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Original Article

LOX-1 in the maintenance of cytoskeleton and proliferation in senescent cardiac fibroblasts

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ABSTRACT

Lectin-like oxidized low-density lipoprotein receptor 1 (LOX-1) is one of the most important receptors for binding and uptake of ox-LDL in endothelial cells, vascular smooth muscle cells and cardiomyocytes. In this study in cultured mice heart fibroblasts, we describe a decrease in LOX-1 expression as these cells go through successive passages. Further, fibroblast aging is associated with significant changes in morphology and proliferation ability. The same phenomena were observed in primary cardiac fibroblasts isolated from the aged mice (130-week). We also noted that the senescent fibroblasts have increased susceptibility to apoptosis and have a disorganized cytoskeleton. To ascertain the contribution of LOX-1 in the decline in proliferative ability and morphological changes in the aged cells, senescent fibroblasts were transfected with h-LOX-1. Transfection with h-LOX-1 resulted in cytoskeleton reorganization and partial restoration of the expression of related proteins, CDC42 and p70 S6 kinase. Upregulation of LOX-1 also significantly enhanced their proliferation potential and restored the expression of related genes Mdm2 and phos-Akt, and modestly reduced the expression of aging markers 4-HNE and β-catenin. These findings suggest that LOX-1 contributes, at least in part, to the process of fibroblast senescence and may be viewed as a new aging maker.

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1. Introduction

Heart failure is a major cause of death and disability, especially in the elderly [1]. Extensive evidence suggests that the aging heart undergoes remodeling process which is, in turn, based on senescence-related alterations in different cells in the heart, especially fibroblasts. Cardiac fibroblasts constitute approximately 25% of myocardial tissue volume and account for nearly 60% of all cells in the heart and play a very important role in the genesis of diastolic heart failure [2]. Fibroblast growth with subsequent release of collagens during ischemia or pressure overload is thought to be a major participant in cardiac remodeling that result in the syndrome of heart failure. Aging is also associated with excess deposition of collagen in the heart that makes the heart non-compliant. Unfortunately, there is paucity of studies on the biology of fibroblasts during aging process.

LOX-1, a lectin-like 52 kD receptor for oxidized low density lipoprotein (ox-LDL), is responsible for binding and uptake of ox-LDL as well as other ligands exhibiting oxidized phospholipids including injured and apoptotic cells. Previous studies, including ours, showed that LOX-1 plays an important role in atherosclerosis and cardiac remodeling following chronic ischemia or sustained hypertension [3–5]. Recently, we observed that presence of LOX-1 is essential in the maintenance of cardiac fibroblast growth [6]. Another study from our group showed a marked decline in LOX-1 expression in late passage endothelial cells [7]. All this information suggests that LOX-1 may be related to aging and cell growth. The purpose of the present study was to analyze LOX-1 expression in cardiac fibroblasts in relation to aging.

2. Methods

2.1. Cardiac fibroblast culture

Fibroblasts were isolated from the hearts of 8-week-old C57BL/6 mice as described previously [8]. All experimental procedures were performed in accordance with protocols approved by the Institutional Animal Care and Usage Committee. The cells were cultured for up to 30 passages. Most experiments were performed in fibroblasts passages 3 and 30 (referred to as P3 and P30) cells. For gene transfection, P30 fibroblasts were seeded in 6-well plates, and transfected with PCI-neo plasmid with human LOX-1 cDNA (h-LOX-1) or PCI-neo empty plasmid (vector). In another set of experiments, fibroblasts were isolated from very young (6-week) and very old (130-week) old mice.

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Table 1
Primers for RT-PCR.

Primer	Sense	Sequence	Product size
P53	Forward	5′-GGATAGCAAAGAGCACAGAGC-3′	472 bp
	Reverse	5′-CCAGTCTTCGGACAAGCGTGAC-3′	
Akt1	Forward	5′-GTCTCTAGGGTCCAGGGCCAAAGTC-3′	338 bp
	Reverse	5′-CATCTAAAAGGACAAGTGCTAGGAG-3′	
β-Actin	Forward	5′-TTCTTTGCAGCTCCTTCGTTGCCG-3′	458 bp
	Reverse	5′-TGGATGGCTACGTACATGGCTGGG-3′	

2.2. Cell counting and cell size measurement

Fibroblasts number was determined using a hemocytometer. The cell area was calculated in several images, at least 100 cells in each passage, using NIH Image J software program. For the transfected fibroblasts, cell counting and cell area measurement were performed after 28 hours of transfection.

2.3. Immunostaining

Immunostaining of the cultured cardiac fibroblasts was performed, as described previously [8]. All samples were imaged with LSM510 Zeiss Laser inverted confocal microscope using LSM510 software.

2.4. Western blotting

Protein was extracted from cardiac fibroblasts using standard protocol. Western blotting was performed, as previously described [8]. Sources of primary antibodies were: 4HNE, LOX-1, CDC42, CD36, MSR1 and β-actin (ABcam, Cambridge, MA, USA), p-Akt and p70 S6 kinase (Cell Signaling Technology, Danvers, MA, USA), β-catenin, Mdm2, Bax and Bcl2 (Santa Cruz Biotechnology, Santa Cruz, CA, USA).

