Research Article

Pulmonary MicroRNA Expression Profiles Associated with Subchronic Aspergillus fumigatus Exposure.

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Abstract
Background: Examination of the miRNA profile post-fungal exposure demonstrated a large number of altered miRNAs, study by Denning support previously reported immune responses following A. fumigatus exposure, furthermore, altered miRNAs may serve as potential biomarkers to evaluate fungal exposure**. objective:** To discuss pulmonary MicroRNA in subchronic Aspergillus fumigates exposure and discuss methods of detection of pulmonary aspergillosis by microRNA. **Summary:** The genus Aspergillus is extraordinary as exemplified by the diversity of its disease manifestations, almost all termed "aspergillosis", Downregulated miRNAs predicted to target Il13, Il33, and Clec7awere identified, suggesting a possible mechanism that contributes in part to an immune response following subchronic exposures to A. fumigatus.These altered miRNAs may serve as potential biomarkers to evaluate fungal exposure.
Keywords: Aspergillus, A.fumigatus, MicroRNAs, Conidiophores, Sabouraud agar

Introduction
Aspergillus fumigates (A.fumigatus) is a saprophytic fungus that plays an essential role in recycling environmental carbon and nitrogen[(1).]

Environmental surveys indicate that all humans will inhale at least several hundreds of A. fumigates conidia per day. For most patients, therefore, disease occurs in the lungs, although dissemination to virtually any organ occurs in the most severely predisposed[(2).]

Because of the increase in the number of immunosuppressed patients, however, and the degree of severity of modern immune-suppressive therapies, the situation has changed dramatically in recent years[(3).]

Examination of the miRNA profile post-fungal exposure demonstrated a large number of altered miRNAs, study by Denning support previously reported immune responses following A. fumigatus exposure, furthermore, altered miRNAs may serve as potential biomarkers to evaluate fungal exposure[(4).]

Aim of the Work
1. To discuss pulmonary MicroRNA in subchronic Aspergillus fumigates exposure.
2. To discuss methods of detection of pulmonary aspergillosis by microRNA.
3. To discuss clinical applications and role of microRNA in diagnosis of pulmo-nary aspergillosis.

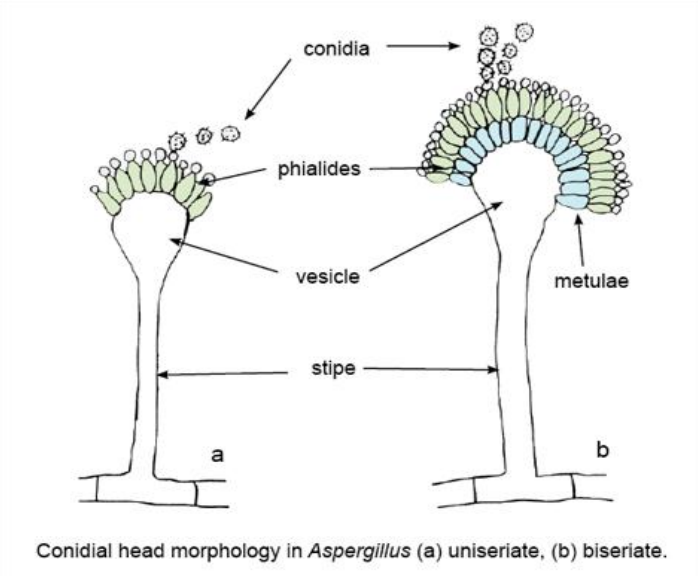
Over View of Pulmonary Aspergillosis
Definition of aspergillosis:
The term aspergillosis refers to diseases caused by fungi belonging to the genus Aspergillus. Aspergillus species have emer-ged as important causes of lifethreatening infections in immune compromised patients that may range from localised conditions to fatal disseminated infections [(5).]

