Airway epithelial-to-mesenchymal transition: Compartmentalized cyclic AMP

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Epithelial-to-mesenchymal transition EMT is characterized by a reduction of the epithelial cell marker E-cadherin and gain of the mesenchymal marker collagen. EMT plays a critical role in pulmonary fibrosis¹. The phosphodiesterase PDE4 inhibitor rolipram has been shown to inhibit transforming growth factor beta 1 TGF-beta1 mediated EMT in A549 cells implicating that cAMP is able to attenuate EMT-associated lung diseases². Signaling by cAMP encompasses compartmentalization via A-kinase anchoring proteins AKAPs. The role of AKAPs and its therapeutic value in EMT is still barely understood.

To induce EMT, lung bronchus epithelial BEAS-2B cells were exposed to TGF-beta1 3ng/ml for 24 hours. Gene expression of a subset of AKAPs (see below) was examined by qPCR. To raise intracellular cAMP or to disrupt AKAP-PKA complexes BEAS-2B cells were pretreated with the beta2-agonist fenoterol the PDE4 inhibitor rolipram the PDE3 inhibitor cilostamide the adenylyl cyclase activator forskolin and st-Ht31 for 30 minutes prior to TGF-beta1. To study the impact of selected AKAPs on EMT induced TGF-beta1 expression of Ezrin AKAP95 Yotiao and AKAP79 was reduced by RNA silencing. Gene (protein) expression of the EMT markers E-cadherin and collagen were analyzed by qPCR and western blot.

In BEAS-2B cells TGF-beta1 changed cell morphology down-regulated E-cadherin and upregulated collagen. The gene expression of AKAP9 AKAP11 and AKAP5 were altered. Fenoterol tended to diminish collagen upregulation by TGF-beta1. Rolipram cilostamide and forskolin diminished both E-cadherin reduction and collagen upregulation by TGF-beta1 pointing to a role of cAMP compartmentalization in EMT. Indeed st-Ht31 alone led to a strong mRNA and protein E-cadherin reduction, but further augmented E-cadherin reduction and largely prevented collagen protein upregulation by TGF-beta1. Silencing of Ezrin AKAP95 Yotiao and AKAP79 primarily diminished collagen upregulation by TGF-beta1.

Our data reveal that AKAP family members regulate EMT by TGF-beta1.