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Heterogeneity of Transcription Factor Expression and Regulation in Human Airway Epithelial and Smooth Muscle Cells

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Abstract

Transcription factors represent a major mechanism by which cells establish basal and conditional expression of proteins, the latter potentially being adaptive or maladaptive in disease. The complement of transcription factors in two major-structural cells of the lung relevant to asthma, airway epithelial and smooth muscle cells, is not known. A plate-based platform using nuclear extracts from these cells was utilized to assess potential expression by binding to oligonucleotide consensus sequences representing >300 transcription factors. Four conditions were studied: basal, β -agonist exposure, culture under pro-asthmatic conditions (IL-13, IL-4, TGF β and LTD₄) and the dual setting of β -agonist with pro-asthmatic culture. Airway epithelial cells expressed 70 transcription factors while airway smooth muscle expressed 110. High levels of multiple transcription factors not previously recognized as being expressed in these cells were identified. Moreover, expression/binding patterns under these conditions revealed extreme discordance in the direction and magnitude of change between the cell-types. Singular (one cell-type displayed regulation) and antithetic regulation (both cell-types underwent expression changes but in opposite directions) dominated these patterns, with concomitant regulation in both cell-types being rare (<10%). β -agonist evoked up-and downregulation of transcription factors, which was highly influenced by the pro-asthmatic condition, with little overlap of factors regulated by β -agonists under both conditions. Taken together, these results reveal complex, cell-type dependent networks of transcription factors in human airway epithelium and smooth muscle that are dynamically regulated in unique ways by β -agonists and inflammation. These factors may represent additional components in asthma pathophysiology or potential new drug targets.

Keywords

Transcription; β -agonist; asthma; inflammation

In eukaryotic cells, transcription factors represent the major elements by which gene transcription by RNA polymerase II is controlled (18, 26). Such factors regulate the basal expression of genes, and serve to dynamically regulate gene expression during normal homeostatic conditions, xenobiotic exposures such as therapeutic agents or toxins, and pathologic conditions, where they may be a basis for, or serve to counteract, aberrant cellular

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physiology (13, 18, 26). In asthma, two stromal cell-types play significant roles in the syndrome, the airway epithelial cell and the airway smooth muscle (5, 9). While some information is known about the regulation and action of selected transcription factors of immune cells of the lung and peripheral circulation (1, 11, 12, 24), there is a paucity of data on the expression of transcription factors in airway epithelial and smooth muscle cells. Furthermore, there are no studies which examine a large complement of transcription factors expressed in these cells and their modulation by inflammatory stimuli or β -agonists, the most common treatment for asthma. In the current work we utilize a platform that quantitates the expression/binding capacity of transcription factors based on binding to 345 consensus oligonucleotide sequences. The two cell-types were studied in the basal state, and after exposure to a β -agonist, a pro-asthmatic culture condition, and the combination of both. We hypothesized that the complement of expressed transcription factors would be markedly different between airway epithelial and smooth muscle cells. And, that there would be significant differences in transcription factor regulation under the above conditions between the two cell-types. We indeed found extensive heterogeneity in the transcription factor expression/binding between airway epithelial and airway smooth muscle cells. This was found in terms of basal expression of individual factors, up- and downregulation by the aforementioned conditions, and marked cell-type dependency in the magnitude and direction of these changes.

METHODS

Cell Culture

BEAS-2B (R1 clone) cells were grown in HAMS F-12 (Gibco) with 10% fetal calf serum, 5 μ g/ml insulin, 5 μ g/ml transferrin, 25 μ g/ml epidermal growth factor, 100 units/ml penicillin and 100 μ g/ml streptomycin (14). Primary human airway smooth muscle cells were obtained from Clonetics (San Diego, CA), and grown in monolayers in SMBM media (Clonetics) (8). Both cell-types were maintained at 37° in a 95% air 5% CO₂ atmosphere. Four treatment conditions were utilized and were for 24 hrs at 37°. Basal conditions consisted of no additives. Isoproterenol exposure was at a concentration of 10 μ M. To mimic some aspects of a pro-asthmatic milieu, cells were incubated with 10 ng/ml IL-4, 10 ng/ml IL-13, 20 ng/ml transforming growth factor β (TGF β), and 100 nM leukotriene D₄(LTD₄). For simplicity, this is termed the “asthma condition.” The fourth condition was concomitant treatments with isoproterenol and the aforementioned agents of the asthma condition. Nuclear extracts were prepared using reagents from the NE-PER extraction system (Pierce, Rockford, IL) as previously described (19). Briefly, attached cells were placed on ice, the media aspirated, and washed three times with cold PBS. Cells were removed by gentle scraping and collected by centrifugation at 12,000 xg for 15 min at 4° and swollen by incubation in CER1 buffer for 10 min. After incubation with the CERII detergent solution for 1 min, preparations were centrifuged at 16,000 xg for 5 min at 4°. The nuclear pellet was resuspended in nuclear extraction buffer and vortexed for 15 sec every 10 min over 40 min, and the preparation centrifuged at 16,000 xg for 10 min at 4°. The supernatant (nuclear extract) was collected, aliquotted, and frozen at –80° until used. All of the above steps included the protease inhibitors leupeptin and aprotinin at 10 μ g/ml.

