Rapid Communication

Parallel Distribution of Bcl-2 and Bax in Hypertrophied Chondrocytes of the Mandibular Condylar Cartilage from the Senescence-accelerated Mouse (SAMP3)

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To demonstrate the distribution of apoptotic cell death in the temporomandibular joint of SAMP3 mice, showing degenerative joint disease resulting in overt deformation of the mandibular condyle, immunohistochemical localization of Bcl-2 and Bax in the condylar cartilage of the animals was examined. Histological changes revealed an apparent deformity of the condyles at around 6 months of age, and thereafter showed severe degeneration in accordance with advancing age until 13 months. The apoptotic changes by the Bcl-2 and Bax expressions were observed in the hypertrophied chondrocytes abutting on the endochondral ossification site, as well as in the synovial membrane, throughout all ages examined. The expression of both of these molecules remained to localize in these sites and increased with advancing age, in which proceeding matrix degradation and chondroid bone formation took place. In addition, the nidus of metaplastic cartilage that protruded into the joint space of the aged animals also demonstrated positive immunoreactivities. The radical production and parallel distribution of Bcl-2 and Bax in the hypertrophied chondrocytes in the affected condyles might indicate that hypertrophic cells may undergo apoptosis, and some others, survive, to regulate the cartilage degradation occurring in the mandibular condyles of SAMP3 mice.

Key words: SAMP3, Mandibular condylar cartilage, Hypertrophied chondrocyte, Apoptosis, Bcl-2/Bax expression

I. Introduction

Various strains of senescence-accelerated mouse (SAM) have been under development by the Chest Disease Research Institute at Kyoto University [21, 22]. At present, there are 12 lines of SAM; the 9 senescence-prone inbred strains (SAMP) and the 3 senescence-resistant inbred strain (SAMR) [21, 22]. Among the former SAMP series, the SAMP3 strain develops degenerative joint disease with the advance of age, resulting in the overt deformation of the temporomandibular joint (TMJ) [8]. Although SAMP3 is a model of degenerative joint disease, with advancing age all strains of SAM show the joint disease initiated by degenerative changes such as roughness, fissure, and erosion on the condylar surface [8]. These pathological changes are consistent in most part with osteoarthritis cartilage (OA) [16]. The primary process of the pathobiology and the progression of OA are not completely understood, but the main consequence of the lesion is a progressive remodeling of bone together with degradation of the cartilage architecture and osteophyte formation, in which progressive apoptosis takes place.

Recent studies have provided evidence of apoptosis in terminally differentiated chondrocytes during endochondral bone formation [1, 7, 12–14, 18–20], and Horton et al. [15] recently reviewed. In several cell types, apoptosis is regulated by the ratio of expression of the cell death...
inhibitor, Bcl-2, and the cell death inducer, Bax [3, 23]. Although levels of Bcl-2 and Bax have been shown to be an immunohistochemical index for apoptosis, little is known about the programmed cell death that occurs in the degenerative joints of mice of the SAM series.

The present study deals with apoptotic cell death in the mandibular condylar cartilage of SAMP3 by using Bcl-2 and Bax as histochemical markers, as a step toward better understanding of human OA in the TMJ.

II. Materials and Methods

Animals

The SAMP3 strain mice from Kyoto University were used. The TMJ from 3-, 6-, 8-, 10-, and 13-month-old animals was dissected under deep anesthesia. The tissue blocks were fixed with 10% formalin at 4°C overnight, and were demineralized in 10% formic acid in 10% formalin for 7 days at room temperature. Following rinsing well, demineralized tissues were dehydrated through a graded series of ethanol and embedded in paraffin.

Immunohistochemistry

The paraffin-embedded tissues were serially sectioned at 4 μm in the sagittal plane, and sections were stained with toluidine blue for histological orientation and matrix modification. Immunohistochemical analyses were performed by the avidin-biotin-peroxidase complex method. The deparaffined sections were treated with 3% hydrogen peroxide to quench endogenous peroxidase activity for 10 min. After elimination of non-specific protein binding by immersion with 1% bovine serum albumin in PBS (BSA-PBS) for 1 hr, sections were incubated sequentially with a 1:100 dilution of mouse antibody against

Fig. 1. Photomicrographs of a sagittal section of the mandibular condylar cartilage from SAMP3 stained by toluidine blue (a, d, g) and reacted with Bcl-2 (b, e, h) and Bax (c, f, i). The condylar cartilage from a 3-month-old SAMP3 showing orderly arranged layers of chondrocytes (a). Bcl-2 (b) and Bax (c) localized in the hypertrophied chondrocytes (arrows) abutting on the endochondral ossification sites. The condylar cartilage from a 6-month-old SAMP3 (d) showing a significant degree of cartilage matrix degradation replaced by chondroid bone. Coexpression of Bcl-2 (e) and Bax (f) can be seen in the hypertrophic cells, as well as in the synovial membrane (arrowheads). The condylar cartilage from a 10-month-old SAMP3 (g) showing remnants of hypertrophied chondrocytes which disclose Bcl-2 (h) and Bax (i) immunoreactivities. A pathologic nidus of metaplastic cartilage (MC) protruding into the synovial space also manifests positive immunoreactivities. Bar=100 μm.
Bcl-2 (N-19, Santa Cruz Bio., USA) or a 1:1,000 dilution of mouse anti-Bax (P-19, Santa Cruz Bio., USA) overnight at 4°C; biotin-conjugated anti-mouse immunoglobulin (1:5,000 dilution in BSA-PBS) for 1 hr; and streptavidin-peroxidase (Nichirei, Japan) for 1 hr. Finally, peroxidase activity was demonstrated with the use of 0.05% 3,3'-diaminobenzidine (Nichirei, Japan) in 0.5 M Tris buffer (pH 7.6) containing 0.1% hydrogen peroxide. Sections were then transferred to distilled water, dehydrated by graded ethanolalcohol, and mounted with xylene.

