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is to examine the role of the Ras signal transduction pathway in the pathogenesis of chronic obstructive pulmonary disease (COPD). COPD is a leading cause of morbidity and mortality in the US. It is important to develop effective therapies for COPD. The Ras pathway is a critical component of signal transduction that regulates cell proliferation, cell differentiation and cell survival. In pilot studies we have demonstrated that Ras activation is increased in lung tissues from COPD patients and in isolated bronchial fibroblasts from COPD patients as compared to controls. We hypothesize that this increased activation of Ras plays an important role in the pathogenesis of COPD through the regulation of apoptotic and growth/survival pathways. Activation of Ras by growth factors and the transcription factor E2F is mediated by the Ras guanyl nucleotide-releasing factor C3G. C3G stimulates Ras through its Ras-GRF and Rho GDI-RhoGDI activities. Ras-GRF activates Ras via its Ras-GTPase-activating protein (GAP) activity, and RhoGDI-RhoGDI acts as an inhibitor of Ras activity through its GDI activity. C3G also promotes activation of the transcription factor E2F through E2F-DP and through its ability to stimulate the kinase activities of E2F and DP. E2F activates transcription of genes by binding to E2F-responsive sites (E2F sites) in promoter regions. In addition, E2F activates the expression of other transcription factors, including c-fos and c-jun, and it regulates cell cycle progression from G1 to S phase. We will examine the hypothesis that the Ras pathway promotes the proliferation and survival of lung fibroblasts through E2F and c-fos-dependent expression of growth and survival factors and through E2F- dependent expression of genes that regulate apoptosis. We will test this hypothesis by: 1) characterizing the Ras signaling pathways and their regulation of apoptotic and growth/survival genes in primary cultures of normal and COPD lung fibroblasts; 2) examining the regulation of Ras signaling and Ras-mediated gene expression in COPD fibroblasts by over-expression and knockdown of E2F activity; 3) evaluating the relationship between Ras activation, E2F transcriptional activation and fibroblast proliferation and survival in COPD; and 4) determining the role of endogenous E2F in COPD by inhibiting DP/E2F transcriptional activation by knockdown of DP and/or E2F and over-expression of dominant negative E2F mutants. We will examine the role of Ras in COPD pathogenesis using cell culture and animal models of the disease. We will also evaluate the role of potential pharmacologic agents that inhibit C3G, Ras-GAP or RhoGDI-RhoGDI activity in COPD using primary cultures of fibroblasts and cell cultures from animal models of the disease. These studies will provide new information about the regulation of apoptosis and cell growth/survival in normal lung and in COPD. 5ec8ef588b

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