Confocal microscopy

To verify the cell-type by characteristic histologic features and antigen expression, and the homogeneity of the cells used for preparing nuclear extracts, confocal microscopy was carried out using methods similar to those previously described (15). Briefly, cells were fixed with 4% paraformaldehyde and permeabilized with 0.5% Triton X100. The antibodies (Sigma) were vimentin conjugated to Cy3 (1:200), and cytokeratin conjugated to FITC (1:50), for identification of smooth muscle and epithelial cells, respectively. 4',6'-diamidino-2-phenylindole dihydrochloride (DAPI, 0.1 µg/ml) was utilized as a fluorescent nuclear counterstain (Molecular Probes, Eugene, OR). Images were obtained on a Nikon Eclipse TE2000-E microscope at a magnification of 20X.

Transcription factor expression arrays

Nuclear extracts from the two cell-types under the four conditions were assessed for the relative expression of 307 transcription factors using the Panomics Combo Array (Panomics, Redwood City, CA) as described in detail elsewhere (10). The transcription factors that are quantitated in the array are found in Supplementary Table 1. Briefly, extracts were preincubated with biotin labeled consensus oligonucleotides for the indicated transcription factors, the free probes separated by washing, and the bound probes extracted and hybridized to the array according to the manufacturer's protocol. Horseradish peroxidase-based chemiluminescence was detected using an AlphaImager (Alpha Innotech, San Leandro, CA) with black, white and gamma values of 0, 65500 and 0.55, respectively. The integrated density values were quantitated using the AlphaEase software provided with the instrument. All plates were imaged under the same conditions. In certain cases, when transcription factors are known to bind to more than one distinctly different positions of a promoter, several oligonucleotide sequences were utilized in separate reactions with the nuclear extracts. The data from these are reported as the transcription factor name followed by a number in parentheses (i.e., AP2 (1), AP2 (2)). Thus while the array has 345 hybridization positions, these represent 307 transcription factors. For the analysis of changes in expression by the various conditions, each hybridization was considered a separate entity.

The lower limit of detection for the array is a density value of 10. Those factors with values <10 are reported as not detected. A relevant change in transcription factor expression was considered an increase or decrease of 50%. In cases where a condition induced expression from undetectable to >10, this is reported as upregulation but a specific percentage of the change is not provided ; similarly, detectable expression that changed to a value <10 is reported as downregulation without a notation as to the percent change. Comparisons were between isoproterenol vs. basal treatment, asthma culture conditions vs. basal, and asthma culture condition with isoproterenol vs. asthma condition alone. Data are presented as mean density ± SD.

Miscellaneous

βAR expression was determined with cell membranes using ¹²⁵I-cyanopindolol (¹²⁵I-CYP) binding as previously described (6, 7). Since neither cell-type expresses the β₁AR subtype, the specific binding from these assays represents the β₂AR subtype. Protein concentrations

of nuclear extract and cell membrane preparations was quantitated by the copper bicinchoninic acid method (23).

RESULTS

Figs. 1 and 2 are representative confocal microscopy images of the cells used in the current study. As shown, they have the characteristic morphologic appearance of epithelial and smooth muscle cells under white light (panels *A, D*). Nuclei are indicated by the blue counterstain from DAPI (panels *B, E*). The epithelial cells revealed intense images from incubation with the antibody to cytokeratin (Fig. 1*C*) but not to vimentin (Fig. 2*C*). In contrast, airway smooth muscle revealed intense signals from vimentin and none from cytokeratin (Figs 2*F* and 1*F*, respectively). As expected, both cell-types expressed β_2 -adrenergic receptors (β_2 ARs). Using ^{125}I -CYP radiolig and binding, these were quantitated at 213 ± 8.4 and 78 ± 12 fmol/mg, respectively, for airway epithelial cells and airway smooth muscle cells ($n=6$).

Tables 1 and 2 show the results of the transcription factor binding assays from the two cell - types, where signals were above the level of detection for any one of the four conditions. The method utilized not only requires expression of the factor, but also any post-translational modifications such as phosphorylation, or other conditions, necessary for binding to the consensus oligonucleotide. This may provide a particular advantage in assessing the regulation of transcription factor activity under relatively short-term conditions. In the intact cell this would represent expression of a “functional factor” capable of altering transcription through such binding. In this paper, for simplicity we refer to this output as representing transcription factor “expression” or “expression/binding”, recognizing that there may be additional aspects that ultimately result in the signal. The complement of transcription factor binding sites queried by these arrays is provided in Supplemental Table 1. For the airway epithelial cells, expression of 70 transcription factors was noted, while 110 factors were detected in airway smooth muscle cells (Fig. 3). In epithelial cells, 10 (~15%) of the transcription factors were unique to these cells as compared to airway smooth muscle (Table 3). In the latter, 49 transcription factors (~45%) were found to be unique. The two cell-types had common expression of 55 transcription factors (Table 3).