2.5. RT-PCR

The expression of p53 and Akt-1 was evaluated by RT-PCR [8]. PCR was performed using a 20 μL reaction volume containing 100 ng cDNA, 10 μL 2X PCR reaction mixture (Sigma-Aldrich, St. Louis, MO, USA) and primers. The primer sequences are shown in Table 1.

2.6. Cytoskeleton analysis

Cardiac fibroblasts were fixed using neutral and buffered 4% formaldehyde and treated with 0.1% Triton-X-100, and then labeled with 2 U Rhodamine phalloidin (Invitrogen, Grand Island, NY, USA) for 30 min in dark. After washing 3 times, fluorescence was viewed with laser inverted confocal microscope.

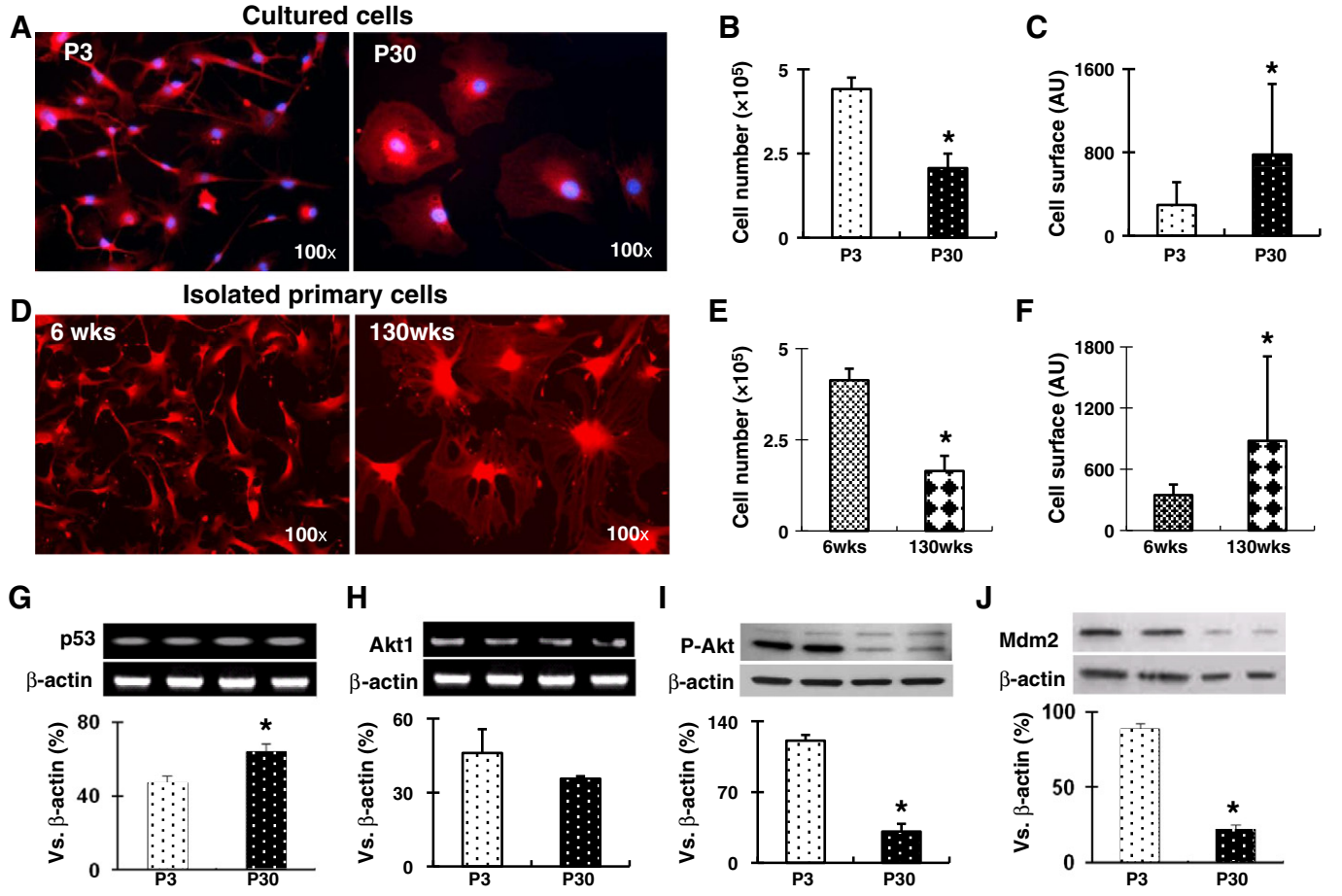

Fig. 1. Morphology and growth of senescent cardiac fibroblasts. A. Morphology of passage 3 (P3) and passage (P30) fibroblasts; B. Proliferation ability of P3 and P30 fibroblasts; C. Average cell surface area of P3 and P30 fibroblasts; D. Cell morphology of primary cardiac fibroblasts isolated from young (6 weeks old) and old (130 weeks old) mice; E. Proliferation of primary fibroblasts from young and old mice; F. Average cell surface area of primary fibroblasts from young and old mice; G. p53 mRNA expression in P3 and P30 fibroblasts; H. Akt1 mRNA expression in P3 and P30 fibroblasts; I. phosphor-Akt protein expression in P3 and P30 fibroblasts; J. Mdm2 protein expression in P3 and P30 fibroblasts. Data are representative of 4 independent experiments. Graphs show data as mean (±SD).*$P < 0.01$ vs. P3 cells or cells from 6-week old mice.