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Structural features of incremental line-like striations in mandibular condylar cartilage of *c-src*-deficient mice

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Summary Mandibular condylar cartilage is sensitive to masticatory force, while mice lacking the *c-src* gene (*c-src*-deficient mice) have osteopetrosis and tooth eruption failure. The purpose of this study was to investigate the morphology of the mandibular condyle in these mice, which were maintained with a soft-food diet for 240 days after birth. The condylar head in the *c-src*-deficient mice showed slight deformity in shape before weaning, but showed remarkable undergrowth after weaning. No significant morphological or histological differences were detected between the mandibular condyle in wild-type mice fed soft food and those fed hard food, indicating that osteopetrosis, as well as abnormal masticatory force, influences the morphology of the mouse mandibular condyle, and that malocclusion rather than dietary consistency may have greater influence. After 70 days, incremental line-like striations consisting of cartilaginous and non-cartilaginous layers were detected in the mandibular condyle of the *c-src*-deficient mice, but not in the tibial growth plate. Immunostaining of aggrecan, collagen types II and X, and osteopontin was detected in the cartilaginous layers, but not in the non-cartilaginous layers showing collagen type I immunostaining. Chondrocyte lacunae were not eroded in the cartilaginous layers, and complete circumferential mineralisation around the lacunae and impaired osteoclast (chondroclast) function can account for this phenomenon. However, repeated cessation of chondrocyte differentiation may be required to completely

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explain the formation of the striations. These results indicate that the mandibular condyle in the *c-src*-deficient mice has unique structural features, adding to its deformity.

Introduction

c-src is first identified as the cellular counterpart of the transforming protein of Rous sarcoma retrovirus, *v-src*. *c-src* is a non-receptor type tyrosine kinase and is involved in cell differentiation via intracellular signal transduction.[1] Although this molecule is expressed in all cells at various levels, the primary defect in mice lacking the *c-src* gene is osteopetrosis, resulting from a deficiency in bone resorption of osteoclasts.[2,3] *c-src*-deficient mice form osteoclasts in normal or increased numbers,[2,4] but osteoclast function is defective due to failure to form ruffled borders.[4,5] The histological features of osteopetrosis, which consist of thickened growth plates, extension of the osseus trabeculae, and decreased bone marrow formation, are well documented in long bones,[2,4] but not in the mandibular condyle. In addition, *c-src*-deficient mice have dental abnormalities associated with tooth eruption failure.[4,6] Because they are toothless, soft food/liquid diets are required for long-term survival.[4]

Mandibular condylar cartilage constitutes the temporomandibular joint and has specific histological features that differ from growth plate cartilage, including existence of a surface fibrous cell zone, arrangement of chondrocyte columns, vascular invading pattern, and method of chondrocyte lacunae erosion.[7] The morphology of condylar cartilage is also affected by dietary consistency, which produces variation in masticatory force.[8–11] Therefore, osteopetrosis, malocclusion (toothlessness) and a soft-food diet may all influence the morphology of condylar cartilage.

Another osteopetrotic mouse model, the *op/op* mouse, is caused by a point mutation in the *colony stimulating factor 1* gene.[12–14] The osteoclasts in these mice are extensively reduced in number,[14] indicating that osteoclast formation rather than osteoclast function is blocked. These mice also have dental abnormalities associated with tooth eruption failure.[6,12,13] Kawata et al.[15] investigated the mandibular condyle in *op/op* mice, and reported undergrowth of the mandibular condyle and mandibular ramus. They considered these deformities to result from not only an osteoclast deficiency, but also insufficient mechanical stress from mastication. These findings led us to hypothesise that the mandibular condylar cartilage of *c-src*-deficient mice has morphological deformities resulting from osteopetrosis, malocclusion and dietary consistency. Further, because the mechanism leading to osteopetrosis in *c-src*-deficient mice is different from that in *op/op* mice, we also hypothesised that the mandibular condylar cartilage in *c-src*-deficient mice may have specific structural features.

Thus, we observed incremental line-like striations in the condylar cartilage of adult *c-src*-deficient mice.

Materials and methods

Animals

All animals were housed in facilities approved by the Central Research Institute of the Electric Power Industry. Our animal-use protocol conformed to the NIH guidelines as stated in the ''Principles of laboratory animal care'' (NIH publication No. 86-23, revised 1985) and was reviewed and approved by the Screening Committee for Animal Research of the Central Research Institute of the Electric Power Industry.

Mice heterozygous for disrupted *c-src* alleles (src^{tm1Sor}) were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). Twenty *c-src*-deficient mice (homozygotes) and 20 *c-src*-wild-type mice were used in present study. Soft food consisting of jelly and crushed food was fed, after weaning, to *c-src*-deficient mice for up to 240 days after birth. *c-src*-wild-type mice were weaned at 21 days after birth, since eruption of molars and occlusion complete at this age.[16,17] To investigate the effects of a soft-food diet, *c-src*-wild-type mice were divided into two groups after weaning (21-day-old): one group was fed normal hard food (wild-type H) and the other was fed soft food (wild-type S).

Tissue preparation

The mice were anaesthetised with ether and sacrificed by cervical dislocation at 17-day-old (before weaning), 37-day-old (16 days after weaning), 70-day-old (7 weeks after weaning), and 240-day-old (32 weeks after weaning). The mandible of each mouse was dissected and immediately immersed in 4% paraformaldehyde (0.1 M phosphate buffer, pH