The three conditions resulted in multiple changes in transcription factor expression/binding in both airway epithelial and smooth muscle cells (Fig. 4*A, B*). For airway epithelial cells, at baseline 50 factors were expressed, and 16 were increased with isoproterenol. For 5 of these, E2F-2, E4F, EGR, PARP, and Pax-4a, isoproterenol resulted in an increase in expression/ binding over detectable basal levels. At baseline 10 factors were not detected, but were induced by isoproterenol exposure. β -agonist exposure reduced expression/binding of 10 factors. In the asthma culture setting, 12 factors were increased over baseline; of these, 10 were undetectable at baseline. Four transcription factors were decreased from baseline under these conditions (Fig. 4*A*). Some studies have shown β -agonists to have anti-inflammatory properties, so for purposes of data presentation the effect of isoproterenol on the transcription factor/binding while in the asthmatic culture conditions was utilized for comparison (i.e., relationship between transcription factor expression in the asthma culture conditions alone vs. expression under the combined condition). Here, we found 18 factors

that were increased by isoproterenol exposure over the asthma culture conditions (Fig. 4A). Of these, 11 were not detectable in the asthma condition alone. Of the 34 (total) factors increased by isoproterenol exposure under either of the two conditions, there was overlap of only six factors (AP-2, Elk-1, E4F, Evi-1, PEBP2alpha and PARP) that were increased by isoproterenol alone and isoproterenol in the asthma culture setting. Sixteen factors were decreased by isoproterenol compared to the asthma + isoproterenol conditions. Six of these (NF-Y, RAR, T3R, USF, CTCF, and HOXD9,10) were also decreased in the isoproterenol alone setting.

For airway smooth muscle cells, a significantly greater number of transcription factors were detected under at least one of the four conditions as compared to airway epithelial cells. For these cells, expression/binding of 110 transcription factors was found, with 96 being expressed under basal conditions. Isoproterenol exposure resulted in an increase in 19 transcription factors, of which 13 were undetectable at baseline (Fig. 4B, Table 2). Sixteen factors were decreased by isoproterenol. Under the asthma culture conditions, 8 factors were increased (7 were not detected at baseline), and 14 were decreased. Expression changes evoked in the isoproterenol with asthma culture conditions compared to the asthma condition alone amounted to 32 factors that were upregulated and 10 that were downregulated (Fig. 4B). Of these 42 total factors that were regulated under the dual condition, 11 also underwent changes in the same direction during β -agonist treatment alone (8 increased, 3 decreased).

As introduced earlier, there were 55 factors that were expressed in both cell-types, and we considered whether these were regulated in a similar manner under the various conditions. We found evidence for regulation that could be classified in three categories (Fig. 5). A singular change denotes that there was a change in expression/binding in either direction but it was found in only one cell-type. Concomitant regulation is defined as a change in expression/binding in the same direction (up-or downregulation) that is observed in both cell-types under a given condition. Antithetic regulation is a change in expression/binding of a transcription factor under a given condition, but the direction of the change is opposite between airway epithelial cells and smooth muscle cells. For the current conditions we found singular changes dominated in this group of transcription factors that were expressed in both cell-types. Of particular interest were the antithetic changes (Fig. 5, red:blue or blue:red), which were observed in ~60% of cases where changes occurred with both cell-types. These factors were AP2, NF-E1, E4F, (observed twice), Elk-1, COUP-TF (observed twice), EGR1, and PU.1. Surprisingly, in all but one case of antithetic regulation, expression of the given factor increased in the airway epithelial cells and decreased in the airway smooth muscle cells. Concomitant regulation was less frequent, with no specific pattern as to a predominance of up-or downregulation. Although airway epithelial cells express >2-fold more β_2 ARs compared to the smooth muscle cells, there was no trend towards a greater number of β -agonist regulated transcription factors (either as an absolute number or percentage of total transcription factors) in these cells vs. smooth muscle.

DISCUSSION

Transcription factor binding to specific sequences in regulatory regions is now recognized as a critical component in establishing gene expression under basal, conditional, and cell-type dependent manners, and transcription factors may be particularly amenable as drug targets in the lung for treatment of asthma and chronic obstructive lung disease (2, 3, 16, 21). As a first step in understanding their role in airway epithelial and smooth muscle cells, we undertook analysis of potential expression/binding of >300 transcription factors, and present this data for other investigators to utilize for specific gene transcription issues. Our global analysis of the data reveals several important findings. There were substantially more transcription factors expressed in airway smooth muscle cells compared to epithelial cells. And, of the epithelial cell factors, less than half were in common with those detected in smooth muscle cells. This underlies the important concept that while cells may express many common proteins, the mechanism(s) by which basal and/or regulated expression is achieved at the transcriptional level may be quite different.

We also show that exposure to β -agonist, a common treatment for obstructive lung disease, alters the binding levels of ~23% of the expressed transcription factors in epithelial cells and ~38% in airway smooth muscle. The impact of these changes is yet to be explored, but it is likely that there are those that are detrimental in terms of the cellular or organ physiology in these two syndromes. Indeed, β -agonist use continues to be controversial in asthma, with multiple reports of increased mortality or other indices of poor asthma control during continuous (chronic) therapy (4, 17, 22, 25). Given the efficacy of β -agonists for relieving bronchospasm, one strategy for new, combined, pharmacologic therapy may involve an additional agent that inhibits dysregulation of certain transcription factors by β -agonists. The therapeutic effect of glucocorticoids in asthma may indeed be in part due to this mechanism (3).

