THE RESPONSE OF COSTAL CARTILAGE TO MECHANICAL INJURY IN MICE

Introduction: The healing potential of articular cartilage in response to injury is poor (1). The main reason is considered to be the lack of blood vessels, articular chondrocytes rarely migrate to sites of injury, and any cells gathering at the defect are easily washed out by joint fluid. In contrast, costal cartilage is covered with perichondrium that has a good supply of blood vessels, and is not subjected to the washing effects of fluid. Therefore, it is considered that costal cartilage has better conditions under which it can heal itself, in comparison with articular cartilage. Clinically, costal cartilage injury has as good a prognosis as simple rib fracture. However, little is known about the biological responses of costal cartilage to mechanical injury. Growth factors, for example bone morphogenetic protein (BMP) and parathyroid hormone-related protein (PTHrP), play crucial roles in chondrogenesis, osteogenesis and fracture healing (2,3,4). Recently, it has been reported that Bcl-2 and Bax are associated with chondrocyte apoptosis, which plays a role in endochondral ossification (5). With regard to the healing response of cartilage to mechanical injury, we are unaware of any studies that have investigated the involvement of factors such as BMP, PTHrP, Bcl-2 and Bax. To address this issue, we histologically investigated the biological response of costal cartilage to mechanical injury in mice.

Methods: Under anesthesia, the left tenth costal cartilage of male mice aged 5 weeks was sharply dissected at a point several millimeters from the growth plate, using microscissors, and only the skin was sutured. At two weeks, and three months after cartilage injury, the mice were sacrificed under anesthesia and the costal cartilage from ribs 9 to 11 were fixed with 4% paraformaldehyde overnight. After demineralization with EDTA for 3 days, paraffin sections were prepared using standard histological procedures. The samples of costal cartilage were coronally sectioned at a thickness of 4μm, and the sections were stained with hematoxylin-eosin and safranin O fast green-iron hematoxylin. Immunohistochemical staining for BMP-2, PTHrP, Bcl-2, Bax, type I collagen and type II collagen was carried out, using antibodies against human BMP-2 (polyclonal), human PTHrP (polyclonal), mouse Bcl-2 (polyclonal), mouse Bax (polyclonal), rat type I collagen, and bovine type II collagen. Reactivity was detected using secondary antibodies and visualized with horseradish peroxidase using DAB as a substrate.

Results: Two weeks after injury, a swollen mass was observed to combine both of the dissected cartilage ends (Fig 1-A). The mass consisted of chondrocyte-like cells, fibroblast-like cells and extracellular matrix that was stained with safranin O, and was covered with a thick perichondrium-like membrane. Within the mass, chondrocyte-like cells were observed near the cartilage ends, and these were positively stained for type II collagen as well as Bcl-2 and PTHrP (Fig 1-B). In contrast, fibroblast-like cells were observed in the center and periphery of the mass, and these were positively stained for BMP-2 (Fig 1-C). No staining for Bax or type I collagen was detected in the mass. Three months after injury, the cartilage ends had not recombined; they were covered with a fibrous membrane, and formed a pseudo-articulation with a cavity (Fig 2). Staining for PTHrP, Bcl-2, Bax and type I collagen was detected in the cells of the pseudo-articulation.

Discussion: Two weeks after costal cartilage injury, both ends of the served cartilage were covered by a swollen mass, in which fibroblast-like cells produced BMP-2 and chondrocyte-like cells produced type II collagen. PTHrP and Bcl-2. BMP-2 may upregulate chondrogenesis by mesenchymal cells (2), PTHrP and Bcl-2 may downregulate hypertrophic changes in chondrocytes, thus contributing to cartilage growth (4,5). These expression patterns observed in the mass were considered to enhance the self-healing of the cartilage. Despite these early biological responses, the newly formed cartilaginous mass had failed to unite with the cartilage ends by three months after injury. These results suggest that re-combination of the type II collagen network in cartilage may be difficult even if early biological healing responses are present. We speculate that the main reason for failure of costal cartilage healing may be repetitive micromovement due to breathing, which causes strain in the cartilage. This study has thus demonstrated the early response of cartilage to mechanical injury and failure of the cartilage to recombine.


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