Quantitative phosphoproteomic analysis defines distinct cAMP signaling networks emanating from AC2 and AC6 in human airway smooth muscle cells

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Human airway smooth muscle (HASM) is the primary target of ssAR agonists used to control airway hypercontractility in asthma and chronic obstructive pulmonary disease (COPD). ss2AR agonism induces the production of cyclic adenosine monophosphate (cAMP) by adenylyl cyclases (ACs), which activates PKA and leads a myriad of cellular effects including bronchodilation. Several other GPCRs expressed in HASM cells transduce extracellular signals through cAMP but these receptors elicit different cellular responses, suggesting cAMP signaling is compartmentized. Previous work has shown these GPCR can couple to specific ACs that are localized in distinct membrane microdomains, partly explaining this compartmentation. However, little is known about the downstream steps of cAMP compartmentation. We developed a proteomic approach to define the downstream signaling networks emanating from specific AC isoforms in HASM. Stable Isotope Labeling of Amino acids in Cell culture (SILAC) was used to identify and quantify phosphorylated proteins in HASM overexpressing AC2 or AC6 after a short stimulus of AC activity by forskolin. Phosphopeptides were analyzed by LC-MS/MS and the quantitative difference between light and heavy labeled SILAC pairs represented the change in abundance of that peptide caused by forskolin treatment. Forskolin-stimulated cAMP production in control (lacZ) HASM displayed a logEC50 of -8.14 ± 0.04 and an Emax of 0.328 ± 0.010. In AC2-overexpressing cells, forskolin potency as efficacy was moderately increased (logEC50 of -8.79 ± 0.02 and an Emax of 0.498 ± 0.007). AC6 overexpression led to similar increases in forskolin responses (logEC50 of -8.67 ± 0.12 and an Emax of 0.509 ± 0.030). Using a subtractive analysis to identify effects of just the overexpressed AC isoform (over that natively expressed in control HASM), we found 14 differentially phosphorylated proteins (DPPs) linked to AC2 signaling and 34 DPPs linked to AC6 signaling. AC2 and AC6 groups showed in common 4 up- and 3 down-regulated phosphorylated proteins (using fold change ≥ 1.5 or ≤ 1/1.5 of the light/heavy ratio with p-values < 0.05, n=3). The Gene Set Enrichment Analysis (GSEA) was used to identify statistically significant pathways with a p-value < 0.05 reflected with enrichment scores. We identified 11 and 78 significant pathways using AC2 and AC6 differentially regulated proteins. Analysis of the phosphorylated peptides with the STRING protein interaction tool showed that AC2 signaling is more associated with modifications in RNA/DNA binding proteins and microtubule/spindle body proteins while AC6 signaling is associated with proteins regulating autophagy, calcium-calmodulin signaling, Rho GTPases and cytoskeletal regulation. One protein, OFD1, was regulated in opposite directions, with serine 899 phosphorylation increased in the AC6 condition 1.5-fold while in the AC2 condition it decreased to 0.46-fold. In conclusion, quantitative phosphoproteomics is a powerful tool for deciphering the complex signaling networks resulting from discreet signaling events that occur in cAMP compartments. Our data show key differences in the cAMP signaling networks generated by AC2 and AC6 and imply that distinct cellular responses are regulated by these two compartments.

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