# Distinct Epithelial mRNA and miRNA Expression Profiles During Differentiation and Between EIB(+) and EIB(-) Asthma Phenotypes


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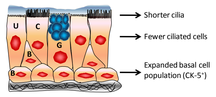
## Introduction

- Asthma is a respiratory syndrome characterized by periods of reversible airflow obstruction, often in response to inhaled stimuli. In addition to symptoms, there is also significant structural airway remodeling associated with the disease.
- 30-50% of asthmatic patients experience exercise-induced bronchonstriction (EIB)[3,4].
- EIB is thought to involve defective epithelial water transport[5], and EIB(+) patients have more pro-inflammatory mediators and columnar epithelial cells in induced sputum[4] and greater numbers of intraepithelial mast cells compared to EIB(-) patients[6]. These findings suggest epithelial differences between disease phenotypes.
- The airway epithelium in asthma has been shown to be remodeled with loss of columnar epithelial cells[1], and an expanded basal cell population[2], suggesting chronic damage or impaired differentiation. Many of the phenotypic airway epithelial cell differences observed *in vivo* are also evident *in vitro*, when cells are cultured at an air-liquid interface (ALI) to induce mucociliary differentiation[2].
- In poster A4885, we demonstrate that both EIB(-) and EIB(+) ALI cultures had impaired ciliary differentiation, but only EIB(-) had increased numbers of cytokeratin 5-expressing basal cells, suggesting epithelial differences in specific asthma phenotypes.
- This study aims to understand the mechanisms of RNA expression and its effect on mucociliary differentiation of airway epithelial cells from EIB(+) and EIB(-) asthma.

**Impaired epithelial cell differentiation in asthma**




C – Ciliated; G – Goblet; U – Undifferentiated; B – Basal

## Hypothesis

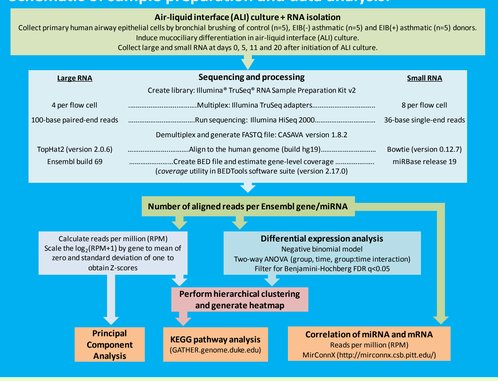
Distinct miRNA and mRNA expression profiles are responsible for defective mucociliary differentiation of airway epithelial cells from asthmatic donors with and without exercise-induced bronchoconstriction.

## Specific Aims

1. Perform a global analysis of mRNAs and miRNAs that are differentially-expressed between disease groups during differentiation.
2. Identify important miRNA-mRNA interactions that regulate mucociliary differentiation *in vitro*.

## Methods

**Schematic of sample preparation and data analysis:**




## Conclusions

- Several biological pathways were dysregulated during differentiation of epithelial cells from EIB(-) and EIB(+) asthmatic compared to non-asthmatic donors. Our findings suggest:
  - Deficient cellular energy production by oxidative phosphorylation and nucleotide metabolism/transcription in EIB(-) cultures and delayed induction of these pathways in EIB(+). This may have implications for differentiation of specialized cell types with high energy requirements, such as ciliated cells.
  - Impaired cell polarization and adhesion via actin-related pathways in EIB(-) cultures.
  - Altered growth factor and MAPK signaling in asthmatic ALI cultures.
  - Overall, airway epithelial cells from asthmatic donors did not induce key pathways required for mucociliary differentiation.
- MiRNAs regulate a complex network of mRNA targets in airway epithelial cells, which are consistent with a transition from proliferation to differentiation over 20 days of ALI culture.
- We did not identify any miRNAs that were dysregulated between disease phenotypes during mucociliary differentiation *in vitro*.
- These data provide insight into the molecular mechanisms that regulate epithelial differentiation *in vitro*, and identify key pathways that are altered in asthma.

