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LR8 expression in fibroblasts of healthy and fibrotic human tissues

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ABSTRACT

LR8 gene was first reported in a subpopulation of cultured human lung fibroblasts expressing the receptor for C1q-globular domain, and it was not detectable in cultured endothelial cells and smooth muscle cells. LR8 mRNA levels were higher in fibrotic lungs. In this study we assessed LR8 production in human tissues and determined if the distribution of fibroblasts producing LR8 is affected in fibrosis. Normal and fibrotic tissue sections from human liver, lung and kidneys were immunostained with antibodies to LR8 and examined for the presence of fibroblasts staining positively and negatively. The cells were also examined for co-expression of α-smooth muscle actin (SMA), a marker for myofibroblasts. The results showed that LR8 was expressed by fibroblasts, smooth muscle cells, endothelial cells, bile duct cells, pulmonary alveolar cells and distal and proximal kidney tubule cells. Connective tissues of normal and fibrotic tissues contained fibroblasts staining positively and negatively with anti- LR8 antibody. The number of LR8-positive cells was higher in fibrotic tissues, but differences were not statistically significant. Fibroblasts producing both LR8 and SMA were present in higher numbers in fibrotic tissues as compared to normal tissues and the differences were statistically significant ($p < 0.05$). Our results show that fibroblast subtypes differing in LR8 expression are present in human tissues, and that in fibrotic tissues cells co-expressing LR8 and SMA are present. Our results indicate that LR8 expressing cells may participate in the early stages of fibrotic diseases and that fibroblasts expressing LR8, not LR8 negative cells, have potential to become myofibroblasts in fibrotic tissues.

1. Introduction

Fibrosis is a pathological phenomenon in which excessive deposition of collagen and other extra cellular matrix (ECM) components leads to loss of normal tissue architecture and function. Fibrosis is believed to be due to dysregulated wound healing response to chronic and progressive tissue injury, and inflammation is believed to play a significant role in many types of fibrosis. The degree of inflammation and repair varies depending on the etiology, and host and tissue responses. Injury activates inflammation and in most cases ongoing chronic inflammation is the major cause for the progression of fibrosis. Patients respond poorly to anti-inflammatory therapies because there is little or no inflammation in advanced stages of fibrosis. In certain types of fibrosis, intrinsic defects in the wound healing can also lead to chronic fibrosis. Fibroblasts are the major cell type responsible for the synthesis of ECM components in normal and fibrotic connective tissues. Fibroblasts from normal and diseased tissues and cells from different anatomic locations have been shown to manifest phenotypic differences, and fibroblast subpopulations have been separated based on differences in the expression of thymocyte 1 antigen (Thy 1) and receptors for the collagen- and globular-domains of C1q [1–8]. The fibroblasts expressing receptors for C1q-globular domain have the phenotype expected of cells participating in inflammation and wound healing [7].

A gene product, LR8 (accession no. AF115384), has been identified in human lung fibroblast subpopulation with the receptor for C1q-globular domain [9]. This product is either not detectable or only minimally expressed in other fibroblasts, and it was not detected in cultured endothelial cells, epithelial cells or alveolar macrophage. The LR8/TMEM176B gene is mapped to chromosome 7q32 in humans and it is located on chromosome 6 in the mouse genome [10,11]. Human LR8 gene is about 12 kb long and contains a 772 base pair long open reading frame. The LR8 protein belongs to the CD-20 superfamily (NCBI Conserved Domain Database), and it appears to be involved in the control of dendritic cell maturation, differentiation of myoblasts into an osteoblast lineage and regulation of immune cells [10,12,13].

LR8 expression is upregulated in human lungs with idiopathic pulmonary fibrosis and bleomycin-induced fibrotic mouse lungs [9].

