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Special article

Aerially transmitted human fungal pathogens: What can we learn from metagenomics and comparative genomics?

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ABSTRACT

In the last few decades, aerially transmitted human fungal pathogens have been increasingly recognized to impact the clinical course of chronic pulmonary diseases, such as asthma, cystic fibrosis or chronic obstructive pulmonary disease. Thanks to recent development of culture-free high-throughput sequencing methods, the metagenomic approaches are now appropriate to detect, identify and even quantify prokaryotic or eukaryotic microorganism communities inhabiting human respiratory tract and to access the complexity of even low-burden microbe communities that are likely to play a role in chronic pulmonary diseases. In this review, we explore how metagenomics and comparative genomics studies can alleviate fungal culture bottlenecks, improve our knowledge about fungal biology, lift the veil on cross-talks between host lung and fungal microbiota, and gain insights into the pathogenic impact of these aerially transmitted fungi that affect human beings. We reviewed metagenomic studies and comparative genomic analyses of carefully chosen microorganisms, and confirmed the usefulness of such approaches to better delineate biology and pathogenesis of aerially transmitted human fungal pathogens. Efforts to generate and efficiently analyze the enormous amount of data produced by such novel approaches have to be pursued, and will potentially provide the patients suffering from chronic pulmonary diseases with a better management.

This manuscript is part of the series of works presented at the "V International Workshop: Molecular genetic approaches to the study of human pathogenic fungi" (Oaxaca, Mexico, 2012).

Hongos de transmisión aérea patógenos para el ser humano: conocimientos adquiridos a partir de su metagenómica y genómica comparativa

Palabras clave:
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RESUMEN

En las últimas décadas se ha reconocido cada vez más la influencia de los hongos patógenos para el ser humano, y cuya transmisión es aérea, en el curso clínico de afecciones pulmonares crónicas, como el asma, la fibrosis quística o la enfermedad pulmonar obstructiva crónica. Gracias al desarrollo reciente de métodos de secuenciación de alto rendimiento, que no requieren cultivo, en la actualidad los análisis metagenómicos permiten detectar, identificar e incluso cuantificar comunidades de microorganismos procariotas o eucariotas que habitan en las vías respiratorias del ser humano, y acceder a la complejidad de las comunidades microbianas cuya población es de baja densidad, que posiblemente desempeñan un papel en las enfermedades pulmonares crónicas. En la presente revisión examinamos cómo los estudios metagenómicos y genómicos comparativos pueden ayudar a superar los obstáculos de los cultivos de hongos, mejorar nuestros conocimientos sobre la biología fúngica, desvelar el diálogo cruzado (*crosstalk*) entre el pulmón del huésped y la microbiota fúngica asociada, y adquirir información sobre la influencia patogénica de estos hongos transmitidos por el aire que afectan al ser humano. Revisamos los estudios metagenómicos y los análisis genómicos comparativos de microorganismos cuidadosamente seleccionados, y confirmamos la utilidad de estas estrategias para definir mejor la biología y la patogenia de

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hongos de transmisión aérea que son patógenos para el ser humano. Los esfuerzos por generar y analizar eficientemente la ingente cantidad de datos obtenidos con estos nuevos métodos deberán continuar, y es posible que ofrezcan un mejor tratamiento de los pacientes portadores de enfermedades pulmonares crónicas.

Este manuscrito forma parte de la serie de artículos presentados en el «V International Workshop: Molecular genetic approaches to the study of human pathogenic fungi» (Oaxaca, México, 2012).

Although plant disease epidemics caused by fungi are recognized as having major impacts on food security, pathogenic fungi are emerging as major threats to animal health.[27] Indeed, several severe wildlife declines have recently occurred in bats or amphibians pointing to previously unknown fungi as causes of mass mortalities. In humans, the contribution of fungi to the global burden of diseases is largely unrecognized. Over 600 fungal species have been reported to affect multiple organs and to cause mild-to-severe infections in immunocompetent or otherwise healthy individuals.[10]

In the last few decades, fungal infections have become a leading cause of mortality and morbidity especially in patients with severe immunological impairments.[41] Indeed, the number of patients susceptible to fungal infections has increased dramatically as a consequence of underlining diseases (HIV infection, leucopenia, etc.) or treatments (including biotherapy) affecting their immunological status in the case of organ transplantation, surgery or cancer.[41] In developing countries, invasive mold infections (especially aspergillosis and mucormycosis) are also more frequently reported both in a large economically deprived portion of the population but also in the limited but more prosperous portion, for which access to modern medical interventions (i.e. organ transplantation, intensive therapy for malignancies) is possible.[17] The mortality due to mycoses still ranges from 30 to 50% in spite of recently discovered and more potent antifungal drugs as well as better diagnosis tools, which both need to be improved.[61]