## Results

### Figure 1. Global analysis of RNA expression during epithelial differentiation *in vitro*

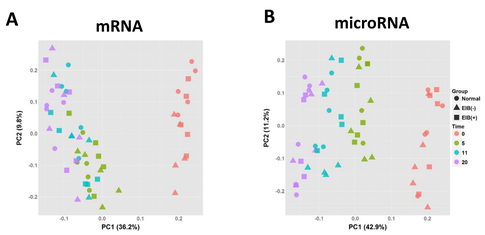


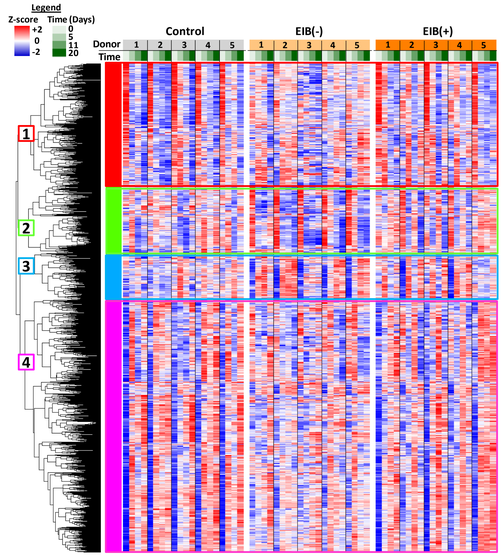

**Figure 1.** Principal component analysis was used to assess variance in expression of **(A)** mRNA and **(B)** miRNA between all samples, using reads per million (RPM) of all features that passed filtering criteria. Donor groups are differentiated by symbol, and timepoints by colour. X-axes are principal component (PC) 1, y-axes PC2, and the contribution to overall variance is given as a percentage in brackets.

### Figure 2. Summary of differentially-expressed transcripts between donor groups during ALI culture

| | mRNA | microRNA |
|---|---|---|
| **Unique genes/miRNA identified** | 55,841 | 901 |
| Negative binomial model<br>Filter: 1) >1 read in every sample<br>2) Median reads across all samples > 20<br>3) Negative binomial model converges | | |
| **Filtered genes/miRNA identified** | 21,876 | 437 |
| Statistical analysis by two-way ANOVA | | |
| **FDR q<0.05 for each two-way ANOVA term** | | |
| Group | 2561 | 62 |
| Time | 18,527 | 382 |
| **Group:Time Interaction** | **1564** | **0** |

**Figure 2.** Summary of analyses of differential expression. The number of ENSG gene IDs (mRNA) or mature miRNAs is given for each step. Total aligned sequencing reads were analyzed by two-way ANOVA and filtered as described. To understand the processes underlying impaired epithelial differentiation in asthma, the 1564 genes with a false discovery rate (FDR) <0.05 were selected for further analysis.

### Figure 3. Biological pathways related to cytoskeleton dynamics and cellular metabolism are aberrantly expressed in asthmatic-derived ALI cultures




B

| Cluster | Total # genes | KEGG Pathway | Annotation | # genes in pathway | Bayes factor | FDR |
|---|---|---|---|---|---|---|
| 1 | 390 | hsa04810 | Regulation of actin cytoskeleton | 12 | 69 | 0.016 |
| | | hsa04530 | Tight junction | 8 | 24 | .022 |
| | | hsa04510 | Focal adhesion | 11 | 9 | .045 |
| 2 | 218 | hsa00190 | Oxidative phosphorylation | 4 | 26 | .052 |
| 3 | 148 | hsa04910 | Insulin signaling pathway | 6 | 270 | .0041 |
| | | hsa04010 | MAPK signaling pathway | 6 | 18 | .033 |
| 4 | 808 | hsa00190 | Oxidative phosphorylation | 18 | $1x10^{-7}$ | $8.5x10^{-8}$ |
| | | hsa00240 | Pyrimidine metabolism | 12 | 9228 | $5.1x10^{-5}$ |
| | | hsa00230 | Purine metabolism | 11 | 14 | .025 |

