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Hostile takeover: fungal protein promotes host cell invasion

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The ability to suppress the immune system has led to great advances in transplant technology and treatment of autoimmune diseases. Unfortunately, the immunosuppression of these patients has led to the rise of opportunistic infections by organisms that are recalcitrant to current prophylactic strategies. One such example is the increase of mucormycosis, an invasive infection caused by filamentous fungi of the order Mucorales. In this issue of the JCI, Gebremariam and colleagues determined that spore coat homolog (CoTH) proteins are required for angiogenesis and that these proteins are unique to Mucorales. Their findings provide a potential therapeutic target for prevention and treatment of mucormycosis.

The rise of mucormycosis

Recent medical advances have made remarkable progress in treating previously refractory conditions. More aggressive and targeted cancer chemotherapies have vastly improved outcomes for many malignancies. Inhibition of TNF- α activity now affords better control of various autoimmune disorders. Moreover, advances in solid organ transplantation have dramatically improved the lives of many patients

with organ failure syndromes. However, these revolutionary therapies considerably impair patient immunity.

Because of the increased infection risk in patients with highly immunocompromised states, clinicians have adopted concrete strategies for infectious disease prevention in many of these patient populations. Unfortunately, as our ability to suppress infections by the most common microbial pathogens has improved, other, less well-characterized infectious agents have begun to fill this clinical void. One very important example of this phenomenon is the increasing incidence of mucormycosis, an invasive infection

caused by the Mucorales order of filamentous fungi (1, 2). Human pathogens in this fungal group include *Rhizopus*, *Mucor*, and *Cunninghamella* species.

The increased incidence of mucormycosis has been attributed to many factors, including the fact that Mucorales are much less susceptible to current antifungal agents than other fungal pathogens. Therefore, the use of standard antifungal drugs in prophylactic strategies is unlikely to successfully prevent this type of infection (3, 4). Mucormycosis occurs in association with a wide range of disorders. In addition to classical immunocompromised states, such as prolonged neutropenia and organ transplantation, conditions such as diabetic ketoacidosis (DKA), undernutrition, and iron chelation therapy also predispose patients to mucormycosis (2). Once established, invasive infections due to Mucorales frequently take an aggressive clinical course characterized by rapid tissue destruction. These infections are difficult to treat, requiring high-dose antifungal therapy and surgical debridement. Mortality in mucormycosis remains high, despite

Conflict of interest: The author has declared that no conflict of interest exists.

Citation for this article: *J Clin Invest.* 2014; 124(1):74–76. doi:10.1172/JCI73585.

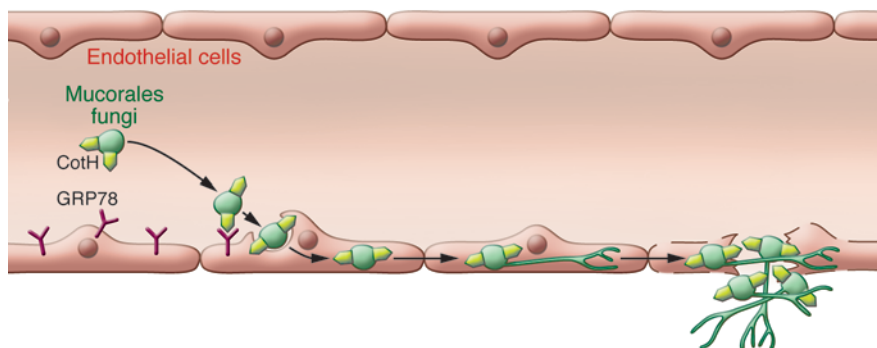


Figure 1

CotH proteins on the fungal surface promote angiogenesis. CotH proteins on the surface of Mucorales specifically bind to GRP78 on the surface of host endothelial cells. This interaction facilitates fungal invasion of the cell. Damage to the endothelial cells promotes angiogenesis and dissemination.

combined medical/surgical therapy (5, 6). Therefore, new insights into the pathogenesis of this fungal infection are needed in order to envision innovative strategies for prevention and treatment.

Focus on angiogenesis

The study in this issue by Gebremariam et al. (7) began with a classical clinical observation about mucormycosis that was used to infer unique aspects regarding the establishment and propagation of this disease. This infection tends to display angiogenesis, or progression along the micro- and macrovasculature (8). Clinically, angiogenesis results in tissue infarction, which complicates the infection and explains many of the principal manifestations of this disease. The authors decided to pursue the angiogenic behavior of Mucorales to determine whether this fungal feature could offer insight into approaches to altering disease progression. Thus, they sought to determine whether specific molecular interactions exist between the fungal cell surface and the mammalian endothelium. Historically, there has been some debate among clinicians and pathologists as to whether fungal angiogenesis is the result of a specific interaction between host and fungus, or whether this process simply involves microbial growth along the path of least resistance within the infected tissue. The data now presented by Gebremariam et al. (7) strongly argue that the interaction of *Rhizopus* species and endothelial cells involves targeted molecular recognition between fungal and host cells, promoting microbial invasion and disease progression.

The authors previously identified glucose-regulated protein 78 (GRP78) as

a component of mammalian cells that bound *Rhizopus oryzae* hyphae (9). GRP78 colocalized with the fungal hyphae during invasion of endothelial cells. Additionally, anti-GRP78 antibodies effectively prevented the adherence and endocytosis of *R. oryzae*, but not other fungal pathogens. Expression of GRP78 was induced in mice during DKA and in cultured epithelia exposed to excess glucose and iron, conditions that favor mucormycosis. Blocking this putative endothelial cell receptor with antibodies protected DKA mice from experimental mucormycosis (9).

