Endothelial-derived Endothelin-1 Inhibits Lymphangiogenesis and Promotes Fibrosis in Mice Model of Pulmonary Fibrosis

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Background: Abnormal lymphangiogenesis is observed in human pulmonary fibrosis, suggesting the presence of soluble factors and cells as substrates for lymphatic development in these microenvironment. Lymphatic vessel area is higher in fibrosis which emphasize that fibrosis is a millieu of lymphangiogenesis. Current concepts of lymphangiogenesis in pulmonary fibrosis implicate hyaluronan deposition and active macrophage as substrates for lymphangiogenesis. The interplay between fibrosis and lymphangiogenesis may involve endothelin-1 (ET-1), since ET-1 is a profibrotic peptide and has a capacity to promote lymphangiogenesis in malignancy. Using mice model of pulmonary, we hypothesized that ET-1 has role in both fibrosis and lymphangiogenesis.
Methods: To produce pulmonary fibrotic lesion, bleomycin was given into the lung of ET-1$^{flox/flox}$;Tie2-Cre (+) mice, which has conditional deletion of ET-1 gene in endothelial cells, and their wild-types. The fibrotic lesion, fibrotic markers and fibroblast accumulation were assessed four weeks later. The lymphangiogenesis was assessed by calculating lymphatic density inside and at surrounding area of fibrotic lesion. The level of VEGFc protein and number of active macrophages were evaluated.
Results: ET-1$^{flox/flox}$;Tie2-Cre (+) mice have less fibrotic lesion than their wild-types. In accordance, quantitative assessment showed lower number of fibroblast-specific protein-1 (FSP-1) expressing cells and TGF-β level in ET-1$^{flox/flox}$;Tie2-Cre (+) mice than their wild-types. In the contrary, lymphatic density was increased in ET-1$^{flox/flox}$;Tie2-Cre (+) mice both inside fibrotic lesion and at surrounding area. The VEGFc level did not differ between mice. Active macrophage (MAC3(+) cells) were increased inside fibrotic lesion of ET-1$^{flox/flox}$;Tie2-Cre (+) mice. In this lesion, we find evidence of MAC3(+) cell transdifferentiation into lymphatic endothelial cells in ET-1$^{flox/flox}$;Tie2-Cre (+).
Conclusion: Endothelial-derived endothelin-1 inhibits lymphangiogenesis and promotes profibrotic effect in bleomycin-induced pulmonary fibrosis.