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 OA [(1)], and in recent years, cellular senescence is widely known as a major risk of OA [(2)]. Senescence is a cell fate characterized by permanent cell cycle arrest and the release of harmful pro-inflammatory molecules. Our previous study suggests that TNIIIA2 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 TNⅢA2 and FNⅢ14.

METHODS:
TNIIIA2 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. TNIIIA2 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 TNIIIA2 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 between 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 TNIIIA2 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 day5 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 TNIIIA2-treated group.

DISCUSSION:
To our knowledge, there are no reports discussing the relationship between cellular senescence and FNⅢ14 in chondrocytes. We observed significant increase of multiple senescent marker such as SA-β-gal, p16, p21 and p53 by the addition of FNⅢ14. 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 proliferation [(3)] and induces apoptosis of chondrocytes [(4)], 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.

REFERENCES: (1) Loeser R.F. et al. Arthritis Rheum. 2012 (2) Loeser R.F. et al. Nat Rev Rheumatol. 2016
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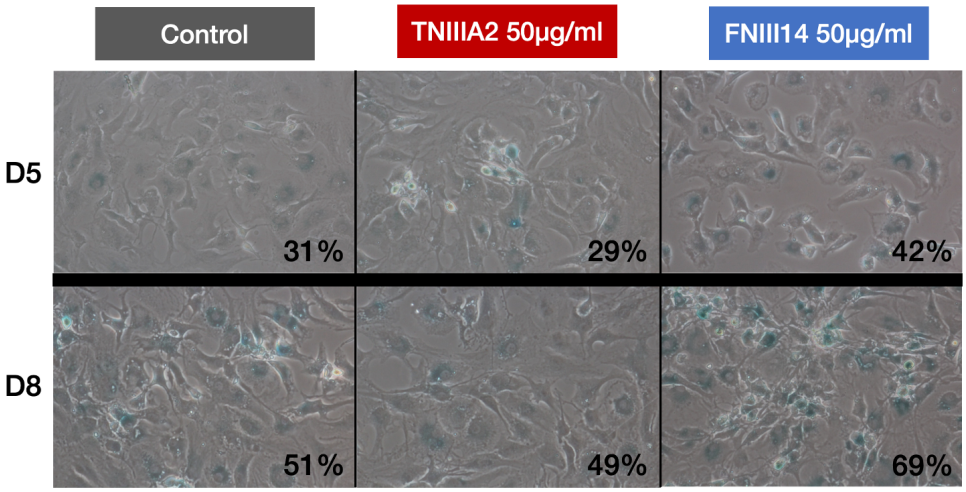

Figure 1. Increase in the percentage of SA-β-gal positive cells with the passage of days. Higher detection of SA-β-gal in FNⅢ14-treated chondrocytes on day5 and 8.

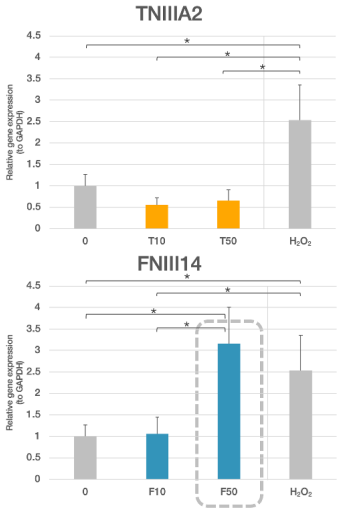

Figure 2. Elevated expression of p16 in the group treated with 50 μg/ml of FNIII14 and H_2O_2.