MicroRNA-targeted proteomic profiling predicts potential biomarkers of *Aspergillus versicolor* exposure

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Abstract

Rationale: Personal exposure to fungal bioaerosols within damp indoor environments is associated with adverse respiratory health effects. Pathway analysis of proteomic and RNA alterations following exposure to *Aspergillus versicolor*, a prominent indoor fungal contaminant, identified proteins and microRNAs not previously associated with fungal exposure.

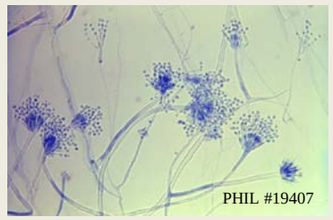
Methods: Mice inhaled *A. versicolor* conidia ($3.5x10^5$ estimated pulmonary deposition) or air twice a week for 4 and 13 weeks. Lungs harvested 24 hours post-exposure were processed for proteomic and microRNA analyses. Molecular pathways were analyzed utilizing Qiagen's Ingenuity Pathway Analysis software

Results: After a 4-week exposure, microRNA-targeted proteomic analysis identified proteins not previously associated with fungal exposures, such as phosphodiesterase 3A, which is involved in pulmonary hypertension and cardiac disease, and troponin T-type 2, a contributor to cardiac failure and fibrosis. Decreased G protein-coupled receptor kinase 5 expression is associated with respiratory and neurological damage. The 13-week exposure revealed decreased protein tyrosine kinase 2 expression, which is involved in abnormal morphology of the vasculature, cardiac tissue, and neuroepithelium, as well as interstitial fibrosis. Additionally, increased miR-138 and miR-142 expression, both involved in pulmonary fibrosis and respiratory disease, has not been previously reported with *A. versicolor* exposure.

Conclusions: Pathway analysis identified proteins and microRNAs not previously associated with fungal exposures. Further, these proteins and microRNAs contribute to adverse responses, such as pulmonary hypertension and neurological damage, consistent with recently completed studies that revealed arterial remodeling and neuroinflammation following exposure. These proteins, and their regulating microRNAs, provide insight into potential mechanisms associated with adverse health effects following inhalation of fungal spores.

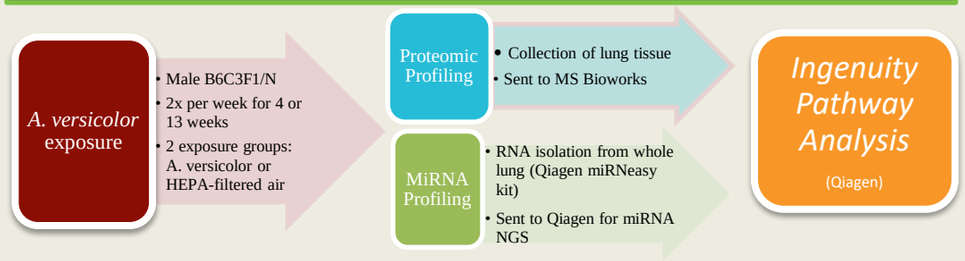
Background

- Associations between personal exposure to bioaerosols from damp indoor environments and adverse respiratory health effects have been identified.
- *Aspergillus versicolor* is a ubiquitous hydrophilic fungal species predominantly found in damp indoor environments.
- *A. versicolor* produces respirable sized conidia that are deposited within the lung following inhalation.



- Preliminary data demonstrated that *A. versicolor* exposure led to an inflammatory Th2-biased immune in the lungs of exposed mice. Histological assessment identified pulmonary inflammation and tissue remodeling, as well as neuroinflammation following exposure.
- The objective of this study was to further characterize responses to repeated *A. versicolor* exposure and identify molecular biomarkers associated with exposure.