Aspergillus species:
Aspergillus is a large genus of common contaminants containing more than 180 species. Most Aspergillus species of clinical importance are anamorphs (asexual states) related to the following teleomorph (sexual states) genera in the Ascomycota phylum: Emericella, Eurotium, Fennellia, Hemicarpenteles, Neopetromyces, Neosar-torya and Petromyce [(5).]
Aspergillus fumigatus causes the majority of cases of aspergillosis, followed by A. flavus, A. niger, A. terreus and A. nidulans [(6)(7)]

Morphology of aspergillosis:
Most species in the anamorph genus Aspergillus have non-melanised structures, although

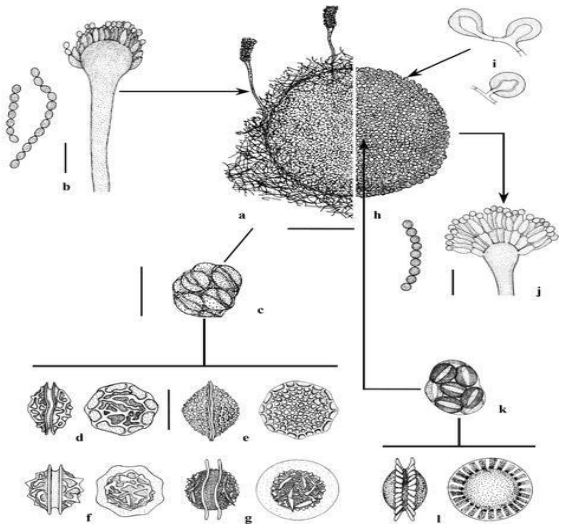
some unusual species may produce blackish conidia, when grown in culture.

Aspergillusspecies form colonies that usually develop quickly to display different colours, which are in turn very useful for the phenotypic identification of some species. Aspergilli produce two types of propagules: the conidia, formed by mitosis, and the ascospores that are produced by meiosis **(Fig:1)** [8].



(Fig:1): Diagram of terminology used for the identification of Aspergillus species

When grown in culture, the teleomorphs of Aspergillus form the typical fruiting bodies of the ascomycetes, called ascomata, ascomata are spherical structures containing globose asci usually with 8 ascospores, the ascospores are one-celled, subglobose to lenticular and have different types of ornamentation on their surface.**(Fig : 2)** [7].



(Fig: 2): Neosartorya spp. (a) part of an ascoma with conidiophores associated; (b) conidiophore and conidia; (c) ascus; (d) ascospore of N. fischeri; (e) ascospore of N. hiratsukae; (f) ascospore of N. pseudofischeri; (g) ascospore of N. udagawae; (h) Emericella nidulans – part of an ascoma; (i) Hülle cells; (j) conidiophore of A. nidulans; (k) ascus; (l) ascospore [8].

Habitat and Distribution of aspergillus:

Aspergilli are typical soil fungi common in warm climates but they can be found in extreme climates[9]. A number of species of Eurotium are osmotolerant and can grow on materials with low water activity and others of the fumigati section are thermotolerant, they have tolerated temperatures of pasteurization,this high temperature tolerance differentiates A. fumigatus from the closely related species[10].

Virulence factors of aspergillus:

Fatal experimental infections leading to multiple organ involvement have been most frequently produced in mice with an intravenous injection of conidia that compared the virulence of different species, in general, A. flavus seemed to be more virulent than other Aspergilli of clinical interest [10].

In a study byBowmanthe virulence of isolates of A. terreus, A. flavus and A. nidulans were compared in DBA/2 mice, using an inoculum of 105 colony forming units (CFU), A. flavus killed 90% of animas at day 10, whilst 40% mortality was observed with a higher inoculum of A. terreus (106 CFU) [11].

Prevalence of aspergillosis:

Invasive aspergillosis is the most common filamentous fungal infection in immune compromised patients, this is a severe infection with a 57% increase in death rates reported in the United States of America from 1980 to 1997 [12].

Fumigatus is by far the most frequent species present in such infections, followed at some distance by A. flavus, A. niger, A. terreus, A. nidulans and A. ustus, and with a lower percentage Neosartorya spp, Significantly, in recent years the proportion of infections caused by non-fumigatus Aspergillus species has increased considerably **(table 1)** [13].