Incubation of the tissue sections with omission of the primary antibodies was performed as a negative control.

III. Results

Histological changes as revealed by toluidine blue (TB) staining from the SAMP3 mice in different ages showed degenerative changes in the TMJ which is divided into three stages according to the histopathological grading by Chen et al. [8]. The first stage, i.e., 3-month-old animals, showed no apparent morphological alterations of the condylar cartilage compared with their normal counterpart of the same age (Fig. 1a). The second stage (6-~8-month-old animals) exhibited an overt deformity of the entire condyle with cartilage degeneration and with chondroid bone formation in the deep layer (Fig. 1d). An increased metachromasia by TB staining was also seen in the joint cartilage. The third stage (10-~13-month-old animals) demonstrated peripheral remodeling of circumferential tissues including synovial chondromatosis (Fig. 1g), in combination with the changes seen in the second stage.

Positive immunostaining of both Bcl-2 and Bax was seen in the hypertrophied chondrocytes throughout the samples from the different ages examined, but the positive sites of Bax were larger in number than that of Bcl-2. In the relatively intact condylar cartilage around 3 months after birth, the immunoreactions were restricted to the layer of hypertrophied cells adjacent to the calcified cartilage (Fig. 1b, c). During the onset of cartilage deformation seen around the period of 6 months of age, the immunoreactivities of Bcl-2 and Bax were detected likewise in the excessive numbers of hypertrophied chondrocytes (Fig. 1e, f), but not in the calcified cartilage. In addition, the positive immunoreactivity of two molecules was also seen in the synovial membrane. The severely affected cartilage from aged animals, manifesting an overt deformity of the condylar contour with metaplastic cartilage that protruded into the synovial space, also expressed both Bcl-2 and Bax in the hypertrophied chondrocytes (Fig. 1h, i).

IV. Discussion

The present study reported the parallel distribution of Bcl-2 and Bax in the hypertrophied chondrocytes of the mandibular condylar cartilage from SAMP3 mice. These molecules were characterized by existence firstly in the condylar cartilage of SAMP3 mice of 6 months of age, and the highest incidence of severe changes was found in SAMP3 mice of 8 to 13 months of age. The expression of both Bcl-2 and Bax in the hypertrophic cells suggests the existence of local regulatory factors for apoptosis in the joint. Evidence for apoptosis in terminally differentiated chondrocytes has been reported [19, 20, 23]. In fact, when chondrocyte DNA is end-labeled, using the terminal deoxynucleotidyl transferase (TUNEL) method, there is preferential staining of hypertrophied and terminally differentiated chondrocytes [1, 7, 12-14, 18, 24]. Although substantial numbers of late hypertrophied chondrocytes undergo apoptotic cell death [7], Roach and co-workers [19, 20] suggested that some chondrocytes undergo apoptosis, while other cells manifest osteoblastic transdifferentiation. The expression of both Bcl-2 and Bax in the hypertrophied chondrocytes lend support to this assumption, since Bax heterodimerizes with Bcl-2, and the ratio of Bcl-2 and Bax determines cell survival after the apoptotic stimulus [3]. The regulation of apoptotic cell death in the region is probably an interplay between these two proteins. In either event, it was conceivable that the region of hypertrophied chondrocytes is the regulatory site of apoptotic cell death during degradation of the SAMP3 cartilage.

Irrespective of the degenerative process in the joint of SAMP3 develops and subsequent condylar deformity occurs, the morphologic alterations seen in the TMJ of SAMP3 were not unlike from those of OA cartilage, in which an extensive apoptosis of the chondrocytes takes place [6, 10]. The main consequence of OA is a progressive remodeling of bone with cartilage matrix degradation [16]; i.e., a loss of collagen with associated fibrillation of the cartilage is a prominent characteristic of OA. Recent studies have provided evidence for the important role of matrix metalloproteinases (MMPs) in the degradation of the cartilage matrix in OA [5, 11, 17]; a disturbance of the physiological balance between MMPs and their inhibitor (TIMPs) results in the disease [9]. Alternatively, the role of PTH and PTHrP for the induction of MMPs during apoptotic cell death has been described [2, 3]. These peptides may bear a pivotal role in development of the cartilage degradation in the SAMP3, in which cartilage simultaneous apoptosis (as expressed by Bcl-2 and Bax) takes place in the region of the hypertrophied chondrocytes.

In OA cartilage, thickening of the synovial membrane can be observed in the early stage of the disease [16]. In the present study using SAMP3, a synovial chondromatosis (membranous cartilaginous metaplasia) was likewise seen at late stages of the degenerative joint disease, in addition to the overt condylar deformity. This phenomenon might be due to an alteration and/or modification of synovial tissues under the peculiar pathologic condition present in SAMP3. In general, the etiology of synovial
chondromatosis includes trauma, OA, and other inflammatory and non-inflammatory joint disease [4]. The accurate explanation of the expression of both Bcl-2 and Bax in the synovial membrane remains unknown. The possibility may arise that the synovial membrane plays a role in the development of the TMJ degeneration in SAMP3, and a paracrine regulation of the cartilaginous metaplasia of the synovial tissue is quite plausible. The occurrence of synovial chondromatosis, as a primary or secondary phenomenon [4], is associated with the nidus of cartilaginous metaplasia in the synovial space of SAMP3 which may be one of the pathologic products linked to the overall degradation of the TMJ in the aged animals.

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VI. References