Mucorales-specific ligand promotes invasion

In the present study, the authors identified fungal ligands that bind GRP78 (7). The data presented here compellingly argue that bacterial spore coat protein homolog (CotH) proteins present on the *R. oryzae* cell surface mediate a specific interaction with GRP78, promoting fungal adherence and invasion (Figure 1). Heterologous expression of the *R. oryzae* CotH proteins resulted in mammalian cell invasion by the typically nonpathogenic yeast *Saccharomyces cerevisiae*. Additionally, interruption of CotH function in *R. oryzae* disrupted its invasive potential. Interference of the CotH/GRP78 interaction with anti-CotH antibodies resulted in a protective effect in a murine DKA model of mucormycosis. Interestingly, these CotH proteins were present on the surface of Mucorales, but do not appear to be produced by other fungal pathogens (7). This may explain the more extensive degree of angiogenesis observed in

mucormycosis compared with other invasive fungal infections.

Perspectives and future directions

Many questions naturally arise when considering the results of these investigations. Mucorales are not adapted human pathogens, but rather environmental molds that infect patients with specific defects in immunity; therefore, the native role of CotH proteins remains to be determined. Do these fungal proteins promote interaction with potential food sources in the environment? Alternatively, do these fungal surface proteins provide some sort of protection against natural predators?

In addition to the important questions in fungal biology raised by these findings, there are equally important clinical implications. The specificity of the CotH proteins to Mucorales suggests that they could be effective therapeutic targets for preventing and treating mucormycosis. Would therapies directed against fungal angiogenesis be effective once these infections are well established in tissues? It would also be interesting to study whether disruption of fungal adherence and invasion could be used in various prevention strategies, in addition to targeted therapies of active Mucorales infections.

These studies also highlight the larger context of recent investigations into the interaction of the fungal cell surface with the infected host. Fungal surface molecules that act as adhesins, invasins, and immunogens are being increasingly characterized in diverse human pathogens. Vaccine strategies against various fungal surface epitopes have been explored, and clinical trials of resulting fungal vaccines are now being pursued (10). Additionally, the fungal cell surface often determines the nature and degree of immune activation during invasive fungal infections. Exposure of highly immunogenic fungal surface components may be beneficial in some settings, allowing infected hosts to recognize and rapidly clear invading microorganisms. Conversely, excessive immune activation could potentially accelerate host damage, which would result in allergic responses for superficial fungal conditions and worsen morbidity in invasive mycoses (11–13). Therefore, continued investigations are warranted into this important intersection of microbiology and host immune response.



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More than skin deep: connecting melanocyte pigmentation and angiogenic diseases

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Epidemiological studies have identified racial differences in susceptibility to numerous diseases, including several ocular and skin diseases characterized by increased vascular growth. In most cases, the specific mechanisms and genetic variants responsible for these differences have remained elusive. In this issue of the JCI, Adini et al. explore a direct connection between skin pigmentation and susceptibility to angiogenic diseases and identify an extracellular matrix protein that is regulated by melanogenesis and potently modulates angiogenesis.

Skin pigmentation and angiogenesis dysfunction

An important and ongoing challenge in the postgenomic era is the successful identification of gene variants that render certain individuals susceptible to specific diseases. For example, it is well known that individuals with less skin pigmentation have a higher incidence of angiogenic ocular and skin disease compared with darker-skinned individuals. Genetic variation drives differences in pigmentation; however, a genetic link between skin color and angiogenic disease has not been identified. Studies on ocular diseases indicate that individuals of European descent are twice as likely to develop age-related macular degeneration (AMD) as African-American

or Asian-Americans (1). Furthermore, ocular melanoma is almost 20 times more common in light-skinned individuals (2), and several skin diseases, including melanoma and hemangioma, are more common in those of European descent (3, 4). The fact that these diseases are all characterized by increased vascular growth suggests a potential unexplored link between skin pigmentation and angiogenesis.

In this issue of the *JCI*, Adini and colleagues investigate the connection between skin color and disease susceptibility (5). The authors hypothesized that pigmented cells from light- or dark-skinned individuals differentially express a proangiogenic factor. They observed that angiogenesis in the cornea and in healing wounds is considerably increased in C57-albino mice, which have decreased melanin synthesis due to a mutation in the tyrosinase gene, compared with their pigmented counterparts (C57BL/6 mice).

Using a cell-culture model of sprout formation, Adini et al. performed a detailed assessment of the effects of melanocyte-conditioned media on EC migration. Conditioned media were prepared by culturing melanocytes from albino and pigmented mice and, in a complementary fashion, from European- and African-Americans. The authors demonstrated that nonpigmented melanocytes secrete a factor that promotes microvascular EC migration and proliferation in vitro. These data are consistent with a melanocyte-secreted factor playing a role in promoting blood vessel growth.

Fibromodulin: a potent angiogenic factor

In order to identify the specific factor or factors responsible for the observed effects, the authors performed a targeted microarray that compared expression of genes predicted to encode secreted proteins in pigmented and nonpigmented melanocytes (5). Fibromodulin (FMOD), an ECM protein of the small leucine-rich proteoglycan (SLRP) family, was expressed to a much greater degree in nonpigmented melanocytes both in vitro and in vivo. Silencing or antibody blocking of FMOD substantially inhibited the angiogenic effects of conditioned media from nonpigmented melano-

Conflict of interest: The author has declared that no conflict of interest exists.

Citation for this article: *J Clin Invest.* 2014; 124(1):76–79. doi:10.1172/JCI73559.