Table (1): Percentageof aspergillosis involving immunocompromised patients [13].

Species	Percentage
Aspergillus fumigatus	68.7
Aspergillus flavus	17.0
Aspergillus niger	5.7
Aspergillus terreus	3.9
Aspergillus nidulans	1.1
Aspergillus ustus	1.1
Neosartorya spp.	0.7
Each of the rest of species	≤ 0.5

Dignosis of aspergillosis

Culture Methods:

Colonies cultured on defined laboratory media such as Sabouraud agar (glucose peptone agar), Czapek agar, Czapek yeast autolysate agar, oat meal agar, potato dextrose agar or malt extract agar with or without additional glucose [14].

The temperature of incubation can also help to distinguish between species, although many are recognised as being thermotolerant, a temperature of 42◦C can help to distinguish the more pathogenic Aspergillusspecies such as Aspergillus fumigatus group[15].

Diagnostic specimens should routinely be cultured at 3o◦C and 37◦C but for more accurate identification prolonged incubation at 25◦C may be necessary for some species [16].

Generic Description of Aspergillus Species:

Aspergillus species usually produce rapidly growing, more or less powdery or granular white, green, black, brown or yellow colonies[17].

Features of Colonial Morphology Useful for Identification:

- Colour of surface and reverse
- Rate of growth (colony diameter)
- Texture (velvety, powdery, granular, floccose)
- Zonation

- Form of sporing heads (columnar, radiate)
- Diffusing pigment
- Sclerotial bodies
- Cleistothecia
- Coremia
- odour (suitable only for corroborative evidence)[18]

Features of Microscopic Morphology Useful for Identification:

- Size and shape of vesicle.
- Colour, surface roughening and length of conidiophores.
- The foot cell.
- Presence or absence of metulae.
- Formation of sporing heads.
- Size and shape of spores.
- Surface ornamentation of spores.
- Cleistothecia.
- Sclerotial bodies.
- Size, shape and surface ornamentation of ascospores.
- Hülle cells [19].

MicroRNAs

Definition of MicroRNA:

MicroRNAs (miRNAs) are single-stranded RNAs (ssRNAs) of 19–25 nucleotides in length that are generated from endogenous hairpin-shaped transcripts [20]

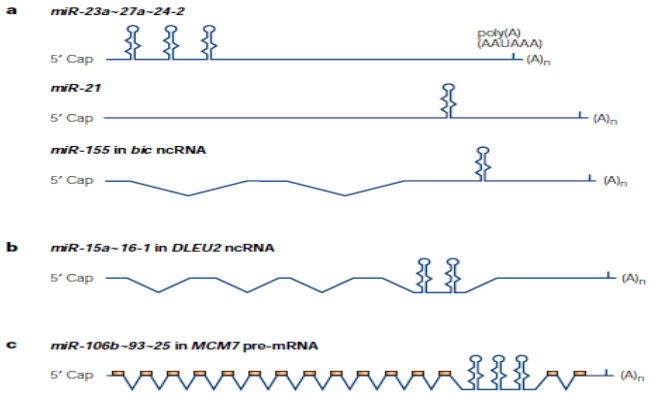
MicroRNA function:

MicroRNAs (miRNAs) have important roles in diverse regulatory pathways [21].

The paradigm for the function of miRNAs has been provided by nematode lin-4 and let-7 RNAs, they function as post-transcriptional repressors of their target genes when bound to the specific sites in the 3' untranslated region (UTR) of the target mRNA [22].

Nuclear processing by Drosha:

Transcription of miRNAgenes yields primary transcripts, pri-miRNAs, that areusually several kilobases long and that contain a localhairpin structure **(Fig: 2),** the stem-loop structure iscleaved by the nuclear RNase III Drosha to release theprecursor of miRNA (pre-miRNA)



(Fig: 2): The structure of five pri-miRNAs.

Primary transcripts that encode miRNAs, primiRNAs,contain 5′□cap structures as well as 3′□poly(A) tails, miRNAs can be categorized intothree groups according to their genomic locations relative to their positions in an exon or intron.

a) Exonic miRNAs in non-coding transcripts.
b) Intronic miRNAs in non-coding transcripts.
c) IntronicmiRNAs in protein-coding transcripts.