The role of transcription factors in immune cells has revealed a number of important pathways that initiate or maintain inflammation (1, 3, 11, 12, 16, 24). Here we show that these two resident structural cells, airway epithelial and smooth muscle, also undergo changes in expression of transcription factors in an inflammatory environment. These may provide the link, or the “amplification,” necessary for the pro-asthmatic signals that reach these cells to evoke pathologic sequelae. These would include the altered mucous content and production from epithelial cells, increased smooth muscle mass, hyperresponsiveness to contraction by smooth muscle, and resistance to β -agonist promoted relaxation. As such, transcription factor “agonists” and “antagonists,” inhibitors of protein kinases that phosphorylate transcription factors, and chromatin modifying agents have been proposed as therapeutic agents for asthma (20).

It is also intriguing to consider the effect of β -agonist in the context of the asthmatic culture conditions. In airway epithelial cells a number of transcription factors displayed increased as well as decreased expression/binding under these conditions. However, of the 43 factors that under went expression changes under the dual condition, only 11 of these displayed the same changes with β -agonist alone. In airway smooth muscle, about 3-times more transcription factors were upregulated under the dual conditions compared to β -agonist

exposure alone. And again, there was little overlap in the factors that underwent up-or downregulation between the two conditions. This clearly adds an additional layer of complexity in terms of new pathway and drug discovery, in that the advantageous or deleterious effects of β -agonists on transcription factor expression in these cells may be dependent on the severity and characteristics of the inflammation. Our findings with the transcription factors that are in common with the two cell-types also raise some intriguing issues. Of particular note is the heterogeneity of the directional changes by cell-type. There were multiple instances where a factor from one cell-type was up-or downregulated by a given condition with no change, or the opposite change, observed in the other cell-type. In fact, concomitant regulation in the same direction in both cell-types was rare. This further highlights the cell-type specificity of the regulation of transcription factors, and cautions against over-generalization about such processes when studying a single cell-type.

In summary, we have identified transcription factor expression/binding in human airway epithelial and smooth muscle cells under basal conditions, as well as three other conditions relevant to asthma. We found substantial differences in the complement of transcription factors expressed between the two cell-types. In addition, β -agonist exposure, a pro-asthmatic inflammatory milieu, and the combination of both conditions, evoked distinct regulatory events that differed by transcription factor, and the direction and magnitude of the change between the cell-types. The identification of these transcription factors, and their regulation, provides additional insight into potential adaptive and maladaptive events in asthma and may represent novel therapeutic targets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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GRANTS

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Fig. 1.

Confocal microscopy of airway epithelial and smooth muscle cell cultured cells used for transcription factor expression studies. Cells were prepared as described in Methods. Morphologic characteristics (panels *A, D*) and signals from cytokeratin-FITC (panels *C, F*) are consistent with cell-type, with airway epithelial cells (AEC) but not airway smooth muscle cells (ASM) expected to stain positive. The signals in panels *B, E* are from the fluorescent nuclear counterstain DAPI. Magnification = 20X.

Fig. 2. Confocal microscopy of airway epithelial and smooth muscle cell cultured cells used for transcription factor expression studies. Cells were prepared as described in Methods. Morphologic characteristics (panels *A, D*) and signals from vimentin-Cy3 (panels *C, F*) are consistent with cell-type, with smooth muscle, but not epithelial cells, expected to stain positive. The signals in panels *B, E* are from the fluorescent nuclear counterstain DAPI. Magnification = 20X.

Fig. 3. Global expression of transcription factors in airway epithelial and smooth muscle cells based on a query of >300 factors. Shown is the number of transcription factors detected by consensus oligonucleotide binding under any of the four culture conditions (see Methods). Factors expressed only in one cell-type are denoted as unique, and when detected in both as common.

Fig. 4. Conditional changes in transcription factor expression/binding in airway epithelial and smooth muscle cells. Cells were treated with the β -agonist isoproterenol, a pro-asthma cocktail (see Methods), or both for 24 hrs, and nuclear extracts prepared. The y-axis represents the number of transcription factors that changed by condition, with the number of upregulated factors in the black bars and the number of downregulated factors in the white bars.

	iso		asthma		iso+asthma			iso		asthma		iso+asthma	
	AEC	ASM	AEC	ASM	AEC	ASM	Factor	AEC	ASM	AEC	ASM	AEC	ASM
AP-2							L-III BP						
EGR							USF						
MEF-1							Sp1 (1)						
YY1							Sp1 (2)						
p53							NF-Y						
T3R							p300						
HSF							PARP						
ADR1							Pax-4a						
AhR							Pax-6						
NRF-1							Pax-8						
RAR-beta							CACCC-BF						
CEF2							CTCF						
COUP-TF							HNF						
c-Rel							HMG-1						
E47							HOXD8,9,10						
E4F, ATF							HOXD9,10						
Elk-1							LF-A1						
FOXO4							PEBP2alpha						
FOXF2							PPYR						
USF							WT1						
GATA-1							NF-4FA						
GATA1, 2							PU.1						
GATA-2							RPGalpha						
HFH-1							TEF-1						
FOXD3							TFEB						
FOXI1							Thy-1BP						
ISGF							XBP1						
TFE3-L							YB-1						

Fig. 5.

