論　文　内　容　要　旨

The role of tetraspanin CD9 in osteoarthritis using three different mouse models

（3 個のマウス実験モデルで解析した

テトラスパニン CD9 の変形性関節症における役割）

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INTRODUCTION

Key pathological features of all forms of arthritis include joint inflammation with infiltration of leukocytes in the synovium, causing joint tissue remodeling and destruction. Osteoarthritis (OA) is the most prevalent joint disease. It is characterized by degradation of articular cartilage and alterations in other joint tissues such as subchondral bone and the synovium. The critical risk factors are aging, trauma and inflammation, and these factors impair the homeostasis of joint tissues through dysregulation of intracellular signaling. The understanding of the mechanisms of OA pathogenesis remains limited.

CD9 is localized in the cell membranes and partly in the endosomes of all types of mammalian cells. It is a four membrane-spanning protein and a member of the tetraspanin family, which also includes CD63, CD81, CD82, and CD151. However, CD9 has unique functions in angiogenesis through cell adhesion, migration, and signal transduction, and it is involved in cell fusion processes linked to fertilization, osteoclastogenesis and myogenesis. Regarding arthritis, previous reports have shown that CD9 is abundantly expressed in the OA synovium, in activated osteoclasts in osteoporosis, and in the sites of bone erosion in arthritic lesions of collagen-induced arthritis. However, the role of tetraspanins in OA pathogenesis remains unclear. The purpose of this study is to examine the OA development in $CD9^{-/-}$ mice using three different mouse models of arthritis: an aging model of primary OA, a surgical model of posttraumatic OA, and antigen-induced arthritis (AIA) as a transient inflammation model.

MATERIALS AND METHODS

This study examined the role of CD9 in OA development in three different mouse models: an aging model, a surgical model and an antigen-induced arthritis (AIA) model, using CD9 deficient mice. The severity of cartilage degeneration and inflammation in was evaluated.

To determine whether CD9 in cartilage is important for the degradation of the cartilage-related matrix, we quantified proteoglycan release from mouse femoral head cartilage explants using proteoglycan release assay.

Furthermore, we analyze the expression of inflammatory related genes and chondrocyte related genes in chondrocytes stimulated by IL-1ß using real-time PCR.

The data were analyzed by the Mann-Whitney U test, Steel or Steel-Dwass.

RESULTS

Our study showed that CD9 deficiency reduced the severity of hallmarks of OA including cartilage degradation and soft tissue inflammation in aged mice. In the AIA model, cartilage damage and inflammation was also reduced in $CD9^{-/-}$ mice. This was in contrast to the surgical OA model where disease severity was similar in wild-type and $CD9^{-/-}$ mice.

The expression of ADAMTS-5 and MMP-13, main cartilage degradation enzymes, was

characterized by immunohistochemistry in the articular cartilage of mice with AIA. Although ADAMTS-5 was not significantly different, the number of MMP-13 positive chondrocytes was significantly lower in the CD9$^{-/-}$ mice than in the wild-type mice.

we quantified proteoglycan release from mouse femoral head cartilage explants. Cartilage explants from wild-type and CD9$^{-/-}$ mice treated with IL-1ß showed significantly increased proteoglycan release. However, it was not significantly different between the wild-type and CD9$^{-/-}$ mice with or without IL-1ß .

The levels of cartilage matrix genes *Col2a1* and *Aggrecan* in control were significantly higher in CD9$^{-/-}$ chondrocytes than in wild-type chondrocytes. In response to IL-1ß stimulation, the levels of *Col2a1* and *Aggrecan* were significantly reduced in wild-type and CD9$^{-/-}$ chondrocytes but even under IL-1 treatment remained higher in CD9$^{-/-}$ chondrocytes .

DISCUSSION

This is the first study to demonstrate a role of CD9 in OA development in three different animal models. CD9 deficiency did not lead to apparent abnormalities in joint development and joints of CD9$^{-/-}$ mice were completely normal on histological analysis at 6 months. However, intra-articular inflammation and cartilage degeneration during aging was attenuated in the CD9$^{-/-}$ mice compared with the wild-type mice. Our study also showed CD9 deficiency had certain protective effects in the AIA model where it reduced the severity of cartilage degradation, some inflammation markers and the total AIA score. By contrast, the severity of the surgical OA model was not significantly different between wild-type and CD9$^{-/-}$ mice. Thus CD9 deficiency has certain protective effect in the aging and AIA models but not in the surgical model.

In the present study, cartilage degradation was significantly reduced in CD9$^{-/-}$ mice in aging and in the AIA model. Thus we compared chondrocytes from CD9$^{-/-}$ and wild-type mice. *Col2a1* and *Aggrecan* expression was increased in chondrocytes of CD9$^{-/-}$ mice compared with wild-type mice. IL-1ß-induced proteoglycan release from cartilage explants, and *Adamts-5* and *Mmp-13* expression were not significantly different between CD9$^{-/-}$ and wild-type chondrocytes. Thus, these results suggest that the suppression of cartilage degradation in CD9$^{-/-}$ could be in part related to an increase in the expression of the two main cartilage extracellular matrix proteins aggrecan and type II collagen.

The present study showed that CD9 deficiency reduces the severity of OA such as cartilage degradation in aged mice, as well as in AIA transient inflammation model mice. Thus, CD9 might have potential to open new insight for the mechanism and protection of OA.