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Accelerated osteoarthritis in the temporomandibular joint of biglycan/fibromodulin double-deficient mice

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Summary

Objective: To investigate whether the absence of biglycan and fibromodulin, two proteoglycans expressed in cartilage, bone and tendon, resulted in accelerated osteoarthritis in the temporomandibular joint (TMJ).

Methods: Histological sections of TMJ from 3-, 6-, 9- and 18-month-old wild-type (WT) and biglycan/fibromodulin double-deficient (DKO) mice were compared. Immunostainings for biglycan, fibromodulin and proliferating cell nuclear antigen (PCNA) were performed.

Results: Biglycan and fibromodulin were highly expressed in the disc and articular cartilage of the TMJ. At 3 months of age, both WT and DKO presented early signs of cartilage degeneration visible as small acellular areas under the articular surfaces and superficial waving. From 6 months of age, DKOs developed accelerated osteoarthritis compared to WT. At 6 months, small vertical clefts in the condylar cartilage and partial disruption of the disk were visible in the DKO. In addition, chondrocytes had lost their regular columnar organization to form clusters. At 9 months, these differences were even more pronounced. At 18 months, extended cartilage erosion was visible in DKOs when by comparison the thickness of the articular cartilage in WT controls was basically intact. PCNA staining was stronger in 3-month-old WT TMJ fibrocartilage than in 3-month-old DKO TMJ fibrocartilage suggesting that chondrocyte proliferation might be impaired in DKOs.

Conclusion: The biglycan/fibromodulin double knock-out mouse constitutes a useful animal model to decipher the pathobiology of osteoarthritis in the TMJ.

Key words: Temporomandibular joint (TMJ), Biglycan, Fibromodulin, Proliferation, Osteoarthritis, Mice.

Introduction

Temporomandibular joint disorders (TMD) are comprised of a number (over 20) of pathologic conditions. Whether some of these conditions are part of the natural history of the same pathological process or each condition exists as a separate entity is unknown. One of the most prevalent conditions of TMD is osteoarthritis of the temporomandibular joint (TMJ OA)[1,2]. Currently, there are no biomarkers or treatments for TMJ OA. Hence, diagnosis of TMJ OA is made by imaging techniques only after there is irreversible damage to the joint. Due to the limited capacity of joints to regenerate, it would be beneficial if one could detect and treat TMJ OA at an early stage. Since it is practically impossible to obtain human pre-osteoarthritic TMJ samples, the generation of animal models, covering the whole disease process is necessary.

The TMJ is composed of two bones, the mandibular condyle and the glenoid fossa of the temporal bone, separated by a fibrocartilaginous disc, the TMJ disc. Unlike most diarthrodial joints in which the articular surfaces are covered by pure hyaline cartilage, the articulating surfaces of the bones are made of fibrocartilage[3]. Interspersed among these tissues are members of the small leucine repeat proteoglycan (SLRP) family. Members of this family are characterized by a small protein core that consists predominantly of the repetition of leucine-rich regions[4]. Four of them, biglycan (Bgn), fibromodulin (Fmod), lumican and decorin are found in bone, cartilage and tendons, have been shown to be able to bind transforming growth factor (TGF)-beta and play a role in regulating collagen fibrillogenesis (see Ameye and Young[5] for a review).

We have previously shown that mice double deficient in Bgn and Fmod have severe and premature knee OA, while mice single deficient in Bgn or Fmod have a milder form of it[6]. Mice single deficient in lumican and mice double deficient in lumican and fibromodulin have also been shown to develop premature knee OA but again more slowly than the mice double deficient in Bgn and Fmod[7]. Mice single deficient in decorin, in contrast to mice single deficient in Bgn, Fmod or lumican, do not develop OA. We have previously shown that knees from mice double deficient in Bgn and Fmod displayed a progressive degeneration of the articular cartilage from early fibrillation to complete erosion, subchondral sclerosis, osteophytes and bone cysts, the hallmarks of human OA[6]. In this study, we investigated

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whether the absence of Bgn and Fmod resulted in accelerated TMJ OA. We chose to use the Bgn/Fmod double-deficient mouse instead of the other SLRP deficient mice not only because they were available to us, but also because the more rapid progression of the disease in this mouse makes it a more convenient animal model to study. We found by histology, that mice that were deficient in both Bgn and Fmod have accelerated OA in their TMJ compared to control mice. We also characterized mandibular condylar cartilage proliferation and found that the double-deficient mice had a significant decrease in condylar chondrocyte proliferation compared to WT before TMJ OA was overt.