C

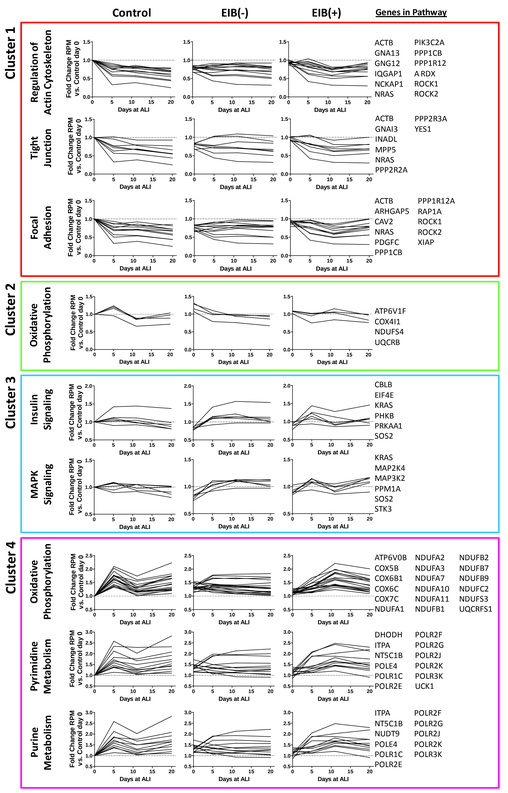



**Figure 3. (A)** The 1564 differentially-expressed genes for group:time term were hierarchically clustered using the complete linkage method into four groups as indicated by numbered boxes on the dendrogram at left. Each row of the heatmap represents one gene, while each column represents one sample. Red indicates expression (by Z-score) above the median for a given gene, and blue indicates expression below the median. **(B)** KEGG pathways enriched in each cluster of differentially-expressed genes using GATHER (gather.genome.duke.edu). The Bayes factor and false discovery rate (FDR) for the enrichment of each pathway are provided. **(C)** Kinetics of pathway expression in each cluster are expressed as mean fold change compared to the mean RPM in control day 0 (dashed horizontal line) for control (left column), EIB(-) (middle column) and EIB(+) (right column) groups. Genes in each pathway are listed at right.

### Figure 4. MicroRNA-mRNA networks regulate the transition from proliferation to differentiation in ALI culture

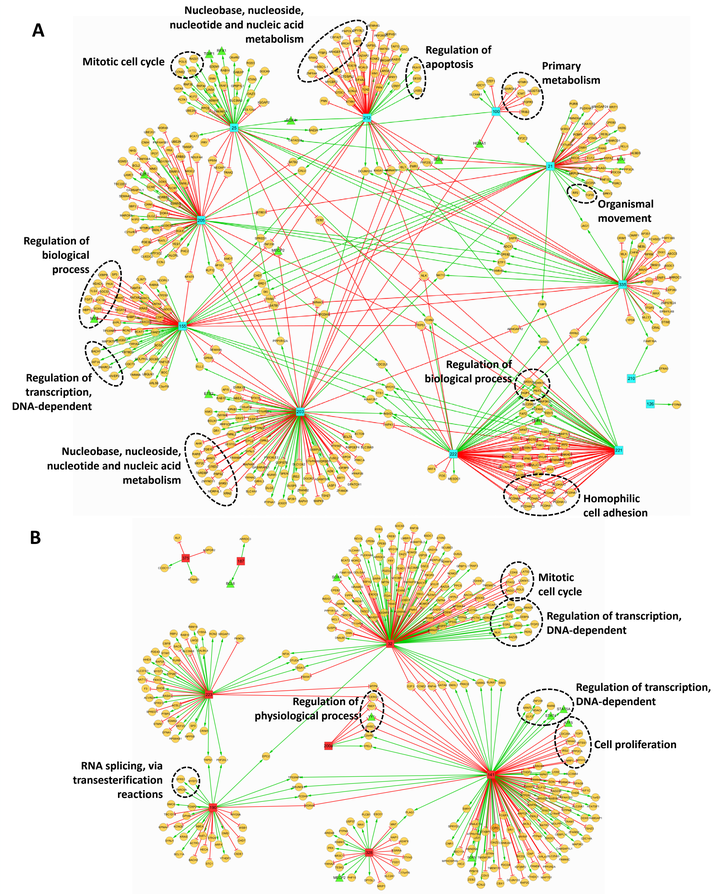



**Figure 4.** Networks regulated by miRNAs that **(A)** decreased by ≥2-fold (blue squares) and **(B)** increased by ≥2-fold (red squares) between day 0 and 20 of differentiation in air-liquid interface culture. Green triangles denote transcription factors, while yellow circles denote genes. Red lines indicate negative regulation (suppression), while green lines indicate positive regulation (activation) of targets. Targets that were enriched for specific gene ontologies, as determined by GATHER, are shown in dashed circles and labeled accordingly.

## References


[1] Barbato et al. AJRCCM 2006 [2]Hackett, Warner et al. AJRCCM 2009 [3]Crapo, Casaburi et al. AJRCCM 2000 [4]Hallstrand, Moody et al. AJRCCM 2005 [5]Anderson and Daviskas JACI 2000
[6]Hallstrand, Lai et al. JACI 2013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