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Original Article

Molecular characterization of gene regulatory networks in primary human tracheal and bronchial epithelial cells☆

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Abstract

Background: Robust methods to culture primary airway epithelial cells were developed several decades ago and these cells provide the model of choice to investigate many diseases of the human lung. However, the molecular signature of cells from different regions of the airway epithelium has not been well characterized.
Methods: We utilize DNase-seq and RNA-seq to examine the molecular signatures of primary cells derived from human tracheal and bronchial tissues, as well as healthy and diseased (cystic fibrosis (CF)) donor lung tissue.
Results: Our data reveal an airway cell signature that is divergent from other epithelial cell types and from common airway epithelial cell lines. The differences between tracheal and bronchial cells are clearly evident as are common regulatory features. Only minor variation is seen between bronchial cells from healthy or CF donors.
Conclusions: These data are a valuable resource for functional genomics analysis of airway epithelial tissues in human disease.

Keywords: Airway epithelium; Primary human bronchial cells; Primary human tracheal cells; Molecular signature; Open chromatin; DNase-seq; RNA-seq; Transcriptional networks

1. Introduction

Major advances in understanding mechanisms of human lung disease have arisen through the development of methods to culture primary cells from the airway epithelium [1,2]. These cells may be differentiated on permeable supports to generate a polarized cell layer that at air-liquid interface are a robust model for investigating many aspects of airway epithelial function. Cells of nasal, tracheal and bronchial epithelial tissue are all amenable to these protocols, though the bronchial cells have been most extensively studied. Moreover, the latter are the desired endpoint of recent methods to differentiate human induced pluripotent stem cells (IPSCs) into airway epithelium [3,4]. Use of these primary cell cultures to study normal human airway epithelial biology [5] and dysfunction in diseases such as cystic fibrosis (CF), asthma [6] and chronic obstructive pulmonary disease (COPD) [7] among other disorders has provided important insights into disease mechanisms. The precise cellularity of each tracheal and bronchial culture will depend on many factors, including substantial donor-to-donor

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variation, both genetic and environmental, and specific culture protocols. Our goal was to compare the molecular signatures of primary human tracheal (HTE) and bronchial epithelial (HBE) cells to determine functional differences between them. We used genome-wide analysis of open chromatin, which is associated with active regulatory elements, combined with gene expression profiles of each cell type. The signature of HBE cells derived from healthy and CF donor lungs was also compared. The data revealed a clustering of open chromatin profiles in primary airway cells, which was distinct from that observed in lung epithelial cell lines and other epithelial cell types. Though there was substantial overlap between the tracheal and bronchial cells, distinct features of each cell population were evident and correlated well with divergent gene expression profiles. Also of note were the very limited differences between HBE and CFHBE primary cells, with only a few disease-related pathways identified.

2. Methods

2.1. HTE, HBE, CF HBE and NHBE cells

Human tracheae were collected post mortem from healthy donors and human tracheal epithelial (HTE) cells isolated and grown as described previously [1]. Human bronchial epithelial (HBE) cells were obtained (at passage 1, P1) under protocol #03–1396 approved by the University of North Carolina at Chapel Hill Biomedical Institutional Review Board [2]. Informed consent was obtained from authorized representatives of all organ donors. Normal human bronchial epithelial (NHBE) cells were purchased from Lonza. Primary HTE, HBE and NHBE cells were grown on collagen-coated plastic in bronchial epithelial growth medium (BEGM, Lonza) [2]. Cells were grown on plastic rather than permeable supports to provide adequate cell numbers for the DNAse-seq protocol and to ensure efficient DNase I digestion. See Supplemental Methods for detailed culture protocols.

2.2. DNase-seq and RNA-seq

DNase-seq and RNA-seq libraries were prepared according to standard protocols. See Supplemental methods for detailed analysis methods.

3. Results and discussion

3.1. DNase-seq in HTE, HBE and NHBE cells reveals substantial similarities between the open chromatin landscapes of the three cell types

Regulatory elements within the genome are usually associated with regions of open chromatin, which can be readily detected by enzymatic or chemical methods. In order to compare open chromatin profiles in human bronchial (HBE) and tracheal (HTE) cells, we performed DNase-seq on three cell types: primary HBE cells cultured from three different donors, primary HTE cells from two different donors, and NHBE cells (Lonza CC-2541, a mixture of HBE and HTE cells) from two donor codes. After mapping reads to the human genome, we used the differential peak calling function of the MACS2 peak caller to identify subclasses of DNase-I hypersensitive sites (DHS) as follows: “common” DHS are present in all three (HBE/HTE/NHBE) cell types, “shared” DHS are present in pairs of samples, and “cell type selective” DHS are present in only one of the three cell types. A total of 56,649 peaks were seen in NHBE cells, 76,759 peaks were evident in HBE cells, and 104,384 peaks were called in HTE cells. Of these, 16,053 were common to all three of the cell types (Fig. 1A). Only a small percentage of peaks were cell type selective. NHBE showed 453 (0.8%) cell type-selective DHS, HBE had 1923 (2.5%) such sites, and HTE 5495 (5.3%). Pairwise comparison of cell type-selective and shared DHS revealed that HBE and HTE were the most similar, with high reciprocal overlaps of 87% (HBE) and 64% (HTE). In contrast, NHBE DHS appeared to represent a subset of HTE DHS, with 85% of NHBE DHS found in HTE, but only 46% of HTE DHS present in NHBE. NHBE and HBE were the least similar, with <50% of DHS in either cell type present in the other (HBE: 31%, NHBE: 43%). Since NHBE cells are established as mixture of tracheal and bronchial epithelial cells these profiles suggest that in the lot numbers that we analyzed cells of tracheal origin were predominant. Though the HBE cultures may have a small (1–5 cm of trachea) contribution of tracheal cells these are a minor component of the population. Comparison of normalized DNase-seq signal across replicates (Fig. 1B–C) confirmed the cell type-specificity of DNase-I sensitivity at HTE- and HBE-selective DHS. The “NHBE-selective” DHS, however, were detectable exclusively in one NHBE replicate, while the other NHBE sample appeared most similar to the HTE profile.

Next, we used gene annotations to classify DHS as either promoter-associated (within 2 kb upstream of an annotated transcription start site [TSS]), gene-body associated (between 20 kb upstream and 20 kb downstream of an annotated gene), or intergenic (at least 20 kb from the nearest annotated gene). Pairwise comparison of cell type-selective and shared DHS in promoter regions, gene bodies and intergenic regions were largely consistent with the patterns observed when comparing all DHS (data not shown). Notably, nearly 50% reciprocal overlap was seen between NHBE and HBE DHS at promoters, in contrast to many fewer common sites in the overall and intergenic (HBE: 22%, NHBE: 36%) comparisons. This suggests that where the same genes are active in the 2 cultures types, they may often recruit different *cis*-regulatory elements to drive their promoters. Alternatively, more of the intergenic sites are not directly associated with the transcriptional program of the cells. To further compare the distribution of overlap between cell types, we next visualized the proportion of cell type-selective, shared (between 2 cell types) and common (seen in all 3 cell types) DHS by annotation in HBE (Fig. 2A), HTE (Fig. 2B) and NHBE (Fig. 2C) cells. In each cell type, the highest proportion of shared sites was found in the promoter-associated DHS (second column), supporting a hypothesis of a shared airway epithelial chromatin landscape at gene

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