Cytoplasmic processing by Dicer:

Following their exportfrom the nucleus, pre-miRNAs are subsequentlyprocessed into ~22-nucleotide miRNA duplexes by the cytoplasmic RNase III Dicer [24][25].

Because Dicer was originally found to function in generating siRNAs, which are similar in size (21–25 nucleotides) to miRNAs it was predicted that Dicer also functionsin the processing of ~70-nucleotide stem-loop RNAs into mature

miRNAs, indeed, it was laterproven that immune precipitated Dicer generates ~22-nucleotide miRNAs from in vitro synthesized ~70-nucleotide let-7stem-loop RNAs [25][26].

Regulation of microRNA biogenesis:

MiRNA expression might be regulated at multiple steps of RNA biogenesis, although it remains to be determined which step is controlled and how this control is achieved, transcriptional regulation is probably the main control mechanism, for example, the temporal regulation of let-7RNA in C. elegansis dependent on a transcriptional enhancer element, known as the temporal regulatoryelement (TRE) [27].

The role of MicroRNA in diagnosis of fungal infections and aspergillus

Invasive candidiasis, mostly caused by Candida albicans, and invasive aspergillosis, mostly caused by Aspergillus fumigatus, are the main types of invasive fungal infections [28][29].

Guided by the microRNA, the miRISC binds to a target mRNA in its 3′-UTR, thereby inducing mRNA destabilization or translational repression [30]. In a few cases, the binding can also happen in the 5′-UTR or in the coding region [31].

MicroRNAs are involved in many physiological processes like cell differentiation and organ development [32]. Also play crucial roles in the development and functioning of the immune system [33].

They contribute to the regulation of the immune system by influencing the function and differentiation of various immune cells of both the innate and the adaptive immune responses [34].

The innate immune system, mainly neutrophilic granulocytes and resident macrophagesrepresent the first line of defense against invasive fungal infections, denditic cells (DCs) are key for the immune defense against fungi as they build a bridge between the innate and the adaptive immune system [35].

These antigen-presenting cells are involved in pathogen recognition and are solely able to prime naïve T cells in the lymph nodes, thereby determining the fate of the immune response.

Thus, an extended understanding of the regulations may give rise to new therapeutic approaches in anti-fungal therapy, after investigate in vitro the role of microRNAs in response to fungal infections by C. albicans and A. fumigates thus, Monk and others contribute new knowledge to the role of microRNAs in the response to fungal infections [36].

Studies by Buskirk and Nayak showed germination of A. fumigatus conidia in the lungs of mice exposed to viable A. fumigatus conidia these are further supported by increased Clec7A expression in the viable versus HIC group at both time points,the decrease in miR-29a-3p is one potential mechanism that could contribute to the increase in Clec7A [37].

Genes and miRNAs are color-coded (red or green for up- and downregulation, respectively) for the expression of Clec7a, TGF-β3, SMAD2/3 and miRNAs in the viable versus HIC group 48 hours post-exposure [38].

Summary

The genus Aspergillus is extraordinary as exemplified by the diversity of its disease manifestations, almost all termed “aspergillosis”.

All forms of aspergillosis are sapronoses, that is transmissible form an abiotic environment, and not communicable from person to person, or zoonoses.

Aspergillus fumigatus is a saprophytic mold naturally found in the environment that produces respirable asexual conidia.

The first case of aspergillosis described was probably that of a fungus ball of the sinuses.

Mice exposed to viable A. fumigatus conidia highlighted the influence of conidia germination on gene expression controlling the ensuing pulmonary immune responses.This was specifically supported by data that showed increased Clec7a expression and its association with IL-13 and IL-33.

Downregulated miRNAs predicted to target Il13, Il33, and Clec7awere identified, suggesting a possible mechanism that contributes in part to an immune response following subchronic exposures to A. fumigatus.

These altered miRNAs may serve as potential biomarkers to evaluate fungal exposure.

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