Regulation of transcription factors that are expressed in both airway epithelial and smooth muscle cells. Shown are the transcription factors where expression was common to both cell-types under at least one of the four conditions. The red bars indicated an increase and the blue bars a decrease in expression/binding by the indicated condition. The yellow bars represent the scenario where expression was detectable, but there was no change with the condition. White bars indicate that there was no detectable expression for the transcription factor under the given condition.

Table 1

Transcription factor expression in airway epithelial cells. Blanks indicate that no expression was detected. I, increased by >50%; D, decreased by >50%, compared to the indicated reference.

Trans. Factor	Basal	SD	Iso	SD	Asthma	SD	Iso+ asthma	SD	Iso Effect on Basal	Asthma Effect on Basal	Iso Effect on Asthma
AP2 (1)			14.65	1.49			25.26	4.46	I		I
NF-Y (2)	13.52	1.87			12.11	1.05			D		D
E2F-2	16.01	0.57	26.59	2.74	14.18	1.48			I		D
EGR	39.11	0.57	58.03	0.45	26.11	2.58	18.44	2.92	I		
MEF-1					13.94	0.69				I	D
NF-1 (1)					13.68	2.20				I	D
YY1 (1)			24.84	1.44	16.73	2.12			I	I	D
NF-E2 (1)	15.76	0.40	20.76	0.21	14.11	0.85					D
p53 (1)	29.71	1.52	20.38	2.09	17.88	0.63	24.56	2.04			
RAR	10.78	3.57			18.48	1.51			D	I	D
SRF					14.01	1.08				I	D
T3R (2)	14.47	1.88			13.74	0.64	15.34	2.60	D		
T3R (3)	13.80	1.58			14.44	0.14			D		D
USF1	14.88	2.12			19.41	1.58			D		D
VDR					11.88	0.04				I	D
HSF	10.38	5.84			14.03	0.00	11.92	1.86	D		
ADRI	19.56	7.58	13.86	0.37	13.49	0.00	11.40	6.99			
AbR (1)	82.92	12.98	83.35	6.05	74.56	3.89	77.11	4.26			
NRF-1 (2)	61.35	11.99	64.94	8.24	41.33	2.38	68.63	3.86			I
AP3 (1)	12.27	0.90	13.49	1.81	11.00	0.76					D
RAR-beta	22.62	1.80	22.69	2.35	22.30	0.98	33.27	4.76			
CEF2	33.01	5.03	30.10	1.94	21.89	3.08	15.92	2.29			
COUP-TF (3)					13.83	0.72				I	D
c-Rel			21.61	2.09	29.92	4.22	26.89	1.82	I	I	
E47	47.26	4.95	58.43	2.93	51.83	4.55	36.81	2.98			
E4F, ATF	10.23	0.56	17.52	2.76			15.29	0.86	I	D	I

Trans. Factor	Basal	SD	Iso	SD	Asthma	SD	Iso+ asthma	SD	Iso Effect on Basal	Asthma Effect on Basal	Iso Effect on Asthma
Elk-1			13.04	1.82			13.21	1.29	I		I
Evi-1			10.51	1.18			11.52	0.02	I		I
FOXO4							11.43	1.32			I
FOXF2 (1)			21.66	2.51	24.47	0.36	20.43	4.00	I	I	
USF (1)	30.74	0.86	22.90	1.05	32.55	8.00	32.45	2.19			
GATA-1 (1)	35.58	5.27	34.68	4.57	34.77	4.59	46.57	5.18			
GATA1, 2	68.26	11.76	68.33	2.96	55.11	5.74	58.03	2.51			
GATA-2	74.55	11.12	81.07	3.80	61.56	5.61	68.32	3.71			
HFH-1	13.06	0.86			12.93	0.00	18.91	3.41	D		
FOX D3	44.65	1.04	34.85	1.67	60.96	4.00	70.44	0.23			
FOXII	77.98	1.59	78.23	3.43	90.94	3.97	88.78	4.07			
ISGF (3)			23.85	1.82					I		
L-III-BP	32.07	1.13	24.11	4.24	22.58	3.03	34.75	0.35			I
USF (3)	52.40	10.13	34.64	6.21	33.44	4.33	34.15	4.89			
Sp1 (1)	41.43	2.31	30.46	2.62	44.77	3.34	60.62	4.79			
Sp1 (2)	51.16	5.57	55.31	10.63	70.10	1.83	72.87	3.91			
NF-Y (1)							14.53	1.87			I
p300			13.65	2.31					I		
PARP	27.27	1.75	60.90	0.55	25.57	2.99	59.23	7.54	I		I
Pax-4a	22.36	0.05	37.47	2.90	47.32	2.37	47.64	0.19	I	I	
Pax-6 (1)	18.72	2.04	20.74	4.96	13.28	0.62	38.96	0.49			I
Pax-8	48.87	3.07	31.10	2.29	33.33	0.00	31.97	1.80			
CACCC-BF	20.99	3.47	22.00	2.70	23.39	4.10	23.89	0.30			
CTCF	12.96	0.31			11.22	0.40			D		D
HNF	21.13	3.95	17.70	3.47	16.55	2.07	42.32	4.65			I
HMG-1	18.66	3.04	18.73	1.70	10.28	0.00	33.56	0.06			I
HOXD8,9,10	12.74	0.55	12.27	1.75			16.87	2.07		D	I
HOXD9,10	13.40	1.56			10.32	0.85			D		D
LF-A1 (1)							14.02	5.20			I