Methods



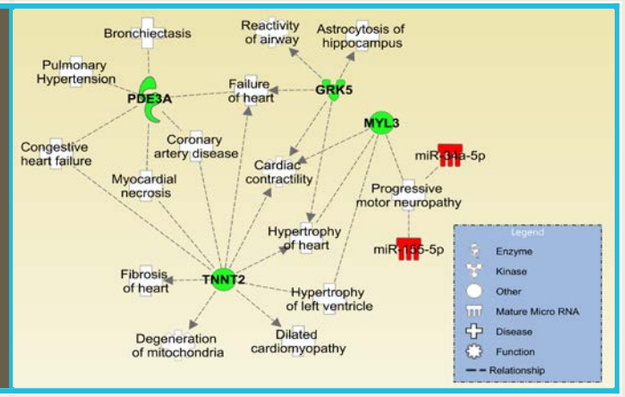
Results

Table 1. MicroRNA-targeted proteomic analysis using Ingenuity Pathway Analysis

Protein	Gene	Fold change	Pathway	MiRNA	Fold change
4 weeks					
Phosphodiesterase 3	PDE3A	-2.85*	Cardiac dysfunction Respiratory dysfunction	miR-155-5p	2.71[t]
G protein-coupled receptor kinase 5	GRK5	-6.51**	Cardiac dysfunction Respiratory dysfunction Neurological damage	miR-135b-5p	11.18[t]
Myosin light chain 3	MYL3	-2.94*	Cardiac dysfunction Neuropathy	miR-122-5p miR-449c-5p	3.16 3.26[t]
Cardiac muscle troponin T	TNNT2	-3.94**	Cardiac dysfunction	miR-122-5p	3.16
13 weeks					
Protein tyrosine kinase 2	PTK2	-1.98*	Cardiac dysfunction Neurological damage	miR-135b-5p	304.95[t]
Syndecan-4	SDC4	-6.9**	Neurological damage		
Glycogen branching enzyme	GBE1	-2.43**	Cardiac dysfunction	miR-135b-5p	304.95[t]

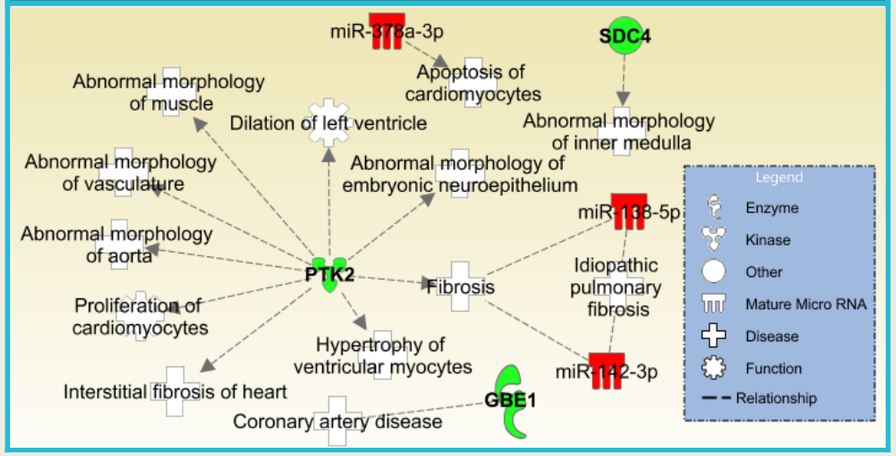
*p < 0.05; **p < 0.01; [t]p < 0.001

Figure 1. Networks identified following 4 weeks of *A. versicolor* exposure. Network map generated by IPA depicting proteins associated with increased miRNAs following 4 weeks of exposure. Proteins and miRNAs are color-coded (red or green for up- and downregulation, respectively) 24 hours post-exposure.



Results

Figure 2. Identified associations following 13 weeks of *A. versicolor* exposure. Network map generated by IPA depicting proteins associated with increased miRNAs following 13 weeks of exposure. Proteins and miRNAs are color-coded (red or green for up- and down-regulation, respectively) 24 hours post-exposure.



Conclusions

- Pathway analysis identified proteins and microRNAs not previously associated with fungal exposures.
- Decreased proteins targeted by increased miRNAs following *A. versicolor* exposure were involved in cardiac and respiratory dysfunction, and neurological damage which was consistent with recent preliminary studies examining histological, immunological, and neurological endpoints.
- Not previously shown to be involved with fungal exposures, miR-135 targets proteins heavily involved in cardiac dysfunction.
- These proteins, and their regulating microRNAs, provide insight into potential mechanisms associated with adverse health effects following inhalation of fungal spores.

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