Methods and materials

GENERATION OF Bgn AND Fmod SINGLE AND DOUBLE-DEFICIENT MICE

All experiments were performed under an institutionally approved protocol for the use of animals in research (#NIDCR-IRP-98-058 and 01-151). Mice deficient in Bgn or Fmod were generated by gene targeting in embryonic stem cells as described previously[6]. Heterozygous Bgn/Fmod-deficient mice were produced by breeding a homozygous Bgn-deficient female (*Bgn−/−/Fmod+/+*) with an Fmod-deficient male (*Bgn+/0/Fmod−/−*); Bgn males are designed as Bgn−/0 since the Bgn gene is located on the X chromosome and absent from the Y chromosome. F2 Bgn/Fmod double-deficient (male *Bgn−/0/Fmod−/−* and female *Bgn−/−/Fmod−/−*) mice were obtained by interbreeding F1 heterozygous Bgn/Fmod mice and will be referred to hereafter as Bgn/Fmod DKO mice.

GENOTYPING

All mice were genotyped for Bgn and Fmod alleles by polymerase chain reaction (PCR) analysis as described previously[6]. PCR products were resolved by electrophoresis through 1.8% agarose gels, yielding bands of 212 bp for the WT Bgn allele, 310 bp for the disrupted Bgn allele, 280 bp for the WT Fmod allele, and 603 bp for the disrupted Fmod allele.

HISTOLOGY AND IMMUNOHISTOCHEMISTRY

For each genotype and age, heads from at least three animals were dissected in two halves. After removal of the brain, the specimens were fixed for 2 weeks at room temperature in 10% formalin. After being washed in tap water for 5 min, they were decalcified in formic acid bone decalcifier solution (Immunocal from Decal Corporation, Tallman, NY, USA) for 4 weeks. Specimens were then washed in tap water for 5 min and fixed for 3 days in buffered zinc formalin (Z-fix from Anatech Ltd, Battle Creek, MI, USA) before being classically processed for histology and sectioned sagittally. Sections were stained by hematoxylin and eosin (H&E) or by Safranin O[8].

Tissue sections were deparaffinized with xylene. Following rehydration, with graded ethanol, endogenous peroxidase activity was blocked by treating the sections in 3% peroxide in methanol for 20 min. In order to expose the antigen, sections were predigested in chondroitinase ABC (cat# KE01502, Seikagaku Corp, Tokyo, Japan) at a concentration of 0.015 units/ml for 1 h at 37°C. Non-specific binding was reduced by incubating the sections in 10% goat serum for 30 min. Sections were then incubated for 1 h at 37°C with primary antibody at a 1: 200 dilution. Fibromodulin and biglycan antibodies (LF 150 and LF 106, respectively) were kind gifts from Larry Fisher[9], whereas proliferating cell nuclear antigen (PCNA) antibody clone PC 10 was purchased from DAKO Cytomation (Carpinteria, CA, USA). Biotinylated goat anti-rabbit secondary antibody was used and visualized by a streptavidin–peroxidase solution in presence of AEC chromagen.

The data presented in this paper were reproduced in at least three different animals of the same genotype at each age. Serial sections through the whole joints were obtained for each animal and observations were confirmed in different interspaced serial sections chosen to cover the whole joint. In this way, it was ensured that the reported observations were genuine and not local random abnormalities. In order to minimize experimental variability, WT and DKO were processed in parallel at the same time for histology and immunohistostainings.

EVALUATION OF PCNA STAINING

Images from sections immunostained with PCNA were captured using an AxioCam MR camera (Zeiss). A rectangular box was constructed to evaluate the percentage of PCNA positive cells in the mandibular condylar cartilage. The number of PCNA positive cells were counted and divided by the total number of cells within the box. For each section, three areas were analyzed, corresponding to anterior, center and posterior portions of the mandibular condylar cartilage. Eleven sections from three different 3-month-old DKO mice and 10 sections from three different 3-month-old WT mice were evaluated.

Results

Immunohistochemistry revealed that Bgn and Fmod were abundantly expressed within the TMJ of 3-month-old WT mice (Fig. 1). Both were found in the disc, and in the articular cartilage of the mandibular condyle. In the disc, fibromodulin was found in the anterior and posterior attachment parts, while biglycan was found predominantly in the anterior attachment portion of the disk. Regarding their expression in the articular cartilages of the glenoid fossa and the mandibular condyle, both proteoglycans were more highly expressed in the anterior and posterior parts of these cartilages than in the central parts [Fig. 1(a and b)]. At the same time, they were also more highly expressed in the superficial zone than in the deeper zones. This pattern of differential expression was more marked for Bgn than for Fmod: Bgn was not expressed in the central parts and in the deepest zones of these articular cartilages, while Fmod was expressed although at a relatively low level [Fig. 1(c and d)]. As the mice aged, the localization of Bgn and Fmod remained the same; however, there was an overall decrease in the intensity of their immunohistochemical staining (data not shown).

In order to examine the function of Bgn and Fmod in the TMJ, the morphology of TMJ from WT and double-deficient mice was compared at 3, 6, 9 and 18 months of age. At 3 months, early and similar signs of degeneration were present in the articular cartilage of the mandibular condyle from deficient and WT TMJs. These defects consisted of the presence of small acellular areas under the articular surfaces and superficial waving (Fig. 2). While little differences were apparent at 3 months of age between the WT and deficient TMJs, they became obvious at 6 months of age due to the accelerated degeneration of the articular