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Trans. Factor	Basal	SD	Iso	SD	Asthma	SD	Iso+ asthma	SD	Iso Effect on Basal	Asthma Effect on Basal	Iso Effect on Asthma
NF-4FA	54.90	5.28	45.18	6.32	44.40	2.64	55.89	5.81			
PEBP2alpha (2)			11.85	5.91			11.70	5.95	I		I
PPYR	56.58	4.47	57.91	3.93	73.32	0.00	90.07	1.60			
WT1 (2)	34.45	3.50	39.27	4.84	43.03	3.38	44.62	6.48			
EGR1			11.97	2.38					I		
NF-A3			13.05	1.80	13.28	1.35	11.27	2.96	I	I	
NF-E2 (2)					12.37	2.74				I	D
PU.1	16.58	7.97	12.71	4.41	10.64	0.79	17.20	2.26			I
RPGalpha	50.64	6.83	42.72	6.72	36.10	2.72	49.57	5.42			
TEF-1	85.31	2.61	71.73	12.26	80.42	0.00	84.88	1.45			
TFE3-L	11.03	0.76					10.67	1.94	D	D	I
TFEB	15.67	1.65	10.36	0.96	11.69	0.81	13.02	0.88			
Thy-1-BP	23.14	2.11	14.50	1.17	18.68	0.00	22.46	2.48			
XBP1 (3)	15.05	11.71	12.23	1.85			17.56	2.75		D	I
YB1-1	22.44	2.24	20.44	2.48	18.23	0.00	22.16	0.91			

Table 2

Transcription factor expression in airway smooth muscle cells. Blanks indicate that no expression was detected. I, increased by >50%; D, decreased by >50%, compared to the indicated reference.

Trans. Factor	Basal	SD	Iso	SD	Asthma	SD	Iso+ Asthma	SD	Effect on Basal	Asthma Effect on Basal	Iso Effect on Asthma
AP2 (1)	36.36	1.33	15.95	1.06	11.76	0.91	11.49	1.82	D	D	
EGR	63.24	1.78	59.95	9.26	72.88	5.35	67.86	2.04			
GATA	12.09	0.83	19.45	0.78			28.60	2.12	I	D	I
MEF-1					11.01	1.45				I	D
YY1 (1)	26.65	0.35	12.05	0.78	26.15	2.76			D		D
PPAR	28.02	0.45	35.50	4.67	24.47	2.88	13.32	0.68			
Sp1 (2)	28.22	1.39	25.25	4.88	39.62	1.39	21.48	2.72			
TBR (2)	30.24	2.50	12.45	0.07	25.88	3.36	14.40	2.69	D		
HSF	14.43	1.45	16.50	2.26						D	
ADRI	38.48	2.37	41.20	2.40	43.20	3.12	29.97	1.80			
AHR (1)	78.85	6.86	63.80	7.64	87.51	5.23	67.84	4.05			
NRF-1 (2)	48.27	7.16	32.85	3.46	41.71	3.97	39.75	3.05			
RAR-beta	13.64	1.65	12.50	0.14						D	
CEF2	31.14	4.44	29.25	3.18	25.45	3.61	30.23	2.79			
COUP-TF (3)	14.73	2.73	22.00	0.57			16.58	0.18	I	D	I
c-Rel	62.18	5.77	58.60	7.07	60.90	7.08	66.83	4.77			
E47	67.01	12.88	64.75	5.87	68.37	2.60	71.75	12.80			
E4F, ATF	11.53	1.17			11.27	0.81			D		D
Elk-1	17.77	2.22			13.57	1.65	10.58	2.29	D		
FOXO4	21.53	4.07	21.70	1.56	19.31	1.43	19.27	1.46			
FOXF2 (2)	42.28	2.51	39.85	2.19	29.50	0.28	45.16	11.24			I
FOXDI	34.48	5.62	37.60	7.21	30.76	3.17	37.96	3.32			
USF (1)	74.63	9.51	81.35	2.76	71.70	9.90	81.32	10.73			
GATA-1 (1)	76.26	14.65	70.85	5.59	77.20	6.23	67.96	14.06			
GATA1, 2	73.38	13.12	85.90	0.71	93.05	2.62	84.41	4.38			
GATA-2	71.04	2.60	75.25	4.45	81.99	5.11	75.68	10.36			

