INTRODUCTION: Recent epidemiological and clinical studies have reported that intervertebral disc (IVD) degeneration is associated with the occurrence of low back pain (LB[P]) [1]. A previous study evaluated the association between DNA methylation and disc degeneration by genome-wide association analysis of human nucleus pulposus (NP) tissues and identified 220 differentially methylated loci (DMLs) associated with human IVD degeneration [2]. Caspase recruitment domain-containing protein 14 (CARD14), one of the DMLs, have been identified as the essential causative genes of psoriasis, and its mutation causes activation of NF-kB, which causes inflammation [3]. Psoriatic arthritis, included in spondyloarthritis, develops as a complication of psoriasis and causes LBP [4]. The function and expression of CARD14 in psoriasis have been reported; however, the expression of CARD14 in IVD remains unknown. The purpose of this study was (1) to examine the expression of CARD14 in human nucleus pulposus (NP) cells, and (2) to evaluate the expression of CARD14 and NF-kB in human NP tissues in early and advanced stages of degeneration.

METHODS: Human NP cells obtained from spine surgeries were isolated from IVD tissues (Pfirrmann’s classification [5]: grades 2-4, n=5) by sequential enzyme digestion. The cells were cultured in a monolayer at 4.0×10^4 cells/ml in 5% CO2 and 95% air in complete medium (DMEM/F12 containing 10% fetal bovine serum, 25 μg/ml ascorbic acid, 10,000 units/ml penicillin, and 10,000 μg/ml streptomycin). To examine the effect of proinflammatory cytokines on the expression of CARD14, human NP cells were cultured in the presence of interleukin-1β (IL-1β); 0.1, 1, and 10 ng/ml for 48 h.

Real-time PCR analysis: mRNA was isolated from NP cells in monolayer culture using Isogen (Nippon Gene) according to the manufacturer’s instructions. The mRNA was reverse transcribed using a first strand cDNA synthesis kit (Roche Applied Science) with a DNA thermal cycler. The assay was calibrated using 18s ribosomal RNA as an internal control. mRNA expression of CARD14 was quantified by real-time PCR analysis.

Western blotting: After pre-culturing, NP cells were lysed with RIPA buffer to release the proteins of interest. Gel electrophoresis, transfer from the gel to the membrane, and immunostaining of the blot were performed with anti-CARD14 antibodies. β-actin served as a positive control. Protein bands were visualized with a LAS4000 mini (Fujifilm, Tokyo, Japan) using the electrogenerated chemiluminescence (ECL) method.

Immunohistochemistry: Human NP tissues obtained from spine surgeries were divided into the early degeneration (ED) group (Pfirrmann’s grade 2-3, n=15) or advanced degeneration (AD) group (Pfirrmann classification grade 4, n=15). After epitope retrieval with citrate buffer (pH 6.0), the sections were incubated with a primary antibody against CARD14, mouse monoclonal antibody (Novus Biological). The primary antibody was visualized using the Histofine Simple Stain MAX-PO (MULTI) kit (Nichirei Bioscience, Tokyo, Japan) according to the manufacturer’s instructions, with some modifications. Peroxidase activity was detected with 3,3′-diaminobenzidine-tetrahydrochloride (DAB; Dojindo, Toyama, Japan). Sections were counterstained with Mayer’s hematoxylin. The isotype control was processed using mouse IgG. Five views of each section of the NP area at 200× magnification were randomly captured, and the number of positive cells for each was counted. The percentage of immunopositive cells and 2+ positive cells in the ED group (Fig. 3) and that in the AD group were calculated from the immunopositive cells and 2+ positive cells in the ED group and AD group.

RESULTS: The results of this study showed, for the first time, that human NP cells constitutively express CARD14 at both mRNA and protein levels. CARD14 expression in NP cells was shown to be significantly stimulated by inflammatory stimuli, and its expression was upregulated in human NP tissues at the advanced stage of degeneration. Since CARD14 is associated with inflammatory activation, the enhanced expression of CARD14 in degenerated IVDs might be associated with the process of tissue degeneration.

SIGNIFICANCE/CLINICAL RELEVANCE: We have shown for the first time that human NP cells express CARD14 at both mRNA and protein levels. CARD14 expression in NP cells was shown to be significantly stimulated by inflammatory stimuli, and its expression was upregulated in human NP tissues at the advanced stage of degeneration. Thus, CARD14 may be a key molecule responsible for IVD degeneration.


Fig. 1 Gene expression of CARD14
Fig. 2 The percentage of immunopositive cells
Fig. 3 The concordance rate of p65 and CARD14-positive cells

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