Identification of proteomic changes in primary human bronchial epithelial cells in response to chronic formoterol treatment

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Introduction: Bronchial asthma is characterised by bronchoconstriction, airway hyper-responsiveness and elevated mucus secretion. Pathophysiological changes observed in asthma involve altered expression of inflammatory mediators and reflect genetic heterogeneity and environmental influences (1). Current therapies include long-acting β_2-adrenoceptor (β_2-AR) agonists (LABAs), which exploit the bronchodilatory action of β_2-ARs on airway smooth muscle cells (2). Short-term use of these treatments causes beneficial airway relaxation, but chronic use exacerbates asthma symptoms (2-3). Identification of proteomic changes in response to chronic LABA treatment could therefore provide insight into the molecular mechanisms involved in LABA-induced exacerbations and might allow the design of more effective asthma therapies.

Method: Primary human bronchial epithelial cells (HBEC) were subjected to the long-acting β_2-AR agonist formoterol (100 nM) for 48h. Cells were lysed and subjected to SWATH-MS (label free quantification by data independent acquisition; 4) to identify differentially expressed proteins. The identified proteins were processed for network analysis using MetaCore^{TM} pathway analysis suite and the formoterol-mediated changes in expression of proteins present in the network were confirmed by western blotting. Furthermore, to investigate changes in protein expression following more chronic β_2-AR stimulation, HBECs were subjected to formoterol (100 nM) for 7 days and protein expression evaluated by western blotting. One-way ANOVA followed by Dunnett's post-hoc test was employed to assess statistical significance.

Results: SWATH-MS analysis identified 9 differentially expressed proteins in response to formoterol treatment in HBECs. Network analysis revealed that the top ranked network included calreticulin, peroxiredoxin-1 and macrophage migration inhibitory factor (MIF). Expression of these three proteins under control and formoterol-stimulated conditions at 48 h and 7 days were confirmed using western blotting (Table 1).

Table 1: Calreticulin, peroxiredoxin-1 and MIF expression in HBECs under control and formoterol (100 nM; 48h and 7 day)-stimulated conditions. *p<0.05 and **p<0.01 versus control cells. Data represent means ± S.E.M. obtained from three independent experiments.

	48 h		7 days	
	Control	Formoterol	Control	Formoterol
Calreticulin	65.2 ± 7.7	149.4 ** ± 15.1	129.8 ± 22.1	118.5 ± 12.8
Peroxiredoxin-1	55.6 ± 8.0	95.3 * ± 9.5	95.2 ± 3.9	90.9 ± 9.7
MIF	68.1 ± 3.0	22.0 ** ± 4.3	46.4 ± 8.8	88.9 * ± 12.3

Conclusion: Calreticulin and Peroxiredoxin-1 are both up-regulated in HBECs following 48h exposure to formoterol, but return to control levels within 7 days. In contrast, MIF is down-regulated at 48h, but substantially up-regulated upon more chronic exposure to formoterol. These changes in MIF levels could contribute to asthma exacerbations observed upon chronic use of formoterol.

References:

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