Bach1 Deficient Mice Reduce Severity of Age-related Osteoarthritis Through the Maintenance of Autophagy and SOD2

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Introduction:
Osteoarthritis (OA), the most prevalent aging-related joint disease, 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 homeostatic balance through dysregulation of extracellular regulators and gene expression network in several cells including joints cells. Oxidative stress is elevated in joint tissues including cartilage in aging and OA (1). Reactive oxygen species (ROS), major oxidative stress, are known to cause activating catabolic factors such as inflammatory cytokines and cartilage degradation-related proteases. Antioxidant enzymes such as Heme oxygenase-1 (HO-1) and superoxide dismutase family (SOD1, 2, 3) are an important to prevent an accumulation of ROS. HO-1 is the enzyme of heme catabolism that has a variety of physiological activities collectively referred to as cytoprotection and plays an important role in maintenance of homeostasis. The expression of HO-1 gene (HMOX-1) is negatively regulated by Bach1 (BTB and CNC homology 1) and thus Bach1 deficient mice exhibit constitutively high levels of HO-1 in various tissues under normal physiological conditions (2). This deficient mice show reduced pathogenesis from several experimental models (3, 4). However, the beneficial effects and molecular mechanisms of HO-1 remain to be unclear in OA. The objective of this study was to define the function of HO-1 in cartilage homeostasis involved in OA progression using Bach1-/- mice.

Methods: The expression of HO-1 in the articular cartilage was evaluated using primary chondrocytes from femoral heads of Bach1-/- and wild type mice. The expression of hmox1 (HO-1) and cartilage related genes were measured by real-time PCR using TaqMan probe (ABI). HO-1 protein in these cells was detected by western blotting. To clarify the expression of HO-1 in articular cartilage with aging, the knee joints of newborn, 3, 12 and 22 month-old (m-o) wild-type mice were obtained and assessed by immunohistochemistry using HO-1 antibody (ab52947).
To elucidate the role of HO-1 involved in OA progression with aging, we used two experimental models; age-related OA models and experimental OA models. As age-related OA model, we prepared 22 m-o Bach1-/- mice (n=16, 22 knees) and wild-type mice (n=8, 14 knees). Experimental OA was induced in 12 week-old (w-o) Bach1-/- mice (n=13) by transection of the medial meniscotibial ligament and the medial collateral ligament in the right knees and sacrificed 12 weeks later. Wild-type mice (n=11) were subjected to the same surgery and were used as a control. Each knee was stained with Safranin O and the modified OARSI and Mankin’s scoring systems that we previously developed were used to validate the histological changes.
To observe autophagy and the expression of SOD2 in articular cartilage, immunohistochemistry for microtubule-associated protein 1 light chain 3 (LC3), a main marker of autophagy, and SOD2 was performed with specific antibodies (LC3, ABGENT; SOD2, StressMarq).
To investigate the interaction between HO-1 and Autophagy or SOD2, mice articular chondrocytes were transfected with HO-1 siRNA (siHO-1) or negative control siRNA and treated with or without IL-1 for 24 h. HO-1, LC3 and SOD2 protein expression were detected by Western blot analysis.
To define the anti-inflammatory effect in aged-Bach1-/- mice and surgical OA-Bach1-/- mice, the levels of cytokines (IL1β, IL2, IL4, IL5, IL10, GM-CSF, IFN-g and TNFα) in serum were measured by using BioPlex a mouse 8 plex bead assay (BioRad).

Results: The knee joints from wild-type mice at neonatal, 1 to 22 m-o showed an aging-related significant reduction in HO-1 positive cells compared with 1 m-o mice. Whereas articular cartilage and meniscus in Bach1-/- mice showed significantly more HO-1 positive cells than 22 m-o wild-type mice. Bach1-/- mice inhibited the development of an age-related OA-like pathology compared with the wild-type mice at 22 m-o. Histological scores indicated that Bach1-/- mice were significantly decreased in the severity of the OA-like changes (Figure 1). Bach1-/- mice inhibited not only degradation of cartilage, but also arthritic changes of other joint tissues such as meniscus degeneration, osteophyte formation and synovitis (Figure 2). In OA surgical model, Bach1-/- mice reduced the severity of OA-like changes than wild-type mice. In aging model, proinflammatory cytokines in serum of aged-mice (22 m-o) in wild-type mice were significantly increased compared with that in young mice (10 w-o), however, cytokines in Bach1-/- mice did not significantly change between young- and aged-mice. In aged-mice, IL-1b, IL-2, IFNg and TNFα in Bach1-/- mice were significantly reduced compared with that in 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 expression of LC3 and SOD2 by siHO-1 were slightly reduced in chondrocytes.

**Discussion:** Bach1/−/− mice showed to reduce the severity of aging-related OA-like changes. These results suggest that maintenance of HO-1 expression play an important role in OA pathogenesis. Suppression of proinflammatory cytokines by HO-1 might be involved in the prevention of degradation with aging. Our present study showed that activation of autophagy and the expression of SOD2 were decreased with aging as well as HO-1 expression, and they were maintained in articular cartilage of aged-Bach1/−/− mice. From these results, HO-1 likely reduces the severity of age-related OA-like changes of articular cartilage via activation of autophagy and the expression of SOD2. These studies indicate a new network that SOD2 and autophagy via HO-1 regulate age related-OA pathogenesis. Thus, control of HO-1 might be an effective treatment for OA prevention.

**Significance:**

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Figure 1. Bach1/−/− mice inhibited the development of OA-like pathology compared with wild-type mice. (A and B) Knee joints were analyzed by staining with Safranin O. Magnification: 40×. (C) Histological scores for aging models. **p<0.001.

Figure 2. Comparison of histological scores. Bach1/−/− mice inhibited not only degradation of cartilage, but also arthritic changes of other joint tissues such as meniscus, osteophyte formation and synovitis. *p<0.05. **p<0.01. **p<0.001.

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