Asthma- and COPD-related Differential Gene Expression in Primary Human Lung Fibroblasts

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Introduction

With the prevalence of asthma and chronic obstructive pulmonary disease (COPD) growing worldwide, the availability of primary human cells from these respiratory diseases is critical to increase research and knowledge about the disease at a cellular level. In this study, we sought to identify genes differentially regulated in asthma and COPD lung fibroblasts (DHLF-asthma and DHLF-COPD). Fibroblasts are the most common cells of connective tissue in humans. Insufficiency of tissue repair by lung fibroblasts may contribute to the decrease in elastic fibers in COPD[1]. Lung fibroblasts are also the major producers of extracellular matrix (ECM) components within the lung and may initiate, regulate and contribute to the airway remodeling in asthma[2].

Primary lung fibroblasts are isolated from normal, asthma and COPD human donors in accordance with all informed consent rules and regulations. The cells were cultured for seven passages in optimized media. Gene expression analysis was performed using the Human Immune System Phenotyping 96 StellARray™ qPCR Array. The StellARray™ Gene Expression System is a quantitative polymerase chain reaction (qPCR)-based method and provides reliable profiling of biologically focused gene sets. This article summarizes a study comparing normal versus COPD lung fibroblasts and normal versus asthma lung fibroblasts to explore differences in gene expression among the donor samples.

Materials and Methods

In this study, we grew primary lung fibroblasts from normal, asthmatic and COPD tissues in standard submerged culture (see Table 1) to assess gene expression changes associated with diseased states.

Cell Isolation

Fibroblasts were isolated from normal and diseased lung tissues. Isolated cells were expanded in standard submerged culture in FGM™-2 Growth Medium (Lonza, cat. no. CC-3132) and then cryopreserved after the second passage. The vials were stored in liquid nitrogen until further use.

	NHLF: Normal Human Lung Fibroblasts	DHLF-As: Diseased (Asthma) Human Lung Fibroblasts	DHLF-COPD: Diseased (COPD) Human Lung Fibroblasts
Cat. no.	CC-2512	194912	195277
Lot no.	0000120188	0000188312	0F3100
Age	45 Years	27 Years	55 Years
Sex	Male	Male	Female
Race	Caucasian	Caucasian	Caucasian

Table 1
Donor characteristics of primary cells used.

Cell Harvesting and Cell Lysis

Each lot of normal and diseased lung fibroblasts was thawed and plated at a density of 2,500 cells/cm^2 in FGM™-2 Growth Medium (Lonza, cat. no. CC-3132). Growth media were changed after 24 hours and the cells were subcultured through seven passages. At P7, cells were pelleted and cell lysates were obtained using the QIAshredder™ column (Qiagen, cat. no. 79654).

qPCR Experimental Design

To generate data with biologically relevant variance, three replicate samples were independently assayed for each cell type.

RNA Isolation and cDNA Synthesis

Each cell lysate was transferred to the Qiagen RNeasy® Mini Kit (cat. no. 74104) and RNA was extracted. cDNA was synthesized with 2 µg of RNA per sample using SuperScript® II Reverse Transcriptase and dNTP mix (Life Technologies, Inc., cat. no. 18064-014 and 10297-018, respectively). For primers, random decamers and oligo dT primers (Life Technologies, Inc., cat. no. AM5722G and 18418-012) were used. cDNA synthesis reactions were performed according to the specifications of the supplier.

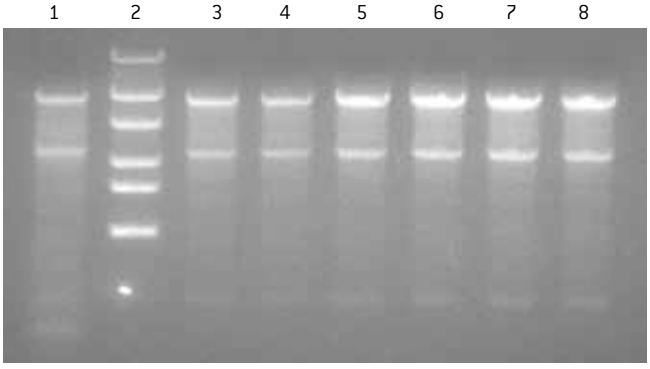

Figure 1

Lane	Sample
1	Reference RNA
2	Marker
3	NHLF Normal Prep 1
4	NHLF Normal Prep 2
5	NHLF Asthma Prep 1
6	NHLF Asthma Prep 2
7	NHLF COPD Prep 1
8	NHLF COPD Prep 2

For Each qPCR Plate, a Reaction Mix Was Prepared in the Following Manner	
2 x SYBR® Green Master Mix (Fast SYBR® Green Master Mix; Life Technologies, Inc.)	1031 µl
H_2O	RPMI, 10% FBS
cDNA template: 40 µl cDNA synthesis reaction mix + 310 µl H_2O	DMEM, 10% FBS

Table 2
Real-time qPCR.

20 µl of the reaction mix was distributed into Human Immune System Phenotyping 96 StellARray™ qPCR Array Plate (Bar Harbor BioTechnology, Inc.). The master mixes contained AmpliTaq® Fast DNA Polymerase (Life Technologies, Inc.), designed to allow instant hot start. Arrays were run on the BioRad CFX 96 using a standard qPCR program.
Post-run data collection involved the setting of a common threshold (Ct) across all arrays within an experiment, exportation and collation of the Ct values, and analysis via GPR.

