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Research: Small Interfering RNAs Targeting Airway Chemokines - (Regular Score Project)

Hereditary and environmental factors are involved in the onset of asthma, a chronic inflammatory disorder of the airways. Prevalence of these disorders has increased in developed and underdeveloped countries in the past two decades. In the general sequence of disease events, the inciting trigger activates cells that release signals to Th2 lymphocytes and eosinophils. Chemokine release results in recruitment and infiltration of activated leukocytes that perpetuate inflammation. Airway epithelial cells, an important source of cytokines and the eotaxin family of chemokines (CCL11, CCL24 and CCL26), may play pivotal roles in the continuation of airway inflammation. Thus, an alternative view of asthma, which encompasses both allergic/immunologic and nonimmunologic has been developed and formulated into the following experimental paradigm: The primary pathology in asthma, both immunologic and non-immunologic forms, is an aberrant response of the airway epithelium which leads to cyclic target cell/effector cell proinflammatory responses via chemokine receptor-ligand interactions. The experimental hypothesis is as follows: The airway epithelium CCR3 receptor for the eotaxins CCL11, CCL24 and CCL26 may be an important target for development of novel siRNA-based adjunctive therapies designed to interrupt the underlying chronic inflammation in allergic and inflammatory disorders. The experimental hypothesis will be addressed by the following specific aims: specific aim 1 - establish CCR3 targeted short interfering RNA methods for alveolar and bronchial airway epithelial cell lines; specific aim 2 assess CCR3 gene silencing by siRNA; and specific aim 3 - analyze the siRNA induced CCR3 loss-of function phenotype on CCL11, CCL24 and CCL26 synthesis, storage and release in bronchial and alveolar airway epithelial cells. Experimental models will consist of A549 alveolar and 16HBE14₀- bronchial human epithelial cells followed by proof-of-concept demonstrations in NHBE (normal human bronchial epithelial cells) and SAEC (normal small airway epithelial cells). Immunocytochemistry, western blotting, ELISAs, flow cytometry, RT-PCR and real-time RT-PCR assays will be used to assess transfection efficiency, duration and loss-of-function biological effects. The Global Initiative for Asthma member scientists recently indicated the need for increased research to understand molecular and cellular events, which initiate, direct and perpetuate airway inflammation to identify new treatment targets and develop new therapies. Successful completion of these investigations may assist in this important quest and support the development of interfering RNAs for development of gene-specific therapeutics.

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