CCI-15

Cellular dynamics and activation signature of fibroblasts in bleomycin-induced lung fibrosis

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Abstract

Fibrotic diseases are characterized by an excessive deposition of extracellular matrix (ECM) produced by activated fibroblasts in fibrotic lesions. Previous studies revealed increased in vitro proliferative capacity and ECM production of activated fibroblasts isolated from fibrotic organs. However, technical limitations of conventional histological approaches and lack of fibroblast-specific surface markers have made it difficult to reveal precise activation mechanisms of fibroblasts during fibrosis in vivo. In order to develop a specific anti-fibrotic therapy, the property of fibroblasts in vivo should be more investigated. Here we elucidated the changes in the number and activation status of collagen I-producing fibroblasts by using flow cytometry in a bleomycin-induced lung fibrosis model. Collagen I-producing fibroblasts were isolated from transgenic mice harboring enhancer/promoter sequences of α2(I) collagen gene linked to EGFP. Flow cytometric analysis of enzymatically-dissociated lung tissue cells showed the number of fibroblasts did not increase even at the peak of fibrosis. Freshly isolated fibroblasts from bleomycin-treated mice had activated phenotype with the increase of collagen I expression and intracellular organelle complexity. These fibroblasts up-regulated expression of α-smooth muscle actin at day 7 and 14 but not 21 after bleomycin treatment. Both proliferation and apoptosis of fibroblasts slightly increased at the height of inflammation. We also analyzed gene expression profiles of normal and activated fibroblasts by second generation DNA sequencing, identifying a variety of important signatures of activated fibroblasts including novel activation markers. These findings suggest that qualitative change, rather than quantitative change, is a hallmark of activated fibroblasts in bleomycin-induced lung fibrosis.