* related to the endocytotic pathway of phagocytes, a confocal fluorescence microscopy study was carried out on several phagocytic cell types such as murine-derived human neutrophils, different types of macrophages and those derived from alveolar and human monocytes (49). The results revealed that *A. fumigatus* could reduce the acidification of phagolysosomes without modifying the form of conidia containing the phagosomes lysosomal complex. By further investigation, it was revealed that DHN-melanin was able to block phagolysosomal vATPase that is essential for the proper function of phagocytosis. This finding was supported by an experiment showing that DHN-melanin ghosts were also able to supress the phagolysosomal vATPase activity through being treated with bafilomycin.

In the same way, in another study, a *pksP* mutant was unsuccessful in reducing phagolysosomal acidification. This finding suggests that the initial step in DHN-melanin synthesis catalysed by *pksP* is essential and that the first DHN-melanin precursor, naphthopyrone, is responsible for the reduction of phagolysosomal acidification (43).

Another component that reduces phagolysosomal acidification is the anti-apoptotic effect of *A. fumigatus* conidia. These conidia inhibit both the intrinsic and extrinsic apoptosis pathways of macrophages by preventing the activation of caspases (50). In turn, this mechanism is dependent on the survival signalling pathway of PI3K/Akt. This pathway is dependent on DHN-melanin that induces the direct phosphorylation of PI3K/Akt kinase.

Based on these findings, a hypothesis is proposed for the survival mechanism of fungi from phagocytes using a two-step process: (i) phagocytosed conidia prevent phagolysosomal acidification by inhibiting the activity of the vATP-ase transport system of the host phagocyte to reduce the fungal death by macrophages, and (ii) conidia inhabiting non-

acidified phagolysosomes supress phagocyte apoptosis, thus providing an intracellular microenvironment for conidial survival (43).

A study was carried out to investigate the mechanism of the ROS scavenging activity of melanin in *A. fumigatus*. The results revealed that *pksP* conidia were significantly more sensitive than the wild-type conidia, yet the same was not applicable to the conidial colour mutants *ayg1*, *arp2* and *abr2*. This finding suggested that the precursors of melanin, like naphthopyrone synthesised by *pksP* contribute to *A. fumigatus* virulence (43, 51).

Pyomelanin

A. fumigatus produces another melanin pigment, i.e. pyomelanin. The biosynthesis of pyomelanin occurs by the polymerisation of the homogentisic acid, an intermediate of L-tyrosine catabolism. During the infection period, *A. fumigatus* secretes several proteases (52) that trigger the transcription of the tyrosine degradation gene cluster at the presence of tyrosine (53). When the fungal cell wall is exposed to the stress, there is an increase in the transcription of the tyrosine degradation gene cluster (54). This increase, in turn, influences the pyomelanin formation via the MpkA mediated cell wall integrity pathway (55). Moreover, several genes (*hppD*, *hmgX*, *hmgA* and *fahA*) of the tyrosine degradation cluster were found to be differentially regulated when *A. fumigatus* was exposed to the immune cells of the host.

Studies show that pyomelanin plays a protective role against ROS. Germlings of the non-pyomelanin producing mutant *hppD* showed increased sensitivity to hydrogen peroxide and the thiol-oxidising agent di-amide at the presence of tyrosine. Hence, pyomelanin production might be involved in dodging the immune system and the survival of the fungus (43).

Secondary Metabolites

Secondary metabolites are tiny molecules with low molecular weight synthesised by numerous microorganisms. They are not essential for normal growth, yet they play an important role in the well-being of the organism by initiating defence mechanisms and signalling processes (56). Some secondary metabolites are non-toxic, such as various pigments and molecules involved in interspecies communication, whereas others are toxic, being responsible for eliminating competitors, such as antimicrobial substances. The genes involved in the production of secondary metabolites are generally present in clusters. In *A. fumigatus*, about 30 secondary metabolism clusters have been identified, yet the functions of the majority of gene clusters have not been characterised (57).

These metabolites are synthesised as polyketides or as nonribosomal peptides; they are catalysed by the polyketide synthase (PKS) and nonribosomal peptide synthetase (NRPS) systems, respectively. It has been reported that the expression of at least 50% of these genes is regulated by a global regulator of a secondary metabolite generation, i.e. *LaeA* (58).

From among the secondary metabolites identified, one can refer to melanin, pseurotin A, gliotoxin, fumitremorgins, fumagatin, helvolic acid, gibberellin and aflatoxin. These metabolites play an important role in the virulence of *A. fumigatus*.