GPR Algorithm

Data input for GPR consists of a list of Ct values, derived directly from real-time PCR instruments, for each sample (normal or diseased). After designating the control and experimental sets of Ct values, GPR filters expression data to separate genes into two groups – genes considered for analysis (G) and genes that can be used as potential normalizers (N). Genes that are not expressed in either sample are not considered further. After filtering the data into two sets, GPR performs a proprietary global normalization and statistical analysis by comparing each G to each N. The magnitude of change in expression ("fold change") for each gene is subsequently determined using the ten best N genes, as defined within each experiment.

Results and Discussion

In each diseased cell type, statistically relevant differences in gene expression were detected in the diseased cells compared to the normal cells. Genes with P-values of <0.05 or better are reported.

Asthmatic

The asthmatic fibroblast cells had two upregulated genes as compared to the normal. CD14 was upregulated 4.97 fold and IL7R was upregulated 2.8 fold. Both of these genes play a role in the innate immune system which is activated in response to stimuli in asthmatic patients[3]. CD14 is a surface antigen that is expressed on several immune cells and IL7R is critical in the development and activation of lymphocytes[4,5].
The two downregulated genes in the asthmatic donor as compared to the normal donor were CD22 and CD40 (3.2 fold and 4.6 fold respectively). CD40 encodes for a protein involved in a variety of immune and inflammatory responses[6].

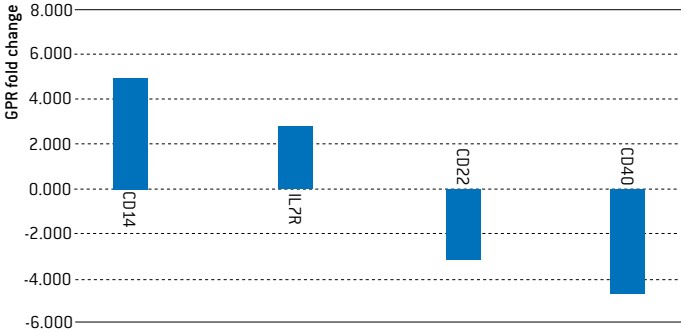

Figure 2
Genes up- or downregulated in asthmatic sample vs. normal cells.

COPD

The COPD sample had several upregulated genes compared to the normal sample. Two of these genes, TNFSF4 and TNFSF18, encode for proteins that are cytokines and part of the tumor necrosis factor (TNF) ligand family. TNFSF4, upregulated 15.1 fold, is involved in T cell antigen-presenting cell interactions and is reported to mediate adhesion of activated T cells to vascular endothelial cells[7]. TNFSF18, upregulated 10.4 fold, has been observed to modulate T lymphocyte survival in peripheral tissue and is expressed in endothelial cells. It is thought to be important for interactions between the two cell types[8]. CD40 was upregulated 4.2 fold and encodes for a protein belonging to the TNF-receptor family. The receptor is critical in mediating a broad variety of immune and inflammatory responses[6]. CD36 was upregulated 15.1 fold and the resulting protein of this gene serves as a receptor for thrombospondin in platelets[9]. One study found that long-term cigarette smoke exposure is associated with fibrosis and inflammation via increased levels of thrombospondin[10]. KLRD1, expressed by natural killer cells that mediate cytotoxic activity and secrete cytokines upon immune stimulation, was upregulated 7.1 fold[11].

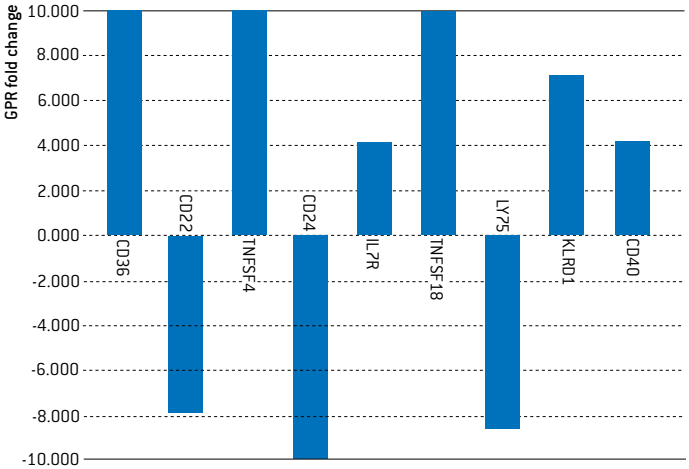

Figure 3
Genes up- or downregulated in COPD sample vs. normal cells.

One significantly downregulated gene in the COPD donor sample was CD24 (25.3 fold) which encodes a sialoglycoprotein that is expressed on mature granulocytes and also in many B cells[12]. Other downregulated genes included CD22 (7.9 fold) and LY75 (8.7 fold).

Summary

We have described a method in which normal, asthmatic, and COPD diseased lung fibroblasts were expanded and gene expression was analyzed using an immune system qPCR array. The resulting data revealed differences in gene expression between the normal and diseased cell types. Several of the detected genes were implicated in previously published work relating to asthma and COPD research which indicates that although this study involved cells from single donors, the resulting data is valuable and relevant.

Both asthma and COPD are characterized by inflammation of the lungs and involve immune responses. Most drug targets are enzymes or receptors aimed at regulating these pathways. Access to these diseased cell types provides a convenient, biologically relevant model to assess the genetic pathways involved in the disease and may help to indicate other potential drug targets.

Lonza currently offers a selection of normal, asthmatic, and COPD human bronchial epithelial cells, human lung fibroblasts, and human bronchial smooth muscle cells. The cells are tested and guaranteed to perform with the suggested Clonetics™ Media Kits and Reagents. More in-depth donor information can be obtained through Lonza's Scientific Support Team.

References

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