FNIII14 has the potential to induce cellular senescence in chondrocytes

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INTRODUCTION:
Osteoarthritis (OA) is a type of degenerative joint disease that is characterized by articular cartilage destruction. It has been reported that various factors are involved in the pathogenesis of OA1,2, and in recent years, cellular senescence is widely known as a major risk of OA3. Senescence is a cell fate characterized by permanent cell cycle arrest and the release of harmful pro-inflammatory molecules. Our previous study suggests that TNIII A2 which is domain peptide of Tenascin C promotes cartilage repair by stimulating β1 integrin conformational changes in chondrocytes, while FNIII14 which is a fibronectin peptide is involved in the pathogenesis of OA by inhibiting chondrocyte proliferation and inducing apoptosis. We hypothesized that FNIII14 is involved in the pathogenesis of OA through the cellular senescence of chondrocytes and investigated the role of TN III A2 and FN III 14.

METHODS:
TNII A2 and FNIII14 were synthesized through a solid-phase method combined with the Fmoc and Boc chemistry. Chondrocytes were isolated and cultured from articular cartilage tissue of OA patients. TNII A2 and FNIII14 were added to the cultures at concentrations of 0, 10, and 50 μg/ml, and SA-β-gal staining was performed in 1, 5, and 8 days to assess the rate of senescent cells. Additionally, cultured chondrocytes were treated with TNII A2 and FNIII14 at each concentration and collected 24 hours later for RT-qPCR to measure the expression levels of p16, p21, and p53, which are cell cycle arrest factors. Kruskal-Wallis test was performed to test the significance of the treated sample and the control sample. A calculated P value of less than 0.05 was considered significant.

RESULTS:
The SA-β-gal assay showed an increase in the percentage of SA-β-gal positive cells with the passage of days in both the TNII A2 and FNIII14-treated groups. A percentage of SA-β-gal positive cells was significantly higher in the group treated with 50 μg/ml of FNIII14 than in the group of control on day 5 and 8 (Figure 1). The expression level of p16, p21 and p53 was significantly upregulated in the group treated with 50 μg/ml of FNIII14 by RT-qPCR (Figure 2). Furthermore, the expression level of p21 is slightly increased even at a dose of 10 μg/ml of FNIII14. It was suggested that cellular senescence of chondrocytes was induced by FNIII14 in a dose-dependent manner. No significant changes were observed in either SA-β-gal assay or RT-qPCR in the TNII A2-treated group.

DISCUSSION:
To our knowledge, there are no reports discussing the relationship between cellular senescence and FN III 14 in chondrocytes. We observed significant increase of multiple senescent marker such as SA-β-gal, p16, p21 and p53 by the addition of FN III 14. These results suggest that FNIII14 is a senescence-promoting factor as well as oxidative stress. Previous studies have shown that the addition of FNIII14 to tumor cells and mouse fibroblasts inhibits cell proliferation10 and induces apoptosis of chondrocytes11, which are consistent with some of the characteristics of cellular senescence. Our data support the hypothesis that FNIII14 may be involved in the pathogenesis of OA through the cellular senescence of chondrocytes.

SIGNIFICANCE/CLINICAL RELEVANCE:
Elucidating the promoting factor of cellular senescence in chondrocytes may lead to drug discovery such as disease-modifying therapies for OA.


Figure 1. Increase in the percentage of SA-β-gal positive cells with the passage of days. Higher detection of SA-β-gal in FNIII 14-treated chondrocytes on day 5 and 8.

Figure 2. Elevated expression of p16 in the group treated with 50 μg/ml of FNIII 14 and H2O2.