論　文　内　容　要　旨

Bach1 deficiency reduces severity of osteoarthritis through upregulation of heme oxygenase-1

（Bach1 ノックアウトマウスは加齢性および実験的変形性関節症の症状を軽減する）

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INTRODUCTION

Osteoarthritis (OA) is characterized by degradation of articular cartilage and alterations in other joint tissues. The most important risk factors are aging, mechanical stress and inflammation, and these factors impair tissue homeostasis through dysregulation of intracellular signaling mechanisms and extracellular matrix (ECM) remodeling.

Increased oxidative stress results from increased reactive oxygen species (ROS) generation and from reduced anti-oxidants, and is accompanied by a progressive accumulation of damaged molecules and organelles, leading to activation of catabolic factors such as inflammatory cytokines and ECM-degrading proteases. Antioxidant enzymes such as Heme oxygenase-1 (HO-1) and superoxide dismutase 2 (SOD2) are an important defense against ROS-mediated damage.

Previous studies revealed that induction of HO-1 has beneficial effects in several diseases. The expression of HO-1 gene (*Hmox-1*) is negatively regulated by BTB and CNC homology 1 (Bach1) and thus Bach1 deficient mice exhibit constitutively high levels of HO-1 in various tissues under normal physiological conditions.

The objective of this study was to define the role of Bach1 in cartilage homeostasis and OA development using in vitro models and Bach1$^{-/-}$ mice.

METHODS

HO-1 expression in Bach1$^{-/-}$ mice and wild-type mice was analyzed by real-time PCR, immunohistochemistry and immunoblotting.

To elucidate the role of HO-1 involved in OA progression with aging, knee joints from Bach1$^{-/-}$ and wild-type mice with age-related OA and surgically-induced OA were evaluated by OA scoring systems. Knee joints were harvested at 6 months (n=7), 12 months (n=11), and 22 months (n=14) to monitor spontaneous age-related OA. Experimental OA was induced in 10 week-old, wild-type mice (n = 13) and Bach1$^{-/-}$ mice (n = 11) by transection of the medial meniscotibial ligament and the medial collateral ligament in the right knees.

To determine whether the increase in HO-1-positive cells in articular cartilage was associated with changes in SOD2 expression and autophagy, the expression of SOD2 and microtubule-associated protein 1 light chain 3 (LC3), a main marker of autophagy, was characterized by immunohistochemistry.

To investigate the interaction between HO-1 and autophagy or SOD2, mice articular chondrocytes were transfected with small interfering RNA (siRNA) for HO-1 (siHO-1) or negative control siRNA.

RESULTS

The expression of HO-1 gene and protein were significantly increased in Bach1$^{-/-}$ chondrocytes compared with wild-type chondrocytes. HO-1 expression decreased with aging in articular cartilages and menisci of mouse knees.

Histological scores indicated that Bach1 -/- mice were significantly decreased in the severity of the OA-like changes. Bach1 -/- mice inhibited not only degradation of cartilage, but also arthritic changes of other joint tissues such as meniscus degeneration, osteophyte formation and synovitis. In surgically-induced OA model, Bach1$^{-/-}$ mice reduced the severity of OA-like changes than wild-type mice.

The number of LC3-positive chondrocytes in Bach1-/- mice was significantly higher than wild-type mice. SOD2-positive chondrocytes were also higher in Bach1-/- mice than wild-type mice.

The increased SOD2 expression in Bach1$^{-/-}$ mice was reduced by HO-1 knockdown. Bach1$^{-/-}$mouse chondrocytes had significantly decreased numbers of caspase-3/7-positive cells following treatment with the oxidant t-BHP. Compared with wild-type mice, the number of positive cells was significantly increased in chondrocytes treated with siHO-1. These results suggest that the expression of SOD2 and the suppression of apoptosis in Bach1$^{-/-}$ chondrocytes were mediated by HO-1.

CONCLUSIONS

Bach1 deficiency reduces the severity of OA-like changes. This may be due to maintenance of cartilage homeostasis and joint health by antioxidant effects through HO-1 and downregulation of ECM degrading enzymes. These results suggest that inactivation of Bach1 is a novel target and signaling pathway in OA prevention.