Trans. Factor	Basal	SD	Iso	SD	Asthma	SD	Iso+ Asthma	SD	Effect on Basal	Asthma Effect on Basal	Iso Effect on Asthma
GATA-3	12.97	1.79			16.67	0.52	26.43	1.94	D		I
GATA-6	12.88	2.23					17.14	4.33	D	D	I
HFH-1	51.31	8.63	43.35	5.30	41.09	2.24	43.45	0.07			
FOXO3	82.84	4.86	80.35	3.18	90.47	7.82	86.06	4.02			
FOXO1	88.08	7.61	97.00	3.11	97.16	4.02	91.89	7.09			
HIF-1	32.32	2.15	35.00	4.24	19.34	2.35	33.92	1.81			I
HNF-4 (2)	12.73	2.86							D	D	
ISGF (3)	62.10	6.22	62.90	5.09	59.81	1.14	69.40	5.94			
L-III-BP	46.73	6.68	54.35	0.92	44.07	4.20	64.68	6.26			
KKLF-15	11.57	1.36	15.75	3.04	12.41	2.42	19.02	2.23			I
USF	49.47	7.68	45.20	5.94	55.61	4.23	70.57	3.44			
Sp1 (3)	56.47	7.03	80.30	0.99	68.55	2.19	79.10	11.04			
Sp1 (4)	73.47	5.32	86.25	6.43	74.37	5.98	83.90	8.35			
MZF1 (1)			14.05	1.63			13.63	0.66	I		I
NF-Y (1)							13.24	0.80			I
p300	21.01	1.56	25.30	3.39	21.72	1.24	20.25	0.78			
PARP	70.39	1.39	55.25	7.28	53.90	6.50	57.34	3.91			
Pax-2							12.07	1.18			I
Pax-4a	35.08	1.10	22.25	1.63	28.56	2.48	23.72	1.95			
Pax-6 (2)	15.43	2.64					16.98	1.09	D	D	I
Pax-8	29.17	1.89	15.70	0.57	37.78	2.93					D
RSRFC4	25.84	3.31	21.20	2.69	27.65	5.73	30.39	0.58			
USF (2)	23.62	4.55	18.45	3.46	24.32	2.95	29.92	1.68			
SREBP-1c	10.98	1.10	17.20	2.12	11.02	0.45			I		D
AFP1	29.97	3.30	26.10	0.71	24.15	2.33	28.70	4.11			
COUP-TF2 (2)	30.32	1.67	26.95	1.77	35.73	4.00	43.17	5.70			
AML1			13.35	2.47			11.87	2.31	I		I
C/ACCC-BF (1)	11.47	1.93	16.95	2.90	10.15	0.93	17.44	1.36	I		I
CCAAT-BF	11.66	2.06	13.50	1.98			21.59	3.84		D	I

Trans. Factor	Basal	SD	Iso	SD	Asthma	SD	Iso+ Asthma	SD	Effect on Basal	Asthma Effect on Basal	Iso Effect on Asthma
CACCC-BF (2)	41.77	5.04	44.55	5.02	54.16	6.72	66.54	6.03			
CD28RC (1)			14.80	1.84					I		
Cdx2 (1)	25.43	1.17	27.95	6.43	27.48	3.08	26.47	1.37			
CREB2	11.27	2.16	11.43	2.93			13.80	0.43		D	I
CTCF	58.03	1.66	64.20	1.41	70.47	1.04	72.42	4.64			
E12	51.94	1.22	60.20	1.41	53.27	5.70	67.63	0.74			
EBP40-45	43.51	4.53	56.90	3.82	51.86	1.48	76.60	0.70			
EKLF (1)	18.84	3.73	21.00	2.12	18.94	1.36	24.88	2.23			
EKLF (2)	29.80	0.56	50.50	6.65	32.63	3.57	37.00	6.50	I		
HiNF	54.21	4.94	60.95	4.60	60.53	3.49	53.40	12.45			
HMG	67.71	6.80	73.35	3.46	52.09	7.05	68.16	5.86			
HNF-1alpha-A	25.90	0.29			17.73	2.31	15.16	2.33	D		
HOXD9							14.56	3.17			I
HOXD8,9,10	72.83	5.06	67.50	9.05	71.66	4.32	70.48	2.58			
HOXD9,10	37.97	0.33	47.05	1.77	32.88	4.13	48.88	0.11			
IRF-8			13.15	1.34					I		
LF-A1 (1)	26.49	1.53	34.60	3.54	30.47	2.88	33.48	1.59			
LR1	15.72	0.54	20.35	0.21	13.44	0.93	23.36	3.03			I
Ikaros (2)	18.70	1.70	18.20	1.84	15.14	0.91	18.31	3.97			
Ikaros (4)	59.92	11.77	44.35	2.90	47.40	2.54	56.26	3.17			
MAZ	63.21	2.11	56.80	5.09	52.92	3.71	67.97	0.61			
MTB-Zf	12.24	0.93	12.27	4.57			16.17	3.44		D	I
MTF-1			13.70	0.71					I		
MZF1 (2)			13.45	0.35					I		
MZF1 (3)	24.13	0.61	28.45	3.75	17.84	0.79	35.41	0.86			I
NF-4FA	68.09	7.76	63.50	5.66	64.07	0.89	74.25	6.15			
NF-IL-2A			10.33	0.81			13.19	4.54	I		I
p53 (2)	14.27	0.24	13.25	2.19	10.23	2.69	22.74	5.71			I
PEBP2alpha (2)	50.10	5.66	34.05	2.19	37.82	3.42	30.39	1.15			