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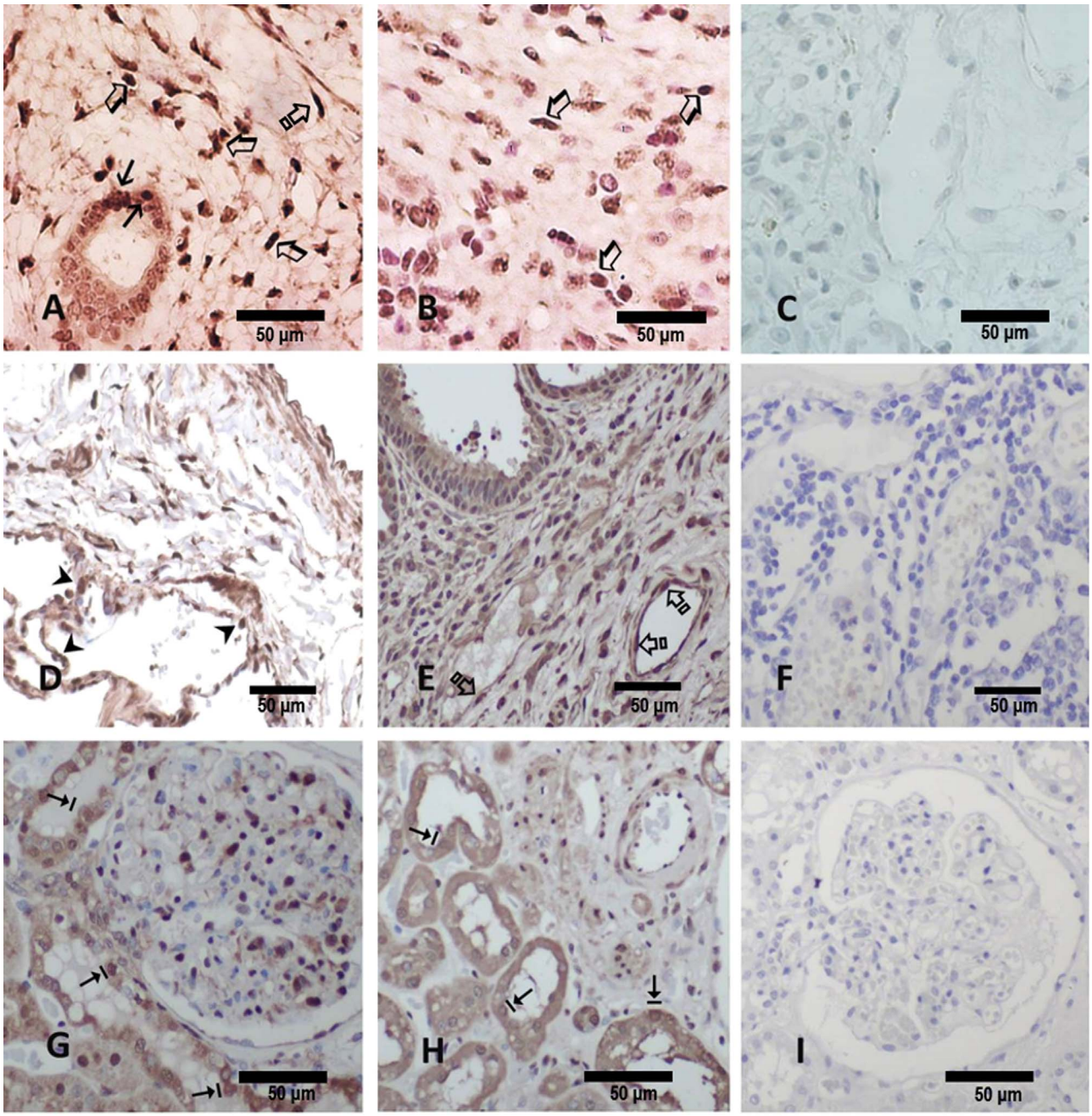

Fig. 1. Photomicrographs of human tissue sections immunostained with anti-LR8 antibody. A. Normal liver B. Fibrotic liver C. Control, preimmune serum D. Normal lung E. Fibrotic lung F. Control, preimmune serum G. Normal kidney F. Fibrotic kidney I. Control, preimmune serum. Cells staining positively are shown. ⇦- Hepatocytes; ←- Bile duct cells; ◀-Alveolar cells; ⇪- Endothelial cells; ⭳ - Nephrons.

LR8 expression is not detectable in gingival fibroblasts cultured from some human patients [14], whereas cells from all patients with phenytoin induced gingival overgrowth express LR8 [data not shown]. These observations indicate that fibroblasts are heterogeneous with respect to LR8 expression and that LR8-expressing cells may participate in the evolution of fibrosis. In order to examine these possibilities, we determined LR8 expression in normal and fibrotic human tissues. Our objectives were to determine if fibroblasts in tissues are heterogeneous in LR8 expression, and if the distribution of LR8 expressing cells is affected in fibrosis. LR8 expressing gingival fibroblasts also express α-smooth muscle actin (SMA) and there is a positive correlation between the expression of LR8 and SMA [14]. The SMA is a component of microfilaments of myofibroblasts, which are believed to be activated fibroblasts and associated with excessive connective tissue synthesis in fibrosis and inflammation [15]; therefore we also determined if LR8 expressing fibroblasts in tissues express SMA.

2. Materials and methods

2.1. Materials

Rabbit polyclonal antibody produced against carboxyl terminus of LR8 protein was obtained as a generous gift from Dr. Math Cuajungco, California State University, Fullerton [16]. Paraffin embedded normal and fibrotic human lung, liver and kidney tissue sections were obtained from the Department of Pathology, University of Washington Medical Center, after approval by University of Washington Human Subjects Committee. Fibrotic liver tissues were obtained from patients with HCV