Role of Toxins

Gliotoxin

One of the most studied secondary metabolites produced by *A. fumigatus* is gliotoxin. Gliotoxin ($C_{13}H_{14}N_2O_4S_2$) belongs to the epipolythiodioxopiperazine (ETP) class of toxins, characterised by the presence of a disulfide bridge across a dioxopiperazine ring (59). Biosynthesis of gliotoxin occurs through the condensation reaction of serine and phenylalanine amino acid, catalysed by a NRPS (59).

Studies show that 96% of virulence caused by *A. fumigatus* is due to the presence of gliotoxin that facilitates the fungal growth and the colonisation of the host tissue through the initiation of a local or generalised immunosuppression mechanism. Various roles played by gliotoxin include the inhibition of various enzymes, such as creatine kinase, alcohol dehydrogenase, farnesyltransferase and NADPH oxidase enzymes; the inhibition of macrophages and polymorphonuclear cell function by the direct inactivation of essential protein thiols; so does the inhibition of NF-B, a transcriptional regulator of the host pro-inflammatory response (60, 61). Gliotoxin induces the apoptotic cell death of various cells involved in the immune system by causing DNA fragmentation and adduct formation. It has also been shown to inhibit the respiratory burst in neutrophils by

preventing the assembly and activity of the NADPH oxidase enzyme complex, which in turn facilitates in vivo fungal dissemination (59, 60).

In *A. fumigatus*, 13 genes constituting a multi-gene cluster, are responsible for encoding all enzymes involved in gliotoxin metabolism. These genes are co-ordinately and uniformly expressed during the gliotoxin biosynthesis process (60). The gene *gliP*, encoding a bimodular NRPS, is responsible for synthesising a Phe-Ser dipeptide, i.e. a precursor of gliotoxin. *gliA,* a putative gliotoxin transporter, facilitates gliotoxin efflux and elicits tolerance to exogenous gliotoxin. *gliZ*, encoding a binuclear cluster domain transcription factor, is responsible for gliotoxin production. Studies indicate that the disruption of any of these genes leads to the reduced production of gliotoxin. Moreover, the disruption of *gliZ* results in the complete inhibition of all the gliotoxin cluster gene expression, thereby diminishing gliotoxin production (60).

Surprisingly, gliotoxin is capable of inhibiting fungal growth, including *A. fumigatus*. Hence, to protect itself, *A. fumigatus* also contains *GliT*, a gliotoxin sulfhydryl oxidase involved in gliotoxin synthesis. This enzyme provides protection by preventing the generation of ROS and maintaining gliotoxin in the sulfur bridge (62).

Other toxins

Another important toxin produced by *A. fumigatus* during the hyphal development period is fumagillin, having a molecular weight of 458.6 Da (63). Fumagillin suppresses the generation of ROS, thereby preventing the immune response, neutrophil function and angiogenesis through a covalent interaction with methionine aminopeptidase-2 (MetAP-2) and facilitating hyphal growth (63).

Apart from the items mentioned, *A. fumigatus* produces various toxic metabolites like verruculogen, pseurotin, sulochrin, asterric acid, trypacidin and fumiquinazolins. Verruculogen is a potent tremorgen capable of modifying the electrophysical properties of the human nasal epithelial cells. Pseurotin A inhibits immunoglobulin E's function in response to hypoxic conditions. Sulochrin inhibits eosinophil activation, whereas the related asterric acid inhibits vascular endothelial growth factor-induced tube formation. Another two metabolites, fumiquinazolins and trypacidin, are known to be cytotoxic. Hence, these toxins contribute to virulence in pathogenic *A. fumigatus* (64).

Conclusions

A. fumigatus relies on the synergetic expression of multiple genes involved in various aspects of fungal growth, cell wall assembly, nutrient obtainment and resistance to detrimental conditions, such as oxidative stress, pH and temperature variation. The substantial advancement in molecular techniques and bioinformatics has enabled the identification and detailed functional analysis of genes involved in the interaction between pathogenic fungi and their hosts. Yet, many questions related to the pathobiology of *A. fumigatus* are unanswered. For a better understanding of pathogenicity, further studies should be conducted in order to clarify how far fungal compounds facilitate the escape of fungi from the host immune system and their response to fluctuations in pH, temperatures and ROS. Other interesting questions include 'what is the carbon source for the fungal development?', 'What is the mechanism behind the inhibition of caspase3', 'which is involved in apoptosis?', 'What is the melanin's role in inhibiting the assembly and action of the vATP-ase of host phagocytes to supress the acidification of phagolysosomes?', 'What is the action mode of gliotoxin in causing pathogenicity?' The knowledge to be gained in studying these processes will enhance the development of novel therapies for fungal infection.

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