Depending on the fungal burden and the immune status of the host, air-borne fungal pathogens may be harmless or cause invasive or non-invasive diseases, inflammation or even allergy.[52] Indeed, in the case of *Pneumocystis*,[16,60] a low fungal burden, defined as a colonization or carriage state with no clinical symptoms, could play a major role in the transmission of the disease from immunocompetent to susceptible patients or in the worsening of prognosis in chronic obstructive pulmonary disease (COPD) patients. Recent development of non-culture highthroughput sequencing methods enables detection, identification and even quantification of prokaryotic or eukaryotic microorganism communities inhabiting human digestive or respiratory lumina. Indeed, the lungs of healthy never-smoker individuals are inhabited by communities of bacteria that are few in number but composed of diverse genera, thus showing that the healthy lung is not as sterile as it first appeared.[7,19,24] Although most recent studies focused mainly on bacterial communities, investigations have been initiated on viruses and fungi.[18,21] These *omics* approaches are powerful enough to access the complexity of even low-burden microbe communities that modulate the immune system and are likely to play a role in chronic pulmonary diseases and pulmonary transplantation system.

In this review, we will focus on new generation technologies such as metagenomics or comparative genomics and try to approach how they can alleviate fungal culture bottlenecks and bring more knowledge about fungal biology and cross-talks between the host lung and the fungal microbiota. The scope of this review is restricted to aerially transmitted micromycetes and their impact on chronic pulmonary diseases such as asthma, cystic fibrosis (CF) or COPD.

Clinical impact of aerially transmitted micromycetes on chronic pulmonary diseases

Fungi and lung allergy

Sensitization to fungi is an important factor in patients with allergic respiratory tract diseases, playing a major role in the development, persistence and severity of low airway diseases, particularly asthma.[44]

Allergic bronchopulmonary mycosis (ABPM) can be caused by several fungi including *Candida*, *Penicillium*, *Scedosporium*, and *Curvularia* species. But the most common form of ABPM is allergic bronchopulmonary aspergillosis (ABPA) that is a well-recognized fungal complication of asthma and is caused by chronic or intermittent bronchial colonization with *Aspergillus fumigatus*.[44] Severe asthma with fungal sensitization (SAFS) is less recognized but has been appropriately named to highlight that patients suffering from severe asthma are highly sensitive to fungal antigens and respond to oral antifungal therapy.[22] The prevalence of ABPA ranges from 0.7% to 3.5% in asthmatic patients while it reaches 7–9% in CF patients.[31,44]

Fungi and chronic obstructive pulmonary diseases

Isolation of fungal conidia in the sputum of immunocompetent individuals often represents colonization with no clinical consequences. However, isolation of fungal conidia in sputum of immunocompromised or severely ill patients is highly predictive of invasive disease.[45] For example, invasive pulmonary aspergillosis (IPA) primarily occurs in a context of neutropenia but patients with severe COPD or from intensive care unit have also emerged to be at risk for IPA.[2] Advanced COPD stages, as well as prolonged use of corticosteroids, are correlated with a higher risk of developing IPA. The use of broad-spectrum antibiotics to prevent bacterial exacerbation episodes may also promote the emergence of fungal populations in airways.[2]

It is now widely accepted that infection is the predominant cause of exacerbation in COPD patients and most probably contributes to the pathogenesis of COPD.[71] Even microorganism communities developing at low-burden in the lungs may perpetuate inflammatory and lung-remodeling responses that lead to an increased severity of COPD.[59,60] This is illustrated by *Pneumocystis jirovecii*, a micromycete colonizing patients suffering from severe COPD. The *Pneumocystis* colonization state is defined as low number of organisms present in the lungs without clinical symptoms of pneumonia. The prevalence of colonizing *P. jirovecii* in COPD patients is increased, reaching 55% when compared to patients with other pulmonary diseases (reviewed in Morris and Norris[57]). Whether the installation of *P. jirovecii* is secondary to COPD or a risk factor for the establishment of this chronic disease remains to be elucidated. Several animal and human studies report *Pneumocystis* colonization as being a risk factor (i) to develop more severe COPD in non-HIV patients[58] or (ii) to suffer from airway obstruction in HIV patients.[55]