Trans. Factor	Basal	SD	Iso	SD	Asthma	SD	Iso+ Asthma	SD	Effect on Basal	Asthma Effect on Basal	Iso Effect on Asthma
PPYR	71.87	6.55	86.23	3.08	86.88	4.00	73.48	7.25			
RFX1,2,3 (1)			11.95	2.47					I		
NeuroD1			11.20	1.13					I		
Sp1 (1)	17.81	1.42	25.65	2.90	18.95	1.91	27.44	2.03			
TGIF	14.39	1.25					18.34	2.18	D	D	I
TFE3							15.56	1.62			I
TF-LF (1)	15.65	0.07	14.35	1.77	11.64	1.61	19.42	1.39			I
WT1 (1)	34.51	4.37	70.05	11.95	44.07	8.81	66.89	5.11	I		I
WT1 (2)	63.19	4.93	81.25	12.37	66.46	8.97	74.86	3.03			
WT1 (3)			14.40	0.14			13.27	1.32	I		I
Ahr (2)			13.95	0.21	13.91	0.30	12.82	2.15	I	I	
EGR1	13.99	2.83					14.92	2.43	D	D	I
FOXMIa, b (1)			14.35	0.07	12.35	3.04			I	I	D
FOXMIa, b (2)					14.55	0.21	13.92	0.82		I	
KBF-A			11.42	2.94	12.59	0.72	12.55	2.62	I	I	
v-Myb (2)	10.72	1.82	12.95	2.33	14.47	2.73	13.31	0.56			
HOXA5					12.52	0.59				I	D
NF-AT1	25.96	2.18			25.33	1.94	26.78	2.15	D		
Pax-6 (1)	24.72	2.29	18.98	2.23	16.9	1.84	11.72	0.59			
YY1 (2)							11.99	1.57			I
CTF							12.15	1.34			I
PRDI-BFc	11.40	1.28							D	D	
PTF1-beta							13.75	0.63			I
PU.1	25.25	0.07	15.30	2.40	40.65	4.04	14.63	2.87		I	D
Pur							13.91	0.58			I
RVF	18.03	2.37	18.05	2.76	24.29	2.81	21.44	4.02			
Spm-1					12.02	0.25				I	D
STAT1,3	11.03	2.08			10.85	0.06			D		D
RPGalpha	35.82	5.82	31.95	0.35	45.31	6.07	46.28	1.52			

Trans. Factor	Basal	SD	Iso	SD	Asthma	SD	Iso+ Asthma	SD	Effect on Basal	Asthma Effect on Basal	Iso Effect on Asthma
T3R (1)							14.69	0.26			I
TEF-1	93.73	4.43	96.45	3.32	95.57	1.03	93.50	3.97			
TFE3-L	72.72	1.53	65.80	7.92	85.63	2.51	74.88	5.27			
TFEB	19.82	0.68	20.05	2.76	19.81	0.42	26.79	2.71			
Thy-1-BP	46.66	5.15	40.15	2.76	43.66	0.64	50.88	0.18			
Tf-LF (2)	38.10	5.52	32.65	2.76	42.12	4.22	34.53	4.56			
TxREF	24.37	1.09	22.65	2.33	24.01	1.82	27.92	0.88			
XBp1 (3)	23.00	3.12	18.85	0.78	26.88	0.11	36.23	2.22			
YB-1	13.15	1.49			12.55	0.77	10.45	2.20	D		

Table 3

Common and uniquely expressed transcription factors in airway epithelial and smooth muscle cells.

Common		AEC-unique	ASM-unique	
AP2	L-III-BP	Evi-1	GATA	NF-AT1
EGR	USF	E2F-2	FOXD1	HOXA5
MEF-1	Sp1	NF-1	GATA-3	Pax-2
YY1	RPGalpha	NF-E2	GATA-6	NF-IL-2A
p53	NF-Y	RAR	HIF-1	LR1
T3R	p300	SRF	HNF-4	RFX1,2,3
HSF	PARP	NF-A3	KKLF-15	NeuroD1
ADR1	Pax-4a	USF1	MZF1	Ikaros
AhR	Pax-8	VDR	RSRFC4	TGIF
NRF-1	YB-1	AP3	MTB-Zf	TFE3
RAR-beta	CACCC-BF		SREBP-1c	Tf-LF
CEF2	CTCF		AFP1	MAZ
COUP-TF	HINF		COUP-TF2	IRF-8
c-Rel	HMG-1		AML1	TxREF
E47	HOXD8,9,10		Sprm-1	PPAR
E4F, ATF	HOXD9,10		CCAAT-BF	FOXM1a,b
Elk-1	LF-A1		CD28RC	kBF-A
FOXO4	NF-4FA		Cdx2	v-Myb
TFE3-L	PEBP2alpha		CREB2	RVF
USF	PPYR		E12	STAT1,3
GATA-1	WT1		EBP40-45	CTF
GATA1, 2	PU.1		EKLF	PRDI-BFc
GATA-2	TEF-1		MTF-1	PTF1-beta
HFH-1	TFEB		HNF-1alpha-A	Pur
FOXD3	Thy-1-BP		HOXD9	
FOX11	XBP1			
ISGF	FOXF2			
Pax-6				